AMMONIA-BASED PRETREATMENT FOR LIGNO-CELLULOSIC BIOMASS CONVERSION – AN OVERVIEW

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Abstract

Lignocellulosic biomass is generically known to describe a plant-based biomass, typically agricultural residues that forming a complex structure of cellulose, hemicellulose and lignin. In Malaysia, there is about 168 million tons of lignocellulosic biomass been produced locally and provide an opportunity for industries to make a breakthrough in biomass utilization. Currently, several pretreatment technologies have been progressively developed, preparing it for efficient downstream utilization such as acid or enzymatic hydrolysis. Ammonia-based pretreatment has become as one of the most attainable chemical options due to its high potential in the post- pretreatment effect and future commercial utilization. The most promising feature of this pretreatment owes to the ability of ammonia to be selective and effective in removing the lignin and allowing the carbohydrates to be easily digested in bioconversion. This paper intends to provide an overview on the biomass conversion using the ammonia-based pretreatment focusing on the fundamental concept on the lignocellulosic biomass and ammonia itself, aspect of the ammonia-based pretreatment, the chemical reaction of carbohydrates, the process from the aspect of chemical and physical changes in biomass and hydrolysis performance. This review also discusses the ammonia handling in the operation and safety precautions requirement during the development of EFB pretreatment process.

Keywords: Ammonia, Biorefinery, Cellulose, Hemicellulose, Lignin, Lignocellulosic biomass, Pretreatment technologies.

1.Introduction

Bioconversion of lignocellulosic biomass to energy and biochemical products has gained attention from many researchers due to the technical feasibility of the developed alternative process as well as being renewable and sustainable in nature. The high energy demand globally has push researcher to find more option for energy supply. This scenario, therefore, opens up the potential of the lignocellulosic biomass to be an important energy resource for human economic activities. Research conducted in the previous prove that lignocellulosic biomass has the ability to create and supply environmentally technology for energy production. The bioconversion of lignocellulosic biomass for energy, chemicals and products begins with the pretreatment process to purposely degrade the amorphous hemicellulose, break down the crystallinity of cellulosic materials and remove the recalcitrant lignin prior to downstream process such as the enzymatic hydrolysis process that uses an enzyme to produce fermentable sugar for further bioconversion to produce biohydrogen, bioethanol and biosuccinic acid [1, 2]. In order to fully utilize the bioconversion of biomass, the pretreatment is an essential process required to prepare the complex and recalcitrant biomass for efficient utilization in downstream processing.

Pretreatment will render and make the cellulose fibre more easily for the enzyme to bind and hydrolyze the fibre [3, 4]. The best pretreatment technology should able to render lignocellulosic biomass completely and allow the action of cellulase enzymes. There are numerous technologies have been developed thus far for pretreatment, but recent studies prove that ammonia-based pretreatment has the most promising technologies in converting the recalcitrant biomass to fermentable sugars without significantly affecting the carbohydrates content. When compared to others pretreatment technologies, ammonia-based pretreatment is very efficient in removing the lignin from the biomass. An effective process pretreatment technologies should have restricted the formation of inhibitors which will disturb and impede the enzymatic hydrolysis of carbohydrates, recovered value-added product, required low energy input as well as low capital cost [5]. This review focusses on the chemistry of lignocellulosic biomass, the utilization of biomass, the main principles behind each of ammonia-based pretreatment, the effectiveness of using ammonia toward lignocellulosic biomass structure as well as handling and safety on ammonia-based pretreatment during operation.

2. Why Lignocellulosic Biomass?

The most abundant and attractive material of lignocellulosic biomass such as agricultural residue, wood and energy crops are easy to find for many purposes. This renewable material is very useful for further utilization because it always considers as waste and it became cheap to obtain. The demand for lignocellulosic biomass from agricultural is increasing yearly because it can be utilized to produce a substantial energy, biochemical and bioproducts. Lignocellulosic biomass from agricultural residue can be a high potential for renewable and sustainable energy to 1.6 billion people in developing countries which still did not get the full access of electricity. These developing countries are the major producer for most of the materials and with the improvement of technology, they will gain the profit and develop a better nation. As raw materials, biomass residues offer attractive potentials for large-scale commercial utilization and community-level enterprises [6-8].

A basic requirement for preparing and utilizing the agricultural biomass in the future industrial bioeconomy is to control and minimize the waste volume. It is known that only a minor portion of a given crop's total biomass is actually used productively, and the remaining is wasted as residue. Typically, this biomass is left over after the crop have been harvested. Some of the leftover may be disposed by landfill method or burning due to space limitation and to save time and energy [9-14]. The major portion of the under-utilized waste stream mostly come from agricultural and plantation residues [15], the residues are a readily available source of lignocellulosic biomass, which can be obtained from the current feedstock and harvesting activities without the need for additional land cultivation [16]. The best concept is to create a zero-waste production for the crop utilization to improve the bioeconomy, which eventually will uphold the value of the residues. With the right platform, this concept can be sustained for the long term instead of searching new alternative raw materials [15, 17].

Due to the availability and to minimize the waste production, lignocellulosic biomass is very attractive for further utilization is mainly because of the low environmental impacts [18, 19]. This is also coming together with the global attempt in controlling the climate change issue, which leads the research and development team to opt for alternative energy so we can reduce the emission of greenhouse gas. Production of biofuels from lignocellulosic biomass proved to significantly minimize the carbon dioxide release to the environment [20, 21].

Lignocellulosic biomass offers considerable potential for renewable and sustainable energy resource without causing a feud of food-fuel supply since the biomass itself is primarily an agricultural residue derived from non-edible matter [21, 22]. Therefore, utilizing the biomass as the feedstock in bioconversion plants could avoid competition with food production. Moreover, this potential utilization of lignocellulosic biomass could be economically beneficial to smallholder farmers since they could increase their incomes through selling the residues to the processing plants. Currently, some agricultural residues have negative economic value since the farmers have to pay to eliminate the residues from their land. Therefore, by using the bio-based economic approach, this biomass residues can be sold, utilized and converted into high-value products which create additional income opportunities for the farmers. The biomass utilization by the bio-based economic approach could offer considerable potentials to improve the development of the rural area and increase the overall financial situation as well as developing regions to emerging [23]. Lignocellulosic biomass only causes problems to a human when it is not in use. Therefore, the challenge is to utilize and convert the biomass as a renewable source for energy and other productive uses.

2.1. Chemistry of lignocellulosic biomass

2.1.1. Cellulose

The most abundant naturally occurring organic and high molecular weight polymer in the world is cellulose. It is the major structural material in plant cell wall because a large fraction of the cell wall is made from cellulose and this structure provides the plant to be stable even in the absence of water as well as being renewable, and biodegradable [24]. The basic structure of cellulose is a linear, consisting of a homopolymer of D-anhydroglucopyranose monomeric units that connected through

β-(1-4)-glycosidic linkages. The standard structure of cellulose is a long chain polymer with D-glucose, a sugar, as its repeating units that formed unbranched chains [17, 25] as shown in Fig. 1. Depending on the source, the degree of polymerization of plant cellulose could be in between of 7000 to 15000 glucose molecules [26]. The strong hydrogen bonds hold together the microfibrils that formed cellulose chain. The cellulose fibre that consists of microfibrils is attached together via van-der-Waals forces interaction [27]. Due to the crystalline structure, the cellulose fibres are very difficult to be degraded. Natural cellulose is hydrophilic in nature and changes of their characters will cause loss of mechanical properties on moisture absorption which is very important in paper and board application.
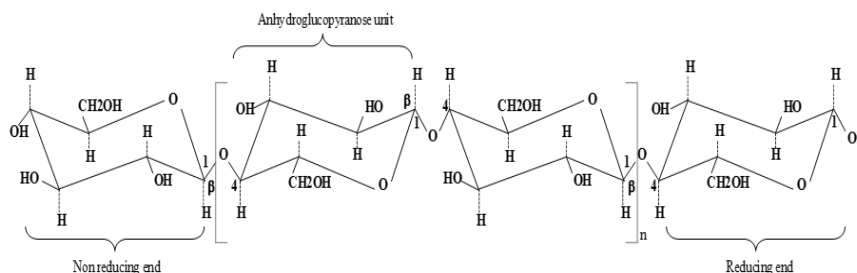

Fig. 1. Molecular structure of cellulose [17].

Cellulose is a highly polar polymer and is compatible with the polar polymer. The hydroxyl groups (OH-) is known as the functional groups of cellulose chain. Interaction of the OH- groups with each other or with N-, O- and S- group are via hydrogen bonds. The bond in between of OH- groups of cellulose and water molecules are hydrogen bonds. Hydrogen bonds also exist between OH-groups of cellulose and water molecules. Because of these, the cellulose surface becoming hydrophilic due to the hydroxyl groups. Both ends of the cellulose chain hold OH-groups [28]. The C_1 -end, a free anomeric carbon, is in hemiacetal form, termed as the reducing end. It has the reducing properties, which can result in the formation of an acetal when the hydroxyl group of hemiacetal becomes protonated and is lost as water [17].

The strong hydrogen bonds along the direction of the chain help the cellulose chain to be stable. In general, cellulose is surrounded by a substance that acts as matric, which is the hemicellulose, and encrusting substance which is lignin materials [28]. Cellulose is a hydrophilic component but the physical properties of it are insoluble in water and in organic solvents due to the existent of hydrogen bond and adhesion of lignin to the polysaccharides [17, 29].

The complex structure arrangement has caused material such as enzymes and even water can hardly penetrate in these fibres. However, the structures of cellulose are not completely crystalline. The structure characteristic that has micropores with amorphous will be the point of attack for the cellulolytic enzymes [8].

2.1.2. Hemicellulose

The degree of polymerization of hemicelluloses is in between 80-200 of different pentose and hexose sugars. Its structure is branched polymers with low molecular

weight [17, 30, 31]. Every plan dry weight consists of hemicellulose, which is about 20 to 35%, therefore it is the second most available polymer in the world. Typically, hemicellulose is formed by a group of homo- and heteropolymers. In this group, it is mostly consisting of anhydrous β-(1-4)-xylopyranose, mannopyranose, glucopyranose, and galactopyranose which is the main chains or backbones with a number of substituents. The principal sugars in hemicelluloses are D-xylose, L-arabinose, D-glucose, D-mannose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid, D-galacturonic acid, and to a lesser extent L-rhamnose, L-fucose, and various O-methylated neutral sugars. [8, 9, 17].

Low hydration hemicelluloses stabilize the cell wall through the hydrogen bonding interaction with cellulose. Arabinoxylans, glucuronoxylans, and xyloglucans are the example of low hydration hemicelluloses. Another interaction that stabilizes the cell wall is in between of covalent interaction with lignin and water-soluble stabilize in their raw state. [18].

A high degree of side-chain substitutions on hemicellulose main chain binds less tightly to cellulose and becomes more water-soluble. Hemicellulose is commercially being extracted via aqueous alkali [32-34]. Even with alkaline extraction, hemicelluloses like galactoglucomannans, glucomannans, galactomannans, and β-glucans tend to be heavily hydrated and have fewer, if any, ester-linked side chains do not change to insoluble. Alkaline extraction does not usually render them insoluble [18]. Both celluloses primary and secondary cell wall is associated with xylan-rich hemicelluloses [29, 32, 35]. Most of the finding reported that hemicellulose is chemically linked or cross-linked with other polysaccharides, proteins or lignin. The major interface of lignin and other carbohydrates is xylan [36, 37].

Different types of plants such as hardwoods (angiosperms), softwoods (gymnosperms) and grasses (graminaceous plants) contain different constituents on the hemicellulose and lignin. The graminaceous plant such as rice straw consists of a main β-(1-4)-D-anhydroxylopyranose backbone with substituents of α-(1-2)-4-O-methyl-D-glucuronic acid (MeGlcA) or α-(1-2)-D-glucuronic acid, and α-(1-3)-L-arabinofuranosyl attached at C2 and C3 position of the main chain [29, 36]. Figure 2 shows the molecular structure of hemicellulose for typical graminaceous plants with main β-(1-4)-D-anhydroxylopyranose backbone.

In addition, the graminaceous xylan and other naturally occurring hemicelluloses contain O-acetyl groups located at some of the hydroxyl groups in the hemicellulosic backbone. It is well accepted for the isolation of hemicellulose by aqueous alkali from straw [32, 38] and in the same time, O-acetyl groups also are frequently removed during this isolation process [29, 30]. Xiao et al. [29] characterized the hemicellulose prepared from rice straw and found that it is composed with the significant amount α-glucan and β-(1-3)-L-arabinofuranosyl-(-α (1-2)-4-O-Methyl-D-glucorono)- β-(1-4)-D-xylan. Xiao et al. [29], Sun et al. [32] and Sun et al. [38] reported that comparison on hemicelluloses extracted from rice straw using alkali-soluble (NaOH) and alkaline-peroxide (H_2O_2) extractions shows that the former extraction solubilizes the glucose which inside the hemicellulose and probably came from α-glucan while the latter extraction increases more dissolution for larger molecular sizes of hemicellulose. The larger molecular is rich in xylose and based on theory, this is resulting from L-arabino-(4-O-Methyl-D-glucorono)-D-xylan.
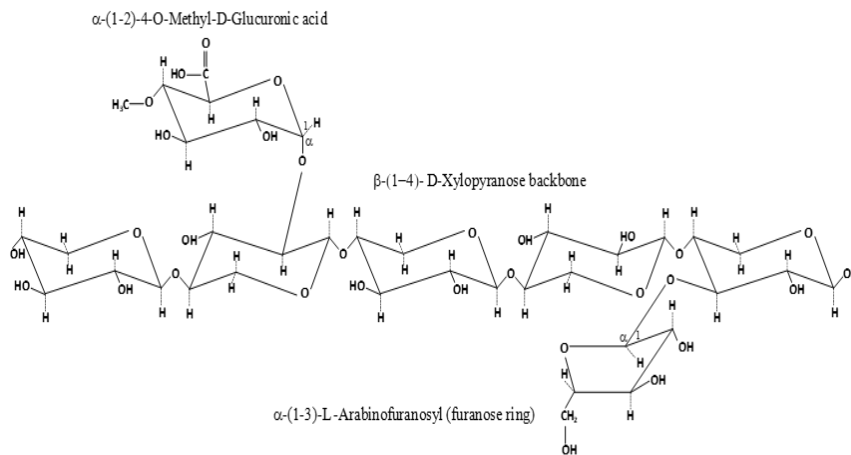
Fig. 2. Molecular structure of β-(1- 4)-D-xylopyranose backbone attached with α- (1-2)-4-O-MeGlcAand α-(1- 3)-L-arabinofuranosyl (furanose ring) [17, 36].

2.1.3. Lignin

Globally lignin is estimated about 300 billion MT with a yearly biosynthetic production rate of 20 billion MT. As the third most available natural polymer after cellulose and hemicellulose, lignin is can become very useful material for the global bio-based economic specifically in biofuels and production of bioproduct [39].

Lignin is a heterogeneous, complex and highly cross-linked large aromatic polymer with substituents connected by both ether and carbon-carbon linkages [28, 36, 40]. Lignin is an amorphous polymer consisting of phenylpropane units, and their precursors are three aromatic alcohols (monolignols) namely p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol as shown in Fig. 3. The respective aromatic constituents of these alcohols in the polymer are called p-hydroxylphenyl propanol (H), guaicyl propanol (G), and syringyl propanol (S) [28].

These polymers are deposited largely in the cell wall of the secondary thickened cell [41], and this characteristic makes the cell wall become rigid, function as internal transport of nutrients and water as well as a good defence system against microorganism attack. Despite the lignin structure itself made it as the most recalcitrant polymer to process, the energy content in lignin is very high and upon separation, the lignin can be burned to produce electricity as well as to produce other chemicals for further used [9].

There are three types of monomers that made lignin complex macromolecules namely; p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol.

2.1.4. Lignin-carbohydrate complexes (LCC)

In graminaceous plants or herbaceous crops, such as wheat straw and rice straw, lignin and carbohydrates (hemicellulose) are attached by ferulic bridges via ester-linked ferulic acids. Therefore, they are often referred to as "lignin/phenolics-carbohydrate complexes" or LCC. Ether bond helps ferulic acid attached to lignin and attached to carbohydrates via ester bond [41] as shown in Fig. 3.
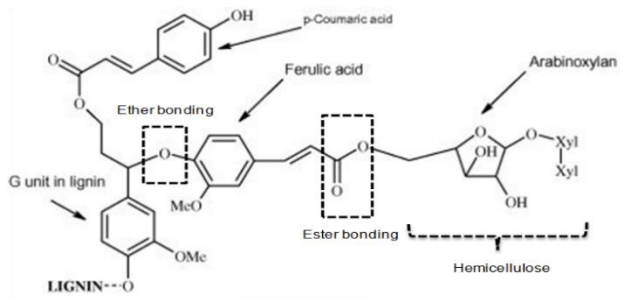
Fig. 3. Lignin/phenolic-carbohydrate complexes [39].

The previous study on isolated LCC from energy crop *Arudo donax* Linn confirmed that LCC was xylan-rich, exhibited narrow molecular weight distribution and the carbohydrate and lignin constituents were chemically bonded via covalent bonds. A more detail characterization of lignin demonstrated that β-O-4' alkyl ether linkages (77-100%) were predominant [42]. Native LCC linkages are likely to exist throughout the cell wall structure.

2.2 Utilization of lignocellulosic biomass in Malaysia

Geographically Malaysia is located at the equator and make the country blessed with a tropical climate, which is hot and humid. This climate condition making Malaysia rich with agriculture resources such as palm oil and rubber plantation that give this country to fully discover the tropical forest potential [43, 44].

Lignocellulosic biomass is one of the cheapest and high potential of raw materials to produce a biobased product such as fuel, energy, refine chemicals and many more. The National Biofuel Policy encourages the production of biofuels, specifically from oil palm waste so that it be used locally as well as improve the local economy. Since 2011, the Malaysia government has launched a goal for the year 2020 in promoting the biofuels through the National Biomass Strategy. The aim of this goal is to strategize and develop better technology and utilization for the local biomass and the related economy activities that eventually will increase Malaysia gross national income (GNI) as well as creating more jobs to the nation [44].

In 2013, over 100 million tonnes of biomass have been generated from the palm oil industry (Table 1) and the amount is expected to be approximately about 100 million dry tonnes by the year 2020. The strategy also suggests that 10% of bioethanol produced locally will be mix with current petrol in the country by 2020 in order to reduce the greenhouse gasses (GHG) emission. As a result, many industries sector show their interest in improving the bioethanol production plant from oil palm biomass. In addition to this, one of the states in Malaysia, Sabah with the Green Agenda on Investment Opportunities in Bio-based Materials has a plan for the commercial scale biomass-ethanol plant. The key findings include Sabah's potential to mobilize up to 5 million dry tonnes of biomass alone and this giving the availability of vast opportunities in the state for the longer term [45]. Table 2 shows that the oil palm tree itself has approximately 5.8 million hectares total of planted area in 2017 for both peninsular and east Malaysia.

Even though the numbers look promising but there is still much more to be done in Malaysia to maximize the utilization of oil palm residues for cogenerating in Malaysia. Many studies conducted show that the used of lignocellulosic biomass as a new source of energy is one of the ways to consume and reused the residues properly [44].

Table 1. Availability of Malaysia biomass in year 2013.

Type of Biomass	Source	National total (million tonnes wet biomass)	Reference
Fronds	Palm Oil	46.51	[45]
Trunks		6.58	[45]
EFB		22.39	[45]
Shells		2.28	[45]
Fiber		3.36	[45]
POME		59.30	[45]
Rice husk	Paddy	0.38	[46]
Rice straw		2.79	[46]
Sugarcane bagasse	Sugar cane	1.64	[46]

Table 2. Performance of Malaysia agriculture industries in year 2017.

Industries	Total planted area (hectares)	Production (tonnes)	References
Oil Palm Tree	5,811,145	19,919,331	[47]
Paddy	708,148	627,642	[48]
Sugarcane	330,800	-	[48]
Pineapple	13,280	413,800	[48]
Maize	11,217	75,246	[48]

3. Pretreatment of Lignocellulosic Biomass

Lignocellulosic biomass has calories and carbon building blocks such as cellulose, hemicellulose and lignin "locked-up" in their plant cell walls. Owing to the chemical structure of the biomass that made up of carbohydrate and lignin, it is not an easy task to utilize the native biomass. Unlike corn and sugarcane, both are easy to depolymerized to simple sugars, however, carbohydrates fraction in lignocellulosic biomass are not easy to be hydrolyzed by enzyme due to its recalcitrant and complex structure. The hydrolysis performance process is depending on the efficiency and accessibility of the enzyme to cellulose [49].

Therefore, pretreatment process is necessary to alter and exposing the structure of the cell wall, particularly the lignin matrix, to hydrolyze the hemicelluloses (arabinoxylan), to reduce crystallinity of the cellulose, and finally to make the cellulose more susceptible to enzymes in the hydrolysis process to produce fermentable sugars [19, 50, 51]. Figure 4 shows percentage of general composition of lignocellulosic biomass feedstock Pretreatment, even conducted in mild

condition will slightly alter in the structural and chemical composition of the lignocellulosic biomass. It can be conducted at very severe condition employ high pressure or temperature or combination of both treatment with additional acid or alkaline chemicals to further aggravate the internal cell wall structure which can remove the recalcitrant lignin and hemicellulose to a certain limit and making more porosity of amorphous in the cellulose structure [17, 24, 52]. Figure 5 shows the structure of lignocellulosic biomass before and after the pretreatment.

Currently, there is a variety of pretreatment method that alters the structure of both physical and chemical composition of the lignocellulosic biomass. Most of the technologies have improved and furthest developed. Many methods have been shown to be feasible than the others but in the end the need for end product and acceptability rate of any disadvantages that make the technology best for each pretreatment. Pretreatment technologies vary in their efficiency of sugar (xylose oligomers) recovery and to reduce the formation of unwanted byproduct that might become an inhibitor in fermentation process [24].

Pretreatment can be grouped into four main categories namely: physical, chemical, physicochemical and biological pretreatment. The pretreatment process can be solely or in a combination process with another type of pretreatment as long as the pretreatment process can enhance the lignin removal and increase the digestibility of cellulose. Even though pretreatment is not a cheap process for biomass utilization, but it has a great potential to alter the lignocellulosic biomass chemical structure and enhance the pretreated biomass utilization in the downstream process which can eventually reduce the operating cost on the conversion technology the costing by further exploration [53]

Several pretreatment processes, such as mechanical pretreatment, chemical, liquid hot water, irradiation beam or fungal pretreatment [54, 55], or combination of this pretreatment are commonly applied to pretreat biomass from agricultural residues. However, among these pretreatments, mechanical and chemical pretreatments have been used aggressively to pretreat rice straw. Typically, the particle size and the operating pretreatment conditions vary with a different structure of the biomass, and therefore optimization work on pretreatment process, tailored for specific biomass, is performed to enhance conversion and yield in the enzymatic hydrolysis conversion or any related downstream processing.
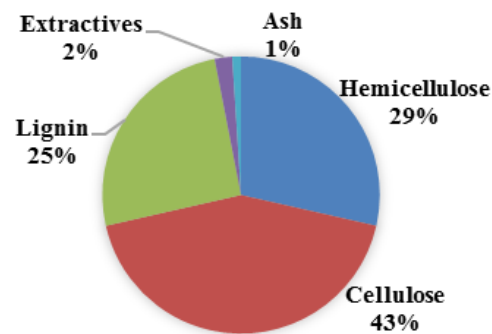

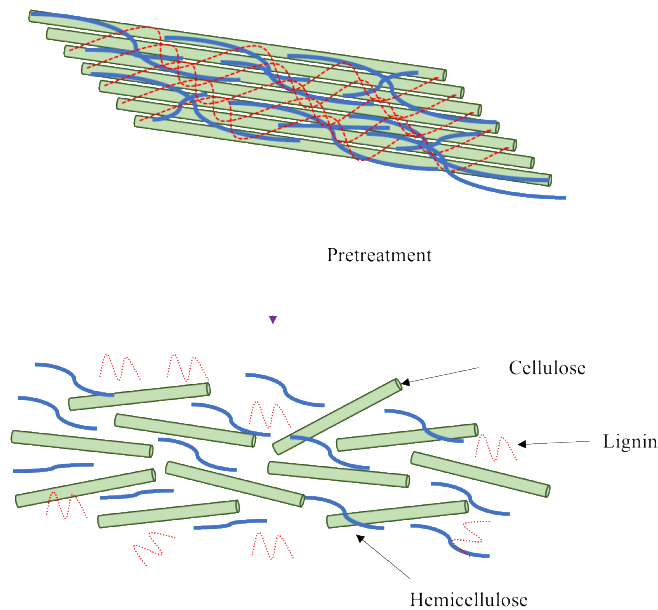**Fig. 4. General structure composition of lignocellulosic biomass [43].**

Fig. 5. Effect of pretreatment on lignocellulosic biomass.

3.1. Ammonia-based pretreatment

Ammonia has the ability as an effective pretreatment reagent that can make the lignocellulosic biomass swells [56]. Ammonia, having an alkaline characteristic, acts by breaking down crystallinity of cellulose and acetyl linkages in the chemical structure of the cell wall of lignocellulosic biomass [57]. It is one of the most potential and efficient pretreatment because ammonia is highly volatile and recyclable [56]. Dale [58] reported that the ammonia residue from pretreatment process can improve bacteria growth in fermentation due to high nitrogen content in the pretreated biomass.

3.1.1. Chemistry of ammonia

The most abundant nitrogen-containing compound in the environment is ammonia. Ammonia is well known with the strong pungent smell and colourless gas. The gas is lighter than the air and it is highly soluble in water, but only about 30% (weight %) of the gas can be solubilized in the water. By applying a certain pressure, ammonia can be liquefied at room temperature. Ammonia is a stable compound, however, it is still can be decomposed into hydrogen and nitrogen by heated up through electric discharge or by heated catalyst [57, 59].

Generally, alkaline pretreatment, the initial reaction involves solvation of hydroxyl groups by hydroxyl ions that causing the biomass to swell. Depending on the reaction temperatures, these are the possible chemical reactions that can occur [26, 59, 60], including:

- Dissolution of un-degraded polysaccharides (hemicellulose) and lignin.

- Peeling of the end- groups and the formation of alkali stable end-groups.
- Alkaline hydrolysis of glycosidic bonds and acetyl groups, and
- Degradation and decomposition of dissolved polysaccharides and peeled monosaccharide.

Ammonia, added during biomass pretreatment, generally reacts with moisture (water) to form ammonium and hydroxyl ions. This is known as exothermic reaction, where it will increase the temperature of biomass inside the system. Compare to pure alkaline pretreatment without the presence of ammonia, this ammonia pretreatment has ammonium and hydroxyl ions that can react with some reactive linkages such as ester linkage in a lignin-carbohydrate structure in the biomass. At high temperature, many chemical reactions can occur, loss of polysaccharides and declining in the degree of polymerization are in large part due to peeling and hydrolytic reactions. Other low molecular weight acids like acetic acid and formic acid are formed during this pretreatment [17, 56, 59].

3.1.2. The benefit of using ammonia

Several chemicals have been used in pretreatment process, of which ammonia is much more desirable. Ammonia is being very attractive to the industry due to selective delignification occurrence without significantly carbohydrate loss. The properties of ammonia that is highly volatile, makes it is easier to be recovered and reused [61, 62]. This feature indirectly reduces the cost of operation if ammonia is utilized in the pretreatment system in a well-design process. In addition to this, ammonia utilization will also create anti-microbial effect due to ammoniation that will allow a long-term storage of pretreated lignocellulosic biomass prior to downstream processing.

Meanwhile, as for aqueous ammonia that contains 70% of water with the remaining is ammonia, thus no water should be added to the dry biomass during pretreatment process. Similarly, with anhydrous ammonia, the pretreated biomass can be used without any extraction once all the ammonia has been evaporated [63].

3.2. Pretreatment method for lignocellulosic biomass

There are many methods of pretreatment to disrupt and change the structural and chemical compositional of lignocellulosic biomass. It can be via high temperature or high-pressure process condition, treatment with corrosive chemicals like acids and alkali or exerting a combination of both conditions. Novel pretreatments have been well explored by many researchers and some have scaled up the pretreatment technology from laboratory to pre-commercial or commercial scale. This section will further discuss the ammonia-based pretreatment as well as other leading pretreatment technology, for comparison purpose, in pretreating empty fruit bunch (EFB), one of the highly produced local biomass residue. Table 3 summarizes and compares the conditions of different pretreatment parameters for these ammonia-based, steam explosion and acid pretreatments with respect to EFB pretreatment.

Table 3. Comparison on different pretreatment condition.

Pretreatment method	AAS	ARP	AFEX	EA	SE	Acid
Chemical form	NH_4OH	NH_4OH	NH_3	NH_3	Saturated steam	H_2SO_4, HNO_3, HCI, H_3BO_3
Chemical loading to biomass	2:1	2:1	1:1	3:1	-	2:1
Temperature	27°C-60°C	90°C-180°C	80°C-140°C	27°C -120°C	180°C-240°C	100°C-200°C
Pressure	no pressure	1-3 bar	10-30 bar	10-30 bar	2-10 bar	1-3 bar
Reaction time	1-12 hour	10-60 min	5-45 min	5-30 min	5-60 min	15-45 min
Reference	[64]	[65]	[17, 66]	[66]	[52, 62]	[67, 68]

*- Data not available

3.2.1. Aqueous ammonia soaking (AAS)

Soaking in aqueous ammonia at low temperature and the ambient condition can remove lignin efficiently in lignocellulosic biomass by reducing the interaction with hemicellulose. This is due to the increase of pore size, surface area and retained hemicellulose that can be hydrolyzed to produce sugar as a supplement of carbon source in the fermentation process. According to Kim and Lee [65], which started the soaking ammonia pretreatment with corn stover, evaluated that approximately 83% was recovered and about 75% delignification was achieved with 92% of hydrolyzed xylan. The pretreatment consisted two-stages percolation process where it washed the biomass with water and followed by soaking with aqueous ammonia. More recently, Kim et al. [69] evaluated AAS pretreatment with barley hull. In this study, barley hull was pretreated with 15 % aqueous ammonia at 75°C for 48 hours with solid to liquid ratio 1: 12 and result show about 66 % of lignin was solubilized, 83 % of glucan and 63 % of xylan were hydrolyzed [70].

The purpose of using aqueous ammonia is due to high lignin removal from lignocellulose biomass. OPEFB contain 30% higher of lignin than any other lignocellulosic biomass. The EFB pretreatment experiments were conducted by varying different temperature (40°C, 60°C and 80°C) with the same retention time of 12 hours, and aqueous ammonia loading of 21%. The lignin removal ranged from 36.4 % to 43.6 % and this amount improved with an increase in the temperature. However, the glucan yield was not increased as the reaction temperature increased. This is a typical chemical change of lignin when using ammonia as pretreatment materials [62, 71]. The result found that the most optimal conditions for AAS were at 60°C, 12 hours of retention time and 21% ammonia loading, showed 41.4 % glucose yield during an enzymatic digestibility test for 96 hours using 60 FPU of cellulase. More recently, Zulkiple et al. [63] evaluated OPEFB with AAS at room temperature condition with 6.25% of ammonia and 14 hours of soaking time produce 168.58 g L^{-1} of glucose. This study also indicated that glucose and xylose yield did not increase even with longer pretreatment time. The same result was observed when they increased the concentration of ammonia

to 20 % where this concentration increase did not yield as much glucose and xylose when pretreated with 6.25 % of ammonia [64].

3.2.2. Ammonia recycle percolation (ARP)

Ammonia recycles percolation (ARP) pretreatment was performed to pretreat the herbaceous biomass. Many studies have been conducted particularly with corn stover and switchgrass. In ARP pretreatment, biomass was pretreated with aqueous ammonia through a percolation reactor (packed -bed, flow-through type). This aqueous ammonia cause swelling and efficiently delignified the lignocellulosic biomass at high temperature [70].

The process was able to solubilize almost half of the xylan, and yet about 92% of the cellulose content still remained in the pretreated biomass. After the completion of the reaction, the liquid was separated from the biomass and sent to recovery system by using steam heated evaporator. Then, the ammonia was reused meanwhile the rest of fraction was sent to the crystallizer. Based on this study, ARP was applied as biomass fractionation method in two stages process. The first stage of ARP separated the xylan about 84 % from the biomass followed by lignin separation of about 75 % in the second stage [70].

In general, ARP gave a high degree of delignification but still able to preserve the cellulosic component in biomass. According to Kim and Lee [70], two stages of the process of dilute acid or hot water treatment followed by ARP would provide a better yield. As a result, the xylan was hydrolyzed up to 90% conversion and the lignin removal was up to 75% with the remaining solid contained approximately 78% cellulose after the two-stages pretreatment. Overall, this ARP enhanced the enzymatic hydrolysis process to almost 95% of glucan conversion.

3.2.3. Ammonia fibre explosion (AFEX)

Ammonia Fiber Explosion (AFEX) is regarded as one of the high potential for biomass pretreatment technology. The process involves pressurized liquid anhydrous ammonia to pretreat lignocellulosic biomass at a mild temperature (60°C-100°C) at high pressure (250-300 psi) for 5-30 minutes of retention time [58]. The pressure in the vessel is released instantaneously after the pretreatment process. The reaction combining effect of ammonia and pressure causes the biomass to swell and disrupt the fibres physically while the lignin remains unaffected during the process. Ammonia molecule penetrates the lignocellulosic cell wall structure during AFEX pretreatment and promotes partial decrystallization of cellulose, depolymerisation of hemicellulose, deacetylation of acetyl groups, cleaving lignin-carbohydrate linkages with no inhibitor formation [17, 59, 72, 73].

About 97% of ammonia can be recovered and recycled despite its high volatility [59]. This is a dry-to-dry process as there is no liquid stream produced after the pretreatment process. Lignocellulosic biomass is well preserved and stable for a long time after the pretreatment as there is no degradation of cellulose and hemicellulose. Thus, the biomass is easy to be densified, and pelletized for a longer storage time and transported on a commercial scale. The only drawback of this pretreatment is that it cannot be conducted on the biomass that contains a high amount of lignin inside the cell wall. Additionally, there is a concern about using the ammonia as it could have a negative impact towards the environment [17]. It also requires tight safety controls due to the high-pressure requirement in

maintaining the liquid ammonia in the system.

There are many pretreatment technologies that have been tested on pretreating the EFB. Pretreatments are aimed to produce pretreated EFB that can be hydrolyzed easily by enzymes. Some of the technologies are reported to produce high sugar yield but the pretreatment causes sugar degradation product like furfural and hydroxymethyl furfural (HMF) such as acidic pretreatment. These inhibitor products can cause low yield in the fermentation process [72, 74]. Therefore, EFB was pretreated with AFEX to determine the effect of its physical and chemical structure changes [72]. This type of pretreatment is highly recommended for biomass with low lignin content. AFEX pretreatment is one of the ammonia-based pretreatment that shows a positive result in terms of lignin depolymerization.

Despite that, at optimum process conditions, more than 90% of sugars was released from the AFEX pretreated biomass after enzymatic hydrolysis. The optimum pretreatment conditions were reported to be 1:1 ratio of ammonia to biomass loading, 1:1 ratio of moisture content, 45 minutes retention time and 130°C of pretreatment temperature [74]. Physical observation has shown that EFB has black brownish colour and aroma in comparison with the native untreated. AFEX pretreated biomass appeared to be more fragile and its colour charred slightly to a darker brown tone. Moreover, there are significant differences on the chemical composition of lignin, extractive and nitrogen contents. Significant reductions were observed particularly in the lignin and hemicellulose compositions owing to the delignification and hemicellulose deconstruction of the lignin-carbohydrate complex. Formation of organic acids such as acetic acid, ferulic acid and coumaric acid showed the cleavage of the lignin-carbohydrate complex. The increase in nitrogen content was primarily due to the ammonia pretreatment where some ammonia reacted in the biomass structure to produce some amide-based product [17, 72].

3.2.4. Extractive ammonia (EA)

A recent and latest pretreatment method known as Extractive Ammonia (EA) was developed by using liquid ammonia to simultaneously convert the native crystalline cellulose I (C_I) to cellulose III (C_{III}) and the selective extraction of lignin from the plant cell wall [74]. The newly modified cellulose can doubly increase the hydrolysis rate and significantly saves the enzyme loading during hydrolysis. Currently, this EA pretreatment can extract almost half of the lignin content in the corn stover which is beneficial towards the enzymatic hydrolysis. Removal of the lignin during EA pretreatment also helps to improve the yield in enzymatic hydrolysis due to the reduction in enzyme inhibition.

The structural carbohydrates combine with the ammonia-insoluble lignin which can be found in the un-hydrolyzed solid residue from enzymatic hydrolysis process. C_{III} is believed to increase the hydrolysis rates up to fold compared to native C_I when lignin is known to be as a major inhibitor for both enzymes and microbes. In addition, ester bonds play a vital role in cross-linking lignin and carbohydrate and cleavage of the bonds results in breaking down the complex structure of the cell wall and allowing sugars to be easily hydrolyzed [75].

EA reaction process occurs when biomass and liquid ammonia are combined in a reactor at the adequate amount of reactant loading at the required pretreatment conditions. The process continues with filtration of the EA-pretreated biomass to separate the residual solid from ammonia-soluble components and continuously

remove ammonia from biomass into an extraction-collection vessel. Finally, the ammonia is evaporated from the extractives, in a form of dark brown colour viscous liquid. Similar to AFEX pretreatment, about 2% of ammonia retained in the pretreated biomass due to the reaction between ammonia and the biomass [75].

In comparison with AFEX pretreatment, EA pretreated corn stover gives a higher yield of fermentable sugar with 60 % reduction in enzyme loading. This EA pretreatment maintains the extracted lignin and could be further upgraded to achieve high biofuels and chemical in the biorefinery using green process technology [74, 75].

3.2.5. Steam explosion (SE)

Steam explosion is one of the leading pretreatment that uses steam in the process. During this pretreatment, the lignocellulosic biomass is subjected to high pressure saturated steam at high temperature for various residence time. Similar to AFEX, upon reaching the required residence time, the pressure in the reactor is released instantly to give an explosion effect towards the biomass. The explosion effect deconstructs and opens up the cell structure due to the sudden drop in pressure [52].

A steam explosion has been successfully utilized to pretreat several lignocellulosic materials in ethanol production. These biomasses were pretreated from 180°C to 220°C, with residence time ranging from 5 to 10 minutes. This pretreatment shows the decrease of 75 to 90% of xylose content in the biomass with extensive solubilisation of the hemicellulose sugars in the condensed water. The steam penetrates into the biomass and expands the cell walls. During this process, acetyl residues from xylan hemicellulose are liberated in the form of acetic acid and catalyse the chemical reaction which is known as autohydrolysis [62].

Steam explosion becomes an attractive technology because it does not cause excessive dilution of the sugars produced, no chemicals required in the pretreatment and low energy input without any environmental impact. This is one of the most cost-effective processes as compared to mechanical milling, however, the lignin-carbohydrates matrix is not completely disrupted which causes incomplete recovery of the hemicellulose. The drawback of this pretreatment is the inhibitory compounds production that inhibits the subsequent fermentation process [62].

3.2.6. Acid pretreatments

The main purpose of using acid in the pretreatment is to solubilize lignin and hemicellulose and to make an easy access for cellulose. The main acid reagents are sulfuric acid, hydrochloric acid, boric acid, and nitric acid. The degree of lignin and hemicellulose degradation depends on strength of the acid, either concentrated or diluted, used in the pretreatment. However, a strong concentration of acid causes severe degradation of cellulose and also a high concentration of inhibitors that affect the subsequent enzymatic hydrolysis process [67].

Many studies have investigated the effect of dilute acid pretreatment conditions on the recovery of both monomeric xylose and glucose. The conditions of this pretreatment are usually in between of 100°C to 200°C with sulfuric acid concentration at 0.5 to 2.5% for 15-45 minutes of reaction time. One of the results shows that this pretreatment leads to 87% of the overall cellulose recovery and 35% xylose recovery [67]. The high temperature is vital for acid hydrolysis of cellulose.

However, if the reaction time takes more than one hour, xylan may degrade and causes a decrease in the xylose concentration.

Even with the promising result, the major drawback of acid pretreatment is the formation of a different type of inhibitors such as furans, carboxylic acid and phenolic compounds. Glucose and xylose can be degraded to furfural and hydroxymenthyfurfural at high temperature and pressure, and this will form formic acid. All compounds act as inhibitor on the downstream process [68, 76].

4. Efficacy of Ammonia-Based Pretreatment

Most of the ammonia-based pretreatment preserves the carbohydrate after the pretreatment. The glucan is well-preserved in alkaline pretreatment due to high crystallinity and low alkali reactivity with cellulose [77]. There are few key factors influence the efficacy of the pretreatment. The technology should have low operational cost and low capital investment. The ammonia-based pretreatment is efficacy promoting more sugar production and limits the lignin degradation product during fermentation process [78, 79].

Another feature in ammonia-based pretreatment is that the generation of higher-value lignin co-products with a high concentration of hemicellulosic sugars obtained in the liquid phase from hemicellulose after pretreatment [64]. The best pretreatment process results in a good deconstruction of biomass while preserving the cellulose component that results in high yield of sugar during hydrolysis. It is very important to control the production of by-product that will be an inhibitor in the downstream process such as in the fermentation process. Pretreatment efficacy is also assessed by analysing the sugar production of both monomers and oligomers released after the pretreatment as that indicates the total amount of recoverable carbohydrates, the hydrolysate performance in fermentation or any other additional biotechnological potential of pretreated fraction [80].

4.1. Morphology and chemical changes during pretreatment

4.1.1. Crystallization vs amorphous

A major factor that influences the enzymatic hydrolysis performance of pretreated biomass is the physical cellulose structure. The crystalline cellulose is formed by long chains of (1,4) ß-glycosidic linked D-glucose molecules bonded together by hydrogen bonds. The hydrogen bond chains in the cellulose structure prevent cellulose from enzymatic and microbial attack [74]. Additionally, crystallinity index indicates the relative amount of crystalline material in the cellulose structure. Removal of the amorphous fraction in lignocellulosic biomass increases the value of crystallinity index. However, an increase of crystallinity index does not indicate the increment of crystal structure inside of cellulose structure. According to Kim and Lee [65] when the intensity of the amorphous phase reduces, this leads to increment in crystallinity index which finally gives a positive response during enzymatic hydrolysis with the increase in crystallinity index.

4.1.2. Hemicellulose changes

Hemicellulose structure is different from cellulose. It is a chemically heterogeneous polymer of pentose and hexose sugars with significant chemical branches. Due to its low molecular weight, hemicellulose is easily hydrolyzed. During ammonia-

based pretreatment, hemicellulose undergoes the most chemical and thermal changes. As hemicellulose surrounds the cellulose fibrils in the cell wall structure, efficient removal of hemicellulose during ammonia-based pretreatment increases the accessibility of the enzyme to the cellulose that results in more cellulose digestibility [62, 63]. However, pretreatment parameters must be optimized accordingly to avoid the hemicellulose degradation to furfurals and hydroxymethyl furfural which can reduce the sugar yield and inhibit the downstream processes.

4.1.3. Lignin changes

Lignin is well-known as an adhesive material that protects the cell wall structure of lignocellulosic biomass. The lower amount of lignin increases the enzymatic digestibility of cellulose. Different biomass contains different amount of lignin, and during pretreatment, lignin 'melts', coalesces upon cooling and sometimes precipitates [81, 82]. This changes leads to increment of the pore size of the cell wall structure and reduces the non-productive binding adsorption of the enzyme to lignin. Ammonia pretreatment causes delignification and makes the biomass structure to swell which partly deconstructs the lignin structure and increases more internal surface area. The changes enhance the cellulolytic enzymes to digest the cellulose fibres [83].

Only certain pretreatment results in high delignification as well as alteration in the chemical properties of lignin. The study shows that the pretreated biomass becomes more digestible than the native biomass although both may have approximately the same amount of lignin content [83].

4.3. Effectiveness of ammonia-based pretreatment on lignocellulosic biomass

Generally, ammonia-based pretreatment reduces some content of lignin and remove a portion of hemicellulose and decrystallizing the cellulose. The effect of removing lignin from lignocellulosic biomass increases the reaction activity of the remaining polysaccharides. Ammonia pretreatment also swells the cell wall structure of the biomass and improves the enzyme accessibility to the cellulose in the cell wall during the downstream processing in enzymatic hydrolysis.

Mechanism of this action is known as saponification of intermolecular ester bonds crosslink hemicellulose and lignin. This action leads to the breakdown of the lignin-carbohydrates complex (LCC) linkages and the exposes the cellulose microfibrils and enhances the enzymatic digestibility of cellulose. According to Kumar et al. [56], ammonia-based pretreatment did not alter the structure of hemicellulose significantly but it changed some linkages and functional groups in hemicellulose structure significantly. The results from FTIR spectroscopy revealed that the decreased in the hydroxyl stretching and C-OH bonding peaks of hemicellulose hydroxyl groups as well as the decrease in the carbonyl stretching region that representing hemicellulose acetyl and uronic ester groups [84, 85].

Furthermore, due to the swelling of cellulose after pretreatment, the degree of polymerization decreases and more surface area was made available for the enzymatic reaction. Ammonia-based pretreatment caused the amorphous regions suffered greater peeling reaction than the crystalline regions [77, 80]. Thus, the occurrence of the peeling action on the amorphous region led to an increase of cellulose crystallinity. Table 4 compares the major effect of the pretreatment on the physical and chemical changes of the biomass structure while Table 5 shows the effect of pretreatment on the carbohydrate conversion during enzymatic hydrolysis.

Table 4. Major effect of pretreatment on lignocellulosic biomass structure.

Pre-treatment	Removing lignin	Removing hemicellulose	De crystallizing cellulose	Swelling effect	Ref.
AFEX	√√	√	√√√	√√√	[59, 68]
EA	√√	√√	√√	√√√	[75]
ARP	√√√	√√	√√	√√√	[63]
AAS	√√√	√√√	√√	√√	[70]
SE	√	√√√	√	-	[62]
Acid	√√	√√√	-	-	[86]

*√: Low effect; √√: Medium effect; √√√: High effect; -: data not available

Table 5. Major effect of pretreatment on carbohydrates conversion during hydrolysis.

Pre-treatment process	Biomass	Pretreatment conditions	Enzymatic condition	Carbohydrates conversion	Ref.
AFEX	EFB	1:1 (BAL), 135°C, 45 min, 50% MC	35 FPU/g glucan, 24 hours	78% glucan, 62% xylan	[74]
AFEX	EFB	1:1 (BAL) 135°C, 45 min, 50% MC	13.8 FPU/g glucan, 48 hours	90% glucan, 75% xylan	[72]
AAS	EFB	21% ammonia, 12 hours, 60°C	60 FPU/g glucan	80% glucan	[66]
AAS	EFB	6.75% ammonia, 12 hours, Room temperature	15 FPU/g glucan, 24 hours	168.58 g L^{-1} of glucose	[63]
AAS	Barley Hull	15% ammonia, 48 hours, 75°C	30 FPU/g glucan, 96 hours	83 % glucan	[64]
ARP	Corn Stover	15% ammonia, 10 min, 170°C	60 FPU/g glucan, 72 hours	82.1% glucan, 66.9 % xylan	[65]
EA	Corn Stover	1:6 BAL, 120°C, 30 min, 10% MC	7.5 FPU/ g glucan, 24 hours	90% glucan, 75% xylan	[87]

* BAL: Biomass to Ammonia Loading, MC: Moisture content

5. Handling and Safety of Ammonia-Based Pretreatment

Handling and storing of ammonia as well as disposing the ammonia residue upon completion of the pretreatment is a very crucial issue. Ammonia volatiles at atmospheric pressure and poses a high risk of health hazard towards skin, lung or eyes. Due to its hydrophilic properties, (high solubility in water) ammonia is easily absorbed into water and water-retaining body parts particularly human eyes and lungs. Direct or indirect inhalation of concentrated ammonia can cause serious damage and can be fatal if no immediate action is taken [88]. As ammonia is hazardous chemical in both liquid and gaseous forms, even use in small quantities tight safety control has to be implemented during pretreatment.

A multi-step approach must be taken to reduce the risk of operational and even during ammonia storage. Among the steps taken are inclusive of personal protective equipment, administrative controls (sign and warning) as well as engineering control of the system [88]. As for personal protective equipment, a full-face respirator with ammonia compatible materials, ammonia compatible gloves, non-permeable aprons must be worn by the personnel at all times working with ammonia-based pretreatment [88]. Every critical parameter needs to be evaluated by specially trained personnel so that every risk and hazard is under proper assessment.

The primary concern when handling the ammonia is its volatility. It is very important that the reaction vessel is designed according to the specification needed to reduce any potential risk or problem. A proper ventilation is needed so that residues ammonia can be safely released (small amount) according to the proper and safe method.

Ammonia pretreatment is operated at certain pressurized conditions. The design of the vessel must withstand the pressure in the vessel and must include 'fool-proof' pressure relief system consists of primary and secondary safety relief to prevent any unwanted circumstances. The purpose is to make sure, the operation is always under tight safety control and to avoid any malfunction of primary control measure.

A proper documentation of Hazard Identification, Risk Assessment and Risk Control (HIRARC) must be prepared in order to understand the process identify the potential risk, evaluate the risk and finally control the risk as a preventive measure. This administrative control is very important because the document is the guideline for the implementation safety control operation. Regular safety review and safety audit are highly recommended to maintain safety performance and identify items for continuous safety improvement.

6. Conclusion

Lignocellulosic biomass is a plant-based biomass, typically agricultural residues that forming a complex structure of cellulose, hemicellulose and lignin. It is the most plentiful natural bio-resources in the world and becoming an attraction for a fossil-fuel alternative because it is renewable and sustainable. It is underutilized as energy resource due to the complex and recalcitrant plant cell wall structure to biodegradation. Hence, lignocellulosic biomass pretreatments offer some opportunity to overcome the existing problems. There are many pretreatment technologies are developed to utilize and convert lignocellulosic biomass to high-value energy, chemical and product.

Among the pretreatment technologies, ammonia-based pretreatment has become known as one of the most attainable chemical options due to its high potential in the post- pretreatment effect and is economically viable for future commercial utilization. The most promising feature of this pretreatment owes to the ability of ammonia to be selective and effective in removing the lignin and allowing the carbohydrates to be easily digested in bioconversion. Several fundamental concepts on the lignocellulosic biomass and ammonia itself, an aspect of the chemical pretreatment, types of ammonia-based pretreatment, the chemical reaction of carbohydrates, the process performance from the aspect of chemical and physical changes in biomass and hydrolysis conversion are presented in this article, and advantages as well as disadvantages of ammonia-based pretreatment. The ammonia handling and safety concern during pretreatment is also briefly presented.

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