Glucose and xylose synthesis by hydrolysis reaction of water hyacinth using p-TSA as acid catalysts

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การสังเคราะห์กลูโคสและไซโลสด้วยปฏิกิริยาไฮโดรไลซิสของผักตบชวาโดยใช้กรด p-TSA

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Abstract

This work is devided into two main parts, which are pretreatment of water hyacinth by alkaline and hydrolysis reaction of water hyacinth by acid hydrolysis. The first part, pretreatment is aimed to remove lignin for more accessible of acid to hydrolyze cellulose and hemicellulose into glucose and xylose. The important parameters, which will affect the pretreatment of water hyacinth were concentration of NaOH and pretreatment time, concentration of NaOH in range of 1 to 2 %w/v (g/mL) and reaction time of 60 to 120 minutes were studied. In part of hydrolysis reaction of water hyacinth, the important variables were investigated, the temperature of 77 and 100 °C, concentration of catalyst of 0.25 to 1 M and reaction time of 2 to 4 hours. The products were analyzed using high performance liquid chromatography (HPLC). The appropriate condition for pretreatment of water hyacinth were at concentration of 1.5 %w/v NaOH and pretreatment time of 60 min. At this condition, lignin was removed by 4.7%. For the hydrolysis reactions, the appropriate conditions that gave the highest yield is at the temperature of 100 °C, 1 M of p-TSA and reaction time of 4 hours. The highest yield of the glucose product was 10.8% and xylose product was 6.3%. The results showed that the water hyacinth was the alternative material to produce glucoses and xylose.

Keywords: Glucose, Hydrolysis, Water hyacinth, Acid catalyst

เรื่อง	การสังเคราะห์กลูโคสและไซโลสด้วยปฏิกิริยาไฮโดรไลซิสของผักตบชวาโดยใช้กรด p-TSA
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บทคัดย่อ

งานวิจัยนี้มีสองส่วนหลักคือการปรับสภาพผักตบชวาด้วยด่างและปฏิกิริยาไฮโดรไลซิสของผักตบชวาด้วยกรด ในส่วนของการปรับสภาพมีจุดประสงค์เพื่อกำจัดองค์ประกอบของลิกนินเพื่อเพิ่มการเข้าถึงของกรดเพื่อสลายเซลลูโลสและเฮมิเซลลูโลสเป็นกลูโคสและไซโลส ตัวแปรที่สำคัญที่มีผลต่อการปรับสภาพของผักตบชวาคือความเข้มข้นของโซเดียมไฮดรอกไซด์และเวลาในการทำปฏิกิริยา โดยใช้ความเข้มข้นของโซเดียมไฮดรอกไซด์ในช่วง 1 ถึง 2 ร้อยละโดยมวลต่อปริมาตร(กรัมต่อมิลลิลิตร)และที่ช่วงเวลาการปรับสภาพ 60 ถึง 120 นาที ในส่วนของปฏิกิริยาไฮโดรไลซิสใช้ผักตบชวา ตัวแปรสำคัญหลายตัวแปรที่ถูกศึกษาได้แก่อุณหภูมิที่ใช้ในการทำปฏิกิริยาที่ 77 และ 100 องศาเซลเซียส ความเข้มข้นของตัวเร่งปฏิกิริยาที่ 0.25 ถึง 1 โมลาร์ และระยะเวลาในการทำปฏิกิริยาที่ 2 ถึง 4 ชั่วโมง วิเคราะห์ผลผลิตภัณฑ์ด้วยเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูง ภาวะที่เหมาะสมที่สุดของการปรับสภาพผักตบชวาคือที่ความเข้มข้นของโซเดียมไฮดรอกไซด์เป็น 1.5 ร้อยละโดยมวลต่อปริมาตรและเวลาในการทำปฏิกิริยาที่ 60 นาที โดยสามารถกำจัดลิกนินร้อยละ 4.7 สำหรับการทำปฏิกิริยาไฮโดรไลซิสพบว่าภาวะการทำปฏิกิริยาที่ให้ผลผลิตสูงสุดคือ อุณหภูมิ 100 องศาเซลเซียส ความเข้มข้นกรดพาราโทลูอีนซัลโฟนิค 1 โมลาร์ และเวลาในการทำปฏิกิริยา 4 ชั่วโมง ที่ให้ร้อยละของผลิตภัณฑ์กลูโคสสูงสุดอยู่ที่ร้อยละ 10.8 และไซโลสสูงสุดที่ร้อยละ 6.3 ผลการทดลองจะพบว่าผักตบชวาเป็นหนึ่งในทางเลือกสำหรับการผลิตเป็นกลูโคสและไซโลส

คำสำคัญ: กลูโคส; ปฏิกิริยาไฮโดรไลซิส; ผักตบชวา; ตัวเร่งปฏิกิริยากรด

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CHAPTER I
INTRODUCTION

1.1 Background

Thailand is predominantly an agriculture-based country. A large proportion of land about 47 percent is an agriculture sector [1]. Because of that there are a lot of agricultural residue causes environmental problem. One problem in Thailand is the fast growing of water hyacinth. The amount that have to be disposed is more than 7 million tons per year [2]. Since the water hyacinth is rapidly growing, it affects the economic, social and environment by obstruct the navigation and cause the filthy water. The enactment for the disposal of water hyacinth effectively in use since 1913 to control the amount of water hyacinth [3]. By this reason, this project is interested in using water hyacinth to produce chemical with various utilization such as glucose. It will be the solution for the disposal of water hyacinth and also add value to the waste.

Glucose can be used as intermediate substance in many industries such as in the food industries, the pharmaceutical industries or even the chemical industries. Glucose can be produced by many routes mostly use the biological hydrolysis or sometimes called enzymatic hydrolysis and the other route is chemical hydrolysis. But the enzymatic hydrolysis has the disadvantages of higher cost of the initial substance like enzyme, long reaction time and also sensitive to pH and temperature used and still this method gives the low yield of glucose [4]. For chemical hydrolysis is more attractive due to the advantages of can operate at mild condition, lower cost of catalyst and also shorter reaction time and some disadvantages such as corrosive and difficult to recovery.

Before the hydrolysis process of lignocellulosic biomass, pretreatment methods are required in the purposes of more accessibility of acid catalyst to hydrolyze cellulose into glucose, which lead to the improvement in hydrolysis and then give the greater yield of glucose. Moreover, it can decrease the lignin content, which is cover cellulose and hemicellulose in form of complex crystalline structure.

Glucose production from agricultural residue by hydrolysis reaction using acid catalyst have recently caught attention to many researchers. Two types of acid catalyst are widely used are homogenous catalyst and heterogeneous catalyst. The heterogeneous catalyst usually used in form of solid catalyst. This type of catalyst can reduce the environmental problem because it can be recycled, decreased the degree of corrosion. It also reduces the cost of product purification because it can easily be separated out of product and can be recovered [5]. But some disadvantage is low yield of glucose product compared to the homogeneous catalyst. The homogeneous catalyst will give proton (H^+) to destroy the β-1,4- glycosidic bonds of cellulose before convert to glucose. The reaction is usually used the temperature in range of 160-190°C. When reaction occurs at mild condition of 100 °C might help avoiding the formation of undesired product from glucose and also reduce cost of energy.

This senior project focuses on the study of glucose synthesis by hydrolysis reaction of water hyacinth using homogeneous acid catalyst p-Toluenesulfonic acid (p-TSA) to find the optimum condition to achieve the highest yield of glucose.

1.2 Objectives

1) To find the optimum of pretreatment condition to eliminate lignin in the water hyacinth.

2) To find the optimum conditions for glucose synthesis from hydrolysis reaction of water hyacinth with p-TSA acid catalyst.

1.3 Scopes of work

1) The important parameters, which will affect the pretreatment of water hyacinth, are studied with alkaline pretreatment with ultrasonic method as follows:

- Concentration of NaOH 1, 1.5 2 %w/v
- Reaction time 60, 90, 120 min

2) The important parameters, which will affect the hydrolysis reaction, are studied as follows:

- Temperature 77 and 100 °C
- Concentration of p-TSA acid catalyst 0.25, 0.5, 1 M
- Reaction time 2, 3, 4 hours

CHAPTER II
LITERATURE REVIEW

The glucose production generally used two synthesis paths, which are fermentation (enzymatic hydrolysis) and chemical hydrolysis. The enzymatic hydrolysis has many disadvantages including high operating cost of the enzyme, temperature and pH sensitive and long reaction time and some advantages are highly specific catalytic properties and can be operated at mild condition. In contrast, chemical hydrolysis is more attractive because of many advantages are can operate at mild condition, lower cost of catalyst and also shorter reaction time and some disadvantages such as corrosive and difficult to recovery. By this reason the chemical hydrolysis was usually used. The glucose synthesis via the hydrolysis reaction using acid as a catalyst, the heat added, acid catalyst will give proton to destroy the β-1,4-glycosidic bond of the cellulose the reaction is easily occurred. By this way, the hydrolysis with the acid catalyst has the lower reaction time and can easily control the condition and give higher yield of products. The reactants used can be cellulose or agricultural residue. The yield of glucose depends on several factors, the important will be extensively reviewed in the following section 2.4.

2.1 Agricultural residues

Biomass is the raw material, mainly comprise of lignocellulose. Lignocellulose is consist of cellulose, hemicellulose and lignin, which mostly found in plants cell wall for example, scraps from the softwood, hardwood and the agriculture residue such as corncobs, corn fibers, bagasse, husks and rice straws and the waste from the food processing. In each agriculture biomass has the different combination of cellulose, hemicellulose and lignin as shown for example in the table 2.1.

Table 2.1 The composition of cellulose, hemicellulose and lignin in agriculture biomass (Percent weight dry basis).

Lignocellulosic material	Cellulose	Hemicellulose	Lignin	Reference
Pine wood	44.6	5.3 - 8.8	27.2	[6]
Eucalyptus wood	49.5	13.1	27.7	[6]
Corn cops	45.0	35.0	15.0	[6]
Rice Straw	35 – 41	14.8 – 25.0	9.9 -12.0	[6]
Bagasse	25.0 – 50.0	25.0 – 14.8	9.9 – 12.0	[6]
Sugarcane husk	55.6-57.4	23.9-24.5	24.3-26.3	[7]
Banana peel	60.2-65.2	48.2-59.2	5.55-10.35	[7]
Coconut (coir)	36.6-43.2	0.12-0.25	41.2-45.3	[7]
Pineapple shell	70.5-82.3	18.7-21.9	5.3-12.3	[7]
Water hyacinth	25.0-35.0	33.5-35.0	10.0-15.5	[8]

The most abundant polymer in lignocellulosic biomass is cellulose, which has the repeating unit of disaccharide cellobiose. Cellulose consists of the glucose unit tightly bind with each unit by glycosidic bond at the β-1,4 position, which becomes the long chain that has more than 2,000 molecules of glucose. The structure of cellulose is shown in figure 2.1 [9].

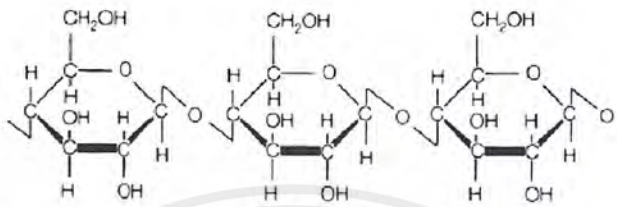


Figure 2.1 Structure of cellulose [9].

The second most abundant polymer is hemicellulose, which is heteropolysaccharide. Hemicellulose composed of various type of sugar including xylose that has β-1,4 linked backbones with an equatorial configuration in the main chain, perhaps composed of manose, galactose or glucose also in the main chain and has the others sugar like arabinose and glucaric in the side chain. Different type of plants will also differ in composition of hemicellulose. For example, the hardwood hemicellulose is mainly xylans, the softwood hemicellulose contains mostly glucomannans. The structure of hemicellulose has a random repeating unit unlike the cellulose [10]. The structure of hemicellulose is shown in figure 2.2 [11].

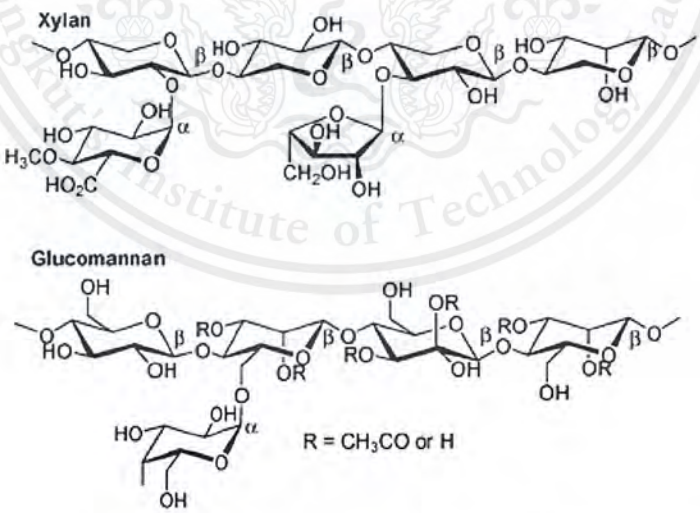


Figure 2.2 Example of polymers in hemicellulose, xylan and glucomannan monomer in hemicellulose [11].

Finally, lignin is a polymer of phenylpropanoid units, which has a function as the cellular glue that provides compressive strength for plant tissue. The phenylpropane has different three building blocks consist of p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol [12]. The structure of lignin is shown in figure 2.3 [13].

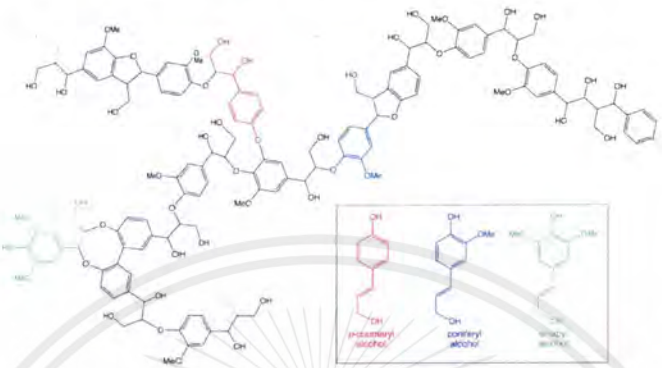


Figure 2.3 The structure of lignin [13].

2.2 Derivative of glucose into variety of high-value chemicals.

The conversion of biomass into numerous chemicals has become one of the most interesting and considerably research in the last decade. Hydrolysis of cellulose as abundant feedstock in nature can become one of renewable resource for chemical production [14]. The hydrolysis of lignocellulosic biomass produce glucose, which can be converted to various chemicals and alternative fuels such as levulinic acid and 5-hydroxymethylfurfural. The example of glucose converted to chemicals as shown in figure 2.4 [15].

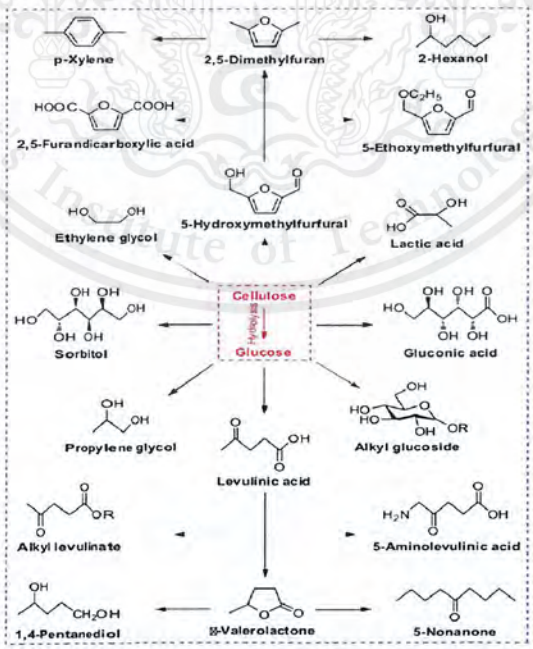


Figure 2.4 The various chemicals and fuel from glucose [15].

2.3 Pretreatment of lignocellulose biomass.

Before the hydrolysis process of lignocellulosic biomass, pretreatment is required to remove lignin in the purposes of more accessibility of acid to hydrolyze cellulose into glucose, which lead to improve the rate of reaction and give the higher yield of glucose [16]. Various method can be used in pretreatment. Three methods commonly used are mechanical, physicochemical and chemical pretreatment. These common pretreatment techniques of biomass are described in the following section.

2.3.1 Mechanical Pretreatment

Mechanical pretreatment aim is to reduce size of feedstock and disrupting the structure of biomass in order to increase surface area for hydrolysis reaction. An example of mechanical pretreatment is milling, grinding and chipping. The size of feedstock materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding [17].

2.3.2 Physicochemical Pretreatment

Physicochemical pretreatment used both physical and chemical method for biomass. digestibility Physicochemical has several methods including steam explosion, which is the most commonly used for pretreatment by treat the biomass with steam at high temperature of 160–260 °C and decrease pressure suddenly in order to separate lignin and degradation of hemicellulose at high temperature but it has some drawback such as production of some inhibitor like hydroxymethylfurfural and cause partial degradation of hemicelluloses, required high energy consumption and also the formation of toxic components [18]. The more attractive method is ultrasonic pretreatment. The ultrasonic generate the small bubble and cavitation effect that can destroy and fragment the cellulose particles into shorter fiber and also reduce the crystallinity of
cellulose [19].

Svetlana et al. [20] studied the utilization of ultrasonic pretreatment using corn meal was carried out in sonicator for 1 to 10 min. They reported that the ultrasonic pretreatment effectively increased the glucose yield about 6.82% compared to untreated control sample.

Sivakumar et al. [21] studied on the yield of glucose by using ultrasound assisted in pretreatment of saw dust by using sonication for an hour. Their result show inn case of sonication almost 25% increased yield compared to the case without sonication and it gave highest yield of glucose with sonication pretreatment of 38.9% glucose at 20 min of sonication.

Wasinton et al. [22] studied the effects of ultrasonic by employing cassava solid waste dispersed in 250 mL of deionized water and using 5% H_2SO_4 solution to adjust pH of sample in ranging from 1 to 4 and pretreatment time of 30 to 300 min and temperature of 50-90 °C. They reported the highest yield of reducing sugar was obtained at pH of 2, temperature of 80 °C, pretreatment time of 90 min. The reducing sugar from control sample without sonication was only 71.2 mg/L but in case of using sonication gave the yield of reducing sugar of 801 mg/L, which is more than 11 times compared with untreated sample.

Waesarat et al. [23] studied effects of alkaline combined with ultrasonic pretreatment by using five agricultural wastes consist of corn cob, pineapple waste, bagasse, rice straw and water
hyacinth and using 1%w/v and 2%w/v NaOH with ultrasound for 60 and 90 min and compared with alkaline pretreatment without using ultrasound. They reported that the

highest reducing sugar 1%w/v NaOH and ultrasound for 60 min gave total reducing sugar of 36.21%. The ultrasonic assistance reduced processing time, significantly decreased lignin content more than in case without ultrasonic in all samples and also gave higher yield of reducing sugar.

2.3.3 Chemical Pretreatment

Chemical pretreatment has the main objective to remove hemicellulose and lignin in the raw material to make the cellulose much more accessible to hydrolyzed by acid in the next process. In addition, chemical pretreatment usually used acid pretreatment and alkaline pretreatment to remove hemicellulose and lignin.

Several types of acid such as sulfuric acid, hydrochloric acid, phosphoric acid and nitric acid were used in acid pretreatment. Concentrated acid or dilute acid can be used but use of dilute acid is more attractive due to the concentrate acid can cause the formation of by-product including furfural, 5-hydroxymethylfurfural, phenolic acids and aldehydes and main the disadvantage is corrosive to the equipment [24]. Dilute acid pretreatment can use high temperature approximately above 160 °C for shorter reaction time or lower temperature approximately below 160 °C for longer reaction time (30-90 min).

Eufrozina et al. [25] employed wheat straws treated by acid pretreatment method by using sulfuric acid at concentration ranging of 0.5 to 4 wt% at temperature of 120 °C, 130 °C, 160 °C and 170 °C. They achieved an increase of total reducing sugar content of 63.37% compared without pretreated sample.

Zhang et al. [26] studied about the dilute acid pretreatment of cattails by treated with different sulfuric acid concentration in the range of 0.1% to 1% in temperature in the range of 140-180 °C for 15 min. They reported that the highest yield of glucose achieved at 0.5% sulfuric acid 180 °C for 5 min. This result show that the dilute acid required high temperature and also used short reaction time.

Jhalique et al. [27] studied about the optimization condition of acid pretreatment of cogon grass by using sulfuric concentration of 0.2 wt% to 0.6 wt% in temperature range of 115 to 127 °C for 30 min. Their result gave the optimum condition of dilute pretreatment acid of 0.6 wt% sulfuric acid, 126 °C for 20 min gave 71.29% of reducing sugar.

Another approach of chemical pretreatment is alkaline pretreatment. Alkaline pretreatment is used to remove lignin from lignocellulose. Sun et al [28] reported that alkaline hydrolysis can cause the saponification of intermolecular ester bonds crosslinking of xylan, hemicellulose and lignin.

Nuntika et al. [29] studied about the alkaline pretreatment of sweet sorghum bagasse by using sodium hydroxide concentration of 5%wt to 15%wt at 121 °C for 25 min. They reported that at the optimum condition of 10%wt NaOH gave the highest reducing sugar yield of 34.97% and the cellulose content of 90.37%, lignin and hemicellulose remaining are 5.97% and 3.56%, respectively. This result showed that the alkaline pretreatment can reduce the hemicellulose and lignin content from lignocellulose and also gave high yield of product.

Waesarat et al. [30] studied the optimization of alkaline pretreatment of rice straw for reducing sugar production by different concentration of NaOH in ranging from 1%wt to 3%wt, duration ranging of 1 to 3 hours at 60 °C. They reported that the rice straw without pretreatment gave the lower yield than with alkaline pretreatment and the optimum

condition was 1.12%wt NaOH at 2.08 hours gave the yield of reducing sugar of 31.24%, compared to yield of reducing sugar of 7.74% of untreated raw material.

2.4 The hydrolysis reaction of celluloses and agricultural residues.

The hydrolysis reaction of cellulose and agricultural residues as a raw material usually used acid catalyst to produce glucose. The yield of glucose depends on type of agricultural residues and catalysts, which can be heterogeneous catalyst or homogeneous catalyst. The advantages and disadvantages of both types will be reviewed in the next topic

2.4.1 The hydrolysis reaction using homogeneous catalyst.

The reaction using homogeneous catalyst refers to reaction, which has the same phase of the substrate and catalyst in the system and mostly occurred in the liquid phase [31]. The homogeneous catalyst was used for a long time for hydrolyze cellulose to glucose. Advantages of the homogeneous catalyst includes less expensive of operating cost because reaction can be operated under mild condition. The cost of homogeneous catalyst is lower than the heterogeneous catalyst. But they have some disadvantages such as the corrosive from the harsh mineral acid and difficultly of separation and recovery because these reaction is in one phase and affect in the large amount of waste water in the nautralization step [32]. The homogeneous catalyst act upon the substrate, which dissociate to give proton (H^+) in aqueous solution result in conversion of cellulose to glucose. The homogeneous catalyst, which widely used in hydrolysis reaction are sulfuric acid, phosphoric acid and hydrochloric acid. When heat is added into the reaction, the β-1,4-glycosidic bonds of cellulose are splited by the proton (H^+) and water molecules [31]. The *p*-Toluenesulfonic acid (p-TSA) is a type of homogeneous catalysts act upon the substrate which dissociate to give proton (H^+) in aqueous solution and destroy the β-1,4-glycosidic bonds of cellulose. The *p*-toluenesulfonic acid (p-TSA) is more interesting catalyst for convert cellulose to glucose because higher yield of product and strong acid with of pK_a of -2.5. The homogeneous catalyst in aqueous solution can convert cellulose into glucose by mechanism as shown in figure 2.5 [33].

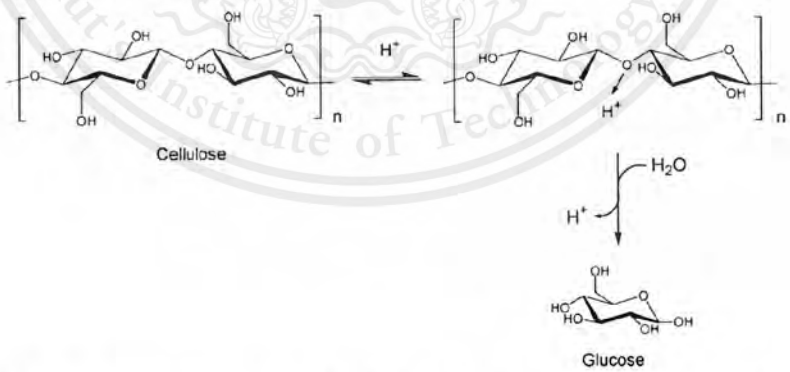


Figure 2.5 The hydrolysis reaction of cellulose by the homogeneous catalyst [33].

Ananda et al. [34] studied the hydrolysis of cellulose by using the homogeneous catalyst. They employed cellulose 0.03 g and suspended in 2 mL of aqueous acid catalyst, which has a concentration of 0.0321 M at the temperature ranging from 140 to 190 °C,

both sulfuric acid and p-toluenesulfonic acid (p-TSA) were tested as catalysts for the reaction. They reported a maximum yield of glucose was obtained at 170 °C of both catalysts that were 21% glucose using p-toluenesulfonic acid (p-TSA) catalyst and 16% glucose using sulfuric acid catalyst. They found that p-toluenesulfonic acid (p-TSA) catalyst produce higher glucose yield compared to sulufuric acid catalyst solutions of the same acid strength because of it tends to give higher catalytic activities in the degradation of cellulose.

Silvia et al. [35] studied the same reaction by using p-toluenesulfonic acid (p-TSA) and sulfuric acid as the homogeneous catalyst. The cellulose 0.5 g were mixed with 10.00 mL of acid solution at concentration of 0.2 M, 140 °C for 5 hours. They observed the yield of glucose were 18% glucose and 9% using sulfuric acid and p-toluenesulfonic acid (p-TSA), respectively. This result was different from the previous studied due to the different acid strength of sulfuric acid and p-toluenesulfonic acid (p-TSA). They reported that the amount of glucose produced increases with increasing acid strength.

Ananda et al. [36] employed the p-TSA and sulfonic acid to hydrolysis cellulose and compare with the same acid strength of catalysts at 0.0321 M by adding 0.03 g of cellulose in to 2 mL of an aqueous acid solution at 160 °C for 3 hours. They reported the yield of glucose are 21.5% using p-toluenesulfonic acid (p-TSA) and 17.4% using sulfuric acid catalyst and as a catalyst at 166 °C. They found that the increasing hydrophobicities of sulfonic acid can increase in cellulose degradation. Moreover, the sulfonic acid tends higher thermal stability than the sulfuric group. In addition, the glucose yield will increase in the temperature range at 140-170 °C and decrease rapidly at beyond 170-180 °C for all the catalyst because the decomposition of glucose to other products such as 5-hydroxymethulfurfural (HMF), levulinic acid, and formic acid at high temperature.

Silvia et al. [37] studied the hydrolysis of cellulose to glucose 0.5g cellulose and 40 mL of water were mixed with 10 mL of acid solution at concentration of 0.2-0.5 M in the temperature range at 120-160 °C. They obtained the highest yield of glucose at 160 °C by using 0.2 M of sulfuric acid and p-toluenesulfonic acid (p-TSA) as catalyst achieved 18.7% glucose despite its different acid strength.

The optimum condition of cellulose hydrolysis is shown in Table 2.2 it showed that the sulfuric acid and p-toluenesulfonic acid (p-TSA) are often used as catalysts in glucose synthesis, both of these two catalysts can be operated at moderate temperature for the degradation of cellulose. The p-toluenesulfonic acid (p-TSA) is more interesting catalyst for convert cellulose to glucose because higher yield of product and less corrosive than the sulfuric acid.

Table 2.2 Optimum conditions of hydrolysis reaction of cellulose to glucose by homogeneous acid catalyst.

Temperature (°C)	Water to cellulose mass ratio	Catalysts type	Amount of cellulose (g)	Amount of catalyst (mL)	Reaction time (hour)	Glucose yield (%)	Reference
170	66:1	p-TSA	0.03	2.0	3	21.0	[34]
140	80:1	H_2SO_4	0.5	10.0	5	18.0	[35]
166	66:1	p-TSA	0.03	2.0	3	21.5	[36]
160	80:1	H_2SO_4, p-TSA	0.5	10.0	2	18.7	[37]

2.4.2 The hydrolysis reaction using heterogeneous catalyst.

The heterogeneous catalyst by definition is a catalyst that is in a different phase from the reactants, usually be solid catalyst. The advantages of heterogeneous catalyst including easy to separate and recover, stable at high temperature and less corrosive problem. But there are disadvantages of very poor selectivity of product resulting in low yield of glucose and produce by-product in the reaction, higher cost of the catalyst required, high catalyst to substrate mass ratio and longer reaction time [33]. Various types of solid catalyst are used for the cellulose hydrolysis to glucose production. A summary of the studied from the literature employing heterogeneous catalysts is provided in table 2.3.

Table 2.3 Optimum conditions of hydrolysis reaction of cellulose to glucose by heterogeneous acid catalyst.

Temperature (°C)	Water to cellulose mass ratio	Catalysts type	Amount of cellulose (g)	Amount of catalyst (g)	Reaction time (hour)	Glucose yield (%)	Reference
150	111:1	Amberlyst-15 Activated-carbon Silicon dioxide	0.045	0.05	24	26.0	[38]
150	10:1	Amberlyst-15 γ-Al_2O_3	1.5	1.5	3	15.0	[39]
180	123:1	Amberlyst-70 Silicon dioxide Titanium dioxide	0.324	0.05	0.33	6.3	[40]
130	50:1	Amberlyst-15	0.1	0.2	12	3.8	[41]

Ayumu et al. [38] studied the hydrolysis of cellulose into glucose over various types of solid acid catalysts. They employed the cellulose 45 mg and catalyst 50 mg mixed with distilled water 5.0 mL for 24 hours at 150 °C. The result of reaction achieved the low yield of glucose, 26.0% using Amberlyst-15 catalyst, 6.3% using activated-carbon (AC) and only 3.2% using silicon dioxide (SiO_2) as solid acid catalysts.

Da-ming et al. [39] used the 1.5 g of cellulose and 1.5 g of solid catalyst mixed with 15 mL of water at 150 °C for 3 hours of studied the hydrolysis of cellulose to

glucose. They observed the yield of glucose only 15.0% and 3,0% using Amberlyst-15 and γ-Al_2O_3, respectively.

Yabushita [40] studied the catalytic conversion of cellulose using the hydrolysis reaction by employed cellulose 0.324 g, 0.05 g of solid catalyst mixed with 40 mL of distilled water at 150 °C for 20 mins. The result obtained the low glucose yield at 6.3%, 2.1% and 2.3% using Amberlyst-70, silicon dioxide (SiO_2), titanium dioxide (TiO_2), respectively.

Atsushi et al. [41] studied hydrolysis of cellulose by using layered transition-metal oxide ($HNbMoO_6$) and Amberlyst-15. They reported the highest yield of glucose of 3.8% by operated under mild condition at 130 °C for 12 hours using cellulose 0.1 g mixed with 0.2g of solid acid catalyst using Amberlyst-15.

All of report showed that Amberlyst-15 and Amberlyst-70 are suitable catalyst for synthesis of glucose due to high catalytic performance as a solid acid catalyst for the hydrolysis of cellulose. The heterogeneous catalyst in aqueous solution can be converted cellulose to glucose by a mechanism as shown in figure 2.6 [42].

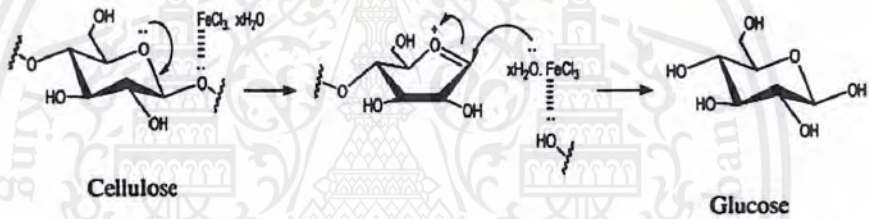


Figure 2.6 The hydrolysis reaction of cellulose by the heterogeneous catalyst [42].

From the result, the heterogeneous catalyst achieved very poor yield of catalyst and also used long reaction time. By this reason, the homogeneous catalyst is the better catalyst for hydrolysis of cellulose and agricultural residue to produce glucose due to high yield of glucose and lower reaction time and also lower cost of catalyst and can be operated at milder condition. The p-toluenesulfonic acid (p-TSA) is selected as a catalyst for study.

CHAPTER III
RESEARCH METHODOLOGY

This senior project aimed to study the pretreatment of water hyacinth prior to the hydrolysis reaction. The various concentration of NaOH and pretreatment time were investigated. And to study the synthesis of glucose by hydrolysis reaction of water hyacinth using p-TSA as an acid catalyst, in order to find the optimum condition. The parameters studied were temperature, concentration of catalyst and reaction time.

3.1 Raw material preparation

3.1.1 Equipments

1) Dry blender
2) Hot air oven

3.1.2 Raw material

1) Water hyacinth

3.1.3 Experimental procedure

1) Washed the water hyacinth with tap water.
2) Separated and dice stem and leaf of water hyacinth.
3) Dried the water hyacinth in a hot air oven, keep temperature at 105 °C for 24 hours.
4) Reduced size by spinning in dry blender.
5) Collected dried water hyacinth in silica gel box for moisture prevention.

3.2 Analysis of chemical components in water hyacinth

The analysis of chemical components of cellulose, hemicellulose and lignin in water hyacinth can use klason lignin method in TAPPI T222 om-98 standard. The method in brief is as followed:

3.2.1 Equipments

1) Autoclave
2) Beaker 100 mL
3) Analytical scale 4 digits
4) Pipette 10 mL
5) Pipette bulb
6) Filter paper 11 μm
7) Suction flask
8) Buchner funnel
9) Vacuum pump
10) Graduated cylinder 100 mL
11) Volumetric flask 100 mL
12) Laboratory bottle for autoclave
13) Ultrasonic bath

3.2.2 Chemicals

1) Dried water hyacinth
2) 98 vol% concentrated sulfuric acid (H_2SO_4)
3) Distillated water

3.2.3 Experimentatal procedure

1) Weigh the dried water hyacinth 0.2g into 100 mL beaker.
2) Prepared 72 vol% H_2SO_4 from 98 vol% H_2SO_4 using distillated water to adjust the volume in volumetric flask capacity 100 mL.
3) Add 2 mL of 72% H_2SO_4 acid into the prepared beaker from step 1.
4) Put the breaker in the step 3 into ultrasonic bath for 2 hours.
5) Add 75 mL distillated water into beaker for adjusting concentration of H_2SO_4 to 3% H_2SO_4 and pour into laboratory bottle.
6) Put laboratory bottle into an autoclave machine and keep temperature at 121 °C for an hour and took the sample out of the autoclave and wait until it becomes room temperature.
7) Set up the suction flask with Buchner funnel and connect with vacuum pump for filter the solid part from step 6.
8) Pour the solution in step 6 pass filter paper on the Buchner funnel and suction flask equipped with vacuum pump.
9) Bring the filter paper with solid to dry in a hot air oven keep temperature at 105 °C for 24 hours and weigh the remaining solid. Calculate the remaining lignin by this equation,

$$\text{percent of lignin}(\%)=\left(\frac{(W_S+W_{FP}+W_{FL})-(W_{FP}+W_{FL})}{W_{WH}}\right)\times 100,$$

whereas W_S is weight of solid part
W_{FP} is weight of filter paper
W_{FL} is weight of foil
W_{WH} is weight of initial water hyacinth

10) Collected the liquid part sample for analysis using high-performance liquid chromatography (HPLC). The mobile phase used is the distillation water with flow rate 0.7 milliliters per minute. The column used was phenomenex (rezex RHM Monosaccharide H^+ (8%)) in the size of 300 × 7.8 milliters operate at 80°C and the refractive index (RI) detector was used. Cellulose and hemicellulose can be calculated by area under curve in chromatogram of HPLC.

3.3 Pretreatment of water hyacinth

3.3.1 Equipments
1) Sample bottle 20 mL
2) Spatula
3) Beaker 50 mL
4) Stirring rod
5) Pipette 10 mL
6) Pipette bulb
7) Ultrasonic bath
8) Filter paper 11 μm
9) Suction flask
10) Buchner funnel
11) Vacuum pump

3.3.2 Chemicals
1) Dried water hyacinth

2) Distillated water
3) Sodium hydroxide (NaOH) 99 vol%

3.3.3 Experimentatal procedure

1) Weight dried water hyacinth 0.2g into 50 mL beaker.
2) Prepared NaOH, as studied concentration shown in table 3.1.
3) Add the NaOH 40 mL into the prepared beaker in step 1.
4) Put the beaker into ultrasonic bath.
5) Stop the reaction at the required time as studied shown in table 3.1.
6) Set up suction flask with Buchner funnel and connect with vacuum pump.
7) Pour the solution in the beaker pass filter paper size 11 μm on the Buchner funnel and suction flask equipped with vacuum pump to filter the solid part of the sample.
8) Washed the sample using distilled water until it reaches neutral pH.
9) Bring the filter paper with solid to dry in a hot air oven keep temperature at 105 °C for 24 hours.
10) Dried pretreated water hyacinth was collected in the silica gel box for use in the synthesis of glucose. The compositions of dried pretreatment water hyacinth were also analyzed using Klason lignin method.

3.3.4 Conditions

Table 3.1 Condition use in the pretreatment of water hyacinth

Parameter	Conditions
Water hyacinth (g)	0.2
NaOH (mL)	40
Reaction time (min)	60,90,120
Concentration of NaOH (%w/v)	1,1.5,2

3.4 Synthesis of glucose : Hydrolysis of water hyacinth

3.4.1 Equipments

1) Round bottle neck 20 mL
2) Hotplate stirrer
3) Condenser
4) Magnetic stirrer
5) Thermocouple
6) Spatula
7) Stirring rod
8) Pipette 10 mL
9) Pipette bulb
10) Rubber tubes
11) Pumps

3.4.2 Chemicals

1) Dried pretreated and untreated water hyacinth
2) Distillated water
3) *p*-Toluenesulfonic acid (p-TSA)

3.4.3 Experimentatal procedure

1) Weigh dried pretreated and untreated water hyacinth 0.33g into round bottom flask 20 mL.
2) Prepare p-TSA, as studied concentration shown in table 3.2.
3) Add the p-TSA solution of 7 mL into round bottom flask 20 mL from step 1.
4) Heat an oil bath and keep temperature at 77 or 100 °C.
5) Add the magnetic bar into the round bottom flask.
6) Connect the round bottom flask with condenser, rubber tube and pump. Preparation set up for the reaction was as in figure 3.1.
7) Heat the substance at the temperature at 77 or 100 °C and stirrer at 300 rpm.
8) Stop the reaction at the required time.
9) Collect the glucose sample for product analysis using high-performance liquid chromatography (HPLC).

3.4.4 Conditions

Table 3.2 Condition use in hydrolysis reaction

Parameter	**Conditions**
Cellulose (g)	0.33
p-TSA(ml)	7
Temperature (°C)	77, 100
Reaction time (h)	2,3,4
Concentration of catalyst (M)	0.25,0.5, 1

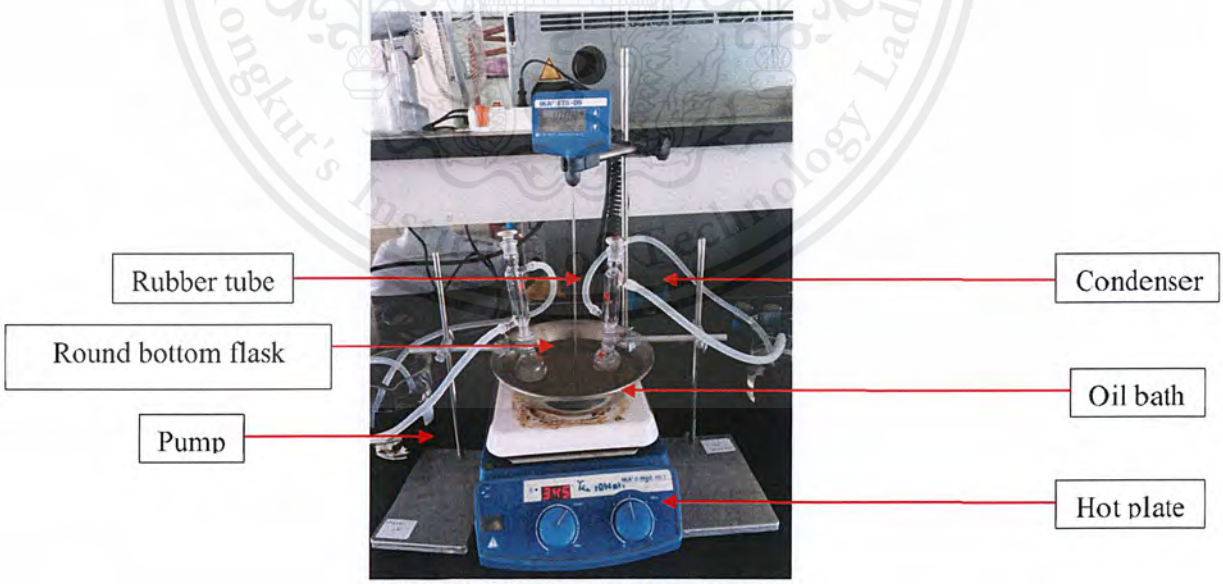


Figure 3.1 Set-up the experimental

3.5 Analysis of the sample

The quantity of glucose product can be analyzed using high performance liquid chromatography (HPLC) as following:

3.5.1 Equipments

1. Syringe 3 mL
2. Syringe filter 0.22 μm
3. Micro syringe 1 mL
4. Phenomenex Rezex RHM-Monosaccharide H+ (8%) column size 300 x 7.8 mm.
5. Security guard of HPLC column
6. High performance liquid chromatography, HPLC

3.5.2 Analysis

The concentration of glucose in part per million (ppm) were analyzed by using high performance liquid chromatography. The mobile phase used is the DI water with flow rate of 0.7 milliliters per minute. The column used was phenomenex (rezex RHM-Monosaccharide H^+ (8%) in the size of 300 × 7.8 milliters operate at column temperature of 80°C and the refractive index (RI) detector was used. The procedures were as follows:

1) Turn on the high-performance liquid chromatography and computer. Get access to YL-Clearity program, which is a software used for manipulating the HPLC.
2) Get the sampling substance 1 mL by using the syringe with filter and place into the sampling bottle.
3) Cleanse the micro syringe with the substance. Then, use the syringe to draw up the substance about 60 microliters.
4) Inject the solution to the HPLC injection port. Then, turn the injection port down. Turn the red bottom below the injection port and hold the needle for 10 seconds. Turn the injection port up. Take the syringe out of the injection port.
5) The peaks presented belong to the concentration of reactants and the products which were analyzed on the display of the computer. The concentration of reactants and the products can be calculated from the calibration curve of standard substances.
6) Calculate, the percent of each component in the sample and the yield of products using equation (3.1) and (3.2).

$$\text{Percent components}(\%) = \frac{C_{component} \times V}{W_{substrate}} \times 100 \qquad (3.1)$$

$$\text{Yield of products}\ (\%) = \frac{C_{product} \times V}{W_{substrate}} \times 100 \qquad (3.2)$$

Where

$C_{component}$	is concentration of required component from HPLC (mg/L)
$C_{product}$	is concentration of required from HPLC (mg/L)
$W_{substrate}$	is weight of substrate used in experiment (g)
V	is volume of solution (mL)

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Pretreatment of water hyacinth

The pretreatment method using NaOH in order to remove lignin for more accessible of acid to hydrolyze cellulose and hemicellulose into glucose and xylose. This might lead to improvement in hydrolysis and give the greater yield of glucose and xylose. The study of pretreatment of water hyacinth using NaOH and ultrasonic pretreatment without heat were investigated. The reaction condition of water hyacinth 0.2 g and NaOH 20 mL with various concentration of NaOH from 1 to 2 %w/v (g/mL) and various pretreatment time from 60 to 120 min in ultrasonic bath.

4.1.1 Effects of concentration of NaOH

The study on the effects of concentration of NaOH for water hyacinth pretreatment and observe the lignocellulose component in water hyacinth, which change in range of NaOH concentration from 1 to 2 %w/v found that when NaOH, increase, more lignin content will be removed. In case of cellulose, which is intensively affects to yield of glucose, the results show that at the concentration of 1.5 %w/v NaOH, cellulose and hemicellulose loss is less than at 1 and 2 %w/v NaOH. Sodium hydroxide could disrupt the lignin, On the contrary, the higher NaOH at 2% w/v, the cellulose and hemicellulose might be destroyed. In this study, the concentration of NaOH at 1.5% was selected because of suitable cellulose, hemicellulose and lignin content of pretreated water hyacinth, as shown in figure 4.1, figure 4.2 and figure 4.3.

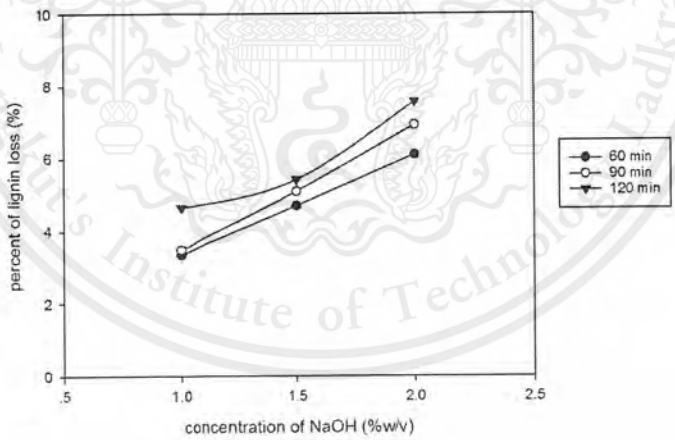


Figure 4.1 Effect of concentration of alkaline to the percent of klason lignin loss of water hyacinth using NaOH pretreatment with ultrasonic bath at various concentration of NaOH. Reaction condition: water hyacinth 0.2 g, NaOH 20 mL.

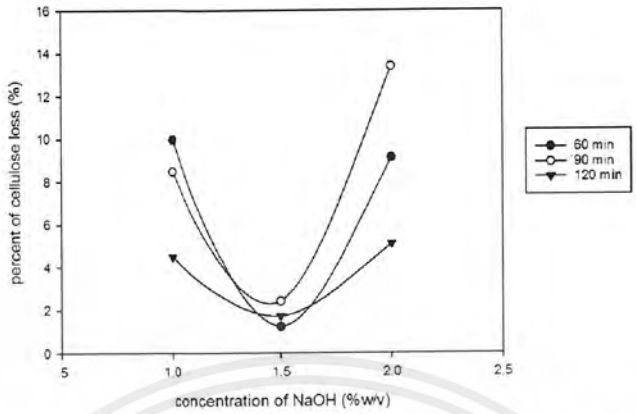


Figure 4.2 Effect of concentration of alkaline to the percent of cellulose loss of water hyacinth using NaOH pretreatment with ultrasonic bath at various concentration of NaOH. Reaction condition: water hyacinth 0.2 g, NaOH 20 mL.

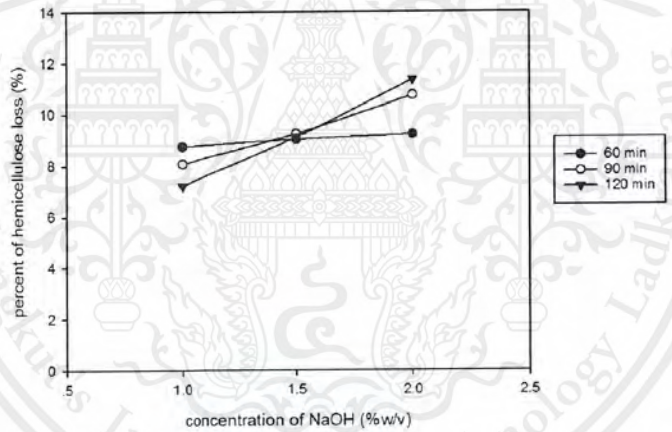


Figure 4.3 Effect of concentration of alkaline to the percent of hemicellulose loss of water hyacinth using NaOH pretreatment with ultrasonic bath at various concentration of NaOH. Reaction condition: water hyacinth 0.2 g, NaOH 20 mL.

This senior project studied the synthesis of glucose and xylose at moderate conditions of 77 and 100°C and using homogeneous catalyst (p-TSA) to find the optimum condition to obtain high glucose yields

4.2 Analysis of chemical components in water hyacinth

The analysis of cellulose, hemicellulose and lignin component in water hyacinth substrate using klason lignin method. The results of initial substrate of water hyacinth and after pretreatment are shown in the Table 4.1 and 4.2, the cellulose, hemicellulose and klason lignin component of initial substrate are 53.4, 24.2 and 15.2, respectively. After

pretreatment gave the cellulose, hemicellulose and lignin component of substrate remaining are 52.2, 15.2 and 10.5, respectively.

Table 4.1 The percent components of initial substrate

Substrate	Percent cellulose	Percent hemicellulose	Percent klason lignin
Water hyacinth without pretreatment	53.4	24.2	15.2

Table 4.2 The percent components of substrate after pretreatment

Substrate	Percent cellulose	Percent hemicellulose	Percent klason lignin
Water hyacinth after pretreatment	52.2	15.2	10.5

4.3 The hydrolysis reaction of water hyacinth into glucose with p-TSA as acid catalyst

4.3.1 Effects of temperature

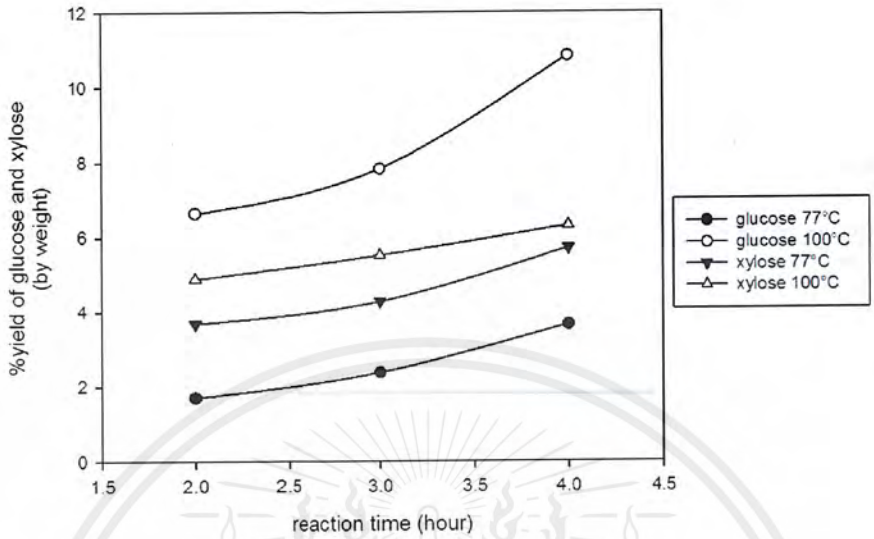
This study uses moderate temperature of 77 and 100°C. Both yield of glucose and xylose increases with increases temperature as expected as shown in figure 4.4 (a) the yield of glucose and xylose in weight and (b) the percent recovery of glucose and xylose. At the both temperature, the yield of xylose is a lot more than the yield of glucose because hemicellulose is easier react than cellulose. In this case, glucose product might come from glucomannan, which is one type of hemicellulose in biomass not glucose from cellulose. Because cellulose has stronger bond than hemicellulose and the bond might not be broken at this mild condition.

4.3.2 Effects of concentration of p-TSA

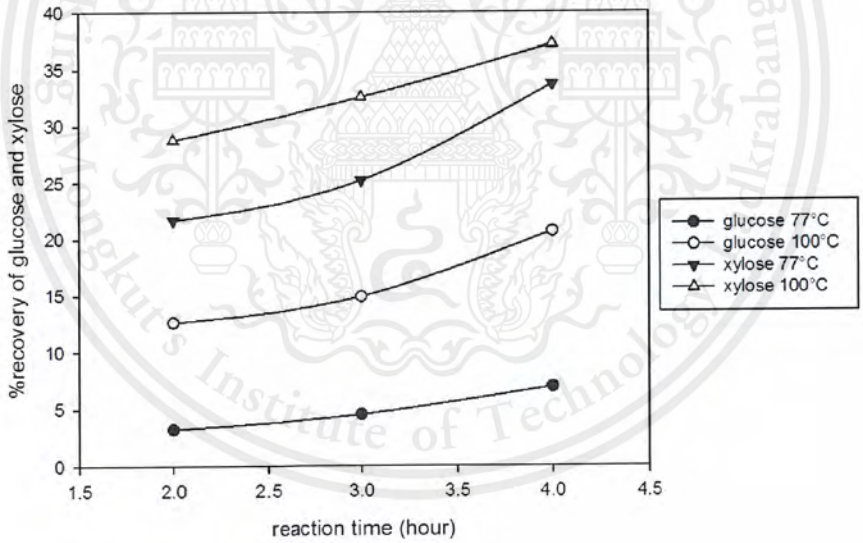
The result showed that increase catalyst concentration will increasing the yield of glucose. In case of xylose the concentration of 1M p-TSA gave the lower yield of xylose than 0.5 M p-TSA might cause of at higher concentration of catslyst, xylose weight react to form other by-product. The glucose and xylose yield reached the maximum of 10.8wt% and 6.3wt% and the glucose and xylose the percent recovery reached the maximum of 20.7% and 37.2% using 1M of *p*-Toluenesulfonic acid (P-TSA) at 100°C, as shown in figure 4.5 (a) the yield of glucose and xylose in weight and (b) the percent recovery of glucose and xylose.

4.3.3 Effects of reaction time

The decomposition of cellulose using *p*-Toluenesulfonic acid (p-TSA) as a catalyst increases as a course of the reaction time. The yield of glucose and xylose gradually increased as the reaction progressed, glucose and xylose yield reached the maximum of 10.8% and 6.3% after 4 hours at 100°C, as shown in figure 4.5 (a) the yield of glucose and xylose in weight and (b) the percent recovery of glucose and xylose.



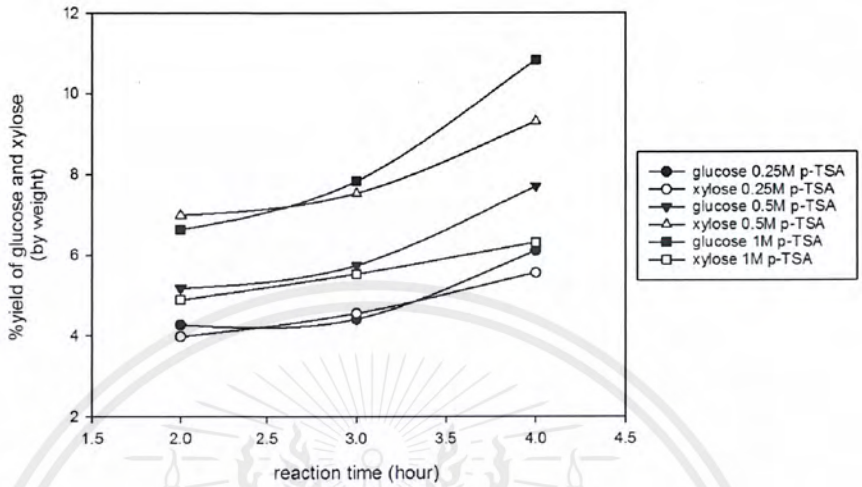
(a)



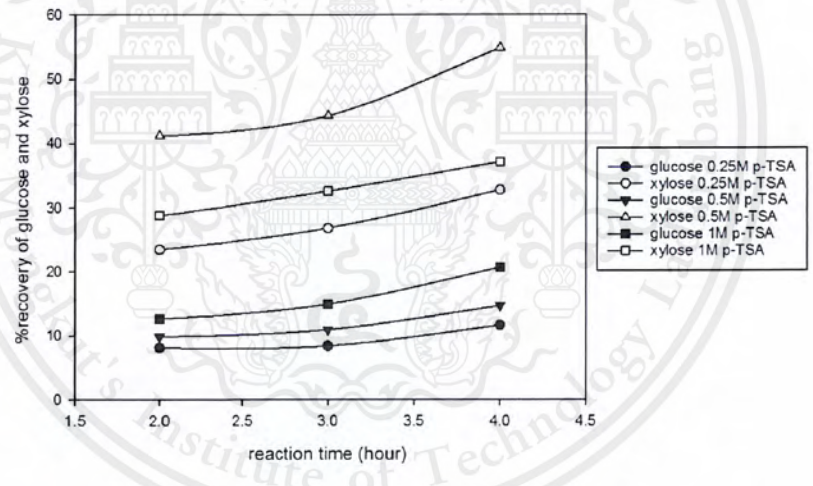
(b)

Figure 4.4 (a) Effect of temperature and reaction time to the yield of glucose and xylose of pretreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C and 100°C, water hyacinth 0.33 g, 1M p-TSA 7 mL.

(b) Effect of temperature and reaction time to the percent recovery of glucose and xylose of pretreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C and 100°C, water hyacinth 0.33 g, 1M p-TSA 7 mL.



(a)



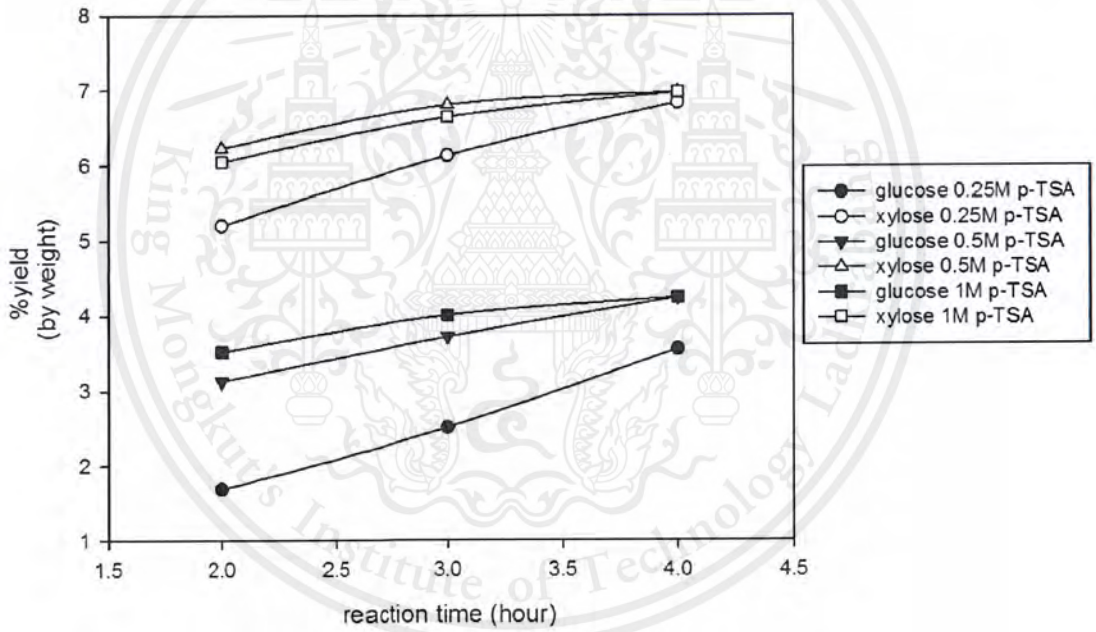
(b)

Figure 4.5 (a) Effect of concentration of p-TSA and reaction time to the yield of glucose and xylose of pretreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 100°C, water hyacinth 0.33 g, p-TSA 7 mL.

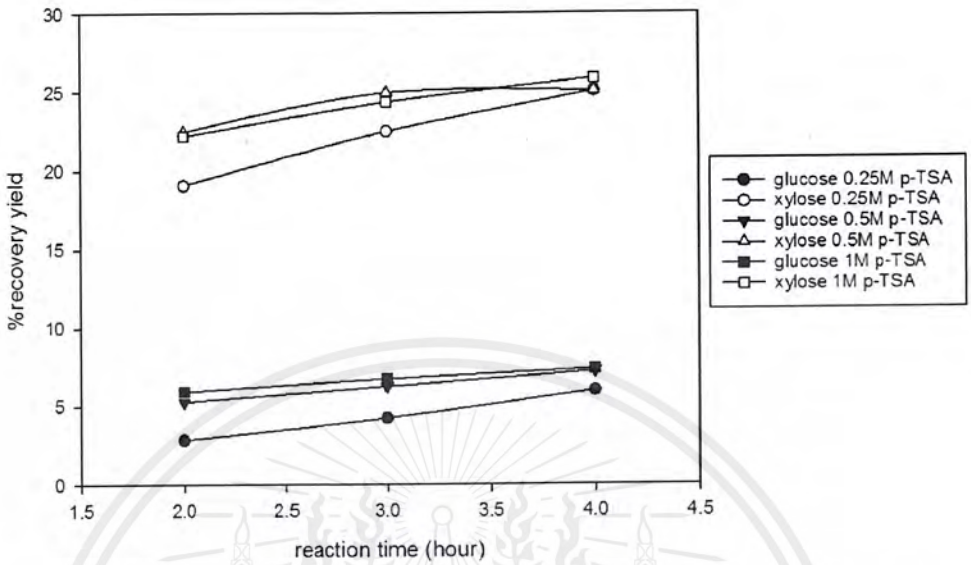
(b) Effect of concentration of p-TSA and reaction time to the percent recovery of glucose and xylose of pretreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition:100°C, water hyacinth 0.33 g, p-TSA 7 mL.

4.3.4 Effects of pretreatment

The effect of pretreated and untreated water hyacinth was compared at the temperature 77°C, at concentration of 1 M p-TSA for 4 hours. The result showed that the untreated water hyacinth gave the higher yield of xylose but gave a little lower yield of glucose than pretreated water hyacinth. This might cause by, the hemicellulose was release in the pretreatment of water hyacinth and it becomes easier to break bond to xylose than the untreated case. Thus, the untreated water hyacinth gave yield of glucose of 4.2% and xylose 7.0% whereas the pretreated water hyacinth gave the yield of glucose of 3.6% and xylose of 9.4%. In case of the percent recovery, the untreated water hyacinth gave the yield of glucose of 7.4% and xylose 25.9% whereas the pretreated water hyacinth gave the yield of glucose of 7.0% and xylose of 33.7% at the same condition of hydrolysis of 1 M p-TSA for 4 hours at 77°C, as shown in figure 4.6 (a) the yield of glucose and xylose by weight and (b) the percent recovery of glucose and xylose of untreated water hyacinth and figure 4.7 (a) the yield of glucose and xylose by weight of pretreated water hyacinth and (b) the percent recovery of glucose and xylose The pretreatment use 1.5%w/v NaOH for 60 min.



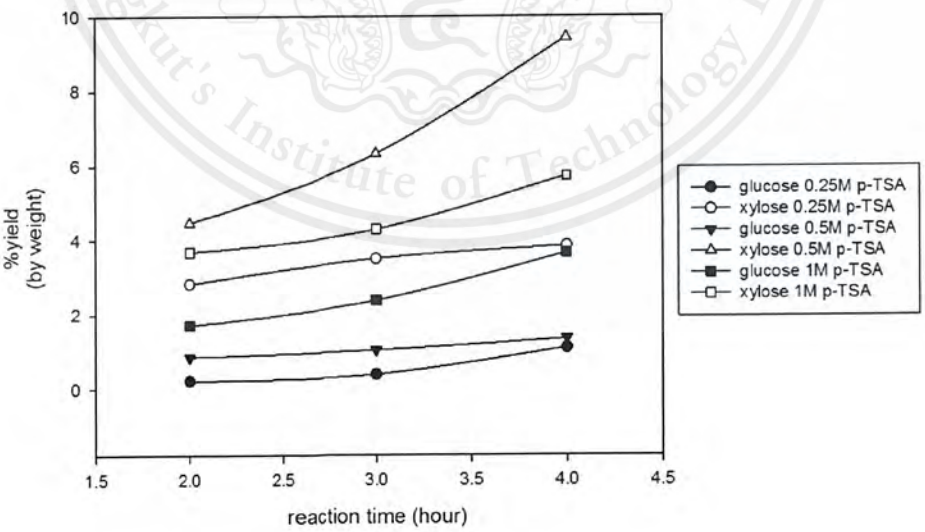
(a)



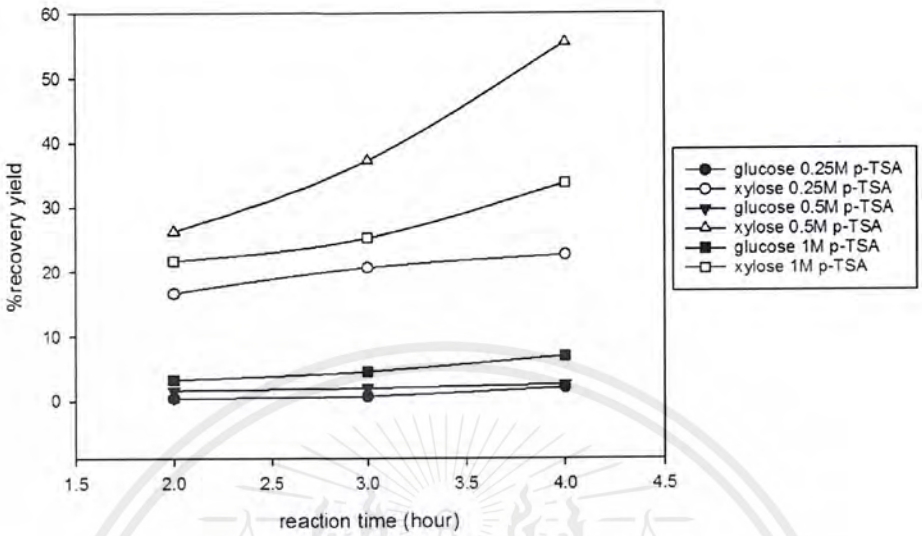
(b)

Figure 4.6 (a) Effect of reaction time to the yield of glucose and xylose of untreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C, water hyacinth 0.33 g, p-TSA 7 mL.

(b) Effect of reaction time to the recovry yield of glucose and xylose of untreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C, water hyacinth 0.33 g, p-TSA 7 mL.



(a)



(b)

Figure 4.7 (a) Effect of reaction time to the yield of glucose and xylose of pretreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C, water hyacinth 0.33 g, p-TSA 7 mL.

(b) Effect of reaction time to the recovry yield of glucose and xylose of pretreated water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C, water hyacinth 0.33 g, p-TSA 7 mL.

4.4 The hydrolysis reaction of cellulose powder into glucose with p-TSA as acid catalyst

This part compared the yield of glucose product between the hydrolysis reaction of pure cellulose and untreated water hyacinth from the appropriate condition of hydrolysis of untreated water hyacinth by using p-TSA acid catalyst at 77°C 1 M of p-TSA for 2 to 4 hours. The results showed that both of cellulose powder and water hyacinth almost gave the same yield of glucose product at this condition, as shown in figure 4.8 (a) the yield of glucose from cellulose powder and water hyacinth using and (b) the percent recovery of glucose from cellulose powder and water hyacinth.

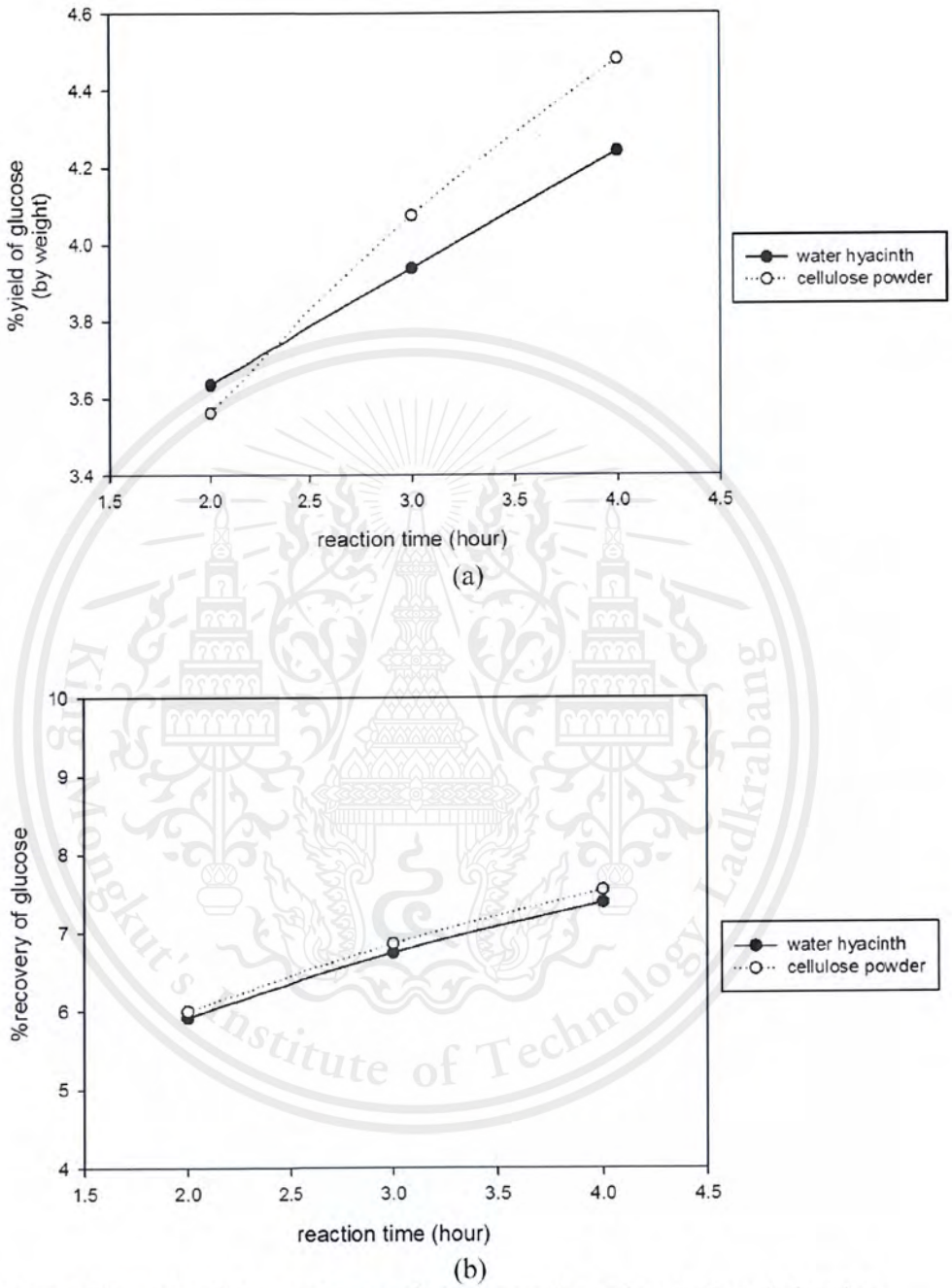


Figure 4.8 (a) The yield of glucose from cellulose powder and water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C, water hyacinth and glucose powder 0.33 g, p-TSA 7 mL.

(b) The percent recovery of glucose from cellulose powder and water hyacinth using p-TSA as acid catalyst at various time. Reaction condition: 77°C, water hyacinth and glucose powder 0.33 g, p-TSA 7 mL.

From the results in section 4.2 and 4.3 obtained the appropriate condition of pretreatment and hydrolysis of water hyacinth into glucose and xylose. In pretreatment, sodium hydroxide (NaOH) as alkaline pretreatment, the condition at concentration of 1.5% NaOH for 60 min was used because low loss in cellulose and hemicellulose and partially removed lignin. In case of hydrolysis, the water hyacinth without pretreatment the glucose was not significant different yield but xylose gave the higher yield than the pretreated water hyacinth at temperature 77°C. For the temperature at 100°C, the appropriate conditions are at reaction time of 60 min and 1.5 %w/v of NaOH for pretreatment and reaction time of 4 hours and 1M of *p*-Toluenesulfonic acid (p-TSA) for hydrolysis reaction gave the highest yield of glucose and xylose was 10.8% and 6.3%, respectively and the percent recovery of glucose and xylose was 20.7% and 37.2%, respectively.

CHAPTER V
CONCLUSIONS

5.1 Summary of the results

This senior project studied the appropriate condition of alkaline pretreatment using ultrasonic prior to the hydrolysis and the possibility of glucose and xylose synthesis from hydrolysis reaction of water hyacinth with p-TSA acid catalysts and find the appropriate conditions for glucose and xylose synthesis. The results can be summarized as follows:

The important parameters, which will affect the pretreatment of water hyacinth are the concentration of sodium hydroxide (NaOH) and reaction time. The increases of concentration of NaOH increases the lignin removal. The pretreatment time in the sonicator bath did not have a significant effect on the result. The appropriate condition of NaOH pretreatment was the concentration of 1.5% NaOH for 60 min. At this condition, 4.7% of lignin was removed and low loss in cellulose and hemicellulose of 1.2% and 9.0%, respectively.

For the hydrolysis of water hyacinth, the important parameters are temperature, the concentration of *p*-Toluenesulfonic acid (p-TSA) and reaction time. The yield of glucose and xylose gradually increased as the reaction temperature increases and prolong reaction time. When concentration of p-TSA increased, the yield of glucose increased as expected. But in case of xylose, the yield of xylose increases to a certain point of p-TSA concentration and then decrease. This might be because the xylose was reacted to form other product. The appropriate condition of hydrolysis reaction of water hyacinth with p-TSA acid catalysts gave the yield of glucose of 10.8wt% and the yield of xylose of 6.3wt% and the percent recovery of glucose and xylose was 20.7% and 37.2%, respectively at the condition temperature at 100°C, 1M p-TSA for 4 hours.

5.2 Suggestion

5.2.1 Further study on how to separate product from substances should be studied.

5.2.2 The appropriate condition for hydrolysis reaction in this project occurred when used water hyacinth as substrate, other substances might give the appropriate condition different from water hyacinth.

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APPENDIX

APPENDIX A

CALCULATION OF SUBSTRATE AND PRODUCT

A.1 Calculation of percent component in substrate

Calculation of percent component in substrate 0.2 g by inject the solution from filtration of klason lignin method using the high-performance liquid chromatography to find the amount of glucose and xylose in order to convert to amount of cellulose and hemicellulose, respectively. In part of solid from the filtration of klason lignin method will find the amount of lignin by the calculation in each step as follows:

Table A.1 The results of injection the solution from filtration after klason lignin method

Experiment	Area under curve of glucose (mV.s)	Area under curve of xylose (mV.s)
Run 1	402.056	190.236
Run 2	394.950	183.453

The area under curve of glucose and xylose are equivalent to concentration compared to the standard graph to find concentration of glucose and xylose in unit of parts per million unit (ppm) or milligram per liter (mg/L) as shown in the figure A.1 and A.2 are respectively.

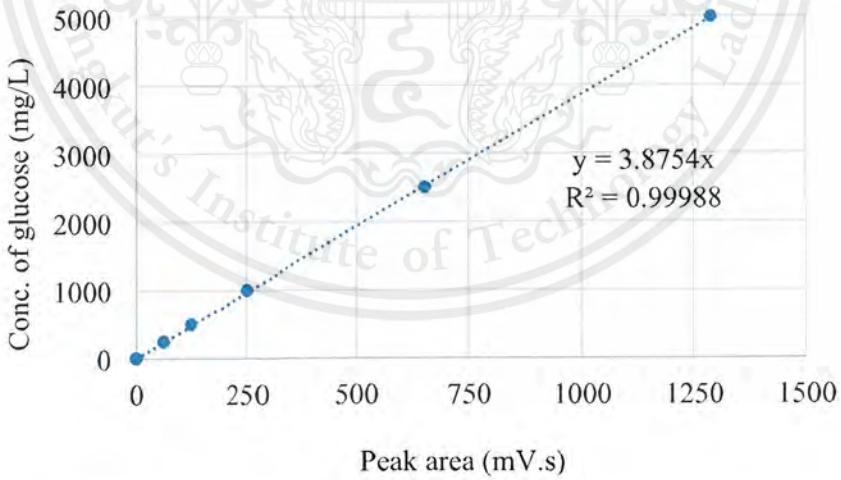

Figure A.1 Calibration curve of glucose concentration (mg/L)

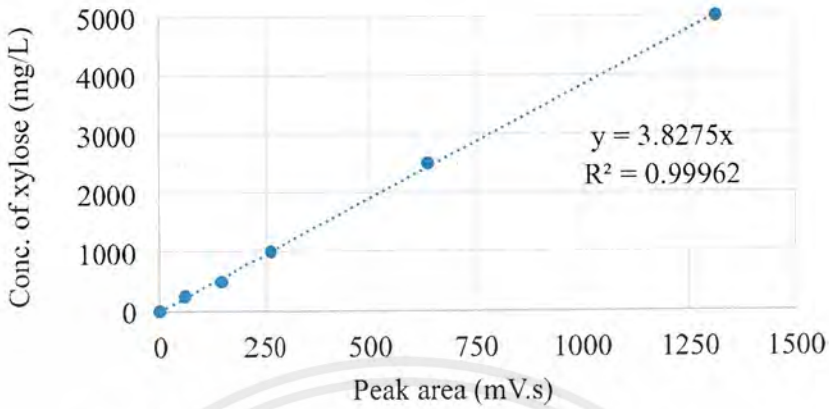

Figure A.2 Calibration curve of xylose concentration (mg/L)

% of cellulose and % of hemicellulose were calculated using equation (A.1) – (A.9).

Concentration of glucose (g) = (slope glucose of standard graph)(area of glucose) (A.1)

$$\text{Weight of glucose (g)} = \frac{(\text{concentration of glucose})(\text{initial volume of solution})}{1{,}000{,}000} \quad \text{(A.2)}$$

$$\text{Percent of glucose (\%)} = \frac{(\text{weight of glucose})}{(\text{weight of initial substrate})} \times 100 \quad \text{(A.3)}$$

Percent of cellulose (%) = (0.9)(percent of glucose) (A.4)

Concentration of xylose (g) = (slope xylose of standard graph)(area of xylose) (A.5)

$$\text{Weight of xylose (g)} = \frac{(\text{concentration of xylose})(\text{initial volume of solution})}{1{,}000{,}000} \quad \text{(A.6)}$$

$$\text{Percent of xylose (\%)} = \frac{(\text{weight of xylose})}{(\text{weight of initial substrate})} \times 100 \quad \text{(A.7)}$$

Percent of hemicellulose (%) = (0.88)(percent of xylose) (A.8)

$$\text{Percent of lignin(\%)} = \left(\frac{\text{weight of solid with filter and foil-weight of filter and foil}}{\text{initial weight of water hyacinth}}\right) \times 100 \quad \text{(A.9)}$$

Example of calculation: water hyacinth 0.2g from klason lignin method using sulfuric acid (H_2SO_4) 77 mL for 2 hours in ultrasonic bath and 1 hour in autoclave.

Concentration of glucose (g) = (3.875)(402.056) = 1558.128 mg/L

$$\text{Weight of glucose (g)} = \frac{(1558.128)(77)}{1{,}000{,}000} = 0.120 \text{ g}$$

$$\text{Percent of glucose (\%)} = \frac{(0.120)}{(0.2)} \times 100 = 59.895$$

Percent of cellulose (%) = (0.9)(59.895) = 53.906

Concentration of xylose (g) = (3.828)(190.236) = 728.128 mg/L

$$\text{Weight of xylose (g)} = \frac{(728.128)(77)}{1{,}000{,}000} = 0.056 \text{ g}$$

$$\text{Percent of xylose (\%)} = \frac{(0.056)}{(0.2)} \times 100 = 27.990$$

Percent of hemicellulose (%) = (0.88)(27.990) = 24.631

$$\text{Percent of lignin (\%)} = \frac{(1.556\text{-}1.526)}{0.2} \times 100 = 15.124$$

A.2 Calculation the yield of product from hydrolysis reaction

$$\text{Yield of glucose (\%)} = \frac{\text{(weight of glucose)}}{\text{(weight of glucose in initial substrate)}} \times 100 \quad \text{(A.10)}$$

$$\text{Yield of xylose (\%)} = \frac{\text{(weight of xylose)}}{\text{(weight of xylose in initial substrate)}} \times 100 \quad \text{(A.11)}$$

$$\text{The percent recovery of glucose (\%)} = \frac{\text{(weight of glucose)}}{\text{(weight of initial substrate)}} \times 100 \quad \text{(A.12)}$$

$$\text{The percent recovery of xylose (\%)} = \frac{\text{(weight of xylose)}}{\text{(weight of initial substrate)}} \times 100 \quad \text{(A.13)}$$

Example of calculation: unpretreated water hyacinth 0.33g from hydrolysis reaction using 0.25 M p-TSA
7 mL for 2 hours.

Concentration of glucose (g) = (3.875)(205.45) = 796.201 mg/L

$$\text{Weight of glucose (g)} = \frac{(796.201)(77)}{1{,}000{,}000} = 0.006 \text{ g}$$

$$\text{Yield of glucose (\%)} = \frac{(0.006)}{(0.196)} \times 100 = 2.844$$

Concentration of xylose (g) = (3.828)(640.79) = 2452.624 mg/L

$$\text{Weight of xylose (g)} = \frac{(2452.624)(77)}{1{,}000{,}000} = 0.017\text{g}$$

$$\text{Percent of xylose (\%)} = \frac{(0.017)}{(0.09)} \times 100 = 19.076$$

APPENDIX B
EXPERIMENTAL DATA

Table B.1 The result from alkaline pretreatment of water hyacinth

Concentration of NaOH (%w/v)	Time (min)	Percent cellulose (%)	Percent hemicellulose (%)	Percent lignin (%)
1	60	43.470	15.442	11.864
	90	44.952	16.117	11.730
	120	48.951	16.977	10.556
1.5	60	52.196	15.158	10.506
	90	51.014	14.940	10.105
	120	51.714	15.079	9.787
2	60	44.305	14.965	9.094
	90	40.045	13.452	8.276
	120	48.343	12.840	7.632

- The results from used 0.2g of water hyacinth with 20 mL NaOH for various concentration of NaOH and reaction time in ultrasonic bath.

Table B.2 The result from hydrolysis reaction of untreated water hyacinth

Temp. (°C)	Concentration of p-TSA (M)	Time (h)	Glucose yield (%)	Xylose yield (%)	Glucose recovery yield (%)	Xylose recovery yield (%)
77	0.25	2	2.844	19.076	1.689	5.203
		3	4.232	22.484	2.513	6.132
		4	5.984	25.051	3.554	6.832
	0.5	2	5.263	22.451	3.126	6.123
		3	6.263	24.960	3.720	6.807
		4	7.228	25.070	4.242	6.863
	1	2	5.921	17.701	3.517	6.045
		3	6.749	24.365	4.008	6.645
		4	7.384	25.861	4.242	6.970

- The results from used 0.33g of untreated water hyacinth with 7 mL of p-TSA for various concentration and reaction time at 77 °C

Table B.3 The result from hydrolysis reaction of pretreated water hyacinth

Temp. (°C)	Concentration of p-TSA (M)	Time (h)	Glucose yield (%)	Xylose yield (%)	Glucose recovery yield (%)	Xylose recovery yield (%)
77	0.25	2	0.218	2.824	0.415	16.642
		3	0.385	3.506	0.735	20.658
		4	1.090	3.841	2.080	22.632
	0.5	2	0.848	4.453	1.617	26.243
		3	1.032	6.318	1.969	37.231
		4	1.336	9.420	2.548	55.512
	1	2	1.706	3.683	3.254	21.706
		3	2.378	4.279	4.537	25.217
		4	3.653	5.714	6.968	33.674
100	0.25	2	4.267	3.978	8.140	23.439
		3	4.410	4.553	8.413	26.828
		4	6.103	5.554	11.642	32.729
	0.5	2	5.171	6.988	9.864	41.178
		3	5.740	7.530	10.950	44.373
		4	7.703	9.312	14.694	54.876
	1	2	6.638	4.884	12.662	28.780
		3	7.831	5.527	14.937	32.572
		4	10.841	6.309	20.679	37.178

- The results from used 0.33g of pretreated water hyacinth with 7 mL of p-TSA for various concentration and reaction time at 77 and 100 °C

APPENDIX C
ANALYSIS OF GLUCOSE

The concentration of glucose in part per million (ppm), analyzed by high performance liquid chromatography (HPLC) at column temperature of 80°C flow rate of 0.7 milliliters per minute. The mobile phase was DI water. The column used was phenomenex (rezex RHM-Monosaccharide H^+ (8%)) in the size of 300 × 7.8 milliters. The procedures were as follows:

C.1 Prepared a mobile phase

1) Place DI water in a 1 liter bottle.

2) Remove bubbles in mobile phase solution with ultrasonic bath at room temperature for 30 minutes.

C.2 Prepared sample to analysis

1) Take the sample from sample bottle in a beaker with a 3 milliliter syringe.

2) Get the sampling by using the syringe with filter, size 0.2 microns. Then inject the sample through the filter into a clean container.

C.3 Setup the HPLC

1) Turn on switch of pump system on (1-A)

2) Turn on switch of column system on (1-B)

3) Turn on switch of detector on (1-C)

Figure C.1 High-performance liquid chromatography-on / off switch.

4) Wait the status of pump, column and detector are ready

5) Open the computer

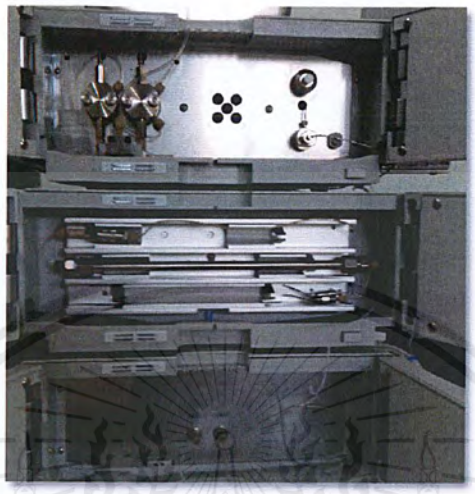

Figure C.2 Composition of equipment in High-performance liquid chromatography

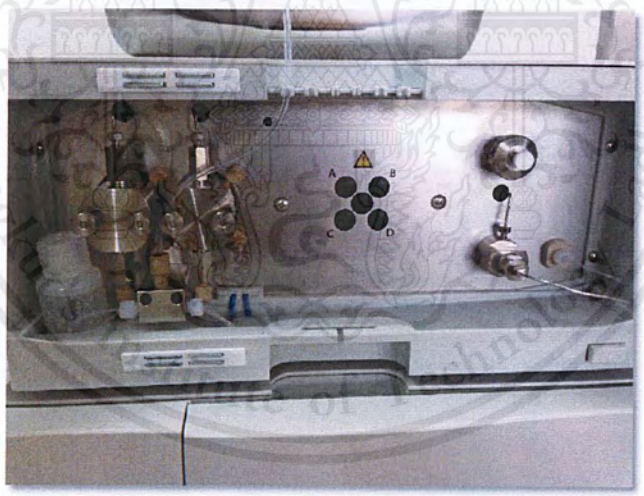

Figure C.3 Composition of pump in High-performance liquid chromatography

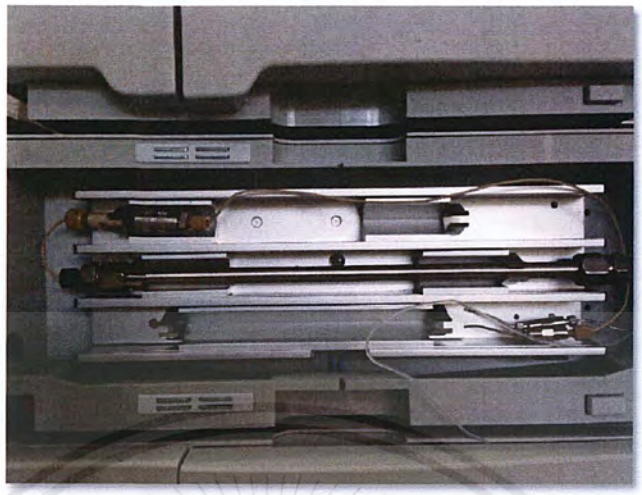

Figure C.4 Composition of column in High-performance liquid chromatography

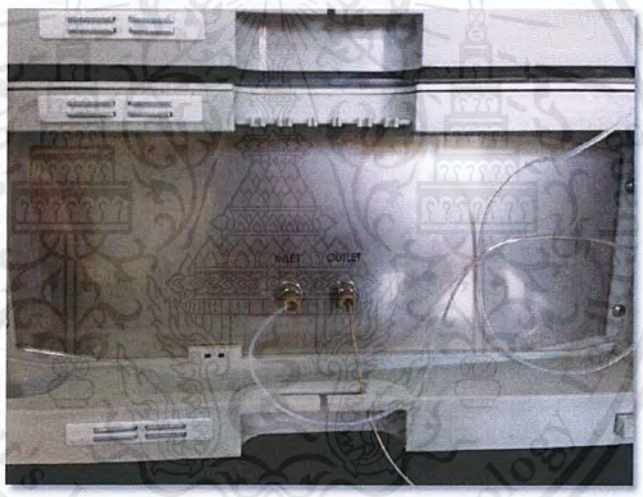

Figure C.5 Composition of detector in High-performance liquid chromatography

C.4 Program YL-Clarity

1) Selected the icon of program on the desktop of computer
2) Tab of chromatogram is appeared (2-A), login to system
3) Tab of HPLC system, which can be set the method and folder for save (2-B)
4) Tab of HPLC-LC Gradient to set the flow rate (2-C)
5) Tab of HPLC-Acquisition to set the temperature of detector (2-D)
6) Tab of HPLC-Thermostat to set the temperature of column (2-E)

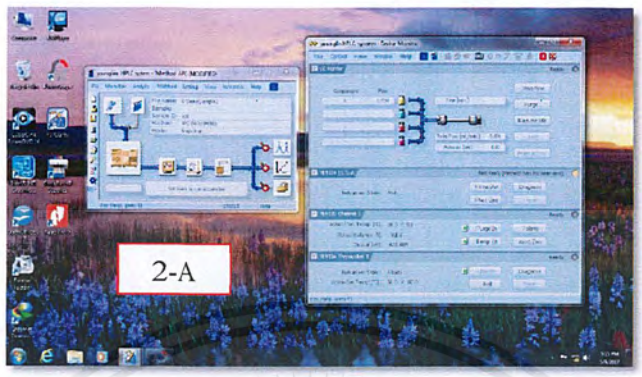

Figure C.6 Setup the program

Figure C.7 Setup the program (cont.)

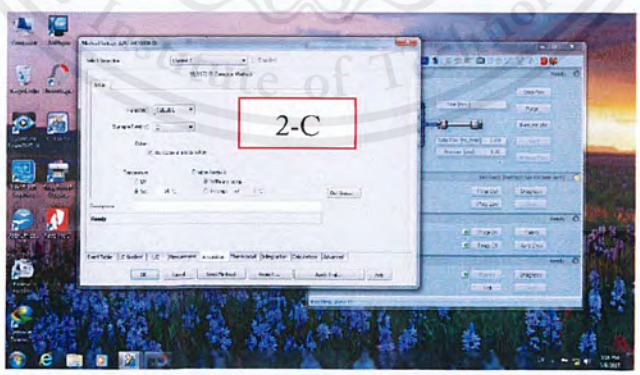

Figure C.8 Setup the program (cont.)

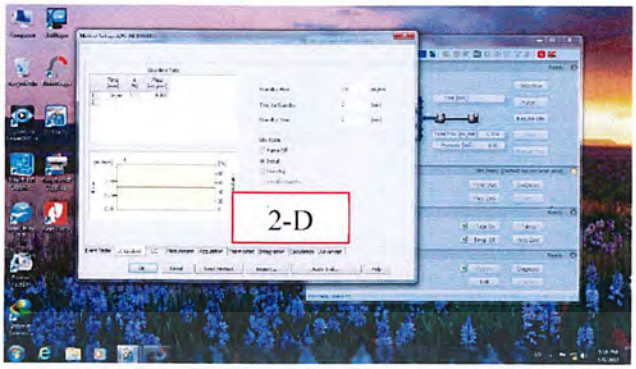

Figure C.9 Setup the program (cont.)

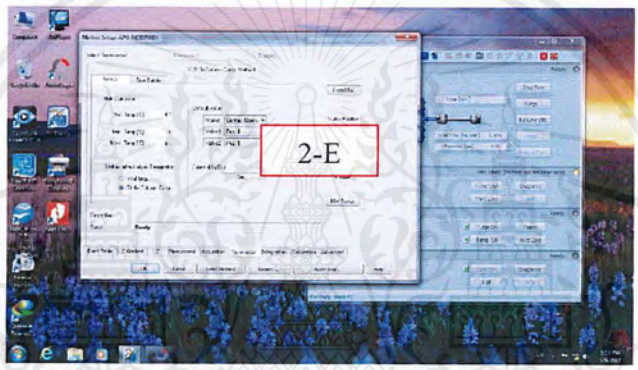

Figure C.10 Setup the program (cont.)

C.5 Analysis of product

1) Turn on the high-performance liquid chromatography and computer. Get access to YL-Clarity program, which is a software used for manipulating the HPLC.
2) Get the sampling by using the syringe with filter, about 1 milliliter in the sample bottle and label the sampling.
3) Cleanse the micro syringe with the substance. Use the syringe to draw the substance about 60 microliters. Avoid bubble in the micro syringe.
4) Inject the solution to the injection port. Pull it down around 6 seconds then pull it up. (1-D)
5) Take the syringe out of injection port. (1-E)

6) Consider the information detected and showed on the screen of the computer representing each substance. The peaks presented belong to the concentration of reactants and the products. (3-A)

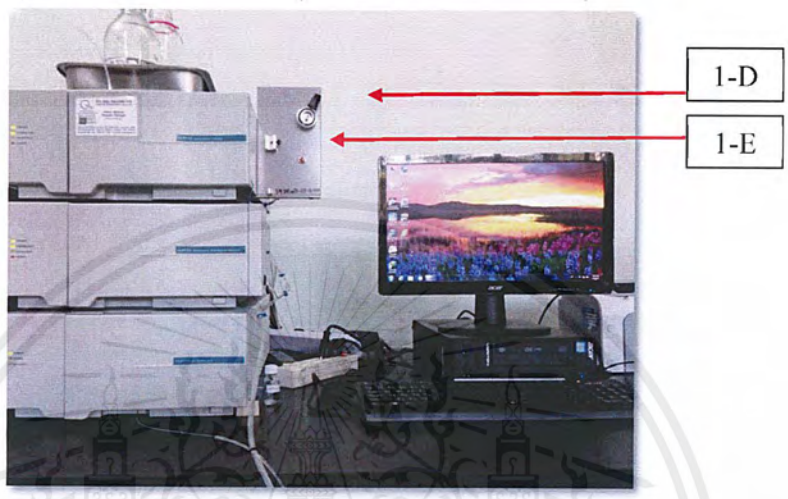

Figure C.11 Analysis of product

Figure C.12 Analysis of product (cont.)

C.6 Chromatography

1) Tab of chromatogram to analysis of product. (3-B)

2) When analyze to time limit, stop the program and linked to HPLC-Chromatogram to analysis of product and preview the chromatogram. (3-C)

3) Save the results, click of File >> Save As >> selected the folder to save

4) Analysis of others sample

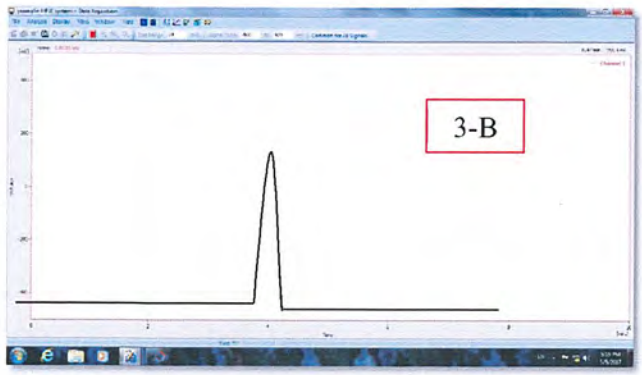

Figure C.13 HPLC chromatogram

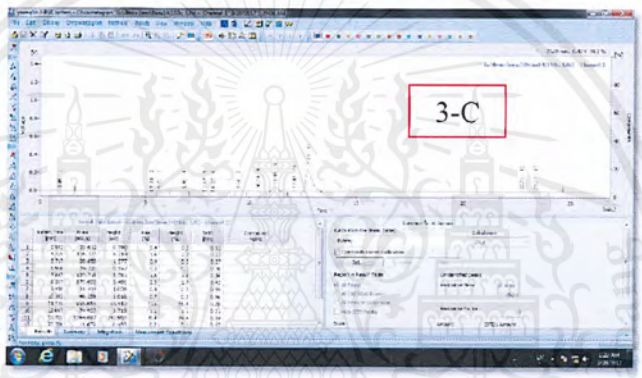

Figure C.14 HPLC chromatogram (cont.)

C.7 Cleaning of column and detector

For the clean of column and detector used mobile phase as DI water to remove residuals and contaminants about 30 minutes.

C.8 Shutdown of HPLC

1) Close all Chromatogram windows that appear on the computer screen.
2) Shut down of computer
3) Shut down of pump
4) Shut down of column and keep column
5) Shut down of detector

BIBLIOGRAHPY

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