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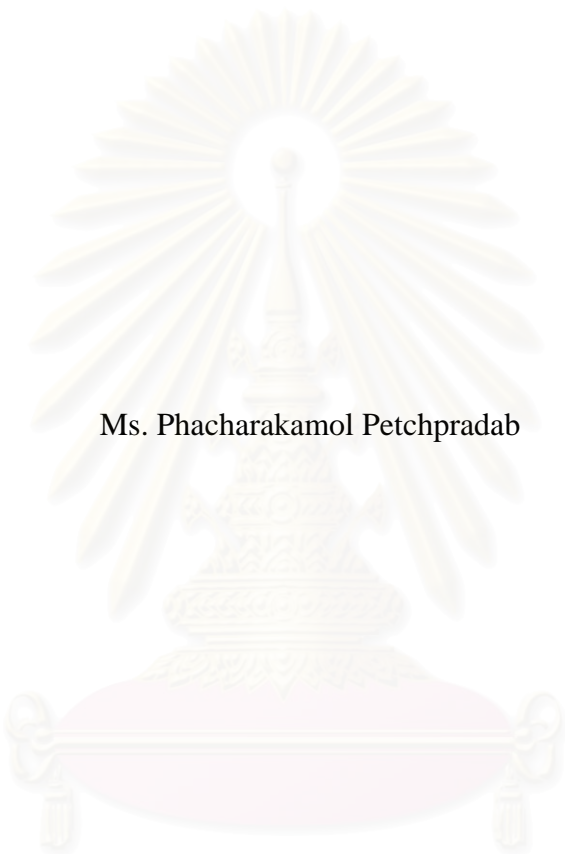
HYDROTHERMAL PRETREATMENT OF RUBBER WOOD RESIDUE
FOR GLUCOSE PRODUCTION

Ms. Phacharakamol Petchpradab

A Thesis Submitted in Partial Fulfillment of the Requirements

for the Degree of Master of Engineering Program in Chemical Engineering

Department of Chemical Engineering

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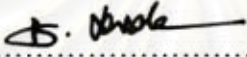
Thesis Title   Hydrothermal pretreatment of rubber wood residue for
                    glucose production

By   Ms. Phacharakamol Petchpradab
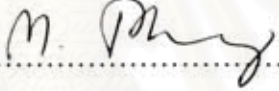Field of study   Chemical Engineering
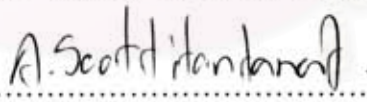Thesis Principal Advisor   Associate Professor Tawatchai Charinpanitkul, D.Eng.

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พชรกมล เพชรประดับ : กระบวนการไฮโดรเทอร์มอลพรีทรีตเมนต์ของกากไม้ยางพาราเพื่อผลิตกลูโคส (HYDROTHRMAL PRETREATMENT OF RUBBER REIDUE FOR GLUCOSE PRODUCTION) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. ธวัชชัย ชรินพาณิชกุล, 113 หน้า.

พลังงานชีวะมวลจากวัตถุดิบที่เหลือจากธรรมชาติ   เพื่อนำไปประยุกต์ใช้ในการผลิตพลังงานทดแทน เช่น เอทานอลต่อไปนั้น กำลังได้รับความสนใจ จากหลายหน่วยงาน โดยเฉพาะประเทศไทย ซึ่งเป็นประเทศเกษตรกรรม และ มีขยะที่เหลือจากเกษตรกรรมมากมาย กากไม้ยางพาราจึงเป็นอีกหนึ่งทางเลือกที่น่าสนใจ เนื่องจากประเทศไทยได้เป็นผู้ส่งออกผลิตภัณฑ์จากไม้ยางพารารายใหญ่ของโลก กากไม้ยางพาราจึงเป็นอีกหนึ่งทางเลือกที่น่าสนใจ นำมาผลิตพลังงานทดแทน ดังเช่น ในงานวิจัยนี้ ได้เลือกเลือกกระบวนการไฮโดรเทอร์มอลพรีทรีตเมนต์ของกากไม้ยางพาราเพื่อผลิตกลูโคส

ในงานวิจัยนี้จึงตั้งเป้าหมายที่จะศึกษาหาความเหมาะสมจากปัจจัยต่างๆ ได้แก่ อุณหภูมิ,ความเข้มข้นของสารตั้งต้นคือกากไม้ยางพารา, เวลาในการเกิดปฏิกิริยา และ การเติมกรดน้ำส้มสายชู เมื่อผ่านกระบวนการไฮโดรเทอร์มอล พรีทรีตเมนต์จากนั้นจะเติมเซลลูเลสเอมไซน์ เพื่อช่วยในการเร่งปฏิกิริยาในการแตกเซลลูโลสให้เป็นกลูโคสต่อไป

ภาควิชา........วิศวกรรมเคมี................. ลายมือชื่อนิสิต.....พชรกมล เพชรประดับ.......

สาขาวิชา.......วิศวกรรมเคมี.................ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก..........................

ปีการศึกษา.....2551........................ ลายมือชื่อ อ.ทีปรึกษาวิทยานิพนธ์ร่วม...............................

PHACHARAKAMOL PETCHPRADAB: HYDROTHERMAL PRETREATMENT OF RUBBER WOOD RESIDUE FOR GLUCOSE PRODUCTION. THESIS PRINCIPAL ADVISOR: ASSOC. PROF. TAWATCHAI CHARINPANITKUL, D.Eng., 113 pp.

Many agricultural countries including Thailand are the producers of various rubber wood products supplied to the whole global market. Rubber wood is a typical lignocellulose, resourse available for production of ethanol. Because of it excessive supply the rubber wood residues can be considered as a promising energy renewable source. Although direct combustion or gasification accompanying with burning has been employed as general means of such waste utilization there is a great deal of interest in utilizing and converting the lignocelluloses fraction to ethanol and other chemicals

In this thesis, rubber wood was employed as a biomass raw material in the hydrothermal pretreatment process accompanying with enzymatic hydrolysis. Pulverized rubber wood was treated at 130–280°C using an autoclave with inner volume of 96 cm^3. The glucose content in the treated liquid product was measured. The solid product was also analyzed for cellulose content and further treated with cellulase. The glucose yield was considered as an indicator for the effectiveness of the hydrothermal pretreatment. A reaction network model for the hydrothermal pretreatment was proposed with respect to the experimental results. Finally the reaction parameters which are rate constants were determined..

Department........Chemical Engineering...... Student's signature...

Field of study.....Chemical Engineering.......Principal Advisor's signature.............................

Academic year.......2008................

ACKNOWLEDGEMENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1.1 Background

Biomass ethanol is the major renewable energy source. It would provide an alternative liquid fuel which is derived from domestic resource and then utilized as partial substitute for petroleum based fuel in many developing countries. The use of ethanol as a substitute for the petroleum products (such as gasoline, diesel and naphtha) would complement dependence on coal, wood and hydroelectric power which used as the replacement of the some parts of the petroleum resource. The basic technology for producing ethanol from a number of biomass raw materials has been investigated for appropriate means to transfer and utilize. Even though many technical improvements are currently being develop to make the technology more economical. It should be note that ethanol production requires medium-scale industrial units that can be located in rural areas and can become an important additional source of permanent rural incomes, and help stem the migration of rural population to the urban centers.

In general, as a gasoline blend and/ or substitute for gasoline ethanol has drawn the most attention both because its application can take advantage of physical/ chemical characteristics which is more environtal friendly. When used in an internal combustion engine, ethanol significantly changes combustion efficiency and also

results in changes of octane rating, in turn resulting in engine performance characteristics such starting, carburetion and emissions. Ethanol can be used as automobile fuel either as "gasohol" in which case anhydrous (99.8%) ethanol is mixed with gasoline up to a 20% ratio, or as hydrous or straight alcohol, in which case hydrated ethanol (94% pure) is used directly (World bank, 1980). The economic value of alcohol as gasoline additive is about 15-20 % higher than as a direct gasoline substitute.

Ethanol can be produced from three main types of biomass material: (a) sugar bearing materials (such as sugarcane, molasses, sweet sorghum, etc.): (b) starches (such as cassava, corn, babassu mesocarp, potatoes, etc.) and (c) cellulose (such as wood, agricultural residues, etc.). Production of ethanol from these materials includes, except in the case of sugars, three stages: first, conversion of carbohydrates into water soluble sugars, then fermentation of these sugars into ethanol, and finally separation of ethanol from water and other fermentation products by distillation. (World bank, 1980)

The production of bioethanol requires fermentation of the sugar (mono- and polysaccharides) could be achieved by using many all kinds of biomass. Today there are primarily two types of process technology called first and second generation technology.

Figure 1.1 Wheat - sugar cane – corn

Generation of bioethanol from sugar (a dimer of the monosaccharides glucose and fructose) and starch-rich (polysaccharides of glucose) crops such as grain and corn has been a current issue of interest for tackling energy crisis. The fermentation technology is well-known as an effective mean to transfer sugar to etahnol. But high prices of the raw material and the ethics about using food products for fuel are two major problems. Therefore, there is requirement of other alternative technologies for production of bioethanol instead a new technology is required.

Figure 1.2 Wood - corn stover – straw

Raw materials in containing lignocellulosic compounds such as straw, wood and agricultural residues, are often available as wastes. These kinds of materials are cheap but required the more advance process technology for converting sugar and

starch to ethanol. The major cause is the lignin which binds together pectin, protein and the two types of polysaccharides, which are cellulose and hemicellulose. Lignin is capable of resisting microbial attack and to add strength to the plant. It also a key portion which gives some difficulties for decomposition of these biomass. Pre-treatment is therefore used to open the biomass by degrading the lignocellulosic structure and releasing the polysaccharides.

Pre-treatment is generally followed by treatment with enzymes which could hydrolyse cellulose and hemicellulose. The cellulose fraction releases glucose (C_6 monosaccharide – sugar with six carbon atoms) and the hemicellulose fraction releases pentoses (C_5 monosaccharide – sugar with five carbon atoms) such as xylose. Out of carbohydrate monomers in lignocellulosic materials xylose is the second most abundant after glucose. Glucose could easily be fermented to produce ethanol, but another fermentation process is required for handling xylose.

Countries of Southeast Asia including Thailand are the biggest producers of various rubber wood products in the world, shown in Figure 1 shown the market share of rubber wood exporting of the world.
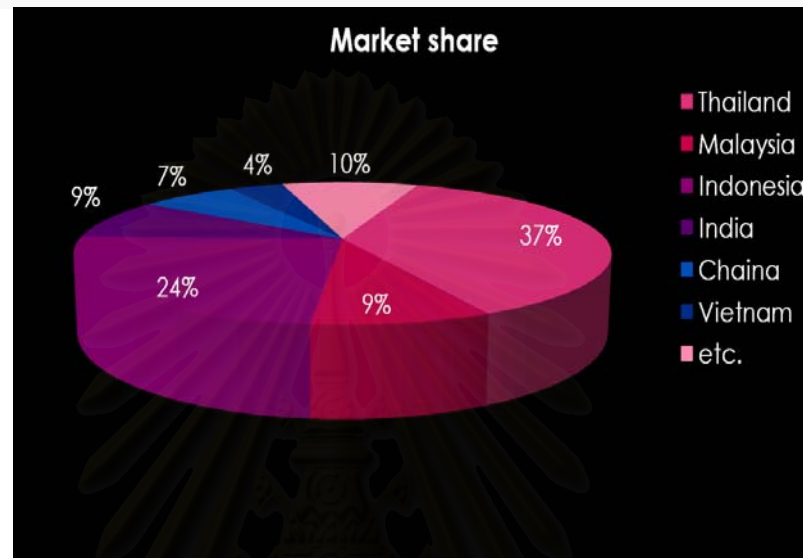
Figure 1.3 Rubber wood exporting market share

Furniture and particle board industries are the main consumers of the rubber wood. In these industries, generally only 10% of the wood ends up as the end product. In other word, 90% of rubber wood turn to residues, which is comprised of small branches (54%), small wastes (32%), and other (4%). This leads to annual availability of rubber wood residues of 6.9 Tg (6,900,000 t) in the plantation and 4.1 Tg (4,100,000 t) at the sawmill (Prasertsan and Sajjakulnukit., 2006). Rubber wood is typical lignocelluloses, mean while, production of ethanol from lignocellulosics has been studied all over the world Therefore, the rubber wood chips are excessive waste 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 (Sreenath et al., 1999)

Thus, in this study, glucose production from rubber wood residue is studied. The concept of this work is to hydrolyze the cellulose and hemicellulose to recover C6 and C5 sugars, and to ferment the obtained sugars into ethanol. Various treatments have been proposed and studied, but to achieve a high sugar yield, two-stage hydrolysis is often employed. Lignocellulosics are first treated by various chemicals or under severe condition (pretreatment) and then the product is treated with the enzyme, cellulose (Wyman et al., 2005).The pretreatment included ammonia explosion, aqueous ammonia recycle, controlled pH, dilute acid, flowthrough, and line approaches etc. Among the various pretreatment, hydrothermal pretreatment enjoys the advantages of using no toxic or harmful chemicals, of employing relative mild conditions, and of being free from costly treatment of wastewater or byproducts.

The main function of the hydrothermal pretreatment is to decrease the crystallinity of cellulose, thus improving the effectiveness of the cellulase treatment, while degradation of carbohydrate are to be avoided (Yoshida et al., 2005; Jonathan et al., 2007). Too high temperature and too long treatment time is not desirable from the viewpoint of avoiding the carbohydrate degradation. Then, only hydrothermal pretreatment cannot achieve complete hydrolysis of cellulose to glucose. Cellulase is became it works at a temperature lower than 80°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 (Adschiri at el., 1993).

Hydrothermal treatment has also been studied as the pretreatment without the following cellulase treatment by several researchers (Kumagi et al., 1996; Sasak et al., 2004). Aquasolve process is one of the early studies (Allen et al., 1996; Laser et al., 2002).In this process, Allen 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. Similar idea was studied by Sakaki et al., and they also reported that inhibition of byproducts on ethanol fermentation was small (Sasaki et al., 2002). Hydrothermal pretreatment for cellulase hydrolysis was studied by Ballesteros et al., (2002). They employed an autoclave to treat olive residue.

Though there are piles are previous studies, the optimum conditions for the hydrothermal pretreatment has not been clearly understood. This is due to the variety of biomass. In some biomass species, crystallinity of cellulose would not be high, and hydrothermal pretreatment would become less importance. The optimum condition would also depend on the physical from of the biomass. If the biomass is in the form of fine powder, the reaction taking place in the hydrothermal pretreatment should be faster than when biomass in large pieces is treated. The content of hemicelluloses and lignin should also affect, since they attaches and covers cellulose, and prevents the easy access of cellulase to cellulose. Establishment of the guideline to obtain the optimum conditions for the hydrothermal pretreatment is wanted.

This research is focusing on finding the guideline for the optimum conditions for the hydrothermal pretreatment. As the first step, a network of the "apparent" reactions have been proposed. Without hydrothermal pretreatment, only few glucose is obtained even if the biomass feedstock is treated with cellulase. Thus, it is

reasonable that cellulose in the biomass as non-reactive form in terms of cellulase treatment. This is attributed to the high crystallinity, due to the lignin and hemicellulose. After hydrothermal pretreatment, part of the product can be converted into glucose by cellulase treatment. This is a complicated physical and chemical phenomena related to dissolution of hemicellulose and part of lignin in the hot compressed water, reduction in crystallinity of the cellulose, destruction of cell structure, and penetration of water molecules into the piece of biomass, etc. Reaction engineering has been taken into account for analysis of overall treatment phenomena. In addition to this change, part of the cellulose is hydrolyzed into glucose. Produced glucose is further decomposed in the hydrothermal conditions.

For the optimal design of the hydrothermal pretreatment process, the kinetics of this network of the "apparent" reactions will be elucidated. The purpose of this study is to treat rubber wood hydrothermally at 130 – 280°C in an autoclave reactor in order to check the validity of the proposed reaction model, to determine the kinetic parameters, and to predict the optimal condition for the hydrothermal pretreatment.

1.2 Objectives

The objective of this research is to investigate the optimal condition of saccharification process of rubber wood residue.

1.3 Scopes of Research

1. Rubber wood residue will be used as a biomass feed stock under hydrothermal pretreatment conditions

2. The optimal conditions for saccharification process of rubber wood residue by hydrothermal pretreatment will be focus on reaction temperature in a range of 130 – 280 ^{O}C

3. Other parameters that affect on glucose product will be investigated such as

   Size of ground rubber wood

   Addition of acetic acid

   Concentration of rubber wood residue in a range of 10 –20 wt%

   Treatment time on hydrothermal pretreatment between 0 and 30 minute

4. Effect of enzymatic hydrolysis will also be investigated for exploring the possibility to convert the saccharified product to glucose.

1-4 EXPECTED BENEFIT

1. To get the guideline for the optimal conditions for the hydrothermal pretreatment.

2. To support biomass technology in Thailand for the future to used renewable energy sources.

CHAPTER II

LITERATURE REVIEW

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

Adschiri et al. (1993) describes the non-catalytic conversion of cellulose in supercritical water and subcritical water. First, it was demonstrated that even without any acid catalyst, cellulose was rapidly converted to water soluble species with a relatively high glucose yield in near critical water and glucose yield increased with elevating temperature. Then the rate constants for cellulose decomposition and glucose deposition were evaluated at a pressure of 25 MPa over a temperature ranging from 200- 400 °C by using semi-batch reactor and flow reactor, respectively. From the reported cellulose pyrolysis rate constant and the evaluated cellulose decomposition rate constant, cellulose hydrolysis rate constant was evaluated By using the cellulose hydrolysis rate, cellulose pyrolysis rate and the glucose decomposition rate, glucose yield in the semi-batch experiment was reasonably explained.

Sasaki et al (1996) The noncatalytic decomposition characteristics of cellulose in near-critical water were examined by heating a sealed reactor in which the cellulose and water were charged in a salt bath kept at 305, 355, or 405 °C. Cellulose was

rapidly decomposed to water solubles (WS), and the WS was further decomposed after the WS yield reached nearly 80%. The heating time giving the maximum WS yield was shortened to under 15 s by increasing the treatment temperature to over 355 °C.In the WS formation process, hydrolysis preferentially occurred, and the glucose yield reached 40% by the treatment for 15 s in the bath kept at 355 °C. On entering the second decompositionprocess, the WS was converted to gaseous products and methanol-soluble products, and charlike solid products were formed from the liquid phase. The hydrolysate of cellulose obtained in the WS formation process was subjected to a fermentation test, and the formed glucose was confirmed to be converted to ethanol.

Zhang et al (2007) Studied by used batch pressure vessels commonly for hydrothermal liquefaction have typical heating times in the range of 30 to 60 min. Thermodynamically, the complex set of reactions are path dependent, so that the heating rate can possibly affect yields and the composition of the resultant liquid products. It is postulated that the mode of heat transfer becomes an uncontrolled variable in kinetic studies and can seriously impact scale-up. To confirm this hypothesis and minimize these heat-transfer-related artifacts, we designed a batch pressure vessel equipped with an induction heating system, which allows the reduction of heat-up times by about two orders of magnitude to several seconds, compared to tens of minutes with standard pressure reactors. This system was used to study the direct liquefaction of corn stover and aspen wood with a pretreatment. The heating rate was found to have no significant effect on the composition of the liquid products. However, the liquid yields are dependent on the heating rate. Varying the

cooling rate does not show obvious effects. The results confirm that the heating rate, as governed by the mode of heat transfer, is an important factor that needs to be considered during scale-up.

Kruse and Dinjus (2007) investigated on hot compressed water (HCW, here water above 200 ◦C) owns interesting properties. The impact of the unique properties is discussed exemplary for the thermal degradation of *tert* butylbenzene and the oxidation of methanol. Both reactions have been conducted not only in HCW but also in other high-pressure media and from the comparison the impact of the special properties of HCW can be settled. In addition the degradation of glycerol, a model substance for carbohydrates and biomass in HCW was studied. This reaction shows a strong dependence on the properties of HCW. The examples picture an increased specific impact of HCW with rising polarity of the reactants and intermediates. The studies also points to higher importance of microscopic properties for understanding reactions in HCW than assumed in the past. They found the water accelerates biomass depolymerization by hydrolysis.The glycosidic bond of cellulose and hemicellulose is polar and is hydrolyzed very fast in hot compressed water. The cellulose and hemicellulose is split in sugar units and the whole biomass structure breaks down. This improves a further attack by water molecules. At subcritical conditions HCW supports water eliminations from carbohydrates and alcohols because of the high ionic product increasing the reaction rate of this usually acid-catalyzed reactions. In addition, other reactions like for example Aldol splitting is enhanced by the special properties of subcritical water.

Eiji Kamio et al (2008) proposed on the effect of heating rate on the liquefaction of a microcrystalline cellulose particle was investigated by using a batch type reactor with a temperature controller. Experiments were carried out over the temperature range of 443–553 K. The temperature profile of the heating was controlled to obtain a proportional relationship with time. Heating rates ranged from 0.0167 to 0.167 K/s. The resulting concentration profiles of cellulose, oligosaccharides, monosaccharides and pyrolysis products were analyzed using a theoretical model which considered temperature variation during the reaction. The calculated results exhibit a reasonable fit to the experimental data. The predicted cellulose concentration profiles over a wide range of heating rates indicate that liquefaction of cellulose is affected when the heating rate is below 1 K/s. It is concluded that consideration of the heating process is necessary in the modeling of liquefaction when using reactors which cannot achieve fast heating.

2.2 Hydrothermal treatment with non catalytic conversion of lignocellulosic residue

Stephen et al (1996) studied by used sugar-cane bagasse and leaves (10-15 g oven-dry basis) were fractionated without size reduction by a rapid (45 s to 4 min), immersed percolation using only hot (190-230 °C), compressed ($P > P$ sat), liquid water (0.6-1.2 kg). Over 50% of the biomass could be solubilized. All of the hemicellulose, together with much of the acid-insoluble lignin in the bagasse (>60%), was solubilized, while less than 10% of the cellulose entered the liquid phase. Moreover, recovery of the hemicellulose as monomeric sugars (after a mild

posthydrolysis) exceeded 80%. Less than5% of the hemicellulose was converted to furfural. Percolation beyond that needed to immerse the biomass in hot liquid water did not result in increased solubilization. The yield of lignocellulosic residue was also not sensitive to the form of the sugar cane used (bagasse or leaves) or its moisture content (8-50%). Commercial applications for this fractionation process include the pretreatment of lignocellulosics for bioconversion to ethanol and the production of pulp and paper products.

2.3 Hydrothermal treatment of lignocellulosic residue to enzymic saccharification

Hassan et al (1999) proposed liquid hot water (LHW) at a high temperature has been advocated as a pretreatment for herbaceous and lignocellulosics materials prior to enzymic saccharification. The focus of our research was the suitability of LHW pretreatment of alfalfa (*Medicago sali6a*) fibre in the presence and absence of mild acid for optimum saccharification using enzymes. Enzymic saccharification was optimised in terms of substrate and enzyme concentrations. The main components of the enzymic hydrolyzates were sucrose, glucose, xylose, arabinose, and low amounts of uronic acid. After LHW pretreatment, both the soluble hemicellulose-rich 'extract' fraction and the insoluble residue 'raffinate' fraction (which contains predominantly cellulose) of alfalfa were recovered; the yield was 48 and 41%, respectively. Enzymic saccharification released more reducing sugars from pretreated fibre than from untreated fibre. For untreated alfalfa, a commercial pectinase and cellulase mixture caused maximum release of reducing sugars; using 2 and 4% (w:v) enzyme, a

maximum of 51 g:l reducing sugars was released from 100 g:l untreated alfalfa. The soluble extract, LHW-pretreated alfalfa, was clarified and saccharified with the same cellulase and pectinase mixture releasing 8.4 g:l reducing sugars from 15.5 g:l solids. Release of reducing sugars from the insoluble raffinate ranged from 59 to 65 g:l from 100 g:l substrate, using 2 and 4% (w:v) cellulase. Addition of 0.07% sulphuric acid to the LHW pretreatment facilitated hemicellulose solubilisation; 9.9 g:l reducing sugars were released from the alfalfa extract by enzymic saccharification. However, pretreatment with sulphuric acid reduced sugar release and decreased fibre degradation of the raffinate.

Rebecca et al. (2006) The effectiveness of sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), and ozone pretreatments for conversion of cotton stalks to ethanol was investigated. Ground cotton stalks at a solid loading of 10% (w/v) were pretreated with H_2SO_4, NaOH, and H_2O_2 at concentrations of 0.5%, 1%, and 2% (w/v). Treatment temperatures of 90 _C and 121 _C at 15 psi were investigated for residence times of 30, 60, and 90 min. Ozone pretreatment was performed at 4 _C with constant sparging of stalks in water. Solids H_2SO_4, NaOH, and H_2O_2 pretreatments (at 2%, 60 min, 121 _C/15 psi) showed significant lignin degradation and/or high sugar availability and hence were hydrolyzed by Celluclast 1.5 L and Novozym 188 at 50 _C. Sulfuric acid pretreatment resulted in the highest xylan reduction (95.23% for 2% acid, 90 min, 121 _C/15 psi) but the lowest cellulose to glucose conversion during hydrolysis (23.85%). Sodium hydroxide pretreatment resulted in the highest level of delignification (65.63% for 2% NaOH, 90 min, 121 _C/15 psi) and cellulose conversion (60.8%). Hydrogen peroxide pretreatment

resulted in significantly lower (p 6 0.05) delignification (maximum of 29.51% for 2%, 30 min, 121 _C/15 psi) and cellulose conversion (49.8%) than sodium hydroxide pretreatment, but had a higher (p 6 0.05) cellulose conversion than sulfuric acid pretreatment. Ozone did not cause any significant changes in lignin, xylan, or glucan contents over time. Quadratic models using time, temperature, and concentration as continuous variables were developed to predict xylan and lignin reduction, respectively for sulfuric acid and sodium hydroxide pretreatments. In addition, a modified severity parameter (logM0) was constructed and explained most of the variation in xylan or lignin reduction through simple linear regressions.

Jonathan et al. (2007) studies on the effect of substrate concentration and enzyme loading indicated the inhibition pattern for the lime-pretreated corn stover–cellulase reaction system was non-competitive, which agrees with the inhibition pattern used to develop the HCH-1 Model. The inhibition pattern was identified for a reaction system composed of Trichoderma reesei cellulase enzyme complex and lime-pretreated corn stover. Also, the glucose inhibition effect was quantified for the aforementioned reaction system over a range of enzyme loadings and substrate concentrations.

2.4 Studied the reaction kinetics of cellulose hydrolysis to glucose

Sasaki et al. (2004) experiments of microcrystalline cellulose conversion in subcritical and supercritical water were conducted at temperatures between 290 and

400°C, a pressure of 25 MPa, and residence times of 0.02–13.1 s using a continuous-flow-type microreactor. First, the reaction mechanism of microcrystalline cellulose in subcritical and supercritical water was proposed on the basis of detailed product analyses. Next, the kinetic description of this reaction in subcritical and supercritical water using a grain model was carried out to verify the proposed reaction mechanism and consequently found that the reaction-rate models were able to express the reaction of microcrystalline cellulose at identical conditions.

Yoshida et al. (2005) investigated the hydrothermal treatments can be applied to pretreatment of ethanol fermentation process. The important in the pretreatment step is that lignocelluloses material could be effectively hydrolyzed to smaller saccharides and not be further convert to other substances. They developed reaction kinetics of cellulose hydrolysis to glucose for evaluation of hydrothermal treatment condition. They have examined the reaction of cellulose and glucose in hydrothermal pretreatment at temperature 150-250 °C. The glucose yield indication that higher operating temperature.

CHAPTER III

FUNDAMENTAL THEORY

3.1. Biomass

Biomass refers to living and recently dead biological material that can be used as fuel or for industrial production. Most commonly, biomass refers to plant matter grown for use as biofuel, but it also includes plant or animal matter used for production of fibers, chemicals or heat. Biomass may also include biodegradable wastes that can be burnt as fuel. It excludes organic material which has been transformed by geological processes into substances such as coal or petroleum.

Biomass is grown from several plants, including miscanthus, switchgrass, hemp, corn, poplar, willow, sugarcane, and oil palm (palm oil). The particular plant used is usually not very important to the end products, but it does affect the processing of the raw material. Production of biomass is a growing industry as interest in sustainable fuel sources is growing. Although fossil fuels have their origin in ancient biomass, they are not considered biomass by the generally accepted definition because they contain carbon that has been "out" of the carbon cycle for a very long time. Their combustion therefore disturbs the carbon dioxide content in the atmosphere. Plastics from biomass, like some recently developed to dissolve in seawater, are made the same way as petroleum-based plastics, are actually cheaper to manufacture and meet or exceed most performance standards. But they lack the same water resistance or longevity as conventional plastics.

Biomas*s* is part of the carbon cycle. Carbon from the atmosphere is converted into biological matter by photosynthesis. On death or combustion the carbon goes back into the atmosphere as carbon dioxide (CO_2). This happens over a relatively short timescale and plant matter used as a fuel can be constantly replaced by planting for new growth. Therefore a reasonably stable level of atmospheric carbon results from its use as a fuel. It is accepted that the amount of carbon stored in dry wood is approximately 50% by weight.

Though biomass is a renewable fuel, and is sometimes called a "carbon neutral" fuel, its use can still contribute to global warming. This happens when the natural carbon equilibrium is disturbed; for example by deforestation or urbanization of green sites. When biomass is used as a fuel, as a replacement for fossil fuels, it still puts the same amount of CO_2 into the atmosphere. However, when biomass is used for energy production it is widely considered carbon neutral, or a net reducer of greenhouse gasses because of the offset of methane that would have otherwise entered the atmosphere. The carbon in biomass material, which makes up approximately fifty percent of its dry-matter content, is already part of the atmospheric carbon cycle. Biomass absorbs CO_2 from the atmosphere during its growing lifetime. After its life, the carbon in biomass recycles to the atmosphere as a mixture of CO_2 and methane (CH_4), depending on the ultimate fate of the biomass material. CH_4 converts to CO_2 in the atmosphere, completing the cycle. In contrast to biomass carbon, the carbon in fossil fuels is locked away in geological storage forever, unless extracted. The use of fossil fuels removes carbon from long-term storage, and adds it to the stock of carbon in the atmospheric cycle.

Energy produced from biomass residues displaces the production of an equivalent amount of energy from fossil fuels, leaving the fossil carbon in storage. It also shifts the composition of the recycled carbon emissions associated with the disposal of the biomass residues from a mixture of CO_2 and CH_4, to almost exclusively CO_2. In the absence of energy production applications, biomass residue carbon would be recycled to the atmosphere through some combination of rotting (biodegradation) and open burning. Rotting produces a mixture of up to fifty percent CH_4, while open burning produces five to ten percent CH_4. Controlled combustion in a power plant converts virtually all of the carbon in the biomass to CO_2. Because CH_4 is a much stronger greenhouse gas than CO_2, shifting CH_4 emissions to CO_2 by converting biomass residues to energy significantly reduces the greenhouse warming potential of the recycled carbon associated with other fates or disposal of the biomass residues.

The existing commercial biomass power generating industry in the United States, which consists of approximately 1,700 MW (megawatts) of operating capacity actively supplying power to the grid, produces about 0.5 percent of the U.S. electricity supply. This level of biomass power generation avoids approximately 11 million tons per year of CO_2 emissions from fossil fuel combustion. It also avoids approximately two million tons per year of CH_4 emissions from the biomass residues that, in the absence of energy production, would otherwise be disposed of by burial (in landfills, in disposal piles, or by the plowing under of agricultural residues), by spreading, and by open burning. The avoided CH_4 emissions associated with biomass energy production have a greenhouse warming potential that is more than 20 times greater

than that of the avoided fossil-fuel CO_2 emissions. Biomass power production is at least five times more effective in reducing greenhouse gas emissions than any other greenhouse-gas-neutral power-production technology, such as other renewables and nuclear.

Currently, the New Hope Power Partnership, owned by Florida Crystals Corporation, is the largest biomass cogeneration energy facility in the U.S. The 140 MWH facility recycles sugar cane fiber and urban wood waste, generating enough electricity to power its large milling and refining operations as well as renewable electricity for more than 40,000 homes. The facility reduces dependence on approximately 800,000 barrels of oil per year and by recycling sugar cane and wood waste, preserves landfill space in urban communities in Florida.

Despite harvesting, biomass crops may sequester (trap) carbon. So for example soil organic carbon has been observed to be greater in switchgrass stands than in cultivated cropland soil, especially at depths below 12 inches.The grass sequesters the carbon in its increased root biomass. But the perennial grass may need to be allowed to grow for several years before increases are measurable.

3.2 Cellulose

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$ consisting of a linear chain of several hundred to over nine thousand $\beta(1\rightarrow4)$ linked D-glucose units as shown in figure 3.1 Cellulose is the structural component of the primary cell wall

of green plants. Some species of bacteria secrete it to form biofilms. Cellulose is the most common organic compound on Earth. About 33 percent of all plant matter is cellulose (the cellulose content of cotton is 90 percent and that of wood is 50 percent)
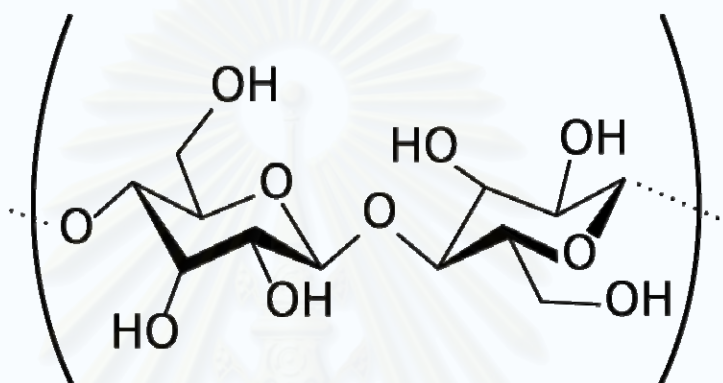
Figure 3.1 Cellulose, a linear polymer of D-glucose linked by β(1→4)-glycosidic bonds (Stamm, 1964)

For industrial use, cellulose is mainly obtained from wood pulp and cotton. It is mainly used to produce cardboard and paper; to a smaller extent it is converted into a wide variety of derivative products such as cellophane and rayon. Some animals, particularly ruminants and termites, can digest cellulose with the help of symbiotic micro-organisms that live in their guts. Cellulose is not digestible by humans and is often referred to as 'dietary fiber' or 'roughage', acting as a hydrophilic bulking agent for feces. 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. Cellulose is a

straight chain polymer: unlike starch, no coiling occurs, and the molecule adopts an extended and rather stiff rod-like conformation. The multiple hydroxyl groups on the glucose residues from one chain form hydrogen bonds with oxygen molecules on another chain, holding the chains firmly together side-by-side and forming *microfibrils* with high tensile strength. This strength is important in cell walls, where they 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 25 MPa to become amorphous in water.

Chemically, cellulose can be broken down into its glucose units by treating it with concentrated acids at high temperature. Many properties of cellulose depend on its degree of polymerization or chain length, 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 break down 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 contaminated with hemicellulose, lignin, pectin and other substances, while microbial cellulose is quite pure, has a much higher water content, and consists of long chains.

3.3 Hemicellulose

A hemicellulose can be any of several heteropolymers (matrix polysaccharides) present in almost all plant cell walls along with cellulose. While cellulose is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. It is easily hydrolyzed by dilute acid or base as well as myriad hemicellulaseenzymes

Hemicellulose contains many different sugar monomers. In contrast, cellulose contains only anhydrous glucose. For instance, besides glucose, sugar monomers in hemicellulose can include xylose, mannose, galactose, rhamnose, and arabinose. Hemicelluloses contain most of the D-pentose sugars, and occasionally small amounts of L-sugars as well. Xylose is always the sugar monomer present in the largest amount, but mannuronic acid and galacturonic acid also tend to be present.

Unlike cellulose, hemicellulose (also a polysaccharide) consists of shorter chains - 500-3000 sugar units as opposed to 7,000 - 15,000 glucose molecules per polymer seen in cellulose. In addition, hemicellulose is a branched polymer, while cellulose is unbranched.

Hemicelluloses are embedded in the cell walls of plants, sometimes in chains that form a 'ground' - they bind with pectin to cellulose to form a network of cross-linked fibres

3.4 Glucose
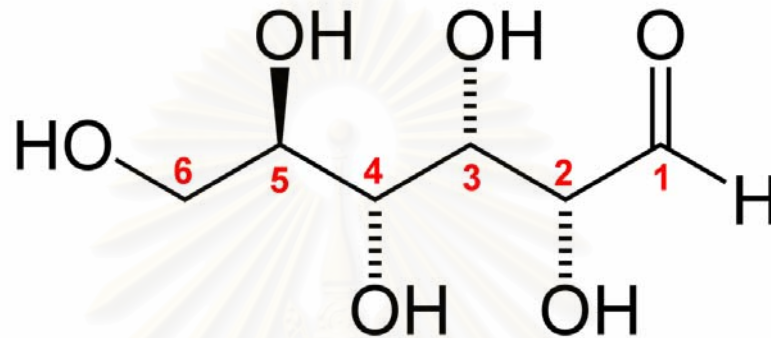
Figure 3.2 The chain form of D-glucose (Stamm, 1964)

Glucose is a monosaccharide (or simple sugar), is an important carbohydrate. The living cell uses it as a source of energy and metabolic intermediate. Glucose is one of the main products of photosynthesis and starts cellular respiration in both prokaryotes and eukaryotes. Two stereoisomers of the aldohexose sugars are known as glucose, only one of which (D-glucose) is biologically active. This form (D-glucose) is often referred to as dextrose monohydrate, or, especially in the food industry, simply dextrose (from dextrorotatory glucose). This article deals with the D-form of glucose. The mirror-image of the molecule, L-glucose, cannot be metabolized by cells in the biochemical process known as glycolysis.

Glucose ($C_6H_{12}O_6$) contains six carbon atoms, which is part of an aldehyde group. In solution, the glucose molecule can exist in an open-chain (acyclic) form and a ring (cyclic) form (in equilibrium). The cyclic form is the result of a covalent bond between the aldehyde C atom and the C-5 hydroxyl group to form a six-membered cyclic hemiacetal. At pH 7 the cyclic form is predominant. In the solid phase, glucose assumes the cyclic form. Because the ring contains five carbon atoms and one oxygen atom, which resembles the structure of pyran, the cyclic form of glucose is also referred to as glucopyranose. In this ring, each carbon is linked to a hydroxyl side group with the exception of the fifth atom, which links to a sixth carbon atom outside the ring, forming a CH_2OH group. Glucose is commonly available in the form of a white substance or as a solid crystal. It can also be dissolved in water as an aqueous solution.

Although glucose is produced commercially via the enzymatic hydrolysis of starch, agricultural waste containing cellulase could also be considered as an alternative source for glucose preparation. Many researcher works have paid increasing attentions on utilization of such cellulosic waste for glucose production due to its abundant.

3.5 Cellulase

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. Spano et al showed that 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. (Wilke et. al, 1983)

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 action of each of these being:

(1) Endo-p-glucanase, 1,4-ß-D-glucan glucanohydrolase, CMCase, Cx: "random" scission of cellulose chains yielding glucose and cello-oligo saccharides.

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

(3) ß-glucosidase, cellobiase: hydrolysis of cellobiose to glucose.

Reese et al. (1950) proposed that exo-ß-glucanase could result in a disruption in cellulose hydrogen bonding, followed by hydrolysis of the accessible cellulose with endo-ß-gucanase. Although cellulase isolation techniques have not been fully developed, the hypothesis depicted in Fig. 3-2 is now accepted. According to this hypothesis, 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 (Table 3-1). 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.
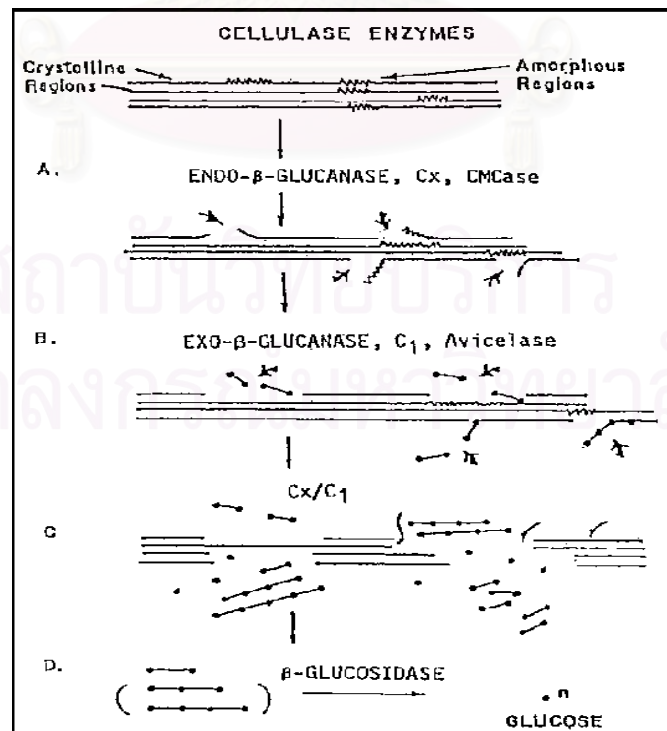
Table 3.1 Representative Cellulase-producing Microorganism (Kazuhisa, 1997)

	Microorganism		Microorganism
Fungi	Acremonium cellulolyticus	Bacteria	Clostridium thermocellum
	Aspergillus acculeatus		Ruminococcus albus
	Aspergillus fumigatus		Streptomyces sp.
	Aspergillus niger		
	Fusarium solani		
	Irpex lacteus		
	Penicillium funmiculosum		
	Phanerochaete chrysosporium	Actinomycetes	Streptomyces sp.
	Schizophyllum commune		Thermoactinomyces sp.
	Sclerotium rolfsii		Thermomonospora curvata
	Sporotrichum cellulophilum		
	Talaromyces emersonii		
	Thielavia terrestris		
	Trichoderma koningii		
	Trichoderma reesei		
	Trichoderma viride		

3.6 The reaction rate constant

In the chemical reaction considered in the following paragraphs, we take as the basis of calculation a species A, which is one of the reactants that is disappearing as a result of the reaction. The limiting reactant is usually chosen as our basis for calculation. The rate of disappearance of A, r_A, depends on temperature and composition. For many reaction it can be written as the product of a *reaction rate constant k* and a function of the concentrations (activities) of the various species involved in the reaction: The rate law gives the relationship between reaction rate and concentration as shown in equation (3.1)

$$-r_A = [k_A\,(T)]\,[\text{fn } C_A,\, C_B, \ldots] \tag{3.1}$$

The algebraic equation that relates $-r_A$ to the species concentrations is called the kinetic expression or **rate law**. The specific rate of reaction, k_A, like the reaction rate $-r_A$, is always referred to a particular species in the reactions and normally should be subscripted with respect to that species. However, for reactions in which the stoichiometric coefficient is 1 for species involved in the reaction.

The reaction rate constant k is not truly a constant, but is merely independent of the concentrations of the species involved in the reaction. The quantity k is also referred to as the specific reaction rate (constant). It is almost always strongly dependent on temperature. In gas- phase reaction, it depends on the catalyst and many be a function of total , pressure , and in addition can depend on other parameter s, such as ionic strength and choice of solvent. These other variables normally exhibit much less effect on the specific reaction rate than dose temperature , so for the purposes of the material presented here it will be assumed that k_A depends only on

temperature. This assumption is valid in most laboratory and industrial reactions and seems to work quite well.

It was the great Swedish chemist Arrhenius who first suggested that the temperature dependence of the specific reaction rate k_A, could be correlated by an equation of the type .

$$k_A = Ae^{(E/RT)} \qquad (3.2)$$

Where A = preexponential factor or frequency factor

E= activation energy, J/mol or cal/mol

R= gas constant = 8.314 J/mol•K = 1.987 cal/mol•K

T= absolute temperature, K

Equation (3.2) , known as the *Arrhenius equation*, has been verifield empirically to give the temperature behavior of most reaction rate constants within experimental accuracy over fairly large temperature ranges (Foglor, 1999)

CHAPTER IV

EXPERIMENTAL

4.1. Hydrothermal pretreatment

4.1.1 Autoclave reactor

Rubber wood residue was pretreated at the Hiroshima University using autoclave reactor
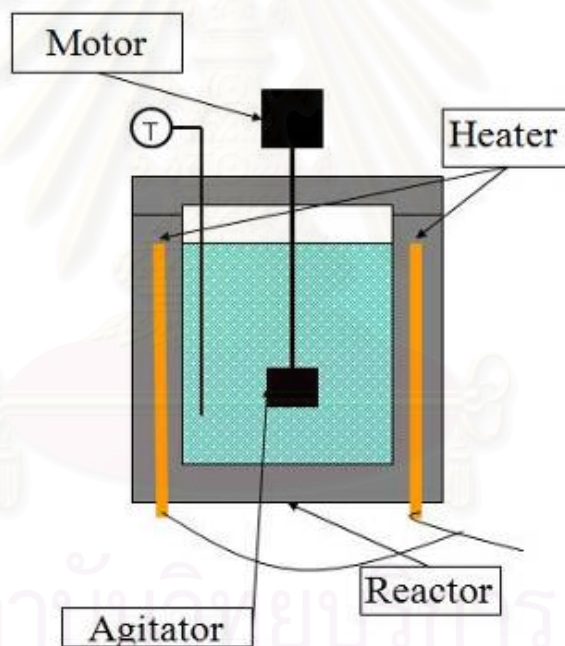
Figure. 4.1 Autoclave reactor

As seen in figure 4.1, the autoclave employed in this study. The inner volume of the autoclave is 96 cm^3. Rubber wood powder 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 4.2. 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 4.3.

Figure 4.2 Autoclave reactor and 8 embedded heaters

Figure 4.3 Temperature profile reordered

The sample after pretreatment was separated to liquid sample and solid sample. The solid sample was used for enzymatic hydrolysis and measured for the content of cellulose after pretreatment. The liquid sample was analyzed by HPLC with SUGAR KS-802 (Shodex) column operated at 60°C with water at 0.8 cm^3/min as an elute. Figure 4.4 shows the High performance liquid chromatography (HPLC) analyzer. Figure 4.5 shows scheme of the different steps followed in hydrothermal pretreatment, enzymatic hydrolysis experiments

Figure 4.4 High performance liquid chromatography.

4.2. Enzymatic hydrolysis

Cellulase from ***Aspergillus niger powder*** ($\geq$ 0.3 units/mg-solid) was used in this

study. The samples after the hydrothermal pretreatment was separated to solid sample

and liquid sample and the solid sample were used as reactant in this step. The

pretreated sample, cellulase, and buffer fluid were placed in a conical flask as shown

in Table 1, and the flask was shaken at 250 rpm at 37°C for 2 days by SHAKING

INCUBATOR SI-300R as shown in figure 4.6. 15 ml of pretreated samples was load

in the steam autoclave, about 10-15 minute for reach to the 105 °C temperature. Stop

the enzyme reaction by using steam autoclave (Autoclave SP200, YAMATO) as

shown is figure 4.7. In each run, the liquid product was sampled every 24 h, and was

analyzed by HPLC. Buffer fluid was prepared from acetic acid (0.01 mol/lit), sodium

hydroxide (0.01 mol/lit) and de-ionized water, whose pH was set at 5

Table 4.1 Conditions for cellulase treatment

Reactant [g]	1
Buffer fluid [cm^3]	60
10 g/dm^3 cellulase solution [cm^3]	5
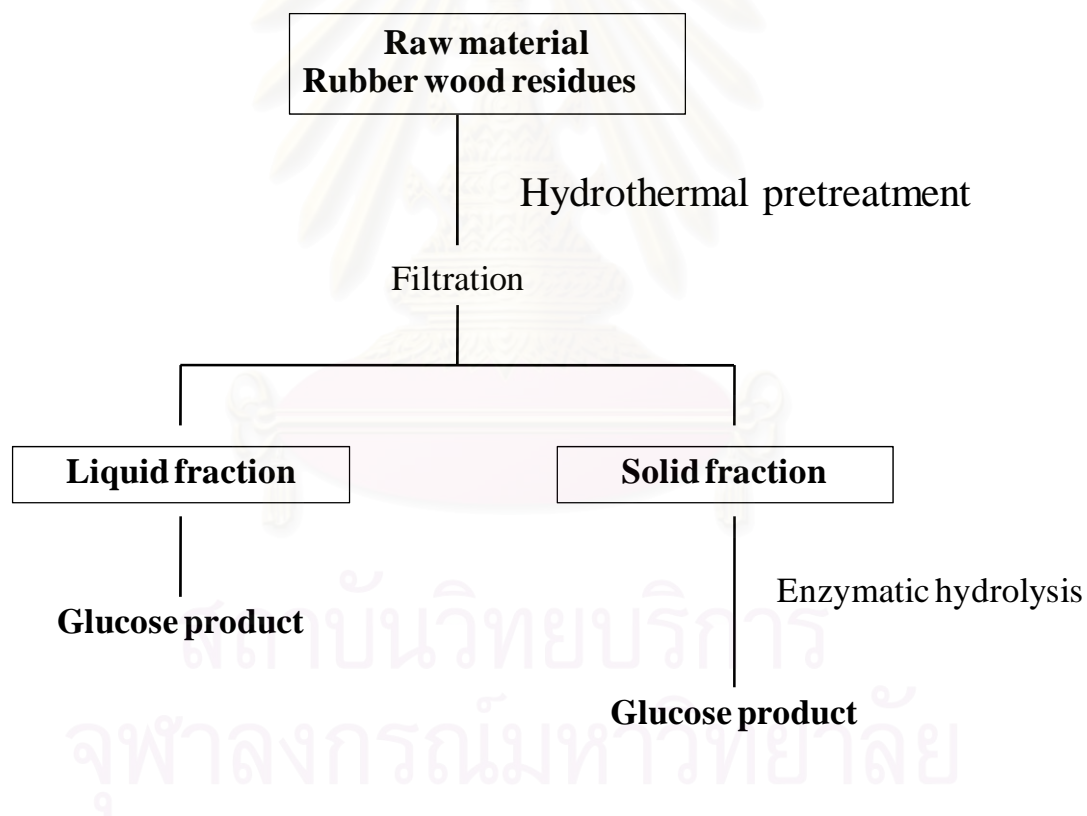
Figure 4.5 Scheme of the different steps followed in hydrothermal pretreatment,

enzymatic hydrolysis experiments

Figure 4.6 Shaking incubator

Figure 4.7 Steam autoclave

4.3. Component analysis

The cellulose content of the product of hydrothermal pretreatment was determined following the procedure recommended by the United States Department of Agriculture. The theory of this method is removed by preparing acid-detergent fiber, which is chiefly composed of lignin, cellulose, and insoluble minerals. Lignin is oxidized with an excess of acetic acid-buffered potassium permanganate solution, containing trivalent iron and monovalent silver as catalysts. Deposited manganese and iron oxides are dissolved with an alcoholic solution of oxalic and hydrochloric acid, which leaves cellulose and insoluble minerals. Lignin is measured as the weight lost by these treatments; whereas, cellulose is determined as the weight loss upon ashing.

4.4. Feedstock

Figure 4.8 Rubber wood residues

The rubber wood residue was pulverized with the ball mill equipment into rubber wood powder, and used in all experiments. Figure 4.8 shows the rubber wood residue before crushing and figure 4.9 shows the rubber wood powder after crushed

by ball mill. The average particle size was 28 μm on average shows in figure 4.10, measured by Master sizer 2000.

Figure 4.9 Rubber wood powder and rubber wood residues
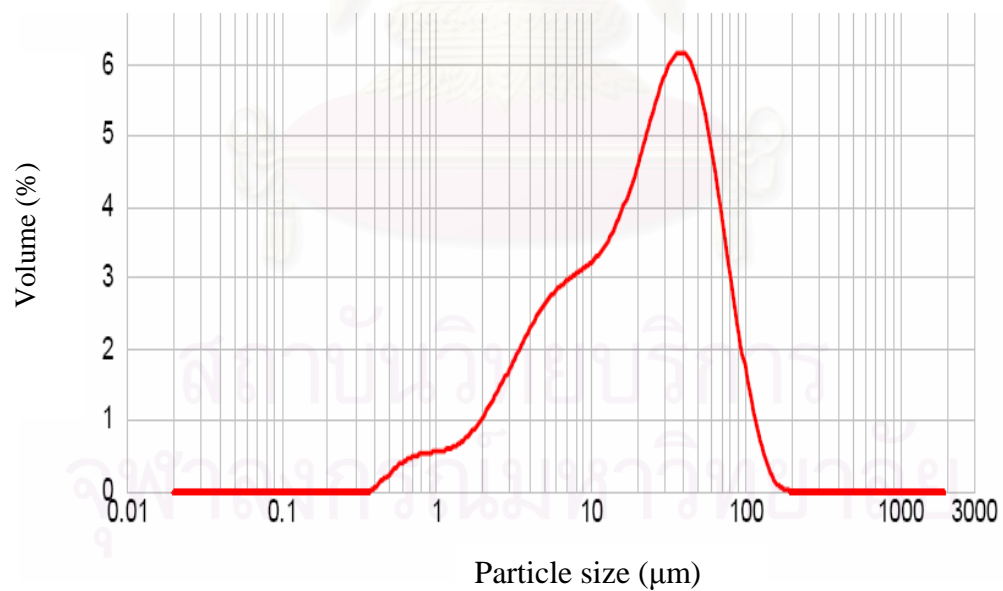
Figure 4.10 Particle size distributions

Table 4.2 shows the components of the rubber wood powder (dry weight basis) determined by the USDA's method.

Table 4.2 Overall analysis of rubber wood components

hemicelluloses	29 %
Lignin	28 %
Cellulose	39 %
Ash	4 %

4.5 Experimental condition

4.5.1 Reaction temperature in a range of $130 - 280\ ^{O}C$

The temperature is important parameter for determine the reaction rate parameter. In this studied, first will studied on the effect of temperature on hydrothermal pretreatment of rubber wood residue. By varies in a range of $130 - 280\ ^{O}C$ for find the optimal temperature for this process. The rubber wood powder was used in this studied was crushed by ball mill for 48 hr. The average particle size was 28 μm on average. Table 4.3 shows the condition for studied on effect of temperature.

Table 4.3 Temperature conditions for hydrothermal pretreatment.

Temperature (°C)	130, 150, 170, 190, 200, 210, 240, 260 and 280
Rubber wood powder (g)	7
De-ionized water (g)	63

4.5.1.1 Apparent reaction network for the hydrothermal pretreatment

This study is working to get the guideline to optimum the conditions for the hydrothermal pretreatment. However, the phenomena that are taking place in the hydrothermal reactor are very complex. Thus, as the first step, we have proposed the network of the "apparent" reactions taking place in the hydrothermal reactor as shown in Fig. 4.11. In figure. 4.11, C denotes cellulose that cannot be converted into glucose by cellulase treatment. This "cellulase-resistant" cellulose is the starting material, because without hydrothermal pretreatment, almost no glucose is obtained even if the rubber wood feedstock is treated with cellulase. Thus, we can consider cellulose in the rubber wood as practically non-reactive form in terms of cellulase treatment. This may be due to the high crystallinity, to the lignin and hemicellulose covering the cellulose, or due to the large size of the pieces of biomass being treated. By the hydrothermal pretreatment, a part of the "cellulase-resistant" cellulose is converted into"cellulase-hydrolyzable" cellulose, because after the hydrothermal pretreatment, the treated rubber wood produces glucose by applying cellulase in the second stage. In Fig. 4.10, C* denotes cellulose that can be converted into glucose by cellulase treatment, produced by hydrothermal pretreatment. This change can be treated as a kind of reaction. Of course, this change is a complex of physical and chemical phenomena: dissolution of hemicellulose and part of lignin in the hot compressed water, reduction in crystallinity of the cellulose, destruction of cell structure, introduction of water molecules into the piece of biomass, etc. However, these phenomena are too complex to be treated as it is. Reaction engineering tells us that overall treatment is effective when complex phenomena are to be discussed. This is the approach we take here. In addition to this change, part of the cellulose is directly

hydrolyzed into glucose hydrothermally. In Fig. 4.11, this reaction is shown by the arrows from C and C* to G, where G denotes glucose. Part of the produced glucose is further decomposed in the hydrothermal reactor. In Fig. 4.10, this reaction is shown by the arrow from G to D. D denotes decomposition products, which is a mixture of various compounds. It is known that hydrothermal decomposition of glucose results in production of various compounds, but that the reaction rate can be expressed by a single overall reaction rate equation (Matsumura et al., 2006).To help readers to understand what are taking place in hydrothermal pretreatment and enzymatic hydrolysis processes, change of cellulose in the feedstock is shown in Fig. 4.12. The reaction network shown in Fig. 4.11 and the reaction kinetics discussed in the following part of this paper are for the hydrothermal pretreatment step in Fig. 4.11.For the optimal design of the saccharification process, the kinetics of this network of the "apparent" reactions that are taking place in the hydrothermal pretreatment are to be elucidated. In this study, rubber wood is treated hydrothermally at 130 – 280°C in an autoclave reactor, in order to check the validity of the proposed reaction model shown in Fig. 4.11, to determine the kinetic parameters, and to predict the optimal condition for the hydrothermal pretreatment. The visual basic of Microsoft program was use for calculated the reaction rate parameter, while this program will be calculating every 0.l s. Fig. 4.11 was determined as follows. Glucose yield, X_G , was determined by dividing glucose amount in the liquid phase obtained from HPLC n_G by the theoretical amount of glucose in the cellulose in the feedstock n_{G0}

$$X_G = \frac{n_G}{n_{G0}} \qquad\qquad (1)$$

The yield of cellulose that can be hydrolyzed by cellulase X_{C*} was determined by dividing the amount of glucose after cellulase treatment n_{G*} by the theoretical amount of glucose in the cellulose in the feedstock n_{G0}.

$$X_{C*} = \frac{n_{G*}}{n_{G0}} \tag{2}$$

The yield of cellulose that cannot be hydrolyzed by cellulase X_C was determined by

$$X_C = \frac{n_C - n_{C*}}{n_{C0}} \tag{3}$$

where n_C denotes the amount of cellulose after hydrothermal pretreatment. Then, the yield of decomposed product X_D was determined by
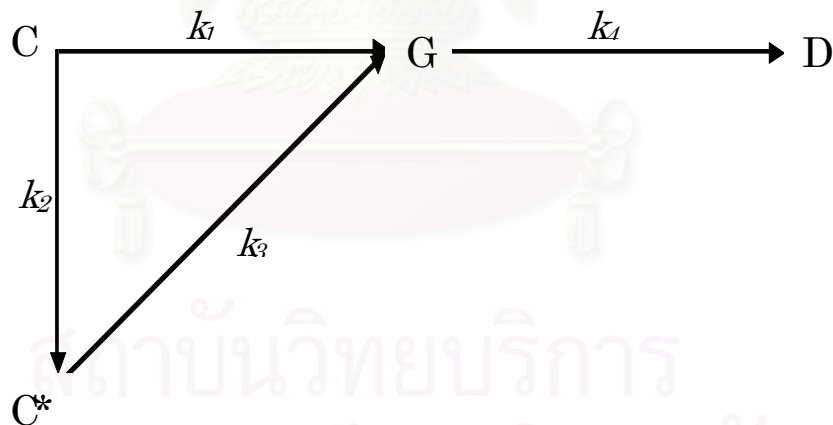
$$X_D = 1 - X_G - X_{C*} - X_C \tag{4}$$

Figure. 4.11 Reaction scheme
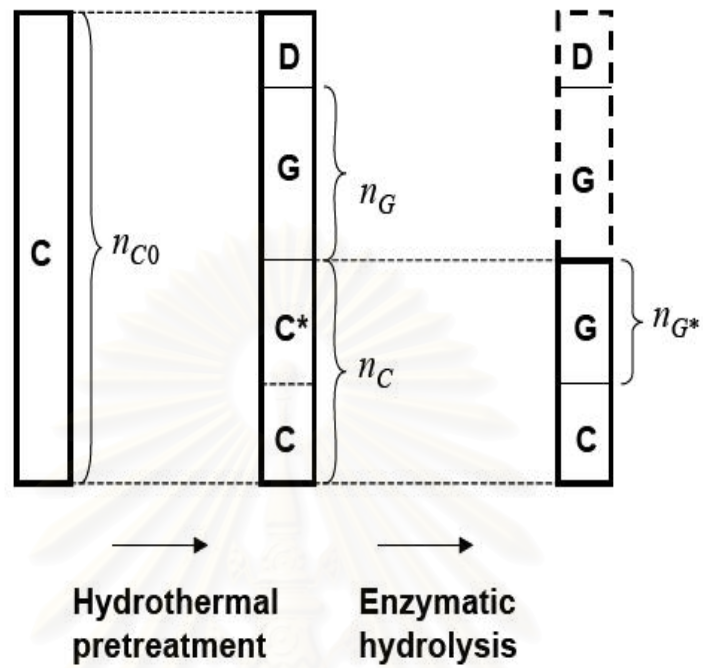
Figure. 4.12 Change of cellulose in the hydrothermal pretreatment and enzymatic hydrolysis processes.

4.5.2 The effect of treatment time on hydrothermal pretreatment

Varies the treatment time of 0 and 30 minute. Compared with the previous condition, when we know the optimal temperature for hydrothermal pretreatment. The optimal temperature was used in this condition for studied for the effect of treatment time on hydrothermal pretreatment with rubber wood powder. Table 4.4 shows the treatment time condition.

Table 4.4 Treatment time condition for hydrothermal pretreatment

Treatment time (min)	0 and 30
Rubber wood powder (g)	7
Deionised water [g]	63
Temperature (°C)	170, 210 and 250

4.5.3 The effect of adding acetic acid on hydrothermal pretreatment

We have found a good result in the previous work using acetic acid as shows in the figure 4.13 In addition, sulfuric acid is too strong, and they are suffering from post treatment. Acetic acid is organic, produced by fermentation, and can be decomposed by fermentation. It is much good catalyst, but we have to be quantitative. Table 4.5 shows the adding acetic acid condition for hydrothermal pretreatment.

Table 4.5 Adding acetic acid conditions for hydrothermal pretreatment.

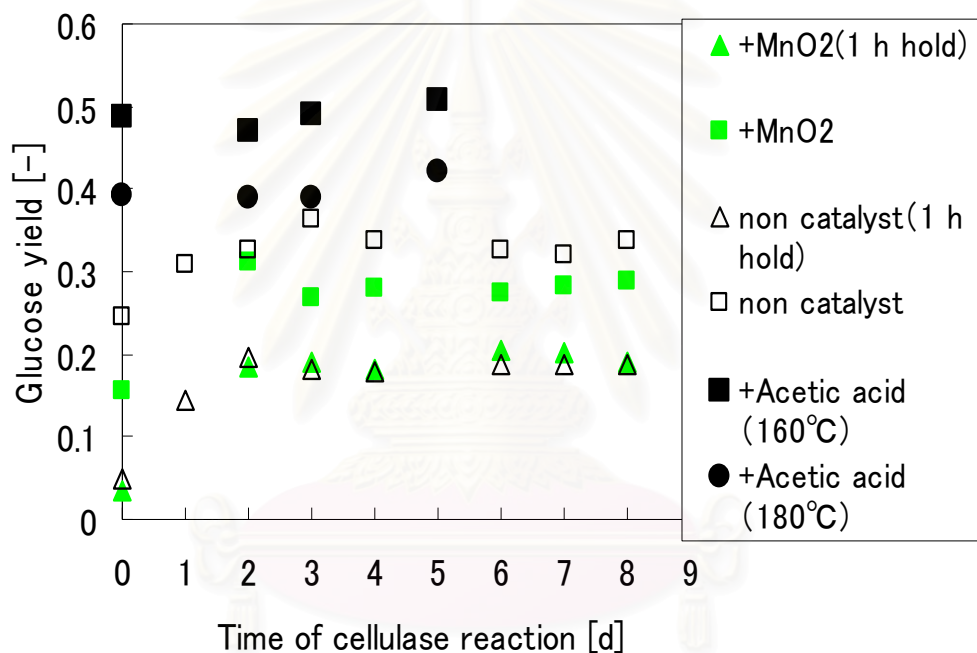|	
Acetic acid 0.025 mol/lit (g)	63
Rubber wood powder (g)	7
Temperature (°C)	130, 170, 190, 210 and 250

Figure. 4.13 Time dependence of amount of glucose generated from solid residue
treated of cabbage in each catalyst (Okahisa , 2007).

Figure. 4.13 shows the dependence of glucose yield on time of cellulose hydrolysis.

The presence of acetic acid the glucose yield could be increased to the level of 50%

by used cabbage for raw material. (Okahisa , 2007).

4.5.4 The effect of concentration of rubber wood powder on hydrothermal

pretreatment

Table 4.6 Concentration conditions of hydrothermal pretreatment.

Concentration of rubber wood powder (% wt)	10, 15 and 20
De-ionizer water [g]	63, 59 and 56
Temperature (°C)	170, 210 and 250

Rubber wood powder was varies concentrations in ranging from 10%- 20 % for hydrothermal pretreatment. In this studied assumed the reaction of rubber wood powder in hydrothermal pretreatment is first order reaction. To check the reaction order in each concentration, the glucose yield should be same. Table 4.6 shows the concentration condition of hydrothermal pretreatment.

4.5.5 The effect of size of ground rubber wood

In this studied, the ground rubber wood from varies on crushing time of ball mill was focus. The idea is, when the rubber wood powder was crushed to very small size the surface area was increased compared with bigger size and then the reaction should be good. Table 4.7 shows the ground rubber wood conditions.

Table 4.7 Ground rubber wood conditions

Crushing time (hr)	12, 24 and 48
Rubber wood powder (g)	7
De-ionizer water [g]	63
Temperature (°C)	170, 210 and 250

CHAPTER V

RESULTS AND DISCUSSIONS

5.1   Effect of temperature on glucose yield.

First, rubber wood powder were hydrothermally treated under conditions of temperature from 130 to 280 °C. Figure 5.1.shows the temperature history inside autoclave reactor.
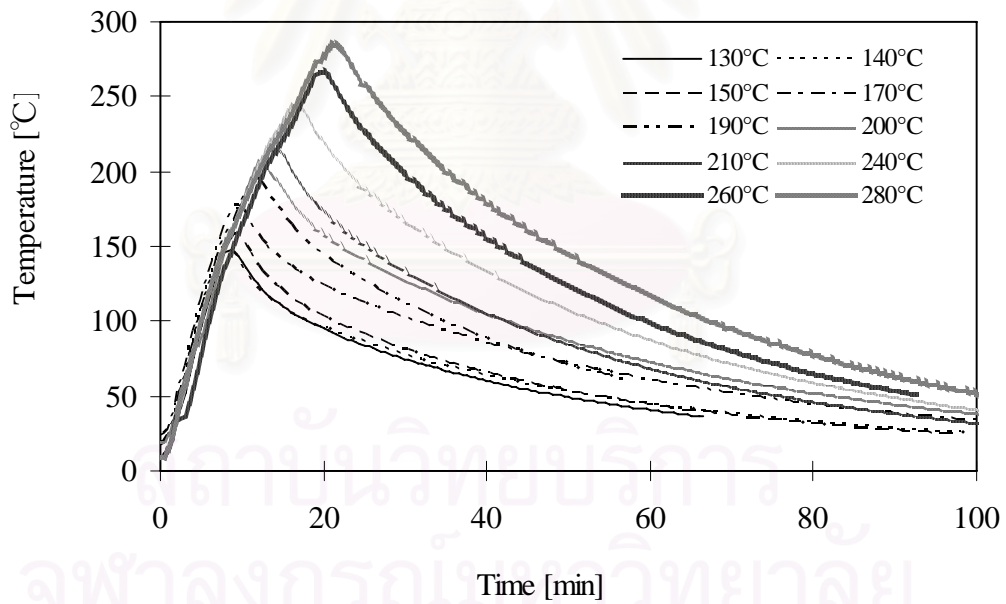
Figure.5.1. Temperature history regarding different target temperature.

After hydrothermal pretreatment within the autoclave liquid products with distinctively different appearance could be obtained the experimental conditions for hydrothermal pretreatment which includes temperature, weight of rubber wood and de-ionized water are summarized in Table 4.3. At least two separate experiment were conducted for confirming repeatability. In each experiment, solid sample and liquid sample were separated as shown in the figure 5.2-5.7. Base on visualization, the higher the operating temperature, the darker the color of the liquid product.

In this studied, one gram of solid samples was sampled for examination on enzymatic hydrolysis. The liquid extract obtained from solid sample was analyzed by HPLC analysis. Figure 5.8 shows the glucose yield with respect to the cellulase reaction time.

Figure 5.2 Total samples after hydrothermal pretreatment at temperature of (A) 130, (B) 140, (C) 150, (D) 170, and (E) 190 °C

Figure 5.3 Total samples after hydrothermal pretreatment at temperature of (A) 200,

(B) 210, (C) 240, (D) 260, and (E) 280 °C

Figure 5.4 The liquid samples after hydrothermal pretreatment at temperature of

(A) 130, (B) 140, (C) 150, (D) 170, and (E) 190 °C
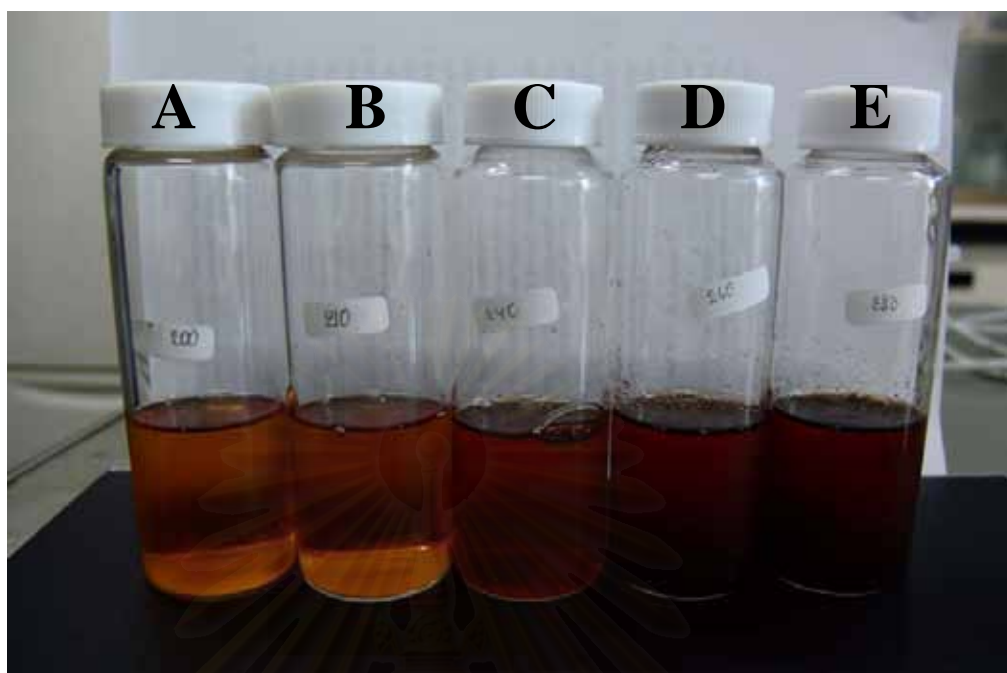
Figure 5.5 The liquid samples after hydrothermal pretreatment at temperature of

(A) 200, (B) 210, (C) 240, (D) 260, and (E) 280 °C

Figure 5.6 The solid samples after hydrothermal pretreatment

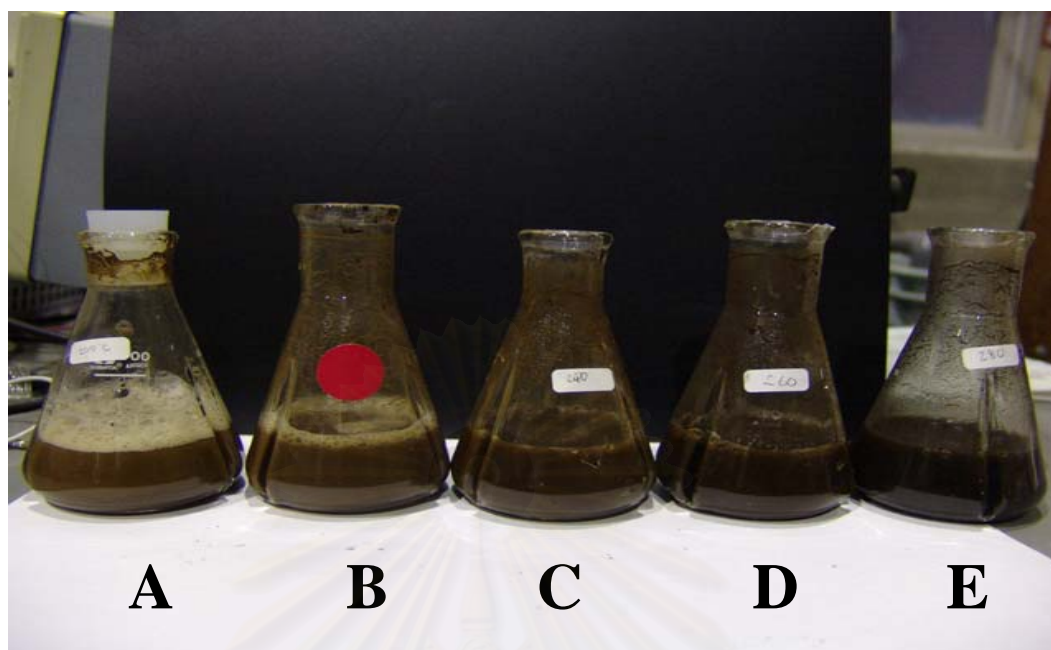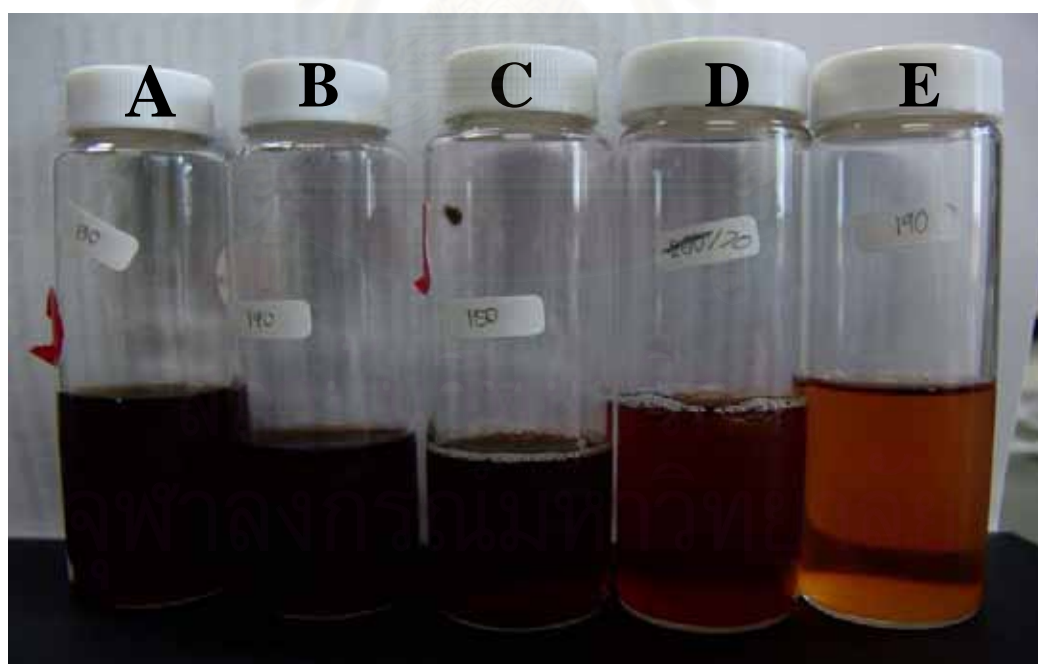at temperature on (A) 130, (B) 140, (C) 150, (D) 170, and (E) 190 °C
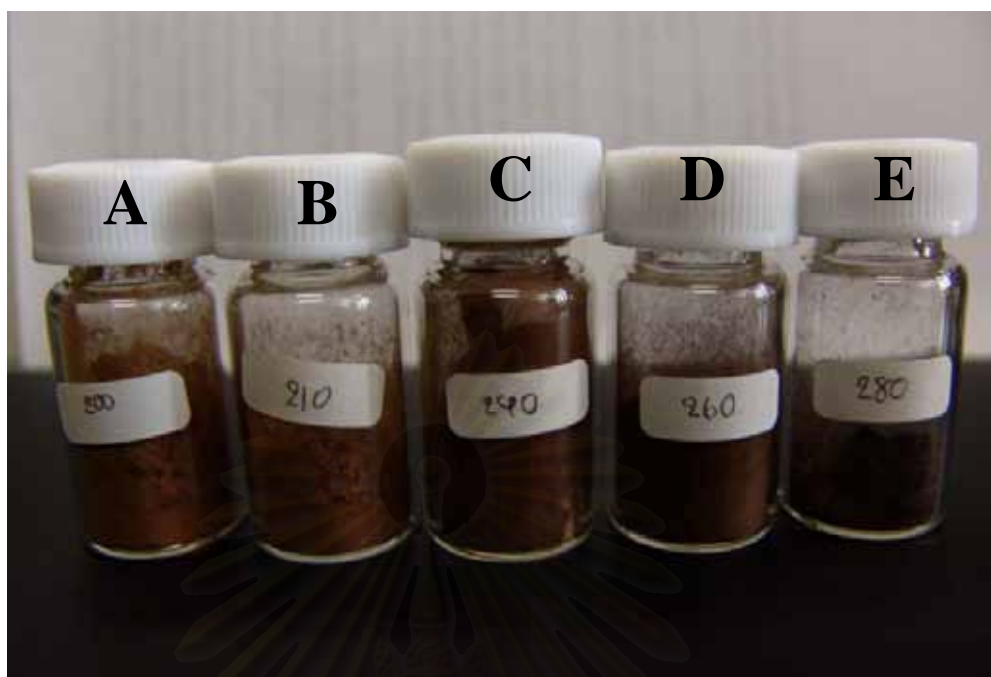
Figure5.7 The solid samples after hydrothermal pretreatment

at temperature on (A) 200, (B) 210, (C) 240, (D) 260, and (E) 280 °C

Figures 5.8 – 5.9 shows the glucose yield with respect to the cellulase reaction time. The glucose yield obtained from hydrothermally pretreated solid increased with an increase in cellulase reaction time. However, it is note that glucose yield did not exceed the level of 14% while the initial value of glucose yield at t=0 was that of sample without enzymatic treatment. Okahisa, 2006 investigated on hydrothermal pretreatment of pure cellulose within a similar autoclave reactor. They also reported that even using the pure cellulose, the maximal yield of glucose was only 20%

Amount of glucose generated from raw material with time on varied with different treatment time and different cellulase reaction time, average particle size 28 μm, treatment time 0 min. (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis

Figure 5.8 Amount of glucose generated from raw material with time on varied with different temperature in the range of 130 – 200 °C, average particle size 28 μm, treatment time 0 min. (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis)
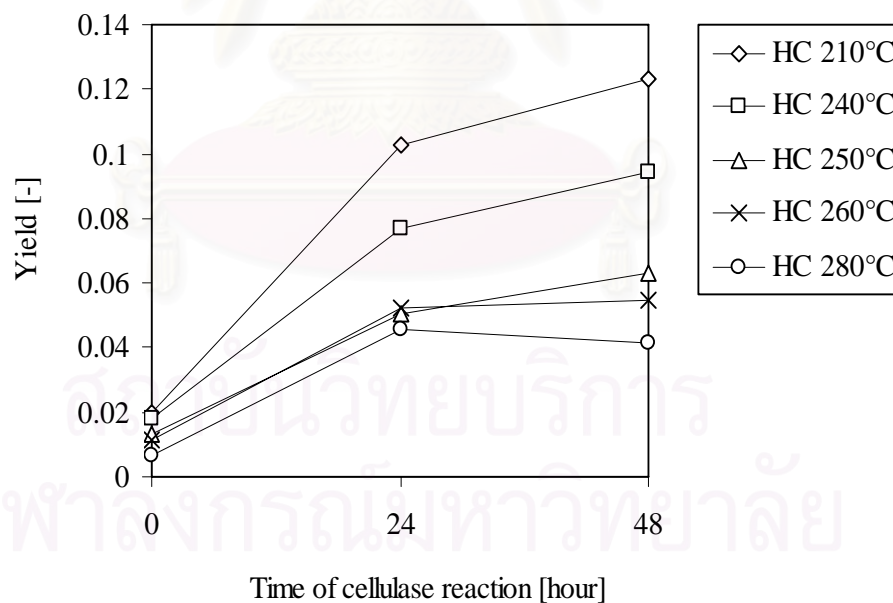
Figure 5.9 Amount of glucose generated from raw material with time on varied with different temperature in the range of 210 – 280 °C, average particle size 28 μm, treatment time 0 min. (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis)
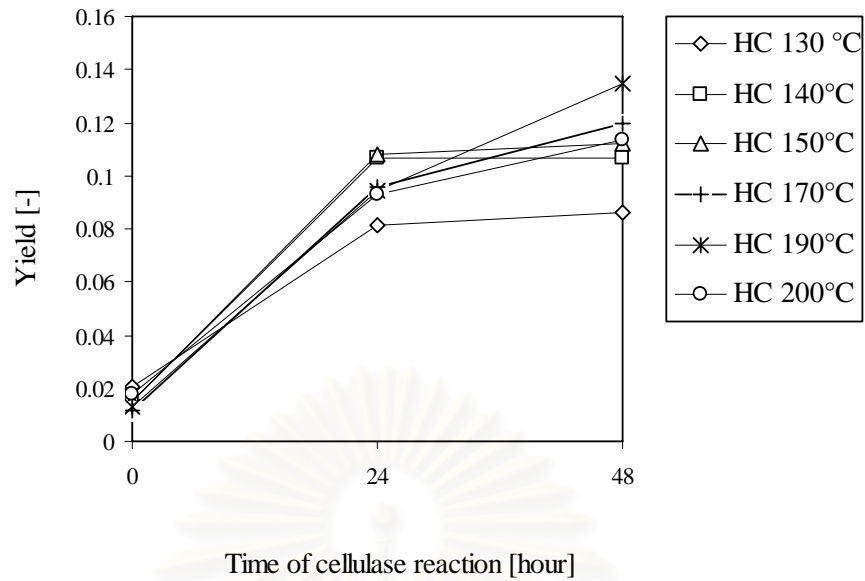
The effect of temperature hydrothermal pretreatment in temperature on the yield of glucose obtain by enzymatic is shown in Fig. 5.11 Most of the glucose decomposed future with longer elapsed time, resulting in a decreased in the glucose concentration. It should be note that the final glucose yield increased when the target temperature of the hydrothermal pretreatment are increased from 230 to 260°C However at 270°C the glucose was further decomposed leading to the glucose content in the autoclave. The highest value for the glucose yield was about 20 %, the result is attributed that the glucose content obtain from the hydrothermal pretreatment was easily future decomposed in the autoclave (Yoshida et al , 2005).
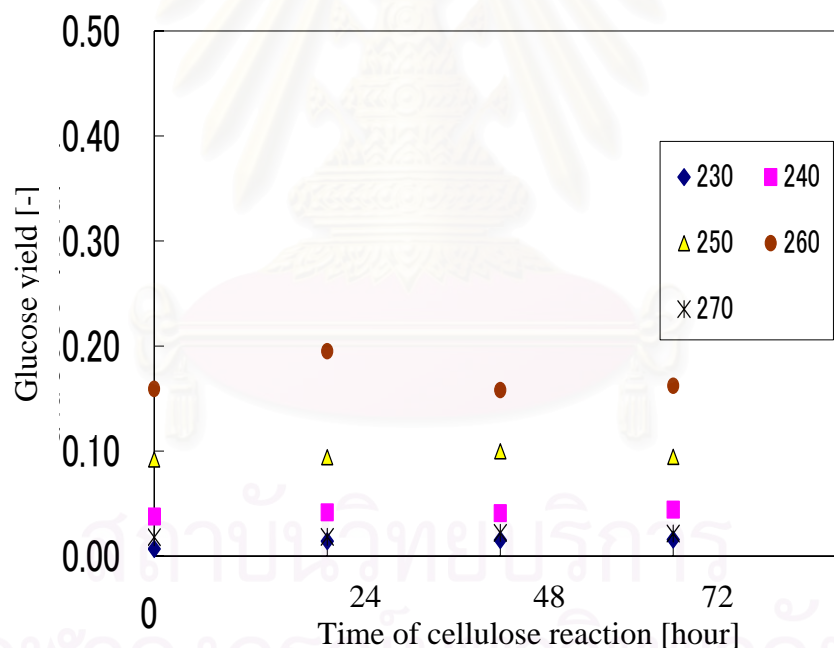
Figure 5.10 Time dependence of amount of glucose generated from solid residue (pure cellulose used as raw material) treated temperature 230 – 270 °C (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis) (Okahisa, 2006)
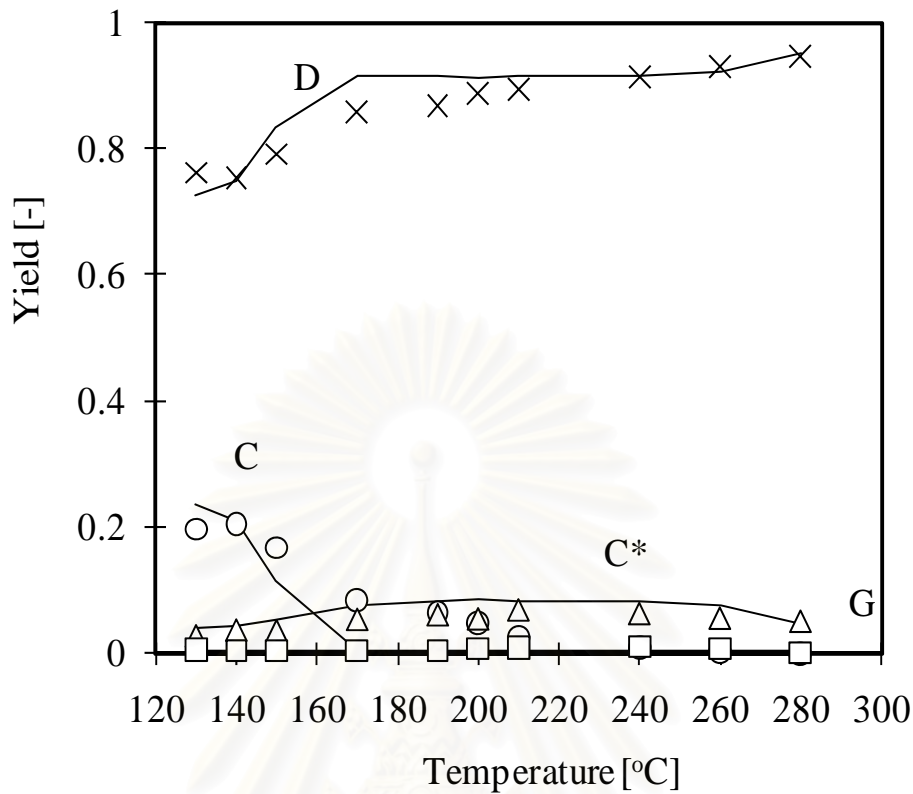
Figure 5.11 Comparison with experimental value and calculated values

(symbol: experiment, line: calculation value).

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

$$\frac{d[G]}{dt} = k_3[C^*] + k_2[C] - k_4[G] \tag{6}$$

$$\frac{d[C^*]}{dt} = k_1[C] - k_3[C^*] \tag{7}$$

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

For the investigation of reaction kinetic, its has been reported that the

Arrhenius rate law is applicable (Foglor, 1999) Each reaction rate constant, could be

obtained from analysis of concentration change with respect to the reciprocation of

temperature as shown in Fig. 5.12. 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. There values are summarized in Table 5.1.These rate constants were analyzed by using a set of multiple reactions which are expressed by equation (1) – (4) Figure 5.12 compares the experimental and calculated yields of each constituent in this calculation. Good agreement could be obtained and the effectiveness of applying the "apparent" reaction network to hydrothermal pretreatment of rubber wood could reasonably be validated. It should be noted that .since k_2 is much larger than k_1 , the cellulose (C) would reasonably be converted to the form C* radical that can be hydrolyzed by cellulose. Such radical further be decomposed to glucose (G). However, it should also be noted that k_4 is greater than k_3 , and the produced glucose would be further decomposed to form carbon compounds with smaller molecular sizes.
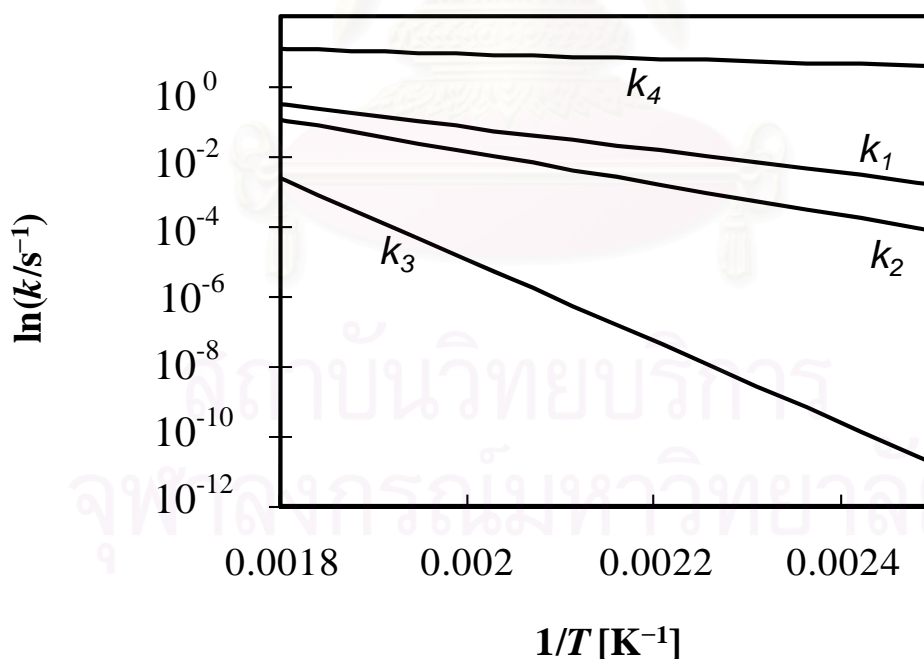
Figure. 5.12. Arrhenius plot of each reaction rate parameters for rubber wood

powder

Table 5.1. Parameters of reaction rate

	Preexponential factor [1/s]	Activation energy [kJ/mol]
k_1	1.87×10^5	62.0
k_2	2.02×10^7	87.7
k_3	1.80×10^{18}	222.2
k_4	2.88×10^2	14.4

Based on these kinetic parameters the optimal condition of continuous hydrothermal treatment of rubber wood powder would be able to be estimated. Within the autoclave reactor, the optimum target temperature was found to be 235.5°C, with the heating rate of 8°C/min and cooling rate of 4°C/min. The expected glucose yield under this condition was 0.183. This result indicates that other slow on heating rate and cooling rates was not suitable for hydrothermal pretreatment of our rubber wood residues However, at the moment we have a limiting of our experimental set up which could not provide higher rate of heating and cooling.

5.2   Effect of varied treatment time on hydrothermal pretreatment

In this studied, we focus on the varied treatment on hydrothermal pretreatment. As shown the condition in table 4.4. Figure 5.3.shows the temperature history inside autoclave reactor.
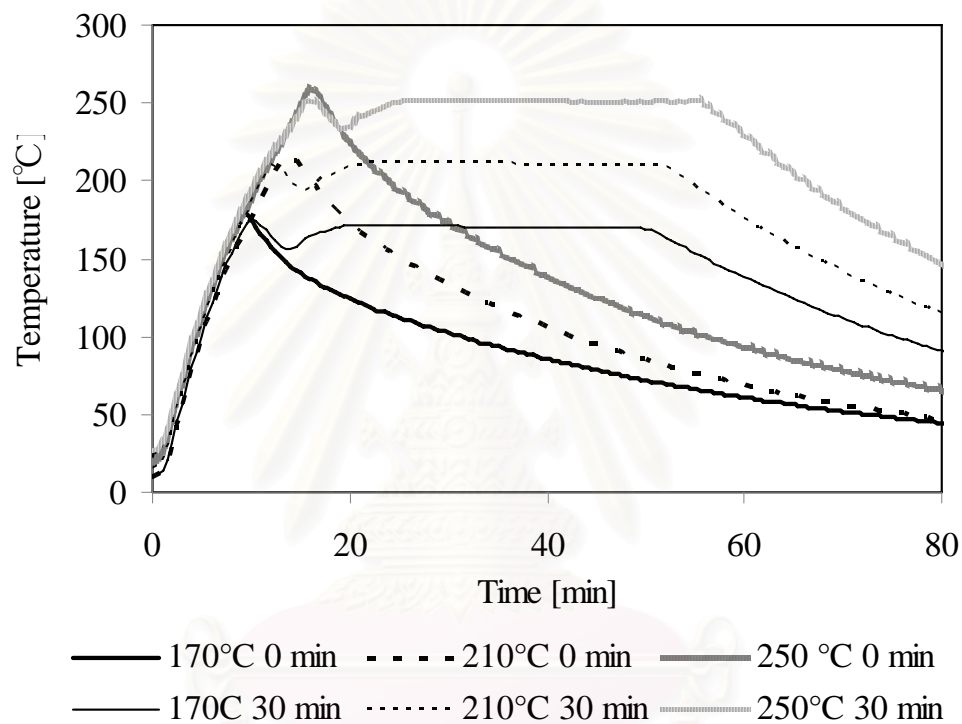
Figure 5.13. Temperature history regarding different treatment time in each target temperature.
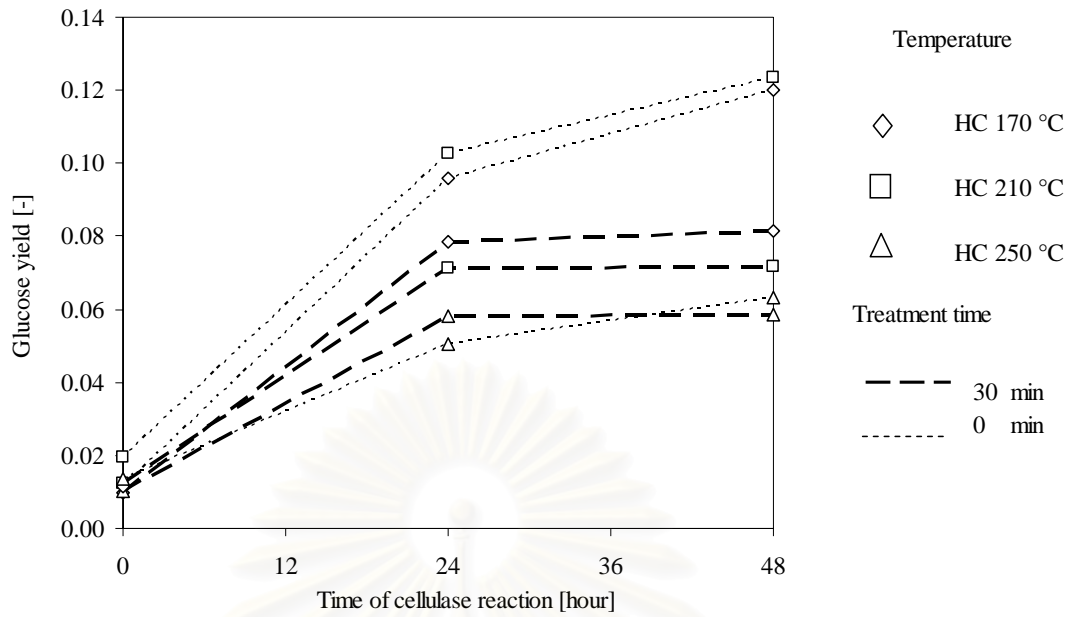
Figure 5.14 Amount of glucose generated from raw material with time on varied with different treatment time and different cellulase reaction time, average particle size 28 μm, treatment time 0 min. (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis).
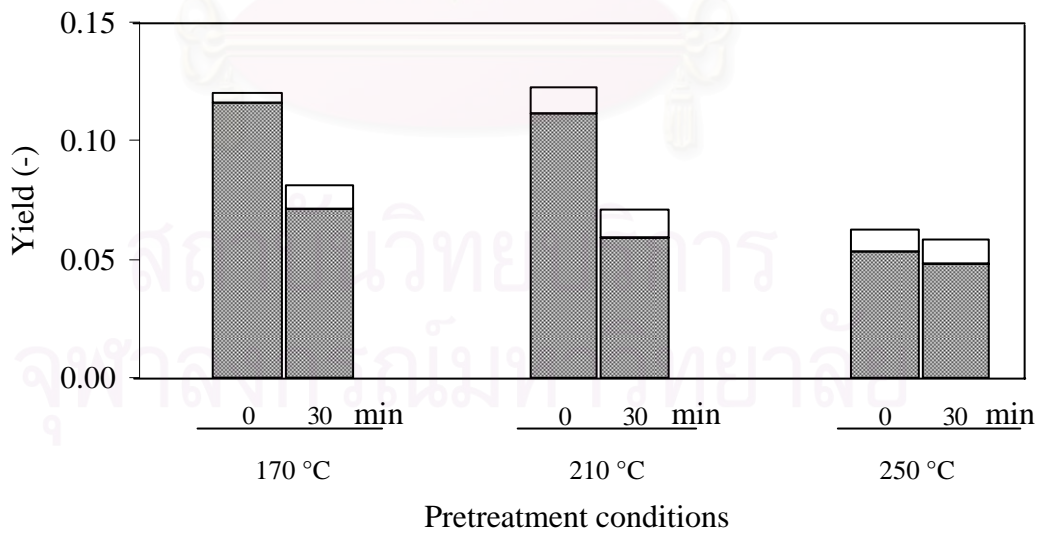
Figure.5.15 Total glucose yield at different treatment time condition at 2 day of enzymatic

From the previous studied, we known the range of temperature which highest glucose yield, Therefore in this studied we scoped the range of temperature are 170, 210 and 250 °C. By varied the treatment time between 0 and 30 min. Figure 5.14 shows the amount of glucose generated with time on the treatment time 0 and 30 min. In addition, the glucose yield became higher with the increased treatment time up to 15 min but at 30 min, the glucose is further decomposed in all temperature. Figure.5.16 shows total glucose yield at different treatment time condition. Compare the results with the innitial conditions (Treatment time 0 min and 10 % wt).
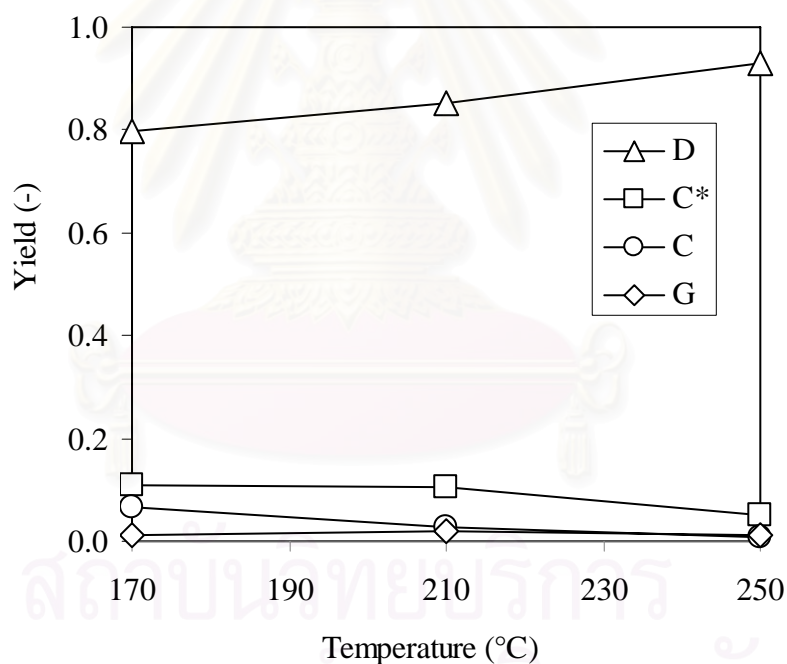
Figure 5.16 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at treatment time 0 min.

From the figure 5.16-5.17 shows the components of rubber wood powder (D, C, C* and G) after hydrothermal pretreatment in each treatment time (0 and30 min). It is shown that most of the cellulose is over decomposed under the conditions

employed here from the high value of X_D. The final glucose yield becomes higher

when temperature for the hydrothermal pretreatment is increased from 170 to 210°C

but at 250°C, the glucose is further decomposed
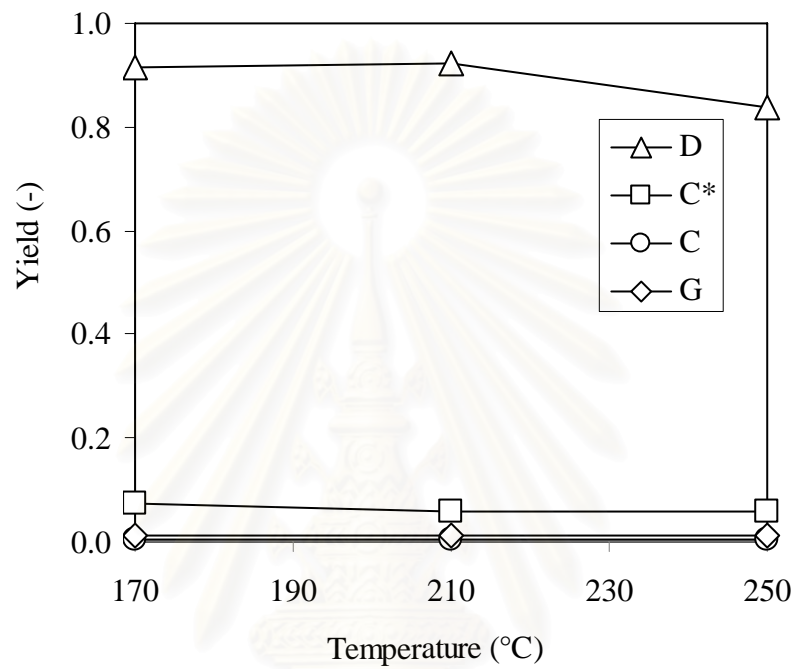
Figure 5.17 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at treatment time 30 min

5.3   Effect of varied concentration of rubber wood powder on hydrothermal pretreatment
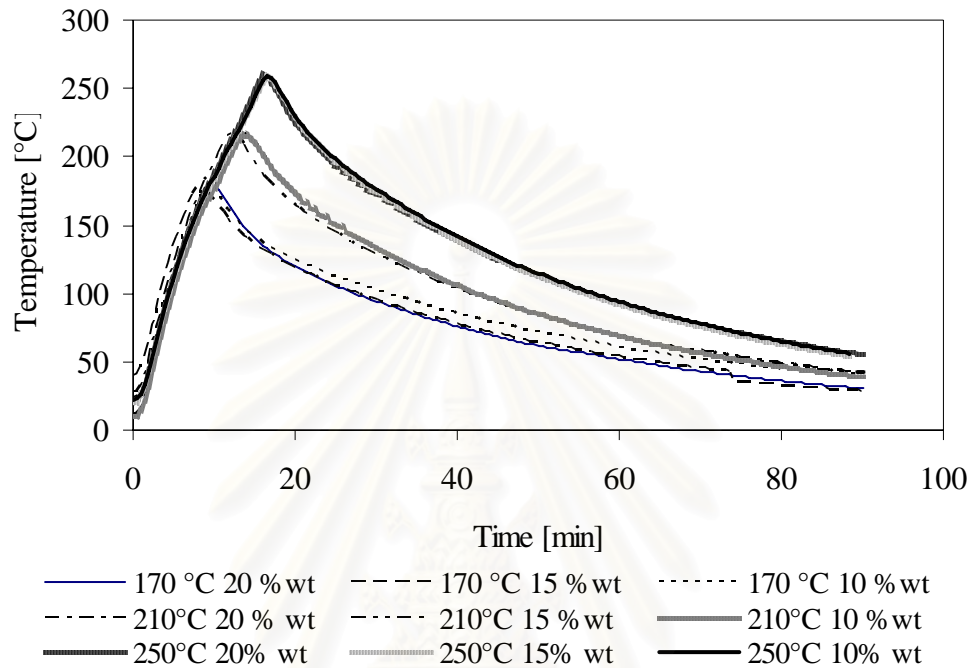
Figure.5.18. Temperature history inside the reactor with time in each concentration of raw material.

In this studied, we focus on the varied the concentration of raw material (rubber wood powder) on hydrothermal pretreatment. The target temperature still studied on 170, 210 and 250 °C Figure 5.18 shows the temperature profile of this studied. Adschiri et al,1993 assumed that decomposition rate of cellulose in near critical water was evaluated by the first order reaction with respect to the residual amount of cellulose that assumption was agreed with our experimental results.
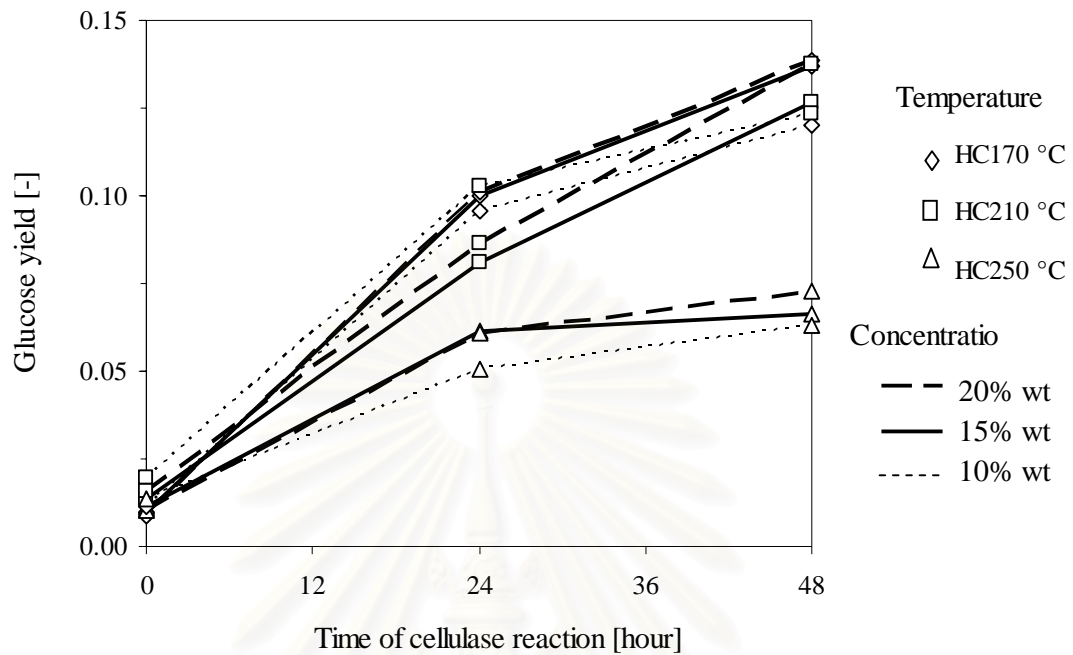
Figure 5.19 Amount of glucose generated from raw material with time on varied with

different concentration of raw material and different cellulase reaction time, average

particle size 28 μm, treatment time 0 min. (HC denote the solid sample from

hydrothermal pretreatment used with enzymatic hydrolysis).

In each temperature (170, 210 and 250 °C), we varied the concentratio of raw

material at 10, 15 and 20 %wt. Figure 5.19 shows that glucose yield are increased

when temperature was increased and the glucose yield was changed with initial

concentration. Figure 5.20 show the total glucose yield at different concentration of

raw material condition at 2 day of enzymatic. The tread of glucose yield are increased

with higher temperature (170 to 210) and also decreased at 250 °C) The totally

glucose yield in each concentration are same. Figure 5.21 – 5.23 shows the shows the

components of rubber wood powder (D, C, C* and G) after hydrothermal

pretreatment in each conditions. It is shown that most of the cellulose is over

decomposed under the conditions also employed here from the high value of X_D.
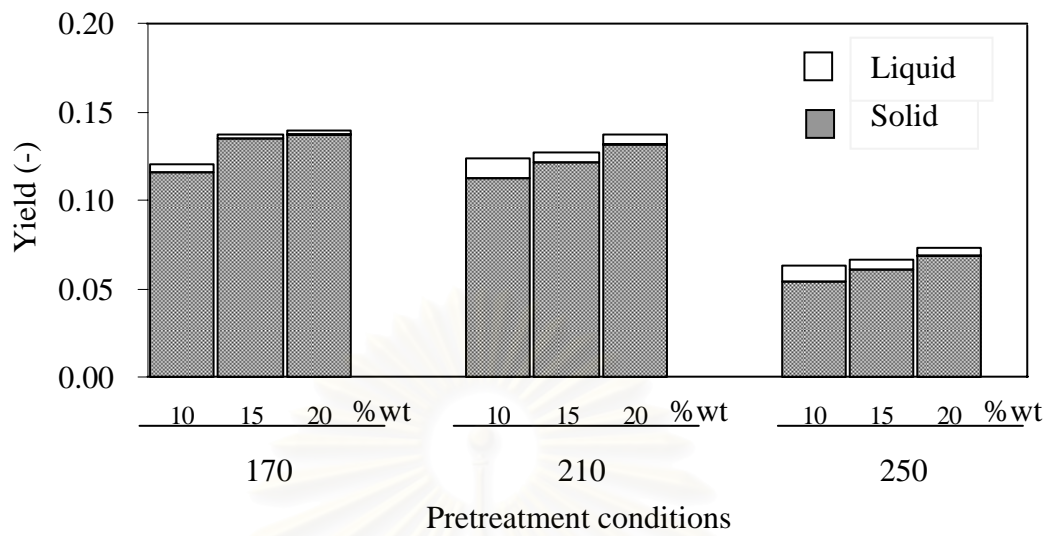
Figure. 5.20 Total glucose yield at different concentration of raw material at 2 day of enzymatic..

Figure 5.21 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at concentration 10 %wt of raw material.

Figure 5.22 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at concentration 15 %wt of raw material.

Figure 5.23 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at concentration 20 %wt of raw material

5.4   Effect of varied crushing time of rubber wood powder on hydrothermal pretreatment
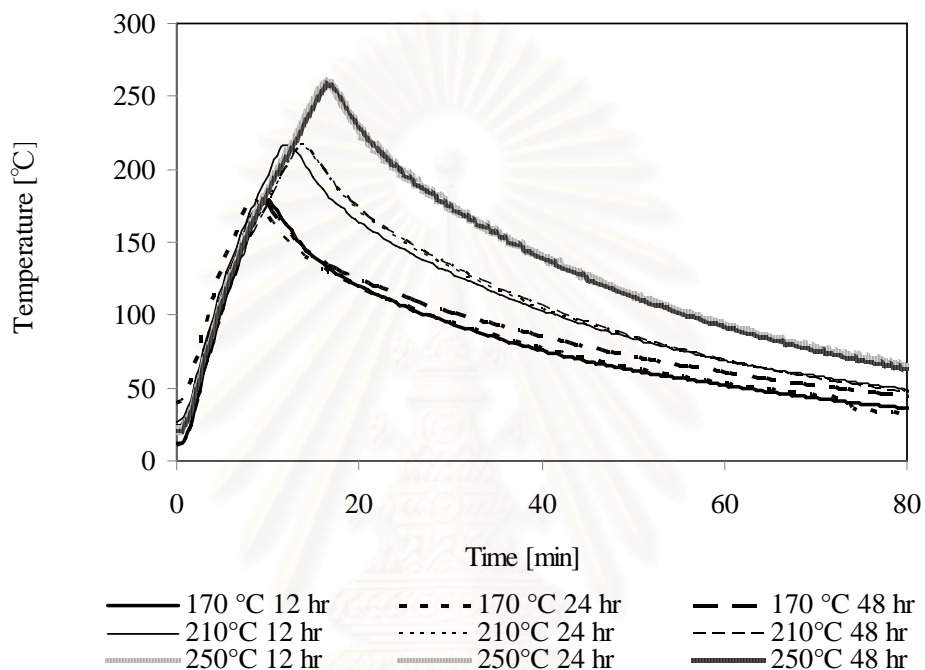
Figure 5.24 Temperature change inside the reactor with time by varied the treatment time

Varied crushing time for 12, 24 and 48 hour were studied in this case. Figure 5.24 shows the temperature profile inside the reactor. In this studied, we used 10 %wt concentration of rubber wood as raw material and 0 min for treatment time.
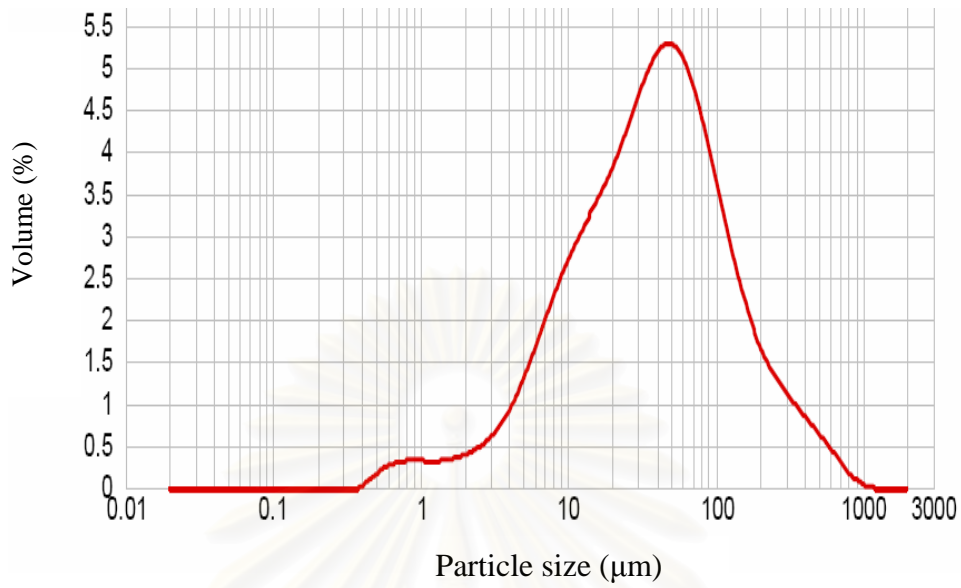
Figure 5.25 Particle size distribution of rubber wood powder, crushing for 12 hr by ball mill.
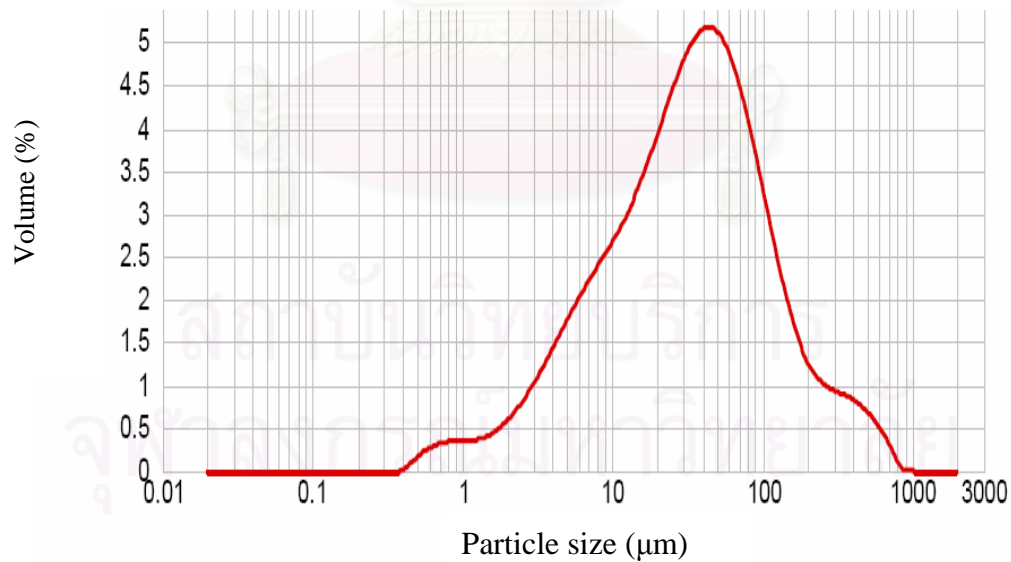
Figure 5.26 Particle size distribution of rubber wood powder, crushing for 24 hr by ball mill
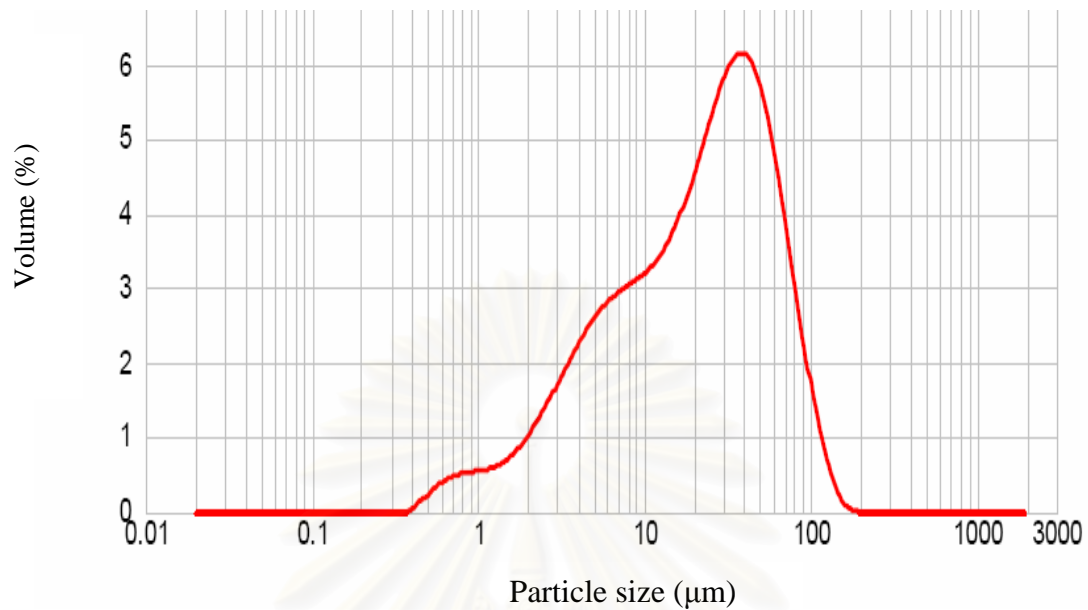
Figure 5.27 Particle size distribution of rubber wood powder, crushing for 48 hr by

ball mill

Figure 5.25- 5.27 shows the particle size distribution of rubber powder, which

crushed for 12, 24 and 48 hour by ball mill and measured by Master sizer 2000. Table

5.2 shows the average particle size in each condition.

Table 5.2 Average particle size with crushing time

Crushing time (hour)	Average particle size (μm)
12	37
24	33
48	28
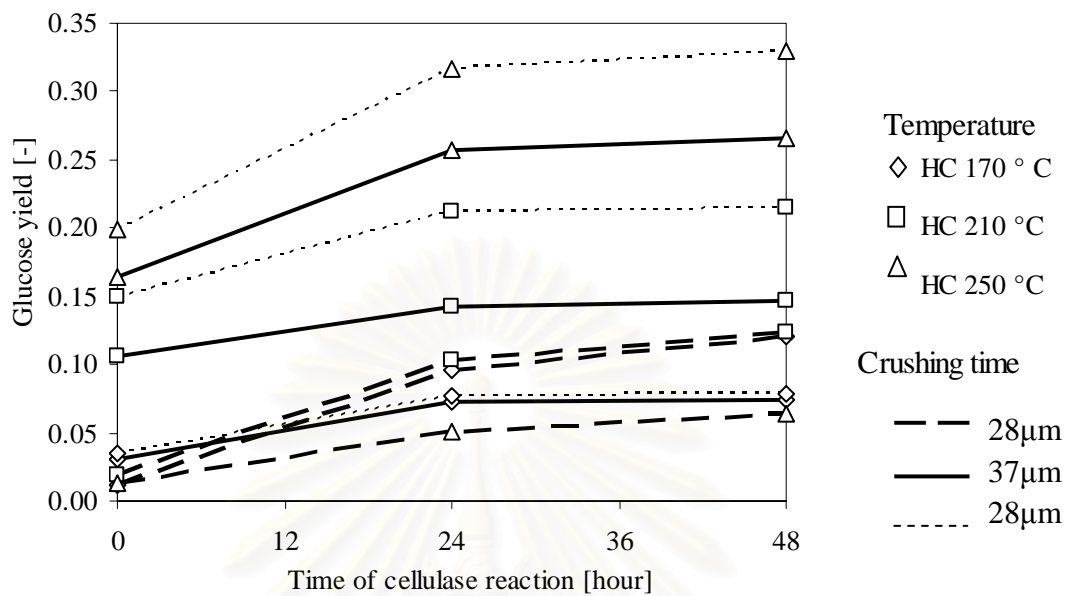
Figure 5.28 Amount of glucose generated from raw material with time on varied with different particle size and different cellulase reaction time, 10 % wt of concentration of raw material, treatment time 0 min. (HC denote the solid sample from hydrothermal pretreatment used with enzymatic hydrolysis)
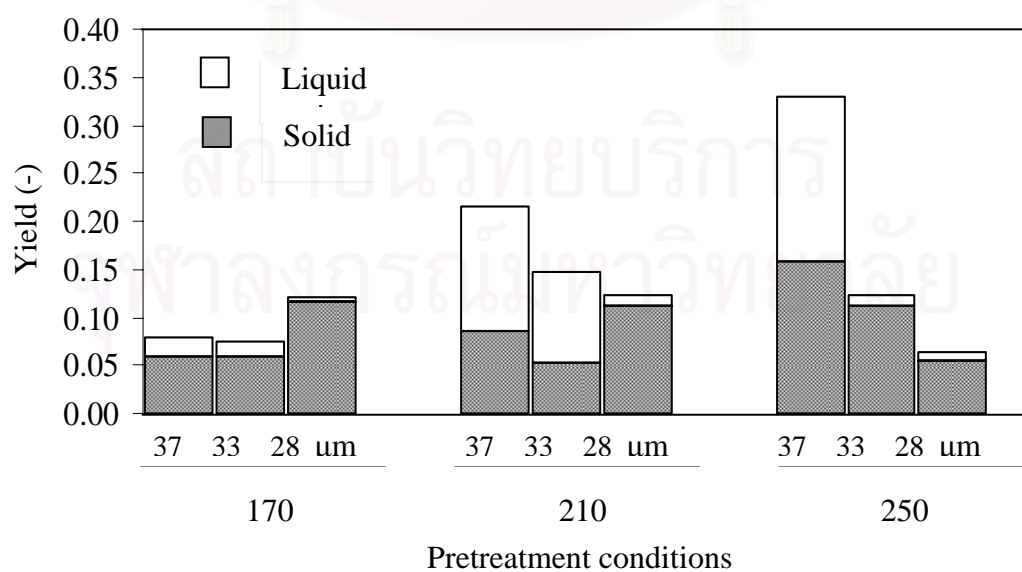
Figure. 5.29 Total glucose yield at different concentration of raw meterial.

Figure 5.28 shows the amount of glucose generated with time by varied the crushing time of raw material (rubber wood powder).The trend of glucose are same in previous conditions that higher when the temperature was increased. As expected the ground rubber wood with the smallest of particle size distribution could provide the increased yield of product. Base on the experimental results, in 48 hr crushing time the glucose yield was decreased while decomposed product yield was increased. Moreover, in 12 hr crushing time the glucose yield are highest. From this results, we found that, the particle size have effected for hydrothermal pretreatment. In the smallest particle size of raw material, the decomposition rate of cellulose was well. Agreed with Negro et al,2003 experiment. They studied on liquid hot water by used poplar as raw material. They varied the particle size between 2-5 and 12 – 15 mm. The highest glucose product was obtained at 220 °C and large particle size and no significant effect of particle size on enzymatic hydrolysis. Fig. 5.26 shows the total glucose yield at different concentration of raw meterial condition The highest glucose in this studied are 32 % at 250 °C, 12 hr crushing time.
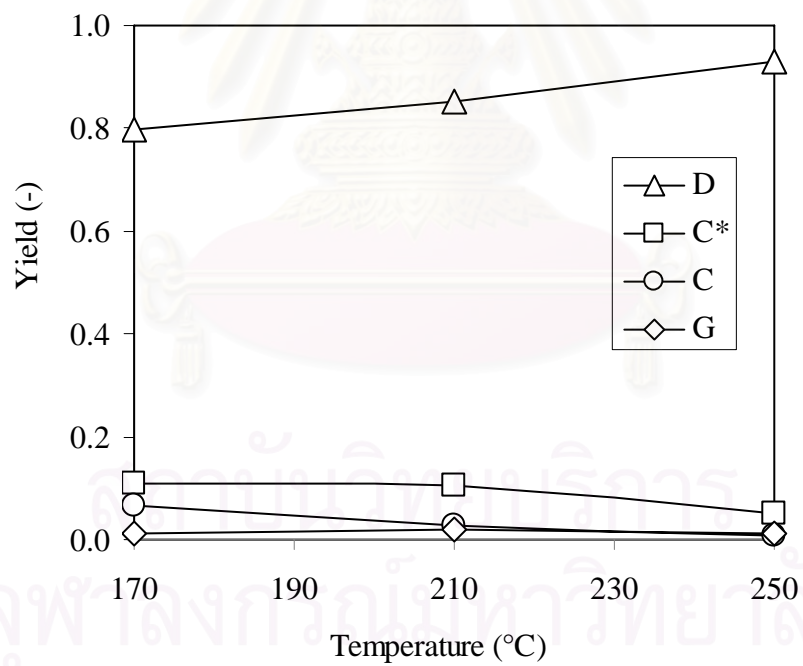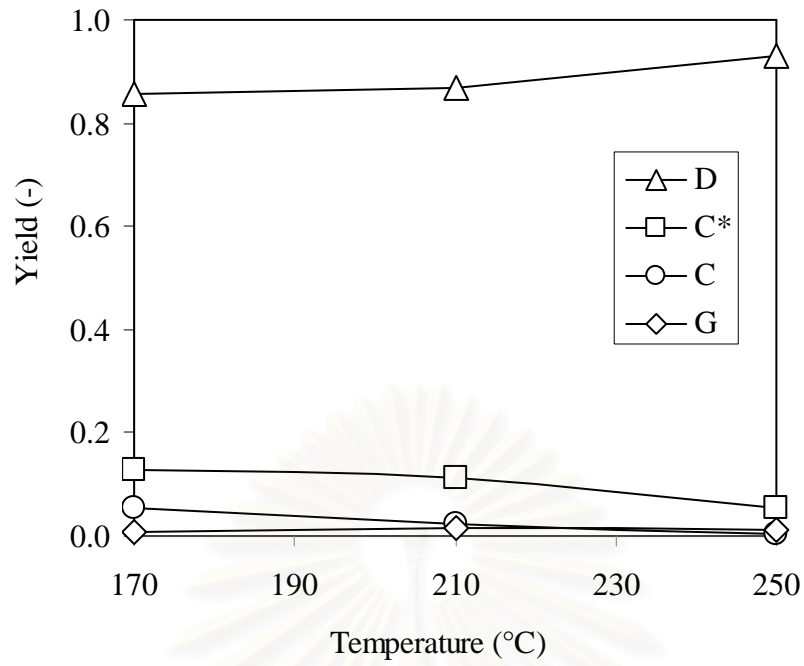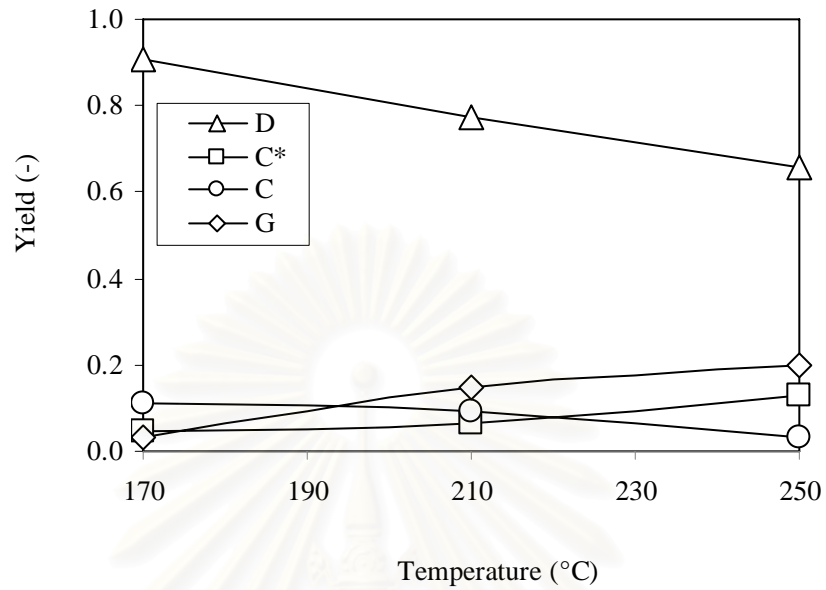
Figure 5.30 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at crushing time 12 hr of raw material
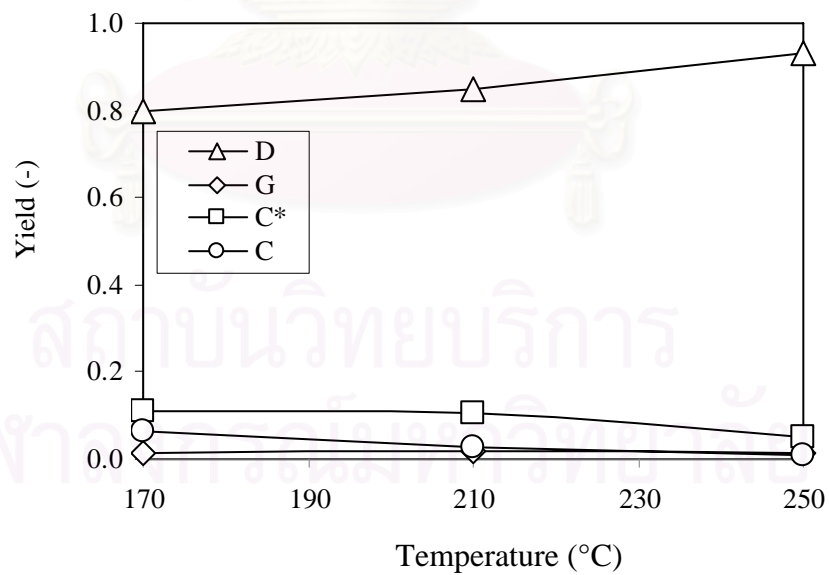
Figure 5.31 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at crushing time 24 hr of raw material
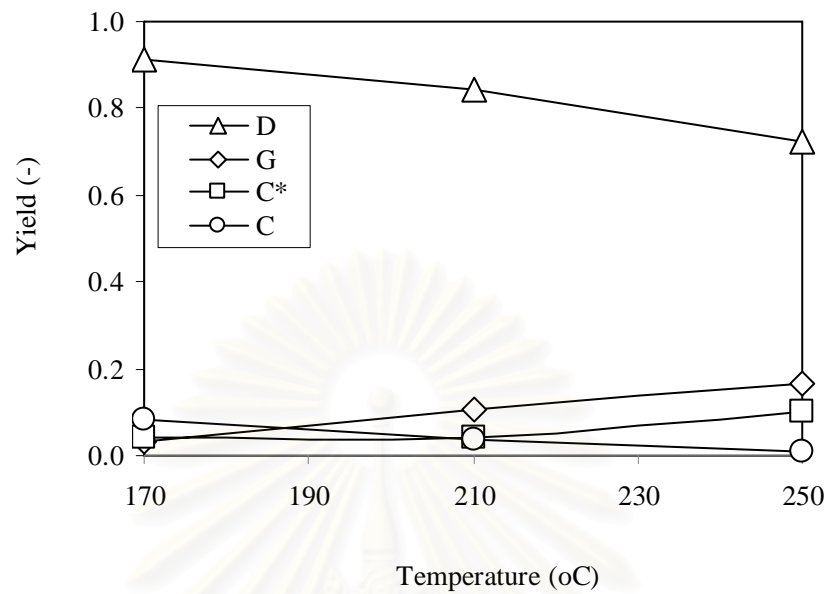
Figure 5.32the components of rubber wood powder (D, C, C* and G) after

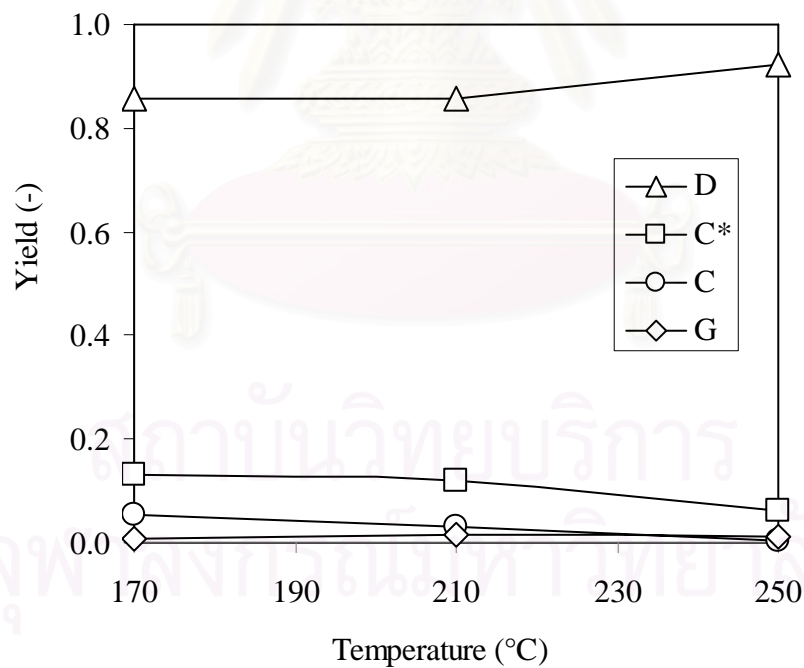hydrothermal pretreatment at crushing time 48 hr of raw material.

Figure 5.30- 5.32 shows the components of rubber wood powder (D, C, C*
and G) after hydrothermal pretreatment in each crushing time of raw material. It is
shown that most of the cellulose is over decomposed under the conditions also
employed here from the high value of X_D.

5.5 Effect of adding acetic acid on hydrothermal pretreatment
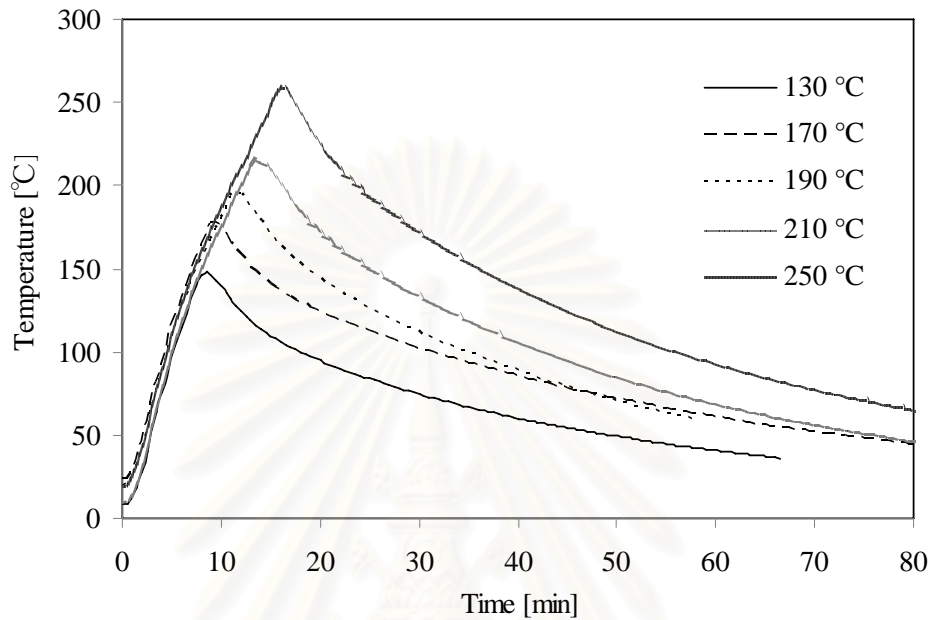
Figure.5.33. Temperature history inside the reactor under condition of acetic acid
addition

In this studied, we are interested is employing added acetic acid for
hydrothermal pretreatment of rubber wood. The condition listed in the table 4.5.
figure 5.33 shows the temperature profile in side autoclave reactor. Figure 5.33 shows
amount of glucose generated with time on varied the crushing time of raw material.
The results shows that adding acetic acid did not provide effect on this process
regardless of temperature.
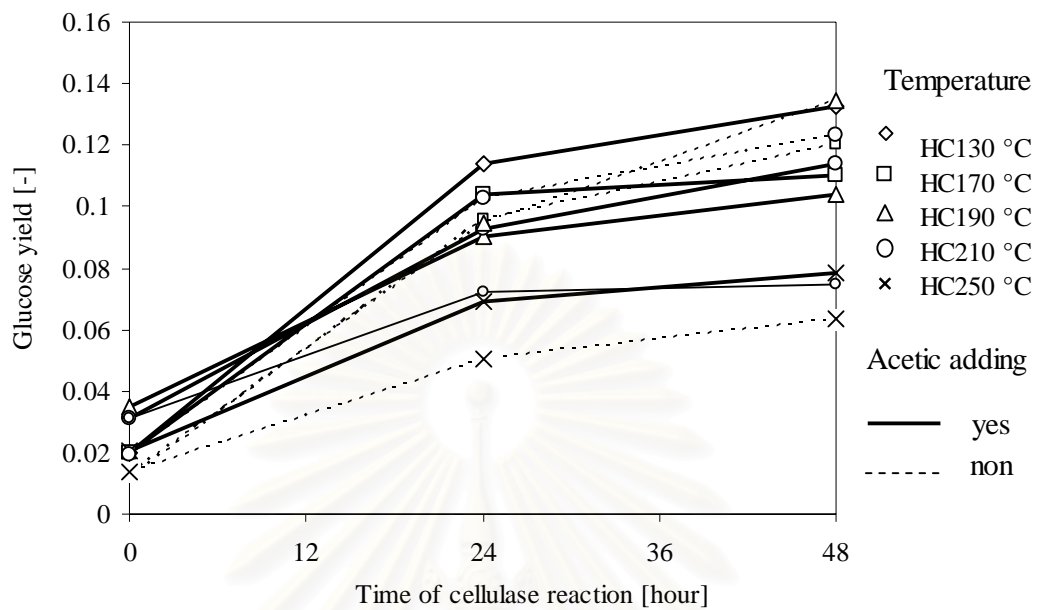
Figure 5.33 Amount of glucose generated from raw material with time on varied with

addition acetic acid at different temperature in the range of 130 – 250 °C, average

particle size 28 μm, treatment time 0 min. (HC denote the solid sample from

hydrothermal pretreatment used with enzymatic hydrolysis)
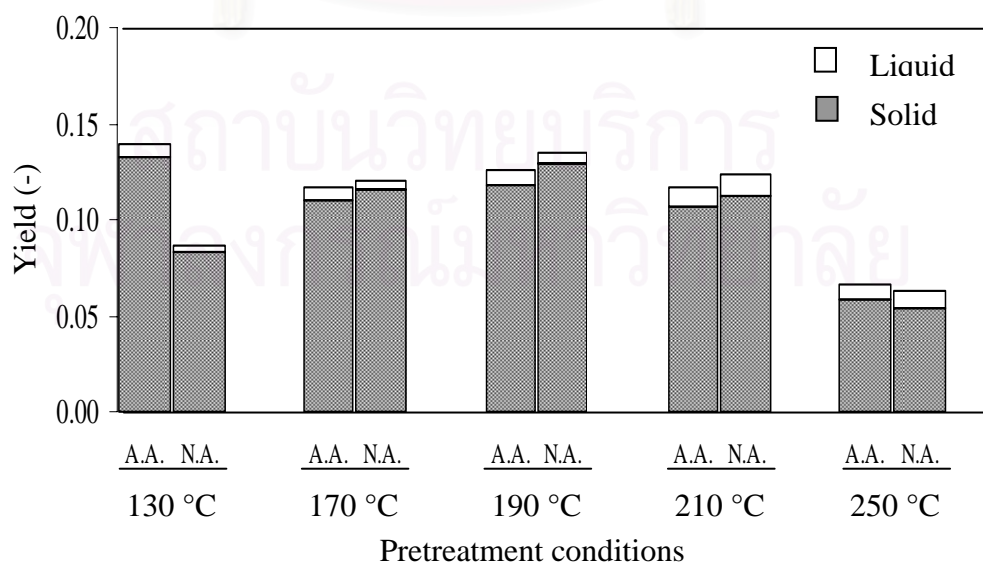
**Figure. 5.34** Total glucose yield at adding acetic acid condition.
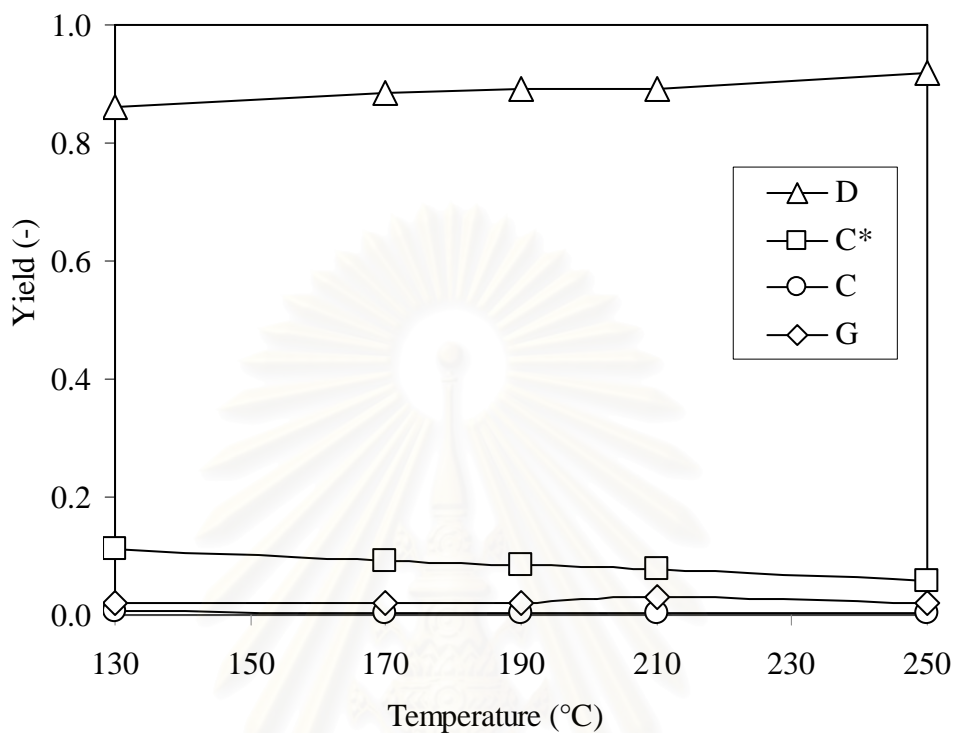
Figure 5.35 the components of rubber wood powder (D, C, C* and G) after

hydrothermal pretreatment at crushing time 48 hr of raw material

Figure. 5.34 shows total glucose yield at adding acetic acid conditio. Adding

acetic acid have effected only at temperature 130 °C, the glucose yield was improved

in this condiotion Figure 5.35 shows the components of rubber wood powder (D, C,

C* and G) after hydrothermal pretreatment. It is shown that most of the cellulose is

over decomposed under the conditions also employed here from the high value of X_D.

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

Rubber wood was hydrothermally treated in the autoclave reactor without catalyst. The pretreatment of cellulase could provide for glucose with some content. Enzymatic hydrolysis was also employed to improve glucose synthesis. It was found that the optimal treatment time was almost constant at 2 day. The effect of hydrothermal pretreatment temperature on the final yields was found to be complied with the Arhenius law. In the lower range (T< 190 C°) the higher treatment time provided higher value of X_D . Because of competitive decomposition of cellulose to glucose and glucose to decomposition product, the glucose yield increased with the treatment temperature. However, at a higher temperature, glucose was further decomposed in the autoclave. The reaction rate parameters were then determined for the investigation of the optimal condition of the hydrothermal pretreatment of rubber wood. The optimum conditions for the hydrothermal pretreatment were predicted, and the highest yield of 0.183 is expected. This result shows that there exists the limitation of the hydrothermal pretreatment in the autoclave which could provide a certain heating and cooling rate.

With the variation of the treatment time of 0 and 30 min with 10 % of rubber wood powder at 170,210 and 250 °C. the higher treatment time (30 min) also provide higher value of X_D. For the effect of ground rubber wood size which was expressed in term of the grounding time the glucose yield of 32 % could be obtained under the condition of using powder ground for 12 hr. It was also found that addition of acetic

acid could not enhance the reaction of hydrothermal pretreatment, leading to the same level of the glucose yield although the different concentration of rubber wood powder was employed.

Recommendations

Due to bigger size of rubber wood powder was obtained higher glucose yield. Therefore, the effect of particle size of raw material on hydrothermal are interested point from this work. The method for remove hemicellulose or lignin from rubber wood before hydrothermal pretreatment should be consider in future work.

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APPENDIX A

USDA method and Calibration curve of glucose solution

APPENDIX A1

Calibration curve of glucose solution
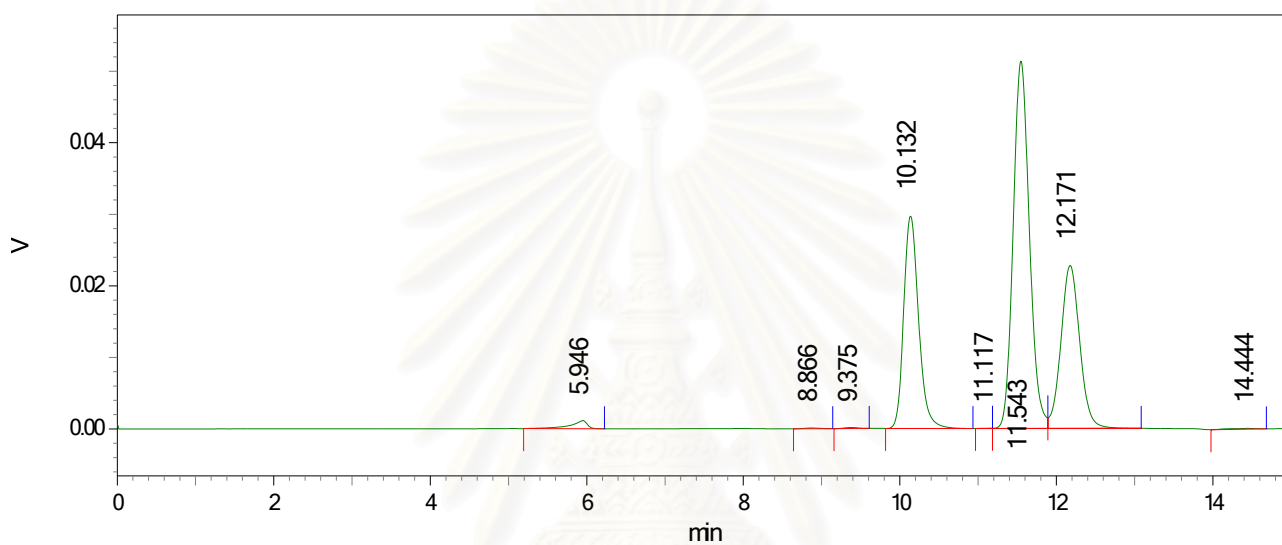
Figure A1-1 Chart of High Performance Liquid Chromatography of glucose solution

0.203 g/50 mL
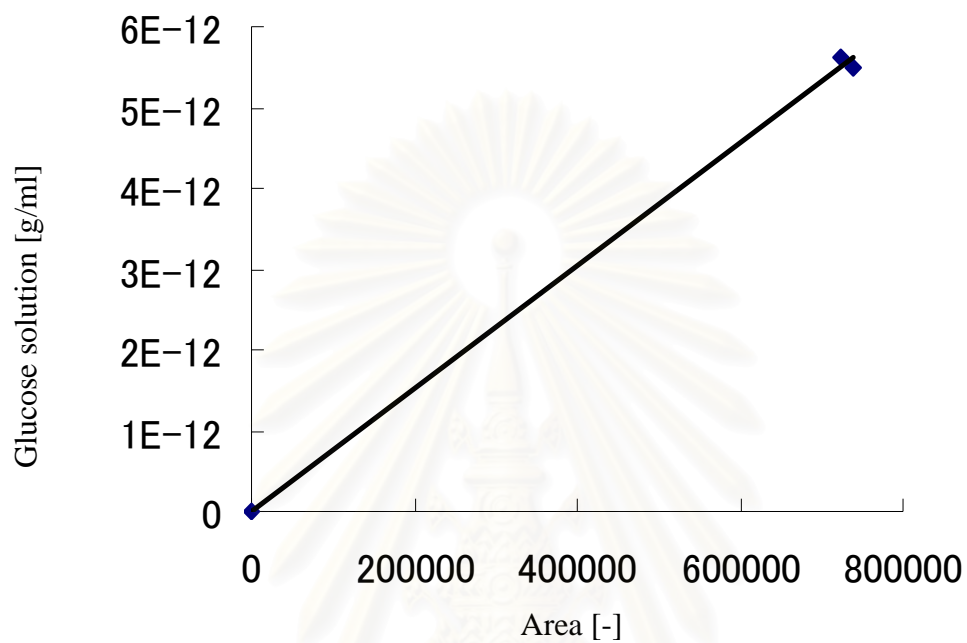
Figure A1-2 Calibration curve of glucose solution

APPENDIX A2

USDA method

Equipment:

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

   Cetyl trimethylammonium 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. 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.

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.

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. (purple)

8. *Demineralizing solution* (1 L)

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

Dissolve oxalic acid dehydrate in ethanol .Add concentrated

hydrochloric acid and distilled water and mix.

9. *Ethanol 80 %*

95 ethanol	845	ml
Distilled water	155	ml

Step;

1. Dry sample at less than 65 °C. And grind through 20-30 mesh

    (1 mm). Add *acid-detergent fiber* to 1(g) samples in crucibles in a shallow

    enamel pan containing cold water 1 cm.

    Acid-detergent fiber:

    1)   Weight 1 g air dry sample ground to pass 1 mm

    2)   Add 100 ml acid detergent solution and 2 ml

    decahydronaphthalene. Heat to boiling in 5 to 10 minutes. Reflux 60

    min.

    3)   Filter on a previously tared crucible and suck dry. Break up the

        filtered and wash twice with hot water (90-100 °C)

    4)   Repeat wash with acetone until remove no more color : break

        up all lumps.

    5)   Optional wash with hexane. Suck dry the acid detergent fiber

        free of hexane. Dry at 100 °C. overnight.

2. 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. Stir contents to break lump and

    draw *permanganate solution* up on side of crucibles to wet all particles.

3. 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 time.

4. Remove crucibles to filtering apparatus. Suck dry. Do not wash. Place in a clean enamel pan, and fill crucibles no more than half full with *Demineralizing solution ,* maybe added directly to crucible . Care must be taken foaming.

   After 5 min, suck dry on filter and refill haft full with *Demineralizing solution.* Repeat after second interval of solution is very brown. Rinse side crucible with solution from a wash bottle with a fine stream. Treat until fiber is white. (20-30 min)

5. Fill and thoroughly wash crucible and contents with ethanol. Suck dry and repeat two time. Wash twice in similar manner with acetone. Suck dry.

6. Dry at 100 °C overnight. And weigh. Calculate lignin content as loss weight from ADF

7. Ash at 500 °C. for 3 hr, cool , and weigh. Calculate residual ash as the difference between the weigh original tare of crucible. Calculate cellulose by weight loss upon ashing.
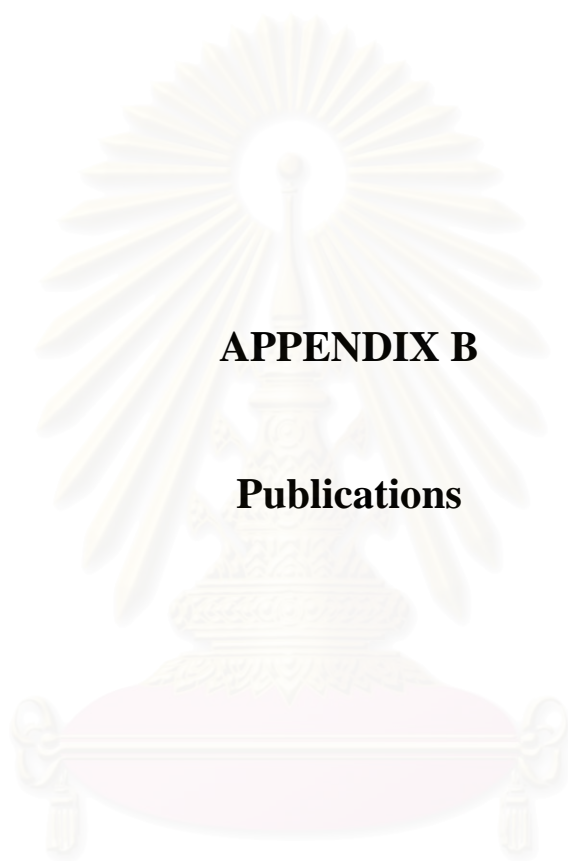
APPENDIX B

Publications

PUBLICATIONS

International Proceedings

P. Petchpradab, Y. Matsumura, and T. Charinpanitkul, "Optimal condition for continuous saccharification process of rubber wood chip" *Proceedings of Japan Institute of Energy*, January 15-16, 2007, kyoto, Japan.

P. Petchpradab, T. Charinpanitkul, N. Suankaew, T.Yoshida and Y.Matsumura, "Saccharification of rubber wood residue by hydrothermal pretreatment" *Proceeding of International System on Envirinmental Management* September 22-23,2008, Nakron nayok Thailand.

P. Petchpradab, T.Yoshida, T. Charinpanitkul and Y.Matsumura, "*Hydrothermal pretreatment of rubber wood for saccharification process*" *Journal of Industrial & Engineering Chemistry Research*. (Submits)

社団法人 日本エネルギー学会
The Japan Institute of Energy
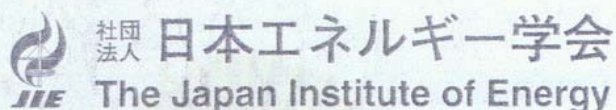
第3回 バイオマス科学会議
発表論文集

開催日：平成20年1月15日（火）〜1月16日（水）
会　場：キャンパスプラザ京都

主催：社団法人　日本エネルギー学会　バイオマス部会

協賛：アルコール協会 エネルギー・資源学会、環境経済・政策学会、科学技術振興機構、化学工学会、紙・パルプ技術協会、下水道協会、触媒学会、新エネルギー・産業技術総合開発機構、新エネルギー財団、森林バイオマス利用学会、森林利用学会、石油学会、日本ガス協会、日本機械学会、日本森林学会、日本太陽エネルギー学会、日本農学会、日本木材加工技術協会、日本木材学会、日本有機資源協会、農業土木学会、農林水産技術情報協会、バイオインダストリー協会、バイオマス利用研究会、廃棄物学会、木質バイオマス利用研究会

P-233 ゴムの木の連続式水熱処理における最適条件

（広島大）○Phacharakamol PETCHPRADAB、松村幸彦、

（チュラロンコン大）Tawatchai CHARINPANITKUL

Optimal conditions for continuous saccharification process of rubber wood chip

○Phacharakamol PETCHPRADAB, Yukihiko MATSUMURA*,

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SYNOPSIS

Rubber wood chip was employed as a biomass raw material for the hydrothermal saccharification process. The glucose production from rubber wood chip was experimentally studied at 130-280°C. Hydrothermal pretreatment and following cellulase hydrolysis was conducted using an autoclave reactor. Reaction parameters for the related reactions were determined.

1. Introduction

Thailand is one of the biggest producers of various products made from rubber wood in the world. Therefore, the rubber wood chips are excessive waste and can be used as a feedstock for ethanol production. Hydrothermal pretreatment of lignocellulosics can be employed for this purpose. The first step of conversion is sugar production from cellulose via this hydrothermal pretreatment and the following cellulase treatment, and the second step is the fermentation of sugar into alcohol. The purpose of the pretreatment is to decrease the crystallinity of cellulose, thus improving the effectiveness of the cellulase treatment, while avoiding carbohydrate degradation (1-3). For the optimal designing of the process, reaction rates on the hydrothermal pretreatment are to be determined. The purpose of this study is to treat rubber wood chips hydrothermally at 130-280°C in an autoclave-type reactor to determine the needed reaction parameters.

2. Experimental methods

2.1 Feedstock

The rubber wood chip was pulverized with a ball mill equipment into rubber wood powder, used in all experiments. The average particle size was 28 μm. On average, the rubber wood powder contained 29% hemicelluloses and cell wall, 28% lignin, 39% cellulose and 4% ash (dry weigh basis). (Agriculture Handbook No. 379)

2.2 Pretreatment

Figure 1 shows the autoclave employed in the experiments (inner volume: 96 mL). Seven grams of rubber wood powder was loaded in the autoclave with 63 g of de-ionized water. The autoclave was heated up by the embedded heaters. When the temperature reached the target value, the heaters were turned off and the reactor was cooled with the air fan. Figure 2 shows the temperature profile of the reactor. Five target temperatures are 130, 170, 200, 240, and 280 °C. Based on this temperature profile, the reaction parameters were determined in the following section.
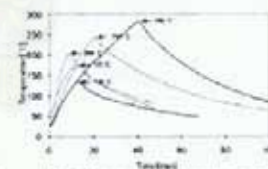

Fig. 1 Autoclave reactor   Fig. 2 Temperature change inside the reactor with time

2.3 Enzymatic hydrolysis

Cellulase from Aspergillus niger powder (≥0.3 units/mg solid) was used in this study. The samples after the hydrothermal pretreatment were used as reactant in this step. The samples were shaken at 250 rpm at 37 °C for 3 days.

Table 1 shows the conditions of cellulase

reaction of rubber wood powder. In each experiment, the liquid product was analyzed by HPLC with SUGAR KS-802(Shodex) column operated at 60 °C with water at 0.8mL/min as an elute.

Table 1. Cellulase reaction conditions.

Reactant [g]	30
Buffer fluid [mL]	60
10 g/L cellulase solution [mL]	5

3. Results and discussions

Figure 3 shows the glucose yield with respect of time of cellulase reaction. The glucose yield obtained becomes better when temperature is increased from 130 to 240°C but at 280°C the glucose seemed to be further decomposed in the autoclave. The related reaction scheme is shown in Fig. 4, where C denotes cellulose, G denotes glucose, C* denotes cellulose hydrolyzed by cellulase after treatment, and D denotes decomposition products of glucose. In this study, the reaction rate constants (k_1, k_2, k_3 and k_4) are determined, assuming the first order reactions following the Arrhenius equation, using the least-square method. Table 2 shows the reaction parameters thus determined.
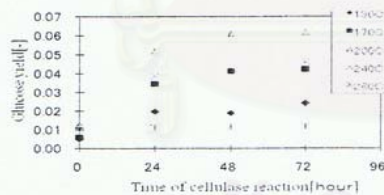
Fig. 3 Amount of glucose generated with time
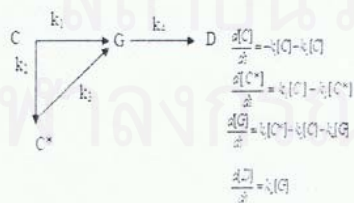
Fig. 4 Reaction scheme

Table 2 Parameters of reaction rate

	A(1/s)	E(kJ/mol)
k_1	144180.8	75.15423
k_2	5×10^{15}	150.7961
k_3	3×10^{16}	198.0688
k_4	2014.016	44.4395

Figure 5 presents the relationship between experimental values and calculated values, and Fig. 6 presents the Arrhenius plot for reaction coefficients. At higher temperatures, higher glucose yields are available. The obtained parameters can be used to determine the optimum condition of continuous treatment
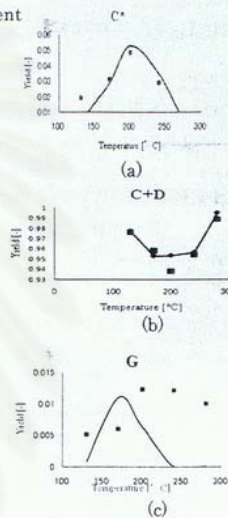
Fig. 5 Comparison with experimental values and calculated values (symbol: experiment, line: calculation value)
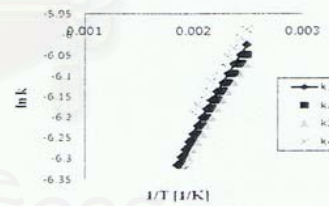
Fig. 6 Arrhenius plot for reaction rate of rubber wood powder in autoclave.

4. Conclusions

The amount of glucose obtained is quite good in the temperature range of 130-240°C, but at 280°C, the glucose seems to be further decomposed. The reaction parameters were determined.

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Saccharification of rubber wood residue by hydrothermal pretreatment

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Abstract

Global energy crisis has stimulated research attempts in the search for renewable energy sources. In developing countries many agro-industrial products could be employed as the new sources of renewable energy. Among them, rubber wood has been recognized as a potential biomass for producing alcohol by the hydrothermal pretreatment apn,ssisted by the enzymatic hydrolysis because it consists of lignocellulosic structure. In this study, production of ethanol from rubber wood residue is examined on the concept of hydrolyzing cellulose and hemicellulose in the rubber wood to C6 and C5 sugars, which would be further fermented to produce ethanol. Pulverized rubber wood was treated at 130–280°C using an autoclave with effective volume of 96 cm^3 for examining effect of particle size distribution of ground rubber wood and reaction time in the hydrothermal pretreatment on the glucose yield. The glucose content within the hydrothermally treated liquid product was analyzed by HPLC. The solid product was analyzed for cellulose content and further treated with cellulase. The yield of glucose was determined to indicate the effectiveness of the hydrothermal pretreatment.

Based on our experimental results, it was found that the glucose yield become higher when the temperature was increased from 130 to 240°C but at 280°C the glucose could further be decomposed, resulting in the lower yield. In addition, the glucose yield became higher with the increased treatment time up to 15 min. As expected the ground rubber wood with the biggest of particle size distribution could provide the increased yield of glucose. A reaction network model for the in hydrothermal pretreatment was proposed for determining the reaction parameters with the expectation to verify the optimal condition for the hydrothermal pretreatment.

Key words: Hydrothermal pretreatment, Saccharification of rubber wood

Hydrothermal pretreatment of rubber wood for saccharification process

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Abstract: Rubber wood was employed as a biomass raw material for the hydrothermal pretreatment process for the enzymatic hydrolysis. Pulverized rubber wood was treated at 130–280°C using autoclave with inner volume of 96 cm^3. The glucose content of the treated liquid product was measured. The solid product was analyzed for cellulose content and further treated with cellulase. The product glucose yield indicated the effectiveness of the hydrothermal pretreatment. A reaction network model for the hydrothermal pretreatment was proposed, and applied successfully. The reaction parameters were determined.

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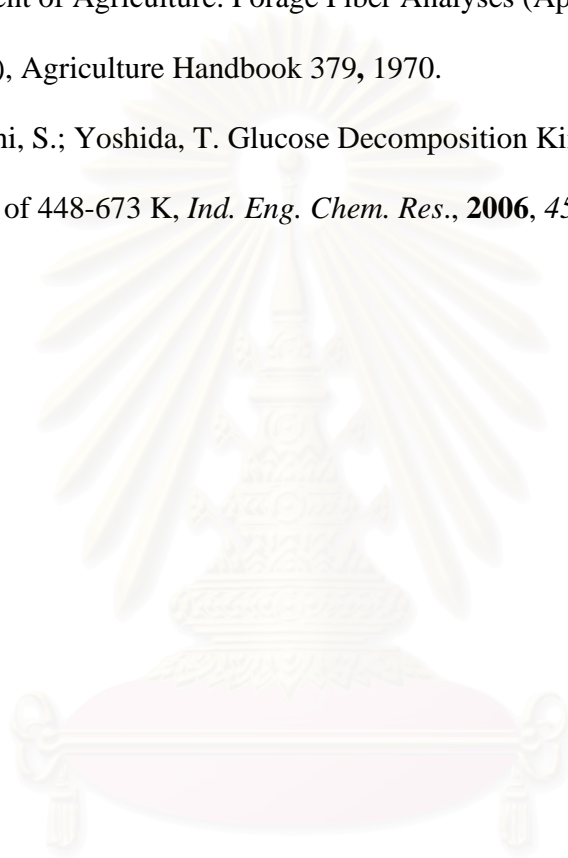
List of Tables

Table 1. Conditions for enzymatic hydrolysis.

Reactant [g]	1
Buffer fluid [cm^3]	60
10 g/dm^3 cellulase solution [cm^3]	5

Table 2. Composition of rubber wood feedstock.

Hemicelluloses and cell wall	0.29
Lignin	0.28
Cellulose	0.39
Ash	0.04

Table 3. Rate parameters for the hydrothermal reactions.

	Preexponential factor [1/s]	Activation energy [kJ/mol]
k_1	1.87×10^5	62.0
k_2	2.02×10^7	87.7
k_3	1.80×10^{18}	222.2
k_4	2.88×10^2	14.4

List of Figures

Fig. 1. Autoclave reactor

Fig. 2. Proposed reaction network.

Fig. 3. Change of cellulose in the hydrothermal pretreatment and enzymatic hydrolysis processes.

Fig. 4. Temperature change inside the reactor with time.

Fig. 5. Amount of glucose generated with time of enzymatic hydrolysis.

Fig. 6. Effect of target temperature of hydrothermal pretreatment on each yield. (symbol: experiment, line: calculation value).
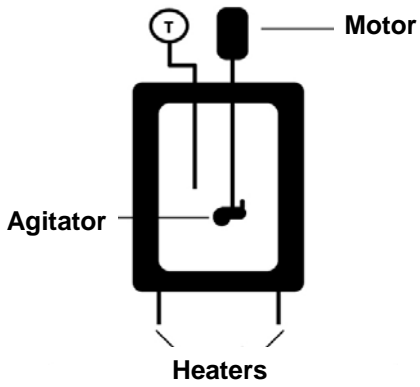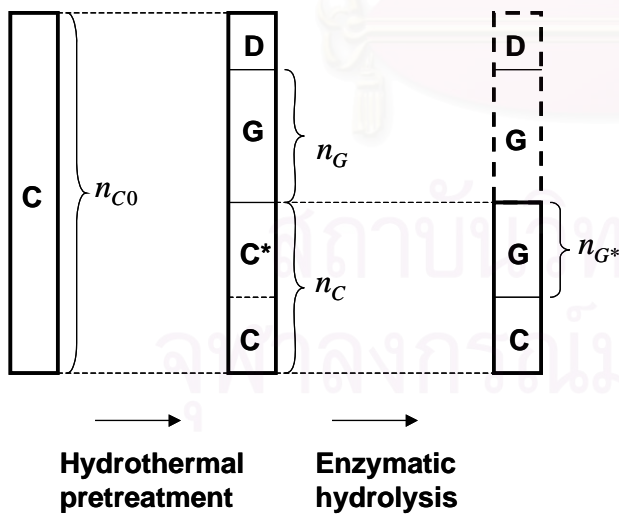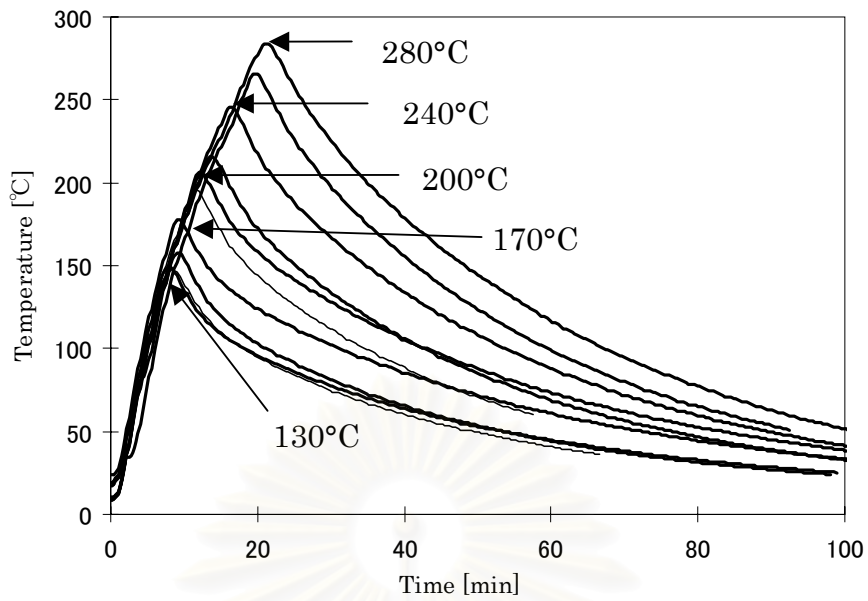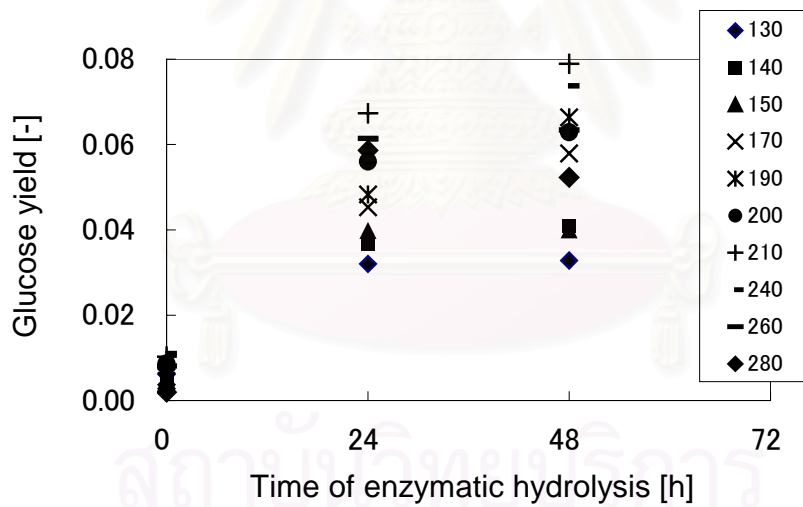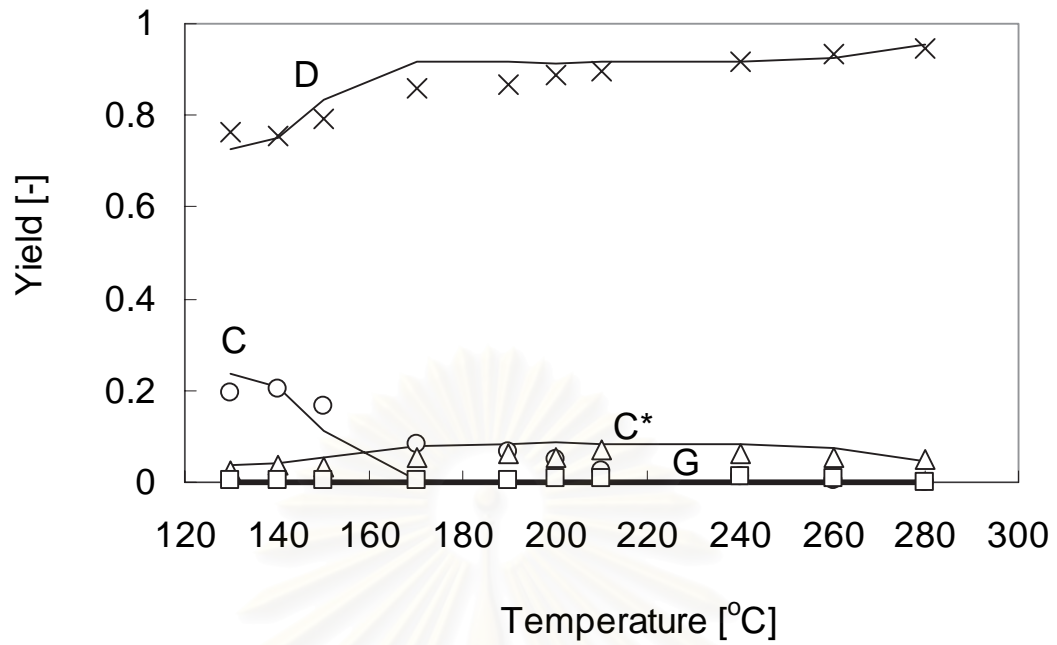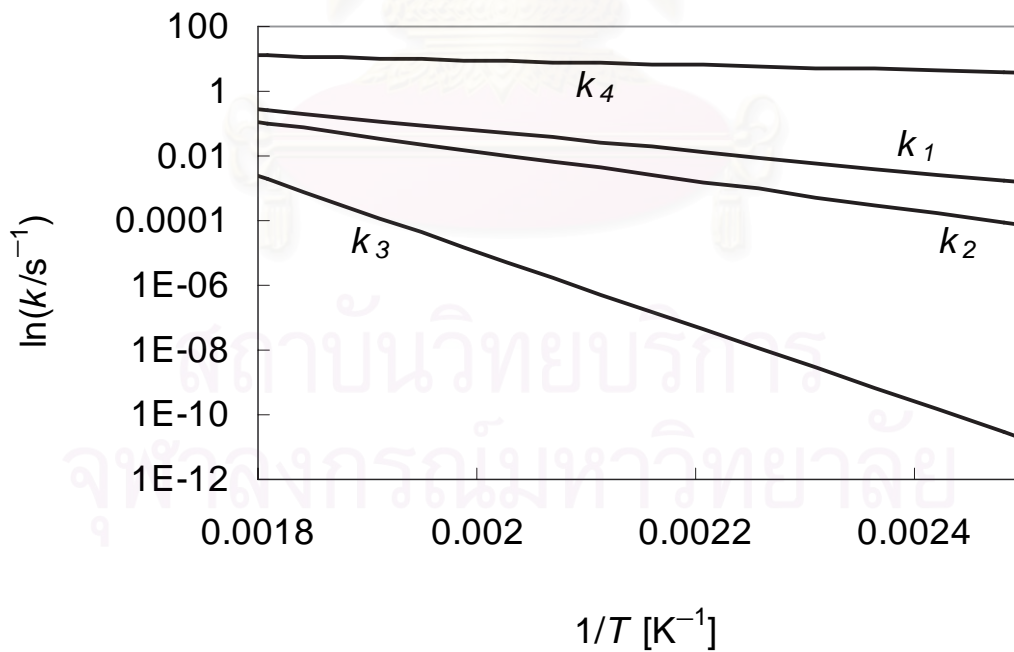

Fig. 7. Arrhenius plot.

1. Introduction

Countries of Southeast Asia including Thailand are the biggest producers of various rubber wood products in the world. Furniture and particle board industries are the main consumers of the rubber wood. In these industries, generally only 10% of the wood ends up as the end product. In other word, 90% results in residues, which is comprised of 54% from small branches, 32% from wastes at sawmill, and 4% from furniture factories. This leads to annual availability of rubber wood residues of 6.9 Tg (6,900,000 t) in the plantation and 4.1 Tg (4,100,000 t) at the sawmill[1]. Therefore, the rubber wood chips are excessive waste 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[2].

Thus, in this study, ethanol production from rubber wood residue is studied. Rubber wood is a typical lignocellulosics, and production of ethanol from lignocellulosics has been studied all over the world. The concept is to hydrolyze the cellulose and hemicellulose to recover C6 and C5 sugars, and to ferment the obtained sugars into ethanol. Various treatments have been proposed and studied, but to achieve a high sugar yield, two-stage hydrolysis is often employed. Lignocellulosics are first treated by various chemicals or under severe condition (pretreatment) and then the product is treated with the enzyme, cellulase[3]. The pretreatment included ammonia explosion, aqueous ammonia recycle, controlled pH, dilute acid, flowthrough, and line approaches. Among the various pretreatment, 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.

Hydrothermal pretreatment for cellulase hydrolysis was studied by Ballesteros et al.[4]. They 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[5, 6]. Aquasolve process is

one of the early studies[7, 8]. In this process, Allen 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. Similar idea was studied by Sakaki et al., and they also reported that inhibition of byproducts on ethanol fermentation was small[9]. However, their glucose yield was limited due to the decomposition of product glucose. Thus, only hydrothermal pretreatment cannot achieve complete hydrolysis.

Cellulase works 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[10]. Considering the results from these previous studies, we decided to concentrate on the two-stage hydrolysis of rubber wood using hydrothermal pretreatment in this study.

After the hydrothermal pretreatment in the first stage, the lignocellulosic feedstock is much more easily hydrolyzed by cellulase in the second stage. However, it is also known that hydrothermal pretreatment under too severe condition results in degradation of carbohydrate, which is to be avoided[11, 12]. Namely, too high temperature or too long treatment time is not desirable from the viewpoint of avoiding the carbohydrate degradation. Then, there should be an optimum condition for this hydrothermal pretreatment.

Unfortunately, in spite of this pile of studies, the optimum condition for the hydrothermal pretreatment has not been determined. This is due to the variety of biomass. In some biomass species, crystallinity of cellulose may not be high, and hydrothermal pretreatment may be of little importance. The optimum condition should also depend on the physical form of the biomass. If the biomass is in the form of fine powder, the reactions taking place in the hydrothermal pretreatment should be faster than when biomass in large pieces is treated. The content of hemicellulose and lignin should also affect, since they attaches and covers cellulose, and prevents the easy access of cellulase to cellulose. Establishment of the guideline to obtain the optimum conditions for the hydrothermal pretreatment is wanted. The purpose of this study is to treat rubber wood hydrothermally at 130–280°C in an autoclave

reactor so that the effect of temperature on the reaction of cellulose in the rubber wood during the hydrothermal pretreatment is determined, that overall reaction model and kinetic parameters are derived, and that the optimum condition for the hydrothermal pretreatment of rubber wood is determined.

2. Experimental

2.1 Hydrothermal pretreatment

Figure 1 shows the autoclave employed in this study. The inner volume of the autoclave was 96 cm^3. Seven grams of rubber wood powder was loaded in the autoclave with 63 g of de-ionized water. After starting the agitator, the autoclave was heated up by the embedded heaters. When the temperature reached the target value, the heaters were turned off and the reactor was cooled with the air fan. The sample after pretreatment was separated to liquid sample and solid sample. The solid sample was used for enzymatic hydrolysis and measured for the content of cellulose after pretreatment. The liquid sample was analyzed by HPLC with SUGAR KS-802 (Shodex) column operated at 60°C with water at 0.8 cm^3/min as an elute.

Ten target temperatures were 130, 140, 150, 170, 190, 200, 210, 240, 260 and 280°C. The pretreatment agitator was set at 500 rpm.

2.2 Enzymatic hydrolysis

Cellulase from Aspergillus niger powder ($\geq$ 0.3 units/mg-solid) was used in this study. The samples after the hydrothermal pretreatment were used as reactant in this step. The pretreated sample, cellulase, and buffer fluid were placed in a conical flask as shown in Table 1, and 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. Buffer fluid was prepared from acetic acid, sodium hydroxide and de-ionized water, whose pH was set at 5.

2.3 Component analysis

The cellulose content of the product of hydrothermal pretreatment was determined following the procedure recommended by the United States Department of Agriculture[13].

2.4. Feedstock

The rubber wood chip was pulverized with the ball mill equipment into rubber wood powder, and used in all experiments. The average particle size was 28 μm on average. Table 2 shows the components of the rubber wood powder (dry weight basis) determined by the USDA's method[13].

3. Apparent reaction network for the hydrothermal pretreatment

Our group is working to get the guideline for the optimum conditions for the hydrothermal pretreatment. However, the phenomena that are taking place in the hydrothermal reactor are very complex. Thus, as the first step, we have proposed the network of the "apparent" reactions taking place in the hydrothermal reactor as shown in Fig. 2. In Fig. 2, C denotes cellulose that cannot be converted into glucose by cellulase treatment. This "cellulase-resistant" cellulose is the starting material, because without hydrothermal pretreatment, almost no glucose is obtained even if the rubber wood feedstock is treated with cellulase. Thus, we can consider cellulose in the rubber wood as practically non-reactive form in terms of cellulase treatment. This may be due to the high crystallinity, due to the lignin and hemicellulose covering the cellulose, or due to the large size of the pieces of biomass being treated.

By the hydrothermal pretreatment, a part of the "cellulase-resistant" cellulose is converted into "cellulase-hydrolyzable" cellulose, because after the hydrothermal pretreatment, the treated rubber wood produces glucose by applying cellulase in the second stage. In Fig. 2, C* denotes cellulose that can be converted into glucose by cellulase treatment, produced by hydrothermal pretreatment. This change can be treated as a kind of reaction. Of course, this change is a complex of physical and chemical phenomena: dissolution of hemicellulose and part of lignin in the hot compressed water, reduction in crystallinity of the cellulose, destruction of cell structure, introduction of water molecules into the piece of biomass, etc. However, these phenomena are too complex to be treated as it is. Reaction engineering tells us that overall treatment is effective when complex phenomena are to be discussed. This is the approach we take here.

In addition to this change, part of the cellulose is directly hydrolyzed into glucose hydrothermally. In Fig. 2, this reaction is shown by the arrows from C and C* to G, where G denotes glucose.

Part of the produced glucose is further decomposed in the hydrothermal reactor. In Fig. 2, this reaction is shown by the arrow from G to D. D denotes decomposition products, which is a mixture of various compounds. It is known that hydrothermal decomposition of glucose results in production of various compounds, but that the reaction rate can be expressed by a single overall reaction rate equation[14].

To help readers to understand what are taking place in hydrothermal pretreatment and enzymatic hydrolysis processes, change of cellulose in the feedstock is shown in Fig. 3. The reaction network shown in Fig. 2 and the reaction kinetics discussed in the following part of this paper are for the hydrothermal pretreatment step in Fig. 3.

For the optimal design of the saccharification process, the kinetics of this network of the "apparent" reactions that are taking place in the hydrothermal pretreatment are to be elucidated. In this study, rubber wood is treated hydrothermally at 130 – 280°C in an autoclave reactor, in order to check the validity of the proposed reaction model shown in Fig. 2, to determine the kinetic parameters, and to predict the optimal condition for the hydrothermal pretreatment.

In the analysis of the experimental results, the yield of each compound shown in Fig. 2 was determined as follows. Glucose yield, X_G, was determined by dividing glucose amount in the liquid phase obtained from HPLC n_G by the theoretical amount of glucose in the cellulose in the feedstock n_{C0}.

$$X_G = \frac{n_G}{n_{C0}} \tag{1}$$

The yield of cellulose that can be hydrolyzed by cellulase, X_{C*}, was determined by dividing the amount of glucose increased by the cellulase treatment, n_{G*}, by the theoretical amount of glucose in the cellulose in the feedstock n_{C0}.

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

The yield of cellulose that cannot be hydrolyzed by cellulase X_C was determined by

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

where n_C denotes the amount of glucose unit it the cellulose left after hydrothermal pretreatment. Then, the yield of decomposed product X_D was determined by

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

4. Results and discussions

Figure 4 shows the temperature profiles in the reactor. Based on this temperature profile, the reaction parameters were determined in the following section.

Figure 5 shows the glucose yield with respect of the cellulase reaction time. The glucose yield after hydrothermal pretreatment is shown at time of cellulase reaction of 0 s. It is clear that glucose yield is less than 0.01 after the hydrothermal pretreatment. The glucose yield increases with time during the cellulase treatment. In this study, we employ the yield after 48-h treatment with cellulase as the final yield, since in the previous study, yield at this time showed the maximum value.

The effect of temperature of hydrothermal pretreatment on the final yields is shown in Fig. 6. It is shown that most of the cellulose is overdecomposed under the conditions employed here from the high value of X_D. The final glucose yield that is expressed by the sum of X_G and X_{C*} becomes higher when the target temperature of the hydrothermal pretreatment is increased from 130 to 240°C but at 280°C the glucose is further decomposed in the autoclave. The highest value for the glucose yield is as low as 0.1, and this low value indicates that produced glucose is easily decomposed in the autoclave.

Assuming the first order reaction for each reaction in Fig. 2, the following set of equations are obtained

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

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

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

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

We also assumed that the Arrhenius rate law is applicable to each reaction rate constant, and integrated the differential equations numerically, following the temperature profiles shown in Fig. 4. By fitting the parameters k_1, k_2, k_3, and k_4 so that integrated result reproduces the experimental yields using the least-square method, the reaction rate parameters are determined as shown in Table 3. Figure 6 compares the experimental and calculated yields of each constituent in this calculation. Good agreement is obtained and the effectiveness of applying the "apparent" reaction network to hydrothermal pretreatment of rubber wood is validated. Figure 7 presents the Arrhenius plot for the reaction rate

constants. Since k_1 is smaller than k_2, cellulose (C) is mainly converted to glucose (G), not to hydrolysable form (C*). However, k_4 is greater than k_1, and the produced glucose is further decomposed.

The obtained parameters can be used to determine the optimum condition of continuous hydrothermal treatment of rubber wood powder. For the autoclave reactor, the optimum target temperature is found to be 235.5°C, by setting the heating rate 8°C/min and cooling rate 4°C/min. The expected glucose yield under this condition is 0.183. This result indicates that the autoclave, which only allows limited, slow heating rate and cooling rates is not a suitable reactor for hydrothermal pretreatment. Reactors that allow different heating and cooling rates are needed. Reaction rate parameters determined here can be employed to predict the optimum condition for this kind of reactor, which, however, is beyond the scope of this study.

5. Conclusions

Rubber wood was hydrothermally treated as a pretreatment of the cellulase hydrolysis for ethanol production. The network shown in Fig. 2 is effective to express the result of this hydrothermal pretreatment. The reaction rate parameters were determined for the hydrothermal pretreatment of rubber wood. The optimum conditions for the hydrothermal pretreatment were predicted, and the highest yield of 0.183 is expected. This result shows the limitation of the hydrothermal pretreatment using the autoclave where only slow heating and cooling rate is available.

VITA

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