ASSESSMENT OF PRE-TREATMENT AND FERMENTATION TECHNIQUES FOR THE ENHANCEMENT OF BIOETHANOL YIELD FROM CORN (*ZEA MAYS* L.)

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MASTER OF ENGINEERING IN RENEWABLE ENERGY ENGINEERING

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ชื่อเรื่อง การประเมินเทคนิคของการปรับสภาพและการหมักเพื่อเพิ่มผลผลิตไบโอเอทานอลจากข้าวโพด

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บทคัดย่อ

วัสดุเหลือทิ้งจากต้นข้าวโพดมีศักยภาพในการเป็นวัตถุดิบทางเลือกสำหรับการผลิตไบโอเอทานอล ข้าวโพดนับว่าเป็นพืชเศรษฐกิจที่สำคัญของประเทศไทยและผลิตได้ 5.68×10^6 ตันต่อปี แต่ชีวมวลเหล่านี้ไม่ได้ถูกนำมาใช้ประโยชน์ แต่กลับถูกเผาทำลายหลังการเก็บเกี่ยว ซึ่งส่งผลให้เกิดปัญหาร้ายแรง เช่น มลพิษจากหมอกควัน โดยเฉพาะอย่างยิ่งในพื้นที่ภาคเหนือ เพื่อที่จะแก้ไขปัญหามลพิษที่เกิดจากการเผาวัสดุเหลือทิ้งของข้าวโพด รวมทั้งให้ทางเลือกแก่เกษตรกรในการกำจัดผลผลิตทางการเกษตร รวมทั้งเสนอวัตถุดิบทางเลือกสำหรับการผลิตไบโอเอทานอล ในการศึกษานี้ได้ทำการประเมินศักยภาพของข้าวโพดสองสายพันธุ์สำหรับการผลิตไบโอเอทานอล ได้แก่ ข้าวโพดสายพันธุ์ Hi-brix 53 และสายพันธุ์ Sugarstar × Hi-Brix 53 ทำการศึกษาน้ำจากต้นข้าวโพด ชานต้นข้าวโพด และใบ โดยใช้ ยีสต์ (Saccharomyces cerevisiae) ในการหมัก น้ำที่สกัดจากต้นข้าวโพดใช้วิธีการหมักสองวิธี ในส่วนของวัสดุประเภทลิกโนเซลลูโลส ได้แก่ ชานต้นข้าวโพดและใบ จะถูกนำไปผ่านการปรับสภาพ การย่อยสลาย และการหมัก ได้มีการทดสอบการปรับสภาพ 3 วิธี ที่แตกต่างกัน เพื่อให้ทราบการปรับสภาพที่เหมาะสมสำหรับวัสดุเหล่านี้ ได้แก่ การปรับสภาพทางกายภาพ ไม่ผ่านการปรับสภาพ และการปรับสภาพด้วยด่าง กระบวนการหมักแบบ SHF และ SSF ได้ถูกนำมาใช้เพื่อกำหนดวิธีการหมักที่มีประสิทธิภาพมากที่สุดสำหรับวัสดุเหล่านี้ ผลการศึกษาพบว่า น้ำที่สกัดจากต้นข้าวโพดสายพันธุ์ Hi-brix 53 และสายพันธุ์ Sugarstar × Hi-brix 53 มีน้ำตาลที่หมักได้ง่าย ในการหมักแบบแบทช์พบว่าทั้งสองสายพันธุ์สามารถผลิตไบโอเอทานอลสูงสุด 62.12 กรัมต่อลิตร (7.87 เปอร์เซ็นต์) จากนั้นทำการทดลองอีกครั้งเพื่อเพิ่มผลผลิตไบโอเอทานอล โดยนำน้ำที่สกัดจากต้นข้าวโพดซึ่งเก็บไว้เป็นระยะเวลา 6 เดือน มาผ่านการหมักอย่างต่อเนื่องที่ยาวนานถึง 5 รอบ ได้ปริมาณไบโอเอทานอล 27.62-29.98 กรัมต่อลิตร (3.5-3.9 เปอร์เซ็นต์โดยปริมาตร) หลังจากการกลั่นพบว่ามีปริมาณไบโอเอทานอลเท่ากับ 126.24 (16 เปอร์เซ็นต์โดยปริมาตร) สำหรับส่วนของลิกโนเซลลูโลสในข้าวโพดพบว่าการปรับสภาพด้วยด่างโดยใช้โซเดียมไฮดรอกไซด์เป็นวิธีการปรับสภาพที่เหมาะสมที่สุด เมื่อเปรียบเทียบกับการปรับสภาพโดยใช้หม้อนึ่งฆ่าเชื้อและการปรับสภาพทาง

กายภาพ ใช้ RSM ในการประเมินสภาวะที่เหมาะสมสำหรับการปรับสภาพด้วยด่าง สำหรับการใช้ชานต้นข้าวโพดและใบในวิธีการหมักแบบ SHF และ SSF พบว่าไม่มีความแตกต่างอย่างมีนัยสำคัญในแง่ของการผลิตไบโอเอทานอล ชานต้นข้าวโพดสายพันธุ์ Sugarstar × Hi-brix-53 พบว่าสามารถผลิตไบโอเอทานอลได้สูงกว่าเมื่อเปรียบเทียบกับสิ้นส่วนอื่น ๆ ของต้นข้าวโพด สำหรับการเพิ่มขนาดการผลิตไบโอเอทานอลจากต้นและใบของข้าวโพดโดยใช้การหมักแบบ SHF พบว่าสามารถผลิตไบโอเอทานอลประมาณ 27.77 กรัมต่อลิตร (2.9 เปอร์เซ็นต์โดยปริมาตร) เมื่อเปรียบเทียบกับวัสดุทั้งหมดแสดงให้เห็นว่าน้ำสกัดจากต้นข้าวโพดนับว่าเป็นวัตถุดิบที่เหมาะสมสำหรับการผลิตเอทานอลและควรศึกษาเพิ่มเติมเกี่ยวกับน้ำสกัดจากต้นข้าวโพด สำหรับส่วนของลิกโนเซลลูโลสควรใช้วิธีการย่อยสลายและวิธีการหมักแบบอื่น ๆ การวิเคราะห์ทางเศรษฐกิจและเทคโนโลยีของโรงงานอุตสาหกรรมพลังงานและเคมีชีวภาพขนาดเล็กพบว่า วัสดุเหลือทิ้งจากต้นข้าวโพด (ก้านและใบข้าวโพด) เป็นวัตถุดิบที่เป็นไปได้สำหรับการผลิตเอทานอลทางชีวเคมี ยังแนะนำให้ศึกษาเพิ่มเติมเกี่ยวกับวัสดุเหล่านี้

คำสำคัญ : ข้าวโพด (Zea mays L), ชีวมวล Lignocellulosic, น้ำข้าวโพด, เอทานอล, ยีสต์ (Saccharomyces cerevisiae), ขยะเป็นพลังงาน

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Degree Master of Engineering in Renewable Energy Engineering

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ABSTRACT

Corn residue as an alternative feedstock has a potential for bioethanol production. Corn is one of the major crops of Thailand and it produces about 5.68 × 10^6 dry tones of residue per year. Unfortunately, some of this biomass were not utilized but rather burned on the field due to a lack of post-harvest control. This leads to serious problems like haze pollution, especially experienced in the Northern area. In order to alleviate the pollution problem brought by combustion of corn residues it was determined to give farmers some options for agricultural by-product disposal, as well as offering an alternative feedstock for bioethanol production. This study evaluated two corn varieties for their potential as a viable option for bioethanol production, Hi-brix 53 and Sugarstar × Hi-Brix 53 corn. Corn stalk juice, stalk bagasse and leaves were studied. Yeast (*Saccharomyces cerevisiae*) microorganisms were used for fermentation. Two method of fermentation were used using corn stalk juice. Lignocellulosic part: stalk bagasse and stalk leaves did undergo pretreatment, hydrolysis and fermentation. Three pretreatments were tested in order to know the suitable pretreatment on these materials: physical, control and alkaline. SHF and SSF fermentation process were also applied in order to determine the most effective mode of fermentation in these materials. Hi-brix 53 and Sugarstar × Hi-brix 53 stalk juice contains readily fermentable sugar. Both varieties produce bioethanol with a highest yield of 62.12 g/L (7.87%) in batch fermentation. Another experiment was done in

order to improve the ethanol yield. 6-month old stalk juice underwent continuous fermentation that lasted up to 5 cycles. Bioethanol content was from 27.62-29.98 g/L (3.5-3.9% v/v). After distillation ethanol content was found to be 126.24 (16% v/v). As for the lignocellulosic part of corn, alkaline pretreatment using sodium hydroxide was found to be the most suited pretreatment compared to autoclave and physical. Using RSM, the optimal condition for alkaline pretreatment was predicted. For the mode of fermentation, SHF and SSF fermentation using stalk bagasse and leaves does not show any significant difference in terms of bioethanol production. Stalk bagasse of Sugarstar × Hi-brix-53 found to yield higher bioethanol compared to other material and variety. For the scale up production using SHF, mix stalk and leaves were used as feedstock generated about 27.77 g/L (2.9% v/v) of bioethanol. Comparing all used materials, stalk juice shows the most promising feedstock for bioethanol production. Further study on the juice is highly recommended. As for the lignocellulosic part, application on other hydrolysis and fermentation method was suggested. Techno-economic analysis on a small pilot scale biorefinery found that the corn residue (corn stalk and leaves) were a feasible feedstock for biochemical ethanol production. Still further study about these materials was recommended.

Keywords : Corn (Zea mays L), Lignocellulosic biomass, Corn Juice, Bioethanol, Yeast (S. Cerevisiae), waste-to-energy

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Katherine Bautista

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CHAPTER I

INTRODUCTION

Principles, Theory, Rationale and Background of the study

In 2010, the world energy demand was at 5.5×10^{20} J and was predicted to increase up to 6.6×10^{20} J in 2020 and 8.6×10^{20} in 2040 (USEIA, 2013). Almost 80% of the world's total energy supply comes from a non-renewable source. According to Goldemberg, (2007), the known reserves of oil, natural gas and coal will last around 41 years, 64 years, and 155 years, respectively, in the current constant state of production and consumption. Furthermore, fossil fuel use has been related to some alarming environmental problems such as global warming and climate change. These increasing demands for energy, alongside with the diminishing and limited supply of fossil fuels, together with the negative impacts in the environment, are the reasons industries and governments worldwide are seeking renewable alternatives. Bioenergy, renewable energy sources, draws attention due to its availability and low carbon dioxide emission (Guo et al., 2015).

Bioenergy refers to the stored chemical energy in biomass (Ehrlich, 2013). Biomass includes plants, trees, woods, and agricultural or forest residues (Kumar et al., 2009). The bioenergy that can be harvested and utilized each year is estimated at 190×10^{18} J, almost 35% of the world's energy demand (Haberl et al., 2013). Bioenergy can be in solid, liquid and gas form just like fossil fuels; these refer as biofuels. Ehrlich, (2013) stated that one advantage of biofuels over fossil fuel, in principle, biofuels are carbon-neutral meaning it can be used without adding any carbon dioxide in the atmosphere. Bioethanol and biodiesel are the two liquid biofuels that have been widely used worldwide alternative to gasoline (Ehrlich, 2013). Bioethanol can be produced by fermentation using feedstock like sugar, starch, lignocellulosic materials and algae (Mielenz, 2001). According to the paper published by Guo et al., (2015), 42% of U.S. corn grain has been used to produce 49 billion liters of ethanol; in 2012, this is about 94% of the liquid biofuel produced (52.2 billion liters) and has replaced a total

of 10% of the nation's gasoline demand. Almost all the world's bioethanol supply is produced by corn grain in the United States and sugarcane in Brazil (Mielenz, 2001).

Corn, also known as Maize, is one of the most important crops in the world due to its high carbohydrate content that can be used as a raw material in different products (Semenčenko et al., 2015). Corn has been used for food, animal feed and various industrial products (Zabed et al., 2016). Semenčenko et al., (2015) noted that the most important part of the corn is its kernel/grain. Its kernel contains 84% carbohydrates, 10.9% protein, 4.5 % fat and 1.3 % mineral (Du Plessis, 2003). Since corn grain is made up of 70% starch, it is also utilized as a feedstock for bioethanol production (Semenčenko et al., 2015). According to Mussoline et al., (2017), in the United States, one of major bioethanol producers which contribute 60% of the world's bioethanol supply, almost 90% of its biorefineries use corn grain as feedstock for the production of ethanol. Although corn is a sufficient feedstock for bioethanol production, there is a growing concern regarding the use of food source to energy production (Rass-Hansen et al., 2007). To avoid conflicts to the food supply, the lignocellulosic part of the corn is also a viable option for ethanol production.

Bioethanol can be produced from different feedstocks such as sugar, starch, and lignocellulosic materials that are rich in hexoses and pentoses (Mohapatra et al., 2017). Lignocellulosic materials contain lignin, cellulose, and hemicellulose. These materials are identified as a structural framework of plant cell walls; thus it is available in different parts of plants in varying amounts (Jørgensen et al., 2007). However, ethanol production from lignocellulosic biomass differs than that of the starch and sugar. Lignocellulosic materials have to undergo pretreatment before hydrolysis. They need extensive processing to release the polymeric sugars in cellulose and hemicellulose which contributed about 20-53% of plant materials. Cellulose is a beta-linked glucose polymer; meanwhile, hemicellulose is a highly branched chain of xylose and arabinose that also consists of glucose, mannose, and galactose (Mielenz, 2001). The goal of this process (pretreatment) is the following: (1) to improve the formation of sugars or the ability to form them, and (2) to avoid the formation of products that can inhibit the hydrolysis and fermentation process (Sanchez and Cardona, 2008). The next step will be hydrolysis (saccharification); this process converts cellulose and

hemicellulose into simple sugars ready to be fermented into bioethanol (Mohapatra et al., 2017).

The aim of this study was to used non-food plant source of sweet corn for bioethanol production such as the leaves, stalk juice, and stalk bagasse. This main study aim was to assess different pre-treatment and fermentation techniques through experimentation and evaluate each process by techno-analysis for the enhancement and improvement of bioethanol yield from corn agricultural by-products.

Objectives of the study

1. To compare bioethanol production from stalk juice, leaves and stalk bagasse of two sweet corn cultivars: hi-brix 53 and sugarstar x hi-brix 53.
2. To assess and evaluate different pretreatment and fermentation techniques for bioethanol yield enhancement.
3. To evaluate different pretreatment and fermentation using RSM and SPSS statistical program, respectively; performed energy and techno-economic analysis.

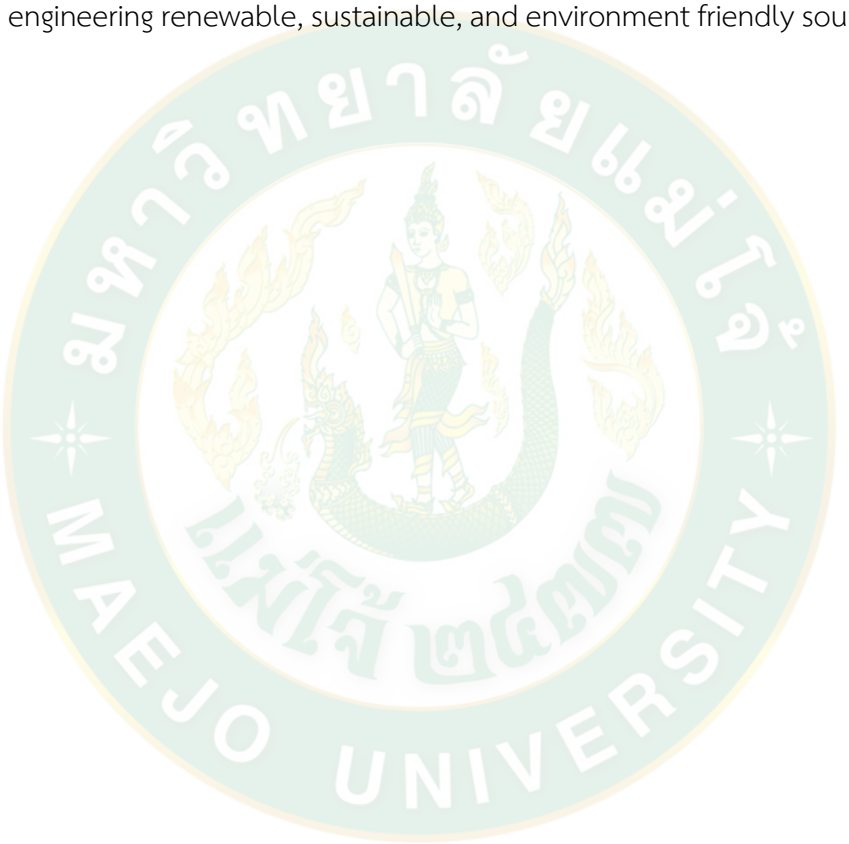
Scope and limitation of the study

1. This study used two sweet corn (*Zea mays* L.) cultivars (hi-brix 53 and sugarstar x hi-brix 53 hybrid) and three lignocellulosic parts such as leaves, stalk juice and stalk bagasse for bioethanol production.
2. Three different pretreatments: physical, alkali, and steam, and two different fermentation techniques (SSF and SHF) were applied suited for optimization and maximization of the bioethanol yield and energy value.
3. Different pretreatment and fermentation techniques were evaluated using RSM and ANOVA, respectively and the optimal conditions were applied for the techno-economic analysis of bioethanol production.

Benefits of the study

1. The result of this study was benefitted not just co-researchers but also ethanol industries, especially in Thailand, for finding the ideal and suitable pretreatment and fermentation process for corn.

2. This study contributed to agricultural waste turn energy, in which reducing the waste input in the environment and use it to something useful; a feedstock for bioethanol, a renewable fuel.
3. The industrial sector can have baseline data on the cost of production of bioethanol using corn through the techno-analysis results of this study.
4. Lastly, the major benefit of this study was its contribution to the growing renewable energy engineering sector, in Thailand, in terms of discovering and engineering renewable, sustainable, and environment friendly source of energy.



CHAPTER II

LITERATURE REVIEW

Global Sustainability and Energy

Sustainability, especially in the energy sector, has been in focus of concern due to the declining supply fossil fuel, rapidly increasing oil price, global warming and energy security (Chovau et al., 2013). The world's energy demands keep increasing through time, as it is estimated to increase 6.6×10^{20}J in 2020 and 8.6×10^{20}J in 2040, the supply of fossil fuels such as petroleum, natural gas, and coal will only last for 41,64,155 years respectively, (USEIA, 2013; Goldemberg, 2007). Additionally, extreme consumption of fossil fuels in the past few years, especially in developed countries, held responsible for the huge amount of greenhouse gases (GHGs) in the atmosphere. With the increased of GHG on the biosphere, environmental problems such as global warming and climate change emerge. These following concerns in energy security and environment stimulate worldwide interest to utilize an alternative, clean, sustainable and renewable energy such as solar, wind, water, geothermal and biomass (Gupta and Verma, 2015). The world's total primary energy supply by fuel in the year 2015 is illustrated in figure 1.

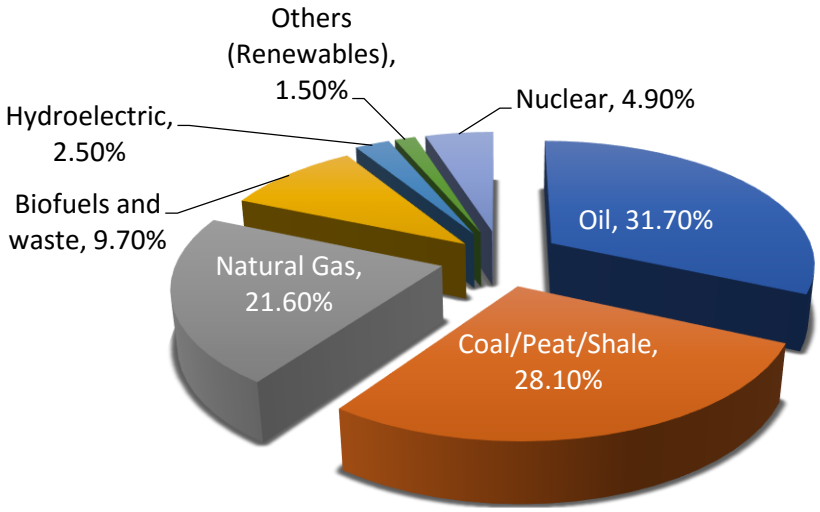


Figure 1 World total primary energy supply (TPES) by fuel, year 2015 (source: IEA, 2017)

Bioethanol as a renewable fuel

Bioethanol is one of the renewable fuels made from biomass, the fourth largest source of energy after conventional fuels like petroleum, coal and natural gas (Gupta and Verma, 2015). It has a huge potential to replace gasoline and be sustainable transport (Kim and Dale, 2004). According to Gupta and Verma, (2015), bioethanol could reduce about 90% of CO_2 and 60-80% SO_2 emission when blended with 95% gasoline. Chovau et al., (2013) stated that reducing emissions of these pollutants would help fight climate change. Due to these advantages, bioethanol becomes the most significant produced liquid biofuel in the world. As of 2016, the total of world's bioethanol production has been 26,583 million of gallons, being the USA the top producer with 15,329 million of gallons (see Table 1).

Table 1 World's production of bioethanol in 2016

Country	Millions of Gallons
1. USA	15,329
2. Brazil	7,295
3. European Union	1,377
4. China	845
5. Canada	436
6. Thailand	322
7. Argentina	264
8. India	225
9. Rest of the world	490

*data from: http://www.ethanolrfa.org/ resources/industrystatistics/# 1454099103927-61e598f7-7643

Furthermore, the Renewable Fuels Association, (2017) reported that the production of ethanol in America also produced 42 million metric tons of co-products that had a significant economic impacts, like $42 billion contributions to GDP, $23 billion in household income, $9 billion in tax revenue and also created 74,420 direct

jobs and 264,756 indirect and induced jobs. With this, the production of bioethanol not just helps the energy and environmental sectors but also the economic sectors.

Feedstock for bioethanol production

Bioethanol, renewable energy from biomass, is a liquid fuel that can replace gasoline (Dahnum et al., 2015). It can be produced from different materials such as starch-containing material, sugar-based feedstock, lignocellulosic materials and algae (Semenčenko et al., 2015). The different feedstock for bioethanol production from different countries is shown in Table 2.

Table 2 Different feedstock for bioethanol production (source: Woiciechowski et al., 2016)

Feedstocks	Country
Corn, soybean oil, sorghum	EUA
Sugarcane, soybean, palm oil	Brazil
Rapeseed, sunflower, wheat, sugar beet, barley, sewage, manure, food wastes, landfill	EU
Corn, cassava, sweet potato, rice, jatropha	China
Corn, wheat	Canada
Wheat, sugarcane, molasses, palm oil, cotton oil	Australia

Currently, corn is the major feedstock used for bioethanol production in the US (Wu et al., 2010). The USA is one of producers of bioethanol contributing nearly 60% of the world's bioethanol in 2015, and almost 90% of the products used corn as feedstock (Mussoline et al., 2017).

Corn (*Zea mays*)

Corn, also known as Maize, is one of the most important crops in the world due to its high carbohydrate content that can be used as a raw material in different products (Semenčenko et al., 2015). In terms of cultivation, it is a warm-weather crop

so it can be grown in regions where the mean daily temperature is higher than 19 ^{o}C. For the water usage, Du Plessis, (2003) estimated that in every millimeter of water used can produce almost 10 to 16 kg of grain. For its morphological structure, corn stem can grow up to 0.6 m to 5.0 m, depends on some genotypes. It consists of eight to twenty leaves that arranged spirally on the stem. Its leaf blade can be described as long, narrow, undulating and tapers towards the tip. Its kernel contains 84% carbohydrates, 10.9% protein, 4.5 % fat and 1.3 % mineral (Du Plessis, 2003). Semenčenko et al., (2015) noted that the most important part of corn is its kernel/grain.

Corn has been used for various food products like cereals, for animal feed and for other industrial products (Zabed et al., 2016). Since corn grain is made up of 70% starch, it is also being utilized as a feedstock for bioethanol production (Semenčenko et al., 2015). According to Woiciechowski et al., (2016) the USA's corn production reached almost 13.8 billion bushels of corn in 2013-2014, and 40% is used to produce ethanol.

With the growing technology and increasing demand for bioethanol production, lignocellulosic parts of corn such as the corn stover have been subjected to different research as well (Mielenz, 2001). Corn stover has high carbohydrate content and a residue feedstock for the lignocellulose-to-ethanol process. While corn kernel has starch, corn stover contains huge quantities of cellulose (Woiciechowski et al., 2016). The composition of the corn kernel and corn stover has been compared in Table 3.

Table 3 Comparison of the corn kernel and corn stover compositions (adapted from Woiciechowski et al., 2016)

Corn Kernel	% Dry Basis	Corn Stover	% Dry basis
Starch	72	Cellulose	37.3
Hemicellulose/cellulose	10.5	Galactan/mannan	1.4
Protein	9.5	Xylan	20.6
Oil	4.5	Arabinan	2.1
Sugars	2.0	Lignin	17.5
Ash	1.5	Ash	6.1
		Acetate	2.0
		Extractives	13.0

A maximum of 2.74 gallons (98 gallons per ton at 15% moisture or 115 gallons per dry ton) of ethanol can be made from a bushel of corn, depends on the starch content whereas the maximum theoretical yield from corn stover is 107 gallons per dry ton (or 91 gallons per ton at 15% moisture (Woiciechowski et al., 2016).

Lignocellulosic source

One way to produce bioethanol is through lignocellulosic biomass, second-generation biofuels. Kim and Dale, (2004) stated that it has a major potential feedstock for bioethanol production since it is a rich source of chemicals, biopolymers, and sugar. Lignocellulosic materials are derived from plant cell walls that are mainly composed of cellulose, hemicellulose and lignin that will undergo a different process to convert into ethanol (refer to figure 2) (Chen, 2014).

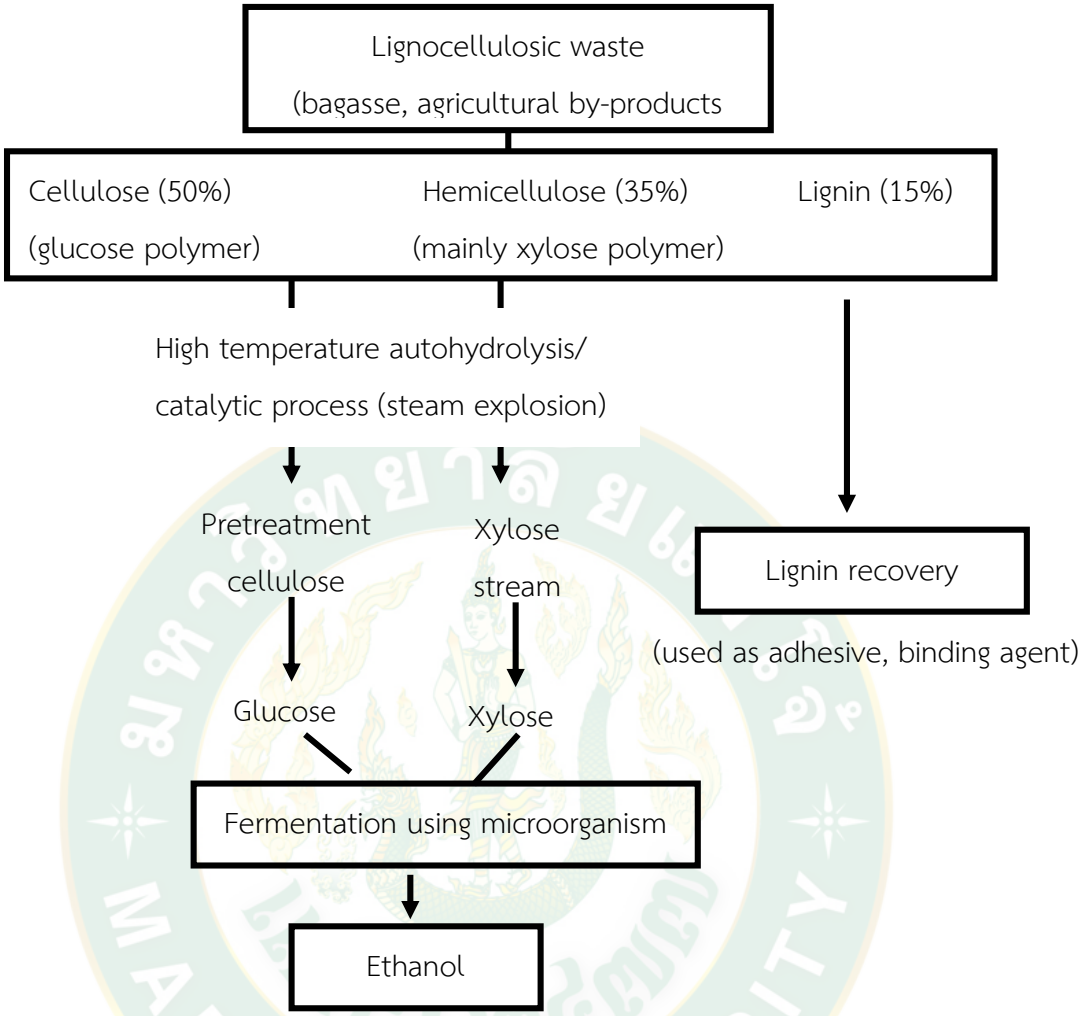


Figure 2 Conversion process of lignocellulosic biomass into ethanol (source: Balat et al., 2008)

In order to obtain bioethanol from lignocellulosic materials, it will undergone pretreatment (for delignification and release the cellulose and hemicellulose fractions), hydrolysis of cellulose and hemicellulose to obtained fermentable sugars (glucose, xylose, galactose, mannose, and arabinose) (Woiciechowski et al., 2016). Although various sources pointed out the positive impacts of the use of lignocellulosic biomass not just in the bioethanol ethanol industry but also for the environment, it is still not the main feedstock for the production like corn and sugarcane. One of the reasons is the process of converting lignocellulosic biomass to bioethanol is the cost of production.

However, the advantage of lignocellulosic biomass is its practically everywhere meaning the supply is abundant and the feedstock for production will be potentially cheap; it includes all plants, agricultural residues, herbaceous crops, forestry wastes, wastepaper, and other wastes (Wheals et al., 1999; Kim and Dale, 2004). Additionally, the use of lignocellulose materials for fuel production eradicates the competition between food versus fuel in grain-based bioethanol production (Sarkar et al., 2012). Wheals et al., (1999) estimated that the use of wastes such as agricultural, forest and municipal could replace about 40% of the US gasoline market inequivalent. Also, bioethanol produced from lignocellulosic materials can be up to 442 billion liters per year according to Balat, (2011).

Pretreatment or first stage hydrolysis

The main challenge of producing bioethanol from lignocellulosic materials will be the feedstock pretreatment. This step needs extensive processing to release the polymeric sugars in cellulose and hemicellulose which contributed about 20-53% of plant materials. Cellulose is a beta-linked glucose polymer; meanwhile, hemicellulose is a highly branched chain of xylose and arabinose that also consists of glucose, mannose, and galactose (Mielenz, 2001). The primary function of the pretreatment process is to remove lignin and hemicellulose around cellulose, to make it more accessible for further hydrolysis and fermentation (see figure 3) (Chovau et al., 2013).

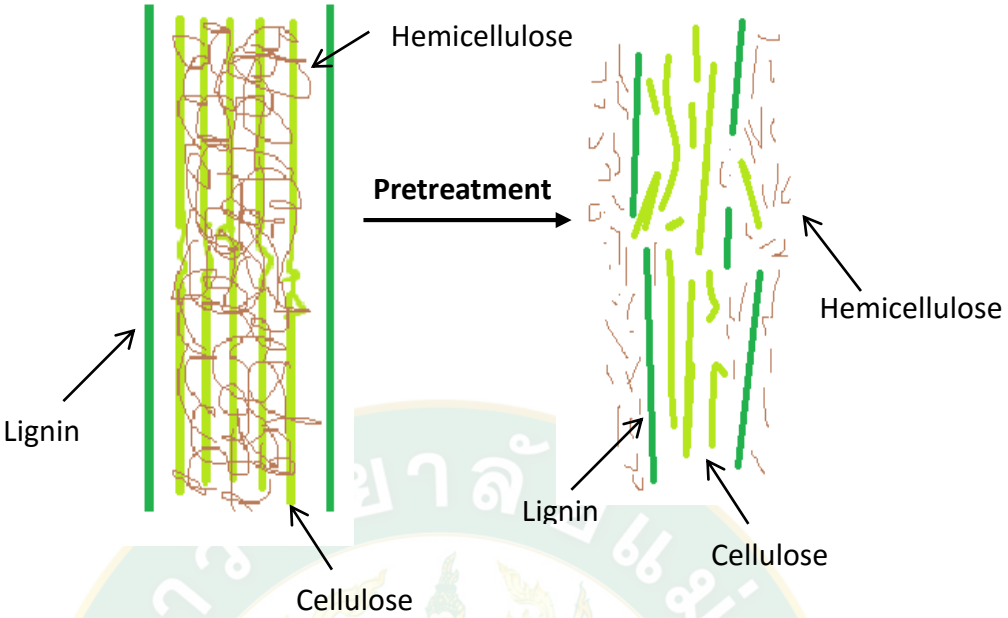


Figure 3 Role of the pretreatment process in bioethanol production from lignocellulosic materials (Adapted from Kumar et al., 2009).

The goal of pretreatment process is the following: (1) to improve the formation of sugars or the ability to form them, and (2) to avoid the formation of products that can inhibit the hydrolysis and fermentation process (Sanchez and Cardona, 2008). According to Sanchez and Cardona (2008), the yield after pretreatment exceeds 90% of the theoretical compared to 20% of the theoretical when pretreatment is not carried out. Hence, the pretreatment process for bioethanol production from lignocellulosic biomass is vital. However, different pretreatment methods have their advantage and disadvantages. These advantages and disadvantages of different pretreatment processes are illustrated in Table 4.

Table 4 Summary of different pretreatments use for lignocellulosic biomass (source: Kumar et al., 2009)

Pretreatment Process	Advantages	Limitations and Disadvantages
Mechanical comminution	reduces cellulose crystallinity	power consumption usually higher than inherent biomass energy
Steam explosion	causes hemicellulose degradation and lignin transformation; cost-effective	destruction of a portion of the xylan fraction; incomplete disruption of the lignin-carbohydrate matrix; generation of compounds inhibitory to microorganisms
AFEX	increases accessible surface area, removes lignin and hemicellulose to an extent; does not produce inhibitors for downstream processes	not efficient for biomass with high lignin content
CO_2 explosion	increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	does not modify lignin or hemicelluloses
Ozonolysis	reduces lignin content; does not produce toxic residues	large amount of ozone required; expensive high
Acid hydrolysis	hydrolyzes hemicellulose to xylose and other sugars; alters lignin structure	high cost; equipment corrosion; formation of toxic substances

Alkaline hydrolysis	removes hemicelluloses and lignin; increases accessible surface area	long residence times required; irrecoverable salts formed and incorporated into biomass
Organosolv	hydrolyzes lignin and hemicelluloses	solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Pyrolysis	produces gas and liquid products	high temperature; ash production
Pulsed electrical field	ambient conditions; disrupts plant cells; simple equipment	process needs more research
Biological	degrades lignin and hemicelluloses; low energy requirements	rate of hydrolysis is very low

Hydrolysis (Second stage hydrolysis)

Hydrolysis describes as the process of releasing sugars that are usually linked together in complex chains (Sheehan, 2000). The hydrolysis process attacks the cellulose chains to produce more fermentable sugars. This process usually catalyzed by dilute acid, concentrated acid or enzymes (cellulase). The different type of cellulose hydrolysis processes that were being done at present was listed in Table 5.

Table 5 Three different cellulose hydrolysis processes (source: Hamelinck et al., 2005)

	Consumables	Temperature (°C)	Time	Glucose Yield
Dilute acid	<1% H_2SO_4	215	3 min	50-70%
Concentrated acid	30-70% H_2SO_4	40	2-6 h	90%
Enzymatic	Cellulase	70	1.5 days	75% → 95%

The biochemical conversion of cellulose and hemicellulose through hydrolysis (eq. 2.1 -2.2) can be expressed by the reaction of hexose (eq. 2.1) and pentose (eq. 2.2) with water (Chovau et al., 2013) :

$$(C_6H_{10}O_5)_n \begin{pmatrix}\text{starch, cellulose,}\\ \text{sugar}\end{pmatrix} + nH_2O \longrightarrow nC_6H_{12}O_6 \begin{pmatrix}\text{glucose,}\\ \text{fructose}\end{pmatrix} \quad \text{(eq. 2.1)}$$

$$(C_5H_8O_4)_n \ (\text{hemicellulose}) + nH_2O \longrightarrow nC_5H_{10}O_5 \begin{pmatrix}\text{xylose, mannose,}\\ \text{arabinose, etc.}\end{pmatrix} \quad \text{(eq. 2.2)}$$

Chovau et al. (2013) stated that the hexose and pentose maximum theoretical yield per kg of glucan and xylan is 1.136 kg and 1.111 kg, respectively.

Fermentation

Fermentation is the biological process that involves microorganisms usually bacteria, yeast or fungi to convert sugars (hexoses and pentoses) into ethanol (Chovau et al., 2013) Ethanol produced from different biomass through fermentation (eq. 2.3-2.4) involves the following biochemical reactions (Guo et al., 2015):

$$C_6H_{12}O_6 \longrightarrow 2CH_3CH_2OH\ (\text{ethanol}) + 2CO_2 \quad \text{(eq. 2.3)}$$

$$C_5H_{10}O_5 \longrightarrow 5CH_3CH_2OH\ (\text{ethanol}) + 5CO_2 \quad \text{(eq. 2.4)}$$

According to Chovau et al., (2013), the maximum theoretical yield of per kg sugar (hexoses and pentoses) for ethanol and CO_2 is 0.511 kg and 0.489 kg, respectively. Therefore, the overall ethanol theoretical yield at 20 ^{o}C becomes 0.719 and 0.7361 L per kg of glucan (C_6) and xylan (C_5), respectively.

Separate hydrolysis and fermentation (SHF)

Separate hydrolysis and fermentation, also known as SHF, is a configuration employed in the fermentation of biomass hydrolysates. This involves a sequential process of hydrolysis (saccharification) and fermentation that carried in separate units (Sanchez and Cardona, 2008). This process can optimize each independent step. Additionally, the use of different microorganism for fermenting different sugars is possible. However, one of the drawbacks of this process is the cost since it requires two separate reactors and the high glucose concentrations can inhibit fermenting organism (Chovau et al., 2013).

Simultaneous saccharification and fermentation (SSF)

Simultaneous saccharification and fermentation (SSF) performed the hydrolysis (saccharification) and fermentation at the same time (Sanchez and Cardona, 2008). Unlike SHF, SSF only needed one reactor; hence the cost for constructing two reactors will lessen. Also, the inhibition of glucose on the fermenting organism will diminish. Nevertheless, the temperature that will work on both enzymatic hydrolysis and fermentation must be chosen carefully due to the different temperature required by the two process (Chovau et al., 2013).

Yeast *(Sacchomyces cerevisae)*

Yeast (*Sacchomyces cerevisae)* have been the subject of various research due to their importance in biotechnology areas such as environmental technologies, fermentation, food, chemical, and pharmaceutical industries (Díaz-Nava et al., 2017). This microorganism played an important role in the production of bioethanol because it has the ability to ferment a wide range of sugars to ethanol (Mohd Azhar et al., 2017). It is often used in research since it can be easily manipulated and culture.

Immobilized yeast

Since yeast has been mainly used in fermentation, several ways have been invented to optimize it for fermentation. Yeast immobilization, unlike the traditional yeast systems that use freely suspended yeast cells in the reactors that can only do one-time fermentation, offers continuous fermentation. Higher conversion rates, faster fermentation rates, improved product consistency, reduced product losses, and environmental advantages are the benefits of continuous fermentation. There are various immobilization techniques (as seen in figure 4) based on the physical mechanism of the cell localization and the nature of support mechanisms. Attachment to the surface, entrapment within a porous matrix, containment behind a barrier and self-aggregation are some of the basic ways to immobilized yeast for continuous fermentation (Verbelen et al., 2006).

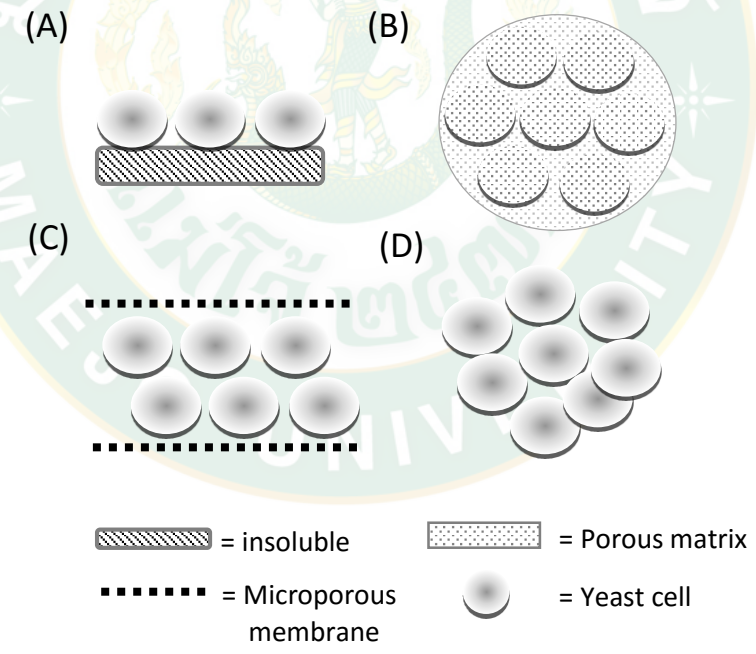


Figure 4 Simple methods for yeast immobilization: (A) attachment to the surface, (B) entrapment within porous matrix, (C) containment behind a barrier and (D) self-aggregation (Adapted from Verbelen et al., 2006).

Distillation process

Bioethanol produced through fermentation contains not only ethanol but also water. The goal of distillation is to remove water from the mixture in order to obtain high ethanol concentration (Chovau et al., 2013). Distillation process can be done through boiling, since ethanol had low boiling point (78.3 °C) compare to water (100 °C); ethanol will then have vaporized then condensed and separated from the water. The distillation of ethanol removes the remaining water through the different process such as chemical dehydration process, dehydration by vacuum, distillation process, azeotropic distillation process, extractive distillation process, membrane process, adsorption process, and diffusion distillation process (Cutzu and Bardi, 2017).

Kinetic model for Bioethanol Production

Fermentation kinetic model consists of different mathematical equations have been made to predict the phenomena occurring during the fermentation process. It can be divided into three: the growth model, substrate model, and product model. The following equations were from the paper published by Wang et al., (2004) and the definition of the terms where describe in Table 6:

$$\frac{dx}{dt} = \mu_m x \left(1 - \frac{x}{x_m}\right) \quad \text{(eq. 2.5)}$$

For equation 2.5, it is a logistic model derived for cell concentration, X, where μ_m refers to the maximum specific growth rate concerning the fermentation conditions such as: t=0 ∴X=X_0, S= S_0, P=0

$$x = \frac{x_o x_m e^{\mu_m t}}{x_m - x_0 + x_o e^{\mu_m t}} \quad \text{(eq. 2.6)}$$

Equation 2.6 is a kinetic model formulated by the integration of Eq. 2.5. This refers to as the biomass production rate yields where the relationship between the biomass and fermentation time is shown. Two parameters such as μ_m and X_m can be estimated by using a mathematical software SAS system experimental data.

$$\frac{dP}{dt} = Y_{p/x} \frac{dX}{d(t-\Delta t)} \qquad \text{(eq. 2.7)}$$

The equation 2.7 recognized that there is a delay of ethanol production during a yeast lag growth phase thereby parameter lag time, Δt, was introduced in the equation.

$$P = Y_{p/x} \left[\frac{x_o x_m e^{\mu_m (t-\Delta t)}}{x_m - x_0 + x_o e^{\mu_m (t-\Delta t)}} - \frac{x_o x_m e^{-\mu_m \Delta t}}{x_m - x_0 + x_o e^{-\mu_m \Delta t}} \right] \qquad \text{(eq. 2.8)}$$

Equation 2.8 was developed from the integration of the parameters μ_m and X_m from eq. 2.6.

$$-\frac{dS}{dt} - \frac{1}{Y_{x/s}} \cdot \frac{dX}{dt} + m \cdot x \qquad \text{(eq. 2.9)}$$

Meanwhile, equation 2.9, which describe the substrate consumption rate for the alcoholic fermentation process, has taken two aspects into account: sugar consumption in the formation of biomass and the maintenance of biomass.

$$S = s_o - \frac{1}{Y_{x/s}} \left[\frac{x_o x_m e^{\mu_{m^i}}}{x_m - x_0 + x_o e^{\mu_{m^t}}} - X_0 \right] - \frac{x_m m}{\mu_m} \ln \frac{x_m - x_0 + x_0 e^{\mu_{m^t}}}{x_m} \qquad \text{(eq. 2.10)}$$

Lastly, equation 2.10 which refers to the sugar consumption equation is a combination of Eq. 2.5 and 27 plus the estimated parameters. For parameters estimation, initial values such as X_0 and S_o were remained fixed by experimental conditions. Whereas, parameters: μ_m, X_m, Δt, *m* and some yield coefficient were estimated from the experimental data by SAS 8.01 system.

Table 6 Equations definition of terms and symbols (source: Akpan et al., 2008)

Term/Symbol	Definition	Unit
X	Biomass concentration	g/L
X_m	Maximum biomass concentration	g/L
X_0	Initial biomass concentration	g/L
M_s	Maintenance coefficient	g sugar/g biomass h
Δt	Lag time	H
T	Time	H
P	Produced ethanol concentration	g/L
S	Fermentable sugar concentration	g/L
S_0	Initial fermentable sugar concentration	g/L
$Y_{p/x}$	Yield coefficient of ethanol on biomass	g ethanol/g biomass
$Y_{x/s}$	Yield coefficient of biomass on sugar	g biomass /g sugar
M	Specific growth rate	h^{-1}
μm	Maximum specific growth rate	h^{-1}

Chapter III

MATERIALS AND METHODS

This study was divided into two: the experimental part and the analysis part. The complete method for both experimental and analysis part is illustrated in figure 5.

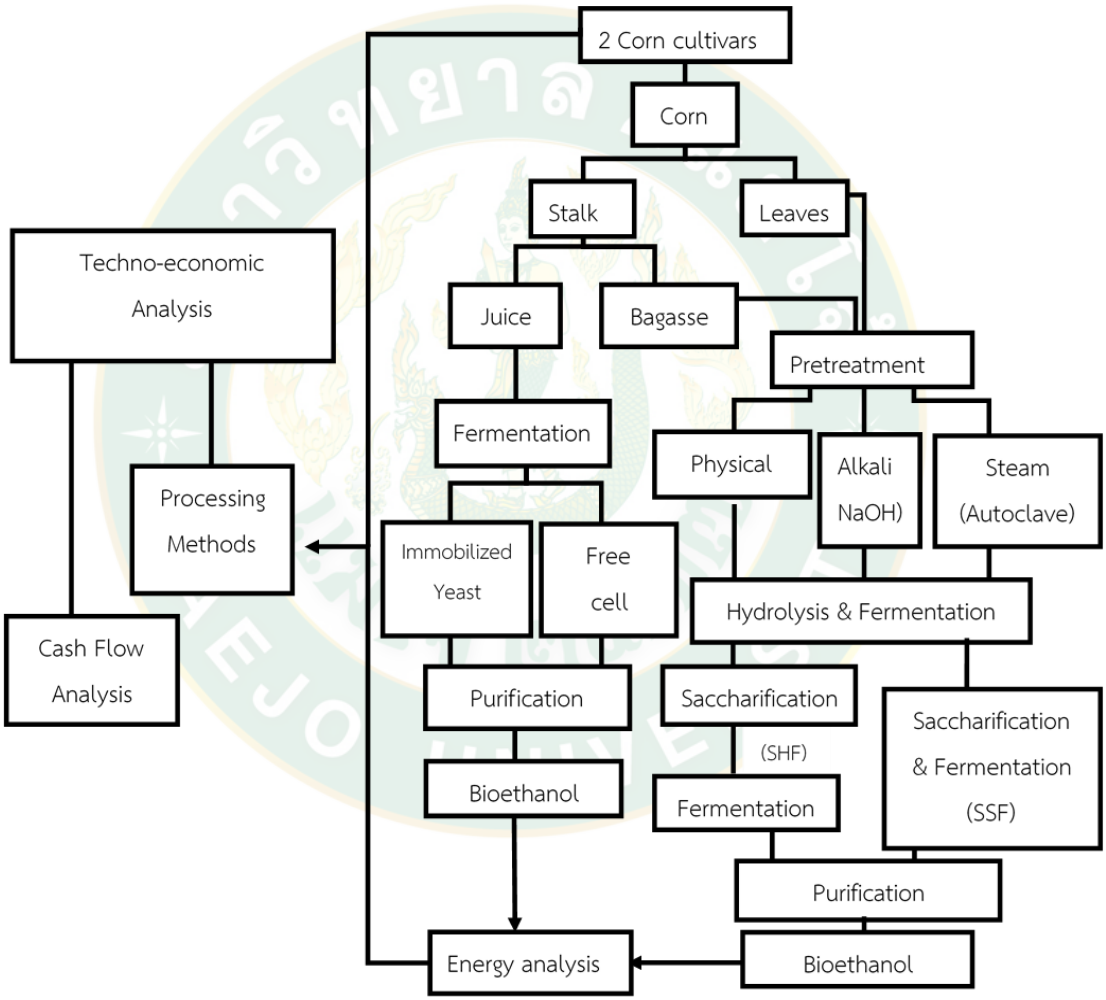


Figure 5 Experimental design of the study

Material collection and preparation

Corn materials were collected at Ban Hong, Bang Hong District, Lamphun 51130 (18°18’37” N, 98°47’34” E) and transported back to Energy Research Center, Maejo University, Sansai, Chiang Mai 50290. Sample collection was done on November 2017, February and November 2018 (figure 6).

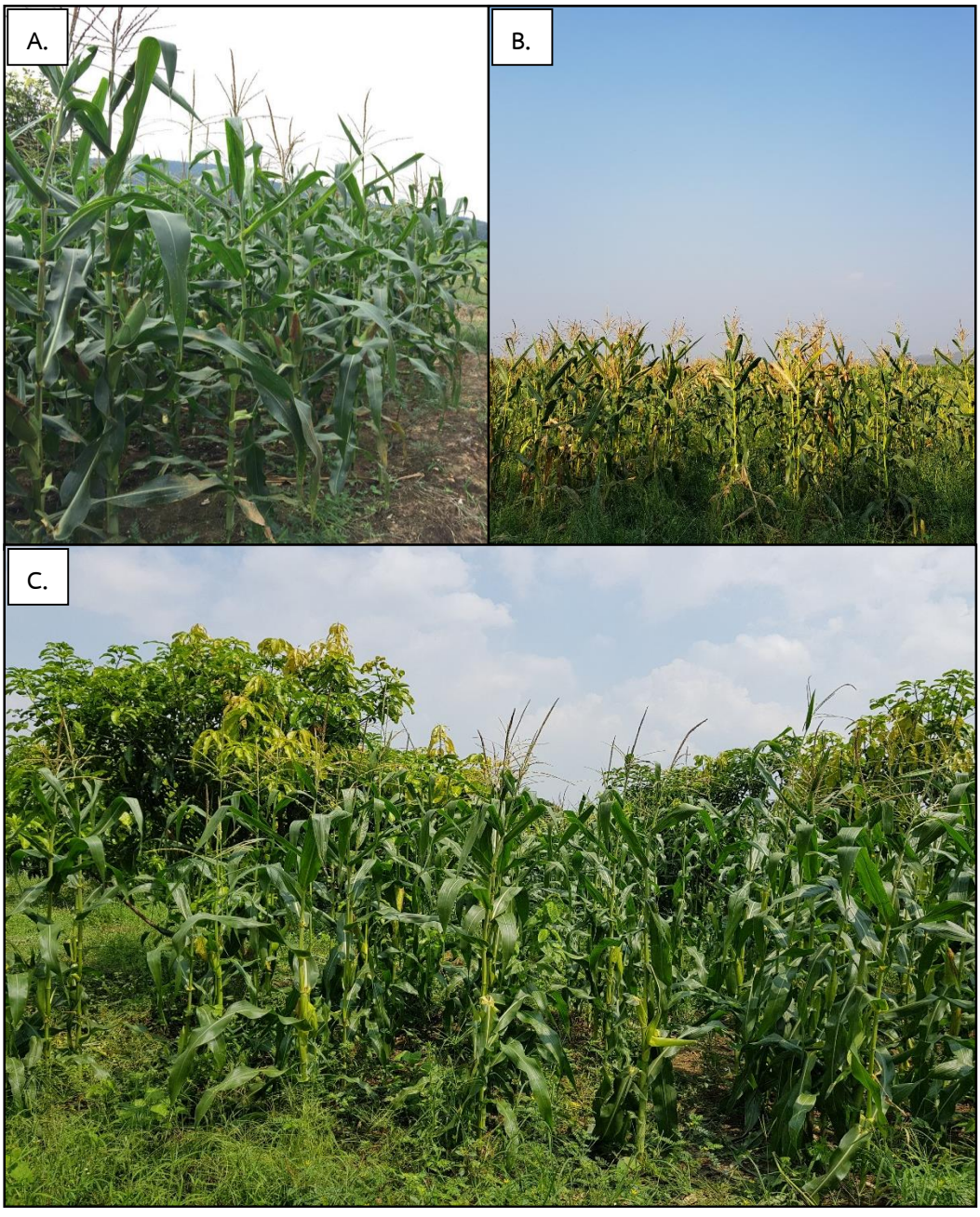


Figure 6 Sample location and collection: (A) November 2017, (B) February 2018, (C) November, 2018.

This study used the lignocellulosic part or corn, as well as its corn stalk juice for bioethanol production. Corn materials were separated by stalk and leaves (figure 7) It was then transported to Energy Research Center, Maejo University. The stalk was undergone juice extraction using sugarcane juice extractor machine. Meanwhile, the stalk bagasse and the leaves were dried, powdered and placed to an oven at 50 °C for 4 hours. The extracted juice, powdered stalk bagasse, and leaves were the desired condition for bioethanol production

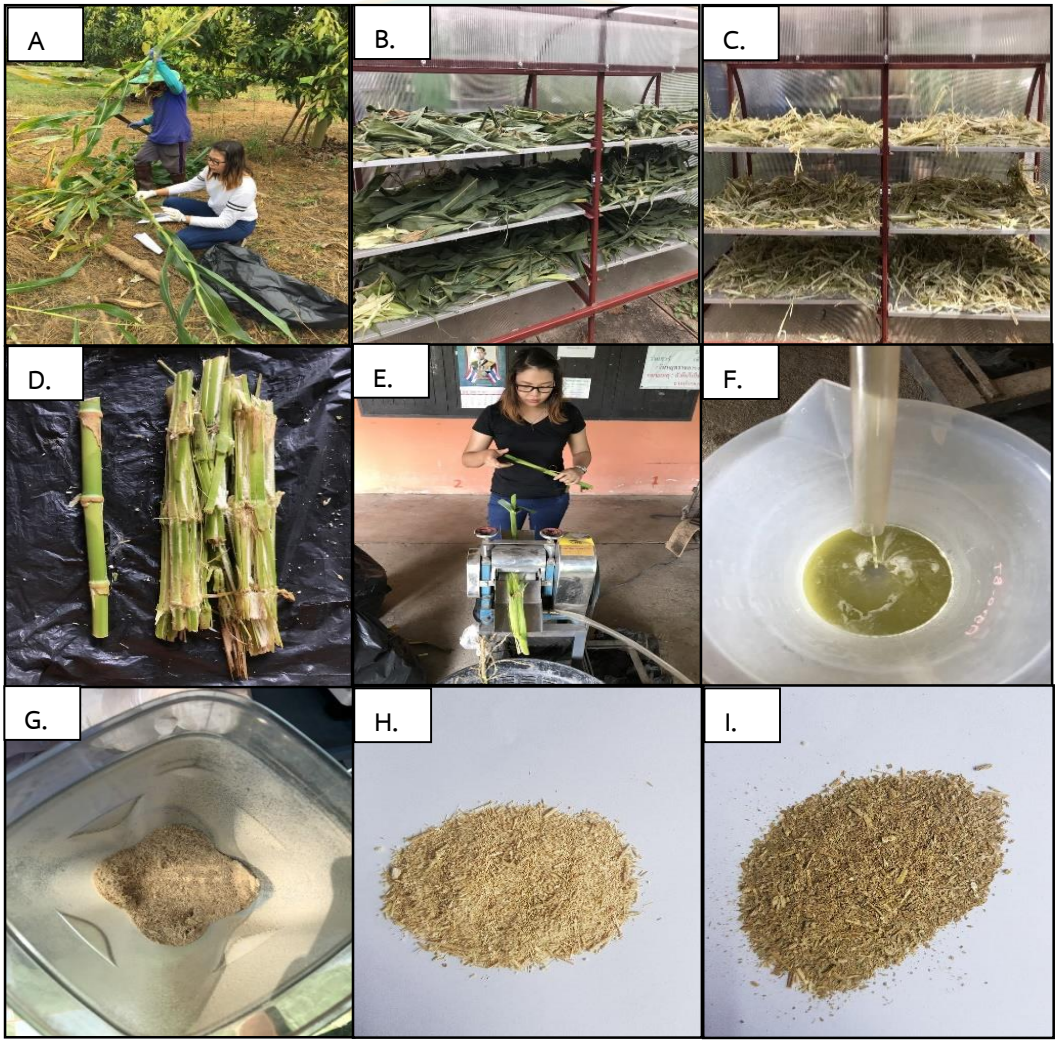


Figure 7 Sample preparation: (A) leaves and stalk separation, (B) drying of leaves (C) dying of stalk bagasse, (D) stalk and stalk bagasse, (E) juice extraction, (F) stalk juice, (G) powdered sample, (H) stalk bagasse sample, and (I) leaves sample.

Experiment 1: Stalk juice bioethanol production

The stalk juice was boiled in the laboratory to sterilize the samples. After cooling, the juice pH was adjusted to 5.6 using sodium hydroxide (Merck kGaA, Germany). Two methods were tried for juice fermentation: free cell yeast and immobilized yeast (figure 8).

Figure 8 Readily fermentable sugar from corn stalk juice (top)
free cell yeast used for fermentation (lower left)
and immobilized yeast (lower right).

Yeast preparation

Yeast strain, *S. cerevisiae* TISTR 5020, were used in this study. It was then grown in a YPD (Yeast Extract-Peptone-Dextrose) medium using 20 g/L glucose (Union Science Co., Ltd), 10 g/L yeast extract (Himedia Laboratories, India). and 10 g/L peptone (Himedia Laboratories, India). It was then sterilized at 121 °C for 15 min using autoclaved. The seed culture of *S. cerevisiae* was grown at room temperature and was agitated using a magnetic stirrer for 24 h. The broth was then used for free cell yeast

fermentation. Meanwhile, the remaining medium was 1000 rpm for 15 min to separate the *S. cerevisiae* cell to the remaining medium for yeast cell immobilization.

For the preparation of immobilized yeast, concentrated yeast cell was injected in a substrate. The substrate used for cell immobilization was 2 cm cotton balls wrapped using cloth mesh and string thread. A total of 2mL of *S. cerevisiae* were injected inside the cotton ball using a sterilized syringe.

Fermentation of the corn stalk juice

The fermentation was carried out in a 1 L bottle (figure 9). For free cell yeast fermentation, 10% of the yeast (*S. cerevisiae* TISTR 5020) with 1×10^7 cell mL^{-1} has been added to the juice. The mixtures were then incubated with a maintaining temperature of 36 ^{o}C for 120 hours. For immobilized yeast, 15% of immobilized yeast were added to each bottle. It was also incubated at 36 in a span of 120 hours. Then, the immobilized yeast was used again for another batch of fermentation to test the effectivity of the substrate to hold the yeast cell. The immobilized yeast was used for 3 batch of fermentation each lasted for 120 hours. All experiments were performed in triplicates. The ethanol content, sugar concentration, and pH were monitored for every 24 hours.

Figure 9 Stalk juice fermentation: immobilized yeast (left) and free cell yeast (right).

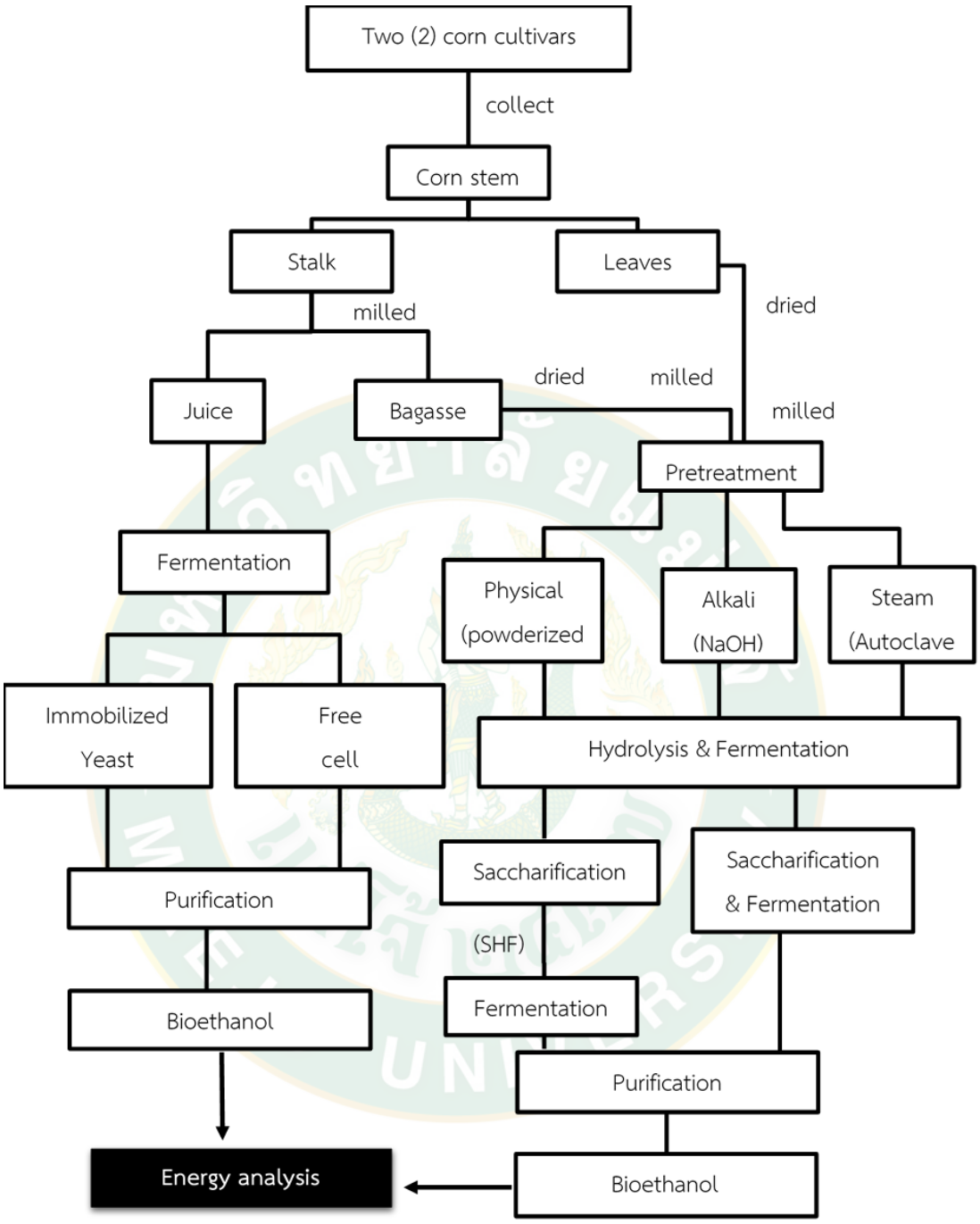


Figure 10 Experimental process of bioethanol production from corn leaves, stalk and juice.

Experiment 2: Lignocellulosic materials (leaves and bagasse) fermentation

Once the leaves and stalk were dried, these two materials were pulverized in a blender. The experiment process explained in figure 10. The leaves and stem bagasse

were undergone different pretreatment methods: physical, alkali, and steam. This process removes the lignin and hemicellulose structure around cellulose for better access to the following steps. After the pre-treatment process, the samples were undergone hydrolysis and fermentation. During hydrolysis, the released cellulose is converted into glucose. The conversion of cellulose and hemicellulose can be described as the reaction of glucan (hexose) (eq. 3.1) and xylan (pentose) with water (3.2) (Chovau et al., 2013):

$$(C_6H_{10}O_5)_n + nH_2O \longrightarrow nC_6H_{12}O_6 \quad \text{(eq. 3.1)}$$

$$(C_5H_8O_4)_n + nH_2O \longrightarrow nC_5H_{10}O_5 \quad \text{(eq. 3.2)}$$

Fermentation is a biological process in which the sugars (hexoses and pentoses) are converted into bioethanol using microorganisms such as bacteria, yeast or fungi. In this experiment, yeast (*Saccharomyces cerevisiae*) were used for the fermentation. This process involves anaerobic conditions where microorganisms were allowed to obtain energy and grow. The conversion reactions for hexoses (eq. 3.3) and pentoses (eq. 3.4) are express as (Chovau et al., 2013):

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \quad \text{(eq. 3.3)}$$

$$3C_6H_{10}O_5 \longrightarrow 5C_2H_5OH + 5CO_2 \quad \text{(eq. 3.4)}$$

For this experiment, separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) were used. The SHF configuration can be independently optimized each step and process, and the used of different microorganism for fermentation is possible. Meanwhile, SSF configuration can reduce the number of reactors by integrating both hydrolysis (saccharification) and fermentation in one system (Chovau et al., 2013). While both configurations have offered some advantage from each other, they also pose some drawbacks. Each drawback and advantage of each configuration were evaluated in the techno-analysis part. Another step is the distillation; the fermented solution was undergone distillation

using an Megahome distiller (Nutriteam Inc., Vermont, USA). One advantage of distillation is the high ethanol recovery (Chovau et al., 2013).

Batch Pretreatment method

Twenty grams (20g) of powdered sample (Hi-brix 53 stalk and leaves, and Sugarstar x Hi-brix 53 stalk and leaves) were used to test the effectivity of the three (3) pretreatment methods: physical (control), autoclave and alkaline. For physical pretreatment, this one acted as a control to test the effectiveness of mechanical pretreatment alone on lignocellulosic biomass. For autoclave pretreatment, powderized were subjected in 121°C at 15 psi for 15 min in an autoclave machine, this was done to test if heat and pressure can break the lignin crystalline structure. Finally, for alkaline pretreatment, the sample were mixed with 2 % (w/v) sodium hydroxide anaerobically with a ratio of 1:5 (w/v) for 24 hours. After pretreatment, the samples were subjected into enzymatic hydrolysis.

Optimization of Alkaline pretreatment using Response Surface Methodology

Out of all three pretreatments applied to four (4) different lignocellulosic biomass alkaline pretreatment (prior to enzymatic hydrolysis) got the highest reducing sugar and total sugar concentration. In this case, alkaline pretreatment was subjected to optimization using Design Expert version 11 (free trial) (Stat-Ease Inc., Minnesota, USA) were reducing sugar and total sugar concentration was used as a response. The independent variable tested was reaction time (X_1) with three (3) levels: 24, 48 and 72 hours. All four lignocellulosic materials were subjected to optimization, this was done to determine each pretreatment factor suited for each material.

Enzymatic hydrolysis

Hydrolysis is another vital process for lignocellulosic bioethanol production. Enzymatic hydrolysis was done using commercial cellulase enzyme (Union Science Co., Ltd, Thailand) (2398 units/g, 577 units/g beta glucosidae, pH of 4). After the pretreatment process, the pH of the sample was adjusted to 5.0. After that, a total of 2% cellulase (v/v) were added to the mixture and incubated at 50 °C for 24 hours.

SHF AND SSF Fermentation

After the determination of the optical pretreatment and pretreatment reaction time, the next step was the determination of suited fermentation technique. Ten (10) grams of sample were subjected to two different fermentation routes.

For SHF or separate hydrolysis and fermentation, after the pre-treatment the sample underwent enzymatic hydrolysis. Adjusting the pH of the solution to 5.0 was the first step, then addition of 2% cellulase (v/v) incubated at 50 °C for 24 hours were performed. After this process, the pH of the solution was adjusted to 5.6, 2% (w/v) glucose supplement and 0.5% (w/v) of alcohol active dry yeast, *S. cerevisiae, (*Angel Yeast Co., Ltd., Hubei, China) were soon added. The solution then incubated at 38 °C for 96 hours.

For SSF or simultaneous saccharification and fermentation, enzymatic hydrolysis and fermentation were done at the same time after the pretreatment process. The pH of the solution was adjusted to 5.3. Then, 2% (w/v) glucose supplement, 2% (v/v) cellulase and 0.5% (w/v) of alcohol active dry yeast, *S. cerevisiae,* were added all together to the mixture. The solution was incubated at 38 °C for 96 hours. The alcohol content and sugar concentration were checked after the 4^{th} day of incubation.

Analytical method

The parameters measured in this experiment were pH, total sugar, reducing sugar and bioethanol content. pH was checked using Multi-parameter PCSTestr 35 tester model Oakton 35425-10 (Oakton Instruments, Illinois, USA) and bioethanol content was checked using ebulliometer (Laboratoires Dujardin-Salleron, France) (figure 11). Total sugar and reducing sugar concentration were analyzed using phenol-sulfuric acid and DNS standard method, respectively (.

The ebulliometer used the different boiling point of distilled water compare to water-alcohol solutions. Bioethanol ethanol content was checked using as described by Vu et al. (2018). A calculating dial (figure 11) were used to calculate the percentage of ethanol in the solution by comparing two different boiling points from distilled water and the solution.

Total sugar content was analyze using the method of Dubois et al., (1956) with minor modification. A total of 0.5 ml of the sample were mixed with 0.5 ml of 5% (w/v) phenol (Qrec, New Zealand) and 2.5 mL of 98% sulfuric acid (RCI Lab Scan). The mixed solution was read using Spectrophotometer model DV-8000 (Drawell, Shianghai, China) at 490 nm. Reducing sugar determination were adopted from Miller, (1959) with minor modifications. A total of 0.5 ml of sample were added with 0.5 ml of DNS solution (3,5-dinitrosalicylic acid) (Sigma Aldrich). The solution was mixed using a vortex and was boiled for 15 minutes. After boiling, the solution was added with 4 ml of distilled water. The solution was read at 540 nm using Spectrophotometer model DV-8000. Glucose was used as a standard for both total and reducing sugar.

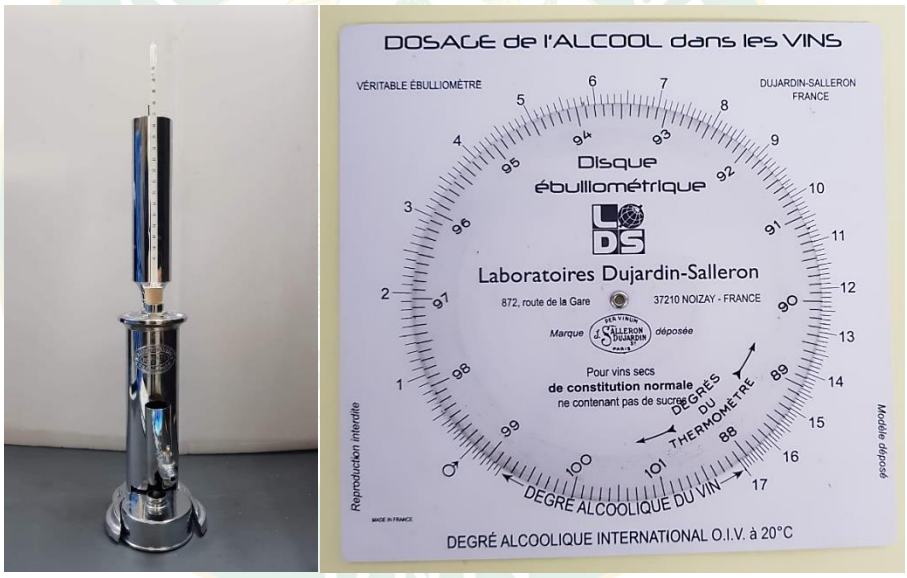


Figure 11 Ebulliometer (left) and calculating dial (right)

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 23. Data are reported as mean ±sd (n=3). ANOVA and Tukey's test were used to determine the differences between the parameters. The difference between the values were considered significant when $p<0.05$.

Analysis 1: Energy analysis

In order to determine the efficiency of the produced bioethanol from corn, an energy analysis was done. The ethanol produced was evaluated by its co-generated electricity. This was performed using literature reviews and calculations referencing to Luo et al. (2009) ethanol energy analysis study from cellulosic feedstock.

Analysis 2: Techno- economical comparison of different pretreatment and fermentation techniques for bioethanol production

This techno-economical study evaluated the different process for bioethanol production from lignocellulosic materials. This study focused on the technologies used in the experiment part. According to Chovau et al., (2013), a techno-economic model assess the potential of research developments to reduce the production cost by process designs. Also, it can be used to estimate absolute production cost of ethanol production from lignocellulosic materials by defined process and plant design assumptions. Factors and processes to create a techno-economic analysis are illustrated in figure 12.

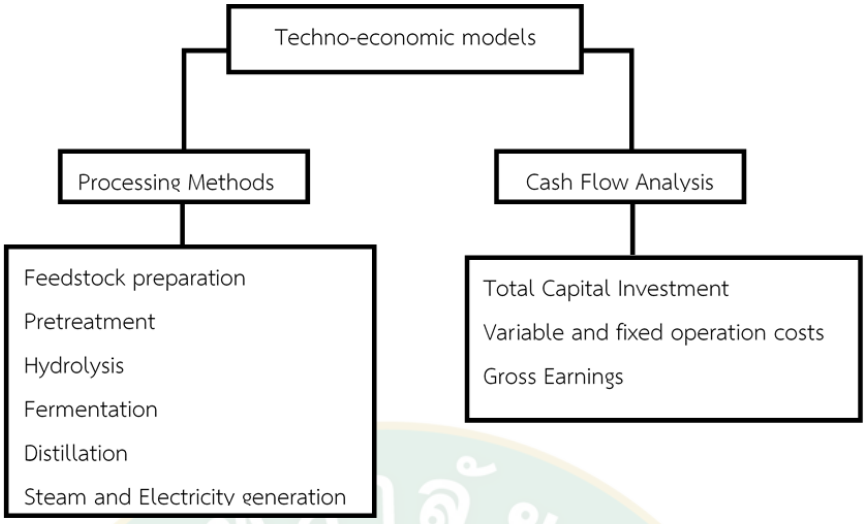


Figure 12 Techno-economic models
(Adapted from Chovau et al., 2013).

The assumptions that were used in this study were calculated using the following:

- A small pilot plant with a capacity of 15,000-25,000 L/year
- Operation of 2000h/year
- Biomass fuel required 40-60 dry tonnes/year
- Publicly available and experimentally validated reaction conversions and parameters were used.
- Equipment, chemical and labor costs were indexed to dollars
- The average corn stalk and leaves composition were based on this study or some available experimental data.

CHAPTER IV
RESULTS AND DISCUSSION

Experiment 1: Stalk juice bioethanol production

During the 120-h fermentation process, changes in the pH, sugar concentrations and ethanol content were recorded every 24 h.

Free cell yeast fermentation

Hi-brix 53 stalk juice initially contained a total sugar of 161.19±10.87 g/L and reducing sugar of 35.06±0.77 g/L (Table 7). Sugar consumption was observed at first 24 hours of fermentation. Alongside the declining sugar concentration, production of ethanol was also observed on the first 24 h of fermentation. The substrate pH level decrease from the initial value of 5.6 to 4.94±0.08 fermentation on the first 24 h of fermentation then, it increases back up to 6.15±0.09 at 120 h. These results proved that Hi-brix 53 stalk juice could produce bioethanol even without adding a supplement. Razmovski and Vučurović (2012) attained the same result: Instant pH decay in the early part of fermentation. This change in pH level may suggest the formation of other by-products, other than ethanol, that was not identified in this study. The final bioethanol production from hi-brix 53 stalk juice during the 24-120hr of fermentation ranged from 43.79±2.73 ─ 47.87±0.91g L^{-1} (5.55 %-6.01 % v/v) (Table 7). The highest final ethanol concentration was from the 24 h of fermentation; however, we found no significant differences between the values from 24-120 h of fermentation. These results on ethanol production were expected from a batch fermentation process. Zabed et al. (2014) discussed longer fermentation time affect the microbial growth due to prolonged exposure to ethanol while Nuanpeng et al. (2011) mentioned batch fermentation can have negative effect on the microorganism growth.

Table 7 Hi-brix 53 free cell fermentation. Data are presented as mean ± sd (n=3).

Incubation Time	Reducing Sugar (g/L)	Total Sugar (g/L)	Ethanol Content (g/L)	Bioethanol (%)	pH
0	35.06±0.77[a]	161.19±10.87[a]	-	-	5.6
24	3.46±0.58[b]	8.37±0.82[b]	47.87±0.91[a]	6.01±0.19[a]	4.94±0.08
48	1.21±0.10[c]	6.72±1.04[b]	47.35±1.34[a]	6.00±0.17[a]	4.88±0.07
72	1.17±0.04[c]	5.87±1.09[b]	47.65±4.58[a]	6.04±0.58[a]	5.04±0.07
96	1.34±0.13[c]	6.04±0.62[b]	46.65±1.33[a]	5.91±0.17[a]	5.66±0.04
120	2.20±0.34[c]	9.94±1.03[b]	43.79±2.74[a]	5.55±0.35[a]	6.15±0.09

*values with the same letters are not significant ($p<0.05$)

The second variety of corn used for stalk juice free cell yeast fermentation was sugarstar x hi-brix 53 hybrids with an initial sugar concentration of 61.83±0.60 g/L reducing sugar and 118.57±2.62 g/L of total sugar (Table 8). Sugar consumption was observed on the 24th h of incubation time. Compare to hi-brix 53, this variety attained the highest ethanol concentration on the 72 h of fermentation and have a slightly higher ethanol concentration. Still, no significant difference was found among the values of ethanol content (g/L).

Table 8 Sugarstar x Hi-brix53 stalk juice free cell fermentation. Data are presented as mean ± sd (n=3)

Incubation Time (h)	Reducing Sugar (g/L)	Total Sugar (g/L)	Ethanol Content (g/L)	Bioethanol (%)	pH
0	61.83±0.60^{a}	118.57±2.62^{a}	-	-	5.6
24	2.59±0.31^{b}	6.578±0.15^{b}	47.05±5.02^{a}	5.96±0.64^{a}	4.95±0.10
48	2.20±0.17^{bc}	6.97±0.35^{b}	44.08±1.55^{a}	5.59±0.20^{a}	4.89±0.02
72	2.23±0.07^{bc}	6.87±0.18^{b}	48.71±1.00^{a}	6.17±0.13^{a}	4.86±0.03
96	2.14±0.18^{bc}	6.81±0.38^{b}	48.08±0.24^{a}	6.09±0.03^{a}	4.84±0.05
120	1.57±0.24^{c}	5.66±0.31^{b}	47.34±0.00^{a}	6.00±0.00^{a}	4.36±0.11

*values with the same letters are not significant ($p<0.05$)

As for the change of pH of the solution, Lin et al. (2014) mentioned the influence of pH in terms of ethanol production and by-products formation. It can also be used as an indicator of the products that have been formed in the process of fermentation. For example, in the pH of 5.5-6.0 the main product would be ethanol and butyrate, whereas pH lower than 5.0, the main product would be acetic acid.

The study of Laopaiboon et al. (2007) shows improves ethanol production and overall efficiency rate on the fed-batch fermentation compare to batch fermentation. Phukoetphim et al. (2017) found a 51% increase in ethanol concentration and ethanol productivity on fed-batch fermentation with continuous feeding compare to batch fermentation. With this, different fermentation techniques and process may be applied in improving the overall efficiency of hi-brix 53 stalk juice for bioethanol production.

Immobilized yeast cell fermentation

In this study, corn stalk juice straight-up undergone fermentation without adding any supplement. Each batch fermentation (a total of 3 batch fermentation) incubated for 120 h. The difference in the bioethanol concentration on each batch may due to some parameters that affect fermentation that wasn't analyzed in this study. Munnecke (1981) explained that factors such as temperature, pH, sugar, and

ethanol could influence the fermentation process. Kang and Lee (2015) added that a change in these parameters might hinder the ability of the microorganisms to convert sugar into ethanol. Nevertheless, these result shows immobilized yeast cell reusability and cotton as an effective support material for cell immobilization (Table 9 and Table 10).

For hi-brix 53, the highest ethanol concentration was observed to be at the highest on the 120 h or fermentation in each batch (Table 9). Sugar consumption found to be directly proportional to the ethanol content concentration.

Table 9 Hi-brix 53 immobilized yeast fermentation. Data are presented as mean ± sd (n=3).

	Incubation Time (h)	Batch 1	Batch 2	Batch 3
Reducing	0	20.00±0.44^{a}	25.56±1.67^{cd}	31.83±1.59^{c}
Sugar (g/L)	24	13.33±2.03^{b}	86.45±5.27^{a}	65.56±4.86^{a}
	48	6.18±2.32^{c}	68.06±13.79^{b}	48.72±3.13^{b}
	72	1.58±0.15^{d}	55.83±1.92a^{b}	31.22±1.97^{c}
	96	1.57±0.04^{d}	28.50±7.95^{c}	20.39±5.55^{d}
	120	1.77±0.28^{d}	6.50±3.21^{d}	17.72±1.78^{d}
Total	0	122.24±7.74^{a}	143.57±13.97^{a}	156.10±4.16^{a}
Sugar	24	26.86±1.44^{b}	107.76±5.31^{b}	93.14±5.35^{b}
(g/L)	48	10.02±2.88^{c}	81.81±15.54^{bc}	68.81±6.60^{c}
	72	4.17±0.78^{c}	76.14±7.89^{c}	45.28±2.20^{d}
	96	4.20±0.15^{c}	43.10±9.59^{d}	35.14±9.04^{de}
	120	4.63±0.51^{c}	14.79±2.27^{e}	24.57±1.08^{e}
Ethanol	0	-	-	-
Content	24	22.23±2.38^{b}	5.87±0.75^{d}	2.31±0.47^{d}
(g/L)	48	34.03±2.16^{a}	8.52±1.96c^{d}	7.88±1.02^{c}
	72	30.51±0.91^{a}	13.62±2.96^{c}	13.72±2.85^{b}

	96	34.98±1.98[a]	22.99±1.90[b]	17.75±3.37[b]
	120	34.45±1.64[a]	33.81±2.88[a]	23.80±0.60[a]
Bioethanol	0	-	-	-
(%)	24	2.82±0.30[b]	0.74±0.09[d]	0.29±0.06[d]
	48	4.31±0.27[a]	1.08±0.25[cd]	1.00±0.13[c]
	72	3.87±0.12[a]	1.73±0.38[c]	1.74±0.36[b]
	96	4.43±0.25[a]	2.91±0.24[b]	2.19±0.43[b]
	120	4.37±0.21[a]	4.29±0.36[a]	3.02±0.08[a]
pH	0	5.6	5.6	5.6
	24	4.18±0.05	3.84±0.17	3.98±0.07
	48	3.79±0.10	3.46±0.10	3.60±0.04
	72	3.63±0.30	3.30±0.10	3.54±0.04
	96	3.62±0.20	3.35±0.04	3.55±0.02
	120	3.60±0.06	3.41±0.04	3.55±0.03

*values with the same letters are not significant ($p<0.05$)

The maximum bioethanol concentration—7.87% (v/v), were achieved on the 24 h of the first batch fermentation (Table 10). The second batch of fermentation achieved its highest bioethanol concentration with 0.67% (v/v) on the 120 h of incubation. Meanwhile, 3rd batch of fermentation achieved 5.26% of bioethanol concentration at 120 h. Initial total sugar concentration from each batch fermentation ranges from 137.95-180.62 g/L. The first batch of fermentation operated with 137.95 g/L of total sugar and resulted with 62.12 g/L of ethanol production on the 24th h of incubation time. Ethanol production steadily declines from this point of fermentation. This may be due to a lower sugar concentration as per 24 h, sugars were reduced up to 8.66 g/L. Sugar concentration and ethanol concentration relationship were directly proportional. Another, we found no significant difference between the values of 24 h, 48 h, and 72 h, and also the values from 48-120 h of fermentation.

Table 10 Sugarstar x Hi-brix 53 immobilized yeast fermentation. Data are presented as mean ± sd (n=3)

	Incubation Time	Batch 1	Batch 2	Batch 3
Reducing Sugar (g/L)	0	42.06±3.71[a]	68.00±1.37[c]	22.05±2.88 [b c]
	24	2.70±0.14[b]	125.78±1.07[a]	100.22±2.27[a]
	48	2.52±0.08[b]	125.55±4.60[a]	52.56±14.52 [b]
	72	2.48±0.13[b]	124.33±6.81[a]	23.11±14.26 [b c]
	96	2.53±0.23[b]	132.89±3.37[a]	20.42±21.61 [b c]
	120	2.13±0.45[b]	128.33±14.67[a]	2.44±0.71 [c]
Total Sugar (g/L)	0	137.95±1.87[a]	180.62±20.16[a]	146.95±16.02 [a]
	24	8.66±0.76[b]	142.81±11.91 [b]	128.48±17.68 [ab]
	48	8.10±0.21[b]	134.90±2.65 [b]	114.33±21.33 [ab]
	72	8.25±0.38[b]	133.19±4.78 [b]	108.43±15.16 [ab]
	96	9.49±0.57[b]	137.00±5.85 [b]	99.38±8.08[b]
	120	9.60±0.15[b]	132.67±3.79 [b]	11.07±1.31[c]
Ethanol Content (g/L)	0	-	-	-
	24	62.12±4.04[a]	4.74±2.37[a]	1.13±1.95
	48	58.97±1.18[ab]	4.20±0.33[a]	11.35±13.24
	72	57.00±0.98[b]	4.36±0.81[a]	19.97±22.72
	96	56.40±0.03[b]	3.84±1.24[a]	19.83±22.88
	120	54.13 ± 0.98[b]	5.26±1.27[a]	44.97±0.79
Bioethanol (%)	0	-	-	-
	24	7.87±0.51[a]	0.60±0.30[a]	0.14±0.25
	48	7.47±0.15[ab]	0.53±0.04[a]	1.44±1.68
	72	7.22±0.12[b]	0.55±0.10[a]	2.53±2.88
	96	7.15±0.01[b]	0.49±0.16[a]	2.51±2.90
	120	6.89±0.09[b]	0.67±0.16[a]	5.70±0.10

pH	0	5.60	5.60	5.60
	24	4.39±0.05	5.43±0.10	4.92±0.09
	48	5.26±0.01	5.62±0.04	4.84±0.26
	72	5.26±0.02	5.64±0.04	4.78±0.20
	96	5.28±0.04	5.59±0.04	4.54±0.13
	120	5.28±0.02	5.46±0.10	4.45±0.08

*values with the same letters are not significant ($p<0.05$

Initial total sugar concentration from each batch fermentation ranges from 137.95-180.62 g/L (Table 10). The first batch of fermentation operated with 137.95 g/L of total sugar and resulted with 62.12 g/L of ethanol production on the 24th h of incubation time. Ethanol production steadily declines from this point of fermentation. This may be due to a lower sugar concentration as per 24 h; sugars were reduced up to 8.66 g/L. Sugar concentration and ethanol concentration relationship were directly proportional. Another, we found no significant difference between the values of 24 h, 48 h, and 72 h, and also the values from 48-120 h of fermentation.

Second batch fermentation shows a poor production of bioethanol with 5.6 g/L on the 120 h. All values from 24-120 h are found to be not significant to each other. This value was lower compared to the 1st batch fermentation. One reason for low ethanol productivity may be due to its due high initial sugar concentration (Table 2). Thus, high initial sugar concentration doesn't necessarily mean high ethanol concentration. Sridee et al. (2012) revealed that high sugar concentration may inhibit yeast metabolism due to the increase of osmotic pressure, that may result in low ethanol concentration. They offered a solution to this problem by acclimatizing inoculum under high sugar concentration.

On the other hand, Laopaiboon et al. (2007) mentioned that this case was expected in batch fermentation. Laopaiboon and Laopaiboon, (2012) revealed that microorganisms in batch fermentation were greatly affected by product inhibition. A different fermentation system such as fed-batch or continuous system should be investigated.

The third batch of fermentation offers a different scenario: Ethanol production was seen to increase through longer incubation time (Table 10). The values from 48, 72, 96 and 120 h are found to be not significant to each other while benefits from 24 and 120 h have a considerable difference. This time it may be due to catabolite repression, where various sugars were present, resulting in the slower conversion of sugar to ethanol (Munnecke, 1981). This sequential sugar metabolism degrades glucose first before other sugars (Kang and Lee, 2015).

Stalk juice from both corn variety contains a high amount of readily fermentable sugar—production of ethanol is possible without the addition of a supplement. Additionally, corn (*Zea mays*) juice can compete with other energy crops juice as feedstock for bioethanol production. The researchers suggested two things from this paper: application of different fermentation techniques for stalk juice and further study of different corn cultivars as bioethanol feedstock.

Continuous fermentation using immobilized yeast

Improvise fermenter was created and design to performed a scale-up continuous fermentation using immobilized yeast (figure 13). The improvise fermenter consists of three openings: inlet, outlet, and bubbler. It has a volume 0f 1.25 L. The inlet and outlet were sealed while the bubbler kept the system anaerobic while letting carbon dioxide production through fermentation escape from the system. There was a 700 mL working volume with 10% immobilized yeast (*S. cerevisiae)* with an incubation time of 24 h. The immobilized yeast was used up to 5 cycles.

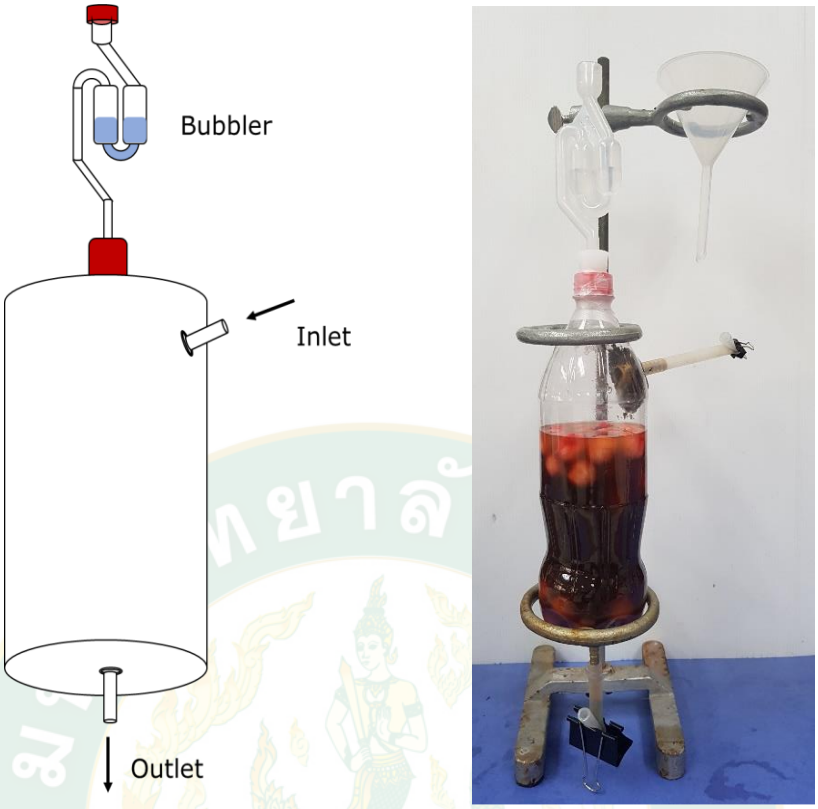


Figure 13 Improvise fermenter for stalk juice continuous fermentation: design (left) and actual device (right)

The six-month-old stalk juice produces an average of 29.04 g/L (3.68 % v/v) (Table 11). The ethanol production was lower compared to the batch fermentation; however, the consistency of the values of ethanol suggest that the juice fermentable sugar may be degraded from the fresh one. This result suggests that a six-month-old stalk juice can still be used for bioethanol fermentation. After distillation, the ethanol content was found to be 126.24 g/L (16% v/v).

Table 11 Bioethanol from immobilized yeast continuous fermentation

Cycle	% Alcohol (v/v)	Produce ethanol (g/L)
1	3.50	27.62
2	3.80	29.98
3	3.70	29.19
4	3.70	29.19
5	3.70	29.19
Average	**3.68**	**29.04**
Distillation	**16.0**	**126.24**

Experiment 2: Lignocellulosic bioethanol production

Batch Pretreatment

The effectivity of each pretreatment method was evaluated based on the sugar concentrations before hydrolysis (figure 14). Pretreatment process breaks down lignin barriers making it easy for the enzymes to access hemicellulose and cellulose. Three pretreatments were performed in this study: physical (control), autoclave and alkaline. As the sample particle size affects greatly the enzymatic hydrolysis, physical pretreatment was performed. Autoclave and alkaline were also done with the same particle size. The physical pretreatment, powdered sample straight up gone enzymatic hydrolysis, this also acts the control of the group. Alkaline pretreatment (powderized sample were added with NaOH) is the most suitable pretreatment to use with the highest sugar content observed in all plant materials that were tested. Autoclave method is not sufficient enough to disrupt lignin structure shows a poor result of reducing sugar and total sugar concentration. Values for the physical and autoclave were found no significant difference from each other. With this, the suitable pretreatment particle applied for corn stalk bagasse was NaOH with powderized size.

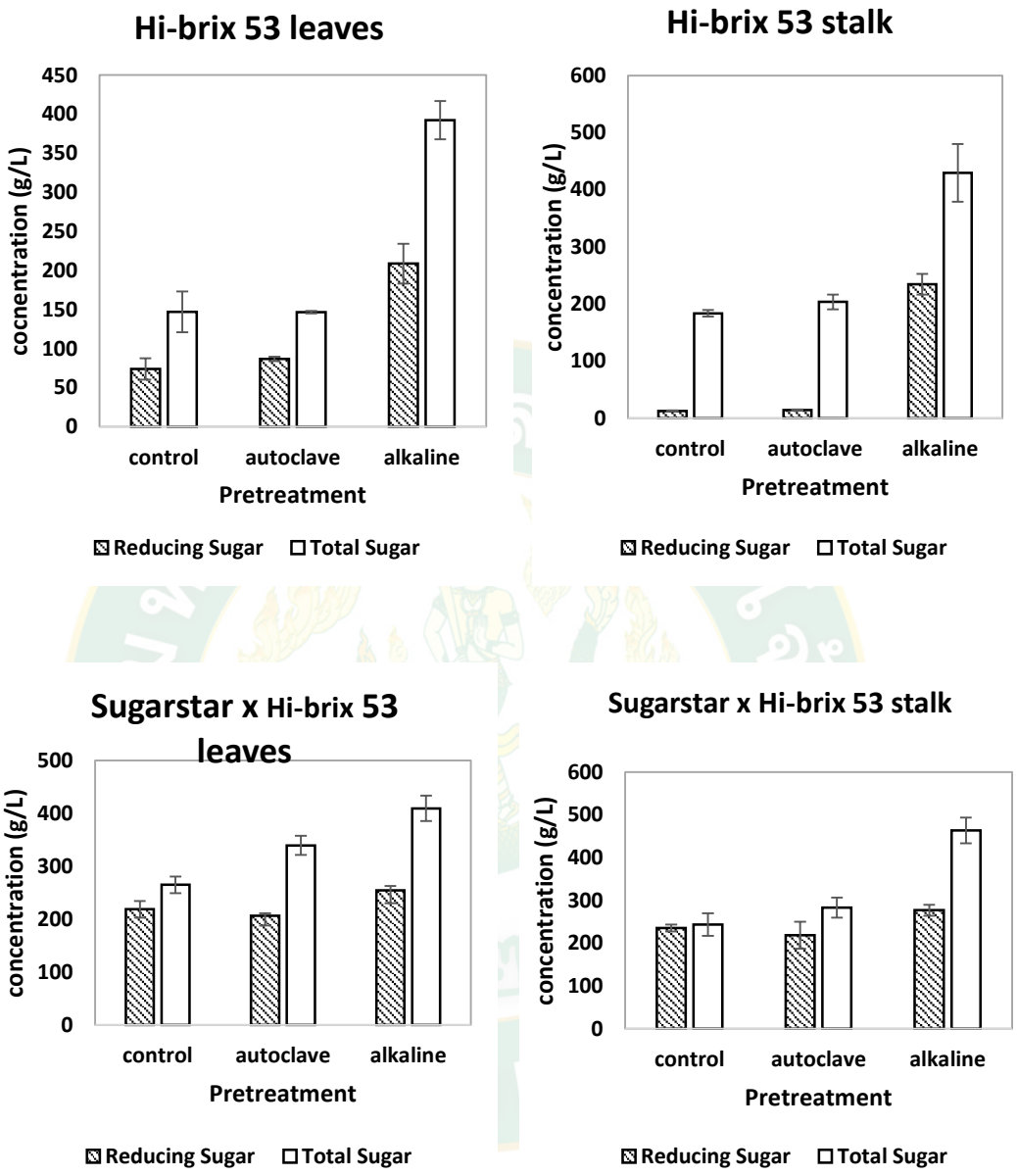


Figure 14 Effect of different pretreatment methods on different plant material reducing sugar and total sugar concentration. Data were presented as mean, error bar as sd (n=3).

Optimization of alkaline pretreatment using RSM

Response surface method was used for the optimization of alkaline pretreatment. Design type is I-optimal point exchange and randomized sub-type. Design model used was linear and quadratic based on analysis of the software. Designs

were allowed 6 runs on the reaction time conditions. A total of 10 g of sample were undergone alkaline pretreatment with one factor involve: reaction time, *X* (h). The variable has 3 level: 24-72 h reaction time. Reducing sugar concentration, $\hat{y}$ (g/L) and total sugar concentration (g/l) were used as the dependent variable (outcome). Table 12 shows the fit summary of materials; ANOVA was performed to ensure the reliability of the model ($p<0.05$). The Lack of fit f-value of <0.05 implies that the lack of fit is not significant relative to the pure error. Non-significant lack of fit is good because we want the model to fit.

Table 12 Fit summary for lignocellulosic biomass materials

Material *	Sequential p-value	Lack of Fit	Adj R^2	Pred. R^2	Equation
Hi-brix 53					
Leaves					
RS	0.0064	0.6216	0.8403	0.7646	$\hat{y}$=182.24- 20.86 x
TS	0.0780		0.7871		$\hat{y}$=297.33-12.50 x-20.75 x^2
Stalk					
RS	0.0093		0.8081	0.7182	$\hat{y}$=182.75+20.20 x
TS	0.0248	0.6085	0.6929	0.6032	$\hat{y}$=323.75+14.92 x
		0.9349			
Sugarstar x Hi-brix 53					
Leaves					
RS	0.0093	0.1623	0.8087	0.5647	$\hat{y}$=193.75-18.71 x
TS	0.0693		0.9831		$\hat{y}$=405.76-22.53 x-5.04x^2
Stalk					
RS	0.0259	0.7754	0.6868	0.6097	$\hat{y}$=193.22-12.18 x
TS	0.0139		0.8454		$\hat{y}$=436.63+9.98 x-25.82x^2

*RS = reducing sugar (g/L); TS = total sugar (g/L)

All materials, whether a RS and TS, R^2 were in reasonable agreement with the Adjusted R^2 (Table 13). Adeq precision measures the signal to noise ratio. A ratio greater than 4 is desirable. All materials have >4 adeq. precision indicates an adequate signal meaning this model can be used to navigate the design space.

Table 13 Fit statistics lignocellulosic biomass materials

Material	St Dev	mean	CV %	R^2	Adj R^2	Predicted R^2	Adeq Precision
Hi-brix 53							
Leaves							
RS	6.56	185.63	3.53	0.8722	0.8403	0.7646	10.75
TS	4.85	294.03	1.65	0.8723	0.7871		7.292
Stalk							
RS	7.24	186.11	3.89	0.8465	0.8081	0.7182	9.665
TS	7.17	326.23	2.20	0.7543	0.6929	0.6083	7.212
Sugarstar x Hi-brix 53							
Leaves							
RS	6.69	190.63	3.51	0.8469	0.8087	0.5647	9.682
TS	2.16	404.53	0.53	0.9898	0.9831		29.53
Stalk							
RS	5.93	191.19	3.10	0.7494	0.6868	0.6097	7.118
TS	5.91	425.38	1.39	0.9073	0.8454		8.569

The system runs both reducing sugar and total sugar concentration, the goal was to find the optimal condition to achieve the ideal concentration on both sugars

showed in figure 15-18 (summarize in Table 14). Different reaction time was simulated on four materials ranging from 38-98-72 h. Desirability closer to 1 is the most ideal.

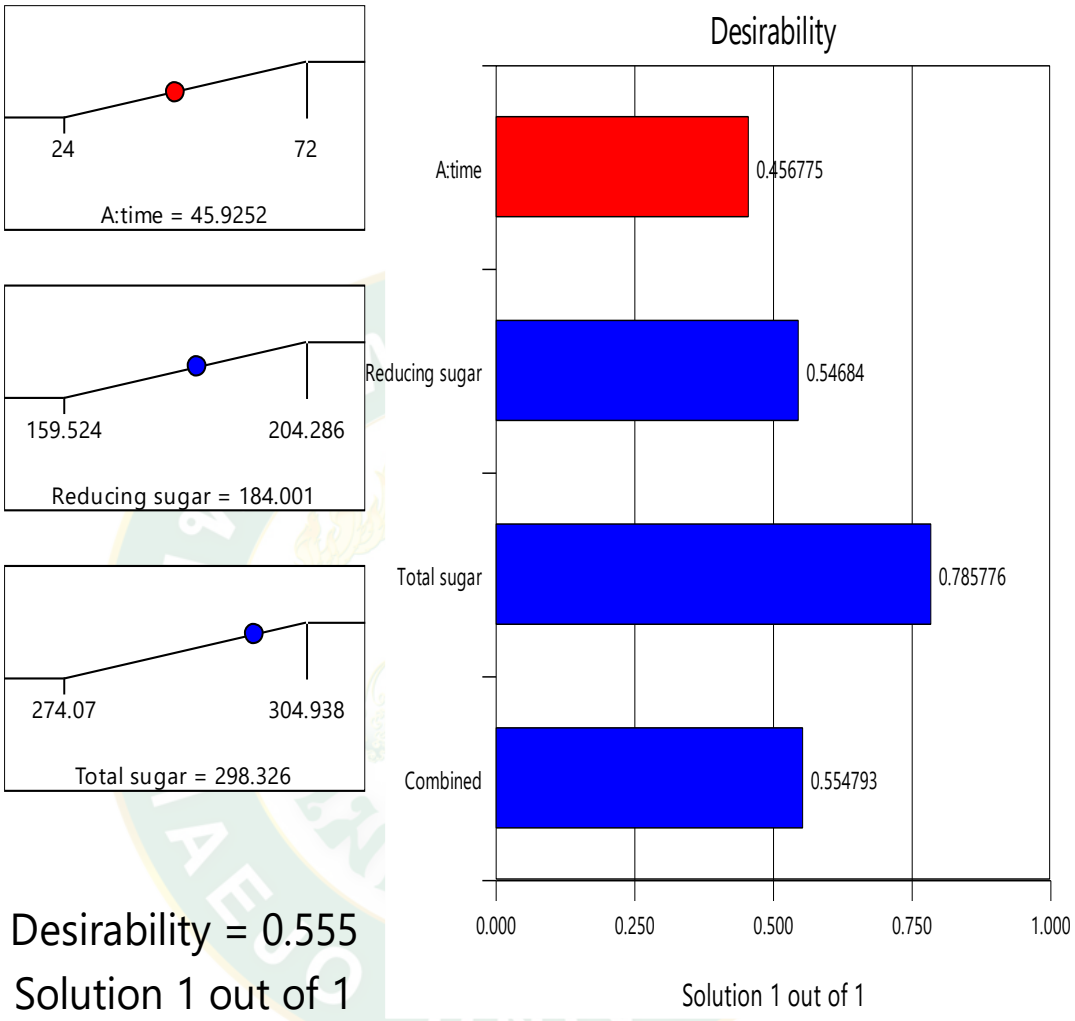


Figure 15 Optimal pretreatment reaction time for hi-brix leaves sugar concentration.

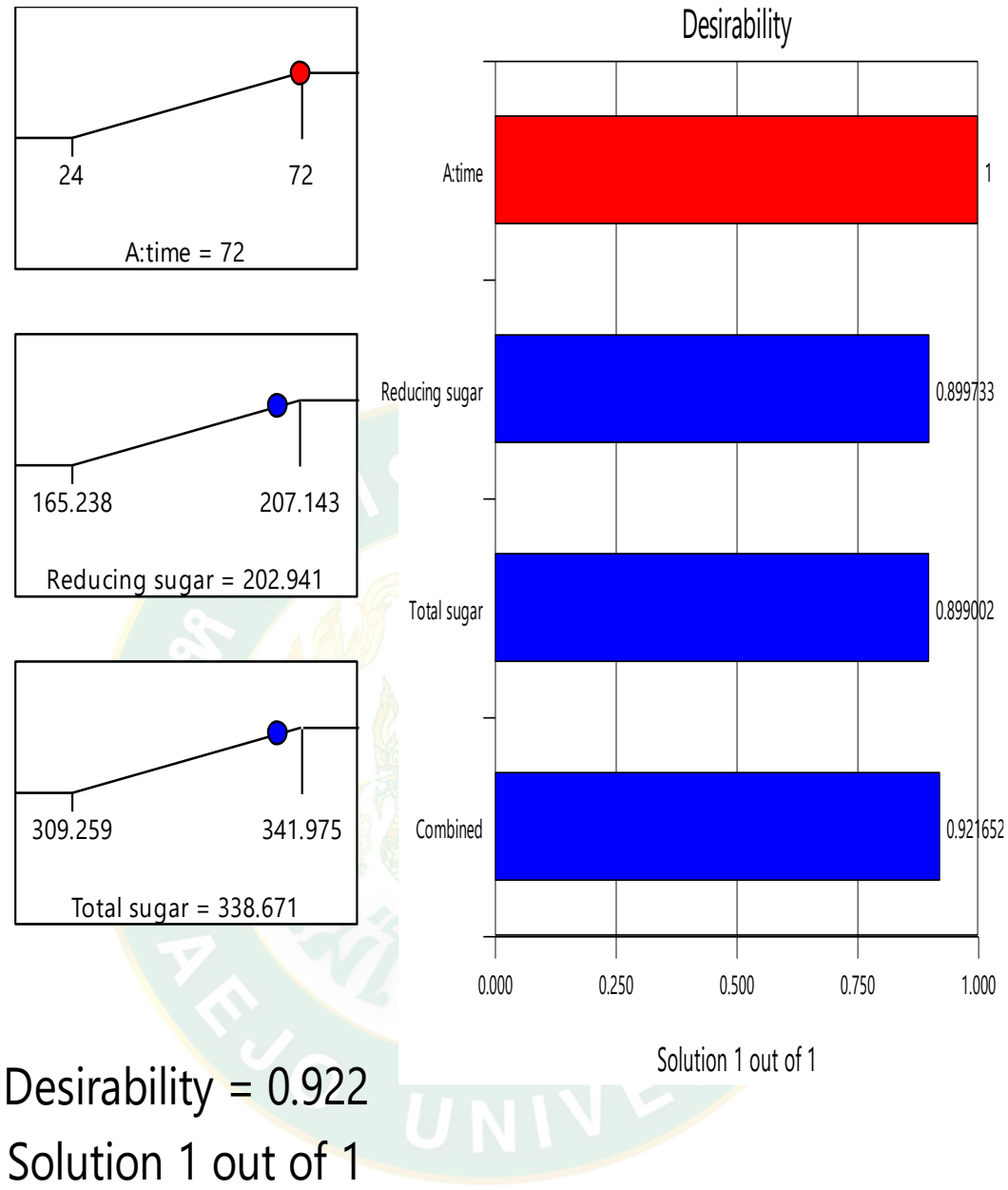


Figure 16 Optimal pretreatment reaction time for hi-brix leaves sugar concentration.

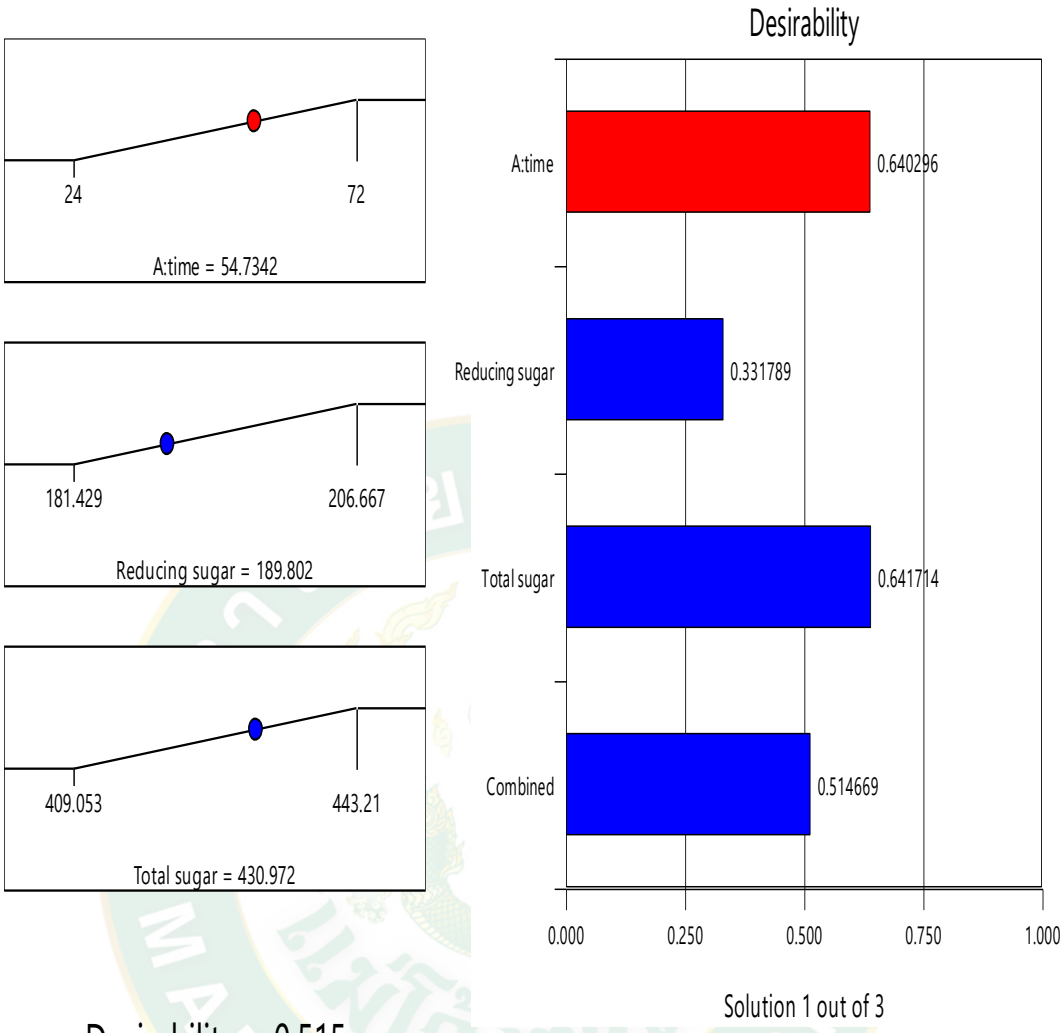


Figure 17 *Optimal pretreatment reaction time for sugarstar x hi-brix leaves sugar concentration.*

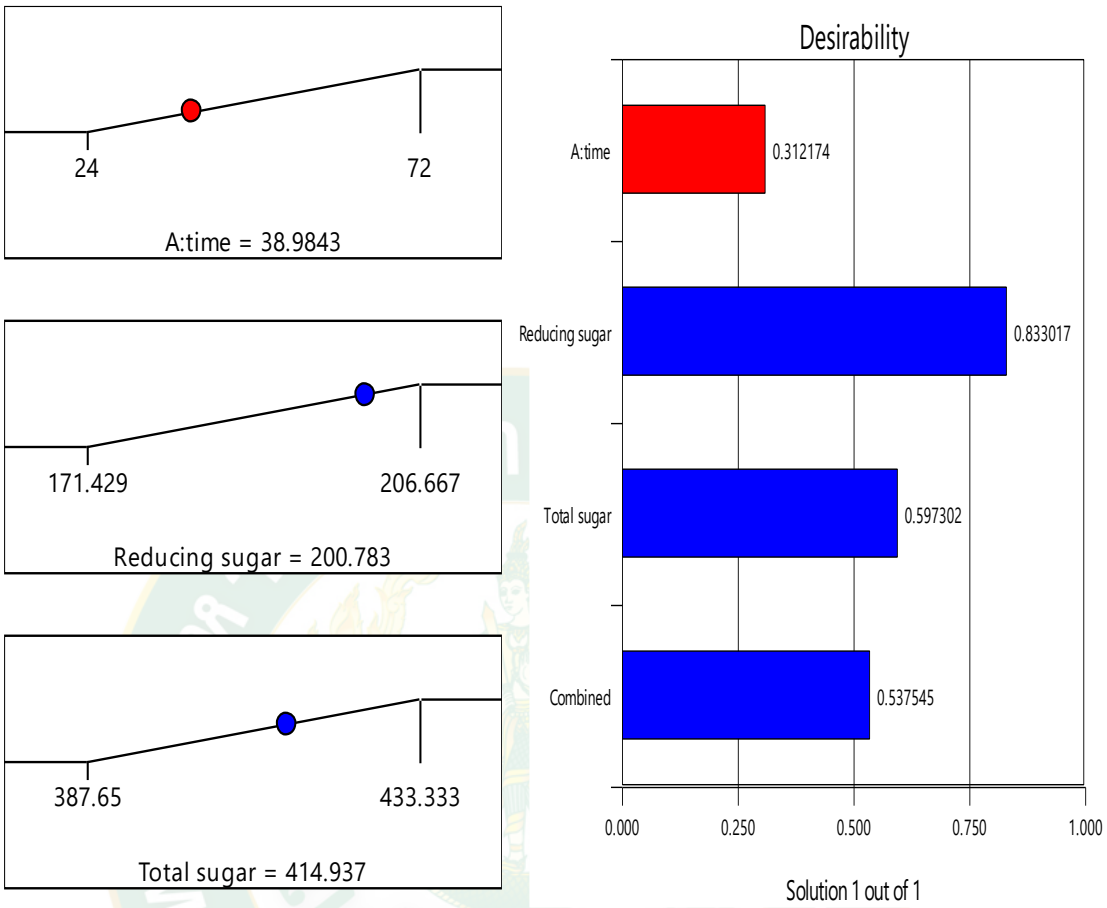


Figure 18 Optimal pretreatment reaction time for sugarstar x hi-brix leaves sugar concentration.

Table 14 Optimal reaction time and predicted values

Material	Time (h)	Reducing Sugar	Total Sugar	Desirability
Hi-brix 53				
Leaves	45.92	184.00	298.33	0.55
Stalk	72	202.94	338.67	0.92
Sugarstar x Hi- Brix 53				
Leaves	38.98	206.67	414.94	0.53
Stalk	54.73	189.90	430.98	0.52

Table 15 shows the comparison between the predicted and experimental values obtain through the stimulation of the software and experimentation. Hi-brix stalk with the highest desirability among the materials got the closest predicted to experimental values. This indicates that the model needs to be improved in order to increase the accuracy of the predicted values. More runs on the experiment were suggested to improve the prediction and simulation of the software.

Table 15 Predicted vs experimental fermentable sugars

Material	Predicted		Experimental	
	RS	TS	RS	TS
Hi-brix 53				
Leaves	184.00	298.33	263.33	378.95
Stalk	202.94	338.67	222.22	333.77
Sugarstar x Hi- Brix 53				
Leaves	206.67	414.94	239.44	398.68
Stalk	189.90	430.98	196.11	354.39

SSF and SHF Fermentation Process

Two types of fermentation process were applied in this study: SSF and SHF. SSF or simultaneous saccharification and fermentation was done by doing hydrolysis and fermentation at the same time. While SHF or separate hydrolysis and fermentation done hydrolysis and fermentation on a different time and container. After the pretreatment process, the four different materials were subjected into the SHF and SSF. SSF process produced 1.37-1.83% (10.79-14.46 g/L) of ethanol while SHF process 1.43-1.82% (11.31-14.33 g/L). Sugarstar x Hi-brix 53 (Table 16). These values were 10 times higher compared to the result obtained by Kanophorn et al. (2011) were pretreated leaves (*Acacia auriculiformis Cunn.)* undergone SHF and SSF with 1.00-1.08 g/L produced bioethanol.

Table 16 Bioethanol from SSF and SHF fermentation method on corn lignocellulosic materials

Fermentation	Material	Bioethanol Content	
		(g/L)	%
SSF			
	Hi-brix 53		
	Leaves	10.79±0.91[b]	1.37±0.12
	Stalk	11.31±0.46[b]	1.43±0.06
	Sugarstar x Hi-brix 53		
	Leaves	13.15±01.46[ab]	1.67±0.06
	Stalk	14.46±0.46[a]	1.83±10.06
SHF			
	Hi-brix 53		
	Leaves	11.31±0.46[b]	1.43±0.10
	Stalk	12.10±1.20[ab]	1.53±0.15
	Sugarstar x Hi-brix 53		
	Leaves	12.89±1.64[ab]	1.63±0.21
	Stalk	14.33±0.82[a]	1.82±0.10

Comparison of SSF and SHF

Four materials have undergone SSF fermentation where pretreated materials were added with 2% cellulase plus 0.5% yeast and were incubated for four days. After the fourth day incubation, ethanol content of the solution was checked. The highest bioethanol produced on SSF and SHF method were from sugarstar stalk with 1.83% (14.47 g./L) and 1.82 % (14.33 g/l) (figure 19).

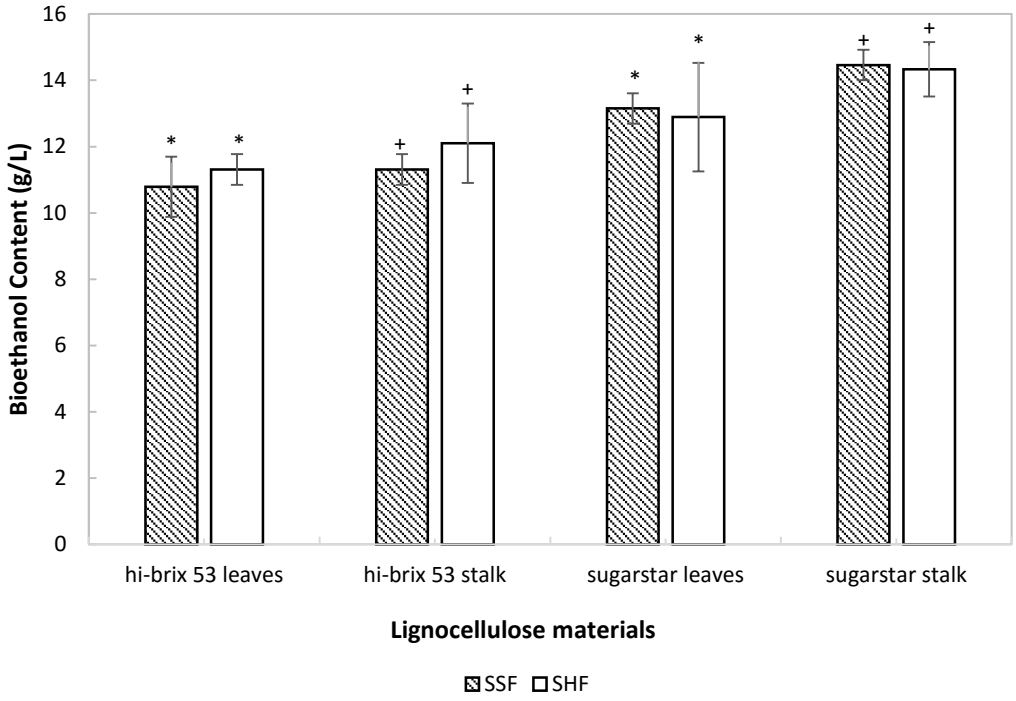


Figure 19 SHF vs SSF of corn lignocellulosic materials

Based on the post-hoc test applied, there is no significant difference between the two values. It is also the same case on the other material use, between the produce bioethanol values using SSF and SHF, there is no significant difference on the values of hi-brix 53 leaves, hi-brix 53 stalk, and sugarstar leaves. Even though both processes yielded at the same results, each process has its own advantage and disadvantages. Mohapata et al. (2017) stated the advantage of SHF over SSF is the ability to optimize the two processes (hydrolysis and fermentation). For example, cellulase enzyme optimum temperature ranges from 45-50 °C; this kind of condition may compromise yeast growth as they typically survive at 30-38° C.

On the other hand, Dahnum et al. (2015) study showed SSF as a better process compares to SHF based on its ability to produce a much higher ethanol concentration in a short time. Another study pointed out that SSF is a better process than SHF, however this time, they used repeated batch using immobilized yeast. The process lasted until 7 cycles with a 79% fermentation efficiency on the 5 consecutive cycles

(El-Dalatony et al., 2016). With this, different fermentation techniques and the process can be applied to these materials to improve ethanol production. The researchers suggest further study on these materials on different fermentation process and methods that wasn't tried on this study.

Scale-up Lignocellulosic Fermentation

Scale up lignocellulosic was performed using 10 kg of mix stalk and leaves the material. All parameters and material added were shown in Table 17. A total of 50 L of water were added plus 80 L of 2% NaOH for the pretreatment that lasted for three weeks. It followed by hydrolysis using cellulase. The fermentation was performed using dry yeast for 72 hours using a fermenter with 18 hz of agitation (figure 20). All process was done on ambient temperature to reduce the cost of production

Table **17** Parameters of scale-up lignocellulosic biomass fermentation

Material: **mix stalk and leaves** **tap water**	 10kg 50 L
Pretreatment: **2% Sodium Hydroxide (3 weeks)**	80 L
Hydrolysis: **Cellulase (48 h)**	 2% (v/w)
Fermentation: **Dry Yeast (72 h)**	 1 kg
Ethanol content	6.31 g/L (0.8 % v/v)
After Distillation	22.88 g/L (2.9% v/v)

A total of 6.31 g/L (0.8 %) of ethanol produced on the span of 72 h fermentation. Bioethanol content was increased after distillation with 22.88 g/L (2.9%).

Figure 20 Ethanol production operating system capacity of 150 L per production (AC 400 V 50 Hz; 1.2 m width; 1.7 m length; 1.5 m height and 1,000 kg weight).

Energy Return Investment (r_E)

This study energy return investment was adapted from Hammerschlag (2006). Energy return investment (r_E) refers to the ratio of energy in a liter of the ethanol produced to the renewable energy required to make the same amount of ethanol production. Ethanol energy investment can be calculated using the formula:

$$r_E = \frac{E_{out}}{E_{in,\ nonrenewable}}$$

where E_{out} is the energy of the ethanol output and $E_{in,\ nonrenewable}$ is the nonrenewable energy input to the ethanol manufacturing process. If $r_E < 1$, total energy from ethanol is less than the non-renewable used on making it. If $r_E > 1$, energy release from ethanol is higher than the energy needed to produce it.

The term $E_{in,\ nonrenewable}$was derived from fuel and electricity and upstream energy. Fuel and electricity refer to fuels and electricity used by the farmer from the start of the feedstock production, transportation, and through the processing facility. Upstream energy refers to the fuels and electricity used by the supplier or commodities the farmer adds to the whole production like fertilizers and pesticides.

Table 18 shows the ethanol energy investment of corn stalk, alongside another study for corn stover and corn grain. This study excludes the fuel and electricity used on the production of corn stalk due to its agricultural by-product nature. Transportation cost were both adapted from Sheehan et al., (2004) and Kim and Dale (2005) study report. Upstream energy is excluded for the same reason. Cornstalk was cultivated for the sole purpose of producing corn grain for food production. Often farmers disregard the corn stalk after corn harvest. For the processing cost, data from Manmai, (2018) were considered as a baseline for alkaline pretreatment, hydrolysis, and fermentation. All values were at MJ/ L; ethanol gross output was its HHV equivalent.

The R_e calculated is at 1.81 meaning that the produced ethanol from corn stalk meaning corn stalk on this process was able to capture some renewable energy using the nonrenewable investment. This value is slightly higher than the study of Kim and Dale (2005) however, Kim and Dale, (2005) got high nonrenewable energy use on its process. In comparison to Sheehan et al. (2004) (re of 4.40).with the use of corn stover as a material, this study r_E is lower.

Table 18 Energy renewable investment

	Corn stalk (this study)	**Corn stover Sheehan et. al 2004**	**Corn grain (Kim and Dale 2005)**
FUEL AND ELECTRICITY			
Agriculture			
Fuel	0.8	0.8	0.8
Electricity			0.1
Feedstock transport	0.5	0.5	0.5
Process			
Fuel	2.10	0.3	12.5
Electricity	7.12		2.2+0.6*
TOTAL FUEL AND ELECTRICITY	10.52	1.5	16.8

UPSTREAM ENERGY			
Agriculture			
Fertilizer	2.0	4.0	2.0
Biocides	0.4		0.4
Others	0.1	0.3	0.1
Total	2.5	4.3	2.5
CALCULATION FOR			
r_E	13.02	5.8	19.3 (-4.8)
Gross energy input			
Gross energy output	23.6	25.5	23.6
r_E (unitless)	1.81	4.40	1.62
REFERENCE DATA			
Upstream fuel included?	No	Yes	Yes
Feedstock yield (Mg/ha-yr)	6.06	8.2	9.0
	0.10	0.32	0.39

Techno-economic analysis

Data obtained from this analysis were those from literature. A small pilot plant with a capacity of 15,000 L-25,000 L and an operation of 2,000 h/ year were used. Table 19) A small scale plant with a capacity of 25,000 L per year. Cost consists of feedstock cost, collection cost, transportation cost, production cost (rate for biochemical fermentation). The plant process 83.33 dry tonnes of corn reside per year. A collection cost refers to labor or those who collect the residue or materials. The feedstock cost is null, due to its agricultural by-product nature. Transportation cost cover the transport of materials within the 20km distance. One truck can load up to 4

tonnes. In this simulation, the biorefinery is at 40km distance. Processing cost rated as a biochemical process using enzyme. The bioethanol price was based on Thailand price converted into US dollar. A total yield of 25,000 L based from the conversion of 300 L / dry tonnes. This small scale biorefinery has gross earnings of 12,877.28$ per year.

Table 19 Techno-economic analysis using small pilot plant

Capacity	**25,000 L**	
Operation	2000 h / year	
Land area to produce biomass	1-3% within 1 km radius	
Production	25,000 L	
Bioethanol yield	300 L/dry tonnes	
COST		
Feedstock cost	0	
Collection cost	19.95$/ton	1,662.43 $
Transportation Cost	16.65 $/ ton every	693.72
(1 truck= 4 tonnes)	20 km	
Processing cost	30.2$/ton	2,516.57
Bioethanol price	0.71 $/L	17,750 $
GROSS EARNING		12,877.28 $

CHAPTER V

SUMMARY, CONCLUSION, AND RECCOMENDATION

Corn materials were disregarded on the field, and some were eradicated through combustion. In order to alleviate the growing problem for solid waste problem due to the accumulation of these agricultural by-products, one way to use these materials is to turn them into something useful. The government of Thailand proposes the waste-to-energy project where these materials were used as feedstock for bioethanol production. Possible corn residues such as corn juice, stalk bagasse and leaves were tested and studied for their potential to be a viable option for bioethanol production. Different methods were applied and studied order to know the optimal ethanol yield from these materials. For corn stalk juice, fermentation using free cell yeast and immobilized yeast was compared.

Additionally, batch and continuous fermentation were also applied to determine the most effective mode of fermentation using corn stalk juice. Fermentation using Immobilized yeast showed promise by lasting up to 3 cycles of batch fermentation each lasted for 5 days in order to determine the highest ethanol production within the incubation time. For the up-scale experiment, continuous fermentation using immobilized yeast were performed. The cycle lasted for 5 days and produced ethanol for 3.5-3.9%. After distillation, the ethanol content was up to 16%.

Lignocellulosic materials were tested for the pretreatment, hydrolysis, and fermentation. Three pretreatments were studied. Among these three, alkaline pretreatment yields the highest fermentable sugar among autoclave and physical. Sodium hydroxide was used and RSM was applied to optimized the reaction time. Each material, hi-brix 53 stalk and leaves, and sugarstar x hi-brix 53 stalk and leaves, showed different time for reaction time on alkaline pretreatment. This proves that each material was differ to each other and need further study in order to find the suitable pretreatment for each materials. Next step was the hydrolysis and fermentation, were two steps SHF and SSF were both studied and applied. Both materials yielded 1.3-

1.9% of bioethanol. Based on the statistical analysis there is no significant difference between the SHF and SSF process. However, sugarstar -x hi-brix53 stalk yield the highest ethanol among all the materials. Scale up bioethanol production produce about 2.9% of bioethanol after distillation. Energy and techno-economic analysis showed the feasibility of the corn as a feedstock for a small scale biorefinery.

The researchers recommended to further study different corn varieties and materials. Corn juice produces the most ethanol compared to the other two materials. Further study on this material should be done.

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APPENDIX

APPENDIX A ANOVA/STATISTICAL ANALYSIS

ANOVA for linear model of reducing sugar concentration of hi-brix 53 leaves

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1174.98	1	1174.98	27.30	0.0064	**significant**
A-time	1174.98	1	1174.98	27.30	0.0064	
Residual	172.15	4	43.04			
Lack of Fit	15.69	1	15.69	0.3008	0.6216	not significant
Pure Error	156.46	3	52.15			
Cor Total	1347.13	5				

ANOVA for quadratic model hi-brix 53 leaves total sugar concentration

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	481.86	2	240.93	10.24	0.0456	**significant**
A-time	416.80	1	416.80	17.72	0.0245	
A^2	163.24	1	163.24	6.94	0.0780	
Pure Error	70.55	3	23.52			
Cor Total	**552.42**	**5**				

ANOVA for linear model of reducing sugar concentration of hi-brix 53 stalk

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1155.66	1	1155.66	22.05	0.0093	significant
A-time	1155.66	1	1155.66	22.05	0.0093	
Residual	209.60	4	52.40			
Lack of Fit	20.49	1	20.49	0.3250	0.6085	not significant
Pure Error	189.12	3	63.04			
Cor Total	1365.27	5				

ANOVA for linear model of total sugar concentration of hi-brix 53 stalk

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	631.03	1	631.03	12.28	0.0248	**significant**
A-time	631.03	1	631.03	12.28	0.0248	
Residual	205.54	4	51.38			
Lack of Fit	0.5379	1	0.5379	0.0079	0.9349	not significant
Pure Error	205.00	3	68.33			
Cor Total	836.57	5				

ANOVA of Linear model of sugarstar x hi-brix 53 leaves reducing sugar concentration

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	992.01	1	992.01	22.13	0.0093	**significant**
A-time	992.01	1	992.01	22.13	0.0093	
Residual	179.27	4	44.82			
Lack of Fit	95.26	1	95.26	3.40	0.1623	**not significant**
Pure Error	84.01	3	28.00			
Cor Total	1171.28	5				

ANOVA of Linear model of sugarstar x hi-brix 53 leaves total sugar concentration

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1362.99	2	681.50	146.28	0.0010	**significant**
A-time	1353.84	1	1353.84	290.59	0.0004	
A^2	35.86	1	35.86	7.70	0.0693	
Pure Error	13.98	3	4.66			
Cor Total	1376.97	5				

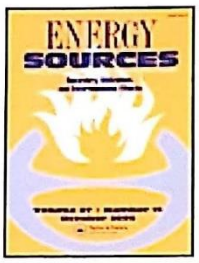
ANOVA of Linear model of sugarstar x hi-brix 53 leaves reducing sugar concentration

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	420.67	1	420.67	11.96	0.0259	**significant**
A-time	420.67	1	420.67	11.96	0.0259	
Residual	140.67	4	35.17			
Lack of Fit	4.50	1	4.50	0.0992	0.7734	**not significant**
Pure Error	136.17	3	45.39			
Cor Total	561.34	5				

ANOVA for linear model of sugarstar x hi-brix 53 stalk total sugar concentration

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1024.71	2	512.36	14.67	0.0282	significant
A-time	265.57	1	265.57	7.61	0.0703	
A^2	941.41	1	941.41	26.96	0.0139	
Pure Error	104.74	3	34.91			
Cor Total	1129.46	5				

APPENDIX B PUBLICATION



Energy Sources, Part A: Recovery, Utilization, and Environmental Effects

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Bioethanol production from corn stalk juice using *Saccharomyces cerevisiae* TISTR 5020

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ABSTRACT

This study aimed to use sweet corn hybrid hi-brix53 stalk juice for bioethanol production, to give a solution to the growing problem of food vs. fuel and to utilize waste for cheaper production. Hi-brix 53 stalk juice contained 112.07 ± 2.99 g L^{-1} of total sugars and 21.83 ± 1.09 g L^{-1} of reducing sugars. Through fermentation (24–120 h) using yeast (*Saccharomyces cerevisiae*), it produced 6.01% (v/v) bioethanol. The final ethanol produce (g L^{-1}) yield efficiency and volumetric ethanol productivity were at the highest at 24 h with 47.87 L^{-1}, 87.62% and 1.97 ± 0.06 (g L^{-1} h^{-1}). These results suggest that hi-brix 53 stalk juice is an ideal substrate for bioethanol production.

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KEYWORDS
Corn (*Zea mays*) juice; bioethanol production; *Saccharomyces cerevisiae*; free cell yeast; agri-waste to energy

Introduction

Thailand is becoming one of the rising global players in terms of bioethanol production (Figure 1). Bioethanol, a liquid biofuel, is a transportation fuel that can be substituted for or blended to gasoline (Sridee et al. 2012). E85 (85% bioethanol and 15% gasoline) is one of the most popular blends for light vehicles. In several countries like Australia, China, Columbia, Canada, Peru, Thailand, and the United States, gasohol or E10 with 10% bioethanol were the most common blends (Balat, Balat, and Oz 2008). Bioethanol can be produced from microbial fermentation by converting sugar to ethanol. Through this, bioethanol substrates are almost found everywhere because sugars exist in every plant tissue. Corn, potato, sugar cane, sugar beet, and grains are only a few examples of agricultural crops rich in starch or sugar that can be utilized for bioethanol production. Wood, straw, newspaper, wastes from industries or manure, and other agricultural by-products, called as cellulosic materials, are also substrates for bioethanol production (Munnecke 1981). Sugar (monosaccharides and disaccharides), starchy (reserve polysaccharides), and lignocellulosic (structural polysaccharides) crops are the three classifications of bioethanol substrates (Barros-Rios et al. 2015). In Thailand, the main feedstock for bioethanol production are sugarcane molasses and cassava, with 1.17 million liters/day and 0.33 million liters/day of production in 2011 (Kumar et al. 2013). However, based on the report of Sridee et al. (2012), these substrates may not be able to meet the country's continual production. Thailand aims to increase 20% of biofuel substitution in the transport sector by 2036 (IEA 2015). In order to meet this goal, the alternative substrate for bioethanol production was being pursued.

Corn grain (*Zea mays* L.) is one of the materials used for bioethanol production due to its high starch content. USA, one of the major producers of bioethanol, primarily use corn grain as the substrate. According to the data from US Department of Energy, 2016, from 2010 to –2016,

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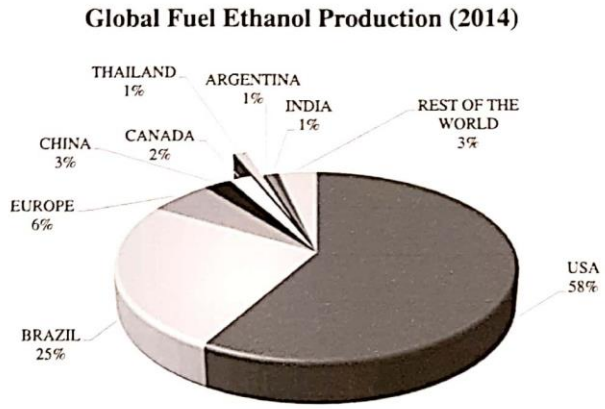

Figure 1. Share of global production of ethanol by country (million gallons: 1 gallon = 3.785 L) (Source: ata from Renewable Fuels Association 2015).

almost 40% of total corn production goes for ethanol production. This fueled the issues on food sources being used for fuel. Pimentel, Patzek, and Cecil (2007) revealed that this practice will only exaggerate both food and fuel shortages. As an alternative to the current substrates, corn non-food parts with readily fermentable sugars such as corn stalk juice were used (Gomez-Flores et al. 2018). Corn can produce readily fermentable sugars in its stalk juice (Widstorm et al). Corn stalk consists of soluble sugars like sucrose, glucose, and fructose (Gomez-Flores et al. 2018).

Corn is widely cultivated in Thailand with a 32.73% of a national production. About 71.33% of these corn came from the Northern Region of Thailand (Phonin, Likasiri, and Dankrakul 2017). Hi-brix 53 is a newly developed sweet corn hybrid in Thailand. It has an average yield of 22,431 kg/ha and reaches up to 200 cm in height. This corn is ready for harvest at 68–75 days of planting. It is also resistant to the northern corn leaf blight that is prominent to areas like Chiangmai, Chiangrai, and Lampang in Northern Thailand. It is popular with the farmers because of its ability to produce high-quality grains ideal for canning industry (Pacific Seeds (Thai) Ltd. 2018). However, cultivation of these corn leaves a huge amount of agricultural waste. Often, corn farmers get rid of these wastes by burning the fields, a practice that aggravates the Northern Thailand haze pollution (Chantara 2012). Phonin, Likasiri, and Dankrakul (2017) explained the incorporation of these biomass into energy. Transforming waste into energy will help the farmer's extra income, and eradication of these wastes will lessen some pollution brought by land incineration.

Hence, this study aimed to investigate the potential of sweet corn hi-brix 53 stalk juice as substrates for bioethanol production, with an aid of yeast strain, *S. cerevisiae* TISTR 5020. As this sweet corn hybrid is newly developed, only a little research is available regarding the use of Hi-brix 53 stalk juice in bioethanol production. Since this cultivar was appealing to the farmers, tons of biomass were available especially in Northern Thailand. The main objectives of this study were to find an alternative feedstock for bioethanol production, to give a solution to the growing problem of food vs. fuel. and to utilize waste for cheaper energy production.

Materials and methods

Material preparation

Sweet corn hybrid hi-brix 53 stalks were collected in a corn farm located at Bang Hong District, Lamphun 51130, Chiang Mai, Thailand. The leaves and husk were removed from the stalk. It was then chopped and fed through a sugarcane juicer for juice extraction. The juice then refrigerated until further use.

Yeast preparation

Saccharomyces cerevisiae TISTR 5020 yeast strain was used in this study. YPD medium was prepared using 10% yeast extract (Himedia Laboratories, Telangana, India), 20% peptone (Himedia Laboratories, India), and 20% dextrose (Union Science Co., Ltd, Chiang Mai, Thailand). The medium was sterilized in an autoclave for 15 min at 120°C. Finally, the yeast was added to the medium under aseptic conditions and cultivated in a room temperature at 150 rpm for 48 h.

Fermentation assay

Hi-brix 53 stalk juice has been boiled for 15 min for sterilization. Then, it was cooled down and adjusted the pH to 5.6 using sodium hydroxide (Merck kGaA, Darmstadt, Germany). The fermentation was carried out in a 1-L bottle with a working fluid of 300 mL. Ten percent (10%) of yeast (*S. cerevisiae* TISTR 5020) with 1×10^7 cell mL^{-1} was added to the juice. It was then put in an incubator with a maintaining temperature of 36°C for 5 days. The experiment was done in triplicate. Ethanol and sugar concentrations were checked for every 24 h.

Kinetic parameters

The following kinetic parameters of fermentation were calculated using the equation from Laopaiboon et al. (2007):

$$Q_P = \frac{P}{t} \tag{1}$$

where Q_P is the volumetric ethanol productivity (g L^{-1} h^{-1}), P is the final ethanol concentration (g L^{-1}), and t is the time of fermentation (h).

$$E_y = \frac{Y_{ps} \times 100}{0.51} \tag{2}$$

where E_y is the yield efficiency (%), Y_{ps} is the ethanol yield expressed as the g ethanol per g sugar utilized (g g^{-1}), and 0.51 derived from the maximum theoretical ethanol yield per 1 g of glucose consumption.

Experimental analysis

Total sugar and reducing sugar were determined using Phenol/Sulfuric method and DNS (3,5-dinitrosalicylic acid) method by Dubois et al. (1956) and Miller (1959) with minor modifications:

For total sugar determination, 0.5 mL of the sample, 0.5 mL of 5% phenol (w/v) (Qrec, Selangor, New Zealand), and 2.5 mL of 98% H_2SO_4 (RCI Lab Scan, Bangkok, Thailand) were mixed together using a vortex. The solution was left for 10 min and then read at Spectrophotometer model DV-8000 (Drawell, Osaka, Japan) at 490 nm.

For reducing sugar determination, 0.5 mL of the sample was added with 0.5 mL of DNS (3,5-dinitrosalicylic acid) (Sigma Aldrich, Missouri, USA) solution. The solution was mixed using

Table 1. Residual sugar, final ethanol concentration, etOH yield, yield efficiency, and volumetric ethanol productivity of corn juice in response to its varying fermentation time.

Fermentation time (h)	% Bioethanol (v/v)	Final o\ethanol concentration (g L^{-1}) **	EtOH Yield (g g^{-1}) **	Yield efficiency (%) **	Volumetric ethanol productivity(g L^{-1} h^{-1}) ***
24	6.01	47.39 ± 1.50	0.38 ± 0.01	74.85 ± 4.45	1.97 ± 0.06
48	6.00	47.36 ± 1.35	0.38 ± 0.01	74.11 ± 5.75	0.99 ± 0.03
72	6.04	47.66 ± 4.58	0.38 ± 0.05	73.89 ± 2.22	0.66 ± 0.06
96	5.91	46.65 ± 1.33	0.37 ± 0.01	72.74 ± 4.95	0.49 ± 0.01
120	5.55	43.79 ± 2.73	0.36 ± 0.02	69.82 ± 6.63	0.36 ± 0.02

** No significant difference was found between the values.
***All values have significant differences.

declining sugar concentration, production of ethanol was also observed on the first 24 h of fermentation. These results proved Hi-brix 53 stalk juice can produce bioethanol even without adding a supplement. The final bioethanol production from hi-brix 53 stalk juice during the 24–120 h of fermentation ranged from 43.79 ± 2.73 to 47.66 ± 4.58 g L^{-1} (5.55%–6.01% v/v) (Table 1). The highest final ethanol concentration was from 72 h of fermentation; however, we found no significant differences between the values from 24 to 120 h of fermentation. These results on ethanol production were expected from a batch fermentation process. Zabed et al. (2014) found that longer fermentation time affects the microbial growth due to prolonged exposure to ethanol, while Nuanpeng et al. (2011) mentioned that batch fermentation can have a negative effect on the microorganism growth. Meanwhile, we attained highest volumetric ethanol productivity (1.97 ± 0.06 g L^{-1} h^{-1}) and ethanol yield (g g^{-1}) at 24 h of fermentation with yield efficiency of 74.45%. (Table 1). The study of Laopaiboon et al. (2007) shows improves ethanol production and overall efficiency rate on the fed-batch fermentation compared to batch fermentation. Phukoetphim et al. (2017) found a 51% increase in ethanol concentration and ethanol productivity on fed-batch fermentation with continuous feeding compared to batch fermentation. With this, different fermentation techniques and process may be applied in improving the overall efficiency of hi-brix stalk juice for bioethanol production.

Comparison between sugar energy crops

Hi-brix 53 got the lowest total sugar concentration among the other sugar energy crops (Table 2). Despite this, its yield efficiency is close to sweet sorghum and sugar beet juice. As stated above, various fermentation processes may be applied in improving the overall efficiency rate of hi-brix 53 stalk juice.

In comparison to sugar corn, Canada's variety of sweet corn, hi-brix 53, shows higher yield efficiency by 13%. This shows a variation within different sweet corn (*Z. mays)* cultivars and variety.

Table 2. Total sugar, final ethanol concentration, and yield efficiency of different sugar crops juice.

Substrate	Total Sugar (g L^{-1})	Final ethanol concentration (g L^{-1})	Yield Efficiency * (%)	Reference
Sugarcane Juice *(Saccharum officinarum)*	176.25	89.02	99.03	Liang et al. (2008)
Sweet Sorghum Juice *(Sorghum bicolor)*	191.0	82.30	84.48	Guigou et al. (2011)
Sugar Beet Juice *(Beta vulgaris)*	190.0	80.00	82.56	Tan et al. (2015)
Canada Sugar Corn stalk Juice *(Zea mays)*	145.0	45.60	61.66	Gomez-Flores et al. (2018)
Thailand Hi-brix 53 stalk Juice *(Zea mays)*	130.62	47.39	74.85	This study

*Yield efficiency was calculated using Equation (1).

a vortex and was boiled for 15 min. After boiling, the solution was added with 4 mL distilled water and was mixed again using a vortex. The solution was read at 540 nm.

Lastly, the alcohol content was checked using an Ebulliometer (Laboratoires Dujardin-Salleron, Noizay, France) as described by Vu, Unpaprom, and Ramaraj (2018). All experiments were done in triplicates.

Statistical analysis

Statistical analysis was done using IBM SPSS Statistics 23. Data are reported as mean ± SD (n = 3). Analysis of variance and Tukey's test were used to determine the differences between final ethanol concentration (g L^{-1}) and different fermentation parameters. The difference between the values was considered significant when $p<0.05$.

Results and discussion

During the 120-h fermentation process, changes in the pH, sugar concentration, and ethanol content were recorded every 24 h. The substrate pH level decreased from the initial value of 5.6 to 4.94 ± 0.08 fermentation on the first 24 h of fermentation and then, it increases back up to 6.15 ± 0.09 at 120 h (Figure 2). Razmovski and Vučurović (2012) attained the same result: instant pH decay on the early part of fermentation. This change in pH level may suggest the formation of other by-products, other than ethanol, that were not identified in this study. Lin et al. (2014) mentioned the influence of pH in terms of ethanol production and by-product formation. It can also be used as an indicator of the products that have been formed in the process of fermentation. For example, in the pH of 5.5–6.0, the main product would be ethanol and butyrate, whereas for pH lesser than 5.0, the main product would be acetic acid.

Sugar and bioethanol concentration ethanol

Hi-brix 53 stalk juice contained initial total sugar concentration of 130.62 g L^{-1} and reducing sugar of 21.83 g L^{-1} (Figure 2). Sugar consumption was observed at first 24 h of fermentation. Alongside the

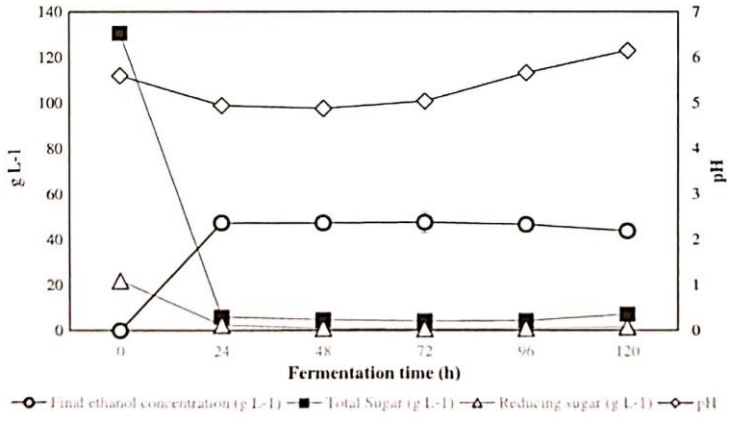

Figure 2. Trends of final ethanol concentration, sugar consumption (in total sugar and reducing sugar), and pH level during the 120 h of fermentation. Points are expressed as mean; error bars as standard deviation (n = 3).

On that note, different juice extraction method and post-harvest treatment should be considered in these variations. In the research of Guigou et al. (2011) different methods of juice extraction on three different sweet sorghum varieties were evaluated. The research showed that cultivars and post-harvest treatments affect the overall ethanol production.

Conclusion

Hi-brix 53 stalk juice contains a high amount of readily fermentable sugar which is a good candidate for bioethanol substrate. It has higher yield efficiency compared to a known juice substrate for bioethanol production. The researchers suggested two things from this paper: application of different fermentation techniques for stalk juice and further study of different corn cultivars as bioethanol feedstock.

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Statistical Modeling and Optimization of Corn Stalk Bagasse Pretreatment for Fermentable Sugar Production

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Abstract:

Lignocellulosic materials were known feedstocks for bioethanol production. One of the processes for lignocellulosic bioethanol fermentation is the pretreatment process. Pretreatment process frees up the cellulose that then will be accessible for hydrolysis. In this study, different pretreatment: physical, autoclave and alkaline, were evaluated in the improvement of sugar yields from corn stalk bagasse. Alkaline pretreatment method proved to be an effective pretreatment in terms of sugar yield. Central-composite design (CCD) was employed in the optimization of the alkaline process. Reaction time (X_1, h) and sodium hydroxide (NaOH) concentration (X_2, %) were the two independent variables while reducing sugar concentration ($\hat{y}$, g/L) was the dependent variable (response). Reducing sugar concentration was found at the highest (196.67 g/L) at 24 h using 2% NaOH experimentally. For the optimum level derived using the statistical model, a total of 203.13 g/L of reducing sugar can be produced from 26.93 h of reaction time (X_1) and 2.56 % of NaOH (X_2).

Keywords: pretreatment, alkaline, autoclave, corn stalk bagasse, response surface methodology

1. Introduction

Corn is one the most major agricultural product of Thailand. There's a roughly over 10.4 million hectares of corn plantation with a total of 4.06 million of tons of corn production per year (Ariyajaroenwong et al., 2015). Corn plantations also generates huge amount of agricultural waste. In search of finding a suitable feedstock for bioethanol production, agricultural-based lignocellulosic biomass, like corn stalk bagasse, were utilized.

Sugar is one of the major components for biofuel products through bioprocesses (Chandrasekaran and Sivamani, 2018). Lignocellulosic materials contain lignin, hemicellulose and cellulose (Vu et al., 2018). Lignocellulosic materials undergone intensive pretreatment process followed by hydrolysis and fermentation (Mupondwa et al., 2017). Pretreatment process is a crucial step for cellulosic ethanol and amounts to roughly 40% of the total processing cost (Sindhu et al., 2016). The pretreatment process breaks down the protective lignin barrier and frees up the hemicellulose and cellulose for hydrolysis where it can be converted into fermentable sugar (Kumar et al., 2009). Through years of research, different pretreatment process has been developed such as physical, chemical, biological and combination of these processes (Vu et al., 2018). The effectiveness of the pretreatment process depends largely on the biomass structure and treatment conditions (Sindhu et al., 2016).

With this, the main objective of this study was to determine the pretreatment process suitable for corn stalk bagasse. Another was to optimize treatment conditions using response surface methodology (RSM). Response surface methodology analyze different variables and their interactions (Luo et al., 2014). RSM reduces the number of experiments therefore lessen the cost on the analytical method (Wang and Blaschek, 2011). This study aimed to improve efficiency by doing the least amount of work and getting the most amount of information on the pretreatment process for corn stalk bagasse.

2. Materials and Methods

2.1 Sample Preparation

Corn materials were collected at Bang Hong District, Lamphun 51130, Chiang Mai, Thailand (18°18'37" N, 98°47'34" E). It was transported to the lab then cleaned up. Sugarcane juicer was used to extract the juice from the corn stalk. The juice was stored in a freezer for other research purposes. After juice extraction, corn stalk bagasse was collected and dried. The dried sample were then powderized and then stored in a desiccator until further experimentation.

2.2 Batch Pretreatment

A total of 20g of powderized sample were subjected into different pretreatment method: physical (control), autoclave and alkaline. Autoclaving was carried out at 121° C at 15 psi for 15 min. In alkaline pretreatment, 2% (w/v) of sodium hydroxide with a ratio of 1:5 (w/v) were added to sample. Lastly, for the physical pretreatment, also acts as control, the sample where mix with water in a 1:10 ratio to create a slurry density. After pretreatment process, the samples were undergone enzymatic hydrolysis. Difference of the means where analyze using ANOVA and Tukey's test ($p<0.05$).

2.3 Enzymatic Hydrolysis

After pretreatment process, each sample were added with 2% (v/v) of cellulase for enzymatic hydrolysis. This process indicates if the pretreatment methods is an effective process for opening up the lignin in order for the cellulase have access to the cellulose inside the stalk bagasse.

2.4 Optimization of Alkaline Pretreatment using Response Surface Methodology

Central composite design (CCD) was used for optimization of alkaline pretreatment. A total of 10 g of sample were undergone alkaline pretreatment with two factors: reaction time, X_1 (h) and NaOH concentration, X_2 (%, w/v). Each variable has 3 level: 1-3% for NaOH concentration and 24-72 h for reaction time (Table 1). Reducing sugar concentration, $\hat{y}$ (g/L) were used as the dependent variable (outcome).

Table 1. Coded levels and actual level of the variables

Factor	Name	Units	Type	Minimum	Maximum	Coded Low	Coded High
X_1	Reaction Time	h	Numeric	12.00	36.00	-1 ↔ 12.00	+1 ↔ 36.00
X_2	NaOH concentration	%	Numeric	1.00	3.00	-1 ↔ 1.00	+1 ↔ 3.00

The number of experiments required in CCD is $N= 2k + 2k + C_0$ where k is the number of factors (k=2) and C_0 is the number of central points (Shukla and Nishkam, 2014). Nineteen experiments were performed to optimize two variables with three replications at center point (n=3) and duplicates on the axial point. Second order polynomial model were used and calculated using the Eq (1).

$$\hat{y} = \beta_0 \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{i \neq j=1}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (1)$$

where $\hat{y}$ is the response, β_0 is the constant or the intercept, β_i is the slope or linear effect of the factor X_i, β_{ii} is the quadratic effect of the factor X_i, β_{ij} is the interaction effect between the input factors X_i and X_j, and ε is the residual term (Koech et al, 2017). Second-order model is the most suitable, highly structured, flexible and diversified in order to locate the optimum point (Shukla and Nishkam, 2014). The analysis of variance (ANOVA) and regression analysis were performed to define the coefficients of the predictive model and significant terms using Design Expert version 11 (State-Ease, Inc., Minnesota, USA). The model obtained by regression was for the maximum reducing sugar concentration. The value where considered significant with p-values less than 0.05.

2.5 Analytical methods

The parameter measured in this experiment was reducing sugar were analyze using DNS (3,5-dinitrosalicylic acid) method by Dubois et al. (1956) with minor modification. A total of 0.5 mL of sample plus 0.5 mL of DNS (Sigma Aldrich, Missouri, USA) solution were mixed together. The solution was boiled for 15 mins and then added with 4.0 mL of distilled water and finally read using Spectrophotometer model DV-8000 (Drawell, Osaka, Japan) at 540 nm.

3. Results and Discussion

3.1 Pretreatment Process

Pretreatment process breaks down lignin barriers making easy for the enzymes to access hemicellulose and cellulose. Three pretreatments were performed in this study: physical (control), autoclave and alkaline. As the sample particle size affects greatly the enzymatic hydrolysis, physical pretreatment was performed. Autoclave and alkaline were also done with the same particle size. The physical pretreatment, powderized sample straight up gone enzymatic hydrolysis, this also acts the control of the group. Alkaline pretreatment (powderized sample were added with NaOH) is the most suitable pretreatment to use with the highest sugar content (Figure 1) compare to the other two pretreatments applied. It contains 225.17 g/L of reducing sugar after hydrolysis. Autoclave method is not sufficient enough to disrupt lignin structure shows a poor result of reducing sugar. Values for the physical and autoclave were found no significant difference. With this, the suitable pretreatment particle applied for corn stalk bagasse was NaOH with powderized size.

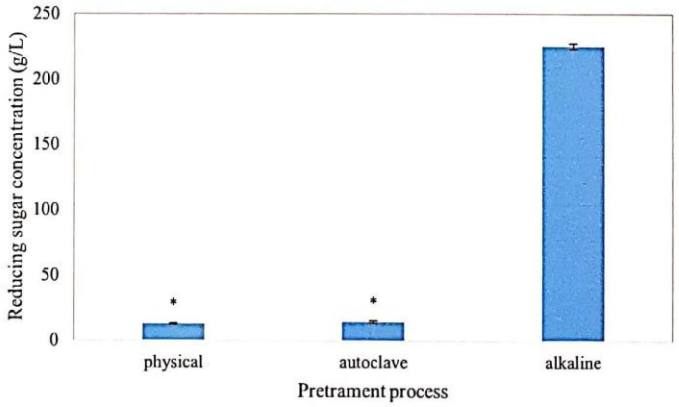

Figure 1. Effect of different pretreatment methods on the reducing sugar concentration. Data were presented as mean, error bar as sd (n=3). (* indicates no significant difference among the values $P<0.05$)

3.2 Optimization of sugar production employing response surface methodology (RSM)

CCD was applied to determine the effect and interaction of each variables to the reducing sugar concentration. The statistical model obtained showed first order (linear), second order (quadratic) and interactions of the faction to each other (eq. 2).

$$\hat{y} = 195.07 - 2.08\,X_1 + 34.58\,X_2 + 17.29\,X_1X_2 - 12.21\,X_1^2 - 39.71\,X_2^2 - 14.79\,X_1^2X_2 + 6,87\,X_1X_2^2 \quad \text{.....(2)}$$

The results shows that the model was highly reliable (R^2= 0.99) (Table 1). The lack of fit with 0.94 was considered not significant. This prove that the model fitted well to the experimental data. The highest concentration of reducing sugar was obtained at 24 h using 2% NaOH experimentally.

Table 1. Central composite design applied on the alkaline pretreatment of corn stalk bagasse

Std	Run	Reaction time, X_1 (h)	NaOH concentration, X_2 (%, w/v)	Reducing sugar concentration, $\hat{y}$ (g/L)		Residual
				Experimental	Predicted	
2	1	12	1	133.33	135.86	-2.53
17	2	24	2	195.00	195.07	-0.0725
16	3	24	3	191.67	189.95	1.72
6	4	12	3	141.67	140.86	0.8062
3	5	36	1	108.33	110.86	-2.53
9	6	12	2	180.00	184.95	-4.95
7	7	36	3	185.00	185.03	-0.0272
1	8	12	1	138.33	135.86	2.47
15	9	24	3	188.33	189.95	-1.61
11	10	36	2	180.00	180.78	-0.7790
5	11	12	3	140.00	140.86	-0.8605
4	12	36	1	113.33	110.86	2.47
18	13	24	2	196.67	195.07	1.59
13	14	24	1	121.67	120.78	0.8877
14	15	24	1	120.00	120.78	-0.7790
10	16	12	2	190.00	184.95	5.05
19	17	24	2	193.33	195.07	-1.74
8	18	36	3	185.00	185.03	-0.0272
12	19	36	2	181.67	180.78	0.8877

R^2= 0.99, R^2 (adjusted) = 0.99, R^2 (predicted)= 0.98.

Table 2. **Analysis of Variance (ANOVA) of the results for the response from alkaline pretreatment of corn stalk bagasse**

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	18549.74	7	2649.96	322.73	< 0.0001	significant
X_1= Time	17.36	1	17.36	2.11	0.1739	
X_2=NaOH concentration	4784.03	1	4784.03	582.62	< 0.0001	
$X_1 X_2$	2392.01	1	2392.01	291.31	< 0.0001	
X_1^2	653.15	1	653.15	79.54	< 0.0001	
X_2^2	6908.30	1	6908.30	841.33	< 0.0001	
$X_1^2 X_2$	583.45	1	583.45	71.06	< 0.0001	
$X_1 X_2^2$	126.04	1	126.04	15.35	0.0024	
Residual	90.32	11	8.21			
Lack of Fit	0.0453	1	0.0453	0.0050	0.9449	not significant
Pure Error	90.28	10	9.03			
Cor Total	18640.06	18				

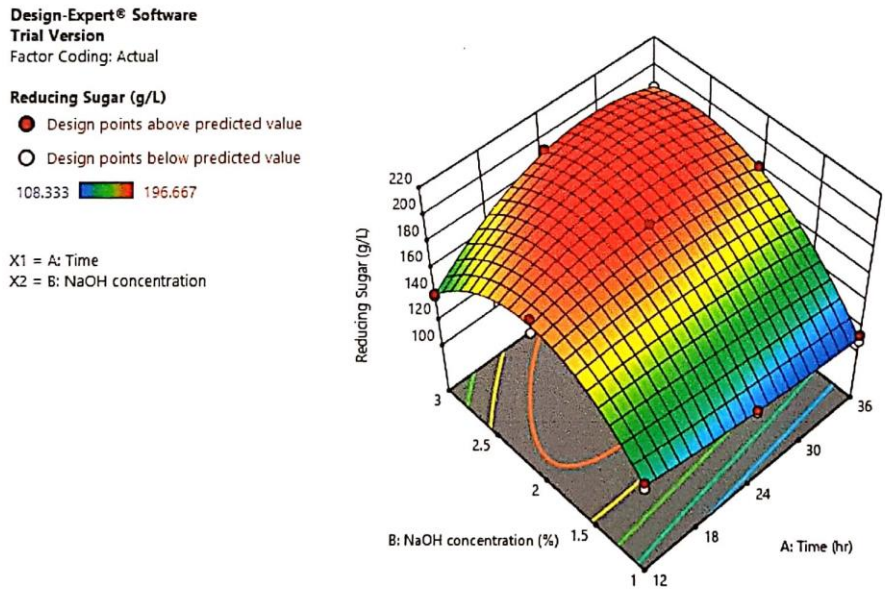

Figure 2. Response surface plots for reducing sugar concentration by alkaline pretreatment

3.3 Optimization of Alkaline pretreatment using the statistical model

Base on the constructed model (Eq 2 and Table 2), the optimal condition to achieved the maximum reducing sugar concentration are within the range of 12-36 h for reaction time and 1-3% for NaOH concentration. A total of one hundred solutions with a random varying levels of reaction Time, X_1(h) and NaOH concentration, X_2 (Table 3) were run using the statistical model derive using CCD (Table 3).

Table 3. Hundred solution runs using the statistical model with the different level and variable

Number	Reaction Time, X_1 (h)	NaOH concentration, X_2 (%)	Reducing Sugar concentration, $\hat{y}$ (g/L)	Desirability
1	20.082	2.165	197.824	1.000
2	32.400	2.700	198.139	1.000
3	22.800	2.100	198.025	1.000
4	32.700	2.625	198.170	1.000
5	21.328	2.390	200.358	1.000
6	22.200	2.100	197.868	1.000
7	21.038	2.330	200.041	1.000
8	26.400	2.800	199.593	1.000
9	21.600	2.400	200.640	1.000
10	30.869	2.476	200.641	1.000
11	23.759	2.411	202.447	1.000
12	27.708	2.577	202.974	1.000
13	31.126	2.832	197.835	1.000
14	19.551	2.340	198.170	1.000
15	20.819	2.564	197.892	1.000
16	18.935	2.354	197.163	1.000
17	18.937	2.187	196.899	1.000
18	19.391	2.186	197.395	1.000
19	30.003	2.692	200.965	1.000
20	29.730	2.717	200.862	1.000
21	29.418	2.695	201.323	1.000
22	32.252	2.606	198.942	1.000
23	21.454	2.614	198.017	1.000
24	20.562	2.548	197.714	1.000
25	24.399	2.597	201.894	1.000
26	26.898	2.605	202.859	1.000
27	21.642	2.480	200.240	1.000
28	23.822	2.370	202.348	1.000
29	24.453	2.719	199.899	1.000

30	20.278	2.096	196.820	1.000
31	25.497	2.267	201.599	1.000
32	28.114	2.794	200.164	1.000
33	25.343	2.255	201.426	1.000
34	25.445	2.668	201.641	1.000
35	26.119	2.842	198.320	1.000
36	30.256	2.770	199.729	1.000
37	24.414	2.678	200.683	1.000
38	27.314	2.501	203.229	1.000
39	24.561	2.714	200.105	1.000
40	32.089	2.630	199.096	1.000
41	32.819	2.583	198.081	1.000
42	18.859	2.176	196.705	1.000
43	20.528	2.569	197.270	1.000
44	21.513	2.533	199.479	1.000
45	21.965	2.454	200.827	1.000
46	18.551	2.254	196.752	1.000
47	31.894	2.451	199.100	1.000
48	23.710	2.168	199.720	1.000
49	28.529	2.879	198.164	1.000
50	29.393	2.590	202.147	1.000
51	31.387	2.620	200.091	1.000
52	20.851	2.245	199.401	1.000
53	31.506	2.296	197.618	1.000
54	24.884	2.199	200.422	1.000
55	25.127	2.286	201.885	1.000
56	20.309	2.476	198.344	1.000
57	20.686	2.303	199.584	1.000
58	19.134	2.371	197.390	1.000
59	19.898	2.218	198.235	1.000
60	30.166	2.859	198.073	1.000
61	23.073	2.738	197.723	1.000
62	28.184	2.516	202.955	1.000
63	18.799	2.293	197.140	1.000
64	28.618	2.430	202.401	1.000
65	26.933	2.563	203.133	1.000
66	21.260	2.560	198.688	1.000
67	21.626	2.390	200.688	1.000
68	21.584	2.494	200.040	1.000
69	28.437	2.873	198.303	1.000
70	23.595	2.784	197.220	1.000
71	21.247	2.354	200.297	1.000

72	23.111	2.420	202.018	1.000
73	21.028	2.077	196.865	1.000
74	28.041	2.746	201.065	1.000
75	27.829	2.547	203.051	1.000
76	19.446	2.312	198.063	1.000
77	28.899	2.725	201.197	1.000
78	32.520	2.564	198.583	1.000
79	26.865	2.218	200.322	1.000
80	23.162	2.133	198.867	1.000
81	25.854	2.412	203.101	1.000
82	24.729	2.254	201.410	1.000
83	23.133	2.273	201.240	1.000
84	23.728	2.694	199.635	1.000
85	24.838	2.801	198.303	1.000
86	23.600	2.443	202.348	1.000
87	22.427	2.524	200.791	1.000
88	24.263	2.053	196.748	1.000
89	27.345	2.738	201.190	1.000
90	21.197	2.327	200.199	1.000
91	29.472	2.516	202.171	1.000
92	27.655	2.141	197.982	1.000
93	22.677	2.332	201.483	1.000
94	27.936	2.629	202.572	1.000
95	20.232	2.434	198.654	1.000
96	28.987	2.707	201.403	1.000
97	28.139	2.400	202.438	1.000
98	29.302	2.193	197.993	1.000
99	21.260	2.292	200.114	1.000
100	25.692	2.628	202.320	1.000

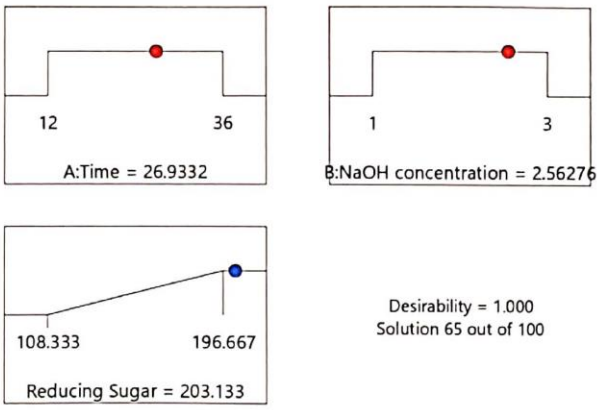

Figure 3. Optimum level of each factor determined using response optimization

Optimum conditions of 26.93 h X_1 and 2.56% X_2 were predicted under which a maximum reducing sugar yield of 203.13 g/L was estimated (Fig .3). This predicted result is 4% higher compare to the experimental value obtained. By running these predicted values using statistical model, RSM proved to be an efficient tool for researchers. A hundred experiments were deemed expensive to execute. Postulating a model needing only small number of experiments saves up the cost of the performing expensive analytical method. To ensure the accuracy of the model, verification analysis was recommended.

4. Conclusions

Alkaline pretreatment (NaOH) suited applied on corn stalk bagasse among the other pretreatment tested based on the sugar yield concentration after the hydrolysis application. NaOH concentration found to have a high influence on the outcome while other factor, reaction time, have low influence on the outcome. However, interaction of these two factors brings positive effect on the sugar yield. RSM proved to be an effective tool for optimization by predicting optimum conditions of two factors resulting to a higher reducing sugar concentration compare to the experimental value. Verification of the RSM model were recommended to verify the predicted versus experimental reducing sugar concentration.

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BIOETHANOL PRODUCTION FROM SWEET CORN JUICE (*Zea mays* L.) CULTIVATED IN CHIANG MAI PROVINCE, THAILAND

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ABSTRACT: Thailand's energy plan proposed an increase on the use of renewable fuel in the country. Their aim is to lessen the country's dependency to non-renewable fuel and to minimize its carbon footprint. One goal of the plan is to increase the biofuel substitution by 25%. Biofuels are considered carbon-neutral and comes from renewable source. The aimed of this paper is to produce bioethanol from corn stalk juice, an agricultural waste in Northern Thailand. Combustion of agricultural waste is a problem in Northern Thailand; this practice worsens the haze pollution in the region. Corn stalk was pressed in a sugarcane presser to get the juice and set aside the bagasse for further study. Corn stalk juice contained 118.57 g/L of total sugar and 53 g/L of reducing sugar. During the 5-day fermentation period, using *S. cerevisiae*, highest produced bioethanol with 6.17 % (v/v) or 48.71 g/L was attained on the 72 h of fermentation. This was achieved without adding any supplement to the corn stalk juice; therefore, scale-up production can saved up a great deal on the capital cost compared to feedstock from lignocellulose source. This study found corn stalk juice as a compelling feedstock for bioethanol production.

1. INTRODUCTION

Thailand plans to increase its renewable energy source by 30% in its 20 year alternative energy development plan. The country targets to lessen its dependency to conventional fuel and to increase renewable and clean energy source [1]. One of its main goals is to increase biofuel substitution by 25%. Biofuels are made from biomass and weighed up as carbon-neutral [2].

Bioethanol, a liquid biofuel, viewed as an alternative or additive fuel to gasoline. It can be made from a variety of resources: starch-based, lignocellulose-based, algal-based etc [3]. Thailand is an agricultural country with huge source of biomass and materials for biofuel production. With this, finding a good material for bioethanol production is being pursued.

Corn is food plant product that's been grown all over the world. It is one of the major crops cultivated in Northern Thailand with a total of 383,790 hectares of plantation [4][5]. Corn plantations were one of the main contributors of agricultural waste in the area. Farmers resort in burning their fields as a way to eradicate corn residues to make way for the new planting season. However, this particular farming practice elicited some negative environmental impacts. One research study mentioned farm practice release a variety of air pollution in both gases and particulate forms [6]. Another pointed out agricultural waste combustion is one of the factors that aggravate haze in Northern Thailand [5]. Therefore, the aim of this paper was to use corn waste material in Chiang Mai province, Thailand for bioethanol production. Another aimed, was to lessen waste generated from agricultural sector and alleviates combustion of agricultural waste contributing to the haze pollution experienced in Northern Thailand.

2. MATERIALS AND METHODS

2.1 Raw material

The corn variety that was used in this experiment was a hybrid of Hybrix 53 and Sugarstar corn cultivated at Chiang Mai Province, Thailand. The sample was collected at Bang Hong District, Lamphun 51130, Chiang Mai, Thailand (Fig. 1).

Fig. 1 Corn Plantation in Chiang Mai, Thailand

It was then transported to the lab and cleaned by stripping out the leaves and panicles, leaving only the stalk. The stalk was then feed to a sugarcane presser to extract the juice. The stalk bagasse was set aside for further study. The extracted juice was then frozen until further use.

2.2 Fermentation assay

The inoculum was composed of yeast extract (10g/L), peptone (10 g/L) and glucose (20g/L). It was prepared in a 200mL erlenmeyer flask and was then autoclaved to sterilize the mixture. After cooling, the yeast was added using aseptic techniques to avoid contamination. The culture was then leaved at room temperature while it was mixed by a working magnetic stirrer for 24 h.

The fermentation was done in a 1L bottle, in triplicates (Fig. 2). The juice was boiled for 15 min to lower the chance of contamination. After cooling, the juice pH was checked and adjusted to 5.6, suitable for yeast fermentation, using sodium hydroxide (NaOH). Ten percent (10%) of yeast (*S. cerevisiae* TISTR 5020) was added to the juice with a 350mL working volume. The bottle was then incubated at 36 °C for 5 days. The fermentation was done in triplicates.

Fig. 2 Fermentation assay

2.3 Analytical methods

Sugars (total sugar and reducing sugar) were determined using Phenol-Sulfuric [7] and DNS method [8] and read in a spectrophotometer DV-8000 at 490nm and 540 nm, respectively. Bioethanol content was checked using an ebulliometer. Fifty (50) mL aliquot was extracted from the sample every 24 h to monitor the change of bioethanol and sugar content of the mixture.

2.5 Statistical analysis

All statistical analysis was done using the software IBM SPSS Statistics 23. ANOVA and Tukey's test were perfumed on the produce bioethanol to determine the significance between the values. The values were considered significant to each other at $p<0.05$.

3. RESULTS AND DISCUSSION

This experiment checked produced bioethanol within the 5-day fermentation, as well as, the sugar content and pH.

3.1 Concentration of sugars

Corn stalk juice contained 118.57 g/L of total sugar and 53.00 g/L of reducing sugar (Fig. 3). Sugar concentration dwindled during the fermentation period. Almost all reducing sugars were consumed by yeast in the first 24 h of fermentation whereas total sugars reduce in half. Total sugar refers to the summation of all the sugars present in the solution meanwhile reducing sugars refers to monosaccharide sugar like glucose and fructose which is the main sugars reduce by yeast to alcohol. Sucrose is a non-reducing sugar that yeast digests for ethanol production [9]. Thiruvengadathan [10] reported corn stalk juice contained sucrose, glucose and fructose; all known readily fermentable sugars. Gomez-Flores et al. [11] pointed out that juice can directly produce bioethanol through fermentation.

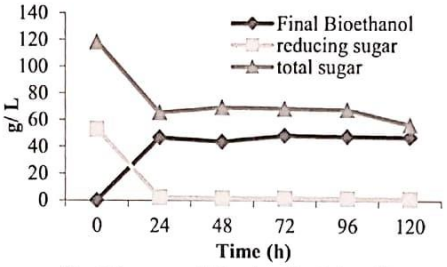

Fig. 3 Sugars and Bioethanol relationship

Sugar concentration decreased significantly during the fermentation period coinciding with the increase of bioethanol production (Fig. 3). Zabed et al. [12] stated the fermentation rate is dependent to the initial sugar concentration. Almost all reducing were used up on the first 24 h of fermentation. This was also observed from the study of Chen et al. [13].

In theory, 1 g of glucose can produce 0.511 g of ethanol [14]. But, the practical yield efficiency is at 92%. Sucrose is a non-reducing sugar that comes mainly from stalk [9]. Equation (1) and (2) shows the conversion of sucrose into simple sugars and its fermentation [15].

$$\underset{sucrose}{C_{12}H_{22}O_{11}} + H_2 \xrightarrow{invertase} \underset{glucose}{C_6H_{12}O_6} + \underset{fructose}{C_6H_{12}O_6} \quad (1)$$

$$\underset{glucose\ or\ fructose}{C_6H_{12}O_6} \xrightarrow{zymase} \underset{ethanol}{2CH_3CH_2OH} + 2CO_2 \quad (2)$$

3.2 Bioethanol production

A total of 5.96% (47.05 g/L) of bioethanol were recorded on the first 24 h of fermentation (Table 1). Meanwhile, at the 72 h of fermentation attained the highest bioethanol content with 6.17±0.13% (48.71±1.00 g/ L) in the 5-day fermentation process; no significant differences were found among the values of % bioethanol (v/v) and final bioethanol (g/L) in varying period of fermentation (Table 1). This value is lower compare to the 8.1% (v/v) of bioethanol from tropical maize [13]. Nevertheless, this confirms the potential of corn stalk juice (hybrix x sugarcorn hybrid) as a raw material for bioethanol production.

Table 1 Percent Bioethanol and pH

Time (h)	% Bioethanol (v/v) *	pH	Final Bioethanol (g/L) *
24	5.96±0.64	4.95±0.10	47.05±5.02
48	5.59±0.20	4.89±0.02	44.08±1.55
72	6.17±0.13	4.86±0.03	48.71±1.00
96	6.09±0.03	4.84±0.05	48.08±0.24
120	6.00±0.00	4.36±0.11	47.34±0.00

*no significant different between the values

3.3 Changes in the pH

There is a noticeable change in the pH of the solution during fermentation (Table 1). The pH turned acidic from the original pH of 5.6, which was adjusted before fermentation. This change in the pH may due to the by-products (weak acids) of sugar metabolism [11].

3.4 Energy balance

Bhatia [16] showed the energy conversion efficiency rate of sugar to ethanol in Eq. (3).

$$\begin{array}{lll} \text{Glucose} \rightarrow & 2\ \text{ethanol} + 75\ \text{kJ} & (3) \\ 180\ \text{g} & 2 \times 46\ \text{g}\ (92\ \text{g}) & \\ 2.82\text{J} & 2 \times 1.37\ (2.74\ \text{MJ}) & \end{array}$$

Applying this equation in the result obtained from this study, we get 1,857 J from the total sugar (expressed as sucrose, glucose and fructose) and 1,450 J of bioethanol produced. A total of 78% energy conversion was observed in this study. From 1,450 J of ethanol can light a 30W LED light bulb for 48 hrs.

4. CONCLUSION

Corn (Hybrix 53 × Sugarstar hybrid) stalk juice contained high readily fermentable sugar and a suitable feedstock for bioethanol production. It has been proven to be a compelling feedstock for bioethanol production by its ability to produce high bioethanol content without any additional supplement and treatment.

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"Make Natural Products the next trend"

APPENDIX C CERTIFICATES AND AWARDS

25th TRI-U2018
@ Chiang Mai University, THAILAND

This is to certify that

Katherine Bautista

Maejo University

THAILAND

Attended The 25th Tri-University International Joint Seminar and Symposium
November 4-8, 2018
Hosted by Faculty of Engineering, Chiang Mai University, THAILAND

Given on November 8, 2018

Assistant Professor Nat Vorayos, Ph.D.
Dean, Faculty of Engineering
Chiang Mai University, THAILAND

25th TRI-U2018
@ Chiang Mai University, THAILAND

Certificate of Best Oral Presentation

For Graduate Student

Katherine Bautista

Maejo University

THAILAND

Your oral presentation

BIOETHANOL PRODUCTION FROM SWEET CORN JUICE (*ZEA MAYS L.*) CULTIVATED IN CHIANG MAI PROVINCE, THAILAND

has been recognized as one of the best in The 25th Tri-University International Joint Seminar and Symposium
November 4-8, 2018
Chiang Mai University, THAILAND

Given on November 8, 2018

Assistant Professor Nat Vorayos, Ph.D.
Dean, Faculty of Engineering
Chiang Mai University, THAILAND

Certificate of Participation

This certificate is presented to

Katherine Bautista, Yuwalee Unpaprom, and Rameshprabu Ramaraj

on the manuscript entitled:

Statistical Modeling and Optimization of Corn Stalk Bagasse Pretreatment for Fermentable Sugar Production

for attanding 2nd Maejo - Engineo International Conference on Renewable Energy
14 - 15 December 2018, International Education and Training Center
Maejo University, Chiang Mai, Thailand
This activity was awarded by:

Dr. Thanud Katpradit
Engineo Co. Ltd.
General Co - Chair

Asst. Prof. Dr. Nutthawud Dusadee
School of Renewable Energy, Maejo University
General Chair

Best Oral Presentation

This certificate is presented to

Katherine Bautista, Yuwalee Unpaprom, and Rameshprabu Ramaraj

on the manuscript entitled:

Statistical Modeling and Optimization of Corn Stalk Bagasse Pretreatment for Fermentable Sugar Production

for the best oral presentation at
2nd Maejo-Engineo International Conference on Renewable Energy
14 - 15 December 2018, International Education and Training Center
Maejo University, Chiang Mai, Thailand
This activity was awarded by:

Dr. Thanud Katpradit
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General Chair

บัณฑิตวิทยาลัย มหาวิทยาลัยแม่โจ้

GRADUATE SCHOOL MAEJO UNIVERSITY

เกียรติบัตรประกาศเกียรติคุณฉบับนี้ให้ไว้เพื่อแสดงความยินดี เนื่องในโอกาสที่

Miss Katherine Bautista

ได้รับรางวัล Best Oral Presentation ในงาน The 25th Tri-University International Joint Seminar and Symposium, November 4-8, 2018 ณ Chiang Mai University, Thailand จากผลงานเรื่อง "Bioethanol Production from Sweet Corn Juice (*Zea Mays L.*) Cultivated in Chiang Mai Province, Thailand"

ให้ไว้ ณ วันที่ 4 กุมภาพันธ์ พ.ศ. 2562

(รองศาสตราจารย์ ดร.เกรียงศักดิ์ แม่งอำพัน)
คณบดีบัณฑิตวิทยาลัย

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Certificate of MJU-TEP Results

This is to certify that

Miss Katherine Bautista

took the Maejo University Test of English Proficiency (MJU-TEP) on

December 22nd, 2017

The test results are as follows (see reverse side for more details):

1. Listening Comprehension Skill	21/30 marks
2. Semi-speaking Skill	19/25 marks
3. Semi-writing Skill	21/30 marks
4. Reading Comprehension Skill	27/45 marks
Total Score	**88/120 marks**

Given on December 28th, 2017

(Asst. Prof. Dr. Kittinoot Chulikavit)
Director, Maejo University Language Center

มหาวิทยาลัยแม่โจ้
MAEJO UNIVERSITY

CURRICULUM VITAE

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