FEASIBILITY ASSESSMENT OF

BIOETHANOL FROM *ZEA MAYS* AND *NICOTIANA TABACUM* STALKS

KARN SOPHANODORN

MASTER OF ENGINEERING IN RENEWABLE ENERGY ENGINEERING

MAEJO UNIVERSITY

2021

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT

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ชื่อเรื่อง	การประเมินความเป็นไปได้ในการผลิตไบโอเอทานอลจากต้นข้าวโพดและยาสูบ
ชื่อผู้เขียน	ว่าที่ร้อยโทกาณฑ์ โสภโณดร
ชื่อปริญญา	วิศวกรรมศาสตรมหาบัณฑิต สาขาวิชาวิศวกรรมพลังงานทดแทน
อาจารย์ที่ปรึกษาหลัก	Dr. Rameshprabu Ramaraj

บทคัดย่อ

กรณีเกี่ยวกับการเปลี่ยนเชื้อเพลิงฟอสซิล วัสดุจากป่า และวัสดุอื่น ๆ ให้กลายเป็นวัตถุดิบ เช่น แป้งและแหล่งวัตถุดิบอื่น อันเนื่องมาจากมาจากจำนวนประชากรที่เพิ่มขึ้น และต้องการผลิตเชื้อเพลิงพลังงานทดแทน วัสดุเหลือทิ้งจากต้นยาสูบและต้นข้าวโพดอาหารสัตว์ปริมาณหนึ่งตันสามารถใช้เป็นวัตถุดิบในการแปรรูปเป็นเชื้อเพลิงได้ ดังนั้นจึงนำต้นยาสูบและต้นข้าวโพดอาหารสัตว์ใช้ในการทดลองนี้เพื่อผลิตไบโอเอทานอล ในต้นพืชมีส่วนประกอบทางเคมีจำนวนมาก ได้แก่ เซลลูโลส เฮมิเซลลูโลส และลิกนิน ตามลำดับ และใช้วิธีฟีนอล – ซัลฟิวริก และ DNS วิเคราะห์น้ำตาลทั้งหมดและน้ำตาลรีดิวซ์เพื่อตรวจสอบน้ำตาลที่ได้ก่อนและหลังกระบวนการหมักไบโอเอทานอล นอกจากนี้ยังได้ใช้วิธีปรับสภาพร่วมให้ชีวมวลสามารถเปลี่ยนเป็นน้ำตาลให้มากขึ้นเพื่อเพื่อผลผลิตไบโอเอทานอล ซึ่งได้เลือกวิธีการที่ได้น้ำตาลสูงที่สุดในการทำไฮโดรไลซิสและหมักไบโอเอทานอล น้ำตาลทั้งหมดและน้ำตาลรีดิวซ์ของต้นยาสูบที่ได้จากผลการทดลองคือ 27.97 กรัม / ลิตรและ 5.43 กรัม / ลิตร ผลการทดลองพบว่าที่ 48 ชั่วโมงของการหมัก ผลผลิตเอทานอลสูงสุดที่ 75.74 กรัม / ลิตร ในศึกษานี้มีวัตถุประสงค์ในการประเมินการผลิตไบโอเอทานอล โดยใช้วัตถุดิบที่มีต้นทุนต่ำ ได้แก่ ต้นยาสูบหลังการเก็บเกี่ยวใบ ในขณะเดียวกันต้นข้าวโพดอาหารสัตว์ก็มีลักษณะดังกล่าว ซึ่งพบว่าความเข้มข้นของน้ำตาลสูงสุดหลังกระบวนการไฮโดรไลซิส น้ำตาลทั้งหมดและน้ำตาลรีดิวซ์ของต้นข้าวโพดมีค่าเท่ากับ 191.667 กรัม / ลิตร และ 84.625 กรัม / ลิตร ผลการทดลองพบว่าที่ 120 ชั่วโมงของการหมักซึ่งได้ผลผลิตเอทานอลสูงสุดคือ 158.59 กรัม / ลิตร การใช้ต้นยาสูบเหลือทิ้ง เป็นแนวทางเปลี่ยนพืชผลทางการเกษตรที่เหลือทิ้งเป็นแหล่งชีวมวลที่สำคัญสำหรับอุตสาหกรรมปิโตรเคมีเพื่อผลิตไบโอเอทานอลในราคาที่เหมาะสม กระบวนการแยกไฮโดรไลซิสและการหมัก (SHF) การผลิตเอทานอลด้วยถังหมักระบบคอมพิวเตอร์ถูกนำมาใช้ในกระบวนการหมักชีวมวลของต้นยาสูบและข้าวโพดสายพันธุ์ดั้งเดิมของไทย โดยลำต้นถูกย่อยหลังจากปรับสภาพด้วยวิธีทางกายภาพ / เคมีที่มีประสิทธิภาพสูงที่สุด ในการปรับสภาพได้ใช้สารเคมีอัลคาไลน์ที่มีต้นทุนต่ำที่สุด (แคลเซียมออกไซด์ 2%) และในการหมักเอทานอลได้ใช้จุลิทรีย์ที่ใช้สำหรับการผลิตเอทานอลทางอุตสาหกรรม คือ

Saccharomyces cerevisiae จากการศึกษาในครั้งนี้ แสดงให้เห็นว่า การปรับสภาพชีวมวลด้วยวิธี
ทางกายภาพ / เคมี และการใช้เอนไซม์ในการไฮโดรไลซิส มีส่วนช่วยทำให้ผลผลิตเอทานอลจากต้น
ยาสูบและต้นข้าวโพดสูงขึ้น อย่างไรก็ตามในการขยายขนาดการผลิตไบโอเอทานอลจากทั้งต้นยาสูบ
ทั้งต้นและต้นข้าวโพดอาหารสัตว์ยังคงต้องมีการทำวิจัยเพิ่มขึ้น และเป็นความท้าทายในการศึกษา
ต่อไป

คำสำคัญ : ไบโอเอทานอล, ต้นยาสูบ, ต้นข้าวโพดอาหารสัตว์, RSM

Title	FEASIBILITY ASSESSMENT OF BIOETHANOL FROM *ZEA MAYS* AND *NICOTIANA TABACUM* STALKS
Author	SUB LIEUTENANT Karn Sophanodorn
Degree	Master of Engineering in Renewable Energy Engineering
Advisory Committee Chairperson	Dr. Rameshprabu Ramaraj

ABSTRACT

Extending the debate about removing fossil fuels, forest and field materials, and other materials into things like starches and conventional sources inevitably increases the number of people who want to produce renewable fuels. One ton of different bio-refinery waste streaming can be used as cultivated tobacco stalk and animal corn stalks. Hence, tobacco stalks and animal corn stalks were used in this experiment to create bio-ethanol. The stalks contain huge chemical compounds, including cellulose, hemicellulose and lignin, respectively. The total and reducing sugar utilizing phenol-sulfuric and DNS methods were used to confirm before and after the bioethanol fermentation process. Furthermore, it was incorporated into the collective pretreatments to significantly affect biomass and more accessibility to available sugars to improve bioethanol yield. Proceed with the bioethanol fermentation with the highest sugar concentration and separate the product using hydrolysis. The total and reducing sugar levels of tobacco stalks were obtained from experimental results of 27.97 g/L and 5.43 g/L. The results indicated that at 48 hours of fermentation, the maximum ethanol yields were 75.74 (g/L). In this report, an attempt has been made to investigate bioethanol production using low-cost feedstock, namely after leaf harvest tobacco waste. In a similar study using animal corn stalks, it was found that the highest sugar concentration after hydrolysis process. The total sugar and reducing sugar levels of the corn stalks were obtained from the experimental results of 191.667 g/l and 84.625 g/l. The results showed that

at 120 hours of fermentation which achived the highest ethanol yield was 158.59 g/L. The use of a plant's tobacco stalk residue will turn a leftover agricultural crop, a significant source of biomass for the petrochemical industry, into an affordable supply of bioethanol. Separate hydrolysis and fermentation (SHF) in ethanol production with a computerized fermenter were used in the biomass process with traditional Thai tobacco and corn. The stalks were successfully hydrolyzed due to the use of an efficient physical/chemical pretreatment. Since it was the least costly alkaline chemical (2% CaO), it was used for pretreatment. The best choice for industrial ethanol production was *Saccharomyces cerevisiae*, which was chosen for the project. These findings support the hypothesis that biomass pretreatment would allow ethanol from animal starch and corn stalk via both physical and enzymatic hydrolysis. While the expansion of bioethanol production using whole tobacco and animal corn stalks is a potentially positive aspect still being studied, some challenges must be addressed.

ACKNOWLEDGEMENTS

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CHAPTER 1

INTRODUCTION

Principles, theory, rationale and/or hypothesis

The growing oil demand would dramatically impact gasoline prices in the transportation market, primarily due to the price of oil being pushed higher by global warming. I like to use it at home, during company transactions, and on the day when on the go. The total energy demand was rising because numerous energy sources were needed to satisfy it. The planet is on the brink of running out of renewable energy, on which the oil industry is based. Because there is such competitiveness in energy supply, many countries are pressured to explore more effective ways of improving their economies. Generally speaking, this climate change is being triggered by the Greenhouse effect's extension would have a worldwide impact. To accomplish these targets, we pursue sustainable and environmentally safe solutions that avoid energy shortages while minimizing emissions, and we must first find ways to do so (Sophanodorn et al., 2020).

A source of supply that is looked to meet increasing energy demand in Thailand. Several initiatives and policies supported the use of alternative energy in various ways to expand on this idea, meaning the use of domestically-produced energy sources such as solar, wind, geothermal, biomass, and hydropower. Concerning energy crops, most of them now being made from field crops, the most widely used source of energy is biomass (Sophanodorn et al., 2020; Vu et al., 2018). Many people in the world are malnourished, so there is a controversy on whether bioenergy should be produced only for human consumption, but, unquestionably, growing crops for consumption will significantly improve people's nourishment. More intensively than previously because of the rise in fertilizer prices, alternative feedstocks for renewable energy (specifically, namely, oil) are needed (Onuru, 2018). In addition, bioethanol has an important benefit over green energy producers: It's an emerging technique that's currently far more widespread in the world. Bioethanol is also known as ethylic alcohol or ethanol. Its molecular formula is CH3CH2OH, which

is the same organic compound that is used in alcoholic beverages. In recent years, ethanol has emerged as the most widely used liquid biofuel; it can use agricultural waste to develop renewable energy. Ethanol is produced by the fermentation of both sugary and starchy plant matter and starch-sugary material and cellulose and hemicellulose production, used as automotive fuel and petrochemical feedstock. It is relatively well understood and often used using the same methods in varying bioethanol concentrations with petroleum. The alternative is to this is it can be used as a source of energy instantly. Currently, it can be shown that the gasoline the water combined with ethanol is called gasohol which can be seen by the beginning of the oil with "E" and followed by a number showing the amount of ethanol blended, such as E10 means oil that contains 10% ethanol by volume of the oil.

Even if all traces of water are removed, the final ethanol or fuel percentage is about 90%." E85 is one of the most widely used gasoline/petrol mixtures in light-duty engines. Bioethanol (a mixture of E10 gasohol) was among the most common options in many countries, including Australia, Columbia, Canada, Peru, and the United States. Although (Balat, 2011) and many others believe that reducing body mass helps improve physical ability, some others argue the reverse. Ethanol use is promoted, as the latest, 91-oil fuel ethanol producers plan to begin a program to use it as a feedstock in the production of gasoline in October 2012, as increased demand for gasoline would otherwise suggest, reflects in a rise in the world's eye price of renewable energy. In 2013, an increase in ethanol was used, which was caused by an increase in fuel consumption. While bioethanol can be derived from various biomass, including sugar, starch, and lignocellulose, it does not occur naturally in this form. As a bioethanol source, succulent biomass has a long growth period and a plentiful supply, indicating that it may be a viable substitute substrate for ethanol production. Plant material is used to produce lignocellulose, hemicellulose, hemicellulose, and cellulose. Lignin is a massive and complex phenol monomer. It is conifer-type alcohol, similar to conifer alcohol or sinapyl alcohol (Hassan et al., 2018; Ramaraj and Unpaprom, 2019).

(Rastogi and Shrivastava, 2017) remarked that the substance was primarily made up of cellulose and lignin after their conversion from lignocellulose biomass, in

which they continued to do more treatment with pretreatment to turn it into green energy. Sugar from hemicellulosic or hydrolyzed compounds is vital to get the chains and covers lifts [mucilizes] separated in order to get total fermentable sugar from the cells. Recently, researchers have done a lot of pretreatment work on feedstock; for instance, physical, chemical, and biological treatments all yield increasing while biological approaches have become more specific. It is also possible to either mill, break up or combine small biomass pieces to provide an increased surface area and porosity in wood. Following a high treatment temperature or no-up to no chemical treatment, or microorganisms are added to the powder or parts, the powders are compacted and allowed to expand. When extreme conditions are applied, the feedstock architecture is separated into its components (Kim et al., 2016; Rastogi and Shrivastava, 2017; Vu et al., 2018). Primary characteristics of alkalinity pretreatment are that it targets lignin while avoiding the degradation of carbohydrates and is rich in porosity and pore size and generalizing benefits on enzymatic hydrolysis. It is much less harmful to pretreat with alkaline pretreatments, such as sulfuric acid and sulfite, for acid. We find evidence that specific therapies are carried out in milder conditions even though they are done at room temperature. We found that soaking in a combination of sodium hydroxide or a solution of ammonium hydroxy produces the same result.

Biomass, such as wheat straw, corn cobs, can also be sold and used in the food supply chain and does not compete with food; and also, since it is available, is both cheap and non- and efficient in biofuel production. In Thailand, corn (maize, which is widely known as "Indian corn" in the U.S.) and tobacco (called "Nicotiana tabacum" elsewhere) are among the most important crops. Tobacco è uno dei prodotti agricoli più preziosiutos dellosi al màso ilèislditc. Cultivated for cigarette leaf extends the [green, prolongs] the life of tobacco leaf used. About 4 million hectares of land were used to cultivate tobacco in Thailand and exported to 125 countries worldwide. All of the stalks are incinerated until the leaves are removed, so the field becomes dangerous. Tobacco (Shakhes et al., 2011) states that the composition of cellulose, hemicellulose, and lignin is the main constituents of fibrous biomass, which comprises 5% or less of tobacco stems/stalks. Waste resulting from extensive

and large amounts of high concentrations of nicotine in stalks face solid waste disposal problems (Acda and Cabangon, 2013).

For example, in March 2019, the Northern Thailand city of Chiang Mai was measured as the most polluted city in the world, displayed in figure 1. In order to satisfy the demand for additional energy, Thailand is an essential source of supply to be looked at and to expand on this idea, several initiatives and policies supported the use of alternative energy in various ways, meaning the use of domestically-produced energy sources such as solar, wind, geothermal, biomass, and hydropower (Wipatayotin, 2019). Concerning energy crops, most of them now being made from field crops, the most widely used source of energy is biomass (Lin, 2018).

TOPS FOR TOXICITY

At some points, Chiang Mai ranked as the worst major city in the world for air quality, according to the AirVisual app. Bangkok was also in the top 10.

Major city	US AQI
❶ Chiang Mai, Thailand	230
2. Dhaka, Bangladesh	170
3. Kabul, Afghanistan	164
4. Chengdu, China	163
5. Hanoi, Vietnam	163
6. Kolkata, India	161
7. Chongqing, China	160
❽ Bangkok, Thailand	160
9. Lahore, Pakistan	153
10. Port Harcourt, Nigeria	151

According to the AirVisual app as of 4pm.

PM2.5 levels in Thai provinces moved into the red, meaning the air quality posed a health hazard.

Cities with the highest PM2.5 levels

1. Chiang Rai	163
2. Phrae	148
3. Lampang	143
4. Chiang Mai	127
5. Nan	109

The PCD's 'acceptable' PM2.5 standard is not in excess of 50 µg/m³, while the World Health Organisation standard is 25 µg/m³.
According to the Pollution Control Department (PCD) at 6pm

Unit: microgrammes per cubic metre (µg/m³)   BANGKOK POST GRAPHICS

Figure 1 Northern Thailand city of Chiang Mai was measured as the most polluted city in the world, March, 2019

Particulate contamination is a recurring issue that is typically exacerbated by the burning of crop residues in conventional farmland and highland agricultural systems. Crop-residue control and calculating the volume of pollutant emissions from burning crop residues for each land-use trend (grain corn, seed maize and integrated cultivation, tobacco stalks burning), as well as the chemical compositions of PM 2.5 emissions from agricultural burning in Chiang Mai Province, Thailand. For over a decade, haze has become a seasonal issue in the North. It typically occurs from January to April, but it peaks in March due to the arid conditions that increase residue-burning fires' severity. As a result, this research is interested in studying two types of plants that are predicted to be further grown to grow at-bed, namely Zea mays and Nicotiana tabacum stalks. Both plants are currently burned by incineration after harvesting. If the study concludes that it is acceptable, it would add value to the remaining agricultural materials and increase ethanol production raw materials.

Objectives of research

1.   To investigate the potential of bioethanol production from *Zea mays* and *Nicotiana tabacum* stalks.

2.   To recognize that the time effect alkaline pretreatment process of each stalks

3.   To evaluate the optimization of pretreatments for the fermentation method by the mathematical model (Response Surface Methodology, RSM).

4.   To analyze the energy and production cost of bioethanol production from these two stalks.

Scopes of research

1.   Zea mays and *Nicotiana tabacum* stalk wastes from the croplands will be used as a feedstock for possible bioethanol production.

2.   Low-cost alkaline (calcium oxide) pretreatment designs with include stem-assisted, will be applied.

3.   The highest sugar concentration method from this experimental optimized method will be chosen and proceed to bioethanol fermentation for three days.

4.   For scale study, the optimizing process will be applied using response surface methods (RSM) for the design of experiments.

Benefits of study

1.   Reduce the open field burning of crop residues (*Zea mays* and *Nicotiana tabacum* stalks) transforms to gaseous form which is released to the atmosphere and making haze.

2.   Avoid the adverse impact (tobacco stalks which is harmful for soil microorganisms) on the subsequent plantation.

3.   Increase the value-added approach and waste to energy production along with economic benefits from the waste biomass of *Zea mays* and *Nicotiana tabacum* stalks.
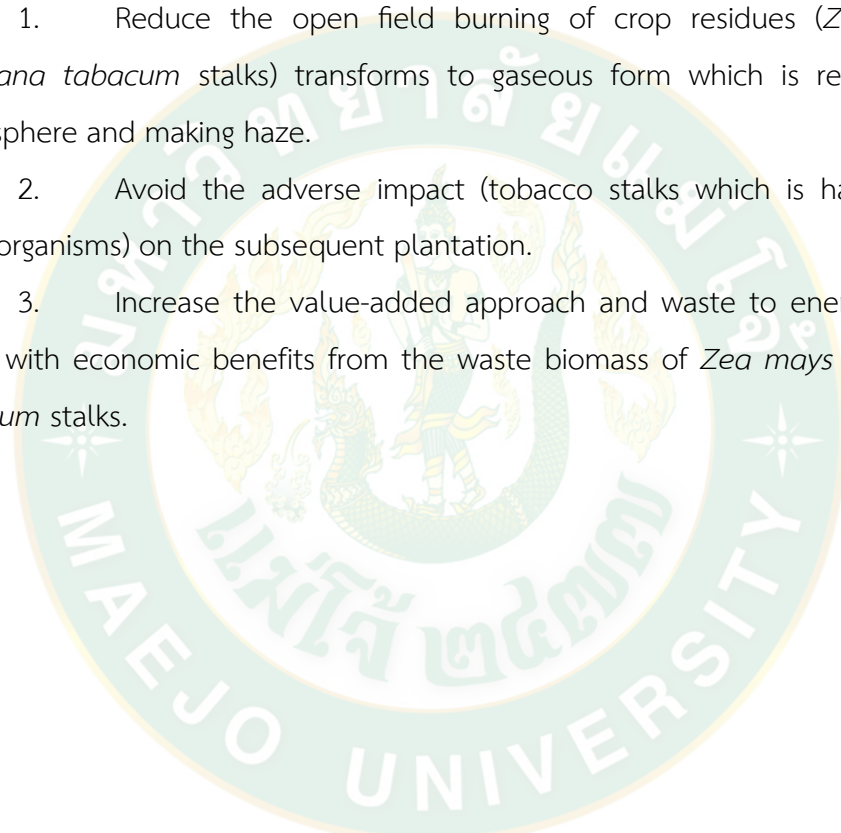
CHAPTER 2

LITERATURE REVIEW

The more I read about it, the more I remember it was never meant to be addictive or drinkable in the first place. One hundred and thirty-seven proof/high octane alcohol has a light color, is very easy to distill, and is valuable in various industries, including the food and beverage industries, but it is highly flammable. It, therefore, can only be manufactured in a limited number of distilling plants. The polycyclic aliphatic resin could be used as diesel fuel and is/was being used to stable plastics for water filtration in vehicles and vessels, among other applications. Ethanol is used as a fuel in the process, for example, cassava [to be ground into a pulp]: Using ethanol as a grinder, cassava is converted into a solid gasoline source. Boil the liquid until it reaches the specific gravity specified in the recipe, convert it to sugar and finish the decomposition with an enzyme. A more incredible amount of sugar can be processed using yeast to produce alcohol. The ethanol would then be distilled at atmospheric pressure, yielding 95.6 percent pure ethanol. The ethanol would then be refined at atmospheric pressure, yielding an additional 95% purity. Ethan can be used as diesel fuel in vehicles if it contains at least 99.5 percent pure ethanol. Ethanol is commonly used as a petroleum additive, a truck fuel, and a fuel in the manufacture of biofuels.

After distillation, the ethanol purity is improved to 99.6%, and the ethanol is separated into parts depending on relative molecular weight. Purity and re-distilled into gasoline with a lower weight percentage Ethanol can be used as a fuel in three ways. Similar concentrations of oil, kerosene, or diesel may be combined. Octane booster is a diesel additive that is often used. The amount of ethanol blended into fuel increases as diesel octane consumption grows. It produces no smoke and is thus safer for the atmosphere. In the case of complete combustion, whether or not conventional fuels are used.

Nonetheless, sugarcane ethanol can be used successfully in Brazil. The nation that manufactures about half of the corn consumed in the United States is also a

major producer of many of the cars we use. Fuels are now being tested to see how they can be used in aircraft.

World ethanol production

With this demand reaching 105.9 billion liters, the world's ethanol supply is anticipated to rise in 2018 (4.62 percent YoY). The growth in the production of ethanol in Brazil has correspondingly increased the global supply, which is contributing to an overall increase in the world supply. as a result of the government's initiative to raise the volume of ethanol mixing in gasoline by five billion liters, a total of 5 billion liters of ethanol was produced worldwide (Table 1).

Table 1World ethanol production (million liters)

Country	2013	2014	2015	2016	2017	2018
Argentina	473	642	815	890	1,105	1,180
Australia	300	230	210	225	210	220
Brazil	23,369	24,477	29,662	26,506	25,804	27,645
Canada	1,720	1,720	1,700	1,650	1,790	1,880
China	2,790	3,200	3,000	2,650	3,500	5,000
Colombia	388	407	456	434	395	4,512
EU	4,623	5,214	5,107	4,791	5,221	5,324
India	270	304	783	1,010	778	117
Paraguay	230	215	225	235	225	220
Peru	240	210	215	196	100	120
Philippines	72	116	168	230	234	264
Thailand	949	1,058	1,174	1,195	1,347	1,480
US	50,398	54,286	56,051	58,032	59,984	60,300
Other	610	664	695	580	542	597
Total	86,432	92,743	100,261	98,624	101,235	105,915

Source: ISO Ethanol Yearbook 2017: ISO forecasts

Production and use of ethanol in Thailand

There was an increase in the average ethanol output in Thailand in 2017 from the 3.32 million liters daily liters that were reported in 2016 to 3.4 million liters (111.13 million liters). Daily, the amount of ethanol generated from C-April 2018 is approximately 4.32 million liters. It was estimated that the consumption of ethanol in 2017 was 3.19 million liters a day. The amount of liquefied petroleum rose from 3.67 million liters per day in 2016. It was projected that in 2018, ethanol consumption was expected to be at 4.11 million liters per day. One million liters a month (i.e., an average of approximately 4.12 million liters a year) has been consumed between January and April 2018. the Year 2015-2017 was when no ethanol was exported out of the United States (Table 3).

Table 2 World ethanol consumption (million liters)

Country	2013	2014	2015	2016	2017	2018
Argentina	221	476	653	804	910	950
Australia	265	220	200	175	194	210
Brazil	17,790	21,456	24,085	82,796	26,200	25,370
Canada	2,585	2,943	3,106	3,000	3,000	3,000
China	2,509	2,890	3,050	3,200	3,200	3,200
Colombia	368	394	418	460	430	560
EU	5,718	5,430	5,387	5,246	5,100	5,400
India	268	211	454	706	925	750
Japan	305	340	485	550	700	775
Paraguay	165	180	195	205	215	225
Peru	123	125	165	165	180	180
Philippines	307	363	442	550	540	545
Thailand	509	948	1,186	1,302	1,430	1,450
US	9,405	50,280	50,900	52,276	54,250	54,700
Other	606	595	629	630	809	885
Total	81,144	86,851	91,355	98,499	97,235	89,200

Source: ISO Ethanol Yearbook 2017: ISO forecasts

The Alternative Energy and Alternative Energy Development Plan 2015-2036 (AEDP 2015) aims to encourage the use of ethanol 11.3 million liters a day by 2036. However, a second DOE and alternative energy development plan issued in 2017 reduced the original goal of renewable biofuel such as ethanol use from 11.3 million liters to 7 million liters per day, as part of an effort to find alternative renewable sources of energy to the DOE anticipated in 2036 to 4.3 million liters per day.

Table 3 Ethanol data for the years 2007-2018

year	Ethanol content			
	Production (Million liters / day)	Use (Million liters / day)	Export (Million liters)	Manufacturer stock (Million liters)
2007	0.53	0.64	14.90	19.26
2008	0.95	0.93	65.80	4.01
2009	1.09	1.25	15.62	24.59
2010	1.17	1.24	48.18	38.62
2011	1.43	1.63	139.28	50.83
2012	1.80	1.39	303.87	19.85
2013	2.61	2.60	63.78	38.69
2014	2.90	3.15	8.18	22.58
2015	3.22	3.33	-	51.76
2016	3.23	3.67	-	19.77
2017	4.00	3.91	-	63.24
2018				
- Jan	4.653	97	-	75.05
- Feb	4.69	4.17	-	114.58
- Mar	4.44	4.09	-	123.90
- Apr	3.51	4.25	-	93.33
	4.32	4.12	-	101.72

Source: Department of Alternative Energy Development and Efficiency Ministry of Energy

Raw materials for the production of ethanol

It was hoped that as more cassava capacity was added to the system in 2017, the new percentage of ethanol generated from it would increase, but growth was slower than expected, so growth in 2018 was slower than expected. On the other hand, the molasses-based ethanol processing process benefits from not requiring any limited raw materials. The manufacturer is typically the enterprise, and the business that started first in the rural region farthest from the sugar factory has the most opportunities. Due to the overall demand for primary raw materials, which is also decreasing, cassava starch production has trouble interfering [plague] with other industries. The industry's supply is influenced by overall fluctuations in raw material markets and procurement questions, such as government subsidies for fixed or variable production costs, as shown in Table 4.

Table 4 Comparison of raw materials used in the production of 1 liter of ethanol.

Material type	Raw material quantity (kg./ Ethanol ๑ liter)
Sugar cane just	10.0
Sugar cane	12.7-14.3
Molasses	4-6
Sweet sorghum	14
Sugar beet	10.3
Cassava	6.50-5.45
Potatoes	8.50
Wet milling	3.68
Dry milling	2.58
Wheat	2.60
Millet	2.30
Paddy rice	2.25
Wood	3.85

Source: Department of Alternative Energy Development and Efficiency Ministry of Energy

The raw material situation for ethanol production

Sugar cane

Sugarcane production area in 2017/18 was 11.54 million rai (5 percent YoY), spread across 47 provinces, with 134.93 million tons (45.16 percent YoY) of sugarcane capable of processing 14.71 million tons of sugar (46.81 percent YoY). The factory received 11.19 million rai (96.97 percent) of sugarcane plantation area, and 5.49 million tons of molasses. Furthermore, according to data from the Office of Agricultural Economics, the volume of sugarcane produced was 86.25 million tons in January to March 2018, up from 79.77 million tons in the same timeframe last year, and the price was reduced from 768 baht per ton to 768 baht per ton. The ministry of industry, which plans to increase the area of sugarcane to replace inadequate rice fields in the amount of 6 million rai, is responsible for 968 baht per ton charged by sugar cane factories (including the existing sugarcane plantation at 10 million rai, totaling 16 million rai of sugarcane by 2026).

Cassava

The second-largest worldwide producer of cassava, but also the second in global terms, generates over 2 billion lbs, and million [and] 700 million pounds of cassava exports per year. According to the World Wildlife Research Bureau's predictions, 2017/2018 (October 2017 to September 2018) is expected to see the rice paddy landscape grow to be 7.87 million rai. According to recent reports, the 2017 harvest was projected to be 27.24 million tons of crude oil produced, relative to the 27.82 million tons from the previous years, which indicates an annual decline of 9.70% yield and 10.67% in production per acre. A large portion of the crop is devoted to exporting; the rest of the cassava is used. Another 25% of the nation's gross domestic product is used. Cassava is used as raw materials for ethanol processing. This ratio ranges from 5-8 percent for culinary use to ethanol, as high as 8 percent for non-food purposes. Farmers' prices have fallen for two consecutive years, causing problems of lowered desire for them to sell the roots, resulting from the trend toward buying cheaper food from traders.

Since this process results in greater returns, farmers may become accustomed to farming sugarcane or animal husbandry rather than cultivating other cash crops, such as maize. This can be noted in some locations where there is a great deal of rainfall, where wetness is achieved. Heavily damaged cassava roots Productivity decreases with each round, so rai must expand. Additionally, farmers have difficulty obtaining funds for maintenance projects, leading cassava plots to be small and of low quality. Thus, the gross amount of output was reduced, too. The analysis predicts that cassava prices would be good for the time period 2010-2015. However, over the year from 2016 to 2017, the average Thai tapioca price was 0.20 bah per kilogram, but in June 2017, it was lower at 0.2 bah.20 kg. The price of the commodity is 1.56 Baht per kg. This is smaller than one can expect. As a lot of Thai cassava is being shipped to China, the top market, consumption in the Asian country has been cut in half. China has adopted policies to return stored corn to a low price, resulting in less corn being used by the agriculture sector.

In this case, Chinese factories decide to use locally sourced corn to manufacture ethanol rather than importing cassava from Thailand because of the economic benefits of ethanol production and its trade restrictions on cassava. In addition to the aforementioned Vietnam and Cambodia, which are considered essential to consumers, cassava is more available and can be purchased to serve those in the country directly than those abroad. Causing a decrease in the supply of Thai tapioca to lower prices and according to the Economic Research Service forecasts, farmers are likely to earn a price that will cover their costs for growing and selling cassava. In comparison to other commodity price predictions, June 2018 rises will result in an average price increase of 2.51 Baht per kilogram over other months. They were particularly beginning in the first quarter of 2018 when China's cassava products' export was impacted by increased demand due to Chinese corn prices, combined with a significantly higher overall global price. Moreover, the need for a larger volume of exporting entrepreneurs experienced a surplus, with enough of the overall crop in partner supply could not meet those needs, resulting in higher prices.

Lignocellulose material

In simpler terms, lignocellulose is the raw material for bioenergy production. Phlegms of the second generation are more efficient and environmentally friendly than phlegms of the first generation. Three essential components exist: hemicellulose, which provides the material with its weight, and cellulose, which provides the material with its stiffness. In this sense, the word "lignocellulose" refers to plant walls consisting of ligninan and cellulose. This happens as two fragments of hemicellas and cellulose, a sugar polymer, are mixed to form hemicellulos me sodonim endo peutaine.
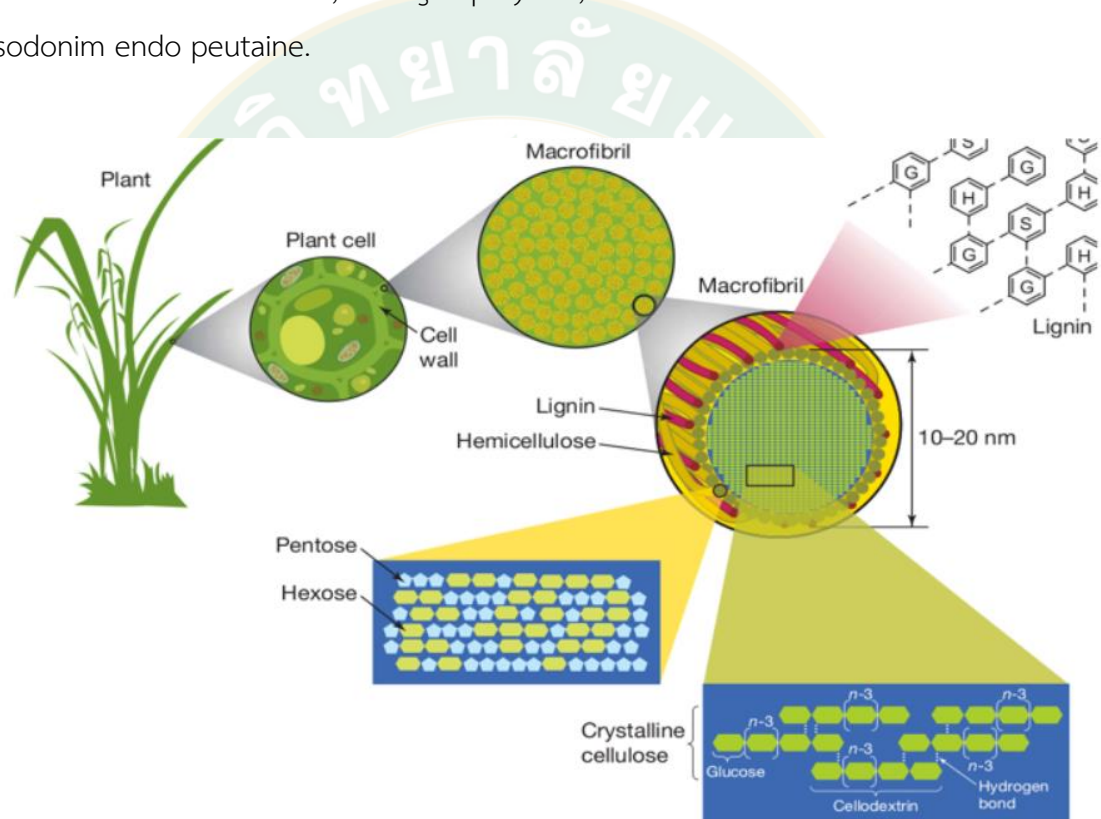
Figure 2 Structure of plant cellwall

Source: http://www.promma.ac.th/main/chemistry/boonrawd_site/lignin.htm

The ratio of lignin, hemicellulose, and cellulose will vary according to the type of plants, as shown in the table. Different parts of plants and harvesting periods affect the composition of lignocellulose materials:—the proportion of lignin, hemicellulose and cellulose (percentage range) presented in Table 6.

Table 5 shows the proportion of lignin, hemicellulose and cellulose (percentage range)

Types of lignocellulose materials	cellulose	Hemicellulose	Lignin
straw of grains	30-40	20-30	10-25
Materials in the grass group	5-40	25-50	1030
Perennial material Eucalyptus or pine	40-55	11-40	10-30

Lignin

Some hardwoods also yield compounds, whereas softwoods extract the least. Then, half of the non-fossil cular carbon was found in lignin 1819, and its use has spread since then. It is equal to one-third of the total mass of dry wood in the fire. Even because it is made of two polyhydroxyalkylene glycols, it has supposed to develop. While other tissues, including wood, have only simple structures, unorganized cellular material, this interpretation does not apply to xylem, and it is believed that the primary cause of its structure is different: Certain material is present in the complicated cells of which this xylem is composed, causing it to grow. Scleritis and tracheids commonly associate with hemicellulose, a part of lignans that can be found in most plant cells and the cell wall of screen throws, and they unite to form a typical mix of polysaccharides.

A cell's strength depends on many other cells, while they bring another tissue's strength. It is an accumulation of plants and trees and vegetation that can be harmed by compression. Primarily nonstress The timber used in natural wood is of no use in applications requiring great stress. The environment for plant growth and development is water is derived from the breakdown of the hemicelluloses (cellulose matrices from wood). The components of the polysaccharide wall cause water to reach the water. While water molecules have been known to inhibit wall expansion, the mechanism which relies on this distinction is not yet fully understood. This means that the plants can be subjected to vascular expansion, the movement of water throughout the tissues, which facilitates plant growth. Other plants, other

than woody plants, include lignin, except crop plants. Due to lignin's action in regulating water movement, it cannot be elaborated on in bytes. Which evolutionary cycle is most likely to be the most widely accepted by plants? This question can only be answered if it is discovered if it can be shown that plants and algae have similar origins, although it could be possible that earlier, in which case, the relation between the two still exists. Moreover, anatomical structures take advantage of the general development of red caladium. Farm carbon is important to the carbon cycle since large quantities of it readily dissociate into carbon dioxide, escaping the atmosphere as carbon dioxide. Because it decomposes too slowly, this is a bichloride substance that is not easily recycled, and when treated with strong acid, it transforms into humus, a kind of decaying plant matter.

Found source

Plant cell wall polysaccharide is essential since it aids in structure building and chemical stabilization. It is also present in the wall, utilizing the cells' growing force to stabilize the wall fiber, mainly made of cellulose and hemicellulose. Generally, the material was made from the lowest of the tree roots and a stem's tip. It is in the process of expanding, is being used to make the final product's entirety. In addition to the development, the amount of lignin produced will increase as they age. It occurs when a plant is mature and has started to lose its fruit's outer layers, revealing a tender, well-developed core. More than raw fruit, the structure of lignin can be shown in Figure 3.
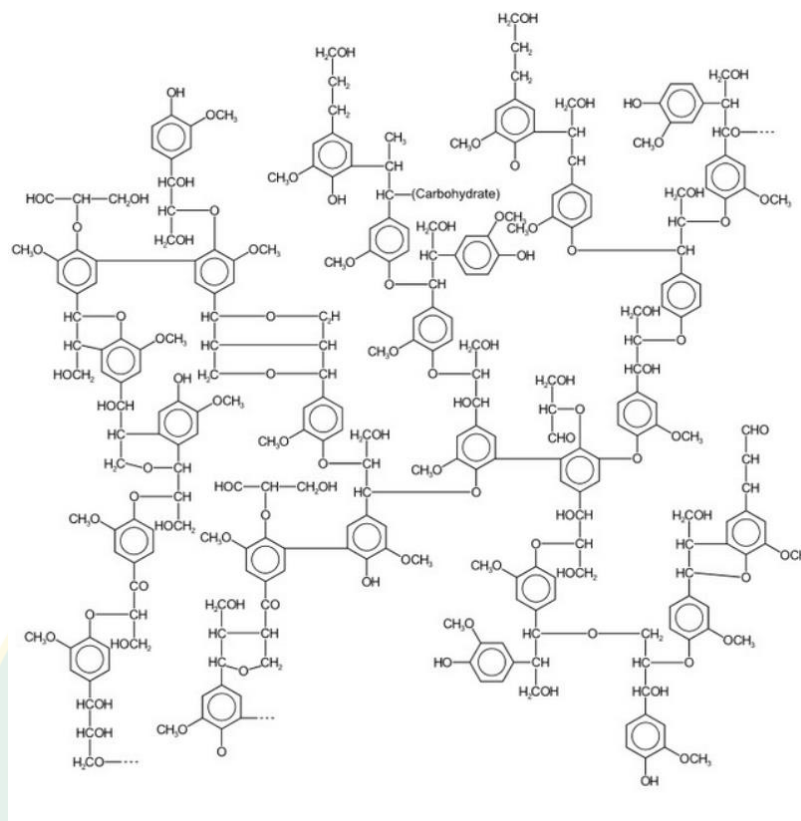
Figure 3 Structure of lignin

Source: http://www.promma.ac.th/main/chemistry/boonrawd_site/lignin.htm

Not only is lignin made from long-chain alcohol, but even it is free of polymer. This can draw molecules of bacteria and fibrous debris together: The mid-lamellar crypt epithelial layer enfolds bacteria and porous debris. Additionally, there are various food and nutrition products present. The molecular composition of phenylpropane is similar to that of phenolic, or hydrocarbon compound having 9 carbon atoms: The Hydrocarbon Compound Formula: Phenylpropane, or about 65-67% of the molecule, has a Phenyl group throughout it. Currently, it is not possible to isolate lignin. Since the graphite network composition is still unknown, the structure of the graphite structure cannot be studied. However, people are studying the chemical formula Can be analyzed to $C_9H_{8.83}O_{2.37}$ (OCH3) 0.96 with a molecular weight between 3,000-30,000.

Benefits of lignin

Lignin and lignin derivatives can be utilized extensively as follows:

1. They are used in the paper industry as adhesives such as scales, binders, and coatings.

2. They were used as farm chemicals (agricultural chemicals), including soil enhancement chemicals, dust management chemicals, fertilizers, pesticides, fermentable herbicides, and animal feed additives.

3. It is used to manufacture chemicals used in water purification and wastewater treatment, such as ion exchange compounds.

4. Apply cement to improve properties, such as aiding with the well-hardening of the cement.

5. They are used in the oil drilling industry as drilling fluids and sealants for oil wells.

6. They are used as a catalyst, elastomer, and plasticizer in the rubber industry.

7. Lignin was used to disperse the particles. It is helpful in a wide variety of industries, including removing ink from printing, plating, and garment industries such as dyeing.

They are also used in other markets, such as tanning and battery manufacturing.

Cellulose

Cellulose has a molecular formula which is ($C_6H_{10}O_5$). Cellulose is a polymeric compound. Long, slow hydrolysis reaction foods are highly carbohydrate-rich foods with low hydrolysis polysaccharides, polysaccharides, and high molecular weight. The molecular formula of glucose (a polymer composed of glucose molecules) is containing with a hydroxyl group. It is the primary functional group pinch-of-off molecule (also known as an Organoglycosan formation) of 1,000 glucose molecules is cross-linked to the next linker at the b-end by a chain of CH with an ordered 1000 glycosidic molecules (as opposed to random bonds, W: The pinch-of model glycos linker phage network is organized into 1,000 glycosyls. Extend the figure from 1 to The molecule contains a replicated unit called cellobiose, and each second member of cellobiose is capable of rotationally connecting the hydroxyl

groups of the following units. The strength of cellulose is improved by the addition of glucose to the molecule. In addition, it has a higher melting point, but it will not dissolve in any kind of solution. (Hao et al., 2000). Figure 3 shows the structure of the cellulose.
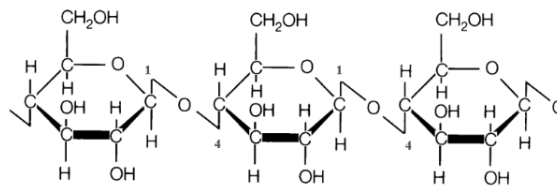
Figure 4 the structure of cellulose.

Carboxymethyl cellulose (CMC) is a cellulose derivative that is a hydrophilic hydroxylated polymer. Both are hydrocolloids formed by altering or strengthening the properties of cellulose, a part of plant cell walls. CMC or general cellulose derivatives can be prepared from cellulose membranes containing high-quality cellulose or alpha-cellulose by replacing the original structure with methyl groups and carboxymethyl groups, as seen in Figure 4. As a stabilizer, CMC is employed. Viscous additives that aid in adhesion or may be used as a coating Laundry, adhesives, paper, food, and pharmaceuticals, to name a few industries. (Pejo et al., 2008). The molecular structure of carboxymethylcellulose displayed in Figure 4.
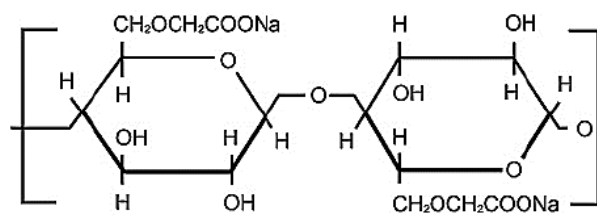
Figure 5 the molecular structure of carboxymethylcellulose.

Source: http://micro.magnet.fsu.edu/cells/plants/cellwall.html

Cellulose is the main structure (Figure 5) of plant cell walls such as vegetables, fruits, and grains, combined with hemicellulose. Furthermore, pectin cellulose is a form of dietary fiber. It is insoluble in water and cannot be digested by enzymes in the human digestive system or by animals with a single stomach. Cellulose is also present in molds' cell walls and is produced by bacteria such as Acetobacter xylinum, which is found in coconut jelly. A compound that is not soluble in liquids. Furthermore, enzymes in the human digestive system and single stomach animals cannot absorb it. Cellulose can also be used in mold cell walls. Furthermore, cellulose, which is derived from bacteria such as A. xylinum and used in coconut jelly, is known as dietary fiber. One species is water-insoluble. Humans cannot digest with enzymes that digest carbohydrates in the body, such as amylase, because they are immune to the reaction of diluted enzymes, acids, and bases. When cellulose in wood is charred, it produces a smoke odor, which is used in the preparation of smoking food.
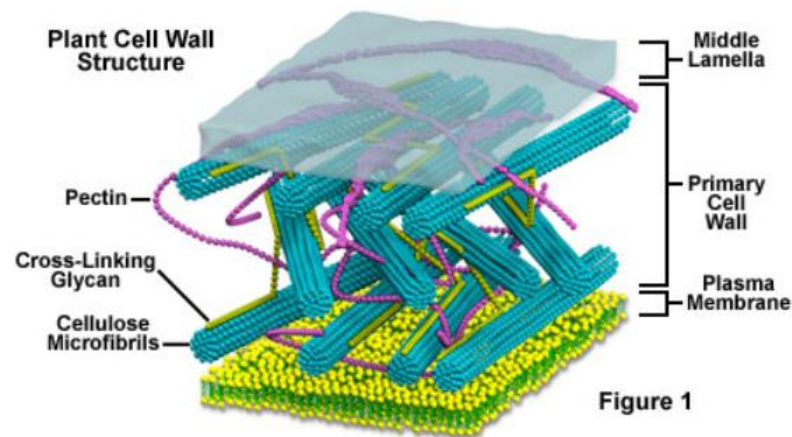
Figure 6 shows a plant cell wall structure consisting of cellulose and pectin.
Source: http://micro.magnet.fsu.edu/cells/plants/cellwall.html

Hemicellulose

Hemicellulose (Figure 6) is a branched heteropolymer polymer consisting of various branched heteropolymer, such as D-Xylose, L-Arabinose D-Manose D-Glucose

D-Ka. Uronic acids and lactose can also be present in L-rhamnose and L-fucose sugars in the structure. It can, though, only be present in trace quantities. Furthermore, the acetylene group will replace the hydroxyl group of sugar in the hemicellulose structure. In this case, eurocic acid is a sugar acid that contains both a carbonyl and a carboxylic group in its form. Euroic acid is an acid present in hemicellulose. Glucuronic acid (D-glucuronic acid) is a type of sugar D-4-O-Methyl galactonic acid and D-galactonic acid are two types of galactonic acid.
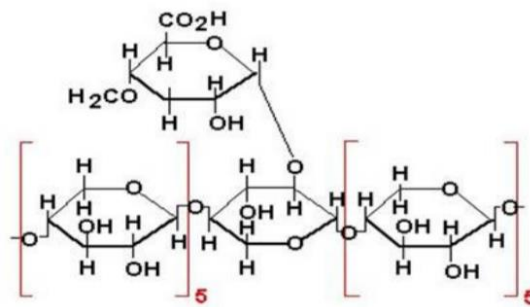
Figure 7 shows the molecular structure of the hemicellulose.

Source : http://www.foodnetworksolution.com/vocab/wordcap/cellulose, http://en.wikipedia.org/wiki/File:Hemicellulose.png, http://2008.igem.org/Team:Wisconsin / Project

Pretreatments

There are many methods of physical conditioning. Lignocellulose materials can be grouped into 4 groups: physical pretreatment, chemical pretreatment, pretreatment by physical chemistry, and pretreatment using biological methods.

Physical Pretreatment

The use of instruments or devices was applied for chipping, milling, and grinding lignocellulose to maximize the sample surface area and minimize particle size. It also decreases the size of cellulose crystals. (Harun et al., 2011) It can be minimized in general. After grinding, the raw material is roughly 1-3 cm in height. Moreover, the dimensions are 0.2-2 mm. The energy needed to mill raw materials

after the target biomass's final size determines fine grinding. Physical strengthening techniques are often used in combination with other pretreatment processes.

Physicochemical pretreatment

Stream explosion. Botanicals are compressed under pressure and exposed to many solvents before being sent into the treatment system at lower temperatures. At that point, reduce the strength of the applied pressure. Temperature is transformed and hemicellulose at a lower pressure. Owing to can temperatures and increasing cellulose breakdown capacity, individuals will reduce their caloric content as easily as they accumulate it. Many different things included time, temperature, the volume of the biomass, and the heat treatment intensity (Pejo et al., 2008). It has the advantage of only needing one-or little-grinding compared to other computer processing methods: the use of lower energy. Although it is a smart practice to use to condition freshly milled hardwood and agriculture waste, conditioning forestry waste is more complicated. In the other side, softwood is just half as effective as hardwood when used together." One drawback of this system is the degradation of the polysaccharides present in plant cell walls, impeding the microbe's capacity for action in the subsequent processes (Cheng, 2017).

Ammonia fiber explosion (AFEX) entails exposing biomass to liquid ammonia at elevated temperatures and pressures for a prolonged period. The strain is then decreased. For this method to be reliable, four critical variables must be adjusted: ammonia load, temperature, and pressure. Water load, temperature, and reaction time are all variables to remember. In general, the AFEX process uses 1-2 kilograms of liquid ammonia per kilogram of dry biomass for 30 minutes at 60-120oC and 1.72-2.0 megapascals pressure (Kumar et al., 2009). This process will aid in the conversion of starch to sugar. However, when used to condition biomass with high lignin content, such as newspapers (18-30% lignin) or wood chips, this method is less successful (25-35 percent lignin). Furthermore, AFEX has been found to be more costly than steam blasting (Cheng, 2017).

Chemical pretreatment

Ozonolysis

Ozone can decompose lignin and hemicellulose in lignocellulose materials such as wheat straw, bagasse, grass, plants, nuts, pine trees. Ozone is a good oxidant that is water-soluble. It can break down the structure of lignin and release soluble and low molecular weight compounds such as acetic acid, flinoic acid. The efficiency of enzymatic degradation of biomass increases with the ozone processing. The advantages of pretreatment with this method are (1) effective in eliminating lignin (2) not producing residues that are toxic to further processes and (3) reactions can be carried out under temperature and pressure conditions. Rooms, however, require large amounts of ozone in the pretreatment process, resulting in high costs (Cheng, 2017).

Acid hydrolysis

Ozone will release the hemicellulose materials found in these products by breaking down the sign and hemicellulolytic used in wheat, rice, corn, and pine trees. Since ozone can dissolve in water, it is extreme. Like cellulose, ligninophin increases the molecular scale, allowing smaller, non-aqueous molecules of low molecular weight to escape, such as formic acid and acetic acid because of the increased enzymatic activity of ozone, the efficacy of biomass deoxidation increases. Not compromise subsequent treatment with the lignin extraction; does the use of this procedure offer an effective means to remove lignin; is efficient; and must not be achieved at high temperatures or under pressure, which is helpful for processing afterward; it may also be conducted at room temperature and pressure. The contrast between rooms on the one side was made less difficult by the need for large amounts of pretreatment, on the other hand increasing care costs (Cheng, 2017).

Alkaline hydrolysis

In the alkali pretreatment, the most widely used, the following compounds are used: sodium hydroxide and lime. The different inorganic compounds can cause a reduction in cellulose and assist in breaking down the lattice structure of the

biomass (Sun and Cheng, 2002). Alkaline therapy is simpler to grasp and use than acid treatment because it is almost zero. In this concentration increase [can] be achieved by expanding the reaction time without sacrificing the catalyst's original properties [Polsášer et al., 2014b]. Additionally, (Cheng, 2017) observed that 70% of hemicellulose is broken down in 4 weeks under alkaline conditions at room temperature and with mixed enzymes.

A mild oxidative treatment after delignification allows the following step to gain by breaking down lignin with hydrogen peroxide to function more efficiently. As (Hao et al., 2000; Harun et al., 2011) if a g steam blasting the raw materials beforehand is performed in the presence of iron (Fe) and peroxide (PrO3) react with each other to form hydroxyl radicals (H2O) to be more efficient in the fermentation. Since the Fenton reactions would break down toxic compounds, such as Furan, it was used by (Hao et al., 2000) to do take apart plant phenolic. (Jain and Vigneshwaran, 2012) used phenolic components (PFNA) reactions were run on cellulose (PHAN in this instance). Moreover, further studies were carried out on its impact on Furan (dismantioxidant capacity). It was discovered that the Fenton reaction was effective in modifying the raw material so the enzyme could successfully reach and degrade it. Also, Fenton reaction experiments (at room temperature (28-30ºC) with H2:C:COD ratio: Fe of 20:1) showed that industrial wastewater treated with the solution containing 140 g/L to produce POD after sitting for 14 days at 45° for 60 days. (Sinnaraprasat and Fongsatitkul, 2011). Like other microbeerythomas, the diatogenic fruits and vegetables contain carbohydrates, from which are available to be fermented to generate alcohol (Sinnaraprasat and Fongsatitkul, 2011).

Empowering blasting can increase raw material production and provide more efficiency for the final product. Owing to such toxins, including furan and this reaction is an alternative for pyridine degradation. Accommodatements "Everyone has free will except for those people who are governed under an authority, those who are ruled by a certain principle have a special authority," said (Jain and Vigneshwaran, 2012), who experimented with a solvent and studied how to change the cellulose content in their food solutions. The findings indicate that the Fenton reaction

effectively broadened the spectrum of a hydrolysis enzyme in raw material, allowing for better access and biodegradation. Also, at room temperature of 26-30 degrees H2:Fe2+O:C (expressed as the ratio of H2 to Fe2+ to COD): Under these conditions, the Fenton reaction had a Fe2+O to H2O ratio of approximately 130:20 in wastewater obtained from palm oil factories (Sinnaraprasat and Fongsatitkul, 2011). The maximum amount of sugar is given to produce alcohol) is needed for yeast, and each form of yeast (the four yeasts mentioned above) has its fermenting potential (the four yeast strains described here vary in the amount of raw sugar they use) (Sinnaraprasat and Fongsatitkul, 2011).

Biological pretreatment

Bio-degradable polymers such as the fungi produce brown-mold, white-mold, and soft-rotting Microbial decomposition treatments such as mold and brown-mold are used to decompose in the lignin and lignins. (Mold like Trichoderma rei and Trichoderma asper AG1) is associated with subclinical vitamin B deficiency. While cellulolytic bacteria and anaerobicetes are able to produce enzymes, various aeobacterial groups also produce cellulolytic enzymes. These cellulase and hemicellulase enzymes can completely break down lignocellulose substrates. Instead of merely expanding the world economy, (Lin, 2018; Polprasert, 2014) is arguing that now the international community should redistribute economic power to sub-Saharan Africa to facilitate greater global growth. The energy efficiency of this method is lower than standard filtering techniques. Do not use toxic substances in the production sector to serve the world. The decomposition rate is high but does not take a long time, requiring much processing space and producing very little residual material.

Hydrolysis by enzyme technology

It is then followed by enzymatic hydrolysis after pretreatment, after which benzoic acid hydrolysis is used to hydrolyze the cellulose for ethanol production. While global R&D activities have been ongoing for decades, two important issues remain that impede the process's potential. Pretreatment of biomass by enzymes

causes enzymatic hydrolysis, and this is a necessary step in the transition of cellulose, from R-5-sulphated glucose to its stereoisomer R-5-mono-cose. to carry out the bioconversion of cellulose under moderate conditions of 30° to 50 ℃, and even in a pH of 4.5 to 5.0 to help diminish corrosion issues

The major biotransformation enzymes that catalyze the hydrolysis of carbohydrates in bioreactors are amylases and cellulases. They are glycosyl hemohydrolases, having up to 115 isomerases, or belong to the largest of the bi-, mono-, di- and triangulated disaccharidases families (Lin, 2018; Polprasert, 2014). Comparable to enzymes for starch ammonolysis, the enzyme is widely available for cellulose cleavage,gu John T. Hodgkin's solution is much more costly for cellulose hydrolysis. for more than 70 years, Trichoderma reese has been determined to be the best for cellulase development and has been tested for more than any other plant. Because several enzymes synergistically perform cellulose hydrolysis, there is no way to alter these enzymes' properties without increasing the efficiency of the others; making better enzymes and improved enzyme ratios to hydrolyze effectively the most efficiently is the only realistic path to take. The heterogeneous nature of the enzymatic hydrolysis reduces the reaction rate, and the enzyme must be at a dosage of one to two units of enzyme per catalysis is required for it. However, because glucose is readily emitted from lignocellulosic biomass, there is no decrease in livestock feed cost, even though the overall biomass is cheaper. In terms of ligninolytic and hemicellulization, this process's usefulness is limited to how much biomass can be dissolved and solubilized by the pretreatment; its duration depends on hydrolysis and enzyme loading.

Types of fermenters

A bioreactor may be any structure or process that makes the system work, holds in place, or extends. Reactions such as fermentation or cell growth in living organisms are the most commonly seen types of bioreactors. There are two primary types of experimental system-embedded scales: a small one placed on a desk or around a lab and a large building-dependent. Bioreactors may be configured to regulate environmental variables such as temperature, pH, strain, light emission, oxygen levels, and aerobic or anaerobic environments. Bioreactors have been divided

into two general types in biology: batch (also known as bulk) and continuous. The biological process is also known as a single- Vessel, for the name of a batch bioreactor, which is a means process occurring in a single vessel. Batch fermentations were seen as alcoholic fermentations at present.

- What batch bioreactors have to offer: This technique is commonly used in contemporary businesses and technologies, which has been enhanced
- An optimal solution that is well suited for several forms of economic reactions.
- Enlarge is often used for distillation, crystallization, liquid-liquid separation, and also [that is] for.
- A container used item that is designed to hold a small amount of something.
- Capable of [of handling] perhaps the most complicated materials.

Biological emulsifiers can also produce larger emulsified water droplets at slower and constant rates, whereas a bioreactor can make bigger emulsified water droplets at a slower and constant rate over time. In continuous bioreactors, a flow of reactants causes biochemical reactions to occur and the sample, therefore, results are fed out by the same mechanism as the reaction.

In the continuous bioreactor, there are several advantages, which include:

- As long as digital media is being implemented, products and waste should be eliminated while products and waste are introduced.
- They are favoring further separation between the reactants and the better separation between products which yields better product separation.
- Smaller grains can be handled more efficiently, leading to improved mixing rates.
- The amount of product that is manufactured is increased by changing the duration of the process, thereby controlling the production.
- There are two fundamental ways that we can expand our operational freedom for producers

Ethanol production

The production of bioenergy, such as bioethanol or biodiesel, will be divided according to the type of raw materials used in production into 3 models, which are First-generation biofuels are bio-energy produced from raw materials in the form of starch, sugar, or vegetable oil. These raw materials are agricultural products and also human food. Second-generation biofuels are bio-energy produced from raw materials that are not human food sources. Such as manufactured from materials in the lignocellulose group, including agricultural and industrial wastes. It may also be agricultural products and waste from the forestry industry. Examples of second-generation energy production raw materials are bagasse, corncobs, rice straw, napier grass, etc.

Third generation biofuels are bio-energy produced from raw water plants which here means algae in the 3rd generation bio-energy production study, large algae use and the use of microalgae. in the production of ethanol from lignocellulose materials, there are more steps and processes for ethanol production than the production of sugar and flour ethanol, that is, the process of preparation and conditioning. Therefore, the ethanol production process from lignocellulose material can be divided into 5 main steps, consisting of preparation and reduction of the lignocellulose material, the adjustment of raw materials Raw material digestion, fermentation and separation for purifying products.

The study of various aquaculture methods for the production of ethanol from lignocellulose materials, including the development of microbes for the production of integrated bio-ethanol, provides production options. Dry yeast S. cerevisiae via separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) could perform for bioethanol fermentation industries. SHF is a fermentation process based on the separation of hydrolysis to degrade the feedstock into monosaccharides continuously by fermentation process with a fermentative microorganism that converts fermentable sugars into ethanol. The advantages of this process are low cost of chemicals, short residence time and simple equipment system. The advantages of this process are low cost of chemicals, short residence time and simple equipment system. In SSF, carbohydrate polymers are converted to

fermentable sugars by cellulases and xylanases. SSF process requires a compatible condition with similar pH, temperature and optimum substrate concentration.

Ethanol can take many forms. In Figure 7, a summary of possible ethanol production processes from lignocellulose materials takes into consideration existing and currently developing technologies. In Figure 7, a production process using pre-fermentation digestion methods. This process will have the most operational steps. They are focusing on the digestion of lignocellulose materials to obtain sugar before going into the fermentation process to convert into ethanol. Therefore, the key points that will affect this production process are the efficiency of digestion of lignocellulose materials, which is related to the efficiency of the material pretreatment to make it easier to digest cellulase enzyme. The problems encountered for the digestion of the material into sugar are some of the sugar that is digested is in the form of oligomers that microbes can not be used. Also, the digestion process may require large amounts of enzymes as the sugar released from the digestion inhibits the end-product inhibition effect.
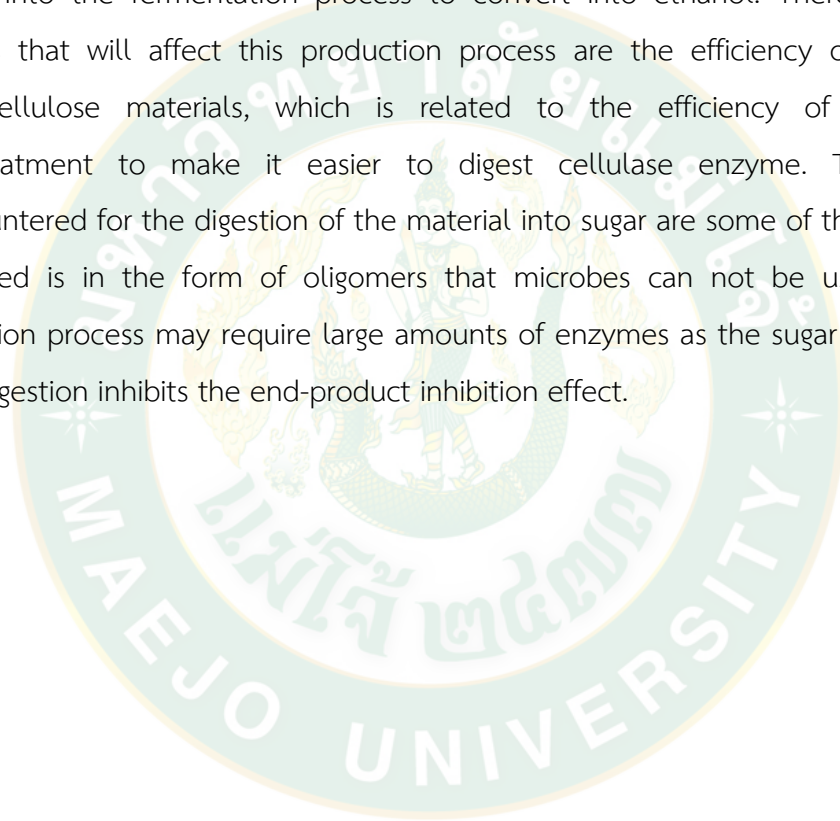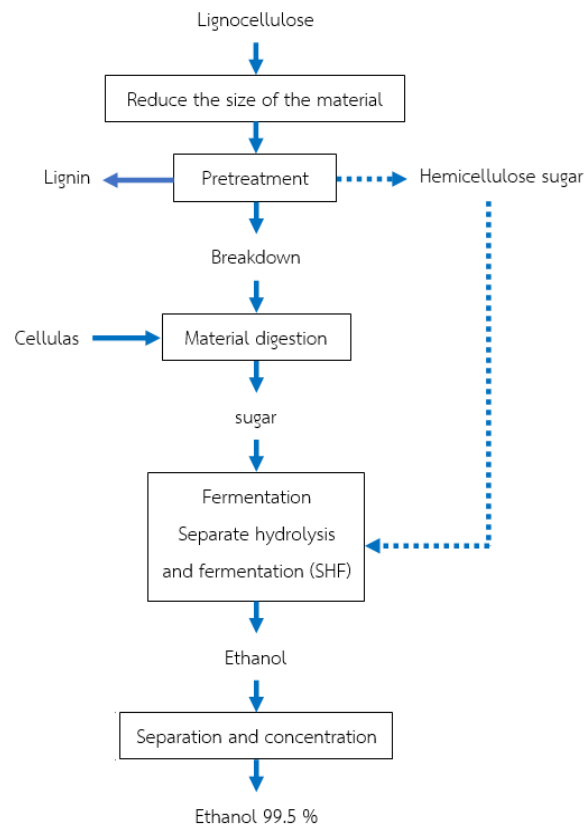
Figure 8 shows the process of fermentation of lino-cellulose sub-fermented materials

Source : Balat et al., 2008

The addition of fermentation may also use sugars resulting from hemicellulose digestion before the grain begins to germinate. The most effective way to improve this formulation's fermentation could be to select microbes that use five-carbon molecules as a fuel source or have evolved to use five-carbon molecules as fuel. This is represented in Figure 7, where lino-cellulose is colonized by lactic acid bacteria and then poly-substrate milk.

Under an expansion technique, another potential processing phase is a sub-fermenting ready ferment. The expansion and the author's steps to it are illustrated in Figure 8. Same treatment: The material this production cellulariophane goes through is pretreated just like the material lignocellulose galactanide, which is produced. Liquified and made permeable The cellulose content undergoes after

being sub-expanded via the lignified through a three-proceduretation stage. The added cellulase acts to reduce syrup accumulation caused by high-BEGF, then goes through the fermentation process to help reduce sugar. Because the structure breaks it down, its immediate consumption by microbes that produce ethanol-producing microbes has much of the work of fermenting sugar being done during the sugar build phase. Thus, the fermentation liquor's decreased pressure during the whole procedure is used in the fermentation as those used in the operation of initial or primary fermentation.
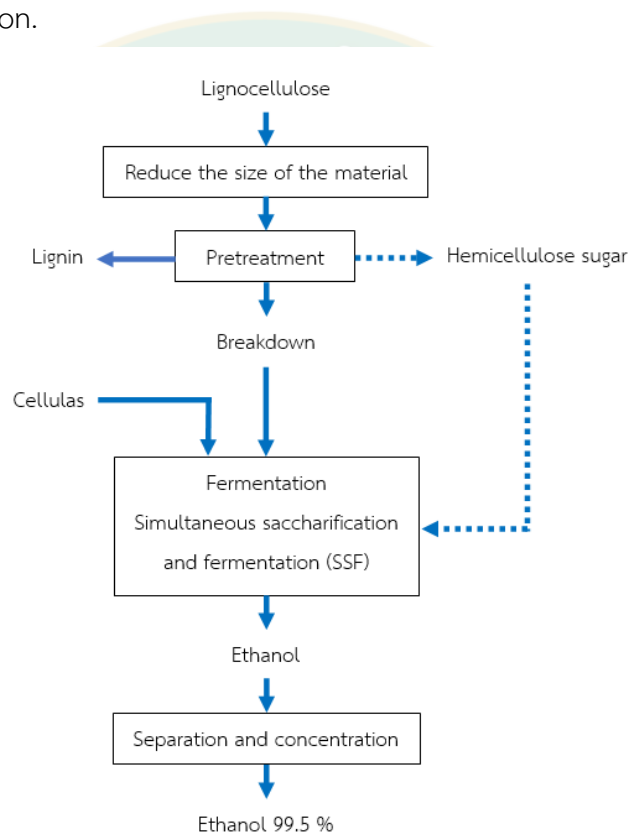
Figure 9 shows the fermentation process for liquefied cellulose materials, digested, ready to ferment

Source : Balat et al., 2008

Additionally, the issue with sub-free culture is mass transfer and agitation, limiting their capacity to produce certain types of beer to very small quantities. The object's size can cause problems when beginning to ferment, so it has to be treated with caution when getting ready to add to the fermenter. When the ethanol

fermentation mechanism is combined, less complicated means are typically associated with expansion, common to a large undertaker and general undertaker. Even so, this technology is still in its early stages of growth. What makes this procedure effective is the rate of CBP microbe growth, whether it is the CBP I or CBP II microbes. The fermentation process for lignocellulose materials in an integrated bio-process is anticipated in Figure 9.
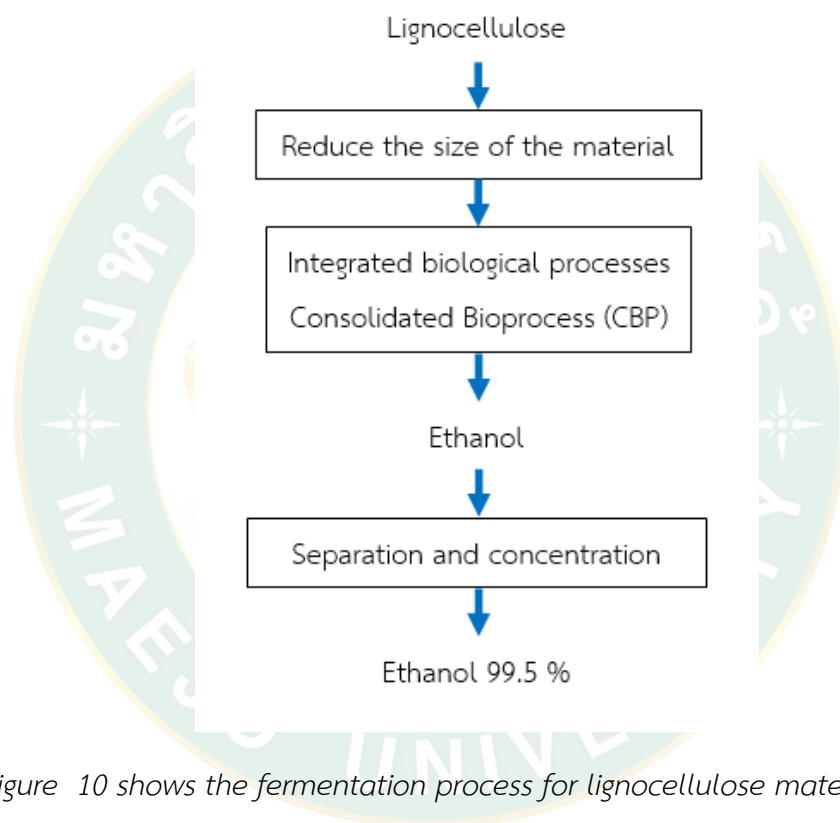
Figure 10 shows the fermentation process for lignocellulose materials in an integrated bio-process.

Source : Balat et al., 2008

CHAPTER 3

MATERIALS AND METHODS

Conceptual framework and methodology

Figure 11 depicts the conceptual framework and methodology for the study
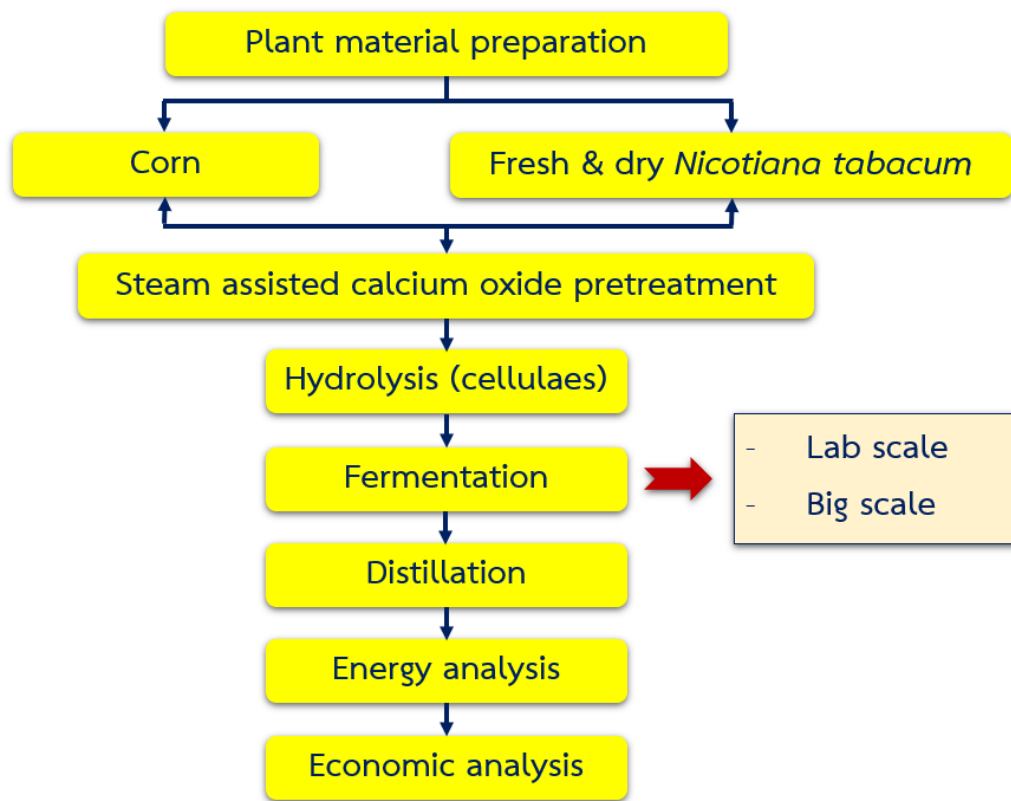
Figure 11 Conceptual framework and methodology

Samples preparation and composition analysis

Corn samples (Zea mays) harvested at area Rong Wua Daeng, San Kamphang, Chiang Mai, Thailand (Latitude: 18°15'17.2"N, Longitude: 99°58'56.3"E) presented in Figure 11 Nicotiana tabacum stalks are collected from Ban Laen area, Vieng Ta, Long, Phrae, Thailand (Latitude: 18°44'33.4"N, Longitude: 99°10'32.1"E) shown in Figure 12 Within 2 hours, both samples were delivered to the School of Renewable Energy (Energy Research Center), Maejo University, Sansai, Chiang Mai-50290 for identification and analysis. The sample was also used following the research schedule. In order to exclude systemic changes, a fresh sample of Nicotiana stalkes was studied for a short

period. Both plant samples were also dried and stored for component analysis and further treatment. Air drying and solar drying are used to dry the samples.

Figure 12 Tobacco plants are prepared to be delivered to the laboratory.

Figure 13 Making powder by blender about 1-2 mm of samples

Figure 14 Size reduction of samples.

Figure 15 Corn sample location and available materials.
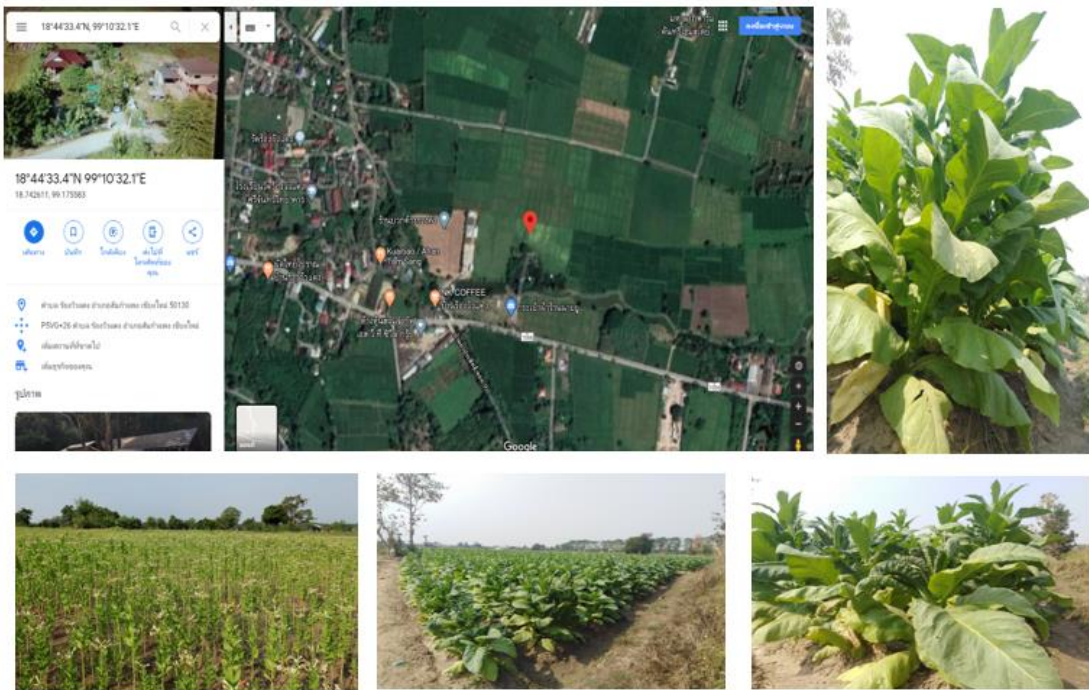
Figure 16 Nicotiana stalks sample location and available materials.

After that sample size reduction for pretreatment and SEM analysis (Figure 17).
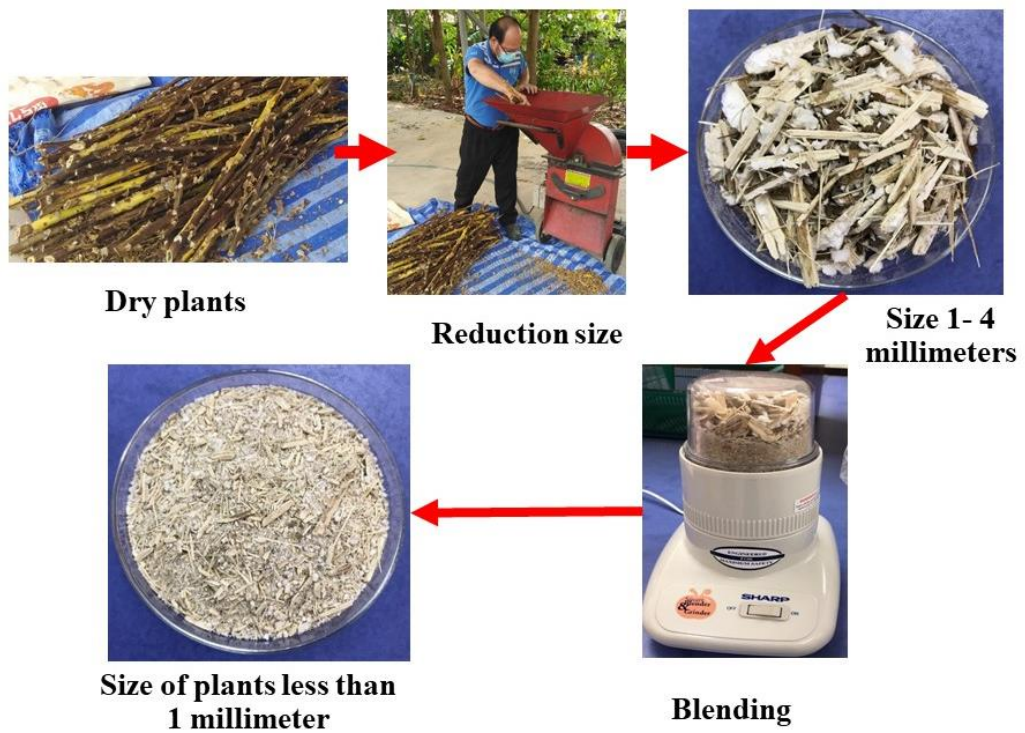
Figure 17 Sample size reduction for pretreatment

Determination of lignocellulosic components and sample preparation for scanning electron microscopy

According to (Vu et al., 2018), the percentages of cellulose, hemicellulose, and lignin in the samples were calculated using the normal methods of neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). In a reflux device, 1 g of dried tobacco and corn residue, 100 mL of NDF, and 0.5 g of sodium sulfite were boiled for two hours. Using the cold extraction machine (FT 121 FibertecTM, Denmark), the cauldrons and test samples were washed three times with hot water and acetone. After cleaning, the pots and samples were continuously immersed in ADF solution and heated for 1 hour and 30 minutes in a hot extraction unit (FT 122 FibertecTM, Denmark). After the reaction, the crucibles were washed with boiling water, purified water, and acetone and dried for 4 hours at 105 °C, held in a desiccator, and weighted until steady for ADL. The percentages of NDF, ADF, and ADL are used to measure the cellulose and hemicellulose rates. The sample preparation for scanning electron microscopy (SEM) and the sample characteristics measurement technique followed (Ramaraj and Unpaprom, 2019). SEM was used to analyze the morphologies of raw and pretreated tobacco (JSM-5410LV). The photographs were taken at a magnification of 500 times with a 15 kV acceleration voltage.

Pretreatment procedure

Figure 18 depicts the stalk pretreatment process. Dried tobacco and corn stalks were pretreated at 100 °C for varying periods of time ranging from 0 to 15 minutes. Ten (10) grams of biomass were combined with 200 ml of purified water to create the reactions. Following the procedure, the samples were cooled to room temperature and washed to separate the liquid from repeated solids. The liquid solutions were centrifuged to isolate the residual solid deposit, and the sugar concentration was measured using the phenol-sulfuric method and the DNS method.
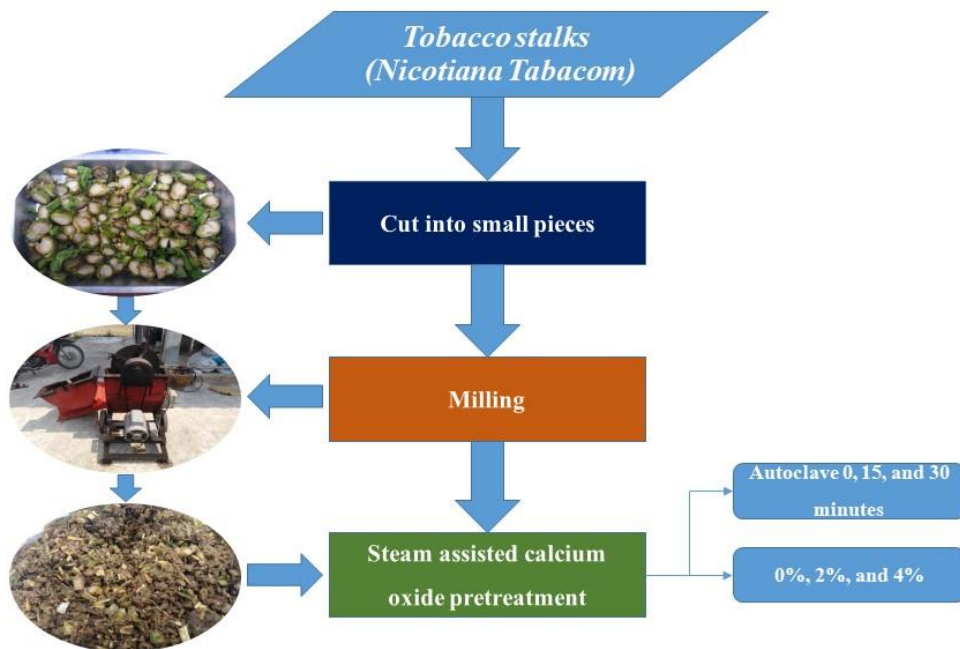
Figure 18 Pretreatment process of stalks

The adapted process was used to further delignify pretreated tobacco and corn stalks residue at 60 ℃ for 24 hours using 0%, 2%, and 4% (v/v) calcium oxide (CaO). In a 250 ml flask, the solution was prepared with a pretreated solid residual to a calcium oxide ratio of 1:6 (w/v) separately with varying alkaline concentrations. After that, the flasks were put in a low-cost laboratory oven (Binder ED-115, Germany). Total sugar and reducing sugar determination were performed on the CaO pretreated sample. Hydrolysis was conducted on the pretreated solid residue recovered from pretreated samples.

Pretreatment optimization and sugar analysis

RSM (response surface methodology) is a statistical and computational framework. It is normally sufficient for newly developed, adapted, and improved yields and manufacturing processes. When there are several variables to consider when doing

trials, the impact on yield, efficiency, and a specific method that may be aided for variable and fixing is extremely beneficial. RSM, which begins with the design of experiment (DOE), is used to evaluate important variables that influence experimentation in order to reduce the number of investigational runs while optimizing performance from the data generated. The knowledge can be used to advance observed models that relate the response to the experimental variables. The models' aid in the discovery of a stronger solution mechanism that has been studied. The model is replicated until it determines the required method or approaches the experimental data source's limit (Polprasert, 2014; Wang, 1999). The MINITAB application is utilized to examine the experimental results applying a full quadratic response surface model given by the following Equation 1 (Abdu Yusuf and L Inambao, 2019; CELLULOSE, 2006).

$$Y = \beta_{\circ} + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_i X_i^2 + \sum_{i<j} \sum \beta_{ij} X_i X_j$$

(eq.1)

The recommended procedure was used to determine the amounts of reducing and total sugars (Dashtban et al., 2009; Shakhes et al., 2011). Glucose was used as a control. The samples were diluted with purified water before being analyzed.

Enzymatic hydrolysis

The liquid hydrolysates (solid and residue liquid solution) from pretreated alkaline solution were used. Until enzymatic hydrolysis, the pH of each pretreated dried tobacco and corn stalks residue was set to 5. Then, two percent (2 percent v/v) of cellulase enzyme (Union Science Company, Chiang Mai, Thailand) with 2398 units/g, -glucosidase 5.77 units/g, and pH 4 was applied to each solution. The mixture was then incubated for 24 hours at 50° C. To separate the fluid hydrolysate from the

insoluble solids, the hydrolyzed samples were filtered via cheesecloth. The total and minimum sugar is calculated and the hydrolysate from the better pretreatment and hydrolysis, which was then fermented.

Pretreatment and hydrolysis for large scale

Alkaline pretreatment (2 kg of 2% CaO v/v) was carried out for 24 hours. Following that, samples were moved to a standard autoclave and sterilized for three hours. This is referred to as a dual pretreatment. Following that, 1 kg of 1% cellulose (commercial grade) was used and stored for 24 hours prior to the hydrolysis process.

Fermentation and ethanol measurement

The hydrolysate recovered from enzymatic hydrolysis in the best pretreatment atmosphere was centrifuged (1000 rpm, 4°C, 15 mins) to remove any residual solids. To prepare the fermentation in a 5000 ml Erlenmeyer flask, pretreated tobacco and corn stalk hydrolysates were used (Figure 20). The hydrolysate pH was changed to 5.6 using 1N H_2SO_4, then sterilized for 15 minutes at 121°C. Following that, 2 percent (v/v) S. cerevisiae (Angel Yeast Co., LTD, P.R. China) was aseptically added to the sterilized hydrolysates and incubated for 72 hours at 30° C in an economy incubator (GallenKamp, UK). Samples for ethanol and sugar analysis were taken every 24 hours. This approach's principle is based on the different boiling points of pure water (distilled water) and water-alcohol solutions (Vu et al., 2018). The percentage of ethanol in 50 mL fermented samples taken from the fermenter after 24, 48, 72, 96, and 120 hours was determined using an Ebulliometer (Dujardin-Salleron, Alcohol Burner, France).

Figure 19 Fermentation system for lab scale

Pretreatment and hydrolysis

The samples in this analysis were pretreated with steam-assisted calcium oxide. Each solution received two percent (2 percent v/v) cellulase enzyme with 2398 units/g, -glucosidase 577 units/g, and pH 4 (Union Science Company, Chiang Mai Thailand).

Fermentation and distillation for large scale

In this analysis, Saccharomyces cerevisiae, a common and commonly used commercial yeast, was used. The dry yeast was acquired (Alcohol Yeast, Xinjiang Shengli Biotechnology Co., Ltd, China). Figure 20 depicts a computerized pilot-scale fermentation machine and a fermenter with a gross volume of 150 L, a working volume of 100 L, and a total volume of 150 L. Maejo University's School of Renewable Energy conducted a 48-hour fermentation. The next move developed bioethanol was permitted for distillation at Maejo University's Faculty of Engineering and Agro-Industry. The commercial-scale distillation unit (Figure 21).

Figure 20 Pilot-scale computerized fermentation system
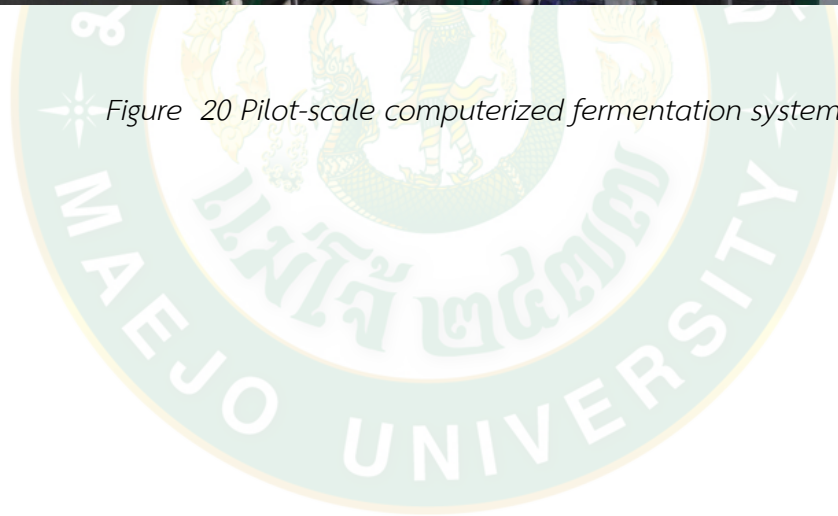
Figure 21 Distillation unit

Analytical methods and fermentation

The samples were sampled in order to consider the properties of raw material feedstock. Table 6 shows the techniques used in the experiments.

Table 6 The experiments of methods.

Parameter	Equipment or methods
Moisture	AOAC protocol
pH	pH meter
Total sugar	Phenol-Sulfuric acid
Reducing Sugar	DNS method
Degree of polymerization	-

To monitor the change in the mixture, the sugars and ethanol were measured. Ethanol content was measured in triplicates using an ebulliometer. The ebulliometer relied on the fact that distilled water has a lower boiling point than water-alcohol solutions. By comparing two different boiling points from distilled water and the solution, the percentage of ethanol in the solution was calculated using a measuring dial. The total sugar and reducing sugar levels were determined using the phenol-sulfuric acid and DNS standard methods, respectively, before and after the pre-treatment step. During the 5-day incubation period, the sugars and ethanol content were also monitored (Vu et al., 2018). Fermentation was carried out using free cell yeast (Figure 22). Separate hydrolysis and fermentation were used in the fermentation process used in the analysis.
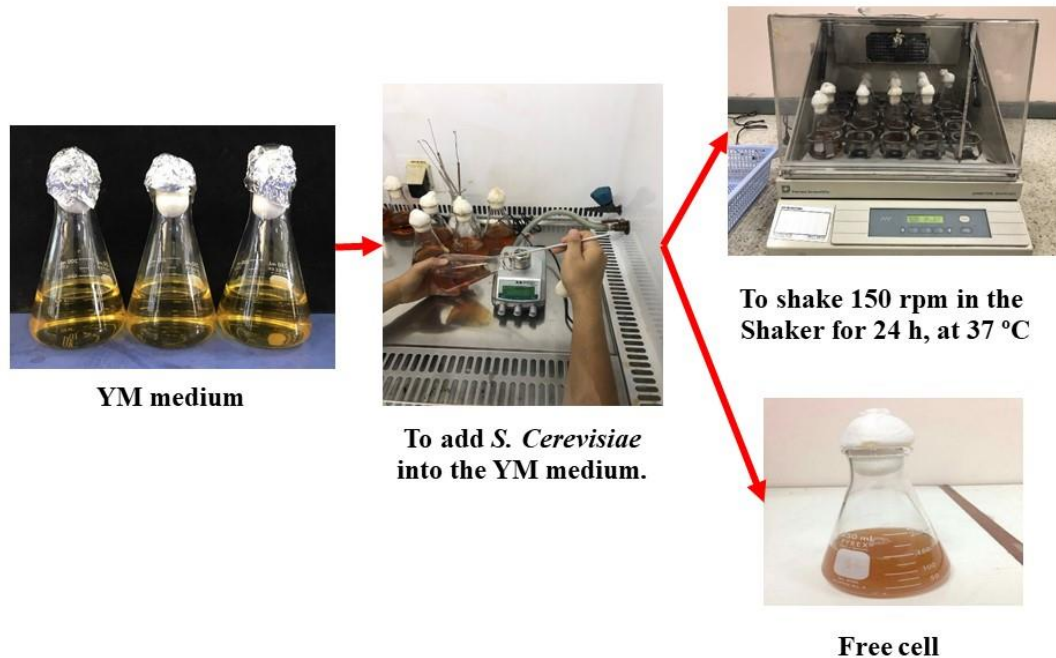
Figure 22 Yeast culture preparation

Statistical analysis

The data was presented in triplicates as a mean sd (standard deviation). When $p<0.05$ is used to determine significance between ways, the discrepancies between them are considered important. SAS and SPSS version 20.0 were used to conduct all statistical analyses.

Energy analysis

A conversion analysis was performed to calculate the various tested samples' performance to find out how much energy they will be provided to the country by using ethanol as fuel.

Optimization of Bioethanol production

Ethanol production, especially from this study samples, comprises a series of biochemical reactions with various factors involved in the process. Response surface

methodology (RSM) has been widely applied to optimize ethanol production from various substrates.

Mass balance calculation

The following equations were used to measure total solids' biomass conversion on a dry basis in tobacco, and they were adopted from (Pilot-scale study on steam explosion and mass balance for higher sugar recovery from tobacco stalks). The contents of both total sugar and enzymatic hydrolyzed CaCO3 pretreatment were determined by the techniques above. These equations help to give an idea of pretreatment and hydrolysis.

$$TS\ conversion\ (P)\ \% = \frac{TS\ Pretreament\ (kg)}{Theoretical\ sugar\ (kg)} \times 100$$

$$RS\ conversion\ (P)\ \% = \frac{RS\ Pretreament\ (kg)}{Theoretical\ sugar\ (kg)} \times 100$$

$$TS\ conversion\ (H)\ \% = \frac{TS\ Hydrolysis\ (kg)}{Theoretical\ sugar\ (kg)} \times 100$$

$$RS\ conversion\ (H)\ \% = \frac{RS\ Hydrolysis\ (kg)}{Theoretical\ sugar\ (kg)} \times 100$$

Statistics analysis

All of the tests were repeated three times, and the findings are presented as mean and standard deviation. Minitab 15.0 program was used for all statistical analysis (Minitab, State College, PA). The study of variance (ANOVA) was conducted with a 95% confidence level.

Techno-economical analysis

   The different processes and technologies for bioethanol production from the study materials were compared in this techno-economical study. The technologies used in the experiment were the subject of this review. The entire techno-economic research was based on selecting appropriate software for the study results.

CHAPTER 4

RESULTS AND DISCUSSION

Bioethanol from Nicotiana stalks

Role of agricultural crop residues and depiction of tobacco stalks

Field residues and processing residues are examples of agricultural crop residues. Crop wastes are an abundant and renewable resource. Agricultural field residues are products that remain in the cultivation area after the crop has been harvested. Straw and stalks, leaves, and seed pods make up the majority of them. Crop residues are produced in large quantities during the processing of cereals, vegetables, and fruits. Biomass can be obtained from either dedicated crops or residues primarily found in the agricultural sector. Furthermore, the residues are composed of three major components (cellulose, hemicellulose, and lignin), each of which can be processed into various finished products using a mixture of technical processes. According to Khat-udomkiri et al. [26], turning agricultural waste into a useful commodity reduces agricultural residues' management, treatment, and disposal.

Agricultural residues rich in lignocellulosics, such as corn cobs, corn stalks, sugarcane bagasse, cotton stalks, wheat straw, sunflower stalks, green coconut husks, pigeon pea stalks, and tobacco stalks, were used in Thailand's biofuel industry. Using all residues produced from harvesting and processing residues as raw materials for biofuels production may be appropriate for the countries involved. Thailand still has much promise when it comes to using green raw materials. Despite the growing importance of industry and services, according to Ramaraj and Dussadee [27], agriculture remains a major source of income for Thais. It is one of the main agricultural producers, resulting in a significant amount of residues and waste. Traditional Thai tobacco is produced in large quantities throughout Thailand, especially in the northern provinces. Several authors investigated the technical feasibility and potential of agricultural waste conversion.

Table 7 Comparison of the chemical composition of tobacco stalks and other crops stalks

Crop stalks	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Tobacco stalks	35.45	43.9	18.16
Cotton stalk	40.1	13.6	29.4
Corn stalk	36.4	30.3	6.9
Sunflower stalk	34.0	20.8	29.7
Sorghum stalk	57.99	17.41	14.95
Pearl millet stalk	52.49	25.42	10.54
Hemp stalk	37.3	19.79	12.35
Pigeon pea stalk	42.71	18.33	2.31
Wheat stalk	42.7	24.9	22.3
Jerusalem Artichoke stalk	41	22	20

Table 8 Proximate and ultimate analyses of tobacco stalks

Sample Description	Amount
Proximate analysis (wt.%)	
Moisture	6.07
Volatile matter	71.75
Fixed carbon	18.11
Ash	5.46
Ultimate analysis (wt.%)	
C	44.92
H	7.62
N	3.51
O	42.61
S	1.33

Post-harvesting biomass sources of tobacco stalks include litter, dried manure, pet waste, coffee grounds, and crops left on the field after harvest. In other countries, unsold tobacco refuse, which in America is known as "tobsolete," is compacted, recycled, or sent to a waste-to-to-energy facility. Some studies have attempted to extract a profitable commodity from tobacco stalks and fibers. (charcoal, wood, and soy), respectively, as well as a few minor constituents of hemogenic, is and some different types of vegetable material extracted from it such as resin and gas (Table 7).

Furthermore, the dried tobacco stalk fiber revealed that the content of 32.85 ± 1.13 (percent) of the average plant, with 24.38 ± 0.17 (percent being cellulose and 14.66 percent being hemicellulose) and 14.81 percent of lignins were present. Proximate and final analysis results from the same sample of tobacco stalks were included in Table 8 and shown in Figure 23. The carbohydrates in the three macronutrients (proteins, carbohydrates, fat, and lipids) were also measured. A significant is that most of this tobacco crop is pre-exposed to drying, making it a large percentage of the total output suitable for bio-diesel production.
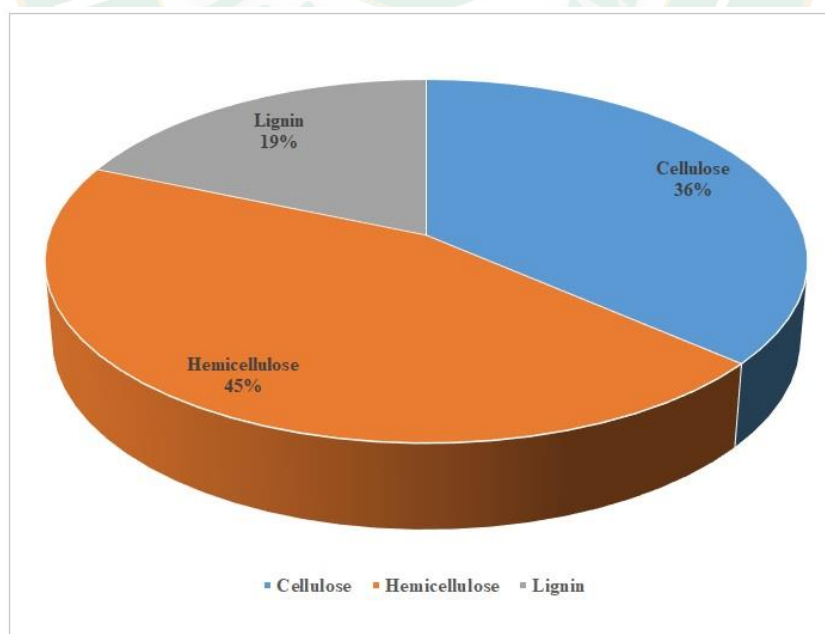
Figure 23 Proximate compositions of the tobacco stalks

Table 9 demonstrates that recent literature compares the composition of many crop stalks and their capacity as feedstocks. These results imply that crop residues and agricultural residues are available as a feedstock in many countries, suggesting that their lignocellulose composition differs depending on the crops generated [11, 28-30]. They concluded that cellulose, a glucan, is responsible for the overall plant strength, whereas hemicellulose, a complex with hexoses and pentoses, is a complex polysaccharide, the main constituent of heterogeneous molecules. Other non-specific chemical forces such as hydrogen, oxygen, and nitrogen hold hemicellulose and cellulose together. Equally, components like coniferyl, sinapyl, and coumarin alcohols, protect the wood from light degradation and serve as a natural sealing agent for lignin.

Table 9 Lignocellulosic component of tobacco stalks residue and other biomasses

Biomass	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	Reference
Shell	33.23	27.77	31.04 ±10	Gonçalves et al. 22]
Husk	39.31	16.15	28.48	Vaithanomsat et al. [23]
Sugarcane bagasse	48.70	21.14	24.81	Bittencourt et al. [24]
Corn husk	49.40	23.20	14.60	Shankar et al. [25]
Rice straw	35.60	28.80	4.10	Elsayed et al. [26]
Wheat straw	31.60	16.20	21.20	Qiu et al. [27]
Tobacco stalks residue	35.45 ± 0.13	43.90 ± 0.26	18.16 ± 0.28	This study

SEM analysis for morphological alterations in the pretreated tobacco stalks

The tobacco stalk biomass's morphological properties untreated and after pretreatment was characterized using scanning electron microscopy (SEM), as shown in Fig. 25. (a-d). When removed, the chopped tobacco stalk appeared to be composed of a sheath surrounding the stalk, having only the middle lamella left (as

if those last cell divisions had sealed the vessel) but little change in form (shown in the picture). I learned from their study that Mohtar et al. [28] said that the untreated biomass usually has smooth and flat morphology and appears to be perfect. Hemicellulose and cellulose-based polysaccharides are likely to contribute to this property because of the close-packed arrangement of fibers.

The SEM picture in Fig. 3b further illustrates an autoclaved example of SEM (Fig. 3a). it showing the cell wall that has been breached and many stalk types are visible, showing various cell types of the wall tissue such as the epidermis and phellemna cells. CaOxygenated 2% of the liginears were found to display highly distorted surfaces, which meant there was a significant area of separation and proliferation where these properties could be located, caused in part by the flow characteristics of the caustic treatment, as well as separation characteristics. These images show SEM micrographs taken through the base of Fig. 24C, so it can be inferred that the majority of the vascular bundles were totally destroyed and only some thick-walled fiber cells remained to observe in Fig. 24D. A rise in porosity helps with usability and enhances the breakdown performance by providing more surface area. Except for the xylem, the cell wall of the parenchyma cells is normally hypertrophied. Fig. 24d shows a scalariform wall in CaO pretreated samples and can see that the scalariform wall will appear. While a molecule split into molecules of different sizes during the creation of small and large pores could be seen, the substrate appeared to be ripped apart. This experiment proves that the variations in surface structure in tobacco stalks occur before and after pretreatment, the images showed clearly show it. Tobacco stalks exhibit changes in the presence of CaO as they become changed and make their sugar offering available to the enzyme.
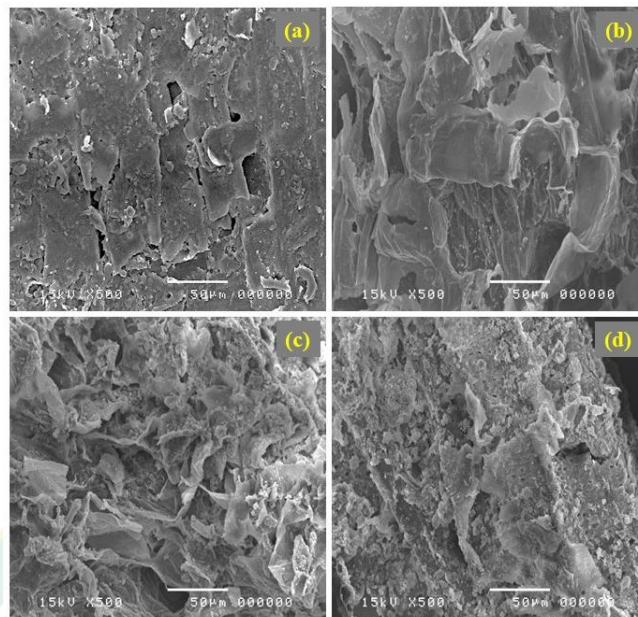
Figure 24 SEM images of tobacco stalks biomass before and after pretreatment (a–d): (a) untreated (b) autoclaved (c) 2% of CaO and (d) 4% of CaO treatment
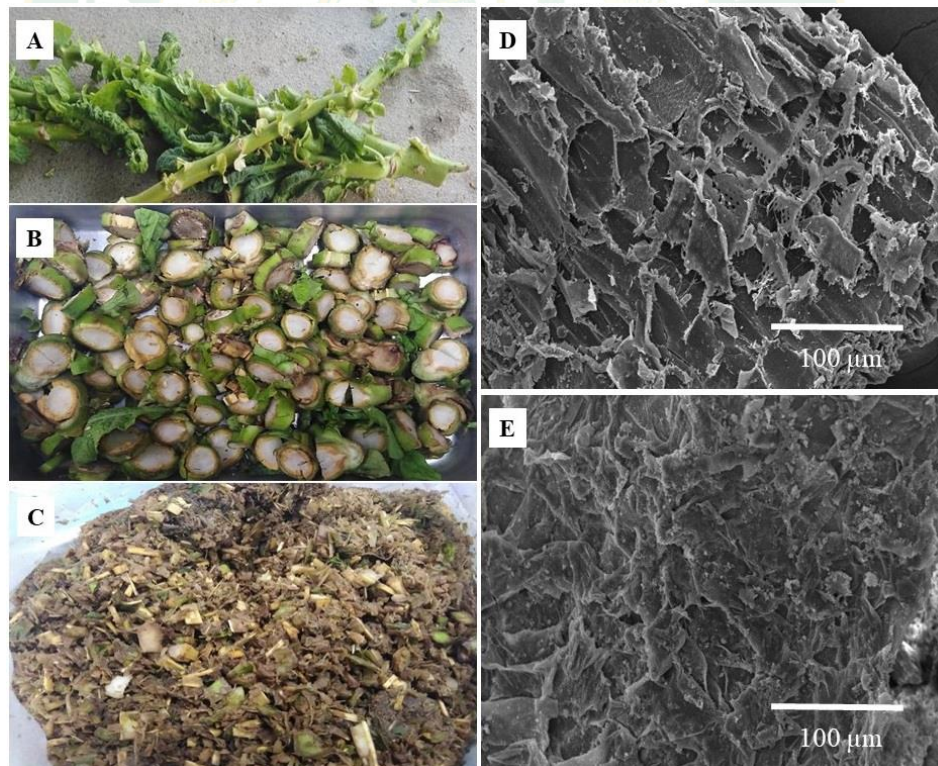
Figure 25 Sample preparation and size reduction (A-C); SEM images showing structural changes of pretreated tobacco stalks: raw tobacco stalks (D), tobacco stalks after physical and chemical pretreatment

SEM analysis for morphological alterations in the pretreated tobacco stalks

The type of biomass used in the Expand process has good grain structure and lignin content, so it is very recalcitrant to biological conversion to bioethanol. Although size reduction is necessary for ethanol production, efficient conversion is impossible. Thus, reducing the stalk size usually entails reducing it to the most miniature, cutting it into smaller and finer shreds, and/or milling it. This is an example of pretreatment, which loosens the crystalline structure and stalk mass to allow greater accessibility to the saccharification enzymes [32]. Enzymes that catalase (lipases, pancreatic enzymes, or extracellular lipases) has to be utilized in decreasing the lignin content of facilitating hydrolysis yields after they have been reduced in size using physical and chemical treatment (Figure 25, B), the treatment results are highly simplified by applying whole stalks (physical and chemical treatment alike) Generally, the use of diluted alkaline/ acid cuttation or sample-based farming techniques is needed to generate ethanol from agricultural crops. The findings suggested that using physical-chemical methods decreased the total particle size, thereby making the substrate significantly more available for uptake of nutrients and nutrients increased on the total substrate made available for absorption and raising photos-nutrient levels (Figure 25 D and E).

Pretreatment of the physical-chemical changes has proven to be the best approach to improving the substrate's quality for hydrolysis. Most of the lignin causes annotation with the opposite reactivity against the alkaline action, and some of the hydroxyl groups in cellulose cause inactivate or severe alginate inhibition, while all of the remaining uronic acid dissolves lignin contact with catase. In addition, fast lime (a basic compound that quickly releases calcium from calcium oxides) is the least expensive option; it is a good pretreatment for use with higher lignin (a slower calcium release mechanism) [6]. Also, acid treatment has been shown to facilitate plant cell wall biodegradation, causing the formation of hemicellulose and lignin linkages to be broken. Making the cell wall a more accessible, this enzyme treatment

increased the cell membrane area. This was advantageous since the amount of wall space did not decrease. The use of physical-chemical pretreatments is commonly employed during the bioethanol production process [33]. To expand on this: After pretreatment, the cellulose content was found to be at 57.02%, and it was expanded by 20.45%. As opposed to the endosperm and germ, hemicellulose and lin-brin contents decreased to 33.86 and 10.24 percent; this represents a reduction in as a decrease in calories from the 10.04 and 7.92 to 5.56 (about 33 percent each). Combined chemical and temperature and mechanical pretreatment efforts, typically on the flocks, are more efficient in preventing biomass collapse, which means pretreatment with chemicals needs to be particularly severe in order to be effective [34]. It has multi-lamina layer sandwiched between lignate structures in which the laminate is made up of alternating layers of lignin and hemiin (or hemi-)cellulose (or hecicen). a study showed that the impact of using a physico-chemical pretreatment was greater than the extraction efficiency of Lignin, which is a recognized level of excellence at 43.6% Early removal of lin also reduces lignification and biochemical regeneration and refluxing/recycling and can both simplify and shortens distillation.

Optimization of combined pretreatment process and enzymatic hydrolysis

The term "pretreatment" refers to the process of removing the natural carbohydrate-lignin shield that prevents cellulose and hemicellulose from being accessed [2]. Figure 26 depicted the impact of pretreatment briefly. The pretreatment primarily breaks down the feedstock's lignin and hemicellulosic components, resulting in the conversion of simple cellulose [7, 8]. The pretreatment process is used to extract lignin and hemicelluloses, increasing the porosity of lignocellulosic materials and decreasing cellulose crystallinity. Pretreatment should be low in cost, successful on a wide range of samples and lignocellulosic materials, result in the recovery of the majority of lignocellulosic components in usable form, require minimal size

reduction, and most significantly, prevent the development of inhibitory compounds [7, 12].
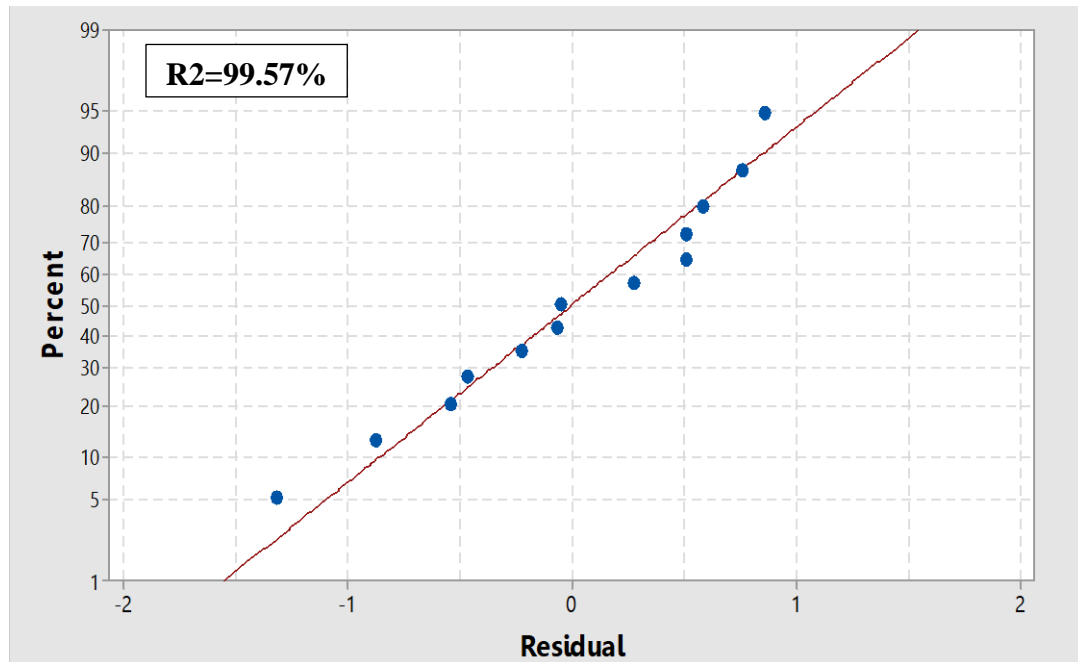
Figure 26 Actual and predicted plot of reducing sugar (%)

As a result, choosing the right pretreatment for biomass is critical. There are various pretreatments available, which are divided into two categories: 1) Physical and 2) Chemical. 2) Compounds Biological, Physico-chemical, and Biological. For chemical pretreatment, various chemicals such as acids, alkalis, and oxidizing agents are used. Alkali is the most widely used form for obtaining high glucose yields [7, 12]. Meanwhile, physical pretreatment methods could primarily focus on reducing particle size and crystallinity of lignocellulosic biomass, which aids in increasing the specific surface area and degree of polymerization. It can be done using a mixture of grinding and chipping techniques. In comparison to other pretreatments, this form of pretreatment has a lower rate of lignocellulosic biomass decomposition. It has also known as physic-chemical since it employs a combination of temperature, heat, and chemical agents [2, 7, 8]. Agricultural residues are typically treated with a mixture of

steam explosion and acid or alkali. Recent studies the microorganisms, such as brown-white, and soft rot fungi are also used in biological pretreatment.

This form of pretreatment is more cost-effective, gentle, and environmentally friendly than others. The effectiveness of pretreatment is, however, based on the form of biomass used. Alkaline pretreatment is one of the most common pretreatment methods for obtaining high sugar yield from lignocellulosic biomass. By solubilizing the hemicellulose in the biomass, primarily increases the accessibility to cellulosic fractions. For complex lignocellulosic feedstock, many studies have used mild and concentrated alkaline. Photographs taken with a scanning electron microscope were verified. The pretreatment procedure went off without a hitch, as planned. The photographs were taken before and after treatment, and the pretreatment was visible. However, when it comes to toxicity, corrosiveness, and reactor requirements, mild alkaline has an advantage over concentrated alkaline.

Furthermore, mild or dilute alkaline produces few or no inhibitors that affect bioethanol development during the saccharification stage. Various alkalides have been used in the pretreatment of lignocellulosic biomass, according to studies. Several lignocellulosic biomasses have been treated with traditional diluting alkalies such as NaOH, CaO, CaOH, and KoH [29]. CaO, on the other hand, is the most commonly used chemical for industrial applications due to its low cost, high operation, availability, and safety and environmental concerns. The optimum glucose is reached at 170°C, 30 minutes incubation time, and a 2:2 CaO concentration. Sugar concentrations in dried tobacco stalk as a result of the hydrothermal pretreatment process.

Tobacco was examined as a test subject for hydrothermal preparation, which would increase yield by allowing higher temperatures. Unprocessed tobacco stems had as many as much as sugar until they were stripped of their epidermis. According to the study, the total sugar concentration of tobacco stems was found to be 27.97 g per liter, while the reducing sugar concentration was found to be 5.43 g per liter. After

hydrothermal treatment, the total sugar content of the solution (total sugar) increased. By contrast, total sugar (simple sugars) in the treated biomass from 4.07 g/L to 4.55 g/mL is lower than in untreated biomass, as the result of several studies conducted. The presence of hemicellulose in the residue indicates that hemicellulosic sugar complexes are likely to present and that they may provide soluble or methanogenic substrates. This is important because the reducing sugars may not yield such positive HPLC results [30].

Lignocellulosic materials with high lignin and hemicellulose content benefit greatly from sequential pretreatment. Until saccharification, various pretreatments are applied sequentially, and all of the hydrolysates are combined. Just a few novel pretreatments have been studied in recent years. According to studies, sequential and combined pretreatments could fully hydrolyze the hemicellulose, eliminate the lignin, and expose the cellulose to enzymes, resulting in a higher sugar extraction rate [31, 32, 33]. Boiling and steam explosion are two typical pretreatments for lignocellulosic biomass, both of which require heat transfer. Water is widely used in these pretreatment procedures because, at high temperatures, it behaves as a dilute acid. High-temperature pretreatment opens up the structure of the biomass and primarily extracts the hemicelluloses [30]. During such pretreatments, acetic acid and other acids are thought to hydrolyze hemicelluloses.

It's thought that extracting hemicelluloses from microfibrils exposes the cellulose surface, increasing sensitivity to the enzyme. However, lignin is only eliminated to a small degree [31, 34]. The sequential and combined thermal and alkaline pretreatments must be an excellent combination since they can target hemicellulose and lignin, respectively, to expose the cellulose to enzymatic hydrolysis. Polysaccharides are broken down into simple sugars during hydrolysis. Hydrolysis can be done in three ways: (1) dilute acid hydrolysis (2) concentrated acid hydrolysis (3) enzymatic hydrolysis. Among these, enzymatic is the most commonly used method nowadays. As compared to acid, enzymatic hydrolysis is less expensive in terms of

recovery and wastewater. Furthermore, it yields a higher yield, and producers have significantly reduced prices, which are still relatively low as compared to acid. The enzymatic hydrolysis/saccharification process converts liberated polymeric sugars from the pretreatment process to mono sugars in solution in a green way [18]. The sugar concentrations of this study's post-alkanie pretreatment performance. The most common method for converting cellulose to glucose and hemicellulose to pentoses is this method (xylose arabinose, hexoses, glucose, galactose, and mannose).

Hemicellulase, aldolase, the analytic and cellular, are used together in the cellulose-to-to-hydrolyase and hemecyclase biosynthesis processes. The enzymatic hydrolysis is normally conducted in neutral conditions, with a temperature of about 45-50 °C [35], at a pH of 4.8 to prevent precipitates from forming in the solution. It is important to the overall fermentation that this step yields great amounts of glucose, which is later used and converted into ethanol. The study concluded that enzyme activity had the greatest effect on tobacco dry stalk concentrations after enzymatic hydrolysis. Based on the analyses performed pretreatments and the enzymatic treatment, the combined method and hydrolysis treatment are novel and highly effective fractionation and treatment processes for biomass.

RSM was used to refine the process of reducing sugar production. The CCD method was used to optimize sugar concentration from dried tobacco stalk pretreatments. Table 10 shows the results of the experimental runs obtained from the CCD. Due to the occurrence of treatment mixtures at the middle points of the box's experimental space, the CCD is a characteristic trial design. The first and second order coefficients are calculated using CCD as a result of this experiment. In this analysis, the use of CCD resulted in a non-linear second order model between the input factors (CaO concentration and pretreatment time), with the output being a reducing in sugar concentration. The significance and adequacy of the RSM model shown in Equation 1.

Reducing sugar (g/L) = 10.163

+ 19.732 [CaO (%)] 0.3731 [Time of pretreatment (Mins)]   -

4.281 [CaO (%)]^2 – 0.00632 [Time of pretreatment (Mins)]^2 (Eq.1)

Table 10 Experimental runs of actual and predicted results from tobacco stalk pretreatment

Std	Run	CaO (%)	Time of pretreatment (Mins)	Reducing sugar (g/L)		
				Actual value	Predicted value	Residual
9	1	2	15	35.362	36.677	-1.315
3	2	0	30	15.125	15.666	-0.541
12	3	2	15	36.216	36.677	-0.462
10	4	2	15	37.189	36.677	0.511
5	5	0	15	14.292	14.338	-0.046
7	6	2	0	31.635	32.503	-0.868
11	7	2	15	37.189	36.677	0.511
4	8	4	30	25.875	26.096	-0.211
1	9	0	0	10.750	10.163	0.587
6	10	4	15	24.708	24.768	-0.060
13	11	2	15	37.537	36.677	0.860
8	12	2	30	38.768	38.006	0.762
2	13	4	0	20.875	20.594	0.281

Minatab 17 is the statistical software is used for calculating the regression analysis of experimental resulted and to plot the contour and 3D response surface graphs [15]. The resulted statistical parameters were calculated by using ANOVA. It was showed in Table 11.

Table 11 Analysis of variance of quadratic model for reducing sugar production.

Source	DF	Adj SS	Adj MS	F-value	P-value	
Model	4	1,222.55	305.64	460.52	<0.0001	significant
Linear	2	208.61	104.31	157.16	<0.0001	
A:CaO (%)	1	163.19	163.2	245.9	<0.0001	
B:Time of pretreatment (Mins)	1	45.42	45.42	68.43	<0.0001	
Square	2	1,013.94	506.97	763.88	<0.0001	
A^2	1	809.92	809.92	1,220.36	<0.0001	
B^2	1	5.59	5.59	8.43	0.02	
Error	8	5.31	0.66			
Lack-of-Fit	4	2.11	0.53	0.66	0.653	not significant
Pure Error	4	3.2	0.8			
Total	12	1,227.86				
Std. Dev.	0.814661	R^2(adj)	99.35%			
R^2	99.57%	R^2(pred)	99.90%			

The F-values of the linear and square models are 460.52, 157.16, and 763.88, respectively. Furthermore, it can refer to the model as a rectangle and a square. This coefficient can go as low as a hundredth decimal place (one-hundred thousandth of a percent), resulting from Manmai et al. [5] experiments Pretreatment with sodium hydroxide the previous results found the F-value of the pretreated sunflower stalk to be zero, but due to noise, the previous study had demonstrated that it to be much less than one percent. Additionally, only when the model's P-values are under 0.1 can this relationship be used, regardless of the number of samples in the dataset. This study can only be based on combining the following models (A, B, and A2).

Regression and individual model coefficients of fit were tested on the F-value of 0.66 and any possible regression to achieve a proper accuracy measurement of a non-significant F-test. There is a 65.3% chance of a Lack of Fit F-value; this large could occur due to noise. The actual and the predicted percentage of reducing sugar is shown in Figure 26. It was found that the values of R^2 (adj) and R^2 (pred) were 98.35% and 99.90%, respectively are the difference is less than 0.2. It is in reasonable agreement.
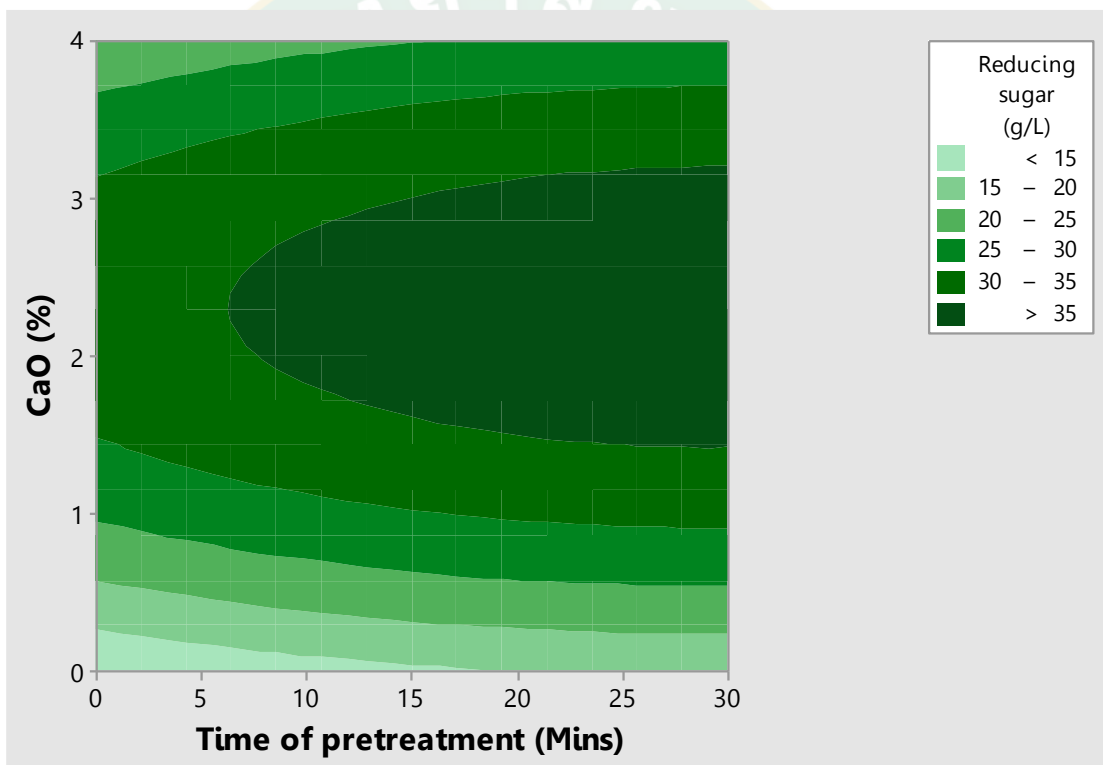
Figure 27 Contour interacted effectjp of CaO concentration and time of pretreatment on reducing sugar production from tobacco stalk
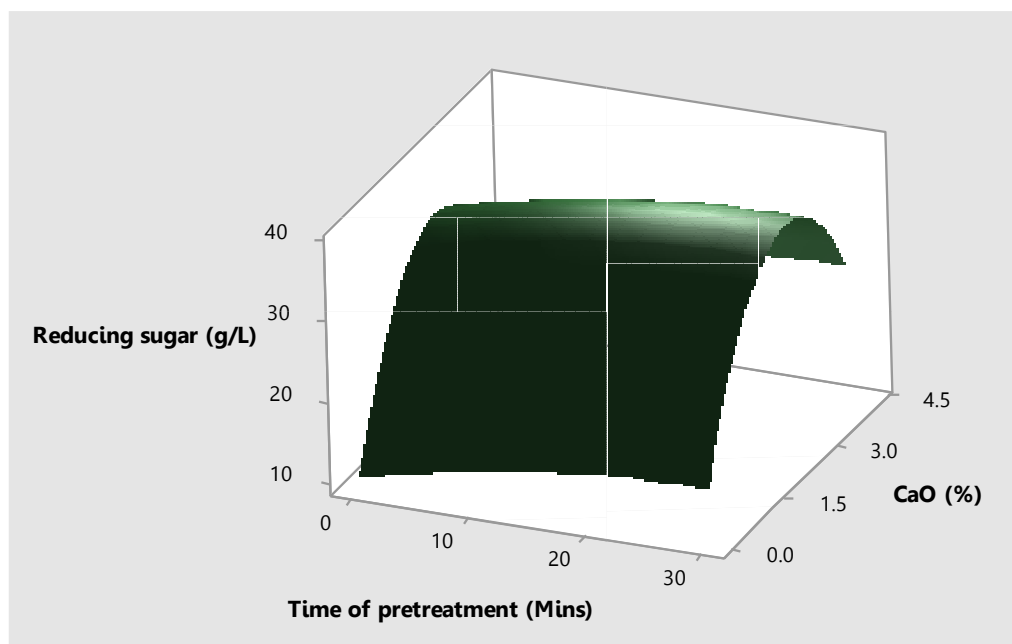
Figure 28 3D interacted effect of CaO concentration and time of pretreatment on reducing sugar production from tobacco stalk

Figure 28 depicts the relationship between CaO concentration and pretreatment time in terms of a contour plot; this figure depicts the strength of green in each hue. The shade's strength correlates to the increased sugar concentration. Using a pretreating time of 7 to 30 minutes, the highest peak of reducing sugar production during CaO 1.5 to 3% ranges. However, in Figure 28, it can be seen in greater detail than in Figure 27. Three-dimensional plots are used to demonstrate the relationships of two separate variables. Consequently, two factors are designated as research variables, while the remaining factors are regarded as constant values, with the interaction of various factors influencing the response value. Individually, a deeper detailed CaO concentration and pretreating time for reducing sugar output at 2% CaO and a pretreating time range of 20 to 30 minutes could be seen in this figure.

Enzymatic hydrolysis

Enzymatic hydrolysis was used to release fermentable sugar from the biomass of pretreated tobacco stalks. When C5 and C6 sugars are consumed, effective

conversion of lignocellulosic biomass into bioethanol is required. In this case, the microbial strain chosen to transform the sugars is critical. As a result, the most effective S. cerevisiae was chosen and used for fermentation as traditional glucose-fermenting yeasts. Kumar et al. [31] stated that the enzyme cellulase and β-glucosidase were reported earlier to hydrolyze the cellulosic polymers to monomeric glucose units for further use fermentation. The total and reducing sugar concentrations rose after hydrolysis to 69.594 and 36.708 g/L, respectively, relative to pretreatment (total and reducing sugar concentrations were 49.744 and 28.417 g/L). Overall, the enzymatic hydrolysis process proved to be an effective mechanism for improving tobacco stalk biomass's saccharification process. Fermentation, biomass concentration, and mixing speed all significantly reduced sugar yield during the hydrolysis process.

Bioethanol production of tobacco stalks (lab scale)

More and more recent examples of the fact that biofuels' general availability are making the spotlight on bioethanol extremely attractive because of their energy stability and environmental integrity [3, 36, 37]. It is a truly eco-friendly process because it contains 34.7% oxygen instead of the typical conventional gas, much less. Since it contains oxygen and bioethanol, the combustion efficiency is improved by 15 percent, resulting in lower pollution produced by reducing nitrogen oxide emissions. Such hazardous gases released by gasoline, such as sulfur oxide and carbon monoxide, can also be minimized by adding ethanol to gasoline. These toxic gases lead to acid rain and contaminate potable water supplies, all of which harm health [38, 39]. Fermentation is the metabolic mechanism by which microorganisms turn soluble sugars into alcohol. Some bacteria and yeast can metabolize carbohydrates such as monosaccharides and disaccharides in the absence of oxygen, producing ethanol and emitting carbon dioxide [40].

Conventional yeast is used for the ethanol fermentation process in almost every refinery. In alcohol production, mainly S. cerevisiae yeast species have been used, especially in the brewery and wine industries. Since it produces a high ethanol yield, is efficient, and can withstand high ethanol concentrations, this yeast lowers distillation costs. The ethanol fermentation is depicted in Figure 29. The sugars, especially glucose, are converted to ethanol. Glucose and fructose are fermented by yeast but not pentose sugars [5, 41]. Batch, continuous, and fed-batch fermentation are the three types of fermentation modes.
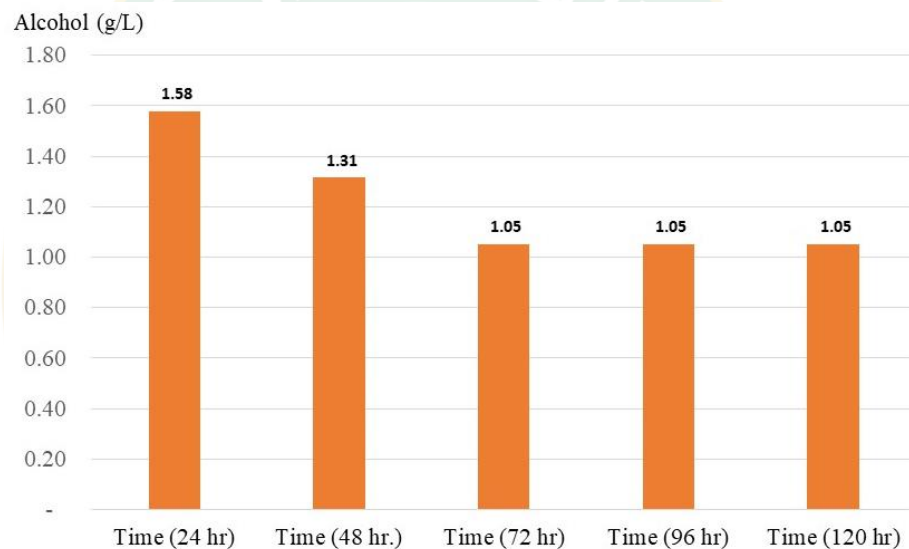
Figure 29 Bioethanol production from dried tobacco stalks

Batch fermentation is the most straightforward of the fermentation modes since no medium is needed. It takes place in a closed system, with the fermentation medium supplemented with required ingredients at the start and yeast or another bacterial inoculum added to the medium before fermentation begins. The pH and temperature in this system are kept constant to enable the microorganism to expand [10, 42]. It follows a lag, log, and stationary phase, in which yeast cells expand in a lag phase, then an exponential phase, then cells compete for the limited amount of nutrients in the medium and eventually enter a stationary phase the nutrients are

depleted. After sequential pretreatment and hydrolysis results, the ethanol concentration, volumetric ethanol productivity, and fermentation efficiency obtained during (SHF) fermentation mode were described; additionally, reducing sugar and alcohol concentrations during fermentation data were presented in Figure 29. At 84 hours of fermentation, the maximum ethanol yield of 75.74 (g/L) was achieved, which remained stable after 72 hours. Previous research has shown that using a combined pretreatment design to generate and synthesize high sugar from dried tobacco stalks aided efficient bioethanol production. This study's findings could help the ethanol industry secure a long-term supply of feedstock to meet the growing demand for bioethanol.

Bioethanol production of tobacco stalks (large scale)

To release fermentable sugar, the pretreated tobacco stalk biomass was subjected to enzymatic hydrolysis. When C5 and C6 sugars are consumed, efficient conversion of lignocellulosic biomass into bioethanol is required. In this case, picking the right microbial strain to transform these sugars is critical. As a result, the most effective S. cerevisiae was chosen as traditional glucose-fermenting yeasts and used for fermentation. According to Kumar et al. [20], the enzymes cellulase and -glucosidase have previously been identified to hydrolyze cellulosic polymers to monomeric glucose units for use in fermentation. The total and reducing sugar concentrations after hydrolysis were 69.594 and 36.708 g/L, respectively, compared to 49.744 and 28.417 g/L before hydrolysis. Overall, the enzymatic hydrolysis process proved to be an effective mechanism for improving tobacco stalk biomass's saccharification process. Fermentation, biomass concentration, and mixing speed all affected the reducing sugar yield during the hydrolysis process. Table 4 indicates bioethanol production from tobacco stalks and other biomass. When compared to other biomass feedstocks, tobacco stalks had a higher ethanol content. Ethanol was used in the distillation unit after fermentation. It had an alcohol content of 8%.

Enzymatic hydrolysis and fermentation are performed separately in SHF. Although the end-product inhibition of the cellulolytic enzymes is a limiting factor, this allows each process to operate at its best. SHF also allows for cell recycling, while SSF does not allow for the separation of cells from solid raw material particles [20-22]. Table 4 indicates bioethanol production from tobacco stalks and other biomass. When compared to other biomass feedstock tobaccos, the stalk showed a higher ethanol yield. In comparison to the 48-hour fermentation, the alcohol content was higher during the 24-hour fermentation. The number of living yeast cells decreases after 24 hours of yeast entering the death process. The alcohol and total sugar levels decreased as a result of contamination by other bacteria that used glucose in anaerobic fermentation growth and produced acetic acid. Ethanol was used in the distillation unit after fermentation. It had an alcohol content of 8%.
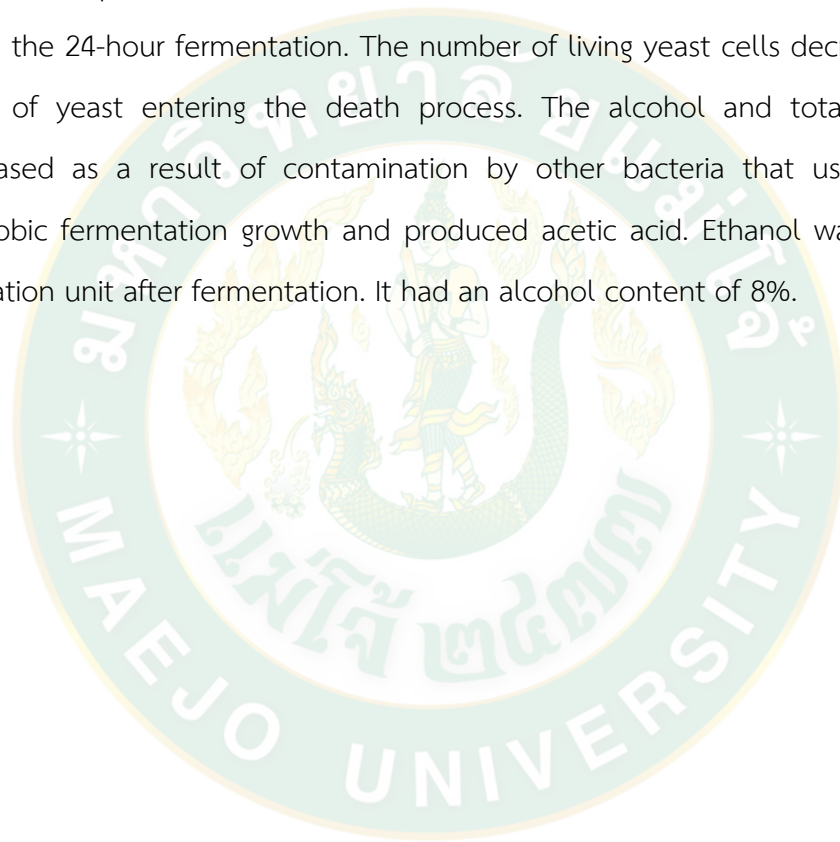
Table 12 Bioethanol production from tobaccos stalks and other biomass

Factors	Total sugar (g/L)	Reducing sugar (g/L)	Alcohol (g/L)	Reference
Pretreatment	49.744	28.417	-	This study
After hydrolysis	69.594	36.708	-	This study
Fermentation 24 hr.	40.288	17.29	7.101	This study
Fermentation 48 hr.	35.512	16.315	5.523	This study
After distillation 24 hr. bioethanol)	-	-	63.12	This study
Compared with other feedstock				
Sargassum sagamianum	-	-	1-2	Lee et al. [35]
Laminaria digitata	-	-	5.16	Adams et al. [36]
Sago pith	-	-	2.8	Sunarti et al. [37]
Municipal solid waste	-	-	0.15	Mtui and Nakamura [38]

Mass balance calculation

This research established the mass balance for this method, including chemical combination with thermal pretreatments and enzymatic hydrolysis, using arrangement evaluations after the sugar production stage, as shown in Figure 6. Calculating the feasibility of extracting sugar from tobacco stalks based on their cellulose and hemicellulose percentage since these two polymers contain monomers of several sugars. As a result, in 10 kg of tobacco stalk, sugar production's fusibility is 7.935 kg. Biomass was transformed to 4.97 kg of total sugar and 2.84 kg of reducing sugar after chemical and thermal pretreatments. The enzymatic hydrolysis step, 6.97 kg of total sugars and 3.67 kg of reducing are obtained in 10 kg of tobacco

stalk with 2% CaO 100 by spending cellulase enzyme 1 kg. The detailed calculations results were presented in Table 13 and Figure 30.

Table 13 Mass balance of pretreatment and hydrolysis

Mass balance	
Theoretical sugar (kg)	7.935
RS Pretreament (kg)	2.842
TS Pretreament (kg)	4.974
RS Hydrolysis (kg)	3.671
TS Hydrolysis (kg)	6.959
RS conversion in pretreatment (%)	35.812
TS conversion in pretreatment (%)	62.689
RS conversion in hydrolysis (%)	46.261
TS conversion in hydrolysis (%)	87.705
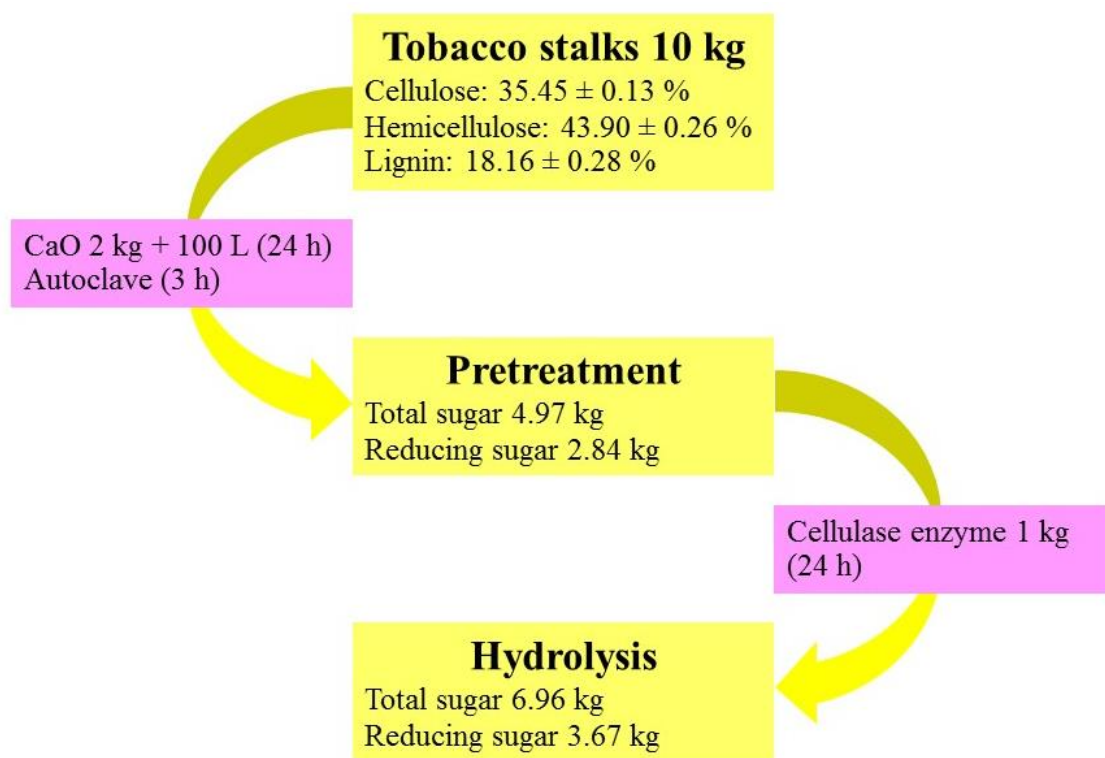
Figure 30 Mass balances for pretreatment and hydrolysis conditions

The total sugar and reducing sugar were pretreated and hydrolyzed at pretreatment and hydrolysis to determine the mass balance. The cellulose content of tobacco stalks was 35.45% and the hemicellulose content was 43.9 percent (w/w). As a result, tobacco stalk biomass conversion can be sugar 79.35 percent of 7.395 kg sugar (theoretical sugar) from biomass 10 kg in. As seen in Table 5, sugar and total sugar conversions in the pretreatment phase were reduced by 35.812 percent and 62.689 percent, respectively. After adding a cellulase enzyme 1 kg for 24 hours, the mass balance at the end of the hydrolysis cycle showed 46.261 percent in total sugar conversion and 87.705 percent in reducing sugar conversion.

Economic analysis

Studies or analysis in projects to determine if planned projects will be able to achieve their goals, analyze whether the proposals will produce an economic or monetary return, or price gains when taking into account the effects on the environment and community, and meet the targets set with a smaller budget and a shorter timeline. A broad range of subjects, such as technical availability and market demand for the product, must also be covered in the feasibility report. As seen in corporate and economic and monetary management, there are two general analytical targets: (or that have two common characteristics).

Objectives of financial analysis

- To assess the cost of investment of the project
- To analyze project financial status and funding sources
- To consider the financial management of the project
- To assess the project's cost-effectiveness in the efficient use of resources.
- To analyze the impact of the project on economic development and social welfare.
- Factors used in financial and economic analysis.
- Projections or projections of project financial statements

Tables 14-16 have been analyzed previously in several experiments, the primary experimental research and the initial economic analysis involving fresh tobacco, the latter involving dried tobacco, and finally, these previous studies on dry corn. Since the investment generates large profits regardless of how much spend on it, the returns are small, the cost of investment is low, and the risk of the outcome is also high; the savings are higher.

Table 14 The unit cost of bioethanol production from fast tobacco

Vessel total	100 Liters		
Items	Units	Quantity	Baht
Biomass	1.00 baht/ kg	10 kg	100.00
Water	0.50 baht/L	100 L	50.00
Enzyme cellulase	3,950.00 baht	1 kg	3,950.00
Yeast	130.00 baht/kg	1 kg	130.00
Sulphuric acid	190.00 baht/L	0.48 L	91.20
Milling machine	3.25 baht/Unit	0.15 kw.	4.90
Distillator	28.25 baht/L	100 L.	2,825.00
Centrifuge	3.25 baht/Unit	0.55 kw	3.573.00
Human	350 baht/ day	4 days	1,400.00
TOTAL			12,124.10

Table 15 The unit cost of bioethanol production from dry tobacco

Vessel total	100 liters		
Items	Units	Quantity	Baht
Biomass	2.00 baht/ kg	37 kg	74.00
Water	0.50 baht/L	100 L.	50.00
Enzyme cellulase	3,950.00 baht	1 kg	3,950.00
Yeast	130.00 baht/kg	1 kg	130.00
Sulphuric acid	190.00 baht/L	1 L.	190.00
Milling machine	3.25 baht/Unit	0.15 kw	4.90
Distillator	28.25 baht/L	100 L	2,825.00
Centrifuge	3.25 baht/Unit	0.55 kw	3.573.00
Human	350 baht/ day	4 day	1,400.00
TOTAL			12,196.90

Table 16 The unit cost of bioethanol production from dry animal corn.

Vessel total		100 liters	
Items	Units	Quantity	Baht
Biomass	2.00 baht/ kg	82 kg	164.00
Water	0.50 baht/L	100 L	50.00
Enzyme cellulase	3,950.00 baht	1 kg	3,950.00
Yeast	130.00 baht/kg	1 kg	130.00
Sulphuric acid	190.00 baht/L	1 L.	190.00
Milling machine	3.25 baht/Unit	0.15 kw	4.90
Distillator	28.25 baht/L	100 L	2,825.00
Centrifuge	3.25 baht/Unit	0.55 kw	3.573.00
Human	350 baht/ day	4 days	1,400.00
TOTAL			12,196.90

A tentative calculation of the cost-effectiveness of ethanol investment in wood-based cellulosic ethanol, assuming the outcome is higher, gives ethanol a relative to be seen concerning. The study's findings can be found in Table 17 - Table 22. While the preliminary costs and assumptions used in estimating fixed investment per-only numbers in the calculation tables have only provided rough estimates of the total costs, more detail can be found in the underlying processes and components. Interestingly, in investments in Lignocellulose-produced bioethanol plants, besides the type of plant used as a production factor, in that case, the price of the enzyme will also be affected by bioethanol production from tobacco and maize. However, environmental issues such as ethanol distillation and employee labor costs (Kang et al., 2019) affect the ethanol production cost equal to 75% of the total ethanol production cost. Key project feasibility analysis tools include Payback

Period: PB, Net Present Value: NPV, Internal Rate of Return: IRR, and Benefit-Cost Ratio: B / C ratio.

Payback Period: PB in general, the determined by how long the payback period will be in order to break even, known as the acceptable payback period; a project may have criteria that differ significantly from the point of initial feasibility to its planned expansion. Small businesses use a five-year planning cycle for investment purposes, with the principal left after the investment period used to assess project performance. If the payback period is ≤ 5 years, then the investment decision is made. However, if the payback period> 5 years, then decide not to invest, it was found that this project Payback Period 5.11 years means that this project is slow to return, less liquidity and very risky. Despite these constraints, the time value of money is not overlooked, and the incentive received during the repayment period is often taken into account.
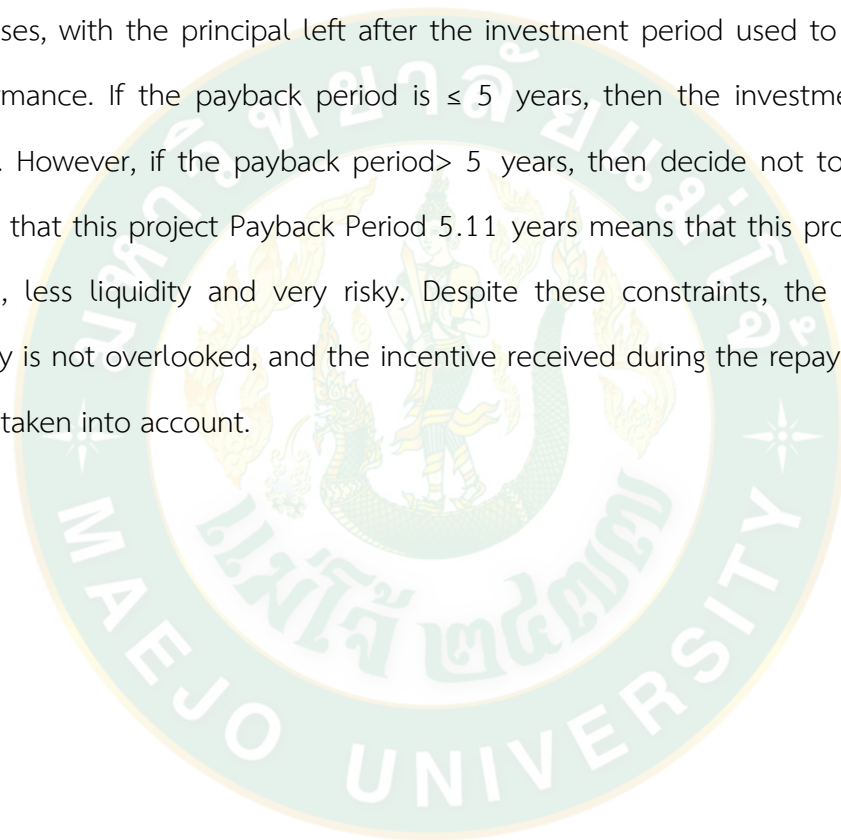
Table 17 Shows the budget of operating cash flows.

Cash flow statement	year 0	1st year	2nd year	3rd year	4th year	year 5	year 6
Operating cash flow							
Cash income from sales.	0	4,775,192	6,135,790	6,497,252	6,826,080	7,171,627	7,534,748
Expenses are cash from cost of sales and inventory.	0	(2,805,419)	(3,341,901)	(3,475,926)	(3,650,079)	(3,836,989)	(4,033,554)
Selling and Administrative Expenses.	0	(2,040,819)	(2,139,292)	(2,472,812)	(2,987,585)	(3,133,699)	(3,287,251)
income tax.	0	0	(15,828)	(85,451)	(50,679)	(8,251)	(11,218)
Depreciation and amortization expenses	0	0	0	0	0	0	0
Net cash received (paid) from operations.	0	(71,046)	638,768	463,063	137,736	192,689	202,724
Cash flows from investing activities							
Investing in fixed assets.	(1,353,700)	0	0	0	0	0	0
Pre-operating expenses other than fixed assets.	(30,000)	0	0	0	0	0	0
Net received (paid) from investments	(1,383,700)	0	0	0	0	0	0

Table 18 Shows the budget of operating cash flows. (continues)

Cash flow statement	year 0	1st year	2nd year	3rd year	4th year	year 5	year 6
Cash flows from financial activities							
Owner capital.	562,115	0	0	0	0	0	0
Disbursement of the principal of the long-term loan.	1,311,602						
Repay the principal of a long-term loan.		(154,201)	(163,698)	(174,046)	(185,325)	(197,624)	(211,037)
Interest paid.		(87,440)	(77,943)	(67,595)	(56,316)	(44,017)	(30,604)
Cash received (paid), net from financial activities.	1,873,717	(241,641)	(241,641)	(241,641)	(241,641)	(241,641)	(241,641)
Net cash received (paid) from operating, investment and financial activities.	490,017	(312,687)	397,127	221,422	(103,905)	(48,952)	(38,917)
Cash at the end of the period.	490,017	177,330	574,458	795,880	691,975	643,023	604,105

Table 19 shows cash flows from operations and investments. To calculate IRR and payback period

Cash flow statement	year 0	1st year	2nd year	3rd year	4th year	year 5	year 6
Cash income from sales	0	4,775,192	6,135,790	6,497,252	6,826,080	7,171,627	7,534,748
Expenses are cash from cost of sales and inventory.	0	(2,805,419)	(3,341,901)	(3,475,926)	(3,650,079)	(3,836,989)	(4,033,554)
Selling and Administrative Expenses	0	(2,040,819)	(2,139,292)	(2,472,812)	(2,987,585)	(3,133,699)	(3,287,251)
income tax	0	0	(15,828)	(85,451)	(50,679)	(8,251)	(11,218)
Investing in fixed assets	(1,353,700)	0	0	0	0	0	0
Pre-operating expenses other than fixed assets	(30,000)	0	0	0	0	0	0
Remnant value of fixed assets at the end of business	0	0	0	0	0	0	0
Operating cash flows and net investments	(1,383,700)	(71,046)	638,768	463,063	137,736	192,689	202,724
Operating cash flows and accumulated investments	(1,383,700)	(1,454,746)	(815,978)	(352,915)	(215,178)	(22,490)	180,234
Internal rate of return (IRR)	9.4%						
The difference between return and financial costs (IRR - WACC)	2.8%						
Payback Period		1.00	2.00	3.00	4.00	5.00	5.11
Payback Period (year)	5.11						

If the person wants to invest in this project, the total investment in the project is 1,147,953.00 baht, divided into funds for investing in fixed assets and pre-operating expenses 898,494 and working capital 249,459 baht.

Table 20 Financial Ratio Analysis

Year	1	2	3	4	5	6
1 Liquidity Ratio						
1.1 Current Ratio	5.2	7.6	9.3	9.2	9.1	9.1
1.2 Quick Ratio	2.6	4.8	5.8	5.1	4.7	4.4
2. Activity Ratio						
2.1 Receivable Turnover	17.1	17.1	17.1	17.1	17.1	17.1
2.2 Receivable Day	21.3	21.3	21.3	21.3	21.3	21.3
2.3 Inventory Turnover	17.5	17.5	17.5	17.5	17.5	17.5
2.4 Inventory Day	20.8	20.8	20.8	20.8	20.9	20.9
2.5 Payable Turnover	28.4	31.5	32.1	32.1	32.0	32.0
2.6 Payable Day	12.9	11.6	11.4	11.4	11.4	11.4
3. Leverage Ratio						
3.1 D/E Ratio	1.6	0.8	0.5	0.4	0.3	0.2
3.2 D/A Ratio	0.6	0.5	0.4	0.3	0.2	0.2
3.3 Interest coverage ratio	3.8	9.8	8.6	3.8	5.2	8.0
4. Leverage Ratio						
4.1 Gross Profit Margin	46.9%	46.9%	46.8%	46.8%	46.8%	46.7%
4.2 Operating Margin	6.6%	12.4%	8.9%	3.2%	3.2%	3.2%
4.3 Net Profit Margin	4.6%	9.7%	7.1%	2.2%	2.4%	2.6%
4.4 ROA	10.8%	32.2%	15.9%	5.3%	6.1%	7.0%
4.5 ROE	28.6%	42.6%	24.5%	7.5%	7.9%	8.3%

Table 21 Forecasting Balance Sheet: B/S

Year	1	2	3	4	5
Asset					
Current assets					
Cash	177,330	574,458	795,880	691,975	643,023
Trade accounts receivable	295,808	361,769	380,074	399,310	419,524
Inventory	289,151	353,771	371,846	390,852	410,838
raw material	139,433	170,686	179,394	188,550	198,177
Work in progress	37,429	45,771	48,113	50,576	53,165
Finished product	112,288	137,314	144,339	151,727	159,496
Other current assets	*167,590*	*199,853*	*336,510*	*480,825*	*579,743*
Total current assets	**929,879**	**1,489,850**	**1,884,310**	**1,962,962**	**2,053,128**
Non-current assets					
Land, plant and equipment					
Land	300,000	300,000	300,000	300,000	300,000
Plant	80,000	80,000	80,000	80,000	80,000
Machine	360,000	360,000	360,000	360,000	360,000
Vehicle	500,000	500,000	500,000	500,000	500,000
Office supplies	50,000	50,000	50,000	50,000	50,000
Expenses before operation	30,000	30,000	30,000	30,000	30,000
Total Land, plant and equipment	**1,320,000**	**1,320,000**	**1,320,000**	**1,320,000**	**1,320,000**
Less accumulated depreciation	(101,886)	(203,771)	(305,657)	(407,543)	(509,429)
Net Land, plant and equipment	**1,218,114**	**1,116,229**	**1,014,343**	**912,457**	**810,571**
Other non-current assets					
Total Non-current assets	**1,218,114**	**1,116,229**	**1,014,343**	**912,457**	**810,571**
Total assets	**2,147,994**	**2,606,079**	**2,898,653**	**2,875,419**	**2,863,699**

Table 22 Forecasting Balance Sheet: B/S (continues)

Year	1	2	3	4	5
Liabilities and owners' equity **Debt** **Current liabilities**					
Overdraft loan					
promissory note					
Trade payable	178,649	196,896	203,181	213,551	224,455
Current liabilities	**178,649**	**196,896**	**203,181**	**213,551**	**224,455**
Non-current liabilities					
Long term loan	1,157,401	993,703	819,657	634,331	436,708
Non-current liabilities	**1,157,401**	**993,703**	**819,657**	**634,331**	**436,708**
Total liabilities	**1,336,050**	**1,190,599**	**1,022,837**	**847,882**	**661,162**
Owner's equity					
Owner capital	579,948	579,948	579,948	579,948	579,948
Retained earnings	231,996	835,532	1,295,868	1,447,590	1,622,589
Total ownership	**811,943**	**1,415,480**	**1,875,816**	**2,027,537**	**2,202,537**
Total liabilities and owner's equity	**2,147,994**	**2,606,079**	**2,898,653**	**2,875,419**	**2,863,699**

For determining the net present value: The difference between the cumulative present value of net cash inflows over the life of a project and the present value of the investment, using one of the discount rates to change the value of the cash flows produced at each time, is known as net present value (NPV). To arrive at the same point in time, the decision criterion is that if the estimated net present value of the project is greater than 0, the project is accepted or invested in.

If the net present value is less than zero or negative, do not invest in the project because it is not worthwhile.

Table 23 shows the net present value (22.3) of the study with an interest rate (5%); Positive net present value (NPV> 0) Factory gain is earned over a period of time. To equal the total present value of 1,147,953.00 baht, ethanol production capacity 182,500 liters/year), the price of ethanol per unit calculated in accounting is 57.42 baht/liter, but economically, the price is 43.84 baht/liter.

Table 23 The net present of value of investment

Year	Cash flow (Baht)	Present value (Baht)
0	1,147,953.00	1,147,953.00
1	1,201,530.42	1,177,191.32
2	1,297,663.92	1,153,400.65
3	1,317,104.25	1,174,544.61
4	1,332,.562.66	1,201,359.46
5	1,413,140.42	1,287,674.03
NET PRESENT VALUE		20.26

The price of ethanol is equal to $ 1.7. The price of ethanol from the corn shed reached $ 2.25, according to (Humbird et al., 2011). Ethanol was also discovered to be the total annual manufacturing expenditure. The cost of lignocellulose biomass is $1.92 per gallon (Alvarado-Morales et al., 2009). In this study, the processing time was five years with an internal rate of return of 5%.

Internal Rate of Return: IRR is a discount rate that compares the present value of the project's net cash inflow to the net expenditure cash. To put it another way, the discount rate at which the project's net present value equals zero. Throughout the project's existence, investors should expect to gain a 9.4% annual return on their

money, the project's annual return on investment (IRR). A low-yielding, high-risk investment was described as one with a difference between return and capital costs (IRR - WACC) of 2.8%.

If previous studies' findings are taken into account, the study's findings will estimate the importance of the gain that expenditure in the effects of trying to provide the industry within the economic net result will provide. Table 4.6 - Table 9 summarizes the economic analysis of the results of Tables 4 through 9. On the other hand, the tables only provide an estimation of the overall cost arising from the procedures and components used to calculate the fixed cost. Investing in plants that generate bioethanol from lignocellulosic waste Additionally, the commodity form of raw materials, location, and the scale of manufacturing facilities play a large roles in business expansion. The problem here is that the enzyme pricing model will cause issues with bioethanol development from these two sources (Kang et al., 2019). Equivalent to about 75% of the overall cost of ethanol production; the processes, namely, the production costs, includes ethanol distillation and labor, equal to total production costs; work for ethanol distillation, even, equ about production cost 75%.

CHAPTER 5

SUMMARY

Traditional Thai tobacco and animal corn stalks offer many advantages over lignocellulosic biomass rich in cellulose, hemicellulose, and lignin for bioethanol processing. Physical and chemical pretreatments (boiling and autoclaving) rupture stalk cell walls, resulting in smaller particles that can be hydrolyzed. After enzymatic therapy, the hydrolyzed stalk yields higher sugar concentrations in the hydrolysate. By combining physical and alkaline pretreatments with enzymatic hydrolysis, this study was able to achieve higher ethanol concentrations and productivity. As compared to raw bioethanol, the ethanol percentage after distillation is much higher. Tobacco stalks are an appealing feedstock for large-scale biological bioethanol production because of the concentration and availability of low-cost raw materials, reducing greenhouse gas emissions and improving food security. A continuing economic study includes further research into ethanol fermentation.

This study produced a combined pretreatment design to aid in the creation of efficient bioethanol production by generating and synthesizing high sugar from dried tobacco stalks. Chemical compounds such as cellulose, hemicellulose, and lignin are abundant in the stalks, with 35.45 0.13 percent, 43.90 0.26 percent, and 18.16 0.28 percent, respectively. The findings of this study could help the ethanol industry secure a long-term supply of feedstock to meet the growing demand for bioethanol. Since the techniques used in this study are simple and inexpensive, even a small business may produce biofuel. Before and after the fermentation, phenol-sulfuric and DNS methods were used to calculate the total and reducing sugar used. In addition, the combined pretreatment method was used to increase bioethanol output by degrading the biomass and improving accessibility to usable sugars. The hydrolysate with the highest sugar content was chosen and fermented for 72 hours in a bioethanol reactor. The total and reducing sugar concentrations of dried tobacco stalks were 27.97 g/L and 5.43 g/L, respectively, according to the experimental

results. At 48 hours of fermentation, the maximum ethanol yield of 75.74 (g/L) was achieved. Ethanol production can choose a suitable production process or adapt one to meet specific needs in order to minimize costs and boost productivity. It can also use agricultural waste biomass such as tobacco and animal corn stalks. As a result of the findings of this report, the majority of bio-refinery wastes can be recycled, reducing environmental impact and increasing the importance of waste as a potential energy and economic resource.

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APPENDIX A

PUBLICATION

Original Research Article

THERMOCHEMICAL PRETREATMENT METHOD FOLLOWED BY ENZYME HYDROLYSIS OF TOBACCO STALKS FOR BIOETHANOL PRODUCTION

KARN SOPHANODORN [1], YUWALEE UNPAPROM [2], NIGRAN HOMDOUNG [1], NATTHAWUD DUSSADEE [1], RAMESHPRABU RAMARAJ [1]

ABSTRACT

Energy use from fossil fuels increases, causing an energy crisis, increasing greenhouse gases, and other environmental issues. In this study, obtaining renewable energy sources from biomass to replace fossil fuels is vital for future energy supply. Ethanol production from lignocellulosic materials was gain more attention recently. It is an interesting process and an alternative way countries with agricultural waste can be recycled as energy. To convert such waste biomass source into energy in ethanol needed to adjust cellulose conversion to different suitability. Therefore, to obtain the fermentable sugars for bioethanol production, the pretreatment process involved a vital role. In this experimental study, 4% of calcium oxide (CaO) was applied. Moreover, a scanning electron microscope (SEM) distinguished the characteristics of untreated and pretreated samples. In this study, the separated hydrolysis and fermentation (SHF) method was used for bioethanol production. Total and reducing sugars yield confirmed that tobacco stalks are suitable feedstock for bioethanol production.

Keywords: Tobacco stalks, thermochemical pretreatment, calcium oxide, hydrolysis, SEM, SHF.

AUTHOR AFFILIATION

[1]School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand.
[2]Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand.

CORRESPONDENCE

Rameshprabu Ramaraj, School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand
Email: rrameshprabu@gmail.com
    rameshprabu@mju.ac.th

1. INTRODUCTION

The distinction between supply and demand has resulted in a lack of fossil fuel resources. The search for an alternative source of renewable energy with the potential to replace conventional nonrenewable fuel sources is urgently required [1,2]. Due to this worrying issue, bioethanol has been determined as an excellent alternative fuel. Unlike fossil fuels, bioethanol is a clean, renewable resource source [3,4].

Among the crucial choices to diminishing nonrenewable energies is eco-friendly bioenergy resources, which may minimize the world's reliance upon non-sustainable, notorious, nonrenewable fuel source-based power resources [5-8]. Bioethanol is one of the eco-friendly bioenergy sources considered one of the possible options for petrol-located fossil gas, specifically in the transport market. Bioethanol is produced through glucose fermentation and its usage as energy happens with both ecological and social perks that consist of significantly reduced emission of CO_2 and the creation of work for the nearby neighborhoods due to the development and transformation of renewable biomass to bioethanol [9].

Nonetheless, bioethanol creation presents specific difficulties: use of meal crops grown on the agricultural property as feedstock like corn, cassava, sugar beetroot, potato, etc., which leads to both deficiency and also rate walking of these meals products [10,11]. These plant's potential to collect high carbohydrates content in its biomass under varying development states has recorded scientists' interest. Plants-gathered starch could be fermented for the creation of bioethanol. Bioethanol made from tobacco stalks can easily remove the requirement of food plants and also agricultural property utilization. The stalks containing lignocellulosic biomass components; make up many eco-friendly substrates for bioethanol and various other biofuels development that certainly not compete with food manufacturing and creature feed [9].

These cellulosic components additionally help in environmental sustainability [12]. Also, lignocellulosic biomass may be supplied massively from various affordable resources like domestic and industrial rubbishes, timber and agricultural residues [13]. Tobacco stalks, which mainly consist of lignocellulose, possess the possibility to work as a low-cost feedstock to increase the manufacturing of energy ethanol. Direct saccharification or biotransformation of tobacco stalk is remarkably complicated because of the stubborn attributes of lignocellulosic material. Therefore, this study aimed to apply the thermochemical pretreatment method followed by enzyme hydrolysis of tobacco stalks for bioethanol production.

2. MATERIALS & METHODS

2.1. Raw Material and Reagents

Tobacco stalks were obtained from crops land directly and the material was transferred to Energy Research Center, Maejo University, Thailand. Before compositional analysis, the biomass, which consisted primarily of stalks, was ground to a particle size of 3 mm. Commercial cellulase was used. All chemicals were analytical grade, provided by Sigma S.A. (USA).

Figure 1. Tobacco planta cultivation area, harvesting leaves, leftover stalks, size reduction

2.2. Pretreatments and Enzymatic hydrolysis

Aqueous calcium oxide (CaO) solutions at a concentration of 4% (w/v) were used to pretreat tobacco stalks samples. The concentration of 4%CaO per 100 g of tobacco stalks (w/w, g CaO/100 g of dry tobacco stalks). Treatments were performed in triplicate in an autoclave at 121 °C with 15 psi (103.4 kPa) pressure for the residence time of 60 min. The pretreated solids were filtered, washed thoroughly with deionized water, dried in an air-circulated oven for 16 h at 105 °C, and used for the subsequent hydrolysis and fermentation experiments.

Enzymatic hydrolysis experiments were conducted at 1% of cellulase (w/v) in 100 mM citrate/phosphate buffer (pH 5.0) containing 0.01% (w/v) sodium azide (to prevent microbial contamination). All the experiments were conducted in triplicate. Samples were withdrawn periodically, centrifuged (10.000×g for 10 min) and analyzed for total and reducing sugars.

2.3. Scanning Electron Micrograph Characterization

Tobacco stalks were subjected to morphological examination by Scanning Electron Microscope (JSM-5410LV). SEM images of the material were taken at a magnification of × 500×, with an EHT of 15 kV and a working distance of 10.5 mm.

2.4. Yeast culturing and fermentation

For the efficient development of bioethanol originating from lignocellulosic biomass, the demand to enhance fermentation capability and make durable fungus tensions together with tolerant-ability to repressive materials discharged from pretreatment is critical.
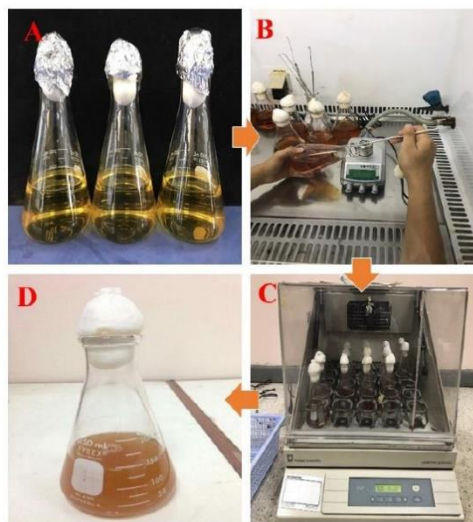
Figure 2. YM medium (A), adding yeast into the YM medium (B), shaking and free cell yeast culture (D)

Besides improving the metabolic feedback efficiency with the sensible concept, it is necessary to adapt microbial tissues used for metabolite production to strengthen and enhance the metabolic operation's feature. The robust yeast Saccharomyces cerevisiae was used in this study and routinely maintained at Program in Biotechnology, Faculty of Science, Maejo University, Thailand. S. cerevisiae was maintained on YM medium pH 5.6 and shake 150 rpm for 24 h, at 37ºC (Figure 2). Optimum pH for S. cerevisiae was found to be 4.0-5.0.

In this study, a separate hydrolysis and fermentation (SHF) design was adopted from Vu et al. [12,13]. In SHF, enzymes hydrolyze solid residues into fermentable sugars, and then the yeast ferments the sugars to bioethanol in the following step. Fermentation setup is presented in Figure 3 with triplication units (R1, R2 and R3).


Figure 3. Fermetation setup

2.5. Analytical methods and statictical analysis

The total sugar content was determined quantitatively using the Phenol-sulphuric acid method as outlined by Dubois et al. [14]. Moreover, the reducing sugar analyzed using the DNS (dinitrosalicylic acid) method [15]. All the experiments were done in triplicate conditions, and the standard deviation was examined.

3. RESULTS & DISCUSSION

Biomass from aquatic and the lignocellulosic product is viewed as an interesting source of raw material for sale into biofuels, biochemicals, as well as biomaterials that are coproduced using biomass upgrading [16]. The primary reason is that biofuels, including bioethanol and brand-new bio/co-products with the higher added value, may result in lasting growth. Ultimately, each is desirable in industry and the bioeconomy concerning a combined biorefinery for second and third generations. The incorporated biorefinery idea is fixated on economic as well as environmental elements. As a result, the pretreatment procedure takes on a crucial function in an integrated biorefinery, because this stage makes it possible for the fractionation of the primary components of the lignocellulosic and aquatic biomass [17].

Tobacco stalks provide an enormous biomass resource for bioethanol production, but its characteristic recalcitrance towards catalysis results in inefficient cellulose hydrolysis, with lower bioethanol yield than other major crop straws. The majority of category, premium examination, or grading of the flue-cured tobacco leaves are manually run, which counts on professionals' judgmental knowledge and is undoubtedly confined by the individual, ecological, and tangible factors. The distinction as well as the premium evaluation are consequently individual and also experientially based. In this particular study, the stalks were used for an automatic distinction technique of tobacco places based on availability and obtained biomass. The plant classification and stalks sugar composition was presented in Figure 4.
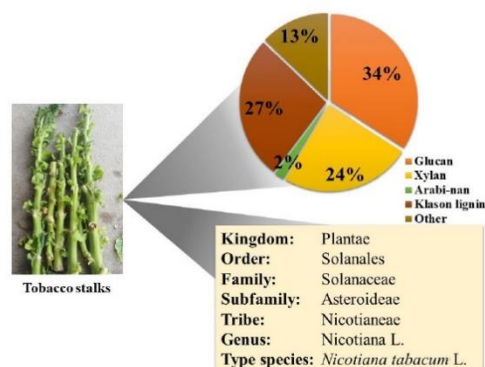

Figure 4. The tobacco plant classification and stalks sugar composition

High-resolution electron microscopic techniques, like scanning electron microscopy (SEM) or transmission electron microscopy (TEM), are commonly used to analyze the plant cell wall morphology [18]. SEM is an ideal instrument for determining structural features and cellular and nanometer-scale resolution of biomass degradation. SEM microscopic, as well as nanoscopic analyses of the lignocelluloses and evaluations along with the pretreated samples, may be used to qualitatively recognize the susceptibility and anticipate of the lignocellulosic materials to succeeding hydrolysis. Nevertheless, acquiring a successful characterization utilizing the surface area image resolution is actually challenging.

Scanning electron microscopy (SEM) images of raw and pretreated tobacco stalks depicted the lignocellulosic biomass's morphology (Fig. 5A, B). The untreated tobacco stalks were composed of a compact arrangement of cells, as exposed in Figure 5A, giving an intricate appearance that indicated tight binding of lignin to the cells. The soft area, contaminations in the form of white-colored coatings and rectangular form, surface area texture were actually noticed in the fiber surface. This is due to the hemicellulose, lignin, wax, and various other impurities covered over the fiber area. Image of pretreated tobacco stalks treated with CaO (Fig. 5B) confirmed slight loosening of fibers and distortion of cellular arrangements, which suggested removing lignin from the biomass preparation and increased porosity increasing surface area. The alkaline concentration enhances the separation of the vascular bundles, which is more pronounced in samples treated with 4% CaO (Figure 5B).
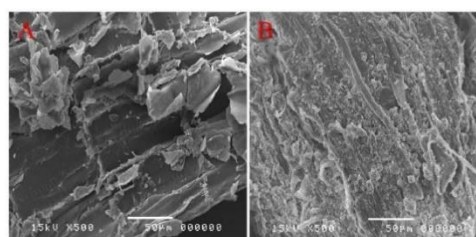
Figure 5. SEM untreated tobacco stalks (A) and pretreated tobacco stalks (B)

This is in agreement with the preferential localization of lignin in the middle lamellae, the membrane delimiting neighboring cells. Consequently, images of treatments with CaO revealed further disintegration of cellular integrity, increasing the external surface area and porosity, as well as pretreated biomass, which has revealed the loss of structural order due to the exposure and separation of microfibrils.

Chemical pretreatments are frequently made use to solubilize polymers. Lignocellulosic biomass can solubilize hemicellulose by breaking the hyperlinks between lignin-carbohydrate complexes, while alkalis act lignin's structure. Just in the case of biomass nevertheless, alkali addition involves the solubilization of healthy proteins and carbohydrates. Chemical pretreatments of microalgal biomass need to have mild conditions for launching the organic component because of lignin's absence. Chemical pretreatment, among the concerns associated with these pretreatments, is the feasible development of biofuel production processes. The sugar concentrations of different stages during fermentation was shown in Figure 6.

Reliable pretreatment possesses a great addition in enhancing the price connected with bioethanol creation. The price of manufacturing is actually mainly dependent on the pretreatment method. This pretreatment method of the tobacco stalks was found to be the most efficient and resulted in the highest quantities of fermentable sugars. Biomass can be hydrolyzed enzymatically to produce glucose which can be converted to liquid fuel, ethanol. Hydrolysis of biomass can be accomplished by fungal and bacterial biomass hydrolyzing enzymes. Several enzymes are required for complete hydrolysis of biomass, such as cellulase, xylanase, ligninase, pectinase, etc., among which

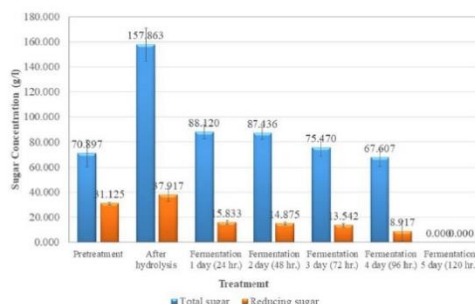cellulase is the most essential one biomass contains about 40% or above cellulose.

Figure 6. Sugar concentrations of different stages during fermentation

Table 1. Ethanol production from different biomass resources

Sample/ Substrate	Enzyme	Microoragnism	Fermentation type	Ethanol concertation (g/L)	Reference
Chlamydomonas mexicana	Cellulase	*S. cerevisiae*	SHF	10.5	[19]
Chlamydomonas reinhardtii UTEX 90	α-amylase	*S. cerevisiae* S288C	SHF	11.73	[20]
Corn stalks	Cellulase	*S. cerevisiae*	SHF	3.32	[21]
Cotton gin trash	Genencor Inc., Accellerase 1500, Accellerase XY.	*S. cerevisiae*	SHF	5.5	[22]
Water hyacinth	Cellulase	*Kluyveromyces marxianu strain*	SSF	7.34	[23]
Tobacco stalks	Cellulase	*S. cerevisiae*		12.47	This study

Cellulase is a multi-enzyme complex of three different enzymes; exoglucanase, endoglucanase and beta-glucosidase. These are act synergistically for complete hydrolysis of cellulose [19]. The highest amount of sugar contents has resulted from the pretreatment of the tobacco stalks with CaO followed by cellulase. The total amount of fermentable sugar released during enzymatic hydrolysis was gauged to figure out each tobacco stalk's ability before and after the pretreatment. Alternatively, the stalk's biomass digestibility was considerably strengthened through CaO pretreatment. The saccharification experiments allowed us to confirm that fermentable sugar production and its following conversion into bioethanol is an option to add value to tobacco stalks. Experiments were performed to assess further the potential of producing ethanol from tobacco stalks and bioethanol yield to 12.47 g/L. Produce ethanol from cellulosic feedstock using SSF (simultaneous saccharification and fermentation) and SHF (separate hydrolysis and fermentation) processes were compared and results prested in Table 1. This research study prospects can be used in future studies to enrich bioethanol production in the continuous process of bioenergy generation.

4. CONCLUSION

Tobacco stalks may be a promising feedstock for bio-ethanol generation and ideally matched to the biorefinery method because of its all-over excellent world quantity, high fermentable sugar content, and simple fact. It does indeed not compete with the land readily available for food items as well as feed production. The alkaline and autoclave combine pretreatment had significant effects in supporting enzymatic saccharification of tobacco stalks. After pretreatment and enzyme hydrolysis process, results were achieved high fermentable sugar yield. As a result, fermentable sugars yield increased after enzymatic hydrolysis and the bioethanol yield was reached 12.47 g/L. This study result is a contribution to developing a feasible bioethanol production from tobacco stalks.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest related to the publication of this article.

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THERMOCHEMICAL PRETREATMENT METHOD FOLLOWED BY ENZYME HYDROLYSIS OF TOBACCO STALKS FOR BIOETHANOL PRODUCTION

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ORIGINAL ARTICLE

A biorefinery approach for the production of bioethanol from alkaline-pretreated, enzymatically hydrolyzed *Nicotiana tabacum* stalks as feedstock for the bio-based industry

Karn Sophanodorn[1] · Yuwalee Unpaprom[2] · Kanda Whangchai[3] · Akksatcha Duangsuphasin[4] · Numchok Manmai[5] · Rameshprabu Ramaraj[1]

Abstract

In this study, an attempt was made to investigate bioethanol production using low-cost feedstock, namely, tobacco wastes obtained after the leaves harvesting. Tobacco stalks, an abundant biomass source of the leftover agricultural crop field, are a promising feedstock for bioethanol production. Traditional Thai tobacco (*Nicotiana tabacum* L.) is known as non-Virginia type tobacco stalks and was used as biomass feedstock for ethanol production by separate hydrolysis and fermentation (SHF) with a computerized fermenter. Tobacco stalks were efficiently hydrolyzed after a mild physical-chemical pretreatment. The economically cheapest alkaline chemical (2% CaO) was used for pretreatment. The robust yeast *Saccharomyces cerevisiae* was utilized, and it is suitable for industrial ethanol production. These data suggest that tobacco stalks are potential candidates for ethanol production from physical alkali-pretreated biomass with enzymatic hydrolysis on the SHF system.

Keywords Biorefinery approach · Tobacco stalks · Pretreatment · Hydrolyzed · Bioethanol · Bio-based industry

1 Introduction

Thailand is a country with a diverse range of agricultural products. As a result of agricultural activities, many wastes are available as biomass [1, 2]. Conversing to the waste to wealth concept of the circular economy, agriculture products and waste from the agriculture industry can be processed as a biomaterial or other highly valuable substances in the biomass and biofuel industry. Biomass waste represents the excellent replacement of fossil fuels for energy recovery and valorization into value-added products [3–5]. Among biochemical and thermochemical conversion techniques of biomass, bioenergy appears to be most appealing due to its low pollutant emission and diverse product formation [6, 7]. Therefore, agricultural waste is a resource for sustainable energy in local communities.

Furthermore, air pollution from various sources, including agricultural wastes open burning, is a significant risk of public health problems. The World Health Organization (WHO) valuations that a third of all global deaths from stroke, lung cancer, and respiratory diseases can be interconnected to air pollution. Mueller et al. [8] stated that exposure to particulate matter emitted from biomass burning increases mainly in Southeast Asia. Open burning for agricultural purposes is a common practice in many Asian countries, including Thailand. Air pollution is an impactful problem in Northern Thailand, making many people nauseous, harms the environment, and affects the tourism industry.

According to Moran et al. [9], the Northern Thai provinces face air pollution annually due to its geographical features, climate, and agricultural customs. It is recorded distinguished from March to May because it reached the highest haze pollution in the world. Here, yearly occurred the dry season (November to February), and the wet or rainy season (June – October). These

✉ Rameshprabu Ramaraj
rameshprabu@mju.ac.th; rrameshprabu@gmail.com

1 School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand

2 Program in Biotechnology, Faculty of Science, Maejo University, 50290 Chiang Mai, Thailand

3 Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

4 Faculty of Engineering, Rajamangala University of Technology Lanna, Chiang Mai 50300, Thailand

5 Department of Forestry, National Chung Hsing University, Taichung 402, Taiwan

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seasons are circumscribed by the hot season or the haze period from March to May, which are also the hottest months. The air pollution in northern Thailand has long been recognized as a seasonal smog effect or haze crisis. During this season, the agricultural area is occupied by field crops, including maize, cassava, wet rice, low land rice, sugarcane, soybeans, and tobacco.

Nan and Chiang Mai provinces, northern Thailand, are among the most famous areas well known for cultivating traditional Thai tobacco plants. Traditional Thai tobacco (*Nicotiana tabacum* L.) is known as non-Virginia type tobacco [10]. The traditional tobacco cultivation areas increased by 50% due to favorable prices and increased profits, which encouraged farmers to cultivate more. Besides, tobacco stalks are biomass waste after tobacco harvesting. The yield of tobacco stalks in tobacco production is around 20%, far more than any other tobacco waste, covering tobacco stems, discarded tobacco leaf, tobacco debris, etc. This massive tobacco production leads to large quantities of tobacco waste, which is harmful to the environment and is generated during the cultivation and manufacturing processes [11]. Cong et al. [12] stated that the major waste of tobacco stalks of 1.2 million tons is produced every year and much of it is subjected to burning or returning to the field. Therefore, significant economic profits can be accomplished by appropriate utilization of the waste tobacco stalks. Crop stalks are potential biomass for bioethanol production, but direct conversion without pretreatment continually results in a low yield because of lignocellulose's rebellious nature [13].

Lignocellulose conversion into ethanol involved three main processes: (1) a pretreatment to remove the barrier of lignin and expose plant cell wall polysaccharides, (2) enzymatic saccharification of sugars with a cellulolytic or hemicellulolytic enzyme, and (3) fermentation of the sugars with ethanol-producing microorganisms [14–16]. Pretreatment involves the use of acids, alkalis, and organic solvents. Many pretreatment strategies have been developed, such as physical treatment, chemical treatment, and biological treatment, combined with physicochemical treatment [17, 18]. You et al. [19] suggested that calcium oxide (CaO), which is commonly called quicklime or burnt lime, is cheaper in the market, compared with sodium hydroxide (NaOH). Moreover, the future biorefinery will be an integrated complex that makes several products (e.g., biofuels, chemicals, power, and protein) from various feedstocks. Studies on bioethanol produced from various non-edible feedstocks have recently expanded significantly because such production can avoid the conflict between food and fuel. The bioethanol production from the lignocellulosic biomass methods can be varied in many specific approaches: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bioprocessing. The SHF approach

was applied because this process optimizes hydrolysis and fermentation conditions, which are vital for new material [20, 21]. The total ethanol yield and final ethanol concentration were found to be higher in SHF [22]. This work's objective was to evaluate the environmental benefits, leftover agricultural wastes utilization for ethanol production with SHF, and bioenergy usage. Therefore, in this study, physicochemical pretreatment was carried by traditional autoclave with a combination of CaO for bioethanol production from tobacco stalks using a pilot-scale fermenter.

2 Materials and methods

2.1 Biomass

The tobacco stalks were a kind gift from the agricultural farmers, Phrae Province, Thailand. This study procedure and outline is shown in the schematic diagram of Fig. 1. The stalks were dried in a local solar drying facility at 60–70 °C until the stalks became fragile at Energy Research Center, Maejo University, Thailand. Ten kilograms of dried tobacco stalks was milled to reduce the particle size to less than 3 mm using a biomass crusher shredder machine and then stored under dry and air-tight conditions.

2.2 Pretreatment and hydrolysis

Pretreatment, alkaline (2 kg of 2% CaO v/v), was carried out 24 h. Afterward, samples were transferred to a traditional autoclave and used for 3 h. This process is called a combined
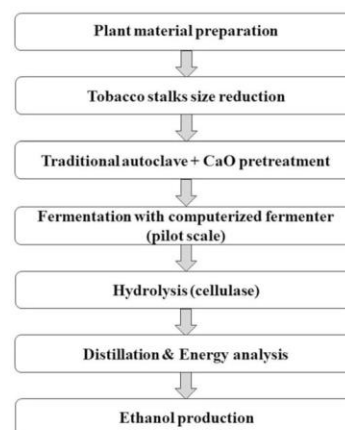

Fig. 1 Schematic diagram of this study

pretreatment. Subsequently, 1 kg of 1% cellulose (commercial grade) was used and kept 24 h for the hydrolysis process.

2.3 Fermentation and distillation

The popular and widely used commercial yeast, *Saccharomyces cerevisiae*, was utilized in this study. The dry yeast was purchased (Alcohol Yeast, Xinjiang Shengli Biotechnology Co., Ltd., China). Fermenter's total volume was 150 L, and working volume was 100 L. Also, the computerized pilot-scale fermentation system is shown in Fig. 2. The fermentation was carried for 48 h at the School of Renewable Energy, Maejo University. The next step produced bioethanol was allowed for distillation at the Faculty of Engineering and Agro-Industry, Maejo University. The commercial-scale distillation unit was used in this study.

2.4 Scanning electron microscope analysis

The sample preparation procedure was adopted from Nong et al. [18]. The morphologies of raw and pretreated tobacco were observed with scanning electron microscope (SEM) (JSM-5410LV). The images were acquired under 15 kV acceleration voltage with a magnification of × 500.

2.5 Analytical methods

The compositions of the sample were determined following the method from Van Soest et al. [23]. The tobacco stalks total sugar and reducing sugars were determined by the phenol-sulfuric acid method [24] and dinitrosalicylic acid (DNS) method [25].

Standard curves were made using D-glucose (Merck, USA). The ethanol concentration was determined by the method adopted from Vu et al. [20].

Fig. 2 Pilot-scale computerized fermentation system

2.6 Mass balance calculation

The biomass conversion of total solids on a dry basis in tobacco was calculated from equations, and they were adopted from pilot-scale study on steam explosion and mass balance for higher sugar recovery from tobacco stalks. The sugar contents of total sugar and reducing sugar-rich CaO pretreatment and enzymatic hydrolyzate were analyzed by the methods described above. The following equations calculated the conversion of pretreatment and enzymatic hydrolysis.

$$\text{TS conversion } (P)\% = \frac{\text{TS Pretreament (kg)}}{\text{Theoretical sugar (kg)}} \times 100$$

$$\text{RS conversion } (P)\% = \frac{\text{RS Pretreament (kg)}}{\text{Theoretical sugar (kg)}} \times 100$$

$$\text{TS conversion } (H)\% = \frac{\text{TS Hydrolysis (kg)}}{\text{Theoretical sugar (kg)}} \times 100$$

$$\text{RS conversion } (H)\% = \frac{\text{RS Hydrolysis (kg)}}{\text{Theoretical sugar (kg)}} \times 100$$

2.7 Statistical analysis

All experiments were done in triplicate, and results are presented as mean and standard deviation. All statistical analysis was accomplished using Minitab 15.0 software (Minitab, State College, PA). Analysis of variance (ANOVA) was performed at a confidence level of 95%.

3 Results and discussion

3.1 Role of agricultural crop residues and depiction of tobacco stalks

Agricultural crop residues include field residues and processing residues. Crop wastes are renewable and plentiful resources. Agricultural field residues represent materials left in the cultivation area after harvesting the crop. They mainly consist of straw and stalks, leaves, and seed pods. Harvesting of cereals, vegetables, and fruits generates vast amounts of crop residues. The biomass can be supplied either from dedicated crops or residues predominantly available in the agricultural sector. Moreover, the residues are made of three main components (cellulose, hemicellulose, and lignin), which can be refined into different final products using a set of together applied technological processes. Khat-udomkiri et al. [26] stated that converting agricultural waste into a useful product reduces agricultural residues' management, treatment, and disposal.

In Thailand, the lignocellulosic rich agricultural residues such as corn cob, corn stalks, sugarcane bagasse, cotton stalks,

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wheat straw, sunflower stalks, green coconut husks, pigeon pea stalks, and tobacco stalks were applicable in the biofuel industry. The use of all residues generated from harvesting and processing residues as raw materials for biofuels production could be necessary for the countries involved. Also, Thailand has excellent potential to use renewable raw materials. Ramaraj and Dussadee [27] specified that agriculture is still mainstream for Thai citizens, despite the share of industry and services increasing constantly. It is one of the largest producers of agriculture, which produces large amounts of residues and wastes. Especially in the northern provinces of Thailand, there is a vast amount of traditional Thai tobacco production yearly. Several authors analyzed the technological feasibility and potential of converting agricultural waste biomass.

The chemical composition of tobacco stalks and other crop stalks showed in Table 1. The potential feedstocks and agricultural residues are a common lignocellulosic biomass source available in many countries; also, the lignocellulosic composition varies depending on the crops [11, 28–30]. Proximate and ultimate analyses of samples tobacco stalks were presented in Table 2 and Fig. 3. Kumar et al. [31] stated that cellulose is a glucose polymer responsible for the plant's mechanical strength, while hemicellulose is a heteropolysaccharide of hexoses and pentoses. Cellulose and hemicellulose are bound to one another by non-covalent attractions. Equally, lignin comprising various alcohols, such as coniferyl, sinapyl, and coumaryl alcohols, acts as a protective seal around holocelluloses.

3.2 SEM analysis for morphological alterations in the pretreated tobacco stalks

Crop stalk biomass has a strong structure and high lignin content, making it very recalcitrant to microbial bioconversion to bioethanol. Without size reduction, stalks cannot be efficiently converted to bioethanol. Therefore, the size reduction of stalks typically involves cutting as smaller, shredding and

Table 2 Proximate and ultimate analyses of tobacco stalks

| Sample description	Amount
Proximate analysis (wt%)	
Moisture	6.07
Volatile matter	71.75
Fixed carbon	18.11
Ash	5.46
Ultimate analysis (wt%)	
C	44.92
H	7.62
N	3.51
O	42.61
S	1.33

milling. Pretreatment can loosen the crystalline structure and stalks' lignin, making them more accessible to the saccharification enzymes. It is necessary to reduce the lignin content in facilitating carbohydrate hydrolysis by enzyme systems [32]. Physical-chemical pretreatment uses whole tobacco stalks after several involved stages of size reduction (Fig. 4a–c), and this procedure simplifies the tobacco stalks pretreatment. Generally, physical requires diluted alkaline/acid chemicals or sample cutting to produce ethanol from agricultural crop stalks. SEM images indicated that physical-chemical treatment reduced whole tobacco stalks' particle size and consequently increased the substrate surface area (Fig. 4d and e).

Physical (thermal)-chemical pretreatment of biomass has been the pretreatment of select to enhance substrate accessibility for effective enzymatic hydrolysis. Alkaline pretreatment dissolves most of the lignin and various uronic acid substitutions responsible for inhibiting cellulose accessibility to enzymatic saccharification and eliminates part of hemicellulose, swelling cellulose microfibrils. Furthermore, lime was the least expensive. It is called quick lime (CaO) pretreatment with this chemical provides a low-cost alternative for lignin [33]. Alkaline pretreatment has been reported to increase plant cell walls' biodegradability through cleavage of the lignin

Table 1 Comparison of the chemical composition of tobacco stalks and other crop stalks

| Crop stalks	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Tobacco stalks	35.45	43.9	18.16
Cotton stalk	40.1	13.6	29.4
Corn stalk	36.4	30.3	6.9
Sunflower stalk	34	20.8	29.7
Sorghum stalk	57.99	17.41	14.95
Pearl millet stalk	52.49	25.42	10.54
Hemp stalk	37.3	19.79	12.35
Pigeon pea stalk	42.71	18.33	2.31
Wheat stalk	42.7	24.9	22.3
Jerusalem artichoke stalk	41	22	20

Fig. 3 Proximate compositions of the tobacco stalks
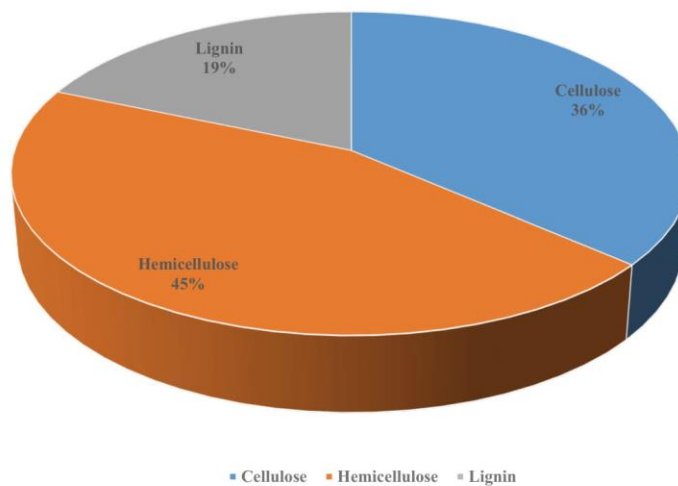
bonds with hemicellulose and cellulose [34]. The increased cell wall surface area improved accessibility of cell wall-degrading enzymes, and the reduced particle size was economically beneficial. Physical-chemical pretreatments are generally applied during bioethanol production. After pretreatment, the cellulose content was reached 57.02%, and it was increased by 20.45%. Hemicellulose and lignin contents were 33.86 and 10.24%; they were reduced to 10.04 and

Fig. 4 Sample preparation and size reduction (**a**–**c**); SEM images showing structural changes of pretreated tobacco stalks: raw tobacco stalks (**d**) and tobacco stalks after physical and chemical pretreatment (**e**)
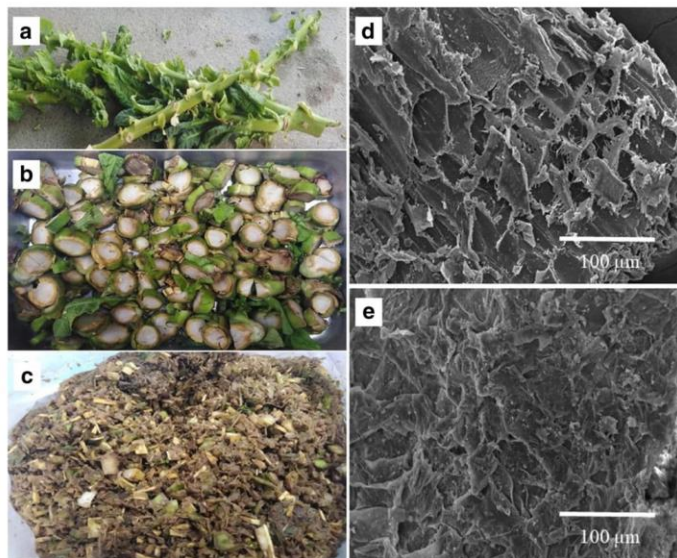
Table 3 Chemical composition of untreated and pretreated tobacco stalks

Chemical composition	Untreated tobacco stalks	Pretreated tobacco stalks	Remarks	Removal efficiency
Cellulose (%)	35.45	55.9	20.45 (increased)	–
Hemicellulose (%)	43.9	33.86	10.04 (reduced)	22.87
Lignin (%)	18.16	10.24	7.92 (reduced)	43.61

7.92%. The stalks biomass condensed lignin layers are usually more resistant to pretreatment chemistry and require combined mechanical, temperature, and chemistry efforts, namely, high severity. The lignin–hemicellulose and cellulose–hemicellulose layers are alternately located in the wall, forming sandwich-like multi-lamellae structures. In this study, the physicochemical pretreatment was performed significantly, and the removal efficiency of lignin was 43.61%. In addition, early removal of lignin can also simplify cell and enzyme recovery and recycle and shorten distillation (Table 3).

3.3 Enzymatic hydrolysis and fermentation

The pretreated tobacco stalk biomass was further subjected to enzymatic hydrolysis for the release of fermentable sugar. The efficient biochemical conversion of lignocellulosic biomass into ethanol be needed upon the consumption of C5 and C6 sugars. In this circumstance, the selection of microbial strain for converting these sugars is of crucial importance. Therefore, the most efficient *S. cerevisiae* as conventional glucose-fermenting yeasts were selected and used for fermentation. Kumar et al. [31] stated that the enzyme cellulase and β-glucosidase were reported earlier to hydrolyze the cellulosic polymers to monomeric glucose units for further use fermentation. After hydrolysis, the total and reducing sugar concentration increased to 69.594 and 36.708 g/L than pretreatment (total and reducing sugar concentration was 49.744 and 28.417 g/L). Overall, the enzymatic hydrolysis process proved to be an effective mechanism to enhance tobacco stalk

biomass's saccharification process. The reducing sugar yield during the hydrolysis process was considerably influenced by fermentation, biomass concentration, and mixing speed.

In SHF, enzymatic hydrolysis and fermentation are carried out in separate steps. This makes it possible to run each process under its optimum conditions, although the cellulolytic enzymes' end-product inhibition is a limiting factor. Moreover, SHF offers cell recycling, whereas SSF is not possible to separate cells and solid raw material particles [20–22]. Bioethanol production from tobacco stalks and other biomass is shown in Table 4. Compared to other biomass feedstock tobaccos, stalk illustrated the higher ethanol yield. The alcohol content was higher during 24-h fermentation compared with the fermentation of 48 h. After 24 h of yeast going to the death phase, living the yeast cell decreases. Results of contamination of other bacteria using glucose in anaerobic fermentation growth and producing acetic acid made the alcohol and total sugar decrease. After fermentation, ethanol was applied in the distillation unit. It reached 8% alcohol.
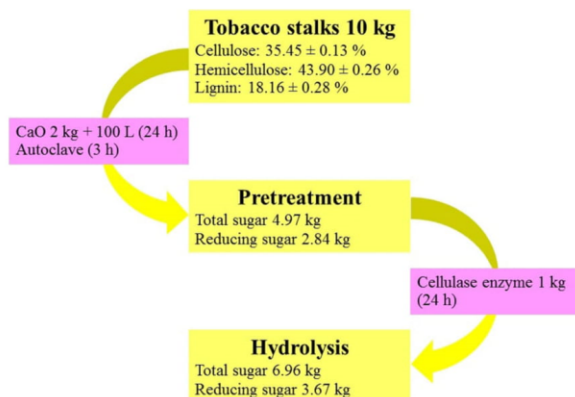
3.4 Mass balance calculation

SHF's mass balance data would help find reasons for the ethanol yield and seek out corresponding procedures. After the sugar production step, this research developed the mass balance for this process, including chemical combination with thermal pretreatments and enzymatic hydrolysis are shown in Fig. 5. Also, calculating the feasibility of tobacco stalk's sugar production from cellulose and hemicellulose is very

Table 4 Bioethanol production from tobacco stalks and other biomass

Factors	Total sugar (g/L)	Reducing sugar (g/L)	Alcohol (g/L)	Reference
Pretreatment	49.744	28.417	–	This study
After hydrolysis	69.594	36.708	–	This study
Fermentation 24 h	40.288	17.29	7.101	This study
Fermentation 48 h	35.512	16.315	5.523	This study
After distillation (24 h bioethanol)	–	–	63.12	This study
Compared with other feedstock				
Sargassum sagamianum	–	–	1–2	Lee et al. [35]
Laminaria digitata	–	–	5.61	Adams et al. [36]
Sago pith	–	–	2.8	Sunarti et al. [37]
Municipal solid waste	–	–	0.15	Mtui and Nakamura [38]

Fig. 5 Mass balances for pretreatment and hydrolysis conditions

Tobacco stalks 10 kg
Cellulose: 35.45 ± 0.13 %
Hemicellulose: 43.90 ± 0.26 %
Lignin: 18.16 ± 0.28 %

CaO 2 kg + 100 L (24 h)
Autoclave (3 h)

Pretreatment
Total sugar 4.97 kg
Reducing sugar 2.84 kg

Cellulase enzyme 1 kg
(24 h)

Hydrolysis
Total sugar 6.96 kg
Reducing sugar 3.67 kg

important; because two polymers make cellulose and hemicellulose. These polymers consist of the monomer of many sugars. Consequently, the fusibility of sugar-producing in 10 kg of tobacco stalk is 7.935 kg. After chemical and thermal pretreatments, biomass converted to 4.97 and 2.84 kg of total sugar and reducing sugar. In the enzymatic hydrolysis step, 6.97 kg of total sugar and 3.67 kg of reducing are obtained in 10 kg of tobacco stalk with 2% CaO 100 by spending cellulase enzyme 1 kg. The detailed calculation results are presented in Table 5 and Fig. 5.

The mass balance was carried out by determining the total sugar and reducing sugar which were pretreated and hydrolyzed at the end of pretreatment and hydrolysis. Tobacco stalks contained 35.45% of cellulose and 43.9% (w/w) of hemicellulose. Therefore, biomass conversion of tobacco stalks can be 79.35% of 7.935 kg of sugar (theoretical sugar) from biomass 10 kg. This resulted in reducing sugar and total sugar conversions in the pretreatment process of 35.812% and 62.689%, respectively, as shown in Table 5. At the end of the hydrolysis step, the mass balance showed increasing sugar

conversion of total sugar and reducing sugar of 46.261% and 87.705% after adding a cellulase enzyme 1 kg for 24 h.

4 Conclusion

Traditional Thai tobacco stalks are an attractive source of biomass for bioethanol production and have several advantages over lignocellulosic biomass rich in cellulose, hemicellulose, and lignin. Physical (boiling and autoclave) and chemical (2% CaO) pretreatments rupture stalk cell walls, producing smaller particles suitable for hydrolysis. After enzymatic treatment, the hydrolyzed feedstock was produced a higher sugar yield. This study obtained higher ethanol concentration and ethanol productivity by combining physical and alkaline pretreatments also enzymatic hydrolysis. After distillation, the ethanol percentage is increased much higher compared to raw bioethanol. For the large-scale biological production of bioethanol, tobacco stalks are considered an attractive feedstock because of the concentration and abundance of low-cost raw materials, reducing greenhouse gas emissions and improving food security. Further investigations concerning ethanol fermentation are part of a continuing economical analysis.

Table 5 Mass balance of pretreatment and hydrolysis

Mass balance	
Theoretical sugar (kg)	7.935
RS pretreatment (kg)	2.842
TS pretreatment (kg)	4.974
RS hydrolysis (kg)	3.671
TS hydrolysis (kg)	6.959
RS conversion in pretreatment (%)	35.812
TS conversion in pretreatment (%)	62.689
RS conversion in hydrolysis (%)	46.261
TS conversion in hydrolysis (%)	87.705

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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ORIGINAL ARTICLE

Environmental management and valorization of cultivated tobacco stalks by combined pretreatment for potential bioethanol production

Karn Sophanodorn[1,2] · Yuwalee Unpaprom[2,3] · Kanda Whangchai[4] · Nigran Homdoung[1] · Nattawud Dussadee[1] · Rameshprabu Ramaraj[1,2]

Abstract

Expanding concern over exhausting fossil fuel and nursery gas limits must lead the more intrigued in renewable fuel-making from biomass sources counting sugars, starches, and lignocellulosic materials. Cultivated tobacco stalk is one of the massive amounts of available biorefinery wastes. Therefore, tobacco stalk was used for bioethanol production in this study. It contains abundant chemical compounds including cellulose, hemicellulose, and lignin 35.45 ± 0.13 (%), 43.90 ± 0.26 (%), and 18.16 ± 0.28 (%), respectively. The total and reducing sugar utilizing phenol-sulfuric and DNS methods were carried out before and after the bioethanol fermentation process. Also, the combined pretreatment process was used for the degradation of the biomass and better accessibility to available sugars to increase the bioethanol production. Hydrolysate with the highest sugar concentration was selected and proceeded to bioethanol fermentation for 72 h. From the experimental results obtained, the total and reducing sugar concentration of tobacco stalk was 27.97 g/L and 5.43 g/L, individually. The results revealed the highest ethanol yields 75.74 (g/L) was reached at 48 h fermentation. Consequently, this form of combined pretreatment technique is a promising method of increasing the overall yield in the dried tobacco stalks to the bioethanol production process.

Keywords Bioethanol · Cultivated tobacco stalk · Valorization · Pretreatment · Fermentation

1 Introduction

Exhausting fossil fuels lead the researcher to investigate alternative sources of energy. Carbon dioxide emissions, global warming, acid rain, and urban smog are one of those damages generated using fossil fuels [1–3]. Moreover, several countries are seeking new forms of alternative energy sources, which is the guarantee for long-term energy security and reducing carbon dioxide from the use of fossil fuels [4]. Hence, this

phenomenon had shifted toward the production of biofuels from renewable resources [5]. These materials aim to produce biofuels in an environment-friendly approach that will limit the effect on climate change and global oceanic acidification and, more importantly, decrease the dependence on fossil fuel [6].

Biomass, like agricultural crop residues such as wheat straw, corn stover, rice straw, and cotton stalks, forestry residues, grasses, sawdust, and wood chips, offers a promising source for biofuels [7, 8] because it is abundant, inexpensive, and does not compete with food and feed applications. Tobacco (*Nicotiana tabacum*) plants are important agricultural crops in the northern part of Thailand. Tobacco is one of the most valuable agricultural products in the world. It is an annual plant culturally managed for quality leaf for cigarette production. Tobacco is grown in over 125 countries, including Thailand, on over four million hectares of land. After the leaves are harvested, the stalks are most of the place burned in the field. Shakhes et al. [9] specified that waste tobacco stalk is fibrous biomass consisting basically of cellulose, hemicellulose, and lignin. The significant volume and presence of nicotine in waste stalks pose rising solid waste

✉ Rameshprabu Ramaraj
  rrameshprabu@gmail.com; rameshprabu@mju.ac.th

1   School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand

2   Sustainable Resources and Sustainable Engineering Research Lab, Maejo University, Chiang Mai 50290, Thailand

3   Program in Biotechnology, Faculty of Sciences, Maejo University, Chiang Mai 50290, Thailand

4   Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

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disposal and pollution problems [10]. Using tobacco stalks as a carbon source in the fermentation of valuable organic chemicals contributes to reducing the negative environmental problems and the cost of production. FAO statistics show that in 2015, developing world farmers grew 544 million tons of corn defined as cob, husk, and kernel and therefore grew 1.5 billion tons of corn stalk left in their fields as waste. Many authors studying specific areas conclude that farmers burn between 50 and 90% of crop waste.

Burning fields for haze appear to be a few particular actions which people are engaging in, which continue cyclical haze. One is the practice of slash-and-burn agriculture where, after harvesting their crops, farmers burn the dried organic matter, which remains in the field [11]. This farming method is widely used in tropical regions all over the world. Particulate pollution is a continual problem that is usually caused by the burning of crop residues in normal agricultural land and highland agricultural systems. Therefore, crop-residue management and estimated pollutant emissions were originated from burning crops for each land-use pattern (grain maize, seed maize and integrated farming, tobacco stalk burning), and the chemical compositions of PM2.5 emissions from agricultural burning in Chiang Mai Province, Thailand. Haze has been a seasonal problem in the North for over a decade. It usually appears from January to April but peaks in March as the arid conditions increase the magnitude of residue burning fires. Therefore, this research is interested in studying two types of plants that are expected to be further developed to produce at-bed, consisting of tobacco stalks. Both plants are currently destroyed by incineration after harvesting. If the study finds that it is appropriate, it will add value to the remaining agricultural materials and increase raw materials in bioenergy production. The utilization of biofuels is a developing elective solution to decrease the utilization of fossil fuels and its byproducts that adversely influences the environment.

Bioethanol is one of the most attractive fuel being renewable and sustainable, having higher oxygen content and octane number, among other fuels [6]. Bioethanol production comes in three fundamental stages: (i) pretreatment, (ii) enzymatic hydrolysis, and (iii) fermentation design development. Pretreatment is a mandatory step to decrease the recalcitrance of the lignocellulosic material in order to have more accessibility to polymers in the biomass, reduce the cellulose crystallinity, porosity, and surface area of the material [2, 7].

On the other hand, enzymatic hydrolysis follows as the enzyme further reduces the cellulose and converts it into fermentable sugars [7, 8]. The combined come hand in hand to produce a high sugar concentration before fermentation. In the fermentation stage, the sugar from feedstock is being converted into ethanol. The higher-sugar-content of the materials is expected to have a higher ethanol yield. Typically, *Saccharomyces cerevisiae* is used in the fermentation to convert glucose into ethanol [12]. *Saccharomyces cerevisiae*

tolerates a wide range of pH, thus making the process less susceptible to infection. Moreover, *S. cerevisiae* is the cheapest and readily available compared to another type of yeast [13].

Morone and Pandey [14] mentioned that the feedstock is one of the main important factors that should be considered. It should be cheap and contains enough fermentable sugars such as energy crops, food processing residues, and forest waste to produce substantial ethanol. Corn, sugarcane, wheat, and barley were commonly used in the production of biofuel. Mostly, bagasse, stalk, leaves, and other waste from processing were widely utilized as feedstock for biofuel. One of the undervalued lignocellulosic materials is tobacco stalk wastes. Therefore, this research is interested in studying tobacco plant that is expected to be further developed to produce at-bed, consisting of stalks. The plants are currently destroyed by incineration after harvesting. If the study finds that it is appropriate, it will add value to the remaining agricultural materials and increase raw materials in ethanol production. In most research studies, the bioethanol production process, mostly single pretreatment, is being applied in the feedstock for the degradation, and only a few have well researched the combination of two or more pretreatments to enhance the sugar production of the biomass. Therefore, this research investigation focused on the possibility of cultivated tobacco stalks (leftover waste biomass) as a potential feedstock for bioethanol production through a series of combined pretreatment designs were optimized for bioethanol production.

2 Materials and methods

2.1 Raw material preparation

Nicotiana tabacum (tobacco) stalks are obtained from cultivated cropland of Ban Laen area, Vieng Ta, Long, Phrae, Thailand (Latitude 18°44′33.4″N, Longitude 99°10′32.1″E). The samples were transported to the School of Renewable Energy (Energy Research Center), Maejo University, Sansai, Chiang Mai-50290 within 2 h for further experiments and analysis. Additionally, the plant samples were dried by air dry then solar dryer and stored for components analysis and further treatment (Fig. 1).

2.2 Determination of lignocellulosic components and sample preparation for scanning electron microscopy

The standard method such as neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) was used to calculate the percentage of cellulose, hemicellulose, and lignin in the samples as described by [2, 15]. The mixture of 1 g of dried tobacco residue with 100 mL of NDF and 0.5 g of sodium sulfite was boiled in a reflux system for

Fig. 1 Tobacco plant (**a**), leftover biomass stalks (**b**), dried stalks crushed into small particles by machine (**c**–**d**)

2 h. The cauldrons and test samples were then washed with hot water and acetone three times, utilizing the cold extraction unit (FT 121 Fibertec™, Denmark). After washing, the pots and tests persistently added with ADF solution and heated by hot extraction unit (FT 122 Fibertec™, Denmark) for 1 h and 30 min. For ADL, the residues recovered in ADF were in this way, treated with 72% H_2SO_4 for 3 h. After the reaction, the crucibles were washed with boiled water, distilled water, and acetone and dried for 4 h at 105 °C, kept in a desiccator, and weighed until steady. The rate of cellulose and hemicellulose are calculated based on the percentage of NDF, ADF, and ADL. Sample preparation for scanning electron microscopy (SEM) and sample characteristics measurement procedure were adopted from Unpaprom et al. [16].

2.3 Hydrothermal pretreatment

Dried tobacco stalks were hydrothermally pretreated at 100 °C and various residence times from 0, 15, and 30 mins. Reactions were prepared by combining ten (10) grams of biomass with 200 ml of distilled water. After treatment, the samples were then cooled at room temperature and filtered to separate the liquid from residual solids. The liquid solutions were centrifuged to separate the remaining solid residue and sugar concentrations were examined using the phenol-sulfuric method and the DNS method. Solid residues recovered proceed to post-alkaline pretreatment.

2.4 Alkaline pretreatment/hydrolysis

The pretreated solid residue recovered from hydrothermal pretreatment was subjected to alkaline pretreatment/hydrolysis. The modified method was applied for further delignification of pretreated tobacco stalk residue using 0%, 2%, and 4%

(v/v) calcium oxide (CaO) at 60 °C for 24 h. The solution was prepared in a 250-ml flask with a pretreated solid residue to a calcium oxide ratio of 1:6 (w/v) separately with different concentrations of alkaline. The flasks were then placed in an economy laboratory oven (Binder ED-115, Germany). The alkaline pretreated residue was analyzed for total sugar and reducing sugar determination.

2.5 Pretreatment optimization and sugar analysis

Response surface methodology (RSM) is mathematical and numerical systems. It usually is suitable for developed, optimized, and improved yields and manufacturing processes. When there are many factors of doing experiments, the effect on yield, production, and particular process, it could be assisted for variable and fixing are highly useful. RSM, beginning with the design of experiment (DOE), is used to determine important factors that affect the testing to decrease the number of investigational runs while maximizing output through the data created. The data can be utilized to advance observed models that associate the response to the experimental factors. The models assist in finding a better response process that has been tested. The model is repeated until it identifies the appropriate process or reaches the limit of the experimental data source [17].

The MINITAB application is utilized to examine the experimental results applying a full quadratic response surface model given by the following Eq. 1 [18].

$$Y = \beta_0 + \sum_{i=1}^{k}\beta_i X_i + \sum_{i=1}^{k}\beta_i X_i^2 + \sum_{i<j}\sum \beta_{ij} X_i X_j. \tag{1}$$

The prescribed method was used to analyze the reducing and total sugar concentrations [19, 20]. Glucose was used as a standard. The aliquot of samples was diluted with distilled

water and used for analysis. And 0.5-ml was withdrawn from the dilution and 0.5 mL of DNS (3,5-dinitrosalicylic acid) solution was added. The mixture was immediately boiled for 15 min, after which, 4 ml of distilled water was added and allowed to cool. The absorbance of each sample mixture was measured using UV–vis spectrophotometer (Drawell Artist of Science, China) at a wavelength of 540 nm.

On the other hand, the total sugar was measured using the same method by replacing the DNS with 5% phenol and 98% sulfuric acid. 0.5 ml of 5% phenol and 2.5 ml of sulfuric acid was added to each diluted 0.5 ml of samples. The solution was mixed vigorously and cooled for 10 min. The absorbance was read at a wavelength of 490 nm.

2.6 Enzymatic hydrolysis

The liquid hydrolysates from the hydrothermal pretreatment combined with each pretreated alkaline solution (solid residue and liquid solution) were used. The pH of each pretreated dried tobacco stalks, tobacco stalk residue, was adjusted to 5 before enzymatic hydrolysis. Then 2% (2% v/v) of cellulase enzyme with 2398 units/g, β-glucosidase 577 units/g, and pH 4 (Union Science Company, Chiang Mai Thailand) was added to each solution. Then the mixture was incubated at 50 °C for 24 h. The resulting hydrolyzed samples were filtered using cheesecloth to separate the liquid hydrolysate from insoluble solids. Total and reducing sugar were measured and the hydrolysate from the best pretreatment and hydrolysis proceeds to fermentation.

2.7 Fermentation and ethanol measurement

The hydrolysate from the best pretreatment condition recovered from enzymatic hydrolysis was centrifuged (1000 rpm, 4 °C, 15 mins) to remove remaining residual solids. The fermentation was prepared using pretreated tobacco stalk hydrolysates in a 5000-ml Erlenmeyer flask (Fig. 2). The pH value of the hydrolysate was adjusted to 5.6 using 1 N H_2SO_4, then

Fig. 2 Fermentation system

sterilized at 121 °C for 15 min. Thereafter, 2% (v/v) of *S. cerevisiae* (Angel Yeast Co., LTD, P.R. China) was aseptically added to the sterilized hydrolysates and placed in an economy incubator (GallenKamp, UK) for 72 h at 30 °C. Samples were withdrawn every 24 h for ethanol and sugar determination. The principle of this method is based on the different boiling points of pure water (distilled water) from water-alcohol solutions [2, 5, 6, 10]. The sampling of 50 ml fermented sample from the fermenter was done after 24, 48, 72, 96, and 120 h to measure the percentage of ethanol using Ebulliometer (Dujardin-Salleron, Alcohol Burner, France).

3 Results and discussion

3.1 Characterization of tobacco stalks

Tobacco stalks, which is obtained after leaf harvesting, is an inedible biomass source. These are considered waste and usually burned vast quantities are left to rot on the field as waste material [21]. Several researches have attempted to create an economically valuable product from tobacco stalks. The stalk biomass in nature consists of the three major components of lignocellulose, cellulose, hemicellulose, and lignin (Table 1). Also, the dried tobacco stalk fiber and analysis showed that it contains 32.85 ± 1.13 (%) cellulose, 24.38 ± 0.17 (%) hemicellulose, and 14.66 ± 1.81 (%) lignin. Moreover, total sugars as carbohydrates were determined, which makes this dried tobacco stalks is the good candidate for bioethanol production.

3.2 SEM analysis for morphological alterations in the pretreated tobacco stalks

Scanning electron microscopy (SEM) was used to characterize the morphological properties of the tobacco stalk biomass untreated and after pretreatment displayed in Fig. 3(a–d). When untreated, the anatomy of the harvested, size-reduced chopped tobacco stalks is simply identifiable, with sheath surrounding the stalk itself (Fig. 3a) with completed cell wall and middle lamella appeared as strongly bundled, rigid, and highly ordered. Mohtar et al. [28] stated that the untreated biomass surface normally had smooth and plane morphology, and seems to be complete. This probably is due to the dense lignin coating on hemicellulose and cellulose fibers.

The autoclaved sample of SEM micrograph is illustrated in Fig. 3b. It shows that the cell wall is partially destroyed and several cell types of the stalk wall can be seen, including epidermis cells and parenchyma cells. The tobacco stalks were treated with a 2% dose of CaO displayed a highly distorted surface, which reason fiber separation and which, in turn, proliferations the reachable surface area and porosity of stalks. Accordingly, the cell wall is almost destroyed and vascular bundles and thick-walled fiber of vessel cells can be seen in SEM micrograph

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Table 1 Lignocellulosic component of tobacco stalk residue and other biomasses

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Reference
Shell	33.23	27.77	31.04 ± 10	Gonçalves et al. [22]
Husk	39.31	16.15	28.48	Vaithanomsat et al. [23]
Sugarcane bagasse	48.70	21.14	24.81	Bittencourt et al. [24]
Corn husk	49.40	23.20	14.60	Shankar et al. [25]
Rice straw	35.60	28.80	4.10	Elsayed et al. [26]
Wheat straw	31.60	16.20	21.20	Qiu et al. [27]
Tobacco stalks residue	35.45 ± 0.13	43.90 ± 0.26	18.16 ± 0.28	This study

presented in Fig. 3c. Also, the increase in porosity improves breakdown efficiency by increasing the accessible surface area. Cell wall of parenchyma cells collapsed except xylem. Then, the scalariform wall of vessel is exposed clearly in Fig. 3d, which is 4% of CaO pretreated samples. It showed many cracks on its surfaces, became noticeably shredded and broken along with formation of pores of variable sizes. Therefore, the images clearly demonstrate significant differences in the surface structure of the untreated and pretreated tobacco stalks. This performance specifies that the structure of tobacco stalks is modified with the presence of CaO and made the structure more accessible to the enzyme to produce reducing sugars by completely opening the bundles.

3.3 Combined pretreatment process and enzymatic hydrolysis

The pretreatment generally refers to the breaking down of the natural resistant carbohydrate-lignin shield that prohibits the accessibility to cellulose and hemicellulose [2]. The effect of pretreatment was briefly shown in Fig. 4. The pretreatment mainly breaks down the lignin and hemicellulosic part of the feedstock which convert to simple cellulose [7, 8]. The pretreatment process is carried out to remove the lignin and hemicelluloses and it will help to increase the porosity of the lignocellulosic materials and also reduce the crystallinity of cellulose. The pretreatment should be at a low operational cost, effective on a variety of samples and

Fig. 3 SEM images of tobacco stalks biomass before and after pretreatment (**a–d**): **a** untreated, **b** autoclaved, **c** 2% of CaO, and **d** 4% of CaO treatment
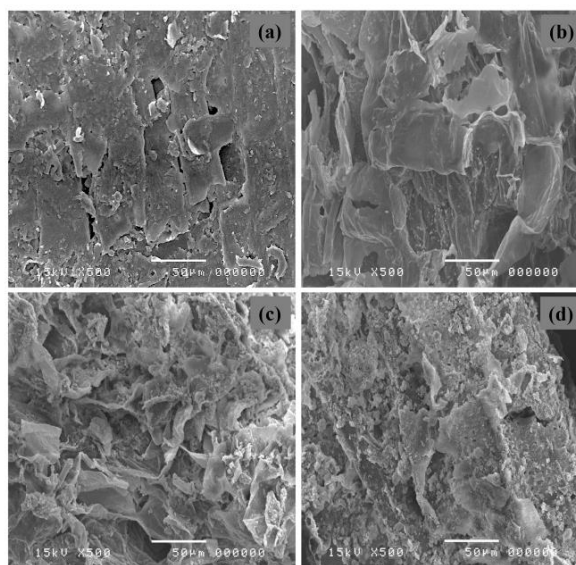
Fig. 4 Actual and predicted plot of reducing sugar (%)
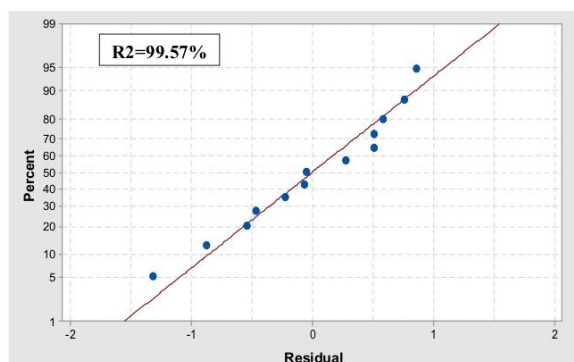
loading lignocellulosic materials; should result in the recovery of most lignocellulosic components in usable form, requiring size reduction which should be minimized; and most importantly, to avoid the formation of inhibitory compounds [7, 12].

Hence, the selection of pretreatment for biomass is crucial. Various pretreatments are available and classified into (1) physical, (2) chemical, (3) biological, and (4) physico-chemical. Different chemicals are used such as acids, alkali, and oxidizing agents for the chemical pretreatment. Mostly, alkali is the most commonly used method which produces high glucose yields [7, 12]. Meanwhile, the physical methods of pretreatment could be mainly focused on the reduction of particle size and the crystallinity of lignocellulosic biomass to which helps to increase the specific surface and the degree of polymerization. It can be performed by a combination of grinding and chipping. This type of pretreatment has lower lignocellulosic biomass decomposition rates compared to other pretreatments. There is also called physic-chemical where it uses a combination of temperature, pressure, or chemical [2, 7, 8]. Usually, a combination of steam explosion and either acid or alkali is applied mostly on agricultural residues. Recent studies on the microorganisms, such as brown-white and soft rot fungi, are also used in biological pretreatment.

Comparing to others, this type of pretreatment is cost-effective, mild, and environment friendly. Nonetheless, the effectiveness of pretreatment is dependent on the type of biomass used. One of the most desired pretreatment methods to attain high sugar yield from the lignocellulosic biomass is the alkaline pretreatment. It mainly increases the accessibility to cellulosic fractions by solubilizing the hemicellulose in the biomass. Several studies have utilized the mild and concentrated alkaline for complex lignocellulosic feedstock. Scanning electron microscopy photographs were confirmed. The pretreatment process was successfully done as observed. The images were taken prior to treatment and were clearly evident after the pretreatment. However, the findings revealed that mild alkaline has more edge than that

of concentrated considering the toxicity, corrosiveness, and reactor requirements. Moreover, mild or dilute alkaline develops only a little or no inhibitors that affect the saccharification stage of bioethanol production. Various alkalines have been reported to be utilized in the pretreatment of lignocellulosic biomass. The conventional diluting alkalines, such as NaOH, CaO, CaOH, and KoH, are among those that have been applied to several lignocellulosic biomasses [29]. However, CaO is the most widely exploited due to low cost, highly active, and readily available chemical for industrial application along with safety and environmental concerns. Noticeably at 170 °C, 30 min incubation time, and 2:2 concentration of CaO, the maximum glucose is attained. Sugar concentrations of dried tobacco stalks from the hydrothermal pretreatment step is described.

The effects of hydrothermal pretreatment on dried tobacco stalks were presented. Initially, the sugar concentration of raw (untreated/0 min) dried tobacco stalks was determined. Based on the results, the total and reducing sugar concentration of dried tobacco stalks were 27.97 g/L and 5.43 g/L, respectively. When hydrothermal pretreatment was applied, the total sugar (complex sugar) concentration increased. Meanwhile, the reducing sugar (simple sugars) observed to be lower compared to the untreated biomass with values ranging from 4.07 to 4.55 g/L. The total higher sugar concentration on treated residue indicates that the hemicelluloses are solubilized and is available in a complex form of sugars, which also explains the low yield of reducing sugars (simple/fermentable sugars) [30].

Sequential pretreatment is significantly beneficial to lignocellulosic materials containing high lignin and hemicellulose. The process involves different pretreatments applied consecutively and combining all the hydrolysates before saccharification. Limited numbers of novel pretreatment have been researched nowadays. According to reports, the sequential and combination of pretreatments were able to hydrolyze the hemicellulose completely, remove the lignin, and expose the cellulose to enzymes, which lead to an increase in sugar extraction [31–33].

One of the conventional pretreatments used in lignocellulosic biomass is boiling and steam explosion which all involve heat transfer. Commonly, water is used in these pretreatment procedures as it acts as dilute acid at high temperatures. Pretreatment with high temperatures opens up the structure of the biomass and removes mainly the hemicelluloses [30]. Hemicelluloses are thought to be hydrolyzed by acetic acid and other acid releases during those pretreatments.

The removal of hemicelluloses from the microfibrils is believed to expose the cellulose surface and increase susceptibility to the enzyme. However, lignin is only removed to a limited extent during this heat-related pretreatment [31, 34]. The sequential and combination of thermal and alkaline pretreatment must be a great combination as it could target the hemicellulose and lignin, respectively, to expose the cellulose to the enzymatic hydrolysis. The hydrolysis involves the breakdown of polysaccharides to their simple sugars. Three commonly known methods are used in hydrolysis: (1) dilute acid hydrolysis, (2) concentrated acid, and (3) enzymatic hydrolysis. Among these, enzymatic is the prominent route that is being used nowadays. Enzymatic hydrolysis is less costly in recovery and wastewater compared to acid. Moreover, it produces better yield, and manufacturers reduced the costs substantially and still relatively lower compared to acid. The enzymatic hydrolysis/saccharification is a green process for converting freed polymeric sugars from the pretreatment process to mono sugars in solution [18]. Sugar concentrations of post-alkaline pretreatment of this study result. The process is mostly used to convert the cellulose into glucose and hemicellulose into pentoses (xylose arabinose, hexoses, glucose, galactose, and mannose).

The conversion of cellulose and hemicellulose is aided by the use of cellulase and hemicellulase enzymes. Usually, the enzymatic hydrolysis is carried out at mild conditions with a pH of 4.8 and a temperature of 45–50 °C [35]. The amount of sugars, mainly glucose, produced in this step is important for fermentation as it will be used and converted into ethanol. Sugar concentrations of dried tobacco stalks after enzymatic hydrolysis data were explored. In conclusion, the combined pretreatment process and enzymatic hydrolysis process is a unique and promising biomass fractionation and pretreatment process for woody biomass.

This study used RSM to obtain the optimum conditions for the maximum reducing sugar production. The optimization of sugar concentration from dried tobacco stalk pretreatments was performed using CCD. The replies of the experimental runs attained from the CCD are presented in Table 2. The CCD is a characteristic trial design due to incidence of treatment mixtures at the middle points of the experimental space of box. Resulting from this experiment, the first- and second-order coefficients are estimated by utilizing CCD. This study applied two factors for reducing sugar production. The first factor is CaO concentration, and the second factor is the pretreatment time. The significance and adequacy of the RSM model shown in Eq. 2.

$$\text{Reducing sugar (g/L)} = 10.163 + 19.732$$
$$[\text{CaO } (\%)] + 0.3731 \text{ [Time of pretreatment (min)]}$$
$$-4.281 \text{ [CaO } (\%)]^2 - 0.00632 \text{ [Time of pretreatment (min)]}^2$$
$$(2)$$

Experimental data were analyzed using the Minitab 15 software. This software applied to calculate the regression analysis of experimental resulted and plotting the contour and 3D response surface graphs [15]. The resulted statistical parameters were calculated by using ANOVA. It was showed in Table 3.

The F value of model, linear, and square are 460.52, 157.16, and 763.88. It can imply that the model, linear and square are significant. There is only a 0.01% accident that this F value may fluctuate. A similar trend and approach were shown by Manmai et al. [5] research. And F value of pretreated sunflower stalk model by using NaOH and resulting from that model also 0.01% chance due to noise. Additionally, the model relationship in this research is significant only when the P values of the model are less than 0.1. All factors in these models (A, B, A^2, and B^2) are significant model terms.

The nonsignificant lack of fit F test ($F = 0.66$) indicates that linear regression is an. Therefore, the regression model and individual model coefficients with a lack of fit test were involved. F value shows the relative to the pure error. There is a 65.3% chance of a lack of fit F value; this could occur due to noise. The actual and the predicted percentage of reduction sugar is showed in Fig. 4. It was found that the values of $R^2(\text{adj})$ and $R^2(\text{pred})$, 98.35% and 99.90%, respectively, have a difference of less than 0.2. It is in reasonable agreement.

In these results, Fig. 5 presented the interaction between the concentration of CaO and time of pretreatment in terms of contour plot; this figure shows the intensity of green in each shade. The intensity of the shade is comparable to the increased sugar concentration. The maximum peak of reducing sugar production was confirmed with the ranges of CaO 1.5 to 3% by using pretreating time 7 to 30 mins. But in Fig. 6, it could show in more detail than Fig. 4. 3D plots are exploited to present the interactions of two independent factors. Therefore, two factors are dignified as study variables and other factors are treated as constant values, with the interaction of variable factors being assessed on the response value. Consequently, the Fig. 6 presents the effectiveness of CaO concentration at 2% and pretreating time range from 20 to 30 mins for reducing sugar production.

3.4 Bioethanol production

The depletion of fossil fuels attracts bioethanol as one of the most beneficial fuels due to its energy security and environmental safety over fossil fuel [3, 33, 36]. It is an environment-

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Table 2 Experimental runs of actual and predicted results from tobacco stalk pretreatment

Std	Run	CaO (%)	Time of pretreatment (Mins)	Reducing sugar (g/L)		
				Actual value	Predicted value	Residual
9	1	2	15	35.362	36.677	−1.315
3	2	0	30	15.125	15.666	−0.541
12	3	2	15	36.216	36.677	−0.462
10	4	2	15	37.189	36.677	0.511
5	5	0	15	14.292	14.338	−0.046
7	6	2	0	31.635	32.503	−0.868
11	7	2	15	37.189	36.677	0.511
4	8	4	30	25.875	26.096	−0.221
1	9	0	0	10.750	10.163	0.587
6	10	4	15	24.708	24.768	−0.060
13	11	2	15	37.537	36.677	0.860
8	12	2	30	38.768	38.006	0.762
2	13	4	0	20.875	20.594	0.281

friendly oxygenated method as it contains 34.7% oxygen, which is absent from gasoline. The presence of oxygen in bioethanol gives 15% higher combustion efficiency over gasoline, resulting in lesser emission of particulate nitrogen oxides. Additionally, other harmful gasses such as sulfur oxide and carbon monoxide being emitted by gasoline can be reduced by mixing ethanol in gasoline. These harmful gases both contribute to acid rain or going to the water and contaminate potable water sources, which causes a detrimental effect on health [37, 38]. Fermentation is the process that converts soluble sugars into alcohol by the metabolic process of microorganisms. In the absence of oxygen, some bacteria and yeast can metabolize carbohydrates such as monosaccharides and disaccharides and produces the ethanol and with the release of carbon dioxide [39].

Mostly in all refinery, the traditional yeast is used for the ethanol fermentation process. Mostly, *S. cerevisiae* species of yeast has been used in alcohol production, especially in the brewery and wine industries. This type of yeast reduces the distillation cost as it gives a high ethanol yield, high productivity, and also can withstand high ethanol concentration. Depicted in Fig. 7 is the ethanol fermentation. The sugars, specifically the glucose, are being converted into ethanol. The yeast ferments glucose and fructose but not pentose sugars [5, 40]. There are three types of fermentation mode, namely, batch, continuous, and fed-batch fermentation.

Batch fermentation is the simplest among the other fermentation modes as there is no need to add in the medium. It is carried out in a closed system, whereas fermentation medium is supplemented with necessary ingredients at the beginning and yeast or another bacterial inoculum is added to the medium before starting fermentation.

The pH and temperature are maintained in this system for the growth of the microorganism [10, 41]. It follows a lag, log,

Table 3 Analysis of variance of quadratic model for reducing sugar production

Source	DF	Adj SS	Adj MS	F value	P value	
Model	4	1222.55	305.64	460.52	<0.0001	Significant
Linear	2	208.61	104.31	157.16	<0.0001	
A:CaO (%)	1	163.19	163.2	245.9	<0.0001	
B:Time of pretreatment (min)	1	45.42	45.42	68.43	<0.0001	
Square	2	1013.94	506.97	763.88	<0.0001	
A^2	1	809.92	809.92	1220.36	<0.0001	
B^2	1	5.59	5.59	8.43	0.02	
Error	8	5.31	0.66			
Lack-of-fit	4	2.11	0.53	0.66	0.653	Not significant
Pure error	4	3.2	0.8			
Total	12	1227.86				
Std. dev.	0.814661	R^2(adj)	99.35%			
R^2	99.57%	R^2(pred)	99.90%			

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Fig. 5 Contour-interacted effect of CaO concentration and time of pretreatment on reducing sugar production from tobacco stalk

and stationary phase such that at the initial stage, growth of yeast cells shows lag phase that follows an exponential phase afterward, thereafter, cells tend to compete with the limited amount of nutrients in the medium and finally enters into a stationary phase as the nutrients are exhausted. Ethanol concentration, volumetric ethanol productivity, and fermentation efficiency obtained during (SHF) fermentation mode after sequential pretreatment and hydrolysis results were described; moreover, reducing sugar and alcohol concentrations during fermentation data were presented in Fig. 7. The highest resulted from ethanol yields 75.74 (g/L) was reached at 48 h fermentation, which was still stable after 72 h. The previous studies were reported that combined pretreatment design was helped in the development of efficient bioethanol production through the generation and synthesis of high sugar from dried tobacco stalks. The results of this study could benefit the

ethanol industry in the sustainable supply of feedstock needed for the impending demand for bioethanol.

Lignocelluloses offer an abundant perspective as a biomass source for bioethanol production. Advanced processes of lignocellulosic ethanol production have the possibility for the reduced life cycle of greenhouse gas emissions when compared with conventional grain-based ethanol, and indeed fossil-based fuels. This present study mainly emphasizes on current knowledge about the characteristics and sources of vegetable biomass, especially leftover from the agriculture field, as well as the development and possibilities for obtaining ethanol from lignocelluloses sources (i.e., tobacco wastes biomass). Spyridon and Willem Euverink [42] suggested that lignocellulosic biomass is a favorable feedstock for biorefineries, reasonable to make up an economic and technical constraint to lignocellulosic-based biofuel production. Bioethanol production could probably be

Fig. 6 3D-interacted effect of CaO concentration and time of pretreatment on reducing sugar production from tobacco stalk
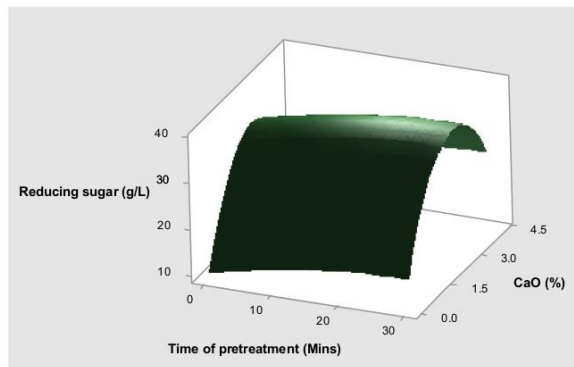
Fig. 7 Bioethanol production from dried tobacco stalks

Alcohol (g/L)
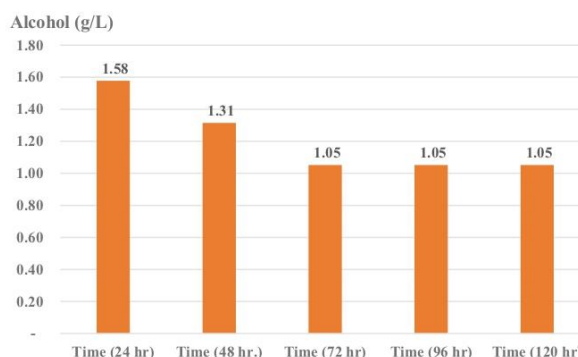
the most successful biofuel because it has plenty of usable forms (heat, power, electricity, or vehicle fuel). Also, plenty of waste biomass, such as leftover tobacco biomass, is helpful to reduce the feedstock cost. Therefore, the study recommended that sustainable and economically feasible large-scale bioethanol production is possible using tobacco stalk biomass as a feedstock.

4 Conclusion

The combined pretreatment design was developed in this study for contribution to the development of efficient bioethanol production through the generation and synthesis of high sugar from dried tobacco stalks. The stalks contain abundant chemical compounds including cellulose, hemicellulose, and lignin $35.45 \pm 0.13 (\%)$, 43.90 ± 0.26 (%), and 18.16 ± 0.28 (%), respectively. The results of this study could be a benefit for the ethanol industry in the sustainable supply of feedstock needed for the impending demand for bioethanol. The techniques applied in this study are simple and cost-effective; hence, a small business can produce their biofuel. The total and reduced sugar use were determined by phenol-sulfuric and DNS methods before and after the fermentation. Also, the combined pretreatment process was used for the degradation of the biomass and better accessibility to available sugars to increase the bioethanol production. Hydrolysate with the highest sugar concentration was selected and proceeded to bioethanol fermentation for 72 h. From the experimental results, the total and reducing sugar concentration of dried tobacco stalks were 27.97 g/L and 5.43 g/L, respectively. The highest result in ethanol yields 75.74 (g/L) was reached at 48 h fermentation. Ethanol production can choose the production process to be suitable or can be developed to suit needs to help reduce costs and increase productivity. Therefore, this study results demonstrated that most of the

bio-refinery wastes could be recycled and help to reduce the impact on the environment and increase the value of waste to be potential energy and economic aspects.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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APPENDIX B

CERTIFICATE

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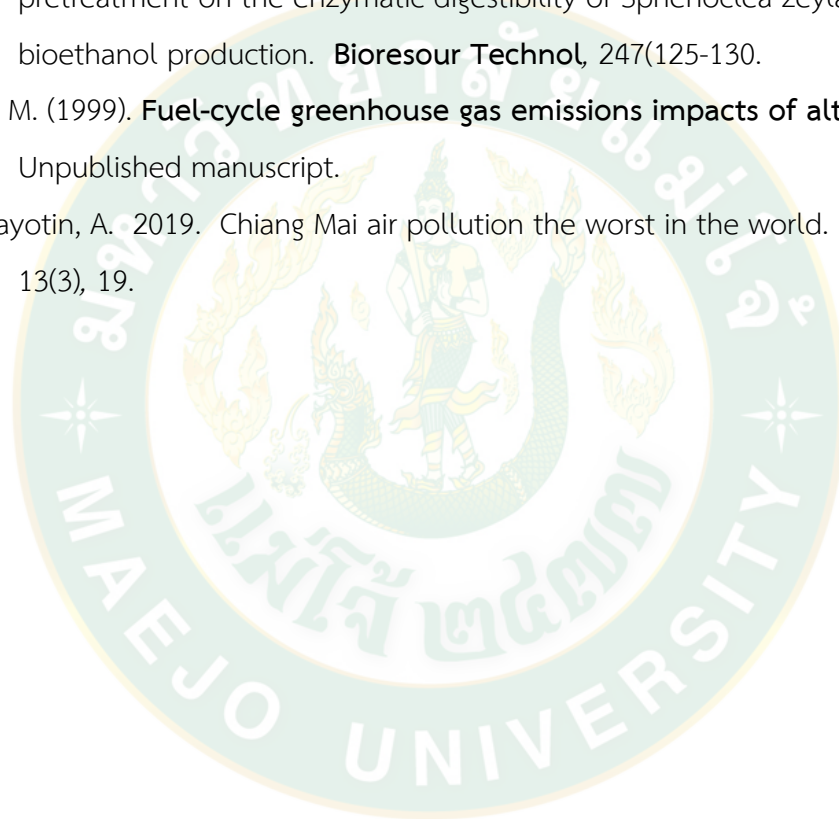WWW.KNOWVEL.COM
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CURRICULUM VITAE

NAME	Acting Lt. Karn Sophanodorn
DATE OF BIRTH	22 Nov 1974
EDUCATION	1988 Junior High School, The Prince Royal's College
	1988-1991 Vocational Certificate of Electrical, Polytechnic Lanna School, Chiang Mai
	1993-1995 High Vocational Certificate of Electrical-Electrical Power, Rajamangala Institute of Technology Tak Campus
	1998-2000 Industrial Education Electrical Engineering - Electrical Power, Rajamangala Institute of Technology Tak Campus
WORK EXPERIENCE	2000-2002 Electrical Measurement and Analysis Engineer, Cresso Corporation Co., Ltd.
	2002- 2010 Electrical engineer, Electric Power And Lighting System Part., Ltd.
	2004-2005 SME's Business consultant, Invigorating Thai Business (ITB) project, Department of Industrial Promotion Ministry of Industry
	2006-2007 SME Business consultant team to increase management and reduce production costs, Department of Industrial Promotion, Ministry of Industry
	2008-2009 SME business consultants to increase management and reduce service sector costs Department of Industrial Promotion, Ministry of Industry
	2009-Now Electrical System Consultant Engineer
	2019-2022 Engineering Owner Consultant, Medical Excellent center Hub Building Construction Project CMU.MED.