CHAPTER 2 THEORY AND LITERATURE REVIEW

In this chapter, the theory of bioethanol process, the definition of lignocellulose material with consideration of sugarcane bagasse, parameters for Lignocellulose pretreatment, the chemical pretreatment method, analysis technology and the Respond Surface Method (RSM) method are presented. Moreover, the literature reviews contains the topic of the chemical pretreatment method consisting of acid pretreatment, alkaline pretreatment as reported by various researchers.

2.1 Theory

2.1.1 Bioethanol Production

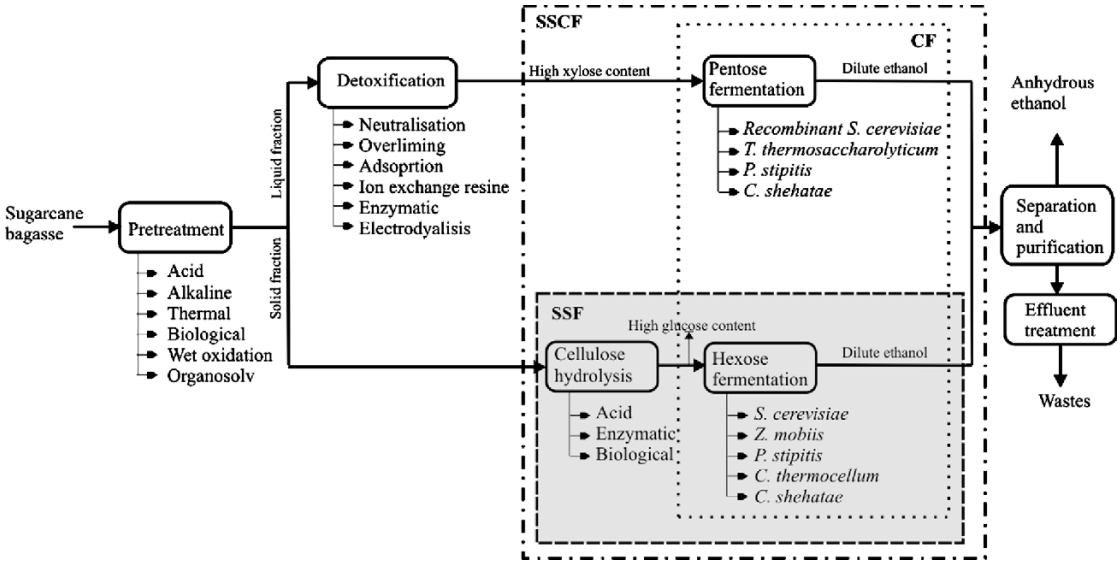

Figure 2.1 Process of Bioethanol Production from Sugarcane Bagasse [1]**.**

The main process for ethanol production from Sugarcane Bagasse consists of 4 steps: sugarcane bagasse pretreatment, enymatic hydrolysis, fermentation and separation and purification as shown in Figure 2.1 [10].

For the first process, pretreatment is requied to remove lignin and hemicellulose from bagasse by chemical method using acid, alkaline, oganic solvent or wet oxidation. Moreover, physical-chemical pretreatment and thermal pretreatment can be applied for delignification process. During pretreatment procees, the inhibitory substreaces which are toxic to enzyme are hydrolyzed into liqiud fraction such as furfural and hydroxymethylfurfural (HMF) which, found in pentose degradation and acetic acid from acetyl group of hemicellulose degradation. Therefore, detoxification process is presented to purify the filtreated by neutralisation, adsorbtion, ion exchange resins and overliming etc. before pentose fermentation [1]. The pretreated bagass in solid phase

that mainly contains of cellulose is hydrolyzed by enzymatic saccharification or dilute acid to form sugar monomer (glucose). Enzymatic hydrolysis is an effective method for cellulose hydrolysis since there is no degration of glucose and no corrosion problem [1]. After hydrolysis process, the fermentation is applied to convert sugar into ethanol product by using yeast or enzyme; nevertheless, the high product yield of ethanol from fermentation depends on the effectiveness of pretreatment process . Finally, ethanol is purified to improve the product value and product specification.

2.1.2 Lignocellulose Material

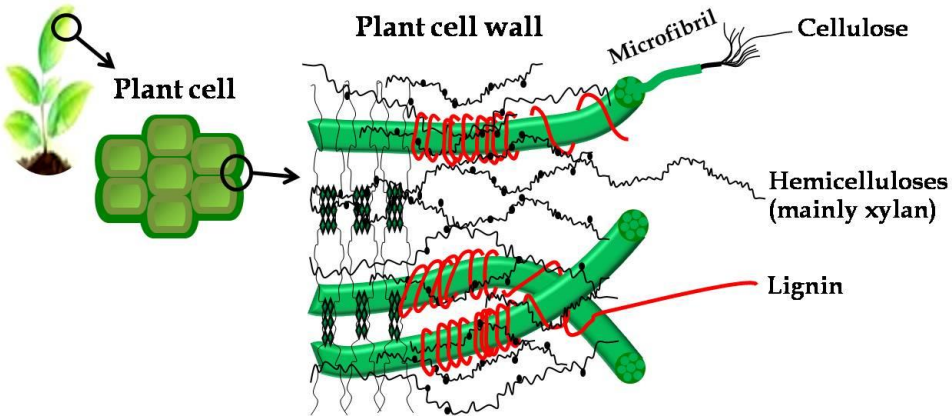

Figure 2.2 The lignocellulose material structure [19]**.**

Lignocellulose is a plant dry matter (biomass) called lignocellulosic biomass. It is the most abundant raw material on earth, which is widely used to produce bio-fuel, mainly bioethanol. Lignocellulose consisted of carbohydrate polymer (cellulose, hemicellulose) and aromatic polymer (Lignin)**.**

1. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundred to over ten thousand that combined together with β(1→4) linked D-glucose units. Cellulose is odorless, hydrophilic and insoluble in water and most of the organic solvents have no teats. There are two regions in cellulose structure that are crystalline and amorphous. The cellulose can be broken down to glucose by treating with strong acid at high temperature. Cellulose is an important structural component of the primary cell wall of green plants, algae and the oomycetes and is the most abundant organic polymer on Earth [33].

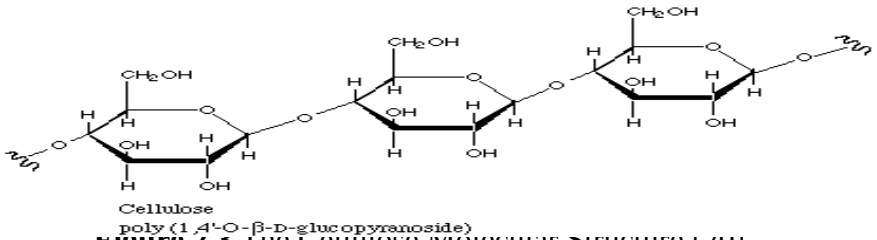

Figure 2.3 The Cellulose Molecular Structure [20].

2. Hemicellulose is a heteropolymers which, presented along with cellulose in almost all plant cell walls. Hemicellulose has a random, amorphous structure with little

strength and easily hydrolyzed by dilute acid or base as well as myriadhemicellulase enzymes. The compositions of hemicellulose consist of xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. After hydrolysis, the hemicellulose is transformed into sugar monomers such as xylose, mannose, galactose, rhamnose, and arabinose. Hemicelluloses contain moslyt of the D-pentose sugars, and occasionally small amounts of L-sugars. The most cases, xylose is the sugar monomer present in the largest amount. Not only sugars can be found in hemicellulose, but also their acidified form, especially glucuronic acid and [18].

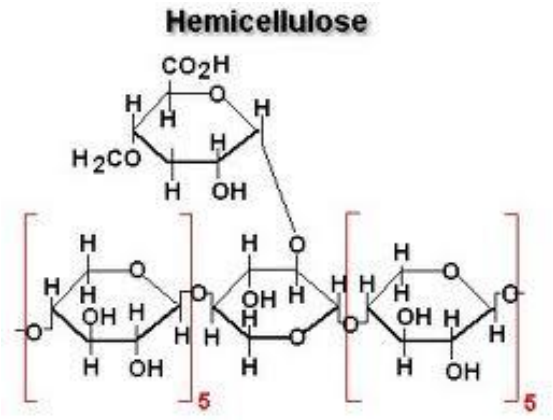

Figure 2.4 The Hemicellulose Molecular Structure [18].

3. Lignin is a cross-linked racemic macromolecule, relatively hydrophobic and aromatic polymer. It is a protective shield around cellulose and hemicellulose for protecting the polysaccharide from enzymatic hydrolyzed which convert sugar to ethanol. There are three monolignol monomers: p-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol. These lignols are incorporated into lignin in the form of the phenylpropanoids p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S), respectively [17].

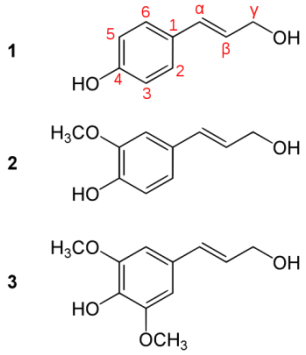

Figure 2.5 The three common monolignols: paracoumaryl alcohol (1), coniferyl alcohol (2), and sinapyl alcohol (3).

In general, lignocellulose materials used for the production of ethanol biofuel can be divided into six groups; *crop residual* (sugarcane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones and pulp), *hard wood* (aspen and poplar), *softwood* (pine and spruce), *cellulose wastes* (newsprint,

waste office paper and recycled paper sludge), *herbaceous biomass* (alfalfa hay, switch grass, reed canary grass, coastal Bermuda grass and timothy grass), and *municipal solid wastes (MSW)* [1].

2.1.3 Chemical Hydrolysis

1. The Mechanism of Cleavage of Glycosidic Bonds

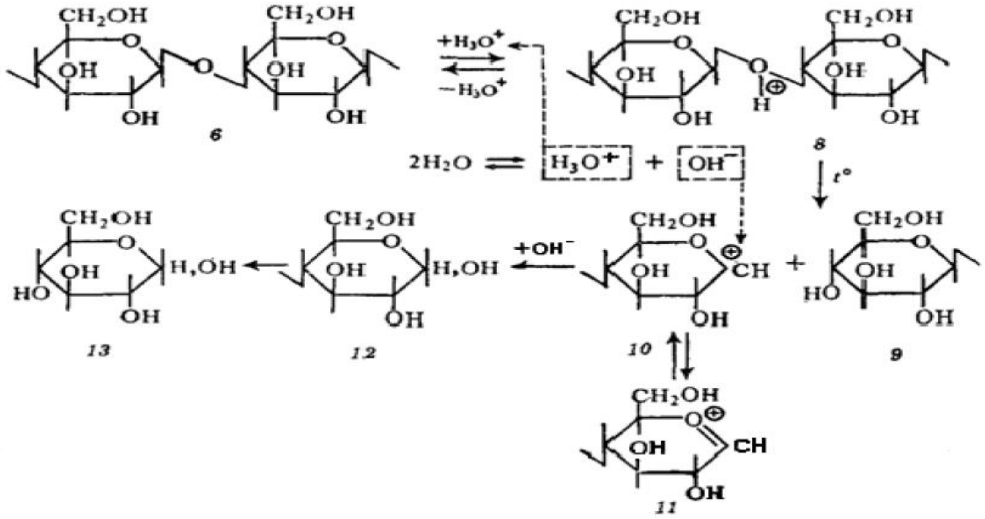

Figure 2.6 The mechanism of hydrolytic cleavage of glycosodic bonds **[**50**].**

The mechanism of acid hydrolysis of lignocellulose material is shown in Figure 2.6 which can be described by the following step: [45, 50]

(1). The diffusion of proton through the wet lignocellulose
(2). The protonation of the glycosidic oxygen bond, which contains the lone pair electron between the monomeric sugar, then the oxonium macroion (8) is formed
(3). The oxonium macroion dissociated, the macromoleculs of cellulose (9) and carbonium macroion (10) are formed and in equilibrium with oxonium ion (11). Since the distribution of the positive charge between carbon and oxygen atom, the system is called carboxonic ion.
(4). The solvation of carboxonic ion with water will form the macromolecule (12)
(5). The macromolecule was hydrolyzed further and the product from full hydrolysis of cellulose is D-Glucose

2. Lignin Reaction Under Acidic Conditions

1. β-O-4 Bond Cleavage

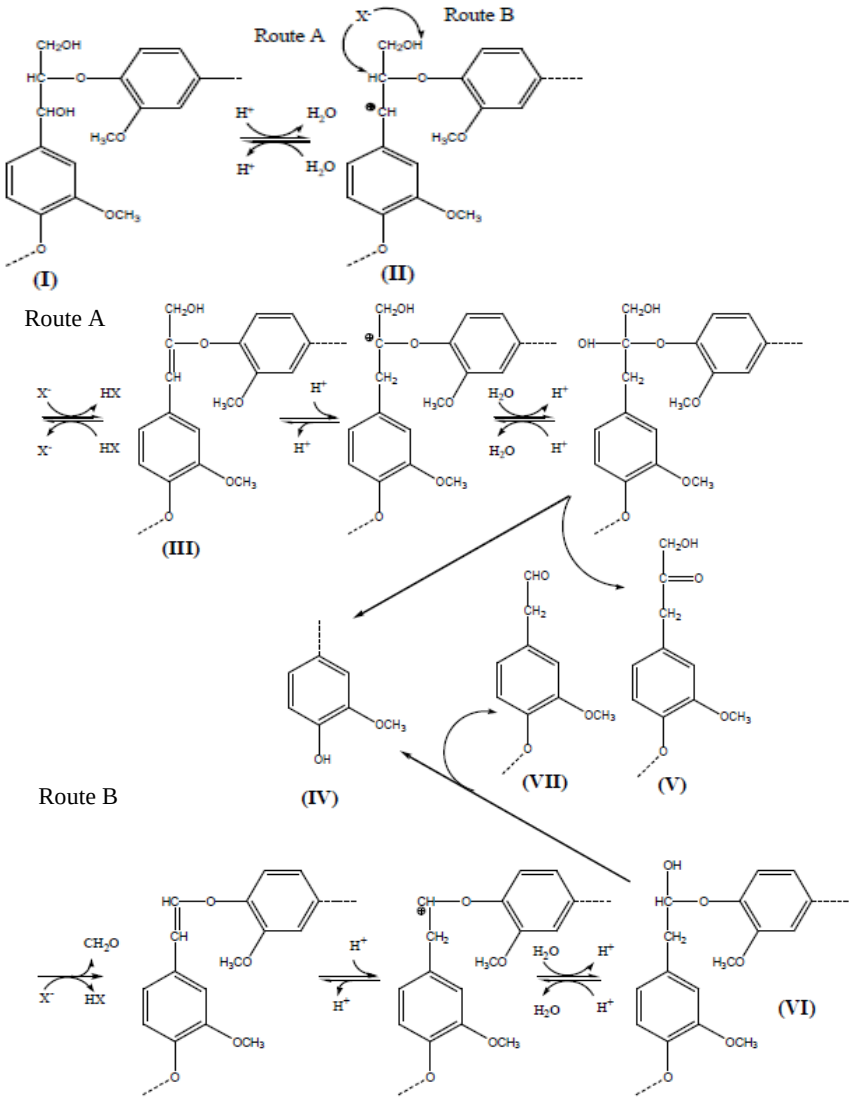

Figure 2.7 Mechanism of the β-O-4 bond cleavage on Lundgren research (1972) [49].

Under the acidic pretreatment condition, the reaction of lignin is the cleavage of the β-O-4 bonds. This reaction has described by several authors (Adler et al. 1957; Lundquist and Lundgren 1972; Lundquist 1973; Ito et al. 1981; Yansuda et al. 1981a, b, 1982, 1985; Yasuda and Terashima 1982; Hoo et al. 1983; Karlaason et al. 1988). The mechanism of β-O-4 cleavage bond is shown in Figure 2.7 (presented by Lundquist and Lundgren). The lignin macromolecules are connected together via a β-O-4 bond in the β position. During the reaction, the β-O-4 bond **I** initially transfers to a benzyl cation type intermediate **II**, and then an enol ether type of structure **III** is formed as shown in the route A. After that, the β-O-4 bond of **III** is hydrolyzed to a new phenolic lignin product **IV** and to from Hibbert's ketone type structure **V**. Another competition reaction is reported in route B, which also leads to β-O-4 bond cleavage. The formaldehyde is released from the γ-position of **II**, the enol ether type substructure **VI** would be formed.

Finally, the β-O-4 bond of structure **VI** is hydrolyzed to the new phenolic unit **IV** and aldehyde **VII** [49].

3. Lignin Reaction Under Acidic Conditions

1. α- Ether Cleavage

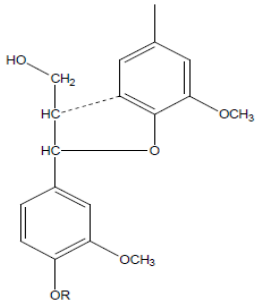

Figure 2.8 Non-cyclic benzyl ether linkage in lignin [49].

The cleavage of α- ether bonds can occur when a free phenolic OH group is presented in the para position of the propyl side chain. Then, the intermediate quinonemethide is formed to facilitate the reaction. Figure 2.8 shows the non- cyclic benzyl ether linkage in lignin such as α-aryl ether bonds. The cleavage bond reaction occurs via the formation of hydroxyl groups by hydroxide ion that causes the content of this functional group to be higher than the actual amount that presented in wood. From the NMR result was suggested that the residual lignin is much less reactive than the wood lignin towards pulping chemicals, because the low content of aryl ether linkages and the prevalence of condensed type structures [49].

2. β-O-4 Cleavage of Non-phenolic and Phenolic Structure

The β-O-4 ether linkage will be cleavage when any adjacent OH or carbonyl group is presented in either the α- or γ- position. Figure 2.9 shows the reaction of the β- ether model compound (1) under alkaline condition, which form guaiacol (2) and veratrylglycerol (3) [49].

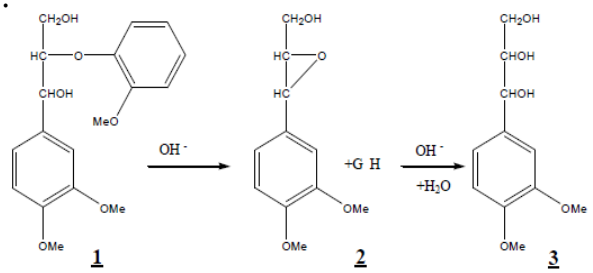

Figure 2.9 Reaction of β-ether model compound to form guaiacol and veratrylglycerol.

2.1.4 Parameters for Lignocellulose Pretreatment

For the bioethanol production, the most important process is the pretreatment step because the lignocellulose material has barrier factors that prevented the digestibility of enzymatic hydrolysis such as crystallinity of cellulose, accessible surface area and protecting of lignin and hemicellulose. However, the performance of pretreatment

depends on type of lignocellulose. The highest yield of glucose can be obtained from the most effective pretreatment that can handle these problems. The parameter is discussed below:

1. Effect of Crystallinity

There are two phases in cellulose structure that are crystalline and amorphous regions. The crystallinity is given by the relative of two regions; however, crystalline is the major regions in cellulose. The amorphous region is more easily digested by enzymatic hydrolysis than crystalline phase. Therefore, the cellulose that consisted of high-crystallinity will resist the enzymatic hydrolysis. Then, the pretreatment is necessary for pretreating cellulose to decrease the degree of crystallinity. There are some studies that reveal the non-effect of degree of crystallinity to the digestibility of enzyme to produce glucose; moreover, the crystallinity can increase the enzymatic hydrolyzed [15]. The crystallinity is the parameter affected to the enzymatic hydrolysis although there are some paradoxical studies of the presented crystallinity. The other parameters such as accessible area, protecting of lignin and hemicellulose are also the important factors for enzymatic hydrolyzed.

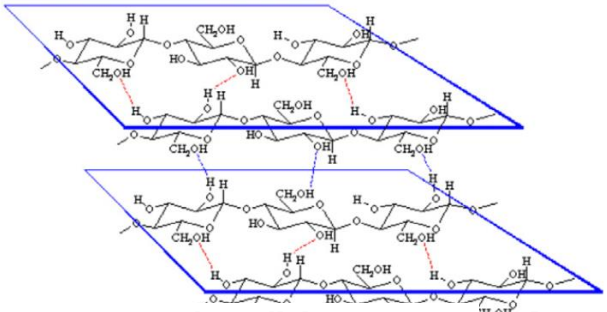

Figure 2.10 The Cellulose Structure [20].

2. Effect of Surface Area

The effects of degree of crystallinity, protecting lignin and hemicellulose have an impact on the accessible surface area of lignocellulose. Therefore, removing lignin and hemicellulose will decrease the crystallinity of cellulose and can improve the pore volume of cellulose. The enzymatic hydrolysis consists of three steps: (1) adsorption of enzymes from liquid phase onto the surface of cellulosic material, (2) enzyme digestibility of cellulose to simple sugars, mainly cellobiose and oligomers, and (3) desorption of enzyme to the liquid phase. From this reason, the accessible surface area of lignocellulose material affects to the enzymatic hydrolysis.

Lignocellulose materials have two types of surface area: external and internal. The external surface area is related to the size and shape of the particles and the internal surface area depends on the capillary structure of cellulosic fibers. The lignocellulose can be swelled in the pretreatment step; in other words, the surface area of cellulosic material will be increased in this process [15].

3. Effect of Lignin

Lignin is a cross-link macromolecule that form protective shield around cellulose and hemicellulose together and protecting the lignocellulose from enzymatic hydrolysis [6, 15]. Since lignin is more complex structure than hemicellulose, the pretreatment process will be difference. For example, hemicellulose can be hydrolyzed by diluted acid but lignin will still be in structure which acts as the inhibitor for cellules, xylanase, and glucosidase. Therefore, removal of lignin can improve the effectiveness of enzyme and help increase the accessible surface area of cellulose [15].

4. Effect of Hemicellulose

Hemicellulose is the part of lignocellulose material which protects the cellulose from enzymatic hydrolysis. However, hemicellulose is amorphous structure that can be easily hydrolyzed by dilute acid in the pretreatment step [15].

2.1.5 Pretreatment Method

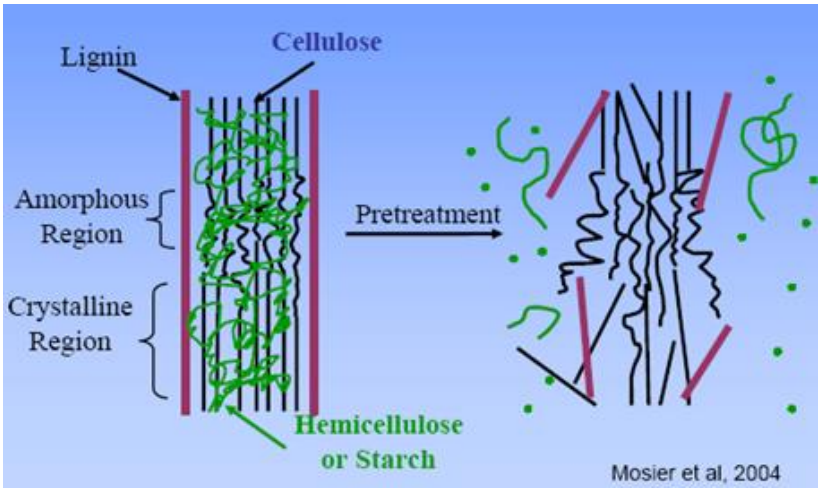

Figure 2.11 Pretreatment process of lignocellulose material [19].

Pretreatment process is the part of bioethanol production which is the most expensive and least technologically mature step. The cost of this process is due to chemicals, recovery process and corrosive resistant equipment [4]. However, pretreatment process is the most important steps for ethanol production process. There are several types of pretreatment including physical process such as milling, irradiation, high pressure stream and extrusion[15]; physical-chemical method, using water or stream explosion [11]; biological pretreatment that used fungi and actinomycetes [15]; and finally, chemical pretreatment, using acid/alkaline, wet oxidation, ozonolysis and organic solvent [15]. In general, the purpose of pretreatment is to remove lignin and hemicellulose, increase the accessible surface area and decrease the degree of crystallinity. Pretreatment will achieve these requirements: (1) improve the digestibility of enzymatic hydrolysis to increase the sugar yield; (2) avoid the degradation of carbohydrate; (3) avoid the formation of inhibitor substrate that harmful to enzyme and (4) be cost-effective [9]. The conclusion of lignocellulose pretreatment is shown in Table 2.1

Table 2.1 Pretreatment process of lignocellulose materials [15].

Pretreatment Method	Processes	Studied Application	Possible changes in Biomass	Notable Remarks
Physical Pretreatments	**Milling:** - Ball milling - Two roll milling - Hammer milling - Colloid milling - Vibro energy milling	Ethanol	- Increase in accessible surface area and pore size - Decrease in cellulose crystallinity - Decrease in degree of polymerization	- Most of the methods are highly energy demanding - Most of them can't remove lignin - It is preferable not to use these method for industrial applications - No chemicals Generally required for these methods
	Irradiation: - Gamma-ray Irradiation - Electron-beam Irradiation - Microwave Irradiation	Ethanol and biogas		
	Others: - Hydrothermal - High pressure steaming - Expansion - Extrusion - Pyrolysis	Ethanol and biogas		
Chemical and Physical-Chemical Pretreatments	**Explosion:** - Steam explosion - Ammonia fiber Explosion (AFEX) - CO_2 explosion - SO_2 explosion	Ethanol and biogas		
	Alkaline: - Sodium Hydroxide - Ammonia - Ammonium sulfite	Ethanol and biogas		

Table 2.1 Pretreatment process of lignocellulose materials [15] (Cont.).

Pretreatment Method	Processes	Studied Application	Possible changes in Biomass	Notable Remarks
Chemical and Physical-Chemical Pretreatments	**Acid:** - Sulfuric acid - Hydrochloric acid - Phosphoric acid **Gas:** - Chlorine dioxide - Nitrogen dioxide - Sulfur dioxide **Oxidizing agents:** - Hydrogen peroxide - Wet oxidation - Ozone **Solvent extraction:** - Ethanol-water extraction - Benzene-water Extraction - Ethylene glycol extraction -Butanol-water extraction - Swelling agent	Ethanol and biogas Ethanol and biogas Ethanol and biogas Ethanol	- Increase in accessible surface area - Partial nearly complete delignification - Decrease in cellulose crystallinity - Decrease in degree of polymerization - Partial or complete hydrolysis of hemicellulose	- These method are among the most effective and include the most promising process for industrial application - Usually rapid treatment rate - Typically need Harsh conditions - There are chemical requirements
Biological Pretreatments	**Fungi and Actinomycetes**	Ethanol and biogas	Delignification Reduction in degree of polymerization of Cellulose Partial hydrolysis of hemicellulose	- Low energy requirement - Low chemical requirement - Mild environment conditions - Very low treatment rate - Did not consider for commercial application

2.1.6 Chemical Pretreatment

Pretreatment of lignocellulose by using chemical is the effective and commercial method. Usually, acid and alkaline pretreatment are the most interesting method that applied to treat the biomass material in ethanol production process. The details of these pretreatments are discussed below:

1. Acid Pretreatment

Acid pretreatment is the commercial method which is considered to be effective for pretreating lignocellulose material [11]. The hemicellulose is easier to hydrolysis than cellulose because of the amorphous character in front of the crystalline of cellulose. Moreover, the furanosidic rings of hemicellulose are hydrolyzed faster than the piranosidic rings of cellulose because five carbon atoms of furanosidic ring have higher tension than six carbon atom of piranosidic ring. However under acid condition, the delignification of lignin is produced by substitution reactions and broken links, controlled by condensation reactions that prevent lignin dissolution then lignin is eliminated in small portions [8]. Therefore, diluted acid is applied to hydrolyze hemicellulose into the liquid phase that consists of main product xylose together with minor amount of lignin [11].

2. Alkaline Pretreatment

Diluted alkali solutions pretreat lignocellulose material by dissolving hemicellulose, lignin and silica, hydrolyzing uronic and acetic acid esters and swelling cellulose [11]. Moreover, alkaline pretreatment was found to increase the biodegradability of lignocellulose due to cleavage of the a-aryl ether bonds from polyphenolic monomers of lignin, while hydrogen bond weakening leads to hemicellulose dissolving and cellulose swelling [3,11]. Sodium hydroxide (NaOH) is the most effective and commercial chemical for improving the performance of fermentation process when compared to other chemicals such as sodium carbonate, ammonium hydroxide, calcium hydroxide and hydrogen peroxide [11].

2.1.7 Enzymatic Hydrolysis

After pretreatment, the pretreated solid is converted into sugar via enzymatic hydrolysis process; cellulases is one of the commercial type of the enzyme used for the cellulose hydrolysis. In general, the enzymatic hydrolysis is carried out under the mild condition at the pH of 4.8 and the temperature of 45 – 50 ºC, which is the optimum condition. The advantage of enzymatic hydrolysis over chemical reaction is that this process is non – corrosive problem; however, it takes the longer times to carry out the reaction. Furthermore, the final product of enzyme hydrolysis inhibits the enzyme and affects the process, if not immediately .One of disadvantage of enzymatic hydrolysis is the cost of enzyme [51].

1. Cellulases

The cellulases are classified in glycosyl hydrolase families based on their sequence homology and hydrophobic cluster analysis by using CAZy classification system (Carbohydrate- Active enzyme). The effective cellulose hydrolysis requires the mixture of different cellulases enzyme. The cellulases are classified into three types. The frist one is endogucanases (EDs) which hydrolyze the internal β - 1, 4 glucosidic linkages in the cellulose chain. Another one is cellobiohydrolase (CBHs or exogucocanases) which further hydrolyzes the cellulose and cleavge of cellobilose to the end product. The last one is β – glucosidase which hydrolyze cellobiose to glucose and also cleavge the glucose unit from cello – oligosaccharides. Fungi are a goog source to produce these enzymes; *Tricoderma reesei* is the one of fungi that usually used for enzymatic hydrolysis of cellulose and degrade the crystalline cellulose. *T. reesei* produces two CHBs, five EGs and and two BGs. The over all of hydrolysis process can be devided into two steps that are primary hydrolysis and secondary hydrolysis. The primary hydrolysis involve endogucanases and cellobiohydrolase that occurs on the surface of solid and releas the sugar with the degree of polymerization up to 6 and this step is the rate determining of the over all process. The secondary hydrolysis occur in liquid phase that hydrolyzes the cellobiose to glucose by β – glucosidase. The diagram of mechanism of cellulose hydrolysis is shown in Figure 2.12

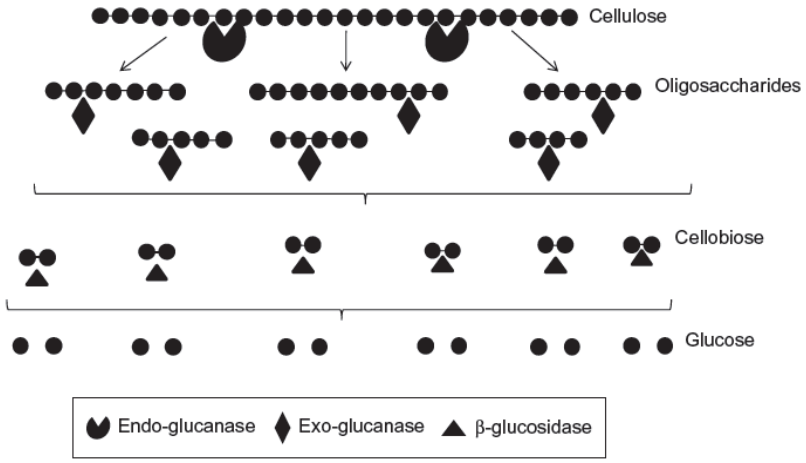

Figure 2.12 Mechanism of enzyme of cellulase.

All *T. reesei* cellulases, except EGs have two domain structures that are catalytic domain (CD) and catalytic binding domain (CBD). The functional group of CBD brings the enzyme closed to the substrate and ensures the correcting position. The CBD and CD are connected together via the glycoslylated flexible linkage which helps them to contact and degrade the crystalline structure of cellulose. The CBDs of CHBs are able to move along the cellulose chain while the CD separates the cellobiose unit. Moreover, the different structure of catalytic domain (CD) of cellobiohydrolase and endogucanases are the action on polymer substrate is controlled by the shape of active site. The endogucanases is open active sites which can able action in the middle of glucan chain while the cellobiohydrolase have the tunnel active site shape that only allows the

enzyme to act at the chain ends. When the process absents the CBD, the ability of the cellobiohydrolase to degrade the crystalline cellulose is decrease. The removal of CBD is little effect to the ability of hydrolysis amorphous region of cellulose and the CBD increase the enzymatic concentration on the surface of solid; moreover, CBD promotes the solubilization of the glucan chain of cellulose.

2.1.8 Analysis Method

After pretreatment, the hydrolyzed product in liquid phase and the solid fraction are characterized to identify the product by using instrumental methods of chemical analysis such as high performance liquid chromatography (HPLC) and UV-Visible spectroscopy. The products from hydrolysis such as glucose, celobiose, xylose, arabinose, acetic acid, furfural and hydroxymethylfurfural (HMF) are analyzed by using HPLC. UV-Visible spectroscopy is used to measure amount of soluble lignin in filtrated. The theories of the instruments are presented in this section:

1. High Performance Liquid Chromatography (HPLC)

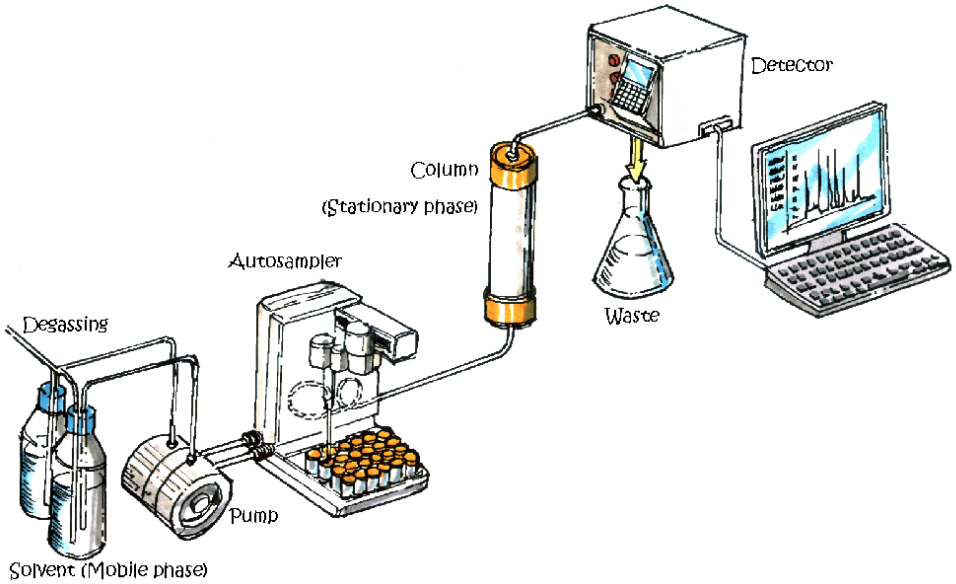

Figure 2.13 High Liquid Performance Chromatography installments [21]**.**

1.1 Principle of High Liquid Performance Chromatography

"A chromatographic separation is achieved by the distribution of substances between two phases, a stationary phase and a mobile phase. Those solutes distributed especially in the mobile phase will move more rapidly through the system than those distributed preferentially in the stationary phase. Thus, the solutes will elute in order to their increasing distribution coefficients with respect to the stationary phase." (R.P.W. Scott, 1996)

For the concept of mobile phase and stationary phase, it tends to disguise the basic process of retention in term of *distribution*

"A solute is distributed between two phase because of the differential molecular force that exist between the solute molecules and those of the two phases. The extent of the distribution will depend on the availability of either phase to the solute molecules." (R.P.W. Scott, 1996)

Therefore, the retention of solute can be controlled by changing the interaction force (for example by changing the type or concentration of mobile phase or nature of stationary phase); moreover, changes the availability of the stationary phase to solutes. Finally, controlled retention of solute cloud be obtained by changing the pore size of the silica or boned phase in stationary phase. Generally, the chiral solute can be changed the retention by modifying the contact area of molecules by applying the appropriate chiral characteristics into the stationary phase.

The stronger force between solute molecules and stationary phase will be spent longer retention to elute from column. In contrary, the stronger interaction between solutes and mobile phase is .more rapidly to pass through the column. Accordingly, solute retention will be controlled by molecular force that divided into 4 types; *ionic interaction, polar interaction, Dispersive interaction and chemical interaction* [23]. HPLC separations have theoretical parameters and equations to describe the separation of components into signal peaks when detected by instrumentation such as by a UV detector or mass spectrometer. Figure 2.14 is shown a chromatogram of sample that analyzed by HPLC

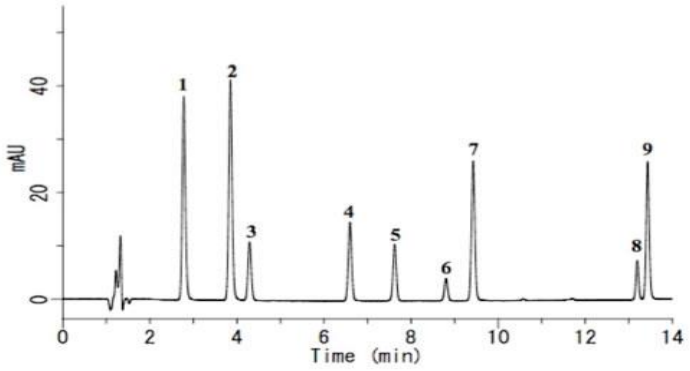

Figure 2.14 A chromatogram of sample that analyzed by HPLC [21].

2. UV-Visible Spectroscopy

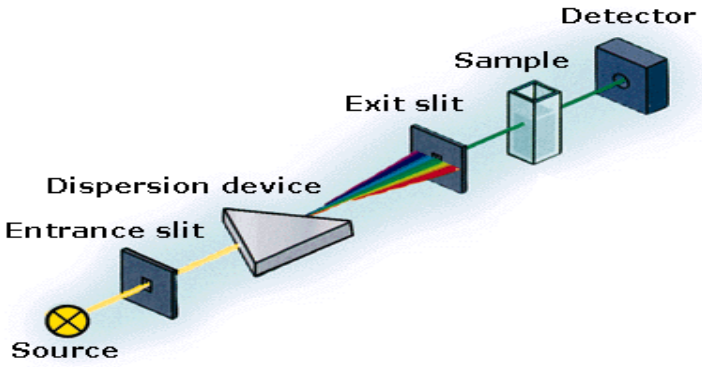

Figure 2.15 UV-Visible Spectroscopy Installments [25]**.**

2.1 Principle of UV-Visible Spectroscopy

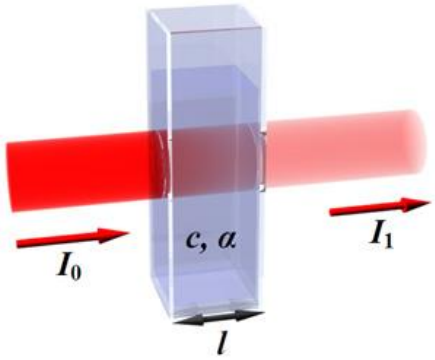

Figure 2.16 The derivation Beer-Lambert's Law [25]**.**

The adsorption of radiant energy by matter can be descried quantitatively through principle of Beer's law. For glass cuvet as shown in Figure 2.16, the losses of reflection at the surface and absorption of grass will be neglected. From Figure 2.16, if the cuvet is filled with the absorbing species which dissolved in non-absorbing solvent, the concentrated solution will be penetrated by the radiation. In generally, the reduction in power radiation is proportional to the number of absorbing molecules. The quantitative statement is related to Beer's law: *"Successive increments in the number of identical absorbing molecules in the path of a beam of monochromatic radiation absorb equal fractions of the radiant power traversing them"* [24].

The shortest term of Beer's law is:

$$A = alc = \log\frac{I_0}{I}$$

Where; A = Absorbance, no unit

a = Absorptivity of the dye, $Lg^{-1}cm^{-1}$

l = the length of the light path, cm

c = the concentration of the dye in solution gL^{-1}

I_0 = power radiation

I = transmitted power

The difference between Absorbance (A) and Absorptivity (a) will be presented as: absorbance is a property of particular sample (am extensive property), while absorptivity is a property of a substance (an intensive property). From Beer's law, the absorptivity is a constant value, independent of concentration, length of path and intensity of radiation. From the equation, is shown that no effect of temperature, nature of the solvent or the wavelength. The temperature is found to have only secondary effect when it not vary over an unusually range. The concentration will vary with a changed of temperature. Commonly, water, alcohol, ether and saturated hydrocarbons are no longer transparent solvents, but aromatic compound, chloroform, carbon tetrachloride, carbon disulfide, acetone absorb too strongly except vary near UV [24].

2.1.9 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) is an integration of mathematical and statistical techniques used to optimize an *output variable* (dependent variable) that is influenced by *input variable* (independent variable). The change of input variables is used to identify the reason for changes in output variables. For example, the scientists would like to study the effect of ammonium sulfate concentration (x_1) and glucose concentration (x_2) that minimize the level of phenol degradation (y). In this case, phenol degradation is a function of ammonium sulfate concentration and glucose concentration. This problem can be expressed follow this equation:

$$y = f(x_1, x_2) + \varepsilon \quad (2.1)$$

Where ε is the noise or error observed in the respond of y, $f(x_1, x_2)$ is called a response surface when x_1 and x_2 is the independent variable. Generally, f is unknown value and to get this term the experiment will be started with low-order polynomial in small region. The respond that can be define by linear function of independent variables, then the function is a **first- order model:**

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \varepsilon \quad (2.2)$$

This equation is shown the relation of response that is a function of 2 independent variables. In the case of respond surface is a curvature the higher order polynomial should be presented. The function with 2 variables is called a **second-order model** [26]:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x^2_{11} + \beta_{22} x^2_{22} + \beta_{12} x_1 x_2 + \varepsilon \quad (2.3)$$

In each model, the levels of each factor are independent of the levels of other factors. The objective of studied RSM is to achieve:

1. Understanding the configuration of the response surface (local maximum, local minimum, ridge lines)
2. Finding the region where the optimal response occurs. The goal is to move rapidly and efficiently along a path to get to a maximum or a minimum response so that the response is optimized.

The response can be presented by graphically, either in 3- dimension or as contour plots that shown in Figure 2.17.

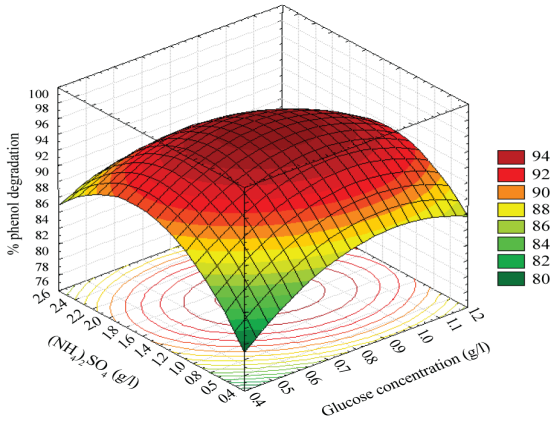

Figure 2.17 The 3 – dimensional response surface and the contour plot for relation of the %phenol degradation and ammonium sulfate concentration (x_1) and glucose concentration (x_2) [32]**.**

The design of experiment is an important step of RSM that compressed as DoE. The objective DoE is to design the points to run the experiment for evaluation of the response. The mathematical model of the process related to optimal design of variable and usually these models are polynomials with an unknown structure. The alternative to design experiments is depended on the accuracy of the approximation and the cost of funding the model.

Design of Experiment (DoE) or screening experiment is performed in early stages of process to identify the independent variable that have large significant effects to the response. The different methodologies (Full Factorial Design, Fractional Factorial Design (FFD) and Central Composition Design (CCD) for performing the experiment design would be discussed in the next section [30].

1. Full Factorial Design

To perform the model that can be shown the interaction between N design variables and this method investigates all possible combinations. The lower and upper bounds of each N design variables would be designed. If the variables are defined at 2 levels of lower

and upper bounds, the experimental design is called 2^N full factorial. In addition, if the midpoint is defined, the design is called 3^N full factorial. The 2^N design is shown in Figure 2.18 [30]

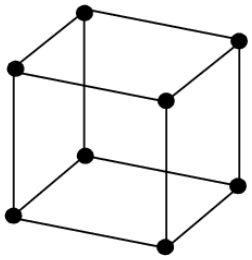

Figure 2.18 2^3 full factorial design (8 points) [44].

Factorial design can be fitted with second order models when the first order model lack of fit in order to interaction between variable and surface carve. The second-order model is defined as:

$$y = a_0 + \sum_{i=1}^{n} a_i x_i + \sum_{i=1}^{n} a_{ii} x_i^2 + \sum_{i=1}^{n} \sum_{i=1}^{n} a_{ij} x_i x_j \quad i<j \qquad (2.4)$$

A full factorial design is usually used for five or fewer variables.

2. Fractional Factorial Design (FFD)

Fractional factorial designs are experimental designs consisting of a carefully chosen subset (fraction) of the experimental runs of a full factorial design. Generally, fractional factorial design is expressed in term of I^{k-p}, where *I* is the number of levels of each factor, *k* is the number of factors studied, and *p* describes the size of the fraction of the full factorial used [26].

The levels of a factor are commonly coded as +1 for the high level, and −1 for the low level. For a three-level factor, the midpoint is coded as 0.

Fractional factorial design is generate from full factorial design by using an *alias structure*. The alias structure determines the effects that are confounded with each other [27].

From the 2^{7-3} design, the code of factors studied are A, B, C, D, E, F and G, when A, B, C and D are the main effects and ABC, ABD and BCD are the generators of E, G and F, respectively. The design of experiment is shown in Table 2.2

Table 2.2 The design of experiment of 2^{7-3} design [27]**.**

Run	A	B	C	D	E=ABC	F=BCD	G=ABD
1	1	1	1	1	1	1	1
2	1	1	1	-1	1	-1	-1
3	1	1	-1	1	-1	-1	1
4	1	1	-1	-1	-1	1	-1
5	1	-1	1	1	-1	-1	-1
6	1	-1	1	-1	-1	1	1
7	1	-1	-1	1	1	1	-1
8	1	-1	-1	-1	1	-1	1
9	-1	1	1	1	-1	1	-1
10	-1	1	1	-1	-1	-1	1
11	-1	1	-1	1	1	-1	-1
12	-1	1	-1	-1	1	1	1
13	-1	-1	1	1	1	-1	1
14	-1	-1	1	-1	1	1	-1
15	-1	-1	-1	1	-1	1	1
16	-1	-1	-1	-1	-1	-1	-1

Defining relation is determined from thee design generators and use to calculate the design structure.

$$E = ABC,\ F = BCD,\ G = ABD$$

The simple algebra is used to perform the defining relation. In this algebra, any letter times itself is the identity, *I*, for example E*E = I. The identity, *I*, times any letter is the same letter, for example E*I = E. From these rules, the three design generator can be transformed into equations shown below: [29]

$$EE = ABCE = I$$
$$FF = BCDF = I$$
$$GG = GABD = I$$

So, I = ABCE = BCDF = GABD, which is the first part of the defining relation. Next step, multiply the four interactions (these are usually called "words") by each other to get:

$I^2 = ABCE\ (BCDF)$ $\quad I = AB^2C^2DEF$ or $I = ADEF$

$I^2 = ABCE\ (ABDG)$ $\quad I = A^2B^2CDEG$ or $I = CDEG$

$I^2 = ABDG\ (BCDF)$ $\quad I = AB^2D^2CFG$ or $I = ACFG$

$I^3 = (ABCE)\ (ABDG)\ (BCDF)$ $\quad I = A^2B^3D^2C^2EFG$ or $I = BEFG$

Therefore, the overall Defining word is:

I = ABCE=BCDF=ABDG=ADEF=CDEG=ACFG=BEFG

The alias structure of each factor can be achieved by multiply the factor with defining word such as, alias of A is:

I(A) = ABCE(A)=BCDF(A)=ABDG(A)=ADEF(A)=CDEG(A)=ACFG(A)=BEFG(A)

A=BCE=ABCDF=BDG=DEF=ACDEG=CFG=ABEFG

Resolution (R) is an important property of a fractional design or ability to separate main effects and low-order interactions from one another. Usually, the resolution of the design is the minimum word length in the defining relation such as:

I = ABCE=BCDF=ABDG=ADEF=CDEG=ACFG=BEFG is resolution IV because the shortest word is 4 words.

Resolutions below III are not useful and resolutions above V this case consist of large number of design experiment [28].

3. Central Composition Design (CCD)

A second- order model can be performed with central composite design (CCD). CCD is the first order (2^N) designs augmented by adding center and axial points to estimate the tuning parameters of a second-order model [30].

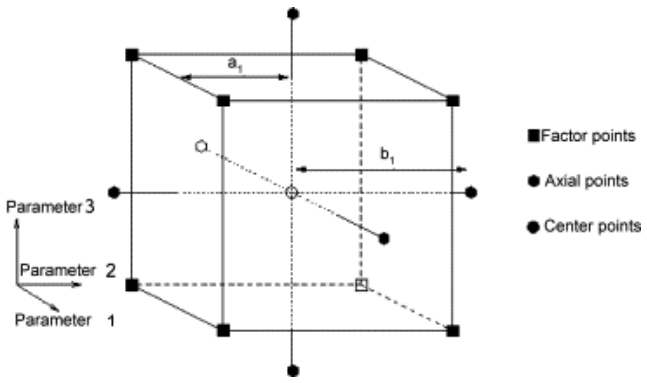

Figure 2.19 A central composition design for 3 variables at 2 levels [31]**.**

From Figure 2.19, the model consists of 2^N factorial points, 2N axial points and 1 central point. CCD is an alternative of 3N designs in the performed of second-order

models because the number of experiment is reduced from full factorial design 27 designs from full factorial reduced to 15 designs in the case of (CCD).

2.1.10 Analysis of Variance (ANOVA)

1. One - Way ANOVA

One - way ANOVA is an analysis of quantitative outcome with only one independent variable. The usual assumptions of this method normality, equal variance and independent error were applied.

The null hypothesis is a key hypothesis referred to *"nothing interesting is happening"*. For one way ANOVA, the null hypothesis uses H_0: $\mu_1 = \ldots = \mu_k$, which mean that all the population is equal. For another hypothesis, alternative hypothesis which expressed as *"at least one of k population means differs from all of the other"* and it can be wrote in term of "H_A: Not $\mu_1 = \ldots = \mu_k$" or *"the population means are not equal"* [40].

The terminologies in ANOVA [43]

$$\text{Sample size} : n_1, n_2, n_3, \ldots, n_k$$
$$\text{Sample means} : \bar{x}_1, \bar{x}_2, \bar{x}_3, \ldots, \bar{x}_k$$
$$\text{Sample variation} : s_1^2, s_2^2, s_3^2, \ldots, s_k^2$$
$$\text{Total sample size} : n = n_1 + n_2 + n_3 + \ldots + n_k$$
$$\text{Grand average} : \bar{\bar{x}} = \text{Average of all } n \text{ responses}$$

Where; **Grand average** is equal to:

$$\bar{\bar{x}} = \left(\frac{n_1}{n}\right)\bar{x}_1 + \left(\frac{n_2}{n}\right)\bar{x}_2 + \left(\frac{n_3}{n}\right)\bar{x}_3 + \ldots + \left(\frac{n_k}{n}\right)\bar{x}_k \tag{2.5}$$

"Between Sample Variations" can be calculated from equation (2.6):

$$SSTr = n_1(\bar{x}_1 - \bar{\bar{x}})^2 + n_2(\bar{x}_2 - \bar{\bar{x}})^2 + n_3(\bar{x}_3 - \bar{\bar{x}})^2 + \ldots + n_k(\bar{x}_k - \bar{\bar{x}})^2 \tag{2.6}$$

Since this equation is the sum of square, so it is called **"Treatment sum of square"**

For **"Within Sample Variations":**

$$SSE = (n_1 - 1)s_1^2 + (n_2 - 1)s_2^2 + (n_3 - 1)s_3^2 + \ldots + (n_k - 1)s_k^2 \tag{2.7}$$

Then, the equation (2.7) is called **"Sum Square of error"**

Therefore, the sum of SSTr and SSE is equal to **"Sum square Total"**

$$SST = SSTr + SSE \tag{2.8}$$

From the words of “Between Sample” and “Within Sample”, the “sample” is referred to “level or treatment”. For “Between” means the information between level while “Within” is the information in the same level.

The degree of freedom is the number of free units of information that use to calculate the mean of “Treatment sum of square” and “Sum Square of Error”.

- The degree of freedom of Sum Square Total (SST) = n-1
- The degree of freedom of Treatment Sum of Square (SSTr) = k-1
- The degree of freedom of Sum Square of Error (SSE) = n-k

Where; n = Total sample size

k = number of level

Then, the new terminologies are presented following these equations:

$$MSTr = \frac{SSTr}{k-1} \tag{2.9}$$

$$MSE = \frac{SSE}{n-k} \tag{2.10}$$

MSTr: Mean square for treatments (Between-sample)

MSE: Mean square error (Within-sample)

Finally, the ***F-statistic*** that uses to analyze the significant factors can be performed follow equation (2.26)

$$F = \frac{MSTr}{MSE} \tag{2.11}$$

Table 2.3 The Summary of One Factors ANOVA.

Source of Variation	**df**	**SS**	**MS**	**F**
Between samples (Treatments)	k-1	SSTr	MSTr	MSTr/MSE
Within samples (Error)	n-k	SSE	MSE	
Total variation	n-1	SST		

2. Two – Way ANOVA

Two - way ANOVA is an analysis of quantitative outcome with two or more independent variables. The usual assumptions of this method normality, equal variance and independent error were applied. Moreover, the null hypothesis and alternative hypothesis have the principle as the same as one – way ANOVA [41]. For the three assumption of two – way ANOVA will be discussed below:

1. Normality

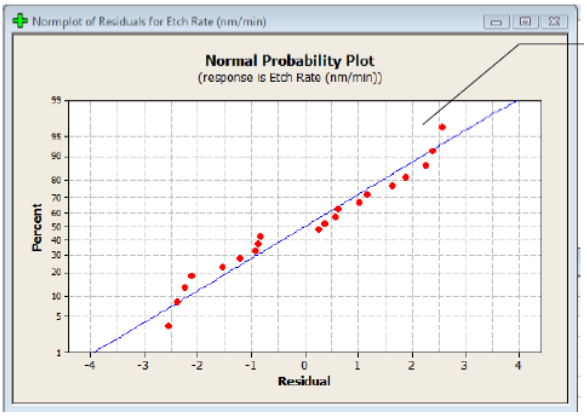

Figure 2.20 Normal Probability Plot.

ANOVA requires population in each treatment to be normally distributed and can be checked with a normal probability plot of residuals. If the distribution of residual is normal, the plot will be a straight line [42].

2. Equal Variance

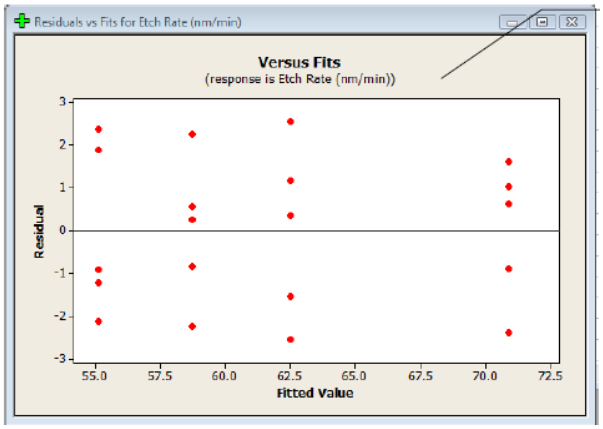

Figure 2.21 Equal Variance Plot**.**

The variance of the observation in each treatment should be equal and can be checked with Residuals vs. Fit Plot. This plot should show a random pattern of residuals on both side of 0 and should not show any recognizable patterns [42].

3. Independent Error

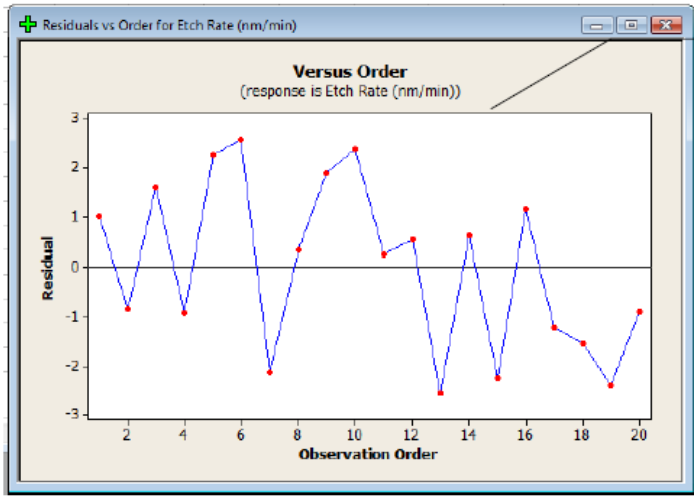

Figure 2.22 Independent Error Plot.

ANOVA requires the observation to be randomly selected from the treatment population and can be checked with the Residuals vs. Order (time order of data collection) plot. A positive correlation or a negative correlation means the assumption is violated. If the plot does not reveal any pattern, the independent assumption is satisfied [42].

The sum of square of 2 Factors can be described in equations (2.12) to (2.16) [43]

$$SS_T = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{y_{...}^2}{abn} \tag{2.12}$$

$$SS_A = \sum_{i=1}^{a}\frac{y_{i..}^2}{bn} - \frac{y_{...}^2}{abn} \tag{2.13}$$

$$SS_B = \sum_{j=1}^{b}\frac{y_{.j.}^2}{an} - \frac{y_{...}^2}{abn} \tag{2.14}$$

$$SS_{AB} = \sum_{i=1}^{a}\sum_{j=1}^{b}\frac{y_{ij.}^2}{n} - \frac{y_{...}^2}{abn} - SS_A - SS_B \tag{2.15}$$

$$SS_E = SS_T - SS_{AB} - SS_A - SS_B \tag{2.16}$$

Where:

T: Total
A: Factor A
B: Factor B
AB: Interaction of Factor A and B
E: Error
a: Number of levels of factor
b: Number of levels of factor B
n: Number of replication per cell

Then, the mean of squares are calculated in equations (2.17) to (2.20)

$$MS_A = \frac{SS_A}{a-1} \tag{2.17}$$

$$MS_B = \frac{SS_B}{b-1} \tag{2.18}$$

$$MS_{AB} = \frac{SS_{AB}}{(a-1)(b-1)} \tag{2.19}$$

$$MS_E = \frac{SS_E}{ab(n-1)} \tag{2.20}$$

Table 2.4 The Summary of Two Factors ANOVA.

Source of Variation	Degree of freedom	Sum of square	Mean square	F-Statistic
Factor A	a-1	SS_A	MS_A	$F_A = MS_A/MS_E$
Factor B	b-1	SS_B	MS_B	$F_B = MS_B/MS_E$
Interaction	(a-1)(b-1)	SS_{AB}	MS_{AB}	$F_{AB} = MS_{AB}/MS_E$
Error	ab(n-1)	SS_E	MS_E	.
Total	abn-1	SS_T	.	.

2.2 Literature Review

2.2.1 Alkaline Pretreatment

Weixing Cao et al. [2] studied the effective of five pretreatment methods for lignin removal and improving the product yield of enzymatic hydrolysis and bioethanol production process. The first method (A) started with 10 grams of solid which was then slurried in 500 ml flask with 100 ml 2% (w/v) of sodium hydroxide for 5 minutes and then autoclaved at 121 ^{o}C for 60 minutes. The solid fraction from filtration was washed by distilled water and dried at 60 ^{o}C. The second method (B), 10 grams of solid was slurried in 500 ml flask with 100 ml 20% (w/v) of sodium hydroxide for 2 hours. The residues solid was washed with distilled water until reach the neutral pH and dried at 60 ^{o}C. The third method (C), 10 grams of solid was slurried in 500 ml flask with 100 ml 2% (w/v) of sodium hydroxide for 5 minutes and autoclaved at 121 ^{o}C for 60 minutes. Cooled down the pretreated sample to room temperature and then added 5% of hydrogen peroxide slurry. The mixture was left in dark and airtight place for 24 hours. After that, the solid fraction from filtration was washed by distilled water until reached neutral pH and dried at 60 ^{o}C. The fourth (D) method, 10 grams of solid was slurried in 500 ml flask with 100 ml 2% (w/v) of sodium hydroxide for 5 minutes and left for 2 hours. The 5% of hydrogen peroxide was added in to the slurry and then kept the mixture in the dark and airtight place. The solid fraction from filtration was washed by distilled water until reached neutral pH and dried at 60 ^{o}C. The fifth method (E), 10 grams of solid was slurried in 500 ml flask with 100 ml of distilled water for 5 minutes and autoclaved at 121 ^{o}C for 50 minutes. The solid fraction from filtration was washed by distilled water until reached neutral pH and dried at 60 ^{o}C. From the analytical results, dilute NaOH solution autoclaving and H_2O_2 immersing pretreatment (C) were considered as the most suitable method because the cellulose contents in method C was the highest one, while acid deterrent lignin (ADL) content and hemicellulose content were the lowest one. Pretreatment at severe condition (high temperature and pressure) with oxidic alkaline method could retain the amount of cellulose and remove ADL lignin and hemicellulose, but was not efficient at retaining dry matter. In conclusion, the cellulose hydrolysis yield, sugar yield and ethanol concentration from method (C) were 74.29%, 90.94% g sugar/100 g dry matter and 6.12 g/L, respectively.

Rishi Gupta et al. [3] presented the effect of varied chemical dosage and pretreatment time on the efficiency of enzymatic hydrolysis and fermentation process. The lignocellulose materials used for studying were *Prosopis juliflora* (PJ), *Lantana camara* (LC) and *Corncob* (CC). The alkaline pretreatment used sodium hydroxide was investigated. 100 grams of solid was added in different concentration of sodium hydroxide from 1-5% (w/v) for 2 hours and autoclaved at 121 ^{o}C for 15, 30, 45 and 60 minutes. The solid fraction from filtration was washed by distilled water until reached neutral pH and dried at 60 ^{o}C. From analytical result, increasing alkaline concentration up to 5% of PJ and LC led to higher lignin removal and high returned of hemicellulose; however, 4% w/v alkaline concentration gives the optimum condition for CC. For the effect of reaction time for all three types of material, the lignin removal increased when

increasing the reaction time up to 30 minutes. After 30 minutes the rate of dissolved lignin becomes constant. For alkaline pretreatment, the lignin content was reduced in the range of 28-38% (w/w), while the hemicellulose content was about 5.2-7.1% (w/w).

Letha Dawson et al. [6] investigated the optimum pretreatment condition for the highest efficiency of ethanol production from sugarcane bagasse by using H_2O_2 together with sodium hydroxide. In the experiments, 3 grams of dry sugarcane bagasse were immersed in 1% H_2O_2 solution and the pH of slurry was adjusted to 8, 11.5 and 13 by adding sodium hydroxide, then continued the reaction for 8, 24 and 48 hours. The pretreatment was repeated by using H_2O_2 concentration for 0%, 2% and 5%. The 0% solution was prepared by using deionized water without adjusting of pH. The solid fraction from filtration was washed triple rinsed for 30 minutes by deionized water and dried at100 ^oC for 12 hours. For the experimental result, 2 % H_2O_2 at pH of 13 reacted for 8 hours was the best condition that can removed 58.97% lignin of total weight of the sample. Then, the pretreated material from this condition subjected to fermentation process and ethanol was produced about 130.5 mg/L ethanol.

Sarita C. Rabelo et al. [4] examined the effect of temperature, chemical concentration and reaction time on the product yield of ethanol production by using different chemicals alkaline hydrogen peroxide and calcium hydroxide. Non-screened bagasse and screened bagasse were used as the sample of pretreatment. Alkaline hydrogen peroxide was prepared by using 1% H_2O_2 solution and adjusted pH to 11.5 with sodium hydroxide and then 4 grams of non-screened and screened bagasse were added into the solution. The sample was allowed to soak for 6, 15 and 24 hours under the temperature of 20, 40 and 60 ^oC. The pretreatment was repeated by using H_2O_2 at the concentration of 3% and 5%. For lime pretreatment, calcium hydroxide was dissolved in distilled water as amount of lime loading about 0.1 g/g and then 4 grams of non-screened and screened bagasse were added into the solution. The sample was allowed to soak for 12, 24 and 36 hours under the temperature of 60, 65 and 70 ^oC. The pretreatment was repeated by using lime loading about 0.25 and 0.4 g/g. Analysis were performed using 2x2x2 factorial design. From analytical results, for non-screened bagasse, the best results for alkaline peroxide pretreatment was 5% H_2O_2 at ambient temperature for 24 hours, whereas the high glucose was obtained from lime pretreatment with 0.40 g lime/g dry biomass at 70 °C for 36 h. Moreover, high TRS (Total reducing sugar) yield was obtained from non-screened bagasse using 0.4 g lime/g dry biomass at 70 ^oC for 36 h. However, for screened bagasse, lime pretreatment could be performed under three conditions for high glucose yields: 0.10 g lime/g dry biomass at 70 °C for 12 h, 0.10 g lime/g dry biomass at 70 °C for 36 h, or 0.40 g lime/g dry biomass at 60 °C for 36 h.

Camila Alves Rezende et al. [11] presented the two- steps pretreatment that included acid step for removing hemicellulose, followed by alkaline pretreatment for lignin removal. For the pretreatment, sugarcane bagasse as 1:10 solid to liquid ratio was initially hydrolyzed by 1% (v/v) of H_2SO_4 at 120 ^oC for 40 minutes. The sample was kept at 1.05 bars. After that, the solid fraction from filtration was washed by distilled water until reached neutral pH and dried at 60 ^oC for 24 hours. Then, the initially

pretreated solid was added into 0.25%, 0.5%, 1.0%, 2.0%, 3.0% and 4.0% w/v of NaOH at 120 ^{o}C for 40 minutes. The pretreated bagasse was washed by distilled water until reached neutral pH and dried at 60 ^{o}C for 24 hours. The result of two-step pretreatment was shown that the most of the hemicellulose fraction was removed by using acid. Especially, acid pretreatment followed by the step with 1% NaOH seemed to be the best pretreatment condition that used for bagasse samples prior to cellulose hydrolysis. In conclusion, 96% and 85% of hemicellulose and lignin were removed from sugarcane bagasse. Total cellulose conversion yield was 72% that increase in comparison to 22% of untreated bagasse.

Rekha Rawat et al. [34] investigated the optimum conditions for reducing sugar production from alkaline pretreatment of *poplar (Populus deltoids)* by studying the effects of alkaline concentration, solid to liquid ratio, pretreatment temperature and pretreatment period. The response surface methodology (RSM) was applied for the design of experiment by using Central Composition Rotary Design (CCRD). The parameters were divided into 5 levels for CCRD method that consists of -2, -1, 0, 1, 2. In the experiment, the poplar was added into 0.5%, 1.5%, 2.5%, 3.5% and 4.5% (w/v) of NaOH for the solid to liquid ratio about 1:4, 1:6, 1:8, 1:10 and 1:12. Then, the samples were heated for temperature 60 ^{o}C, 75 ^{o}C, 90 ^{o}C, 105 ^{o}C and 120 ^{o}C for 20 min, 30 min, 40 min, 50 min and 60 min. After the pretreatment step, the remained solid was hydrolyzed with the enzyme to obtain the reducing sugar as the final product. By using CCRD method for design of experiment, 30 runs were performed. From the experimental result, the optimum condition was 2.8 % (v/w) NaOH with solid to liquid ratio of 1:8 at 94 ^{o}C for 1 h. and the overall second order polynomial equation for scarification was presented as follow:

$$Y = 3.77 + 3.14x_1 + 2.98x_2 + 2.55x_3 + 3.65x_4 - 0.014x_1x_2 - 0.039x_1x_3 - 0.19x_1x_4 - 1.72x_2x_3 - 1.23x_2x_4 - 0.94x_3x_4 - 4.45x_1^2 - 3.92x_2^2 - 2.76x_3^2 + 0.20x_4^2$$

Where, Y = reducing sugar yield (%w/w)

2.2.2 Acid Pretreatment

Rishi Gupta et al. [3] presented the effects of varied sulfuric acid dosage and pretreatment time on the efficiency of enzymatic hydrolysis and fermentation process of *Corncob* (CC), *P. juliflora* (Pj) and *L. camara* (LC). 100 grams of lignocellulose materials were added into 1-5% (w/v) H_2SO_4 solution for 15, 30, 45 and 60 minutes at 121 ^{o}C. The solid fraction from filtration was washed by distilled water until reached neutral pH and dried at 60 ^{o}C. From the analytical result, the optimum condition for hemicellulose hydrolysis of CC was 2.0% (v/v) H_2SO_4 for 30 minutes, while the suitable condition of PL and LC was 3.0% (v/v) H_2SO_4 for 45 minutes. However, increasing acid concentration or pretreatment time over the optimal condition leaded to decrease in sugar yield because of the formation of furfurals and hydroxyl-methyl-furfurals (HMF).

Candido,R.G et al. [8] studied the effect of reaction time of acid pretreatment on percentage of lignin and hemicellulose removal. For the methodology, 1,500 grams of bagasse were soaked in 10% (v/v) H_2SO_4, under a consistency of 10% (w/v), in 4 liters of polypropylene beaker. The reaction continues in water bath and when the temperature reached 100 ^oC the results were recorded at 0, 5, 10, 20, 30, 40, 50 and 60 minutes thereafter. The solid fraction from filtration was washed by distilled water until reached neutral pH. The amount of hemicellulose removed is higher than that of cellulose and lignin because hemicellulose was easily hydrolyzed by sulfuric acid in order to amorphous molecule. The crystallinity of cellulose was not easy to hydrolyze by low concentration mineral acids. Because of the condensation reaction under acid pretreatment then the process was obtained low lignin removal. The hemicellulose was loosen more than 70% for 50 and 69 minutes. The loss of cellulose was satisfied at 50 minutes, after 60 minutes there was increased the loss of cellulose. Nevertheless, the reaction time at 40 minutes was the limit of lignin removal. In conclusion, it was found that reaction time for pretreatment of sugarcane bagasse with H_2SO_4 was 40 minutes.