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ทุนวิจัย
กองทุนรัชดาภิเษกสมโภช

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กระบวนการแปรสภาพและย่อยสลายวัสดุเซลลูโลสให้เป็นน้ำตาลเพื่อผลิตเอทานอล

โดย

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บทคัดย่อ

ชานข้าวฟ่างหวานเป็นวัสดุประเภทลิกโนเซลลูโลสที่ถูกนำมาใช้เป็นวัตถุดิบทางเลือกในการผลิตเอทานอล เนื่องจากสามารถหาได้ง่ายและมีราคาถูก โดยมีเซลลูโลสและเฮมิเซลลูโลสเป็นองค์ประกอบซึ่งสามารถถูกสลายให้เป็นน้ำตาลเพื่อนำไปใช้ในการหมักได้ ชานข้าวฟ่างหวานที่ใช้ในงานวิจัยนี้พบว่ามีองค์ประกอบทางเคมีคือเซลลูโลสร้อยละ 44.51 เฮมิเซลลูโลสร้อยละ 38.12 และ ลิกนินร้อยละ 6.18 โดยต้องมีการปรับสภาพเพื่อปรับเปลี่ยนขนาดและโครงสร้างของวัตถุดิบก่อน งานวิจัยนี้ได้ทำการหาภาวะที่เหมาะสมในการสลายทางเคมีและการย่อยสลายด้วยเอนไซม์ของชานข้าวฟ่างหวาน โดยทำการผสมวัตถุดิบกับสารละลายกรดซัลฟูริกเจือจาง (0-3%โดยปริมาตร) ในอัตราส่วน 10%โดยน้ำหนักต่อปริมาตร แล้วนำไปปรับสภาพที่อุณหภูมิสูง (120 -190 องศาเซลเซียส) โดยใช้ระยะเวลาปรับสภาพนาน 10-30 นาที จากการทดลองพบว่า การปรับสภาพชานข้าวฟ่างหวานที่อุณหภูมิ 120 องศาเซลเซียส สารละลายกรดซัลฟูริกที่ความเข้มข้น 3%โดยปริมาตร ใช้เวลาปรับสภาพนาน 10 นาที จะให้ปริมาณน้ำตาลกลูโคสและไซโลสสูงสุดเท่ากับ 0.234 กรัมกลูโคสต่อกรัมสับสเตรท และ 0.208 กรัมไซโลสต่อกรัมสับสเตรท ตามลำดับ

จากนั้นนำชานข้าวฟ่างหวานที่ผ่านการปรับสภาพด้วยกรดแล้วมาศึกษาหาภาวะที่เหมาะสมในการย่อยด้วยเอนไซม์เซลลูเลสทางการค้า (Celluclast 1.5, Novozyme) โดยศึกษาปัจจัยต่างๆดังนี้ ความเข้มข้นของเซลลูโลส (15–35 ยูนิต/กรัมสารตั้งต้น) ความเข้มข้นของสารตั้งต้น(1–7 เปอร์เซ็นต์), อุณหภูมิ (30-70 องศาเซลเซียส) และค่าความเป็นกรดด่าง (3-7) พบว่าเซลลูเลสสามารถทำงานได้ดีที่สุดเมื่อใช้ชานข้าวฟ่างที่ผ่านการปรับสภาพด้วยกรดแล้ว 1 เปอร์เซ็นต์เป็นสารตั้งต้น ย่อยด้วยเอนไซม์เซลลูเลสปริมาณ 15 ยูนิต/กรัมสารตั้งต้น ที่อุณหภูมิ 40 องศาเซลเซียส และค่าความเป็นกรดด่างเท่ากับ 5 โดยให้ปริมาณน้ำตาลกลูโคสสูงสุดที่ 0.344 กรัมน้ำตาลต่อกรัมสารตั้งต้น

สารละลายน้ำตาลที่ได้จากการปรับสภาพทางเคมีและทางชีวภาพ จะถูกนำไปใช้เป็นแหล่งคาร์บอนในกระบวนการหมักอทานอลด้วยเชื้อยีสต์ *Saccharomyces cerevisiae* ที่อุณหภูมิ 30 องศาเซลเซียส ค่าความเป็นกรด-ด่าง 5.5 และสภาวะการเขย่า 150 รอบต่อนาที พบว่า เมื่อทำการหมักสารละลายน้ำตาลที่ได้จากการย่อยชานข้าวฟ่างหวานที่ผ่านการย่อยด้วยกรดมาแล้ว จะให้ปริมาณเอทานอลสูงสุด 15.40 กรัมต่อ 100 กรัมสารตั้งต้น ที่เวลา 12 ชั่วโมง

Project Title Pretreatment and hydrolysis conditioning process of cellulosic material For bioethanol production

Name of the Investigators Dr. Siriluk Teeradakorn

Year May 2012

ABSTRACTS

Sweet sorghum straw is lignocellulosic material that is promoted as an alternative feedstock for ethanol production because it is available and inexpensive. Due to its composition of cellulose and hemicellulose, that could be hydrolyzed into fermentable sugars. The composition of sweet sorghum straw used in this study consists of 44.51% cellulose, 38.12% hemicellulose and 6.18% lignin. Conversion of this potential feedstock requires a pretreatment step to alter the microscopic size and structure of the lignocellulose. This research was studied in order to find the optimum conditions on hydrolysis of sweet sorghum straw. The biomass was mixed with dilute sulfuric acid (0-3%v/v) with solid loading of 10% w/v and then pretreatment at high temperatures (120-190°C) for 10-30 min of pretreated times. The maximum yield of glucose and xylose from sweet sorghum straw was 0.234 g glucose/g dry substrate and 0.208 g xylose/g dry substrate, respectively, at the pretreatment condition: 120°C, 3%H_2SO_4 for 10 min.

After chemical pretreatment, the pretreated sweet sorghum straw was hydrolyzed with commercial cellulase. Four variables of saccharification condition were investigated ; a substrate concentration (1–7%), cellulase concentration (Celluclast 1.5, Novozyme) (15–35 FPU/g substrate), a temperature (30-70 °C) and a pH (3-7). The optimum conditions were 1% of substrate concentration, 15 FPU/g-substrate of cellulase, at temperature 40°C and pH of 5. Obviously, glucose was the only monosugar detectable with the yield of 0.344 g glucose/g dry solid under this saccharification condition.

Monosugars liberated from the pretreated sweet sorghum straw and the saccharified pretreated sweet sorghum straw was used as carbon source for ethanol fermentation by *Saccharomyces cerevisiae.* Fermentation condition was at 30°C, pH 5.5 and agitation rate of 150 rpm. The high yield of ethanol concentration, of 15.40 g-ethanol/100 g-total sugars after 12 h of cultivation, was obtained when using monosugar liberated from the saccharified acid pretreated sweet sorghum straw as substrate.

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I INTRODUCTION

Use of renewable biomass which contains a significant amount of carbohydrates such as starch, hemicellulose, and cellulose, to produce transportation fuel is well-recognized. In general, biomass from energy crops, such as sweet sorghum. can be used as a raw material for bioethanol production and seems to be the most promising one. Sweet sorghum is a tropical grass grown primarily in semiarid and drier parts of the world. In addition the straw or bagasse of sweet sorghum is an abundant and low-cost lignocellulosic material that can be synthetically used as raw material for ethanol and some byproduct with high additional value.

Success of using renewable biomass for ethanol production depends upon the physical and chemical properties of the biomass, pretreatment method. efficient microorganisms and optimization of the processing conditions. The purpose of the pretreatment is to break the lignin seal, pre-hydrolyze the hemicellulose, and disrupt the crystalline structure of the cellulose. Pretreatment method: such as steam explosion, acid treatment, alkaline treatment, ammonia fiber explosion and organic solvent treatment have been studied. Among these methods, acid pretreatment, such as H_2SO_4 or HCl has been used with a wide range of feedstock ranging from hardwoods to grasses and agricultural residues.

Enzymatic hydrolysis is the second step in the production of ethanol from cellulosic materials. The cellulose usually contains only glucans whereas hemicellulose contains polymers of several sugars such as xylan, glucan. mannan, galactan, and arabinan. Consequently, the main hydrolysis product of cellulose is glucose, whereas the hemicellulose gives several pentoses and hexoses.

The goal of this research was to study the potential and performance of biomass products (sweet sorghum straw) as feedstock for ethanol production. Pretreatment technology using dilute acid following enzymatic hydrolysis was investigated to increase fermentable sugars recovery from sweet sorghum straw. Results from this research will improve the utilization of sweet sorghum straw feedstock for biofuel production.

II. THEORETICAL AND LITERATURE REVIEW

2.1 Ethanol

Ethanol (ethyl alcohol or EtOH) is a clear, colorless liquid with a characteristic, agreeable odor and its molecular formula is C_2H_5OH . Ethanol has been produced both as a petrochemical, through the hydration of ethylene (shown in equation 2.1), and biologically, by fermenting sugars with yeast (e.g., *Saccharomyces cerevisiae*) (shown in equation 2.2). Ethanol has widespread use as a solvent of substances intended for human contact or consumption, including scents, flavorings, colorings, and medicines. In chemistry, it is both an essential solvent and a feedstock for the synthesis of other products. It has a long history as a fuel for heat and light, and more recently as a fuel for internal combustion engines. Which process is more economical is dependent upon the prevailing prices of petroleum and of grain feed stocks. Ethanol is used as an automotive fuel by itself and can be mixed with gasoline to form gasohol, therefore it can reduce the world's dependence on crude oil resources (Gray et al., 2006).

$$C_2H_{4(g)} + H_2O_{(g)} \xrightarrow{H_3PO_4,\ T = 300^\circ C} CH_3CH_2OH_{(l)} \quad \text{..... (2.1)}$$

$$C_6H_{12}O_6 \xrightarrow{\text{Yeast}} 2\ CH_3CH_2OH + 2\ CO_2 \quad \text{..... (2.2)}$$

Ethanol can be produced from lignocellulosic materials, which is the most promising feedstock (Balat et al., 2008). In Figure 2.1 describes the general process for converting the carbohydrates in lignocellulose into ethanol (Keshwani et al., 2009). Pretreatment is required to improve accessibility of enzymes to cellulose and hemicellulose fractions. Following pretreatment, cellulose and hemicellulose fractions can be hydrolyzed into fermentable sugars while lignin can be recovered and used as a fuel to meet some of the energy requirements in a bioethanol production system (Wyman et al., 1994). After hydrolysis, the fermentable sugars are fermented into ethanol, which is then distilled for fuel purposes. Currently, there are technological and economic limitations to ethanol production from lignocelluloses in each step in the conversion process.

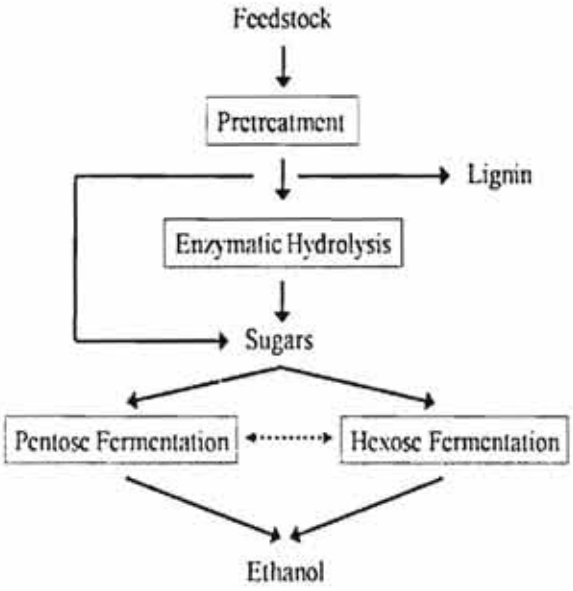

Figure 2.1 The general process to produce ethanol from lignocellulose. (Source : Keshwani et al., 2009)

2.2 Feedstocks for bioethanol production

Bioethanol or ethanol originated from plant oils, sugar beets, cereals, organic waste and lignocellulosic biomass. The biological feedstocks are contained appreciable amounts of sugars or materials that can be converted into sugar (such as starch or cellulose and hemicelluloses) and subsequently fermented to produce bioethanol. Bioethanol feedstocks can be classified into 3 types : (i) sucrose-containing feedstocks (e.g. sugar beet, sweet sorghum and sugar cane), (ii) starchy materials (e.g. wheat, corn, cassava and barley) and (iii) lignocellulosic biomass (e.g. wood, straw, bagasse and grasses). Different feedstocks that can be utilized for bioethanol production and their comparative production potential are given in Table 2.1 (Linoj et al., 2006).

Table 2.1 Different feedstocks for bioethanol production and their comparative production potential

Feedstocks	Bioethanol production potential (l/ton)
Sugar cane	70
Sugar beet	110
Sweet potato	125
Potato	110
Cassava	180
Maize	360
Rice	430
Barley	250
Wheat	340
Sweet sorghum	60
Bagasse and other cellulose biomass	280

(Source : Linoj et al., 2006)

2.3 The composition of lignocellulosic materials

Lignocellulosic materials include wood, grass, forestry waste, agricultural residues(e.g., wheat straw, corn stover, sweet sorghum straw and sugarcane bagasse) and municipal solid waste are composed of three major different types of polymers (Figure 2.2) namely cellulose, hemicellulose and lignin. These components are along with smaller amounts of pectin, protein, extractives (e.g., chlorophyll and waxes) and ash. Cellulose and hemicellulose are carbohydrates constructed from different sugars while lignin is an aromatic polymer synthesized from phenylpropanoid precusors (Sánchez, 2009). The component of these materials can be vary from one species to another species (Table 2.2) (Kumar et al., 2009; and Sánchez, 2009).

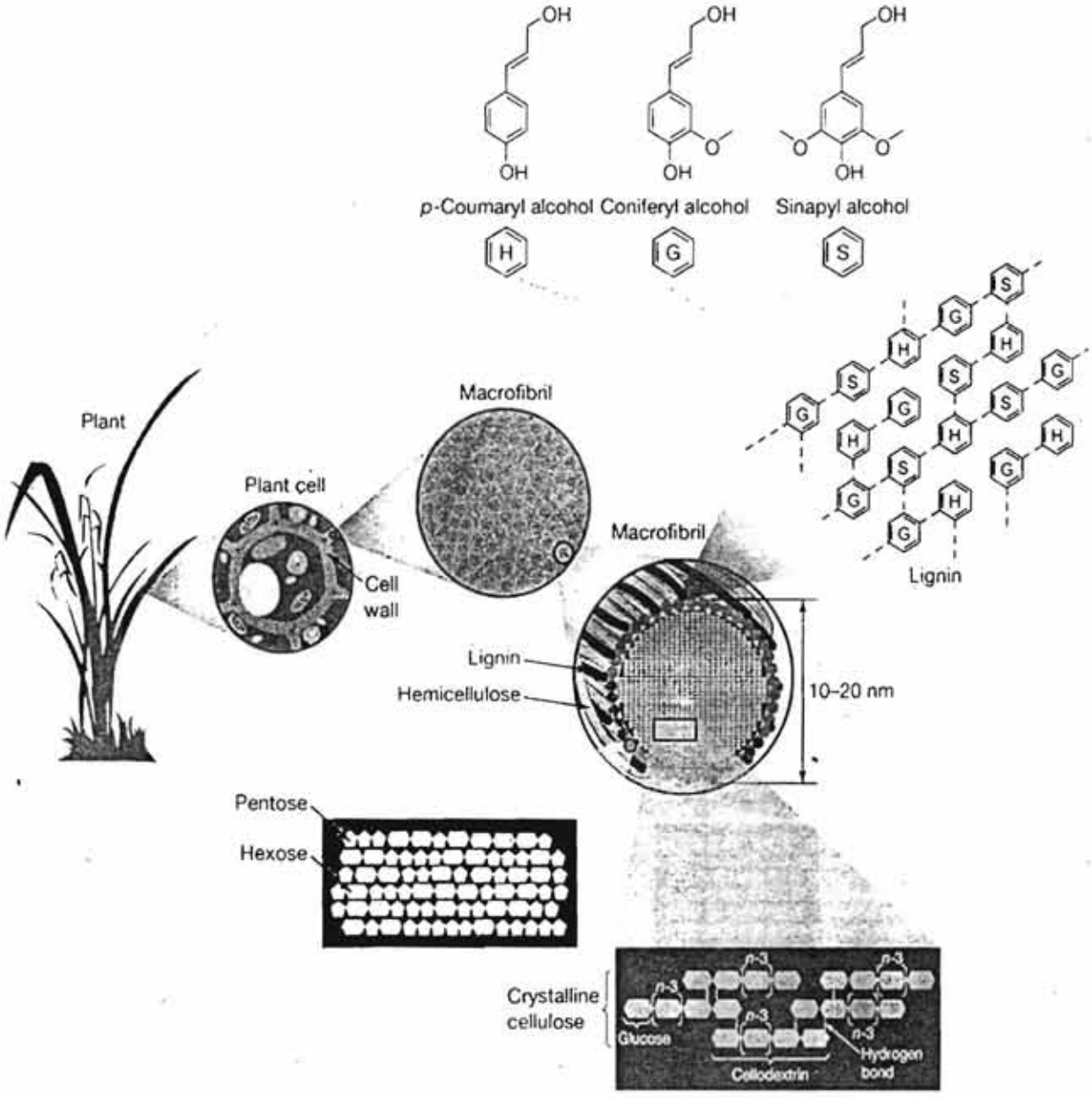

Figure 2.2 Composition of lignocellulosic materials.

(Source : http://www.nature.com/nature/journal/v454/n7206/fig_tab/nature07190_F2.html)

Table 2.2 The composition of lignocellulosic materials

Lignocellulosic material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hardwood stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Wheat straw	29-35	26-32	16-21
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Coastal bermudagrass	25	35.7	6.4
Switchgrass	45	31.4	12
Rice straw	32.1	24	18
Sugar cane bagasse	32–44	27–32	19–24
Barley straw	31–34	24–29	14–15
Oat straw	31–37	27–38	16–19
Rye straw	33–35	27–30	16–19
Bamboo	26–43	15–26	21–31

(Source: Kumar et al., 2009 ; and Sánchez, 2009)

2.3.1 Cellulose

Cellulose is the most abundant component not only of cell walls but also of the plant as a whole. The structure of celiulose was shown in Figure 2.3. It is a linear polymer that compose of D-glucose subunits linked by β-1,4 glycosidic bonds forming the dimmer cellobiose. These form long chains (or elemental fibrils) linked together by hydrogen bonds and van der Waals forces (Sánchez, 2009). This is cause the cellulose to be formed crystalline structures and make them particularly difficult to digest. Starch and cellulose are both long chains of glucose but starch (linked by α-1,4 and α-1,6 bonds) is easily digested by monogastrics, like humans, while the linkages between glucose molecules in cellulose are most commonly broken by enzymes produced by microbial inhabiting the guts of ruminants, such as cattle, sheep and termites Cellulose in biomass is present in both crystalline and amorphous forms (Kumar et al., 2009).

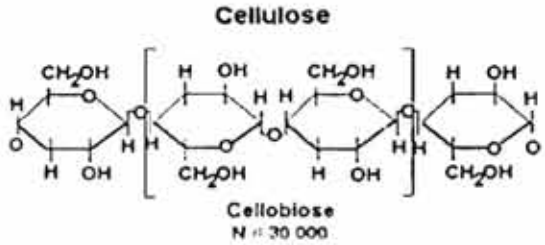

Figure 2.3 Illustration of a cellulose unit
(Source : http://forestproducts.orst.edu/research.php)

2.3.2 Hemicellulose

Hemicellulose is a polysaccharide with a lower molecular weight than cellulose (Sánchez, 2009). The structure of hemicelluloses was shown in Figure 2.4. It is highly branched because of the bonds that form among the sugars that make them up, and they form a network that coats the much larger cellulose microfibrils (structure and function of plants). It consists of different sugars such as pentoses (xylose and arabinose), hexoses (glucose, galactose and mannose) and sugar acids (D-glucuronic and D-galacturonic acids). The backbone of hemicellulose is linked together by β-1,4 glycosidic bonds and sometimes linked by β-1,3 glycosidic bonds (Sánchez, 2009). Hemicellulose is randomly acetylated, with reduces its enzymatic reactivity. The polymer present in hemicelluloses is easily hydrolysable (Kumar et al., 2009).

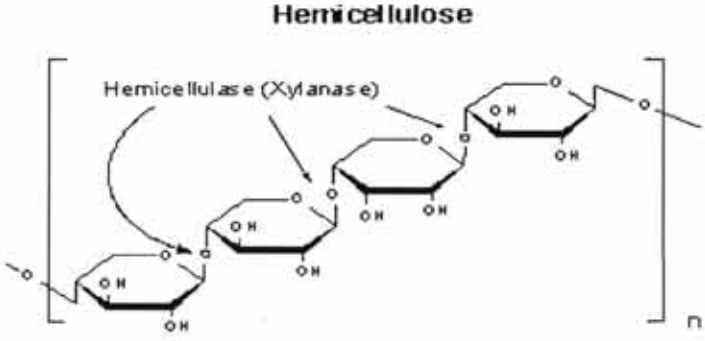

Figure 2.4 The structure of hemicellulose
(Source : http://blogs.princeton.edu/chm333/f2006...try.html)

2.3.3 Lignin

Lignin is a complex, large molecular structure containing cross-linked polymers of phenolic monomers (Kumar et al., 2009). The structure of lignin was shown in Figure 2.5A. It is linked to both hemicellulose and cellulose, forming a physical seal that is an impenetrable barrier in the plant cell wall (Sánchez, 2009). The main function of lignin is to give the plant structural support, impermeability and resistance against microbial attack and oxidative stress (Hendriks and Zeeman, 2009). Lignin is an amorphous heteropolymer, non-water soluble and optically inactive. This polymer is synthesized by the generation of free radicals, which are released in the peroxidase-mediated dehydrogenation of three phenyl propionic alcohols: coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (p-hydroxyphenyl propanol), and sinapyl alcohol (syringyl propanol) (Figure 2.5B) (Sánchez, 2009). These phenolic monomers are linked by alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds (Kumar et al., 2009).

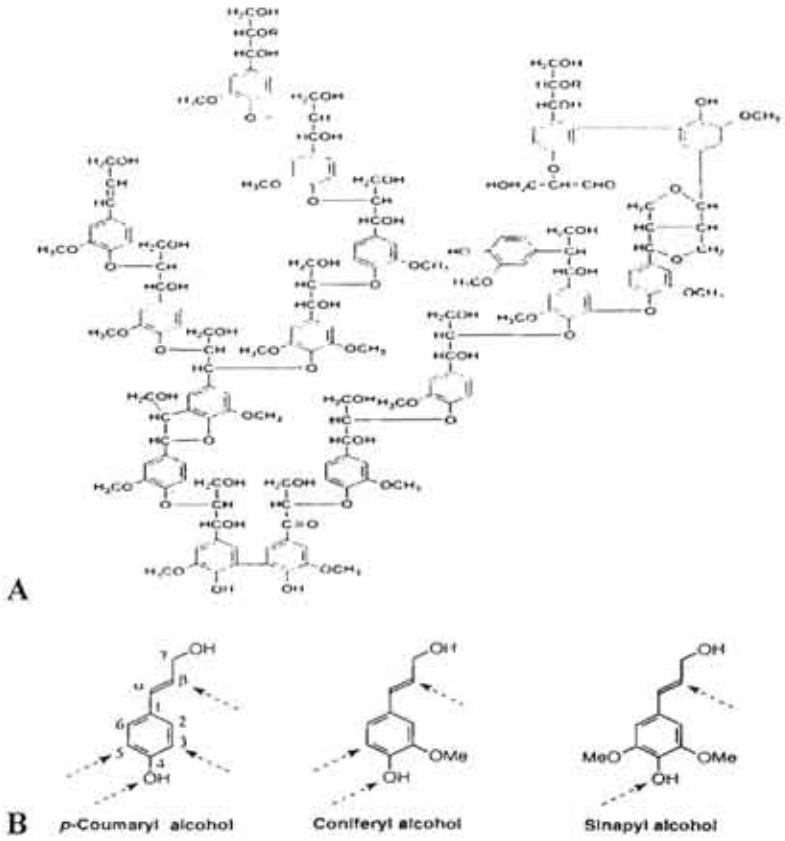

Figure 2.5 The structure of lignin; (A) Phenolic polymer of lignin, (B) The structure of three phenyl propionic alcohols (Source: www.ibwf.de/env%26enz_index.htm)

2.4 Factors limiting the hydrolysis

The hydrolysis of lignocellulose to monomeric sugars is limited by several factors (Hendriks and Zeeman, 2009). The choice of pretreatment technology for a particular raw material depends on several factors, some of them directly related to the enzymatic hydrolysis step such as sugar-release patterns and enzymes employed. Thus, the combination of the composition of the substrate, type of pretreatment, and dosage and efficiency of the enzymes used for the hydrolysis have a great influence on biomass digestibility (Alvira et al., 2010). These factors are described separately although their effect is normally interrelate.

2.4.1 Lignin content

Lignin is the main components in lignocellulose. It limits the rate of enzymatic hydrolysis because of its close association with cellulose microfibrils and prevents enzyme access to the carbohydrate fraction of materials. To enhance digestibility, materials must undergo pretreatment to remove or alter the lignin (Chang and Holtzapple, 2000).

2.4.2 Hemicellulose content

Hemicellulose and lignin are linked by covalent bonds. Acid hydrolysis of hemicellulose can open materials structure as well. Removal of hemicellulose is required to increases .pore size of materials and therefore increases cellulose digestibility (Mosier et al., 2005).

2.4.3 Acetyl content

Degree of acetylation in the hemicellulose is another important factor because lignin and acetyl groups are attached to the hemicellulose matrix and may hinder polysaccharide breakdown (Chang and Holtzapple, 2000).

2.4.4 Cellulose crystallinity

Cellulose crystallinity has been considered as important factors in determining the hydrolysis rates. Several studies have shown that crystallinity prevents the rapid access of enzymes. The lignocellulose was mechanically pretreated,

thus any decrease in crystallinity was accompanied by an alteration of other substrate characteristics such as particle size reduction or increase in available surface area (Alvira et al., 2010).

2.4.5 Degree of polymerization

Degree of polymerization is essentially related to other substrate characteristics. such as cristallinity. Depolymerization depends on the nature of cellulosic substrate. In the enzymatic hydrolysis, endoglucanases cut at internal sites of the cellulose chains, preferentially less ordered, being primarily responsible for decreasing degree of polymerization of cellulosic substrates (Alvira et al.. 2010).

2.4.6 Surface area and porosity (pore size)

Surface area and porosity of the materials are an important factors influencing hydrolysis process. Therefore, the main objectives of the pretreatment is to increase the available surface area and porosity for improve the hydrolysis (Alvira et al., 2010).

2.5 Pretreatment of lignocellulosic materials

2.5.1 Goals of pretreatment

Lignocellulosic materials do not contain monosaccharides readily available for bioconversion. Instead of polysaccharides, they contain cellulose and hemicelluloses, which have to be hydrolyzed, by means of acids or enzymes, to fermentable sugars. Cellulose in lignocellulosic materials are closely associated with hemicelluloses and lignin. The lignin is partly covalently associated with hemicelluloses, thus preventing the access of hydrolytic agents to cellulose. In addition, the crystalline structure of cellulose itself represents an extra obstacle to hydrolysis (Cardona et al., 2010). An effective pretreatment must preserve the utility of the hemicelluloses and avoid the formation of inhibitors (Laser et al., 2002). An economical for pretreatment should use inexpensive chemicals and require simple process and equipment.

The goal of pretreatment process is to alter the physical features and chemical composition of the lignocellulose to improve it more digestible (Mosier et al., 2005; and Sun and Cheng, 2002). Specifically, pretreatment improves enzyme access and

effectiveness (Figure 2.6) by : 1) Removing or altering lignin, 2) Hydrolyzing hemicelluloses, 3) Decrystallizing cellulose, 4) Removing acetyl groups from hemicelluloses, 5) Reducing the degree of polymerization in cellulose and 6) Expanding the structure to increase pore volume and internal surface area

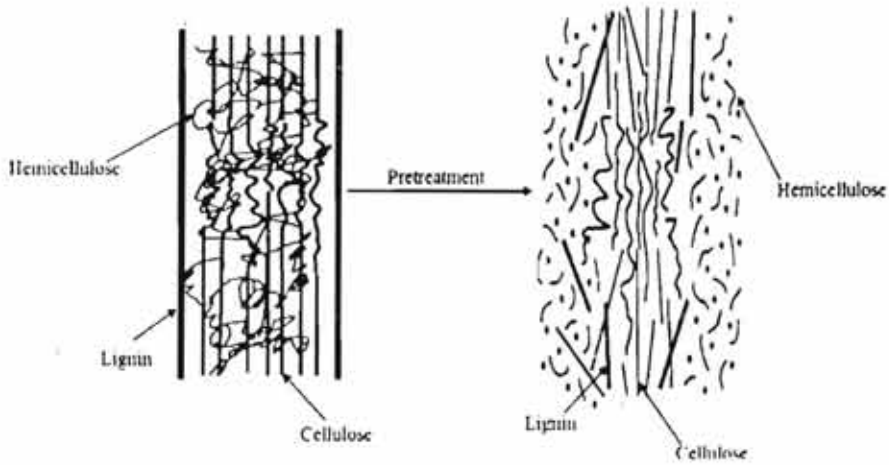

Figure 2.6 Schematic of goals of pretreatment on lignocellulosic material (Source: Mosier et al., 2005)

2.5.2 Pretreatment categories

Pretreatment methods can be classified into 4 method as shown in Figure 2.7: (Sun and Cheng, 2002; and Talebnia et al., 2010)

I. Physical pretreatment :
   - Mechanical comminution (chipping, grinding and milling)
   - Pyrolysis

II. Physico-chemical pretreatment :
   - Steam explosion
   - Ammonia fiber explosion (AFEX)
   - CO_2 explosion
   - Liquid hot water

III. Chemical pretreatment :
   - Acid pretreatment (Acid hydrolysis)
   - Alkaline pretreatment (Alkaline hydrolysis)
   - Ozonolysis

- Oxidative delignification

IV. Biological pretreatment :

- Enzyme from microorganisms (fungi)

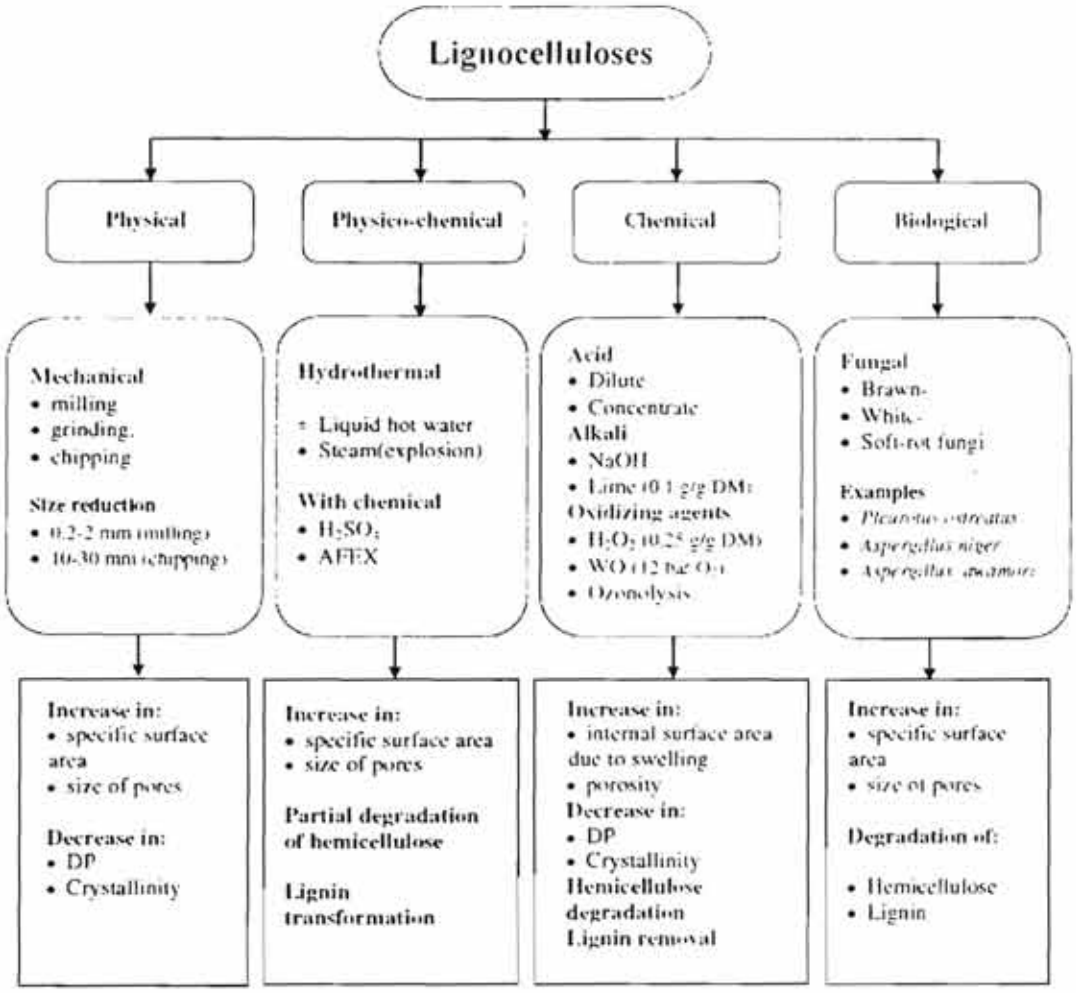

Figure 2.7 The most common pretreatment methods used on lignocelluloses and their possible effects (DP, degree of polymerization; WO, wet oxidation) (Source: Talebnia et al., 2010)

Among all these methods, the applied methods usually use combination of different principles, such as mechanical pretreatment together with chemical pretreatment effects in order to achieve high sugar release efficiencies, low toxicants production, and low energy consumption (Talebnia et al., 2010). Lignocelluloses have

been studied for bioethanol production as summarized in Table 2.3. The advantages and disadvantages of various pretreatment methods were also summarized in Table 2.4 (Alvira et al., 2010).

Table 2.3 The advantages and disadvantages of various pretreatment methods for lignocellulosic biomass

Pretreatment method	Advantages	Disadvantages
Milling	– Reduces cellulose crystallinity	– High power and energy consumption
Steam explosion	– Causes lignin transformation and hemicellulose solubilization – Cost-effective – Higher yield of glucose and hemicellulose in the two-step method	– Generation of toxic compounds – Partial hemicellulose degradatio
AFEX	– Increases accessible surface area – Low formation of inhibitors	– Not efficient for raw materials with high lignin content – High cost of large amount of ammonia
CO_2 explosion	– Increases accessible surface area – Cost-effective – Do not imply generation of toxic compounds	– Does not affect lignin and hemicelluloses – Very high pressure requirements
Wet oxidation	– Efficient removal of lignin – Low formation of inhibitors – Minimizes the energy demand (exothermic)	– High cost of oxygen and alkaline catalyst
Ozonolysis	– Reduces lignin content – Does not imply generation of toxic compounds	– High cost of large amount of ozone needed
Organosolv	– Causes lignin and hemicellulose hydrolysis	– High cost – Solvents need to be drained and recycled
Concentrated acid	– High glucose yield – Ambient temperatures	– High cost of acid and need to be recovered – Reactor corrosion problems – Formation of inhibitors
Diluted acid	– Less corrosion problems than concentrated acid – Less formation of inhibitors	– Generation of degradation products – Low sugar concentration in exit stream
Biological	– Degrades lignin and hemicellulose – Low energy consumption	– Low rate of hydrolysis

(Source : Alvira et al., 2010)

2.5.2.1 Physical pretreatment

The objective of physical pretreatment is a reduction of particle size, crystallinity and degrees of polymerization of cellulose, and increase surface area of materials. In general, mechanical comminution is the initial steps for pretreatment of any lignocellulose which reduces the particle size, through a combination of chipping, grinding and milling (Binod et al., 2010).

2.5.2.2 Physico-chemical pretreatment

Physico–chemical pretreatment methods (such as steam explosion, ammonia fiber explosion, CO_2 explosion and liquid hot water) are considerably more effective than physical. The steam explosion is the most studied method of this type. During this process, the use of saturated steam at high pressure causes autohydrolysis reactions in which part of the hemicellulose and lignin are converted into soluble oligomers.

2.5.2.3 Chemical pretreatment

Chemical pretreatment for lignocellulose employ different chemicals agents such as acids, alkaline, ozone, peroxide and organic solvents. Among these methods. dilute acid pretreatment using sulfuric acid is the widely used method. The effect of structural and components of materials are depended on the type of chemical used for pretreatment. Alkaline pretreatment, ozonolysis, peroxide and wet oxidation pretreatment are more efficient in lignin removal while dilute acid pretreatment is more efficient in hemicellulose solubilization. (Sánchez and Cardona,2008).

2.5.2.4 Biological pretreatment

In biological pretreatment processes, microorganisms such as brown-, white-, and soft-rot fungi that belong to class Basidiomycetes are used to degrade lignin and hemicellulose in waste materials. Brown rots mainly attack cellulose, whereas white and soft rots attack both cellulose and lignin. Lignin degradation by white-rot fungi occurs through the action of lignin degrading enzymes such as peroxidases and laccase. White-rot fungi seem to be the most effective microorganism for biological

pretreatment of lignocellulosic materials (Kumar et al., 2009). The important microbial enzymes for lignocellulose hydrolysis were shown in Table 2.3 (Alper and Stephanopoulos. 2007).

Table 2.4 Important enzymes for hydrolysis lignocelluloses

Enzyme type	Function	Typical sources
Cellobiohydrolase	Solubilizes crystalline cellulose	Fungal systems (especially *Trichoderm* and *Aspergillus* spp.)
Endoglucanase	Hydrolyses the β-(1,4) glycosidic bonds in cellulose	Fungal systems (especially *Trichoderm* and *Aspergillus* spp.)
β -glucosidase	Hydrolyses β-linked disaccharides into monosaccharides	Fungal systems (especially *Trichoderm* and *Aspergillus* spp.)
Xylanase	Hydrolyses β-1,4-xylan into xylose	Fungal systems (especially *Trichoderm* and *Aspergillus* spp.)
Lignin peroxidase	Oxidizes lignin molecules through an H_2O_2 donor	White rot and brown rot fungi
Laccase	Oxidizes phenol groups	White rot fungi

(Source : Alper and Stephanopoulos, 2007)

2.6 Factors affecting on enzyme saccharification

There are several factors that affect enzymatic saccharification of cellulose including substrates, cellulase activity, and reaction conditions (temperature, pH, etc.). To improve yield and rate of the enzymatic saccharification, many researches have focused on the optimization of the hydrolysis process and enhancement of cellulase activity (Ferreiraa, S. et al., 2009).

Fungal cellulase, in general, are stable at 30° C from pH 3 to 8, active from 3.5 to 7. and usually show optimum activity at pH 4.0 to 5.5 in citrate, phosphate, or acetate buffers The activity of cellulase is sometimes doubled on heating to 50 °C for 5 min, presumably due to destruction of a heat labile inhibitor. Crude cellulase from fungi can usually be precipitated by 66 % acetone or 80 %ethanol without loss of activity. They keep indefinitely at 50 °C as solutions or as dry powders.

In case, cellobiohydrolases exhibit considerable synergistic action. Similar to most endoglucanases, catalytic optimum of cellobiohydrolases are situated in a narrow pH range between 4.0 and 5.0. Temperature optimum is between 37 and 60 °C depending on the enzyme and the substrate. The pH optimum for β-glucosidase is usually between 3.5 and 5.5, but the intracellular enzyme from *Phanerochaete chrysosporium* has a neutral pH optimum, the temperature optimum are between 45 and 75 °C(Baldrian. P. et al., 2008).

Enzymes from different sources have different optimal conditions for the activity of cellulolytic enzyme. Summarizes the various factor on saccharification gives an indicative value for a maximum fermentative sugar concentration are shown in Table 2.5

Table 2.5 Summary the optimum conditions for the cellulase activity to hydrolysis lignocellulosic matherial

Source of cellulase	Enzyme concentration (FPU/g DS)	Substrate Concentration (g/g DS)	Temperature (°C)	pH	Reducing sugars (g/g-DS)	References
Trametes hirsute(WR)	30 FPU/g-substrate	2.25% of rice straw	25-35	5	0.685	(Jeya, M. et al., 2009)
Agaricus arvensis	65 FPU/g-substrate	10% of poplar	37	5	0.293	(Jeya, M. et al., 2010)
NS50013 (cellulase complex)	60 FPU/g substrate	0.40 % pretreated rock-rose	50	4.86	0.313	(Ferreiraa, S. et al., 2009)
	60 FPU/g substrate	0.27% of pretreated broom	50	4.5	0.448	(Ferreiraa, S. et al., 2009)
commercial cellulase from *Trichoderma reesei*	40.00 FPU/g substrate	0.22%wheat straw	50	4.8	hydrolysis yield of 51.22%	(Qi, B. et al., 2009)
Commercial cellulase from *Aspergillus niger*	0.16(v/v)	50% food waste	46.3	5.2	0.117	(Kim, J. K. et al., 2008)
Commercial cellulase from Novozymes	15 FPU/g substrate	5% olive tree biomass	50	4.8	0.363	(Cara C. et al., 2008)

Intensive research demonstrates that the efficiency of the enzymatic saccharification of pretreated substrate depends on several process parameters such as enzyme loading, substrate concentration, pH, temperature, etc. These factors often interact with one another, therefore, optimization of the enzymatic saccharification process plays an important role in improving the performance of the process (Kunamneni, A. and Singh, S., 2005).

The traditional optimization method used in the enzymatic saccharification process, a one-factorat-a-time technique which involves changing one independent variable (enzyme concentration, substrate concentration, pH, temperature, etc.) while maintaining other variables at a fixed level, not only is time consuming, laborious and expensive but also often leads to an incomplete understanding of the system behavior,resulting in confusion and a lack of predictive ability(Qi, B. et al., 2009: Jeya, M. et al., 2009).

An alternative and more efficient approach is the use of a statistical method, response surface methodology (RSM), which is an empirical modeling technique derived for evaluation of the relationship of a set of controlled experimental factors and observed results. RSM is a powerful mathematic approach for analyzing the effect of multiple variables or factors, alone or in combination, on a given process rapidly and efficiently with a minimal number of experiments while keeping a high degree of statistical significance in the results. RSM can be used to optimize the enzymatic saccharification of lignocellulosic materials. (Qi, B. et al., 2009) Many research are made to employ to identify the optimum conditions for impove reducing sugars production from lignocellulose by analyzing the relationships among a number of parameters that affect the overall process(Jeya, M. et al., 2009: Jeya, M. et al., 2010: Qi, B. et al., 2009: Kunamneni, A. and Singh, S., 2005: Ferreiraa, S. et al., 2009: Kim, J. K. et al., 2008).

2.7 Ethanol fermentation process

Lignocellulose is often hydrolyzed by dilute-acid treatment; the hydrolyzate obtained is used for bioethanol fermentation by microorganism such as yeast. Lignocellulose hydrolyzate contains not only glucose, but also various monosaccharides, such as xylose, mannose, galactose, arabinose, and

oligosaccharides. Required microorganisms should be efficiently utilized these sugars for the successful production of bioethanol (Balat et al., 2008).

The classic configuration employed for fermenting biomass hydrolyzates involves a sequential process where the hydrolysis of cellulose and the fermentation are carried out in different units. This configuration is known as separate hydrolysis and fermentation (SHF). In the alternative variant, the simultaneous saccharification and fermentation (SSF), the hydrolysis and fermentation are performed in a single unit. The most employed microorganism for fermenting lignocellulosic hydrolyzates is *S. cerevisiae*, which ferments the hexoses contained in the hydrolyzate but not the pentoses (Sánchez et al., 2008).

2.8 Microorganism in bioethanol fermentation process

The hydrolyzates from acid hydrolysis of lignocellulose contain both hexoses and pentoses (if both cellulose and hemicellulose are hydrolyzed).These sugars are released during pretreatment and hydrolysis. Depending on the lignocellulose source, the hydrolyzate typically consists of glucose, xylose, arabinose, galactose, mannose, fructose and rhamnose (Saha, 2003). Glucose and xylose are two dominant sugars in the lignocellulosic hydrolyzates. The best known microorganisms for ethanol production from hexoses are the yeast *Sacchamyces cerevisiae* and the bacterium *Zymomonas mobilis* (Claassen et al., 1999). One of the main problems in bioethanol production from lignocellulosics hydrolyzate is the native strains of *S. cerevisiae* and *Z. mobilis* inability to utilize xylose, the main C5 sugar obtained from hemicelluloses hydrolysis (Rogers et al., 2007).Other approach to this problem is the use of pentose fermenting microorganisms like some species of yeasts and enteric bacteria. In this case, configurations involving the separate fermentation of pentoses and hexoses have been proposed. Yeasts as *Pichia stipitis*, *Candida shehatae* and *Pachysolen tannophilus* can assimilate pentoses but their ethanol production rate from glucose is at least five times less than that observed for *S. cerevisiae*. Moreover, their culture requires oxygen and ethanol tolerance is 2-4 times lower (Claassen et al., 1999; Chandel et al., 2007 ; and Lin and Tanaka, 2006). Among the xylose fermenting yeasts, *P. stipitis* has shown the most promise for industrial application, because it

ferments xylose with a high ethanol yield. Furthermore, *P. stipitis* has no absolute vitamin requirements for xylose fermentation and is able to ferment a wide range of sugars, including cellobiose (Agbogbo et al., 2006).

Pentoses and hexoses are commonly found in lignocellulosic material include xylose (Xyl), arabinose (Ara), glucose (Glc), mannose (Man) and galactose (Gal). These sugars are converted to the phosphorylated forms xylose-5-phosphate (X5P), glucose-6-phosphate (Glu-6P) and fructose-6-phosphate (Fru-6P). These molecules are eventually converted into glyceraldehyde-3-phosphate (Gly-3P) followed by subsequent conversion to ethanol from pyruvate (Pyr) after glycolysis (Alper and Stephanopoulos, G., 2009). A number of possible biofuels can then be produced as shown in Figure 2.15.

In case, xylose utilization can be incorporated into the pentose phosphate pathway through either the three enzyme pathway containing a xylitol intermediate or a second step process that uses a yeast or bacterial and fungi. The second-step process bypasses the need for the reducing power that is incorporated in NAD- and NADP-reducing partners and has been shown to improve ethanol production. Xylulose-5-phosphate is formed by both pathways and can enter into central carbon metabolism(Alper, H. and Stephanopoulos, G., 2009) through the transketolase (Tkl1) catalyzing the formation of sedoheptulose-7-phosphate then transaldolase (Tal1) which converts the products of the transketolase reactions to erythrose-4-phosphate and fructose-6-phosphate and glyceraldehyde-3-phosphate (Gly-3P) from xylulose-5-phosphate and ribose-5-phosphate, followed by subsequent conversion to ethanol from pyruvate (Pyr) after glycolysis as shown in Figure 2.15 resulted in a considerably enhanced growth on xylose(Goshadroua, A., 2011).

The main metabolic pathway involved in the ethanol fermentation is glycolysis (Embden–Meyerhof–Parnas or EMP pathway) under anaerobic conditions through which one molecule of glucose is metabolized, and two molecules of pyruvate are produced then the pyruvate is further reduced to ethanol with the release of CO_2. Theoretically, the yield is 0.511 for ethanol and 0.489 for CO_2 on a mass basis of glucose metabolized. Two ATPs produced in the glycolysis are used to drive the biosynthesis of yeast cells which involves a variety of energy-requiring bioreactions. Therefore, ethanol production is tightly coupled with yeast cell growth,

which means yeast must be produced as a co-product. Without the continuous consumption of ATPs by the growth of yeast cells, the glycolytic metabolism of glucose will be interrupted immediately, because of the intracellular accumulation of ATP, which inhibits phosphofructokinase (PFK), one of the most important regulation enzymes in the glycolysis. This very basic principle contradicts the ethanol fermentation with the yeast cells immobilized by supporting materials, particularly by gel entrapments, which physically restrict the yeast cells and significantly retard their growth are show in Figure 2.16(Bai, F. W. et al., 2008)

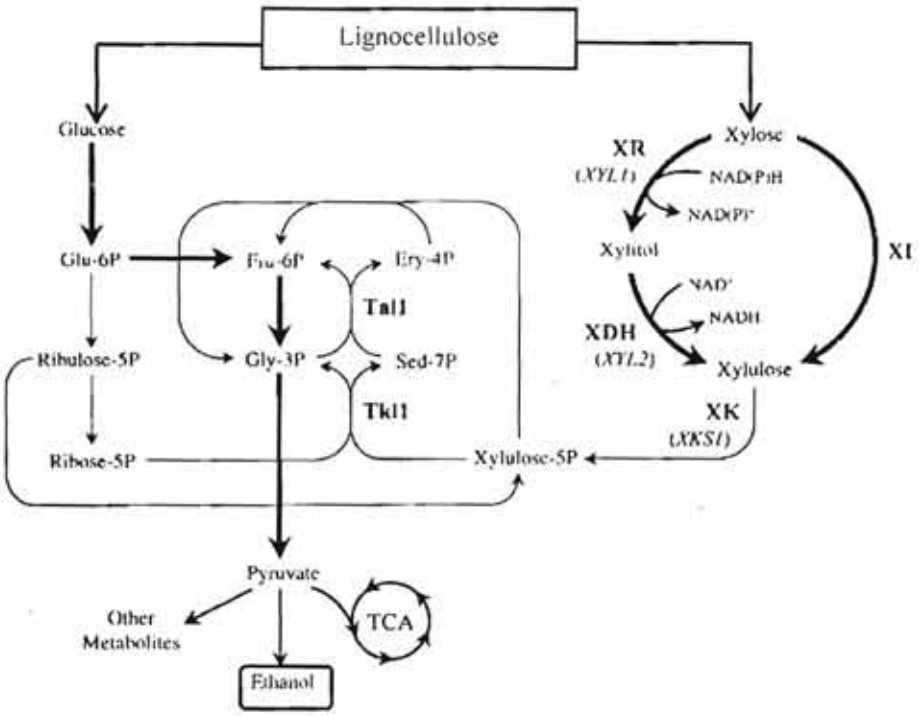

Figure 2.8 Overview of metabolic pathways for glucose and xylose metabolism. The Embden-Meyerhof-Parnas pathway and the xylose utilization pathway are illustrated by thick lines. The PP pathway is indicated by thin lines. Abbreviations: TCA, tricarboxylic acid cycle; Tkl1, transketolase; Tal1, transaldolase; Glu-6P, glucose-6-phosphate; Fru-6P, fructose-6-phosphate; Gly-3P, glyceraldehyde-3-phosphate; Ery-4P, erythrose-4-phosphate; Sed-7P; sedoheptulose-7-phosphate.

(Source: Goshadroua, A., 2011)

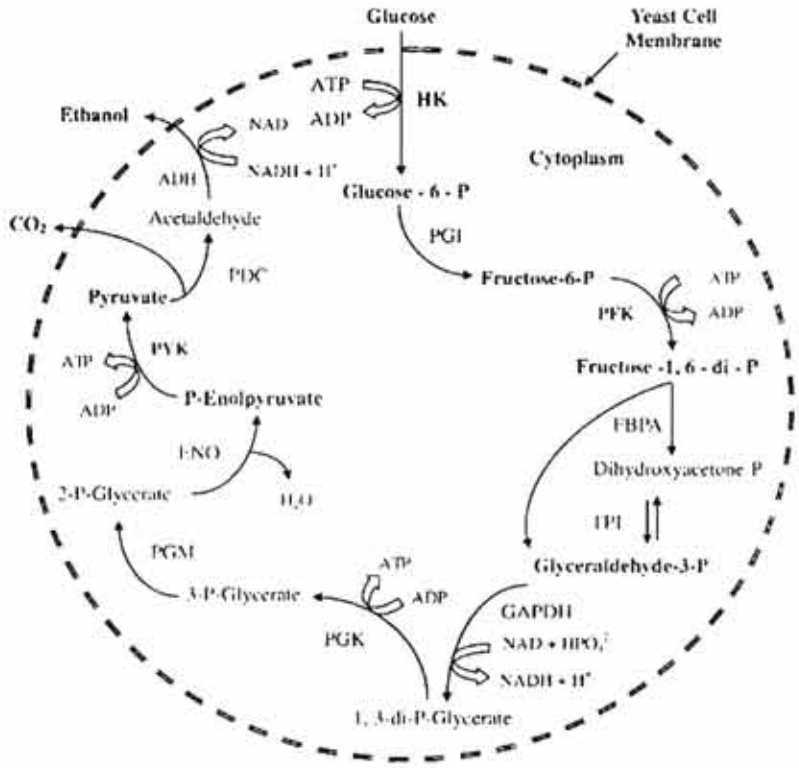

Figure 2.9 Metabolic pathway of ethanol fermentation in *S. cerevisiae*. Abbreviations: HK: hexokinase, PGI: phosphoglucoisomerase, PFK: phosphofructokinase, FBPA: fructose bisphosphate aldolase, TPI: triose phosphate isomerase, GAPDH: glyceraldehydes-3-phosphate dehydrogenase, PGK: phosphoglycerate kinase, PGM: phosphoglyceromutase, ENO: enolase, PYK: pyruvate kinase, PDC: pyruvate decarboxylase, ADH: alcohol dehydrogenase
(Source: Bai, F. W. et al.,

III. PROCEDURES

3.1 Materials

Sweet sorghum straw used in these experiments was obtained from the Suphanburi Field Crop Research Center, Suphanburi province, Thailand. The fresh straw was chopped into a small-size about 10-15 cm and dried in a hot-air oven at 80°C for 24 h. Then, the substrate was milled in a hammer mill to pass through an 8 mm screen. The milled sweet sorghum straw was stored in sealed plastic bags at 4°C for pretreatment study.

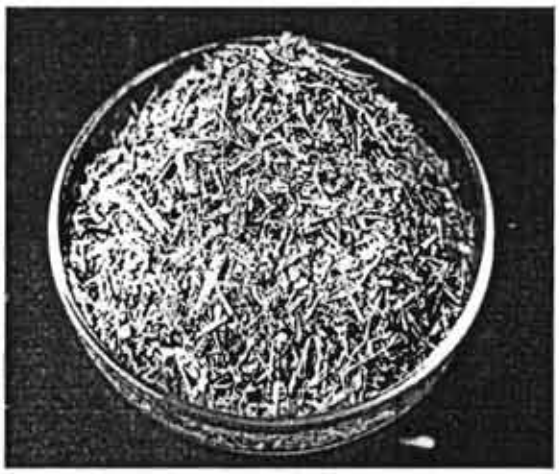

Figure 3.1 Lignocellulosic material used as substrate in this experiment (milled sweet sorghum straw)

3.2 Saccharification reactor

The in-house saccharification reactor (Figure 3.2) consisted of 3 main parts: 1. saccharification unit (reactor); 2.heat generator unit; and 3.temperature controller unit. The pressure of the reactor could be set by a temperature controller. The saccharification reactor was made from iron and equipped with heat generator unit for heating the reactor. Temperature was measured with temperature probe inside the reactor. On the top of the reactor equipped with pressure gage and globe valve for monitoring and controlling the pressure, respectively.

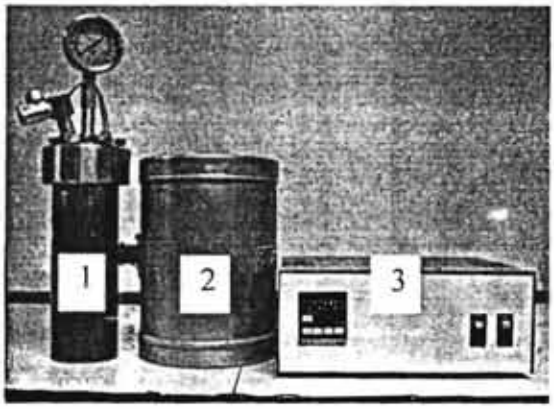

Figure 3.2 In-house saccharification reactor

3.3 Diluted-acid pretreatment of sweet sorghum straw

Milled sweet sorghum straw was mixed with dilute sulfuric acid solution (final concentrations: 0%, 1% and 3% v/v) with solid loading of 10% w/v (30 g of substrate/300 ml of reaction mixture). The mixture was then hydrolysis into the in-house saccharification reactor. The pretreatment was carried out in two types of the diluted-acid pretreatment process: high temperature (T at 150°C, 170°C and 190°C) and low temperature (T at 120°C). Different residence time (10, 20 and 30 min) was used during pretreatment. After pretreatment, the pretreated material was separated into solid and liquid (hydrolyzate) fractions. The hydrolyzates were analyzed for total reducing sugars and monomeric sugar (glucose, xylose, galactose, arabinose, and mannose). The solid fraction was thoroughly washed with distilled water until the filtrate pH about 6-7 and stored at -10°C prior to analysis the composition of pretreated straw. The acid pretreated sweet sorghum straw was dried in the oven at 70°C and used as the substrate for saccharification experiment.

3.4 Hydrolyzate detoxification

Detoxification of acid hydrolyzate was operated by overliming and evaporation. The hydrolyzate of sweet sorghum straw that gave the maximum glucose was selected. This hydrolyzate was overliming by adding 40%w/v $Ca(OH)_2$ solution until the pH of hydrolyzate was about 5-6. During neutralization, salt and toxic

compounds were precipitated and removed by centrifuge at 8,000 rpm for 20 min. The liquid fraction was concentrated by vacuum evaporation until solid content in hydrolyzate increased to about 20°Brix (estimated by using refractometer) and then analyze for total sugars concentration by HPLC. This fraction was used for ethanol fermentation.

3.5 Enzymatic hydrolysis

A typical hydrolysis mixtures consisted of 0.1 g of pretreated sweet sorghum straw, 20 FPU of commercial cellulase enzyme (Celluclast 1.5, Novozyme) /g substrate and 2.0 ml of sodium phosphate buffer (pH 6.0). Microbial contamination was prevented by addition of sodium azide (0.01 mg/ml). The mixture was incubated at 50°C in a rotary shaker at 150 rpm for 7 days. Samples were taken from the reaction mixture at different time intervals. Samples were cooled and then centrifuged at 10,000 rpm for 10 min. The supernatant was determined for total reducing sugars and monomeric sugar.

3.6 Optimum condition for saccharification the acid pretreated sweet sorghum straw

On the basis of initial saccharification results, the commercial cellulase enzyme concentration of 15–35 FPU/g-substrate, a substrate concentration of 1–7%, a pH 3–7, and a temperature of 30–70°C were tested as conditions for optimizing the saccharification process using statistical analysis. The experimental design for saccharification condition by a complete four factor and five level of factorial were shown as code level in Table 3.1.

Table 3.1 The variables and their levels for the central composite experimental design for optimization condition for saccharification of the acid pretreated sweet sorghum straw

Variable	Symbol	Code levels				
		-2	-1	0	1	2
Substrate (%w/v)	A	1%	2.50%	4%	5.50%	7%
Enzyme (FPU/g DS)	B	15	20	25	30	35
Temperature(°C)	C	30	40	50	60	70
pH	D	3	4	5	6	7

Thirty experiments were performed according to Table 3.2 to optimize the parameters. Among them, six replications were at center points (n = 6) and eight axial points, were shown code level in Table 3.2

Table 3.2 Experimental design of four-factor, five-level CCRD

Run	Factor	Factor	Factor	Factor
	A	B	C	D
1	1	-1	-1	1
2	0	0	0	0
3	-1	1	1	-1
4	1	1	1	1
5	0	0	0	0
6	1	1	-1	-1
7	-1	-1	-1	1
8	0	0	0	0
9	1	1	1	-1
10	-1	-1	1	-1
11	-1	-1	-1	-1
12	1	-1	1	1
13	-1	-1	1	1
14	1	-1	-1	-1
15	1	-1	1	-1
16	-1	1	1	1
17	-1	1	-1	1
18	1	1	-1	1
19	-1	1	-1	-1
20	0	0	0	0
21	0	0	0	0
22	0	-2	0	0
23	0	0	2	0
24	0	0	0	2
25	0	0	0	-2
26	-2	0	0	0
27	0	0	-2	0
28	0	2	0	0
29	2	0	0	0
30	0	0	0	0

The collected data were statistically analyzed using SPSS Computer Analysis Programs. Data regarding the saccharification condition formed by various enzyme concentration, substrate concentration, temperature and pH were statistically analyzed using factorial test with a 95% confidence level. Differences in means were judged significant when p values for the null hypothesis were 0.05 or less ($p \leq 0.05$) followed by Tukey's method to demarcate mean differences

3.7 Ethanol fermentation

3.7.1 Yeast strains

Saccharomyces cerevisiae was obtained from the Institute of Biotechnology and Genetic Engineering (IBGE), Chulalongkorn University, Thailand. Yeast strain was maintained on agar slants containing: 10 g/l yeast extract, 10 g/l peptone, 20 g/l agar and 20 g/l glucose as a carbon source.

3.7.2 Inoculum preparation

The inoculum was grown in 50 ml of culture medium that contained: 10 g/l yeast extract, 10 g/l peptone, and 20 g/l glucose as a carbon source. Then, it was incubated in a rotary shaker at 30°C, agitation rate of 150 rpm for 18-22 hr. At the end of incubation, these cells were used for fermentation process.

3.7.3 Ethanol fermentation

Ethanol fermentation was performed under aerobic condition in 250 ml Erlenmeyer flasks with a total reaction volume of 50 ml. The fermentation medium contained: 2 g/l KH_2PO_4, 1 g/l $MgSO_4 \cdot 7H_2O$, 6.4 g/l urea, and 10 g/l yeast extract. Hydrolyzate from the acid pretreatment of sweet sorghum straw (Stage 1), the liquid from saccharification of the acid pretreated sweet sorghum straw (Stage 2), and the combined liquid from both stages (Stage 1+Stage2) were used as carbon source (total sugars equivalent to 20 g/l glucose). Subsequently, adding 10%v/v inoculum to start fermentation process and incubated at the agitation rate of 150 rpm at 30°C. Samples were withdrawn at time intervals and concentrations of ethanol were determined by gas chromatography.

3.8 Analytical methods

3.8.1 Reducing sugars

The reducing sugar concentration of hydrolyzate was determined by the 3,5-dinitrosalicylic acid (DNSA) method applied from Miller (1959), with D-glucose as the standard. In a typical reaction, 100 µl of sample and the reagent are mixed and heated in a boiling water bath for 10 min, then cooled immediately on ice bath and added 1 ml of distilled water. At the end of the reaction, the absorbance was measured by spectrophotometer at 540 nm.

3.8.2 Monomeric sugars

All the samples of hydrolyzate were analyzed for monomeric sugar (glucose, xylose, galactose, arabinose, and mannose) by high-performance liquid chromatography (HPLC). Before injection into a column, all samples were neutralized with 40% NaOH, centrifuged at 10,000 rpm for 15 min and then filtered through a cellulose membrane acetate filter (pore size 0.45 µm). The condition for analysis process was shown below.

Column	Bio-Rad Aminex HPX-87P (300mm x 7.8 mm)
Guard column	Carbo-P micro-guard cartridge
Eluent	H_2O (Milli Q water)
Temperature	85°C
Flow rate	0.6 ml/min
Injection volume	20 µl
Detector	RI detector (Shimadzu Model RID-6A)
Retention times	30 min

Peaks area of samples were indentified and quantified by comparison with retention times (RT) of analytical standards (glucose, xylose, galactose, arabinose and mannose). (Shown in appendix B)

3.8.3 Chemical composition of sweet sorghum straw

The composition of the untreated substrates (sweet sorghum straw) and the solid fraction remaining after acid pretreatment were determined by the Nakhonratchasima Animal Nutrition Research and Development Center. The percentages of cellulose, hemicellulose and lignin in substrate were determined by the procedures of Goering and Van Soest (Goering and Van Soest, 1971).

3.8.4 Calculation methods

Yield of ethanol from fermentation broth of sweet sorghum straw was calculated using the following equation (3.1):

$$\% \text{ Yield ethanol} = \frac{\text{Ethanol concentration (g/l)}}{\text{Initial total sugar concentration}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

3.8.5 Ethanol concentration

Ethanol produced during the fermentation process was analyzed by Gas chromatography (GC). Ethanol was determined using a Hitachi 163 gas chromatography equipped with Porapak Q column and a flame ionization detector (FID) system. The injector and column temperatures were set at 220°C and 190°C, respectively. Nitrogen and helium were used as carrier gas. The flow rate of the carrier gas was 1.0 ml/min. The sample, mixed with 3 mg/ml propanol (ratio of 1:1) about 1 µl was injected manually into the gas chromatography column. The ethanol in fermentation broth was identified and calculated by compare with the peak area ratio of ethanol and propanol relative to various concentrations of ethanol standard. (Shown in appendix B)

3.9 Statistical analysis

All experiments were done in triplicate and the related data were expressed as averages values. The experimental data from acid pretreatment were analyzed using the SPSS for Windows program followed by a factorial test with a 95% confidence level. Differences in means were judged significant when p values for the null hypothesis were 0.05 or less ($p \leq 0.05$). The effects of pretreatment temperature, acid

concentration and residence time were analyzed. The experimental data from enzymatic hydrolysis were statistically analyzed using SPSS program followed by two-way ANOVA. Differences in means were judged significant when p values for the null hypothesis were 0.05 or less.

IV. RESULTS AND DISCUSSION

Generally, glucan and xylan were the major component of sweet sorghum straw followed by acid-insoluble lignin. Arabinan, galactan and mannan accounted for only a small amount of the biomass composition. The composition of sweet sorghum straw used in this study consists of 44.51% cellulose, 38.12% hemicellulose and 6.18% lignin. The composition of this substrate was found to be within the range as other reports (Neureiter et al., 2002).

Chemicals pretreatment process is the old technology for converting lignocellulosic biomass to ethanol. Acid catalyzes the breakdown of long chains hemicellulose to form shorter chain oligomers and then to sugar monomers. After pretreatment at different temperature, sulfuric acid concentrations and residence time the liquid fractions (hydrolyzate) and solid fractions were collected. The separation of monosaccharides in the hydrolyzates of diluted-acid pretreatment of sweet sorghum straw is shown in Appendix B.

4.1 Distilled-water-only hydrolysis of sweet sorghum straw

Sugar yields from pretreatment of sweet sorghum straw using distilled water are shown in Table 4.1. The hydrolyzates contained monomeric sugars glucose, xylose, galactose, arabinose and mannose, Glucose and xylose were the major components. The maximum yield of glucose and xylose were 0.054 and 0.037 g monosugar/g dry substrate, respectively, at a pretreatment temperature of 120°C for 20 min.

4.2 Acid pretreatment of sweet sorghum straw

Results for 1%H_2SO_4 pretreated are shown in Table 4.2. The maximum yield of glucose was 0.221 g glucose/g dry substrate at 170°C for 20 min and the maximum yield of xylose was 0.161 g xylose/g dry substrate at 150°C for 20 min. Glucose and xylose yields increased when the pretreatment temperature increase from 120°C to 170°C in the range 10-20 min. In severe conditions, such as high temperature (T at 190 °C and long residence time (> 20 min), yields of glucose and xylose dramatically decreased.

Results for 3%H_2SO_4 pretreated are shown in Table 4.3. The maximum yield of glucose was 0.234 g glucose/g dry substrate at 120°C for 10 min and the maximum yield of xylose was 0.208 g xylose/g dry substrate at for the same conditions. The experimental data indicate that glucose yields decreased at pretreated temperature above 120°C with increasing residence time. The xylose yield in the hydrolyzate gave similar results with increasing of pretreatment severity.

Table 4.1 Summary of performance for distilled-water-only hydrolysis

Temp (°C)	%H_2SO_4	Time (min)	Yield $_{avg}$ (g monosugar /g dry substrate)			
			Glu	Xyl	Gal, Man, Ara	Total sugar
120	0	10	0.027±0.009	0.012±0.002	0.005±0.006	0.044±0.012
		20	0.054±0.010	0.037±0.031	0.025±0.037	0.115±0.078
		30	0.038±0.010	0.007±0.003	0.006±0.007	0.050±0.008
150	0	10	0.030±0.013	0.009±0.005	0.003±0.002	0.041±0.014
		20	0.043±0.002	0.010±0.008	0.002±0.003	0.054±0.008
		30	0.041±0.008	0.002±0.001	0.005±0.003	0.048±0.008
170	0	10	0.042±0.005	0.005±0.002	0.009±0.002	0.056±0.008
		20	0.041±0.005	0.005±0.004	0.007±0.002	0.053±0.007
		30	0.038±0.005	0.004±0.002	0.013±0.005	0.055±0.010
190	0	10	0.032±0.008	0.012±0.006	0.007±0.004	0.051±0.011
		20	0.029±0.018	0.020±0.011	0.009±0.002	0.057±0.031
		30	0.030±0.017	0.024±0.007	0.009±0.003	0.063±0.025

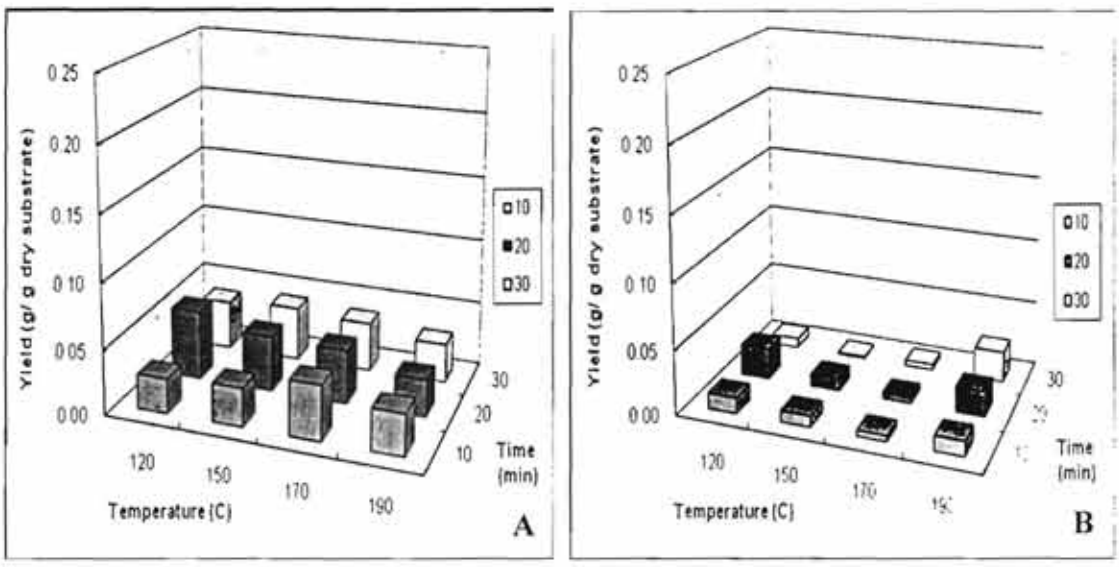

Figure 4.1 Yield of monosugar liberated from sweet sorghum straw when pretreated by distilled-water for 10-30 min (A) Yield of glucose ; (B) Yield of xylose

Table 4.2 Summary of average yields of monosugars using 1% H_2SO_4

Temp (°C)	%H_2SO_4	Time (min)	Yield $_{avg}$ (g monosugar /g dry substrate)			
			Glu	Xyl	Gal, Man, Ara	Total sugar
120	1	10	0.164±0.070	0.146±0.094	0.014±0.004	0.324±0.168
		20	0.175±0.031	0.159±0.037	0.059±0.043	0.393±0.055
		30	0.162±0.053	0.130±0.041	0.086±0.085	0.377±0.103
150	1	10	0.195±0.086	0.106±0.076	0.086±0.039	0.387±0.132
		20	0.215±0.039	0.161±0.029	0.079±0.051	0.454±0.078
		30	0.168±0.038	0.119±0.078	0.060±0.070	0.347±0.148
170	1	10	0.213±0.154	0.054±0.044	0.030±0.031	0.297±0.184
		20	0.221±0.071	0.089±0.037	0.063±0.093	0.373±0.127
		30	0.189±0.069	0.050±0.055	0.070±0.073	0.309±0.032
190	1	10	0.191±0.086	0.050±0.020	0.081±0.063	0.322±0.076
		20	0.155±0.078	0.070±0.019	0.081±0.103	0.307±0.070
		30	0.108±0.098	0.057±0.092	0.074±0.111	0.238±0.025

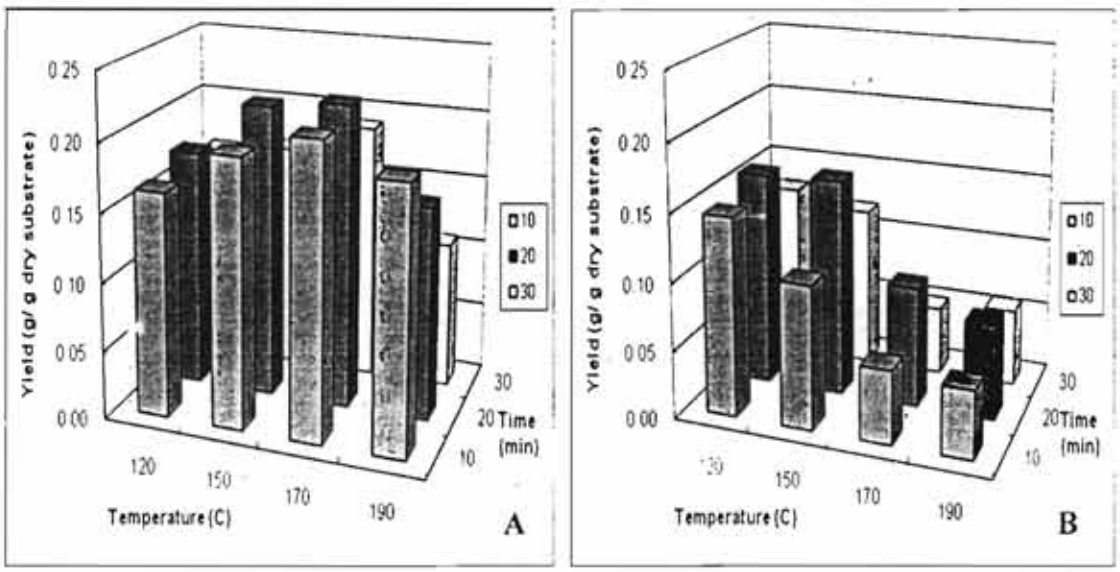

Figure 4.2 Yield of monosugars liberated from sweet sorghum straw when pretreated with 1% sulfuric acid for 10-30 min (A) Yield of glucose ; (B) Yield of xylose

Table 4.3 Summary of average yields of monosugars using 3% H_2SO_4

Temp (°C)	%H_2SO_4	Time (min)	Yield $_{avg}$ (g monosugar /g dry substrate)			
			Glu	Xyl	Gal, Man, Ara	Total sugar
120	3	10	0.234±0.079	0.208±0.073	0.235±0.164	0.676±0.230
		20	0.202±0.047	0.184±0.017	0.288±0.093	0.674±0.068
		30	0.162±0.079	0.161±0.078	0.279±0.280	0.602±0.288
150	3	10	0.227±0.107	0.138±0.047	0.240±0.039	0.605±0.184
		20	0.174±0.072	0.090±0.028	0.201±0.269	0.464±0.356
		30	0.201±0.088	0.091±0.055	0.100±0.173	0.391±0.302
170	3	10	0.134±0.091	0.033±0.021	0.161±0.278	0.327±0.356
		20	0.136±0.088	0.017±0.009	0.235±0.329	0.389±0.405
		30	0.231±0.012	0.049±0.050	0.269±0.379	0.549±0.349
190	3	10	0.045±0.015	0.016±0.015	0.229±0.396	0.290±0.384
		20	0.092±0.067	0.017±0.019	0.315±0.350	0.423±0.400
		30	0.005±0.001	0.002±0.000	0.549±0.483	0.556±0.484

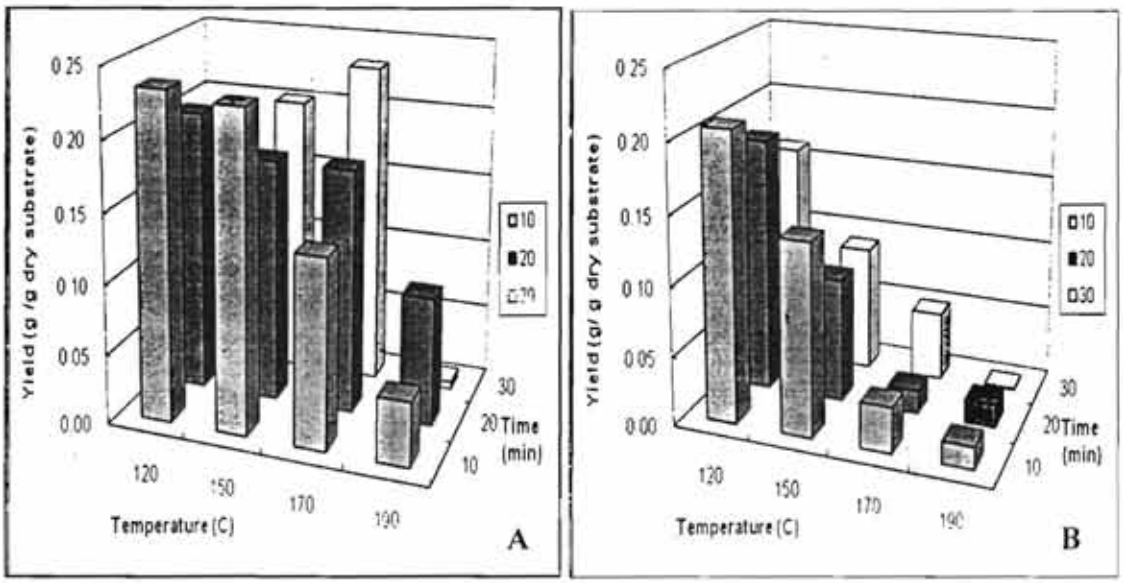

Figure 4.3 Yield of monosugars liberated from sweet sorghum straw when pretreated with 3% sulfuric acid for 10-30 min (A) Yield of glucose ; (B) Yield of xylose

From this study, the maximum yield of glucose was 0.234 g glucose/g dry substrate at pretreatment condition 3% sulfuric acid, 120°C for 10 min and the maximum yield of xylose was 0.208 g xylose/g dry substrate at the same conditions.

In this case, a total of 50.05% of glucan and 76.41% of xylan were converted to glucose and xylose, respectively (data not shown).

All experimental data of sweet sorghum straw were analyzed by factorial design ($p \leq 0.05$).The statistical analysis shown that pretreatment temperature and dilute sulfuric acid concentration had a significant effect on yield of glucose and xylose. By contrast, the residence time for pretreatment did not have a significant effect on the yield of glucose and xylose released from sweet sorghum straw (p = 0.559 and 0.387 respectively). The data were shown in Table C1 and Table C2 (Appendix C).

Based on the experimental results, the pretreatment at severe conditions (high temperature and high sulfuric acid concentration) were not suitable for the hydrolysis because of at these conditions glucose and xylose can be degraded into furfural and hydroxymethylfurfural (HMF), respectively. When furfural and HMF are degraded, formic acid is formed. Levulinic acid is formed by HMF degradation, and phenolic compounds are generated from the partial breakdown of lignin. These compounds are toxic to fermentative microorganisms and inhibit their metabolism (Palmqvist and Hahn-Hägerdal, 2000).

4.3 Enzymatic hydrolysis of the pretreated sweet sorghum straw

Sweet sorghum straw was pretreated with 3% H_2SO_4 prior to enzymatic hydrolysis. This treatment was effective in fractionating the hemicelluloses and lignin components. The pretreated sweet sorghum consisted of 69.50% cellulose, 0.44% hemicellulose, 19.53% lignin and 10.53% ash (data not shown). Comparing the chemical components, acid pretreatment increased the proportion of cellulose by 44.51% and decreased that of hemicellulose by 38.62%. The increment of cellulose content and reduction of hemicellulose content would allow for enhancement of enzymatic saccharification.

The study of experimental design of four-factor, five-level CCRD in Table 3.2 showed that the maximum glucose concentration was obtained at level 19 with a substrate concentration of 2.5%w/v, enzyme concentration 30 FPU/g DS,

temperature at 40°C and pH 4. A maximum of 558 mg of glucose was released after 96 h of hydrolysis.

The statistical significance was also analyzed by checking the *F* test and the analysis of variance (ANOVA) in order to optimize the enzymatic saccharification of the acid pretreated sweet sorghum straw. The result was shown in Table C3 (Appendix C). The model F value of 31.425 and values of probability (P-values) >*F* and $\alpha = 0.000$ ($\alpha < 0.05$) showed that the model terms were significant. It indicated that the model was statistically significant with confidence interval 95% of the coefficient of determination (R^2) of the model was 0.921. The R^2 value is always between 0 and 1. The closer the R^2 is to 1.0, the stronger the model and the better it predicts the response. Normally, a regression model with an R^2 higher than 0.90 was considered to have a very high correlation. According to the result, the differences in means were judged significant when p values for the null hypothesis were 0.05 or less ($p \leq 0.05$). A comparison of difference level of variable of this result showed that the p-value of the various substrate concentration, temperature and pH were less than 0.05, therefore these factors were significance change on sugars liberation but various enzyme concentration had no significance change on sugars liberation because p-value was more than 0.05.

Tukey's method was further analyzed in order to optimize the saccharification condition that was affected by these three variables (substrate concentration, temperature and pH). Results from statistical analysis (Table C4-C7) were shown that the optimum condition for saccharification the acid pretreated sweet sorghum straw were 1% of substrate concentration , 15 FPU/g-substrate of enzyme concentration at temperature 40 °C and pH 5.

Table 4.4 Optimal condition for enzymatic saccharification of the acid pretreated sweet sorghum straw

Variable	Optimum condition
Substrate (%w/v)	1%
Enzyme (FPU/g DS)	15 FPU/g Dry substrate
Temperature (°C)	40°C
pH	5

Table 4.5 showed the partitioning of glucose, xylose and other monosaccharide released from the first pretreatment step with 3% sulfuric acid concentration at 120°C for 10 minutes. Stage 2 was conducted using 15 FPU/g Dry substrate of cellulase (Celluclast 1.5, Novozyme) and saccharification of 1% (w/v) the pretreated sweet sorghum straw at 40°C, pH 5.0 for 96 h. Only glucose was detected from this step. Combined yields of glucose 0.578 g/g dry substrate was obtained from both treatments.

Table 4.5 Combined yields of monosugars liberated from acid pretreatment (stage 1) and enzyme hydrolysis (stage 2) of sweet sorghum straw

Conditions	Yield $_{avg}$ (g monosugar /g dry substrate)			
	Glucose	Xylose	Gal, Man, Ara	Total sugar
Stage 1 Acid pretreatment of sweet sorghum straw at 120°C, 3% H2SO4, 10 min	0.234	0.208	0.235	0.676
Stage 2 Enzymatic hydrolysis of the acid pretreated sweet sorghum straw	0.344	0.000	0.000	0.344
Stage 1 + stage2	0.578	0.208	0.235	1.020

4.4 Ethanol fermentation

Hydrolyzates from the acid pretreated sweet sorghum straw (stage 1), the enzymatic hydrolysis of the acid pretreated sweet sorghum straw (stage 2) and combined hydrolyzates from both stages were used as carbon source for ethanol fermentation process. The fermentation was performed under aerobic condition by *Saccharomyces cerevisiae*.

Results of fermentations were shown in Table 4.6. The high yield of ethanol concentration, of 15.40 g/l after 12 h of cultivation, was obtained when using sugar liberated from saccharification of the acid pretreated sweet sorghum straw (Stage 2)

as substrate. Since glucose is only main monosugar component available in the saccharification liquid (Stage 2). Incase using sugars from hydrolyzate of the acid pretreated sweet sorghum straw (Stage 1), maximum ethanol yield of 9.90 g/l was obtained.

Table 4.6 Summary of maximum ethanol concentration and yield obtained from *Saccharomyces cerevisiae* using total sugars liberated from the acid pretreatment and the saccharified pretreated sweet sorghum straw

Substrate	Ethanol conc. (g/l)	Cultivation time (h)	Productivity (g/l/hr)	Yield [a] max (% EtOH/Total sugars)
Sugars from the hydrolyzate of acid-pretreated SSS (Stage1)	1.98	24	0.0825	9.90
Sugars from saccharification of the acid pretreated SSS (Stage 2)	3.08	12	0.2566	15.40
Combined sugars from Stage 1 + Stage 2	2.17	24	0.0904	10.85

Initial substrate concentration : Total sugar 20 g/l

[a] Yield product/substrate : g ethanol/g total sugar in pretreated sweet sorghum straw.

Sukumaran R. K. et al., 2009 reported that the enzymatic hydrolyzate of rice straw (6% initial reducing sugar concentration) was used as substrate for ethanol production by *Saccharomyces cerevisiae*. The yield of ethanol was 0.093 g per gram of pretreated rice straw and productivity was obtained 0.23 g/l/h.

Buaban, B. et al, 2010 reported that the enzymatic hydrolyzate of sugarcane bagasse (5%w/v)was used as substrate for ethanol production byseparate hydrolysis and fermentation processes using *Pichia stipitis*BCC15191 at pH 5.5, 30 °C resulting in an ethanol concentration of 8.4 g/l after 24 h., ethanol yield of 0.29 g ethanol/g sugars and productivity was obtained 0.35 g/l/h. Comparable ethanol conversion efficiency was obtained by a simultaneous saccharification and fermentation process

which led to production of 8.0 g/l ethanol after 72 h fermentation under the same conditions and productivity was obtained 0.11 g/l/h.

Saha, B.C. et al., 2005reported that the acid and enzyme treated wheat straw hydrolyzate was used as substrate for ethanol production by separate hydrolysis and fermentation processes using recombinant*Escherichia coli* strain FBR5 at pH 6.5, 35 °C. The ethanel concentration was obtained 17 g/l after 39 h., ethanol yield of 0.21 g ethanol/g sugars and productivity was obtained 0.43 g/l/h. Comparable ethanol conversion efficiency was obtained by a simultaneous saccharification and fermentation process which led to production of 17 g/l ethanol after 112 h., ethanol yield of 0.21 gethanol/g sugars, fermentation under the same conditions and productivity was obtained 0.15 g/l/h. Hernández-Salas et al. (2009) reported that maximum ethanol concentration of 12.5 g/l after 48 h and productivity of 0.26 g/l/h.

A critical problem in the fermentation of hydrolyzate from acid-pretreated lignocellulosic materials has been the inability of the fermentative microorganism (such as furfural, HMF, acetic acid and phenolic compounds). These compounds were toxic to microorganisms during fermentation steps. A detoxification step is used to partially or completely remove these inhibitors, consequently improve the fermentation processes (Palmqvist et al., 2000 and Saha et al., 2005). Physical detoxification method by vacuum evaporation lead to decrease volatile compounds (such as acetic acid, furfural and vanillin) and increases hydrolyzate concentration for fermentation. However, this method also moderately increases the concentration of non-volatile toxic compounds (Mussatto and Roberto, 2004). Consistency with this results indicate that fermentation of 20 g/l of total sugars concentration in hydrolyzate is possible to obtain ethanol concentration higher than fermentation of 50 g/l of total sugars concentration in hydrolyzate, because increasing of total sugars concentration in hydrolyzate lead to increases the non-volatile toxic compounds in hydrolyzate.

V. CONCLUSION

Sweet sorghum straw has the potential feedstock for ethanol production. The composition of sweet sorghum straw used in this study consisted of 44.51% cellulose, 38.12% hemicellulose and 6.18% lignin. Chemical dilute acid pretreatment was effective in solubilizing cellulose and hemicellulose in the biomass to fermentable sugars. At the acid pretreatment condition: 120°C, 3%H_2SO_4 for 10 min., the maximum yield of glucose and xylose were 0.234 g glucose/g dry substrate and 0.208 g xylose/g dry substrate, respectively.

The optimum condition for enzymatic saccharification of the acid pretreated sweet sorghum straw by a commercial cellulase enzyme (Celluclast1.5, Novozyme) was 15 FPU/g-substrate, 1% of the acid pretreated sweet sorghum straw at temperature 40°C, pH 5. Glucose was the only monosugar detectable with the yield of 0.344 g glucose/g dry solid.

Fermentation of 20 g/l of total sugars concentration liberated from the saccharified pretreated sweet sorghum straw by *Saccharomyces cerevisiae* gave the highest ethanol concentration of 3.08 g/l and productivity of 0.2566 g/l/hr, at 12 hr of cultivation. The high yield of ethanol concentration was 15.40 g-ethanol/100 g-total sugars.

SUGGESTION FOR FUTURE WORK

Chemical pretreatment of lignocellulosic materials release oligomers and monosaccharides followed by the breakdown of the glucose released to form inhibitors such as furan derivatives (furfural and 5-hydroxymethylfufural), phenolic chemicals and aliphatic acids. These products are generally considered inhibitors for fermentative microorganisms. Further study is needed to investigate improving the yield of monosugars by avoiding further degradation of those sugars, which subsequently increase fermentable sugars for ethanol production.

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APPENDICES

APPENDIX A

CULTURE MEDIA

1. Yeast Peptone Dextrose (YPD) agar

Yeast extracts	10	g
Peptone	10	g
Glucose	20	g
Agar	20	g
Distilled water	1000	ml

Add the yeast extracts, peptone, glucose and agar in distilled water and then dissolve by streaming. The media were sterilized by autoclave at 121°C for 15 minutes.

2. Yeast Peptone Dextrose (YPD) borth

Yeast extracts	10	g
Peptone	10	g
Glucose	20	g
Distilled water	1000	ml

Add the yeast extracts, peptone and glucose in distilled water and then dissolve by streaming. The media were sterilized by autoclave at 121°C for 15 minutes.

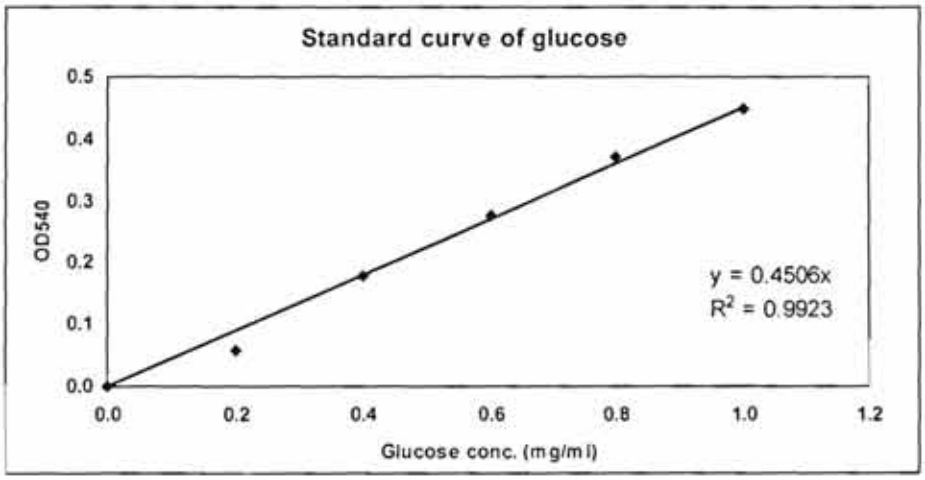
APPENDIX B

B1. Calibration curve for various concentration of glucose by DNSA method

DNSA reagent (Miller, 1959) (per liter)

3,5 –Dinitrosalicylic acid	5.3	g
Sodium hydroxide	9.9	g
Sodium potassium tartratate	153.0	g
Sodium metabisulfile	4.1	g
Phenol (melt at 50°C)	3.8	ml
Distilled water	708	ml

Dissolve 3,5–Dinitrosalicylic acid and sodium hydroxide with distilled water, then add sodium potassium tartratate, sodium metabisulfile and phenol in the mixer. Stir this reagent until homogeneously and store in amber bottle.



Slope = 0.4506

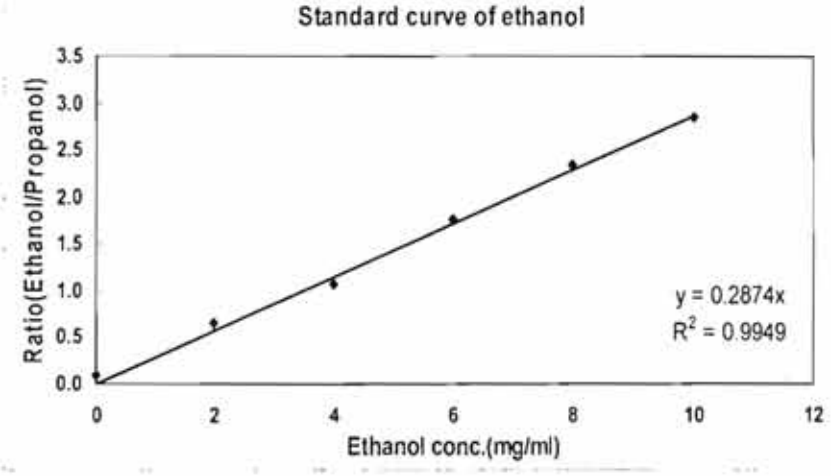
Figure B1 Standard curve of glucose concentration

$$\text{Glucose concentration (mg/ml)} = \frac{1}{\text{Slope}} \times \text{OD } 540$$

B2. Calibration curve for various concentration of ethanol by gas chromatography

Method

1. Prepare standard ethanol at vary concentrations in the range 0–10 mg/ml.
2. Mix sample of standard ethanol 1 ml with 1ml of 3 mg/ml propanol (propanol use as internal standard).
3. Inject 1 µl of mixture solution in gas chromatography to make standard curve.



Slope = 0.2874

Figure B2 Standard curve of ethanol concentration

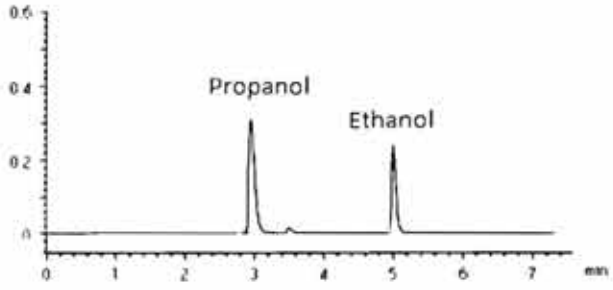
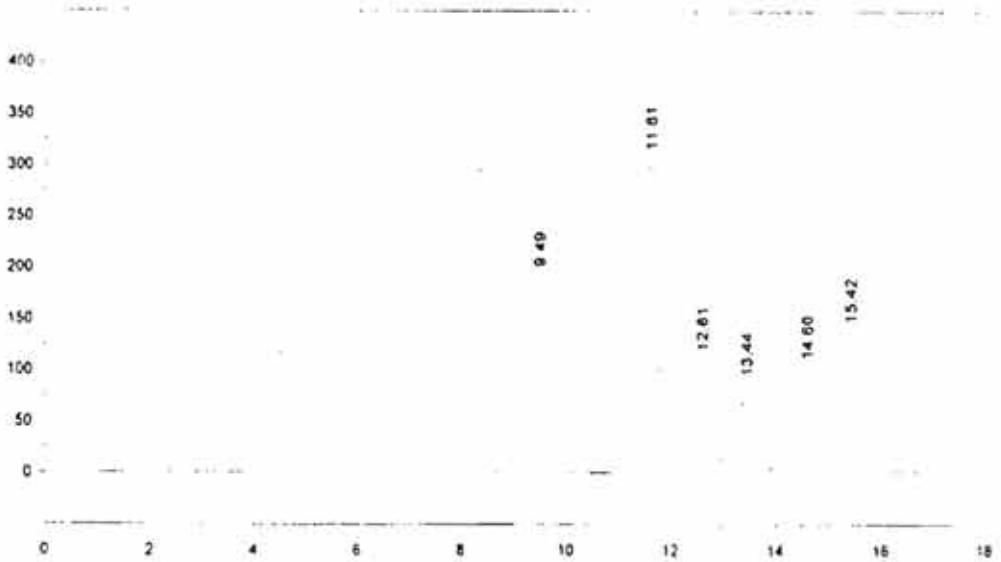


Figure B3 Standard peaks of propanol and ethanol on Porapak Q column

B3. Standard peaks of monosugars determine by HPLC (Aminex HPX-87P Column)



Peak Name	Rel. Time	Plates	Half-Width	Skew 10%	Resolution
Cellobiose	9.49	4174	0.34	0.93	2.14
D-Glucose	11.61	7772	0.31	0.84	3.81
D(+)Xylose	12.61	10031	0.30	0.96	1.95
D(+)Galactose	13.44	10346	0.31	0.69	1.62
D(+) Arabinose	14.60	11035	0.33	0.93	2.14
D(+)Mannose	15.42	5767	0.48	0.91	1.20

Figure B4 Standard peaks of cellobiose and monosugars on the Aminex HPX-87P Column

APPENDIX C

Table C1 Statistical analysis yields of glucose from Sweet sorghum straw

Tests of Between-Subjects Effects

Dependent Variable: Yield of Glucose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6348.326[a]	35	181.381	4.769	.000
Intercept	16460.305	1	16460.305	432.791	.000
Temp	811.587	3	270.529	7.113	.000
Acid	4159.407	2	2079.704	54.682	.000
Time	44.624	2	22.312	.587	.559
Temp * Acid	788.817	6	131.469	3.457	.005
Temp * Time	132.556	6	22.093	.581	.744
Acid * Time	64.242	4	16.061	.422	.792
Temp * Acid * Time	347.093	12	28.924	.761	.688
Error	2738.371	72	38.033		
Total	25547.002	108			
Corrected Total	9086.698	107			

a. R Squared = .699 (Adjusted R Squared = .552)

Table C2 Statistical analysis yields of xylose from Sweet sorghum straw

Tests of Between-Subjects Effects

Dependent Variable: Yield of Xylose

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3956.264[a]	35	113.036	6.491	.000
Intercept	4558.477	1	4558.477	261.773	.000
Temp	1362.479	3	454.160	26.080	.000
Acid	1553.171	2	776.585	44.596	.000
Time	33.511	2	16.756	.962	.387
Temp * Acid	826.612	6	137.769	7.911	.000
Temp * Time	25.966	6	4.328	.249	.958
Acid * Time	88.134	4	22.034	1.265	.292
Temp * Acid * Time	66.390	12	5.532	.318	.984
Error	1253.796	72	17.414		
Total	9768.537	108			
Corrected Total	5210.060	107			

a. R Squared = .759 (Adjusted R Squared = .642)

Table C3 Analysis of Variance (ANOVA) for optimization of enzymatic saccharification the acid pretreated sweet sorghum straw

Dependent Variable: Reducing sugars

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.569(a)	24	.024	31.425	.000
Intercept	2.721	1	2.721	3607.082	.000
Sub	.145	3	.048	64.014	.000
Enz	.010	3	.003	4.269	.08
Temp	.191	3	.064	84.526	.000
pH	.180	3	.060	79.617	.000
Sub * Enz	.003	1	.003	4.530	.037
Sub * Temp	.005	1	.005	6.443	.014
Enz * Temp	3.50E-005	1	3.50E-005	.046	.830
Sub * Enz * Temp	.001	1	.001	1.910	.172
Sub * pH	.001	1	.001	.789	.378
Enz * pH	6.77E-005	1	6.77E-005	.090	.765
Sub * Enz * pH	.000	1	.000	.365	.548
Temp * pH	.013	1	.013	17.718	.000
Sub * Temp * pH	.001	1	.001	.925	.340
Enz * Temp * pH	.006	1	.006	7.381	.008
Sub * Enz * Temp * pH	.003	1	.003	4.309	.042
Error	.049	65	.001		
Total	7.351	90			
Corrected Total	.618	89			

a. R Squared = .921 (Adjusted R Squared = .891)

Table C4 Multiple comparison of various substrate concentration using Tukey's method for optimize the condition of saccharifiction the acid pretreated sweet sorghum straw

Tukey HSD

Substrate	N	Subset			
		1	2	3	4
5.5%	24	.21937			
7%	3	.23500	.23500		
4%	36		.27706	.27706	
2.5%	24			.31042	
1%	3				.40767
Sig.		.845	.058	.201	1.000

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .001.
a Uses Harmonic Mean Sample Size = 6.429.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

Table C5 Multiple comparison of various enzyme concentration using Tukey's method for optimize the condition of saccharifiction the acid pretreated sweet sorghum straw

Tukey HSD

Enzyme	N	Subset
		1
20 U	24	.25321
30 U	24	.27658
25 U	36	.27847
15 U	3	.29033
35 U	3	.33533
Sig.		.100

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .001.
a Uses Harmonic Mean Sample Size = 6.429.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

Table C6 Multiple comparison of various temperature using Tukey's method for optimize the condition of saccharifiction the acid pretreated sweet sorghum straw

Tukey HSD

	N	Subset		
Temp		1	2	3
70 C	3	.15600		
60 C	24		.21263	
30 C	3			.28733
50 C	36			.29367
40 C	24			.31717
Sig.		1.000	1.000	.303

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .001.
a Uses Harmonic Mean Sample Size = 6.429.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

Table C7 Multiple comparison of various pH using Tukey's method for optimize the condition of saccharifiction the acid pretreated sweet sorghum straw

Tukey HSD

	N	Subset		
pH		1	2	3
7	3	.08567		
6	24		.23329	
4	24			.29650
5	36			.29711
3	3			.31633
Sig.		1.000	1.000	.695

Means for groups in homogeneous subsets are displayed.
Based on Type III Sum of Squares
The error term is Mean Square(Error) = .001.
a Uses Harmonic Mean Sample Size = 6.429.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
c Alpha = .05.

ACADEMIC PRESENTATION

Oral presentation and proceedings

1. Apisit Poonsrisawat, and **Siriluk Teeradakorn**; " Conversion of sweet sorghum straw to sugars by dilute-acid saccharification" *in* The 3rd Technology and Innovation for Sustainable Development International Conference (TISD2010). 4-6 March 2010, Royal Mekong Nongkhai Hotel, Nongkhai, Thailand.

2. Sukanya Phuengjayeam and **Siriluk Teeradakorn** : "Saccharification of acid-pretreated sweet sorghum straw by cellulase for bioethanol production" *in* 2011 International Conference on Asia Agriculture and Animal (ICAAA 2011), July 2-3, 2011, Hongkong.

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หัวหน้าโครงการวิจัย

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