# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Banana and Banana Trunk

Bananas are produced in large quantities in tropical and subtropical areas and known as one of the most consumed fruits in the world; according to the world wide analysis, India, China, Philippines, Ecuador, and Brazil are the five largest producers of banana around the world with the production of banana biomass approximately 6.6 – 7.0 million tonnes per year (Food and Agriculture Organization of the United Nations, 2014, 2017). Malaysia also not excluded as one of the country that produces an abundance of banana wastes around 300,000 – 400,000 tonnes yearly from each hectare of the banana crop plantation area which has been imposed a great environmental burden (Agriculture and Agri-Food Canada, 2014).

The banana trunks as shown in Plate 2.1 normally contribute as the major amount of the biomass wastes and they are usually left on the plantation area to avoid soil erosion and act as an organic fertilizer. Those banana trunks are made up of pseudostem which consists of 3 major elements including the cellulose, hemicellulose, and lignin. Therefore, the harvested banana trunk can be known as the lignocellulosic biomass wastes. Figure 2.1, 2.2, and 2.3 shows the chemical structure of cellulose, hemicellulose, and lignin respectively.

**Plate 2.1**: Banana trunks.

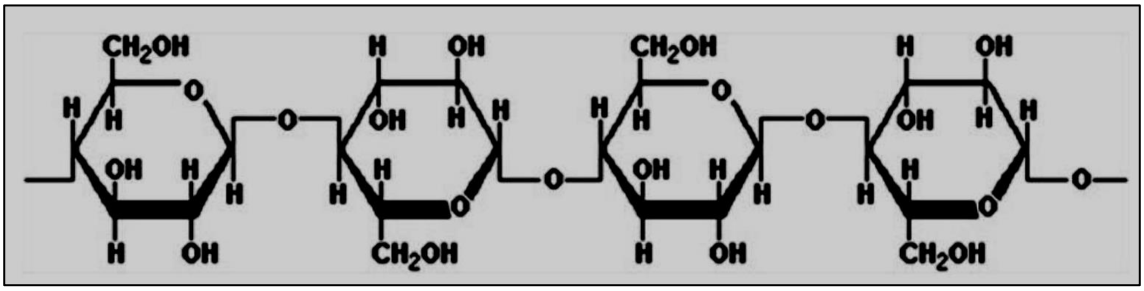

**Figure 2.1**: Chemical structure of cellulose (Bajpai, 2016).

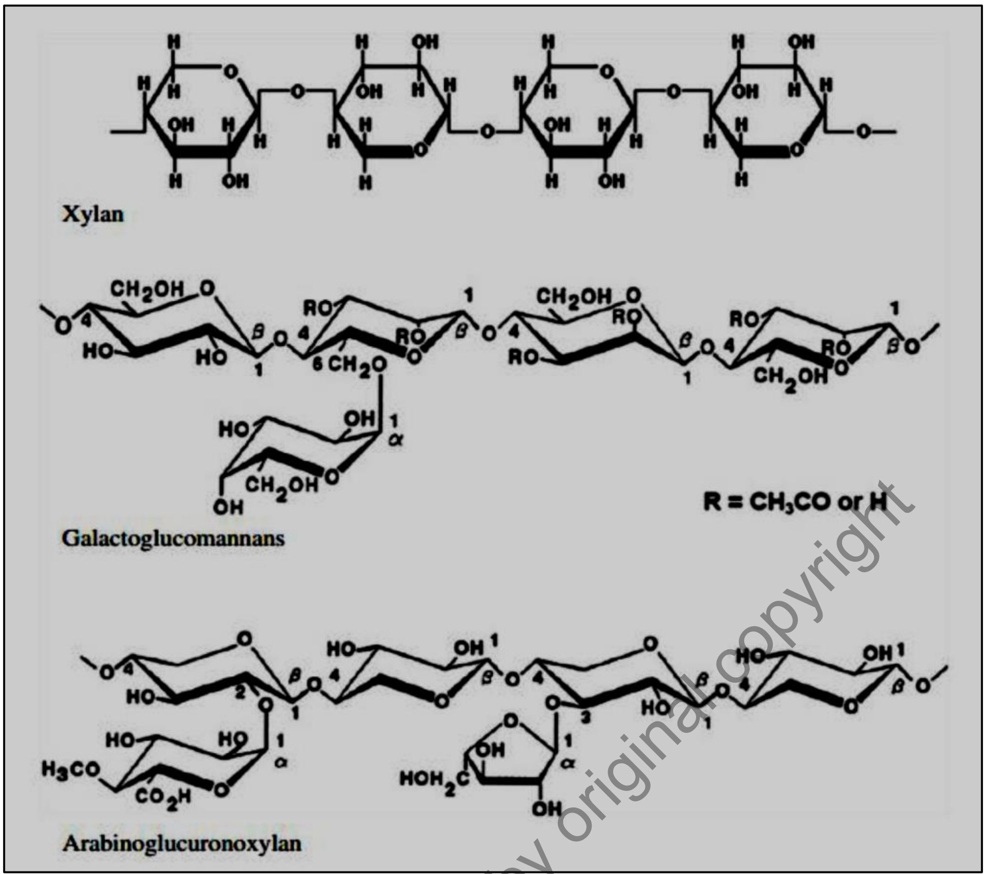


**Figure 2.2**: Chemical structure of hemicellulose (Bajpai, 2016).

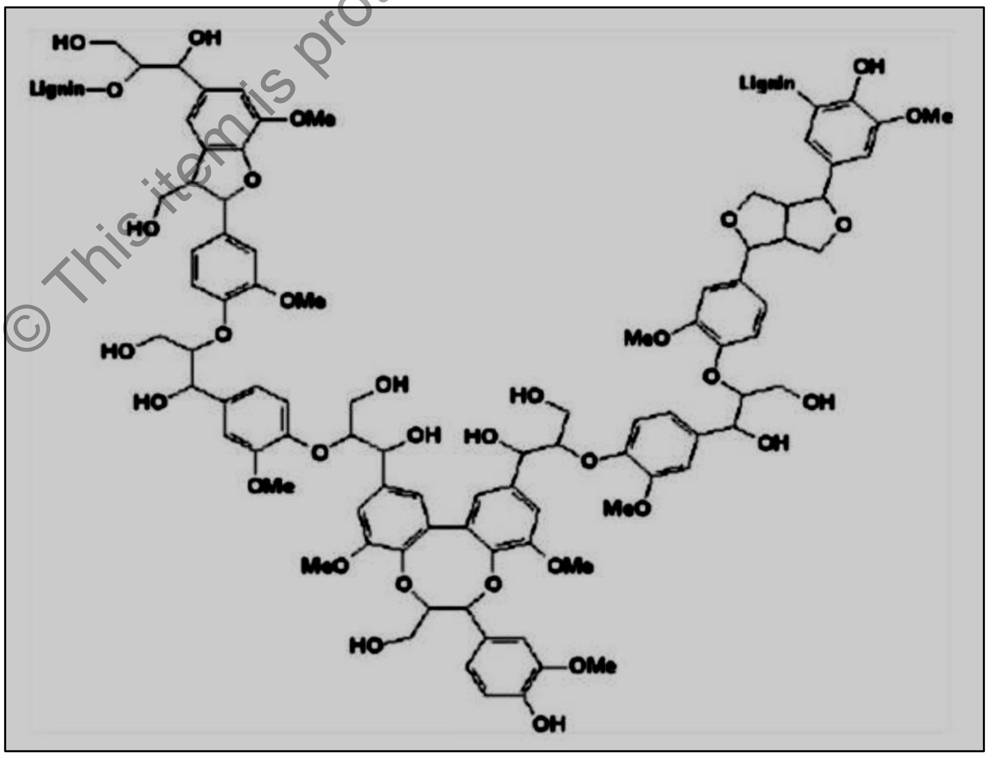

**Figure 2.3**: Chemical structure of lignin with complex cross-linked polymer of aromatic ring (Bajpai, 2016).

Cellulose is the main constituent of plant cell wall conferring structural support. It has long-chain of polymers which linked together by hydrogen and van der Waals bonds as shown in Figure 2.1 (Bajpai, 2016). Cellulose functions to be packed into microfibrils via hydrogen bonding. It can be present in both crystalline and amorphous forms (Bajpai, 2016). It can be hydrolyzed into fermentable glucose by breaking the beta-1,4-glycosidic linkages (Mtui, 2009).

Hemicellulose is the second most abundant polymer of lignocellulose biomass. It has branches with short lateral chains consisting of different types of monosaccharaides include pentoses, hexoses, and uronic acids as shown in Figure 2.2 (Bajpai, 2016). Hemicelluloses within plant cell walls function to bind strongly to cellulose fibrils by hydrogen bonds. It is thermo-chemically sensitive which potentially to be degraded into fermentation inhibitors (e.g. furfurals and hydroxymethyl furfurals) under high temperature (Myat and Ryu, 2015). It should be removed via pretreatment to increase cellulose digestibility by carefully optimizing the pretreatment process.

Lignin is the third most abundant polymer of lignocellulose biomass. It has a complex and large molecular structure containing cross-linked polymers of phenolic monomers as shown in Figure 2.3 (Bajpai, 2016). Lignin functions to bind the different components of lignocellulosic biomass together, thus making it insoluble in water. It present in plant cell walls to impart structural support and impermeable resistance against microbial attack (Bajpai, 2016). It must be removed via pretreatment to enhance biomass digestibility.



## 2.2 Reutilization of Banana Trunk Wastes as the Lignocellulosic Raw Materials

Lignocellulosic wastes normally refer to plant biomass wastes including agricultural residues, pulp mill refuses, municipal solid wastes, and garden wastes which are mainly composed of lignin, cellulose, and hemicellulose (Chidi *et al.*, 2015). Because of their renewability in abundance wastes supply, there has a potential to utilize those wastes in production of second-generation value-added bioproducts such as reducing sugars, ethanol, carbohydrates, amino acids, phenolic compounds, lipids, and surfactants in order to eliminate environmental wastes.

According to Chidi *et al*. (2015), banana trunk is one of the lignocellulosic plant examples which contribute the potential in providing a promising amount of cellulose. A banana trunk consist of moisture content of 95.3% ± 0.2% which can be further converted into dry basis in order to analyse out the lignocellulosic contents of banana trunk (Filho *et al*., 2013). Based on the researchers' report analysis, banana trunk consists of higher cellulose content and lower lignin content compared to most of the other plants (Li *et al*., 2010; Filho *et al*., 2013; Preethi and Balakrishna., 2013). Table 2.1 shows the details of lignocellulosic content analysis according to the literature findings.

**Table 2.1**: Comparison of lignocellulosic content among various agricultural biomasses.

| Biomass | Cellulose | Hemicellulose | Lignin | Reference |
|---|---|---|---|---|
| Banana trunk | 60-65% | 6-8% | 5-10% | Preethi and Balakrishna, 2013 |
| Sugarcane bagasse | 32-48% | 19-24% | 23-32% | Preethi and Balakrishna, 2013 |
| Corn stover | 38-40% | 28-30% | 7-21% | Preethi and Balakrishna, 2013 |
| Paddy straw | 33-38% | 26-32% | 17-19% | Filho *et al*., 2013 |
| Rice straw | 28-36% | 23-28% | 12-14% | Li *et al*., 2010 |



Since the hemicellulose content is low in banana trunk biomass, it is suitable to include the hemicellulose in the glucose production research study as the hemicellulose also includes the glucose unit block in order to achieve more yield of glucose. A research study including the cellulose and hemicellulose can ensure the zero wastes practice in bioconversion technology. Although the high cellulose content of banana trunk biomass believed to contribute the high glucose yield compared to other crops; however, the low lignin content of banana trunk biomass might not give a promise in easier delignification process with lower lignin removal agent (Filho *et al*., 2013).

Currently, there are still lots of research in progress to determine the effectiveness of lignin removal of banana trunk by comparing various selected pretreatment methods.

## 2.3 Glucose Overview and Its Application as Fermentable Sugar

Glucose is a monosaccharide with six carbon atoms and classified as a hexose with chemical formula of $C_6H_{12}O_6$ and molecular weight of 180.16 g/mol (Bruice, 2014). This fermentable glucose with aldehyde group is the building block of cellulose; therefore, cellulose of agricultural biomass can be break down into glucose monomers by using cellulase enzyme.

There are lots of crop choices to be utilized to produce fermentable sugar such as corn, potato, sugarcane, wheat and sugar beet via the enzymatic hydrolysis of starch. The fermentable glucose has been contributing lots of benefits in industrial usage. It is the largest feedstock available to support a bio-based chemicals industry with the utilization to produce biochemical products such as bioethanol, biofuel, acetic acids, amino acids, and antibiotics through the process of fermentation.

According to the previous studies done by Rambo *et al*. (2015), Li *et al*. (2010), and Cordeiro *et al*. (2004), the predominant monomer in banana trunks is glucose and followed by other reducing sugars which have the high suitability to be reutilized for bioconversion purpose. Table 2.2 shows the monosaccharide composition of banana trunk biomass based on the literature review.

**Table 2.2**: Monosaccharide composition of banana trunk biomass.

| Monosaccharide Composition | | | | | Reference |
|---|---|---|---|---|---|
| **Glucose** | **Xylose** | **Arabinose** | **Galactose** | **Mannose** | |
| 64% | 13% | 6% | 2% | 3% | Rambo *et al*., 2015 |
| 72% | 12% | 8% | 3% | 1% | Li *et al*., 2010 |
| 74% | 13% | 9% | 3% | 1% | Cordeiro *et al*., 2004 |

Apart from sugarcane bagasse and straw, other biomass sources from agricultural residues have been considered in glucose conversion to prevent overdependence on a single resource. Rambo *et al*. (2015) made a sugars composition analysis on various selected lignocellulosic biomass to evaluate the best alternative biomass as primary monosaccharaides source. Table 2.3 shows the comparison of glucose composition in various lignocellulosic biomasses. From the observation, banana trunk biomass highlighted to have high potential in glucose production.

**Table 2.3**: Comparison of glucose composition in various lignocellulosic biomasses (Rambo *et al*., 2015).

| **Biomass** | **Glucose composition (%)** |
|---|---|
| Banana trunk | 64 |
| Bamboo | 45 |
| Rice husk | 37 |
| Soy peel | 36 |
| Coconut | 33 |



## 2.4 Pretreatment on Banana Trunk Wastes as Lignocellulosic Materials

All the lignocellulosic materials consist of the structural characteristics of rigid and recalcitrant to provide plant protection and mechanical support (Chidi *et al*., 2015). The target of any pretreatment is to enhance the rate of enzymatic hydrolysis on extracted cellulose in order to improve the production of desired products by removing the structural impediments which made up of lignin and hemicellulose (Mtui, 2009). According to the Filho *et al*. (2013), with a preliminary pretreatment on lignocellulosic biomass may increase the acid hydrolysis yields and enzymatic hydrolysis yields over 8-fold up to 23-fold. The pretreatment of lignocelllosic substrate can be achieved by mechanical and chemical approaches.

### 2.4.1 Mechanical Pretreatment

Mechanical pretreatment involves the reduction of particle size of lignocellulosic materials by using the method of mechanical grinding or milling. Lignin depolymerisation occurs during mechanical pretreatment through the cleavage of uncondensed aryl ether linkages (Inoue *et al*., 2008). This lignin depolymerisation leads to the enhancement of enzymatic digestibility on the extracted cellulose because of the increase of surface area of pulverized materials helping in enhancement of facilitation to subsequent chemical pretreatment on the lignocellulosic materials.

Dry grind milling is chosen in this research study compared to wet grind milling due to the reason for time saving. Although the wet grind milling provides the lower energy consumption and high effectiveness in enzymatic hydrolysis, but it requires approximately ten times repetitions of milling process to complete same amount of sample batch as in dry grind milling (Kumar and Sharma, 2017). Kumar and Sharma (2017) stated that dry grind milling is suitable to pilot or lab scale of research as the high energy consumption would not bring any significant effect in delignification process. Wet grind milling can be considered after the determination on industrial application for effectiveness enhancement in enzymatic hydrolysis.

Most chemical pretreatments are not successful without size reduction (Karimi, 2013). According to Bensah and Mensah (2013), biomass particle size smaller than 1 mm provides the best efficiency in lignocellulosic biomass pretreatment and 500 µm particle size of banana trunk biomass was chosen in this research to ensure higher surface area for biomass wastes in contact with chemical attacks for substrate porosity improvement in subsequent enzymatic hydrolysis steps.



### 2.4.2 Chemical Pretreatment

Chemical pretreatment involves the degradation of lignocellulosic biomass components including lignin, hemicellulose, and cellulose by using chemicals such as acids and alkali. The selection of the pretreatment method used for a particular lignocellulosic biomass is depending on its intrinsic structure of the biomass. There is no one fixed chemical in pretreatment on various lignocellulosic biomasses.

According to Mtui (2009), it has been reported that it was more effective in pretreatment by combining the activity of acids and alkali together rather than they just operating alone. This statement has been explained by researchers that a combination pretreatment method is beneficial in minimizing the probability of enzyme inhibitors production which will influence the glucose yield in enzymatic hydrolysis process later (Kumar and Sharma, 2017).

However, the best and suitable chemical type in particular biomass pretreatment must be determined out first before any further improvement and development of new efficient combination pretreatment method for lignocellulosic biomass. Sulphuric acid ($H_2SO_4$) and sodium hydroxide (NaOH) are chosen to be studied in this research since they are the most commonly used as acid pretreatment agents and alkaline pretreatment agents respectively due to their suitability in product yields and cost effectiveness according to the studies made by researchers (Tutt *et al.*, 2012; Singh and Trivedi, 2013).



#### 2.4.2.1 Acid Pretreatment

Acid pretreatment is one of the chemical pretreatment used to disrupt the hydrogen bonds, covalent bonds, and van der waals forces that hold the biomass components in order to solubilize and recrystallize the lignocellulosic biomass (Li *et al.*, 2010; Chidi *et al.*, 2015). According to Tutt *et al.* (2012), acid pretreatment is best preferred for biomass with high hemicellulose content level based on its functionality in effectiveness of lignin penetration without any preliminary pretreatment of biomass.

The major drawback problem of acid pretreatment application is this method may produce by-products such as hydroxymethylfurfural (HMF) which may act as the enzyme inhibitors for further process of enzymatic hydrolysis of cellulose and hemicellulose in order to obtain high glucose yield (Tutt *et al.*, 2012; Singh and Trivedi, 2013). Singh and Trivedi (2013) stated that acid concentration must be controlled condition for promising of pretreatment effectiveness due to the reason that high concentration of acid may cause the formation of enzyme inhibitors – hydroxymethylfurfural. If the use of concentrated acid forced to do so in high temperature, a shorter time of pretreatment is required to implement (Mtui, 2009; Tutt *et al*., 2012).

### 2.4.2.2 Alkaline Pretreatment

Alkaline pretreatment is another chemical pretreatment used to enable swelling of lignocellulosic biomass via saponification reaction in order to disrupt the lignin structure and separate the structural linkages between lignin and other cellulose component of biomass by increasing the internal surface area of biomass (Singh and Trivedi, 2013; Chidi *et al*., 2015). According to previous study by Tutt *et al*. (2012), alkaline pretreatment is best preferred for biomass with high lignin content level based on its functionality in enhancement of susceptibility of cellulose to enzyme hydrolysis.

The major drawback problem of all alkaline pretreatment application is this method may contribute in slight lower sugar yields in enzymatic hydrolysis process due to the reason that most of the cellulose and hemicellulose are unable to dissolve and soluble with the alkaline pretreatment (Tutt *et al*., 2012; Singh and Trivedi, 2013). Chidi *et al*. (2015) was suggested that alkaline pretreatment should be perform at lower temperature with high alkaline concentration in order to ensure high effectiveness of saccharification by overcoming the low solubility of cellulose and hemicellulose with the alkaline pretreatment.

## 2.5 Factors Affecting Chemical Pretreatment of Lignocellulosic Materials for Efficient Enzymatic Hydrolysis

The critical chemical pretreatment parameters in optimization studies for efficient enzyme hydrolysis include the substrate concentration, treatment duration, treatment temperature, and chemical concentration. All these parameters need to be considered as important influencing factors in order to determine the optimal pretreatment conditions for the purpose of improving the sugars (glucose) production via enzymatic hydrolysis, avoiding the carbohydrate (glucose) degradation during pretreatment, preventing the enzyme hydrolysis inhibitor (furfural) formation during pretreatment, and reducing the cost of pretreatment in consideration of temperature, duration, and substrate loading applications (Kumar *et al*., 2009; Brodeur *et al*., 2011).

### 2.5.1 Effects of Substrate Concentration in Chemical Pretreatment Process

The substrate concentration can be seen as the availability of the lignocellulosic materials to be reacted with the chemical applied, in other words, substrates can act as a limiting reactant in chemical pretreatment process (Lopez-Arenas *et al*., 2010). Karimi *et al*. (2013) stated that optimum hydrolysis is impossible to happen at high substrate concentrations due to the facts of lignin redistribution and the structural modifications within the substrate.

Mass transfer of chemical into biomass matrix plays an important role to the biomass structural disruption; however, mass transfer limitation would occur during the application of saturated substrate concentration in pretreatment process (Karimi *et al*., 2013). Karimi *et al*., 2013 mentioned that saturated substrate concentration will cause the decrease of liquid content due to high viscosity of reaction medium and this will further lead to non-homogeneous distribution between substrate and chemical solution for proper pretreatment reaction. To ensure the high porosity formation of pretreated substrate, it was suggested that to apply low substrate concentration (5 – 10% (w/v)) for temperature greater than 160 ℃ or high substrate concentration (10 – 40% (w/v)) for temperature smaller than 160 ℃ (Harmsen *et al*., 2010).

### 2.5.2 Effects of Treatment Duration in Chemical Pretreatment Process

Time contact between lignocellulosic biomass and applied chemical is the crucial consideration in reduction of cellulose crystallinity and improvement of surface area together with porosity on biomass (Bensah and Mensah, 2013). The longer the pretreatment residence time, the proper the biomass solubilisation and reduction of biomass particle size (Myat and Ryu, 2015). Research by Myat and Ryu (2015) stated that efficiency of the enzymatic hydrolysis always correlated with the pore volume and accessible surface area of cellulose after pretreated.

Lopez-Arenas *et al*. (2010) and Lei *et al*. (2013) reported that every increment of 5 minutes of pretreatment duration would lead to approximately 6 ± 1% increase of total glucose yield in subsequent enzymatic hydrolysis. Although a longer residence time for substrates to contact with the chemicals can promote the greater degree of depolymerization of cellulose via the structural linkage disruption of the lignocellulosic substrates, however, there is always have a limitation to achieve maximum glucose conversion due to the glucose degradation into enzymatic inhibitors such furfurals and HMFs (Karimi and Taherzadeh, 2016). The formation of inhibitors can be prevented by applying proper reaction time under specific temperature condition – 30 minutes under temperature of 120 ℃ as suggested by Singh and Trivedi (2013) and Chidi *et al*. (2015).



### 2.5.3 Effects of Treatment Temperature in Chemical Pretreatment Process

Heating is an essential process to provide additional energy for the deconstruction and disruption of lignocellulosic biomass by altering the physical and chemical structures of the target biomass (Iroba *et al*., 2013). Appropriate application of temperature can enhance the accessibility and digestibility of the cellulose in subsequent enzymatic hydrolysis through creating pores on the biomass matrix and decreasing the crystallinity index of the target fibres during pretreatment process (Myat and Ryu, 2015).

Based on the kinetic studies made by Lopez-Arenas *et al*. (2010), Lei *et al*. (2013), and Iroba *et al*. (2013), they stated that every increment of 1 ℃ of pretreatment temperature would contribute in 1% increase of glucose conversion rate. However, formation of hydrolytic inhibitor products also strongly increases with temperature (Myat and Ryu, 2015). Myat and Ryu (2015) reported that the temperature higher than 160 ℃ will contribute to the production of furfural or hydroxymethylfurfural (HMF) during chemical pretreatment process. Therefore, to prevent glucose degradation to the HMF, lower pretreatment temperatures were aimed to be study in order to achieve optimum glucose formation in this research on banana trunk biomass wastes.

### 2.5.4 Effects of Chemical Concentration in Chemical Pretreatment Process

In order to ensure an effective and efficient subsequent hydrolysis process after pretreatment process, the key factor of toxic compounds elimination must be given the highest consideration (Myat and Ryu, 2015). Chemical concentration plays the most important role to depolymerize the lignocellulosic biomass due to the reason of the glucose production of pretreated biomass was found to be highly depend on chemical concentration compared to others factors such as pretreatment temperature based on the studies made by Bensah and Mensah (2013).

Different concentration of chemical used in lignocellulosic biomass pretreatment process will leads to different effect on the subsequent hydrolysis process in glucose yields. Lignin fraction might release phenolic compounds which considered as the fermentation and hydrolysis inhibitors if a strong acid or strong alkali is used to pretreat lignocellulosic biomass (Myat and Ryu, 2015). According to Myat and Ryu (2015), lignin degradation can be neglected under pretreatment condition of dilute chemicals application at temperature lower than 180 ℃. Therefore, dilute chemicals were used in this research as the constant parameter for optimization studies of glucose production since dilute chemical with concentration of 2% ((w/v) or (v/v)) was believed to have optimum glucose yields in hydrolysis steps without leading any toxic compounds formation effects according to Mtui (2009), Singh and Trivedi (2013), and Chidi *et al*. (2015).

## 2.6 Recovery of Sugars from Cellulase by Enzymatic Hydrolysis

Enzymatic hydrolysis normally been chosen in recovery of sugars after pretreatment steps compared to acid hydrolysis due to its benefits in low energy consumption and environmental friendly (Chidi *et al*., 2015; Wan Azelee *et al*., 2016). Fermentable sugars including glucose, arabinose, galactose, and xylose will come first in the value chain of pretreated lignocellulosic biomass; however, glucose is believed will be the major monosaccharide products in this research study from the banana trunk biomass according to the monosaccharide composition studies carried by Rambo *et al*. (2015), Li *et al*. (2010), and Cordeiro *et al*. (2004) as stated in subtopic 2.3.

Enzyme cellulases are needed in the hydrolysis and conversion of cellulose into the intended products which is glucose as mentioned above. This type of enzymes mainly produces from lignocellulose degrading fungi or bacteria and the well-known example is fungus *Trichoderma reesei* which acts as the benchmark organism for cellulases production for the purpose of biomass conversion in industry usage today due to its high potential in degrading cellulose-rich biomass into fermentable sugars (Paloheimo *et al*., 2016).

The enzyme hydrolysis condition basis in this research was taken according to the optimum hydrolytic parameters studies on lignocellulosic biomass by Kashyap *et al*. (2007), Mtui (2009), Filho *et al*. (2013), and Wan Azelee *et al*. (2016) as stated in Table 2.4.



**Table 2.4**: Subsequent enzymatic hydrolysis conditions after chemical pretreament for determination of glucose formation.

| Hydrolysis condition | Condition level | Reference |
|---|---|---|
| Hydrolysis temperature | 45 ℃ | Filho *et al*., 2013 |
| Hydrolysis duration | 30 minutes | - |
| Enzyme concentration | 7% (v/w) | Kashyap *et al*., 2007 |
| Substrate concentration | 3% (w/v) | Wan Azelee *et al*., 2016 |
| pH of buffer solution | pH 5.0 | Mtui, 2009 |

## 2.7 Product Yield Optimization Studies using Design-Expert Software

By considering all the critical reaction parameters including the treatment temperature, treatment duration, and substrate concentration; optimization on the best chemical pretreatment method for highest production of glucose can be done by using Design-Expert software involves the application of Response Surface Methodology (RSM) (Chi *et al*., 2012). RSM aims to discover the optimum operating conditions by modifying a particular intended response through receiving information on the direct effects, curvilinear variable effects, and pair wise effects from Central Composite Design (CCD) (Khuri, 2017). CCD plays an important role as flexible and efficient response design to provide services in optimization studies by analyzing experiment variable effects and overall percentage error in a number of experimental runs (Olayiwola *et al*., 2011).