HYDROTHERMAL TREATMENT OF WATER HYACINTH LEAVES FOR GLUCOSE PRODUCTION

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ผักตบชวาเป็นพืชน้ำล้มลุกที่ขยายพันธุ์ได้รวดเร็วสามารถพบได้ง่ายตามคลองและแม่น้ำต่างๆในประเทศไทย การแพร่ขยายอย่างรวดเร็วของผักตบชวาส่งผลกระทบต่อการคมนาคมทางน้ำ ทำให้น้ำเน่าเสีย โดยเฉพาะอย่างยิ่งปริมาณผักตบชวาที่มีมากในแม่น้ำลำคลองไปขวางกั้นประตูระบายน้ำ ทำให้การระบายน้ำจากการเกิดอุทกภัยเมื่อปลายปี พ.ศ.2554 ทำได้ล่าช้า เนื่องจากสาเหตุดังกล่าว ผักตบชวาจึงเป็นอีกหนึ่งทางเลือกที่น่าสนใจที่จะนำมาผลิตเป็นพลังงานทดแทน เช่น เอทานอล จากชีวมวล

ในงานวิจัยนี้จึงนำผักตบชวามาเป็นสารตั้งต้นเพื่อผลิตเป็นน้ำตาลกลูโคสโดยนำมาผ่านกระบวนการไฮโดรเทอร์มอลพรีทรีตเมนต์ที่อุณหภูมิช่วง 160-220 องศาเซลเซียส หลังจากนั้นนำตัวอย่างมาผ่านกระบวนการย่อยด้วยเอนไซม์ โดยเอนไซม์ที่ใช้คือเอนไซม์เซลลูเลสเพื่อนำมาย่อยเซลลูโลส ซึ่งปัจจัยที่จะศึกษาเพื่อหาความเหมาะสมของกระบวนการไฮโดรเทอร์มอลทรีตเมนต์คือ อุณหภูมิ, เวลาในการทำปฏิกิริยา และความเข้มข้นของตัวเร่งปฏิกิริยา ซึ่งในที่นี้คือ กรดน้ำส้มสายชูเพื่อพัฒนาปริมาณกลูโคสที่ได้จากกระบวนการไฮโดรเทอร์มอลทรีตเมนต์ ผลิตภัณฑ์ของเหลวจะนำมาวิเคราะห์ปริมาณกลูโคสโดยใช้เครื่อง HPLC และผลิตภัณฑ์ของแข็งนอกจะนำมาวิเคราะห์ปริมาณเซลลูโลส เฮมิเซลลูโลสและลิกนินโดยใช้วิธี United States Department of Agriculture (USDA) จากผลการทดลองพบว่าปริมาณกลูโคสสูงสุดที่ได้จากการทดลองเมื่อเติมกรดน้ำส้มสายชูมีปริมาณมากกว่าปริมาณกลูโคสที่ได้จากเมื่อไม่ได้ใส่กรดน้ำส้มสายชู โดยปริมาณกลูโคสที่ได้สูงสุดคือ 85.5% จากการเติมกรดน้ำส้มสายชูที่ความเข้มข้น 0.75wt% ที่อุณหภูมิ 200°C ซึ่งปริมาณกลูโคสจากที่ไม่เติมตัวทำละลายได้ 26.7% ที่อุณหภูมิ 220°C นอกเหนือจากนั้นได้ศึกษากลไกการเกิดปฏิกิริยาพื้นฐานของเซลลูโลส โดยคำนวณเป็นค่าคงที่ของปฏิกิริยา (k) จากกฎการหาค่าคงที่ของปฏิกิริยาจากกฎของอาร์เรเนียส โดยใช้ปฏิกิริยาอันดับหนึ่งในการอธิบายการเกิดปฏิริยาของเซลลูโลสเปลี่ยนไปเป็นกลูโคส

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Water hyacinth available excessively in various water resources can affect ecological system, leading to many public problems. Especially, excessive of water hyacinth bundles could block the floodway leading to the historical flooding of Thailand in 2011. Water hyacinth is a typical lignocellulosic material which is recognized as a potential source of renewable energy.

In this present study, hydrothermal treatment on water hyacinth (*Eichhornia crassipes*) was performed in a temperature range of 160 to 220 °C to examine an optimal yield of glucose. After treatment, the product was further hydrolyzed by cellulase. The effect of CH_3COOH as an organic catalyst on liquid composition was experimentally investigated. The liquid fraction was characterized by high performance liquid chromatography (HPLC) using refractive index detector (RID) to analyze the amount of glucose. The solid fraction was also analyzed by United States Department of Agriculture's method (USDA's method) to determine the amount of cellulose, hemicellulose, and lignin. The results showed that with the absence of CH_3COOH at 220°C, a glucose yield of 26.7 % was obtained. Meanwhile, the highest glucose yield of 85.5 % was achieved under the condition of 200°C with 0.75wt% CH_3COOH and 10wt% water hyacinth intake. A pseudo-first-order kinetic model with regard to cellulose content was developed to explain the conversion mechanism of cellulose to glucose in the hydrothermal treatment process. Based on the estimated rate constants results, this study was in a good agreement with other pervious investigation.

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LIST OF ABBREVIATIONS

[C]	Non-hydrolysable cellulose concentration (mol/L)
[G]	Glucose concentration (mol/L)
[C*]	Hydrolysable cellulose concentratiosn (mol/L)
[D]	Decomposition products of glucose concentration
(mol/L)	
k_i	Rate constant ((mol/L) $\cdot s^{-1}$)
A_i	Pre-exponential factor [s^{-1}]
E_{ai}	Activation energy [kJ/mol]
T	Target temperature [K]
R	Gas constant; 8.3145 [$JK^{-1}mol^{-1}$]
t	Residence time (s)
e	2.7183

Subscript

Eq	Equation
C	Non-hydrolyzsable cellulose
C*	Hydrolysable cellulose
G	Glucose
D	Decomposed product

CHAPTER I

INTRODUCTION

1.1 Background

Many researchers informed that past and present observations of climate change have many affected to Thailand and other countries around the world the data form the basis for the scientific investigation of this phenomenon and of course it will take in the future. Moreover, this data on the effects of climate change were needful which were important data on its causes, especially on greenhouse gas (GHG) emissions[1].

The records from around the world show that billions of tons of carbon in the form of carbon dioxide (CO_2) were absorbed by oceans and the living in biomass every year. The most serious environmental problems were climate change. Concentrations of CO_2 in the atmosphere will continue rising [2]. Moreover, the anticipated reduction of fossil fuels and the concerns about greenhouse gas (GHG) emissions asked for initiatives to search for renewable energy sources. Particularly interesting in this respect were forms of biomass including agricultural residue, wood, domestic waste, and grass.

In response to the energy crisis and global warming, the renewable and sustainable energy resources gain popularity. This has led to an increasing interest in alternative energy resources such as solar energy, wind energy, hydrogen fuel, tidal energy, geothermal energy, hydropower and biomass. However, Some energy resources, for example solar energy and wind energy, the effective energy conversion of these kinds of energy resources depends on the weather therefore they cannot be reliable energy resources.

The renewable technology such as Biomass Technology attracts world's attention. Because of biomass excessive supply, it will be definitely one of the most promising renewable energy sources. Furthermore, it has many advantages such as carbon-neutral, which can reduce the amounts of carbon dioxide emission leading to global warming issue.

1.1.1 Bioethanol

Bioethanol is an alcohol made by fermenting the sugar components of plant materials which is made mostly from sugar and starch crops. The advanced technology being developed cellulosic biomass such as trees and grasses which are also used as feedstocks for ethanol production. Ethanol can be used as a fuel for vehicles in its pure form. However, it is usually used as a gasoline additive to increase octane and improve vehicle emissions. It comes from a renewable resource for example crops and agricultural residue. Moreover, another benefit over fossil fuels is the greenhouse gas emissions.

The report from The United Kingdom, the transportation network accounts for 22% of all greenhouse gas emissions and through the use of bioethanol and some of these emissions will be decreased like a the fuel crops absorb the CO_2. Furthermore, mixing bioethanol with petrol will help extend the life of the UK's decreasing oil supplies, ensure greater fuel security, and avoiding heavy reliance on oil producing nations. Bioethanol is also biodegradable and far less toxic than fossil fuels. In addition, by using bioethanol in older engines can help reduce the amount of CO produced by the vehicle so that improving air quality. In quantities up to 5%, bioethanol can be mixed with fuel without the need of engine modifications.

In mention that bioethanol can help the environment by decreasing CO_2 on fossil fuels. **Figure 1.1** shows the important difference between bioethanol and gasoline is that bioethanol releases less new CO_2 into the atmosphere by recycling the CO_2 that is already present. Most of greenhouse gases are CO_2 that cause to global warming. Due to the nature's photosynthesis process during the growing of crops and trees can cause the remove of CO_2 to the atmosphere which is then released again because of combustion of the fuel in the car's engine. In addition, the burning of fossil fuels can increase CO_2 levels into the atmosphere. It means that CO_2 levels will continue to increase indefinitely.

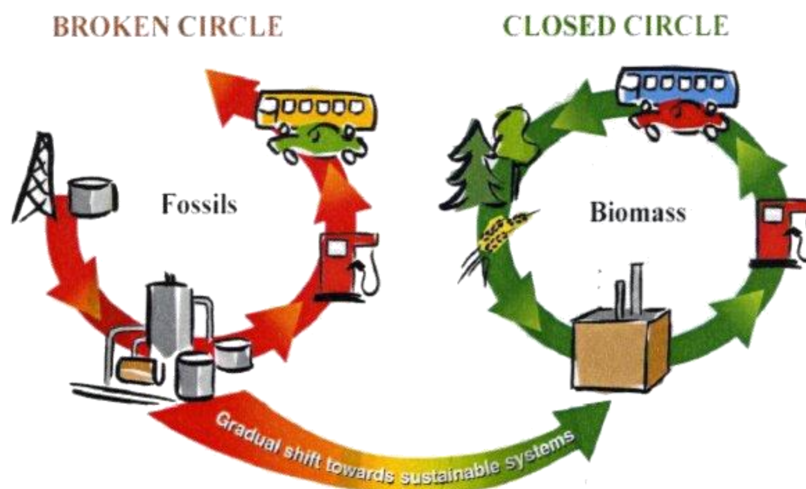


Figure 1.1 The difference between bioethanol and gasoline

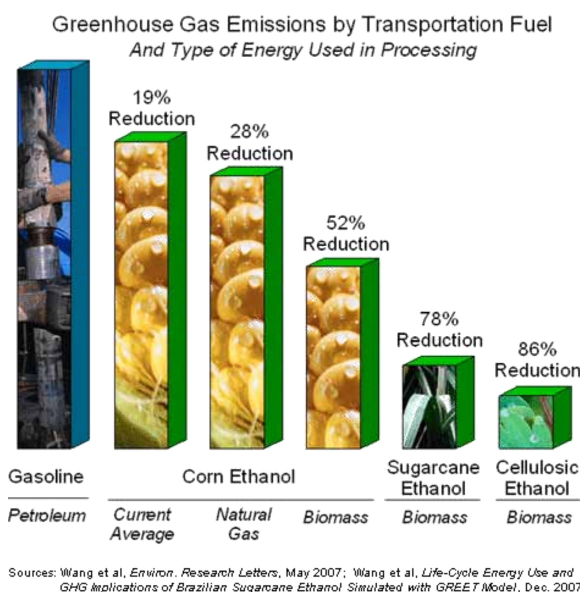


Figure 1.2 The level of greenhouse gas (GHG) emissions

Figure 1.2 shows the level of GHG emissions associated with biofuel depends on the energy used in growing and harvesting the feedstock, as well as the energy used to produce the fuel for example natural gas, coal, and biomass. Ethanol has the ability to decrease the level of greenhouse gas by as much as 52% over petroleum-based fuels. Moreover, ethanol also made from cellulosic feedstocks for example grass, or agricultural residues such as rice straw, has the ability to decrease the level

of greenhouse gas by as much as 86% by comparing to gasoline. Biofuels have the added benefit of providing a "carbon sink". A part from the benefit of crops grow is to produce the feedstocks for making the biofuel. Moreover, they can absorb CO_2 from the atmosphere.

Biomass is a great renewable fuel which can help us solve our earth's greenhouse gas problem. It makes from agricultural waste and other biodegradable waste to originate energy and fuel sources. This is great because the cost of renewable fuel is also cheaper than fossil fuels. Especially, they do not pollute the environment and the use of lignocellulosic biomass such as wood or straw does not compete with the food chain. However, current processes to convert lignocellulosic biomass to useful materials are highly inefficient [3]. In order to learn more about biomass. The topic of biomass and the carbon cycle should be understand first.

1.1.2 Biomass and carbon cycle

The biomass cycle is connected to the carbon cycle which has been known in previous section that to make our environment decrease. However, the better things are that biomass can make the carbon cycle a litter destructive and helps it serve its natural objective as shown in **Figure 1.3**.

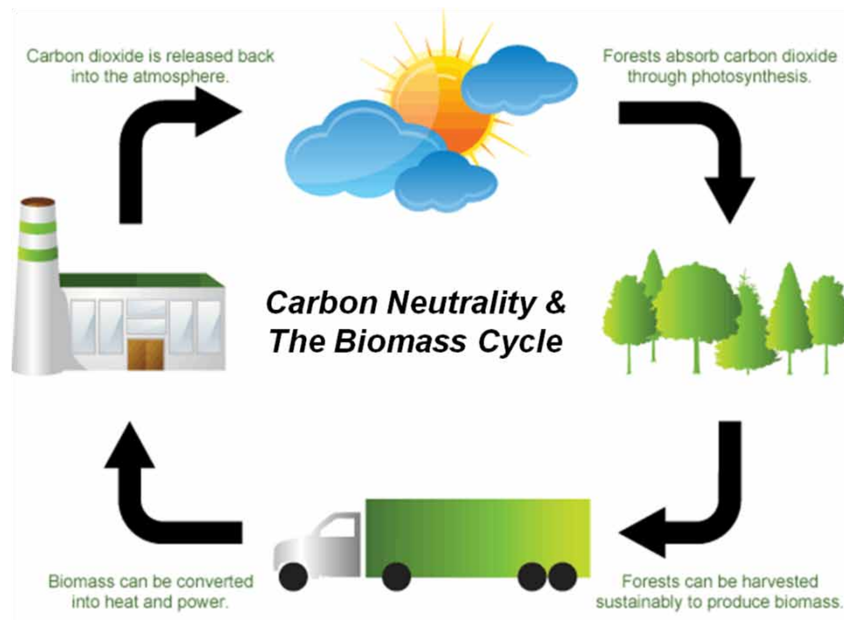


Figure 1.3 Biomass and carbon cycle

Carbon is a natural composition in the environment and does serve its objective on earth. CO_2 feeds plants and they turn it into O_2 for us to breathe. However, if we use carbon which release from fossil fuels, then there will be a lot of carbon in the atmosphere and it will start to create adverse effects seem warmer temperatures which is global warming.

As mention in previous section, biomass is made from biodegradable materials and uses carbon from the carbon cycle for example carbon in the soil is used to grow plants as their primary food source and they exhale O_2 which other creatures use for respiration. When these plants have grown and are eaten, then the carbon in them is used to originate energy for the consumer and the rest of the plant which is not used becomes waste. The waste and the gas released from the consumption of the plants goes into the atmosphere like a carbon. From this carbon cycle, the carbon in the air and soil is used by the plants which can be returned to biomass and biomass fuel. The carbon is used to originate energy and turns back into carbon to be reused. Therefore, creating more biomass can make use of all the carbon which is in the air and turn it into an energy source.

By using biomass energy can reduce the amount of air pollution on the earth. Moreover, plants can help decrease erosion and make a good soil quality. Instead of

adding too much carbon in the carbon cycle such as fossil fuels, biomass fuels can help and recycle it like a clean its natural objective. The crops used to originate biomass are also represented as energy crops and do not necessary pesticides or fertilizers. Therefore, the water supplies for these crops are not contaminated. Moreover, growing of energy crops are near water where chemical runoff is found. They can stop future runoff from going into the water. Therefore, biomass plants can also originate a better environment for wildlife to live in. The energy crops can help small animals and birds for surviving. Also in the streams will be cleaned because of the crops which make the fish in these streams also have a better life. Distinctly, using the rule of biomass and the carbon cycle, we can easily help get the world back to its natural cleanliness and keep pollution away while still creating energy for us to use.

1.1.3 Lignocellulosic biomass

Lignocellulosic material consists of mainly three different types of polymers are cellulose, hemicellulose, and lignin which are associated which each other. Processing of lignocellulosics to convert to ethanol made up of four major unit operations which are pretreatment, hydrolysis, fermentation, and product separation purification.

Cellulosic biomass, sometimes called lignocellulosic biomass, is a heterogeneous complex of carbohydrate polymers and lignin, a complex polymer of phenylpropanoid units. Lignocellulosic biomass typically consists of 55–75% carbohydrates by dry weight as shown in **Table 1**. Cellulose which is like starch is a polymer of glucose. Nevertheless, with unlike starch, the specific structure of cellulose favors the ordering of the polymer chains into tightly packed, highly crystalline structures that is water insoluble and resistant to de-polymerization. The other carbohydrate component in lignocellulosics is hemicellulose which is dependent on the species of biomass. Hemicellulose is a branched polymer of glucose or xylose, substituted with mannose, arabinose, fructose, galactose, glucose, xylose, or glucuronic acid. Some of the side chains might be also contained acetyl groups.

Table 1.1 Percent dry weight composition of lignocellulosic feedstocks

Feedstock	Glucan (cellulose)	Xylan (hemicellulose)	Lignin
Corn stover[a]	37.5	22.4	17.6
Corn fiber[b,c]	14.28	16.8	8.4
Pine wood[d]	46.4	8.8	29.4
Popular[d]	49.9	17.4	18.1
Wheat straw[d]	38.2	21.2	23.4
Switch grass[d]	31.0	20.4	17.6
Office paper[d]	68.6	12.4	11.3

Note: Because minor components are not listed, these numbers do not sum to 100%.

[a] Data from Elander, R. Personal communication, National Renewable Energy Laboratory, Golden, CO, 2002.

[b] Also contains 23.7% by dry weight starch.

[c] Unpublished data from Laboratory of Renewable Resources Engineering, Purdue University.

[d] From Wiselogel et al. (1996).

Hemicellulose links with cellulose microfibrils by hydrogen-bonds so that the forming of a network provides the structural backbone to plant cell wall. The presence of lignin in some cell walls imparts more strength, and provides resistance against diseases and pests. Unfortunately, too much lignin in the cell wall impedes enzymatic hydrolysis of the carbohydrates. Cellulose and hemicellulose are potential sources of fermentable sugars.

1.1.4 Treatment on lignocellulosic material

Hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields when the treatment process is became to alter the biomass microscopic and macroscopic size and structure as well as its submicroscopic chemical composition and structure. Hydrolysis consists of the processing steps that convert the carbohydrate polymers into monomeric sugars. However, a variety of process have been studied for conversion of lignocellulosic biomass into ethanol. Especially, the enzymatic hydrolysis of cellulose provides opportunities to improve the technology thus biomass ethanol is competitive when compared to other liquid fuels on a large scale [4].

Pretreatment process is an important in the first step for practical cellulose conversion, and is the subject of this article. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars as represented in the schematic diagram of **Figure 1.4**. The target of pretreatment process is to disrupt the crystalline structure of cellulose and destroy the lignin seal. Pretreatment has been viewed as one of the most expensive processing steps in cellulosic biomass-to-fermentable sugars conversion with costs as high as 30c/gallon ethanol produced. Pretreatment also has great potential for improvement of efficiency and lowering of cost through research and development [4].

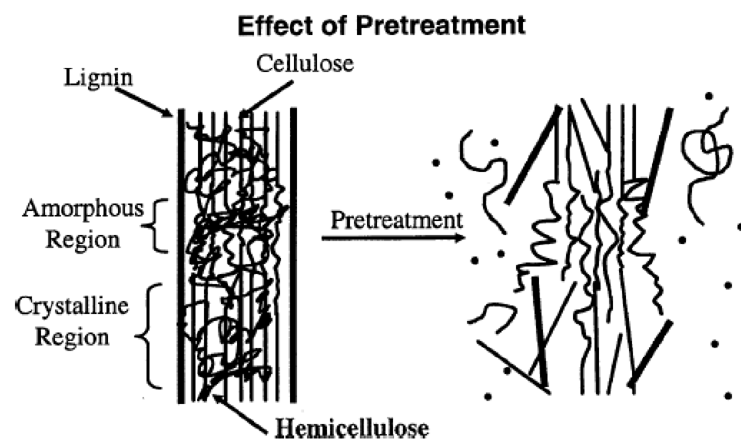


Figure 1.4 Schematic of goals of pretreatment on lignocellulosic material

Several methods have been suggested for pretreatment of lignocellulosesic materials prior to enzymatic hydrolysis or digestion [5]. Various pretreatments have been proposed and studied, however to achieve a high sugar yield, the two-stage hydrolysis is often employed. Lignocellulosics are first treated by various chemicals or under severe pretreatment conditions and after that the product is treated with the cellulase enzyme. The pretreatment consisted of aqueous ammonia recycle, dilute acid, controlled pH, lime approaches, ammonia explosion, uncatalyzed steam explosion, and flow-through acid [4]. Among the various methods, hydrothermal pretreatment enjoys the advantages of not using toxic or harmful chemicals, of employing relative mild conditions, and of being free from costly treatment of

wastewater or byproducts [6]. Acids or bases which promote hydrolysis and improve the yield of glucose recovery from cellulose by removing hemicelluloses or lignin during the pretreatment process. The most commonly used acid and base are H_2SO_4 and NaOH, respectively.

Hydrothermal pretreatment for cellulase hydrolysis has been studied by Ballesteros et al. [7]. They have employed an autoclave to treat olive residue. Hydrothermal treatment has also been studied as the only pretreatment without the following cellulase treatment by several researchers [8]. Aquasolve process is one of the early studies [9]. In the meantime, Kruse et al. added hot compressed water into the vessel of biomass, and after a few minutes, released the pressure of the vessel and removed the water, so that separation of the components was to be achieved [10]. However, their glucose yield was limited due to the decomposition of product glucose. Thus, only hydrothermal pretreatment cannot achieve complete hydrolysis.

Cellulase could be activated at a temperature around 30°C, and it catalyzes only the hydrolysis. However, without pretreatment, the effectiveness of cellulase is largely reduced because of the presence of intermolecular hydrogen bond linkages at the crystalline region in cellulose. In the same idea was studied by Mishima et al. [11], and they also reported that the glucose concentrations in the enzymatic hydrolysate was comparable to or a little higher than that obtained using acid hydrolysis, suggesting that the cellulose and starch in the biomass were sufficiently hydrolyzed by cellulase in this enzymatic hydrolysis stage. Considering the results from these previous studies, we decided to concentrate on the two-stage hydrolysis of water hyacinth leaves by using hydrothermal pretreatment in this study.

1.1.5 Water hyacinth

Growth of water hyacinths has become an environmental problem in many parts of the world. Water hyacinth is a free floating aquatic plant which can be found in every river of Thailand as shown in **Figure 1.5**. It can interfere with processes fisheries, lake biogeochemical, recreational activities such as swimming and boating, and detract from the aesthetic appeal of the system [12]. It can double its size in 5 days and a mat of medium sized plants may contain 2 million plants per hectare that weigh 270 to 400 T. These dense mats interfere with navigation, recreation, irrigation,

and power generation. Many large hydropower schemes have to devote significant time and money in clearing the weed in order to prevent it from entering the turbine and causing damage and power interruptions. The blockage of

Figure 1.5 Growth of water hyacinth in Thailand

canals and rivers can even cause dangerous flooding. On the other hand, increased evapotranspiration due to water hyacinth can have serious implications where water is already scarce. Water hyacinth can also present many problems for the fisherman such as decreased fish population, difficult access to the fishing sites and loss of fishing equipment, resulting in reduction in catch and subsequent loss of livelihood. Water hyacinth is blamed for the reduction of biodiversity as well. These mats competitively exclude native submerged and floating-leaved plants and its associated fauna, thereby causing an imbalance in the aquatic micro-ecosystem. Diversity of fish stocks is also affected. Low oxygen conditions beneath the mats create good breeding conditions for mosquito vectors of malaria, encephalitis and filariasis.[13].

Gunnarsson et al. (2007) [14] report an optimal moisture content of about 60% for the composting process, and an optimal range of moisture content of 50–60%. Gunnarsson et al. (2007) claim that the moisture content of the fresh water hyacinth is too high and hence little additional water is needed during the composting. Therefore the high moisture content is probably not a big problem when composting water hyacinths. Dalzell et al.[15] suggest that compost be produced in pits during dry seasons and, to avoid water logging, in piles during rainy seasons. The compost should be protected from the wind to decrease moisture losses. Placing the compost

pile out of direct sunlight can also reduce the water requirements according to Dalzell et al. [14].

The high water and mineral content of water hyacinth indicates that the nutrients in water hyacinth may be available and suitable to some animals. Boiled and chopped water hyacinth along with vegetable waste, rice bran, copra cake and salt is used to make a suitable feed for pigs in China. In Malaysia, Indonesia, Philippines and Thailand cooked and supplemented water hyacinth is used as feed for pigs, ducks and fish. Dehydrated water hyacinth has been added to the diet of channel catfish fingerlings to increase their growth. Meanwhile, the water hyacinths are excessive waste agriculture and can be considered as a promising energy source. Most of the lignocellulosics can be used directly as fuel either by direct combustion or by first gasifying and then burning the gas. However, there is a great deal of interest in utilizing and converting the lignocelluloses fraction as feedstock material for ethanol and other chemicals.

Characteristic of water hyacinth leaves

The cellulose content of biomass to be subject hydrothermal treatment could be determined following the procedure recommended by the United States Department of Agriculture (USDA). In this work, water hyacinth leaves would also be analyzed by USDA to get preliminary information of its component. **Table 1.1** shows the components of the water hyacinth leaves (dry weight basis) determined by the USDA's method [16].

Table 1.2 Composition of water hyacinth feedstock

Cellulose	0.28
Hemicellulose	0.62
Lignin	0.02
Ash	0.08

The structure of cellulose is a linear polysaccharide polymer with many glucose monosaccharide units. Hemicellulose also has glucose unit in their structure. As a result in **Table 1.2**, the amount of hemicellulose is higher than the

amount of cellulose in water hyacinth leaves which is 2.2 times. However, cellulose consists of 4,000 glucose units. Meanwhile, least than 200 glucose units consist in hemicellulose.It means that the amount of glucose unit in cellulose is higher than the amount of glucose unit in hemicellulose which is around 9 times in water hyacinth leaves.

Therefore, the amount of glucose which consists in hemicellulose could be ignored when compared with those in cellulose.

1.2 Objectives

The objective of this research is to investigate an optimal condition for hydrothermal treatment of water hyacinth leaves in order to convert to glucose.

1.3 Scopes of Research

1. Water hyacinth leaves will be used as a biomass feed stock for hydrothermal treatment.
2. The experimental conditions for hydrothermal treatment process of water hyacinth leaves will be focused by variation of following parameters

    2.1 Reaction temperature in a range of 160 - 220°C

    2.2 Effect of acetic acid on glucose yield

    -Concentration of acetic acid in range of 0.5 – 1.0wt%
3. Effect of enzymatic hydrolysis will also be investigated for exploring the possibility to convert the cellulose to glucose product.

1.4 Expected benefit

1. To get the guideline for the optimal conditions for the hydrothermal treatment.
2. To support biomass technology in Thailand for the future to used renewable energy sources.

CHAPTER II

FUNDAMENTAL KNOWLEDGE AND LITERATURE REVIEW

2.1 Hydrothermal treatment with non-catalyst conversion of cellulose in supercritical water and subcritical water.

(a) Cellulose pretreatment in subcritical water: Effect of temperature on molecular structure and enzymatic reactivity [17].

Kumar et al. (2010) studied on the effect of temperature on cellulose structure and enzymatic reactivity in cellulose pretreatment. Microcrystalline cellulose (MCC) was pretreated with subcritical water in a continuous flow reactor for enhancing its enzymatic reactivity with cellulase enzyme.

MCC was added to continuously stirred hot water (70°C) to prepare 24.1 wt% slurry and charged to the slurry feeder. MCC was treated with subcritical water at temperatures ranging from 200 to 315°C at a constant pressure 27.6 MPa and residence times ranging from 3.4 to 6.2 s.

Enzymatic hydrolysis of all the untreated and treated MCC samples were carried out simultaneously in 35 ml kmax test tubes with rubber stoppers on the top. Enzyme loadings were used for each substrate: 3.5 and 60 FPU/g. Each test tube contained 0.1 g oven dried MCC substrate, 1 ml diluted enzyme (with equivalent activity of 3.5 or 60 FPU/g), 0.07 ml of antibiotics (0.04 ml tetracycline solution + 0.03 ml of cycloheximide solution) and 0.05 N sodium citrate buffer (rest of the volume) for maintaining the pH (4.8) in the reaction. Magnetic stirrer was used for mixing of the reactant in the test tube. All the test tubes were placed in the incubator which maintained constant reaction temperature of 50°C. Samples were collected after 1 and 24 h for measurement of glucose and cellobiose concentration in the liquid. Sugar concentrations were measured using HPLC.

They found that the liquid composition after pretreatment at 300-315°C the cellulose converted to hydrolysis products (oligomers and monomers) and a portion further degraded to the aqueous degradation products of glucose, including

glycoaldehyde, fructose, anhydroglucose, and 5-HMF organic acids, etc., which makes the reaction medium more acidic. The viscosity-average degree of polymerization of cellulose though reduced after the pretreatment, it decreased rapidly at 315°C.

2.2 Hydrothermal pretreatment with non-catalyst conversion of lignocellulosic residue in supercritical water and subcritical water.

(a) Hot compressed water pretreatment of oil palm fronds to enhance glucose recovery production of second generation bio-ethanol (Goh et al, 2010)

The aim of this study was to optimize the pretreatment condition of oil palm fronds using liquid water under elevated temperature and pressure to significantly improve enzymatic hydrolysis for recovery of fermentable sugars. First, the composition of raw oil palm fronds was investigated according to analytical procedures for determination of the extractives in biomass (Ehrman, 1994). Second, a rotational Central Composite Design (CCD) was used for an experimental design to determine the optimal pretreatment condition of oil palm fronds. Variable studies include temperature, residence time and the liquid to solid ration. The glucose yield was evaluated, defined as the percentage of the weight of glucose recovered to the weight of potential glucose in the biomass.

Oil palm fronds was shredded and ground to a particle size smaller than 1 mm and then dried under the sun. (Particle size distribution: 0.500-1.000 mm; 58.68 wt.%, 0.250-0.500 mm; 15.72 wt.%, 0.125-0.250 mm; 2.98 wt.% and <0.125 mm; 22.63 wt.%)

The 500-mL stainless-steel reactor was equipped with electric furnace. The heating rate was app. 5 °C/min. Deionized water was added to a desired solid to liquid ratio. The reactor was pressurized to 10 bars. After reaching target pretreatment temperature and design reaction time, the reactor was quickly cooled down by ice (under 100 °C) and slowly depressurized. The target temperature, reaction time and liquid to solid ratio were varied in a range from 160 -200 °C., 5 - 20 minutes, 8 - 14.4, respectively.

A regression analysis was performed to fit the response function and predict the outcome glucose yield with a mathematic equation. The three-dimension response surface plot of glucose yield was created to investigate the interactive effect of main effects; temperature, reaction time and solid to liquid ratio.

The yield of glucose reaches maximum value at a moderate temperature around 182°. The maximum value of glucose yield was found at around 11 minutes. At the longer reaction time, the biomass would be hydrolyzed to glucose during pretreatment and would be further decomposed to furfurals, acetic acid and so on. (Rogalinski et al, 2008)

Higher yield was observed at a lower liquid-solid ratio. This phenomenon is probably caused by the formation of acidic component in the liquid medium. At the low liquid-solid ratio, the concentration of the acid in the solution was higher. Acidic compounds like acetic acid and formic acid generating from the decomposition of biomass decrease the pH of the liquid and they suspected to enhance the effect of pretreatment on biomass (Negro et al., 2003; Teramoto et al., 2009).

From the calculated model, the predicted optimum conditions were obtained at 178°C, 11.1 min and 9.6 (solid to liquid ratio). The predicted glucose yield was 92.69 wt%, which was verified by carrying out experiment at the suggested optimum conditions. The glucose yield from the experiment was 92.78 wt%, which was in good agreement with the value calculated using model.

(b) Hydrothermal pretreatment of rubber wood for the saccharification process. [18]

The objective of this study is to determine the effect of temperature on the reaction of cellulose in rubber wood during hydrothermal pretreatment. Rubber wood was treated hydrothermally in autoclave reactor and vary the hydrothermal pretreatment temperature at 130-280 °C for studying the effect of temperature on the reaction. The 10 target temperatures were 130, 140, 150, 170, 190, 200, 210, 240, 260 and 280 °C.

Rubber Wood is used as biomass raw material of hydrothermal pretreatment that further converts to Ethanol. The concept of Ethanol production from the rubber wood is to hydrolyze the cellulose and hemicelluloses to recover C5 and C6 sugar and

ferment the sugars into the ethanol. In this experiment they focus on converting rubber wood into glucose.

Hydrothermal pretreatment was conducted by loading 10wt% rubber wood and 90wt% of deionized water in the autoclave reactor. Then the agitator, which was set at 500 rpm, started while autoclave was heated up by the embedded heaters. When the temperature reached the target value, the heater were turned off, the air fan cooled down the reactor. After the pretreatment, the sample was separated into liquid sample, which was analyzed by HPLC with SUGAR KS-802 (Shodex) column operated at 60 °C with water at 0.8 cm3/min as an elute, and solid sample, which was used for enzymatic hydrolysis and measured for the content of cellulose after pretreatment.

Enzymatic Hydrolysis was conducted by placing 1 g of the pretreated sample, 5 cm^3 of cellulase solution (conc. 10g/dm^3), from *Aspergillus niger* powder (≥0.3 units/mg-solid), and 60 cm^3 of buffer fluid, which prepared from acetic acid, sodium hydroxide and deionized water (pH was set at 5), in a conical flask. The flask was shaken at 250 rpm at 37°C for 2 days and the liquid product was sampled every 24 hours in each run and analyzed by HPLC.

The reaction parameters were determined. The change of cellulose in the hydrothermal pretreatment and enzymatic hydrolysis process is described below in **Figure 2.1**.

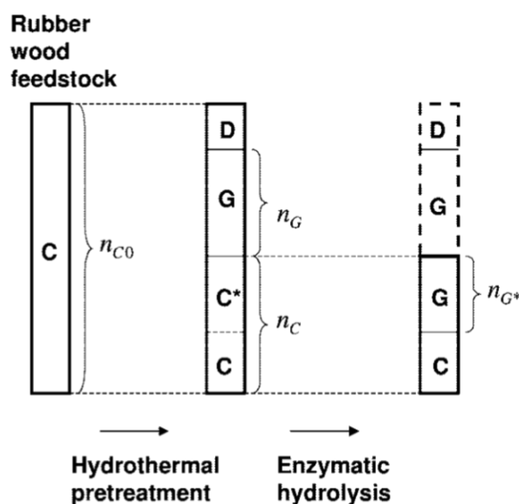


Figure 2.1 The change of cellulose in the hydrothermal pretreatment and enzymatic hydrolysis

Glucose Yield; $X_G = \frac{n_G}{n_{C0}}$ (1)

Where n_G = amount of glucose in liquid phase after hydrothermal pretreatment

n_{C0}= theoretical amount of glucose in the cellulose feedstock

Cellulose, that can be hydrolyzed Yield; $X_{C*} = \frac{n_{G*}}{n_{C0}}$ (2)

Where n_{G*} = amount of glucose increased by the cellulose treatment

Cellulose, that cannot be hydrolyzed Yield; $X_C = \frac{n_c - n_{G*}}{n_{C0}}$ (3)

Where n_c = amount of glucose unit the cellulose left after hydrothermal pretreatment

Decomposed product Yield; $X_D = 1 - X_G - X_{C*} - X_C$ (4)

Reaction Network for the Hydrothermal Pretreatment is shown in **Figure 2.2**.

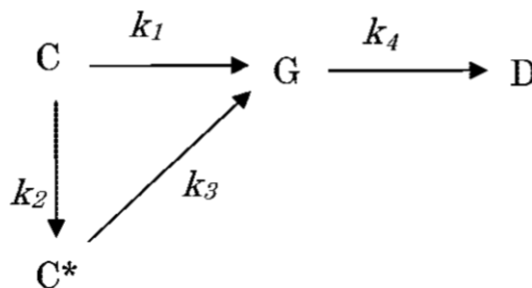


Figure 2.2 Reaction Network for the Hydrothermal Pretreatment

C is (the starting material) cellulose in rubber wood that cannot convert to glucose by cellulose treatment (cellulose resistance).

C* is the cellulose that can be converted into glucose by cellulase treatment, after the hydrothermal pretreatment in the first step. Because the cell structure was destroyed, hemicellulose and part of lignin was decomposed in hot compress water, which also reduced the crystallinity of the cellulose.

G is glucose.

D is decomposition product with is a mixture of various compounds, which catalyzed by acids forms formed during the hydrothermal pretreatment.

By assuming the first order reaction for each reaction, the following equations are obtained.

$$\frac{d[C]}{dt} = -k_1[C] - k_2[C] \tag{5}$$

$$\frac{d[G]}{dt} = k_3[C^*] + k_1[C] - k_4[G] \tag{6}$$

$$\frac{d[C^*]}{dt} = k_2[C] - k_3[C^*] \tag{7}$$

$$\frac{d[D]}{dt} = k_4[G] \tag{8}$$

After assumption that the Arrhenius rate law is applicable to the reaction rate constant, using the least-squares method to find the value of each k parameter.

$k_1 > k_2$ indicated that Cellulose(C) → Glucose (G); mainly, not to hydrolyzed form (C*)

Also, $k_4 > k_1$ indicated that Glucose (G) → Decomposed (D)

Other biomass species different reaction rate parameters have found but the reaction network still remain. Also the autoclave reactor is not suitable for the hydrothermal process because of its slow heating rate and cooling rate, which caused the product to be decomposed in the heating and cooling step.

(c) Hydrothermal pulverization pretreatment for water hyacinth.

The objective of this study to investigate the possibility of simultaneous hydrothermal pulverization pretreatment. In our previous study, particle size was varied. Glucose yield that obtained from smaller particle size of biomass is higher than bigger one. Therefore particle size has a significant effect on hydrothermal reaction. To enhance efficiency of enzymatic hydrolysis process, pulverization of biomass was necessary.

There are various pulverization methods such as disk mill, rotor mill, ball mill, cutter mill, ultracentrifuge mill, mixer mill and so on. . In this study, ball mill is employed as pulverization method due to its advantage. Biomass can be soften easily in hydrothermal pretreatment. It is expected that this method can selectively pulverize

cellulose, because hemicellulose and lignin are removed under hydrothermal condition. Furthermore, the structure of ball mill is very simple, and suitable for hydrothermal pretreatment. Thus, they proposed to conduct simultaneous hydrothermal pretreatment and ball mill pulverization.

Experimental apparatus is shown in **Figure 2.3**. Balls and feedstock solution are loaded in the ball mill reactor. Reactor is rotated by motor controlled by the inverter. The reactor is heated up by the electric furnace. The reactor made of SS316, and the reactor volume is 800 cm^3, where inner diameter is 9 cm. Temperature is measured every 30 seconds.

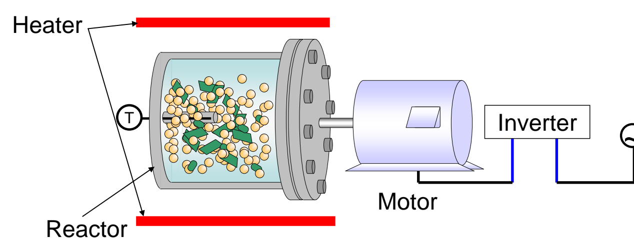


Figure 2.3 Experimental apparatus

Experimental condition under hydrothermal pretreatment is shown in **Table 2.1**. To investigate the effect of pulverization, experiment with ball mill and without ball mill pulverization was conducted.

Table 2.1 Experimental condition

Feedstock	Water hyacinth
Slurry content	100 g
Feedstock concentration	5, 10, 15 wt%
Ball material	ZrO_2
Ball loading	0, 0.25, 0.5, 0.75, 1 kg
Ball diameter	10 mm
Target temperature	160, 180, 200, 220, 230°C
Rotating speed	0, 100, 200, 300 rpm
Pulverizing time	0, 60, 120, 180 min

Enzymatic Hydrolysis was conducted by placing 35 grams of the pretreated sample were enzymatically hydrolyzed. Cellulase from Aspergillus niger powder (≥ 0.3 units/mg-solid) was used in this study. 5 mL of 10-g/L cellulase solution were added to the sample. Buffer fluid was also added, which was prepared from acetic acid, sodium hydroxide and de-ionized water. Its pH was set at 5. The flask was shaken at 250 rpm at 37 °C for 2 days. In each run, the liquid product was sampled every 24 h, and was analyzed by HPLC.

From the results they found that glucose yield decreases above 220 ^{o}C. Too high target temperature, glucose was decomposed. The highest glucose yield was obtained at 10 g of feedstock, 0.5 kg ball loading. When ball loading is increased, balls contacts with each other and the effective pulverization cannot be made. Too small amount of ball loading can be made the effective pulverization. No effect of ball diameter in glucose yield is observed. longer pulverization time increases glucose yield. However, glucose yield of simultaneous treatment is higher than separated treatment. So these results have been confirm the effectiveness of simultaneous hydrothermal pretreatment and ball mill pulverization.

At higher rotational speed, the glucose yield was increased. However, glucose yield decreased over 200 rpm because the rotational speed was exceeded the critical rotational speed. In this study, critical rotational speed is determined by following equation.

$$\mathrm{Nc} = \frac{30}{\pi}\sqrt{\frac{g}{R\sin\theta\sqrt{1-\alpha}}}$$

Where, R: Radius of the reactor

θ: The angle of response of material

α: Volume-containing fraction

Nc: Critical rotational number

Radius of the reactor is 0.045 m. The angle of response of material is 0.29. Volume-containing fraction is 0.21. Nc is around 275 rpm.

Efficiency of simultaneous hydrothermal pretreatment and ball mill pulverization was investigated. Power consumption, production of glucose and

efficiency are shown in **Table 2.2**. It confirms that simultaneous pulverization of biomass can reduce energy consumption.

Table 2.2 Efficiency of Ball mill hydrothermal reactor

	Simultaneous	Separate			
Pulverizing time [min]	0	0	60	120	180
Power consumption [kJ]	7.8×10^2	4.9×10^2	7.7×10^2	1.1×10^3	1.4×10^3
Production of glucose [kJ]	7.5	3.7	5.2	5.2	5.4
Efficiency [J/kJ]	9.6	7.7	6.7	4.9	3.9

2.3 Hydrothermal treatment with catalyst conversion of lignocellulosic residue in supercritical water and subcritical water.

(a) Experimental and kinetic modelling studies on the acid-catalysed hydrolysis of the water hyacinth plant to levulinic acid [19]

Girisuta et al.(2008) investigated the experimental and kinetic modeling on the acid-catalysed hydrolysis of the water hyacinth leaves to levulinic acid in condition with temperature at 150-175°C, water hyacinth intake = 1-5wt% and acid concentration (sulfuric acid) = 0.1-1 M. Subsequently, the LA yields were modeled using a recently developed kinetic model for cellulose. They found that the maximum glucose concentration (8 mM) at 0.1 M was considerably lower than at 1 M sulphuric acid (20 mM).

On the contrary, the rates of formation and decomposition reactions involving the C5-sugars (xylose, arabinose) were not affected by the amount of acid catalyst. Furthermore, at low acid catalyst concentrations the maximum concentration of arabinose was about equal to the value observed at high acid catalyst concentrations. These observations indicate that breakdown of the hemicellulose fraction at low acid concentrations is still very facile. This is in line with earlier investigations on the acid-catalysed decomposition reactions of hemicellulose and is ascribed to the low crystallinity of this fraction.

The highest experimental yield of levulinic adid (Y_{LA}) was 53 mol% (9 wt% based on the mass of oven-dried water hyacinth) and was obtained at T = 175°C,

$X_{WH,0}$ = 1 wt% and C_{H2SO4} = 1 M. The acid concentration had a profound effect on the Y_{LA}, with higher concentrations leading to higher yields. The yield was reduced when performing the reaction at higher temperatures. The kinetic model is the basis for the kinetic model presented here to predict the LA yields and the amounts of glucose for the acid-catalysed hydrolysis of the water hyacinth at different reaction conditions. They found that the rate of depolymerisation of C6-sugars from the water hyacinth matrix is lower than that of pure cellulose. This may be related to matrix effects (e.g. the presence of lignin) and/or difference in the cellulose properties (e.g. crystallinity).

(b) Microwave-assisted organic acid pretreatment for enzymatic hydrolysis of rice straw [20].

The objective of this study to investigate the optimal conditions of microwave-assisted organic acid pretreatment of rice straw under conditions of acid concentration, solid-liquid ratio, microwave intensity, irradiating time, and catalyst concentration on the removal ratio of lignin. After pretreatment, the infrared spectrum of the solution was tested, and micro-morphology was observed by scanning electron microscopy (SEM). The removal ratio of lignin and the yield of reducing sugar were determined by spectrophotometry.

Pretreatment was carried out as follows: straw powder was first placed in a beaker and mixed with a certain concentration of acetic acid or propionic acid. The beaker was radiated at 100-700 W for 2-5 min in the microwave oven. Finally, the products were obtained by pump filter and placed in a vacuum drying box to dry to constant weight.

The pretreated product (adjusted pH to 4-5) was mixed with 4 mL cellulase in a conical flask with 1:10-1:25 solid-liquid ratio, 9 h hydrolysis time, and 50°C reaction temperature. Then the conical flask was placed in a water bath at 50-60°C for saccharification reaction. Finally, the reducing sugar was obtained.

Prior to observation through scanning electron microscopy (SEM), (FEI Sirion, Phillip, Netherlands), the surface of samples of the pretreated materials were metal-sprayed. Following this the micro-morphology of rice straw after pretreatment was observed.

The infrared spectrum of the treating solution after pretreatment was determined by Fourier-transform infrared spectroscopy (Thermo Nicolet NEXUS 670, USA).

According to the influence of acetic acid concentration, they found that The removal ratio of lignin gradually increased with increasing in acetic acid concentration. SEM images as shown in **Figure 2.4** show only a weak erosion of the surface of rice straw when 6% acetic acid was used. Large areas of the cellulose surface were destroyed, and the degree of destruction of the regular cellulose structure that supports the rice straw structure increased when the concentration of acetic acid was increased to 18%. At this concentration, the removal ratio of lignin was improved.

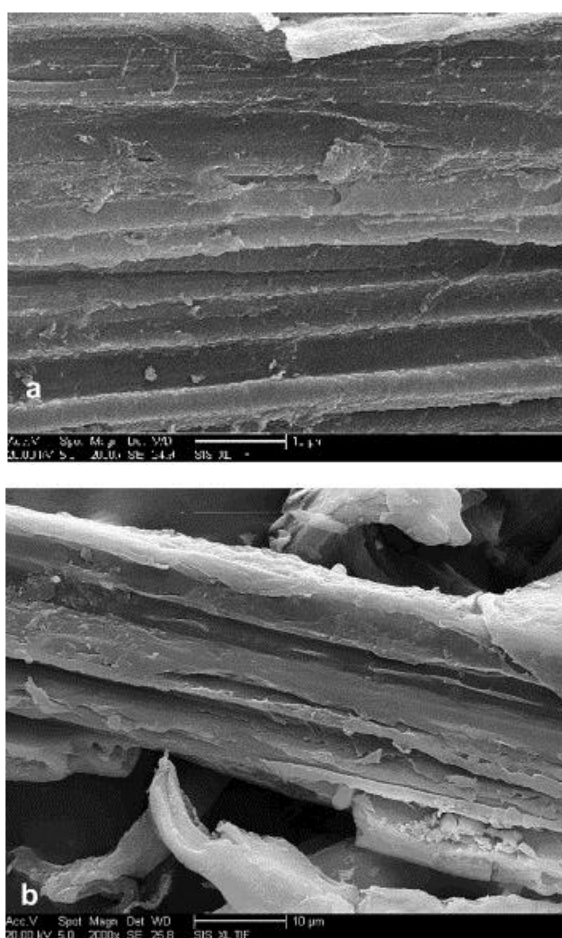


Figure 2.4 SEM images of rice straw pretreated with different concentrations of acetic acid. (a) 6% acetic acid; (b) 18% acetic acid

To further verify the validity of this method, the enzymatic lysis reactions in the samples were studied. The yield of reducing sugar after acetic acid pretreatment, with a maximum value of 71.41%, was obviously higher than that of the blank sample (35.28%).

The sequence of influence strengths of the factors was microwave intensity > solideliquid ratio > acetic acid concentration > microwave irradiating time. The optimal conditions determined were 25% acid concentration, 1:15 solideliquid ratio, 230 W microwave intensity, and 5 min irradiation time. The maximum values of the lignin removal ratios were 46.1% when using acetic acid as solvent. The surface of the samples became markedly more loose and irregular after pretreatment.

(c) Low-temperature catalytic hydrothermal treatment of wood biomass: analysis of liquid products [21].

In this study investigation, the hydrothermal treatment of wood biomass at 280 ◦C for 15 min in the presence of alkaline solutions (NaOH, Na_2CO_3, KOH, and K_2CO_3) was presented. After hydrothermal treatment of wood biomass, an extensive separation and extraction procedure was applied to recover oil products from different portions. The
effects of base solutions on oil products and compounds in oils in three different cuts were discussed. Analyses such as total organic carbon (TOC) content for water phase, GC–MS for oil products, HNMR and CNMR for acetone soluble hydrocarbons were carried out. In final, the volatility distribution of hydrocarbons (ether extract) was carried out.

2.4 Biomass

Biomass energy is a promising renewable energy. Biomass is composed of organic material produced from living creatures. Biomass consists of agriculture crops, animal waste, household garbage, waste from food processing, municipal waste and other waste material such as saw dust and chipping from wood processing. Biomass gets its energy from the sun. All organic matter contains stored energy from the sun during a process called photosynthesis. Furthermore, Biomass is a renewable

energy source because its supplies are not limited because trees and crops can be produced, and waste will always exist. The massive amount of biomass in the world can supply the world energy demand. In addition, Forest residues and agriculture residues are grown to generate electricity or produce heat. The advantage of biomass is carbon dioxide by the amounts of carbon dioxide which release from the combustion of one life will be equal with the amounts of the carbon dioxide that another new life consumes. It is therefore environmental friendly due to carbon neutral, so it can reduce the amounts of carbon dioxide emission, which protect the environment and reduce the global warming.

Biomass includes woody biomass, herbaceous biomass like switchgrass, sugar and starch crops like cassava and sugarcane, oil producing biomass like oil palm, coconut and jatropha, aquatic plant biomass like seaweed and water hyacinth, agricultural residues, wooden residuals, animal waste, sewage sludge, municipal solid waste, black liquor and food processing waste like bagasse, corn cob and molasses.

2.5 Cellulose

Cellulose is an organic compound with the chemical formula of $(C_6H_{10}O_5)_n$ with a polysaccharide consist of a linear chain of several hundred to over ten thousand of β(1→4) linked D-glucose units as shown in **Figure 2.5**.

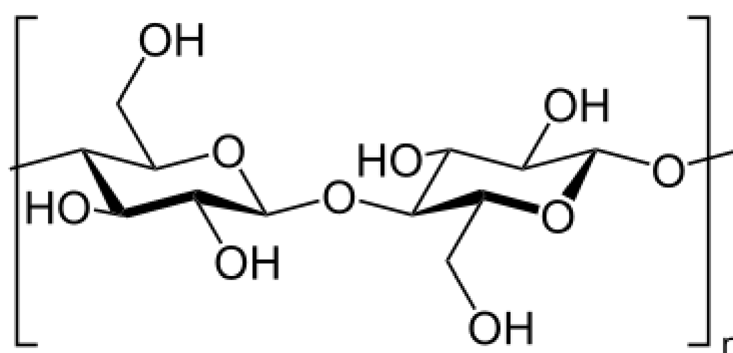


Figure 2.5 Chemical structure of Cellulose

Cellulose is the structural component of the primary cell wall of green plants, many forms of algae and the oomycetes. Some species of bacteria secrete it to

form biofilms. Cellulose is the most common organic compound on Earth. About 33% of all plant matter is cellulose such as the cellulose content of cotton is 90% and that of wood is 40–50%.

For industrial use, cellulose is mainly obtained from wood pulp and cotton. It is mainly used to produce paperboard and paper; to a smaller extent it is converted into a wide variety of derivative products such as cellophane and rayon. Converting cellulose from energy crops into biofuels such as cellulosic ethanol is under investigation as an alternative fuel source.

Some animals, particularly ruminants and termites, can digest cellulose with the help of symbiotic micro-organisms that live in their guts. Humans can digest cellulose to some extent, however it is often referred to as dietary fiber or roughage (e.g. outer shell of maize) and acts as a hydrophilic bulking agent for feces.

Cellulose has no taste, is odourless, is hydrophilic with the contact angle of 20–30, is insoluble in water and most organic solvents, is chiral and is biodegradable. It can be broken down chemically into its glucose units by treating it with concentrated acids at high temperature.

Cellulose is derived from D-glucose units, which condense through β(1→4)-glycosidic bonds. This linkage motif contrasts with that for α(1→4)-glycosidic bonds present in starch, glycogen, and other carbohydrates [22]. Cellulose is a straight chain polymer: unlike starch, no coiling or branching occurs, and the molecule adopts an extended and rather stiff rod-like conformation, aided by the equatorial conformation of the glucose residues. The multiple hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen atoms on the same or on a neighbor chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength. This strength is important in cell walls, where the microfibrils are meshed into a carbohydrate matrix, conferring rigidity to plant cells.

Compared to starch, cellulose is also much more crystalline. Whereas starch undergoes a crystalline to amorphous transition when heated beyond 60-70 °C in water (as in cooking), cellulose requires a temperature of 320 °C and pressure of 25MPa to become amorphous in water.

Many properties of cellulose depend on its chain length or degree of polymerization, the number of glucose units that make up one polymer molecule.

Cellulose from wood pulp has typical chain lengths between 300 and 1700 units; cotton and other plant fibers as well as bacterial celluloses have chain lengths ranging from 800 to 10,000 units. Molecules with very small chain length resulting from the breakdown of cellulose are known as cellodextrins; in contrast to long-chain cellulose, cellodextrins are typically soluble in water and organic solvents.

Plant-derived cellulose is usually found in a mixture with hemicellulose, lignin, pectin and other substances, while microbial cellulose is quite pure, has a much higher water content, and consists of long chains.

Cellulose is soluble in cupriethylenediamine (CED), cadmiumethylenediamine (Cadoxen), N-methylmorpholine N-oxide and litium chloride / dimethylformamide. This is used in the production of regenerated celluloses (as viscose and cellophane) from dissolving pulp.

2.6 Hemicellulose

A hemicellulose is any of several heteropolymers (matrix polysaccharides), such as galactose, glucose, arabinose, xylose and small amounts of rhamnose, glucuronic acid, methyl glucuronic acid, and galacturnic acid, present along with cellulose in almost all plant cell walls [23].

Hemicellulose is a branched, low molecular weight polymer composed of five and six carbon sugars and contains many different sugar monomers as shown in **Figure 2.6**. In contrast, cellulose contains only anhydrous glucose. Xylose is always the sugar monomer present in the largest amount, but mannuronic acid and galacturonic acid also tend to be present.

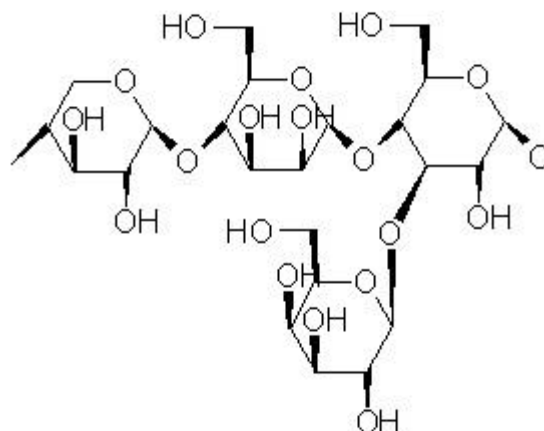


Figure 2.6 Chemical structure of Hemicellulose

The dominant sugars in hemicelluloses are mannose in softwoods and xylose in hardwoods and agriculture residues. In contrast to cellulose, which is crystalline and strong, hemicelluloses have a random, amorphous, and branched structure with little resistance to hydrolysis, and they are more easily hydrolyzed by acids like a acetic acid to their monomer components [24].

2.7 Lignin

Lignin is a very complex molecule constructed of phenylpropane units linked in a three dimensional structure which is, a benzene ring with a tail of three carbons which is particularly difficult to biodegrade as shown in **Figure 2.7**. In their natural unprocessed form, they are so complex that none of them has ever been completely described, and they have molecular weights around 15,000 or more.

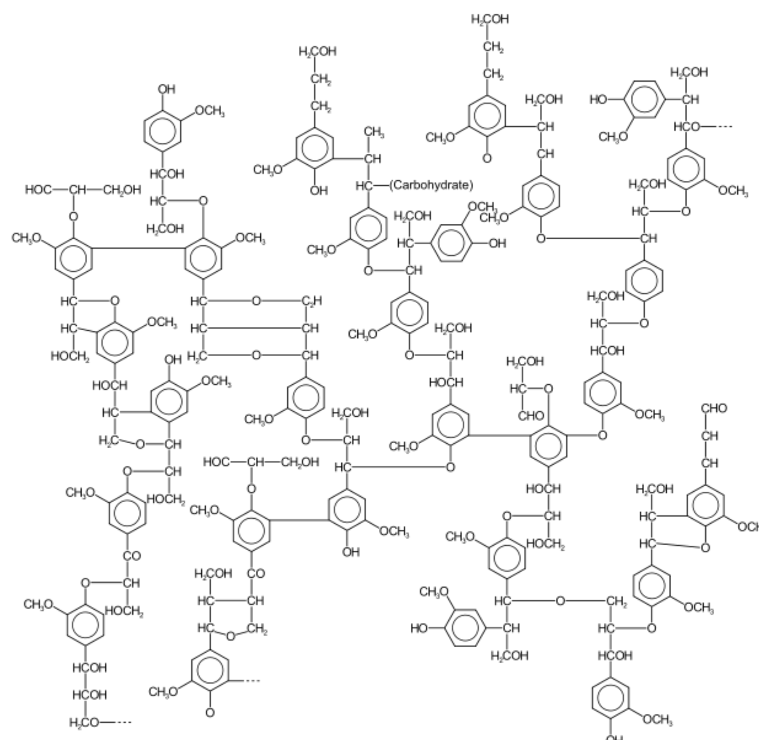


Figure 2.7 Chemical structure of lignin

Lignin is a cross-linked racemic macromolecule with molecular masses in excess of 10,000 u. It is relatively hydrophobic and aromatic in nature. The degree of polymerisation in nature is difficult to measure, since it is fragmented during extraction and the molecule consists of various types of substructures which appear to repeat in a haphazard manner. Different types of lignin have been described depending on the means of isolation.

There are three monolignol monomers, methoxylated to various degrees: *p*-coumaryl alcohol,coniferyl alcohol, and sinapyl alcohol. These lignols are incorporated into lignin in the form of the phenylpropanoids *p*-hydroxyphenyl (H), guaiacyl (G), and syringal (S) respectively. Gymnosperms have a lignin that consists almost entirely of G with small quantities of H. That of dicotyledonous angiosperms is more often than not a mixture of G and S (with very little H), and monocotyledonous lignin is a mixture of all three. Many grasses have mostly G, while some palms have mainly S [23]. All lignins contain small amounts of incomplete or modified monolignols, and other monomers are prominent in non-

woody plants. Thioglycolysis is an analytical technique for lignin quantitation. Lignin structure can also be studied by computational simulation.

Lignin is the most recalcitrant component of the plant cell wall, and the higher the proportion of lignin, the higher the resistance to chemical and enzymatic degradation. Generally, softwoods contain more lignin than hardwoods and most of the agriculture residues. There are chemical bonds between lignin and hemicellulose and even cellulose. Lignin is one of the drawbacks of using lignocellulosic materials in fermentation, as it makes lignocellulose resistant to chemical and biological degradation [25].

Lignin combines with hemicellulose materials to help bind the cells together and direct water flow. Lignin is formed by removal of water from sugars to create aromatic structures. These reactions are not reversible. Lignin resists attack by most microorganisms, and anaerobic processes tend not to attack the aromatic rings at all. Aerobic breakdown of lignin is slow and may take many days. Lignin is nature's cement along with hemicellulose to exploit the strength of cellulose while conferring flexibility.

2.8 Glucose

Glucose is by far the most common carbohydrate and classified as a monosaccharide, an aldose, a hexose, and is a reducing sugar. It is also known as dextrose, because it is dextrorotatory (meaning that as an optical isomer is rotates plane polarized light to the right and also an origin for the D designation.

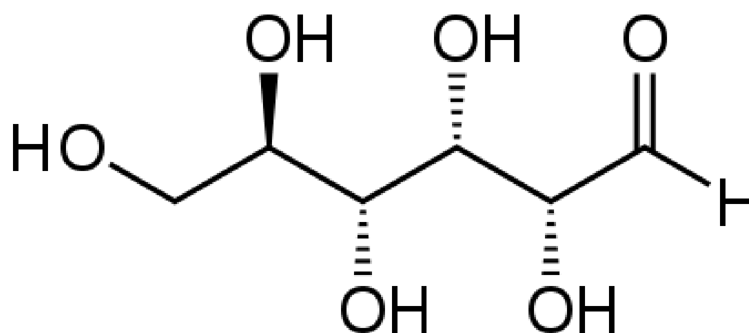


Figure 2.8 Chemical structure of D-glucose

Glucose is a monosaccharide with formula $C_6H_{12}O_6$ or H-(C=O)-$(CHOH)_5$-H, whose five hydroxyl (OH) groups are arranged in a specific way along its six-carbon backbone as shown in **Figure 2.8**. Glucose also known as D-glucose, dextrose, orgrape sugar is a simple and an important carbohydrate in biology. Cells use it as the primary source of energy and a metabolic intermediate. Glucose is one of the main products of photosynthesis and starts cellular respiration.

Glucose exists in several different structures, but all of these structures can be divided into two families of mirror-images (stereoisomers). Only one set of these isomers exists in nature, those derived from the "right-handed form" of glucose, denoted D-glucose. D-glucose is often referred to as dextrose. The term dextrose is derived from *dextrorotatory glucose*. Solutions of dextrose rotate polarized light to the right. Starch and cellulose are polymers derived from the dehydration of D-glucose. The other stereoisomer, called L-glucose, is hardly found in nature. The other open-chain isomer L-glucose similarly gives rise to four distinct cyclic forms of L-glucose, each the mirror image of the corresponding D-glucose.

The rings are not planar but twisted in three dimensions. The glucopyranose ring (α or β) can assume several non-planar shapes, analogous to the 'chair' and 'boat' conformations of cyclohexane. Similarly, the glucofuranose ring may assume several shapes, analogous to the 'envelope' conformations of cyclopentane.

The glucopyranose forms of glucose predominate in solution, and are the only forms observed in the solid state. They are crystalline colorless solids, highly soluble in water and acetic acid, poorly soluble in methanol and ethanol. They melt at 146°C (295 °F) (α) and 150 °C (302 °F) (β), and decompose at higher temperatures into carbon and water.

2.9 Cellulase

Cellulase refers to a class of enzymes produced chiefly by fungi, bacteria, and protozoans that catalyze the cellulolysis (or hydrolysis) of cellulose. However, there are also cellulases produced by other types of organisms such as plants and animals [26].

Successful utilization of cellulosic materials as renewable carbon sources is dependent on the development of economically feasible process technologies for

cellulase production, and for the enzymatic hydrolysis of cellulosic materials to low molecular weight products such as hexoses and pentoses. Cellulase production was the most expensive step during ethanol production from cellulosic biomass, in that it accounted for approximately 40% of the total cost. Significant cost reduction is required in order to enhance the commercial viability of cellulase production technology [27].

A cellulosic enzyme system consists of three major components: endo-ß-glucanase (EC 3.2.1.4), exo-ß-glucanase (EC 3.2.1.91) and ß-glucosidase (EC 3.2.1.21). The mode of enzymatic action of each component in as follows,

(1) Endo-ß-glucanase (1,4-ß-D-glucan glucanohydrolase or CMCase, Cx) could provide "random" scission of cellulose chains to yield glucose and cello-oligo saccharides.

(2) Exo-ß-glucanase (1,4-ß - D-glucan cellobiohydrolase or Avicelase, C1) can exo-attack on the non-reducing end of cellulase with cellobiose as the primary structure.

(3) ß-glucosidase (cellobiase) can be used for hydrolysis of cellobiose to glucose.

According to **Figure 2.9**, in a synergistic sequence of events, endo-ß-glucanase acts randomly on the cellulose chain, while exo-ß-glucanase acts on exposed chain ends by splitting off cellobiose or glucose. Cellobiose is subsequently hydrolysed by p-glucosidase to glucose.

Although a large number of microorganisms are capable of degrading cellulose, only a few of these microorganisms produce significant quantities of cell-free enzymes capable of completely hydrolysing crystalline cellulose *in vitro.* Fungi are the main cellulase-producing microorganisms, though a few bacteria and actinomycetes have also been recently reported to yield cellulase activity. Microorganisms of the genera *Trichoderma* and *Aspergillus* are thought to be cellulase producers, and crude enzymes produced by these microorganisms are commercially available for agricultural use. Microorganisms of the genus *Trichoderma* produce relatively large quantities of endo-ß-glucanase and exo-ß-

glucanase, but only low levels of ß-glucosidase, while those of the genus *Aspergillus* produce relatively large quantities of endo-ß-glucanase and ß-glucosidase with low levels of exo-ß- glucanase production.

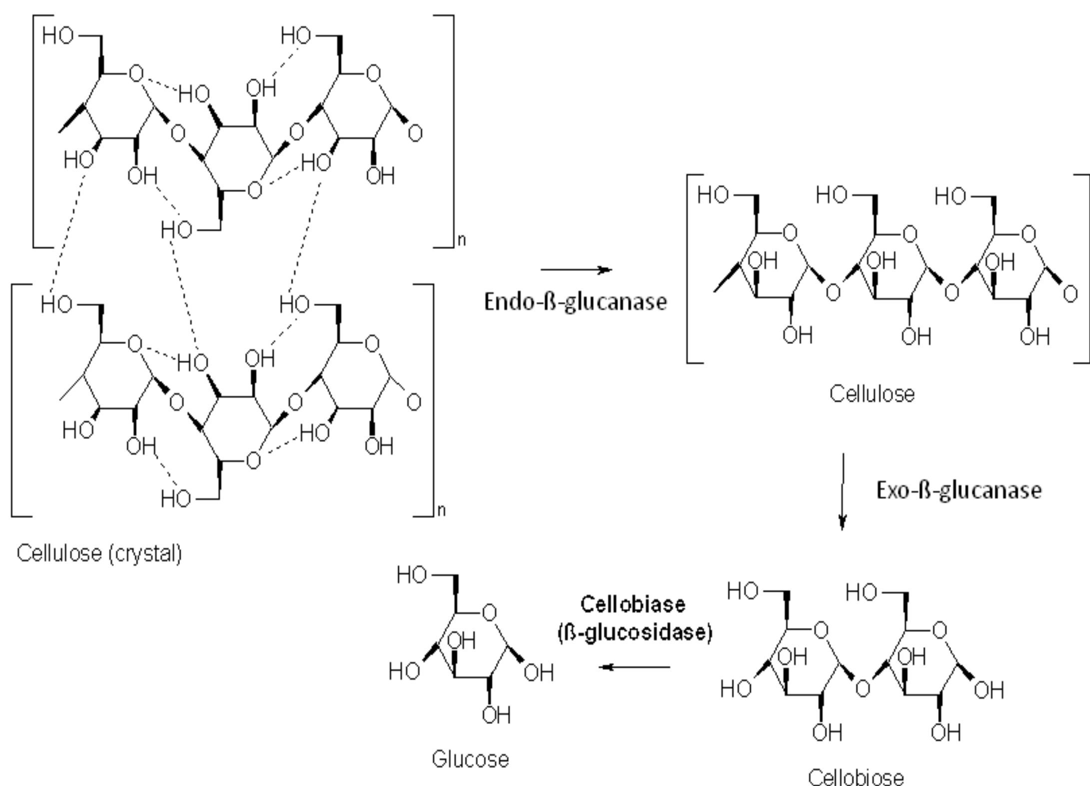


Figure 2.9 Schematic representation of sequential stages in cellulolysis

2.10 The reaction rate constant

In chemical kinetics a reaction rate constant k or λ quantifies the speed of a chemical reaction. For a chemical reaction where substance A and B are reacting to produce C, the reaction rate has the form:

Reaction: $A + B \rightarrow C$

$$\frac{d[C]}{dt} = k(T)[A]^m[B]^n$$

k(T) is the reaction rate constant that depends on temperature.

[C] is the concentration of substance C in moles per volume of solution assuming the reaction is taking place throughout the volume of the solution (for a reaction taking place at a boundary it would denote something like moles of C per area).

The exponents *m* and *n* are called orders and depend on the reaction mechanism. They can be determined experimentally.

A single-step reaction can also be written as

$$\frac{d[C]}{dt} = Ae^{\frac{-E_a}{RT}}[A]^m[B]^n$$

E_a is the activation energy and R is the Gas constant.

Since at temperature T the molecules have energies according to a Boltzmann distribution, one can expect the proportion of collisions with energy greater than E_a to vary with $e^{-Ea/RT}$. A is the pre-exponential factor or frequency factor.

The Arrhenius equation gives the quantitative basis of the relationship between the activation energy and the reaction rate at which a reaction proceeds.

CHAPTER III

EXPERIMENTAL

3.1 Experimental apparatus

3.1.1 Ball-mill hydrothermal reactor (BMHR)

Water hyacinth leaves was pretreated at the Hiroshima University using ball-mill hydrothermal reactor (BMHR) as shown in **Figure 3.1**.

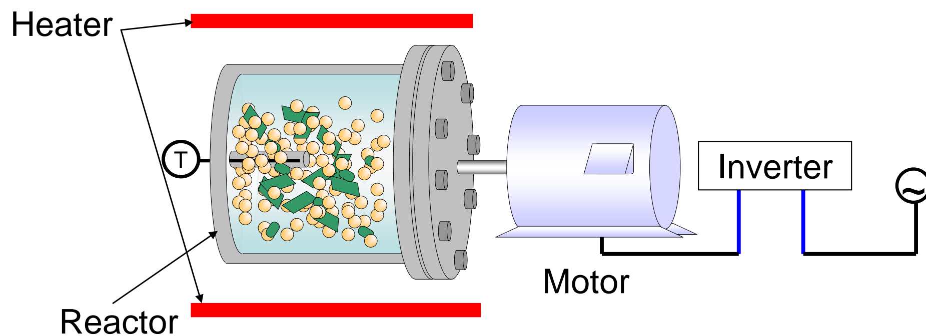


Figure 3.1 Schematic of ball-mill hydrothermal reactor (BMHR)

According to the advantage of hydrothermal treatment in section 1.1.5, the goal of hydrothermal treatment is to break the lignin seal and disrupt the crystalline structure of cellulose. Meanwhile, the grinding method is found to be an effective in reducing the crystallinity of cellulose and breaking the lignin seal. Thus, grinding is performed under hydrothermal treatment conditions. They should be able to improve the characteristics of the hydrothermal reaction by performing the milling and expect to achieve by grinding at low power, while dissolving the hemicellulose and lignin. There are many type of grinding method such as the mill grinding disks, ball mill, cutter mill, and mixer mill. In particular, the ball mill is simple form grinding method. Therefore, it was employed as each thought best to hydrothermal treatment.

As seen in **Figure 3.1**, the ball-mill hydrothermal reactor (BMHR) employed in this study. **Figure 3.2** shows the equipment installation. For the hydrothermal

treatment section, autoclave was a batch reactor as shown in **Figure 3.3**. After connecting reactor, motor, and thermocouple type K together, the reactor was heated up by electric furnace which was controlled by control panel as shown in **Figure 3.4**. For the pulverization section, reactor was rotated by motor as shown in **Figure 3.5** which was controlled by inverter as shown in **Figure 3.6**.

Figure 3.2 Equipment installation

Figure 3.3 Reactor

Figure 3.4 Control panel

Figure 3.5 Motor

Figure 3.6 Inverter

Thermo recorder

The thermo recorder has been measured using temperature data loggers manufactured midi LOGGER GL200A GRAPHTEC as shown in **Figure 3.7**. External USB memory was used to save the temperature using for a computer. The data in the USB memory was installed by using "midi LOGGER GL200A USER GUIDE CD-ROM" software to convert to Excel file.

Figure 3.7 Data logger

3.1.3 Autoclave reactor

Water hyacinth leaves was treated at the Hiroshima University using autoclave reactor which is another type of apparatus as shown in **Figure 3.8**.

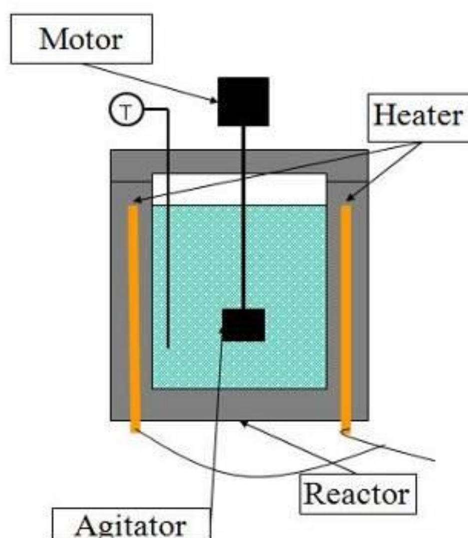


Figure 3.8 Schematic of autoclave

As seen in **Figure 3.8**, the autoclave employed in this study. The inner volume of the autoclave is 96 cm^3. Water hyacinth was loaded in the autoclave with de-ionized water. After starting the agitator, the autoclave was heated up by the embedded heaters as show in **Figure 3.9**. When the temperature reached the target value, the heaters were turned off and the reactor was cooled with the air fan. The sample was mixed well by pretreatment agitator was set at 500 rpm. Studied on the effect of temperature while the temperature profile was recorded by THERMODAC EF Model 5021A as shown in **Figure 3.10**.

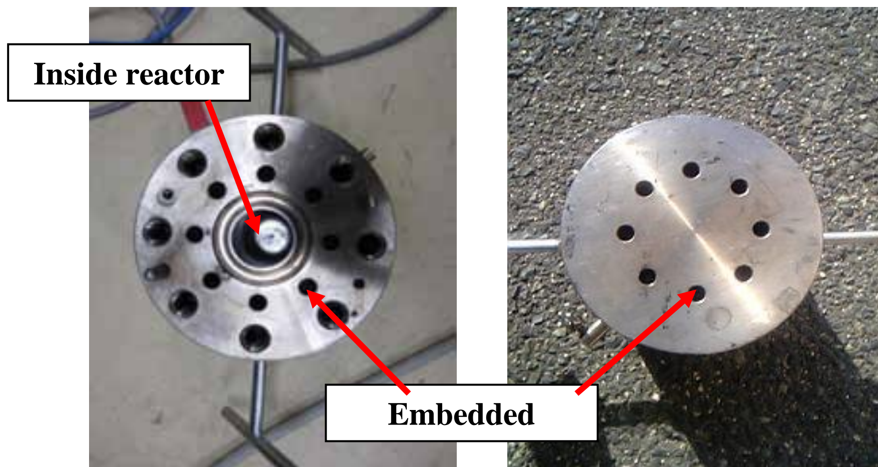


Figure 3.9 Autoclave reactor and 8 embedded heaters

Figure 3.10 Temperature recorder

3.2 Experimental procedure.

Figure 3.11 shows an overview of experimental works which could be divided into 3 parts, which are a) Hydrothermal treatment, b) Enzymatic hydrolysis, and c) Analysis. Summarized procedure of each part is deliberated below.

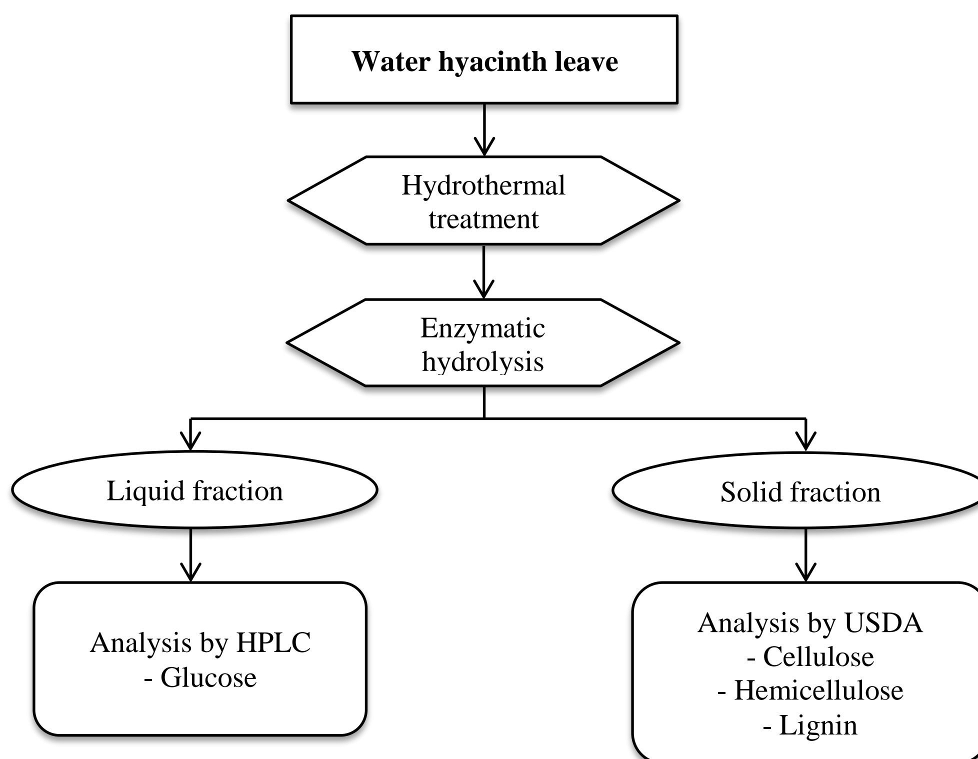


Figure 3.11 Overview of experimental works

3.2.1 Hydrothermal treatment

Figure 3.1 shows the ball mill hydrothermal reactor (BMHR) to be employed in this study. Specific amount of dried water hyacinth leaves and de-ionized water were loaded alternately with a determined amount of ball mill in the reactor. Before start the experiment, the reactor would be linked to the motor. Temperature would be recorded every 30 second by using a data logger and saved into USB. The reactor was heated up by electric furnace. Their target temperatures were 160, 180, 200, and 220°C. The sample solution after hydrothermal treatment would be subject to enzymatic hydrolysis. The sample after enzymatic hydrolysis would be separated to liquid fraction and solid fraction. The liquid fraction would be analyzed by HPLC

analysis in order to find glucose. The solid fraction would be analyzed by USDA method [16] in order to find the amount of cellulose, hemicelluloses, and lignin.

3.2.2 Enzymatic hydrolysis

Cellulase from Aspergillus niger powder (≥ 0.3 units/mg-solid, Sigma-Aldrich product) has been used in this study. **Table 3.1** shows cellulase treatment condition. 35 g of sample solutions after the hydrothermal treatment have been used as reactant in this step. The treated sample, cellulase, and buffer fluid solution will be put into a conical flask and the flask was shaken at 250 rpm at 37°C for 2 days by using SHAKING INCUBATOR SI-300R as shown in **Figure 3.12**. In each run, the liquid product will be sampled every 24 h, and was sterilized immediately after applying to analyzed glucose by HPLC. Buffer fluid solution will be prepared from acetic acid, sodium hydroxide and de-ionized water, of which pH is set at 5.

Table 3.1 Conditions for cellulase treatment

Reactant (g)	35
Buffer fluid (cm^3)	60
10 g/dm^3 cellulase solution (cm^3)	5

Figure 3.12 Shaking incubator

Sterilization process was intended to stop the enzymatic reaction by using steam autoclave (Autoclave SP200, YAMATO) as shown in **Figure 3.13**. The process is summarized below.

Figure 3.13 Steam autoclave

1. Press the start button on the unit steam sterilization prior. The samples were loaded when the temperature inside steam autoclave reached to 100°C. Before running, please make sure there is enough water to flood the base.
2. 2 mL of pretreated samples was loaded in the steam autoclave by injecting into the vessel. Vessel was be dried before loaded the samples into steam autoclave and closed tightly.
3. When the temperature and pressure was about 105 °C and 0.03 MPa, respectively. The sterilization was complete. Sterilized samples were finished and kept it in the freezer.

3.2.3 Product analyses

Figure 3.14 gives an overview of the product analyses used in this study. The reaction products are divided into liquid and solid as follows.

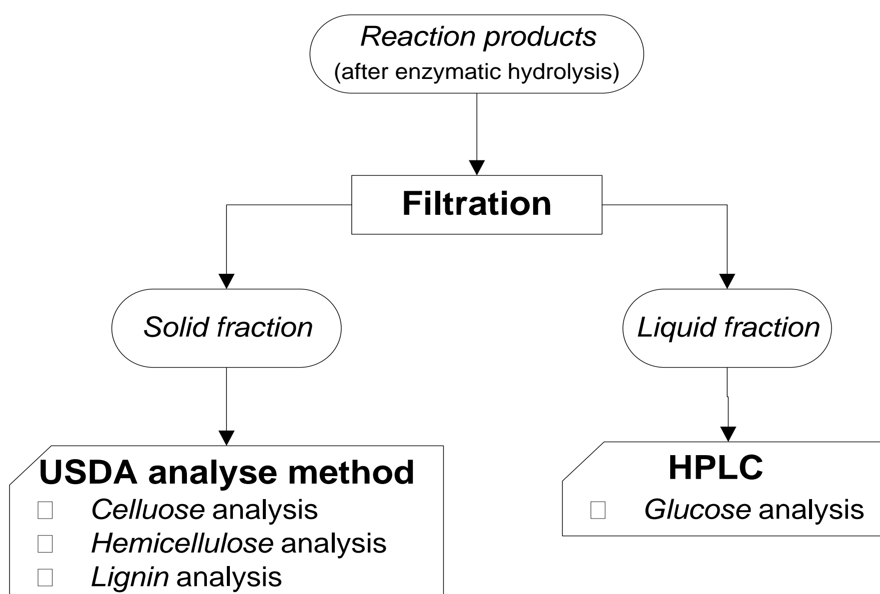


Figure 3.14 Product analyses

3.2.3.1 Liquid fraction

3.2.3.1 (a) pH

The pH of the liquid effluent was measured by a pH meter. A pH meter was calibrated with pH 4.01 and pH 7.01 buffer solution before each use.

3.2.3.1 (b) High Performance Liquid Chromatography (HPLC)

First, the liquid effluent was filtered to obtain a clear liquid product by minisart (RC-membrane 15, pore size: 0.20 µm). Glucose was analyzed by HPLC using a sugar KS-802 column (Shodex) as shown in **Figure 3.15**. The analytical conditions were as follows: flow rate: 0.8mL/min; eluent water; oven temperature: 60°C; RID.

Figure 3.15 HPLC analysis

3.2.3.2 Solid fraction

3.2.3.2 (a) Filtration

The solid product was the particles suspended in the liquid effluent. The solid fraction was obtained by filtering the liquid effluent through a filter paper (Advantec, 0.1 mm pore size; Millipore) and air dried at room temperature and weighed. Deionized water was added several times to ensure that no trace of liquid product was left on the filter paper.

3.2.3.2 (b) USDA analyzes method (United States Department of Agriculture)

After the filtration, the filtered solid particles after hydrothermal treatment and after enzymatic hydrolysis are analyzed to measure the cellulose content, hemicellulose content, lignin content and ash by the procedure recommended by the United States Department of Agriculture (USDA) [16].

The acid-detergent fiber procedure provides a rapid method for lignocellulose determination in the sample. First, the acid fiber is used as a method to measure the hemicellulose for the weight loss by this treatment; however it does include some

protein attached to cell walls. This process is a preparatory step for further lignin determination.

Permanganate lignin is the method to determine lignin and was developed to make it possible for the preparation of cellulose and insoluble ash in the same sample. The advantages of this method over other methods are that it takes shorter procedure time while the residue is reserved for further analysis of cellulose and ash, and that it is less corrosive. However, the disadvantage of this method is that large particles are poorly penetrated by the reagents and yield low values. The sample, therefore, must be dried and ground to a particle size less than 1 mm. (pass through a 20-30 mesh). The excess of acetic acid-buffered potassium permanganate solution, which contains trivalent iron and monovalent silver as catalysts, oxidizes lignin. Deposited manganese and iron oxides are dissolved with an alcoholic solution of oxalic and hydrochrolic acids, which leaves cellulose and insoluble minerals mainly silica. Lignin is measured for the weight loss by this treatment.

Cellulose is determined its weight loss upon the ashing. The sample is loaded in the furnace with the temperature 500-550°C as shown in **Figure 3.16**. Complete ashing of organic matter is accomplished in 2-3 hours. The ash residue is mainly silica.

Figure 3.16 Furnace

3.3 Component analysis

The cellulose content of biomass to be subject hydrothermal treatment could be determined following the procedure recommended by the United States Department of Agriculture (USDA) in part 3.2.3.2 (b). In this work, water hyacinth leaves would also be analyzed by USDA to get preliminary information of its component. **Table 3.2** shows the components of the water hyacinth leaves (dry weight basis) determined by the USDA's method [16].

Table 3.2 Composition of water hyacinth feedstock

Cellulose	0.28
Hemicellulose	0.62
Lignin	0.02
Ash	0.08

3.4 Apparent reaction network for the hydrothermal treatment

In this study, the hydrothermal treatment of cellulose using a ball mill hydrothermal reactor, cellulase reaction is carried further in a subsequent stage.

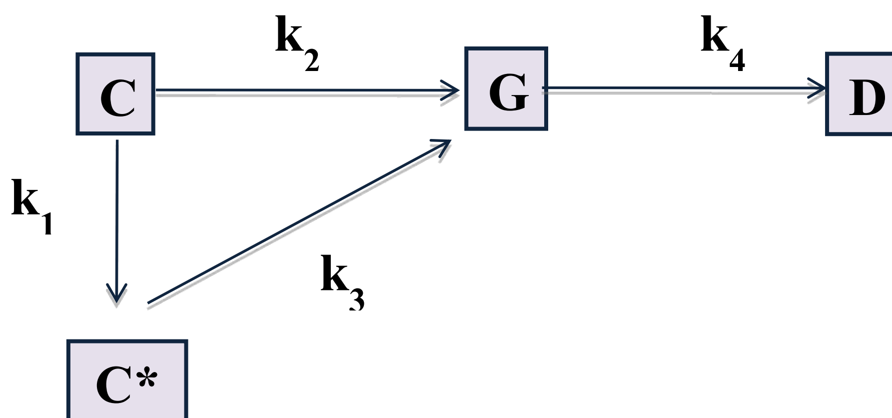


Figure 3.17 Reaction scheme [18]

To explain the complex phenomena that are taking place in hydrothermal treatment, the network of the apparent reactions was proposed as shown in **Figure 3.17** and **Figure 3.18**. In **Figure 3.17**, C denotes non- hydrolyzable cellulose that

cannot be converted into glucose by cellulase treatment. The cellulose is the starting material without hydrothermal treatment; almost no glucose is obtained even if the water hyacinth was treated with cellulase enzyme. Thus the cellulose in the water hyacinth can be considered as a practically nonreactive form in terms of cellulase treatment due to the lignin and hemicellulose covering the cellulose.

By hydrothermal treatment, some part of treated cellulose can be converted into hydrolyzable cellulose in the first stage and further converted into glucose in the second step by cellulase enzyme as shown in **Figure 3.17**. C* denotes treated cellulose that can be converted into glucose by cellulase treatment. This change can be treated as a kind of reaction, combination of physical and chemical phenomena: dissolution of hemicellulose and part of lignin in hot compressed, reduction in crystallinity of the cellulose, destruction of cell structure, introduction of water molecules into the piece of biomass, and so forth[18]. In addition, part of the cellulose can be directly hydrolyzed into glucose hydrothermally. G denotes glucose. However, some of produced glucose can be further decomposed in hydrothermal treatment. D denotes decomposition products, which is a mixture of various compounds.

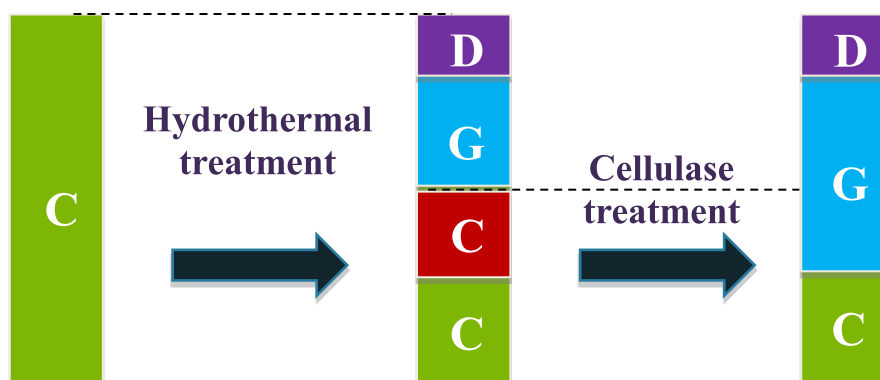


Figure 3.18 Change of cellulose in the hydrothermal pretreatment and enzymatic hydrolysis process

It is suspected that acetic acid appears as a consequence of hydrolysis of acetyl group from hemicellulose fraction of biomass, which decreases the pH of the liquid and enhance the effect of hydrothermal treatment. Unfortunately, the pH of liquid is

not measured in this study. But the reaction rate parameters obtained in this study are the overall value including this effect.

In this study, water hyacinth is treated hydrothermally at 160-220°C in a ball-mill hyreactor, to determine the kinetic parameters and to predict the optimal condition for hydrothermal treatment.

In the analysis of the experimental results, the yields of all compounds shown in **Figure. 3.18** were calculated based on the amount of glucose in the cellulose in the raw water hyacinth as follows.

Glucose yield, X_G was determined by dividing the amount of glucose in the liquid fraction obtained from HPLC, n_G by the amount of glucose in the cellulose in the raw water hyacinth, n_{C0}

$$X_G = \frac{n_G}{n_{C0}} \quad (1)$$

The hydrolysable cellulose yield that can be hydrolysed by cellulase treatment, X_{C*} was determined by dividing the amount of glucose increased by the cellulase treatment in the liquid fraction obtained from HPLC, n_{G*} by the amount of glucose in the cellulose in the raw water hyacinth, n_{C0}.

$$X_{C*} = \frac{n_{G*}}{n_{C0}} \quad (2)$$

The non-hydrolysable cellulose yield that cannot be hydrolysed by cellulase treatment, X_C was determined by

$$X_C = \frac{n_C - n_{C*}}{n_{C0}} \quad (3)$$

Where n_C denotes the amount of glucose unit in the cellulose left after hydrothermal treatment. Then, the yield of decomposition products, XD was determined by

$$X_D = 1 - X_G - X_{C*} - X_C \quad (4)$$

3.5 Experimental condition

3.5.1 Reaction temperature in a range of 160 – 220°C

The temperature is important parameter for determine the reaction rate parameter. In this studied, first will studied on the effect of temperature on hydrothermal treatment of water hyacinth leaves. By varies in a range of 160 – 220°C for find the optimal temperature for this process. The water hyacinth leaves was used in this studied.

In our previous work, Nakashima's research found the optimal condition of the same ball-mill hydrothermal reactor as shown in **Figure 3.19** to **Figure 3.23**. The best condition of his research which are temperature at 220°C, 10wt% of feedstock concentration, 0.5 kg of ball loading, no effect of ball diameter, and 200 rpm of rotation speed. Therefore, in this study, we used the best condition of Nakashima's research to improve the glucose yield and find the optimal condition for this process. Therefore, **Table 3.3** shows the experimental condition for this study on effect of temperature.

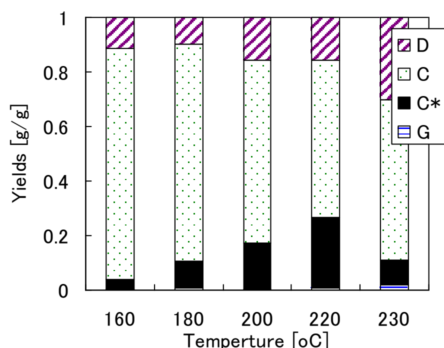


Figure 3.19 Effect of target temperature

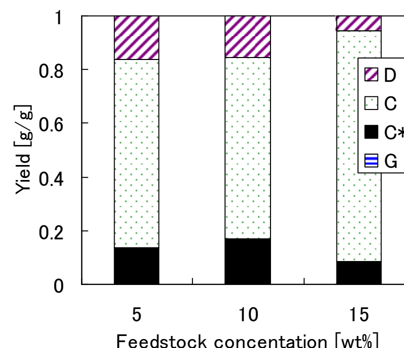


Figure 3.20 Effect of feedstock concentration

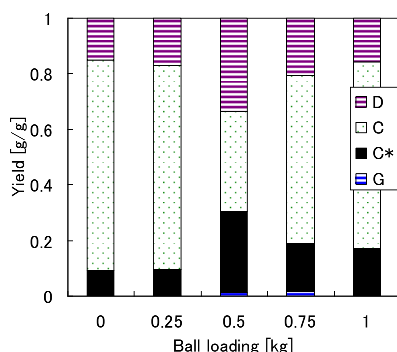


Figure 3.21 Effect of ball loading

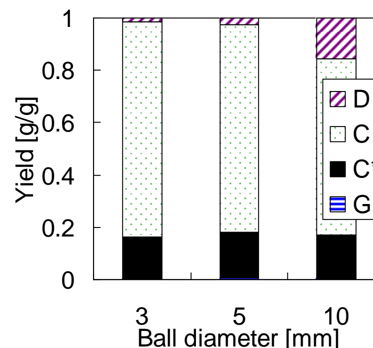


Figure 3.22 Effect of ball diameter

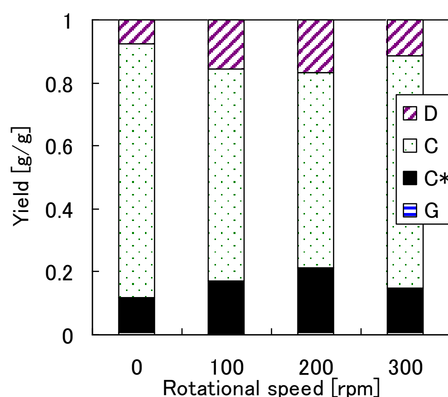


Figure 3.23 Effect of rotational speed

Table 3.3 Temperature conditions for hydrothermal treatment

Feedstock	Water hyacinth leaves
Target temperature	160, 180, 200, 220^oC
Feedstock concentration	10wt%
Ball material	ZrO2
Ball loading	0.5 kg
Ball diameter	10 mm
Holding time	0, 5, 10 min
Rotating speed	200 rpm

3.5.2 The effect of treatment time on hydrothermal treatment

Varies the treatment time of 0, 5, and 10 minute. Compared with the previous condition, when we know the optimal temperature for hydrothermal treatment. The optimal temperature was used in this condition for studied for the effect of treatment time on hydrothermal treatment. **Table 3.4** shows the treatment time condition.

Table 3.4 Treatment time conditions for hydrothermal treatment

Treatment time	0, 5, 10 min
Feedstock	Water hyacinth leaves
Feedstock concentration	10wt%
Target temperature	200^oC

3.5.3 The effect of adding acetic acid on hydrothermal treatment

Acetic acid is organic, produced by fermentation, and can be decomposed by fermentation. They are much good catalyst, but we have to be quantitative. **Table 3.5** shows the adding acetic acid condition for hydrothermal treatment.

Table 3.5 Adding acetic acid for hydrothermal treatment

Acetic acid concentration	0.5, 0.75, 1.0wt%
Feedstock	Water hyacinth leaves
Feedstock concentration	10 wt%
Target temperature	160, 180, 200, 220°C

3.6 Materials

All of the chemicals employed in this study were of high purity and used without further purification. Deionized water was used to prepare all aqueous feedstock (< 1 uS/cm; Organo water deionizer model BB-5A). Water hyacinth (raw material) was dried in the oven as shown in **Figure 3.24** at 105°C to remove the moisture content. The dried water hyacinth was loaded into the reactor follow by zirconium ball as shown in **Figure 3.25** and **Figure 3.26**, respectively. The HPLC analysis was also conducted to reassure its concentration before/after the experiments. Anhydroglucose (99%) of analytical grade is used to prepare the HPLC standard solution.

Figure 3.24 Oven

Figure 3.25 Dried water hyacinth

Figure 3.26 Loading raw material

CHAPTER IV

RESULTS AND DISCUSSION

As mention in previous chapter, glucose is known as product of the enzymatic hydrolysis of the cellulose, which is one of the main components of lignocellulosic biomass. Due to the complex structure of biomass, hydrothermal treatment is employed as a treatment to improve the accessibility of enzyme and decrease the crystallinity of cellulose. Higher glucose yield is usually obtained at the high temperature and moderate catalyst concentration. Our study also revealed that high temperature and moderate catalyst concentration are needed for the effective treatment. In this chapter, the effects of temperature and concentration of catalyst on hydrothermal treatment of water hyacinth leaves were investigated and discussed. Two types of reactors, namely autoclave and ball-mill hydrothermal reactor (BMHR) were employed for converting water hyacinth leaves into glucose. The reaction network of cellulose was developed.

4.1 Effect of target temperature on glucose yield

4.1.1 Autoclave reactor

First, we used an autoclave reactor to acquire basic data for hydrothermal treatment process of water hyacinth leaves. Water hyacinth leaves were chopped and ground by pulverizer. Ground water hyacinth leave with an average particle size of 0.15 to 0.25 mm. Ground water hyacinth leave hydrothermally treated under conditions of temperature from 130 to 270°C.

Figure 4.1 shows the glucose yield after hydrothermal treatment process varied with different temperature in the range of 130–270°C in autoclave reactor. It is clear that the glucose yield after hydrothermal treatment is less than 0.016.

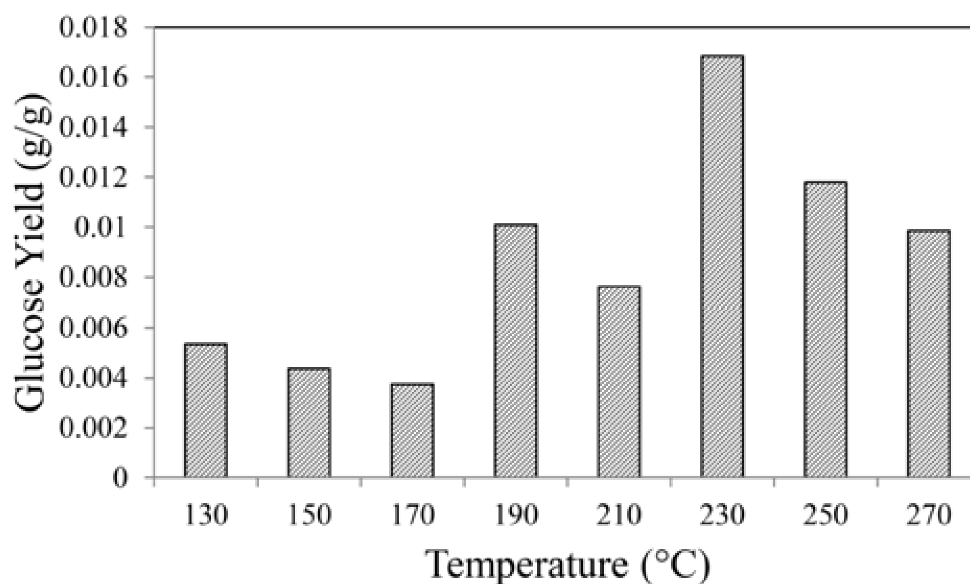


Figure 4.1 Glucose yield obtained from hydrothermal treatment varied with different temperature in the range of 130–270°C in autoclave reactor

After hydrothermal treatment, slurry products with distinctively different appearance could be obtained. 35 grams of slurry products after the hydrothermal treatment were used as reactant in enzymatic hydrolysis.

Figure 4.2 shows the glucose yield with respect to enzymatic hydrolysis (cellulase reaction) time in autoclave reactor. The glucose yield after hydrothermal treatment is shown at the time of cellulase reaction of 0 hour. The glucose yield increases with time during the enzymatic hydrolysis. In this study, the glucose yield after a 48-hours cellulase treatment was employed as the final yield. Since in the previous study, the 48-hours cellulase treatment yield was the maximum value.

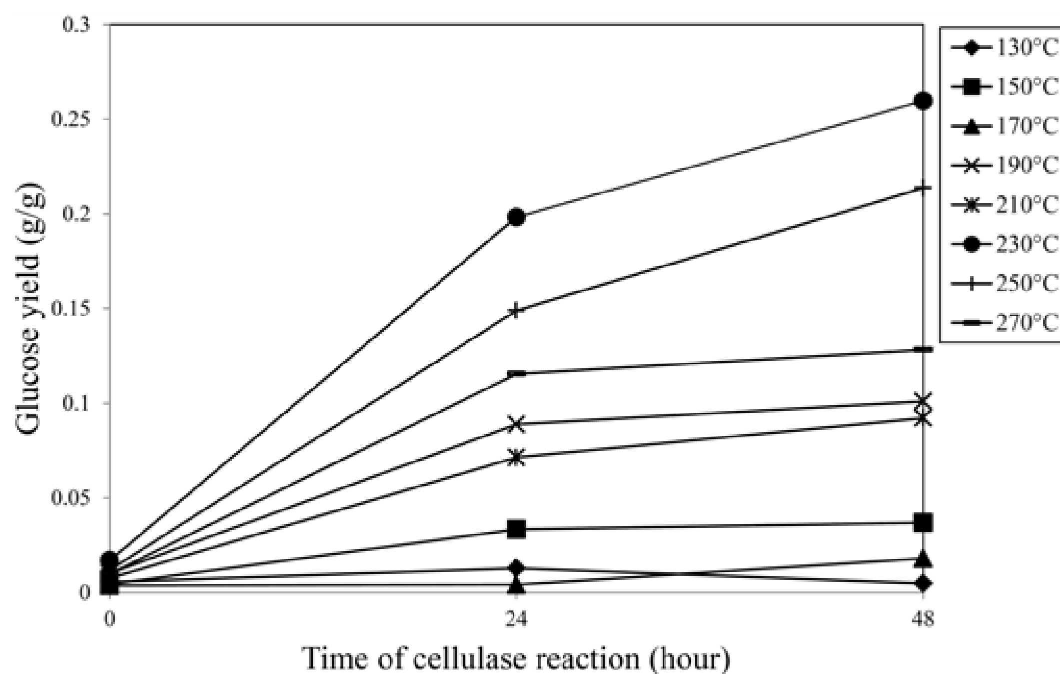


Figure 4.2 Glucose yield obtained from enzymatic hydrolysis varied with different temperature in the range of 130–270°C in autoclave reactor

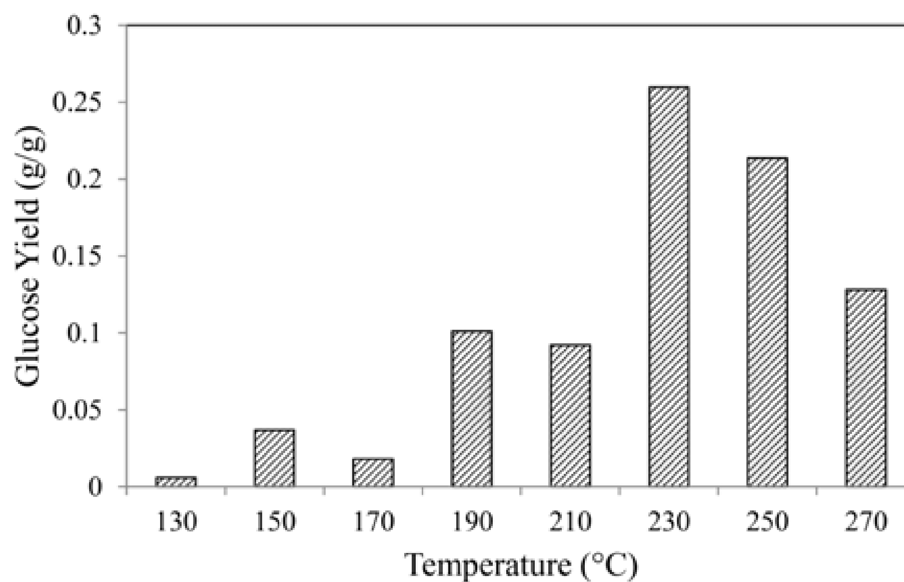


Figure 4.3 Total glucose yield acquired from hydrothermal treatment and enzymatic treatment of water hyacinth pretreated in autoclave at difference temperature

Figure 4.3 shows total glucose yield acquired from hydrothermal treatment and enzymatic treatment of water hyacinth pretreated in autoclave at difference temperature. The experimental results after enzymatic hydrolysis revealed that at the

higher target temperature, the higher glucose yield were obtained. The highest glucose yield of 25.9% was obtained at 230°C. However, decreasing of glucose was occurred at 250°C.

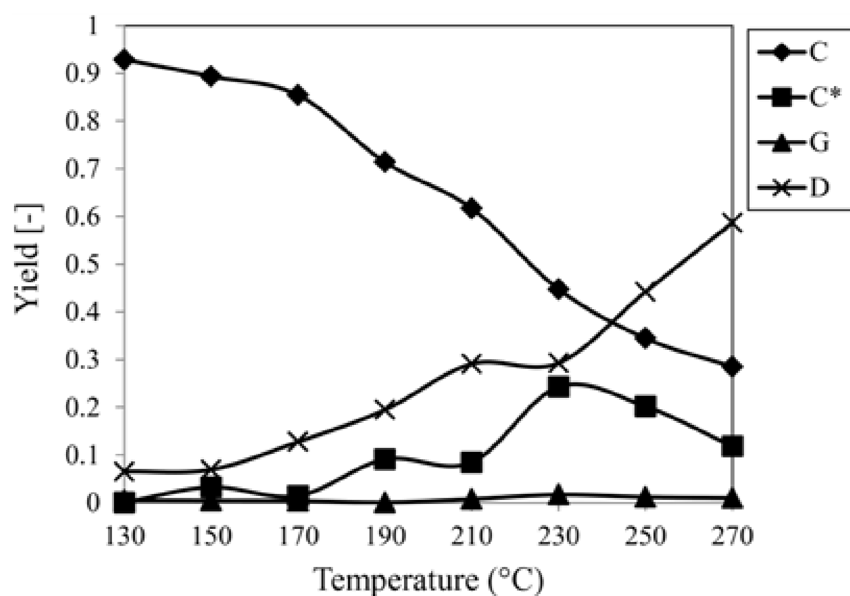


Figure 4.4 Components of final products acquired from hydrothermal treatment at various target temperature in autoclave reactor

Effect of temperature in hydrothermal treatment on the yield of glucose obtained by hydrothermal treatment was shown in **Figure 4.4**. Most of the glucose would possibly be decomposed with a longer elapsed time, resulting in a decreased in the glucose concentration. It should be note that the final glucose yield increased when the target temperature of the hydrothermal treatment were increased from 130 to 230°C. However at 250°C the glucose was further decomposed leading to the glucose content in the autoclave.

The high amounts of decomposition products of glucose such as 5-HMF, furfurals, and acid compounds occurred when the target temperature increased [28], while the amounts of non-hydrolysable cellulose also decreased.

Base on there results, we knew that we could get the glucose from water hyacinth leaves in hydrothermal treatment and enzymatic hydrolysis process. In order to examine the possibility to convert water hyacinth leaves to glucose in a large-sealed process, we employed a new reactor which is ball-mill hydrothermal reactor (BMHR). The similar operating conditions were carried out in the BMHR. It should

be noted that the BMHR could be operated to achieve both hydrothermal treatment and ball mill pulverization simultaneously.

4.1.2 Ball-mill hydrothermal reactor (BMHR)

Dried water hyacinth leaves were hydrothermally treated under conditions of temperature from 160 to 220 °C for ball-mill hydrothermal reactor (BMHR).

After hydrothermal treatment within the BMHR slurry products with distinctively different appearance could be obtained. All experimental conditions including temperature, weight of water hyacinth leaves and de-ionized water are summarized in **Table 3.3**.

Similar to 4.2.1, 35 grams of slurry products after the hydrothermal treatment have been used as reactant in enzymatic hydrolysis. Physical appearance of slurry products obtained after hydrothermal treatment and liquid samples obtained after enzymatic hydrolysis were shown in the **Figure 4.5** to **Figure 4.7**. The characteristics of slurry products after hydrothermal treatment were summarized in **Table 4.1**. With treating temperature of 160°C, 180°C, and 200°C, the solid product had an appearance of coast brown particle. However, with the treating temperature of 220°C, the solid products were transformed to fine brown particle. Change of solid product appearance after the hydrothermal treatment would be resulted from the degradation of cellulose to various carbon derivatives [29]. At low temperature the particle size was bigger than the particle size at high temperature because the crashing time at high temperature was longer because it took longer to achieve the higher target temperature.

Table 4.1 Characteristic of slurry products after hydrothermal treatment

	(a) 160°C	(b) 180°C	(c) 200°C	(d) 220°C
Particle size	rough	rough	rough	fine
Color	brown	brown	brown	dark-brown

Figure 4.5 Sample after hydrothermal treatment

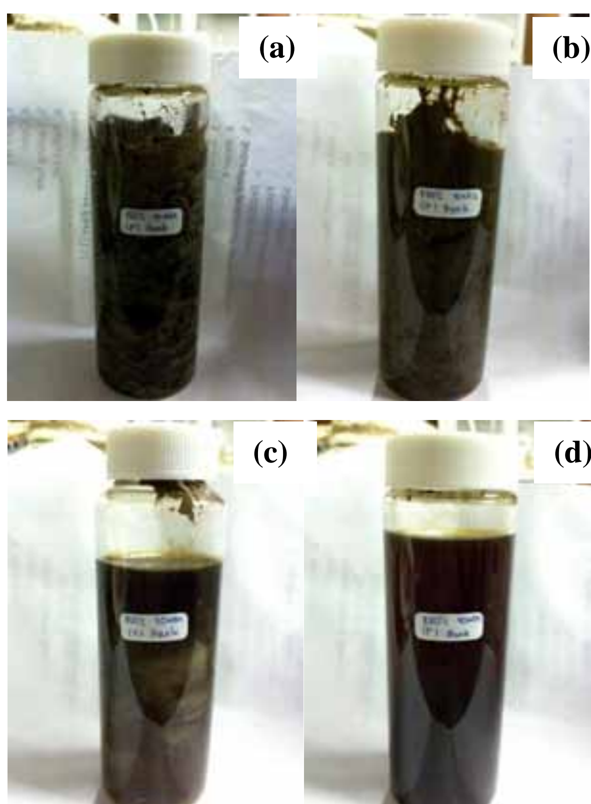


Figure 4.6 Slurry products after hydrothermal treatment at temperatures; (a) 160°C, (b) 180°C, (c) 200°C, and (d) 220°C

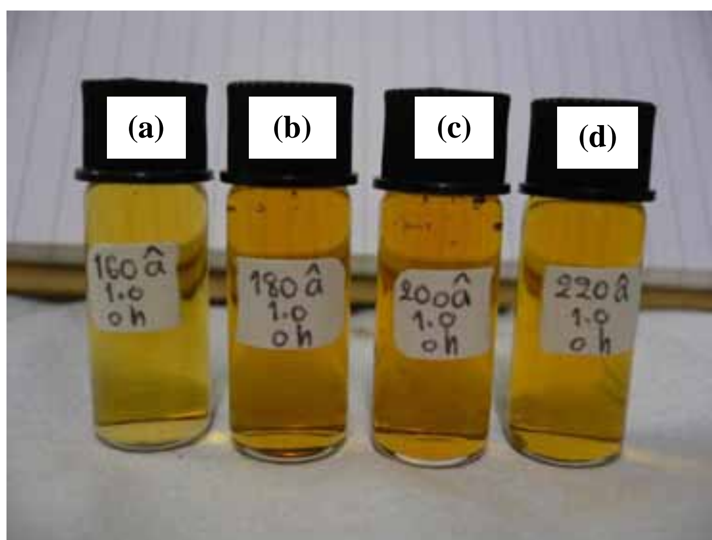


Figure 4.7 Liquid samples after enzymatic hydrolysis at temperatures; (a) 160°C, (b) 180°C, (c) 200°C, and (d) 220°C

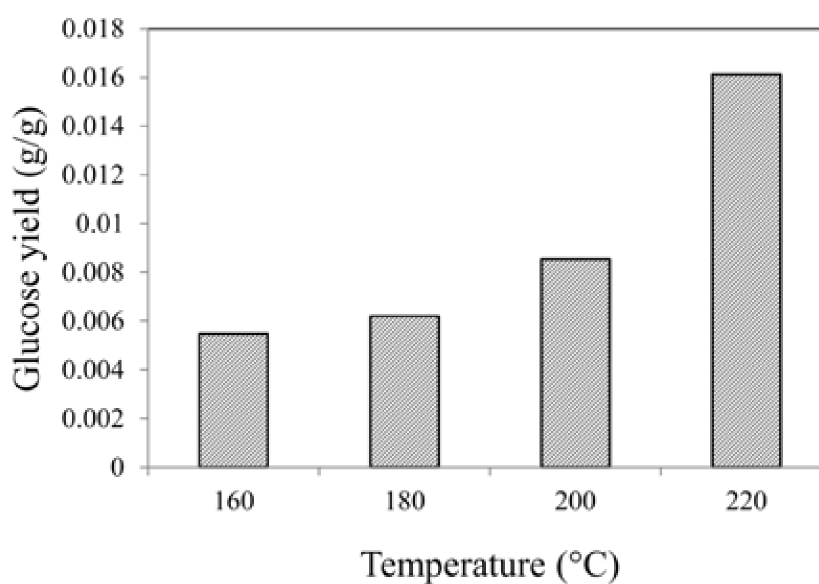


Figure 4.8 Glucose yield obtained from hydrothermal treatment varied with different temperature in the range of 160–220°C in BMHR

Figure 4.8 shows the glucose yield after hydrothermal treatment process varied with different temperature in the range of 160–220°C in BMHR. It is clear that the glucose yield after hydrothermal treatment is less than 0.016.

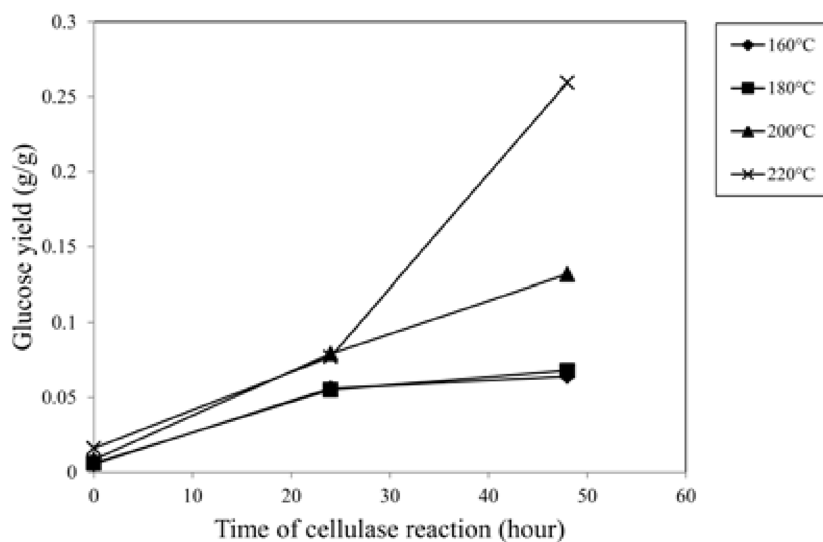


Figure 4.9 Glucose yield obtained from enzymatic hydrolysis varied with different temperature in the range of 160–220°C in BMHR

Figure 4.9 shows the glucose yield obtained from liquid fractions which were further treated by enzymatic hydrolysis (cellulase reaction) in ball-mill hydrothermal reactor (BMHR). The glucose yield after hydrothermal treatment is shown at the time of cellulase reaction of 0 s. The glucose yield increases with time during the enzymatic hydrolysis.

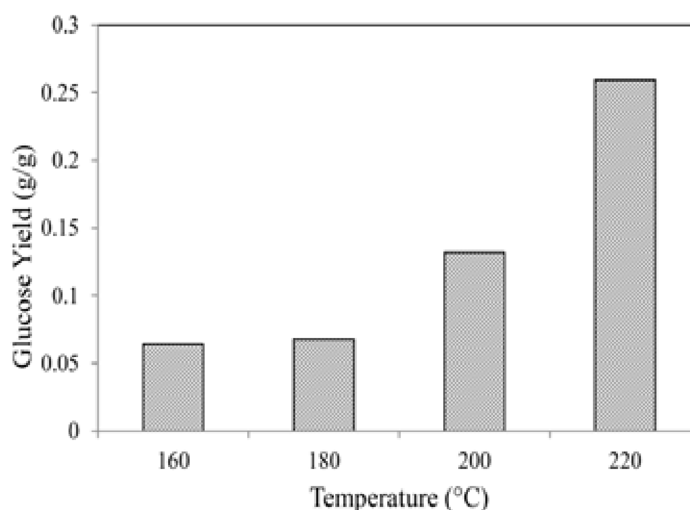


Figure 4.10 Total glucose yield acquired from hydrothermal treatment and enzymatic treatment of water hyacinth leaves treated in BMHR at difference temperature

Figure 4.10 shows total glucose yield at various target temperature of water hyacinth leaves by using BMHR. The experimental results revealed that at the higher target temperature, the higher glucose yield were obtained. The highest glucose yield of 26.7% was obtained at 220°C for BMHR.

However, the high amounts of decomposition products of glucose such as 5-HMF, furfurals, and acid compounds occurred when the target temperature increased, while the amounts of non-hydrolysable cellulose (C) also decreased as shown in **Figure 4.11**.

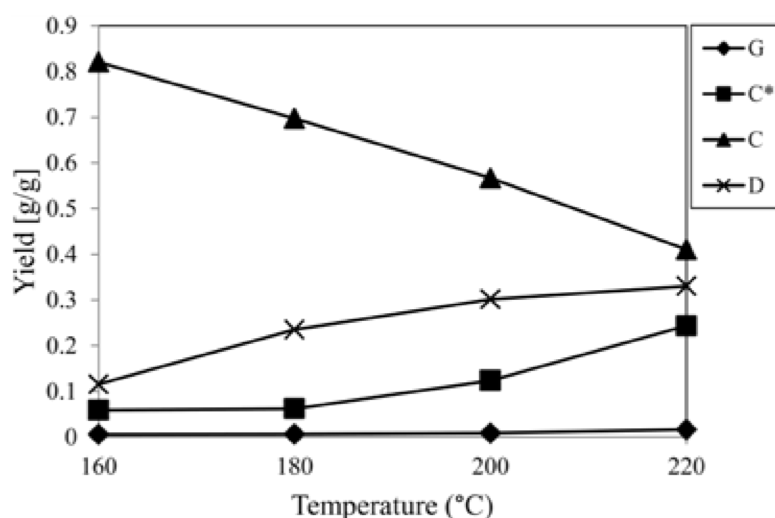


Figure 4.11 Components of final products acquired from hydrothermal treatment at various target temperature in BMHR

Figure 4.11 shows the increase in the hydrolysable cellulose yield with temperature was also well presented for all the cases. It was observed that with increasing target temperature, the hydrolysable cellulose yield (C*) generally increased accompanying with the increasing in decomposition products yield (D) caused by the further decomposition of glucose. The non-hydrolysable cellulose (C) decreased with increasing target temperature, while the glucose yield (G) after hydrothermal treatment increased with increasing target temperature.

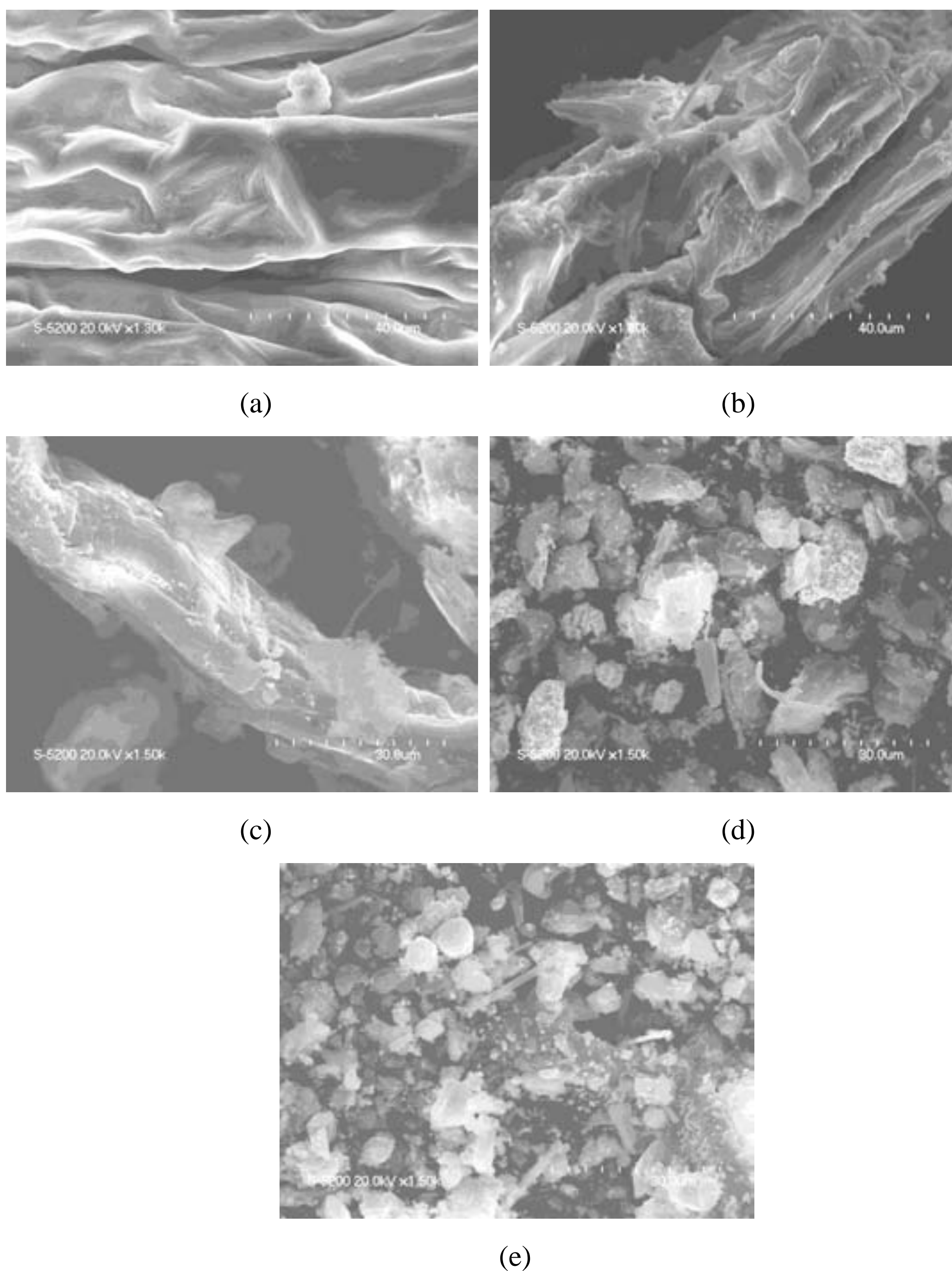


Figure 4.12 SEM images of water hyacinth: (a) raw material and after hydrothermal treatment at: (b) 160°C, (c) 180°C, (d) 200°C, and (e) 220°C in BMHR

Figure 4.12 shows SEM images of the solid fractions after hydrothermal treatment process. The experimental conditions to study the effect of target temperature in range of 160 to 220°C on glucose yield. The results show that the structure of water hyacinth leaves was more destroyed with the temperature increase

when compare to raw material. SEM images show only a weak erosion of the surface of water hyacinth leaves when the target temperature increase. The system becomes acidic because of acetic acid in the solution [20]. The acetic acid in the system are easily hydrolyzed from the acetyl groups in the hemicellulose fraction [19]. This led to the swelling of cellulose, increasing its internal surface area, and possibly reducing cellulose crystalline structure.

4.2 Effect of varied holding time on hydrothermal treatment

In this studied, we focus on the varied treatment on hydrothermal treatment. As shown the condition in **Table 3.3**. 35 grams of slurry products after the hydrothermal treatment have been used as reactant in enzymatic hydrolysis.

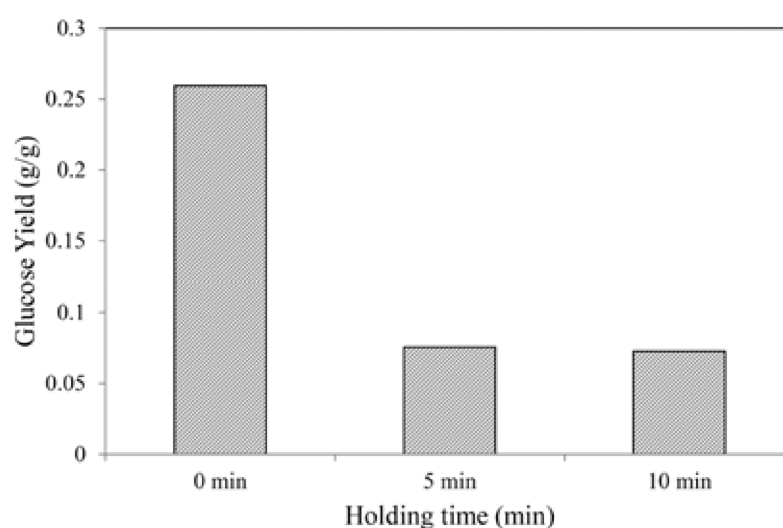


Figure 4.13 Total glucose yield acquired from hydrothermal treatment and enzymatic treatment of water hyacinth leaves treated in BMHR at different holding time in condition of 220°C

From the previous studied, we knew the temperature which got the highest glucose yield. Therefore in this studied we focused in the temperature at 220°C. By varied the holding time in range of 0 to 10 min. **Figure 4.13** shows the amount of glucose generated with time on the holding time 0 and 10 min. The result shows that the glucose yield sharp decreased when kept the holding time from 0 to 10 min. It can

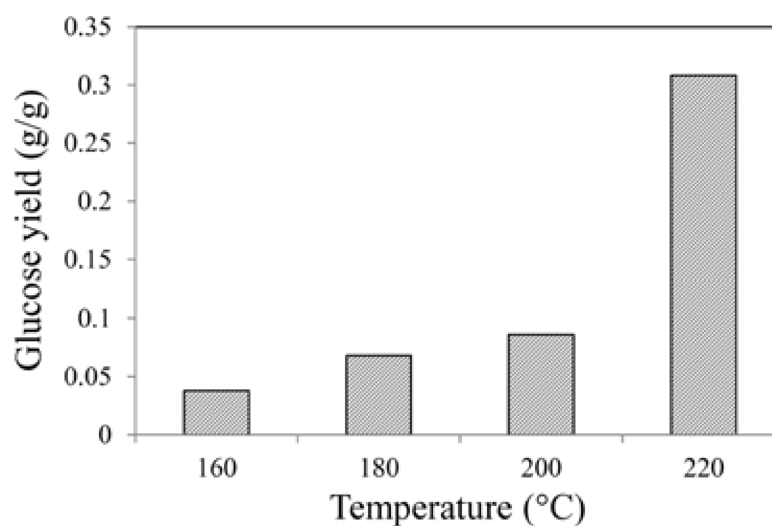
be deduced that the glucose yield was further decomposed to the smaller components when increased the holding time.

From all of this result, we knew that hydrothermal process was an acidic system from acetyl group in the hemicellulose fraction. Moreover, acidic system led to reducing cellulose crystalline structure which is the important point of hydrothermal treatment. That point is a good for hydrolyzing by enzymatic hydrolysis in order to convert to glucose [5].

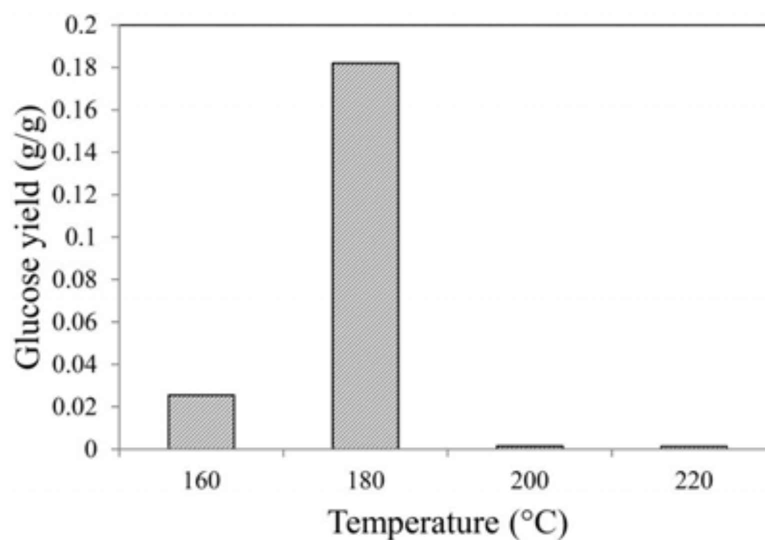
Therefore, we designed to add acetic acid into hydrothermal process in next section. We expected that we can get more amount of glucose from all of process.

4.3 Effect of adding acetic acid on glucose yield

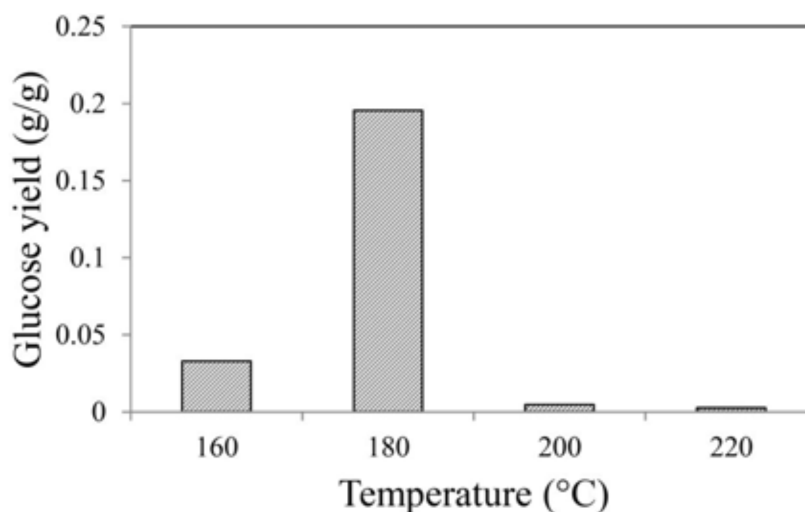
In this studied, we are interested is employing added acetic acid (CH_3COOH) for hydrothermal treatment of water hyacinth leaves. The glucose yield obtained after hydrothermal treatment at various experimental conditions as shown in **Figure 4.16**. The condition listed in the **Table 3.4**.



(a)



(b)

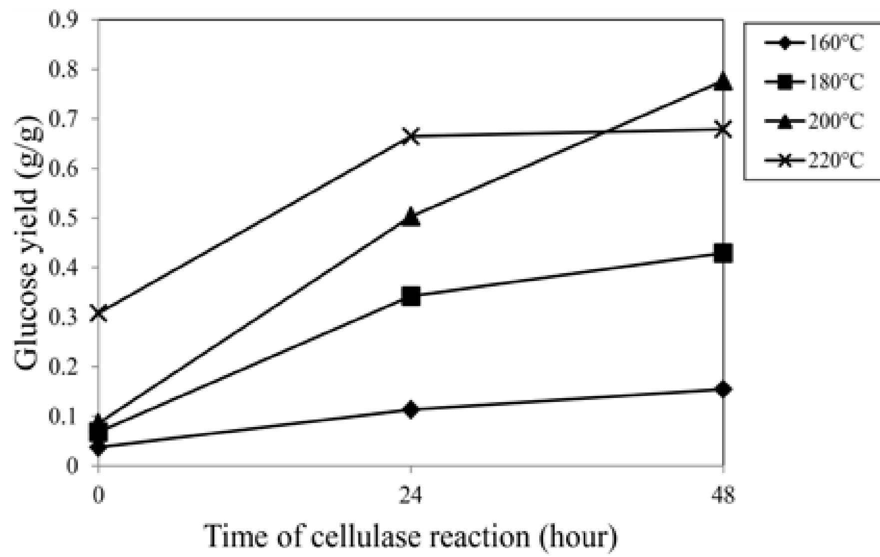


(c)

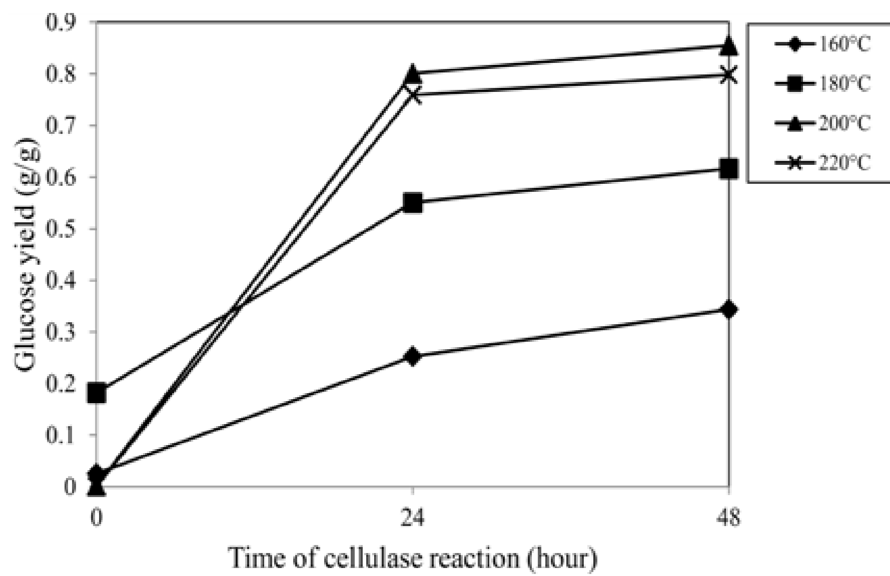
Figure 4.14 Glucose yield obtained from hydrothermal treatment varied with different temperature in the range of 160–220°C in BMHR with: (a) 0.5, (b) 0.75, and (c) 1.0wt% of CH_3COOH

Figure 4.14 shows the glucose yield obtained from hydrothermal treatment varied with different temperature in the range of 160–220°C in BMHR under acetic acid condition. The glucose yield after hydrothermal treatment were at 0.308 (0.5wt% CH_3COOH at 220°C), 0.182 (0.75wt% CH_3COOH at 180°C), and 0.196 (1.0wt% CH_3COOH at 180°C). It is clear that the glucose yield after hydrothermal treatment is less than 0.308 under CH_3COOH condition.

35 grams of slurry products after the hydrothermal treatment have been used as reactant in enzymatic hydrolysis.



(a)



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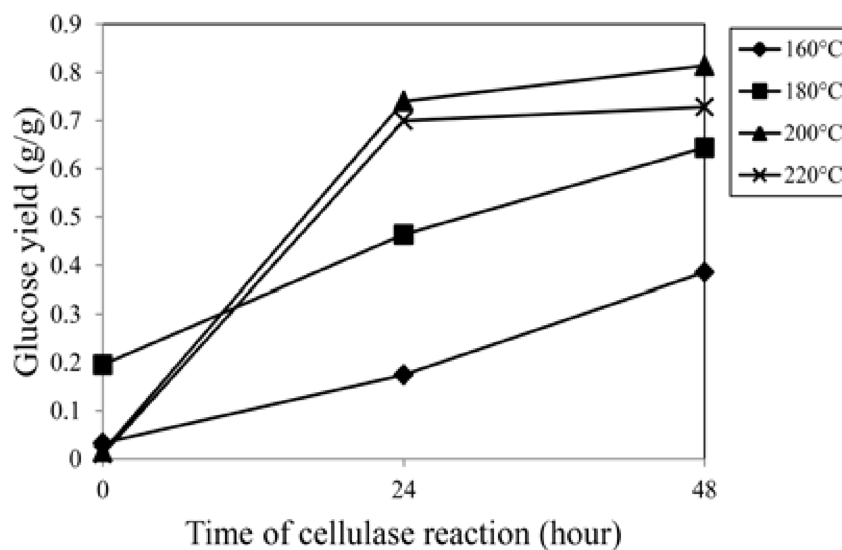


(c)

Figure 4.15 Glucose yield obtained from enzymatic hydrolysis with: (a) 0.5, (b) 0.75, and (c) 1.0wt% of CH_3COOH at conditions; 160°C, 180°C, 200°C, and 220°C in BMHR

Figure 4.15 shows the glucose yield with respect to enzymatic hydrolysis (cellulase reaction) time in BMHR under acetic acid condition. The glucose yield after hydrothermal treatment is shown at the time of cellulase reaction of 0 s. It is clear that the glucose yield after hydrothermal treatment is less than 0.308 under CH_3COOH condition. The glucose yield increases with time during the enzymatic hydrolysis.

Comparing to **Figure 4.9** (without adding acetic acid), The glucose yield after hydrothermal treatment obtained from under CH_3COOH condition even higher than those obtained from **Figure 4.9** (0.016, without adding acetic acid).

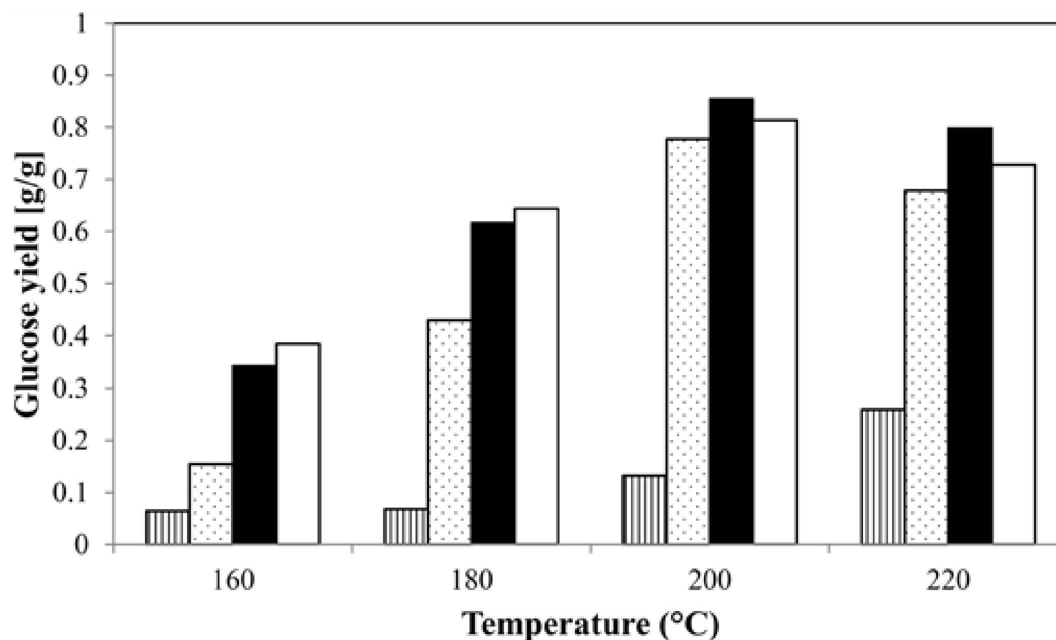


Figure 4.16 Total glucose yield in liquid fraction acquired from hydrothermal treatment and enzymatic treatment at difference pretreatment conditions: Without adding acetic acid (▥), 0.5wt% CH_3COOH (⬚), 0.75wt% CH_3COOH (■),1.0wt%CH_3COOH (□)

The effect of adding acetic acid on glucose yield in water hyacinth leaves is shown in **Figure 4.16** under different treatment conditions. At treatment temperature of 160°C to 200°C, an increase in CH_3COOH concentration from 0.5%wt to 1.0%wt resulted in a sharp increase in the glucose yield. However, a slight decrease in the glucose yield was observed after 200°C. The highest glucose yield of 85.5 % was achieved under the condition of 200°C with 0.75wt% CH_3COOH. Meanwhile, with the absence of ionic solvent at 220°C, a glucose yield of 26.7 % was obtained.

Gong et al. [20] showed that the removal of lignin, and internal surface area were gradually increased with an increase in acetic acid concentration from 6wt% to 18wt% as shown in **Figure 4.17** which implies the effect of acetic acid as a solvent and forming from acetyl group of hemicelluloses [30].

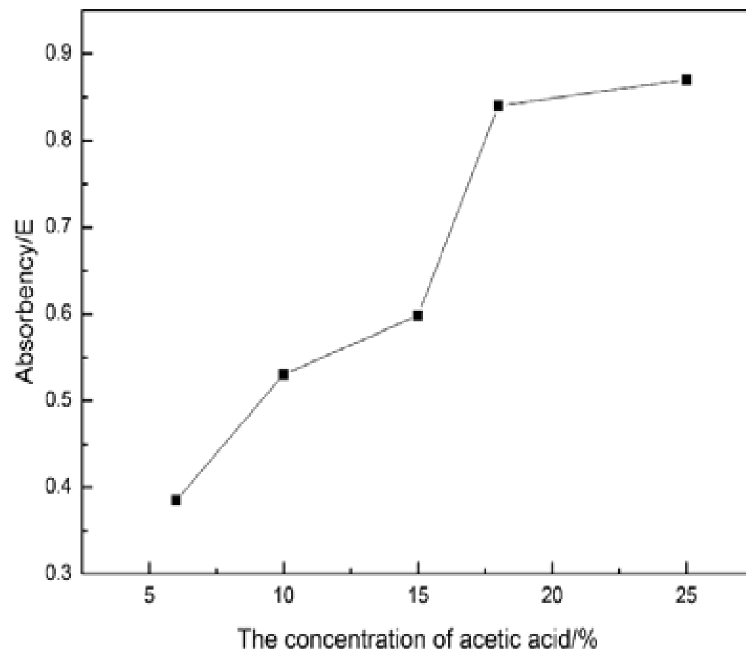
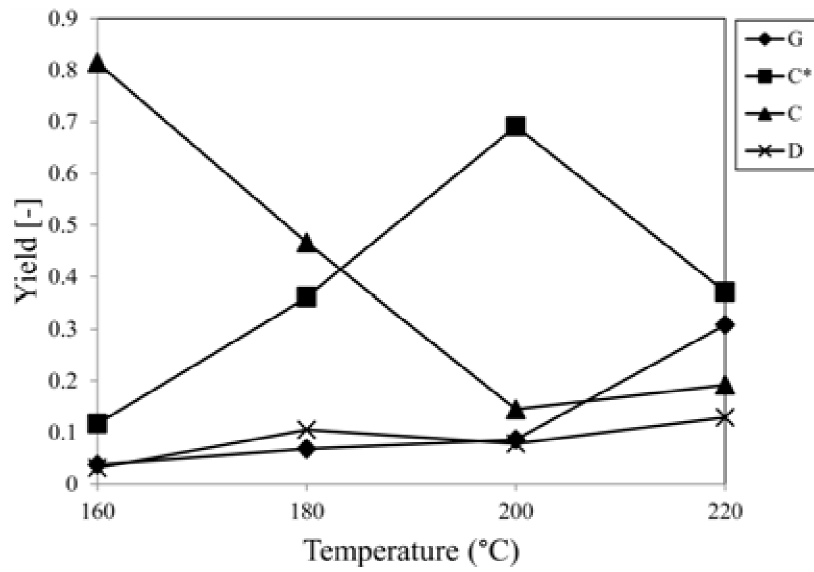
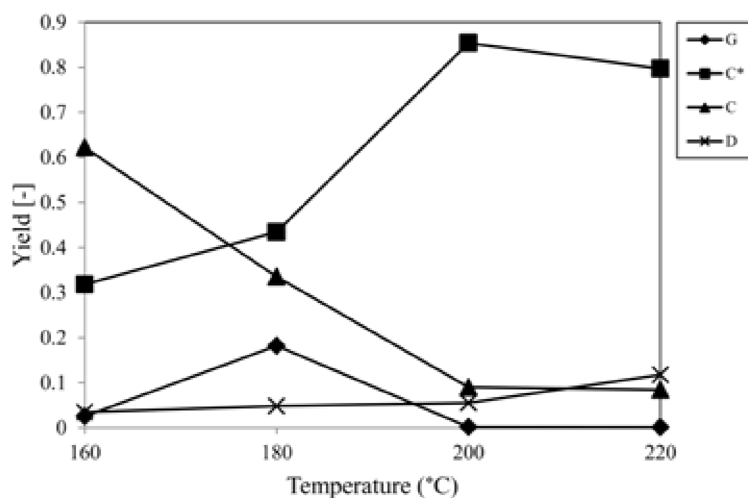


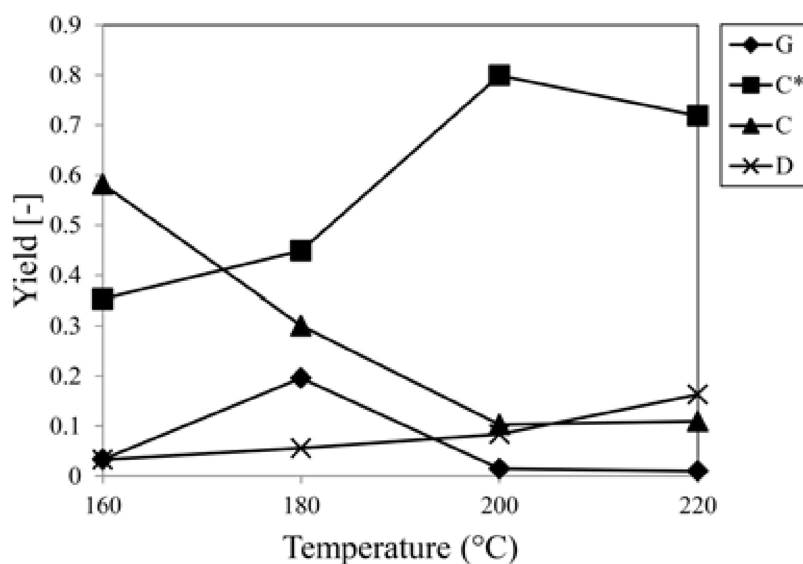
Figure 4.17 Influence of acetic acid concentration on the removal ratio of lignin of rice straw[20]



(a)



(b)



(c)

Figure 4.18 Components of final products acquired from hydrothermal treatment at various conditions: (a) 0.5wt% CH_3COOH, (b) 0.75wt% CH_3COOH, and (c) 1.0wt% CH_3COOH

According to **Figure 4.18**, It was observed that with increasing concentration of CH_3COOH, the hydrolysable cellulose yield (C*) generally increased accompanying with the increasing in decomposition products yield (D). However, the decomposition products yield (D) dramatically increased with increasing the temperature. The statistical analysis of the result in condition at 0.5wt% CH_3COOH, 200°C are reported in **Table 4.2**.

Table 4.2 Statistical analysis

Product yield	Mean±SD
G	0.084±0.002
C*	0.696±0.007

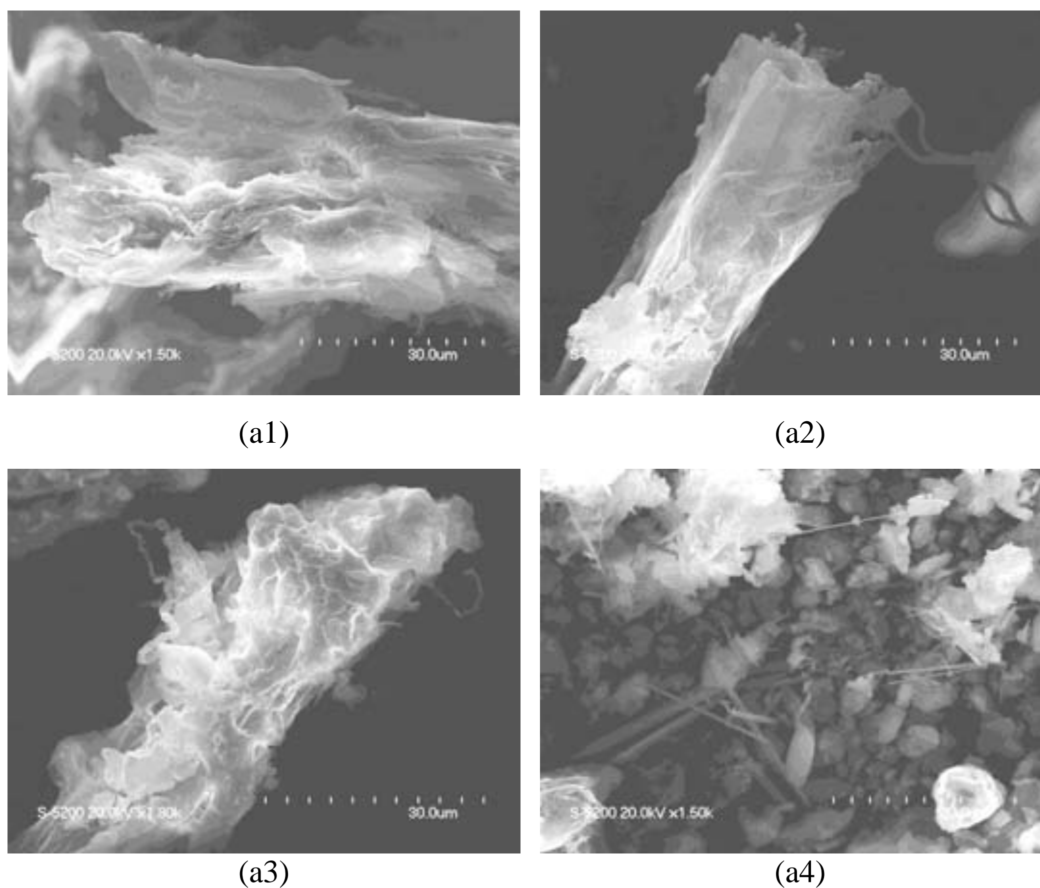


Figure 4.19 SEM images of water hyacinth leaves treated with (a) 0.75wt% CH_3COOH in condition at: (a1) 160°C, (a2) 180°C, (a3) 200°C, and (a4) 220°C in BMHR

Figure 4.19 shows SEM images of the solid fractions after hydrothermal treatment process. The experimental conditions to study the effect of target temperature in range of 160 to 220°C and the effect of adding acetic acid on glucose yield. The results show that the structure of water hyacinth leaves was also more

destroyed with the temperature increase when compare to raw material (Figure 4.11a). The reason can be supported by Girisuta et al, (2008) as shown in **Figure 4.20**.

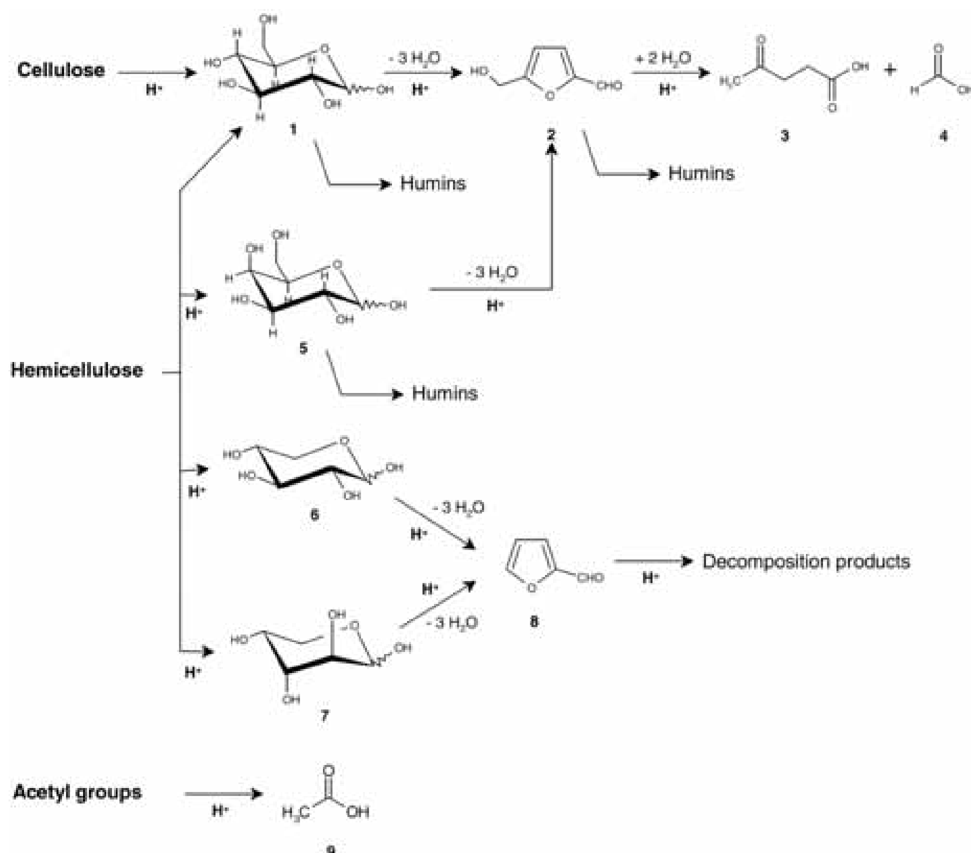


Figure 4.20 Simplified reaction network for the acid-catalysed hydrolysis reaction of the water hyacinth plant (1: Glucose, 2: 5-Hydroxymethylfurfural, 3: Levulinic acid, 4:Formic acid, 5: Galactose, 6: Xylose, 7: Arabinose, 8: Furfural, 9: Acetic acid) [19]

Girisuta, 2008 studied on the effect of acid-catalysed hydrolysis of the water hyacinth plant to levulinic acid. The reactions were conducted at temperature of 175°C, using a water hyacinth leaves intake of 5 wt% and two sulphuric acid concentrations (1.0 and 0.1 M). A well-known approach to convert lignocellulosic material like the water hyacinth to bulk chemicals is treatment of the biomass with a mineral acid like sulphuric acid at elevated temperatures (100–250°C). Upon this treatment, the hemicellulose and cellulose fractions of lignocellulosic materials are converted to soluble low molecular weight components. They proposed the simplified

reaction network for the acid-catalysed hydrolysis reaction of the water hyacinth plant as shown in **Figure 4.20**.

4.4 Pseudo-first order kinetic model

In this section, the pseudo-first order kinetic model for cellulose content in the water hyacinth leaves was developed to explain the reaction mechanism of cellulose in the hydrothermal treatment process.

4.4.1 Model assumption

The assumptions applied in the process of the model development are explained as follows.

- The model is based on cellulose content.

- The pseudo-first order kinetic model is constructed based on the proposed reaction pathway of glucose as shown in **Figure 4.21**. The hydrothermal treatment of cellulose produces a number of compounds in the liquid phase, which are not possible to completely identify. Therefore, only the compounds that are expected to play the important roles are considered in the model formulation. They are glucose (G), the hydrolysable cellulose (C*) and the non-hydrolysable cellulose (C). The rest of the unspecified liquid compounds are referred to as decomposition products (D) of glucose. This type of the model is known as "the lumped kinetic model", which is more suitable for the biomass application than the detailed kinetic model.

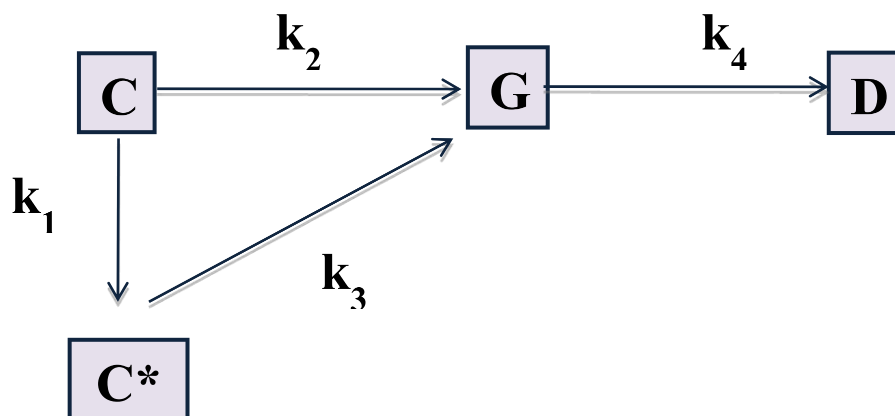


Figure 4.21 Proposed formation pathways of cellulose (first order reactions)

- All the reactions are assumed to be pseudo-first order reactions. These assumptions are reasonable. Petchpradab et al.[18] reported that the phenomena are taking place in the hydrothermal reactor are very complex which are dissolution of hemicellulose and part of lignin in the hot compressed water, reduction in crytallinity of the cellulose, destruction of cell structure, introduction of water molecules into the piece of biomass, and so forth. Therefore, they proposed that the reaction rate of decomposition of glucose in hydrothermal process can be expressed by a first order reaction. In fact, the reaction with the equal stoichiometric number between reactant and product normally has the order of the reaction of unity. These reactions consist of the hydrolyzation of the cellulose to the hydrolysable cellulose (k_1), the hydrolyzation of cellulose to glucose directly (k_2), the further hydrolyzation of the hydrolysable cellulose to glucose (k_3), and the decomposition of glucose (k_4). On the other hand, the hydrolyzation reaction is the process that the larger molecules (oligomers) as the reactant break down into the product with the smaller molecular weight (glucose).

- The change of each product and reactant were measured to determine the kinetic parameters; the pre-exponential factor (A_1, A_2, A_3, and A_4), the activatation energy Ea_1, Ea_2, Ea_3 and $Ea_{4)}$ and to be further used to calculate for the rate constants.

- The rate constants (k_1, k_2, $k_{3,}$ and k_4) obtained from the experiment of the water hyacinth leaves feedstock are used to explain the behavior of cellulose as the reactant in the hydrothermal reaction. In summary, cellulose, as one of the components in lignocellulosic biomass, can be hydrolyzed to yield hydrolysable cellulose, and it can also be directly hydrolyzed to glucose. Furthermore, the hydrolysable cellulose can be further hydrolyzed to glucose. On the contrary, the product, glucose can be further decomposed.

- The rate equations, therefore, can be written as follows:

$$\begin{aligned}\frac{dC}{dt} &= -k_1[C]-k_2[C]\\ \frac{dG}{dt} &= k_2[C]+k_3[C^*]-k_4[G]\\ \frac{dC^*}{dt} &= k_1[C]-k_3[C^*]\\ \frac{dD}{dt} &= k_4[G]\end{aligned} \tag{4-1}$$

We also assumed that the Arrhenius rate law is applicable to each reaction rate constant.

$$k_i = A_i e^{(-\frac{E_{ai}}{RT})} \tag{4-2}$$

where;			
	[C]	=	non-hydrolysable cellulose concentration (mol/L)
	[G]	=	glucose concentration (mol/L)
	[C*]	=	hydrolysable cellulose concentratiosn (mol/L)
	[D]	=	the decomposition products of glucose concentration (mol/L)
	k_i	=	rate constant ((mol/L) $\cdot s^{-1}$)
	A_i	=	pre-exponential factor [s^{-1}]
	E_{ai}	=	activation energy [kJ/mol]
	T	=	target temperature [K]
	R	=	gas constant; 8.3145 [$JK^{-1}mol^{-1}$]
	t	=	residence time (s)
	e	=	2.7183

The excel of Microsoft program was used for calculated the reaction rate parameter, while this program will be calculating every 30 s.

4.4.2 Iteration procedure

The iteration was carried out to determine all the kinetic parameters (the rate constants k_i) in the equations 4-1 that gave the best fitting between the calculated and

experimental values. The criterion of the numerical calculation was the least square of error (LSE):

$$LSE = \min\left(\sum\left([\exp] - [cal]_x\right)^2\right) \quad (4\text{-}3)$$

where; [exp] = the experimental concentration (mol/L)

$[cal]_x$ = the calculated concentration predicted by the set of kinetic parameters (mol/L).

The iteration stops when the set of kinetic parameters *x* that satisfy the equation 4-3 is found.

4.4.3 Pseudo-first-order reaction

A pseudo-first order reaction depends on the concentration of only one reactant because the other one of the reactants in the rate equation is present in great excess over the other in the reaction mixture that its effect is not seen. As section 4.5.1, we assumed that cellulose content in the water hyacinth leaves follows pseudo-first order kinetics as the following equations:

$$\ln C = \ln C_o - kt \quad (4\text{-}4)$$

where;

C_o	=	percentages of cellulose in the water hyacinth leaves at the beginning of reaction
C	=	percentages of cellulose in the water hyacinth leaves after time (t) incubating at a given temperature
k	=	reaction rate constant (min^{-1})
t	=	time (min)

The stability of cellulose in the water hyacinth leaves in hydrothermal treatment process without CH_3COOH at 220°C was determined by USDA method[16]. The plots of natural logarithm of the percentage of cellulose in the water hyacinth leaves remaining against time are shown in **Figure 4.22**. The plot was found to be linear (R^2 = 0.9154), indicating first-order reaction with respect to cellulose concentration. The equation of the curve corresponding to equation 4-5 is:

$$y = -0.0816x + 3.2461 \quad (4\text{-}5)$$

From the slopes of the curves is possible to calculate k, which indicates the rate constants of cellulose degradation.

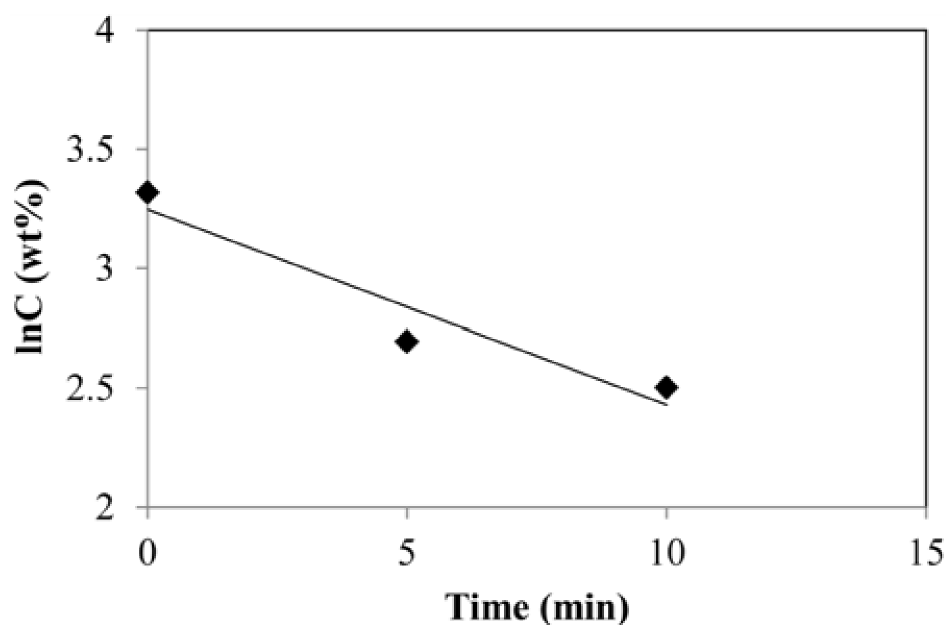


Figure 4.22 First-order plot for the degradation of cellulose in water hyacinth leaves treated without CH_3COOH at 220°C

According to the **Figure 4.23**, the calculated yields obtained from the iteration are comparatively shown with the experimental. The increase in the hydrolysable cellulose yield (C*) with temperature was also well presented for this case. Among those of the product yields, all fitting of products yields seem to be poorer owing to the effect of acetic acid that might have different behaviors at each temperature with presented in the least square of error as shown in **Table 4.3**. It was observed that with concentration of CH_3COOH at 0.75wt%, the hydrolysable cellulose yield (C*) generally increased accompanying with the increasing in decomposition products yield (D) caused by the further decomposition of glucose. The non-hydrolysable cellulose yield (C) dramatically decreased with increasing target temperature, while

the glucose yield (G) after hydrothermal treatment increased with increasing target temperature.

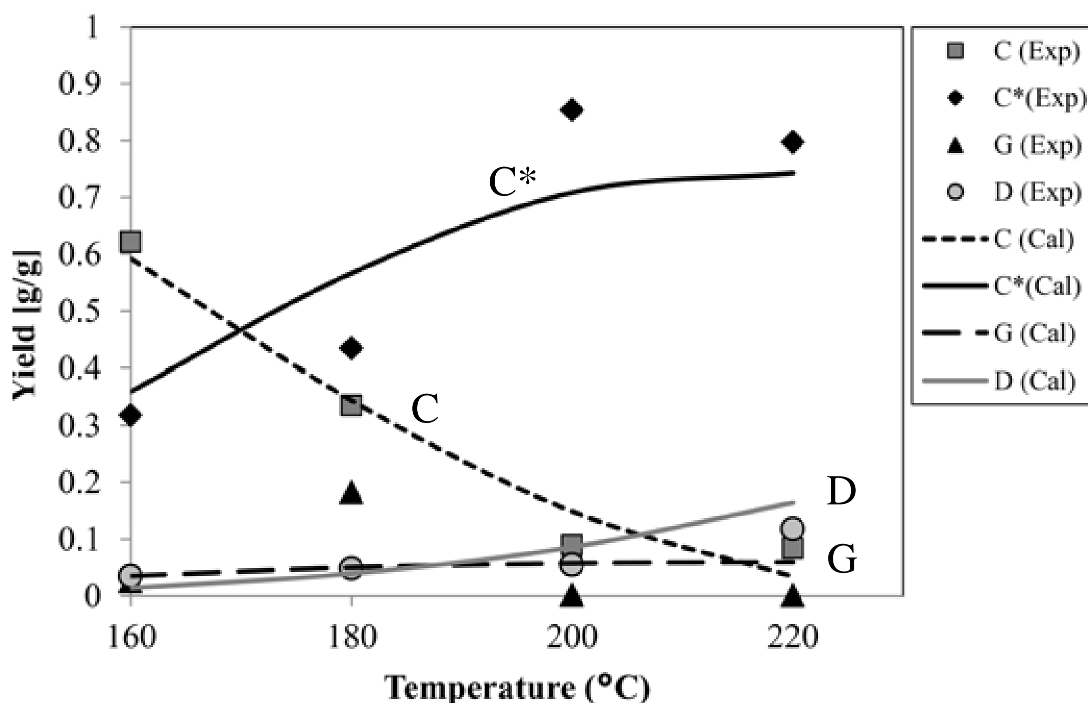


Figure 4.23 Effect of target temperature of hydrothermal treatment on each yield (symbol: experiment, line: calculation value) obtained from an experiment with 0.75wt% CH_3COOH

Table 4.3 least square of error of all fitting products yields

Product	Least Square of Error (LSE)
C	0.0069
C*	0.0431
G	0.0239
D	0.0036

In order to investigate the reaction kinetic, the rate constants k_1 , k_2 , k_3 , and k_4 were determined by the least-square method and assumption of the first order reaction. Their values are summarized in **Table 4.4**.These rate constants were analyzed by using a set of multiple reactions which are expressed by equation 4-1.

Table 4.4 Rate parameters for hydrothermal reaction

	Pre-exponential factor[1/s]	**Activation energy, Ea** [kJ/mol]
k1	66.55	40.37
k2	6.536	39.77
k3	9.160	42.86
k4	12.14	33.50

Figure 4.24 shows an Arrhenius plot for the reaction rate constants with the present of 0.75%wt acetic acid. Within a temperature range of 160 – 220 ˚C, k1 is much higher than k2 which indicates that the non- cellulose (C) is more favorable to be converted to the hydrolysable cellulose (C*). On the contrary, with a higher temperature, k1 is slightly greater than k4 which implies that cellulose can be hydrolysable into C* with a higher temperature. It is supposed that cellulose and hemicellulose can be degraded at temperature range from 200 to 350°C [31]. If we consider in term of activation energy (Ea), Ea4 is the lowest activation energy. It is indicated that the reaction rate of generated decomposition product is the highest rate. This result would be attributed to the barriers of lignin and hemicellulose which could hinder cellulase to hydrolyze cellulose [5]. The first step of hydrothermal treatment would wash away the hemicellulose and some part of lignin from the surface of cellulose, resulting in a more effective hydrolysis of cellulose by the cellulase hydrolysis in the second step.

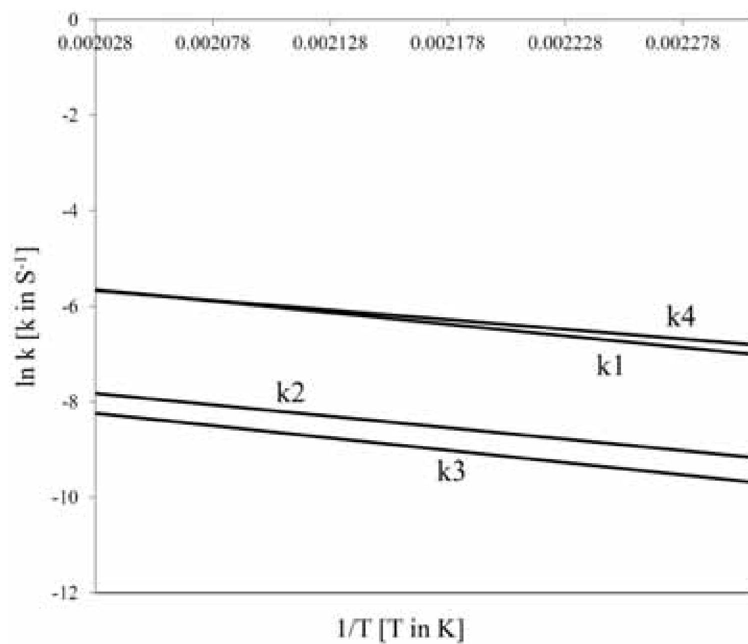


Figure 4.24 Arrhenius plot of each reaction rate constant again 1/T

CHAPTER V

CONCLUSION AND RECOMMENDATION

In this study, water hyacinth leaves was employed as a biomass raw material in the hydrothermal treatment process accompanying with enzymatic hydrolysis. Water hyacinth leaves was treated at 160–220 °C using a ball mill hydrothermal reactor (BMHR) with effective volume of 800 cm^3. The glucose content in the treated liquid fraction was measured by HPLC. The solid fraction was also analyzed for cellulose content by USDA method.

As a treatment of the hydrothermal process and enzymatic hydrolysis in a unique ball-mill reactor was used to convert cellulose in water hyacinth leaves to glucose. The reaction rate parameters of reaction pathways were determined with regard to the effect of acetic acid. The experimental results indicate that the hydrothermal process could provide higher content of glucose with the present of CH_3COOH. The highest glucose yield of 85.5% can be obtained at temperature of 200^oC. Kinetic rate constant of each elementary reaction based on the Arrhenius pseudo 1st order model was also proposed.

Recommendation

As a goal of hydrothermal process is to break the lignin seal and disrupt the crystalline structure of cellulose. Therefore, the crystalline structure of cellulose of raw material and solid fraction after hydrothermal process are interested point from this work. The one of analysis method to analyze crystalline structure is X-ray Diffractrometer (XRD). The method for removing the lignin from water hyacinth leaves before hydrothermal treatment should be considered in the future work.

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APPENDIX A

APPENDIX A1

Calibration curve of glucose solution

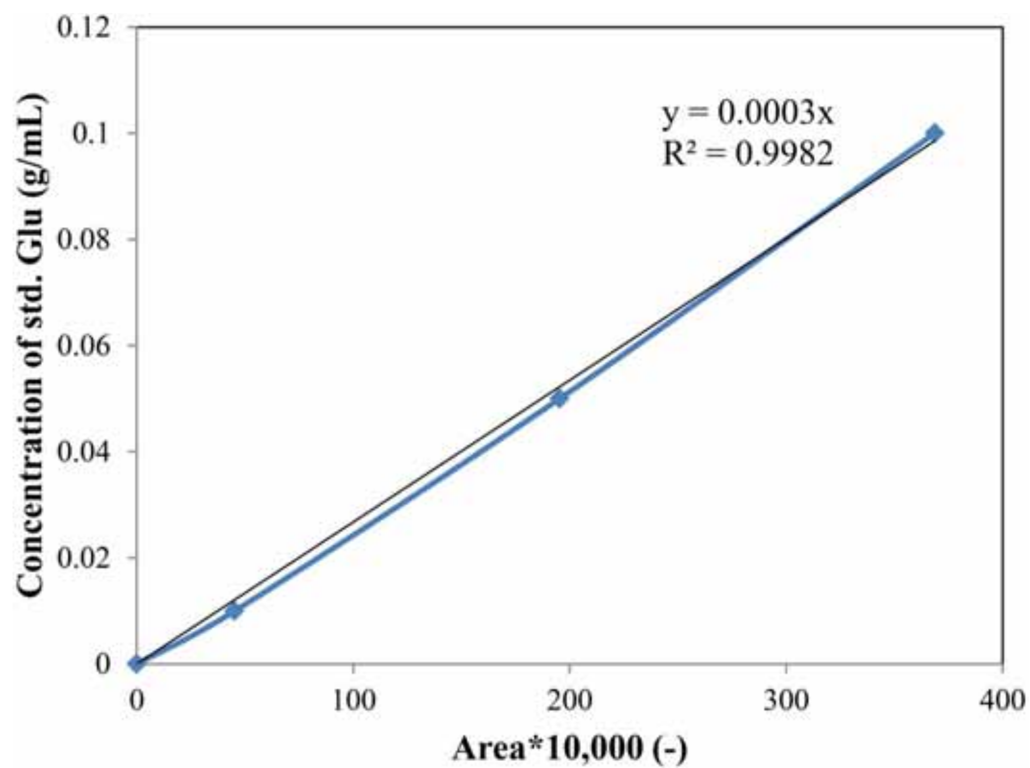

Figure A1 Calibration curve of glucose solution

APPENDIX A2

How to operate the HPLC (in the detailed procedures)

① Check the water level≅ 2400 mL

② Turn on the buttons of degasser, auto sample, oven, RID detector and controller, if the “error” sign appears, push “ce” button

③ Open the drain valve of pump A (clockwise) and push the “purge” button, wait for 5 minutes

③ After 5 minutes, close the drain valve (counter-clockwise)

④ Push the “menu” button, select “sequence”, push the “func” button twice, and then select “clear” and select “ok” (f2)

⑤ Push “F5” button to go back to menu screen, select “analysis”to set the condition; Flow rate max: 0.8 mL/min, Pressure max: 8.0 MPa, and oven temperature: 60 °C.

⑥ Push the “func” button, then select “file” (f1) and change file 1 to file 0 and select “ok” (f2), file 0 is set to gradually increase the flow rate with temperature

⑦ Push the the “func” button twice and push “F5” button to go back to menu screen, then select “sequence” and select “purge” (pump B), wait for 25 minutes, this process and the process in purging pump A can be done simultaneously.

⑧ After 25 minutes, push the “act” button, then push the “run” button instantaneously to prevent error, wait for another 55 minutes

⑨ After 55 minutes, when the “act” sign goes off and the balance at the RID detector is almost near 0, insert the sample

⑩ Select “analysis”, then push the “func” button and select “file” to change file 0 to file 1, file 1 is set to operate at the constant flow rate

⑪ Select “sequence” and set the run no., injection twice, volume 20 mL, run time 20 minutes and then select “ok” (f2)

⑫ Push the “act” button

⑬ Turn on the printer

⑭ Push the “command” button, then “stop time” button to set the run time

⑮ Push the “oper” button, then “D” button, enter the file name, no. sample (2X, in case of 2-time injection) and push the “enter” button to save the file

⑯ After the previous process is done, push the “func” button and select “oven.off” and then go back to menu screen and select “analysis”, then change flow rate from 0.8 to 0.2 mL/min, wait for 30 minutes until the temperature goes down to room temperature.

⑰ After 30 minutes, select “pump off”

⑱ Turn off the buttons of controller, RID detector, oven, auto sample and degasser

⑲ Turn off the printer

Note: If the “error” sign appears, follow the instruction

APPENDIX A3

USDA method

How to analyze hemicellulose, cellulose and lignin by USDA method

Equipments:

1. Crucible
2. Shallow enamel pan
3. Suck dry
4. Oven
5. Cooling bath
6. Reflux set

Reagents:

1. *Acid-detergent fiber (1 L)*

Sulfuric acid	49.04	g
Cetyltrimethylammonium bromide (CTAB)	20	g

Weigh *sulfuric acid* and make up to volume with distilled water at 20°C. Check normality by titration before addition of detergent. Then add *CTAB* and stir.

2. *Decahydronaphthalene*
3. *Acetone*
4. *n-Hexane*
5. *Saturated potassium permanganate (1 L)*

Distilled water	1	L
Potassium permanganate	50	g
Silver sulfate	0.05	g

Dissolve *potassium permanganate* and *silver sulfate* in distilled water. Keep out of direct sunlight. Add *silver sulfate* to dehalogenate the reagent.

6. *Lignin buffer solution*(1 L)

Ferric nitrate nanohydrate	6	g
Silver nitrate	0.15	g

Acetic acid	500	mL
Potassium acetate	5	g
Tertiary butyl alcohol	400	mL
Distilled water	100	mL

Dissolve *ferric nitrate nonahydrate* and *silvernitrate* in distilled water. Combine with *acetic acid* and *potassium acetate*. Add *tertiary butyl alcohol* and mix. Use grades of acid and solvent passing dicromat test (ACS)

7. *Combined permanganate solution*(1 L)

Combine and mix *saturated potassium permanganate* and *lignin buffer* solution in the ratio of 2:1 by volume, before use. Unused mixed solution kept about a week in a refrigerator in the absence of light. Solution is usable if it turns purple and contains no precipitate. Old solution assumes a reddish color and should be discarded.

8. *Demineralizing solution* (1 L)

Distilled water	250	mL
Oxalic acid dehydrate	50	g
Ethanol 95 %	700	mL
Hydrochloric acid	50	mL

Dissolve oxalic acid dehydrate in 95 percent ethanol.Add concentrated hydrochloric acid and distilled water and then mix.

9. *Ethanol 80 %*

95 percent ethanol	845	mL
Distilled water	155	mL

Step-1, Acid-detergent fiber (hemicellulose):

1) Weigh 1 g of air dry sample ground to pass 1 mm (20-30 mesh) screen or the approximate equivalent of wet material into a beaker suitable for refluxing

2) Add 100 ml of *acid detergent solution* (at room temperature) and 2 ml of decahydronaphthalene, heat to boil for 5 to 10 minutes. Reflux heat as boiling begins to avoid foaming. Reflux 60 minutes from onset of boiling; adjust boiling to a slow, even level.

3) Filter on a previous tared Gooch crucible, which is set on the filter manifold; use light suction (suck dry), break up the filtered mat with a rod and wash twice with hot water (90-100 °C), rinse sides of the crucible in the same manner

4) Repeat wash with *acetone* until it removes no more color: break up all lumps so that the solvent comes into contact with all particles of fiber

5) Optional wash with *hexane*,*hexane* should be added while crucible still contains some *acetone*(*Hexane* can be omitted if lumping is not a problem in lignin analysis) Suck the acid-detergent fiber free of hexane and dry at 100 °C

Step-2, Permanganate lignin (lignin):

1. Add 25 ml of combined *saturated potassium permanganate*and *Lignin buffer solution*(2:1 by volume) to crucibles in the enamel pan containing cold water. Adjust level (2-3cm.) of water in pan to reduce flow of solution out of crucibles. Place a short glass rod in each crucible to stir contents, to break lumps and to draw permanganate solution up on sides of crucibles to wet all particles

2. Allow crucible to stand at 20-25 °C for 90-100 min, add more mixed *permanganate solution* if necessary, purple color must be present at all times

3. Remove crucibles to filtering apparatus. Suck dry and do not wash the samples, then place them in a clean enamel pan and fill crucibles no more than half full with *demineralizing solution,demineralizing solution* maybe added directly to crucible in case filtering is difficult, care must be taken to avoid spillage by foaming, after about 5 minutes, suck dry on filter and refill haft full with *Demineralizing solution,* repeat after second interval of solution is very brown, rinse sides of crucibles with solution from a wash bottle with a fine stream, treat until fiber is white, total time required is 20-30 minutes

4. Fill and thoroughly wash crucible and contents with 80 percent ethanol, suck dry and repeat it, wash twice in similar manner with acetone, Suck dry

5. Dry at 100 °C overnight and weigh, calculate lignin content as loss in weight from acid-detergent fiber

6. Ash at 500 °C using a furnace for 3 hr, cool, and weigh, calculate residual ash for its weight difference comparing to the original tare of crucible, calculate cellulose by weight loss upon ashing.

Note: Crucibles containing fiber of high lignin content require more permanganate solution. However, avoid adding more solution than necessary. Appearance of yellow or brown color indicates exhaustion of permanganate. If crucible is full, filter solution off on a vacuum and add more reagents. A yellow color persisting after treatment of fiber with demineralizing solution indicates incomplete removal of lignin. This occurs only in materials with very high lignin content.

APPENDIX A4

Effect of potassium carbonate on glucose yield

In this study, we are interested to study in the effect of potassium carbonate on glucose yield in hydrothermal process as shown in **Figure A2**.

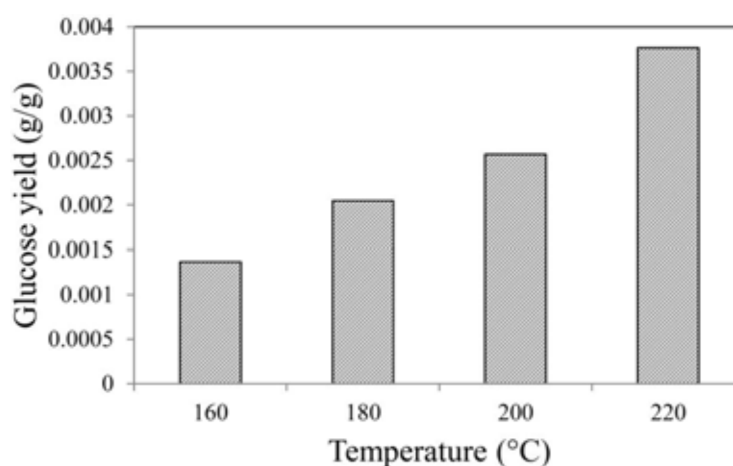

Figure A2 Glucose yield obtained from hydrothermal treatment varied with different temperature in the range of 160–220°C in BMHR with 0.5wt% of K_2CO_3

Figure A2 shows the glucose yield obtained from hydrothermal treatment varied with different temperature in the range of 160–220°C in BMHR K_2CO_3conditions. The glucose yield after hydrothermal treatment was 0.004 in condition at 0.5wt% K_2CO_3, 220°C.

35 grams of slurry products after the hydrothermal treatment have been used as reactant in enzymatic hydrolysis.

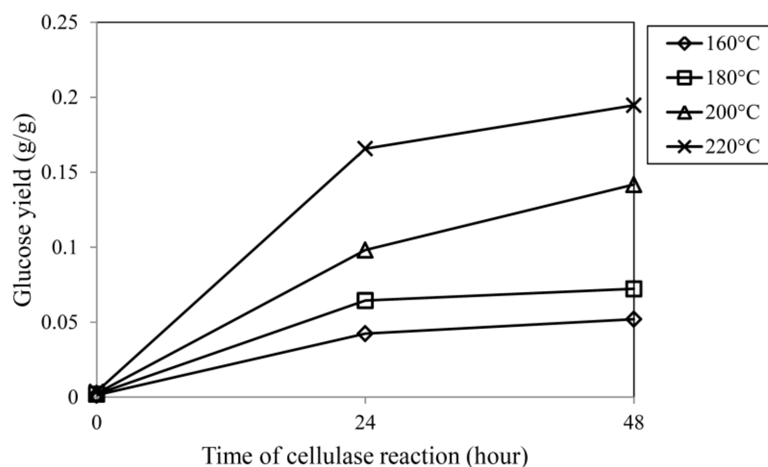

Figure A3 Glucose yield obtained from enzymatic hydrolysis with 0.5wt% of K_2CO_3 at conditions; 160°C, 180°C, 200°C, and 220°C in BMHR

Figure A3 shows the glucose yield with respect to enzymatic hydrolysis (cellulase reaction) time in BMHR under K_2CO_3 condition. The glucose yield after hydrothermal treatment is shown at the time of cellulase reaction of 0 s. The glucose yield increases with time during the enzymatic hydrolysis.

The effect of K_2CO_3 on glucose yield in water hyacinth leaves is shown in **Figure A4** under different treatment conditions. As a result, with the presence of 0.5wt% K_2CO_3, the glucose yield was only 19.5% at 220 °C. Therefore, the least glucose yield was obtained with K_2CO_3 condition when compare with the absence of catalyst and CH_3COOH conditions.

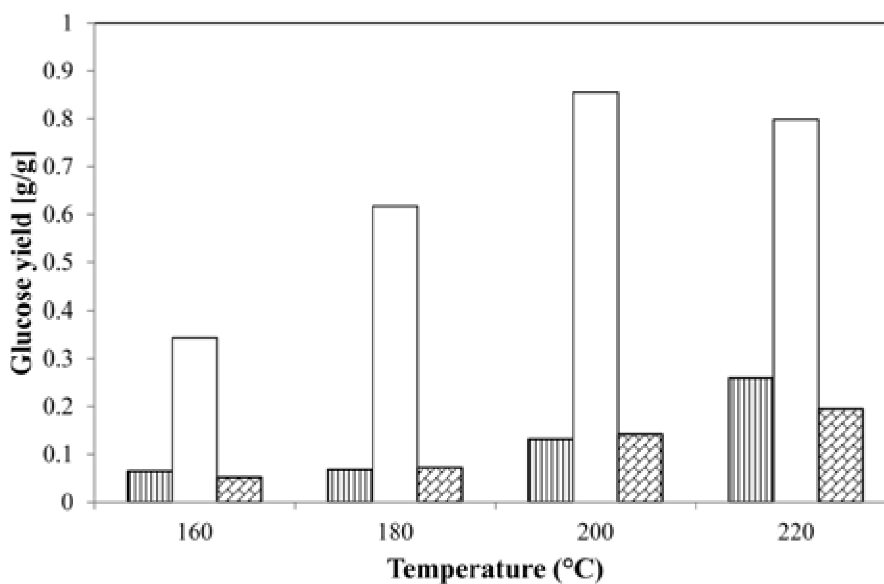

Figure A4 Total glucose yield in liquid fraction acquired from hydrothermal treatment and enzymatic treatment at difference pretreatment conditions: Without adding acetic acid ( ), 0.5wt% CH_3COOH ( ), 0.5wt% K_2CO_3 ( )

Toor et al, (2011) reported that the addition of alkali salts has a positive influence on hydrothermal processes. It improves gasification, accelerates the water-gas shift. Indeed alkali is also known to suppress char and tar formation. Yin et al, they studied on alkaline hydrothermal conversion of cellulose to bio-oil. They proposed the pathways of alkaline hydrothermal conversion of cellulose as shown in **Figure A5**. As many researches, their summary could support this study.

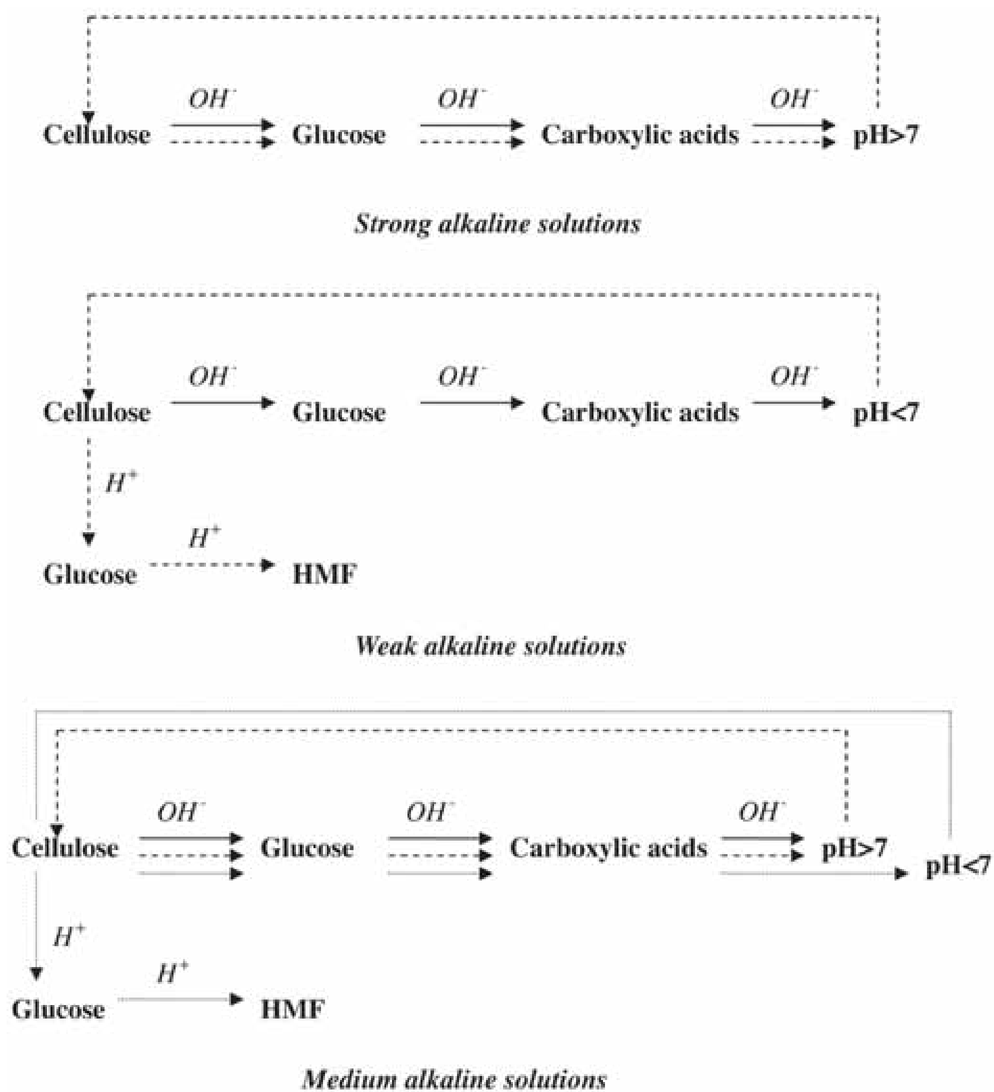

Figure A5 Proposed effects of alkalinity on the pathways of alkaline hydrothermal conversion of cellulose [32]

VITA

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