FERMENTABLE SUGARS BY CHEMICAL HYDROLYSIS OF WATER HYACINTH IN IONIC LIQUID

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ABSTRACT

A chemical process for the hydrolysis of water hyacinth into monosaccharides was reported using dilute acid solution under chloride ionic liquid which was 1-ethyl-3-methyl imidazolium chloride as a co-solvent. Ethanol and ethylene glycol were used as solvents. The optimum conditions which are temperature, acid concentration, hydrolysis time and pretreatment time that gave the highest percent yield of glucose were determined. Glucose concentration was determined by 3,5-dinitrosalicylia acid (DNS) method. It was found that, without pretreatment, hydrolysis of water hyacinth with 15% w/w of dilute H_2SO_4 for 2 hours at 120 °C was the optimum condition that led to highest percent yield of glucose.

Keywords : Water hyacinth, Ionic liquid

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Chapter 1

Introduction

1.1. Motivation

Nowadays, the demand for fossil fuel is increasing while the supply is decreasing which make the price of fossil fuel rising continuously. Reduction in energy consumption seems to be necessary so the alternative energies have become more interesting these days. One of the popular alternative energies used today is gasohol which is a mixture between gasoline and ethanol. Gasohol is cheaper than gasoline and helps reduce the demand for fossil fuel consumption. Unfortunately, ethanol used today is also from petroleum. Ethanol from bio-chemical technologies such as fermentation or enzymatic procedures of biomass gives low yield and takes long time to produce. Moreover, the processes of more efficiently converting biomass into ethanol are difficult and still need more improvement. It is challenging to find the method that gives the best efficiency and can be used in a commercial scale.

Water hyacinth is a biomass that can be found abundantly and is considered as a weed that causes problems to the water sources. Using it as a raw material for producing ethanol will surely not compete with the food crops. Therefore, it is interesting to use water hyacinth as a source of renewable energy by turning it into ethanol but the pretreatment of biomass is needed.

There are many pretreatment processes available. The process that is widely used for hydrolyzing lignocellulosic biomass into fermentable sugar is acid pretreatment using concentrated acid but there are some disadvantages such as corrosion or too high severity of the reaction that may lead to the formation of undesired product. It is better to use dilute acid which also give a satisfied yield of fermentable sugar with lower acid consumption. One of the recent interests is the new pretreatment method using solvent called ionic liquid. Some advantages about ionic liquids are chemical inertness, good thermal stability, low volatility and their unique solvation abilities. It is also considered as green solvent. However, the main disadvantage of ionic liquid is their expensive cost. There are many experiments showing that the chloride ionic liquids are excellent solvents for lignocellulosic biomass. Hua Zhao et.al. (2008) studied pretreatment of lignocellulose using six different ionic liquids at 110 °C with 15-min incubation. It was found that cellulose can be dissolved up to 10 wt%. Swatloski et.al. (2002) studied the dissolution of cellulose in 1-butyl-3-methylimidazolium cations ($[C_4mim]^+$) and varied the cations. Cellulose was heated using heating plate and microwave oven. The result showed up to 25wt% dissolution when 0.5-1.0 g of fibrous cellulose was used with 10 g of $[C_4mim]Cl$ ionic liquid with microwave heating. Binder et. al. (2010) studied hydrolysis of corn stover in 1-ethyl-3-methyl imidazolium chloride ([EMIM]Cl) and hydrochloric acid where 26.7 mg of corn stover is mixed with 502 mg of [EMIM]Cl at 105 °C for 6 hours. After that aqueous HCl is added, followed by adding the aliquot of deionized water periodically. The liquid products were analyzed by HPLC which resulted in 42% yield of glucose and 71% yield of xylose.

In this research, the hydrolysis reactions of water hyacinth in dilute sulfuric acid at the concentration of 10, 15 to 20% w/w together with ionic liquid (1-ethyl-3-methyl imidazolium chloride) at 120 °C for 30, 60, 90, 120, 150 and 180 minutes were carried out. The ratios of ionic liquid to biomass used in all attempts were also reduced to the amount lower than those used in the previous researches by dilution with ethanol and ethylene glycol. The pretreatment of water hyacinth using 1-ethyl-3-methyl imidazolium chloride prior to hydrolysis was also studied. The concentration of glucose for each condition was measured using 3,5 – dinitrosalicylic acid (DNS) method.

1.2. Objectives

1.2.1. To study the hydrolysis of water hyacinth in dilute sulfuric acid and 1-ethyl-3-methyl imidazolium chloride

1.2.2. Determine the optimum condition for hydrolysis of water hyacinth by measuring the concentration of glucose

1.3. Scope

1.3.1 Literature reviews

1.3.2 Preparation of dried water hyacinth by drying in the oven, cutting into small pieces and sieving to collect the size between 150 - 500 µm.

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1.3.4 Study on the hydrolysis of water hyacinth in the presence of 1-ethyl-3-methyl imidazolium chloride and dilute sulfuric acid at 100 °C, 120 °C and 140 °C

1.3.5 Study on the hydrolysis of water hyacinth in 1-ethyl-3-methyl imidazolium chloride together with 10, 15 and 20% w/w sulfuric acid at 120 °C for 30, 60, 90, 120, 150 and 180 minutes

1.3.6 Study on the pretreatment of water hyacinth with 1-ethyl-3-methyl imidazolium chloride at 120 °C for 5, 10, 15 and 20 hours prior to hydrolysis

1.3.7 Determination the optimum condition for hydrolysis of water hyacinth comparing between having and not having pretreatment by determining of glucose concentration in filtrate using 3,5 – dinitrosalicylic acid (DNS) method.

1.4. Expected result

To obtain the optimum condition which gives higher yield of glucose from hydrolysis and pretreatment of water hyacinth comparing with the one without the use of 1-ethyl-3-methyl imidazolium chloride.

Chapter 2

Theoretical Background and Literature Reviews

2.1 Lignocellulosic biomass [1,2]

2.1.1 Introduction

Lignocellulosic biomass refers to plant biomass that is composed of cellulose, hemicellulose, and lignin. The carbohydrate polymers (cellulose and hemicelluloses) are tightly bound to the lignin. Lignocellulosic biomass can be grouped into four main categories: agricultural residues (including corn stover and sugarcane bagasse), dedicated energy crops, wood residues (including saw mill and paper mill discards), and municipal paper waste.

2.1.2 Structure of Lignocellulosic Biomass

Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash. The composition of these constituents can vary from one plant species to another. For example, hardwood has greater amounts of cellulose, whereas wheat straw and leaves have more hemicellulose. In addition, the ratios between various constituents within a single plant vary with age, stage of growth, and other conditions. Cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. The structure of cellulose is shown in Figure 2.1. This linear polymer consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic bonds. Cellobiose is the repeat unit established through this linkage, and it constitutes cellulose chains. The long-chain cellulose polymers are linked together by hydrogen and Van der Waals bonds, which cause the cellulose to be packed into microfibrils. Hemicelluloses and lignin cover the microfibrils. Fermentable D-glucose can be produced from cellulose through the action of either acid or enzymes breaking the β-(1,4)- glycosidic linkages. Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous form.

The main feature that differentiates hemicellulose from cellulose is that hemicellulose has branches with short lateral chains consisting of different sugars. These monosaccharides include pentoses (xylose, rhamnose and arabinose), hexoses (glucose, mannose and galactose), and uronic acids (e.g., 4-omethylglucuronic, D-glucuronic, and D-galactouronic acids). The backbone of hemicellulose is either a homopolymer or a heteropolymer with short branches linked by β-(1,4)-glycosidic bonds and occasionally β-(1,3)-glycosidic bonds. Also, hemicelluloses can have some degree of acetylation, for example, in heteroxylan. In contrast to cellulose, the polymers present in hemicelluloses are easily hydrolyzable. These polymers do not aggregate, even when they cocrystallize with cellulose chains. Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack.16 Three phenyl propionic alcohols exist as monomers of lignin: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol). Alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds link these phenolic monomers together. In general, herbaceous plants such as grasses have the lowest contents of lignin, whereas softwoods have the highest lignin contents.

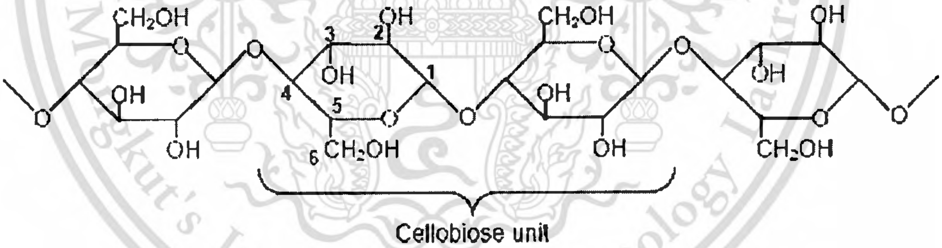


Figure 2.1 Illustration of a cellulose chain.

2.1.3 Applications of lignocellulosic biomass

Pulp and paper industry. Lignocellulosic biomass is the feedstock for the pulp and paper industry. This energy-intensive industry focuses on the separation of the lignin and cellulosic fractions of the biomass.

Biofuels. Lignocellulosic biomass, in the form of wood fuel, has a long history as a source of energy. Since the middle of the 20th century, the interest of biomass as a precursor to liquid fuels has increased. To be specific, the fermentation of lignocellulosic biomass to ethanol is an attractive route to fuels that supplements the fossil fuels. Biomass is a carbon-neutral source of energy: Since it comes from plants, the combustion of lignocellulosic ethanol produces no net carbon dioxide into the earth's atmosphere. Aside from ethanol, many other lignocellulose-derived fuels are of potential interest, including butanol, dimethylfuran and gamma-Valerolactone.

One barrier to the production of ethanol from biomass is that the sugars necessary for fermentation are trapped inside the lignocellulose. Lignocellulose has evolved to resist degradation and to confer hydrolytic stability and structural robustness to the cell walls of the plants. This robustness or "recalcitrance" is attributable to the cross-linking between the polysaccharides (cellulose and hemicellulose) and the lignin via ester and ether linkages. Ester linkages arise between oxidized sugars, the uronic acids, and the phenols and phenylpropanols functionalities of the lignin. To extract the fermentable sugars, one must first disconnect the celluloses from the lignin, and then acid-hydrolyze the newly freed celluloses to break them down into simple monosaccharides. Another challenge to biomass fermentation is the high percentage of pentoses in the hemicellulose, such as xylose, or wood sugar. Unlike hexoses, like glucose, pentoses are difficult to ferment. The problems presented by the lignin and hemicellulose fractions are the foci of much contemporary research.

Figure 2.2 Schematic of the conversion of lignocellulosic biomass to fuel.

2.2 Water hyacinth [3-6]

2.2.1 Introduction

Water hyacinths are a free-floating perennial aquatic plant native to tropical and sub-tropical South America. With broad, thick, glossy, ovate leaves, water hyacinth may rise above the surface of the water as much as 1 meter in height. The leaves are 10–20 cm across, and float above the water surface. They have long, spongy and bulbous stalks. The feathery, freely hanging roots are purple-black. An erect stalk supports a single spike of 8-15 conspicuously attractive flowers, mostly lavender to pink in colour with six petals. When not in bloom, water hyacinth may be mistaken for frog's-bit (Limnobium spongia). One of the fastest growing plants known, water hyacinth reproduces primarily by way of runners or stolons, which eventually form daughter plants. It also produces large quantities of seeds, and these are viable up to thirty years. The common water hyacinth (Eichhornia crassipes) are vigorous growers known to double their population in two weeks.

Figure 2.3 Water hyacinth

2.2.2 Chemical composition

Water hyacinth is low in lignin content (10%) and contains high amounts of cellulose (20%) and hemicellulose (33%) A typical biomass from land plants can have 30-50% cellulose, 20-40% hemicellulose and 15-30% lignins. In plants, lignin (composed of phenylpropanoid groups) acts as a polymer around the hemicellulose microfibrils, binding the cellulose molecules together and protecting them against chemical degradation. Lignin cannot be converted into sugars. Their degradation is a high energy process. Water hyacinth has low lignin, which means the cellulose and hemicellulose are more easily converted to fermentable sugar thus resulting in enormous amount of utilizable biomass for the biofuel industry.

Table 2.1 Average biomass composition of water hyacinth

Components	% composition
1. Lignin	10
2. Cellulose	25
3. Hemicellulose	35
4. Ash	20
5. Nitrogen	03

2.2.3 Problems of water hyacinth

Water hyacinth can cause a variety of problems when its rapid mat-like proliferation covers areas of fresh water. Some of the common problems are listed below:

Hindrance to water transport. Access to harbours and docking areas can be seriously hindered by mats of water hyacinth. Canals and freshwater rivers can become impassable as they clog up with densely intertwined carpets of the weed. It is also becoming a serious hazard to lake transport on Lake Victoria as large floating islands of water hyacinth form, while many of the inland waterways of south east Asia have been

Clogging of intakes of irrigation, hydropower and water supply systems. Many large hydropower schemes are suffering from the effects of water hyacinth. The Owen Falls hydropower scheme at Jinja on Lake Victoria is a victim of the weeds rapid reproduction rates and an increasing amount of time and money has to be invested in clearing the weed to prevent it entering the turbine and causing damage and power interruptions. Water hyacinth is now a major problem in some of the world's major dams - the Kariba dam which straddles the Zambia-Zimbabwe border on the Zambezi River and feeds Harare has pronounced infestations of the weed.

Blockage of canals and rivers causing flooding. Water hyacinth can grow so densely that a human being can walk on it. When it takes hold in rivers and canals it can become so dense that it forms a herbivorous barrage and can cause damaging and dangerous flooding.

Micro-habitat for a variety of disease vectors. The diseases associated with the presence of aquatic weeds in tropical developing countries are among those that cause the major public health problems: malaria, schistosomiasis and lymphatic filariasis. Some species of mosquito larvae thrive on the environment created by the presence of aquatic weeds, while the link between schistosomiasis (bilharzia) and aquatic weed presence is well known. Although the statistical link is not well defined between the presence of aquatic weeds and malaria and schistosomiasis, it can be shown that the brughian type of filariasis (which is responsible for a minor share of lymphatic filariasis in South Asia) is entirely linked to the presence of aquatic weeds (Bos, 1996).

Increased evapotranspiration. Various studies have been carried out to ascertain the relationship between aquatic plants and the rate of evapotranspiration compared with evaporation from an open-surfaced water body. Saelthun (1994) suggests that the rate of water loss due to evapotranspiration can be as much as 1.8 times that of evaporation from the same surface but free of plants. This has great implications where water is already scarce. It is estimated that the flow of water in the Nile could be reduced by up to one tenth due to increased losses in Lake Victoria from water hyacinth.

Problems related to fishing. Water hyacinth can present many problems for the fisherman. Access to sites becomes difficult when weed infestation is present, loss of fishing equipment often results when nets or lines become tangled in the root systems of the weed and the result of these problems is more often than not a reduction in catch and subsequent loss of livelihood. In areas where fishermen eke a meagre living from their trade, this can present serious socio-economic problems. Fishermen on lake Victoria have also noted that, in areas where there is much water hyacinth infestation, the water is 'still and warm and the fish disappear'. They also complain that crocodiles and snakes have become more prevalent.

Reduction of biodiversity. Where water hyacinth is prolific, other aquatic plants have difficulty in surviving. This causes an imbalance in the aquatic micro-ecosystem and often means that a range of fauna that relies on a diversity of plant life for its existence, will become extinct. Diversity of fish stocks is often effected with some benefiting and others suffering from the proliferation of water hyacinth. People often complain of localized water quality deterioration. This is of considerable concern where people come to collect water and to wash.

2.2.4 Applications of water hyacinth

Although water hyacinth is seen in many countries as a weed and is responsible for many of the problems outlined earlier in this fact sheet, many individuals, groups and institutions have been able to turn the problem around and find useful applications for the plant. The plant itself, although more than 95% water, has a fibrous tissue and a high energy and protein content, and can be used for a variety of useful applications. Below we will consider a number of possible uses for the plant, some which have been developed and others which are still in their infancy or remain as ideas only.

Paper. The Mennonite Central Committee of Bangladesh has been experimenting with paper production from water hyacinth for some years. They have established two projects that make paper from water hyacinth stems. The water hyacinth fibre alone does not make a particularly good paper but when the fibre is blended with waste paper or jute the result is good. The pulp is dosed with bleaching powder, calcium carbonate and sodium carbonate before being heated.

Fibre board. Another application of water hyacinth is the production of fibreboards for a variety of end uses. The House and Building Research Institute in Dhaka has carried out experimental work on the production of fibre boards from water hyacinth fibre and other indigenous materials. They have developed a locally manufactured production plant for producing fibreboard for general-purpose use and also a bituminised board for use as a low cost roofing material.

Yarn and rope. The fibre from the stems of the water hyacinth plant can be used to make rope. The stalk from the plant is shredded lengthways to expose the fibres and then left to dry for several days. The rope making process is similar to that of jute rope. The finished rope is treated with sodium metabisulphite to prevent it from rotting. In Bangladesh, the rope is used by a local furniture manufacturer who winds the rope around a cane frame to produce an elegant finished product.

Basket work. In the Philippines water hyacinth is dried and used to make baskets and matting for domestic use. The key to a good product is to ensure that the stalks are properly dried before being used. If the stalks still contain moisture then this can cause the product to rot quite quickly. In India, water hyacinth is also used to produce similar goods for the tourist industry. Traditional basket making and weaving skills are used.

Charcoal briquetting. This is an idea which has been proposed in Kenya to deal with the rapidly expanding carpets of water hyacinth which are evident on many parts of Lake Victoria. The proposal is to develop a suitable technology for the briquetting of charcoal dust from the pyrolysis of water hyacinth. The project is still very much at the idea stage and both a technical and a socio-economic study are planned to evaluate the prospects for such a project.

Biogas production. The possibility of converting water hyacinth to biogas has been an area of major interest for many years. Conversion of other organic matter, usually animal or human waste, is a well established small and medium scale technology in a number of developing countries, notably in China and India. The process is one of anaerobic digestion which takes place in a reactor or digester (an air tight container usually sited below ground) and the usable product is methane gas which can be used as a fuel for cooking, lighting or for powering an engine to provide shaft power. The residue from the digestion process provides a fertilizer rich in nutrients.

Water purification. Water hyacinth can be used to aid the process of water purification either for drinking water or for liquid effluent from sewage systems. In a drinking water treatment plant water hyacinth have been used as part of the pretreatment purification step. Clean, healthy plants have been incorporated into water clarifiers and help with the removal of small flocs that remain after initial coagulation and floc removal or settling. (Haider 1989). The result is a significant decrease in turbidity due to the removal of flocs and also slight reduction in organic matter in the water.

Animal fodder. Studies have shown that the nutrients in water hyacinth are available to ruminants. In Southeast Asia some nonruminant animals are fed rations containing water hyacinth. In China pig farmers boil chopped water hyacinth with vegetable waste, rice bran, copra cake and salt to make a suitable feed. In Malaysia fresh water hyacinth is cooked with rice bran and fishmeal and mixed with copra meal as feed for pigs, ducks and pond fish. Similar practices are much used in Indonesia, the Philippines and Thailand (National Academy of Sciences, 1976). The high water and mineral content mean that it is not suited to all animals.

Fertilisers. Water hyacinth can be used on the land either as a green manure or as compost. As a green manure it can be either ploughed into the ground or used as a mulch. The plant is ideal for composting. After removing the plant from the water it can be left to dry for a few days before being mixed with ash, soil and some animal manure. Microbial decomposition breaks down the fats, lipids, proteins, sugars and starches. The mixture can be left in piles to compost, the warmer climate of tropical countries accelerating the process and producing a rich pathogen free compost which can be applied directly to the soil. The compost increases soil fertility and crop yield and generally improves the quality of the soil.

Fish feed. The Chinese grass carp is a fast growing fish which eats aquatic plants. It grows at a tremendous rate and reach sizes of up to 32kg (National Academy of Sciences, 1979). It is an edible fish with a tasty white meat. It will eat submerged or floating plants and also bank grasses. The fish can be used for weed control and will eat up to 18 - 40% of its own body weight in a single day.

2.3 Pretreatment of lignocellulosic biomass [1]

The beneficial effects of pretreatment of lignocellulosic materials have been recognized for a long time. The goal of the pretreatment process is to remove lignin and hemicellulose, reduce the crystallinity of cellulose, and increase the porosity of the lignocellulosic materials. Pretreatment must meet the following requirements:

(1) To improve the formation of sugars or the ability to subsequently form sugars by hydrolysis.

(2) To avoid the degradation or loss of carbohydrate.

(3) To avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes.

(4) To be cost-effective.

Pretreatment methods can be roughly divided into different categories: physical (milling and grinding), physicochemical (steam pretreatment/autohydrolysis, hydrothermolysis, and wet oxidation), chemical (alkali, dilute acid, oxidizing agents, and organic solvents), biological, electrical, or a combination of these.

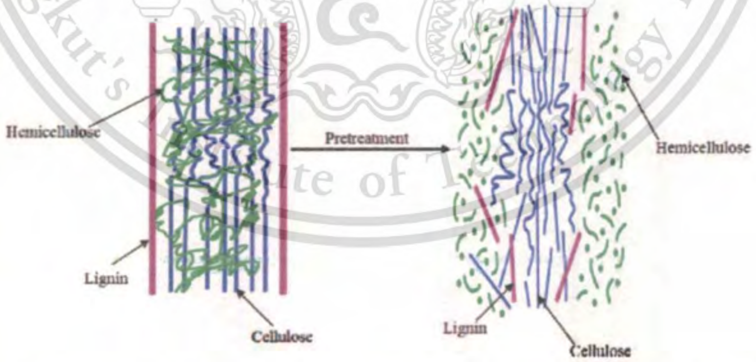


Figure 2.4 Schematic of the role of pretreatment in the conversion of biomass to fuel.

2.4 Hydrolysis [7]

Hydrolysis (from Greek roots hydro "water" + lysis "separation") is a chemical reaction in which molecules of water (H_2O) are split into hydrogen cations (H+, identical to protons) and hydroxide anions (OH^-) in the process of a chemical mechanism. It is the type of reaction that is used to break down certain polymers, especially those made by condensation polymerization. Such polymer degradation is usually catalyzed by either acid, e.g., concentrated sulfuric acid (H_2SO_4), or alkali, e.g., sodium hydroxide (NaOH).

Monosaccharides can be linked together by glycosidic bonds, which can be cleaved by hydrolysis. Two, three, several or many monosaccharides thus linked form disaccharides, trisaccharides, oligosaccharides or polysaccharides, respectively. Enzymes that hydrolyze glycosidic bonds are called "glycoside hydrolases" or "glycosidases".

The best-known disaccharide is sucrose (table sugar). Hydrolysis of sucrose yields glucose and fructose. Invertase is a sucrase used industrially for the hydrolysis of sucrose to so-called invert sugar. Lactase is essential for digestive hydrolysis of lactose in milk. Deficiency of lactase in humans causes lactose intolerance.

The hydrolysis of polysaccharides to soluble sugars is called "saccharification". Malt made from barley is used as a source of β-amylase to break down starch into the disaccharide maltose, which can be used by yeast to produce beer. Other amylase enzymes may convert starch to glucose or to oligosaccharides. Cellulose is converted to glucose or the disaccharide cellobiose by cellulases. Animals such as cows (ruminants) are able to digest cellulose because of symbiotic bacteria that produce cellulases

2.5 Sugar [8]

2.5.1 Introduction

Sugar is the generalized name for a class of sweet-flavored substances used as food. They are carbohydrates and as this name implies, are composed of carbon, hydrogen and oxygen. There are various types of sugar derived from different sources. Simple sugars are called monosaccharides and include glucose, fructose and galactose. The table or granulated sugar most customarily used as food is sucrose, a disaccharide. Other disaccharides include maltose and lactose.

Sugars are found in the tissues of most plants but are only present in sufficient concentrations for efficient extraction in sugarcane and sugar beet. Sugarcane is a giant grass and has been cultivated in tropical climates in the Far East since ancient times. A great expansion in its production took place in the 18th century with the setting up of sugar plantations in the West Indies and Americas. This was the first time that sugar became available to the common people who had previously had to rely on honey to sweeten foods. Sugar beet is a root crop and is cultivated in cooler climates and became a major source of sugar in the 19th century when methods for extracting the sugar became available. Sugar production and trade has changed the course of human history in many ways. It influenced the formation of colonies, the perpetuation of slavery, the transition to indentured labor, the migration of peoples, wars between 19th century sugar trade controlling nations and the ethnic composition and political structure of the new world. The world produced about 168 million tons of sugar in 2011. The average person consumes about 24 kilograms of sugar each year, equivalent to over 260 food calories per person, per day.

In modern times it has been questioned whether a diet high in sugars, especially refined sugars, is bad for health. Sugar has been linked to obesity and suspected of being implicated in diabetes, cardiovascular disease, dementia, macular degeneration and tooth decay. Numerous studies have been undertaken to try to clarify the position but the results remain largely unclear, mainly because of the difficulty of finding populations for use as controls that do not consume sugars.

2.5.2 Types of sugar

Monosaccharides. Glucose, fructose and galactose are all simple sugars, monosaccharides, with the general formula $C_6H_{12}O_6$. They have five hydroxyl groups ($-$OH) and a carbonyl group (C=O) and are cyclic when dissolved in water. They each exist as several isomers with dextro- and laevo-rotatory forms which cause polarized light to diverge to the right or the left.

Glucose, dextrose or grape sugar occurs naturally in fruits and plant juices and is the primary product of photosynthesis. Most ingested carbohydrates are converted into glucose during digestion and it is the form of sugar that is transported round the bodies of animals in the bloodstream. It can be manufactured from starch by the addition of enzymes or in the presence of acids. Glucose syrup is a liquid form of glucose that is widely used in the manufacture of foodstuffs. It can be manufactured from starch by enzymatic hydrolysis.

Fructose or fruit sugar occurs naturally in fruits, some root vegetables, cane sugar and honey and is the sweetest of the sugars. It is one of the components of sucrose or table sugar. It is used as a high fructose syrup which is manufactured from hydrolyzed corn starch which has been processed to yield corn syrup, with enzymes then added to convert part of the glucose into fructose.

Galactose does not generally occur in the free state but is a constituent with glucose of the disaccharide lactose or milk sugar. It is less sweet than glucose. It is a component of the antigens found on the surface of red blood cells that determine blood groups.

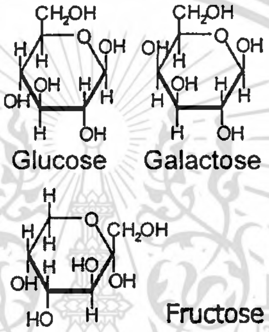


Figure 2.5 Monosaccharides (Glucose, fructose and galactose).

Disaccharides. Sucrose, maltose and lactose are all compound sugars, disaccharides, with the general formula $C_{12}H_{22}O_{11}$. They are formed by the combination of two monosaccharide molecules with the exclusion of a molecule of water.

Sucrose is found in the stems of sugar cane and roots of sugar beet. It also occurs naturally alongside fructose and glucose in other plants, particularly fruits and some roots such as carrots. The different proportions of sugars found in these foods determines the range of sweetness experienced when eating them. A molecule of sucrose is formed by the combination of a molecule of glucose with a molecule of fructose. After being eaten, sucrose is split into its constituent parts during digestion by a number of enzymes known as sucrases.

Maltose is formed during the germination of certain grains, most notably barley, where it is the source of the malt used in the manufacture of beer. A molecule of maltose is formed by the combination of two molecules of glucose. It is less sweet than glucose, fructose or sucrose. It is formed in the body during the digestion of starch by the enzyme amylase and is itself broken down during digestion by the enzyme maltase.

Lactose is the naturally occurring sugar found in milk. A molecule of lactose is formed by the combination of a molecule of galactose with a molecule of glucose. It is broken down when consumed into its constituent parts by the enzyme lactase during digestion. Children have this enzyme but some adults no longer form it and they are unable to digest lactose.

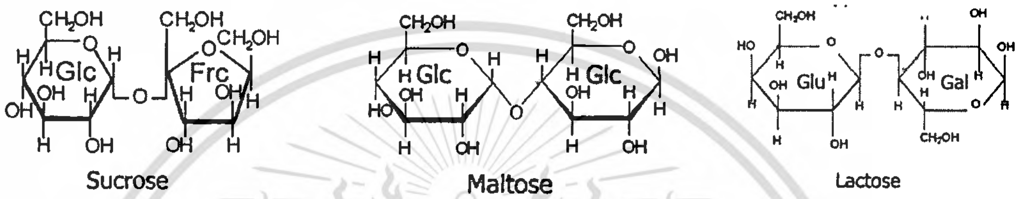
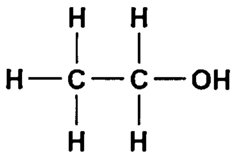


Figure 2.6 Disaccharides (sucrose, maltose and lactose)

2.6 Ethanol [9,10]

2.6.1 Introduction

Ethanol, also called ethyl alcohol, pure alcohol, grain alcohol, or drinking alcohol, is a volatile, flammable, colorless liquid. It is a psychoactive drug and one of the oldest recreational drugs. Best known as the type of alcohol found in alcoholic beverages, it is also used in thermometers, as a solvent, and as a fuel. In common usage, it is often referred to simply as alcohol or spirits. Ethanol is a 2-carbon alcohol with the molecular formula CH_3CH_2OH. Its empirical formula is C_2H_6O. An alternative notation is $CH_3{-}CH_2{-}OH$, which indicates that the carbon of a methyl group ($CH_3{-}$) is attached to the carbon of a methylene group ($-CH_2-$), which is attached to the oxygen of a hydroxyl group (–OH). It is a constitutional isomer of dimethyl ether. Ethanol is often abbreviated as EtOH, using the common organic chemistry notation of representing the ethyl group (C_2H_5) with Et.



$$H-\overset{\displaystyle H}{\underset{\displaystyle H}{C}}-\overset{\displaystyle H}{\underset{\displaystyle H}{C}}-OH$$

Figure 2.7 Molecular formula of ethanol

2.6.2 Physical properties

Ethanol is a volatile, colorless liquid that has a slight odor. It burns with a smokeless blue flame that is not always visible in normal light. The physical properties of ethanol stem primarily from the presence of its hydroxyl group and the shortness of its carbon chain. Ethanol's hydroxyl group is able to participate in hydrogen bonding, rendering it more viscous and less volatile than less polar organic compounds of similar molecular weight.

Ethanol is a versatile solvent, miscible with water and with many organic solvents, including acetic acid, acetone, benzene, carbon tetrachloride, chloroform, diethyl ether, ethylene glycol, glycerol, nitromethane, pyridine, and toluene. It is also miscible with light aliphatic hydrocarbons, such as pentane and hexane, and with aliphatic chlorides such as trichloroethane and tetrachloroethylene. Ethanol's miscibility with water contrasts with the immiscibility of longer-chain alcohols (five or more carbon atoms), whose water miscibility decreases sharply as the number of carbons increases. The miscibility of ethanol with alkanes is limited to alkanes up to undecane, mixtures with dodecane and higher alkanes show a miscibility gap below a certain temperature (about 13 °C for dodecane). The miscibility gap tends to get wider with higher alkanes and the temperature for complete miscibility increases. Ethanol-water mixtures have less volume than the sum of their individual components at the given fractions. Mixing equal volumes of ethanol and water results in only 1.92 volumes of mixture. Mixing ethanol and water is exothermic. At 298 K, up to 777 J/mol are set free. Mixtures of ethanol and water form an azeotrope at about 89 mole-% ethanol and 11 mole-% water or a mixture of about 96 volume percent ethanol and 4% water at normal pressure and T = 351 K. This azeotropic composition is strongly temperature- and pressure-dependent and vanishes at temperatures below 303 K. Hydrogen bonding causes pure ethanol to be hygroscopic to the extent that it readily absorbs water from the air. The polar nature of the hydroxyl group causes ethanol to dissolve many ionic compounds, notably sodium and potassium hydroxides, magnesium chloride, calcium chloride, ammonium chloride, ammonium bromide, and sodium bromide. Sodium and potassium chlorides are slightly soluble in ethanol. Because the ethanol molecule also has a non-polar end, it will also dissolve non-polar substances, including most essential oils and numerous flavoring, coloring, and medicinal agents. The addition of even a few percent of ethanol to water sharply reduces the surface tension of water. This property partially explains the "tears of wine" phenomenon.

When wine is swirled in a glass, ethanol evaporates quickly from the thin film of wine on the wall of the glass. As the wine's ethanol content decreases, its surface tension increases and the thin film "beads up" and runs down the glass in channels rather than as a smooth sheet. Mixtures of ethanol and water that contain more than about 50% ethanol are flammable and easily ignited. Alcoholic proof is a widely used measure of how much ethanol (i.e., alcohol) such a mixture contains. In the 18th century, proof was determined by adding liquor (such as rum) to gunpowder. If the gunpowder still burned, that was considered to be "100 degrees proof" that it was "good" liquor — hence it was called "100 degrees proof". Ethanol-water solutions that contain less than 50% ethanol may also be flammable if the solution is first heated. Some cooking methods call for wine to be added to a hot pan, causing it to flash boil into a vapor, which is then ignited to burn off excess alcohol. Ethanol is slightly more refractive than water, having a refractive index of 1.36242 (at λ=589.3 nm and 18.35 °C).

2.6.3 Applications of ethanol

As a fuel. The largest single use of ethanol is as a motor fuel and fuel additive. More than any other major country, Brazil relies on ethanol as a motor fuel. Gasoline sold in Brazil contains at least 25% anhydrous ethanol. Hydrous ethanol (about 95% ethanol and 5% water) can be used as fuel in more than 90% of new cars sold in the country. Brazilian ethanol is produced from sugar cane and noted for high carbon sequestration. The US uses Gasohol (max 10% ethanol) and E85 (85% ethanol) ethanol/gasoline mixtures.

Alcoholic beverages. Ethanol is the principal psychoactive constituent in alcoholic beverages, with depressant effects on the central nervous system. It has a complex mode of action and affects multiple systems in the brain, the most notable one being its agonistic action on the GABA receptors. Similar psychoactives include those that also interact with GABA receptors, such as gamma-hydroxybutyric acid (GHB). Ethanol is metabolized by the body as an energy-providing nutrient, as it metabolizes into acetyl CoA, an intermediate common with glucose and fatty acid metabolism that can be used for energy in the citric acid cycle or for biosynthesis.

Feedstock. Ethanol is an important industrial ingredient and has widespread use as a base chemical for other organic compounds. These include ethyl halides, ethyl esters, diethyl ether, acetic acid, ethyl amines, and to a lesser extent butadiene.

Antiseptic. Ethanol is used in medical wipes and in most common antibacterial hand sanitizer gels at a concentration of about 62% v/v as an antiseptic. Ethanol kills organisms by denaturing their proteins and dissolving their lipids and is effective against most bacteria and fungi, and many viruses, but is ineffective against bacterial spores.

Treatment for poisoning by other alcohols. Ethanol is sometimes used to treat poisoning by other, more toxic alcohols, in particular methanol and ethylene glycol. Ethanol competes with other alcohols for the alcohol dehydrogenase enzyme, lessening metabolism into toxic aldehyde and carboxylic acid derivatives, and reducing one of the more serious toxic effect of the glycols to crystallize in the kidneys.

Solvent. Ethanol is miscible with water and is a good general purpose solvent. It is found in paints, tinctures, markers, and personal care products such as perfumes and deodorants. It may also be used as a solvent in cooking, such as in vodka sauce.

2.7 Ethylene glycol [11]

2.7.1 Introduction

Ethylene glycol (IUPAC name: 1,2-ethanediol) is an organic compound widely used as an automotive antifreeze and a precursor to polymers. In its pure form, it is an odorless, colorless, syrupy, sweet-tasting liquid. Ethylene glycol is toxic, and ingestion can result in death. Ethylene glycol is not to be confused with diethylene glycol, a heavier ether diol, or with polyethylene glycol, a nontoxic polyether polymer.

2.7.2 Applications of ethylene glycol

The major end uses of ethylene glycol are as an antifreeze, which accounts for over 50% of ethylene glycol's commercial uses, and as raw material in the production of polyester fibers, mainly PET, which accounts for 40% of total ethylene glycol consumption. Because this material is cheaply available, it finds many niche applications.

2.7.3 Toxicity

Ethylene glycol is moderately toxic with an oral LDLO = 786 mg/kg for humans. The major danger is due to its sweet taste. Because of that, children and animals are more inclined to consume large quantities of it than of other poisons. Upon ingestion, ethylene glycol is oxidized to glycolic acid which is, in turn, oxidized to oxalic acid, which is toxic. It and its toxic byproducts first affect the central nervous system, then the heart, and finally the kidneys. Ingestion of sufficient amounts can be fatal if untreated. According to the annual report of the American Association of Poison Control Centers' National Poison Data System in 2007, there were about 1000 total cases resulting in 16 deaths. The 2008 American Association of Poison Control Centers' National Poison Data System annual report lists 7 deaths.

2.8 Sulfuric acid [12]

2.8.1 Introduction

Sulfuric acid (alternative spelling sulphuric acid) is a highly corrosive strong mineral acid with the molecular formula H_2SO_4. The historical name of this acid is oil of vitriol. It is a colorless to slightly yellow viscous liquid and is soluble in water at all concentrations. It is a diprotic acid. The corrosiveness of it is mainly due to its strong acidic nature, strong dehydrating property and if concentrated strong oxidizing property. It has many applications and is a central substance in the chemical industry. Principal uses include lead-acid batteries for cars and other vehicles, mineral processing, fertilizer manufacturing, oil refining, wastewater processing, and chemical synthesis.

2.8.2 Physical properties

Although nearly 99% sulfuric acid can be made, this loses SO_3 at the boiling point to produce 98.3% acid. The 98% grade is more stable in storage, and is the usual form of what is described as "concentrated sulfuric acid." Other concentrations are used for different purposes. Some common concentrations are

Table 2.2 Grades of sulfuric acid

Mass fraction H_2SO_4	Density (kg/L)	Concentration (mol/L)	Common name
10%	1.07	~1	dilute sulfuric acid
29–32%	1.25–1.28	4.2–5	battery acid (used in lead–acid batteries)
62–70%	1.52–1.60	9.6–11.5	chamber acid fertilizer acid
78–80%	1.70–1.73	13.5–14	tower acid Glover acid
98%	1.83	~18	concentrated sulfuric acid

"Chamber acid" and "tower acid" were the two concentrations of sulfuric acid produced by the lead chamber process, chamber acid being the acid produced in lead chamber itself (<70% to avoid contamination with nitrosylsulfuric acid) and tower acid being the acid recovered from the bottom of the Glover tower. They are now obsolete as commercial concentrations of sulfuric acid, although they may be prepared in the laboratory from concentrated sulfuric acid if needed. In particular, "10M" sulfuric acid (the modern equivalent of chamber acid, used in many titrations) is prepared by slowly adding 98% sulfuric acid to an equal volume of water, with good stirring: the temperature of the mixture can rise to 80 °C (176 °F) or higher. When high concentrations of SO_3 gas are added to sulfuric acid, $H_2S_2O_7$, called pyrosulfuric acid, fuming sulfuric acid or oleum or, less commonly, Nordhausen acid, is formed. Concentrations of oleum are either expressed in terms of % SO_3 (called % oleum) or as % H_2SO_4 (the amount made if H_2O were added); common concentrations are 40% oleum (109% H_2SO_4) and 65% oleum (114.6% H_2SO_4). Pure $H_2S_2O_7$ is a solid with melting point 36 °C. Pure sulfuric acid is a viscous clear liquid, like oil, and this explains the old name of the acid ('oil of vitriol').

Commercial sulfuric acid is sold in several different purity grades. Technical grade H2SO4 is impure and often colored, but is suitable for making fertilizer. Pure grades such as United States Pharmacopeia (USP) grade are used for making pharmaceuticals and dyestuffs. Analytical grades are also available.

2.8.3 Applications of Sulfuric acid

Sulfuric acid is a very important commodity chemical, and indeed, a nation's sulfuric acid production is a good indicator of its industrial strength. World production in 2004 was about 180 million tons, with the following geographic distribution: Asia 35%, North America (including Mexico) 24%, Africa 11%, Western Europe 10%, Eastern Europe and Russia 10%, Australia and Oceania 7%, South America 7%.Most of this amount (~60%) is consumed for fertilizers, particularly superphosphates, ammonium phosphate and ammonium sulfates. About 20% is used in chemical industry for production of detergents, synthetic resins, dyestuffs, pharmaceuticals, petroleum catalysts, insecticides and antifreeze, as well as in various processes such as oil well acidicizing, aluminium reduction, paper sizing, water treatment. About 6% of uses are related to pigments and include paints, enamels, printing inks, coated fabrics and paper, and the rest is dispersed into a multitude of applications such as production of explosives, cellophane, acetate and viscose textiles, lubricants, non-ferrous metals and batteries.

Industrial production of chemicals. The major use for sulfuric acid is in the "wet method" for the production of phosphoric acid, used for manufacture of phosphate fertilizers. In this method, phosphate rock is used, and more than 100 million tons are processed annually. This raw material is shown below as fluorapatite, though the exact composition may vary. This is treated with 93% sulfuric acid to produce calcium sulfate, hydrogen fluoride (HF) and phosphoric acid. The HF is removed as hydrofluoric acid. The overall process can be represented as:

$$Ca_5F(PO_4)_3 + 5\ H_2SO_4 + 10\ H_2O \longrightarrow 5\ CaSO_4{\cdot}2\ H_2O + HF + 3\ H_3PO_4$$

Ammonium sulfate, an important nitrogen fertilizer, is most commonly produced as a byproduct from coking plants supplying the iron and steel making plants. Reacting the ammonia produced in the thermal decomposition of coal with waste sulfuric acid allows the ammonia to be crystallized out as a salt (often brown because of iron contamination) and sold into the agro-chemicals industry. Another important use for sulfuric acid is for the manufacture of aluminium sulfate, also known as paper maker's alum. This can react with small amounts of soap on paper pulp fibers to give gelatinous aluminium carboxylates, which help to coagulate the pulp fibers into a hard paper surface. It is also used for making aluminium hydroxide, which is used at water treatment plants to filter out impurities, as well as to improve the taste of the water. Aluminium sulfate is made by reacting bauxite with sulfuric acid:

$$Al_2O_3 + 3\ H_2SO_4 \longrightarrow Al_2(SO_4)_3 + 3\ H_2O$$

Sulfuric acid is also important in the manufacture of dyestuffs solutions.

Sulfur-iodine cycle. The sulfur-iodine cycle is a series of thermo-chemical processes used to obtain hydrogen. It consists of three chemical reactions whose net reactant is water and whose net products are hydrogen and oxygen.

$$2\ H_2SO_4 \longrightarrow 2\ SO_2 + 2\ H_2O + O_2 \qquad (830\ ^\circ C)$$

$$I_2 + SO_2 + 2\ H_2O \longrightarrow 2\ HI + H_2SO_4 \qquad (120\ ^\circ C)$$

$$2\ HI \longrightarrow I_2 + H_2 \qquad (320\ ^\circ C)$$

The sulfur and iodine compounds are recovered and reused, hence the consideration of the process as a cycle. This process is endothermic and must occur at high temperatures, so energy in the form of heat has to be supplied. The sulfur-iodine cycle has been proposed as a way to supply hydrogen for a hydrogen-based economy. It does not require hydrocarbons like current methods of steam reforming. But note that all of the available energy in the hydrogen so produced is supplied by the heat used to make it. The sulfur-iodine cycle is currently being researched as a feasible method of obtaining hydrogen, but the concentrated, corrosive acid at high temperatures poses currently insurmountable safety hazards if the process were built on a large scale.

Cleaning agent. Sulfuric acid is used in large quantities by the iron and steelmaking industry to remove oxidation, rust and scaling from rolled sheet and billets prior to sale to the automobile and major appliances industry. Used acid is often recycled using a spent acid regeneration (SAR) plant. These plants combust spent acid with natural gas, refinery gas, fuel oil or other fuel sources. This combustion process produces gaseous sulfur dioxide (SO_2) and sulfur trioxide (SO_3) which are then used to manufacture "new" sulfuric acid. SAR plants are common additions to metal smelting plants, oil refineries, and other industries where sulfuric acid is consumed in bulk, as operating a SAR plant is much cheaper than the recurring costs of spent acid disposal and new acid purchases.

Catalyst. Sulfuric acid is used for a variety of other purposes in the chemical industry. For example, it is the usual acid catalyst for the conversion of cyclohexanone oxime to caprolactam, used for making nylon. It is used for making hydrochloric acid from salt via the Mannheim process. Much H_2SO_4 is used in petroleum refining, for example as a catalyst for the reaction of isobutane with isobutylene to give isooctane, a compound that raises the octane rating of gasoline (petrol).

Electrolyte. Sulfuric acid acts as the electrolyte in lead-acid (car) batteries (lead-acid accumulator):

At anode: $Pb + SO_4^{2-} \rightleftharpoons PbSO_4 + 2\ e-$

At cathode: $PbO_2 + 4\ H+ + SO_4^{2-} + 2\ e- \rightleftharpoons PbSO_4 + 2\ H_2O$

Overall: $Pb + PbO_2 + 4\ H+ + 2\ SO_4^{2-} \rightleftharpoons 2\ PbSO_4 + 2\ H_2O$

Domestic uses. Acidic drain cleaners usually contain concentrated sulfuric acid which turns a pH paper red and chars it instantly, demonstrating the strong acidic nature and dehydrating property.Sulfuric acid is hygroscopic which readily absorbs atmospheric water vapour and hence it is usually used as a drying agent. Besides, it is used as a general dehydrating agent in its concentrated form due to its strong dehydrating property. Concentrated sulfuric acid is frequently the major ingredient in acidic drain cleaners which are used to remove grease, hair, tissue paper, etc. But they are advised to be used by licensed plumbers because of safety considerations.

2.9 Ionic liquid (1-ethyl-3-methyl imidazolium chloride) [13-15]

The increased interest in ionic liquids by chemists and technologists clearly is due to the utility of ionic liquids as solvents for reaction chemistry, including catalytic reactions. The desire for "green" solvents for industrial processes is partially responsible for this, but also many chemists now realize that ionic liquids offer some unique properties as solvents. Furthermore, ionic liquid solvents have the prospect for custom design of the solvent to meet specific requirements for a particular reaction type. Some barriers to the use of ionic liquids are the bewildering variety of ionic liquids reported in the literature, and the lack of a handbook of these new solvents.

1-Ethyl-3-methylimidazolium chloride or [EMIM]Cl is an ionic liquid that can be used in cellulose processing. The cation consists of a five-membered ring with two nitrogen and three carbon atoms, i.e. a derivative of imidazole, with ethyl and methyl groups substituted at the two nitrogen atoms. Its melting point is 77-79 °C

Figure 2.8 The chemical structure of 1-ethyl-3-methylimidazolium chloride

2.10 3,5 – dinitrosalicylic acid (DNS) [16-17]

3,5-Dinitrosalicylic acid (DNS or DNSA, IUPAC name 2-hydroxy-3,5-dinitrobenzoic acid) is an aromatic compound that reacts with reducing sugars and other reducing molecules to form 3-amino-5-nitrosalicylic acid, which absorbs light strongly at 540 nm. It was first introduced as a method to detect reducing substances in urine and has since been widely used, for example, for quantificating carbohydrates levels in blood. It is mainly used in assay of alpha-amylase. However, enzymatic methods are usually preferred to DNS due to their specificity.

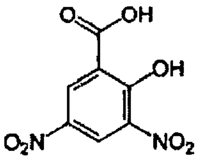


Figure 2.9 Structure of DNS

DNS method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars. This involves the oxidation of the aldehyde functional group present in, for example, glucose and the ketone functional group in fructose. Simultaneously, 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino,5-nitrosalicylic acid under alkaline conditions:

$$\text{aldehyde group} \xrightarrow{\text{oxidation}} \text{carboxyl group}$$

$$3,5-\text{dinitrosalicylic acid} \xrightarrow{\text{reduction}} 3-\text{amino}-5-\text{nitrosalicylic acid}$$

The above reaction scheme shows that one mole of sugar will react with one mole of 3,5-dinitrosalicylic acid. However, it is suspected that there are many side reactions, and the actual reaction stoichiometry is more complicated than that previously described. The type of side reaction depends on the exact nature of the reducing sugars. Different reducing sugars generally yield different color intensities; thus, it is necessary to calibrate for each sugar. In addition to the oxidation of the carbonyl groups in the sugar, other side reactions such as the decomposition of sugar also competes for the availability of 3,5-dinitrosalicylic acid. As a consequence, carboxymethyl cellulose can affect the calibration curve by enhancing the intensity of the developed color.

Although this is a convenient and relatively inexpensive method, due to the relatively low specificity, one must run blanks diligently if the colorimetric results are to be interpreted correctly and accurately. One can determine the background absorption on the original cellulose substrate solution by adding cellulase, immediately stopping the reaction, and measuring the absorbance, i.e. following exactly the same procedures for the actual samples. When the effects of extraneous compounds are not known, one can effectively include a so-called internal standard by first fully developing the color for the unknown sample; then, a known amount of sugar is added to this sample.

The increase in the absorbance upon the second color development is equivalent to the incremental amount of sugar added.

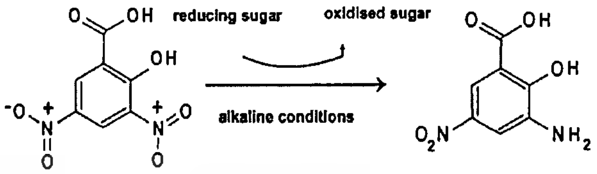


Figure 2.10 Reaction of DNS with reducing sugar which gives 3-amino-5-nitrosalicylic acid as a product

2.11 Literature reviews [3, 18-23]

Ye Sun and Jiayang Cheng (2001) : Lignocellulosic biomass can be utilized to produce ethanol, a promising alternative energy source for the limited crude oil. There are mainly two processes involved in the conversion: hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugars, and fermentation of the sugars to ethanol. The cost of ethanol production from lignocellulosic materials is relatively high based on current technologies, and the main challenges are the low yield and high cost of the hydrolysis process. Considerable research efforts have been made to improve the hydrolysis of lignocellulosic materials. Pretreatment of lignocellulosic materials to remove lignin and hemicellulose can significantly enhance the hydrolysis of cellulose. Optimization of the cellulase enzymes and the enzyme loading can also improve the hydrolysis. Simultaneous saccharification and fermentation effectively removes glucose, which is an inhibitor to cellulase activity, thus increasing the yield and rate of cellulose hydrolysis.

Nathan Mosier, Charles Wyman, Bruce Dale, Richard Elander, Y.Y. Lee, Mark Holtzapple, and Michael Ladisch (2004) : Cellulosic plant material represents an as-of-yet untapped source of fermentable sugars for significant industrial use. Many physio-chemical structural and compositional factors hinder the enzymatic digestibility of cellulose present in lignocellulosic biomass. The goal of any pretreatment technology is to alter or remove structural and compositional impediments to hydrolysis in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose. These methods cause physical and/or chemical changes in the plant biomass in order to achieve this result. Experimental investigation of physical changes and chemical reactions that occur during pretreatment is required for the development of effective and mechanistic models that can be used for the rational design of pretreatment processes. Furthermore, pretreatment processing conditions must be tailored to the specific chemical and structural composition of the various, and variable, sources of lignocellulosic biomass.

Hisashi Miyafuji, Kenji Miyata, Shiro Saka, Fumio Ueda, and Midori Mori (2009) : Reaction of Japanese beech (Fagus crenata) in an ionic liquid, 1-ethyl-3-methylimidazolium chloride ([C2mim][Cl]), which can dissolve cellulose, was investigated. Although both lignin and polysaccharides such as cellulose and hemicelluloses can be liquefied at a treatment temperature of around 100°C, the liquefaction of polysaccharides mainly occurs at the beginning of the treatment with [C2mim][Cl]. Cellulose crystallinity in the wood was gradually broken down as the treatment continued. The solubilized polymers were depolymerized to low molecular weight compounds. The results indicate that [C2mim][Cl] is an effective solvent and reagent for the liquefaction of wood components and subsequent depolymerization of them.

Joseph B. Binder and Ronald T. Raines (2010) : Abundant plant biomass has the potential to become a sustainable source of fuels and chemicals. Realizing this potential requires the economical conversion of recalcitrant lignocellulose into useful intermediates, such as sugars. A high-yielding chemical process for the hydrolysis of biomass into monosaccharides is reported. Adding water gradually to a chloride ionic liquid-containing catalytic acid leads to a nearly 90% yield of glucose from cellulose and 70–80% yield of sugars from untreated corn stover. Ion-exclusion chromatography allows recovery of the ionic liquid and delivers sugar feedstocks that support the vigorous growth of ethanologenic microbes. This simple chemical process, which requires neither an edible plant nor a cellulase, could enable crude biomass to be the sole source of carbon for a scalable biorefinery.

Changzhi Li, Qian Wang and Zongbao K. Zhao (2008) : Acid in ionic liquid was demonstrated as an efficient system for hydrolysis of lignocellulosic materials with improved total reducing sugars (TRS) yield under mild conditions. TRS yields were up to 66%, 74%, 81% and 68% for hydrolysis of corn stalk, rice straw, pine wood and bagasse, respectively, in C_4mimCl in the presence of 7 wt% hydrogen chloride at 100 °C under atmospheric pressure within 60 min. Different combinations between ionic liquids, such as C_6mimCl, C_4mimBr, AmimCl, C_4mimHSO$_4$, and

$SbmimHSO_4$, and acids, including sulfuric acid, nitric acid, phosphoric acid, as well as maleic acid, afforded similar results albeit longer reaction time was generally required comparing with the combination of C_4mimCl and hydrochloric acid. FT-IR spectra and elemental analysis of the recovered residues indicated that modification of lignin occurred during sulfuric acid catalyzed hydrolysis. In addition, kinetic modeling based on experimental data suggested that the hydrolysis likely followed a consecutive first-order reaction sequence, where k_1 and k_2, the rate constants for TRS formation and TRS degradation, were determined as 0.068 min^{-1} and 0.007 min^{-1}, respectively. This novel system may be valuable to facilitate cost-efficient conversion of biomass into biofuels and biobased products.

Anjanabha Bhattacharya and Pawan Kumar (2010) : Biomass is the least expensive and most globally available resource. Therefore, priority should be shifted towards utilizing biomass, leaving aside food for human consumption. New methodologies of fermentation and hydrolysis of biomass have become available, along with development of transgenic varieties amenable for biofuel production. Water hyacinth has long been seen as an invasive species all over the globe and considerable amount of resources have been spent for their control. However, they have certain qualities which can be utilized to produce biofuels (both bioethanol to power vehicles and motors, biogas to generate electricity) as the plants are low in lignin content and have rapid growth rate. This could be of potential interest in south-east Asian countries where this species tends to occur so frequently. Besides they do not compete with food crops for land diversification. They tend to grow well in water bodies even with low nutrient regime and can be easily mechanically harvested. There are so many alternative uses of these plant species and time has come to look at the plant from a different viewpoint and utilize their potential as much as possible.

Jun Seok Kim, Y. Y. Lee, Robert W. Torget (2001) : The kinetics of cellulose hydrolysis under extremely low acid (ELA) conditions (0.07 wt%) and at temperatures >200°C was investigated using batch reactors and bed-shrinking flow-through (BSFT) reactors. The maximum yield of glucose obtained from batch reactor experiments was about 60% for α-cellulose, which occurred at 205 and 220°C. The maximum glucose yields from yellow poplar feedstocks were substantially lower, falling in the range of 26–50%. With yellow poplar feedstocks, a large amount of glucose was unaccounted for at the latter phase of the batch reactions. It was found that a substantial amount of released glucose condensed with nonglucosidic substances in liquid. The rate of glucan hydrolysis under ELA was relatively insensitive to temperature in batch experiments for all three substrates. It was implied that additional factors influence the hydrolysis of glucan under ELA. In experiments using BSFT reactors, the glucose yields of 87.5, 90.3, and 90.8% were obtained for yellow poplar feedstocks at 205, 220, and 235°C, respectively. The hydrolysis rate for glucan was about three times higher with the BSFT than with the batch reactors. The difference of observed kinetics and performance data between the BSFT and the batch reactors was far above that predicted by the reactor theory.

Quang A. Nguyen, Melvin P. Tucker, Fred A. Keller, Delicia A. Beaty, Kevin M. Connors, Fannie P. Eddy (1999) : Whole tree chips obtained from softwood forest thinnings were converted to ethanol via a two-stage dilute acid hydrolysis followed by yeast fermentation. The chips were first impregnated with dilute sulfuric acid, then pretreated in a steam explosion reactor to hydrolyze more than 90% of the hemicellulose and approximately 10% of the cellulose. The hydrolysate was filtered and washed with water to recover the sugars. The washed fibers were then subjected to a second acid impregnation and hydrolysis to hydrolyze as much as 45% of the remaining cellulose. The liquors from both hydrolysates were combined and fermented to ethanol by a Saccharomyces cerevisiae yeast that had been adapted to the inhibitors. Based on available hexose sugars, ethanol yields varied from 74 to 89% of theoretical. Oligosaccharide contents higher than about 10% of the total available sugar appear to have a negative impact on ethanol yield.

Chapter 3

Experimental Details

3.1 Apparatus and equipments

3.1.1 UV/VIS spectrophotometer GENESYS 10 s (Thermo scientific)

3.1.2 Hot air oven (Memmert)

3.1.3 Analytical balance 3 decimals (Startiuos)

3.1.4 Shaker (National)

3.1.5 Laboratory glasswares

3.1.6 Filter paper (Whatman 5C)

3.1.7 Spatula

3.1.8 Oil bath (Fisher scientific)

3.1.9 Refrigerator (Sharp)

3.1.10 Desiccator

3.1.11 Vacuum Filter, Funnel, and rubber cork

3.1.12 Hot Plate (IKA basic safety control)

3.1.13 Rubber tube

3.1.14 Rubber bulb

3.1.15 Magnetic bar

3.2 Chemicals

3.2.1 Water hyacinth

3.2.2 Ethylene glycol (A.R. grade CARLO ERBA)

3.2.3 Methanol (A.R. grade LAB SCAN)

3.2.4 Ethanol (A.R. grade CARLO ERBA)

3.2.5 1-ethy-3-methyl imidazodium chloride, 97% (ACROS organics)

3.2.6 Sulfuric acid conc.98% (H_2SO_4, 98%) (A.R. grade)

3.2.7 3, 5-Dinitrosalicylic acid (DNS) (A.R. grade)

3.2.8 Sodium hydroxide (NaOH) (A.R. grade)

3.2.9 Sodium potassium tartrate (Na-K tartrate) (A.R. grade)

3.2.10 Distilled water

3.2.11 Glucose

3.3 Preparation of dried water hyacinth

The water hyacinth was washed with water then cut into small pieces and sundried for 1 day. After that, it was again dried in the oven at 80 °C until the weight became constant. Next, it was grinded by the blender and sieved in the shaker. The sieved water hyacinth with particle size between 150 - 500 μm was collected and stored in a beaker.

Figure 3.1 Sieve shaker

3.4 Hydrolysis without pretreatment

The water hyacinth was hydrolyzed with dilute sulfuric acid (H_2SO_4) solution in the presence of ionic liquid (1-ethyl-3-methyl imidazolium chloride) and ethylene glycol as solvent.

3.4.1 Study of the suitable weight of ionic liquid

1) 0.5 g of water hyacinth was weighed into a 100-ml round bottom flask.
2) 8 ml of ethylene glycol was added into the same flask.
3) 0.5 g of ionic liquid was added into 2 ml of ethanol and drained into the flask.
4) 5 ml of 10% w/w dilute sulfuric acid was added into the flask.

5) The mixture was refluxed for 180 minutes in the oil bath on the temperature controlled hot plate at 120°C.

6) The hydrolyzate was separated from water hyacinth by using vacuum filtration.

7) Glucose concentration in the hydrolyzate was analyzed using DNS method.

8) The remainder of water hyacinth was dried in the hot air oven until the weight became constant and then recorded.

9) The experiment was then repeated by changing weight of ionic liquid from 0.5 g to 0.25 g, 0.75 g and 1.0 g.

3.4.2 Study of the suitable temperature for hydrolysis

The reactions were done by following the procedure explained in section 3.4.1 using 0.5 g of ionic liquid. The hydrolysis temperature was changed from 120°C to 100°C and 140°C.

3.4.3 Study of the suitable concentration of sulfuric acid (H_2SO_4)

Different concentrations of sulfuric acid were prepared via dilution of 98% sulfuric acid by distilled water. The reactions were done by following the procedure explained in section 3.4.1 using the concentration of sulfuric acid from 10% to 15% and 20% w/w.

3.4.4 Study of the suitable time of hydrolysis

The reactions were done by following the procedure explained in section 3.4.1 using 15% w/w sulfuric acid solution. The hydrolysis temperature of 120°C was used. The experiment was then repeated by changing the hydrolysis time from 180 minutes to 30, 60, 90, 120 and 150 minutes.

Figure 3.2 Reflux equipment set up

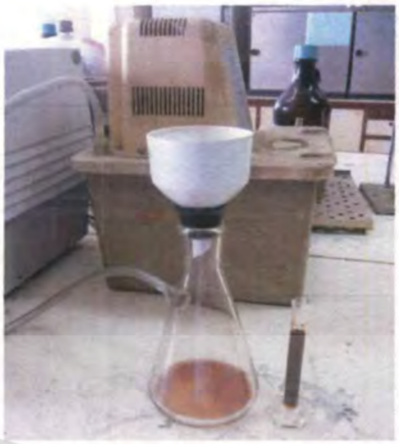


Figure 3.3 Vacuum filtration equipment set up

3.5 Hydrolysis with pretreatment

Pretreatment is the process used prior to hydrolysis with dilute sulfuric acid. To pretreat the water hyacinth, 0.5 g of ionic liquid is firstly dissolved in 2 ml of ethanol, mixed with 8 ml of ethylene glycol and then added to the water hyacinth. The mixture was refluxed for a certain amount of time. After which time, hydrolysis was followed via addition of dilute sulfuric acid into the mixture.

3.5.1 Study of the suitable pretreatment and hydrolysis time

1) 0.5 g of water hyacinth was weighed into a 100-ml round bottom flask.
2) 8 ml of ethylene glycol was added into the same flask.
3) 0.5 g of ionic liquid was added into 2 ml of ethanol and drained into the flask.
4) The mixture of water hyacinth was pretreated by heating in the oil bath on the temperature controlled hot plate for 5 hours at 120°C.
5) After the pretreatment process was finished, 5 ml of 15% w/w sulfuric acid was added into the flask and the mixture was heated for another 30 minutes.
6) The hydrolyzate was separated from water hyacinth by using vacuum filtration.
7) Glucose concentration in the hydrolyzate was analyzed using DNS method
8) The remainder of water hyacinth was dried in the hot air oven until the weight became constant and then recorded.

9) The experiment was then repeated by changing the pretreatment time from 5 hours to 10, 15 and 20 hours and changing the hydrolysis time from 30 minutes to 60, 90, 120, 150 and 180 minutes.

3.6 Determination of glucose concentration using 3,5 – dinitrosalicylic acid (DNS method)

3.6.1 Preparation of 3,5-dinitrosalicylic acid solution (DNS solution) [16]

1) 5 g of 3,5 – dinitrosalicylic acid (DNS) was weighed and dissolved in 125 ml of distilled water.

2) Sodium hydroxide (NaOH) solution was prepared by dissolving 8 g of NaOH in 87.5 ml of distilled water.

3) Sodium hydroxide solution (NaOH) was gradually drained into DNS solution and stirred until it completely dissolved.

4) The solution above was heated in the water bath until it became clear, then 150 g of sodium-potassium tartrate (Na – K tartrate) was gradually added.

5) The final volume was adjusted to be 500 ml and the solution was stored in a brown bottle at room temperature

3.6.2 Analysis of glucose concentration by using 3, 5-dinitrosalicylic acid (DNS) method

1) 5 ml of sample was pipetted into the test tube then 5 ml of DNS solution was added.

2) The solution above was heated in boiling water for 7 minutes then it was put into cooled water for another 7 minutes.

3) 5 ml of distilled water was added and stirred.

4) The concentration of glucose was analyzed by measuring the absorbance using UV spectrophotometer at the wavelength of 540 nm.

5) The absorbance was compared with the standard calibration curve of glucose to obtain the glucose concentration, distilled water was used as a blank.

Figure 3.4 UV spectrophotometer

3.6.3 Preparation of calibration curve of glucose

1) 1 g/L of standard glucose solution was prepared by weighing 0.5 g of glucose and dissolved in 500 ml of distilled water

2) The standard glucose solution was diluted to be 0.2, 0.4, 0.6, 0.8, and 1.0 mg/ml

3) A standard calibration curve of glucose solution was constructed by measuring the absorbance using UV spectrophotometer at the wavelength of 540 nm. The glucose concentration can also be calculated from the equation below;

$$\text{concentration of glucose} = \frac{\text{absorbance at 540 nm} \times \text{dilution factor}}{\text{slope of calibration curve}}$$

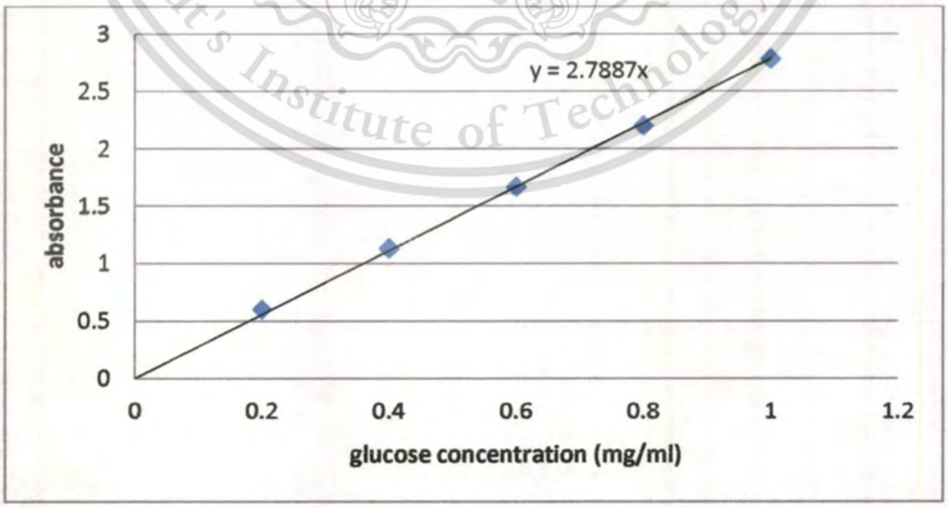


Figure 3.5 Standard calibration curve for determination of glucose concentration

Chapter 4

Results and Discussion

4.1 Study on the effect of ionic liquid on hydrolysis of water hyacinth

The hydrolysis of water hyacinth with 10% w/w of dilute sulfuric acid in the absence and presence of ionic liquid, 1-ethyl-3-methyl imidazolium chloride, was studied. Primarily, 0.5 g of ionic liquid was used together with 2 ml of ethanol as co-solvent. They were poured into 0.5 g of water hyacinth in the rounded bottom flask which contain 8 ml of ethylene glycol, another solvent. The reaction mixture was then refluxed for 4 hours at 120 °C. After the removal of solvents by vacuum filtration, the concentration of glucose in the hydrolyzate was determined using DNS method. Another batch was also done under the same condition except no ionic liquid was added. The results are shown below in Table 4.1

Table 4.1 Glucose concentration from hydrolysis of 0.5 g of water hyacinth using 10% w/w H_2SO_4 with and without 1-ethyl-3-methyl imidazolium chloride at 120 °C for 4 hours

Hydrolysis time (min)	Quantity of 1-ethyl-3-methyl imidazolium chloride (g)	Glucose concentration (mg/ml)
240	0	0.36
240	0.5	0.88

It is noted that the glucose concentrations obtained from the experiments were too high to be used with the standard calibration curve so the inaccuracy might occur. The dark color and high concentration of samples affect the absorbance significantly and hence the error in the measurement using UV spectrophotometer. However, the trend of each experiment is still the same and can be used to observe the effect of variables on hydrolysis.

The value reveals that hydrolysis in the presence of ionic liquid help increasing the glucose concentration significantly. The reason is that the ionic liquid, 1-ethyl-3-methyl imidazolium chloride, used here has the ability to liquefy cellulose by disrupting its hydrogen bonding network and therefore making the cellulose more accessible to hydrolysis and will give out more glucose as the product. This result is consistent with the research of Miyafuji et.al. where Japanese beech wood flour was reacted with 1-ethyl-3-methyl imidazolium chloride. It was found that the residue yield was decreased as the treatment time and temperature increased due to the dissolution of cellulose and hemicellulose into the hydrolyzate.

4.2 Determination of the optimum weight of ionic liquid for hydrolysis

From the previous experiments, it was confirmed that a small amount of 1-ethyl-3-methyl imidazolium chloride was indeed able to boost up the glucose concentration from hydrolysis of water hyacinth. The determination of its optimum weight for hydrolysis was then attempted using 0.25 g, 0.5 g, 0.75 g and 1.0 g of 1-ethyl-3-methyl imidazolium chloride which equals to the weight ratio of 1-ethyl-3-methyl imidazolium chloride to water hyacinth of 1:2, 1:1, 3:2 and 2:1 , respectively. The hydrolysis reactions were carried out under the same condition as described in section 4.1 for 3 hours. The results are shown in Figure 4.1 and listed in Table 4.2.

Table 4.2 Glucose concentration from hydrolysis of 0.5 g of water hyacinth with different weight ratio of 1-ethyl-3-methyl imidazolium chloride to water hyacinth at 120 °C for 3 hours

Weight of ionic liquid (g)	Weight ratio of ionic liquid to water hyacinth	Glucose concentration (mg/ml)
0.25	1 : 2	0.70
0.5	1 : 1	1.53
0.75	3 : 2	1.60
1.0	2 : 1	1.51

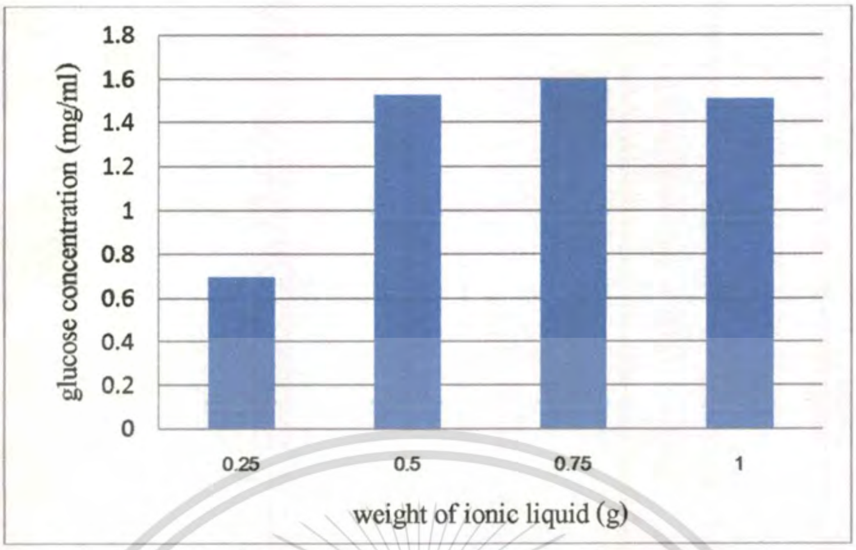


Figure 4.1 Glucose concentration from hydrolysis of 0.5 g of water hyacinth with different amount of 1-ethyl-3-methyl imidazolium chloride at 120 °C for 3 hours

As expected, the higher amount of ionic liquid will promote the reaction and produce more glucose because the ionic liquid has the ability to liquefy cellulose by disruption its hydrogen bonding network, hence easier hydrolysis of the water hyacinth. However, when the amount of the ionic liquid has reached over 0.5 g, the yield of glucose is insignificantly changed as glucose would also consecutively degraded to other undesired products. According to the research of Binder and Raines on cellulose reactivity in 1-ethyl-3-methyl imidazolium chloride, glucose may be further dehydrated to form 5-hydroxymethylfurfural (HMF) and furfural in 1-ethyl-3-methyl imidazolium chloride and sulfuric acid. Therefore, 0.5 g of 1-ethyl-3-methyl imidazolium chloride was chosen as the optimum amount for the following studies.

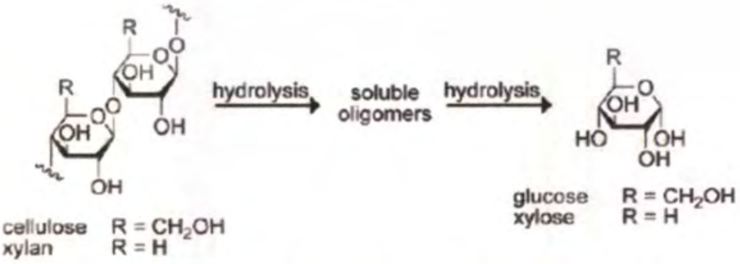


Figure 4.2 Hydrolysis reaction of water hyacinth to glucose

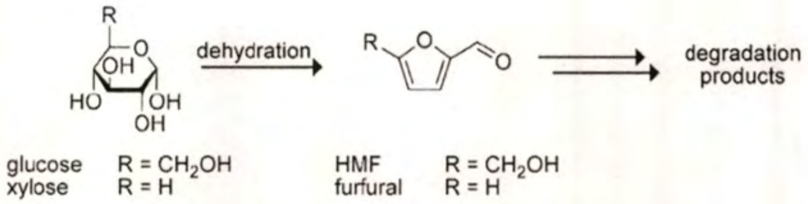


Figure 4.3 Degradation reaction of glucose to 5-hydroxymethylfurfural (HMF) and furfural which are undesired products

4.3 Determination of the optimum temperature for hydrolysis

From section 4.2, the optimum weight of 1-ethyl-3-methyl imidazolium chloride was found to be 0.5 g, and it was used to determine the optimum temperature for hydrolysis of water hyacinth. The various temperatures which were 100°C, 120°C, and 140°C were attempted. The reaction mixture was refluxed for 2 hours in 15% w/w dilute sulfuric acid. The results are shown in Table 4.3 and Figure 4.4 below.

Table 4.3 Glucose concentration from hydrolysis of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at various temperatures for 2 hours

Hydrolysis temperature (°C)	Glucose concentration (mg/ml)
100	1.42
120	1.68
140	1.62

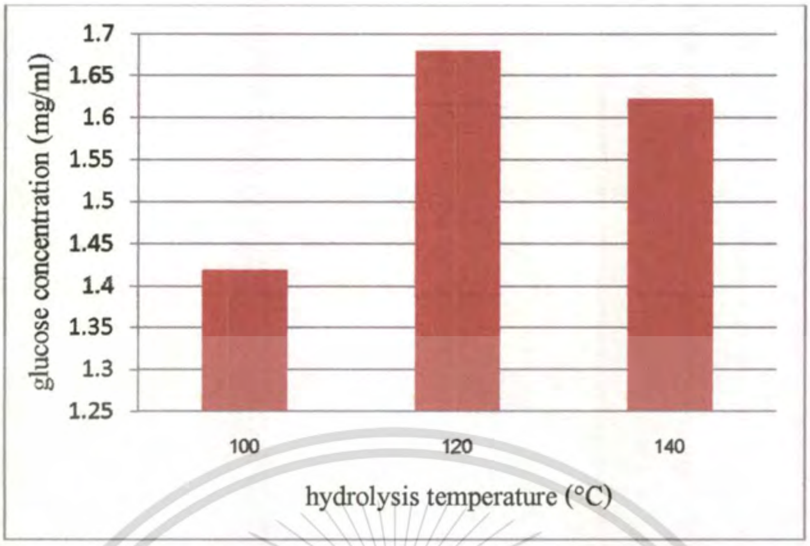


Figure 4.4 Glucose concentration from hydrolysis of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at various temperatures for 2 hours

The optimum temperature that gave the highest glucose concentration was at 120°C. At 100°C, slower reaction rate was to be expected. However, at 140°C, the glucose yield was slightly dropped. This is probable because, not only the rate of desirable reaction increase, but that of undesired reaction also increase to yield undesired products from glucose degradation.

4.4 Determination of optimum acid concentration and hydrolysis time

The optimum weight of ionic liquid and temperature obtained from section 4.2 and 4.3 were used in this section. Water hyacinth was hydrolyzed with various acid concentration and hydrolysis time. The sulfuric acid concentration was varied from 10 to 15 and 20 % w/w. The hydrolysis time was varied from 30 to 60, 90, 120, 150 and 180 minutes. The results are shown below in Table 4.4 and illustrated in Figure 4.5.

Table 4.4 Glucose concentration from hydrolysis of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at 120°C using various hydrolysis time and H_2SO_4 concentration

Hydrolysis time (min)	Glucose concentration (mg/ml)		
	10% w/w H_2SO_4	15% w/w H_2SO_4	20% w/w H_2SO_4
30	-	1.17	1.05
60	-	1.38	1.19
90	0.67	1.22	1.60
120	1.37	1.68	0.75
150	1.16	1.44	1.01
180	1.04	1.53	1.04

Figure 4.5 Glucose concentration from hydrolysis of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at 120°C using various hydrolysis time and H_2SO_4 concentration

From Figure 4.5, each acid concentration shows the similar trend that the glucose concentration increases as time increases. However, after some point, the glucose concentration was dropped presumably because the obtained glucose further degraded to form the undesired products. When 20% w/w H_2SO_4 solution was used, the optimum point was reached faster than the others. This is because the acid plays a dual role in biomass hydrolysis. By disrupting its network of intra- and interchain hydrogen bonds, acid decrystallizes cellulose and makes it accessible to hydrolysis, and by catalyzing the hydrolysis of glycosidic bonds, acids cleave cellulose into glucose. Even though the reaction with 20% w/w H_2SO_4 is faster and give higher glucose concentration at less time but the glucose obtained will also convert to the undesired product at a faster rate. For the effect of hydrolysis time, as it get longer the glucose concentration also get higher because the water hyacinth have more time to react with H_2SO_4 and 1-ethyl-3-methyl imidazolium chloride; however, the degradation of glucose will also occur if the time is too long. The condition that gives the highest glucose concentration is at 120 minutes with 15% w/w H_2SO_4 solution. The variance of the results might as well occur because of the small scale experiments.

4.5 Study on the effect of pretreatment prior to hydrolysis

The effect of pretreatment of water hyacinth was studied by heating the water hyacinth with ionic liquid and ethylene glycol for 5, 10, 15 and 20 hours prior to hydrolysis. After which time, a 15% w/w H_2SO_4 solution was then added for hydrolysis at 120 °C for 30, 60, 90 and 120 minutes. The results are given in Table 4.5 and illustrated in Figure 4.6.

Table 4.5 Glucose concentration at various pretreatment and hydrolysis time of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at 120°C

Hydrolysis time (min)	Glucose concentration at various condition (mg/ml)				
	No pretreatment	Pretreat for 5 hours	Pretreat for 10 hours	Pretreat for 15 hours	Pretreat for 20 hours
30	1.17	0.88	1.28	1.20	1.01
60	1.38	1.12	0.90	1.62	1.03
90	1.22	1.08	0.89	1.38	1.07
120	1.68	0.62	1.38	1.27	1.36

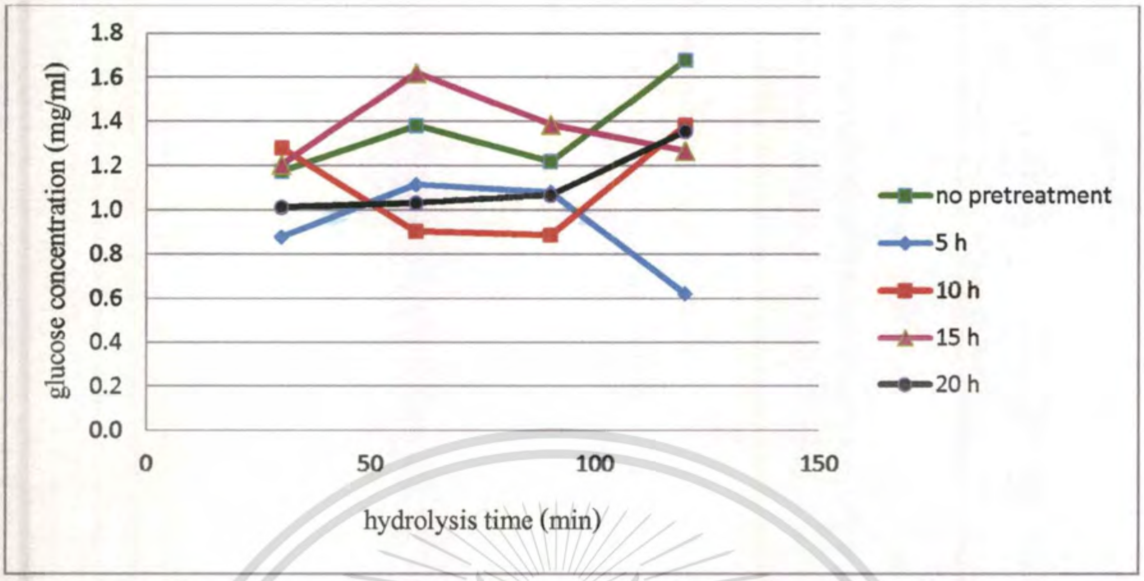


Figure 4.6 Glucose concentration at various pretreatment and hydrolysis time of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at 120°C

Among the pretreatment condition, the duration of 15 hours gave higher concentration of glucose than the others. However, it was still less than that of no pretreatment. The highest glucose concentration was obtained when pretreat the water hyacinth for 15 hours followed by 60 minutes of hydrolysis whereas the highest one for non-pretreatment is at 120 minutes of hydrolysis. From these results, pretreatment of the water hyacinth prior to hydrolysis would help reducing the hydrolysis time for 50% since the pretreatment would make the cellulose more accessible to hydrolysis. When comparing the results between the process having and not having the pretreatment, the acquired glucose concentrations did not differ significantly probably because of the small scale experiments.

Chapter 5

Conclusion and Suggestions

5.1 Conclusion

From the study on the effect of ionic liquid on hydrolysis of water hyacinth, it was found that hydrolysis in the presence of ionic liquid which was 1-ethyl-3-methyl imidazolium chloride at 120 °C helped increasing the glucose concentration significantly. The weight of 1-ethyl-3-methyl imidazolium chloride, temperature, sulfuric acid concentration and hydrolysis time were studied to determine the optimum condition that gave the highest glucose concentration. It was found that 0.5 g of 1-ethyl-3-methyl imidazolium chloride, which equal to the weight ratio of ionic liquid to water hyacinth of 1:1, with hydrolysis temperature of 120 °C, 15% w/w H_2SO_4 and hydrolysis time of 120 minutes were the optimum condition which gave the highest concentration of glucose. In addition, the pretreatment of the water hyacinth prior to hydrolysis was also carried out. It was found that 15 hours pretreatment helped reducing the hydrolysis time for 50%. However, the concentration of glucose obtained did not differ significantly from to the process without having the pretreatment and it also consumed a lot more of energy.

5.2 Suggestions

For measurement of the glucose concentration using DNS method, the standard calibration curve would give the accurate result in the narrow range; therefore, the sample should be diluted before the measurement to obtain the most precise value. In addition, the sample with dark color might cause inaccuracy in the measurement because of the interference in the sample, such as lignin. The measurement using UV spectrophotometer is also sensitive to the light which cause the absorbance to be varied so another method should be used together with DNS method to obtain more accurate result.

This research was done with simple laboratory equipments and using only small amount of ionic liquid as co-solvent with ethylene glycol and ethanol. This might be the reason why the glucose concentration obtained from this research was less than those from previous researches whereas almost all of them used a large amount of ionic liquid when compare to the amount of biomass. This cannot be done commercially due to the expensive cost of ionic liquid. There are also other ways to improve the yield of glucose, for example, the use of the remaining solid from the first hydrolysis for the second hydrolysis or the gradual addition of water during hydrolysis which have been reported to improve the glucose yield. Another important aspect that should also be considered is the recovery of ionic liquid.

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Appendices

Appendix A

Physical properties

Table A.1 Physical properties of sulfuric acid

Molecular formula	H_2SO_4
Molar mass	98.079 g/mol
Appearance	Clear, colorless, odorless liquid
Density	1.84 g/cm^3, liquid
Melting point	10 °C, 283 K, 50 °F
Boiling point	337 °C, 610 K, 639 °F
Solubility in water	miscible
Acidity (pK_a)	- 3, 1.99
Viscosity	26.7 cP (20 °C)

Table A.2 Physical properties of ethylene glycol

Molecular formula	$C_2H_6O_2$
Molar mass	62.07 g mol^{-1}
Density	1.1132 g/cm^3
Melting point	-12.9 °C, 260 K, 9 °F
Boiling point	197.3 °C, 470 K, 387 °F
Solubility in water	Miscible with water in all proportions.
Viscosity	1.61×10^{-2} N•s / m^2

Table A.3 Physical properties of 1-ethyl-3-methyl imidazolium chloride

Molecular formula	$C_6H_{11}ClN_2$
Molar mass	146.62 g mol^{-1}
Melting point	77-79 °C
Flash point	186 °C
Sensitivity	Hygroscopic

Table A.4 Physical properties of ethanol

Molecular formula	C_2H_6O
Molar mass	46.07 g mol^{-1}
Appearance	Colorless liquid
Density	0.789 g/cm^3
Melting point	-114 °C, 159 K, -173 °F
Boiling point	78.37 °C, 352 K, 173 °F
Acidity (pK_a)	15.9
Basicity (pK_b)	-1.9
Refractive index (n_D)	1.36
Viscosity	0.0012 Pa•s (at 20 °C)

Table A.5 Common Water Hyacinth (*Eichhornia crassipes*)

Kingdom	Plantae
Order	Commelinales
Family	Pontederiaceae
Genus	Eichhornia
Species	E. crassipes

Appendix B

Experimental results

Table B.1 Results from hydrolysis of 0.5 g of water hyacinth with different amount of 1-ethyl-3-methyl imidazolium chloride at 120 °C for 3 hours

Weight of ionic liquid (g)	Glucose concentration (mg/ml)	Volume of hydrolyzate (ml)	Quantity of glucose per 500 mg of water hyacinth (mg)	% Yield of glucose
0.25	0.70	10.2	7.14	1.43
0.5	1.53	11.8	18.054	3.61
0.75	1.60	10.7	17.12	3.42
1	1.51	10.3	15.553	3.11

$$\%\ \text{yield of glucose} = \frac{\text{glucose concentration (mg/ml)} \times \text{volume of hydrolyzate (ml)}}{\text{initial weight of water hyacinth (mg)}} \times 100$$

Table B.2 Results from hydrolysis of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at various temperatures for 2 hours

Hydrolysis temperature (°C)	Glucose concentration (mg/ml)	Volume of hydrolyzate (ml)	Quantity of glucose per 500 mg of water hyacinth (mg)	% Yield of glucose
100	1.42	10.6	15.052	3.01
120	1.68	11.3	18.984	3.80
140	1.62	10	16.20	3.24

Table B.3 Results from hydrolysis of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at 120°C using various hydrolysis time and H_2SO_4 concentration

Acid concentration	Hydrolysis time (min)	Glucose concentration (mg/ml)	Volume of hydrolyzate (ml)	Quantity of glucose per 500 mg of water hyacinth (mg)	% Yield of glucose
10% w/w H_2SO_4	30	-	-	-	-
	60	-	-	-	-
	90	0.67	9.6	6.441	1.29
	120	1.37	10.6	14.543	2.91
	150	1.16	10.4	12.083	2.42
	180	1.04	10.6	11.015	2.20
15 w/w H_2SO_4	30	1.17	11.2	13.153	2.63
	60	1.38	10.6	14.626	2.93
	90	1.22	9.2	11.210	2.24
	120	1.68	11.3	18.980	3.80
	150	1.44	10.3	14.881	2.98
	180	1.53	11.8	18.017	3.60
20 w/w H_2SO_4	30	1.05	9.4	9.886	1.98
	60	1.19	10.6	12.570	2.51
	90	1.60	11.4	18.199	3.64
	120	0.75	10.5	7.847	1.57
	150	1.01	10.5	10.599	2.12
	180	1.04	9.2	9.590	1.92

Table B.4 Results from various pretreatment and hydrolysis time of 0.5 g of water hyacinth in 0.5 g of 1-ethyl-3-methyl imidazolium chloride at 120°C

Pretreatment time (h)	Hydrolysis time (min)	Glucose concentration (mg/ml)	Volume of hydrolyzate (ml)	Quantity of glucose per 500 mg of water hyacinth (mg)	% Yield of glucose
0	30	1.17	11.2	13.153	2.63
	60	1.38	10.6	14.626	2.93
	90	1.22	9.2	11.210	2.24
	120	1.68	11.3	18.980	3.80
5	30	0.88	11	9.672	1.93
	60	1.12	9.3	10.382	2.08
	90	1.08	6.8	7.349	1.47
	120	0.62	10.1	6.305	1.26
10	30	1.28	10.6	13.562	2.71
	60	0.90	9.8	8.863	1.77
	90	0.89	8.2	7.286	1.46
	120	1.38	8.2	11.332	2.27
15	30	1.20	10.4	12.523	2.50
	60	1.62	10.8	17.509	3.50
	90	1.38	7.7	10.663	2.13
	120	1.27	10.4	13.172	2.63
20	30	1.01	10.8	10.943	2.19
	60	1.03	10	10.324	2.06
	90	1.07	11.2	11.952	2.39
	120	1.36	9.9	13.423	2.68