USE OF IONIC LIQUID FOR IMPROVEMENT OF GLUCOSE YIELD FOR ETHANOL PRODUCTION

by

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DECLARATION

I declare that this thesis entitled "Use of Ionic Liquid for Improvement of Glucose Yield for Ethanol Production"is the result of my own research except as cited in the references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature   : _____

Name   : _____

Date   : _____

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Use of Ionic Liquid for Improvement of Cellulosic Ethanol Production

ABSTRACT

       Bioethanol production had become one of the popular alternative source of energy to reduce the consumption of fossil fuel based energy. Ionic liquid (IL) are of great interest as solvents for production of fuels from lignocellulosic biomass. The aim of this research is to determine the efficiency of ionic liquid, 1-butyl-3-methylimidazolium (BMIMCl) pretreatment on rice husk (*Oryza sativa*) for improvement of bioethanol production. The pretreatment was conducted by heating 5 % (w/w) rice husk in BMIMCl solution at 80 °C for 48 hours. Rice husk was regenerated by addition of water. The composition of rice husk before and after IL BMIMCl pretreatment were analysed using Technical Association of the Pulp and Paper Industry (TAPPI) test method. The structural changes were also observed and characterized using X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR). It was found that the regenerated rice husk was less crystalline and more high amorphous upon BMIMCl treatment which agreed with finding of XRD and FTIR. The total sugar yield before and after fermentation by *saccharomyces cerevisiae* was analysed using dinitrosalicyclic acid (DNS) method. The regenerated rice husk produces higher total sugar yield compared with untreated rice husk. Therefore, BMIMCl was effective in improvement of bioethanol production.

Penggunaan Cecair Ionik untuk Penambahbaikan Cellulosic Ethanol Pengeluaran

ABSTRAK

Bioethanol telah manjadi salah satu sumber tenaga alternatif yang popular untuk mengurangkan penggunaan tenaga berasaskan bahan api fosil. Cecair Ionik (CI) digunakan sebagai pelarut yang berpotensi untuk penghasilan biobahan api daripada biojisim lignoselulosa. Tujuan kajian ini adalah untuk mengaji kesan pra-rawatan CI 1-butil-3-metilimidazolium klorida (BMIMCl) pada sekam padi (*Oryza sativa*) untuk penambahbaikan pengeluaran bioethanol. Pra-rawatan ini dijalankan dengan memanaskan 5 % (w/w) sekam padi dalam cecair BMIMCl pada 80 °C selama 48 jam. Sekam padi diperoleh semula dengan penambahan air. Komposisi sekam padi sebelum dan selepas pra-rawatan BMIMCl dianalisis dengan kaedah ujian persatuan teknikal pulp dan kertas industri (TAPPI). Perubahan struktur juga diperhatikan dan dianalisis dengan menggunakan belauan sinar-X (XRD) dan spektroskopi inframerah transformasi fourier (FTIR). Ia telah mendapati bahawa sekam padi diperoleh semula selepas pra-rawatan dengan BMIMCl adalah kurang berhablur dan lebih amorfus yang disokong oleh analisis XRD dan FTIR. Jumlah gula hasil sebelum dan selepas penapaian oleh *saccharomyces cerevisiae* dianalisis dengan kaedah DNS. Sekam padi diperoleh semula menghasilkan jumlah gula yang lebih tinggi berbanding dengan sekam padi yang tidak menjalankan pra-rawatan. Oleh itu, BMIMCl adalah berkesan dalam peningkatan pengeluran bioethanol.

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LIST OF ABBREVIATIONS

XRD	X-ray diffraction
FTIR	Fourier transform infrared spectroscopy
BMIMCl	1-butyl-3-methylimidazolium chloride
FAO	Food and Agriculture Organisation
FGB	First-generation bioethanol
SGB	Second-generation bioethanol
IL	Ionic liquid
SHF	Separate hydrolysis and fermentation
SSF	Simultaneously saccharification and fermentation
YPD	Yeast peptone dextrose
BMIM$^+$	1-butyl-3-methylimidazolium cation
CrI	Crystalline index
ODW	Oven dry weight
DNS	3,5-Dinitrosalicyclic acid
TAPPI	Technical association of the pulp and paper industry

LIST OF SYMBOLS

β	Beta
θ	Theta
λ	Lambda
α	Alpha
π	Pi

CHAPTER 1

INTRODUCTION

1.1   Background Study

Fuels such as coal, natural gas, and oil are main sources of energy for our daily life. Nowadays, the energy consumption has been increasing with the population growth. The natural resources have insufficient in meeting the demand of all the user. Depleting of natural resources from nature, mostly is destroyed by humans themselves. The depleting of the natural resources given the awareness for all community according the important to maintain the sustainable growth of natural resources. Therefore, exploring a new alternative energy become significant in order to reduce the total dependency on non-renewable energy sources.

Malaysia is a country that located on the world tropical belt which blessed with fertile soil and abundance of rain. This climate encourages the growth of various types of agricultural products. Therefore, the agricultural activities are usually carried out in many states. However, the agricultural wastes that produced always become the issue since the residues are not disposed properly. As one of the most important agricultural countries in the world, Malaysia creates a huge amount of lignocellulosic agriculture wastes, including palm oil, cocoa and rubber and this provides a great opportunity for producing alternative renewable energy sources that can replace the petroleum as primary fuel source. These agriculture residues are inexpensive and are sustainable sources for biofuel production (Goh *et al.*, 2010).

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Rice is the one of the most important crops in terms of human consumption and is produced in 95 countries across the world. The rising in a number of the local population, cause the requirement towards crops also increase to meet the increasing demand. In Malaysia, especially in Peninsular Malaysia, paddy is cultivated as a rainfed or an irrigated lowland crop. Rice is mainly grown in states such as Kedah, Perak and Kelantan, and which together control more than half of Malaysia's harvested area (Herman *et al.*, 2015). However, the substantial amount of rice husk produces from the paddy usually considered as "waste", which generally burnt or thrown away by the farmers. Most of these wastes is throws away rather than recycle. Hence, the better ways is to turn these surpluses discarded plant material into biofuel, which are eco-friendly and can reduce the emission of CO_2 emissions (Goh *et al.*, 2010).

Nowadays, the worldwide transport sector depends almost totally on fossil fuels. Energy crisis is one of the most serious threats towards the sustainability of human kinds and civilization. Although industrial revolution has changed the world to its sophisticated edge, excessive dependent on fossil fuels as the main source of energy has leads to the minimizing of this non-renewable supply. However, the demand toward petroleum derived fuels is not slowing down but instead increase over the past few decades (Goh *et al.*, 2010). The use of fossil fuels results in many environmental problems and is not economical and effective to use. Furthermore, the resources of fossil fuels are also declining. Bioethanol is one of the main renewable energy sources that can used as a future fuel. There is higher octane number of bioethanol relative to that of gasoline alone, its use as a blender with gasoline which effective in reduces the emission of CO_2, NO_x and

hydrocarbons after combustion. The use of ethanol shows high compression ratio and increased energy production in combustion engine (Shah & Rehan, 2014).

The production of bioethanol is having a long history as a transportation fuel. For examples, it has been used in Brazil since 1925. By that time, the production of bioethanol is 70 times bigger than the production and consumption of petrol (Balat, 2011). The bioethanol production usually from various lignocellulosic residual. The bioethanol feed stocks are generally classified into three types that are starchy materials such as wheat, corn and barley, sucrose containing feedstocks for examples sugar beet, sweet sorghum and sugar cane and lignocellulosic biomass like wood straw and grasses (Shah & Rehan, 2014). The lignocellulosic ethanol production usually can be produce through the process of pretreatment, enzymatic hydrolysis and fermentation (K.S. & Pushpa, 2012).

Recently ionic liquid (IL) a non-volatile solvent has been introduced as a new approach in pretreatment of lignocellulosic biomass. Ionic liquid exhibit excellent physical characteristics such as the ability to dissolve polar and non-polar organic or inorganic as well as polymeric compounds (Pezoa et al., 2010). IL wisely apply in pretreatment of lignocellulosic biomass with the ability to dissolve large amount of cellulose. IL can be recovered after the cellulose regeneration to almost 100 %. Today, ionic liquid is recognized as one of the most promising green chemical solvent due to its desirable property such as non-flammable properties (Vancov et al., 2012).

The study of the production of fuel from the lignocellulosic residue not only provided an alternative to the fossil fuels, moreover and reduce the emission of greenhouse gas. This new bio based industries with low production costs can describe as a method that giving a new life for the lignocellulosic residue rather than being deposit.

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1.2   Problem Statement

The substantial amount of agricultural waste is deposited each day in the environment. Some farmers choose the faster and easier ways to clean all the agriculture waste including rice husk through burning all the waste. This causes the increase the problem of global warming and the environment pollution. Agricultural waste such as rice husk needs to be reduced, and all the waste that does occur needs to be used more constructively. Too often farmers in developing countries lack resources, information and knowledge about the techniques and waste management procedures. All the agricultural waste for example rice husk can provide the value for it by reprocessing them to biofuel that known as bioethanol. Thus, there is a beneficial way to manage all the waste and at the same time can minimize the pollution of our mother earth.

1.3   Significant of Research

Rice husk is one of the agricultural wastes that deposited from the agricultural activities. Rice husk is the lignocellulosic material which has potential for added value by converting it to biofuel that can be the alternatives to the fossil fuel. The demand of biofuels in the market is increasing over year due to the increase of human population. The high demand for biofuel due to the ability to reduce the pollution of the environment and able to recycle the agricultural waste. Apart from that, through this study can demonstrate that rice husk can be utilized wisely in order to reduce the agricultural waste and considered as the bioethanol production in future.

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1.4   Objectives

The objectives of this study are:

1. To determine the composition and structural property (amorphous and crystallinity) of treated and untreated rice husk with ionic liquid 1-butyl-3-methylimidazolium chloride (BMIMCl).

2. To identify the effect of ionic liquid 1-butyl-3-methylimidazolium chloride (BMIMCl) on the production of glucose for treated and untreated rice husk.

3. To analyze the glucose utilization of rice husk after fermented by *saccharomyces cerevisiae*.

1.5   Scope of Study

In this research, the structural properties of the treated and untreated rice husk with the ionic liquid 1-butyl-3-methylimidazolium chloride (BMIMCl) will be elucidated. The total sugar yield hydrolyzed from rice husk after treated with ionic liquid and undergo fermentation process with *saccharomyces cerevisiae* will be analyzed.

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1.6   Hypothesis

The study will show that the structural property of the untreated rice husk will be different from rice husk treated with ionic liquid 1-butyl-3-methylimidazolium chloride (BMIMCl). The treated rice husk will be more amorphous and less crystalline compared untreated rice husk through the changes of the rice husk structural will be elucidated using X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The low crystallinity in the treated rice husk will facilitate the digestibility of lignocellulose during enzymatic hydrolysis of *saccharomyces cerevisiae*. The ionic liquid pretreatment toward rice husk can greatly increase the saccharification rate and the fermentable sugar yield. Thus, it will increase the bioethanol production rate.

CHAPTER 2

LITERATURE REVIEW

2.1   Lignocellulosic Biomass

Lignocellulose is the primary building block of plant cell walls. Lignocellulose is generic term for describing the main constituent in most plants namely cellulose, hemicellulose and lignin biomass (P. Kumar *et al.*, 2009). Biomass is the organic matter derived from the living organism or plant which can be used as a source of energy. Lignocellulosic biomass can define as plant material which are not used for food feed or can know as plant waste. Lignocellulosic biomass is the largest source of hexose and pentose sugars, which can be used for the production of bioethanol Lignocellulosic is present in all agricultural crop residue and is the most abundant biomass on earth. The lignocellulosic is a renewable resource that can be includes various agricultural, forestry residues or industry wastes (K.S. & Pushpa, 2012). Lignocellulosic residue are attractive resources for production of biofuel that can be the alternative to fossil fuels. Lignocellulosic component majority consist of high amounts of carbohydrates such as cellulose, hemicellulose and lignin (Nanda *et al.*, 2014). Lignocellulosic biomass is accumulated every year in large quantities causing environmental problems. However due to their chemical composition based on sugars and other compounds of interest, they could be utilized for the production of a number of value added products such as ethanol, food additives, organic acids, enzymes and others. Therefore, beside environmental problems caused by their accumulation in the nature, the non-use of these material constitutes a loss of potentially valuable sources (Mussatto & Teixeira, 2010). Lignocellulosic biomass may be grouped into different categories such as wood residues, grasses, waste paper,

agricultural residues, non-food seeds or food industry residues. The lignocellulosic biomass which represents the largest renewable reservoir of potentially fermentable carbohydrates on earth is mostly wasted in the form of pre-harvest and post-harvest agricultural losses and waste of food processing industries. The renewability caused lignocellulosic waste has been a great deal of interest in utilizing lignocellulosic biomass for the production and recovery of many value added products (Mtui, 2009).

2.2   Rice Husk

Rice is one of the agricultural crops that continue to be an important source of food and nutrition in Malaysia. According the record mention that in the South-East Asia region from the total agricultural crop biomass produced per annum that approximately 216 million tonnes is comprised of rice (Ang *et al.*, 2011). Rice research play an important role as rice is one of the most important crops in terms of human consumption and is produced in 95 countries across the world. People majority of countries in Asia depend on rice as their main source of nutrition as well as for income and employment. The Food and Agriculture Organisation (FAO) in 2012 stated that current supply of rice outpaces consumption. In comparison to other countries in Asia, Malaysia only produces a small amount of rice. Out of 656.4 million tonnes of rice produced in Asia, only 2.7 million tonnes is from the peninsula and Borneo islands (Herman *et al.*, 2015). Thereby, rice husk becomes one of the byproduct from the rice milling industry.

Rice husk (*Oryza sativa*) is one of the most widely available agricultural wastes in many rice producing countries of the world. Rice husk are the hard protecting coverings

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of grains of rice and can be removed from rice seed as a byproduct during the milling process. Rice husk possess various properties that make them suitable for bioethanol production. Rice husk like others lignocellulosic biomass feedstock has been explored as the cheapest feedstock for bioethanol production. Easy availability and low price of rice husk in rice production countries is an extra benefit towards the use of this material. Therefore, abundantly of rice husk represents a real advantage over source of dwindling fossil fuels for bioethanol production (S. Kumar *et al.*, 2013). Rice husk composition usually will influence the production of biofuel. Rice husk mainly made up of three polymers like cellulose, hemicellulose and lignin. Table 2.1 shows the typical composition of rice husk.

Table 2.1: Typical composition of rice husk (P. S. Kumar *et al.*, 2010)

Composition	Percentage (%)
Cellulose	31.12
Hemicellulose	22.48
Lignin	22.34
Mineral Ash	13.87
Water	7.86
Extractives	2.33

Rice husk has several of application such as in industrial rice husk can be use as alternative fuel for household energy. The application of rice husk is depending upon their physical and chemical properties. For example, rice husk ash with physical property usually small in size and it chemical property which contain high silica content can use in rubber industries as a reinforcing agents, in cosmetics and in food industries as an anti-cracking agent.

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Table 2.2: Element of rice husk on dry basis (Majumder C. B. *et al.*, 2014)

Element	Mass Fraction (%)
Carbon	41.44
Hydrogen	4.94
Oxygen	37.32
Nitrogen	0.57
Silicon	14.66
Potassium	0.59
Sodium	0.035
Sulphur	0.3
Phosphorous	0.07
Calcium	0.06
Iron	0.006
Magnesium	0.003
Zinc	0.006

Rice husk is containing mainly from carbon element (Table 2.2). Therefore, rice husk is a cheap carbon source raw material for the production of bioethanol by using cellulolytic microorganisms such as *saccharomyces cerevisiae*. (S. Kumar *et al.*, 2013).

2.3   Cellulose

Cellulose with molecular formula $(C_6H_{10}O_5)_n$ is a polysaccharide composed of linear glucan chains that link together by β-1,4-glycosidic bond with homopolymer cellobiose as repeated units. Figure 2.1 shows the cellulose structure when glucose units are linked together by β-1,4-glucosidic bonds. Cellobiose is the smallest repetitive unit and it is formed by two glucose monomers. The long chain of cellulose polymers is linked together by the hydrogen and van der Waals forces which cause cellulose to pack into microfibrils. The chains of cellulose tend to arrange in parallel and in form of crystalline structure. Therefore, cellulose microfibrils have both highly crystalline region and less ordered amorphous regions (Zhao *et al.*, 2011). An effective pretreatment method can weaken all these hindrances and exposes cellulose to cellulose enzyme for effective hydrolysis. The proper pretreatment will increase the yield of glucose release from lignocellulosic biomass up to 90 % compared the lignocellulosic biomass without pretreatment (Alizadeh *et al.*, 2005).
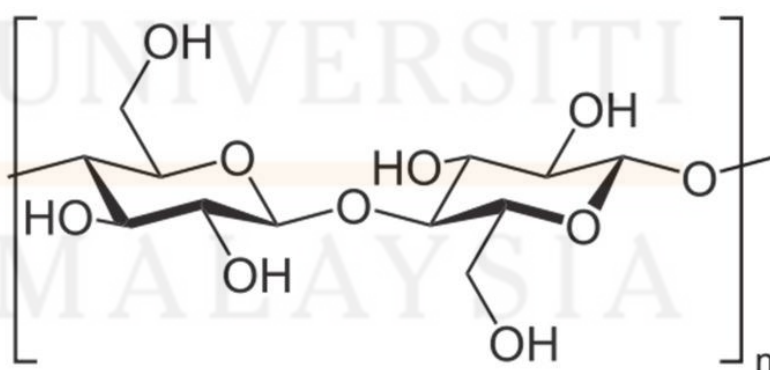
Figure 2.1: The cellulose structure where glucose units are linked together by β-1,4-glucosidic bonds (Ismail, 2011).

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Cellulose contains six types of morphology that are I, II, III_1, III_{11}, IV_1, IV_{11}. The morphology can be interconverted to each other (Figure 2.2). The cellulose I has two types of morphology that are $I\alpha$ and $I\beta$. Cellulose I or native cellulose is form found in nature. Cellulose I can be convert to cellulose II either through regeneration or mercerization. Regeneration is a process to produced cellulose II which the cellulose I is solubilization in a solvent followed by reprecipitation by dilution in water. While the mercerization is which the process of swelling native fibers in concentrated sodium hydroxide, then obtained cellulose II while the removal of swelling agents. Furthermore, Cellulose III_1 and III_{11} are formed in a reversible process from cellulose I and II by undergo treatment with liquid ammonia or some amines and subsequent evaporation of excess ammonia. Polymorphs IV_1 and IV_{11} may be prepared by heating cellulose III_1 and III_{11} to 260 °C in glycerol (O'Sullivan, 1997).
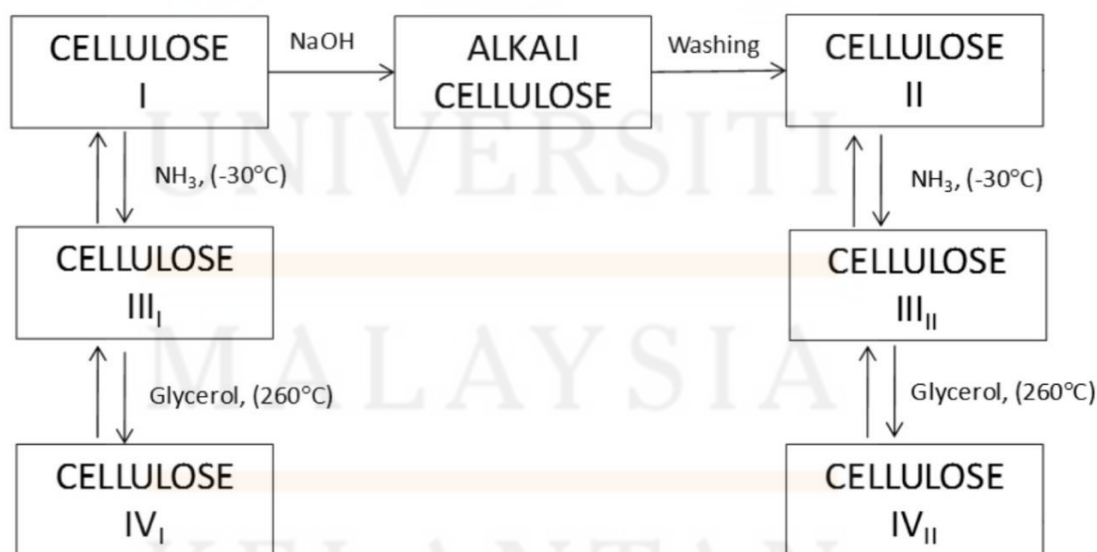
Figure 2.2: Interconversion of polymorphs of cellulose.

2.4   Hemicellulose

Hemicellulose are a heterogeneous group of polysaccharide with the β-(1→4)-linked backbone structure of pentose (C5) sugars like xylose and arabinose and hexose (C6) sugar such as mannose, galactose and glucose as repeating units. Unlike cellulose hemicellulose is random and amorphous in structure. Hemicellulose are embedded and interact with cellulose and lignin which significant in increase the strength and toughness of plant cell wall (Zhao *et al.*, 2011). Hemicellulose is insoluble in water at low temperature. However, it starts hydrolysis at temperature lower than of cellulose. The presence of acid can highly improve the solubility of hemicellulose in water (Harmsen *et al.*, 2010).

2.5   Lignin

Lignin is a non-sugar based polymer with very complex molecule constructed of phenylpropane units that linked in a large three-dimensional structure. Lignin is closely bound to cellulose and hemicellulose and provide rigidity and cohesion to the material cell wall, to confer water impermeability to xylem vessels and to form a physic-chemical barrier against microbial attack. Lignin yield more energy when burned thus lignin is a good selection for combined heat and power production in an environment friendly mode of the biorefinery (Zhao *et al.*, 2011). Lignin is an aromatic and rigid biopolymer covalently bonded to hemicellulosic xylans and responsible for the rigidity and high level of compactness of the plant cell wall. Lignin components are gaining important due to their dilution effect on processes of hydrolysis and fermentation. The phenolic groups will form from the degradation of lignin, substantially deactivate cellulolytic enzymes and

hence hamper enzymatic hydrolysis. However, demonstrated that lignin modification via genetic engineering could considerably reduce lignin formation and improve ethanol yield. Hence lignin always serves as the major plant defense system to pathogen and insects and its modification could disrupt the plants natural protection (Kang *et al.*, 2014).

2.6 Bioethanol

Worldwide, there is a growing concern over the fossil oil prices increase, the security of the oil supply and the negative impact of fossil fuels on environment, particularly greenhouse gas emission. The conversion of lignocellulosic biomass provides best economically feasible and conflict-free second-generation renewable alternatives (Mtui, 2009).

Bioethanol also known as ethyl alcohol, grain alcohol CH_3-CH_2-OH or ETOH is a liquid biofuel, which produces from various types of biomass feedstock and conversion technologies. The awareness of the global warming issue increased the interest in the development of biofuel like bioethanol. Bioethanol is an attractive alternative fuel as it is a renewable bio-based resources and it is oxygenated which potential in reduce emission in compression-ignition engines. Bioethanol has a great advantage compared with gasoline. Bioethanol has a higher octane number, broader flammability limits, higher flame speeds and heats of vaporization compared with gasoline. All these properties allow bioethanol for a higher compression ratio, shorter burning time and leaner burn engine over the gasoline during combustion of the engine (Balat *et al.*, 2008).

Generally, bioethanol converted from edible source is called first-generation bioethanol (FGB). However, the drawback of FGB stems from edible feedstock utilized which includes corns and sugarcane. Therefore, due to this aspect, second-generation bioethanol (SGB) offers great promise to replace fossil fuels without causing the feud of food-fuel supply as the SGB are derived from non-edible sources such as lignocellulosic biomass (Goh *et al.*, 2010).

2.7   Ionic Liquid

Ionic liquid (IL) is a "green" solvent that emerged recently in industrial manufacture of chemicals. Ionic liquid is organic salts that composed of cations and anions. Ionic liquid also has a low melting point that usually below 100 ℃ at room temperature or can be known as salt melt below 100 ℃. The property that causes ionic liquid to get interest are due to ionic liquid has negligible vapor pressure, non-flammable and high thermo-stability. According to previous report the synthesis of ionic liquid usually has been carry out in two step. Firstly, is formation of cation by quaternization reaction and then the anion exchange reaction (Khupse & Kumar, 2010). Ionic liquid usually can category into four types based on their cation segment. These are alkylammonium- ,dialkylimidazolium-, phosphonium- and N-alkylpyridiniumbased ILs (Ghandi, 2014). Nowadays there present many types of ionic liquid that prepared for the chemical application. For examples 1-allyl-3-methylimidazolium chloride, 1-butyl-3-methylimidazolium chloride, 1-ethyl-3-methylimidazolium acetate and so on (Isik *et al.*, 2014).

Ionic liquid is recognized to facilitate more green application in reactions and separations due to their unique beneficial properties, such as negligible vapor pressure and high thermal stability. The very low vapor pressure reduces risk of exposure that is clear advantage over the use of classical volatile solvents. In the last few decades, numerous studies focused on the dissolution off natural polymers in ionic liquid demonstrating a great potential of ionic liquid as solvents. Cellulose was the most studied biopolymer exhibiting a high solubility in variety of ionic liquid (da Costa Lopes *et al.*, 2013).

1-butyl-3-methylimidazolium chloride (BMIMCl) is one of the ionic liquid that used during pretreatment of lignocellulosic biomass to increase of bioethanol production. 1-butyl-3-methylimidazolium cation BMIM$^+$ makes a number of IL with variety properties when combine with different anions. For example, BMIM$^+$ can form BMIMCl by combined with chloride ions. Figure 2.3 show the structural formula of BMIMCl. (Ozawa *et al.*, 2003).

Figure 2.3: 1-butyl-3-methylimidazolium chloride

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2.8 Pretreatment Process of Lignocellulosic Biomass

Pretreatment is a process that implemented during bioethanol production for the lignocellulosic biomass. Pretreatment can be divided into different categories such as physical, physicochemical, chemical, biological, electrical or a combination of the these (P. Kumar *et al.*, 2009). The barrier to the production and recovery of valuable material from lignocellulosic biomass is the structure of lignocellulosic which has evolved to resist degradation due to cross linking between the polysaccharides (cellulose and hemicellulose) and lignin via ester and ether linkage (Mtui, 2009). Thereby the main goals of pretreatment usually purpose to remove lignin and hemicellulose, reduce the crystallinity of cellulose and increase the porosity of the lignocellulosic materials (Figure 2.4). Pretreatment can disrupt the recalcitrant lignocellulosic complex and improve enzymatic digestibility (Ang *et al.*, 2011).
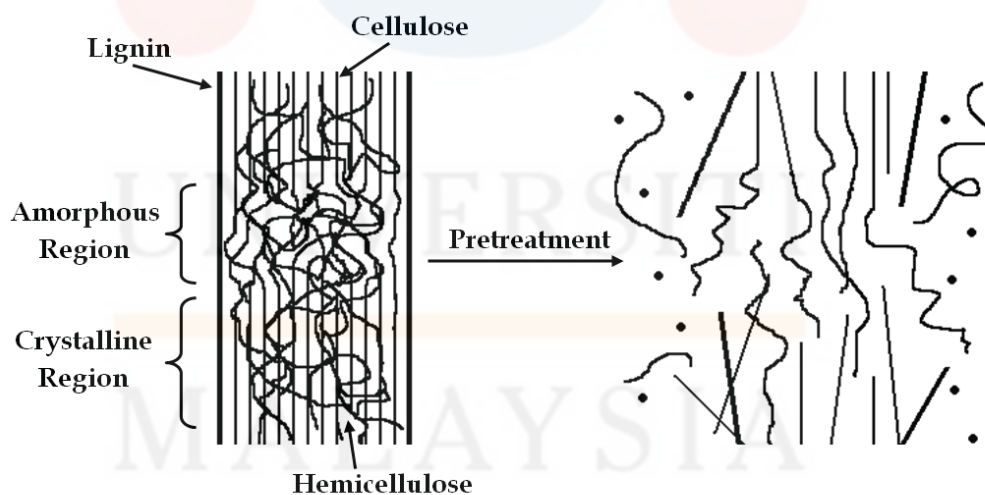

Figure 2.4: Goal of pretreatment process (Ahmad Idi & Mohamad, 2011).

In order to maximize the effectiveness of saccharification of lignocellulosic biomass to sugar the pretreatment choosing must meet the following requirement. Firstly, the pretreatment must improve the formation of sugars or ability to subsequently form sugars by hydrolysis. Secondly, during pretreatment, the degradation or loss of carbohydrate can be avoided. Thirdly is the byproduct formation during pretreatment is avoided. Lastly is the pretreatment choosing must be cost effective to be used (P. Kumar *et al.*, 2009).

2.9   Fermentation Process

The fermentation process is the process involve microorganism that use fermentable sugar for food and in the process produce ethyl alcohol and other byproducts. The fermenting microorganism is effective for conversion of monomeric sugars to ethanol. There is various type of organisms such as bacteria, yeast and fungi. However, the most frequently used organisms in industrial process are the yeast *saccharomyces cerevisiae* (baker's yeast). The fermentation effectiveness depends on several factors. The factors are the choice of microorganism, raw material use, pretreatment method, hydrolysis method and environment factors such as pH, temperature, substrate and ethanol concentration. For example the common conditions for *saccharomyces cerevisiae* are normally pH 5.0 and temperature maximum 37 °C (Axelsson, 2011).

The enzymatic hydrolysis step is often in close collaboration with the following fermentation step in the ethanol production. The fermentation process can be either separate hydrolysis and fermentation (SHF) or by combination of the two step in one step simultaneously saccharification and fermentation (SSF) (Öhgren *et al.*, 2007).

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2.10 Microorganism Use for Fermentation Process

Saccharomyces cerevisiae or common name known as brewer's yeast or baker's yeast is a yeast that normally used in batch fermentation to convert sugars to ethanol for the production of beverages and biofuels (Dombek & Ingram, 1987). The production of ethanol from lignocellulosic feedstock is the high yield and high rate fermentation of biomass hydrolysates to ethanol. A demand on the microorganism used for fermentation are more complicated than other conventional ethanol production from hexoses or their disaccharides (Maris *et al.*, 2006).

Besides that, *saccharomyces cerevisiae* also wisely used as a yeast that capable to anaerobic growth although in the present of added sterol and unsaturated fatty acids. *Saccharomyces cerevisiae* also display the so-called Crabtree effect which the alcoholic fermentation in the presence of oxygen when glucose concentration exceeds a certain level (Verduyn *et al.*, 1990).

2.11   Characterization of Pretreated Rice Husk

After pretreatment of rice husk by BMIMCl the change in the structure in sample rice husk will be observed by using x-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR).

2.11.1 X-ray Diffraction (XRD)

The rice husk obtained from rice mill factory would undergo pretreatment by utilized ionic liquid BMIMCl. The crystallinity of the rice husk sample was analyzed by X-ray diffraction (XRD). A less crystalline regenerated cellulose structures was confirmed by XRD analyzed with occurrence of sharper peak at $2\theta = 18.7\,^{\circ}$ compared with untreated. The lower crystallinity index indicated a higher amount of amorphous cellulose presented in regenerated cellulose (Ang *et al.*, 2012). Figure 2.5 shown the XRD analyzed of untreated and pretreated rice husk.
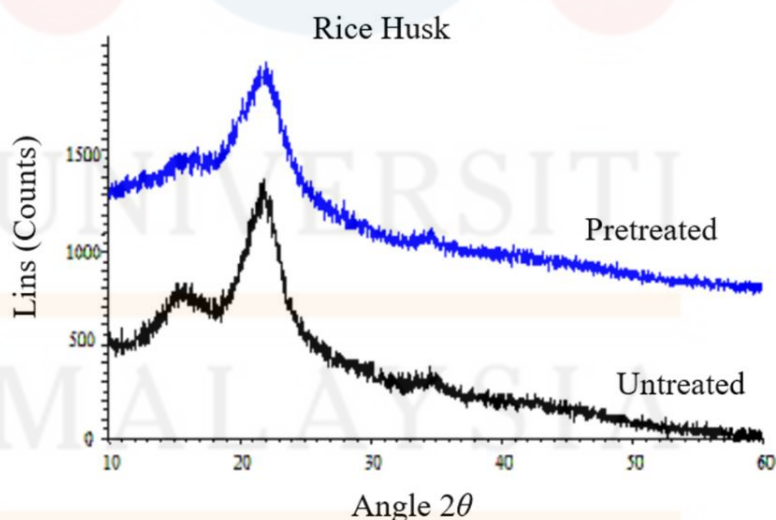
Figure 2.5: XRD analyzed of untreated and pretreated rice husk (Lee *et al.*, 2015)

2.11.2 Fourier Transform Infrared Spectroscopy (FTIR)

Ionic liquid having different anions had different effect on the pretreatment of rice husk, thereby the composition of cellulose regenerated was expected varies according the type of ionic liquid. FTIR was used to investigate the compositional and structural changes of regenerated cellulose. Generally, ionic liquid pretreatment would show capability to reduce the crystallinity of lignocellulosic biomass. The regenerated cellulose was more amorphous compared to untreated rice husk, which interpreted from the band broadening at approximately 800 cm^{-1} (Labbe *et al.*, 2005).

Furthermore, the composition of the regenerated cellulose varied with the type of ionic liquid pretreatment. Certain ionic liquid was more efficient in dissolving cellulose, while some were effective in dissolving lignin or lignocellulose. According to characterization study, the regenerated cellulose from BMIMCl were founded consisted mainly of cellulosic material. Hence the dissolution of cellulose, hemicellulose and lignin was expected to be related to the intrinsic property of ionic liquid (Ang *et al.*, 2011).

2.12   Analyses of Reducing Sugar Concentration

Last two decades, extensive research has been completed on conversion of lignocellulosic material to ethanol (Sun & Cheng, 2002). Degradation of lignocellulosic material to simple utilizable products usually the key step in the process. The degradation of cellulose that produce reducing sugar for fermentation steps usually become the topic of research. There are a number of ways to determine the reducing sugars during cellulose degradation, and used of 3,5-Dinitrosalicyclic acid (DNS) reagent for determination of reducing sugars not only widely been used but it also an assay recommended by the International Union of Pure and Applied Chemistry (IUPAC) (Saqib & Whitney, 2011).

According to Saqib and Whitney (2011) a sugar molecules will acts as reducing agents as long as present of aldehyde group and open chain in the structure. Therefore, the concentration of produce glucose from cellulose hydrolysis was estimated by using 3,5-dinitrosalicylic acid (DNS) method in which the aldehyde group of glucose convert the DNS to its reduce form 3-amino-5-nitrosalicylic acid. The amount of 3-amino-5-nitosalicylic acid form was proportional with the amount of glucose and led to the change in amount of light absorbed (Aboody, 2013).

CHAPTER 3

MATERIAL AND METHODS

3.1   Materials

The raw material agricultural waste (rice husk) was collected from the BERNAS Tumpat rice mill factory. The ionic liquid BMIMCl was purchased from Sigma Aldrich and yeast *saccharomyces cerevisiae* was obtained from Industrial Biotechnology Research Laboratory (IBRL0) University Sains Malaysia.

3.2   Sample Preparation

3.2.1   Physical Treatment

During sample preparation 200 grams of rice husk was dried at 100 ℃ for 24 hours in the oven until constant weight was achieved. This purpose of the drying process was to reduce the moisture content inside the rice husk.

200 grams of dried rice husk was ground to 30 mesh size (500 μm) used a waring blender and stored in a dry beaker prior to experiments (Figure 3.1). The particle size of biomass sample was one of the crucial issue in pretreatment of lignocellulosic because such a parameter would directly impact on contact and diffusion of chemicals into lignocellulosic materials. Thus, smaller particle is suitable to be use in IL pretreatment process (da Costa Lopes *et al.*, 2013).

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Figure 3.1: Rice husk samples with 500 μm

3.2.2 Chemical Treatment

Chemical treatment was a process that undergoes to treat the sample rice husk with ionic liquid BMIMCl in order to break the chemical bonding between the rice husk. During chemical treatment mixture of 5 % (w/w) rice husk and BMIMCl was heated at 80 °C for 48 hours. The set up for BMIMCl treatment was shown in Figure 3.2(a). After dissolution process, rice husk was precipitated from reaction mixture by adding large amount of distilled water. The regenerated rice husk was dried at 60 °C overnight (Ang *et al.*, 2011). The dried IL treated rice husk was shown in Figure 3.2(b).
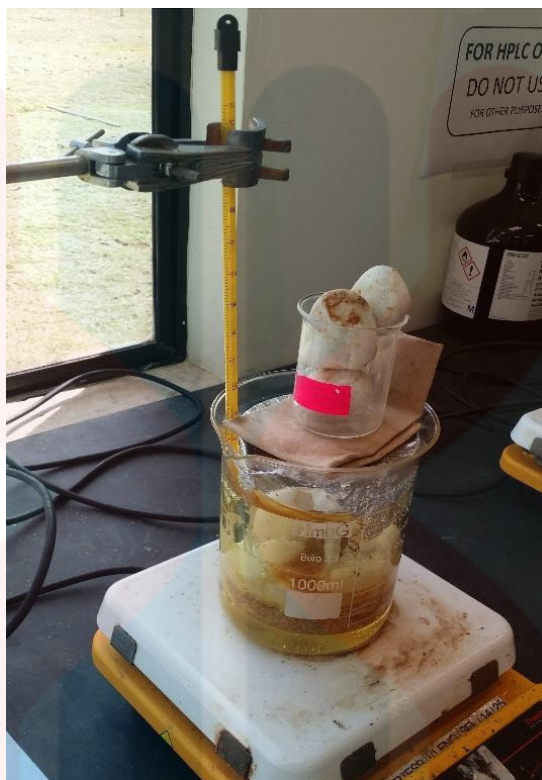
Figure 3.2(a): Set up of BMIMCl treatment for rice husk

Figure 3.2(b): BMIMCl treated rice husk

3.2.3 Acid Hydrolysis Process

10 g dried rice husk was suspended in acid solution, 3 % (v/v) dilute sulfuric acid in ratio 1:10 (w/v) rice husk and dilute sulfuric acid with temperature 200 ℃ for 16 minutes and cold (Ismail, 2011). The solid particles were separated from the liquid by using filtration method. After filtration, the solution was neutralized with NaOH until the pH reach around 5 - 5.5. The liquid was keep in freezer (-20 ℃) for further used.

3.2.4 Media Preparation

Yeast Peptone Dextrose (YPD) was the medium for yeast growth. YPD contained yeast extract, peptone, water and dextrose. It also used as solid medium by including agar in the mixture. The *saccharomyces cerevisiae* was incubated on agar plates (YPD medium including agar) for 24 hours at 30 ℃ (Nanda *et al.*, 2014). One loop from the agar plate was transferred into liquid YPD medium where it was precultivated for 24 h at 30 ℃ and 150 rpm in shaking incubator. The precultivated cultures were centrifuged with speed of 10000 rpm for 10 minutes at temperature 4 ℃. The supernatant and yeast *saccharomyces cerevisiae* formed as shown in Figure 3.3. The yeast pellet then washed repeatedly by using 0.85 % saline water by using micropipette. 1 ml of solution then transferred to cuvette and measured with spectrophotometer with wavelength 575 nm until the yeast was incubated to yield an optical density of 0.5 (Haykir & Bakir, 2013).

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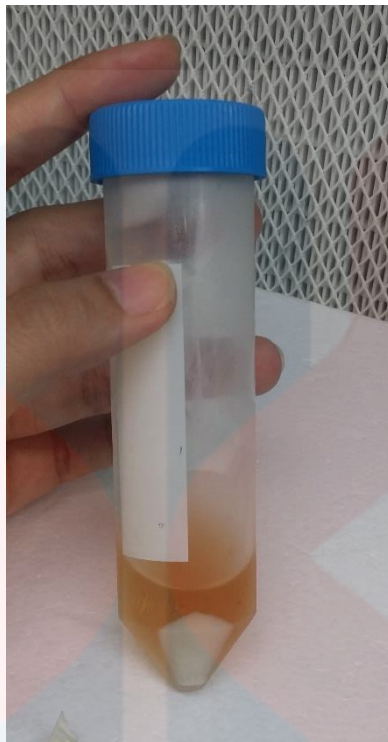
Figure 3.3: Centrifuged *saccharomyces cerevisiae* pellet in liquid YPD medium

3.2.5 *Saccharomyces cerevisiae* **Fermentation Process**

The solution from acid hydrolysis that keep in freezer was autoclaved at 121 °C for 15 minutes. After cooling to room temperature about 3 ml of actively growing *saccharomyces cerevisiae* was added to 97 ml of sterilize media and incubated for 48 hours at 30 °C and 150 rpm in incubator shaker. As shown in Figure 3.4, 1.5 ml of samples was drawn every 12 hours for residual sugar determination until 48 hours reach (Nanda *et al.*, 2014).
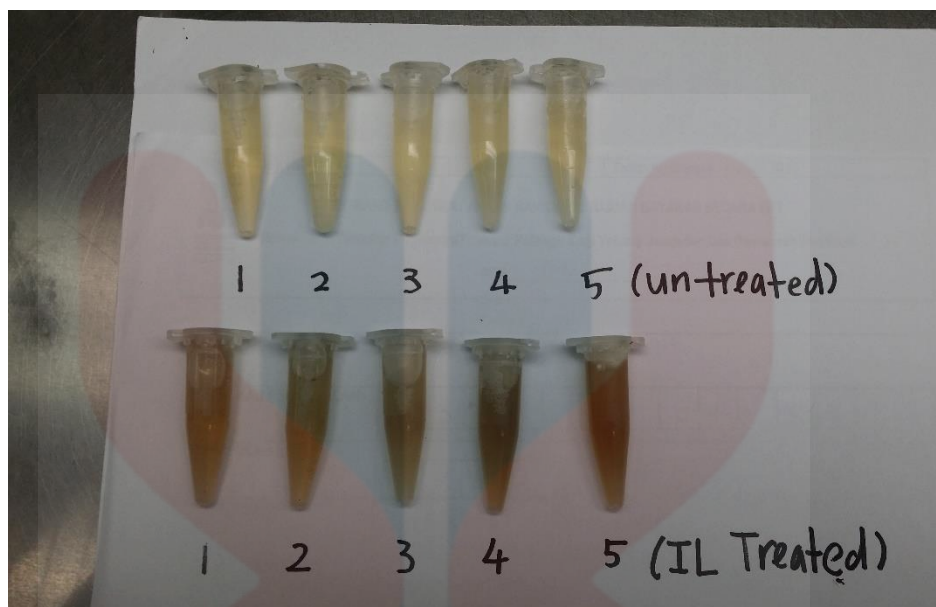
Figure 3.4: Samples that drawn after 48 hours fermentation

3.3   Analytical Method

3.3.1   X-ray Diffraction

XRD was used to analyse the changed of the structural properties (amorphous and crystalline) of the treated and untreated rice husk after pretreatment with ionic liquid. XRD was performed used a Bruker D2-Phaser Benchtop XRD. The pattern was collected from 10° to 70° (2θ) with a Ni-filtered CuKα radiation source ($\lambda = 0.154$ nm) (Hwang & Huynh, 2015).

In order to calculated the crystallinity of the rice husk, the crystalline index (CrI) of the sample rice husk was determined using the equation of Ang *et al.* (2012):

$$\text{CrI (\%)} = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \tag{3.1}$$

Where I_{002} represent the maximum intensity of crystalline peak 002, while I_{am} stand for the height of amorphous peaks 110.

3.3.2 Fourier Transform Infrared Spectroscopy

FTIR was an instrument that use after the pretreatment process by ionic liquid. FTIR was used to determine the structural changes after undergoes pretreatment. The sample rice husk that finely grounded was mixed with potassium bromide, KBr. Then was compressed into pellet form. The FTIR spectral analysis was performed within wave number range of 400-4000 cm^{-1} with 4 cm^{-1} resolution (Johar *et al.*, 2012).

3.3.3 Determination of Glucose Concentration Produce from Hydrolysis of Cellulose

3.3.3.1 Standard Stock Solution of Glucose

Standard stock solution was prepared by dissolving 0.2 g of glucose in 100 ml of distilled water. The standard stock solution of glucose was later used for preparation of glucose standard curve.

3.3.3.2 Preparation of 3,5-Dinitrosalicylic acid (DNS) Reagent

100 ml of DNS reagent was prepared by adding 1 g of 3,5-dinitrosalicylic acid, 20 ml 2 N NaOH and 30 g of potassium sodium tartrate ($KNaC_4H_4O_6.4H_2O$) which later dilute to final volume of 100 ml with distilled water (Saqib & Whitney, 2011) .

3.3.3.3 Preparation of Glucose Standard Curve

There are totally 6 test tube and each test tube was filled with 1 ml of DNS reagent to stabilize the change of color and 3 ml different concentrations of standard glucose which diluted by the different volume of distilled water. The test tube was heated in water bath for 10 minutes at 90 °C to develop red-brown color. Then allowed to cool to room temperature. The absorbance reading was measured and recorded by using

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spectrophotometer with the wavelength of 575 nm. Standard glucose curve was plotted where the glucose concentration on x-axis and corresponding absorbance on y-axis. Which purpose to use dilution of a solution of known glucose concentration to determine glucose concentration of unknown (Saqib & Whitney, 2011).

3.3.3.4 Measurement of Glucose Concentration

The calculate of unknown glucose volume in the fermenting sample, 0.250 ml fermenting sample was collected every 12 hours and the first sample was collected right after the active *saccharomyces cerevisiae* was added to the sterile media. The samples were continued collect until 48 hours reach. Each fermented sample collected was then mixed with 1 ml of DNS reagent and 2.750 ml of distilled water in test tube. Then, heated the test tube in water bath for 10 minutes at 90 ℃ and allowed to cold. The reagent blank sample was prepared with 3.0 ml of distilled and 1 ml of DNS reagent and heated as the samples. The absorbance reading was the measured and recorded by using spectrophotometer with wavelength 575 nm against the reagent blank. The glucose concentration in the sample was calculated by employing the standard glucose curve prepared using the standard glucose (*see* Figure 4.3) (Aboody, 2013).

3.4   Extractive, Holocellulose, *α*-cellulose and Lignin Determinations

3.4.1   Extractive Determination

The 10 g of rice husk samples was weighed and placed in a timber. Then sample was mixed with toluene and ethanol in ratio 1:2 for a period 6 hours at 150 ℃ in Soxhlet apparatus as shown in Technical Association of the Pulp and Paper Industry (TAPPI) test method T240. The extraction procedure purposed for the extraction of any inorganic materials and nonstructural sugars from the rice husk such as lipids and waxes. The

process was stopped after 6 hours then allow to cool and washed with distilled water and acetone (Oksman *et al.*, 2011). The sample was dried in oven for 24 hours at 50 ℃. The dried sample was weighed and known as free extractives that used for next process. The percentage of extractive was calculated based on equation (3.2) (Lai & Idris, 2013):

$$\text{Extractive } (\%) = \frac{\text{Weight}_{\text{Initial sample}} - \text{weight}_{\text{oven dried free extractive}}}{\text{Weight}_{\text{initial sample}}} \times 100 \% \qquad (3.2)$$

3.4.2 Holocellulose Composition Determination

5 g of free extractive samples was placed in a 250 ml of conical flask that contained 100 ml distilled water, 5 ml of 10 % acetic acid (CH_3COOH) and 1.5 g sodium chlorite ($NaClO_2$) and heated to 70 ℃ for 30 minutes. During the heating process the conical flask was closed with a 100 ml conical flask to prevent the released of odor chemical gas. After 30 minutes the 5 ml of 10 % CH_3COOH was added and heated for 30 minutes the process was proceed by adding 1.5 g of $NaClO_2$ and continue heating for another 30 minutes. The 5 ml of 10 % CH_3COOH and 1.5 g $NaClO_2$ was repeated added for another 4 times in 4 hours at constant temperatures 70 ℃. After 4 hours, the conical flask with sample was placed in ice for cooling process (Figure 3.5). Then, the cooled sample was then filter and washed several times by using cold distilled water and acetone. The sample was dried by using vacuum filtration and dried in oven at 50 ℃ for 24 hours. The dried sample was known as holocellullose were weight and used for next process (Oksman *et al.*, 2011).

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Figure 3.5: Colling process for holocellulose extraction process

In order to calculate the percentage of extractives of hemicellulose, the value of total solid and oven dry weight (ODW) was required. The total solid was the amount of solid remaining after heating at 105 ℃ to constant weight. Initial 1 g of holocellulose sample was weight and heated in oven at 105 ℃ for 24 hours. The weight of dried sample was recorded and used to calculate the percentage of total solid by using the equations (3.3) as shown by Sluiter, Payne, *et al.* (2008):

$$\% \text{ Total Solid} = \frac{\text{Weight}_{\text{Dried sample}}}{\text{Weight}_{\text{Initial sample}}} \times 100 \qquad (3.3)$$

Besides that, to calculated the percentage of extractives the oven dry weight (ODW) of extractives was required. ODW was the weight of biomass mathematically corrected for the amount of moisture present in the sample at the time of weighing. The ODW was determined by equation (3.4) by Sluiter, Hames, *et al.* (2008):

$$\text{ODW} = \frac{\text{Weight}_{\text{Air dried sample}} \times \% \text{ total Solid}}{100} \qquad (3.4)$$

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Where the weight of air dried sample was the initial weight of sample used for extraction of holocellulose (5 g). The % of total solid was the percentage of total solid remained after heating at 105 °C as shown by equation (3.3).

Therefore, to determine the percentage of extractive, equation used was shown by Sluiter, Ruiz, *et al.* (2008):

$$\% \text{ Extractive} = \frac{\text{Weight}_{\text{Extractive}}}{\text{ODW}_{\text{Sample}}} \times 100 \tag{3.5}$$

The weight extractive was the weight of dried biomass remained after the extraction and ODW sample was the weight of biomass after mathematically corrected for the moisture present in sample.

Percentage moisture can be determined to indicated the percentage of water content in the biomass. The percentage moisture can be calculated by using equations mentioned by Sluiter, Payne, *et al.* (2008):

$$\% \text{ Moisture} = 100 - \% \text{ Total Solid} \tag{3.6}$$

3.4.3 Alpha Cellulose Composition Determination

2 g of holocellulose and 15 ml of 17.5 % Sodium Hydroxide (NaOH) were placed in 250 ml conical flask and stirred for 1 minutes. Then, 10 ml of 17.5 % of NaOH was added and stirred for 45 seconds. After 45 seconds, 10 ml of 17.5 % NaOH was added and stirred for 3 minutes. The process was proceeded by repeat adding 10 ml of 17.5 % NaOH for 4 times in 10 minutes with each addition within 2.5 minutes. The conical flask was closed with a 100 ml conical flask and continue stirred for 30 minutes. Then, 100 ml of

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distilled water was added and stirred for another 30 minutes. The precipitated was then washed with 8.3 % NaOH and cold distilled water followed by soaking with 2 N of acetic acid. The precipitated was then filtered by using vacuum filtration and dried in oven at 50 °C for 24 hours. The dried sample was known as α-cellulose after eliminating of hemicellulose from holocellulose sample (Oksman *et al.*, 2011).

Moreover, to calculate the percentage total solid of α-cellulose, initial 0.7 g of α-cellulose sample was heating in oven at 105 °C for 24 hours and the weight of dried sample was recorded. The percentage of total solid was calculated with equation (3.3). While % of extractive and % moisture for alpha cellulose was determined by equations (3.5) and (3.6).

3.4.4   Lignin Composition Determination

1 g of free extractives with 25 ml 72 % H_2SO_4 was stirred in 250 ml of beaker surrounded with ice and stirred for 2 hours. The mixture was poured into a l L of conical flask and filled with 560 ml distilled water. The conical flask was heated at 180 °C for 4 hours and the conical flask was attached to the soxhlet set with the both end of water cooled condenser was closed (water in and water out) as shown in Figure 3.6. After that the conical flask with extractives was cooling in fume chamber for 24 hours and two layers was formed. The lignin extracted was washed with hot distilled water, filtered and dried in oven at 50 °C for 24 hours (Teramoto *et al.*, 2009). The weight of dried lignin sample was weighted.

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Figure 3.6: Soxhlet set up for lignin extraction

For lignin, the percentage total solid was calculated by heating initial 0.3 g extracted lignin sample in oven at 105 °C for 24 hours and the weight dried was recorded and calculated with equation (3.3). The % of extractive and % moisture for lignin also can calculated by using the equations (3.5) and (3.6) as mentioned in section 3.4.2.

The percentage composition of extractive, holocellulose, α-cellulose and lignin in rice husk was calculated. The α-cellulose content was considered the cellulose content. The hemicellulose was determined by subtracting the α-cellulose content from holocellulose content. The composition of rice husk was presented in Table 4.1 (Teramoto *et al.*, 2009).

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3.5   Research Flow Chart
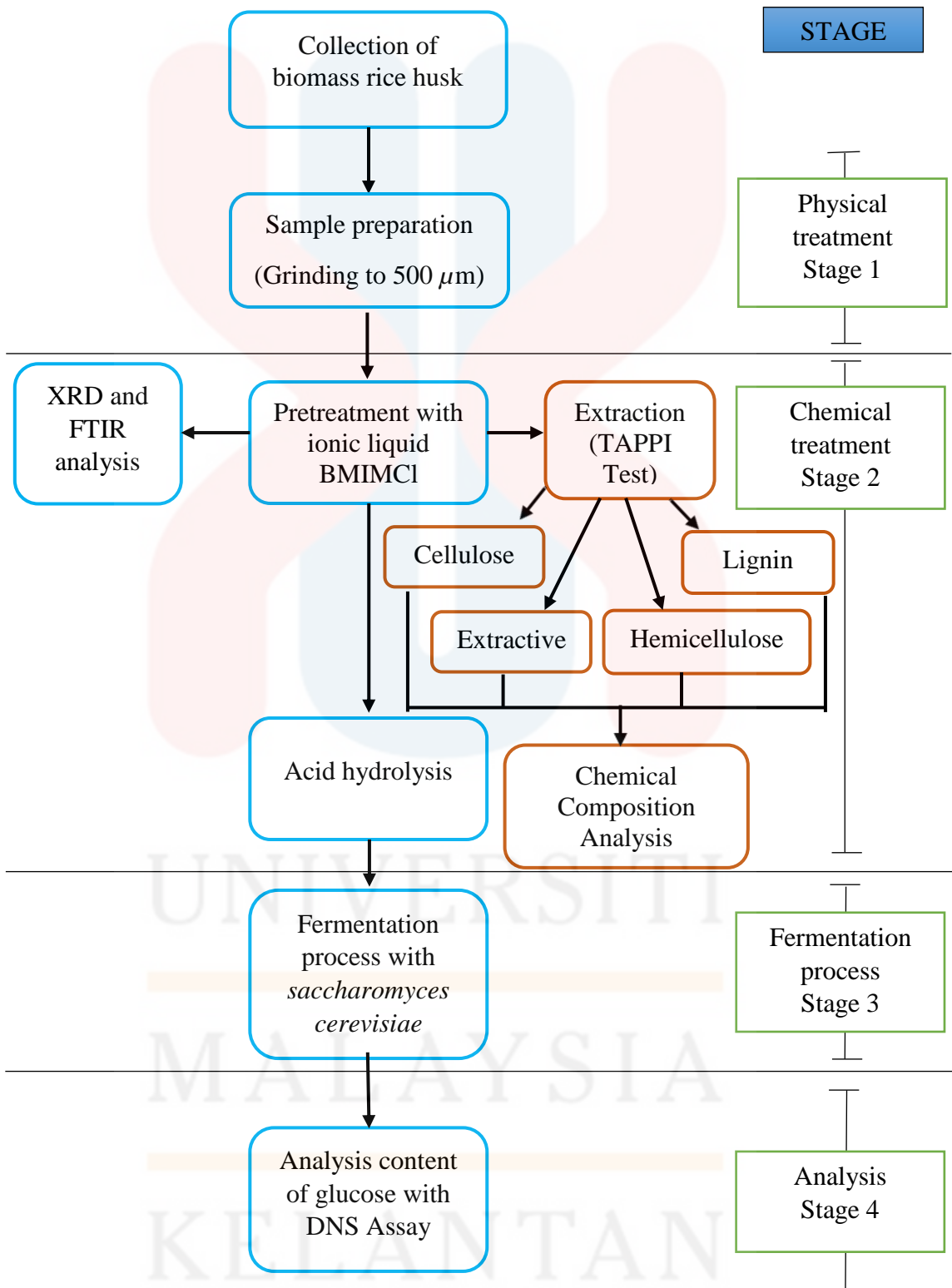
CHAPTER 4

RESULTS AND DISCUSSION

4.1   Structural Characterization

4.1.1   Compositional and Percentage Moisture Analysis

In order to understand the compositional modifications in the rice husk after pretreatment, the composition of the rice husk was analyzed. The results of compositional analysis of rice husk before and after pretreatment are shown in Table 4.1. The cellulose contents and hemicellulose content of untreated rice husk was 27.83 % and 49.55 %. The hemicellulose content was higher if compared with previous studied by Wang *et al.* (2016) which the typical composition of hemicellulose was between 18 to 21 %. This may due to the incomplete of lignin removal during process obtaining the holocellulose. The differences in rice husk cellulose content and hemicellulose content also might due to different geographical locations where crop is grown and the variety of the crop (Ang *et al.*, 2011).

Table 4.1: Composition of untreated and BMIMCl treated rice husk

Composition (%)	Untreated	BMIMCl Treated
Cellulose	27.83	63.18
Hemicellulose	49.55	16.80
Lignin	29.32	37.68
Extractive	11.15	7.20

The amount of cellulose regenerated was dependent on the degree of rice husk dissolution and dissolution ability of ionic liquid in dissolving rice husk (Ang *et al.*, 2011).

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The cellulose content of untreated rice husk was 27.83 % while the cellulose content display a significant increased after pretreated with BMIMCl that 63.18 %. Past studies had shown that the chloride-based IL, BMIMCl was most effective in dissolving cellulose (Ang *et al.*, 2011). The anions of BMIMCl attack the hydroxyl group of cellulose and unfold the intra- and inter-molecular bond in cellulose leading the dissolution (Novoselov *et al.*, 2007). During the interaction, oxygen atoms of cellulose hydroxyl serve as electron pair donor and hydrogen act as electron acceptors which correspondingly interact with IL cation which act as electron acceptors and IL anions as electron donors. The cation with it electron rich aromatic π system only weakly interact with hydroxyl oxygen atom through nonbonding or π electrons, whereas anion was hydrogen bonded to the hydroxyl proton of cellulose. The interaction phenomena caused the separation of oxygen and hydrogen atoms thus disrupted the intra- and intermolecular hydrogen bonds in cellulose that cause it to dissolve (Tan & Lee, 2012). The dissolve cellulose can easily be regenerated in amorphous form by washed thoroughly with distilled water (Ohno & Fukaya, 2009). When anti-solvent (non-solvent) such as water was added in IL and rice husk, the ions of IL form hydrogen bond with water molecules and they displaced into the aqueous phase. The interaction between IL and cellulose are shielded by hydrodynamics shells build up by water molecules around the ions of IL. Hence, cellulose which previously interacted with IL is expelled and rebuilt its intra and intermolecular hydrogen bonds and the precipitated (Tan & Lee, 2012). The dissolution ability of the BMIMCl led to the composition of regenerated cellulose increased after BMIMCl treated as shown in Table 4.1.

Hemicellulose was obtained by difference between holocellulose and cellulose contents. Based on Table 4.1 the hemicellulose content of BMIMCl treated rice husk was lower compared with untreated rice husk. This due to hemicellulose is slightly crosslinked and relatively amorphous which more easily hydrolyzed than cellulose, hence there was a significant loss of hemicellulose when pretreated with IL (Tan & Lee, 2012).

Lignin was complex structure which was irregular and cross-linked as well as strong covalent linkages within polymer and more difficult to be dissolved than cellulose. As shown in Table 4.1 the percentage of lignin for the BMIMCl treated rice husk which was higher compared with the percentage of untreated rice husk. Studies shown that IL such as BMIMCl can partially dissolve lignin. However, although only partial delignification can be achieved by BMIMCl, it was observed that action of IL also can caused lignin delignification and lignin redistribution. Partial delignification and lignin redistribution coupled with reduction of crystallinity can led to an effective biomass conversion process that liberate higher yield of sugar (Kilpeläinen *et al.*, 2007). The efficiencies of BMIMCl although not change much on the lignin content, while resulting significantly high saccharification yields. The efficiency was related to the reduction of the cellulose crystallinity (Shafiei *et al.*, 2015).

While the percentage of extractives for BMIMCl treated and untreated also shown a slightly different. The BMIMCl treated rice husk contained lower extractives while untreated rice husk had higher percentage of extractives. It due to some extractive component was removed during the pretreatment of BMIMCl (Passos *et al.*, 2014).

Table 4.2: Percentage moisture of rice husk before and after pretreatment

Percentage Moisture (%)	Untreated	BMIMCl Treated
Cellulose	6.00	8.29
Holocellulose	6.70	5.30
Lignin	8.67	5.33

The percentage of moisture also was a significant factor during the dissolution process. As shown in Table 4.2 the cellulose after BMIMCl pretreatment shown a higher percentage of moisture compared untreated rice husk. The higher in percentage of moisture was due to the present of amorphous regions of cellulose after BMIMCl treated. Which the amorphous regions of cellulose will absorb water and enzyme faster than crystalline regions cellulose (Shafiei *et al.*, 2015). While for holocellulose and lignin the water concentration after BMIMCl was lower compared untreated. The low in the moisture was significant for the dissolution of cellulose. That because high concentration of moisture will cause water molecules to compete with IL anions to form hydrogen bonds with cellulose thus promoting only partial dissolution of lignocellulosic biomass which reduce the dissolution of cellulose (Silveira *et al.*, 2015).

4.1.2 XRD Analysis
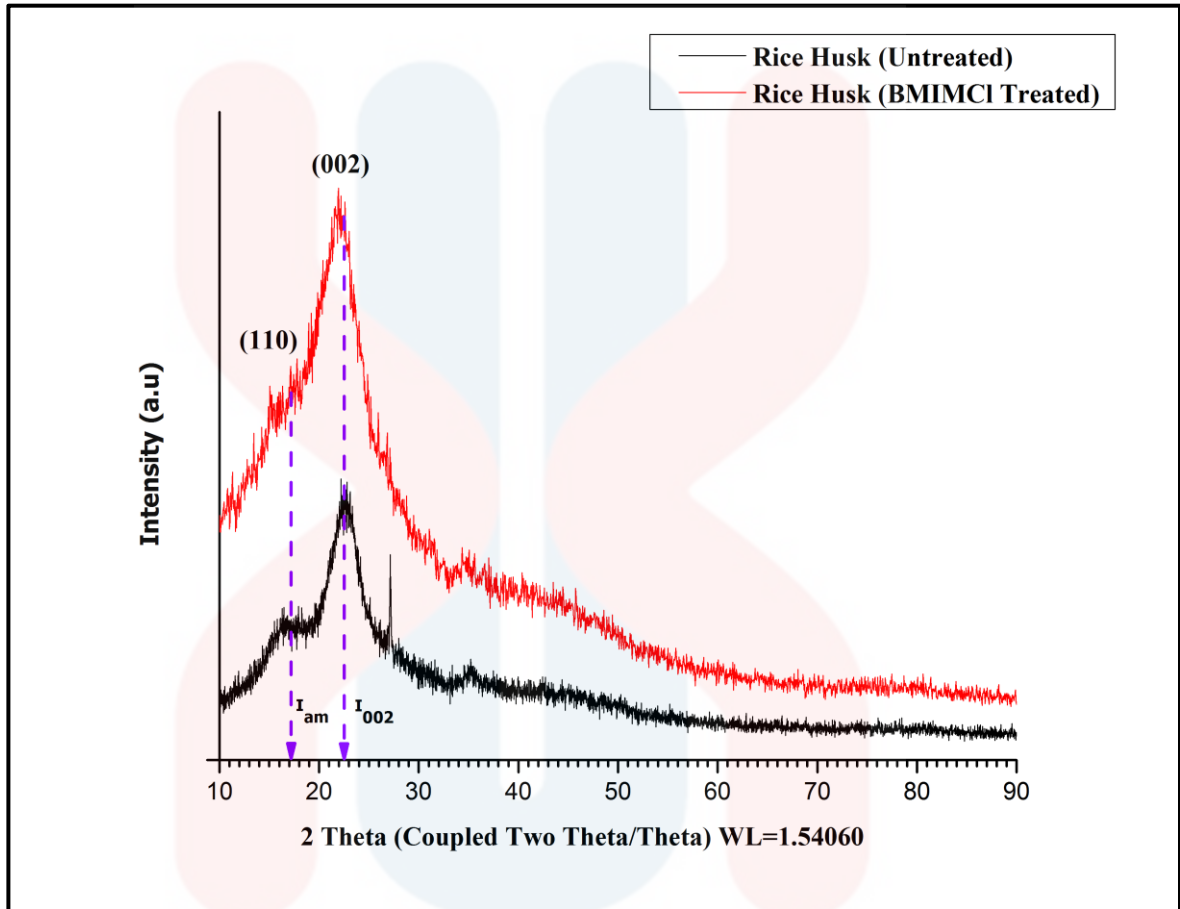
Figure 4.1: The X-ray diffraction diffractogram for untreated and BMIMCl treated rice husk

The changed of the structural properties (amorphous and crystalline) of the BMIMCl treated and untreated rice husk was analysed by XRD.

The diffraction pattern of untreated rice husk showed two peak at $2\theta = 16.38$ ° and 22.43 ° is shown in Table 4.3. The peak at $2\theta = 16.38$ ° indicated the lower peak angle while 22.43 ° was the sharpest peak at the untreated rice husk.

Furthermore, for the BMIMCl treated rice husk the diffractogram showed two peak at $2\theta = 16.34$ ° and 22.45 °. The peak at $2\theta = 16.34$ ° showed the lower peak angle of BMIMCl treated while 22.45 ° indicated the sharpest peak for the diffractogram.

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Table 4.3: The angle diffraction in x-ray diffraction diffractogram of treated and BMIMCl treated rice husk

Diffraction Angle 2θ (°)	Rice Husk	
	Untreated	**BMIMCl Treated**
110	16.38	16.34
002	22.43	22.45

Crystalline index (CrI) was measured by the ratio of intensity of main crystalline plane (002) at 22.4 ° and amorphous plane at 16.3 ° of 2θ (Raj et al., 2015). The Crystalline index (CrI) was calculated (*see* Equation 3.1) and summarized in Table 4.4.

Table 4.4: Percentage of crystallinity and amorphous for untreated and BMIMCl treated rice husk

	Untreated Rice Husk	**BMIMCl Treated Rice Husk**
% of Crystallinity	47.15	35.96
% of Amorphous	52.85	64.04

From Table 4.4 it was clearly seen that the crystallinity of untreated and BMIMCl treated rice husk was different. The BMIMCl treated rice husk had lower crystallinity index compared with untreated rice husk. As reported by Chang *et al.* (2016) the lower in the crystallinity revealed that the crystalline cellulose structure was disrupted and that amorphous cellulose was obtained in regenerated cellulose. The higher content of amorphous cellulose after pretreatment will provided a large surface area that accessible to enzymes and thus readily digestible. The decrease of crystalline index after BMIMCl pretreatment also due to the changed of cellulose I to cellulose II with low crystallinity

which provided benefit for enzymatic hydrolysis which previously reported in literature Liu *et al.* (2015).

4.1.3 FTIR Analysis

The regenerated biomass from the rice husk that treated by BMIMCl altered the chemical and structural characteristics compared to the untreated rice husk. FTIR spectroscopy was used to determine the structural changes in the lignocellulosic biomass upon pretreatment (*see* Figure 4.2) in the region of 4000-400 cm^{-1}, which was commonly used to study the fine structural characteristics of cellulose (Ang *et al.*, 2012).
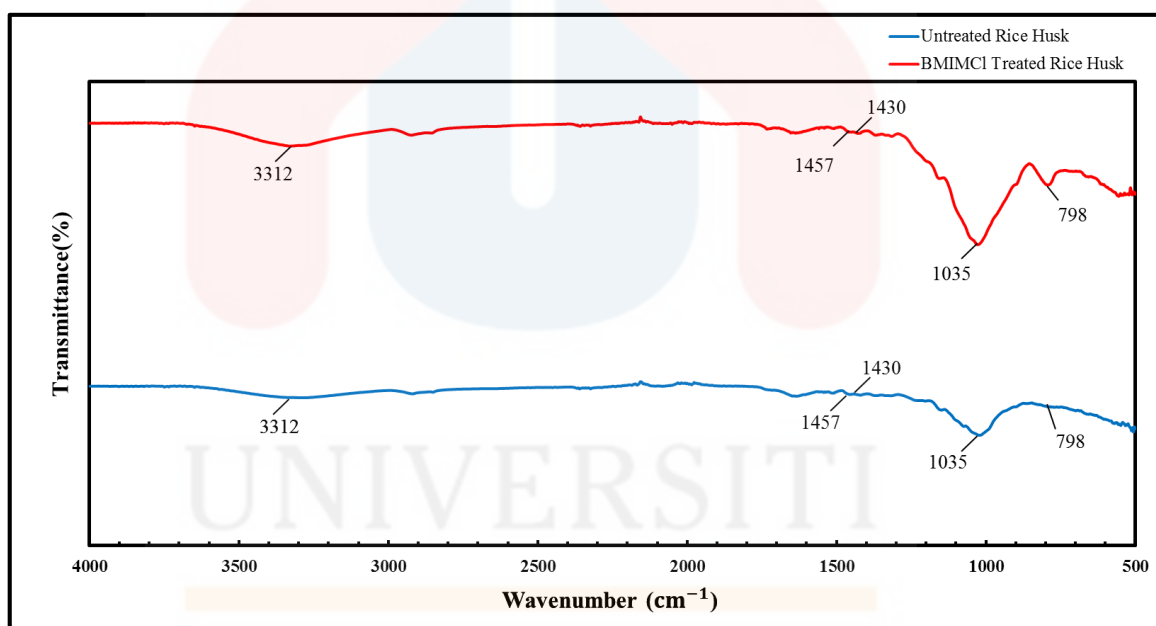
Figure 4.2: FTIR spectra analysis of untreated and BMIMCl treated rice husk

In rice husk both cellulose/hemicellulose- and lignin-associated bands were presented in the spectrum of untreated rice husk, and this indicated the presence of lignin-carbohydrates matrix in rice husk. From the spectra of regenerated cellulose show the strongest absorption band at approximately 1035 cm^{-1}, which corresponded to the C-O stretching vibration in both cellulose/hemicellulose and lignin. While in all spectra the

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peak at 1457 cm^{-1} was presented which corresponded to asymmetric bending of CH_3 and methoxy (-OCH_3) groups presented in lignin this implying that lignin remained in the matrix. Furthermore, a strong absorption band at around 3312 cm^{-1} was observed due to the -OH stretching which could be due to moisture absorption. The band at around 800 cm^{-1} was sensitive to the amount of amorphous cellulose present in regenerated materials, the broadening of this band indicates the higher amorphous of regenerated cellulose (Lee *et al.*, 2015).

There were differences between untreated and BMIMCl treated rice husk where some absorption bands were absent. In the BMIMCl treated rice husk the band in region of around 800 cm^{-1} was broader implying a higher amount of disordered cellulose structure. The disorder of cellulosic structure was caused by hydrogen bond rearrangement and deformation vibration of *β*-glycosidic linkages (Proniewicz *et al.*, 2001). The absorption band at 1430 cm^{-1} corresponding to the motion of CH_2 in C_6 group of more crystalline cellulose (type I) while at the absorption at 800 cm^{-1} identifies the less crystalline cellulose (type II) (Shafiei *et al.*, 2013).

In spectrum of BMIMCl treated rice husk the absorption band at around 1457 cm^{-1} was absent. This indicates the removal of lignin in regenerated biomass of rice husk. Furthermore, there had a strong absorption band at around 3312 cm^{-1} was observed implies that a moisture absorption.

In conclusion, BMIMCl treated rice husk shown a change in the FTIR spectral pattern this indicating that BMIMCl pretreatment was effectively in disruption of hydrogen bonds in the cellulose of rice husk.

4.2 Glucose Standard Curve

After pretreatment, the sample undergo DNS test to compared the amount of glucose concentration produced. In glucose standard curve as shown in Figure 4.3, 0-0.06 mg/ml of glucose was used and the optical density was increased from 0.000 until 1.225 absorbance. The curve regression equation in $y = 20.021x + 0.0004$ with $R^2 = 0.9967$.
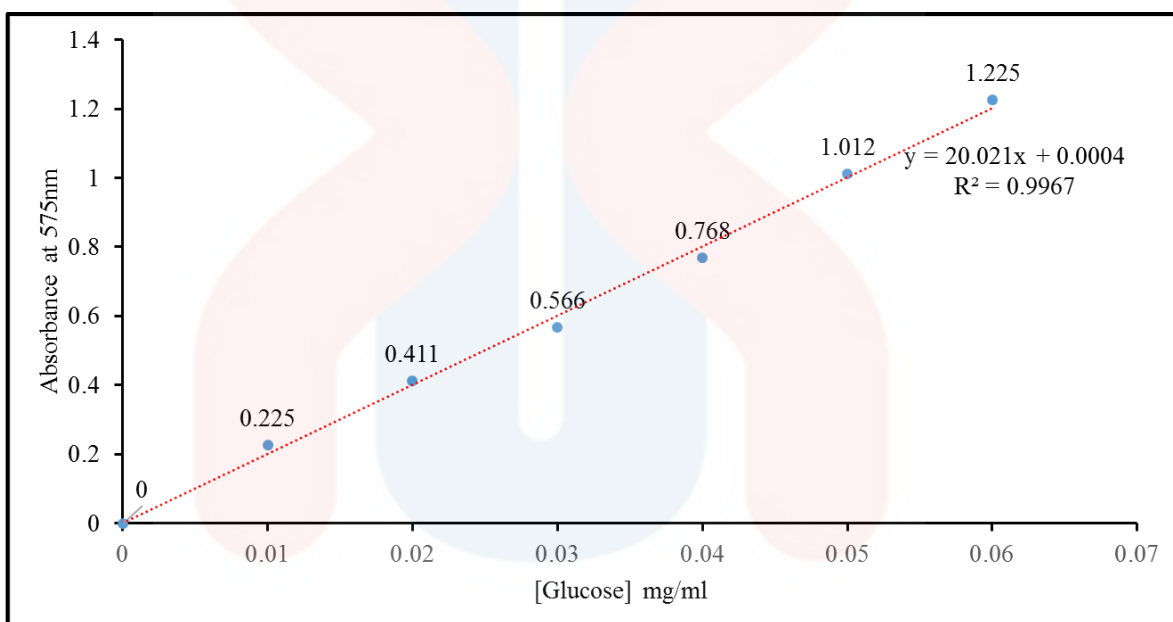

Figure 4.3: Glucose standard curve

4.2.1 Comparison of Untreated and IL-treated Rice Husk with Respect to their Effects on Glucose Yields.
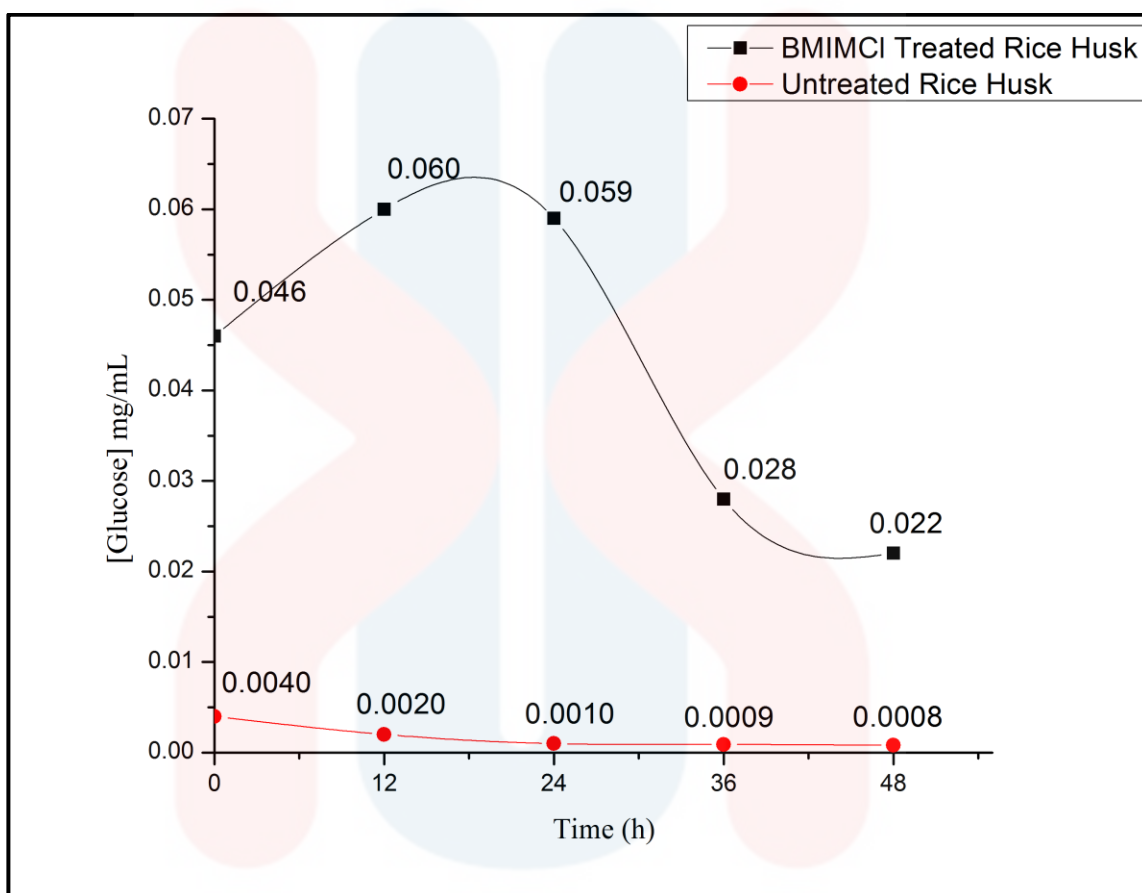
Figure 4.4: Glucose concentration with time of fermentation

Ethanol fermentation was a biological process in which organic material was converted by microorganism to simpler compounds such as glucose. During fermentation glucose that fermented by microorganism produced ethanol and carbon dioxide (Lin & Tanaka, 2006). As shown in Figure 4.4 BMIMCl treated rice husk produced higher glucose compared to untreated rice husk. The increase of sugar concentration was contributed from the effectiveness of hydrolysis of cellulose to glucose. According to Morales-delaRosa *et al.* (2012) the most important bottleneck of commercializing lignocellulosic bioethanol was the discovery of a cost-effective hydrolysis of cellulose.

The *β*-glycosidic linkages of sugar molecules in cellulose and lignocellulosic was strongly protected by tight packing of cellulose chains in microfibers which making hydrolysis process challenging. A harsh conditions and high temperature was needed during hydrolysis of cellulose. However, when IL was used to dissolve the cellulose the cations in the IL solution could more easily disrupt the *β*-glycosidic bonds to hydrolyze the bond. This also mention by Dadi *et al.* (2006) said that IL was effective in disrupt the cellulose structure which could accelerate the subsequent hydrolysis reaction. An efficient hydrolysis process lead to higher conversion of cellulose to glucose which was important for fermentation process.

Surprisingly, it was clearly seen that the glucose yield for BMIMCl treated rice husk shown 30 times higher from untreated rice husk. The effectiveness of IL in saccharification process also due to IL able to reduce the cellulose crystallinity to amorphous cellulose in which ionic liquid opens up the highly ordered cellulose structure and the cellulose molecule freely dispersed in the solvent (Shafiei *et al.*, 2013). The amorphous cellulose was more amiable to enzymatic conversion than closely packed crystalline cellulose in untreated rice husk and resulted in higher glucose and ethanol yields (Goshadrou *et al.*, 2013). While the hydrolysis of untreated rice husk provided a lower glucose yield due to lowest specific surface area and the highest CrI which prohibited accessibility of enzyme (Liu *et al.*, 2015).

Besides, the removal of hemicellulose by IL treatment also caused increased of the internal surface area thus increased the enzymatic access to cellulose and reduces nonproductive binding of cellulase with hemicellulose sugar. Hence, the enzymatic saccharification of cellulose was enhanced (Chang *et al.*, 2016).

The increased of glucose yield in treated rice husk compared with untreated rice husk was due to the high recovery of cellulose percentage after IL treatment (*see* Table 4.1). High percentage of cellulose will produce high glucose yield since more cellulose can be converted to glucose during hydrolysis. In fermentation process, the higher amount of sugar will subsequently produce higher amount of bioethanol since sugar will be converted to bioethanol during fermentation process by *saccharomyces cerevisiae*. Conversion of sugar to bioethanol can be represented by the chemical equation below:

$$C_6H_{12}O_6 \text{ (glucose)} \rightarrow 2C_2H_5OH \text{ (ethanol)} + 2CO_2 \qquad (4.1)$$
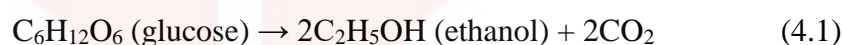
Which 1 mole of glucose will produce 2 moles of ethanol. The glucose consumption was described by the decrease in the glucose concentration during fermentation (Vallet *et al.*, 1996). The glucose yield was consumption when fermentation of *saccharomyces cerevisiae* and result ethanol formation (Bafrncová *et al.*, 1999). As represent in Figure 4.4 the glucose concentration of BMIMCl treated showed a significant decrease at 24 hours of fermentations. While for the untreated rice husk the fermentation was started after the fermentation started until 48 hours reached. The decrease in the glucose yield implied the glucose was consumption during fermentation.

In this research, *saccharomyces cerevisiae* was used in the fermentation to production of ethanol. *Saccharomyces cerevisiae* was used in fermentation process due to it is a promising fermentative microbe that able to produce high concentration of ethanol for about 18 % of fermentation broth which was the preferred one for most ethanol fermentation (Lin & Tanaka, 2006).

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CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1   Conclusion

As a conclusion for the research, the use of BMIMCl IL that during the pretreatment of rice husk was effective to produce more glucose yield and ethanol production. The superior performance of BMIMCl was proven by the effectives of BMIMCl that cause a change in the compositional percentage of BMIMCl treated rice husk. The compositional analysis of the BMIMCl treated rice husk result a significant of increase of cellulose composition from the regenerated cellulose. The high cellulose composition was effective for the production of higher glucose yield which consequently cause higher production of bioethanol when *saccharomyces cerevisiae* was used for fermentation.

Moreover, the structural properties of rice husk shown changed after IL pretreatment. XRD and FTIR indicated the changed of crystallinity in BMIMCl treated rice husk shown a lower crystallinity and high amorphous percentage compared untreated rice husk. This implied that BMIMCl was effective to disorder the crystalline region of cellulose and increased the efficient for enzymatic hydrolysis rate. This provided a benefit resulted the increase of bioethanol production. While the structural change that analysed by FTIR shown some significant absorption changed in some wavelength. The absorption changed in absorption of wavelength implied that the changed in the internal structure after IL treatment. Which the lignin was removed and cellulose was changed from more crystalline cellulose I to amorphous cellulose II.

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The concentration of glucose after IL treatment rice husk also shown 30 times higher than the untreated rice husk through DNS assay testing. The higher glucose yield was due to IL treated rice husk had more cellulose composition that enable to conversion to glucose when the fermentation by *saccharomyces cerevisiae*. The higher in glucose concentration resulted more higher yield of bioethanol produced by consumption of glucose during fermentation.

In terms of the change of composition, crystallinity and glucose concentration production BMIMCl ionic liquid was suitable for utilized as the solvent for pretreatment of rice husk. The right pretreatment was a crucial to obtain regenerated biomass that is more easily digestible by enzymes which able to produce cost-effective bioethanol.

5.2   Recommendation

After completing the research, there are a few recommendations that I would like to suggest in order to obtain a better result for further research. For further study, the focused may be on how to improve the efficiency process for bioethanol production based on some factor that affect the bioethanol production. Based on the research the main obstacle for the production of bioethanol was the formation of inhibitor and incomplete sugar utilization. The further study can focus more on how to overcome the inhibition problems. The factors affected include the enzymatic hydrolysis may be the substrate concentration, cellulase activity and reaction condition such as pH and temperature.

Moreover, two-stage hydrolysis-fermentation process (SSF) also can be utilized for further study on the fermentation of bioethanol production. This due to SSF has more advantages for the production of bioethanol. For examples, SSF can increased of hydrolysis rate by conversion of sugars that inhibit cellulase activity and has a shorter process time (Sun & Cheng, 2002).

Besides that, the fermentation process also affected by the microorganism used. In research yeast *saccharomyces cerevisiae* used is very sensitive to temperature changes for *saccharomyces cerevisiae* the range is between 37 °C to 40 °C. The yeast strains unable to react when temperature reach above 40 °C. This indirectly reduce the bioethanol production during fermentation process. Therefore, for further study the microorganism suggest to use may able to have high tolerance with changing environment and able to react faster during enzyme hydrolysis.

Apart from that, in this research the glucose yield of the rice husk was analysed by DNS test. However, for further studies the content of glucose can be confirm by high

performance liquid chromatography (HPLC) that equipped with refractive index (RI) detector. The application of HPLC due to it can easier monitored and quantitatively analysed the amount of fermentable sugars (dextrin, maltotriose, maltose and glucose) in fermentation booth (Hall & Reuter, 2007).

However, for further study gas chromatography mass spectrometry (GCMS) was suggested for further determine the concentration of bioethanol that produced from glucose for every 12 hours of the fermentation (Trivedi *et al.*, 2015).

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