POTENTIAL STUDY ON BIOETHANOL PRODUCTION FROM LOW GRADE AND DAMAGED LONGANS FRUITS

NGUYEN THUY VY TU

MASTER OF ENGINEERING IN RENEWABLE ENERGY ENGINEERING

MAEJO UNIVERSITY

2021

POTENTIAL STUDY ON BIOETHANOL PRODUCTION FROM LOW GRADE AND DAMAGED LONGANS FRUITS

NGUYEN THUY VY TU

A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING

IN RENEWABLE ENERGY ENGINEERING

ACADEMIC ADMINISTRATION AND DEVELOPMENT MAEJO UNIVERSITY

2021

POTENTIAL STUDY ON BIOETHANOL PRODUCTION FROM LOW GRADE AND DAMAGED LONGANS FRUITS

NGUYEN THUY VY TU

THIS THESIS HAS BEEN APPROVED IN PARTIAL FULFLLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING
IN RENEWABLE ENERGY ENGINEERING

APPROVED BY **Advisory Committee**

Chair .....
(Dr. Rameshprabu Ramaraj)
...../...../ .....

Committee .....
(Assistant Professor Dr. Yuwalee Unpaprom)
...../...../ .....

Committee .....
(Assistant Professor Dr. Nigran Homdoung)
...../...../ .....

Program Chair, Master of Engineering .....
in Renewable Energy Engineering (Assistant Professor Dr. Tanate Chaichana)
...../...../ .....

CERTIFIED BY ACADEMIC .....
ADMINISTRATION AND DEVELOPMENT

(Associate Professor Dr. Yanin Opatpatanakit)
Vice President for the Acting President of Maejo University
...../...../ .....

ชื่อเรื่อง ศึกษาศักยภาพการผลิตไบโอเอทานอลจากลำไยตกเกรดและลำไยเน่าเสีย
ชื่อผู้เขียน Miss Nguyen Thuy Vy Tu
ชื่อปริญญา วิศวกรรมศาสตรมหาบัณฑิต สาขาวิชาวิศวกรรมพลังงานทดแทน
อาจารย์ที่ปรึกษาหลัก Dr. Rameshprabu Ramaraj

บทคัดย่อ

หนึ่งในผลไม้ที่มีความสำคัญทางเศรษฐกิจและอุตสาหกรรมคือลำไย ลำไย (*Dimocarpus longan* Lour.) เป็นไม้ยืนต้นกึ่งเขตร้อนที่อยู่ในวงศ์ Sapindaceae ซึ่งเป็นผลไม้กึ่งเขตร้อนที่รู้จักกันทั่วไปในหลายประเทศ โดยเฉพาะในประเทศจีน ไทย เวียดนาม อินเดีย ออสเตรเลีย และบางบริเวณในพื้นที่เขตร้อนและกึ่งเขตร้อนของสหรัฐอเมริกา อย่างไรก็ตามสถานการณ์ของขยะผลไม้ที่ใช้ในการฝังกลบนั้น ทำให้เป็นเรื่องน่ากังวลของนักวิจัยเป็นเวลาหลายปีที่ผ่านมา ลำไยตกเกรดและของเสียจากผลลำไยไม่มีข้อยกเว้นสำหรับใช้เป็นวัตถุดิบในการผลิตเอทานอลชีวภาพ ในการศึกษานี้ได้ทำการปรับสภาพทางกายภาพโดยการต้ม (30 นาที) และการนึ่งฆ่าเชื้อ (15, 30, 45 นาทีและ 0 นาทีสำหรับชุดควบคุม) หลังจากนั้นนำตัวอย่างมาส่องภายใต้กล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) เพื่อสังเกตความแตกต่างระหว่างวัตถุดิบ ตัวอย่างที่ผ่านการต้ม และตัวอย่างที่นึ่งฆ่าเชื้อ ในกระบวนการไฮโดรไลซิสนั้น มีการไฮโดรไลซิสอยู่สามรูปแบบที่ใช้ในตัวอย่าง ได้แก่ เอนไซม์เซลลูเลสทางการค้าที่ความเข้มข้น 2% เอนไซม์จากสาหร่าย 20% รวมทั้งส่วนผสมของเอนไซม์เซลลูเลสทางการค้าที่ความเข้มข้น 1% และเอนไซม์จากสาหร่าย 10% (C + A) โปรแกรมวิธีการพื้นผิวตอบสนอง (RSM) นำมาใช้เพื่อกำหนดและหาสภาวะที่เหมาะสมที่ดีที่สุดในการปรับสภาพและการไฮโดรไลซิสตัวอย่าง

ในส่วนของลำไยสด สภาวะที่เหมาะสมที่ผลิตน้ำตาลในปริมาณสูงนั้น ใช้เอนไซม์เซลลูเลสทางการค้าที่ความเข้มข้น 2% ในการไฮโดรไลซิส และนึ่งฆ่าเชื้อเป็นเวลา 30 นาที ส่งผลให้เกิดการผลิตเอทานอลสูงสุด (9.25 ± 0.25 กรัมต่อลิตร) หลังจากหมักเป็นเวลา 24 ชั่วโมง ส่วนลำไยอบแห้ง สภาวะที่เหมาะสมที่ผลิตน้ำตาลในปริมาณสูงนั้น ใช้เอนไซม์เซลลูเลสทางการค้าที่ความเข้มข้น 2% ในการไฮโดรไลซิส และนึ่งฆ่าเชื้อเป็นเวลา 15 นาที ทำให้ผลิตเอทานอลได้สูงสุด (16.74 ± 0.62 กรัมต่อลิตร) หลังจากหมักเป็นเวลา 24 ชั่วโมง จากผลการทดลองข้างต้น ลำไยอบแห้งถูกเลือกใช้ในการผลิตขนาดใหญ่ ผลการศึกษาหลังการปรับสภาพตัวอย่างดังกล่าว พบว่ามีปริมาณน้ำตาลทั้งหมดและน้ำตาลรีดิวซ์เท่ากับ 157.19 และ 36.43 กรัมต่อลิตร ตามลำดับ ส่วนหลังจากการไฮโดรไลซิส พบว่ามีปริมาณน้ำตาลทั้งหมดและน้ำตาลรีดิวซ์เท่ากับ 271.07 และ 48.21 กรัมต่อ

ลิตร ตามลำดับ หลังจากนั้น นำตัวอย่างที่ได้มาหมักด้วยยีสต์ *Saccharomyces cerevisiae* ที่ความเข้มข้น 1% เป็นเวลา 24 ชั่วโมง ทำให้ได้ผลผลิตเอทานอลชีวภาพถึง 1.4% และหลังจากการกลั่นจะได้ไบโอเอทานอลเข้มข้นสูงถึง 9% มีค่าความร้อนสูง (HHV) เท่ากับ 4.55 MJ/kg นอกจากนี้ในการทดลองนี้ได้วิเคราะห์สมดุลของมวล พลังงาน และการวิเคราะห์ทางเศรษฐศาสตร์เพื่อพิจารณาการลงทุนเพื่อดำเนินการในโครงการ

คำสำคัญ : ไบโอเอทานอล, ลำไยตกเกรดและลำไยเน่าเสีย, เอนไซม์จากสาหร่าย, โปรแกรมวิธีการพื้นผิวตอบสนอง

Title	POTENTIAL STUDY ON BIOETHANOL PRODUCTION FROM LOW GRADE AND DAMAGED LONGANS FRUITS
Author	Miss Nguyen Thuy Vy Tu
Degree	Master of Engineering in Renewable Energy Engineering
Advisory Committee Chairperson	Dr. Rameshprabu Ramaraj

ABSTRACT

One of the significant fruits in the economy and industry is longan fruits. The longan (*Dimocarpus longan* Lour.) is a subtropical enduring tree belonging to the family Sapindaceae whose name is a well – known subtropical fruit in many countries, particularly in China, Thailand, Vietnam, India, Australia, and some tropical and subtropical regions in the USA. On the other hand, the status of fruit waste, which becomes stuck previous to landfills, is concerned by researchers over the years. Low grade and waste longan fruits are also no exceptions that are feedstock material for bioethanol production. In this study, physical pretreatment has been carried out which include boiling (30 min) and autoclave (15, 30, 45 minutes and 0 min for control). Afterward, samples were scanned by scanning electron microscope (SEM) to recognize differences between raw materials, boiled samples, and autoclaved samples. Hydrolysis process has done with three varied hydrolysis which were utilized in samples: 2% commercial cellulase, 20% algal enzyme and combination of 1% commercial cellulase and 10% algae enzyme (C+A). Response Surface Methodology (RSM) was utilized to determine and optimize condition lead to the best pretreated and hydrolysed ways.

For fresh longan, the optimum condition produce high amount of sugar by using 2% commercial celullase in hydrolysis and 30 min autoclave lead to the highest bioethanol production (9.25 ± 0.25 g/L) after 24 hours of fermentation. For dried longan, the optimum condition produce high amount of sugar by using 2% commercial

celullase in hydrolysis and 15 min autoclave lead to the highest bioethanol production (16.74 ± 0.62 g/L) after 24 hours of fermentation. As result, dried longan has been chosen for a large scale. Results showed that after pretreatment, the total and reducing sugar are 157.19 and 36.43 g/L, respectively; the total and reducing sugar was increased in hydrolysis with 271.07 and 48.21 g/L, respectively. Fermentation with 1% Saccharomyces cerevisiae 24 hours bioethanol production reached 1.4%; alcohol concentration increase to 9% in distillation. The High Heating Value (HHV) was 4.55 MJ/kg. In this experiment, mass balance, energy, and economic analysis were analyzed to consider to invest as a project.

Keywords : Bioethanol, Low grade and damaged longan fruits, Algal enzyme, Response Surface Methodology

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the School of Renewable Energy, Maejo University for the Master degree scholarship. This give me a chance to research and study abroad.

I would like to express my sincerest gratitude to my advisor Dr. Rameshprabu Ramaraj, lecturer of School of Renewable Energy at Maejo University for the continuous support throughout the whole duration. Moreover, I admire his guidance, inspiration, motivation, and encouragement.

I would like to thank my co-advisor Dr. Yuwalee Unpaprom, lecturer of Faculty of Science, Maejo University. Moreover, I admire her patience and her great knowledge. Besides, I would like to thank the rest of my thesis committees: Assistant Professor Dr. Nigran Homdoung and for suggestion that make my thesis improved.

I am very thankful Professor Dr. Kanokwan Tandee in Faculty of Engineering and Agro-Industry, Maejo University about supporting equipment for my experiment. I also want to thank all lab officers, lab managers of School of Renewable Energy and Faculty of Science, Maejo University.

Finally, I am grateful for my family, my friends in Laboratory for their supportion and encouragement.

Nguyen Thuy Vy Tu

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

1.1 Principles, theory, rationale and/or hypothesis

Population growth is directly proportional to the use of fossil fuels. The Word heavily relies on fossil fuels to meet its energy requirements - fossil fuels such as oil, gas, and coal are providing almost 80% of the global energy demands (Asif and Muneer, 2007). However, fossil energy is not only non-renewable but also generates many emissions that affect the environment. Therefore, the mentioned global issues of energy security and environment have boosted the requirement of an alternative and green energy source. Renewable sources like biomass, solar, heat, wind, hydro, wave, geothermal and ocean-thermal have been discovered and applied to replace non-renewable and inhibit climate change. Biofuels can be produced from biomass of plant or animal through a chemical and biological process such as bioethanol, biogas, biodiesel, biohydrogen, biobutanol, etc. Nowadays, many countries in the world such as the USA, Brazil, China, Canada, and Europe have already declared guarantees to biofuel programs as attempts to reduce the dependence on fossil fuels (Subhadrabandhu and Yapwattanaphun, 2000).

One of the biofuels which are used for transportation is bioethanol. There are three main raw materials for bioethanol production: sugar/starch which is called first-generation, lignocellulosic biomass which is called second-generation and algal biomass which is called the third-generation. Bioethanol is obtained after following pretreatment, saccharification, fermentation and distillation steps. Each step has a unique role in bioethanol production, but the most important step is pretreatment. This stage plays vital roles in the destruction of the cell wall which includes cellulose, hemicellulose, and lignin with the tightest association to make quickly the next steps.

Thailand is an agricultural country with agricultural production as a whole account for an estimated 9-10.5 percent of Thai GDP and is also on the way to produce biofuels from edible sources to meet the high demand of the entire nation. Low grade or damaged longans fruits are among the available materials from agricultural activities. Specifically, Lamphun is the most famous producer of longans in northern Thailand. Longan (Dimocarpus longan Lour.) is a tropical tree species that produces edible fruits. Mature longan fruit (Dimocarpus longan Lour.) has a tasty, edible and white aril. Based on fruit weight, skin colour, flesh sugar, acid concentration, sugar: acid ratio, taste, maturity (ripeness) can be calculated (Yang et al., 2011). The production of longans in 1998 was about 238,000 metric tons with the planted area of 41,504 hectares (Department of Agriculture Extension, 1999). The production is confined in the northern provinces. The leading longans growing provinces are Lamphun, Chiang Mai, and Chiang Rai which shared 37.6, 24.1 and 8.0 percent of the total planted area respectively (Subhadrabandhu and Yapwattanaphun, 2000). In Thailand, the main longans production areas are in the Northern area where the main planting areas are in Chiang Mai and Lamphun, accounting for around 75% (Figure 1.1) of the whole yearly production (Sopadang et al., 2012).

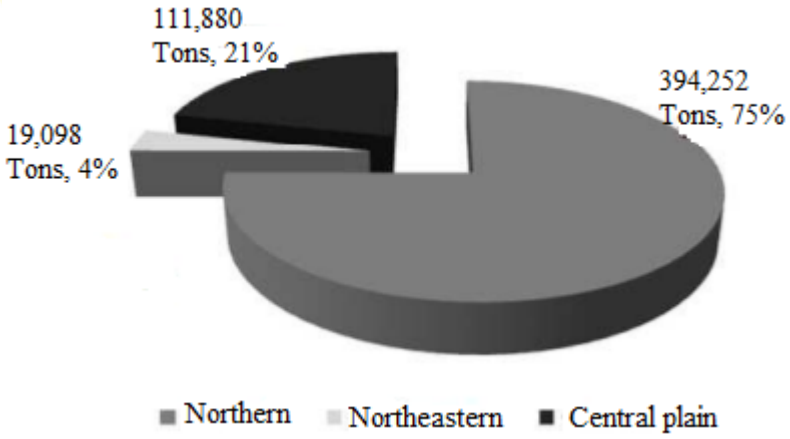


Figure *1. 1* Production of longans by region in Thailand in 2010

The neighbor of Thailand is Vietnam, Vietnam is like the letter S, a long and narrow country. It is located on the eastern edge of the peninsula called Indochina in

Southeast Asia, which is the market for longans processed products, likewise China, Hong Kong, Malaysia, Singapore, USA and France. Key guidelines for the growth of longans export markets have been identified: The research of consumer demand in target markets, the growth of long-term export markets, both old and new, and the production of long-term goods as consumers demand (Jaroenwanit et al., 2001). Moreover, longans are the third most grown fruits in Vietnam and this nation is also the world's second - largest exporter of longans with $62.13 million in 2017 (Tran et al., 2019). Vietnam and Thailand relations through import and export turnover from 2008 to 2016 are shown in Figure 1.2. The Thai government has set a goal for agricultural development orientation in the context of trade liberalization to increase productivity, yield, food security, and farmers' income, restructure, sustainable agriculture growth and promote biofuel production. Thailand's agricultural trade policy reform has increased the flow of trade between Vietnam and Thailand. For the period 2006-2010, Vietnam's annual export turnover of agricultural products to Thailand increased at an average annual rate of 36.8% per year. Meanwhile, Vietnam's agricultural imports from Thailand hit an average speed of 25.5 percent per year from USD 301.6 million in 2006 to USD 779.6 million in 2010 (Phu and Lan, 2018).

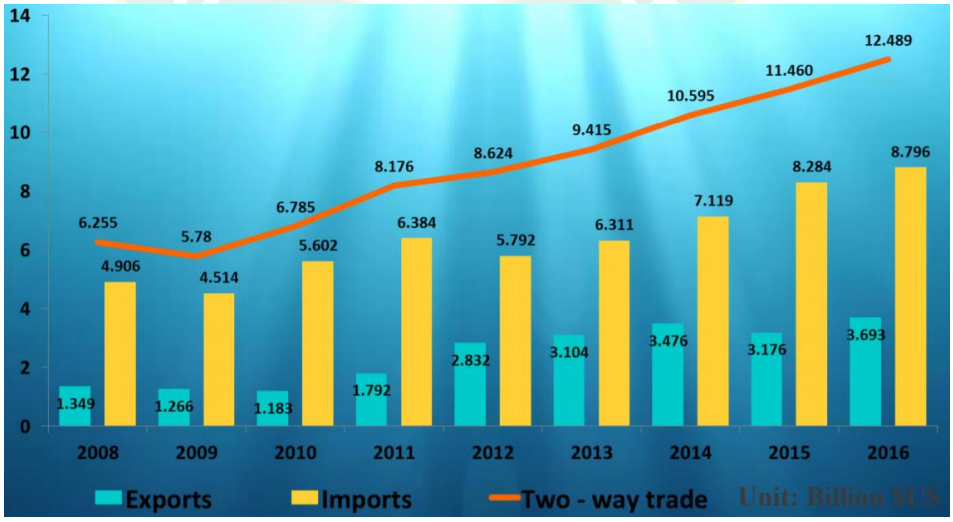


Figure 1. 2 Import and export turnover of Viet Nam – Thailand from 2008 to 2016 (Source: General Statistics Office of Viet Nam, 2017)

1.2 Research objectives

1. To investigate the potential of bioethanol production.

2. To evaluate the influence of autoclaving time to pretreatment process from low grade and damaged longans fruits.

3. To investigate the potential of *cellulase* enzyme and blue green algae in the hydrolysis process.

4. To analyze the energy and production cost of bioethanol production from low grade and damaged longans fruits.

1.3 Scope of research

1. Low grade and damaged longans fruits from the company will use as a feedstock for possible bioethanol production.

2. Pretreatment designs which include steam-explosion and boiling will be applied.

3. Hydrolysate with the highest sugar concentration will be chosen and proceed to bioethanol fermentation for 120 hours.

4. The mathematical model of Response Surface Methodology will be used to optimize the time and enzyme of hydrolysis for bioethanol production from low grade and damaged longans fruits.

5. The analysis of the energy and economy of bioethanol production from low grade and damaged longans fruits.

1.4 Significance of the research

1. Utilization of maximum available materials for bioethanol production.

2. The techniques which are used in this study are uncomplicated, eco-friendly and economic efficiency.

3. Bioethanol from low grade and damaged longans fruits.

4. The suitable method bioethanol production from longans fruits.

5. Added the value of low grade and damaged longans fruits.

CHAPTER 2
LITERATURE REVIEW

2.1 Characteristic of bioethanol

Ethanol also called ethyl alcohol, grain alcohol is a liquid biofuel with the chemical formula C_2H_6O, CH_3-CH_2-OH or EtOH (Figure 2.1). Gasoline and bioethanol have disparities in indicators such as a higher octane number, broader flammability limits, higher flame speeds and higher heats of vaporization detail shown in Table 2.1. These properties allow for a higher compression ratio, shorter burn time and leaner burn engine, which leads to theoretical efficiency advantages over gasoline in an internal combustion engine (Balat et al., 2008).

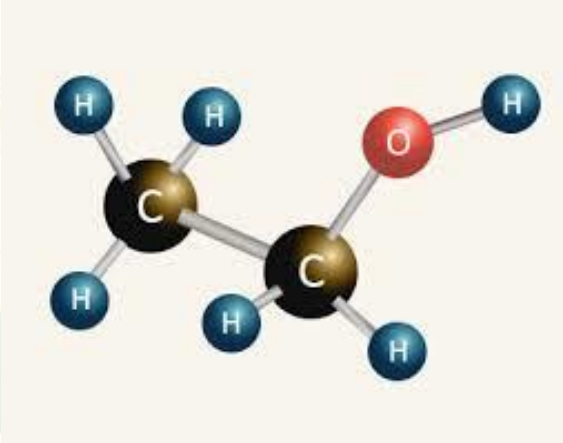


Figure 2. 1 Ethanol molecular formula.
(https://www.thoughtco.com/ethanol-molecular-and-empirical-formula)

Table 2.1 Comparative characteristics of ethanol and gasoline (Tao et al., 2014)

Characteristics	Ethanol	Gasoline
Research Octane number	110	88-98
Lower flammability limit concentration, vol %	3.3	1.4
Upper flammability limit concentration, vol %	19	7.6
Heat of evaporation (MJ/kg)	0.92	0.36

2.2 Feedstocks

Feedstocks, what the origin of plants, are natural materials that convert light energy into chemical energy through the photosynthesis process (Figure 2.2). Plants get the support of sunlight, air, water and nutrients from the soil to form sugars or carbohydrates. In biochemistry, it is a synonym of saccharide, mainly, three types of raw materials, that is starchy crops, sugar juice and lignocellulosic materials are being used for ethanol production (Balat et al., 2008). Photosynthetic biomass is a promising source for the generation of biofuels and other bioproducts.

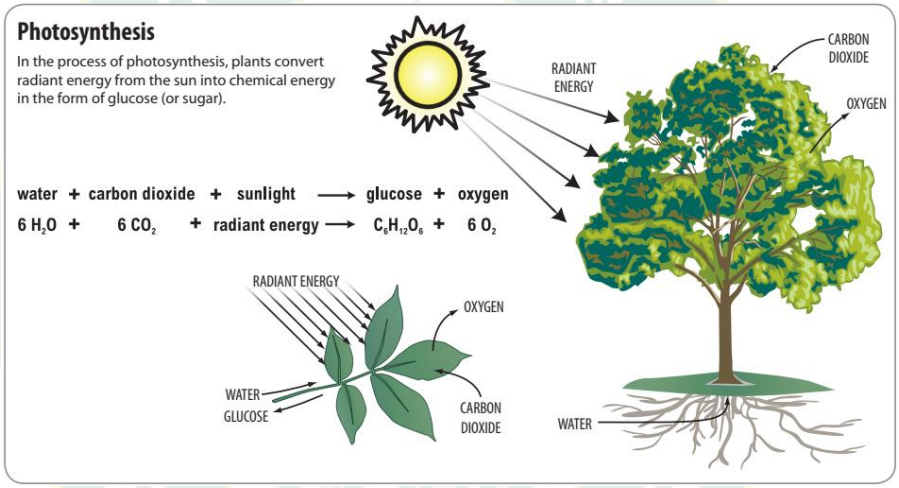


Figure 2. 2 Photosynthesis process
(https://www.need.org/Files/curriculum/infobook/BiomassI.pdf)

2.2.1 Starch

Starch is a polysaccharide carbohydrate. The formula is $(C_6H_{10}O_5)_n$ with **α** - D-glucose. The bioethanol production from starch and sugar crops is developed in the industry. Agricultural wastes are raw material, for instance, sugar beet molasses, thick juice, wheat starch, and cassava starch through fermentable sugars to make bioethanol production. Amylose and amylopectin are two components to create amylum. Amylopectin, which consists of highly branched glucan chains, makes up 70% of starch,

the rest of amylum is amylose account for 30%. Differences between amylose and amylopectin are shown in Figure 2.3.

Amylose

Amylose is a linear polymer with ≈ 200 to 6,000 glucose units (MW 10^5-10^6) linked mainly by **α**-1,4 bonds (≈99%) and few **α**-1,6 bonds (<1%) (Wu et al., 2006). Soluble starch was determined similarly, omitting the initial **α**-amylase digestion before the conversion of dextrins to glucose with amyloglucosidase (Tester and Morrison, 1990).

Amylopectin

Amylopectin is a much more abundant and highly branched polysaccharide with up to $3x10^6$ glucose units and an MW of ≈ $5x10^8$ and linked by ≈ 95% **α**-1,4, and 5% **α**-1,6 bonds (Wu et al., 2006). The latter where enzymes can attach so that resulting in a soluble molecule that can be quickly degraded.

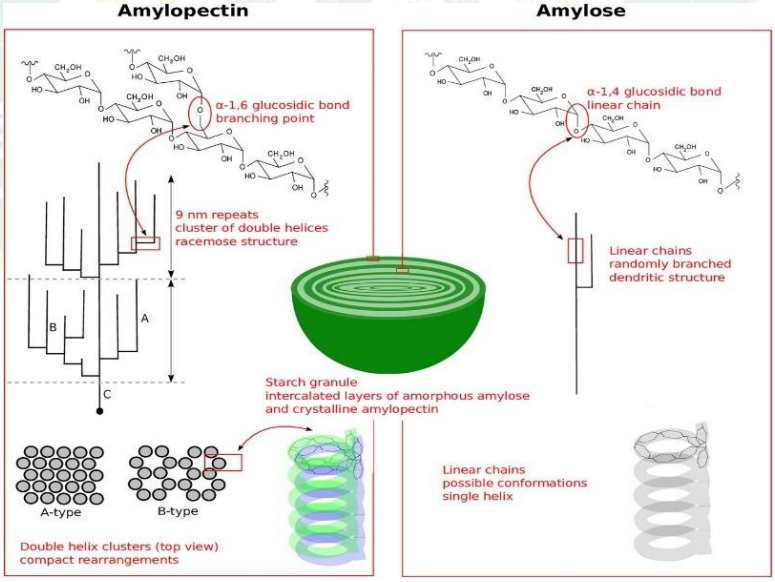


Figure 2. 3 Structure and components of starch granules: amylopectin and amylose (Raguin and Ebenhoh, 2017).

2.2.2 Lignocellulosic biomass

Second-generation bioethanol can be produced from various lignocellulosic biomasses such as wood, agricultural or forest residues (Tester and Morrison, 1990).

Lignocellulose is a widely available, but underutilized resource. Therefore, it is a promising raw material to create bioenergy production. The composition of lignocellulosic biomass can generally be divided into three main types: Cellulose (30-50%), hemicellulose (15-35%) and lignin (10-20%) (Figure 2.4). Cellulose and hemicellulose make up approximately 70% of the entire biomass and are tightly linked to the lignin component through covalent and hydrogenic bonds that make the structure highly robust and resistant to any treatment (Limayem and Ricke, 2012).

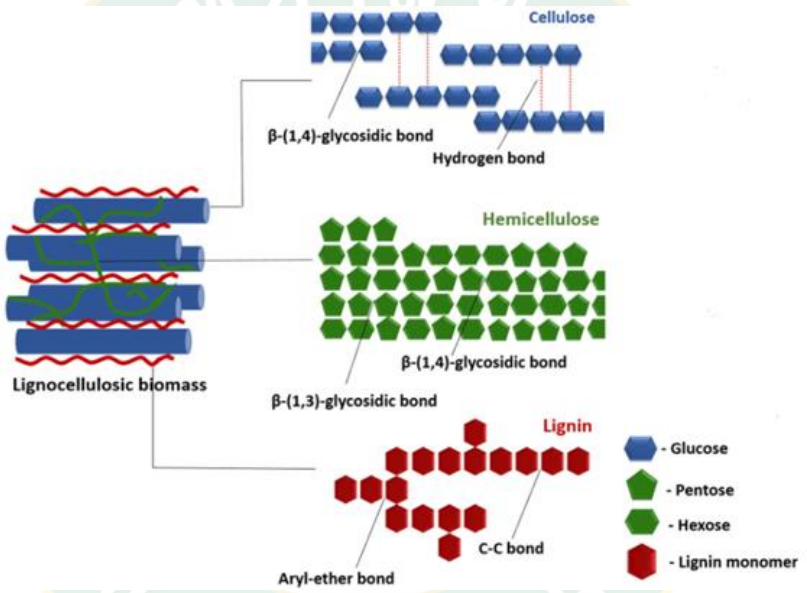


Figure 2. 4 Structure of lignocellulosic biomass (Baruah et al., 2018)

Cellulose content

Cellulose $(C_6H_{10}O_5)_n$, the main constituent of lignocellulosic biomass, is a polysaccharide that consists of a linear chain of D-glucose linked by β-(1,4)-glycosidic bonds to each other (Mood et al., 2013). Cellulose is the most abundant organic compound that can be found in nature possessing a structural function in plant cell walls with a high molecular weight and a maximum of 10,000 monomeric units of D-glucose (Tursi, 2019). Cellulose microfibrils have an amorphous and crystalline region which makes up 30-80% (Figure 2.5). The amorphous region of the cellulose is greater

distances among the molecular chains. Therefore, the cellulose enzyme easily attacked leading to the hydrolysis of the molecule. On the contrary, the crystalline region is hydrophobic does not absorb water. Thus, a reasonable pretreatment is needed to not only make cellulose more accessible to hydrolysis agents but also decrease of crystallization of cellulose.

Hemicellulose content

Hemicelluloses, located in secondary cell walls, have present as the matrix that surrounds the cellulose skeleton, has a random, amorphous structure with little strength. They are easily hydrolyzed by dilute acid or base as well as a myriad of hemicellulose enzymes. While cellulose is composed of glucose units linked by β-1,4-glycosidic bonds, hemicellulose almost entirely consists of sugars with five carbon atoms (xylose and arabinose) and six carbon atoms (glucose, galactose, mannose and rhamnose) with an average molecular weight of < 30,000 (Tursi, 2019).

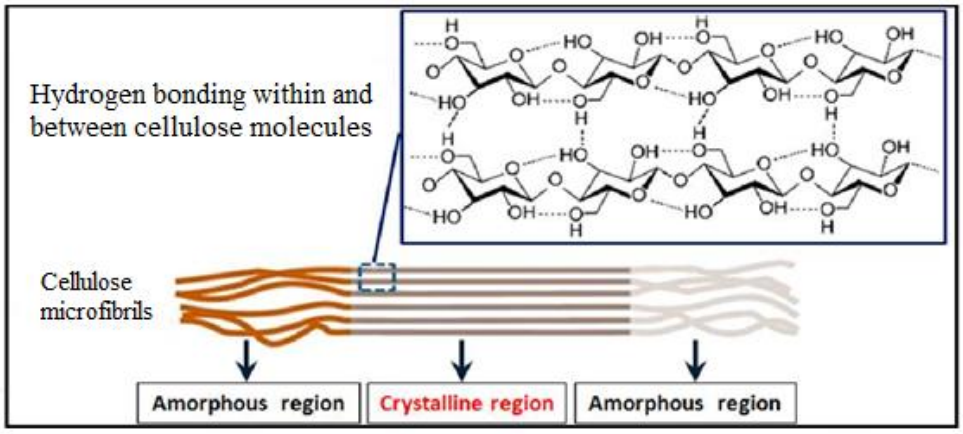


Figure 2. 5 The crystalline and amorphous regions of cellulose

Lignin content

Lignin is also contained in plant cell walls, with the function of binding, cementing and putting the fibers together in order to enhance the compactness and resistance of the plant structure. Therefore, in order to extract cellulosic fibers from

plant materials, lignin degradation is essential (Tursi, 2019). Lignin is a synthesized aromatic polymer from precursors of phenylpropanoid. The major chemical phenylpropane units of lignin consisting primarily of syringyl, guaiacyl and hydroxy phenol are linked together by a set of linkages to make a complicated matrix (Mood et al., 2013). In cell walls, therefore, lignin is the most difficult compound to decompose.

2.3 Pretreatment methods

Pretreatment is an additional step. However, this stage plays vital roles in the destruction of the cell wall which includes cellulose, hemicellulose and lignin with the tightest association (Figure 2.6).

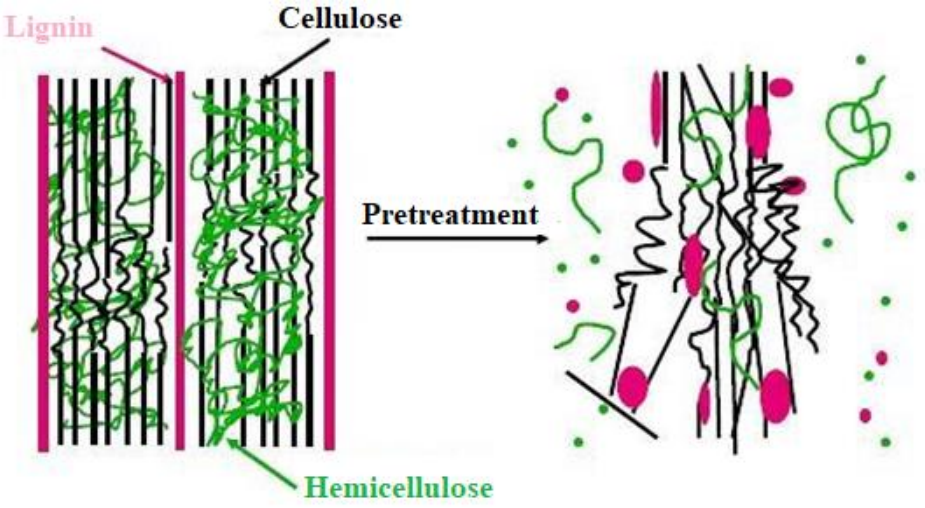


Figure 2. 6 Simplified form of pretreatment of lignocellulosic biomass

The aim of the effective pretreatment should be focused on: increase the accessible surface area and decrystallize cellulose, partial depolymerization of cellulose and hemicellulose, solubilize hemicelluloses and/or lignin, modify the lignin structure, maximize the enzymatic digestibility of the pretreated material, minimize the loss of sugars and minimize capital and operating costs (Maurya et al., 2015). The specific types of materials will appropriate with the different pretreatment methods.

After the whole process, the performance of this step will be evaluated by the number of sugar yields. High sugar yields, for instance, make up above 90% of theoretical for agricultural residues, especially for corn stover (Galbe and Zacchi, 2007). The techniques of pretreatment have been developed and applied in recent decades to improve the deconstruction of the plant cell wall and can be categorized as shown in Figure 2.7 (Baruah et al., 2018).

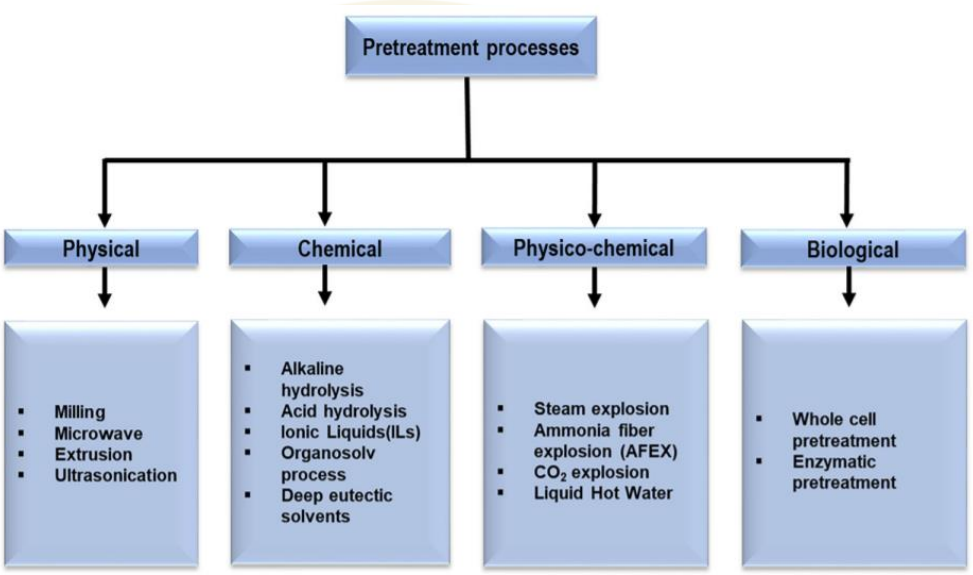


Figure 2. 7 Flow chart diagram of pretreatment processes (Baruah et al., 2018)

2.3.1 Physical pretreatment

The major goal of physical pretreatment is to reduce the biomass into particles of smaller size through mechanical comminution and increase the surface area of biomass where cellulases access to realize hydrolysis step due to improving hydrolysis yields. Drawbacks of this pretreatment method are its high energy consumption depends upon the delignification of the feedstock, the prohibitive cost of its large-scale. After physical pretreatment, morphology inspection and thermal analysis had provided pieces of evidence of structure disruption that led to higher sugar recovery in the hydrolysis process (Harun et al., 2011).

2.3.2 Physico-chemical pretreatment

The physico-chemical methods, such as hydrothermal, superheated water treatments, are mostly based on violent reaction conditions (Zhao et al., 2012). Recent technologies of physico-chemical pretreatment include biochemical and thermochemical conversion. The former takes a short time and is considered to have an eco-friendly method.

2.3.3 Steam explosion

Steam explosion pretreatment is currently the most common pretreatment method in commercial biorefineries. The term “autohydrolysis” has also been used as a synonym for steam pretreatment describing the changes that occur during this process (Agbor et al., 2011). The severity factor is a common term used in steam explosion pretreatment that describes the combined effects of the temperature and duration of the pretreatment (Yu et al., 2012). A higher level of pretreatment can more soften and weaken the lignocellulose structure because of the explosion can help break down the biomass and decrease its particle size, thus improving its digestibility (Pielhop et al., 2016). In this process physically pretreated (chipped, ground or simply raw preconditioned) biomass is usually treated with high pressure saturated steam at temperatures of about 160-240°C and pressure between 0.7 and 4.8 MPa. The pressure is held for several seconds to a few minutes to promote hemicellulose hydrolysis and then released (Agbor et al., 2011). The benefits of pretreatment with steam explosions include low capital expenditure, modest energy requirements and little impacts on the environment. No acid, base or solvent chemicals are required which simplifies the subsequent biorefinery stages and reduces their cost: lower detoxification effort due to less formation of compounds inhibiting enzymatic hydrolysis and fermentation, minimized need for neutralization chemicals and no need for the removal of an organic

lignin solvent, which can be inhibitory to cellulose enzymes and fermentative microorganisms (Pielhop et al., 2016).

2.3.4 Liquid hot water pretreatment

The liquid hot water pretreatment is carried out by cooking the feedstock in process water at temperatures between 160 and 190°C and at a pH of 4-7. No additional chemicals are needed (Kim et al., 2009). The liquid hot water pretreatment is similar to steam explosion but uses water in the liquid state at elevated temperatures instead of steam. Steam explosion and liquid hot water are common hydrothermal pretreatments applied to lignocellulose materials. Although both pretreatment methods do improve cellulose susceptibility to enzymatic hydrolysis, liquid hot water pretreatment results in higher hemicellulose sugar recovery and lower fermentation inhibiting hydrolyzates than steam explosion pretreatment (Cara et al., 2007). Liquid hot water pretreatment under controlled pH at 200°C for 15 min showed a high ethanol yield of 71.8% of theoretical on fermentation. Liquid hot water pretreatment of sugarcane bagasse and achieved 90% glucose recovery on enzymatic hydrolysis at 180°C for 30 min. Liquid hot water pretreatment of corn cobs at 160°C for 10 min provided maximum hemicellulose derived sugar recovery of 58.8% and enzymatic hydrolysis yield of 73.1% with more than 60% lignin removal (Baruah et al., 2018).

2.3.5 Chemical pretreatment

Acid pretreatment

Acidic pretreatment is one of the most advanced engineering among methods of pretreatment for the utilization of feedstocks from lignocellulose. The most commonly used acids for substrate pretreatment are sulfuric (H_2SO_4) and hydrochloric (HCL). Among the two mineral acids used for pretreatment, H_2SO_4 gave higher reducing

sugar (0.437 g/g) than HCL (0.224 g/g) (Sindhu et al., 2011). During acid pretreatment, xylan depolymerizes to form soluble xylose monomers and oligomers.

Beause the xylan found in nature is highly acetylated, xylose monomer formation inv -olves two steps (1) cleavage of xylosid bonds, and (2) cleavage of covalently bonded acetyl ester groups (Yang et al., 2009). Besides, glucan to glucose conversion was enhanced by acid pretreatments, especially those resulting in greater hemicellulose solubilization (Yang et al., 2009). However, this pretreatment is expensive because of acid corrosion on equipment.

Alkaline pretreatment

Besides acidic pretreatment, the alkaline method that also promotes hydrolysis and improves the yield of glucose recovery from cellulose by removing hemicellulose or lignin during pretreatment. On the contrary, more than 95% of the cellulose of lignocellulosic biomass was preserved in alkaline pretreatment, which can be explained by the low reactivity of cellulose with alkali and also its high crystallinity (Chen et al., 2013). The ones in this category are (1) sodium hydroxide, (2) ammonia, (3) calcium hydroxide (Kim et al., 2016). The most commonly used base is sodium hydroxide which is one of the most active base catalysts, with significant effects: 50% hemicellulose dissolution, 60-80% delignification (Kim et al., 2016). Increasing alkali concentration had no further positive effects. The reaction time of the alkaline reagent and lignocellulosic biomass take a long time from an hour to several days. The cost of energy consumption for this method is inexpensive which brings economic benefit if this pretreatment is applied.

2.3.6 Biological pretreatment

Biological pretreatment is one of the inexpensive methods and eco-friendly technique is utilized to breakdown the structure of lignocellulosic materials.

Furthermore, the inhibitor formation is not created during the process. Fungi are the best suited for cellulose, hemicellulose, and lignin degradation applications. Biological pretreatment is used not only to eliminate lignin, but also to remove specific components (Zhou et al., 2017). Table 2.2 presents details of different biological pretreatment strategies involved for pretreatment of lignocellulosic biomass and its advantages (Sindhu et al., 2016).

Table 2.2 Different biological pretreatment strategies involved for pretreatment of lignocellulosic biomass and its advantages (Sindhu et al., 2016)

Microorganism	Biomass	Major effects
Punctualaria sp. TUFC20056	Bamboo culms	50% of lignin removal
Irpex lacteus	Corn stalks	82% of hydrolysis yield
Fungal consortium	Straw	Seven- fold increase in hydrolysis
P. ostreatus/P. pulmonarius	Eucalyptus grandis saw-dust	Twenty- fold increase in hydrolysis
P. chrysosporium	Rice husk	-
Fungal consortium	Corn stover	43.8% lignin removal/seven-fold increase in hydrolysis
Ceriporiopsis subvermispora	Wheat straw	Minimal cellulose loss
Ceriporiopsis subvermispora	Corn stover	2–3-fold increase in reducing sugar yield
Fungal consortium	Plant biomass	Complete elimination of use of the hazardous chemicals

2.4 Hydrolysis

Hydrolysis also called saccharification because components of carbohydrates are decomposed into fermentable sugars which are glucose, fructose, sucrose, maltose and maltotriose. The chemical reaction of hydrolysis is realized when a molecule of water burst one or more chemical bonds to form a hydroxyl group and a carboxylic acid (Figure 2.8). Invertase is one of the crucial factors for hydrolysis which is an enzyme to catalyzes the hydrolysis process. Three main processes of hydrolysis are used to generate a variety of sugars appropriate for the production of ethanol: dilute acid, concentrated acid and enzymatic hydrolysis (Kumar et al., 2009).

Figure 2. 8 Chemical reaction of hydrolysis

2.4.1 Acid hydrolysis

Dilute and concentrated acid is two characteristics of acidic hydrolysis. In the dilute - acid process, the reaction is carried out at high temperature (160-230°C) and pressure (~10 atm) and because of low yields of glucose from cellulose in the hydrolysis step, the ethanol yield is low (Kumar et al., 2009). On the other hand, lower operating temperatures (<50°C) and atmospheric pressures are required during the concentrated acid hydrolysis process. However, the reaction time of the concentrated acid hydrolysis is longer than the dilute acid hydrolysis process due to higher ethanol yields. Cellulose was solubilized in concentrated acid together with hemicelluloses, leaving insoluble lignin. The hydrolysis products were glucose and oligosaccharides without dehydration products (Huang and Fu, 2013). In general, the purposes of the acidic hydrolysis process are the pentose sugar degrade more rapidly compared to

hexose sugars. The drawbacks of dilute and concentrated acid are the creation of waste acid which is required to decline for environmental preservation. Moreover, acidic hydrolysis is able to corrode used equipment during this process lead to a high production cost.

2.4.2 Enzymatic hydrolysis

Enzymatic technology is applied in the hydrolysis process. It means that invertase is utilized to hydrolyzed complex sugars into simple sugars with conditions that can influence hydrolysis productivity such as the nature of substrate present in the solution, available surface area, enzyme complex synergy and physico-chemical modifications to the substrate (Hao et al., 2018). Cellulase is one of a number of enzymes primarily produced by fungi, bacteria. These enzymes synergistically hydrolyze cellulose to cellobiose and glucose. The utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45-50°C) and does not have a corrosion problem (Sun and Cheng, 2002).

2.5 Cellulase enzyme

Fungi, bacteria, and protozoans are the factors that create enzyme. Cellulase which plays an important role in cell breakdown and cellulose degradation. The main task of enzyme cellulase is to break down complex sugars into simple sugars. The response is related to the hydrolysis of the 1,4-beta-D-glycosidic linkages in cell walls include cellulose, hemicellulose, lichenin, and cereal beta-D-glucans.

2.6 Blue green algae

Blue green algae is also known as cyanobacteria or cyanophyta, is a branch of microorganisms. Because of their phototropic performance through plastids which is a membrane-bound organelle. Therefore, they contain pigments chlorophyll a,

biliproteins, β-carotene and are able to photoautotrophs (Fogg, 2012). Therefore, the bacteria are identical in cellular and organismal respects (Stanier et al., 1971). Enzymes from photosynthetic microorganism include cellulases, galactosidases, proteases, lipases, phytases, laccases, amylases, antioxidant enzymes and enzyme associated with carbohydrate accumulation and the carbon concentration (Brasil et al., 2017). In particular, the enzyme for hydrolysis are cellulases, amylases, laccases, galactosidases. Blue green algae can be commercially exploited because of enzymes which create from cyanobacteria. A number of enzymes, such as phosphatase, arylsulfatase, chitinase, L-asparaginase, L-glutaminase, amylase, protease, lipase, cellulase, urease and lactamase producted by blue green algae (Chakdar et al., 2012). The pH range of blue green algae between 7.0 and 8.5 (Fogg, 2012).

2.7 Fermentation

Fermentation is a metabolic process that induces chemical changes in organic substrates in the absence of oxygen through the action of enzymes. Fermentation of ethanol, one glucose molecule is converted into two molecules of ethanol and two molecules of carbon dioxide. Figure 2.9 shows the equation for the reaction of fermentation.

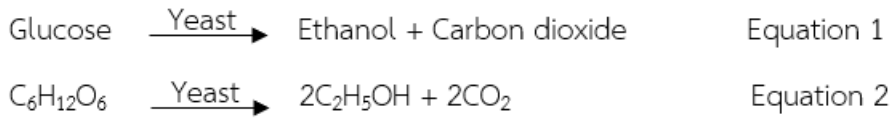


Figure 2. 9 The equation for the reaction of fermentation

The fermentation reaction requires conditions such as temperature, the substrate (the glucose solution), pH, yeast.

The range of fermentation temperature is between 20 and 35°C (Ballesteros et al., 2004). High temperature is an unfavorable factor for microorganisms to multiply and grow. Because of this condition, ribosome of microorganism is inactive and their proteins are shocked by the temperature (McMeekin et al., 2002). The reaction occurs

conveniently and efficiently if the glucose solution is enough offered. In the case, the little substrate is available the rate of the reaction is slowed and cannot increase. According to an investigation of (Lin et al., 2012) about juice fermentation was that sugar and microorganism concentration was found 200 g/l and 30 g/l, respectively. Controlling pH must be conducted to create acid condition which can make some crucial nutrients into the cells by charging of plasma membrane (Zabed et al., 2014). The pH range of *S. cerevisiae* is between 4.0 and 5.0 in bioethanol fermentation (Zhang, 1995). Yeast produces the zymase enzyme that serves as a reaction catalyst.
Therefore, the fermentation of the glucose solution to ethanol cannot take place without the presence of yeast. *Saccharomyces cerevisiae* is a species of yeast. *S. cerevisiae*, which is a very safe microorganism with a typical and important role in the development of industrial bioethanol, has several advantages due to its high efficiency of ethanol and its high tolerance to ethanol and inhibitors (Matsushika et al., 2009). The thermotolerant ability of *S. cerevisiae* to grow and ferment glucose at elevated temperatures and examined the influences of temperature, initial substrate concentration and pH value on ethanol fermentation (Lin et al., 2012).

2.7.1 Simultaneous saccharification and fermentation (SSF)

Ethanol fermentation was investigated using separate hydrolysis and fermentation (SHF) as well as simultaneous saccharification and fermentation (SSF) methods (Szambelan et al., 2018). Simultaneous saccharification and fermentation (SSF) is a process that enzymatic hydrolysis and fermentation are happening at the same time. This means that certain enzymes and fermentative microorganisms are added to lignocellulosic and starchy raw materials which are studied, work the same time and condition for producing higher yields of ethanol. Several advantages of this method are not only cutting both residence times and the capital costs but also reducing inhibitory compounds from enzymatic hydrolysis, which decrease the compatibility of substrate and enzyme. Furthermore, SSF is easily used for the liquid/slurry sample materials to produce higher ethanol concentration. On the other

hand, SSF also has disadvantages in pH and processing temperature. Because the optimal temperature of enzymatic hydrolysis is usually higher than the fermentation temperature. Therefore, it is necessary to find a solution to control the pH and temperature when applying this method. The model predicted SSF processing to be superior. The superiority of SSF over SHF (separate hydrolysis and fermentation) was confirmed experimentally, both concerning ethanol yield on glucose (0.41 g g^{-1} for SSF vs. 0.35 g g^{-1} for SHF) and ethanol production rate, being 30% higher for an SSF type process (Szambelan et al., 2018). A contrast between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stovers. SSF yielded 13% higher overall ethanol yield than SHF (72.4% versus 59.1% theoretical yield) (Öhgren et al.Bura et al.Lesnicki et al., 2007).

Table 2.3 Yield bioethanol using simultaneous saccharification and fermentation

Materials	Yield ethanol (g/l)	References
Banana peels	26.84	(Sharma et al., 2007)
Raw sweet potato	42.9	(Zhang et al., 2011)
Wheat straw	36.2	(Tomás-Pejó et al., 2009)
Slurries	32.0	(Sassner et al., 2006)
Wheat flour	38.6	(Neves et al., 2006)
Corn stover	33.8	(Öhgren et al.Vehmaanperä et al., 2007)

2.7.2 Separate hydrolysis and fermentation (SHF)

Separate hydrolysis and fermentation (SHF) is a process in which enzymatic hydrolysis and fermentation are conducted sequentially with two separate stages. Hence the temperature for the enzymatic hydrolysis can be optimized independently from the fermentation temperature. Materials that are a greater number of solid residues are used for SHF. Since Peng and Chen (2007) investigated and showed that

the rate of conversion of sugar to ethanol was 34.2% and the yield of ethanol was 190 g kg^{-1} of dry paper sludge, leading to a total conversion yield of 56.3% of the carbohydrates available on the initial substrate. The separate hydrolysis process has in charge of hydrolysis through enzymes for further production of convertible sugars from plant components like celluloses and hemicelluloses. On the contrary, the rest of the pretreated materials is fermented directly.

Table 2.4 Yield bioethanol using separate hydrolysis and fermentation

Materials	Yield ethanol (g/l)	References
Wheat straw	12.0	(Singhania et al., 2014)
Sorghum bagasse	28.4	(Shen et al., 2011)
Douglas – fir	20.4	(Shen et al., 2011)
Chlorella vulgaris	11.7	(Ho et al., 2013)

2.8 Distillation

The distillation is a process of removing water in the fermented product after the fermentation process finished to obtain a high ethanol concentration. Boiling is a physical phenomenon that is applied in the distillation process. Because the boiling point of ethanol (78.3°C at 1.013 Pa) is lower than the boiling point of water (100°C at 1.013 Pa). Therefore, ethanol evaporates faster than water, the water is left behind.

2.8.1 Simple distillation

The impure liquid is put in a round-bottomed flask with a distillation head attached to a condenser in turn. The end of the condenser is positioned over a tank that absorbs the distillate. The flask is heated and the liquids (the lowest boiling first) begin to vaporize. The vapor falls through the head of the distillation and moves through the condenser. The vapor is cooled in the condenser by running water which causes it to condense into the liquid phase and drip into the flask of collection. The purpose of this design, the thermodynamic equilibrium between the vapor and the

liquid leaving each stage is presumed to be established (Al-Yaqoobi et al., 2016). Distillations can be controlled by measuring the temperature at the condenser's entrance; as the system heats up, the vapor from the first component (with the lowest boiling point) reaches the thermometer and exits the condenser, while the higher boiling point components condense in the unit (Diwekar, 2011). The temperature will remain constant until all the first liquid is removed at which point the temperature will rise and plateau again until the second liquid is distilled; this pattern will be repeated until all liquids are distilled.

2.8.2 Azeotropic distillation

The mixture boils at a lower temperature which is impossible to use simple distillation to produce ethanol at concentrations higher than 95.6% (Ewell et al., 1944). The use of a third component to distinguish two close-boiling components is azeotropic distillation. This is achieved by creating an azeotropic mixture between one of the original components and the third to increase the boiling point difference and promote distillation separation. It is possible to produce more concentrated ethanol solutions using drying agents such as anhydrous calcium chloride that physically absorb water from an ethanol solution of 95.6%.

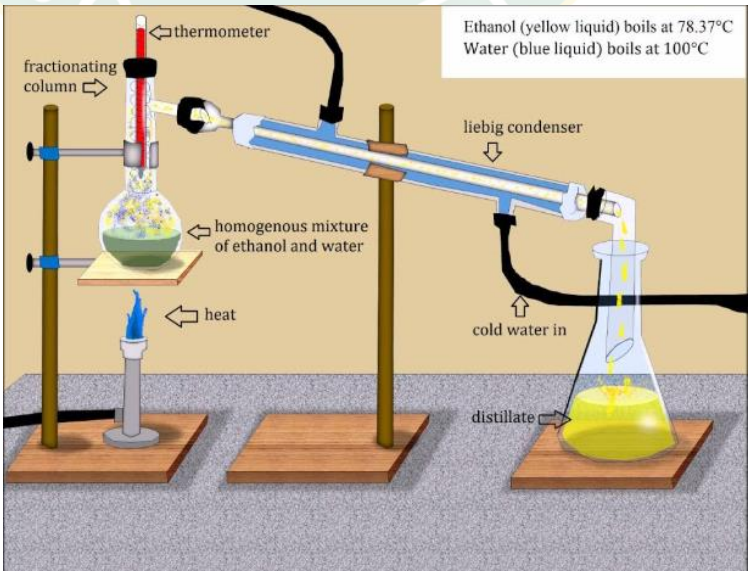


Figure 2. 10 Simple distillation design

2.9 Response surface methodology

The Response Surface Methodology (RSM) is a widely used mathematical and statistical method for modeling and analyzing a process where different variables affect the response of interest and analyze a process where different variables affect the response of interest and the aim of this method is to optimize the response. Dependent variables are the parameters that influence the process (Aydar, 2018). Response Surface Methodology is invented by Box and collaborators in the 50s. RSM is a group of mathematical and statistical techniques based on the suitability of empirical models to the experimental data obtained in relation to experimental design (Bezerra et al., 2008).

RSM's simplest model that can be used in RSM is based on a first-order linear function, a linear regression model with *k* predictor variables, it can be described as in Equation 3.

$$Y = \beta_0 + \sum_{I=1}^{K} \beta_i X_i + \varepsilon \qquad \text{Equation 3}$$

k: Is the number of variables

β_0: Is the constant term

β_i: Regression coefficients of the linear parameters

X_i: Represents the variables

ε: Is the residual related to the experiments

The answer variable was fitted with a second – order model in the form of quadratic polynomial equation to predict the optimal conditions a second – order polynomial function is employed as Equation 4.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i \le ij \le j}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad \text{Equation 4}$$

Y: Is the predicted response to be modeled

k: Is the number of variables

X_i and X_j the represents the variables which influence Y

β_0, β_i, β_{ij} are regression coefficient of the model for intercept, linear, quadratic and interaction terms, the *i* linear coefficient, the quadratic coefficient and the *ij* interaction coefficient, respectively.

2.9.1 Central composite design

The central composite design is employed to fit an empirical, second-order polynomial model. Since it combines a two-level factorial design with a star (axial) and center points, this design allows a greater number of levels without performing experiments at every combination of factor levels which cover the factor space near the center with more points. This means only the center points are replicated to provide excellent prediction capability near the center of the factor space. Therefore, it reduces the total number of experiments needed to determine the best combination of factors for the optimization of a process. This design consists of the following parts: (1) a complete factory or fractional factory design; (2) an additional design, often a star design in which experimental points are at a distance from its center and (3) a central point. Figure 2.11 illustrates the full central composite design for the optimization of two and three variables (Bezerra et al., 2008).

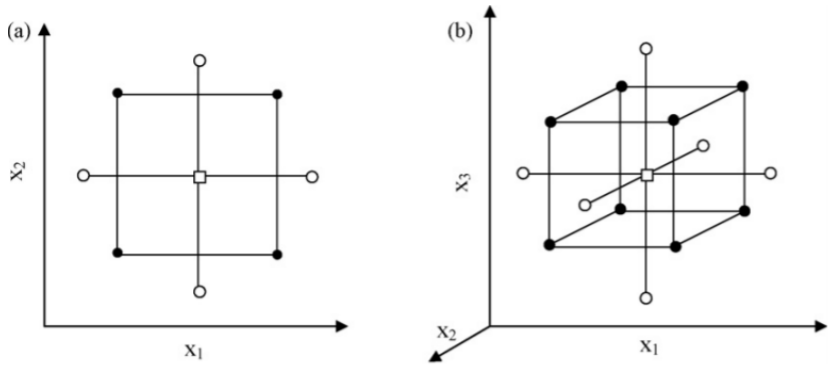


Figure 2. 11 The full central composite design for optimization of two and three variables

2.9.2 Full three-level factorial designs

An experimental matrix of full three-level factorial design has restricted software in RSM when the factor number is higher than 2 because of the number of designed experiments (calculated by expression $N = 3^k$, where N is experiment number and k is factor number) is very huge, thereby losing its efficiency in the modeling of quadratic functions. Figure 2.12 a and b illustrates the representation of the three-level factorial designs for the optimization of two and three variables, respectively (Bezerra et al., 2008).

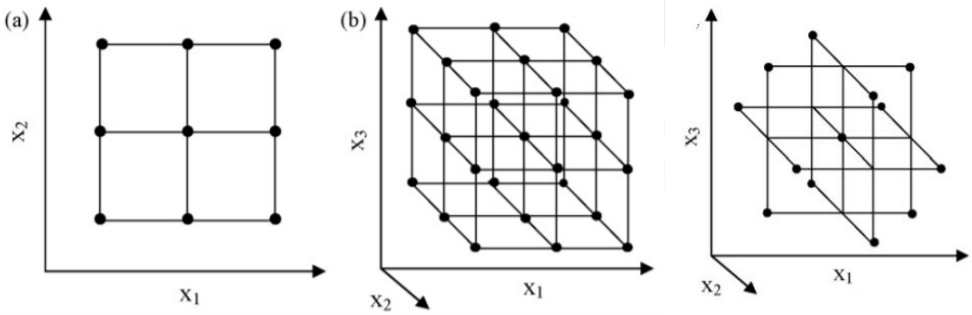


Figure 2. 12 Three-level factorial design for the optimization of (a) two variables, (b) three variables and (c) Box-Behnken design for the optimization of three variables

2.9.3 Box-Behnken design

Box-Behnken design is the other useful tool of response surface methodology for optimizing model. The advantage of Box-Behnken design is in pointing out the issue of where the experimental boundaries should be in general and in particular to avoid the unnecessary combination of treatment. Box and Behnken suggested how to select points from the three-level factorial arrangement, which allows the efficient estimation of the first and second-order coefficients of the mathematical model. These designs are, in this way, more efficient and economical than their corresponding 3^k designs, mainly for many variables. Figure 2.12 C illustrates Box-Behnken design for the optimization of three variables (Bezerra et al., 2008).

CHAPTER 3
MATERIALS AND METHODS

3.1 Sample collection

Low grade and damaged longans fruits are the materials in this study. This material was collected at the company Pratupa Agricultural Cooperative with the location of factory 92 Moo 5, Pratu Pa Subdistrict, Mueang Lamphun District, Lamphun 51000 at coordinates 18°37'44.5"N 98°59'48.5"E.

Figure 3. 1 Low/grade damaged longan fruits from the company

This company buys longan fruits from farmer households at Lamphun. After that, the sorting machine will be used to divide the longan into 4 categories AA, A, B, C to serve the domestic consumption and export to contiguous countries. The rest is low grade and damaged longan fruits will be collected at the back of this machine. Both of these propositions are shown in Figure 3.2. Low grade and damaged longan fruits are shown in Figure 3.1.

Figure 3. 2 Waste longans of company

3.2 Material preparation

After collecting, low grade and damaged longan fruits were brought to the laboratory at Faculty of Science, Maejo University. The leaves, branches, dust, soil and other impurities were removed prior to the experiment. The number of this longan were separated: 1) One-half were directly used for fresh longan experiments. 2) The rest of longan were dried at Energy Research Center (ERC) (see Figure 3.3).

Figure 3. 3 The dried longan at Energy Research Center

3.3 Methodology of experiments

Methodology of experiments performs in Figure 3.4. Both of materials which involve fresh and dried longan were undergone pretreatment, hydrolysis, fermentation, and distillation process to produce bioethanol.

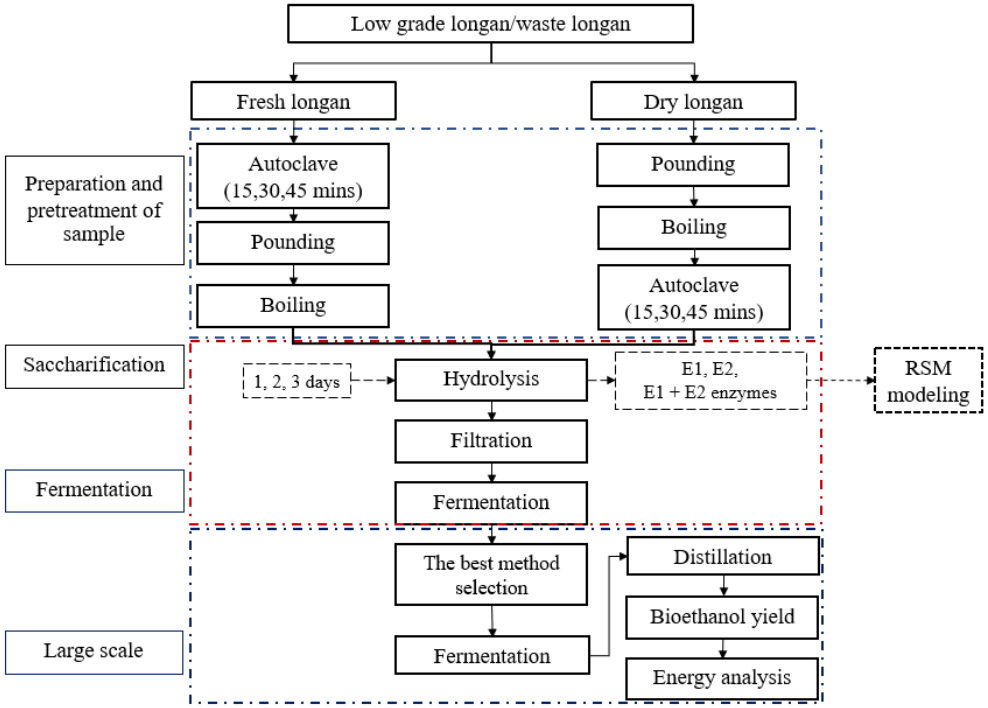


Figure 3. 4 The flow process of bioethanol production

3.4 Laboratory scale experiment for bioethanol production

3.4.1 Pretreatment process

Experiments were carried out in 250 mL Erlenmeyer flasks in boiled and autoclaved conditions. The sample was boiled within 30 minutes, around 95-98°C. After that, the solution was undergone 15, 30, and 45 minutes autoclaving time and 0 min for control.

The steam explosion condition was used at 121°C, 15 psi (see Figure 3.5). Total sugar was checked by phenol - sulfuric acid (Dubois et al., 1956) and reducing sugar was analyzed by DNS acid (Miller, 1959).

Figure 3. 5 Pretreatment process; boiled sample (A); Autoclaved sample (B)

3.4.2 Enzymatic hydrolysis

Commercial cellulase which was bought at Union Science Company, Chiang Mai, Thailand and algal enzymes that was cultivated in ERC were inoculated. Experiments performed including 3 treatments: Treatment 1: Enzymatic hydrolysis using a 2% (v/v) cellulase enzyme. Treatment 2: Enzymatic hydrolysis using a 20% (v/v) algal enzyme. Treatment 3: 1% (v/v) commercial cellulase and 10% (v/v) algal enzymes were added in the samples. The pH was controlled for suitable each treatment. Three treatments underwent hydrolysis process for 1, 2, and 3 days, respectively. The samples were in the incubator which was set at 30°C (see Figure 3.6). By the end of the hydrolysis process, total sugar was checked by phenol - sulfuric acid (Dubois et al., 1956), and reducing sugar was analyzed by DNS acid (Miller, 1959).

Figure 3. 6 Hydrolysis process Adjusting the pH (A); keeping sample in incubator (B)

3.4.3 Fermentation process

The best pretreated and hydrolysis conditions were chosen which were carried out ethanol fermentation. The 2% of *S. Cerevisiae* (dry yeast - Alcohol Yeast, Xinjiang Shengli Biotechnology Co., Ltd, China) was added in the samples. The pH was controlled 5.6 and kept in room temperature in the absence of oxygen. The bioethanol concentration of each samples was measured by Ebulliometer (Dujardin-Salleron, Alcohol Burner, France) after 12 hours.

3.4.4 Response Surface Methodology (RSM)

Response Surface Methodology (RSM) was also used to choose the best pretreated and hydrolysis conditions. Central composite designs which was used in this study, is one of the most common designs used for quadratic models (Makela, 2017). Two factors was run in this software.

Factor 1: Autoclave time 0, 15, 30, 45 mins.

Factor 2: Hydolysis time 1, 2, 3 days.

A central composite design with $\alpha > 1$ which showed in Figure 3.7. Setting α as 1 or $\sqrt[4]{2^k}$ are secure selections. The value $\sqrt[4]{2^k}$ assurances rotatability.

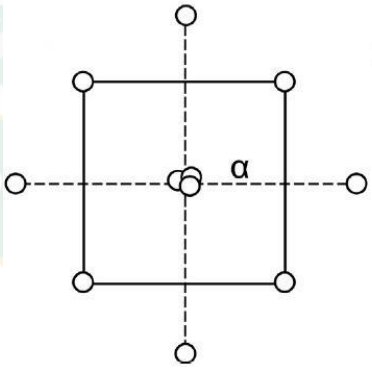


Figure 3. 7 A central composite design with $\alpha > 1$

The software involves organization and accomplishment a set of experiments to determine the effects of experimental variables on that method. Based on empirical data in experiments, the software is a simple mathematical approximation of the response. Quantifying linear and interaction effects through factorial designs can be used. This way suggest the chosen design to determine the level of suitable, detail and complexity that can described with the subsequent model.

A Design-Expert® Software Version 11 trial program from Stat-Ease, Inc., Minneapolis, USA was utilised to optimizate the reducing sugar (g/l) of dry longan was between physical pretreatments and enzymatic hydrolysic time. The independent factors contains the physical pretreatment which contains of autoclaving time (min), and the enzymatic hydrolysis which involves hydrolysis (day). In this proposal, there are three experimental levels: – 1, 0, 1. Table 3.1 showed the range and center point values of the three independent factors which were chosen after a series of primary single factor experiments. Reducing sugar (g/l) enzymatic hydrolysis were selected as the dependent factors by using central composite design (CCD) of RSM which was used to produce 24 runs for design with varied inputs of the factors. The surface roughness was used to investigate influence of the following controllable process parameters in this experimental work. The CCD application and designing methods described by Box and Wilson (Box and Wilson, 2012) were applied for the optimization of the reducing sugar of longan fruits. The CCD in the experimental design consisted of factorial point, axial point ($\alpha > 1$), and five replicates of the central point. Experimental runs were randomized in order to restrain the effects of unpredicted inconsistencies in the practical responses.

Table 3.1 Reducing sugar concentration and their values used for the experiment

Factor	Name	Units	Type	Minimum	Maximum	Low	High
A	Autoclave time	Min	Numeric	0	45	-1	+1
B	Hydrolysis time	Day	Numeric	1	3	-1	+1

3.4.5 Scanning electron microscope (SEM)

The cell wall structure of biomass was affected by the pretreatment process. Because the crystalline structure of biomass was changed into a non-crystalline structure after this process. A scanning electron microscope (SEM) scans the surface

with a focused beam of electrons through interaction between the electrons and atoms in the sample produce surface topography and composition under images. The machine branched JSM-5410LV, USA. The sample was prepared before and after the pretreatment process as a dried powder. Pure gold and dried by a dryer (CPO 7501 Critical Point Dryer, USA) was utilized in 150 seconds at 15 mA. Gold coating plays a role important in conductivity properties for biomass. The qualities of images and interruption of the vacuum were affected by the amount of presence of water in the biomass. Therefore, the powdered biomass needed to do carefully dry. Electron beam at 15000 kV was used to shot gold and samples inside the specimen chamber. The images of the surface in biomass were shown and taken through the secondary electron detector on the monitor screen (Figure 3.8).

Figure 3. 8 Sprinkle of sample in pure gold (A); Scanning electron microscope equipment (B)

3.5 Large scale of bioethanol production

3.5.1 Feedstock preparation

Longan was used in this study as waste fruits. They were collected at Pratupa Agricultural Cooperative company Mueang Lamphun District, Lamphun 51000, Chiang Mai, Thailand (18°37'44.5"N 98°59'48.5"E) not only free of cost but also in enormous quantity due to the small size of longan. Therefore, the expense of the collection of waste/damage longan was cut in economic and energy analysis. These materials were dried at Energy Research Center (ERC), Maejo University, Chiang Mai, Thailand by

sunshine in 1 week until dry. Then the shredder machine was utilized cutting to get the smaller size of particles less than 2 cm.

3.5.2 Pretreatment

Pretreatment for waste/damage longan bioethanol production involves boiling and autoclave. To have an estimate of the energy required for these operations. In theory, 1 kg of gas do can boil 160 kg of water at 100 Celsius degree. In this study, 10 kg of dried longan and 50 liter of water was boiled at 90-95 Celsius degree in 30 minutes by small gas system; so the amount of gas took for boiling around 0.5 kg. Moreover, the steam explosion condition was used by traditional autoclave in 3 hours with 3 kg of gas which was utilized to heat the system.

The total energy used for pretreatment involves the energy input of fuel and labor.

3.5.3 Hydrolysis

The pH of the sample was ajdusted to 5 and 0.5 % of cellulase enzyme (v/v) was inoculated within 24 hours at room temperature. Then the sample was filtered to separate liquid and solid parts.

Hydrolysis for waste/damage longan bioethanol production involves human labor; is usually done manually. To have an estimate of the energy required for these operations. The 68% of human energy is consumed after 8 hours of work by the observation.

3.5.6 Fermentation

40 liter of the liquid sample was conducted fermentation process with 1 % *S. Cerevisiae* (dry yeast - Alcohol Yeast, Xinjiang Shengli Biotechnology Co., Ltd, China) was added at the pH 5.6 condition and kept at 35°C in the absence of oxygen in the fermentor (see Figure 3.9) within 24 hours.

The total energy used for fermentation involves the energy input of machinery and electricity. Fermentation is usually done by the pump which was operated by electricity to stir.

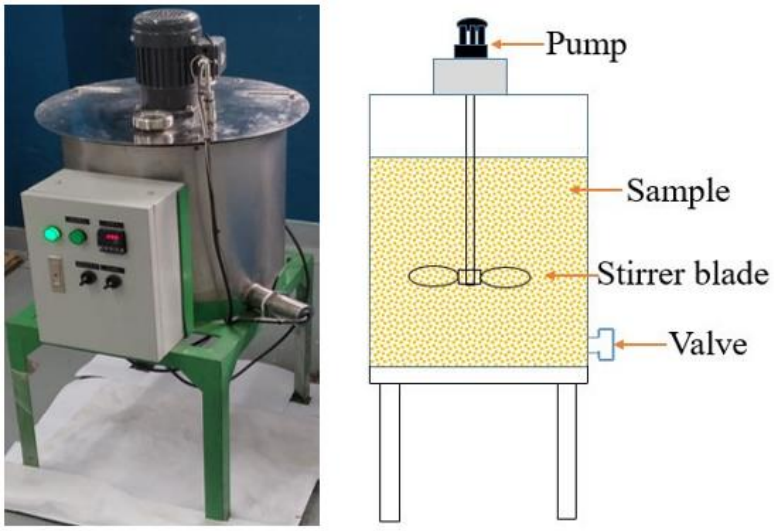


Figure 3. 9 The fermentor machine

3.5.7 Distillation

Then 24 hours, the sample fermented product was distilled by the distillation system to get pure bioethanol. Figure 3.10 showed the distillation system includes 2 parts of the boiler and condenser. The boiler part where the sample was put inside was burned by a gas tank until the temperature reached 80°C. At that time, pure bioethanol across the connection pipe to the condenser where a gaseous substance condenses into a liquid state through cooling. The distillation process was done within 1 hour. Distillation is usually done by the use of gas heat the sample at 78 Celsius degree to get bioethanol.

The purified bioethanol was determined calorific value by bomb calorimeter (Figure 3.11) - (Art. 2060/2070 bomb calorimeter, Thailand). 1 gram pure bioethanol was weighed and put in the little combustion cup which in turn sits on the electrodes. A fuse wire was measured 12 cm by the ruler to set in the center of the little combustion cup. After that, a fuse wire was wired this in wrap it around these electrodes which actually provided the electrical charge to ignite the fuse. The amount of dried oxygen was offered at a pressure of 30 atmospheres (atm) by a particular oxygen cylinder when the system was enclosed. The temperature of the bomb calorimeter rose to measure with the calibrated galvanometer - thermocouple assembly.

The total energy used for distillation and the bomb calorimeter machine involves the energy input of fuel, machinery, and labor.

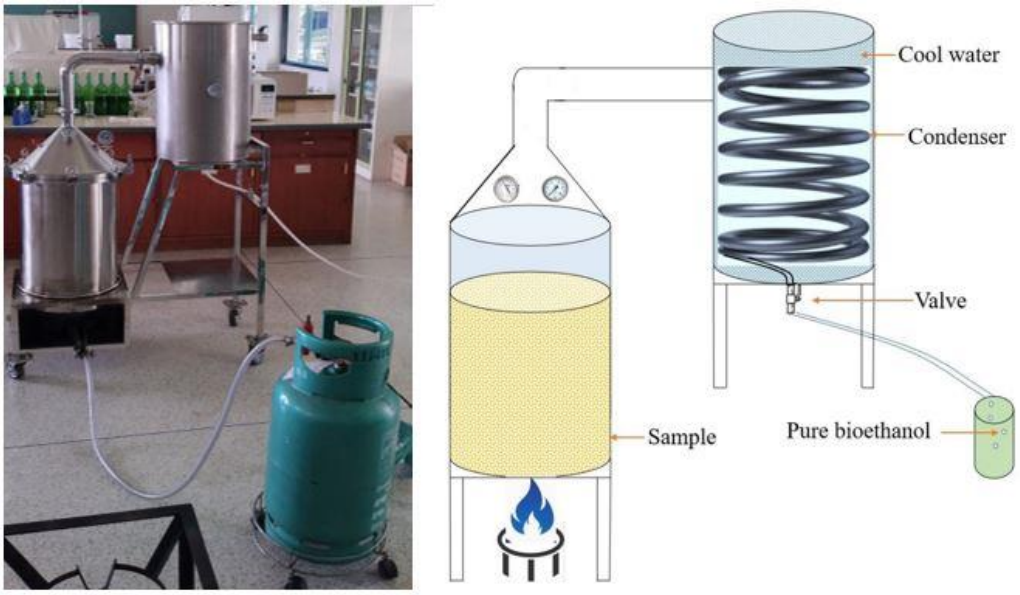


Figure 3. 10 Distillation system

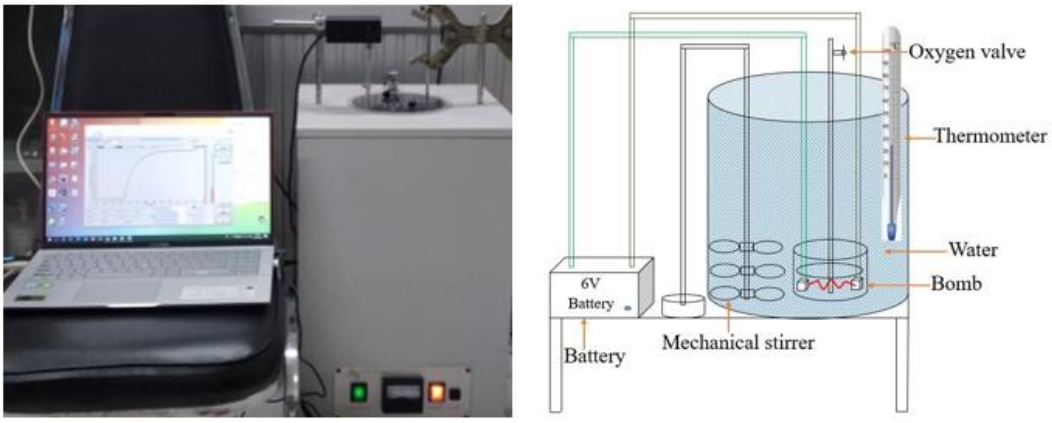


Figure 3. 11 The bomb calorimeter

3.6 Statistical analysis

Data were reported as mean ± SE from triplicate observations. Significant differences between means were analyzed. All statistical analyses were performed using SPSS Version 20.0. A correlation was assumed significant when $P < 0.05$.

3.7 Mass balance

Mass balance was calculated using the equations (Nguyen et al., 2020)

$$Xylan\ recovery\ (\%) = \frac{Xylan\ in\ pretreated\ solid\ (g)}{Xylan\ in\ untreated\ solid\ (g)} \times 100\%$$

$$Xylose\ yield\ (\%) = \frac{Xylose\ in\ hydrolysate\ (g)}{Xylan\ in\ untreated\ solid\ (g)} \times \frac{132}{150}$$

The large molecule is transformed to many smaller monosaccharides or simple sugars equivalent by multiplying the ratio of the molecular weights of polysaccharides to monosaccharide or fermentable sugars (For example, represent C5 sugars: the ratio of the molecular weights of Xylan converted to Xylose 132/150; represent C6 sugars: the ratio of the molecular weights of Glucan to Glucose 162/180). The oligomer is reported in equivalents.

3.8 Energy analysis

Energy analysis is the calculation or determination of the energy flows called input and out or output of a system. Energy can neither be created nor destroyed. The total energy is constant. It can only transfer from one system to another by the Law of Conservation of Energy. There are 2 ways to perform: 1) The basic manual energy analysis approach. 2) A detailed computer-aided simulation. For this study, energy analysis is analyzed and based on energy consumption through processes such as (1) feedstock preparation, (2) pretreatment, (3) hydrolysis, (4) fermentation, and (5) distillation.

Longan fruits bioethanol production's energy efficiency used two conventional indicators the Net Energy Gain (NEG) and the Net Energy Ratio (NER).

NEG is the difference between the output energy and the total energy input to produce one functional unit of longan fruits bioethanol. Besides, NER is the ratio of the output energy to that of the total input energy.

3.9 Economic analysis

The capital budgeting was analyzed by the net present value to get the profitability of the project or an investment (Korzen et al., 2015).

$$PVB = \sum_{t=0}^{T} (\frac{a}{(1+r)^t})$$

$$PVC = \sum_{t=0}^{T} (\frac{b}{(1+r)^t})$$

$$NPV = \sum_{t=0}^{T} \frac{a-b}{(1+r)^t}$$

PVB: is the present value benefit

PVC: is the present value of cost

NPV: The net present value, a: is the benefit, b: is the cost, r: is the discount rate, t: is the number of time periods.

CHAPTER 4
RESULTS AND DISCUSSION

4.1 Raw material characteritics

Longan (*Dimocarpus longan* Lour, syn. *Euphoria longan* Lam.) is commercial fruits in Thailand with an export value of $109 million in 2006 (Office of Agricultural Economics, Thailand, 2007) (Janjai et al., 2011). Flowers of the longan tree bloom at the end of wintertime. Longan fruit harvesting duration is over 4-6 weeks in mid-summer (Jiang et al., 2002). The taste of pulp longan is sweet with translucent of the flesh color. The carbohydrates of longan are in the form of fructose, glucose, and sucrose. After extraction, longan juice's main compositions are 2.77% glucose, 3.91% fructose, and 14.21% sucrose. The pH of longan juice reaches a low acidic level due to the number of organic acids compose, such as gluconic acid, malic acid and citric acid (Surin et al., 2014). Morever, the amount of bioactive compounds such as corilagin, ellagic acid and its conjugates, 4-O-methylgallic acid, flavone glycosides, glycosides of quercetin and kaempferol, ethyl gallate 1-β-O-galloyl-d-glucopyranose, grevifolin and 4-O-α-l-rhamnopyranosyl-ellagic acid contain in longan fruit content. (Yang et al., 2011). According to Li et al., 2004 and Wall, 2006, fresh longans fruit aril contain properties that component are suitable to produce bioethanol production (Table 4.1).

The amount of moisture in fruits affect directly to the amount of carbohydrates or reducing sugar. For example, the composition of the *J. curcas* fruit hull with carbohydrates account for 42.9% of the dry weight, other lignocellulosic materials: wheat straw (59%) and cardoon biomass (52%) (Marasabessy et al., 2012). For longan, moisture account for more than half of the composition of fruit aril. Therefore, the amount of reducing sugar of this longan lower than other lignocellulosic materials. Hemicellulosic sugars (xylose, galactose, mannose, rhamnose, and arabinose) contain xylose as the main hemicellulosic carbohydrate.

Peel of this fruit has brown, thin, and brittle when it ripe. A number of studies previous that fruit maturity was increased by pectin methylesterase and polygalacturonase activities. Longan fruit pulp was easily breakdown at 28 and 3°C,

after 8 and 35 days. The flavour and texture of longan fruits maintain good quality at 0 °C. During ripening, the total sugars increase. After that, the amount of sugar was decreased gradually after harvest. The kind of sugars mainly and presently in longan fruits are sucrose, fructose and glucose (Jiang et al., 2002). The seed is ring shape, dark black color, and white point at the base (Lapsongphol et al., 2007). Phenolic content is the antioxidant which contain in longan seed. Moreover, the longan seed keeps gallic acid, ellagic acid, corilagin and acetonyl-geraniin (Soong and Barlow, 2005).

Table 4. 1 Compositions of fresh longans fruit aril (Li et al., 2004 and Wall, 2006)

Property	Value
Moisture (%)	81.4
Ash (g/100g)	0.7
Total carbohydrate (g/100g)	12.38-22.55
Fiber (g/100g)	0.4
Reducing sugar (%)	3.85-10.16
Protein (g/100g)	1.2

4.2 Effect of physical pretreatment in fresh and dried longan

4.2.1 Hydrothermal process

Pretreatment is a necessary process in the enzymatic hydrolysis of biomass and the production of bioethanol. One of the notable pretreatment steps is hydrothermal pretreatment because that is a way with reduced operational costs and without the use of organic solvents, challenging to handle chemicals, and liquid or solid catalysts (Nitsos et al., 2013). In this study, low grade damaged longan fruits were treated in 90°C -95°C temperature at a heating time of 30 mins. In another case, low severity conditions (temperature and duration) bring unwanted by-products in the experiment process (Nguyen et al., 2020). However, temperature and duration in the experiment of

hydrothermal pretreatment are too severe such as time more than 30 min or a combination of the distinctive heating processes lead to needless compounds, which was called HMF, or the more significant degradation of hemicellulosic sugars (Gabhane et al., 2011). Therefore, in this study, dried low-grade damaged longan fruits were carried out at 100℃ temperature at a heating time of 30 mins that is a suitable condition for longan fruits to assure the amount of sugar of these fruits was produce.

Moreover, during thermal pretreatment, biomass's viscosity was reduced by the destruction of slime extracellular polymers to promote system fluidity. Besides, the volatile dissolved solid increases and volatile suspended solid decreases (Liu et al., 2012).

4.2.2 Steam explosion

The steam explosion was carried out with specific effects such as chemical composition changes of material, practical of accessible enzymatic in saccharification, and the ability of released sugar for fermentation (Shamsudin et al., 2012). (I) After the samples underwent this method, the percentages of cellulose are higher; simultaneously, the percentages of hemicellulose are lower than raw samples; that means steam pretreatment remove hemicellulose, degrade lignin, and increase cellulose content (Ohgren et al., 2007). (II) Moreover, time in steam pretreatment is an important factor. Because steam pretreatment at 60 min reduced the percentage of hemicellulose and lignin compared to 15 min (Alvira et al., 2010), however, 15 min is the best time to conduct a steam explosion because of the peels of longan fruits, which make up a tiny part in the whole fruit. Moreover, the degradation of C5 sugars is, conducting the pretreatment step in a shorter time (30 min) is preferable than a longer pretreatment time if not low pentose recovery is the target (Marasabessy et al., 2012). On the other hand, in case of C6 sugar degradation, the pretreatment time has no significant contribution on the degradation of hexose or the six-carbon polysaccharides (mostly cellulose) of the biomass which was completely depolymerized and degraded during the pretreatment.

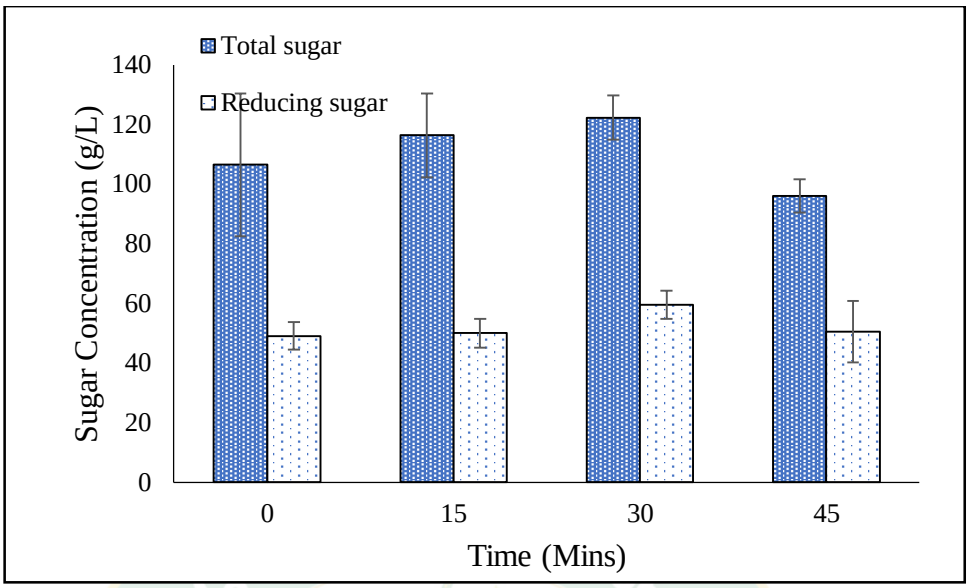


Figure 4. 1 Sugar concentration of fresh longan after pretreatment

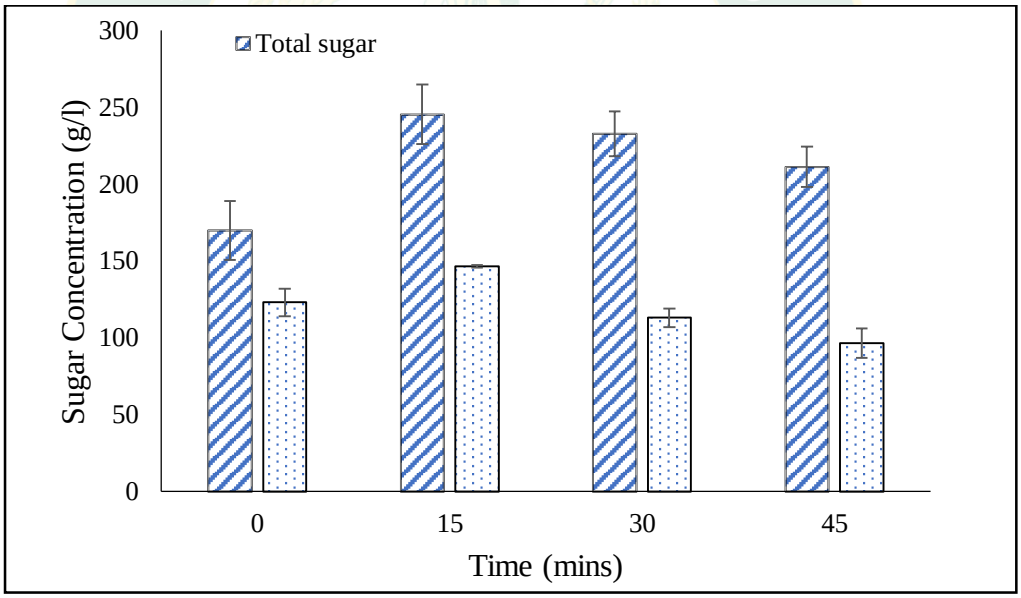


Figure 4. 2 Sugar concentration of dry longan after pretreatment

Figure 4.1 and 4.2 showed sugar concentration of fresh and dry longan after pretreatment at 15, 30, 45 mins autoclaving time with 0 min for control. The amount of total and reducing sugar of dry longan get higher than fresh longan in shorter autoclave time with 245.26 g/L, and 146.41 g/L, respectively. On the other hand, the amount of total and reducing sugar of fresh longan are 122.31 g/L, and 59.52 g/L, respectively. One reason that can explain this result is the amount of moisture in fresh

longan fruits account for 81.4% compare to dry longan (Nguyen et al., 2020). This supposes that dry longan is a promising material for the production of bioethanol.

Physical pretreatment which contains boiling and autoclave in this study affects material structure are temperature and time. Generally, parameters were effective by pretreatment methods and condition, including (1) cellulose's decrystallization and accessible of surface area was increased, (2) Cellulose and hemicellulose content was depolymerized, (3) the solubilization of lignin, (4) the ability of enzymatic digestibility to the material, (5) the minimization loss of reducing sugar, and (6) the decreased formation of inhibitors compound in a tolerant amount for fermentative microorganism or bacteria to grow (Burhani et al., 2017). According to Li et al. (2017) thermal pretreatment also the goal to inactive the enzymes related to waste or damaged fruits (Deng et al., 2019). According to Kumar et al. (2020) lignin a plays as the skeleton factor of a plant cell to cover and hold cellulose so the initial total sugar 60.02 ± 4.12 $mg \cdot g^{-1}$. However, after physical pretreatment, the different reducing sugars released, the contents of glucose, arabinose, galactose, and xylose were 30.35 ± 0.92, 21.44 ± 0.41, 4.54 ± 0.07, and 6.44 ± 0.04 $mg \cdot g^{-1}$, respectively, for the potential of sesame material for the production of bioethanol (Kumar et al., 2020).

4.3 Enzymatic hydrolysis treatments for fresh and dried longan

4.3.1 Influence of type of enzymes as hydrolysis treatments

The pretreatment process supported hydrolysis. Because the pretreatment process degrades the structure of the cell wall to enzyme attack easily. After that, the hydrolysis process occurs after pretreatment to break down the feedstocks into fermentable sugar for bioethanol production. Two pathways involve the enzymatic hydrolysis of cellulosic biomass: conversion of cellulose to cellobiose (intermediate product) and cellobiose to glucose (final product) (Azhar et al., 2017). In this study, 2% commercial cellulase, 20% algal enzyme, and 1% commercial cellulase + 10% algal enzyme (C+A) were utilized to hydrolysis fresh and dried longan.

For fresh longan, 2% commercial cellulase and 1% cellulase + 10% algal enzyme showed the same trend with a gap between total sugar and reducing sugar

9.36 g/l and 9.71 g/l, respectively. Follow by the amount of total with 20% algal enzyme account to 138.90 g/l and reducing sugar 59.81 g/l (Figure 4.3).

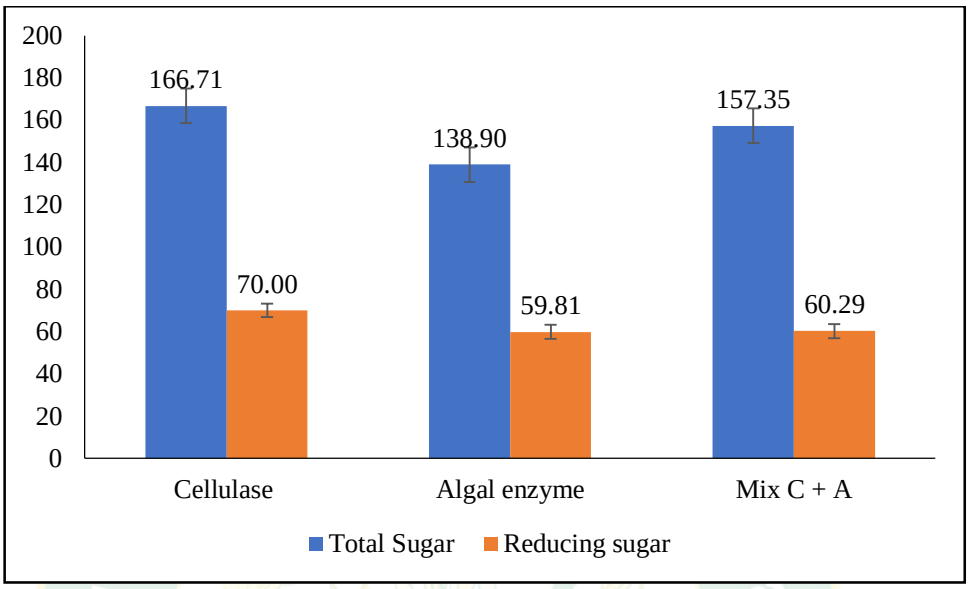


Figure 4. 3 Sugar concentration of fresh longan after hydrolysis

Enzyme hydrolysis is the second essential process to convert biomass into bioethanol. Hexoses and pentose groups in hemicellulose are degraded by the presence of a number of unique enzymes. Moreover, process's main goal is to decrease the degree of polymerization of cellulose by hydrolyzing the large polysaccharides to simplesugars that yeast can use for producing bioethanol.

Hydrolysis is the process of the oligosaccharide in the sample which is entirely hydrolyzed into polysaccharide. Moreover, there is not high contamination at 24 h leading to the efficiency of sugar digestion, yield coefficient (g/g), and productivity (g/L.h) of the total sugar, and reducing sugar is also high. The best result of sorghum stalk total sugar and reducing sugar concentration 151.228 ± 12.470 and 25.600 ± 2.117 g/L, respectively; the best result of sugarcane leaf total sugar and reducing sugar concentration 111.228 ± 5.402 and 17.600 ± 2.117 g/L, respectively at 24h (Manmai et al., 2020).

For dried longan, 2% commercial cellulase and 1% cellulase + 10% algal enzyme showed the same trend with a gap between total sugar and reducing sugar

15.98 g/l and 61.29 g/l, respectively. Follow by the amount of total with 20% algal enzyme account to 336.35 g/l and reducing sugar 189.04 g/l.

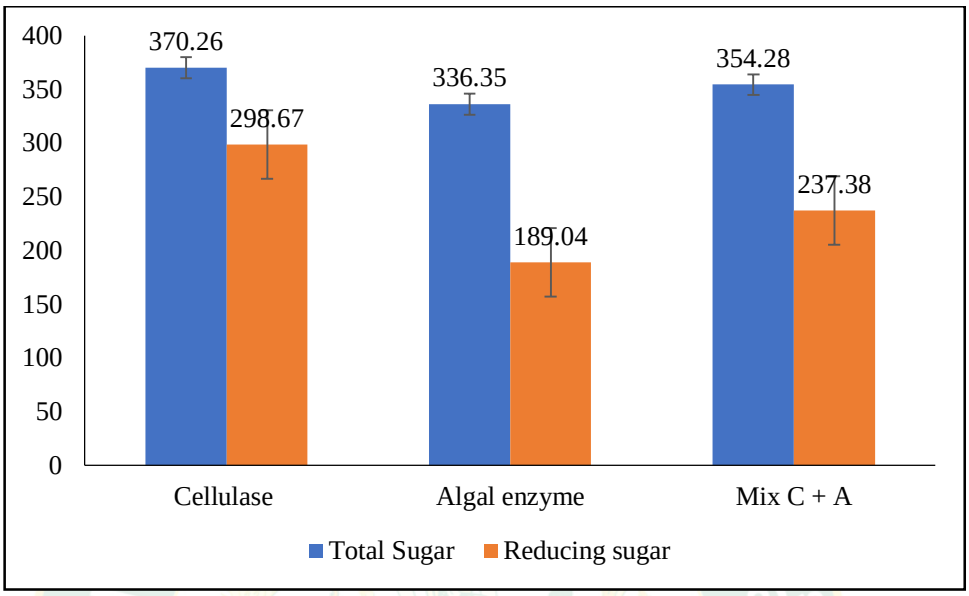


Figure 4. 4 Sugar concentration of dry longan after hydrolysis

The enzyme has disrupted cellulose and hemicellulose; therefore, it produced a significantly higher amount of fermentable sugars (reducing sugars). Performance of hydrolysis after pretreatment often exceeds 90%, whereas without pretreatment, yields of hydrolysis only reach <20% (Khammee et al. 2018).

4.3.2 Effect of hydrolysis time on sugar concentration

Enzymatic hydrolysis was conducted in 1 day, 2 days, and 3 days. Table 4.2 and 4.3 show the highest sugar concentration in 1 day of fresh and dried longan with 70.0 g/L at 30 min autoclave, and 234.60 g/L at 15 min autoclave, respectively. According to Pribowo et al. (2012), the rate of reaction hydrolysis in 24 hour was higher than 48-hour initial hydrolysis. Moreover, xylose of the hydrolysate account for 1.8% wt. Besides, a substrate conversion up to 85% in 24 hour with maximum sugar content of ~5.5% or 55.5 g/L (Yang et al. (2010 & 2011)).

Table 4. 2 Effect of hydrolysis time on sugar concentration of fresh longan

	Total sugar			Reducing sugar		
Autoclave	1 day	2 days	3 days	1 day	2 days	3 days
0 min	147.40	116.89	115.43	55.14	48.38	52.29
15 mins	136.89	121.46	133.01	56.48	57.33	59.29
30 mins	166.71	162.83	163.70	70.00	76.76	77.71
45 mins	132.33	136.53	143.65	70.67	54.86	48.29

Table 4. 3 Effect of hydrolysis time on sugar concentration *of dried longan*

	Total sugar			Reducing sugar		
Autoclave	1 day	2 days	3 days	1 day	2 days	3 days
0 min	237.14	246.11	231.37	131.27	133.98	116.48
15 mins	328.59	343.12	337.35	234.60	244.40	233.15
30 mins	312.99	310.64	351.88	198.56	207.52	234.19
45 mins	242.48	259.36	260.21	152.94	162.94	161.69

4.4 Optimization of physical pretreatment and enzymatic/microbial hydrolysis treatments through Response Surface Methodology

Dried longan was handled by physical pretreatment; the final model in terms of a coded factor for reducing sugar is shown in Equation 5. Table 4.2 finalizes the number of experimental runs of physical pretreatment along with the predicted and observed responses of reducing sugar (RS) concentration.

$$RS\left(\frac{g}{l}\right) = 218.66 - 5.42^{*}A - 10.57^{*}B - 5.85^{*}AB - 55.71^{*}A^{2} - 7.10^{*}B^{2}$$

(Equation 5)

The actual and predicted models were important parts to regulate reducing sugar from physical pretreatment and enzymatic hydrolysis of the experiment. Figure 4.5a show the externally residual versus predicted reducing sugar. All values of the response, the random scatter plot, the variance of original observation is constant.

Table 4. 4 Actual & predicted values of pretreatment and hydrolysis for dried longan

Run	Factor 1 A: Autoclave time (min)	Factor 2 B: Hydrolysis time (day)	Respond: Reducing sugar (g/L) Actual value	Predicted value	Residual
1	0	1	166.27	165.99	0.2846
2	0	2	166.98	168.37	-1.39
3	0	3	156.48	156.56	-0.0783
4	15	1	215.6	215.79	-0.1902
5	15	2	214.4	214.28	0.1195
6	15	3	202.19	198.56	3.63
7	30	1	217.15	216.08	1.06
8	30	2	211.52	210.66	0.8596
9	30	3	185.56	191.04	-5.48
10	45	1	165.69	166.85	-1.16
11	45	2	157.94	157.53	0.4126

12	45	3	135.94	134.01	1.93
13	0	1	166.27	165.99	0.2846
14	0	2	166.98	168.37	-1.39
15	0	3	156.48	156.56	-0.0783
16	15	1	215.6	215.79	-0.1902
17	15	2	214.4	214.28	0.1195
18	15	3	202.19	198.56	3.63
19	30	1	217.15	216.08	1.06
20	30	2	211.52	210.66	0.8596
21	30	3	185.56	191.04	-5.48
22	45	1	165.69	166.85	-1.16
23	45	2	157.94	157.53	0.4126
24	45	3	135.94	134.01	1.93

There is no need for the transformation of response variables. The measured response data include a specific run, and the predicted values evaluated from the model are generated by using the approximating functions in Figure 4.5b. Similar values presented in blue and green points on graphs show as models from physical pretreatment.

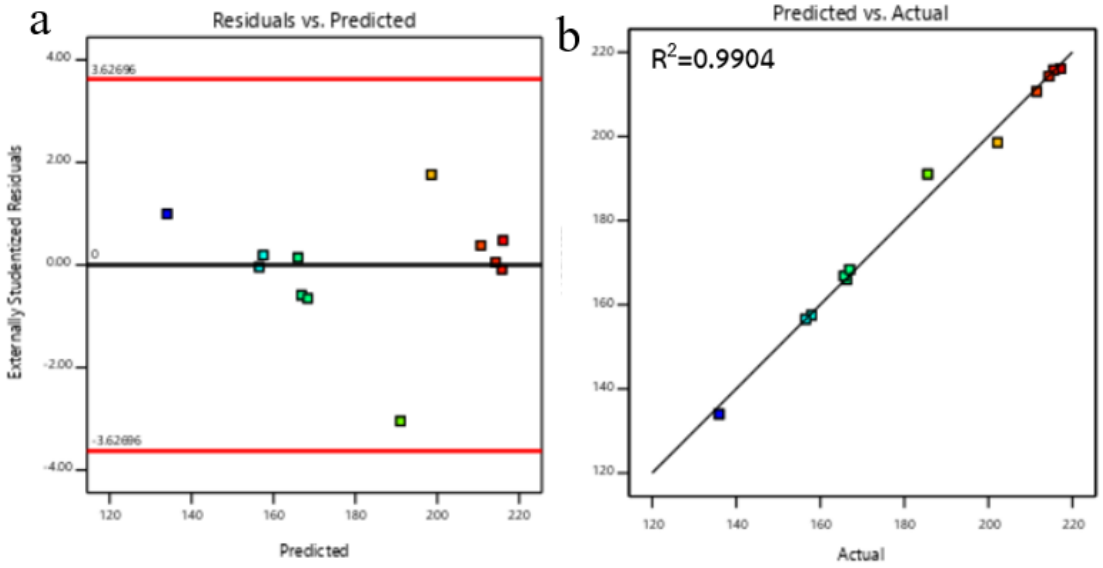


Figure 4. 5 Reducing sugar analysis enzymatic hydrolysis of the experiment.

The prediction of the statistical factors in the evaluation of the model between the procedure variables and the statistical parameters' responeses was utilized by Analysis of variance (ANOVA). The software package Design-Expert, Stat-Ease, Inc (Minneapolis, USA) was used to the trial data regression, so the contour plot and response surface graphs at the optimized condition were plotted. Three-dimensional surface plots and contours were shown to determine the effects of independent factors on reducing sugar concentration in physical pretreatment and enzymatic hydrolysis.

The F value and P-value of 600.10 and lower than 0.05, respectively, have a high statistical significance. The F value of each factor in this model is in the same direction as the reducing sugar ANOVA model. The value of the fortitude coefficient R^2 (0.9904) is presented in Figure 4.5; the representation of 95% of the variation in the average reducing sugar coefficient is connected to the two free factors. The relation between the free factors is A and B with the response Y (reducing sugar). It is evaluated using the contour and response surface plots generated from the predicted model in reducing sugar coded equations. The optimized conditions for saccharification were reducing sugar. Both results are the same as the direction presented.

The highest optimal reducing sugar is predicted and shown in the range of pretreatment time at 15 to 24 min and enzymatic hydrolysis time for 1–1.5 days. The pretreatment time range at 15 to 30 min and the enzymatic hydrolysis time for 1 day. It can be used to produce the highest saccharification productivity significantly. In an antecedent study of Ramaraj and Umpaprom (Manmai et al., 2020), they reported the highest sugar yield coefficient from hydrolysis of pretreated small-flowered nutsedge by using cellulase enzyme for 24 h. The results were reported as total sugar and reducing sugar yield coefficients of 0.196 ± 0.006 and 0.094 ± 0.001, respectively. Based on the experiment methodology design, dried low-grade longan fruit wastes were studied, and the operating conditions were optimized. Strong interactions between parameters on fermentable sugars and solid residue yield were found during pretreatment and hydrolysis.

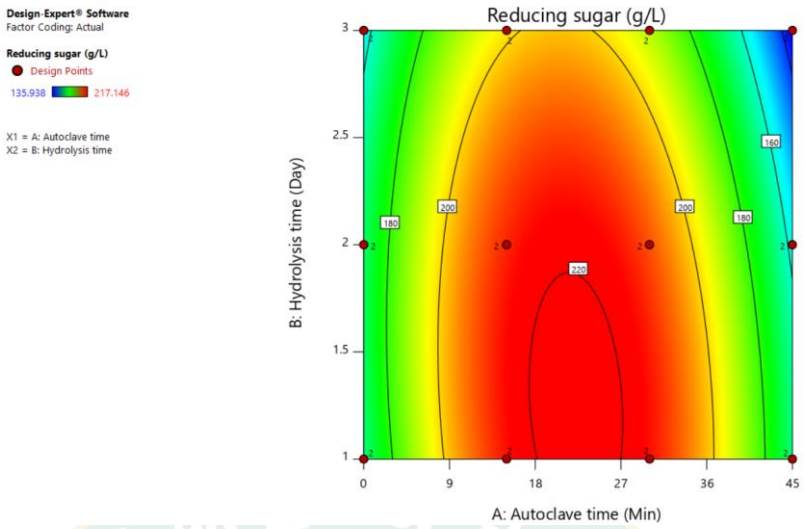


Figure 4. 6 2D contour plot of reducing sugar

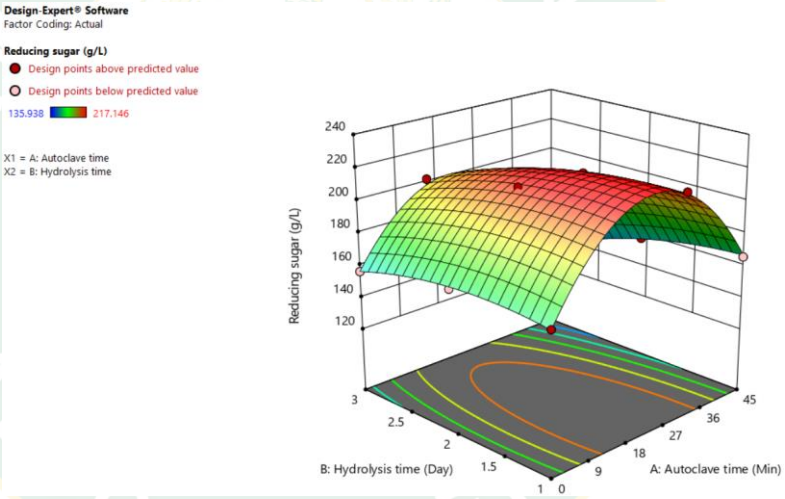


Figure 4. 7 3D diagram plot of reducing sugar

Table 4. 5 ANOVA analysis of model for optimization of RS from physical pretreatment and enzymatic hydrolysis for dried longan

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	17466.03	5	3493.21	600.10	<0.0001	significant
A-Autoclave time	392.07	1	392.07	67.35	<0.0001	
B-Hydrolysis time	1786.80	1	1786.80	306.95	<0.0001	

AB	304.52	1	304.52	52.31	<0.0001
A^2	14713.88	1	14713.88	2527.70	<0.0001
B^2	268.77	1	268.77	46.17	<0.0001
Residual	104.78	18	5.82		
Lack of fit	104.78	6	17.46		
Pure Error	0.0000	12	0.0000		
Cor Total	17570.81	23			
St.Dev	2.41		**R^2**	0.9940	
Mean	182.98		**Adjusted R^2**	0.9924	
C.V. %	1.32		**Predicted**	0.9904	

4.5 Significant influence of physical pretreatment on the structure

Scaning electron microscope (SEM) analysis was conducted to study the effect of physicalpretreatments on the morphology changes in longan fruits before and after physical pretreatment by SEM images taken at × 500 magnification. For the original material, themorphology surface of the longan cell cluster was covered by a polysaccharide layer that contains a complex closedring net structure, which is shown in Figure 4.8 A that can belimited to the attacked ability of enzymes to change cellulose or polysaccharide molecule to fermentable sugars (Lei et al., 2013). However, hydrothermal pretreatment methods hadmade the longan more susceptible to enzymatic attacksby altering the structures, and increasing the porosity ofmorphology leads to some opened holes of cells. The cellwalls were disrupted, allowing the creation of holes withdeep and length scales that lead to the surface runningloose. It can be observed in Figure 4.8 B. Autoclaving pretreatment was second handled time about hydrothermal tochange the more massive structure. The net structure became simple, and all upper parts of the cell walls weredisappeared, and blocks were exposed clearly, as shown in Figure 4.8 C (Cutzu and Bardi, 2017).

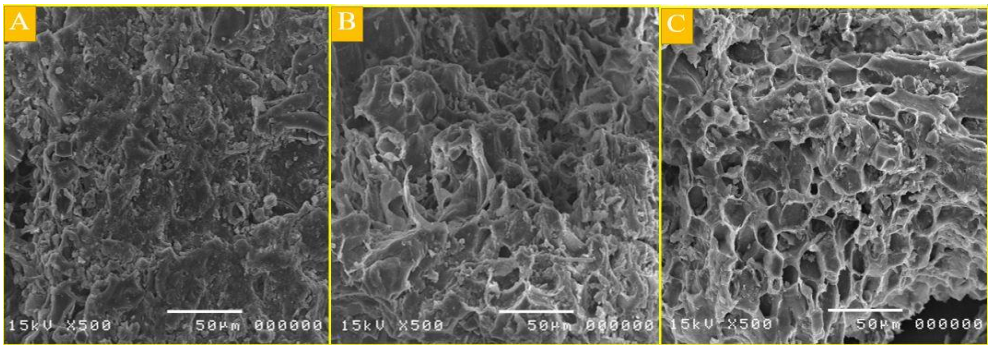


Figure 4. 8 SEM of dried longan (a) Untreated. (b) Boiled. (c) Autoclaved

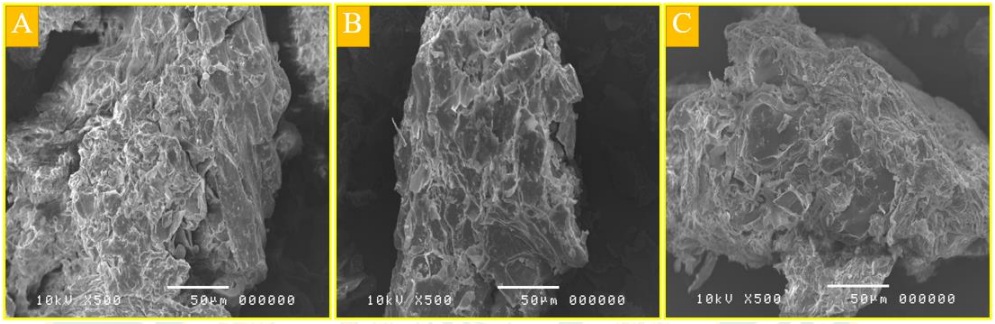


Figure 4. 9 SEM of fresh longan (a) Untreated. (b) Boiled. (c) Autoclaved

4.5 Ethanol fermentation (lab scale)

Each treatment of fresh and dried longan is determined the optimum by RSM to do fermentation process. The fermentation step was conducted to evaluate the performence of bioethanol production from fresh and dried longan. This process was carried out in an anaerobic condition by the hydrolyzed samples with 2% *S. cerevisiae* at pH 5.6. Sugar analysis and ethanol production were measurement was adopted the methods (Nguyen et al., 2020).

The highest ethanol production of fresh longan fruit was observed after 24 hours of fermentation with 9.25 g/L ethanol yield followed by 7.23 g/L at 36 hours. The lowest ethanol production was 6.24 g/L at 72 hours as Figure 4.10. On the other hand, dried longan at 24 hours, the highest ethanol production was observed 16.74 g/L, follow by 14.87 g/L at 12 hours. The lowest ethanol production was 2.38 g/L at 72 hours.

These results recorded moisture of fruits effect on bioethanol production. Bioethanol production from dried low-grade and damaged longan fruit wastes by fermentation using *S. cerevisiae* is given. *S. cerevisiae* showed efficient propagation in

sesame biomass for the initial 24 h, which later become stationary to 108 h. We observed that the volume of bioethanol increased with a decrease in particle size. In a similar fermentation scheme (separate hydrolysis and fermentation), ethanol concentration from low grade and damaged longan fruits exceeded other studies.

Some researchers examined the improvement of bioethanol production using physicochemical pretreatment on rice straw, which produced 26.12 g/L of ethanol (Banoth et al., 2017). Interestingly, the ethanol concentration of low-grade and damaged longan fruit residue is higher than other waste biomass. The higher concentration of alcohol of ethanol produced in the present study is due to the significant amount of reducing sugars hydrolyzed after pre-hydrothermal treatment and post-oven acid treatment.

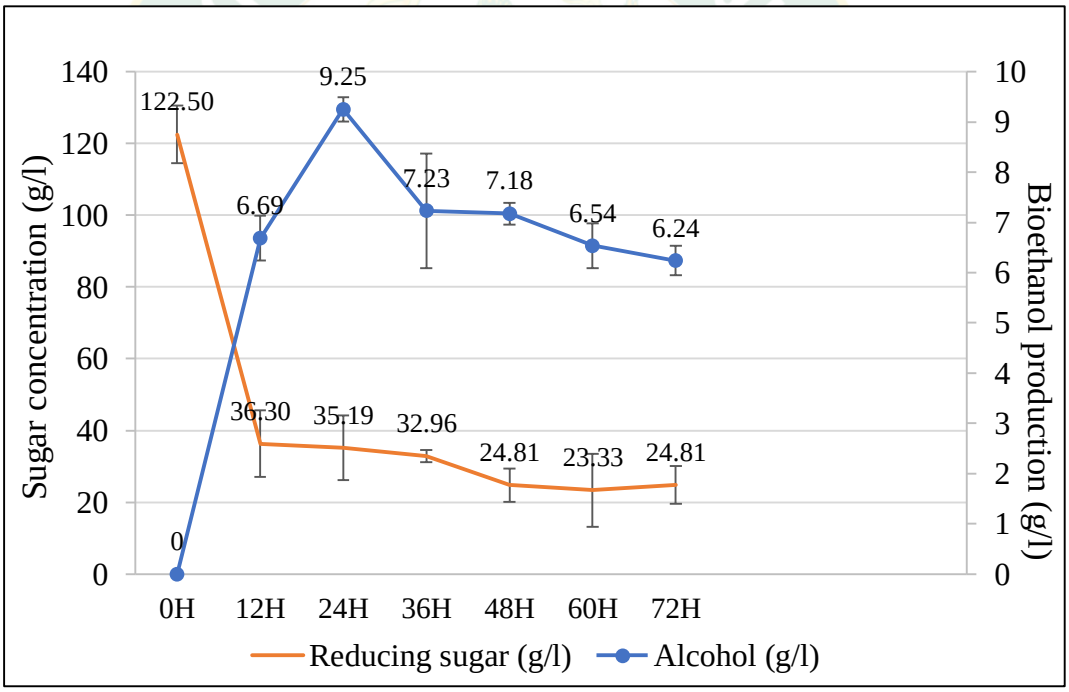


Figure 4. 10 Reducing sugar and alcohol concentration of fresh longan after fermentation

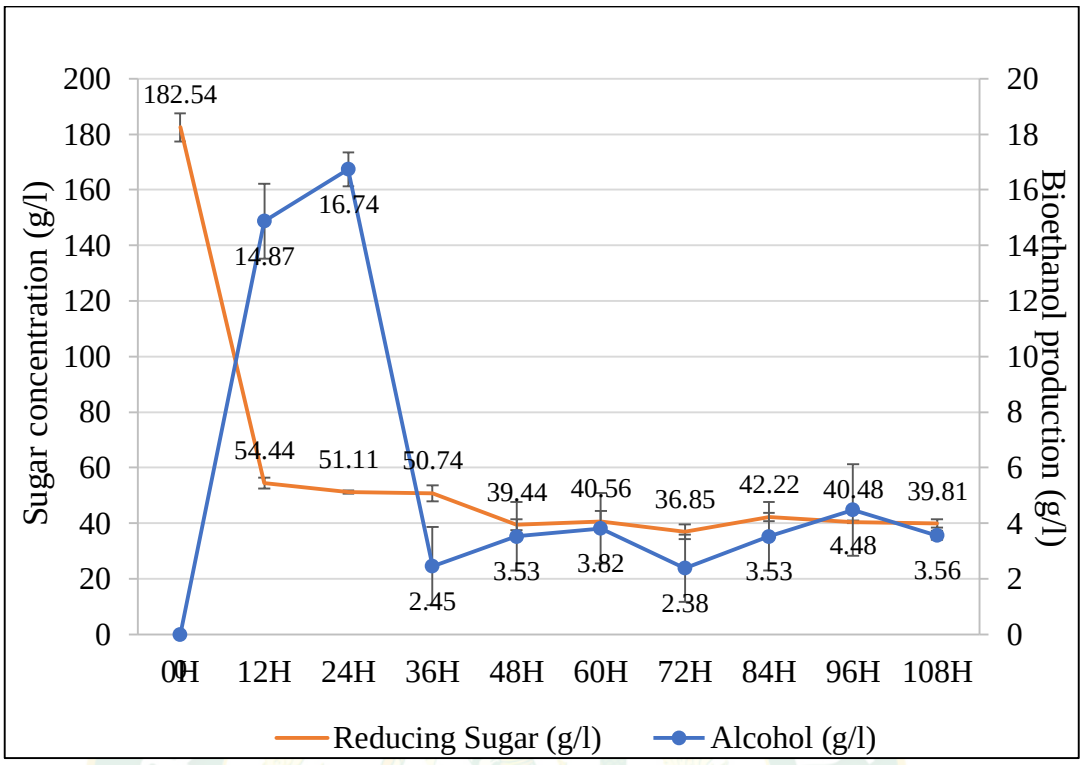


Figure 4. 11 RS and alcohol concentration of dry longan after fermentation

According to Wang et al. (2008), the higher the sugar content the materials have, the higher the ethanol yield. In the present study, it is notable that the reducing sugar was relatively high and therefore resulted in high ethanol from fermentation. More importantly, the study infers the viability of low-grade and damaged longan fruit wastes to produce bioethanol and thus presenting waste to the energy concept. The low grade and damaged longan fruit wastes appear to be a promising and potential feedstock for bioethanol production due to its plentiful availability and attractive composition.

4.6 Sugar concentration of dried longan on scale-up from pretreatment and enzymatic hydrolysis and distillation

The amount of total and reducing sugar after pretreatment are 157.19 g/L and 36.43 g/L, respectively. Hydrolysis step with 1% commercial cellulase enzyme, the amount of total and reducing sugar are 271.07 g/L and 48.21 g/L, respectively. Moreover, the amount of total and reducing sugar after fermentation in 24 hours are 67.09 g/L and 43.39 g/L, respectively. Ethanol yield is the highest ethanol concentration

with 11.17 g/L (1.4 %) after 1 day. Afterward, distilation was conducted to get bioethanol concentration (9 %).

4.7 Mass balance

Mass balance is the conservation of mass to analyzing of physical system, detail, accounting for material entering and leaving a system. The processes and mass balance of pretreatment and hydrolysis of dried longan were accomplished on a large scale and is summarised in Figure 4.12. The experiment was conducted with 1kg of dried longan and 50 liters of water. Chemical compositions contain fructose, arabinose, xylose, mannose, glucose, galactose which were analyzed in every process in this experiment. Mass balance shows the increase of sugar in the pretreatment and hydrolysis process to prepare the fermentation step. Table 4.4 showed the percent of sugar recovery and sugar yield. The sugar recovery comparison between the kind of materials shows that different yields per unit of land area where that crop is cultivated the study's duration. The percent of improvement on sugar recovery up to one percent by agricultural activities, for instance, increases fertilizer use efficiency, drip irrigation, and insect and pest control. Moreover, one of the factors of the effects of sugar recovery is harvest. The products are harvested such as either immature or over-aged but an improper methodology easily leads to loss of quality of products, such as, sugar recovery, yield, or poor juice quality. In addition, humid and warm climate-related to the problem of low sugar recovery (Pathak et al., 2019), (Roy and Chandra, 2018), (Renouf et al., 2008).

According to Sophanodorn et al. (2020), after chemical (2% CaO) and thermal pretreatments, the total sugar and reducing sugar are 4.97 and 2.84 kg, respectively. The amount of the total sugar and reducing sugar increase 6.97 and 3.67 kg in 10 kg of tobacco stalk at the end of hydrolysis by using cellulase enzyme 1 kg. Therefore, the mass balance also determined the total sugar and reducing sugar at the end of pretreatment and hydrolysis. In this study, sugar recovery and sugar yield of fructose are highest at 185.0% and 2.6% in table 4.4. The estimated mass balance from the sample in this study is based on transformation of sugar compounds and is analyzed in Figure 4.13. The production of ethanol from dried longan is based on the supposition

that C-5 and C-6 sugars are co-fermented. Sugars are known as mostly glucose, were converted from 88% of the cellulose fraction of algal biomass by enzymatic hydrolysis. Afterward, 86% of the fermentation efficiency done successfully subsequently (Kumar et al., 2013). Mass balance of palm empty fruit bunches (EFB), 1 kg of dry EFB produced 0.166 kg ethanol with co-production of 0.14 kg of high-purity lignin and 5.26 kg (2.8% xylose concentration) (Cui et al., 2014).

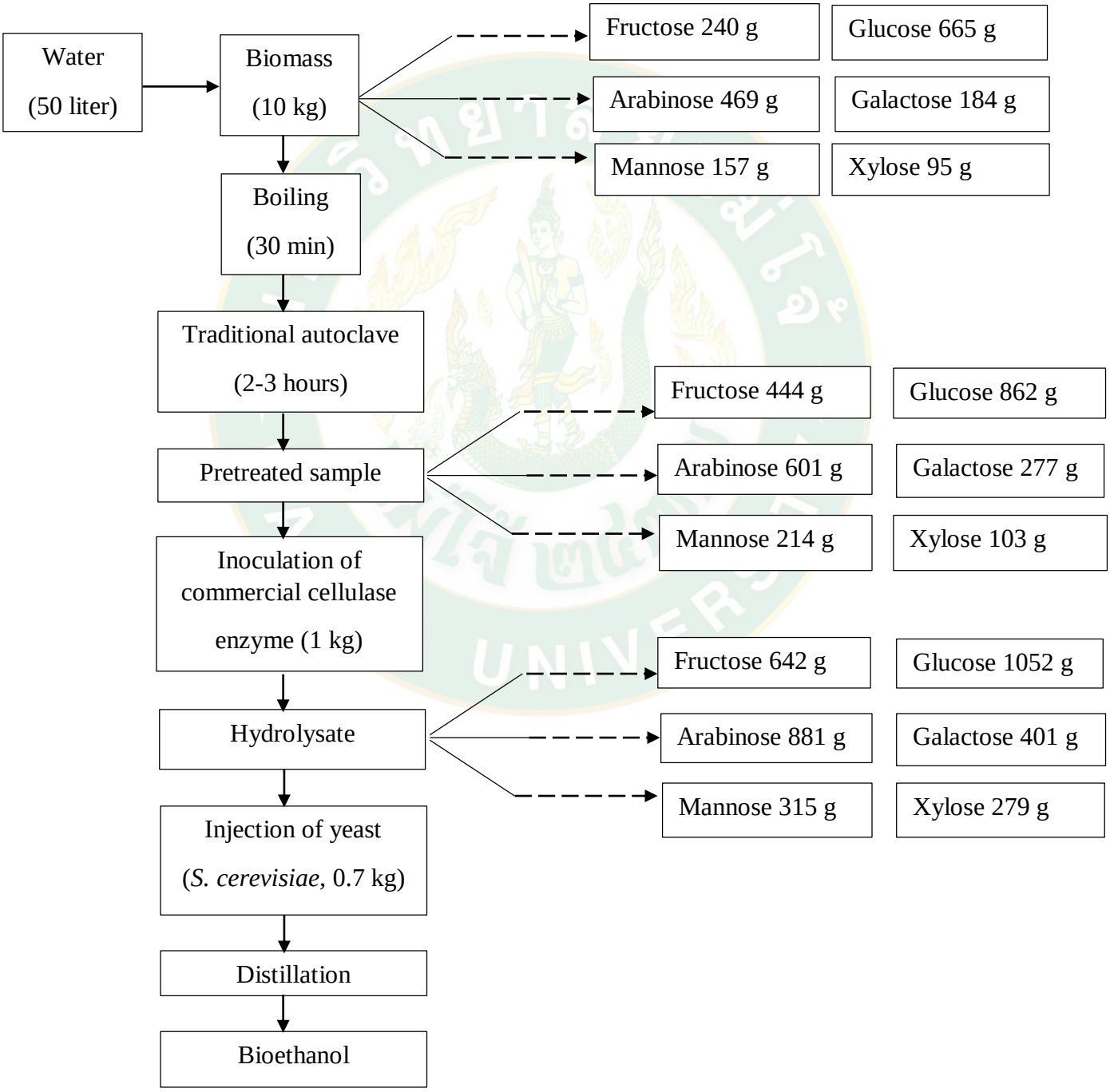


Figure 4. 12 Mass balance of conventional ethanol production from dried longan

Table 4. 6 Sugar recovery, sugar yield

	Glucose	Xylose	Fructose	Arabinose	Mannose	Galactose
Sugar recovery (%)	129.6	108.4	185.0	128.1	136.3	150.5
Sugar Yield (%)	1.4	2.4	2.6	1.7	1.8	2.0

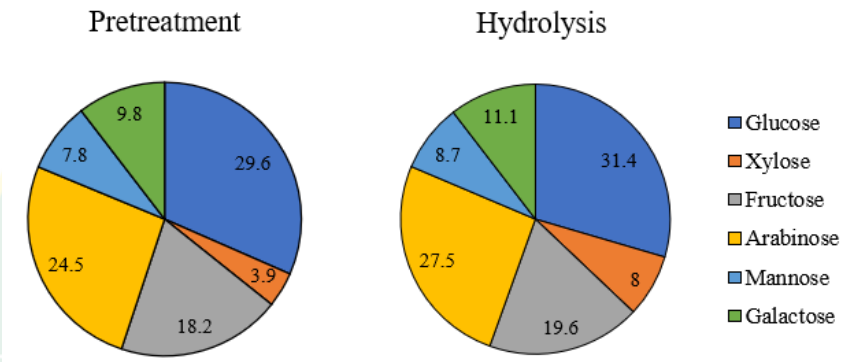


Figure 4. 13 Compositions of pretreated solid and hydrolyzed solid fraction

4.8 Energy analysis

Energy accomplishment of longan bioethanol in Thailand. The value of energy performance was indicated in Table 4.5 that the production of bioethanol of longan in Thailand is effective energy. The Net Energy Gain (NEG) and the Net Energy Ratio (NER) are two common indicators that parameters were used to assess the energy efficiency of bioethanol production from longan. The value of a net energy gain (NEG) reaches a positive amount with 1.51 MJ/L ethanol; a net energy ratio (NER) got 1.49. Two properties were gotten in the energy performance estimation. In this study, the longan tree is a tropical tree that the tree can live and multiply in hot climates or places where it can be very dry. Therefore, those expenses, such as, land preparation, weeding, irrigation, and clearing were reduced. Hence, the energy performance evaluation of ethanol production of longan is effective.

Table 4. 7 Life cycle energy for the production of 1 L of ethanol from longan fruits

Items	Input Energy Sources (MJ/L of ethanol)				
	Machine	Labor	Fuel	Electricity	Total
1. Feedstock preparation	0.355	**0.0009**	**1.809**		2.16
2. Pretreatment	**0.281**	**0.0018**		**0.017**	0.3
Boiling	0.136	0.0008		0.009	
Autoclave	0.145	0.001		0.008	
3. Hydrolysis	**0.082**	**0.0005**		**0**	0.08
4. Fermentation	**0.298**	**0.0007**	**0.806**	**0.136**	1.24
5. Distillation	**0.156**	**0.0009**	**0.908**	**0**	1.06
TOTAL ENERGY INPUT, MJ/L	1.172	0.0048	1.714	0.153	3.04
TOTAL ENERGY OUTPUT, MJ/L					4.55
NET ENERGY GAIN (NEG), MJ/L					1.51
NET ENERGY RATIO (NER), MJ/L					1.49

On the other hand, a perennial plant, such as cassava, energy performance from this plant got a net energy gain (NEG) and a net energy ratio (NER) of cassava were −3.72 MJ/L and 0.85, respectively (Papong and Malakul, 2010). A net energy gain (NEG) reached negative value with −3.72 MJ/L that production of bioethanol from cassava took more energy to create the final product. According to Dong et al. (2008), the overall net energy gain has improved the potential by extracting additional energy through final products in anaerobic digestion or microbial fuel cell with 4.186 kJ/kg. Moreover, fermentation temperatures are higher than that the reason leads to negative net energy gains. The energy input for ethanol production is predominantly consumed in feedstock preparation and fermentation with 2.16 MJ/L and 1.24 MJ/L, respectively. Gallegos et al. (2014), and Dai et al. (2006) who also reported that ethanol conversion utilized the energy input for production, fertilizers and cultivation material remains high spending in ethanol production.

4.9 Economic analysis

Table 4. 8 The unit cost of bioethanol production from dried longan

Vessel total		**7 liter**	
Items	**Units**	**Quantity**	**Baht**
Biomass	0 baht/ kg	10 kg	0
Water	1.02 baht/L	50 liter	51
Enzyme cellulase	3950 baht	1 kg	3,950
Yeast	18.573 baht/kg	0.7 kg	13.001
Sulphuric acid	190 baht/L	1 liter	190
Milling machine	3.2483 baht/Unit	0.015 kw	0.049
Distillator	28.25 baht/L	5 liter	141.25
Centrifuge	3.2483 baht/Unit	0.55 kw	3.573
Human	250 baht/ day	32 hrs	8,000
TOTAL			12349.3

The economic analysis results are summarized in Table 4.6 which demonstrates a summary of the process estimation and the elements used for considering the fixed capital investment. The capital investment of a bioethanol plant depends on the type of feedstock, the location and scale of the plant. In this case, bioethanol production from dried longan was effected by enzyme price, and income of labor. According to Kang et al. (2019), enzyme costs affect on the ethanol production cost; the enzyme cost is 25% of the ethanol production total cost.

Table 4.7 shows the net present value (22.3) of this study with an interest rate (5%); positive net present value (NPV >0), a profit bring from the plant can get in a period of time. In order to be equal to the total net present value of 642,163.6 baht (the investment required to finance a plant, producing 364 liter per year), the ethanol price should be 51.0 baht/L.

Table 4. 9 The net present of value of investment

Year	Cash flow (Baht)	Present value (Baht)
0	642163.6	642163.6
1	511120.6	486781.5
2	42580.6	38621.9
3	40140.6	34675.0
4	50163.6	41269.7
5	52120.6	40837.9
NET PRESENT VALUE		22.3

The value of the ethanol price is equivalent to 1.7 USD. According to Humbird et al. (2011), the price of ethanol from corn stover up to 2.25 USD. Moreover, the total annual industrial charge was found to be 1.92 USD/gal ethanol from lignocellulosic biomass (Alvarado-Morales et al., 2009). In this study, the operation period was assumed to be five years with the internal rate of return 5%.

As a result, the enzyme cost and income of labor had a considerable effect on the overall bioethanol production cost from dried longan; the positive net present value was calculated to consider the operation of plant in five years.

CHAPTER 5

SUMMARY, CONCLUSION AND RECOMMENDATION

Bioethanol production feasibility from low-grade longan fruit waste material was recently studied as a novel substrate for bioethanol production. In this study, bioethanol was created by four steps: Physical pretreatment, enzymatic hydrolysis, fermentation, and distillation. Physical pretreatment is necessary to break down the complex structure of biomass, which contain boiling (30 min), and autoclave; the pretreatment plays a crucial role for improving the productivity of bioethanol. Three types of enzyme hydrolyzed each pretreatment: 2% commercial cellulase, 20% algal enzyme and combine 1% commercial cellulase + 10% algal enzyme (C+A). The variation of sugar amount after each step (pretreatment and hydrolysis) also was calculated by mass balance to recognize the effectiveness of processes. The process of saccharification was done by using *S. cerevisiae*. Distillation and bomb calorimeter was utilized to get higher concentration percent of ethanol and check High Heating Value (HHV) of ethanol. Energy and economic analysis were calculated to get the result of the cost per unit of ethanol.

As a result of energy, physical pretreatment was carried out to bring a higher sugar amount. However, the energy needs to complete this process too high lead to high payment for this project. There are some suggestions for this case, such as reducing the duration of the boil. As a result of economic analysis, the cost of enzyme and humans in this study account for the highest price position. Therefore, the alternative of commercial cellulase into algal enzyme needs to research more depth in the future.

REFERENCES

Agbor, V. B., Cicek, N., Sparling, R., Berlin, A. & Levin, D. B. 2011. Biomass pretreatment: fundamentals toward application. **Biotechnology advances**, 29(6), 675-685.

Al-Yaqoobi, A., Hogg, D. & Zimmerman, W. B. 2016. Microbubble distillation for ethanol-water separation. **International Journal of Chemical Engineering**, 2016.

Alvarado-Morales, M., Terra, J., Gernaey, K., Woodley, J. & Gani, R. 2009. Biorefining: Computer aided tools for sustainable design and analysis of bioethanol production. **Chemical Engineering Research and Design**, 87(9), 1171-1183.

Alvira, P., Tomas-Pejo, E., Ballesteros, M. & Negro, M. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. **Bioresource technology**, 101(13), 4851-4861.

Asif, M. & Muneer, T. 2007. Energy supply, its demand and security issues for developed and emerging economies. **Renewable and Sustainable Energy Reviews**, 11(7), 1388-1413.

Aydar, A. Y. 2018. Utilization of response surface methodology in optimization of extraction of plant materials. **Statistical Approaches With Emphasis on Design of Experiments Applied to Chemical Processes. InTech**, 157-169.

Azhar, S. H. M., Abdulla, R., Jambo, S. A., Marbawi, H., Gansau, J. A., Faik, A. A. M. & Rodrigues, K. F. 2017. Yeasts in sustainable bioethanol production: A review. **Biochemistry and Biophysics Reports**, 10, 52-61.

Balat, M., Balat, H. & Oz, C. 2008. Progress in bioethanol processing. **Progress in energy and combustion science**, 34(5), 551-573.

Ballesteros, M., Oliva, J., Negro, M., Manzanares, P. & Ballesteros, I. 2004. Ethanol from lignocellulosic materials by a simultaneous saccharification and fermentation process (SFS) with Kluyveromyces marxianus CECT 10875. **Process Biochemistry**, 39(12), 1843-1848.

Banoth, C., Sunkar, B., Tondamanati, P. R. & Bhukya, B. 2017. Improved physicochemical pretreatment and enzymatic hydrolysis of rice straw for bioethanol production by yeast fermentation. **3 Biotech**, 7(5), 334.

Baruah, J., Nath, B. K., Sharma, R., Kumar, S., Deka, R. C., Baruah, D. C. & Kalita, E. 2018. Recent trends in the pretreatment of lignocellulosic biomass for value-added products. **Frontiers in Energy Research**, 6, 141.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S. & Escaleira, L. A. 2008. Response surface methodology (RSM) as a tool for optimization in analytical chemistry. **Talanta**, 76(5), 965-977.

Box, G. & Wilson, K. 2012. On the Experimental Attainment of Optimum Conditions. **Breakthroughs in Statistics: Methodology and Distribution**, 270.

Brasil, B. d. S. A. F., de Siqueira, F. G., Salum, T. F. C., Zanette, C. M. & Spier, M. R. 2017. Microalgae and cyanobacteria as enzyme biofactories. **Algal research**, 25, 76-89.

Burhani, D., Putri, A. M. H., Waluyo, J., Nofiana, Y. & Sudiyani, Y. 2017. The effect of two-stage pretreatment on the physical and chemical characteristic of oil palm empty fruit bunch for bioethanol production. **AIP Publishing LLC**.

Cara, C., Romero, I., Oliva, J. M., Sáez, F. & Castro, E. 2007. Liquid hot water pretreatment of olive tree pruning residues. **In Applied biochemistry and biotecnology**, 379-394.

Cui, X., Zhao, X., Zeng, J., Loh, S. K., Choo, Y. M. & Liu, D. 2014. Robust enzymatic hydrolysis of Formiline-pretreated oil palm empty fruit bunches (EFB) for efficient conversion of polysaccharide to sugars and ethanol. **Bioresource technology**, 166, 584-591.

Cutzu, R. & Bardi, L. 2017. Production of bioethanol from agricultural wastes using residual thermal energy of a cogeneration plant in the distillation phase. **Fermentation**, 3(2), 24.

Chakdar, H., Jadhav, S. D., Dhar, D. W. & Pabbi, S. 2012. Potential applications of blue green algae.

Chen, Y., Stevens, M. A., Zhu, Y., Holmes, J. & Xu, H. 2013. Understanding of alkaline pretreatment parameters for corn stover enzymatic saccharification. **Biotechnology for biofuels**, 6(1), 8.

Dai, D., Hu, Z., Pu, G., Li, H. & Wang, C. 2006. Energy efficiency and potentials of cassava fuel ethanol in Guangxi region of China. **Energy Conversion and Management**, 47(13-14), 1686-1699.

Deng, L.-Z., Mujumdar, A. S., Zhang, Q., Yang, X.-H., Wang, J., Zheng, Z.-A., Gao, Z.-J. & Xiao, H.-W. 2019. Chemical and physical pretreatments of fruits and vegetables: Effects on drying characteristics and quality attributes–a comprehensive review. **Critical reviews in food science and nutrition**, 59(9), 1408-1432.

Diwekar, U. 2011. **Batch distillation: simulation, optimal design, and control**. CRC press.

Dong, X., Ulgiati, S., Yan, M., Zhang, X. & Gao, W. 2008. Energy and eMergy evaluation of bioethanol production from wheat in Henan Province, China. **Energy Policy**, 36(10), 3882-3892.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. t. & Smith, F. 1956. Colorimetric method for determination of sugars and related substances. **Analytical chemistry**, 28(3), 350-356.

Ewell, R., Harrison, J. & Berg, L. 1944. Azeotropic distillation. **Industrial & Engineering Chemistry**, 36(10), 871-875.

Fogg, G. 2012. **The blue-green algae**. Elsevier.

Gabhane, J., William, S. P., Vaidya, A. N., Mahapatra, K. & Chakrabarti, T. 2011. Influence of heating source on the efficacy of lignocellulosic pretreatment–a cellulosic ethanol perspective. **Biomass and bioenergy**, 35(1), 96-102.

Galbe, M. & Zacchi, G. 2007. Pretreatment of lignocellulosic materials for efficient bioethanol production. **In Biofuels**, 41-65.

Gallegos, R. K. B., Suministrado, D. C., Elauria, J. C. & Elauria, M. M. 2014. Energy Analysis of Cassava Bioethanol Production in the Philippines. **Journal of the Japan Institute of Energy**, 93(3), 301-309.

Hao, L., Wang, R., Zhao, Y., Fang, K. & Cai, Y. 2018. The enzymatic actions of cellulase on periodate oxidized cotton fabrics. **Cellulose**, 25(11), 6759-6769.

Harun, M., Radiah, A. D., Abidin, Z. Z. & Yunus, R. 2011. Effect of physical pretreatment on dilute acid hydrolysis of water hyacinth (Eichhornia crassipes). **Bioresource technology**, 102(8), 5193-5199.

Ho, S.-H., Huang, S.-W., Chen, C.-Y., Hasunuma, T., Kondo, A. & Chang, J.-S. 2013. Bioethanol production using carbohydrate-rich microalgae biomass as feedstock. **Bioresource technology**, 135, 191-198.

Huang, Y.-B. & Fu, Y. 2013. Hydrolysis of cellulose to glucose by solid acid catalysts. **Green Chemistry**, 15(5), 1095-1111.

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B. & Worley, M. (2011). Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol: dilute-acid pretreatment and enzymatic hydrolysis of corn stover.

Jaroenwanit, P., Srijesdaruk, V. & Ngarmsak, T. 2001. Preliminary study for longan variety and export markets identification. **Warasan Wichai Mokho**.

Jiang, Y., Zhang, Z., Joyce, D. C. & Ketsa, S. 2002. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.)**. Postharvest Biology and technology**, 26(3), 241-252.

Kang, K. E., Jeong, J.-S., Kim, Y., Min, J. & Moon, S.-K. 2019. Development and economic analysis of bioethanol production facilities using lignocellulosic biomass. **Journal of bioscience and bioengineering**, 128(4), 475-479.

Kim, J. S., Lee, Y. & Kim, T. H. 2016. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. **Bioresource Technology**, 199(42-48).

Kim, Y., Hendrickson, R., Mosier, N. S. & Ladisch, M. R. (2009). Liquid hot water pretreatment of cellulosic biomass. In **Biofuels**, 93-102.

Korzen, L., Peled, Y., Shamir, S. Z., Shechter, M., Gedanken, A., Abelson, A. & Israel, A. 2015. An economic analysis of bioethanol production from the marine macroalga *Ulva* (*Chlorophyta*). **Technology**, 3, 114-118.

Kumar, P., Barrett, D. M., Delwiche, M. J. & Stroeve, P. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. **Industrial & engineering chemistry research**, 48(8), 3713-3729.

Kumar, P., Kumar, V., Kumar, S., Singh, J. & Kumar, P. 2020. Bioethanol production from sesame (*Sesamum indicum* L.) plant residue by combined physical, microbial and chemical pretreatments. **Bioresource Technology**, 297, 122-484.

Kumar, S., Gupta, R., Kumar, G., Sahoo, D. & Kuhad, R. C. 2013. Bioethanol production from *Gracilaria verrucosa*, a red alga, in a biorefinery approach. **Bioresource technology**, 135, 150-156.

Khammee, P., Unpaprom, Y., Buochareon, S. & Ramaraj, R. Potential of Bioethanol Production from Marigold Temple Waste Flowers.

Lei, H., Cybulska, I. & Julson, J. 2013. Hydrothermal pretreatment of lignocellulosic biomass and kinetics. **Journal of Sustainable Bioenergy Systems**, 3(04), 250.

Limayem, A. & Ricke, S. C. 2012. Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. **Progress in energy and combustion science**, 38(4), 449-467.

Lin, Y., Zhang, W., Li, C., Sakakibara, K., Tanaka, S. & Kong, H. 2012. Factors affecting ethanol fermentation using *Saccharomyces cerevisiae* BY4742. **Biomass and bioenergy**, 47, 395-401.

Liu, X., Wang, W., Gao, X., Zhou, Y. & Shen, R. 2012. Effect of thermal pretreatment on the physical and chemical properties of municipal biomass waste. **Waste Management**, 32(2), 249-255.

Makela, M. 2017. Experimental design and response surface methodology in energy applications: A tutorial review. **Energy Conversion and Management**, 151, 630-640.

Manmai, N., Unpaprom, Y., Ponnusamy, V. K. & Ramaraj, R. 2020. Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation. **Fuel**, 278, 118-262.

Manmai, N., Unpaprom, Y. & Ramaraj, R. 2020. Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). **Biomass Conversion and Biorefinery**, 1-15.

Marasabessy, A., Kootstra, A. M. J., Sanders, J. P. & Weusthuis, R. A. 2012. Dilute H_2SO_4-catalyzed hydrothermal pretreatment to enhance enzymatic digestibility of *Jatropha curcas* fruit hull for ethanol fermentation. **International Journal of Energy and Environmental Engineering**, 3(1), 15.

Matsushika, A., Inoue, H., Kodaki, T. & Sawayama, S. 2009. Ethanol production from xylose in engineered Saccharomyces cerevisiae strains: current state and perspectives. **Applied microbiology and biotechnology**, 84(1), 37-53.

Maurya, D. P., Singla, A. & Negi, S. 2015. An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. **3 Biotech**, 5(5), 597-609.

McMeekin, T., Olley, J., Ratkowsky, D. & Ross, T. 2002. Predictive microbiology: towards the interface and beyond. **International journal of food microbiology**, 73(2-3), 395-407.

Miller, G. L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. **Analytical chemistry**, 31(3), 426-428.

Mood, S. H., Golfeshan, A. H., Tabatabaei, M., Jouzani, G. S., Najafi, G. H., Gholami, M. & Ardjmand, M. 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. **Renewable and Sustainable Energy Reviews**, 27, 77-93.

Neves, M. A. d., Kimura, T., Shimizu, N. & Shiiba, K. 2006. Production of alcohol by simultaneous saccharification and fermentation of low-grade wheat flour. **Brazilian Archives of Biology and Technology**, 49(3), 481-490.

Nguyen, T. V. T., Unpaprom, Y., Manmai, N., Whangchai, K. & Ramaraj, R. 2020. Impact and significance of pretreatment on the fermentable sugar production from low-grade longan fruit wastes for bioethanol production. **Biomass Conversion and Biorefinery**, 1-13.

Nguyen, T. V. T., Unpaprom, Y., Tandee, K., Whangchai, K. & Ramaraj, R. 2020. Physical pretreatment and algal enzyme hydrolysis of dried low-grade and waste longan fruits to enhance its fermentable sugar production. **Biomass Conversion and Biorefinery**, 1-9.

Ohgren, K., Bura, R., Lesnicki, G., Saddler, J. & Zacchi, G. 2007. A comparison between simultaneous saccharification and fermentation and separate hydrolysis and fermentation using steam-pretreated corn stover. **Process Biochemistry**, 42(5), 834-839.

Ohgren, K., Bura, R., Saddler, J. & Zacchi, G. 2007. Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. **Bioresource technology**, 98(13), 2503-2510.

Ohgren, K., Vehmaanperä, J., Siika-Aho, M., Galbe, M., Viikari, L. & Zacchi, G. 2007. High temperature enzymatic prehydrolysis prior to simultaneous saccharification and fermentation of steam pretreated corn stover for ethanol production. **Enzyme and Microbial Technology**, 40(4), 607-613.

Papong, S. & Malakul, P. 2010. Life-cycle energy and environmental analysis of bioethanol production from cassava in Thailand. **Bioresource technology**, 101(1), 112-118.

Pathak, S., Singh, P., Singh, M. & Sharma, B. 2019. Impact of temperature and humidity on sugar recovery in Uttar Pradesh. **Sugar Tech**, 21(1), 176-181.

Pielhop, T., Amgarten, J., von Rohr, P. R. & Studer, M. H. 2016. Steam explosion pretreatment of softwood: the effect of the explosive decompression on enzymatic digestibility. **Biotechnology for biofuels**, 9(1), 152.

Phu, T. Q. & Lan, N. T. P. 2018. The adjustments in thailand's agricultural trade policy and impact on agricultural trade between vietnam and thailand. **On socio-economic and environmental issues in development**, 119.

Raguin, A. & Ebenhoh, O. 2017. Design starch: stochastic modeling of starch granule biogenesis. **Biochemical Society Transactions**, 45(4), 885-893.

Renouf, M., Wegener, M. & Nielsen, L. 2008. An environmental life cycle assessment comparing Australian sugarcane with US corn and UK sugar beet as producers of sugars for fermentation. **Biomass and Bioenergy**, 32(12), 1144-1155.

Roy, M. & Chandra, A. 2018. Technological and Management options for optimizing sugar recovery in India: A Review. **Intl J Eng Res Mgmt**, 5(4), 14-22.

Sassner, P., Galbe, M. & Zacchi, G. 2006. Bioethanol production based on simultaneous saccharification and fermentation of steam-pretreated Salix at high dry-matter content. **Enzyme and Microbial Technology**, 39(4), 756-762.

Shamsudin, S., Shah, U. K. M., Zainudin, H., Abd-Aziz, S., Kamal, S. M. M., Shirai, Y. & Hassan, M. A. 2012. Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars. **Biomass and Bioenergy**, 36, 280-288.

Sharma, N., Kalra, K., Oberoi, H. S. & Bansal, S. 2007. Optimization of fermentation parameters for production of ethanol from kinnow waste and banana peels by

simultaneous saccharification and fermentation. **Indian Journal of Microbiology**, 47(4), 310-316.

Shen, F., Kumar, L., Hu, J. & Saddler, J. N. 2011. Evaluation of hemicellulose removal by xylanase and delignification on SHF and SSF for bioethanol production with steam-pretreated substrates. **Bioresource technology**, 102(19), 8945-8951.

Sindhu, R., Binod, P. & Pandey, A. 2016. Biological pretreatment of lignocellulosic biomass–An overview. **Bioresource technology**, 199, 76-82.

Sindhu, R., Kuttiraja, M., Binod, P., Janu, K. U., Sukumaran, R. K. & Pandey, A. 2011. Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. **Bioresource Technology**, 102(23), 10915-10921.

Singhania, R. R., Saini, J. K., Saini, R., Adsul, M., Mathur, A., Gupta, R. & Tuli, D. K. 2014. Bioethanol production from wheat straw via enzymatic route employing Penicillium janthinellum cellulases. **Bioresource technology**, 169, 490-495.

Soong, Y. Y. & Barlow, P. J. 2005. Isolation and structure elucidation of phenolic compounds from longan (*Dimocarpus longan* Lour.) seed by high-performance liquid chromatography–electrospray ionization mass spectrometry. **Journal of Chromatography A**, 1085(2), 270-277.

Sopadang, A., Tippayawong, K. Y. & Chaowarut, W. 2012. Application of value chain management to longan industry. **American Journal of Agricultural and Biological Sciences**, 7(3), 301-311.

Sophanodorn, K., Unpaprom, Y., Whangchai, K., Duangsuphasin, A., Manmai, N. & Ramaraj, R. 2020. A biorefinery approach for the production of bioethanol from alkaline-pretreated, enzymatically hydrolyzed *Nicotiana tabacum* stalks as feedstock for the bio-based industry. **Biomass Conversion and Biorefinery**, 1-9.

Stanier, R., Kunisawa, R., Mandel, M. & Cohen-Bazire, G. 1971. Purification and properties of unicellular blue-green algae (*Chroococcales*). **Bacteriological reviews**, 35(2), 171.

Subhadrabandhu, S. & Yapwattanaphun, C. 2000. **Lychee and longan production in Thailand**.

Sun, Y. & Cheng, J. 2002. Hydrolysis of lignocellulosic materials for ethanol production: a review. **Bioresource technology**, 83(1), 1-11.

Szambelan, K., Nowak, J., Szwengiel, A., Jelen, H. & Eukaszewski, G. 2018. Separate hydrolysis and fermentation and simultaneous saccharification and fermentation methods in bioethanol production and formation of volatile by-products from selected corn cultivars. **Industrial Crops and Products**, 118, 355-361.

Tao, L., Tan, E. C., McCormick, R., Zhang, M., Aden, A., He, X. & Zigler, B. T. 2014. Techno-economic analysis and life-cycle assessment of cellulosic isobutanol and comparison with cellulosic ethanol and n-butanol. **Biofuels, Bioproducts and Biorefining**, 8(1), 30-48.

Tester, R. F. & Morrison, W. R. 1990. Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose, and lipids. **Cereal chem**, 67(6), 551-557.

Tomas-Pejo, E., Oliva, J., Gonzalez, A., Ballesteros, I. & Ballesteros, M. 2009. Bioethanol production from wheat straw by the thermotolerant yeast *Kluyveromyces marxianus* CECT 10875 in a simultaneous saccharification and fermentation fed-batch process. **Fuel**, 88(11), 2142-2147.

Tursi, A. 2019. A review on biomass: importance, chemistry, classification, and conversion. **Biofuel Res. J**, 6(2), 962-979.

Tran, H., Van, H. N., Muniappan, R., Amrine, J., Naidu, R., Gilbertson, R. & Sidhu, J. 2019. Integrated Pest Management of Longan (Sapindales: *Sapindaceae*) in Vietnam. **Journal of Integrated Pest Management**, 10(1), 18.

Wang, D., Bean, S., McLaren, J., Seib, P., Madl, R., Tuinstra, M., Shi, Y., Lenz, M., Wu, X. & Zhao, R. 2008. Grain sorghum is a viable feedstock for ethanol production. **Journal of industrial microbiology & biotechnology**, 35(5), 313-320.

Wu, X., Zhao, R., Wang, D., Bean, S., Seib, P., Tuinstra, M., Campbell, M. & Obrien, A. 2006. Effects of amylose, corn protein, and corn fiber contents on production of ethanol from starch-rich media. **Cereal chemistry**, 83(5), 569-575.

Yang, B., Jiang, Y., Shi, J., Chen, F. & Ashraf, M. 2011. Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour.) fruit—A review. **Food Research International**, 44(7), 1837-1842.

Yang, Y., Sharma-Shivappa, R., Burns, J. C. & Cheng, J. J. 2009. Dilute acid pretreatment of oven-dried *switchgrass* germplasms for bioethanol production. **Energy & Fuels**, 23(7), 3759-3766.

Yu, Z., Zhang, B., Yu, F., Xu, G. & Song, A. 2012. A real explosion: The requirement of steam explosion pretreatment. **Bioresource technology**, 121, 335-341.

Zabed, H., Faruq, G., Sahu, J. N., Azirun, M. S., Hashim, R. & Nasrulhaq Boyce, A. 2014. Bioethanol production from fermentable sugar juice. **The Scientific World Journal**, 2014.

Zhang, K. 1995. Alcohol and distilling wine craft. **China Light Industry Press, Beijing**, 246-247.

Zhang, L., Zhao, H., Gan, M., Jin, Y., Gao, X., Chen, Q., Guan, J. & Wang, Z. 2011. Application of simultaneous saccharification and fermentation (SSF) from viscosity reducing of raw sweet potato for bioethanol production at laboratory, pilot and industrial scales. **Bioresource technology**, 102(6), 4573-4579.

Zhao, W., Yang, R., Zhang, Y. & Wu, L. 2012. Sustainable and practical utilization of feather keratin by an innovative physicochemical pretreatment: high density steam flash-explosion. **Green Chemistry**, 14(12), 3352-3360.

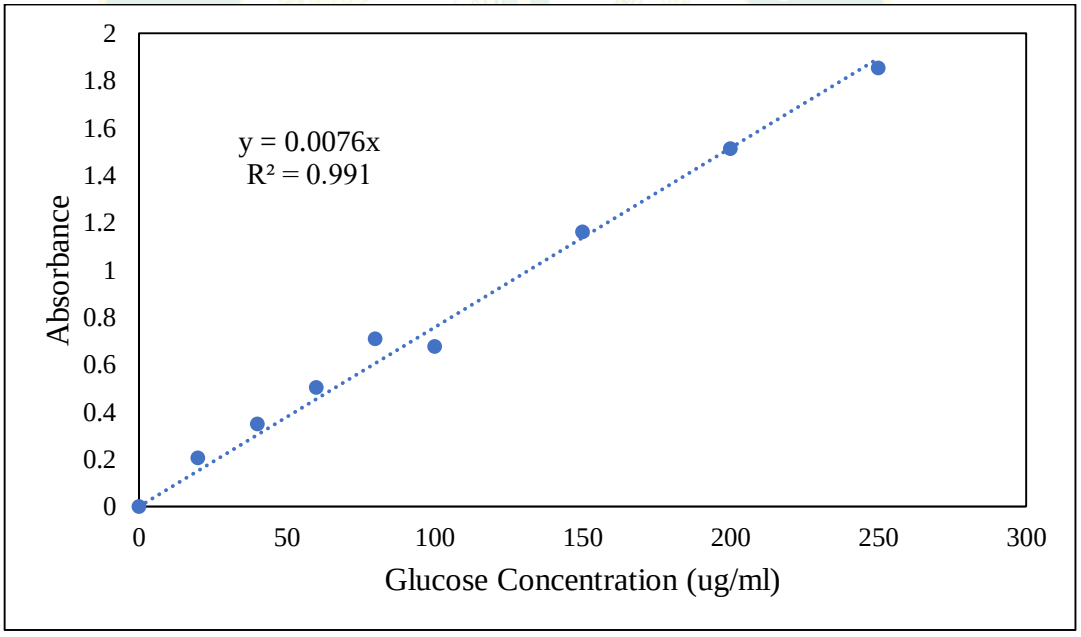
Zhou, X., Li, W., Mabon, R. & Broadbelt, L. J. 2017. A critical review on hemicellulose pyrolysis. **Energy Technology**, 5(1), 52-79.

APPENDICES

APPENDIX A: Total sugar analysis

Glucose was weighed 0.1 gram and dissolved in distilled water in volumetric flask 100 ml.

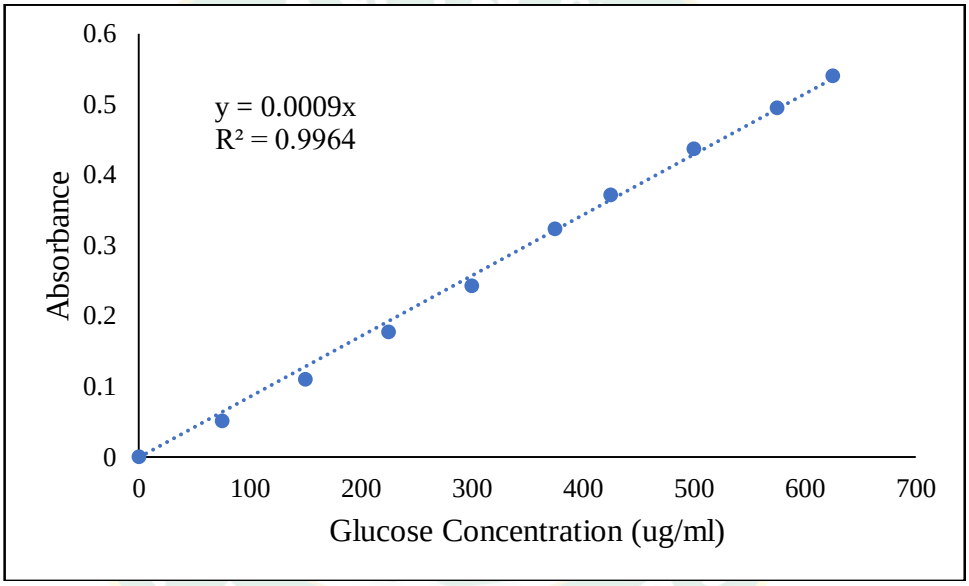
Then 0.5 mL from each concentration of glucose solution was got out to combine 0.5 mL of 5% phenol (w/v) and 2.5 mL of 98% H_2SO_4 by using a vortex to ensure the mixture was mixed well. This mixture was kept in cool water within 10 minutes, checked TS level using a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 490 nm (Dubois et al., 1956).



Standard curve of total sugar

APPENDIX B: Reducing sugar analysis

On the other hand, reducing sugar (RS) analysis. 0.5 mL from every concentration of glucose solution was got out to combine 0.5 mL of 3,5-dinitrosalicylic acid (DNS) through a vortex. Afterward, this mixture was boiled in a water bath within 15 minutes at 90-95°C. Before checking RS, 4mL of distilled water was added. 540 nm was set on a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) to read the absorbance (Miller, 1959).



Standard curve of reducing sugar

APPENDIX C: DETERMINATION OF ETHANOL

For the determination of bioethanol, Ebulliometer (Dujardin-Salleron, Alcohol Burner, France) was utilized to check alcohol of bioethanol. 50 mL of the sample was withdrawn and centrifuged using a centrifuge machine (4 ℃, 1000 rpm, 15 min). The condenser of Ebulliomter, where this sample was poured and boiled until the steady temperature showing in the thermometer of Ebulliomter, has a cylinder shape.

Ebulliometer (Dujardin-Salleron, Alcohol Burner, France)

APPENDIX D: EQUIPMENTS OF EXPERIMENT

a straight-sided saucepan and electric stove

Incubator

Analytical balance

Autoclave machine

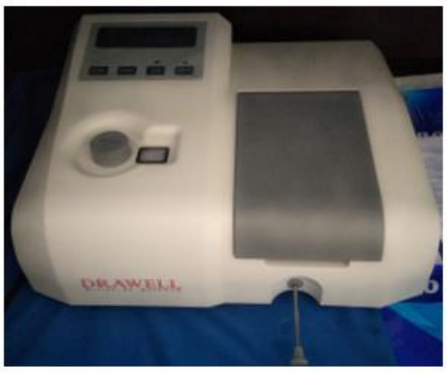
Pestle and mortar

Laminar flow hood

pH meter

Water bath

Centrifuge



Spectrophotometer

Solar dyer

Shredder machine

APPENDIX E: PUBLICATIONS

emergent
Life Sciences Research

Research Article

Enhanced fermentable sugar production from low grade and damaged longan fruits using cellulase with algal enzymes for bioethanol production

Tu Vy Thuy Nguyen, Yuwalee Unpaprom, Rameshprabu Ramaraj

Received: 26 July 2020
Accepted: 02 September 2020 **Online:** 04 September 2020

Authors:

T. V. T. Nguyen, R. Ramaraj ✉
School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand
Sustainable Resources and Sustainable Engineering Research Lab, Maejo University, Chiang Mai 50290, Thailand

Y. Unpaprom
Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand
Sustainable Resources and Sustainable Engineering Research Lab, Maejo University, Chiang Mai 50290, Thailand

✉ rrameshprabu@gmail.com, rameshprabu@mju.ac.th

Emer Life Sci Res (2020) 6(2): 26-31

E-ISSN: 2395-6658
P-ISSN: 2395-664X

DOI: https://doi.org/10.31783/elsr.2020.622631

Abstract

Longan fruits' economic values have been increasing in recent years, for example, nutrients, medicine, cosmetic, and pharmaceutical products, etc. Especially, Southeast Asia countries are the largest longan fruit, producer. The considerable amount of low grade and damaged longan fruits are one of the interesting resources for producing bioethanol. In this study, hydrothermal pretreatment and hydrolysis through added cellulase and algal enzymes were conducted with dried low grade and damaged longan fruits. The total and reducing sugar were achieved 230.70 ± 2.01 g/L and 91.11 ± 1.11 g/L, after the pretreatment process finished, respectively. Subsequently, a rise in the total and reducing sugar in the hydrolysis case was 368.42 ± 13.16 g/L and 297.78 ± 2.94 g/L, respectively. Consequently, longan fruits are valuable edible products, and leftover or low grade/damaged longans are promising bioresources for bioethanol production.

Keywords algal enzymes bioethanol production, cellulose, damaged longan fruits, low grade, pretreatment

Introduction

Over the years, humankind's demand for the use of energy has been increasing. Therefore, the amount of fossil fuel, such as coal, petroleum, and natural gas, becomes limited. Moreover, the phenomenon of environmental pollution happens, for instance, climate change, acid rain, global warming, greenhouse effect, etc. [1-3]. This is the reason that the countries in the world are interested in using renewable energy, for example, biogas, biobutanol, biodiesel, and biohydrogen, etc. One of the potential biofuels that has received much attention is bioethanol [4]. In the world, bioethanol production of the US and Brazil are the highest, in addition to several agricultural-based countries currently focusing on bioethanol production [5].

Bioethanol is a high octane fuel due to the amount of lead that has been replaced, so its ability to combustion is higher with the compression ratio within shorter burning times. Also, bioethanol is a renewable liquid fuel that is utilized transportation, 85% bioethanol and 15% gasoline (E85) is popular [67]. Moreover, bioethanol is lower noxious than because of the reduction of greenhouse gas emissions (e.g., carbon monoxide (CO) and hydrocarbons (HCs).

Therefore, bioethanol is blended to gasoline to oxygenate the fuel mixture using for the internal combustion engines [8]. Ethanol can be blended with gasoline in any ratio, thus obtaining fuels with different properties. Bioethanol is made from biomass (e.g., energy crops, industrial waste, agricultural waste, woody waste and forest biomass, waste from green

areas (parks and gardens), and municipal solid waste) [9]. One of the top agricultural producing countries is Thailand. Hence, agricultural wastes which produced entirely from agricultural activities related to cultivation consist of 17,083,594 tons per year (rice husk, corncob, bagasse, and cassava rhizome) [10]. So the researchers are frequently keeping an eye on this biomass due to its abundance, low cost, but it can produce the amount of significant bioethanol [11]. The low grade/damaged longan fruits are no exclusion.

Longan (*Dimocarpus longan* Lour, syn. *Euphoria longan* Lam.) is commercial fruits in Thailand with an export value of $109 million in 2006 (Office of Agricultural Economics, Thailand, 2007) [12]. Flowers of the longan tree bloom at the end of wintertime. The duration of longan fruit harvesting is over 46 weeks in mid-summer [13]. The Peel of this fruit has brown, thin, and its brittle when it ripe. The taste of pulp longan is sweet with translucent of the flesh color. The seed is ring shape, dark black color, and white point at the base [14]. The carbohydrates of longan are in the form of fructose, glucose, and sucrose. After extraction, the main compositions of longan juice are 2.77 % glucose, 3.91 % fructose, and 14.21 % sucrose. The pH of longan juice reaches a low acidic level due to the number of organic acids compose, such as gluconic acid, malic acid, and citric acid [15].

The microbial fermentation procedure for the production of organic chemicals is greener than the chemically synthesized ones with no release of toxic fumes or chemicals in the environment [16]. Fermentation and production of ethanol from sugar (pure sugar or final product of hydrolysis) are done. For one mole of simple sugar ($C_6H_{12}O_6$) in the presence of yeast or bacteria, two moles of ethanol (C_2H_5OH) and two moles of carbon dioxide (CO_2) are produced [17]. The fermentation process takes three days and is performed at a temperature between 25 and 30 °C [4].

In recent studies, bioethanol production by utilizing the yeast brings high productivity the research of Oberoi et al. [18], the ethanol concentration from Kinnow mandarin (*Citrus reticulata*) waste at 12 h was 42 g L^{-1}, using simultaneous saccharification and fermentation with *S. cerevisiae*. Another research, production of bioethanol using pseudo banana stem by *S. cerevisiae* NCIM 3570 gave maximum ethanol (17.1 g/L) [19]. Bioethanol production with 4.1 to 7.1% was produced from the fermented banana fruit waste, which includes skin and pulp of rotten fruit become convenient for the production of bioethanol as an alternative fuel due to decrease the cost of the original steps [20]. Therefore, in this study that dried low grade and damaged longan fruits were utilized to produce bioethanol using cellulase with algal enzymes.

Methodology

Sample Collection and Material Preparation

Low grade and damaged longans fruits were collected from the Pratupa Agricultural Cooperative company located at Pratu Pa Subdistrict, Mueang Lamphun District, Lamphun 51000 (at coordinate 18°37'44.5"N 98°59'48.5"E). Collected samples were transferred to the laboratory at Faculty of Science, Maejo University, Chiang Mai, Thailand, at which the leaves, branches, dust, soil, and other impurities of low grade/damaged longans fruits were removed, was chosen to experiment. However, this material was dried at the Energy Research Center before experimenting. The chipping disk machine (multi-purpose shredder model MJU-EB8) was used to shred dried low grade/damaged longans fruits until reaching a smaller size.

Pretreatment and hydrolysis

Thermal pretreatment (boiling) was applied with 100g of dried waste longan fruits with a ratio of 1:10 w/v. The sample was boiled at 30 minutes; afterward, this mixture was undergone an autoclaving apparatus at 121°C, 15 psi, 15 mins. After pretreatment, the samples were inoculated with 1% commercial cellulase and 10% algal enzymes for the hydrolysis process. The pH of the combined solution was adjusted at 5.0 and 7.0, respectively. Then the solution was kept in a solar dryer to perform the hydrolysis process. Pretreatment and hydrolysis were carried out step by step as Figure 1.

Figure 1. Pretreatment and hydrolysis process: (A) Boiling, (B) Using autoclave, (C) Adding cellulase enzyme, (D) Adding algal enzyme, (E) Using a solar dryer

Fermentation

The hydrolysate solution was fermented with 2% (v/v) of yeast (*Saccharomyces cerevisiae* TISTR 5020) in 1000 mL fermentor. The pH of the fermented mixture was adjusted 5.6 and kept at room temperature. A small amount of sample was withdrawn every 12 hours to check sugar analysis and alcohol determination.

Sugar analysis and alcohol determination

Total sugar estimation, 0.5 mL of the sample was combined 0.5 mL of 5% phenol (w/v) and 2.5 mL of 98% H_2SO_4 by using a vortex. The solution of total sugar estimation was kept in cold water 10 minutes and use a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 490 nm [21]. Fermentable sugar (i.e., reducing sugar) estimation, the mixture was created by mixing 0.5 ml of the sample and 0.5 ml of 3, 5dinitrosalicylic acid (DNS) through a vortex. After that, the solution was boiled in water bath 15 minutes at 90°C. 4 mL of distilled water was added in this solution. A UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 540 nm was used to read the absorbance [22]. For alcohol determination, 50 mL of the sample was removed and centrifuged before using Ebulliometer (Dujardin-Salleron, Alcohol Burner, France). Then this sample was poured into in the condenser of Ebulliomter and boiled until the steady temperature. A comparison of the resulting temperature recorded and the boiling point of the distilled water was used the Ebulliomter disc.

Statistical analysis

Three replicates were conducted in the present study to report the values. The data were shown as mean ± SE from triplicate. The program SPSS 20.0 (SPSS Inc., Chicago, IL, USA) was used to analyze and statistic data. A significant difference was examined at the level of $p < 0.05$.

Results and Discussion

Influence of physical pretreatment and enzymatic hydrolysis

Peel of longan is one of the parts of this material that plays a role as lignocellulosic biomass, which includes cellulose, hemicellulose, and lignin. Therefore, pretreatment is a necessary step to enhance the accessibility of enzymes to cellulose. This is evaluated through the results of the crystalline structure was decomposed, the lignin was removed, and the amount of total and reducing sugar yields were released [23].

In this study, boiling at 30 min and an autoclave, which was set at 121°C, 15 psi, 15 mins were conducted sequentially. However, according to Gabhane et al. [24], different heating devices were combined with the severe pretreatment of conditions such as high temperature and duration, which get more

Table 1. Sugar concentration of dried longan fruits after physical pretreatment and hydrolysis

	Total sugar (g/L)	Reducing sugar (g/L)	Degree of polymerization
Pretreatment	230.70	91.11	2.53
Hydrolysis	368.42	297.78	1.24

than 30 min. It is those of reasons not only to lead to the more significant degradation of hemicellulosic sugars but also effect to reducing sugar yield due to the conversion of reducing sugar into other compounds such as Hydroxymethylfurfural (HMF) etc.

Hence, 30 min of hydrothermal pretreatment and steam explosion at 121°C, 15 psi, 15 mins are those suitable conditions to reach the high sugar yield. It was clearly shown in Table 1 with the amount of both TS and RS 230.70 ± 2.01 g/L and 91.11 ± 1.11 g/L of sugars, respectively. In addition, it is also expressed through the degree of polymerization; this number after hydrolysis is lower than after pretreatment (Table 1), according to Manmai et al. [25], the degree of polymerization index calculates the number of monosaccharides in a polymer molecule. The higher degree of polymerization of the structure needs handles to produce the simple sugars which are suitable for the fermentation process. Therefore, cellulose with algal enzymes was added within the hydrolysis process; the result of total and reducing sugar reach 368.42 ± 13.16 g/L and 297.78 ± 2.94 g/L.

Bioethanol production

Longan fruit is one of the most significant parts of the recent food industry in the world. The feasibility of bioethanol production from waste longan and low-grade fruits by enzymatic hydrolysis (1% commercial cellulase and 10% algal enzymes) and fermentation were investigated in this study. There are several fruits that several fruits have been suggested for bioethanol fermentation, including rambutan fruit biomass (skin and juice) with different pH and temperature conditions that were researched using similar yeast in this study [26]; the bioethanol highest yields were 7.5% and 9.17%, respectively. Moreover, Shubhra et al. [27] stated that different rotten fruits, such as sapota, papaya, apple, banana, and grapes, were utilized for bioethanol production; the fruits of the grape was reached the highest bioethanol concentration (8.04%) after 4 days using *S. cerevisiae*.

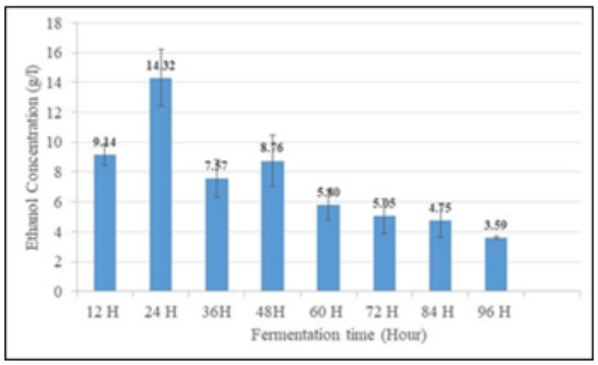


Figure 2. Bioethanol production from dried longan fruits from 96h fermentation

The product of hydrolysis was used to produce bioethanol through anaerobic respiration with 2% v/v *S. cerevisiae* (dry yeast), which was added in the fermenter for 96 h at room temperature. The ethanol concentration was checked every after 12 hours. The result of ethanol concentration was shown in Figure 2 that the highest bioethanol production reaches 14.32 ± 1.89 g/L at 24 hours; the total and reducing sugars were reduced from 103.25 ± 4.94 g/L and 63.52 ± 8.79 g/L to 92.98 ± 5.15 g/L and 55.19 ± 4.66 g/L; this is explained that *S. cerevisiae* consumed fermentable sugars to transform into alcohol, followed by 12 hours with 9.14 ± 0.71 g/L. The results show that the low grades and waste longan fruits can be a suitable feedstock for biochemical conversion into bioethanol for further use as potentially useful products such as fuel, chemical feedstock, or catalyst support.

Conclusion

Low grade and damaged longan fruits, which is agricultural waste, can be used to produce bioethanol. The hydrothermal and steam explosion was applied in the pretreatment process with significant success to enhance the accessibility of enzyme and the high sugar concentration achieved. Hence, the productivity of bioethanol production from low grade and damaged longan fruits was higher significantly.

Acknowledgments

The authors sincerely thank the School of Renewable Energy, Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai, Thailand that provided the research facilities as well as laboratory pieces of equipment during the study.

References

[1] K. Khunchit, S. Nitayavardhana, R. Ramaraj, V. K. Ponnusamy and Y. Unpaprom (2020). Liquid hot water extraction as a chemical-free pretreatment approach for biobutanol production from Cassia fistula pods. Fuel, 279: 118393. doi: 10.1016/j.fuel.2020.118393

[2] B. Saengsawang, P. Bhuyar, N. Manmai, V. K. Ponnusamy, R. Ramaraj, and Y. Unpaprom **(2020)**. The optimization of oil extraction from macroalgae, Rhizoclonium sp. by chemical methods for efficient conversion into biodiesel. Fuel, 274: 117841. doi: 10.1016/j.fuel.2020.117841

[3] Y. Unpaprom, T. Pimpimol, K. Whangchai and R. Ramaraj **(2020)**. Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer. Biomass Convers. Biorefin., doi: 10.1007/s13399-020-00850-7

[4] P. T. Vu, Y. Unpaprom and R. Ramaraj **(2017)**. Evaluation of bioethanol production from rice field weed biomass. Emergent. Life Sci. Res., **3:** 42-49.

[5] M. X. He, Q. Hu, Q. Zhu, K. Pan and Q. Li **(2015)**. The feasibility of using constructed wetlands plants to produce bioethanol. Environ. Prog. Sustain. Energy, **34:** 276-281.

[6] P. Khammee, R. Ramaraj, N. Whangchai, P. Bhuyar and Y. Unpaprom **(2020)**. The immobilization of yeast for fermentation of macroalgae *Rhizoclonium* sp. for efficient conversion into bioethanol. Biomass Convers. Biorefin., doi: 10.1007/s13399-020-00786-y

[7] P. T. Vu, Y. Unpaprom, N. Whangchai and R. Ramaraj **(2017)**. The feasibility of bioethanol production from wetland plant Cyperus difformis. The 1st Maejo - Engineo International Conference on Renewable Energy (MEICRE 2017), The Empress Hotel, Chiang Mai, Thailand, 1-12.

[8] P. T. Vu, Y. Unpaprom and R. Ramaraj **(2018)**. Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production. Bioresour. Technol., **247:** 125-130.

[9] K. Robak and M. Balcerek **(2018)**. Review of second generation bioethanol production from residual biomass. Food Technol. Biotechnol., **56:** 174-187.

[10] P. Thanarak **(2012)**. Supply chain management of agricultural waste for biomass utilization and CO_2 emission reduction in the lower northern region of Thailand. Energy Procedia, **14:** 843-848.

[11] N. Manmai, Y. Unpaprom, V. K. Ponnusamy and R. Ramaraj **(2020)**. Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation. Fuel, **278:** 118262. doi: 10.1016/j.fuel.2020.118262

[12] S. Janjai, N. Lamlert, B. Mahayothee, B.K. Bala, M. Precoppe and J. Muller **(2011)**. Thin layer drying of peeled longan (*Dimocarpus longan* Lour.). J. Food Sci. Technol., **17:** 279-288.

[13] Y. Jiang, Z. Zhang, D. C. Joyce and S. Ketsa **(2002)**. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). Postharvest Biol. Technol., **26:** 241-252.

[14] S. Lapsongphol, B. Mahayothee, S. Phupaichitkun, H. Leis, M. Haewsungcharoen, S. Janjai and J. Mueller (2007). Effect of drying temperature on changes in volatile compounds of longan (*Dimocarpus longan* Lour.) fruit. In Book of abstracts of the conference on International Agricultural Research for Development, Tropentag, Witzenhausen, Germany.

[15] S. Surin, P. Thakeow, P. Seesuriyachan, S. Angeli and Y. Phimolsiripol **(2014)**. Effect of extraction and concentration processes on properties of longan syrup. J. Food Sci. Technol., **51:** 2062-2069.

[16] P. Khammee, Y. Unpaprom, S. Buochareon, and R. Ramaraj **(2019)**. Potential of bioethanol production from marigold temple waste flowers. The 1st Thailand Biorefinery Conference "The Future of Biorefinery for Thailand 4.0". Suranaree University of Technology, Nakhon Ratchasima, Thailand, 18-23.

[17] A. B. Hossain, A. A. Saleh, S. Aishah, A. N. Boyce, P. P. Chowdhury and M. Naqiuddin **(2008)**. Bioethanol production from agricultural waste biomass as a renewable bioenergy resource in biomaterials. In 4th Kuala Lumpur International Conference on Biomedical Engineering, Springer, Berlin, Heidelberg, 300-305.

[18] H. S. Oberoi, P. V. Vadlani, A. Nanjundaswamy, S. Bansal, S. Singh, S. Kaur and N. Babbar (2011). Enhanced ethanol production from Kinnow mandarin (*Citrus reticulata*) waste via a statistically optimized simultaneous saccharification and fermentation process. Bioresour. Technol., **102:** 1593-1601.

[19] S. Ingale, S. J. Joshi and A. Gupte **(2014)**. Production of bioethanol using agricultural waste: banana pseudo stem. Braz. J. Microbiol., 45: 885-892.

[20] A. M. Alshammari, F. M. Adnan, H. Mustafa and N. Hammad **(2011)**. Bioethanol fuel production from rotten banana as an environmental waste management and sustainable energy. Afr. J. Microbiol. Res., **5:** 586-598.

[21] M. Dubois, K. A. Gilles, J. K. Hamilton, P. T. Rebers and F. Smith **(1956)**. Colorimetric method for determination of sugars and related substances. Anal. Chem., **28:** 350-356.

[22] G. L. Miller **(1959)**. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem., **31:** 426428.

[23] P. U. Amadi, M. O. Ifeanacho and E. N. Agomuo **(2018)**. The effects of different heating periods and exclusion of some fermentation conditions on bioethanol production from plantain pseudo-stem waste using the digestive juice of *Archachatina marginata*, garlic and *Saccharomyces cerevisiae*. Biofuels, **9:** 531-539.

[24] J. Gabhane, S. P. William, A. N. Vaidya, K. Mahapatra and T. Chakrabarti **(2011)**. Influence of heating source on the efficacy of lignocellulosic pretreatment–a cellulosic ethanol perspective. Biomass Bioenerg., **35:** 96-102.

[25] N. Manmai, Y. Unpaprom, and R. Ramaraj **(2020)**. Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). Biomass Conv. Bioref. doi: 10.1007/s13399-020-00602-7

[26] A. Hadeel, A. B. Hossain, K. Latifa, H. AL. Naqeb, J. Abear and A. Norah **(2011)**. Bioethanol fuel production from rambutan fruit biomass as reducing agent of global warming and greenhouse gases. Afr. J. Biotechnol., **10:** 10157-10165.

[27] T. Shubhra, S. K. Jadhav, S. Mayuri and K. L. Tiwari **(2014)**. Fermentation of waste fruits for bioethanol production. Asian J. Biol. Sci., **7:** 30-34.

Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-00977-7

ORIGINAL ARTICLE

Impact and significance of pretreatment on the fermentable sugar production from low-grade longan fruit wastes for bioethanol production

Tu Vy Thuy Nguyen [1,2] · Yuwalee Unpaprom [2,3] · Numchok Manmai [4] · Kanda Whangchai [5] · Rameshprabu Ramaraj [1,2]

Received: 2 August 2020 / Revised: 16 August 2020 / Accepted: 19 August 2020

Abstract
The feasibility of bioethanol production from low-grade longan fruit waste material was recently studied as a novel substrate for bioethanol production. First, hydrothermal pretreatment followed by enzymatic hydrolysis converted both the cellulosic and hemicellulosic biomass of the low-grade longan fruit wastes into fermentable sugars, which eventually produced ethanol by yeast fermentation. Therefore, an optimal condition of hydrothermal residence time and pretreatment concentration was determined to obtain the high release of sugars. An experimental design was constructed with the Central Composite Design (CCD) response surface method using factors of the hydrothermal residence time and pretreatment concentration in constant temperature and time.

The results revealed optimum reducing sugar yield of 240.396 g/L (R^2 = 0.9989), sugar productivity of 240.396 g/L.day (R^2 = 0.9763), and sugar yield coefficient of 24.04 g/g (R^2 = 0.9989). The highest ethanol yield (16.74 g/L) was achieved at 24 h of fermenting time. These results show that low-grade longan fruit wastes are an excellent feedstock for producing ethanol that could be either used as biofuel or as a beverage.

Keywords Low-grade longan fruit wastes · Sustainable biomass · Fermentable sugars · Bioethanol production

1 Introduction

Currently, renewable energy has become a popular solution to the energy crisis. It is the potential sustainable and clean energy. Renewable resources of energy are fighting against climate changes, and together they advance economic growth, grow the number of employed people, and afford energetic safety [1–3]. These resources are the most plentiful, available, and inexpensive materials on earth. There are some categories of renewable energy, such as solar, wind, hydro, tidal, geothermal, and biofuel [4, 5]. Biofuel is gaining global attention due to its prospect as an additive for petroleum-derived transportation fuels. One of the biofuels which are used for transportation is bioethanol [6, 7]. There are three primary raw materials for bioethanol production: sugar/starch, which is called first generation; lignocellulosic biomass, which is called second generation; and algal biomass, which is called the third generation [8–10]. Bioethanol is obtained after following pretreatment, saccharification, fermentation, and distillation steps [11]. Each step has a unique role in bioethanol production, but the essential step is pretreatment. This stage plays vital roles in the destruction of the cell wall, which includes cellulose, hemicellulose, and lignin with the tightest association, to make the next steps quicker [12].

Thailand is an agricultural country with an agricultural production [13, 14] that accounts for an estimated 9–10.5% of its GDP and is also on the way to produce biofuels from edible sources to meet the high demand of the entire nation. Low-grade or damaged longan fruits are among the available materials from agricultural activities. Specifically, Lamphun is

✉ 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 Science, Maejo University, Chiang Mai 50290, Thailand

[4] Department of Forestry, National Chung Hsing University, Taichung 402, Taiwan

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

Published online: 06 September 2020

the most famous producer of longans in northern Thailand. Longan (*Dimocarpus longan* Lour.) is a tropical tree species that produces edible fruits. Mature longan fruit has a tasty, edible, and white aril. Based on fruit weight, skin color, flesh sugar, acid concentration, sugar/acid ratio, and taste, maturity (ripeness) can be calculated [15]. The production of longans in 1998 was about 238,000 metric tons, with a planted area of 41,504 ha.

The production is confined in the northern provinces of Thailand. The leading longan-growing provinces are Lamphun, Chiang Mai, and Chiang Rai, which shared 37.6, 24.1, and 8.0% of the total planted area, respectively. In Thailand, the main longan production areas are in the northern area where the main planting areas are in Chiang Mai and Lamphun, accounting for around 75% of the whole yearly production [16]. The neighbor of Thailand is Vietnam; Vietnam is like the letter S, a long and narrow country. It is located on the eastern edge of the peninsula called Indochina in Southeast Asia, which is the market for longan processed products, likewise China, Hong Kong, Malaysia, Singapore, USA, and France. The key guidelines for the growth of longan export markets have been identified: the research of consumer demand in target markets, the growth of long-term export markets, both old and new, and the production of long-term goods as consumers demand. Moreover, longans are the third most grown fruits in Vietnam and this nation is also the world's second most significant exporter of longans with $62.13 million in 2017 [17].

The Thai government has set a goal for agricultural development orientation in the context of trade liberalization to increase productivity, yield, food security, and farmers' income; to restructure sustainable agriculture growth; and to promote biofuel production [13, 14]. Thailand's agricultural trade policy reform has increased the flow of trade between Vietnam and Thailand, as well as other Asian regions. Low-grade and damaged longan fruits were obtained from a local agricultural society economic development center. It was used as a sole feedstock for possible bioethanol production. Therefore, the present study aims to focus on fermentable sugar production from low-grade and damaged longan fruits using autoclaving pretreatment and enzyme hydrolysis process.

Thermal pretreatments have been used for lignocellulosic biomass, including heating to break it down physically at high temperatures and pressures. High-pressure thermal pretreatment is achieved in an autoclave. It is an eco-friendly method to disarrange the cell wall of biomass. Resulting after a thermal pretreatment is a more available material for enzymatic hydrolysis [18, 19]. Cellulase is the appropriate enzyme for cellulose in biomass degradation. Biological enzymatic hydrolysis is a low-cost, low-energy ingesting, and eco-friendly procedure for sugar extraction from lignocellulosic biomass [12, 20]. Separate enzymatic hydrolysis and fermentation (SHF) is a method by which enzymatic hydrolysis and fermentation are achieved successively. In this procedure, enzymatic saccharification of pretreated lignocellulosic biomass is carried out with the proper temperature of the saccharifying enzyme. Later, suitable microorganisms are added to ferment the saccharified solution. The enhancement of the SHF method is the sugar degradability and fermentation process at its own best conditions [1, 21, 22].

Subsequently, the main intentions of this research are the upgrading and converting of fruit waste from the northern part of Thailand for sugar extraction before biofuel production, as well as the comparison of the different thermal and physical pretreatments before enzymatic hydrolysis for fermentable sugar transformation. For this resolution, the impact of two parameters' diversity was studied, with thermal pretreatment time as A by physical pretreatment at different times (0, 15, and 30 min) in an autoclave at 121 °C, 15 psi, and enzymatic hydrolysis time as B by 2% of cellulase enzyme for 1, 2, and 3 days. All results of sugar extracting part were investigated on reducing sugar (g/L), reducing sugar productivity (g/L.day), and reducing sugar yield coefficient (g/g) in the attendance of the two functional parameters previously mentioned. In addition, the effect of different parameters is related to sugar production. A response surface methodology (RSM) was used to optimize the processes of pretreatment and enzyme hydrolysis before fermentation.

2 Materials and methods

2.1 Sample collection and material preparations

Low-grade and damaged longan fruits are the materials in the present study. This material was collected at the company Pratupa Agricultural Cooperative at Pratu Pa Subdistrict, Mueang Lamphun District, Lamphun 51000 (coordinates 18°37′44.5″N, 98°59′48.5″E). Commonly, longan fruits were divided into four categories (i.e., AA, A, B, C) using the sorting machine. Beyond these categories, the rest of them were characterized as low-grade and damaged longan fruits. These fruits were obtained at the back of the machine, and these propositions are displayed in Fig. 1. The low-grade and damaged longan fruits were transferred to the laboratory at Maejo University. The leaves, branches, dust, soil, and other impurities were removed prior to the experiment. The obtained longans were dried at the Energy Research Center (ERC), Maejo University, Thailand (Fig. 2).

2.2 Sample preparation for scanning electron microscopy and sugar analysis

The sample preparation for scanning electron microscopy (SEM) images of the study is in accordance with similar

Fig. 1 Different categories of longan fruits (AA, A, B, C, and low grade/wastes)

studies on pretreated biomass, and the morphological characteristics of dried longans are exhibited in Fig. 3. Overall, the sequential pretreatment applied to the biomass increases the porosity and surface area, which results in better accessibility of cellulose to enzymatic degradation. The total sugar was determined by phenol–sulfuric acid [23], and the reducing sugar was analyzed by DNS acid [24].

2.3 Pretreatment and enzyme hydrolysis

In this experiment, 10 g of the dried longans with 50 mL distilled water were boiled within 30 min, around 90–95 °C. Then these samples underwent physical pretreatment with 0, 15, and 30 min using an autoclave at 121 °C and 15 psi. After pretreatment, 2% cellulase and 0.125% Tween-20 were added in the samples; afterward, these samples were kept in the incubator for 1, 2, and 3 days at 30 °C. Subsequently, total sugar and reducing sugar were determined.

Fig. 2 The longans drying under sunlight

2.4 Optimization of pretreatments using a central composite design

Response surface methodology (RSM) with a standard procedure of central composite design (CCD) was selected to improve the experiments for sugar production runs by using the Design Expert 11 program (trial version). CCD is a significantly optimized method and developed 3D response plots between reducing sugar production, productivity, and yield coefficient responses and functioning parameters to evaluate the effect of pretreatment and hydrolysis times of functioning parameters on the response of plots. The optimum value is attained from the response surface based on the preferred output. The two factors of thermal pretreatment time (A) and enzymatic hydrolysis time (B) were utilized to detect the response on reducing sugar (g/L), reducing sugar productivity (g/L.day), and reducing sugar yield coefficient (g/g) using three levels of CCD. Table 1 presents the reducing sugar estimation parameters, and the values used for the experiment exemplify the ranges of the function of each parameter including 0 to 30 min of thermal pretreatment time and 1 to 3 days of enzymatic hydrolysis time. The high levels of the factors are coded as + 1, and the low levels are coded as − 1. Experimental designs were devolved by CCD and results of reducing sugar (g/L), reducing sugar productivity (g/L.day), and reducing sugar yield coefficient (g/g).

2.5 Sugar kinetic parameters

The kinetic parameters are calculated by reducing sugar productivity and reducing sugar yield coefficient equations (Eqs. 1 and 2) to find the reducing sugar during hydrolysis following Manmai et al. [20].

$$\text{Reducing sugar productivity}\left(\frac{\text{g}}{\text{L.day}}\right) = \frac{\text{Reducing sugar during hydrolysis}\left(\frac{\text{g}}{\text{L}}\right)}{\text{Hydrolysis time (days)}} \quad (1)$$

$$\text{Reducing sugar yield coefficient}\left(\frac{\text{g}}{\text{g}}\right) = \frac{\text{Reducing sugar during hydrolysis}\left(\frac{\text{g}}{\text{L}}\right)}{\text{Dry biomass}\left(\frac{\text{g}}{\text{L}}\right)} \quad (2)$$

2.6 Ethanol fermentation

The best product of hydrolysis, which attained the highest sugar concentration, was chosen to carry out ethanol fermentation. The Duran bottle (1000 mL) was utilized to conduct this process with 760 mL of the hydrolysates, and 2% of

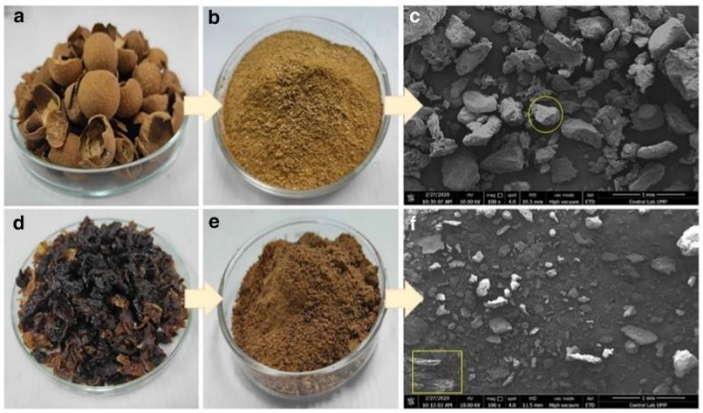


Fig. 3 Morphological characteristics of dried longan. a–c Peel, powdered peel, SEM of peel; d–f pulp, powdered pulp, SEM of pulp

Saccharomyces cerevisiae (alcohol yeast; Xinjiang Shengli Biotechnology Co., Ltd., China) was inoculated. The mixture was kept at room temperature for 4.5 days. The samples in the fermentor were collected every 12 h for sugar analysis and the percentage of ethanol using an ebulliometer (Dujardin-Salleron, Alcohol Burner, France) [6, 11].

2.7 Statistical analysis

Data are reported as mean ± SD from triplicate observations. Significant differences between means were analyzed. All statistical analyses were performed using SPSS version 20.0. A correlation was assumed significant when $P < 0.05$.

3 Results and discussion

3.1 Characteristic of longan fruit waste

Longan fruit waste is a promising carbon source for biofuel fermentation feedstock because of its abundance and low cost. Fruit waste is an attractive biomass substitute for bioethanol production as it has high levels of fermentable sugars. The results of longan nutrition were presented by Yang et al. [15]. One hundred grams of sample contains a moisture of 81.4%, total carbohydrate 12.38%, reducing sugar 3.85%, fat 0.1%, and other extractions carotene 20 µg, vitamin K 196.5 µg, retinol 3 µg, protein 1.2 g, riboflavin 0.14 mg, fiber 0.4 g, ascorbic acid 43.12–163.7 mg, nicotinic acid 1.3 mg, ash 0.7 g, and thiamine 0.01 mg.

Table 1 Reducing sugar estimation parameters and their values used for the experiment

Factors	Symbols	Unit	− 1	0	1
Thermal pretreatment time	A	Min	0	15	30
Enzymatic hydrolysis time	B	Day	1	2	3

3.2 Pretreatments and enzymatic hydrolysis

In the previous study, Manami et al. [20] presented that the highest total sugar and reducing sugar of sunflower stalk degradation by cellulase enzyme is indicated at 1 day of hydrolysis time of 143.86 ± 9.517 and 20.267 ± 3.1200.073 g/L, respectively. The total sugar yield coefficient is important at the first 24 h of 0.143 ± 0.007 g/g. In the same direction as reducing the sugar coefficient, the means were significantly different after 24 h. The highest reducing sugar coefficient was 0.073 g/g; these results were reported by Vu et al. [6].

In the present study, reducing sugar (g/L), reducing sugar productivity (g/L.day), and reducing sugar yield coefficient (g/g) are presented as actual and predicted runs in Table 2. The experimental and predicted yields were obtained by RSM for the sugar production process. The optimization of the factor for the saccharification process is thermal pretreatment time for 10 min and enzymatic hydrolysis time for 1 day. The reducing sugar was 240.396 g/L, reducing sugar productivity was 240.396 g/L.day, and reducing sugar yield coefficient was 24.040 g/g, resulting from degradation that is

Table 2 Experimental and predicted yields by RSM for sugar production process

Std	Run	Factor 1	Factor 2	Response 1		Response 2		Response 3	
		A: thermal pretreatment time (min)	B: enzymatic hydrolysis time (days)	Reducing sugar (g/L)		Reducing sugar productivity (g/L.day)		Reducing sugar yield coefficient (g/g)	
				Actual	Predicted	Actual	Predicted	Actual	Predicted
13	1	15	2	234.604	232.378	117.302	117.781	23.460	23.238
2	2	30	1	210.521	211.067	210.521	218.465	21.052	21.107
10	3	15	2	232.571	232.378	116.286	117.781	23.257	23.238
7	4	15	1	240.396	239.256	240.396	220.235	24.040	23.926
3	5	0	3	111.271	112.303	37.090	33.916	11.127	11.230
5	6	0	2	123.979	122.353	61.990	52.947	12.398	12.235
12	7	15	2	231.315	232.378	115.658	117.781	23.132	23.238
4	8	30	3	198.563	199.546	66.188	58.740	19.856	19.955
11	9	15	2	230.292	232.378	115.146	117.781	23.029	23.238
1	10	0	1	130.438	131.032	130.438	142.655	13.044	13.103
8	11	15	3	226.146	224.131	75.382	86.004	22.615	22.413
6	12	30	2	207.521	205.992	103.761	103.264	20.752	20.599
9	13	15	2	229.952	232.378	114.976	117.781	22.995	23.238

reacted entirely within 24 h in the same direction with previous studies.

3.3 Optimization on saccharification

In the present study, the factors of saccharification processes, productivity, and coefficient were statistically optimized with RSM in Eqs. 3–5 of longan waste pretreatment and hydrolysis. Their equations were expressed in terms of coded factors. It is used to forecast reducing sugar (g/L), reducing sugar productivity (g/L.day), and reducing sugar yield coefficient (g/g) for agreed levels of each factor (A, thermal pretreatment time; B, enzymatic hydrolysis time). The coded equation is beneficial for categorizing the comparative impact of the factors by comparing the factor coefficients.

$$\text{Reducing sugar}\left(\frac{\text{g}}{\text{L}}\right) = +232.38 + 41.82\text{A} - 7.56\text{B} + 1.80\text{AB} - 68.21\text{A}^2 - 0.6849\text{B}^2 \quad (3)$$

$$\text{Reducing sugar productivity}\left(\frac{\text{g}}{\text{L.day}}\right) = +117.78 + 25.16\text{A} - 67.12\text{B} - 12.75\text{AB} - 39.68\text{A}^2 + 35.34\text{B}^2 \quad (4)$$

$$\text{Reducing sugar yield coefficient}\left(\frac{\text{g}}{\text{g}}\right) = +23.24 + 4.18\text{A} - 0.7563\text{B} + 0.1802\text{AB} - 6.82\text{A}^2 - 0.0685\text{B}^2 \quad (5)$$

ANOVA analysis for the quadratic model of reducing sugar concentration, reducing sugar productivity, and reducing sugar yield coefficient are presented in Tables 3, 4, and 5. In the reducing sugar ANOVA model, the model F value of 1238.33 and P values less than 0.0500 indicate that the models are significant. The P values of sources in models A, B, and A^2 are significant model terms. On the other hand, factors of AB and B^2 values greater than 0.05 indicate that the model terms are not significant. The F value of each factor indicated that there are factors that have an effect on the decomposition of biomass into sugar, with the results shown in Table 3, and there are three factors that had a highest effect on reducing sugar concentration, which are the F values of factors A^2, A, and B of 3060.28, 2499.30, and 81.73, respectively. Not a significant lack of fit shows the fitness of the model [22]. F value and P value of lack of fit in this model of 1.39 and 0.3666 are not significant relative to the pure error. The predicted R^2 of 0.9952 was different from the adjusted R^2 of 0.9981; the alteration less than 0.2 is in reasonable agreement. The R^2 of this model is presented in Fig. 4; it was close to 1.

In the reducing sugar productivity ANOVA model, all the outcomes are presented in Table 4. The model F value of 57.72 indicates that the model is significant. There is only a 0.01% chance that an F value this large could occur due to noise. The lower P value less than 0.0500 obtained for almost all factors is significant without an AB factor. The F value of a B factor (enzymatic hydrolysis time) demonstrates the highest effectivity in reducing sugar productivity. The predicted R^2 of 0.7714 is in reasonable agreement with the adjusted R^2 of 0.9594 and R^2 close to 1 in Fig. 5.

Table 3 ANOVA for quadratic model of reducing sugar

Source	Sum of squares	d.f.	Mean square	*F* value	*P* value	
Model	25,995.54	5	5199.11	1238.33	< 0.0001	Significant
A—thermal pretreatment time	10,493.22	1	10,493.22	2499.30	< 0.0001	
B—enzymatic hydrolysis time	343.15	1	343.15	81.73	<0.0001	
AB	12.99	1	12.99	3.09	0.1220	
A^2	12,848.50	1	12,848.50	3060.28	< 0.0001	
B^2	1.30	1	1.30	0.3086	0.5959	
Residual	29.39	7	4.20			
Lack of fit	15.02	3	5.01	1.39	0.3666	Not significant
Pure error	14.37	4	3.59			
Cor total	26,024.93	12				
SD	2.05		R^2	0.9989		
Mean	200.58		Adjusted R^2	0.9981		
CV %	1.02		Predicted R^2	0.9952		
			Adeq precision	91.1995		

Table 5 lists the results of ANOVA, the *F* value, and *P* value in order to estimate the numerical significance of the partial quadratic model. The *F* value and *P* value of 1238.33 and lower than 0.05, respectively, have a high statistical significance. The *F* value of each factor in this model is in the same direction as the reducing sugar ANOVA model. The value of the fortitude coefficient R^2 (0.9989) is presented in Fig. 6; the representation of 99.89% of the variation in the average reducing sugar coefficient is connected to the two free factors. The statistical accuracy value of the adjusted R^2 of 0.9981 and the predicted R^2 of 0.9952 is in reasonable agreement.

The relation between the free factors is A and B with the response Y (reducing sugar, reducing sugar productivity, and reducing sugar yield coefficient). It is evaluated using the contour and response surface plots generated from the predicted model in reducing sugar, reducing sugar productivity, and reducing sugar yield coefficient coded equations. The optimized conditions for saccharification were reducing sugar and reducing sugar yield coefficient. Both results are the same as the direction presented in Figs. 7, 8, 11, and 12. The highest optimal reducing sugar and reducing sugar yield coefficient are predicted and shown in range of pretreatment time at 15 to 24 min and enzymatic hydrolysis time for 1–1.5 days (Figs. 7, 8, 9, 10, 11, and 12).

Table 4 ANOVA for quadratic model of reducing sugar productivity

Source	Sum of squares	d.f.	Mean square	*F* value	*P* value	
Model	37,143.88	5	7428.78	57.72	< 0.0001	Significant
A—thermal pretreatment time	3797.72	1	3797.72	29.51	0.0010	
B—enzymatic hydrolysis time	27,027.21	1	27,027.21	209.99	< 0.0001	
AB	649.88	1	649.88	5.05	0.0595	
A^2	4347.66	1	4347.66	33.78	0.0007	
B^2	3449.07	1	3449.07	26.80	0.0013	
Residual	900.97	7	128.71			
Lack of fit	897.38	3	299.13	333.12	< 0.0001	Significant
Pure error	3.59	4	0.8979			
Cor total	38044.84	12				
SD	11.35		R^2	0.9763		
Mean	115.78		Adjusted R^2	0.9594		
CV %	9.80		Predicted R^2	0.7714		
			Adeq precision	24.1740		

Table 5 ANOVA for quadratic model of reducing sugar yield coefficient

Source	Sum of squares	d.f.	Mean square	*F* value	*P* value	
Model	259.96	5	51.99	1238.33	< 0.0001	Significant
A—thermal pretreatment time	104.93	1	104.93	2499.30	< 0.0001	
B—enzymatic hydrolysis time	3.43	1	3.43	81.73	< 0.0001	
AB	0.1299	1	0.1299	3.09	0.1220	
A^2	128.48	1	128.48	3060.28	< 0.0001	
B^2	0.0130	1	0.0130	0.3086	0.5959	
Residual	0.2939	7	0.0420			
Lack of fit	0.1502	3	0.0501	1.39	0.3666	Not significant
Pure error	0.1437	4	0.0359			
Cor total	260.25	12				
SD	0.2049		R^2	0.9989		
Mean	20.06		Adjusted R^2	0.9981		
CV %	1.02		Predicted R^2	0.9952		
			Adeq precision	91.1995		

On the other hand, the optimization resulting in reducing sugar productivity is shown in Figs. 9 and 10, with the range of the pretreatment time at 15 to 30 min and the enzymatic hydrolysis time for 1 day. It can be used to produce the highest saccharification productivity significantly. In an antecedent study of Ramaraj and Umpaprom [22], they reported the highest sugar yield coefficient from hydrolysis of pretreated small-flowered nutsedge by using cellulase enzyme for 24 h. The results were reported as total sugar and reducing sugar yield coefficients of 0.196 ± 0.006 and 0.094 ± 0.001, respectively. Based on the design of the experiment methodology, dried low-grade longan fruit wastes were studied, and the operating conditions were optimized. Strong interactions between parameters on fermentable sugars and solid residue yield were found during pretreatment and hydrolysis.

3.4 Bioethanol production from low-grade and damaged longan fruit wastes using *S. cerevisiae*

Bioethanol production from low-grade and damaged longan fruit wastes by fermentation using *S. cerevisiae* is

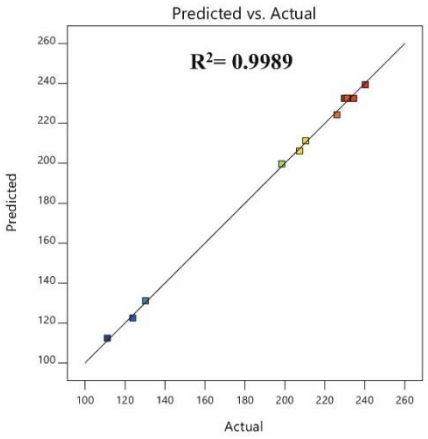


Fig. 4 Predicted vs. actual plot of reducing sugar

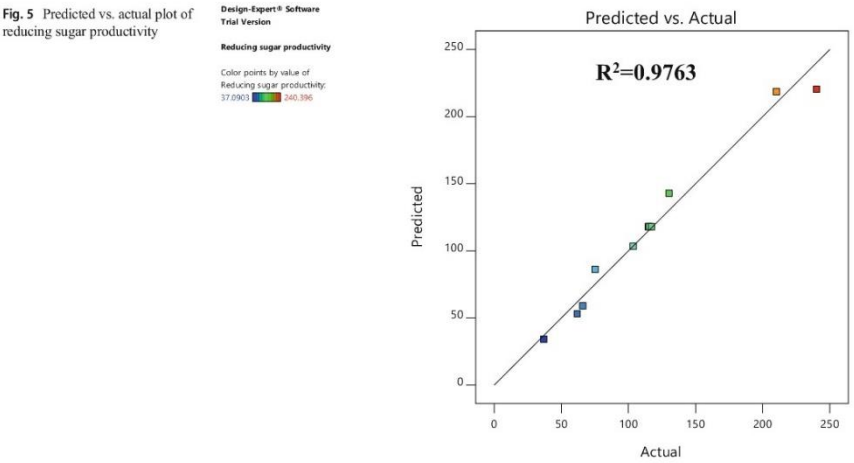


Fig. 5 Predicted vs. actual plot of reducing sugar productivity

given in Fig. 13. *S. cerevisiae* showed efficient propagation in sesame biomass for the initial 24 h, which later become stationary to 108 h. We observed that the volume of bioethanol increased with the decrease in particle size. In a similar fermentation scheme (separate hydrolysis and fermentation), ethanol concentration from low-grade and damaged longan fruits exceeded other studies. Banoth et al. [25] examined the improvement of bioethanol production using physicochemical pretreatment on rice straw, which produced 26.12 g/L of ethanol. Interestingly, the ethanol concentration of low-grade and damaged longan fruit residue is higher than other waste biomass.

The higher concentration of alcohol of ethanol produced in the present study is due to the significant amount

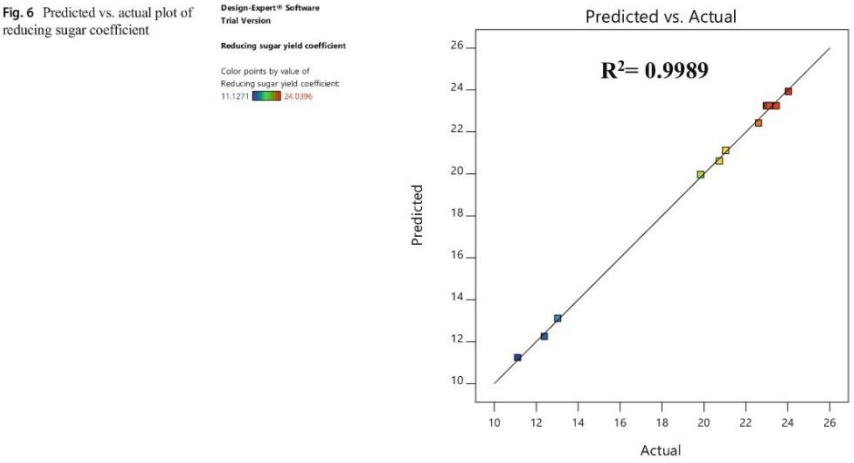


Fig. 6 Predicted vs. actual plot of reducing sugar coefficient

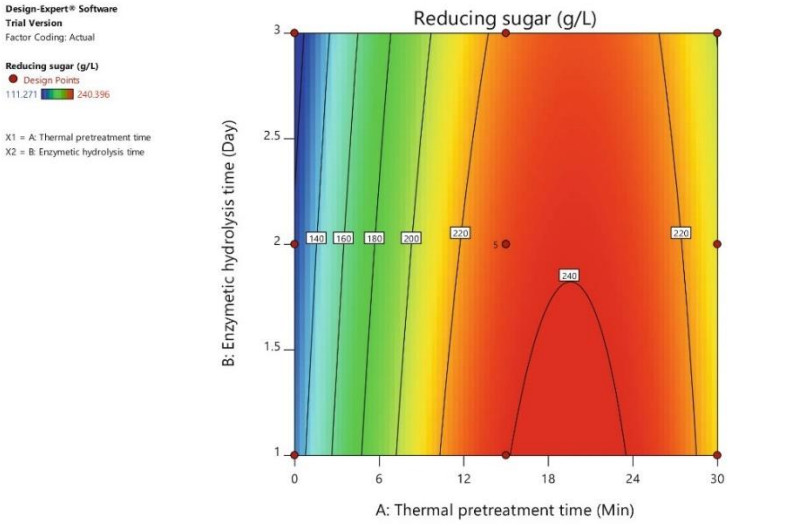
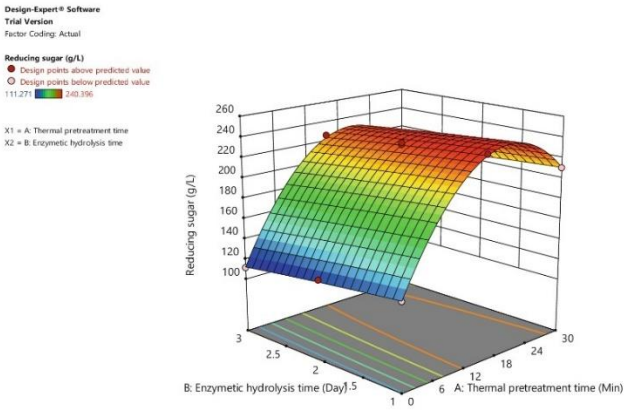


Fig. 7 2D contour plot of reducing sugar

of reducing sugars hydrolyzed after the application of pre-hydrothermal treatment and post-oven acid treatment. According to Wang et al. [26], the higher sugar content the materials has, the higher ethanol yield is expected. In the present study, it is notable that the reducing sugar was relatively high and therefore resulted in high ethanol from

Fig. 8 3D diagram plot of reducing sugar



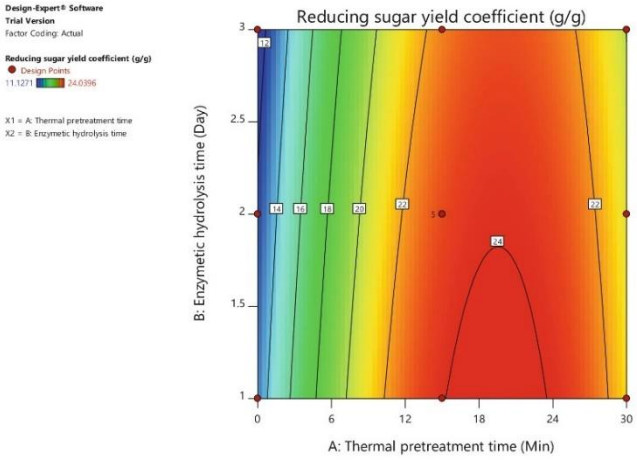


Fig. 11 2D contour plot of reducing sugar coefficient

fermentation. More importantly, the study infers the viability of low-grade and damaged longan fruit wastes to produce bioethanol and thus presenting waste to the energy concept. The low-grade and damaged longan fruit wastes appear to be a promising and potential feedstock for the production of bioethanol due to its plentiful availability and attractive composition.

4 Conclusions

Sugar extraction of longan fruit wastes has considered the effect of pretreatment time interacts with enzymatic hydrolyzing time in thermal and pressure pretreatments and cellulase enzyme hydrolysis on reducing sugar, reducing sugar productivity, and reducing sugar yield coefficient during

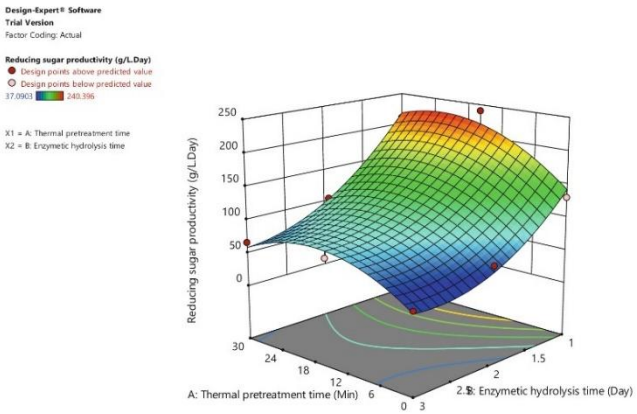


Fig. 10 3D diagram plot of reducing sugar productivity

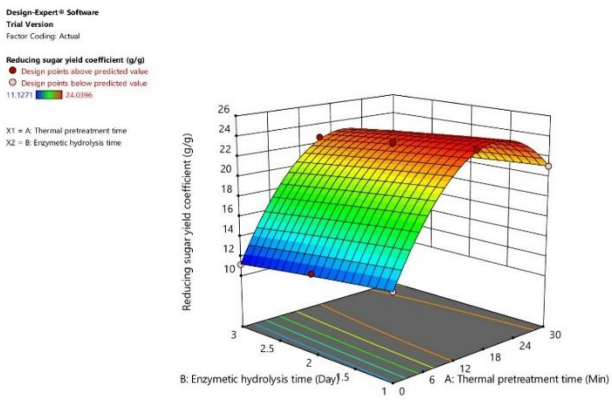


Fig. 12 3D diagram plot of reducing sugar coefficient

saccharification process before fermentation. The process of optimization was attained using response surface methodology and central composite design. The maximum reducing sugar concentration of the longan fruit waste model was attained at the pretreatment time of 15 min and hydrolysis time of 24 h. In addition, the highest ethanol concentration at 48 h was by fermentation with the free cell of *S. cerevisiae* TISTR 5020 from 108 h. Consequently, the results of the present study suggest that it is possible to achieve stable operation using dried low-grade longan fruit wastes as a sole substrate for bioethanol production in pilot- or large-scale biogas plants in the future.

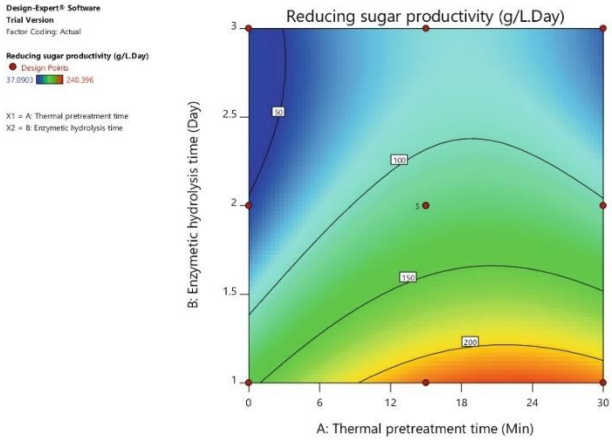


Fig. 9 2D contour plot of reducing sugar productivity

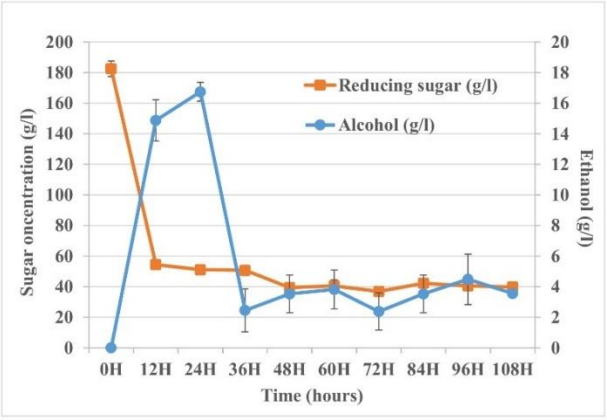


Fig. 13 Trends of final ethanol concentration and reducing sugar consumption after 108 h of fermentation

Acknowledgment

The authors gratefully acknowledge the School of Renewable Energy, Program in Biotechnology, and Energy Research Center, Maejo University, Chiang Mai, Thailand, for the research facilities to accomplish this experimental study. The authors also thank the Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai, Thailand for additional experimental support.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflicts of interest.

References

1. Khammee P, Ramaraj R, Whangchai N, Bhuyar P, Unpaprom Y (2020) The immobilization of yeast for fermentation of macroalgae *Rhizoclonium* sp. for efficient conversion into bioethanol. Biomass Convers Bioref. https://doi.org/10.1007/s13399-020-00786-y
2. Van Tran G, Unpaprom Y, Ramaraj R (2019) Methane productivity evaluation of an invasive wetland plant, common reed. Biomass Convers Bioref. https://doi.org/10.1007/s13399-019-00451-z
3. Saengsawang B, Bhuyar P, Manmai N, Ponnusamy VK, Ramaraj R, Unpaprom Y (2020) The optimization of oil extraction from macroalgae, *Rhizoclonium* sp. by chemical methods for efficient conversion into biodiesel. Fuel 274:117841
4. Ramaraj R, Unpaprom Y, Dussadee N (2016) Cultivation of green microalga, *Chlorella vulgaris* for biogas purification. IJNTR 3: 117–122
5. Ramaraj R, Kawaree R, Unpaprom Y (2016) Direct transesterification of microalga *Botryococcus braunii* biomass for biodiesel production. Emerg Life Sci Res 2:1–7
6. Vu PT, Unpaprom Y, Ramaraj R (2017) Evaluation of bioethanol production from rice field weed biomass. Emerg Life Sci Res 3:42–49
7. Bhuyar P, Sathyavathi S, K Math R, Pragas Maniam G, Govindan N (2020) Production of bioethanol from starchy tuber (*Amorphophallus commutatus*) and antimicrobial activity study of its extracts. Afr J Biol Sci 2:70–76
8. Wannapokin A, Ramaraj R, Whangchai K, Unpaprom Y (2018) Potential improvement of biogas production from fallen teak leaves with co-digestion of microalgae. 3 Biotech 8:1–18
9. Unpaprom Y, Pimpimol T, Whangchai K, Ramaraj R (2020) Sustainability assessment of water hyacinth with swine dung for biogas production, methane enhancement, and biofertilizer. Biomass Convers Bioref. https://doi.org/10.1007/s13399-020-00850-7
10. Chuanchai A, Ramaraj R (2018) Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and biofertilizer. 3 Biotech 8(3):151
11. Vu PT, Unpaprom Y, Ramaraj R (2018) Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production. Bioresour Technol 247:125–130
12. Manmai M, Bautista K, Unpaprom Y, Ramaraj R (2019) Optimization of combined pre-treatments on sugarcane leaves for bioethanol production. Maejo Int J Energy Environ Commun 1(1): 30–39
13. Dussadee N, Reansuwan K, Ramaraj R (2014) Potential development of compressed biomethane gas production from pig farms and elephant grass silage for transportation in Thailand. Bioresour Technol 155:438–441
14. Dussadee N, Ramaraj R, Cheunbarn T (2017) Biotechnological application of sustainable biogas production through dry anaerobic digestion of Napier grass. 3 Biotech 7:47
15. Yang B, Jiang Y, Shi J, Chen F, Ashraf M (2011) Extraction and pharmacological properties of bioactive compounds from longan

(*Dinocarpus longan* Lour.) fruit—a review. Food Res Int 44(7): 1837–1842
16. Sopadang A, Tippayawong KY, Chaowarut W (2012) Application of value chain management to longan industry. AJABS 7(3):301–311
17. Tran H, Van HN, Muniappan R, Amrine J, Naidu R, Gilbertson R, Sidhu J (2019) Integrated pest management of longan (Sapindales: Sapindaceae) in Vietnam. J Integr Pest Manag 10(1):1–10
18. Debiagi F, Madeira TB, Nixdorf SL, Mali S (2020) Pretreatment efficiency using autoclave high-pressure steam and ultrasonication in sugar production from liquid hydrolysates and access to the residual solid fractions of wheat bran and oat hulls. Appl Biochem Biotechnol 190(1):166–181
19. Khunchit K, Nitayavardhana S, Ramaraj R, Ponnusamy VK, Unpaprom Y (2020) Liquid hot water extraction as a chemical-free pretreatment approach for biobutanol production from *Cassia fistula* pods. Fuel 279:118393
20. Manmai N, Unpaprom Y, Ramaraj R (2020) Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM). Biomass Convers Bioref. https://doi.org/10.1007/s13399-020-00602-7
21. Ramaraj R, Unpaprom Y (2019) Enzymatic hydrolysis of small-flowered nutsedge (*Cyperus difformis*) with alkaline pretreatment for bioethanol production. Maejo Int J Sci Technol 13(2):110–120
22. Ramaraj R, Unpaprom Y (2019) Optimization of pretreatment condition for ethanol production from *Cyperus difformis* by response surface methodology. 3 Biotech 9(6):218
23. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3):350–356
24. Miller GAIL (1959) Use of dinitrosalicylic acid reagent for detection of reducing sugars. Anal Chem 31(3):426–428
25. Banoth C, Sunkar B, Tondamanati PR, Bhukya B (2017) Improved physicochemical pretreatment and enzymatic hydrolysis of rice straw for bioethanol production by yeast fermentation. 3 Biotech 7:334
26. Wang D, Bean S, McLaren J, Seib P, Madl R, Tuinstra M, Shi Y, Lenz M, Wu X, Zhao R (2008) Grain sorghum is a viable feedstock for ethanol production. J Ind Microbiol Biotechnol 35:313–320

Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-01176-0

ORIGINAL ARTICLE

Physical pretreatment and algal enzyme hydrolysis of dried low-grade and waste longan fruits to enhance its fermentable sugar production

Tu Vy Thuy Nguyen[1,2] · Yuwalee Unpaprom[2,3] · Kanokwan Tandee[4] · Kanda Whangchai[5] · Rameshprabu Ramaraj[1,2]

Received: 9 October 2020 / Revised: 16 November 2020 / Accepted: 19 November 2020

Abstract
Fruit production in Thailand has been increasing due to the practical farmers' knowledge of agroecology and sustainable farming. Thailand is one of the productions of the most massive fruit in southeast Asian countries. On the other hand, the status of fruit waste, which becomes stuck previous to landfills, is concerned by researchers over the years. Low-grade and waste longan fruits are also no exceptions that are feedstock material for bioethanol production. Accordingly, this study aims to evaluate bioethanol production from dried low-grade and waste longan fruits by using physical pretreatment (boiling and autoclave) and blue-green algal enzymes for hydrolysis. After pretreatment, total and reducing sugar was 227.63 ± 2.63 g/L and 89.26 ± 1.70 g/L, respectively. Algal enzymes were added at a pH of 7.0 in the hydrolysis process; subsequently, total and reducing sugar were achieved 348.68 ± 3.95 g/L and 183.33 ± 14.70 g/L, respectively. The present study shows that using algal enzymes in the hydrolysis process improves fermentable sugar production and applicable for bioethanol production.

Keywords Low-grade longan fruits · Damaged longan fruits · Pretreatment · Algal enzymes · Fermentable sugars

1 Introduction

Nowadays, alternative energy plays an essential energy demand of human in the world. For example, some alternative energy, methanol, biobutanol, biodiesel, biogas, and hydrogen, increased interest in the improvement and development of technologies' production and sourced from natural resources. One biofuel can resolve bioethanol's energy crisis due to this liquid biofuel, for example, octane number (108), evaporation enthalpy, flame speed, and a more extensive range of flammability. Therefore, bioethanol's blended capability with gasoline or diesel at a higher compression ratio and shorter burning time can produce a new solution to decrease the pollution for the environment and raise the engine performance [1–3]. Bioethanol can be produced from biomass through microbial fermentation by converting fermentable sugar to ethanol in anaerobic conditions [4, 5].

Generally, biomass goes through pretreatment because of its complex structure. Therefore, pretreatment is one of the main steps in the fermentation process. Several types of pretreatments can be applicable, including physical, chemical, physicochemical, and biological treatments and a combination of these treatments [6]. The benefits that pretreatment brings to degrade crystallinity into the amorphous structure of cellulose that means to break of cellulose structure in biomass to help enzyme easily attack in the next process, which was called hydrolysis, because the formation of sugars was produced directly or subsequently or limit the formation of inhibitory products [7]. Third-generation biofuel, which can be produced as the most widely used transport biofuel worldwide, is microalgae. It is one of the feedstocks for bioethanol production due to low cost and high energy. Microalgae can also resolve the conflict of edible materials for human consumption and industrial purposes in bioethanol production. Nowadays,

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

[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 Science, Maejo University, Chiang Mai 50290, Thailand

[4] Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai 50290, Thailand

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

Published online: 24 November 2020

Biomass Conversion and Biorefinery
https://doi.org/10.1007/s13399-020-01176-0

ORIGINAL ARTICLE

Physical pretreatment and algal enzyme hydrolysis of dried low-grade and waste longan fruits to enhance its fermentable sugar production

Tu Vy Thuy Nguyen[1,2] · Yuwalee Unpaprom[2,3] · Kanokwan Tandee[4] · Kanda Whangchai[5] · Rameshprabu Ramaraj[1,2]

Received: 9 October 2020 / Revised: 16 November 2020 / Accepted: 19 November 2020

Abstract
Fruit production in Thailand has been increasing due to the practical farmers' knowledge of agroecology and sustainable farming. Thailand is one of the productions of the most massive fruit in southeast Asian countries. On the other hand, the status of fruit waste, which becomes stuck previous to landfills, is concerned by researchers over the years. Low-grade and waste longan fruits are also no exceptions that are feedstock material for bioethanol production. Accordingly, this study aims to evaluate bioethanol production from dried low-grade and waste longan fruits by using physical pretreatment (boiling and autoclave) and blue-green algal enzymes for hydrolysis. After pretreatment, total and reducing sugar was 227.63 ± 2.63 g/L and 89.26 ± 1.70 g/L, respectively. Algal enzymes were added at a pH of 7.0 in the hydrolysis process; subsequently, total and reducing sugar were achieved 348.68 ± 3.95 g/L and 183.33 ± 14.70 g/L, respectively. The present study shows that using algal enzymes in the hydrolysis process improves fermentable sugar production and applicable for bioethanol production.

Keywords Low-grade longan fruits · Damaged longan fruits · Pretreatment · Algal enzymes · Fermentable sugars

1 Introduction

Nowadays, alternative energy plays an essential energy demand of human in the world. For example, some alternative energy, methanol, biobutanol, biodiesel, biogas, and hydrogen, increased interest in the improvement and development of technologies' production and sourced from natural resources. One biofuel can resolve bioethanol's energy crisis due to this liquid biofuel, for example, octane number (108), evaporation enthalpy, flame speed, and a more extensive range of flammability. Therefore, bioethanol's blended capability with gasoline or diesel at a higher compression ratio and shorter burning time can produce a new solution to decrease the pollution for the environment and raise the engine performance [1–3]. Bioethanol can be produced from biomass through microbial fermentation by converting fermentable sugar to ethanol in anaerobic conditions [4, 5].

Generally, biomass goes through pretreatment because of its complex structure. Therefore, pretreatment is one of the main steps in the fermentation process. Several types of pretreatments can be applicable, including physical, chemical, physicochemical, and biological treatments and a combination of these treatments [6]. The benefits that pretreatment brings to degrade crystallinity into the amorphous structure of cellulose that means to break of cellulose structure in biomass to help enzyme easily attack in the next process, which was called hydrolysis, because the formation of sugars was produced directly or subsequently or limit the formation of inhibitory products [7]. Third-generation biofuel, which can be produced as the most widely used transport biofuel worldwide, is microalgae. It is one of the feedstocks for bioethanol production due to low cost and high energy. Microalgae can also resolve the conflict of edible materials for human consumption and industrial purposes in bioethanol production. Nowadays,

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

[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 Science, Maejo University, Chiang Mai 50290, Thailand

[4] Faculty of Engineering and Agro-Industry, Maejo University, Chiang Mai 50290, Thailand

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

Published online: 24 November 2020

researchers are going to keep their eyes to extract cellulases using algal biomass. Because the cost of production can be reduced by 1000–5000 times [8], this study applied algal enzyme for hydrolysis. For producing bioethanol, agricultural substrates and crop residues are cheapest and more natural to access feedstock.

In this study, blue-green algae, known as *Cyanobacteria*, were used as the enzyme in hydrolysis. Blue-green algae are photoautotrophs because they utilize water as an electron donor and have the two photopigments which contain chlorophyll α and β-carotene of plant photosynthesis. Several enzymes such as phosphatase, arylsulfatase, chitinase, L-asparaginase, L-glutaminase, amylase, protease, and lipase cellulase, urease, and lactamase were detected in *Cyanobacteria* by some studies [9, 10]. In nature, species of blue-green algae produce serious nuisance bloom phenomenon to humans and animals. However, the isolation, purification, and culture of blue-green algae under controlled laboratory conditions bring beneficial research that can be applied in many fields [11]. One of these is utilizing as an enzyme in hydrolysis.

On the other hand, there are various lignocellulosic biomass and abundantly available low-grade fruits and fruit wastes with a high sugar content that can be evaluated for bioethanol production. Longan (*Dimocarpus longan* Lour.) trees have many distinct benefits over traditional crops, such as its fruits have high fermentable sugars. It originated from the *Sapindaceae* family, Southeast Asia, where longan production is the most in the world has a tropical zone. Notably, the biggest exporter is currently Thailand, followed by Vietnam (Table 1).

Longan fruit has a thin, light brown color, spherical in shape, leathery edible white aril. Those fruits are not easily preserved at normal conditions after harvesting, only stored in a cold environment (1–5 °C for about 30 days) [11–13]. Therefore, much low-grade and damaged fruits are appropriate for utilizing bioethanol production. Consequently, this study examined dried low-grade/damaged longan fruits to produce bioethanol using blue-green algal enzymes.

Table 1 Planted area, production, and export of longan fruit in the major producing countries

Country	Year	Planted area (ha)	Production (metric ton)	Export (%)
China	1997	444,400	495,800	–
Taiwan	1998	11,808	53,385	–
Thailand	1997	41,434	227,979	50
Vietnam	1999	41,000	365,000	10

2 Materials and methods

2.1 Material preparation

Low-grade and damaged longans, which were utilized as the materials in this study, were collected at Pratupa Agricultural Cooperative Company, Mueang Lamphun District, Lamphun 51000, Chiang Mai, Thailand (18° 37′ 44.5″ N 98° 59′ 48.5″ E); low-grade and damaged longan size was measured after collecting at the company (Fig. 1). Energy Research Center (ERC), Maejo University, Chiang Mai, Thailand, where these materials were dried by sunshine in 1 week until dry. After that, the purpose of the size reduction material was conducted by the shredder machine. Subsequently, mortar and pestle were used again to get the smaller material around sieve 3 to 10 mm (Fig. 2).

2.2 Physical pretreatment

One hundred grams of sample and 1000 ml distilled water were boiled for 30 min in a straight-sided saucepan and electric stove, and the autoclave was carried out at temperature 121 °C, 15 psi in 15 min. Ten milliliters of the pretreatment samples was obtained to estimate the total sugar by phenol-sulfuric acid [14] and reducing the sugar by DNS acid [15].

2.3 Enzymatic hydrolysis

Afterward, the hydrolysis procedure was completed, with pouring 20% algae in the sample at a pH of 7.0. The mixture was kept in a solar dryer for 24 h for the hydrolysis process. The total and reducing sugars were determined by the equivalent of methods [14, 15].

2.4 Mass balance equations

The entire pretreatment and hydrolysis stages were analyzed mass balances by transforming sugar compounds by the below equations. The carbon mass balance of sugar is one crucial factor because its high content accounts for biomass [16].

$$\text{Xylan recovery}\ (\%) = \frac{\text{Xylan in pretreated solid}\ (\text{g})}{\text{Xylan in untreated solid}\ (\text{g})} \times 100\%$$

$$\text{Xylose yield}\ (\%) = \frac{\text{Xylose in hydrolysate}\ (\text{g})}{\text{Xylan in untreated solid}\ (\text{g})} \times \frac{132}{150}$$

The polysaccharides are converted to monosaccharide equivalent by multiplying the ratio of the molecular weights of polysaccharides to monosaccharide or fermentable sugars

Fig. 1 Low-grade and damaged longan size was measured after collecting at the company

(for example, represent C_5 sugars: the ratio of the molecular weights of xylan converted to xylose 132/150; represent C_6 sugars: the ratio of the molecular weights of glucan to glucose 162/180). The oligomer is reported in equivalents.

2.5 Ethanol fermentation

Two percent of *S. cerevisiae* (Alcohol Yeast, Xinjiang Shengli Biotechnology Co., Ltd., China) was inoculated in the hydrolysates [17, 18]. Fermentation was done in 1000-mL Duran bottles, in which 800 mL was fermented. pH in the fermentation process was reduced from 7.0 to 5.6. The mixture was kept at room temperature for 4 days. After 12 h, 50 mL aliquots of fermented samples were obtained to determine the ethanol concentration using an Ebulliometer (Dujardin-Salleron, Alcohol Burner, France) [19].

2.6 Sugar analysis and determination of bioethanol

For total sugar (TS) analysis, 1 mL of the sample was diluted at dilution 1:1000. Then, the mixture of 0.5 mL from dilution 1:1000 was got out to combine 0.5 mL of 5% phenol (*w/v*) and 2.5 mL of 98% H_2SO_4 using a vortex to ensure the mixture was mixed well. This mixture was kept in cold water within 10 min and checked the TS level using a UV spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 490 nm [14].

On the other hand, in reducing sugar (RS) analysis, 1 mL of the sample was diluted at dilution 1:100. Then, 0.5 mL from dilution 1:100 was got out to combine 0.5 mL of 3,5-dinitrosalicylic acid (DNS) through a vortex. Afterward, this mixture was boiled in a water bath for 15 min at 90–95 °C. Before checking RS, 4 mL of distilled water was added. Five hundred forty nanometer was set on a UV spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) to read the absorbance [15].

For bioethanol determination, Ebulliometer (Dujardin-Salleron, Alcohol Burner, France) was utilized to check bioethanol alcohol content. However, 50 mL of the sample was withdrawn and centrifuged using a centrifuge machine (4 °C, 1000 rpm, 15 min). The condenser of Ebulliomter, where this sample was poured and boiled until the steady temperature showing in the thermometer of Ebulliomter, has a cylinder shape [19, 20].

2.7 Scanning electron microscope

The cell wall structure of biomass was affected by the pretreatment process. There is a possibility that the crystalline structure of biomass was changed into a non-crystalline structure after this process. A scanning electron microscope (SEM) scans the surface with a focused beam of electrons through interaction between the electrons and atoms in the sample produce surface topography and composition under images. The machine branched JSM-5410LV, USA. The sample was prepared before and after the pretreatment process as a dried powder. Pure gold and dried by a dryer (CPO 7501 Critical Point Dryer, USA) was utilized 150 s at 15 mA. Gold coating plays a role important in conductivity properties for biomass. The qualities of images and interruption of the vacuum were affected by the amount of water in the biomass. Therefore, the powdered biomass is needed to be carefully dried. An electron beam at 15 kV was used to shot gold and samples inside the specimen chamber.

Fig. 2 **a** Shredder machine. **b** The samples after using the shredder machine. **c** Mortar and pestle. **d** The smaller size samples

2.8 Statistical analysis

Data were reported as mean ± SE from triplicate observations. Significant differences between means were analyzed. All statistical analyses were performed using SPSS Version 20.0. A correlation was assumed significant when $P < 0.05$.

2.9 Formula

Fermentation was calculated using the equation [20].

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

where

Q_P the volumetric ethanol productivity (g/L/h),
P the final ethanol concentration (g/L),
t the time of fermentation (h).

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

where

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

3 Results and discussion

3.1 Composition of low-grade and damaged longan fruits

Low-grade and damaged longan fruits become a promising material for biofuel, especially bioethanol. The compositions of longan fruit, which contains total carbohydrate 12.38–22.55 (g/100 g), reducing sugar 3.85–10.16 (%), protein 1.2 (g/100 g) that are high, are suitable for the production of bioethanol from the created high fermentable sugar level. Moreover, some of the other compositions of longan fruit such as moisture 81.4%, ash 0.7 (g/100 g), and fiber 0.4 (g/100 g) also affect bioethanol production.

3.2 Effect of physical pretreatment

In this study, dried low-grade and damaged longan fruits were treated at 100 °C temperature at a heating time of 30 min. Temperature and duration are not suitable for bringing unwanted by-products in the experiment process [21–23]. For example, temperature and duration in the experiment of hydrothermal pretreatment are too severe such as time more than 30 min or combination of the distinctive heating processes lead to needless compounds, which was called HMF, or the more significant degradation of hemicellulosic sugars [24, 25]. The hydrothermal pretreatment mainly affects the biomass composition partially, but substantial removal of hemicelluloses as the hydronium ion from the water of hydrothermal pretreatment causes hemicellulose depolymerization. Consequently, the overall cellulose content increases lead to produces solid (rich in C-6 sugars) and liquid fractions (rich in C-5 sugars) from the few short glucose chains present in the amorphous section of cellulose bonded to crystalline cellulose through hydrogen bonding [26, 27].

The steam explosion was carried out with specific effects such as chemical composition changes of material, practical accessible enzymatic in saccharification, and the ability of released sugar for fermentation [28]. After the samples underwent this method, the percentages of cellulose are higher; simultaneously, the percentages of hemicellulose are lower than raw samples; that means steam pretreatment removes hemicellulose, degrades lignin, and increases cellulose content [29]. Moreover, time in steam pretreatment is an essential factor. Also, the steam pretreatment at 60 min reduced hemicellulose and lignin percentage compared with 15 min [30–32]. According to Nguyen et al. [23], based on response surface methodology (RSM), the highest optimal reducing sugar and reducing sugar yield coefficient are predicted and shown in the range of autoclave time at 15 to 24 min. Therefore, Fig. 3 shows the result of sugar analysis after pretreatment and hydrolysis processes. After pretreatment, total sugar and reducing sugar were reached 227.63 ± 2.63 g/L and 89.26 ± 1.70 g/L. Fifteen minutes is the best time to conduct a steam explosion.

3.3 Significant influence of physical pretreatment on the structure of dried longan fruits

SEM analysis was conducted to study the effect of physical pretreatments on the morphology changes in longan fruits

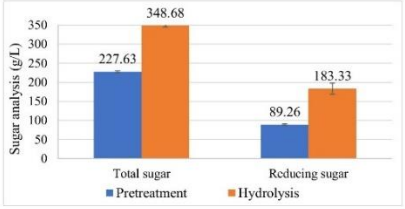


Fig. 3 Sugar analysis after pretreatment and hydrolysis

before and after physical pretreatment by SEM images taken at ×500 magnification. For the original material, the morphology surface of the longan cell cluster was covered by a polysaccharide layer that contains a complex closed ring net structure, which is shown in Fig. 4a that can be limited to the attacked ability of enzymes to change cellulose or polysaccharide molecule to fermentable sugars [33]. However, hydrothermal pretreatment methods had made the longan more susceptible to enzymatic attacks by altering the structures, and increasing the porosity of morphology leads to some opened holes of cells. The cell walls were disrupted, allowing the creation of holes with deep and length scales that lead to the surface running loose. It can be observed in Fig. 4b. Autoclaving pretreatment was second handled time about hydrothermal to change the more massive structure. The net structure became simple, and all upper parts of the cell walls were disappeared, and blocks were exposed clearly, as shown in Fig. 4c [34].

3.4 Effect of enzymatic hydrolysis

The pretreated samples are successful in 30 min of boiling and 15-min autoclave to bring total sugar and reduce sugar. The pretreated samples were inoculated 20% blue-green algae in the hydrolysis process. The hydrolysis process is responsible for the degradation of hemicelluloses and celluloses present in the pretreated material. This process transfers a macromolecule into monosaccharides or simple sugars like glucose connected by β (1–4) glycosidic linkages in the sample exhaustively. Moreover, the degradation of the crystallinity of cellulose and hemicellulose will be changed into an amorphous structure [35, 36]. Based on the reaction, these enzymes are generally termed as endoglucanase, exoglucanase, and cellobiase. The randomly cellulose chains were attacked by the endoglucanase to form glucose, cellobiose, and cellotriose. After that, the exoglucanase attacks the non-reducing end of the cellulose to get the cellobiose units. Finally, cellobiase achieved D-glucose converts cellobiose through yeasts or bacteria into ethanol [37].

Table 2 Compositions of solid fractions and components

Compositions of solid fraction (%)					
Glucan	Xylan	Fructan	Arabian	Mannan	Galactan
40	5	25	35	10	12.5
Compositions of hydrolysates (g/g)					
Glucose	Xylose	Fructose	Arabinose	Mannose	Galactose
10.58	2.85	6.48	8.75	3.09	3.95

The bacterial cell membrane in eukaryotes covered by these membrane-bound organelles carried out all functions such as the secretory and protein synthesis. *Cyanobacteria* cells are single-celled, and so have a more straightforward structure than the multicellular eukaryotic cells. Cells that aggregate at 31 °C may have extracellular substances that mediate cell-cell attachment. Cell aggregates were completely dispersed only with treatment with cellulase. For example, extracellular macromolecules, collagen, enzymes, and glycoproteins are a three-dimensional network [38]. Therefore, *Cyanobacteria* produce a wide array of extracellular polysaccharides (EPSs), taking the form of released polysaccharides.

3.5 Mass balance

The mass balancing of pretreatment and hydrolysis of dried longan was accomplished on the lab scale and is summarized in Fig. 5. The experiment was carried out with 100 g dried longan and 1000 mL distilled water. Chemical compositions, such as fructose, arabinose, xylose, mannose, glucose, and galactose, were analyzed every process in this experiment in Table 2. The amount of xylose is the least sugar. The amount

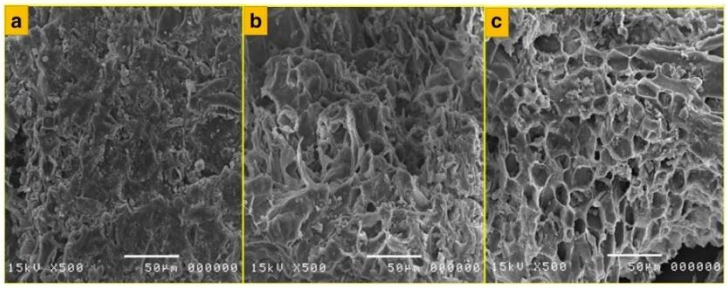


Fig. 4 SEM of pretreatments process: **a** Untreated. **b** Boiling pretreatment. **c** Autoclave pretreatment

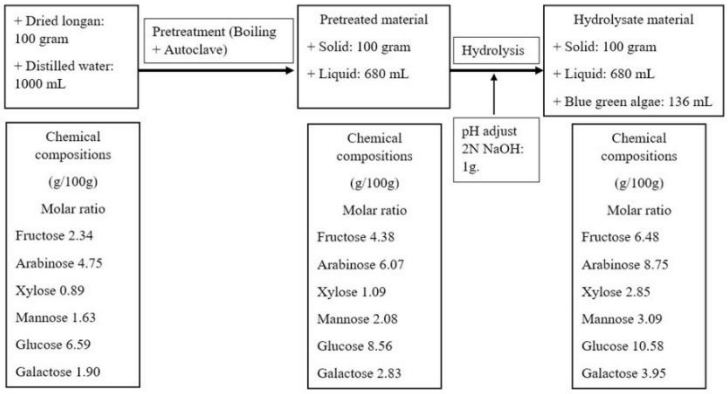


Fig. 5 Flow chart the mass balancing of pretreatment and hydrolysis of dried longan

of least sugar is xylose. Because xylose only can be found in hemicellulose of lignocellulosic biomass. The primary sugar in dried longan fruits is glucose, arabinose, and fructose.

The amount of sugar was degraded, which was reported in various researches. Therefore, the mass balance for the amount of sugar from starting to finish was available and significant to evaluate the pretreatment and hydrolysis effect. Sugar recovery, oligomer yield, and sugar yield for balance closure also are analyzed in Table 3. Fructose recoveries were 187.2%, and xylose yield was 2.82% in an experiment that brings high efficiency for every stage. In contrast, glucose and arabinose yields account for a lower value of 1.44% and 1.62%. However, some researchers showed that, based on glucose conversion efficiency, the hydrolysis step has the lowest glucose yield in the entire process. This discrepancy in glucose conversion might be due to deficient loading of betaglucosidase, inhibition of enzyme complexes, or insufficient lignin removal during pretreatment. Moreover, phenolic esters in material bring the harmful effect, for example, the linkages between lignins and hemicellulose.

Table 3 Sugar recovery, oligomer yield, and sugar yield

Sugars	Sugar recovery (%)	Sugar yield (%)	Total closure (%)
Glucose	129.9	1.44	131.34
Xylose	122.5	2.82	125.32
Fructose	187.2	2.49	189.69
Arabinose	127.8	1.62	129.42
Mannose	127.6	1.71	129.31
Galactose	148.9	1.87	150.77

3.6 Ethanol production

Separate hydrolysis and fermentation (SHF) are one of the fermentation methods that bring effective high bioethanol from biomass, especially residue from agriculture. Along with the resulting hydrolysates from 20%, algal enzymes were used to conduct separate hydrolysis and fermentation in anaerobic respiration conditions with 2% *S. cerevisiae*. Ethanol concentration within 96 h is displayed in Fig. 6. The range of ethanol production starts from 0 to 96 h. The peak of the ethanol production got at 36 h with 5.38 ± 0.54 g/L; the total and reducing sugars were recorded from 348.68 ± 3.95 g/L and 183.33 ± 14.70 g/L to 82.89 ± 5.34 g/L and 42.26 ± 1.99 g/L; after that, the trend of ethanol concentration drops down until the finished experiment at 96 h. From the results assembled, 2% of yeast requests 24 h to familiarize themselves in fermenter conditions until the peak at 36 h. Ethanol yield of jackfruit

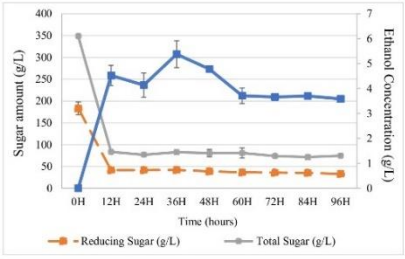


Fig. 6 Ethanol concentration within 96 h

Table 4 Final bioethanol concentration, EtOH yield, yield efficiency, and volumetric ethanol productivity of dry longan fruits

Fermentation time (h)	Final bioethanol concentration (g/L)	EtOH yield (g/g)	Yield efficiency (%)
12	4.53 ± 0.40	0.055 ± 0.0040	10.78 ± 0.7902
24	4.14 ± 0.49	0.051 ± 0.0048	10.00 ± 0.9505
36	5.38 ± 0.54	0.066 ± 0.0054	12.94 ± 1.0556
48	4.78 ± 0.00	0.058 ± 0.0000	11.37 ± 0.0000
60	3.71 ± 0.31	0.045 ± 0.0031	8.82 ± 0.6041
72	3.66 ± 0.06	0.044 ± 0.0006	8.63 ± 0.1265
84	3.70 ± 0.00	0.045 ± 0.0000	8.82 ± 0.0000
96	3.59 ± 0.11	0.043 ± 0.0011	8.43 ± 0.2127

rinds was the maximum at 4.64 g/L, followed by pineapple, muskmelon, and watermelon rinds with 4.38 g/L, 3.08 g/L, and 1.89 g/L, respectively, using the same yeast [39].

EtOH yield and yield efficiency from Table 4 showed that the fermentation time of dry longan fruits reached 36 h with 0.066 ± 0.0054 (g/g) and 12.94 ± 1.0556 (%). After that, these values slowly reduced that means the microorganisms called *S. cerevisiae* activated strongly at 36 h and then decrease their activities. This evidence claim that the survival of *S. cerevisiae*, which has been used to transform reducing sugars (hexose sugars: glucose, galactose, mannose, etc.) to ethanol by their specific metabolism [17], depends on the amount of reducing sugar and fermentation time [40, 41]. Moreover, the fermentation time of longan fruit in 36 h is shorter than a banana and pomelo fruits, jackfruit, pineapple, muskmelon, and watermelon. This supposes that the amount of polysaccharide of longan fruit is higher and easier to decompose than other fruits, except banana and pomelo [42].

The different types of fruit were fermented by using *S. cerevisiae* to produce the amount of different bioethanol. However, the bioethanol concentration of longan is the highest for comparing with other fruits presented in Table 5. Therefore, waste/damaged longan was a choice as a deserving candidate for producing bioethanol.

Table 5 Ethanol concentration from waste fruits

Substrate	Fermentation time (h)	Ethanol concentration (g/L)	Reference
Jackfruit	96	4.64	[39]
Pineapple	96	4.38	
Muskmelon	96	3.08	
Watermelon	96	1.89	
Banana	15	3.13	[42]
Pomelo	24	2.02	[43]
Banana	24	0.32	
Apple pomace	144	1.67	[44]
Longan	36	5.38	This study

4 Conclusions

Although the number of longan fruits serves for consumption, the rest as feedstock is attractive to produce renewable energy, especially bioethanol production. Thirty minutes of hydrothermal combined steam explosion with 15 min, which is the best method for the pretreatment process, released the high reducing sugar. The 20% of the algal enzyme was utilized successfully for hydrolysis through the evidence that the amount of sugar is higher than after pretreatment, and the bioethanol yield was collected after separate hydrolysis and fermentation using 2% *S. cerevisiae*. However, further studies are essential to advance the physical pretreatment and enzymatic hydrolysis process.

Acknowledgments Authors gratefully acknowledged for the School of Renewable Energy and were carried out using equipment from the Faculty of Science, Maejo University, Chiang Mai, Thailand and Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

References

1. Saengsawang B, Bhuyar P, Manmai N, Ponnusamy VK, Ramaraj R, Unpaprom Y (2020) The optimization of oil extraction from macroalgae, *Rhizoclonium* sp. by chemical methods for efficient conversion into biodiesel. Fuel 274:117841
2. Zabed H, Faruq G, Sahu J, Azirun S, Hashim R, Nasrulhaq Boyce A (2014) Bioethanol production from fermentable sugar juice. Sci World J 2014:1–12
3. Manmai N, Unpaprom Y, Ponnusamy V, Ramaraj R (2020) Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation. Fuel 278:118262
4. Ramaraj R, Unpaprom Y (2019) Optimization of pretreatment condition for ethanol production from *Cyperus difformis* by response surface methodology. 3 Biotech 9(6):218

5. Vu P, Unpaprom Y, Ramaraj R (2017) Evaluation of bioethanol production from rice field weed biomass. Emerg Life Sci Res 3: 42–49
6. Nong HT, Whangchai K, Unpaprom Y, Thararux C, Ramaraj R. Development of sustainable approaches for converting the agro-weeds *Ludwigia hyssopifolia* to biogas production. Biomass Conv Bioref 10.1007/s13399-020-01083-47
7. Ramaraj R, Unpaprom Y (2019) Enzymatic hydrolysis of small-flowered nutsedge (*Cyperus difformis*) with alkaline pretreatment for bioethanol production. Maejo Int J Sci Technol 13(2): 110–120
8. Chakdar H, Jadhav S, Dhar D, Pabbi S (2012) Potential applications of blue green algae. JSIR 71:13–20
9. Gerloff G, Fitzgerald G, Skoog F (1950) The isolation, purification, and culture of blue-green algae. Am J Bot 37:216–218
10. Liu S, Liu C, Huang X, Chai Y, Cong B (2006) Optimization of parameters for isolation of protoplasts from the Antarctic Sea ice alga *Chlamydomonas sp*. ICE-L J Appl Phycol 18(6): 783–786
11. Jiang Y, Zhang Z, Joyce D, Ketsa S (2002) Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). Postharvest Biol Technol 26(3):241–252
12. Yang B, Jiang Y, Shi J, Chen F, Ashraf M (2011) Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour.) fruit—a review. Food Res Int 44(7): 1837–1842
13. Tian S, Xu Y, Jiang A, Gong Q (2002) Physiological and quality responses of longan fruit to high O_2 or high CO_2 atmospheres in storage. Postharvest Biol Technol 24(3):335–340
14. Dubois M, Gilles K, Hamilton J, Rebers P, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3):350–356
15. Miller G (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31(3):426–428
16. Qin L, Liu H, Li Z, Dale E, Yuan J (2012) Mass balance and transformation of corn Stover by pretreatment with different dilute organic acids. Bioresour Technol 112:319–326
17. Vu P, Unpaprom Y, Ramaraj R (2018) Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production. Bioresour Technol 247:125–130
18. Azhar S, Abdulla R, Jambo S, Marbawi H, Gansau J, Faik A, Rodrigues K (2017) Yeasts in sustainable bioethanol production: a review. Biochem Biophys Rep 10:52–61
19. Tu V, Yuwalee U, Piyapat C, Rameshprabu R (2020) Improvement of bioethanol production from low grade and damaged longan fruits with thermal pretreatment and different types of the enzymatic hydrolysis. Int J Eng Sci 3:10–15
20. Nguyen T, Unpaprom Y, Ramaraj R (2020) Enhanced fermentable sugar production from low grade and damaged longan fruits using cellulase with algal enzymes for bioethanol production. Emerg Life Sci Res 6(2):26–31
21. Laopaiboon L, Thanonkeo P, Jaisil P, Laopaiboon P (2007) Ethanol production from sweet sorghum juice in batch and fed-batch fermentations by *Saccharomyces cerevisiae*. World J Microbiol Biotechnol 23(10):1497–1501
22. Kader A, Yahia E (2011) Postharvest biology and technology of tropical and subtropical fruits. Postharvest Biology and Technology of Tropical and Subtropical Fruits:79–111
23. Nguyen T, Unpaprom Y, Manmai N, Whangchai K, Ramaraj R (2020) Impact and significance of pretreatment on the fermentable sugar production from low-grade longan fruit wastes for bioethanol production. Biomass convers bioref. https://doi.org/10.1007/s13399-020-00977-7 in 1-13
24. Gabhane J, William S, Vaidya A, Mahapatra K, Chakrabarti T (2011) Influence of heating source on the efficacy of lignocellulosic pretreatment—a cellulosic ethanol perspective. Biomass Bioenergy 35(1):96–102
25. Kristensen J, Thygesen L, Felby C, Jørgensen H, Elder T (2008) Cell-wall structural changes in wheat straw pretreated for bioethanol production. Biotechnol Biofuels 1(1):1–9
26. Yelle D, Kaparaju P, Hunt C, Hirth K, Kim H, Ralph J, Felby C (2013) Two-dimensional NMR evidence for cleavage of lignin and xylan substituents in wheat straw through hydrothermal pretreatment and enzymatic hydrolysis. Bioenergy Res 6(1): 211–221
27. Petersen M, Larsen J, Thomsen M (2009) Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Biomass Bioenergy 33(5):834–840
28. Shamsudin S, Shah U, Zainudin H, Abd-Aziz S, Kamal S, Shirai Y, Hassan M (2012) Effect of steam pretreatment on oil palm empty fruit bunch for the production of sugars. Biomass Bioenergy 36: 280–288
29. Ohgren K, Bura R, Saddler J, Zacchi G (2007) Effect of hemicellulose and lignin removal on enzymatic hydrolysis of steam pretreated corn stover. Bioresour Technol 98(13):2503–2510
30. Alvira P, Tomas-Pejo E, Ballesteros M, Negro M (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour Technol 101(13):4851–4861
31. Cao W, Sun C, Liu R, Yin R, Wu X (2012) Comparison of the effects of five pretreatment methods on enhancing the enzymatic digestibility and ethanol production from sweet sorghum bagasse. Bioresour Technol 111:215–221
32. Ariffin H, Hassan M, Umi Kalsom M, Abdullah N, Shirai Y (2008) Effect of physical, chemical and thermal pretreatments on the enzymatic hydrolysis of oil palm empty fruit bunch (OPEFB). J Trop Agric 36(2):259–268
33. Lei H, Cybulska I, Julson J (2013) Hydrothermal pretreatment of lignocellulosic biomass and kinetics. J Sustain Bioenergy Syst 3(4): 250–259
34. Cutzu R, Bardi L (2017) Production of bioethanol from agricultural wastes using residual thermal energy of a cogeneration plant in the distillation phase. Fermentation 3(2):24
35. Manmai M, Bautista K, Unpaprom Y, Ramaraj R (2019) Optimization of combined pretreatments on sugarcane leaves for bioethanol production. Maejo Int J Energ Environ Comm 1(1):30–39
36. Wang M, Li Z, Fang X, Wang L, Qu Y (2012) Cellulolytic enzyme production and enzymatic hydrolysis for second-generation bioethanol production. In Biotechnology in China III: Biofuels and Bioenergy 1–24
37. Subhedar P, Gogate P (2013) Intensification of enzymatic hydrolysis of lignocellulose using ultrasound for efficient bioethanol production: a review. Ind Eng Chem Res 52(34):11816–11828
38. Whitton B (1965) Extracellular products of blue-green algae. Microbiology 40(1):1–11
39. Bhandari S, Panchapakesan A, Shankar N, Kumar H (2013) Production of bioethanol from fruit rinds by saccharification and fermentation. Int J Sci 2(6):362–365
40. Harun R, Danquah M, Forde G (2010) Microalgal biomass as a fermentation feedstock for bioethanol production. J Chem Technol Biotechnol 85(2):199–203
41. Manmai N, Unpaprom Y, Mariano A, Ramaraj R (2019) Bioethanol production from sunflower stalk: comparison between the impact of optimal chemical and biological pretreatments. The 1st Thailand biorefinery conference "the future of biorefinery for Thailand 4.0" Suranaree University of Technology, Nakhon Ratchasima, Thailand (July 25–26, 2019) 3(8):24–30

42. Jahid M, Gupta A, Sharma D (2018) Production of bioethanol from fruit wastes (banana, papaya, pineapple and mango peels) under milder conditions. J Bioprocess Biotech 8(3):327
43. Chamchoi N (2019) Utilization of fruit waste for bioethanol production by co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. Appl Ecol Environ Res 41(2):63–72
44. Ucuncu C, Tari C, Demir H, Buyukkileci A, Ozen B (2013) Dilute-acid hydrolysis of apple, orange, apricot and peach pomaces as potential candidates for bioethanol production. J Biobased Mater 7(3):376–389

KNOWVEL
TAKE YOUR RESEARCH TO NEXT LEVEL

GLOBAL JOURNAL OF SCIENCE & ENGINEERING
Vol. 03 (2020) 6-11
www.knowvel.com

Original Research Article

IMPROVEMENT OF BIOETHANOL PRODUCTION FROM LOW GRADE AND DAMAGED LONGAN FRUITS WITH THERMAL PRETREATMENT AND DIFFERENT TYPES OF THE ENZYMATIC HYDROLYSIS

TU VY THUY NGUYEN [1,2], YUWALEE UNPAPROM [3], PIYAPAT CHAICHOMPOO [4], RAMESHPRABU RAMARAJ [1,2]

ABSTRACT

Pretreatment is a vital step in the enzymatic hydrolysis of biomass and the successive production of bioethanol. The present study is focused on thermal pretreatment (boiling & autoclave) methods of low grade and damaged longan fruits using three different types of the enzymatic sources from commercial cellulase, an enzyme from algae and mixed enzymes (i.e., commercial cellulase with algal enzyme). Total sugar production after the hydrolysis process from commercial cellulase, the enzyme from algae and mixed enzymes were 326.41 ± 08.97 g/L, 348.68 ± 01.95 g/L and 368.42 ± 01.16 g/L, respectively. Reducing sugar after the hydrolysis process generated from commercial cellulase, the enzyme from algae and mixed enzymes was 182.54 ± 03.05 g/L, 183.33 ± 04.70 g/L and 297.78 ± 02.94 g/L, respectively. Fermentation of these hydrolysate using Saccharomyces cerevisiae TISTR 5020 produced the highest ethanol production from using commercial cellulase, the enzyme from algae and mixed enzymes was 16.74 ± 0.62 g/L, 5.38 ± 0.54 g/L and 14.32 ± 1.89 g/L, respectively. Consequently, this study suggested that suitable pretreatment and hydrolysis processes are performing a significant role in bioethanol production from low grade and damaged longan fruits.

Keywords: Low grade and damaged longan fruits, commercial cellulase, algal enzyme, mixed enzymes, bioethanol

AUTHOR AFFILIATION

[1]*School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand.*
[2]*Sustainable Resources and Sustainable Engineering Research Lab, School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand.*
[3]*Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand.*
[4]*Faculty of Liberal Arts, 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

PUBLICATION HISTORY

Received: June 30, 2020
Accepted: July 28, 2020

ARTICLE ID: GJSE-CP-11

1. INTRODUCTION

The world consumed approximately 98.27 million barrels of crude oil per day in 2019. Sustainability, especially in the energy sector, has been in the focus of concern due to the declining supply of fossil fuel, rapidly increasing oil price, global warming and energy security. The world's energy demands keep increasing through time, as it is estimated to increase 6.6 × 10^{20}J in 2020 and 8.6 × 10^{20}J in 2040, the supply of fossil fuels such as petroleum, natural gas, and coal will only last for 41,64,155 years respectively [1]. Additionally, the extreme consumption of fossil fuels in the past few years, especially in developed countries, held responsible for the massive amount of greenhouse gases (GHGs) in the atmosphere [2–4]. With the increase of GHG in the biosphere, environmental problems such as global warming and climate change emerge. These following concerns in energy security and environment stimulate worldwide interest to utilize an alternative, clean, sustainable and renewable energy such as solar, wind, water, geothermal and biomass [5–7]. Furthermore, fossil fuel use has been related to some alarming environmental problems such as global warming and climate change.

These increasing demands for energy, alongside with the diminishing and limited supply of fossil fuels, together with the negative impacts in the environment, are the reasons industries and governments

worldwide are seeking renewable alternatives [8]. Recently, biofuel's so-called "renewable energy" sources draw attention due to its availability and low carbon dioxide emission. It refers to the stored chemical energy in biomass (for example, plants, trees, woods, and agricultural or forest residues). The bioenergy that can be harvested and utilized each year increasing. Biofuels can be in solid, liquid and gas form just like fossil fuels. The biofuels are carbon-neutral, meaning it can be used without adding any carbon dioxide in the atmosphere. Bioethanol and biodiesel are the two liquid biofuels that have been widely used worldwide alternative to gasoline [9–11].

Bioethanol can be produced by fermentation using feedstock like sugar, starch, lignocellulosic materials and algae. It can be produced from different feedstocks such as sugar, starch, and lignocellulosic materials that are rich in hexoses and pentoses. Lignocellulosic materials contain lignin, cellulose and hemicellulose [12]. These materials are identified as a structural framework of plant cell walls; thus, it is available in different parts of plants in varying amounts. However, ethanol production from lignocellulosic biomass differs from that of the starch and sugar. Lignocellulosic materials have to undergo pretreatment before hydrolysis. They need extensive processing to release the polymeric sugars in cellulose and hemicellulose [13]. Cellulose is a beta-linked glucose polymer; meanwhile, hemicellulose is a highly branched chain of xylose and arabinose that also consists of glucose, mannose, and galactose. Therefore, bioethanol can be produced from carbohydrate-containing substrates by the process of fermentation. For bioethanol production, several microorganisms like bacteria, fungi and yeast were involved. Because fermentation essentials sugar production is faster and adequate when further bacterial and fungal microorganisms are used, which by their rapid consumption of glucose help better develop the fermentation processes of plant materials [14, 15].

Possibly, fruit wastes such as damaged fruits, peels and seeds represent cheap alternative feedstocks for biofuel production. Large quantities of fruit waste are generated from agricultural processes worldwide. This waste is often simply dumped into landfills. Fruit waste has high levels of sugars, including sucrose, glucose, and fructose, that can be fermented for bioethanol production. In some tropical countries like Thailand, longan (*Dimocarpus longan*) is commercially producing, especially in Thailand; longan growing areas cover about 188,574 hectares with a total production of 1,027,298 tons per year. The main production areas in Thailand are located in the northern region consisting of Chiang Mai, Chiang Rai, Lamphun, and Phayao Provinces [16]. In this work, an experimental study was conducted on ethanol production using mixed lowgrade and damaged longan fruits through fermentation by Saccharomyces cerevisiae yeast. The lowgrade and damaged longan fruits were containing a good source of carbohydrate naturally. The longan is suitable for the tropical zone subtopics in heat season. The flesh fruit is much in juicy, low in acid, high in sugar. It has reported that the storability of harvested fruit is associated with the integrity of the cell membrane structure. This study aimed to use bioethanol can be produced from carbohydrate-containing substrates by the process of fermentation.

2. Materials & Methods

2.1. Raw Material Collection and Preparation

This study's conceptual framework and methodology were presented in Figure 1. Low grade and damaged longan fruits were collected Pratupa Agricultural Cooperative company at Pratu Pa Subdistrict, Mueang Lamphun District, Lamphun 51000 (coordinate 18°37'44.5"N 98°59'48.5"E). Then the leaves, branches, dust, soil, and other impurities of low grade/damaged longans fruits were eliminated at the laboratory at Faculty of Science, Maejo University, Chiang Mai, Thailand and the rest of the low grade/damaged longans fruits were dried at the Energy Research Center, MJU, shredded by the chipping disk machine (multi-purpose shredder model MJU-EB8), and used for this study..

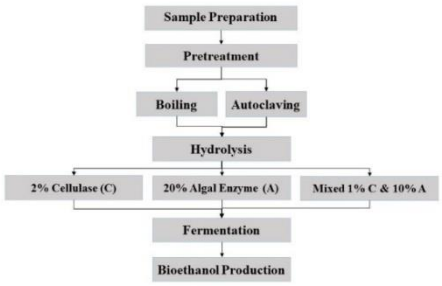


Figure 1. Conceptual framework and methodology

2.2. Pretreatment and Enzymatic Hydrolysis

Physical pretreatment, which includes boiling and autoclaving methods, were applied in this study. The one hundred (100) gram dried low grade and damaged longan fruits with ratio 1:10 of distilled water. Afterward, the mixture was boiled 30 mins by container and electric stove until the temperature of the solution 90-95 °C. Then the mixture was undergone by autoclave at 121°C, 15 psi, 15 mins.

The hydrolysis process was carried out different three treatments (1. 2% cellulase commercial enzyme, 2. 20% algal enzyme and 3. mixed with commercial and algal enzyme). Treatment 1: the 2% cellulase enzyme was added in the pretreated samples in triplicate. However, the pH of these samples was adjusted at 5.0 before adding the cellulase enzyme. A hot air oven was utilized for the hydrolysis process within 1 day. Treatment 2: the pH of these pretreated samples was adjusted at 7.0 in triplicate. Subsequently, a 20% algal enzyme was put in these mixtures. The hydrolysis step occurred in a hot air oven one day. Treatment 3: 1% commercial cellulase and 10% algal enzymes were poured in triplicate. However, before adding enzymes, the pH of these samples was adjusted 5.0 and 7.0, respectively. Subsequently, a certain amount of the pretreated samples and hydrolysate samples were withdrawn to estimate the total sugar and reducing sugar.

2.3. Yeast Preparation

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

2.4. Fermentation

Fermentation of these hydrolysates was carried out at pH 5.6 with 2% (v/v) yeast (Saccharomyces cerevisiae TISTR 5020), which was added in 1000 mL fermentor. These fermentors were kept in a temperature room within 96 hours. After every half-day, a certain amount of sample was withdrawn to analyze sugar and check the alcohol concentration.

2.5. Sugar Analysis and Alcohol Estimation

Total sugar and reducing sugar were analyzed by Dubois et al. [17] and Miller [18]) ways. Total sugar was analyzed by the phenol-sulfuric acid method [17]. 0.5 mL of the diluted sample was combined with 0.5 mL of 5% phenol and 2.5 mL of 98% sulphuric acid. Then the mixture was kept in cold water 10 minutes. Reducing sugar was analyzed by the 3,5-dinitrosalicylic acid (DNS) methods [18]. 0.5 mL of the diluted sample was combined with 0.5 mL of DNS solution. Afterward, the solution was boiled 30 minutes. 4 mL of distilled water was added to this solution. Total and reducing sugar concentration were reach by a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 490 nm and 540 nm, respectively. Alcohol concentration was determined by Ebulliometer (Dujardin-Salleron, Alcohol Burner, France). 50 mL of sample was withdrawn, poured in the condenser of Ebulliomter, and boiled until the stable temperature. The resulting temperature of the fermented sample was reached, compared to the boiling point of the distilled water using the Ebulliomter disc [19–21].

2.6. Statistical Analysis

The values reported in the present study were the mean of three replicates. Moreover, data are reported as mean ± SE from triplicate observations. All Statistical analyses of data were performed using the program SPSS 20.0 (SPSS Inc., Chicago, IL, USA). A significant difference was considered at the level of $p < 0.05$.

3. Results & Discussion

3.1. Longan Characteristics and Cultivation in Thailand

The longan is a perennial fruit tree (Figure 2). It belongs to the Family: Sapindaceae, Genus: Dimocarpus and Species: *Dimocarpus longan* Lour. Thailand is one of the major producers of longan, and it is cultivated mostly in the Northern provinces of Thailand. Longan production in Thailand is confined mainly to the northern provinces of Lamphun, Chiang Mai and Chiang Rai where the planted area makes up 37.6, 24.1 and 8.0 percent of the total planted area, respectively. However, longan cultivation has extended to the eastern and central regions of the country, and they constitute only a small percentage of total production.

Longan fruit is one of the significant economic crops of Thailand with an average annual production volume for the past five years of more than 911,800 ± 53,186 tons. In 2017, Thailand generated the export values of fresh longan fruit totaling $152.74 million or 24.67% of the whole fresh fruits export values, which account for $618.96 million. The flesh fruit is much in juicy, low in acid, high in sugar. Longan fruit has high nutritional and medicinal values and its main functional metabolites include polysaccharides, flavonoids, alkaloids and carotenoids. The mature longan fruit is small (ca. 1.5–2 cm diameter), conical, heart-shaped or spherical in shape and light brown. It has a thin, leathery and indehiscent pericarp surrounding a succulent, edible white aril [22].

The plantation of a longan tree with fruits was exhibited in Figure 2. The fruit size and color is important for export. The small size fruits called lowgrade fruits, which were no market value. These are becoming a waste of fruits possible to damage after the grading separations (by fruit size AA, A, B, C and wastes fruits). A considerable amount of waste is generated from the high amount of their production with significant environmental impact. Therefore, the transformation of these fruits by-products into novel products would reduce the cost of waste treatment and generates additional economic benefits, mainly producing bioethanol due to the high content of sugars. In 100g, longans contain total carbohydrate, fiber, reducing sugar, and protein were 12.38-22.55, 0.4, 3.85-10.16, and 1.2, respectively [23].

Figure 2. Longan tree with fruits

3.2. Pretreatment of the Lowgrade and Damaged Longan Fruits

Vu et al. [24] stated that it has a major potential feedstock for bioethanol production since it is a rich source of chemicals, biopolymers and sugar. Lignocellulosic materials are derived from plant cell walls that are mainly composed of cellulose, hemicellulose and lignin that will undergone different process to convert into ethanol. In order to obtain bioethanol from lignocellulosic materials it will undergone pretreatment, hydrolysis of cellulose and hemicellulose to obtained fermentable sugars. The goal of pretreatment process is the following: 1. to improve the formation of sugars or the ability to form them, and 2. to avoid the formation of products that can inhibit the hydrolysis and fermentation process [25]. Hence, the pretreatment process for bioethanol production from lignocellulosic biomass is vital. The main challenge of producing bioethanol from lignocellulosic materials will be the feedstock pretreatment. This step needs extensive processing to release the polymeric sugars in cellulose and hemicellulose which contributed about 20-53% of plant materials [13,19, 26]. Cellulose is a beta-linked glucose polymer; meanwhile hemicellulose is a highly branched chain of xylose and arabinose that also consists of glucose, mannose, and galactose. The primary function of the pretreatment process is to remove lignin and hemicellulose around cellulose, to make it more accessible for further hydrolysis and fermentation [28]. From the lowgrade and damaged longan fruits, the results obtained for the release of sugars are presented in Figure 3.

The lowgrade and damaged longan fruits gave the highest level of total sugars (234.8 g/L) and reducing sugars (98.33 g/L). Other studies where pineapple peel pretreated biomass or pre-treated Fresh fruit buncheshave been reported [29, 30]. In these studies, single substrates reducing sugars, followed by the pineapple peel autoclaved at 121°C, 60 min (25.47 g/L) and then the fresh fruit bunches autoclaved at 125°C for 120 min (14.11 g/L). Comparatively, the lowgrade and damaged longan fruits achieved highest fermentable sugars by autoclaving pretreatment.

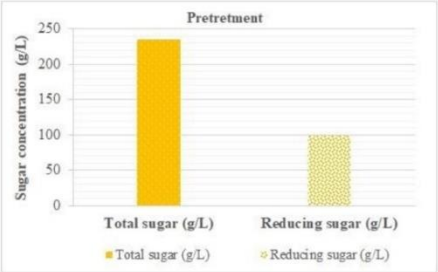


Figure 3. Total and reducing sugars yield after pretreatment

3.3. Hydrolysis the Lowgrade and Damaged Longan Fruits

Hydrolysis describes as the process of releasing sugars that are usually linked together in complex chains [31]. The hydrolysis process attacks the cellulose chains to produce more fermentable sugars. This process usually catalyzed by dilute acid, concentrated acid or enzymes (cellulase). The biochemical conversion of cellulose and hemicellulose through hydrolysis (Eq. 1 and 2) can be expressed by the reaction of hexose (Eq. 1) and pentose (Eq. 2) with water:

$$(C_6H_{10}O_5)_n \left(\begin{matrix}\text{stach, cellulose,}\\ \text{sugar}\end{matrix}\right) + nH_2O \rightarrow nC_6H_{12}O_6 \left(\begin{matrix}\text{glucose,}\\ \text{fructose}\end{matrix}\right) \quad (1)$$

$$(C_5H_8O_4)_n \text{ (hemicellulose)} + nH_2O \rightarrow nC_5H_{10}O_5 \left(\begin{matrix}\text{xylose, mannose,}\\ \text{arabinose, etc.}\end{matrix}\right) \quad (2)$$

According to these equvations that the hexose and pentose maxiumum theoretical yield per kg of glucan and xylan is 1.136 kg and 1.111 kg, respectively. The optimal conditions observed for the highest sugar generation from the hydrolysis of the substrates were used for estimation of bioethanol yield. The enzymatic hydrolysis of lignocellulosic and starch based feedstocks has been investigated previously in several studies [7, 13, 14, 27, 28, 31]. However, due to the different enzymes used in this study, we tried to examine the effect of the most important factors on enzymatic hydrolysis in order to obtain the highest sugar release. For this purpose, the effects of the dosage of the three enzymes were applied, and the solid the lowgrade and damaged longan fruits loading were investigated. The obtained results were presented in Table 1.

Table 1. Different types of enzymatic hydrolysis of the lowgrade and damaged longan fruits

Sugars concentrations	Treatment 1*	Treatment 2*	Treatment 3*
Total sugar (g/L)	326.41 ± 8.97	348.68 ± 1.95	368.42 ± 1.16
Reducing sugar (g/L)	182.54 ± 3.05	183.33 ± 4.70	297.78 ± 2.94

*Note: Treatment 1: 2% Cellulase Enzyme, Treatment 2: 20% Algal Enzymes, and Treatment 3: 1% Cellulase & 10% Algal Enzymes

3.4. Fermentation

In this study, separate hydrolysis and fermentation, also known as SHF, is a configuration employed in the fermentation of biomass hydrolyzates. This involves sequential process of hydrolysis (saccharification) and fermentation that carried in separate units [32–35]. This process has the ability to optimized each independent step. Additionally, the use of different microorganism for fermenting different sugars is possible. The fermentation was carried out in a 1 L bottle (Figure 4). For yeast fermentation, 2% (v/v) of yeast (*S. cerevisiae* TISTR 5020) has been added to the fermenter juice. The mixtures were then incubated with a maintaining room temperature for 96 hours with triplicate fermenters. All experiments were performed in triplicates. The ethanol content, sugar concentration and pH were monitored for every 12 hours.

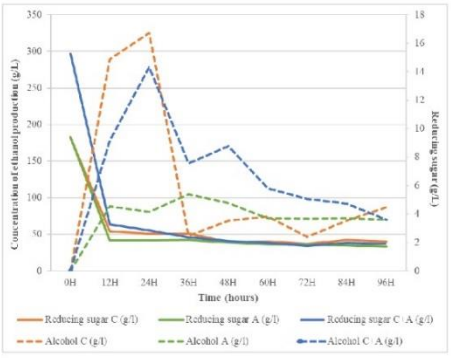


Figure 4. Reducing sugar utilization by S. cerevisiae and ethanol production during SHF process

Hence, the suitable pretreatment is required to improve the efficiency of subsequent breakdown processes and yields of desired products. The results showed that physical (autoclaving) pretreatment was effective in producing high yields with commercial cellulose of the desired products from lowgrade and damaged longan fruits. The autoclaving pretreated lowgrade and damaged longan fruits were hydrolyzed using a cellulase and the total saccharification rate was increased. In addition to the biosugar was converted into bioethanol by separate hydrolysis and fermentation.

4. Conclusions

The present study demonstrated for the first time that lowgrade and damaged longan fruits is a novel potential raw material for the production of bioethanol. The physical pretreatment was effective in producing high yields of the bioethanol from that lowgrade and damaged longan fruits. These fruits were converted into bioethanol using separate hydrolysis and fermentation. The celluase enzyme showed the better performance. The use of popping pretreatment and enzymatic hydrolysis facilitated high yield of fermentable sugar production. This study suggested that economically beneficial processes could be developed from the reuse of these waste materials i.e. low grade/damaged longan fruits. Also, interests in the use of renewable waste biomass are increasing rapidly due to the prospects of converting them into useful products such as bio-ethanol that would help sustain our environment.

Conflict of Interests

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

REFERENCES

[1] Office of agricultural economics, The economical tendency of Thai longan production in 2018, Available at: http://aginfo.oae.go.th/oae_today/report_product.php?product_name=lumyai.xls

[2] R. Ramaraj, Y. Unpaprom, and N. Dussadee, "Cultivation of Green Microalga, Chlorella vulgaris for Biogas Purification," Int. J. New Technol. Res., vol. 2, no. 3, p. 263569, 2016.

[3] R. Ramaraj, R. Kawaree, and Y. Unpaprom, "Direct Transesterification of Microalga Botryococcus braunii Biomass for Biodiesel Production," Emergent Life Sci. Res., vol. 2, no. 2, pp. 1–7, 2016.

[4] K. Khunchit, S. Nitayavardhana, R. Ramaraj, V. K. Ponnusamy, and Y. Unpaprom, "Liquid hot water extraction as a chemical-free pretreatment approach for biobutanol production from Cassia fistula pods," Fuel, vol. 279, 2020.

[5] A. Chuanchai and R. Ramaraj, "Sustainability assessment of biogas production from buffalo grass and dung: biogas purification and bio-fertilizer," 3 Biotech, vol. 8, no. 3, 2018.

[6] R. Ramaraj, N. Dussadee, N. Whangchai, and Y. Unpaprom, "Microalgae Biomass as an Alternative Substrate in Biogas Production," Int. J. Sustain. Green Energy. Spec. Issue Renew. Energy Appl. Agric. F. Nat. Resour. Technol., vol. 4, no. 1, pp. 13–19, 2015.

[7] M. Manmai, K. Bautista, Y. Unpaprom, and R. Ramaraj. "Optimization of combined pre-treatments on sugarcane leaves for bioethanol production," Maejo Int J Energ Environ Comm vol. 1, no. 1, pp. 30–39, 2019.

[8] R. Rameshprabu, R. Kawaree, and Y. Unpaprom, "A newly isolated green alga, Pediastrum duplex Meyen, from Thailand with efficient hydrogen production," Int. J. Sustain. Green Energy Int. J. Sustain. Green Energy. Spec. Issue Renew. Energy Appl. Agric. F. Nat. Resour. Technol., vol. 4, no. 1, pp. 7–12, 2015.

[9] D. N. Ramaraj R., Unpaprom Y., Whangchai N., "Culture of Macroalgae Spirogyra ellipsospora for Long-Term Experiments, Stock Maintenance and Biogas Production," Emer Life Sci Res, vol. 1, no. 1, pp. 38–45, 2015.

[10] Y. Unpaprom, R. Ramaraj, and K. Whangchai, "A newly isolated green alga, Scenedesmus acuminatus, from Thailand with efficient hydrogen production," Chiang Mai J. Sci., vol. 44, no. 4, pp. 1270–1278, 2017.

[11] P. Khammee, Y. Unpaprom, S. Buochareon, and R. Ramaraj, "Potential of bioethanol production from marigold temple waste flowers," Proceeding of The 1st Thailand Biorefinery Conference, The Future of Biorefinery for Thailand 4.0, Suranaree University of Technology, Nakhon Ratchasima, Thailand, pp. 25–26, 2019.

[12] P. Khammee, R. Ramaraj, N. Whangchai, P. Bhuyar, and Y. Unpaprom, "The immobilization of yeast for fermentation of macroalgae Rhizoclonium sp. for efficient conversion into bioethanol," Biomass Convers. Biorefinery, 2020.

[13] N. Manmai, Y. Unpaprom, V. K. Ponnusamy, and R. Ramaraj, "Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation," Fuel, vol. 278, 2020.

[14] B. Saengsawang, P. Bhuyar, N. Manmai, V. K. Ponnusamy, R. Ramaraj, and Y. Unpaprom, "The optimization of oil extraction from macroalgae, Rhizoclonium sp. by chemical methods for efficient conversion into biodiesel," Fuel, vol. 274, 2020.

[15] R. Ramaraj and Y. Unpaprom, "Enzymatic hydrolysis of small-flowered nutsedge (Cyperus difformis) with alkaline pretreatment for bioethanol production," Maejo Int. J. Sci. Technol., vol. 13, no. 2, pp. 110–120, 2019.

[16] A. Pipattanapuckdee, D. Boonyakait, C. Tiyayon, P. Seehanam, and O. U. Ruangwong, "Lasiodiplodia pseudotheobromae causes postharvest fruit rot of longan in Thailand," Australas. Plant Dis. Notes, vol. 14, no. 1, 2019.

[17] M. Dubois, K. Gilles, J. Hamilton, P. Rebers, F. Smith, "Phenol sulphuric acid method for total carbohydrate," Anal Chem, vol. 28, no. 3, pp. 350–356, 1956.

[18] G. Miller, "Use of dinitrosalicylic acid reagent for detection of reducing sugars," Anal Chem, vol. 31. no. 3, pp. 426–428, 1959.

[19] N. Manmai, T. Siriboon, Y. Unpaprom, and R. Ramaraj, "Bioethanol from lignocellulosic biomass: a comparison between sunflower stalk and sorghum stalk," Proceedings of the 24th Tri-University International Joint Seminar and Symposium, Mie University, Japan, pp. 1–4, 2017.

[20] N. Manmai, K. Bautista, Y. Unpaprom, R. Ramaraj, and R. Jayabalan, "Bioethanol production from pretreated–corn stalk waste by separate hydrolysis and fermentation process," Proceeding of The 9th International Graduate Research Conference, Chiang Mai, Thailand, pp. 383–391, 2018.

[21] N. Manmai, K. Bautista, Y. Unpaprom, and R. Ramaraj, "Optimization of combined pre–treatments on sugarcane leaves for bioethanol production," Maejo Int. J. Energy Environ. Commun., vol. 1, no. 1, pp. 30–39, 2019.

[22] K. Rakariyatham, D. Zhou, N. Rakariyatham, and F. Shahidi, "Sapindaceae (Dimocarpus longan and Nephelium lappaceum) seed and peel by-products: Potential sources for phenolic compounds and use as functional ingredients in food and health applications," Journal of Functional Foods, vol. 67. 2020.

[23] M. H. Shahrajabian, W. Sun, Q. Cheng, "Modern pharmacological actions of longan fruits and their usages in traditional herbal remedies," J. Med. Plants Stud, vol. 7, no. 4, pp. 179–185, 2019.

[24] P. T. Vu, Y. Unpaprom, R. Ramaraj, "Evaluation of bioethanol production from rice field weed biomass," Emergent Life Sci. Res., vol. 3, pp. 42–49, 2017.

[25] R. Ramaraj and Y. Unpaprom, "Optimization of pretreatment condition for ethanol production from Cyperus difformis by response surface methodology," 3 Biotech, vol. 9, no. 6, 2019.

[26] P. Khammee, N. Whangchai, B. Prakash, Y. Unpaprom, and R. Ramaraj, "The immobilization of yeast for fermentation of macroalgae *Rhizoclonium sp.* for efficient conversion into bioethanol," Biomass Convers. Biorefinery, 2020.

[27] N. Manmai, Y. Unpaprom, V. K. Ponnusamy, and R. Ramaraj, "Bioethanol production from the comparison between optimization of sorghum stalk and sugarcane leaf for sugar production by chemical pretreatment and enzymatic degradation," Fuel, vol. 278, 2020.

[28] P. T. Vu, Y. Unpaprom, and R. Ramaraj, "Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of Sphenoclea zeylanica for bioethanol production," Bioresour. Technol., vol. 247, pp. 125–130, 2018.

[29] A. Choonut, M. Saejong, and K. Sangkharak, "The production of ethanol and hydrogen from pineapple peel by Saccharomyces cerevisiae and Enterobacter aerogenes," in Energy Procedia, 2014, vol. 52, pp. 242–249.

[30] M. S. M. Kassim, W. I. W. Ismail, A. R. Ramli, and S. K. Bejo, "Oil palm fresh fruit bunches (FFB) growth determination system to support harvesting operation," J. Food, Agric. Environ., vol. 10, no. 2, pp. 620–625, 2012.

[31] N. Manmai, Y. Unpaprom, and R. Ramaraj, "Bioethanol production from sunflower stalk: application of chemical and biological pretreatments by response surface methodology (RSM)," Biomass Convers. Biorefinery, 2020.

[32] R. Ramaraj, and Y. Unpaprom, "Waste to energy: Jasminum sambac temple waste flowers for bioethanol production by different pretreatment methods," Proceeding of The 25th Tri – University International Joint Seminar and Symposium, Chiang Mai, Thailand, pp. 156–159, 2018.

[33] N. Manmai, T. Siriboon, S. Tipnee, Y. Unpaprom, and R. Ramaraj, "Bioconversion of lignocellulosic biomass, sorghum bicolor L. into bioethanol," Proceedings of Academics World 73rd International Conference, Taipei, Taiwan, pp. 41–45, 2017.

[34] N. Manmai, N. Saetang, Y. Unpaprom, and K. T. Wu, "Influential degree of polymerization of sugar extraction on alkali pretreatment from sunflower stalk wastes by applied statistical modelling," Maejo Int J Energy Environ Commun, vol. 1, no. 2, pp. 37–43, 2019.

[35] N. Manmai, Y. Unpaprom, and R. Ramaraj, "Effect of chemical pretreatment on enzymatic hydrolysis and fermentation of corn stalks for ethanol production," Proceedings of the 6th ASCON-IEEChE 2018, Sun Moon Lake, Taiwan, pp. 375–382, 2018

APPENDIX D: CERTIFICATES OF ATTENDANCE

CERTIFICATE OF ATTENDANCE, PARTICIPATION, & PRESENTATION

THIS IS PROUDLY GIVEN TO

TU VY THUY NGUYEN

for attending, participating and conducting an oral presentation at

THE INTERNATIONAL ONLINE CONFERENCE ON INNOVATIVE SCIENCE, ENGINEERING, AND TECHNOLOGY (IOCISET-2020) (JULY 03, 2020 - JULY 05, 2020)

as per the details below:

Submission Type: Full Paper
Title: Improvement of Bioethanol Production from Low Grade and Damaged Longan Fruits with Thermal Pretreatment and Different Types of the Enzymatic Hydrolysis

KNOWVEL
TAKE YOUR RESEARCH TO NEXT LEVEL

KNOWVEL JOURNALS
WWW.KNOWVEL.COM
INFO@KNOWVEL.COM

KNOWVEL JOURNALS TAKE YOUR RESEARCH TO NEXT LEVEL DATE: 06/07/2020

BEST PAPER AWARD

THIS IS PROUDLY GIVEN TO

TU VY THUY NGUYEN

for the best paper (1st position) at

THE INTERNATIONAL ONLINE CONFERENCE ON INNOVATIVE SCIENCE, ENGINEERING, AND TECHNOLOGY (IOCISET-2020) (JULY 03, 2020 - JULY 05, 2020)

on the topic *"Improvement of Bioethanol Production from Low Grade and Damaged Longan Fruits with Thermal Pretreatment and Different Types of the Enzymatic Hydrolysis"*

KNOWVEL
TAKE YOUR RESEARCH TO NEXT LEVEL

KNOWVEL JOURNALS
WWW.KNOWVEL.COM
INFO@KNOWVEL.COM

KNOWVEL JOURNALS TAKE YOUR RESEARCH TO NEXT LEVEL DATE: 13/07/2020

econference

Certificate

BIEC 2020 Business Innovation and Engineering Conference

2nd ICRMIA 2020

The New Dynamics of Business Innovation & Engineering:
Creating and Protecting Value in The Era of Industry 4.0 & Society 5.0

This certificate is awarded to

Tu Vy Thuy Nguyen

as a **Presenter** of a paper entitled:

Enhanced fermentable sugar production from low grade and damaged longan fruits using cellulase with algal enzymes for bioethanol production

Bogor, 28 July 2020

Prof. Dr. Ir. Noer Azam Achsani, MS
Dean of School of Business - IPB University

Dikky Indrawan, PhD
Chair of BIEC & ICRMIA 2020

Sponsored by CRMS

ICBB 2020

INTERNATIONAL CONFERENCE OF BIOMASS AND BIOENERGY 2020

"Advanced Technology and Digital Innovations in Biomass, Bioenergy and Agriculture"

Certificate

This is to certify that

Tu Vy Thuy Nguyen

Has participated as a

Presenter

10-11 August 2020

Chairman of International Conference of Biomass and Bioenergy

Dr. Dwi Setyaningsih, S.TP, M.Si

Certificate of MJU-TEP Results

This is to certify that

Miss Nguyen Thuy Vy Tu

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

June 26th, 2020

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

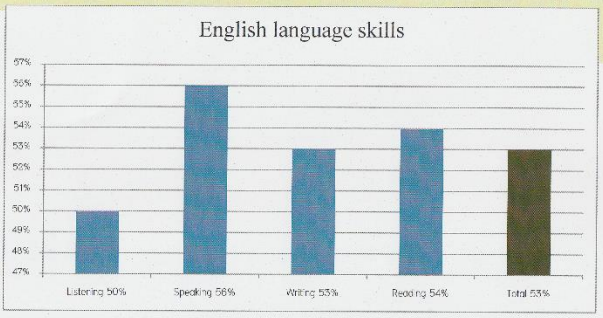
1. Listening Comprehension Skill	15 / 30 marks
2. Semi-speaking Skill	14 / 25 marks
3. Semi-writing Skill	16 / 30 marks
4. Reading Comprehension Skill	19 / 35 marks
Total Score	**64 / 120 marks**

Given on July 2nd, 2020

(Dr. Sutkhet Sakunthong)
Acting Director, Maejo University Language Center

MJU-TEP Scores

of Miss Nguyen Thuy Vy Tu



Meanings

1. Beginning users	0-20%	5. Competent users	61-70%
2. Very limited users	21-30%	6. Good users	71-80%
3. Limited users	31-40%	7. Very good users	81-90%
4. Modest users	41-60%	8. Expert users	91-100%

CURRICULUM VITAE

NAME	Tu Vy Thuy Nguyen
DATE OF BIRTH	17 may 1994
EDUCATION	2013-2018-Bachelor of Engineering in Environmental Engineering Technology, Ton Duc Thang University
WORK EXPERIENCE	Officier - Mechanical and electrical Ky Nguyen Company