THE POTENTIAL OF AQUATIC WEEDS AS FEEDSTOCK SOURCE FOR BIOETHANOL

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MASTER OF ENGINEERING IN RENEWABLE ENERGY ENGINEERING

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
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ชื่อเรื่อง ศักยภาพของวัชพืชน้ำในการเป็นแหล่งวัตถุดิบสำหรับไบโอเอทานอล
ชื่อผู้เขียน MissVu Thi Phuong
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บทคัดย่อ

การผลิตไบโอเอทานอลจากชีวมวลประเภทลิกโนเลลูโลส ได้มีการพัฒนาและทดลองกันมาเป็นระยะเวลานานพอสมควร ซึ่งไม่เพียงแต่เป็นการช่วยลดปัญหาพลังงานโลกแต่ยังช่วยลดสภาวะเครียดทางสิ่งแวดล้อมที่เกิดจากการใช้เชื้อเพลิงฟอสซิลด้วย ประเทศไทยเป็นประเทศที่มีความหลากหลายทางระบบนิเวศอย่างมาก เป็นประเทศที่มีศักยภาพในการผลิตไบโอเอทานอลจากมวลชีวภาพ เช่น วัสดุเหลือทิ้งจากป่าไม้ วัสดุเหลือทิ้งทางการเกษตร ชีวมวลประเภทไม้และอื่นๆ อย่างไรก็ตามงานวิจัยในเรื่องการผลิตเอทานอลนั้นยังมีอยู่น้อยมาก จึงทำให้เป็นข้อจำกัดของการพัฒนาการผลิตไบโอเอทานอลในประเทศไทย ดังนั้นงานวิจัยนี้จึงมุ่งหวังที่จะพัฒนาการผลิตไบโอเอทานอลเพื่อเติมเต็มระหว่างงานวิจัยและการประยุกต์ใช้ได้ทั่วไปและจำเพาะกับงาน โดยแนวคิดเริ่มมาจาก ปัจจุบันนี้แนวโน้มเรื่องผลิตภัณฑ์อินทรีย์เป็นที่นิยมอย่างกว้างขวาง ปัญหาหนึ่งที่สำคัญมากในการปลูกข้าว คือการเติบโตของวัชพืชที่ไม่พึงประสงค์ ในการศึกษาครั้งนี้ได้ใช้วัชพืชสองชนิด ได้แก่ ผักปอดนา (gooseweed, *Sphenoclea zeylanica*) และกกขนาก (small-flowered nutsedge, *Cyperus difformis*) เพื่อผลิตไบโอเอทานอล ในขั้นแรกได้หาสภาวะที่เหมาะสมในการผลิตเอทานอลระดับห้องปฏิบัติการ หลังจากนั้นได้มีการเพิ่มขนาดการทดลองให้ใหญ่ขึ้น ในการปรับสภาพวัตถุดิบนั้นมีทั้งวิธีทางชีวภาพและเคมี วิธีทางชีวภาพทำโดยใช้ปลวกsciencec nameในการย่อยสลายผงวัตถุดิบด้วยอัตราส่วนปลวกต่อผงวัตถุดิบ 2:1 โดยเลี้ยงไว้เป็นระยะเวลา 3 วัน วิธีปรับสภาพทางเคมีทำโดยใช้โซเดียมไฮดรอกไซด์และไฮโดรเจนเพอออกไซด์ที่มีความเข้มข้นแตกต่างกัน จากนั้นศึกษาอัตราส่วนที่เหมาะสมของของแข็งต่อของเหลวและเวลาต่อผลผลิตไบโอเอทานอล ด้วยวิธีการออกแบบการทดลอง Box-Behnken และพื้นผิวตอบสนอง (Response surface methodology) ในการย่อยสลายวัตถุดิบได้ใช้เอนไซม์เซลลูเลสทางการค้าเป็นระยะเวลา 72 ชั่วโมง เพื่อให้ได้น้ำตาลจากวัชพืชทั้งสองชนิด วัตถุดิบที่ถูกย่อยแล้วนำมาหมักด้วย *Saccharomyces cerevisiae* TISTR 5020 ที่อุณหภูมิ 35 องศาเซลเซียส เป็นระยะเวลา 9 วัน พบว่า วิธีการปรับสภาพทางชีวภาพให้ผลผลิตน้ำตาลน้อยกว่าวิธีทางเคมี ซึ่งการใช้โซเดียมไฮดรอกไซด์และไฮโดรเจนเปอร์ออกไซด์ได้ผลผลิตน้ำตาลดีที่สุด ส่วนวิธีการไฮโดรไลซิสโดยใช้เอมไซม์เซสลูเลสเป็นระยะเวลา 24 ชั่วโมง สามารถผลเพิ่มผลผลิตน้ำตาล

ได้มากขึ้น และผลผลิตเอทานอลสูงที่สุดหลังจากหมักเป็นระยะเวลา 3 วัน ผลผลิตเอทานอลสูงที่สุดจากการหมักผักปอดนามีค่าเท่ากับ 11.84 กรัม/ลิตร และจากการหมักกขนากมีค่าเท่ากับ 12.36 กรัม/ลิตร นอกจากนี้ยังได้วิเคราะห์ความสมดุลของมวลชีวภาพเพื่อให้เข้าใจเกี่ยวกับการถ่ายโอนมวล จากนั้นขยายขนาดการทดลองขึ้น กลั่นเอทานอลที่ผลิตได้ และทดสอบค่าความร้อนเพื่อยืนยันผลการทดลอง การทดสอบค่าความร้อนของเอทานอลทำโดยการใช้เครื่องวัดความร้อน บอมบ์แคลอริมิเตอร์ ค่าความร้อนของเอทานอลที่ผลิตได้จากผักปอดนาและกกขนาก มีค่าเท่ากับ 12.61 และ 25.31 กิโลจูล/กรัม ตามลำดับ จึงสรุปได้ว่า ทั้งผักปอดนาและกกขนากสามารถใช้เป็นวัตถุดิบในการผลิตไบโอเอทานอลได้และสามารถประยุกต์ใช้เพื่อสร้างรายได้ในชุมชนท้องถิ่นได้

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ABSTRACT

Bioethanol production from lignocellulosic biomass has been developed and carried out for a long time, not only to reduce the global energy problem but also to reduce the environmental stress caused by the application of fossil fuels. Thailand is a country with much ecosystem diversity, it has a potential in producing bioethanol from biomass such as forestry residues, agricultural wastes, woody and non woody biomass. However, a few research in ethanol production is a limitation for the development of the bioethanol production in Thailand. Therefore, this research was carried out with the aim to develop the bioethanol production and to fill the gap between research and general application, and bioethanol production in particular. The idea arose from the present trend of widely used organic products. A very important problem in rice plantation is the growth of unwanted weeds in the paddy field. In this study, two weeds, namely gooseweed (*Sphenoclea zeylanica*) and small-flowered nutsedge (*Cyperus difformis*), were used to produce bioethanol. Lab-scale experiments were first done to find out suitable conditions for ethanol production and were later scaled up. Both biological and chemical pretreatments were applied. A biological method was carried out using micro -termite to digest powdered raw materials at a ratio 2:1 for 3 days. A chemical method was done by alkaline pretreatment with different concentrations of sodium hydroxide and hydrogen peroxide. The ratio of solid to liquid and time were worked out and optimized by using Box-Behnken experimental design and response surface methodology. Enzymatic hydrolysis using commercial cellulase for 72 hours was applied to produce reducing

sugar from the two weeds. The released reducing sugars were fermented by *Saccharomyces cerevisiae* TISTR 5020 at 35°C for 9 days. It was shown that the sugar yield from biological pretreatment was lower than that from chemical pretreatment. Treatment with both NaOH and H_2O_2 gave the highest amount of sugar. Enzymatic hydrolysis with cellulase for 24 hours produced more sugar and the highest ethanol concentration was obtained after 3 days of fermentation. The highest ethanol yield from gooseweed fermentation was 11.84 g/L and that from small-flowered nutsedge was 12.36 g/L. In order to understand the mass transfer, mass balance analysis of bioethanol production process was conducted. Scale-up experiments were carried out with an addition of distillation step using a distiller. The obtained ethanol was tested for energy content with bomb calorimeter. The higher heating value of bioethanol produced from gooseweed and small-flowered nutsedge were 12.61 KJ/g and 25.31 KJ/g, respectively. In conclusion, both gooseweed and small-flowered nutsedge can be promising materials for bioethanol production and applied to create income in the rural community.

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I want to thank all the faculty members, office staffs, and lab manager of School of Renewable Energy and Faculty of Science, Maejo University, Chiang Mai, Thailand for their kindness and co-operation.

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I hereby declare that this thesis has been composed solely by myself and that it has not been submitted, in whole or in part, in any previous application for a degree.

Vu Thi Phuong

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ABBREVIATION

DOE	Design of experiment
CCD	Central composite design
BBD	Box-Behnken design
PB	Plackett-Burman design
BEP	Breakeven point
NDF	Neutral detergent fiber
ADF	Acid detergent fiber
ADL	Acid detergent lignin
ANOVA	Analysis of variance
HHV	Higher heating value
DP	Degree of polymerization
IGEs	Internal combustion engines

CHAPTER 1
INTRODUCTION

Background

The rapid growth of human population has led to a very high demand of fuel production that nowadays becomes an urgent situation over around the world. Although the current energy types are primarily from fossil sources, its reserve will be exhausted by next 40-50 years (Vohra et al., 2014). The world's consumption of fossil fuels by transportation sector accounts for 60%, which consequently contributes to the massive pollution generation to the environment. Thus, the existent patterns of energy consumption and development are not maintainable in the permanent term. Besides, the exploitation as well as the application of these conventional fuels in a long time have caused severe worldwide environmental effects. To illustrate, the raising of CO_2 emission from industrial activities and transportation has led to significant climate change within a short period. Certainly, due to the main dependence of agricultural activities on weather, there is no doubt that it could have adverse impact on agricultural activities that directly effect on food supply. The mentioned global issues of energy security and environment have boosted the requirement of an alternative and green energy source. Many types of green energy have been discovered and applied to reduce the dependence of traditional hydrocarbon deposit sources. They are derived from natural renewable sources like biomass, solar heat, wind, hydro, wave, geothermal and ocean-thermal which are persistent and sustainable (Twidell and Weir, 2015). The technology of using the above green energy are already well-developed and mature except biofuel (Popp et al., 2011).

Biofuels including bioethanol, biogas, biodiesel, biohydrogen, biobutanol, etc. can be produced from biomass via chemical and biological processes (Guo et al., 2015). The increased attention of biofuel has been started in the early of 2003 with the huge promoting of industrial scale production and consumption in European Union and United States (Azadi et al., 2017). Moreover, many countries, such as USA, Brazil, China, Canada, and several Europe member states have already declared guarantees to

biofuel programs as attempts to reduce the dependence on fossil fuels. It is said that among all major renewable energy technologies examined to date, biomass is the primary source of renewable liquid fuels for vehicle, air, and maritime transportation (Wedges, 2004; Ragauskas et al., 2006). According to the International Energy Agency forecast, fuels from biomass feedstock used for transportation purpose will raise from 2% in 2012 to 20% by 2040 (Birol, 2014). Be back to the history, it is known that ethanol have been used widely in transportation sector as alternative fuel in Europe and The United States the early 1900s (Azhar et al., 2017). In 1984, Germany and France started to use bioethanol as a fuel in internal combustion engines (IGEs) (Demirbas and Karslioglu, 2007). Utilization of bioethanol by Brazil was initiated since 1925. However, the production of ethanol at this period did not draw much attention from government and market due to its high production cost comparing to petrol. As shown in Figure 1, the liquid biofuel production that included bioethanol and biodiesel went up steadily in the period of 2001-2013. In 2011, the two top most countries shared this biofuel production were the United States and Brazil which produced 12. 0 and 8.7 MJ per capita per day. In 2016, the global production of bioethanol achieved 100 billion liters which were mainly from the United States and Brazil (RFA, 2017) (Figure 2). In conclusion, the United States is the largest scale producer of bioethanol from corn in the world, followed by Brazil from sugarcane. However, these crops cannot meet the global demand for bioethanol production as alternative energy. Therefore, a requirement of new materials and upgrade current processes to obtain more ethanol yield to meet the increasing demand is needed.

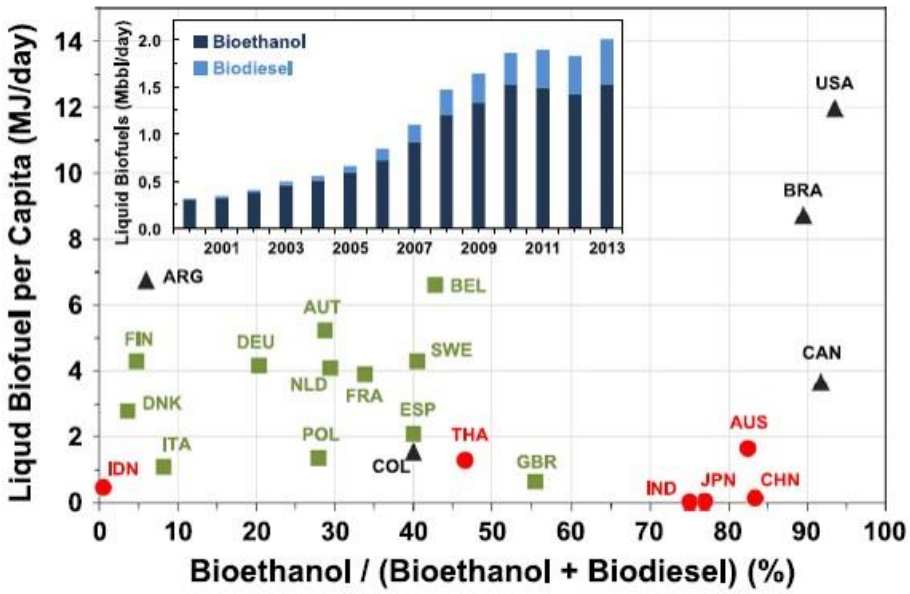


Figure 1 Liquid biofuel production in selected countries (MJ per capita and day). versus the ratio of bioethanol to total liquid biofuels produced in that country in 2011 on an energy basis. The inset plot shows global annual production volume of bioethanol and biodiesel. Different symbols represent different world regions (Azadi et al., 2017)

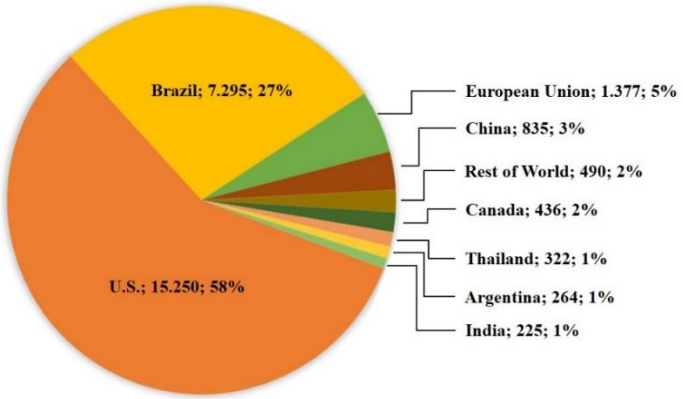


Figure 2 The contribution of ethanol production in 2016 (RFC, 2017)

The main of ethanol production today comes from edible sources (sugar-or starch-based feedstock). As a country with plenty of plants and lands, Thailand is also

on the way to produce biofuels from edible sources (sugarcane and cassava) to meet the high demand of the entire nation. Strategy (2015-2036) target of Thai government to increase the yield of bioethanol to 11.3 billion liters per day in 2036. However, the use of edible source for bioethanol industry exhibit a considerable scale limitation which is relevant to global fuel demand and have led to significant concerns regarding food production (Tilman et al., 2009) (Figure 3). Therefore, the interest in production of ethanol from second generation, so-called lignocellulosic biomass, has been increased recently (Azadi et al., 2017). Although the progress of second-generation bioethanol may not be beneficial as the first generation, the feedstock availability makes it as a gigantic potential if implemented nationally. To further motivate the demand and reduce the conflict of fuel versus food, bioethanol produced from residues, wastes, and non-edible crops should be considered be twice that of other bioethanol. However, this pathway needs more effort from researchers and engineers to overcome the bottleneck of the capital and operation cost for large scale to develop. Bioethanol production from lignocellulosic and cellulosic biomass has been developed and applied in the United State where the very first cellulosic-ethanol factory was built in 2014. Its target was converting 770 ton of biomass per day into ethanol at a rate of 20 million gallons per year (Figure 4).

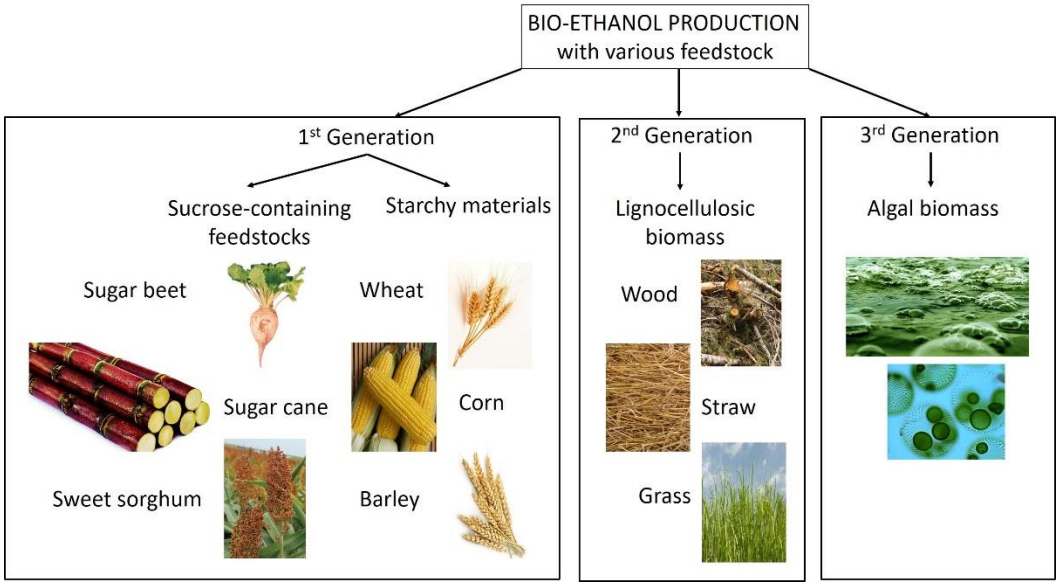


Figure 3 Classification of bioethanol generation

(Wei et al., 2014)

Figure 4 Project Liberty, The first cellulosic-bioethanol factory, the United State (http://poet-dsm.com/)

Gooseweed (*Sphenoclea zeylanica*) and Small-flowered nutsedge (*Cyperus difformis*) are usual and widespread herbaceous weeds of wetland rice (Holm et al., 1977). Gooseweed belongs to the family *Sphenocleaceae* and small-flowered nutsedge is one of species of family *Cyperaceae.* Both plants are able to develop on terrestrial as well as freshwater systems in tropical to temperature areas (Carter et al., 2014). It is instinctive to the Eastern Hemisphere including Thailand, Viet Nam, Indonesia, etc. Since its preferred habitat is wetland and aquatic bodies, these two species has been a problematic non-woody plant on wetland transplanted rice field, and was recognized as one of the worst weed in the world by Holm and his colleagues (Holm et al., 1977). According to Ghosh and Ganguly (1993), dominant gooseweed and other sedges caused 32-50% yield loss in rice field in India because of nutrient and living space competition with rice. Thus, farmers remove this weed by manual, chemical, and biological methods which consume lot of time and efforts without creating any economic benefits (Mabbayad and Watson, 1995). With regards to both economic and energy aspects, gooseweed and small-flowered nutsedge can be promising materials for bioethanol production. Thus, this study was studied to figure out the feasibility of using these weeds to produce a valuable product, bioethanol.

Research Objectives

1. To figure out the pretreatment parameter affecting lignin degradation and yield of reducing sugar.
2. To investigate the potential of producing bioethanol from gooseweed and small-flowered nutsedge.
3. To study the feasibility of digestion by termite colony on gooseweed and small-flowered nutsedge.

Scope of Research

1. The two new materials will be analyzed the compositions and characteristics.
2. Chemical and biological pretreatment will be applied to treat and discover the effect of pretreatment on gooseweed and small-flowered nutsedge.
3. Response surface methodology will be used to optimal the pretreatment condition on the yield of sugar after hydrolysis process.
4. Economic analysis will be conducted to calculate the cost per unit of obtained bioethanol.

Significance of research

Viet Nam, Indonesia, Malaysia, and Thailand etc. are very rich, lignocellulosic plants for bioethanol purpose. In response to the current demand, global bioethanol production has been increased year by year. In Thailand, there is an increasing interest in using ethanol as a neat or blended fuel in the transportation sector as a substitute for fossil fuels such as gasoline. However, a lack of research on those plenty sources and these two weeds in this study have not yet studied for biofuels generally, particularly bioethanol in over the world.

Thailand has approximately 10, 800 hectare of rice area in 2014. These weeds have harmful impacts on rice fields and are often eliminated by herbicide because they compete with rice for essential nutrients. As a result, output of the study will not only have vital contributions in providing new materials in the second generation of bioethanol, but also encourage farmer to remove them manually instead of using chemicals. Secondly, biological pretreatment has been paid more attention from

researcher due to its less energy requirement and environmentally friendly. Using termite to pre-treat samples can reduce the cost for pretreatment and saccharification process. In brief, the study will cover some basic experiments related to termite in order to test the feasibility of the digestion effect of lignocellulosic biomass on ethanol yield. Besides, the study explores the feasibility of building a plant by analyzing economic analysis.

In conclusion, by investigating the potential of these weeds with bioethanol production, not only contributes new materials to list of second generation of bioethanol, but also helps to improve the economy of rural areas.

CHAPTER 2

LITERATURE REVIEW

Characteristic of bioethanol

The three-dimension structure of ethanol compound which is formed of 2 carbon, 6 hydrogen, and 1 oxygen is shown in figure 5. The physical and chemical characteristics of ethanol making it become a promising fuel for transportation sector were listed in the table 1. As a safety and environmental – friendly fuel, ethanol has higher value of octane number, range of flammability limit concentration volume, flash point, and auto ignition temperature comparing to gasoline (Balat and Balat, 2009). Not only the higher octane number allows it to be burnt at a higher compression ratio with shorter burning time, resulting in a lower engine knock, but also higher flash point make it safer to be worked at ambient temperature. Moreover, due to the oxygen contain in ethanol molecule, the combustion efficiency of ethanol is higher (15%) than that of gasoline. In contrast to petroleum fuel, bioethanol is less toxic, readily biodegradable and produces lesser air-bone pollutants (John et al., 2011). Thus, ethanol can enhance the performance of gasoline when blended with ethanol.

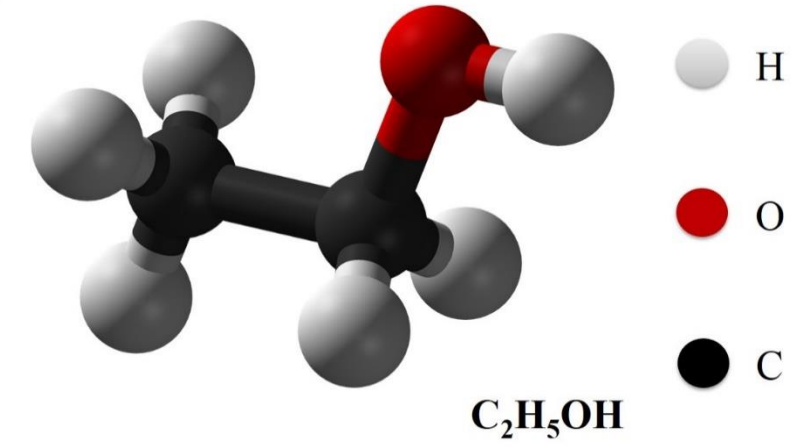


Figure 5 Ethanol molecule in 3D

(https://en.wikipedia.org/wiki/File:Ethanol-3D-balls.png)

Table 1 A comparison of ethanol and gasoline

	Ethanol	**Gasoline**
Energy density (MJ/L)	21.4	30-34
Low heating value (MJ/kg)	26.8	41-44
Research Octane number	90	80-88
Heat of evaporation (MJ/kg)	0.92	0.36
Reid vapor pressure (kPa)	16	54-103
Boiling point (°C)	78	27-225
Solubility at 20 °C	Miscible	Negligible
Kinetic viscosity at 20°C (mm^2/s)	1.5	0.37-0.44
Lower flammability limit concentration volume (%)	3.3	1.4
Upper flammability limit concentration volume (%)	19	7.6
Flash point (°C)	13	-43
Auto ignition temperature (°C)	363	250-300

(Tao et al., 2014)

Lignocellulosic biomass

The potential of lignocellulosic materials as promising feedstock for ethanol production has been draw attention recently due to its abundant availability and cheap cost (Mood et al., 2013; Aditiya et al., 2016). Lignocellulosic materials can be simply divided into different groups including forestry residues, agricultural residues (sugarcane bagasse, corn stover, etc.), aquatic plants, herbs, and energy crops (poplar, switch grass, giant red, elephant grass, etc.) The main portions of lignocellulosic biomass are cellulose, hemicellulose, and lignin which make up 30-50%, 15-35%, and 10-20%, respectively (Limayem and Ricke, 2012). These polymers link together by hydrogen and van der Waals bonds to create recalcitrant matrixes that are stiff and hard (Figure 6) (Volynets et al., 2017). Above all, cellulose chains are the key factor that strongly have positive effects on the sugar yield and ethanol yield. On the other hand, the present of hemicellulose and lignin weak hydrolysis activities and negatively

impact on sugar yield. As a result, understanding of the characteristics of biomass is very essential to design a suitable pretreatment that enhances hydrolysis process.

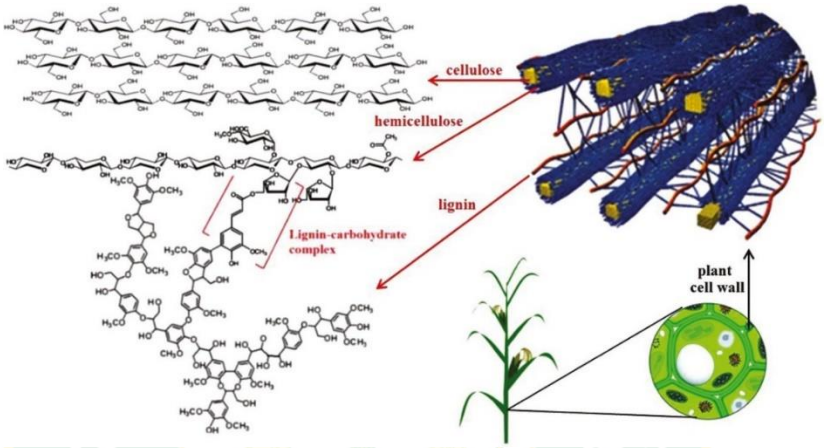


Figure 6 Structure of lignocellulosic materials

(Volynets et al., 2017)

Cellulose

Cellulose is the most abundant organic compound which is around 1.5×10^{12} tons (Klemm et al., 2005). It is composed of many D-glucose molecules linked by β (1→4) - glycosidic bonds and hydrogen bonds. A chain of cellulose is able to reach several thousand glucose units in length that can be formed as crystalline or amorphous regions (Figure 7). While amorphous cellulose is easy to be degraded into monosaccharides by chemical and enzymatic hydrolysis, crystalline cellulose keeps resistant (Hall et al., 2010). Cellulose is recalcitrant to biodegradation so that it is needed to be weaken in a pretreatment step before enzymatic hydrolysis which results to constituent cellobiose units and simpler D-glucose units. It is shown that the enzymatic hydrolysis rate and yields of cellulose crystallization are more than 100 times lower than of amorphous cellulose (Cowling, 1975; Ooshima et al., 1990; Jeoh et al., 2007). Beside, a rising of 10% crystalline cellulose causes 40% decreasing of enzymatic hydrolysis rate (Hall et al., 2010). Therefore, a pretreatment step is needed to not only make cellulose more accessible to hydrolysis agents but also decrease

degree of crystallization of cellulose. In fact, it may require a temperature up to 320°C and a pressure of 25 MPa for the transformation of crystalline-to-amorphous in water (Deguchi et al., 2006).

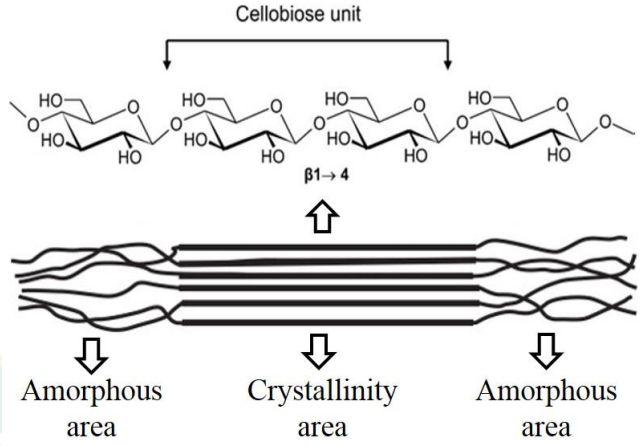


Figure 7 The structure and the structural shape of cellulose

Hemicellulose

Hemicelluloses are cell wall polysaccharides that bind strongly to cellulose microfibrils by hydrogen bonds (McNeil et al., 1984). It contains pentoses (β-D-xylose, α-L-arabinose) and hexoses (β-D-mannose, β-D-glucose, α-D-galactose) (Gírio et al., 2010). It also includes a small amount of other sugars (α-L-rhamnose and α-L-fucose) and uronic acids. However, the most abundant and prominent hemicellulose in the secondary cell walls is xylan which can be made up to 50% of grasses and cereals biomass (Ebringerová et al., 2005).

Lignin

level of lignin which are about 3-30% (Demirbas, 2005). In fact, lignin can be burnt to produce steam or power, pyrolysis, or enzymatically depolymerized to produce mono-aromatic compounds such as gallic and ferulic acids, building clock for phenolic compounds.

The adsorption of lignin to cellulase required a higher enzyme loading. This is because this binding generates a non –productive enzyme attachment and limits the

accessibility of cellulose to cellulase. Moreover, phenolic groups produced from the degradation of lignin substantially deactivate cellulolytic enzymes activities. Therefore, influence enzymatic hydrolysis. Retaining the lignin could have benefits as have demonstrated that lignin components, once recovered from biofuel process may be a potential energy self-sustaining source to retain bio refineries financial solvency.

Process of ethanol production

Generally, the conversion of lignocellulosic biomass into bioethanol includes sequential steps which are namely pretreatment, hydrolysis, fermentation, and distillation (Aditiya et al., 2016). They can be designed differently to optimize the working condition as well as reduce the overall production cost of each stage. Currently, researchers have been created some processes: separate hydrolysis and fermentation (SHF), separate hydrolysis and co-fermentation (SHCF), simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bioprocessing (CBP). Figure 8 performs each process in detail. Co-fermentation (CF) is about the fermentation of both xylose and glucose by a microorganism inside a fermenter. The advantages and disadvantages of each production rout are mentioned as below table (Table 2).

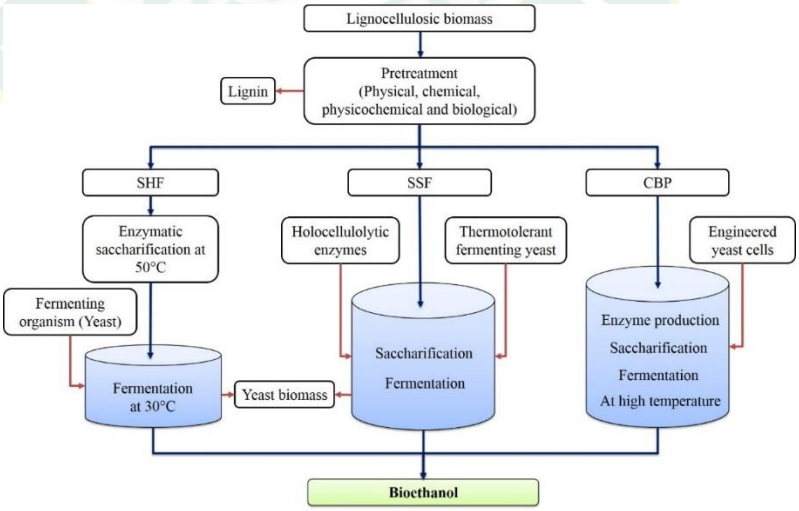


Figure 8 Modified schematic diagram of bioethanol production through different routes

(Choudhary et al., 2016)

Table 2 The pros and cons of different bioethanol production routes

	Advantages	Disadvantages	Reference
SHF (SHCF)	The hydrolysis and fermentation steps can be performed at optimal condition of pH and temperature.	High investment Hydrolysis process can be inhibited by accumulated glucose concentration	(Taherzadeh and Niklasson, 2004)
SSF (SSCF)	Low cost High ethanol yield Short processing time Lessen the negative impact of produced glucose on hydrolysis	Different optimal temperature between saccharification & fermentation	(Baeyens et al., 2015)
CBP	Reduce inhibitors, and operation cost	Suitable microorganism strains for commercial purposes are not yet available	(Fan, 2014)

Pretreatment method

Pretreatment upstream operation include mainly physical and thermochemical processes that involve the disruption of the recalcitrant material of the biomass. Most of current pretreatment technologies produce the by-products that suffer the degraded ethanol productivity. Therefore, an additional detoxification step is required which makes bioethanol production from lignocellulosic biomass economically unfeasible. The turning of lignocellulosic biomass into ethanol begins with the breakdown of the cell wall and release of the starch or sugars in the plants. However, the combination of lignin, hemicellulose, and cellulose is very strong to be split into the simple sugars. That is the main reason for the addition step, pretreatment.

According to some previous researches, the most important factors that affect the hydrolysis efficiency are the accessible surface area, lignin content and the degree

of crystallinity with the cellulose polymer itself (Hendriks and Zeeman, 2009; Alvira et al., 2010; Chiaramonti et al., 2012). Thus, the main target of pretreatment is overcome the physic barriers of the biomass by taking advantages of physical, chemical, biological or the combination of those method. The structure of the cell wall is changed by the attack of physical or chemical agents during pretreatment. In particular, lignin layer is broken, hemicellulose is degraded to monomers, and cellulose is easily exposed to the access of enzyme which converts the cellulose chains to simple sugars (Piccolo and Bezzo, 2009). Furthermore, by undergoing pretreatment process, the crystallinity degree of the cellulose matrix is altered by amorphous shape which reduced the pressure on saccharification step (Aditiya et al., 2016). The change of the structure of cell wall after pretreatment has been proved by many researchers who applied diverse pretreatment methods (Pu et al., 2013). The changes in molecular weights of lignin can be a visible insights during dilute acid and hydrothermal pretreatment (Behera et al., 2014). In general, researchers are able to see the differences between untreated and treated materials by scan electron microscope (SEM) or transmission electron microscope (TEM) (Poornejad et al., 2013; Subhedar and Gogate, 2014).

It is important that application of a suitable pretreatment should be depended mostly on the feedstock characteristics. In general, pretreatment process can be started with physical material's size reduction method that increases the accessible surface of materials, harsh condition (high temperature or pressure and strong chemical) can be used later to totally break the linkage of the cell wall. The more accessible surface leads to a higher efficiency of pretreatment and hydrolysis process. An effective pretreatment is fundamental for optimal successful hydrolysis, and reduces production of inhibitory compounds.

Physical pretreatment

The physical processes of lignocellulosic feedstock pretreatment totally involve in mechanical method, high temperature and pressure without using any chemical reagent. A comparison study was performed and it showed that smaller size of corn stover (about 53-75 um) produces greater outcome by 1.5-fold than the larger

size substrate (Zeng et al., 2007). The main drawback of this method if high energy consumption and high capital investment.

Physico-chemical pretreatment

Behera and his colleagues (2014) pointed out the importance of chemical pretreatment for bioethanol production from lignocellulosic feedstock by listing some of suitable chemical pretreatment methods for industrial scale (Behera et al., 2014). Recent studies conducted have analyzed and compared biochemical and thermochemical conversion pathways based on life cycle assessment studies (Mu et al., 2010). They concluded that despite the equivalent alcohol productivity and energy efficiency performance between the two routes, in the short run biochemical conversion is considered to have more favorable environmental performance than the thermochemical route.

Acid pretreatment

Acid pretreatment has been known as a candidate method for industrial application of bioethanol production from lignocellulosic biomass. The Despite of the high deformed of lignocellulosic structure, using high concentrated acid to damage the cell wall may produce harmful by-product that inactive the activities of downstream microorganism. Due to high temperatures and the acid conditions of the pretreatment, the sugars released by hydrolysis are degraded into two compounds: furfural (degradation of pentoses: xylose and arabinose) and 5-hydroxymethilfurfural or HMF (degradation of hexoses: glucose, mannose, and galactose). While furfural can also degrade into formic acid or polymerize, HMF generates equimolecular quantities of formic and levulinic acid (Bienkowski et al., 1987; Chiaramonti et al., 2012; Dagnino et al., 2013). Acid acetic resulting from dilute acid pretreatment of agricultural residues as well as herbaceous and hardwoods is pH dependent and can reach a high concentration of approximately 10 g/L (Larsson et al., 1999). The acetic acid is produced by the acetyl groups hydrolysis, which is a component of the hemicellulosic fraction, in the shape of substituent of xylose monomers in the solid phase as well as oligomers.

Alkaline pretreatment

Instead of using concentrated/dilute acid at high temperature or pressure, lignocellulosic biomass can be treated with alkaline reagents such as sodium hydroxide, potassium hydroxide, lime, ammonia solution, etc. The reaction of lignocellulosic biomass with alkaline reagent may last for such a long time which ranges from hour to several days (Chiaramonti et al., 2012). Even though there is not necessary to consume more energy, long retention time is one of the main drawback of this alkaline pretreatment which was proved (Kim et al., 2008).

Steam explosion

Steam explosion: high-pressure saturated steam. Pressure is then suddenly reduced, exposing the feedstock to an explosive decompression which opens the biomass structure, increasing enzyme accessibility. The temperature often ranges from 160 - 220°C at 1-2.3 MPa for a short period of time. Steam explosion has been successfully applied for production of ethanol from several lignocellulosic materials. During pretreatment, biomass is heated up by the condensation of steam leading to micro porous structure being filled with liquid hot water. Water acts as a weak acid, which lowers the pH to 3-4 and initiates the depolymerization of hemicellulose. However, this type of pretreatment is quite similar to dilute and concentrated acid method which promote for generation of inhibitor substances for hydrolysis and fermentation (Liu and Chen, 2015).

Liquid hot water pretreatment

Liquid hot water pretreatment is a hydrothermal process which does not employ any catalyst or chemicals. Pressure is applied to maintain water in the liquid state at elevated temperature (160 - 240°C). The main effect of liquid hot water is solubilization and degradation of hemicellulose, making the cellulose more accessible. The pH range can be controlled between 4 and 7 so that the formation of inhibitors can be reduced.

Biological pretreatment

Generally, biological pretreatment is based on the use of microorganisms able to degrade lignin, hemicellulose, and cellulose. Some of the potential microbes are brown-, white-, and soft-rot fungi. The similar microorganism can be found in termite gut and their function as digestive system. The microorganisms living inside its gut are able to break down the lignocellulosic structure. In nature, microorganisms have an important role in degrading lignocellulosic biomass which content lignin, hemicellulose and cellulose. They use many types of different enzyme to break down the polymer chains into simple molecules. In other words, termites can digest lignocellulosic materials like wood, grass, etc. (Sun et al., 2014). Higher termites are on the top for their ability of lignocellulosic biomass degradation. However, the low energy requirement is the main reason for the interest in this kind of pretreatment. In addition, the absence of chemical needed and mild pretreatment conditions are other important advantages of biological pretreatment.

Saccharification

Hydrolysis process takes place after pretreatment to break down the feedstock into fermentable sugar for bioethanol production. There are some characteristic of plant cell wall that significantly effects on the efficiency of hydrolysis process: content of cellulose, hemicellulose, and lignin; cellulose crystallinity, and porosity. Lignin and hemicellulose create a natural physical barrier that prevent the accessibility of enzyme to cellulose chains. Furthermore, the structural order of cellulose such as crystallinity and amorphous also decide the rate of hydrolysis. The more crystallinity shapes appear in cellulose chains, the slower the hydrolysis occurs. In general, the two most commonly used hydrolysis methods are acidic and enzymatic.

Acidic hydrolysis

Acidic hydrolysis can be divided into two types namely dilute and concentrated. Dilute acid hydrolysis is performed at higher temperature using low acid concentration while concentrated acid hydrolysis is carried out at lower temperature using high acid concentration. Acid hydrolysis of lignocellulosic biomass is conducted

into two-stage process as the pentose sugars degrade more rapidly compared to hexose sugars. It generates large amount of inhibitors. However, acid hydrolysis processes have several disadvantages limiting the application to industry. The degradation of sugars to by-products is hard to control the acid is difficult to be separated and recovered from the sugar products, large amounts of acid may contaminate the environment, and dilute acid is corrosive to equipment although corrosion is less of an issue at very high acid concentrations. The disadvantage of acid hydrolysis is the difficulty of performing acid recovery and recycling process which increases the production cost.

Enzymatic hydrolysis

Enzymatic hydrolysis is the preferred saccharification method because of its higher yields, higher selectivity, lower energy cost and milder operating condition than chemical processes (Yang et al., 2011). Cellulose can be hydrolyzed by cellulase enzymes. These enzymes synergistically hydrolyse cellulose to cellobiose and glucose. On the other hand, hemi-cellulose being structurally more complex than cellulose requires much more number of enzymes. The multi enzyme system for xylan hydrolysis includes endoxylanase, exoxylanase, β-xylosidase, α-arabinofuranosidase, α-glucoronisidase, acetyl xylan esterase, and ferulic acid esterase. Most of the solid components of the sample decomposed during the hydrolysis, thereby forming a brown liquid (Küüt, 2013).

Fermentation

Microorganisms such as yeast plays an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. Because the production of bioethanol is founded on the ability of yeasts to catabolize six-carbon molecules such as glucose into two carbon components, such as ethanol, without proceeding to the final oxidation product which is CO_2 (Azhar et al., 2017). They are used in industrial plants due to available properties in ethanol yield (>90.0% theoretical yield), ethanol tolerance (>40.0 g/L), ethanol productivity (>1.0 g/L/h), growth in simple, inexpensive

media and undiluted fermentation broth with resistance to inhibitors and retard contaminants from growth condition (Dien et al., 2003). Certain yeast strains such as *Pichia stipitis, S. cerevisiae and Kluyveromyces fagilis* were reported as good ethanol producers from different types of sugars (Kumar et al., 2009; Pothiraj et al., 2014; Yan et al., 2015; Lewandowska et al., 2016;). There are several factors which influence the production of bioethanol including temperature, sugar concentration, pH, fermentation time, agitation rate, and inoculum size (Zabed et al., 2014). The ideal temperature range for fermentation is between 20 and 35°C. Free cells of *S. cerevisiae* have an optimum temperature near 30°C whereas immobilized cells have slightly higher optimum temperature due to its ability to transfer heat from particle surface to inside the cells (Liu and Shen, 2008). The production of ethanol using free yeast cells is still inefficient due to its higher cost of cell cycling, greater contamination risk, limitation of the dilution rate and susceptibility to environmental variations. Free cells cause substrate or product inhibition from direct contact between the cells and medium. Most of the problem occurred in free-cell systems are reduced by the immobilization method. In addition, there is no significance of ethanol production efficiency between free and immobilized yeast cells (Swain et al., 2007). Although known as the most commonly employed microorganisms, both *Z. mobilis* ad *S. cerevisiae* is incapable to ferment pentose sugars. While *P. stipitis* is recognized in their ability to convert pentose sugar (xylose). However, these bacteria only result low efficiency with high-caring handling; they are vulnerable to acid environment, inhibitors and ethanol with high concentration. The yeast *S. cerevisiae* contain two genes that catalyze not only the reduction of acetaldehyde to ethanol during the fermentation of glucose, but also the reverse action of ethanol into acetaldehyde (Bennetzen and Hall, 1982).

Distillation

Bioethanol obtained from a fermenter requires further separation and purification of ethanol from water through a distillation process (Figure 9). In general, the common and simple distillation technology applied a lot in practice is fractional distillation which is based on the different volatilities of ethanol and other substances inside the fermenter such as water, lignin, unconverted hydrocarbon (Limayem and

Ricke, 2012). Because the boiling point of water (100°C at 1.013 Pa) is higher than the ethanol boiling point (78.3°C at 1.013 Pa), ethanol will be converted to steam before water. The system often is divided into two columns. While the first column is able to remove the dissolved CO_2 and most of the water with the product consist of 37-40 wt% ethanol, the second columns has a role for concentrating the ethanol to a near azeotropic composition (approximately 92.4 wt% ethanol). However, the maximum ethanol concentration obtained after this step can reach to 96% wt only (Cardona and Sánchez, 2007). Therefore, a further dehydrated to 99.5% process by vapor-phase molecular sieve adsorption has to be carried out (Humbird et al., 2011).

Even though this conventional purification method brings a huge benefit of high ethanol recovery, the major drawback of this method are more energy consumption at low ethanol fraction. Thus, there is a need of alternative technologies to reduce used energy and improve the ethanol recovery as well. New advanced distillated technologies with energy and economic efficiency and high ethanol recovery have been investigated and reported previously. Some of them are membrane distillation, liquid/liquid extraction, pervaporative separation, and steam/gas stripping. Membrane distillation is the evaporation process through a hydrophobic membrane whose principle is based on the vapor pressure difference on the both sides of membrane. The main important keys of this process are temperature and compositions of feeding input (Gryta et al., 2000). The pervaporation distillation is the other similar application to membrane distillation which separates the mixtures of liquid by partial vaporization through a solid membrane such as (non)-porous membrane and vapor permeation (Kiss, 2014).

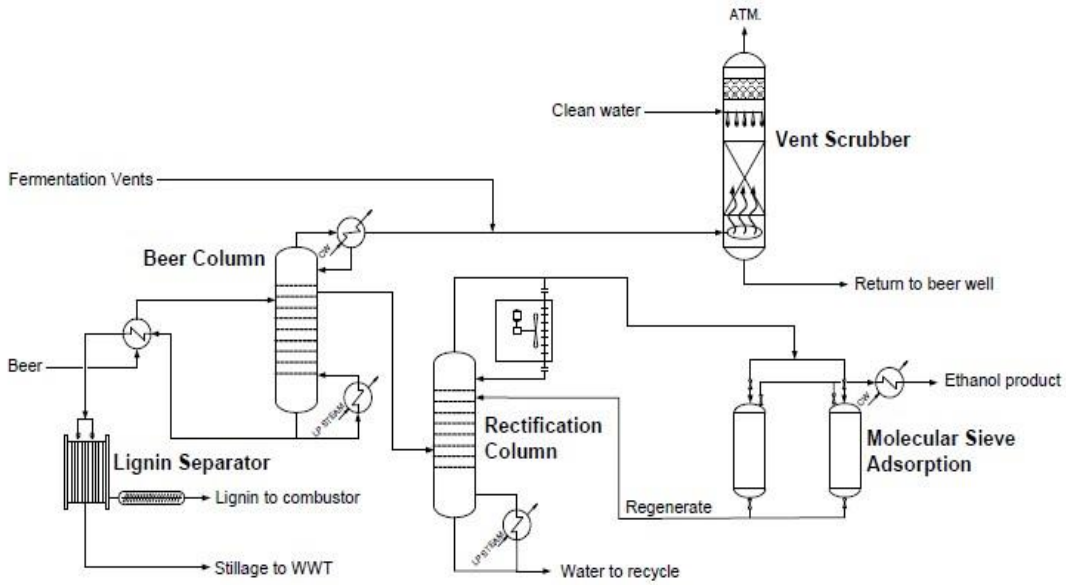


Figure 9 Simplified flow diagram of the separation process

(Humbird et al., 2011)

Design of experiment

The design of experiment (DOE) is a fundamental statistical tool for engineering field (Witek-Krowiak et al., 2014). DOE refers to the process of planning, designing and analyzing the experiment data so that valid and object conclusions can be concluded effectively and efficiently (Antony, 2014). This improves the process by considering only most significant factors, and also to reducing operation costs and saving time (Montgomery and Runger, 2002).There are three type of DOE which includes screening DOE, full factorial DOE, and optimization DOE. A screening DOE is to screen many factors at one time and eliminate insignificant factors of a process by identifying the key factors that significantly affect the process performance or the output. Antony (2014) described screening design was an effective method to take into account a large number of design factors in a lowest number of experimental runs. Full factorial DOE, on the other hand, studies all possible combination of levels of factors to determine statistical significant factors. Last but not least, in order to meet a specific target, the optimal level of significant factors could be set using optimization DOE. Several DOE

methods have been applied for bioethanol optimization including the central composite design (CCD), Box-Behnken design (BBD), Plackett-Burman design (PB), full or fractional factorial design (Figure 10) (Cavazzuti, 2012; Das et al., 2015).

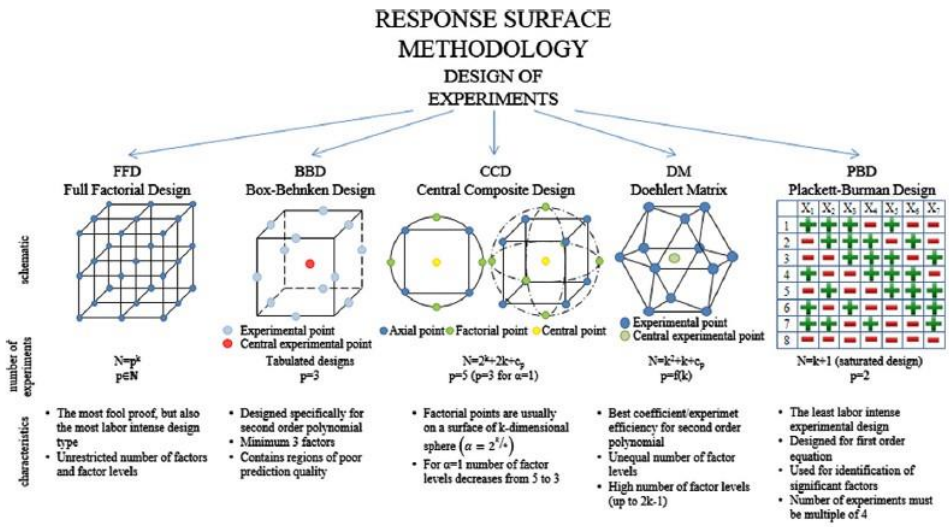


Figure 10 Basic design of experiment models

(Witek-Krowiak et al., 2014)

Optimization design of experiment

Generally, response surface methodology (RSM) is a combination of the mathematical and statistical techniques based on the fit of mathematical models to the experiment results produced from the designed experiment and the confirmation of the model (Antony, 2014). RSM is usually applied for modelling and analyzing a process to study the relation among several independent factors and one or more response and the optimization of a process (Montgomery and Runger, 2002; Ayeni et al., 2013). This combines experimental designs with a method of constructing new data points by first-or second-order polynomial equations in a sequential testing procedure. In fact, RSM has been successfully applied for optimization purpose of some processes of bioethanol production (Saini et al., 2013; Das et al., 2015; Saini et al., 2015). The first step of statistic in optimization is to establish the principles that define experimental factors that have significant effect on the response variables. Many factors may

potentially affect the efficiency of pretreatment, saccharification, and fermentation process of bioethanol production. The two types of RSM including central composite design (CCD) and Box-Behnken design (BBD) were discussed below.

Central composite design

A central composite design is employed to fit an empirical, second-order polynomial model. Since it combines a two-level factorial design with 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 is 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 (Baboukani et al., 2012). A study performed by Ruangmee and Sangwichien (2013) which is about the optimization of enzymatic hydrolysis process of narrow-leaf cattail for bioethanol production by CCD indicated that the predicted and observed glucose amount shared a very high R-squared of 97.72% (Ruangmee and Sangwichien, 2013). This means that the obtained model could be used to predict and optimize the value of significant factors without doing more experiments. Another research of (Avci et al., 2013) used CCD with the total of 20 experimental runs to optimize the acid pretreatment condition to get highest sugar yield from hydrolysis step.

Box-Behnken design

Box-Behnken design is the other useful tool of response surface methodology for optimizing model. The advantage of BBD 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. In fact, the BBD is slightly more labor efficient than the CCD because BBD eliminates all the corner points and the star points which reduces the number of experimental runs required. However, BBD has only two significant limitations. The first is the number of experimental factors has to be equal

or higher than three and the BBD should not be used for fitting other equations but second order polynomial as the below equation (Eq1) (Witek-Krowiak et al., 2014).

$$Y= \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j}^{k} \beta_{ij} X_i X_j + \varepsilon \quad \text{(Eq1)}$$

Where, Y is response (sugar yield), β_0 is the intercept value, β_i (i=1,2...k) is the first order model coefficient, β_{ii} represents the quadratic coefficients of X_i, and β_{ij} is the interaction effects. X_i and X_j are the input variables that influence the response variable and ε represents the random error.

Economic analysis

Economic analysis of biomass chemical conversion technologies is important for its development and commercialization, and one of the key outcomes of an economic analysis is the cost of producing fuels and chemicals. The first generation biofuels (sugar-based and starch-based feedstock) represents a high share of production costs (70%), which is not the case for second generation biofuels, in which the share decreases and becomes less than 40% (Cardona and Sánchez, 2007; Solomon et al., 2007). Lignocellulosic biomass is the most promising feedstock considering its great availability and low cost, but the large-scale commercial production of fuel ethanol from lignocellulosic materials has still not been implemented (Cardona and Sánchez, 2007). Multiple techno-economic analysis (TEA) and life-cycle analysis (LCA) studies have been conducted for various configurations of industrial cellulosic ethanol plants, all of which point toward a path to produce ethanol that is at, or close to, being competitive with petroleum-derived fuels with the potential ability to offset substantial greenhouse gas (GHG) emissions, and indeed, several cellulosic ethanol plants are currently being brought online worldwide (Kazi et al., 2010; Murphy and Kendall, 2015). High production costs and technological uncertainties remain bottleneck for large-scale development of this pathway that depend on environmental and social concerns as well as on economic factors. These challenges, among many others in process integration and yield improvements, must be overcome to cost effectively produce hydrocarbon biofuels from lignocellulosic

biomass. Operation cost, payback period and breakeven analysis are used to investigate the relationships between the planned project cost and the rate of return. The breakeven point (BEP) is the point at which total cost and total revenue are equal, which means there is a balance of the profit and loss. Below figure 11 describes production cost of alcohol and fuels including ethanol reported by Patel et al. (2016). It is obvious that the cost of ethanol production is lower than that of gasoline and hydrogen. This again proves that ethanol as fuel may have advantages over fossil fuels like gasoline in economic aspect.

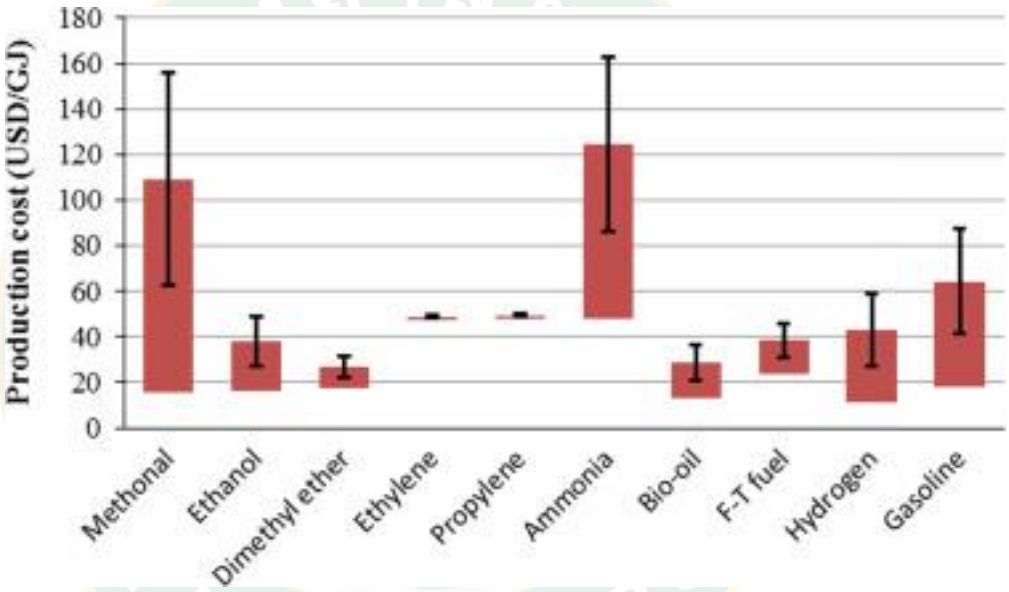


Figure 11 Comparison of chemical/fuels production cost

(Patel et al., 2016)

CHAPTER 3

MATERIALS AND METHODS

The whole experiments carried in this study is described as the bellow diagram (Figure 12). As the materials used are new for bioethanol researches, they firstly were analyzed the compositions by conducting both proximate and compositional analysis. Then, bioethanol was produced in the lab model from these two materials step by step as follow the diagram. After using response surface methodology for optimizing the parameters of pretreatment, testing the suitable time for hydrolysis process, and fermentation time, a scale-up model was done with all the optimal parameters perfectly examined in the lab scale.

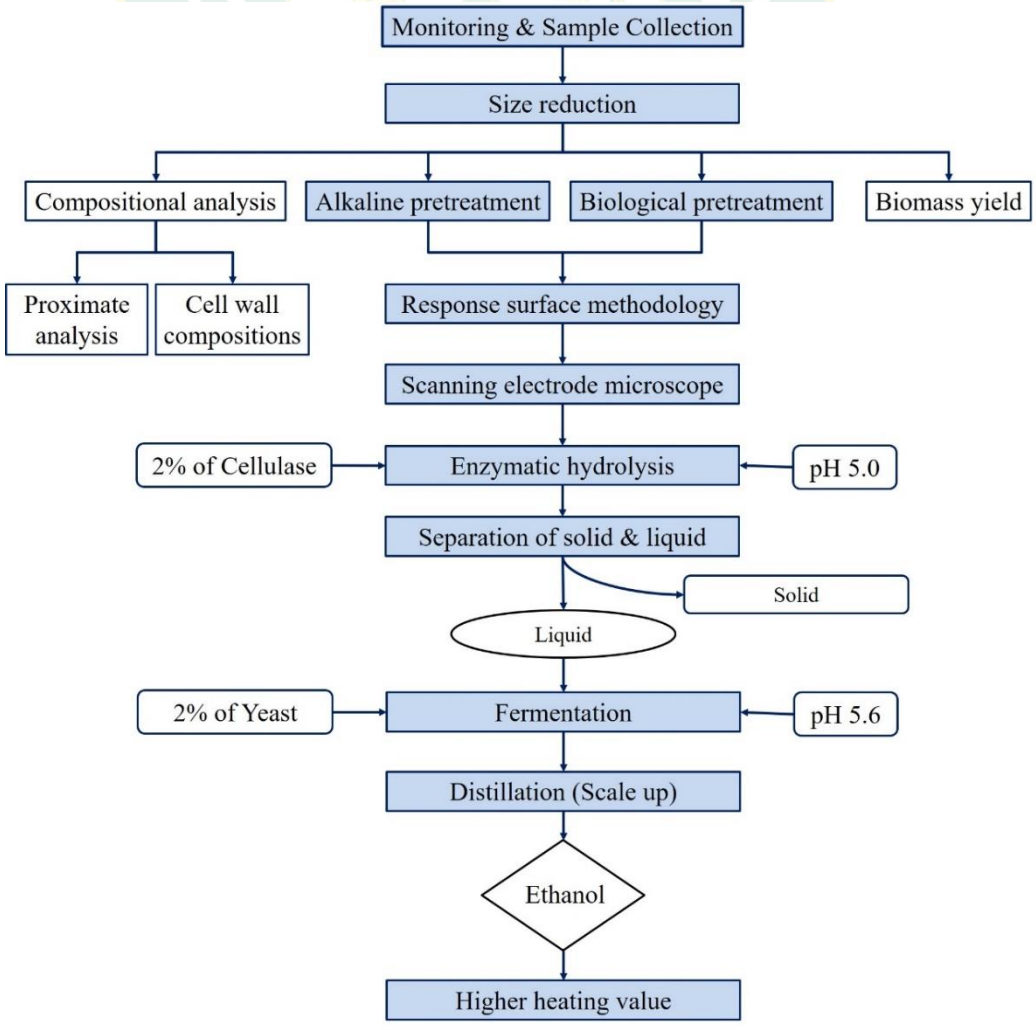


Figure 12 Experimental procedure for throughout study

Sample collection and material preparations

Gooseweed and small-flowered nutsedge were harvested in the organic rice fields located in the campus of Maejo University, Sansai, Chiang Mai, Thailand from September to October 2016 (Figure 13). Fresh samples were moved to the lab of Energy Center Research, Maejo University. Then, they were first washed with tap water to remove dirt, mud and other visible contaminants. A drying rack was used to dried the samples under sunlight for 1 day. The sun-dried samples were placed in a hot air oven at 50°C overnight and were ground to powder that pass the sieve having an aperture size of 1.0 mm (Figure 14). The powdered sample was used for performing experiments shortly after.

Figure 13 Location sampling (Red stars) inside the campus of Maejo University

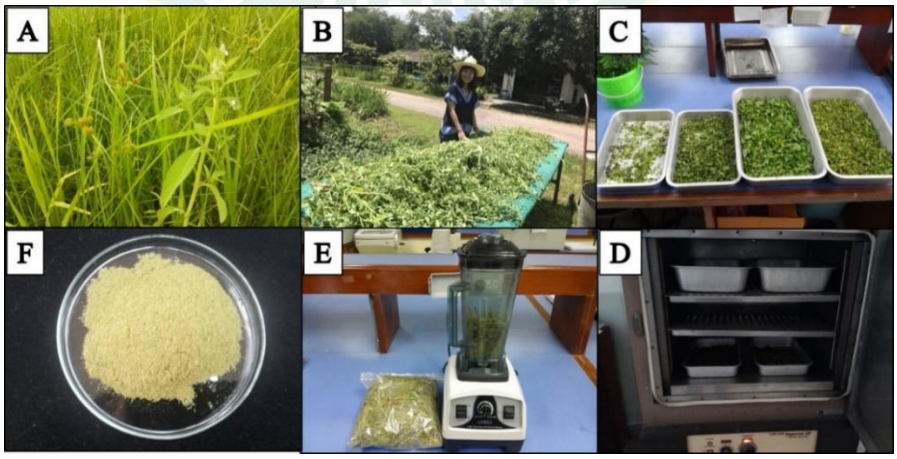


Figure 14 Gooseweed and small-flowered nutsedge in a rice field (A); Sunlight drying (B); Hot air drying (C, D); Powdering process (E, F)

Biomass yield

Biomass yield was calculated by the total mass of plants within a given unit of environment area. Since both gooseweed and small-flowered nutsedge grows in the stagnant area, especially in the rice fields located in Maejo University, Chiang Mai, Thailand (18°53'36.3"N; 99°01'14.4"E). A 1m x 1m quadrat was placed in rice field randomly (Figure 15). The two plants were counted, collected and weighted as fresh samples followed by drying in hot air oven until it reached constant weight. The recorded data was used to calculate density (plant/m^2) and biomass yield (kg/ha).

Figure 15 Counting and collecting sample inside a 1m x 1m quadrat

Lab scale experiment for bioethanol production

The lab scale experiments were carried out to investigate the suitable condition for bioethanol production including pretreatment, enzymatic hydrolysis, and fermentation from these two new materials. The processes are tested in different value of time, concentration, and chemical reagents which are mentioned in detail as the following parts.

Pretreatment

Chemical pretreatment

The chemical pretreatment was adopted and modified from Mishima and Yan (Mishima et al., 2006; Mishima et al., 2008; Yan et al., 2015). In detail, powdered

samples were treated with both sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) at ambient condition for 24 to 72 hours. In order to test the effect and the interaction of chemical concentration, reaction time, and ratio of solid samples to chemical solution on sugar yield during pretreatment process, a study of RSM was carried out using a software, namely design of experiment. According to Myers et al. (2016), RSM is generally used to examine combined effects of several factors and to find optimal conditions for a multivariable system (Myers et al., 2016). The four factors, namely solid/liquid ratio, NaOH concentration (%), H_2O_2 concentration (%), and time (h), were statistically optimized with RSM using Box-Behnken design (Box and Behnken, 1960). The Design-Expert software (Stat-Ease, USA), version 11.0.3.0 was used to build and analyze the experimental design. The low (-1), middle (0), and high (1) levels for each factor were given in table 3. The software displayed totally 27 base runs with 4 runs at the middle points. A repetition of experiments at the central values ensure the accuracy of the data and the reproducibility of model.

Table 3 The low, middle, and high level of the factors by BBD for gooseweed and small-flowered nutsedge

Factor	Unit	Symbol	Coded level		
			-1	0	1
Solid/liquid ratio	-	A	0.05	0.175	0.3
NaOH	%	B	1	1.5	2
H_2O_2	%	C	0.5	1	1.5
Time	Hour (h)	D	24	48	72

The statistical significant of the model was estimated using analysis of variance (ANOVA) with p-value less than 0.05 and insignificance of lack of fit tests. The variables that significantly affected the responses were determined using a confidence level above 95% which p-value less than 0.05. Moreover, the goodness of fit of the model was evaluated by the determination of R-squared, predicted R-squared, and adjusted R-squared coefficients. Three-dimensional surface plots and contours were achieved to demonstrate the effects of independent factors on sugar concentration. A second

order polynomial equation was used to test the effects of independent factors on the response in order to predict the optimal condition divided into linear, quadratic, and interactive components as below (Eq2).

$$Y=\beta_0 + \beta_1 A + \beta_2 B + \beta_3 C + \beta_{12} AB + \beta_{13} AC + \beta_{23} BC + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{33} C^2 \quad \text{(Eq2)}$$

Where Y is the predicted response (sugar concentration g/g); β_0 is the intercept; β_1, β_2, β_3, linear coefficients; β_{11}, β_{22}, β_{33}, squared coefficients; β_{12}, β_{13}, β_{23}, interaction coefficients. A, B, C are coded values for ratio of solid/liquid, NaOH concentration, H_2O_2 concentration, and time.

Biological pretreatment

The biological method, on the other hand, takes advantage of using lower-termite to degrade the materials. Termite colony were collected at School of Renewable Energy campus, Maejo University, Thailand (18°55’34.6’’, 99°1’33.1’’). Termites were separated from the mound and kept in a plastic container for further experiment (Figure 16). A ratio of material to termite in the study was one to two (w:w). The experiment was carried at ambient temperature for three days. The mixture of termite and material were added 100 mL of distilled water and boiled for 1 hour and the solution was determined total and reducing sugar (Figure 17).

Figure 16 Termite collection and preparation

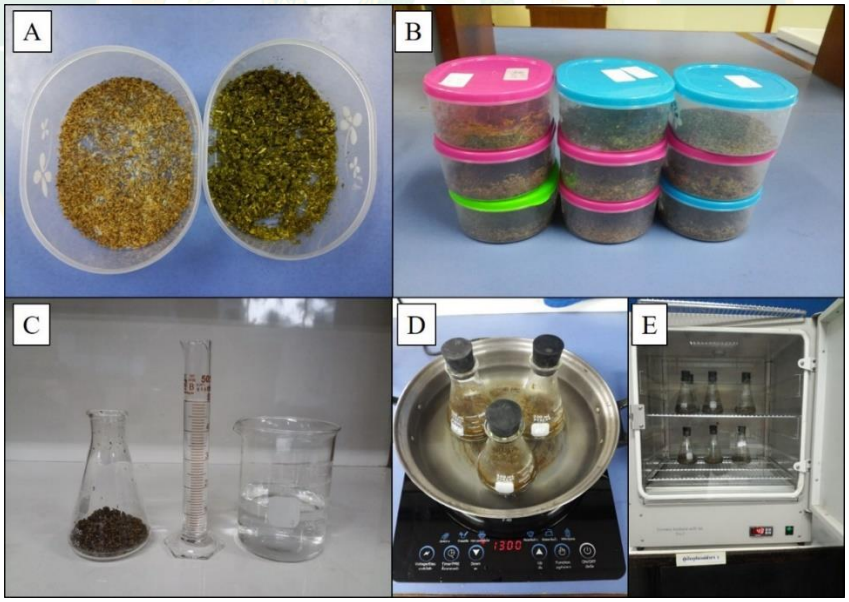


Figure 17 Biological pretreatment; Feeding termite with materials (A and B); Adding 100 mL of distilled water and boiling for 1h (C and D); Enzymatic hydrolysis (E)

Enzymatic hydrolysis

Hydrolysis process was carried out with commercial cellulase enzyme supplied by Union Science Company, Chiang Mai, Thailand. The assay of enzyme are 2398 units/g, beta glucosidase 577 units/g, and pH 4 provided by the supplier. Conical flasks containing 200 mL pretreated sample were adjusted to pH 5 by addition of hydrochloric acid and added 2% (v/v) of cellulase. The mixture was kept at 50°C and agitated at 150 rpm for 24, 48, and 72 hours. The small amount of sample was taken out at each period of time to measure total sugar and reducing sugar following the mentioned methods. The hydrolysis efficiency was calculated by the following formula (Eq3):

$$\text{Hydrolysis (\%)} = \frac{Reducing\ sugar\ released\ (g)}{Total\ sugar\ in\ sample\ (g)}\ x\ 100 \qquad \text{(Eq3)}$$

Fermentation

Microorganism culture

A yeast strain, *Saccharomyces cerevisiae* TISTR 5020, was obtained from Faculty of Science, Maejo University, Chiang Mai, Thailand. This yeast was cultivated in autoclaved (120°C for 15 min) liquid YPD medium (10 g l^{-1} yeast extract, 20 g l^{-1} peptone, 20 g l^{-1} dextrose) at 150 rpm for 24 hours. Then the broth was transferred into centrifuge tubes and centrifuged (7000 rpm, 4°C, 10 min) to separate yeast cells and medium. A same volume of sodium alginate 2% was added to the yeast cell pellet and mixed properly. A syringe was used to drop the mixture into a flask of 150 mL calcium chloride 0.05 M. Finally, immobilized yeast cells were washed with autoclaved distilled water and kept in fridge at 4°C for further using. The cell count of actively growing *S. cerevisiae* was measured using hemocytometer, corresponding to 2.5 x 10^7 cell/mL (Figure 18).

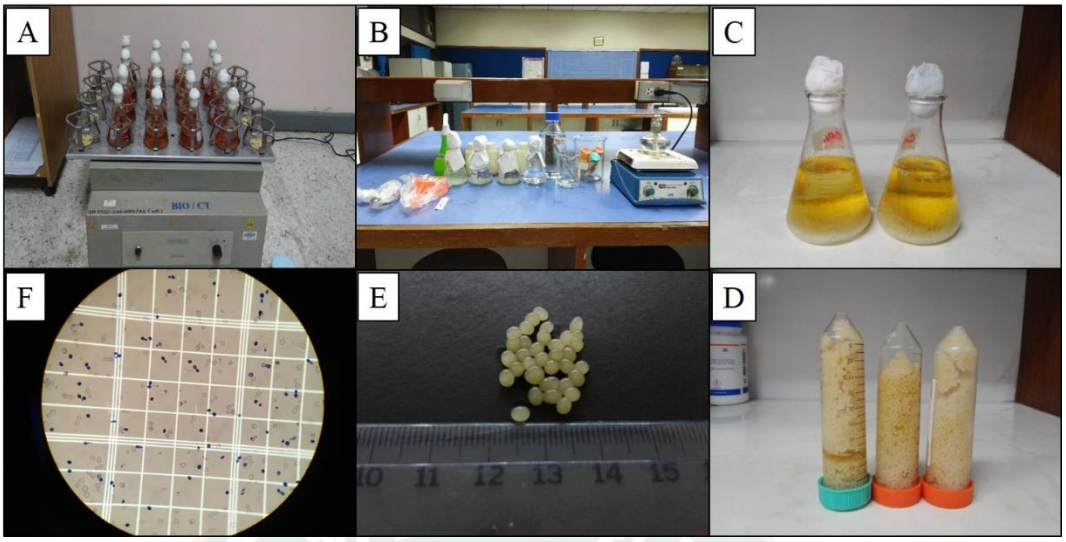


Figure 18 Yeast culturing (A), Producing immobilized yeast (B, C, D, E, F)

This study applied batch fermenters for fermentation process. Hydrolysate solution which was adjusted to pH 5.6, was fermented with 2% of immobilized yeast S. cerevisiae beads in 100 mL working volume fermenter. The mixture was incubated at 35°C from three to nine days (Thangavelu et al., 2014). Aliquots of fermented samples (50 mL) were collected in the fermenter after 3, 5, 7, and 9 days to measure the percentage of ethanol by using Ebulliometer (Dujardin-Salleron, Alcohol Burner, France). The principle of this method is based on the different boiling points of pure water (distilled water) from water-alcohol solutions. The sample solution should be centrifuged in order to be free of suspended solid before measure temperature with Ebulliometer. A calculating dial is used to determine the percentage of ethanol by comparing those two temperatures. Moreover, total sugar and reducing sugar were also determined after 3,5,7, and 9 days to observe the change to sugar comparing to bioethanol produced. The ethanol yield was calculated as follows (Eq4):

$$Y(\%) = [(E \times 0.9)/(G \times 0.51)] \times 100 \qquad \text{(Eq4)}$$

where E is the ethanol concentration in g/L and G is reducing sugar concentration in g/L.

Analytical method

Proximate analysis

Moisture content (%) was determined by drying at 105°C for 4 hours (Singh et al., 2017). The moisture content of sample was estimated by percentage of mass loss at 105°C. Ash content (%) was estimated using muffle furnace at 575°C for 4 hours (NREL, 2008). Moisture, total solids (TS) and ash content were calculated as weight percentage using Eqs. (5), (6), and (7). For estimation of volatile matter (VM), also known as volatile matter, the crucibles and sample were kept in a muffle furnace at 925°C for 7 min (Singh et al., 2017). The percentage of volatile solid was the difference in weight loss at 925°C. The calculation of volatile matter and fixed carbon (FC) were followed Eqs. (8) and (9).

Crucibles and sample in above mention were allowed to cool in a desiccator and recorded the weight using an analytical balance with 4 digits (Ohaus, USA).

$$\%\ \text{Moisture} = \frac{\text{Weight}_{\textit{oven sample and crucible}} - \text{Weight}_{\textit{crucible}}}{\text{Weight}_{\textit{initial sample}}} \times 100 \qquad \text{(Eq5)}$$

$$\%\text{Total solid (TS)} = 100 - \%\text{Moisture} \qquad \text{(Eq6)}$$

$$\%\text{Ash} = \frac{\text{Weight}_{\textit{ash}}}{\text{Weight}_{\textit{initial sample}}} \times 100 \qquad \text{(Eq7)}$$

$$\%\ \text{Volatile matter} = \%\text{TS} - \left(\frac{\text{Weight}_{\textit{initial sample}} - \text{Weight}_{\textit{oven sample}}}{\text{Weight}_{\textit{initial sample}}} \times 100\right) \qquad \text{(Eq8)}$$

$$\%\text{Fixed carbon} = \%\text{TS} - (\%\ \text{Volatile matter} + \%\text{ash}) \qquad \text{(Eq9)}$$

Iodine test for starch

This is a qualitative test using iodine to determine the presence of starch in plant materials. Iodine reagent reacts with amylose chains, one of a main component of starch, and performs deep blue color. The sample is then observed under a microscope. The test was carried out for both gooseweed and small-flowered nutsedge.

Biomass characteristic analysis

The compositions of sample were determined by Van Soest method (Van Soest et al., 1991) in faculty of Animal Science, Maejo University. The percentage of cellulose,

hemicellulose, and lignin are calculated from neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL). Firstly, 1.0 g of milled sample was reacted with 100 mL NDF detergent solution and 0.5 g of sodium sulfite. Since detergent dissolved soluble matters, the left residue was only cell wall which is cellulose, hemicellulose, and lignin. The mixture was boiled in a reflux system for two hours. Then, the crucibles and samples were washed three times with hot water and acetone by a cold extraction unit (FT 121 Fibertec™, Denmark). These above samples and crucibles were continuously added ADF detergent solution and boiled by a hot extraction unit (FT 122 Fibertec™, Denmark) for one and half hour. For ADL, the crucibles and residues from ADF were treated by 72% H_2SO_4 for 3 hours. The equipment used for these experiments was showed in figure 19. After reaction with reagents in above experiments, the sample and crucibles were washed with boiled water, distilled water, acetone and dried at 105°C for 4 hours, kept in desiccator for cooling and weighted out by analytical balance 4 digits (Ohaus, USA). The percentage of NDF, ADF, and ADL were calculated using Eqs. (10), (11), and (12).

$$\%NDF = \frac{Weight_{NDF\ and\ crucible} - Weight_{crucible}}{Weight_{initial\ sample}} \times 100 \quad \text{(Eq10)}$$

$$\%ADF = \frac{Weight_{ADF\ and\ crucible} - Weight_{crucible}}{Weight_{initial\ sample}} \times 100 \quad \text{(Eq11)}$$

$$\%ADL = \frac{Weight_{ADF\ and\ crucible} - Weight_{crucible}}{Weight_{initial\ sample}} \times 100 \quad \text{(Eq12)}$$

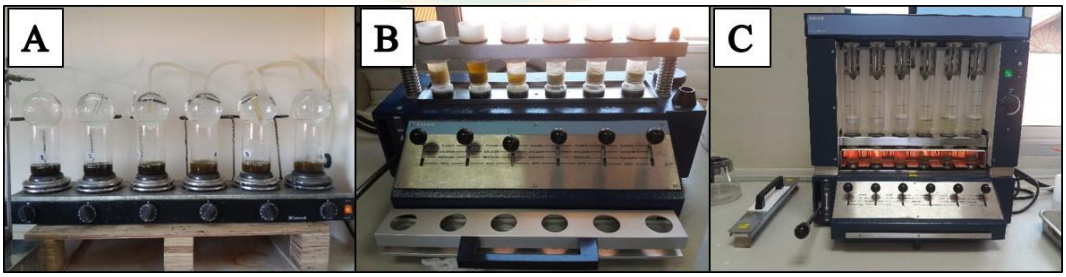


Figure 19 Reflux system (A); Cold extraction unit (B); Hot extraction unit (C)

Scanning electron microscope (SEM)

Pretreatment is expected to have positive effects on the cell wall structure of biomass. Thus, the morphology of gooseweed and small-flowered nutsedge before and after chemical pretreatment was studied using SEM (JSM-5410LV, USA). Powdered biomass and residues after pretreatment were coated in pure gold and dried by a dryer (CPO 7501 Critical Point Dryer, USA) for 150 seconds at 15 mA. Gold coating is required to create conductivity properties for biomass. Since the presence of water interrupts the vacuum and the qualities of images, the samples have to be dried carefully. Both gold and samples were then attached inside the specimen chamber and were shot by electron beam at 15000 kV. The secondary electron detector catches the signal and present an enlarged image of the sample surface on the monitor screen (Figure 20).

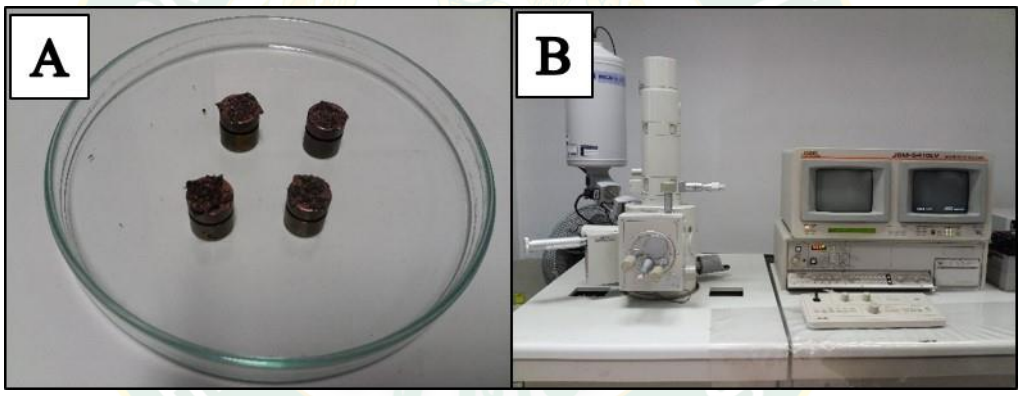


Figure 20 Pure gold coated sample (A); Scanning electron microscope unit (JSM-5410LV, USA) (B)

Scale up for bioethanol production

Figure 21 shows the schematic diagram of scale up experiment. It was carried out in 5 L flask bottle with a working volume of 4 L. An amount of 400 g sample was soaked in 4 L of pretreatment reagents (NaOH/ H_2O_2) for 48 hours (gooseweed) and 72 hours (small-flowered nutsedge). After pretreatment, pH was adjusted to 5.0 and enzymatic hydrolysis was conducted with 2% of cellulase enzyme (v/v) at 50°C for 24 hours. The brown hydrolysate was then fermented with 2% of immobilized yeast (w/v) at 35°C for 3 days. 50 mL was taken to determine percentage of bioethanol by

Ebulliometer when fermentation finished. The separation of solid and liquid using a refrigerated centrifuge (Harrier 18/80, USA) is needed before distillation. A distiller (Mega home 316, Taiwan) was used for distillation. The obtained ethanol was estimated higher heating value (HHV) using a bomb calorimeter (Art.2060/2070 Bomb Calorimeter, Thailand).

1g of bioethanol were weighed into the crucible and placed on the support pillar in the base of the calorimeter. A 12 cm length of steel thread was positioned between the coils of the firing wire with the other end dipped in the center of sample in the crucible. The steel thread works as electrical conductor and ignites the fuel. The system was then enclosed and oxygen was pumped into the chamber at a pressure of 30 atmospheres (atm) to ensure that complete combustion took place. The reaction was occurred for 6 mins. The bomb was then fired and the maximum deflection of the galvanometer was noted. The temperature rise of the bomb calorimeter was measured with the calibrated galvanometer-thermocouple assembly. The HHV of the sample was determined using the calibration factor as calculated using benzoic acid in kJ per division, the mass of sample burnt and the deflection of the sample.

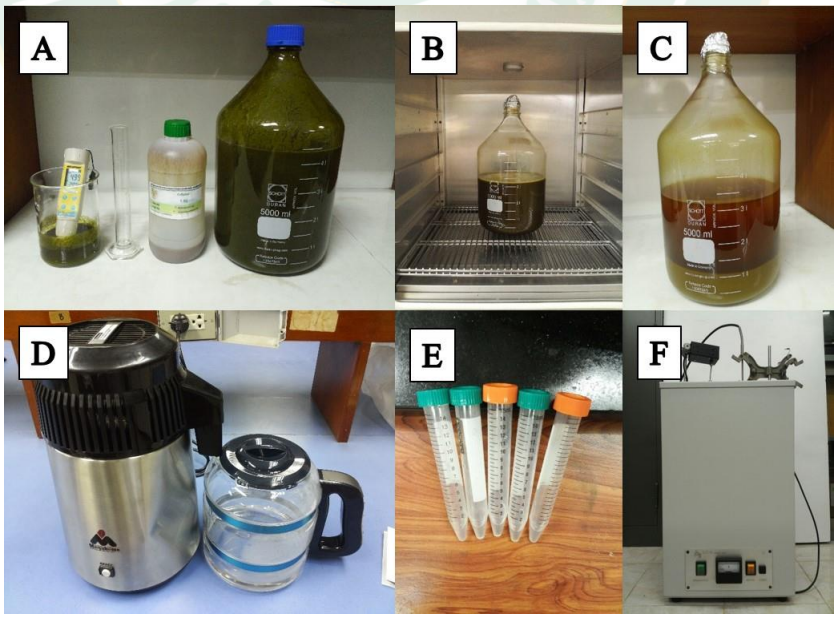


Figure 21 A scale up of 4 L for bioethanol production: Pretreatment/ Hydrolysis (A and B); Fermentation (C); Distillation (D and E); Bomb calorimeter (F)

Economic analysis

In this work, a scale-up bioethanol production from gooseweed and small-flowered nutsedge (4L) were used for evaluation. All of the value of currency used in this test is on the year of 2018. The input of this analysis includes chemicals, equipment, and other utilities (pump, cooling towers, etc.), so-called capital investment cost. The price of these items were referred from one of the largest trade website, namely Alibaba. Furthermore, the variable operating cost is the total of the raw material cost, utilities cost (water, electricity, etc.) (Sadhukhan et al., 2014). However, the materials used in this work were supplied without any charges. The cost per unit of bioethanol in this study is calculated based on the below equation (Eq13):

$$CA = CK + CL + CE + CM + CO - PP \quad \text{(Eq13)}$$

Where CA is the total cost per liter of bioethanol produced from biomass; CK is the cost of raw materials, CO is the cost of operations and maintenance, PP is the credit received for power supplied back to the electrical grid from the processing of lignin. In this study, CK is assumed as zero due to the free available feedstock.

Statistical analysis

All the experiment was performed in triplicate and data were analyzed by analysis of variance (ANOVA) using SPSS software (USA). Simple statistic (means, standard deviations) were computed for each parameter. Results were performed as mean ± SD.

CHAPTER 4
RESULTS AND DISCUSSION

Iodine test for starch

Since the sample chosen in this research are totally new in ethanol production, both gooseweed and small-flowered nutsedge were firstly pre-tested the presence of starch with iodine solution. This method applied to scan the characteristic of a plant that are able to be a promising feedstock for bioethanol production without using any complex analyzing methodology. Thus, it reduces cost and time of the whole research. It was shown that starch is available in both weeds (Figure 22 and 23). High starch content and abundance in numbers makes these two weeds were possible for being raw materials in this study.

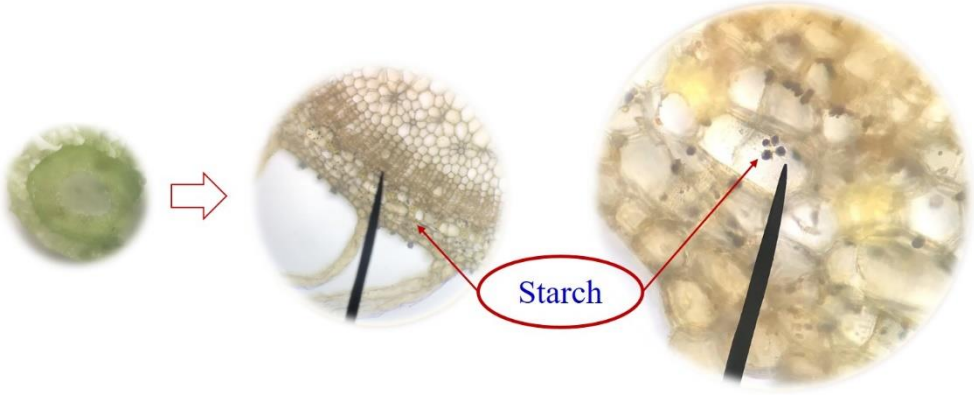


Figure 22 The presence of starch in gooseweed

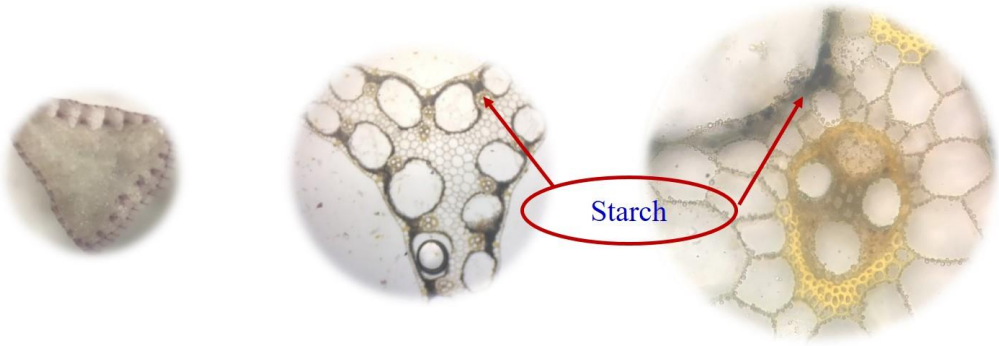


Figure 23 The presence of starch in small-flowered nutsedge

Proximate and compositional analysis

Table 4 shows the results of proximate and compositional analysis of both gooseweed and small-glowered nutsedge. In this study, it can be seen that the moisture content of both samples were quite low compare to other aquatic plants *Impereta cylindrical, Eragrostis airoides, Typha angustifolia , Arundinella khasiana, Echinochloa stagnina* with 8.55%, 8.28%, 13.95%, 10.37%, 10.27%, respectively (Singh et al., 2017). Moisture is an important property because this effects on storage condition of biomass, handling, feeding facilities and conversion processes (Rentizelas et al., 2009; Cai et al., 2017). The physicochemical properties influences to handling, storage, and transportation facilities while the compositions of biomass effects on conversion efficiency of feedstock into energy (Cai et al., 2017). Low moisture content materials (<15%) are often preferred by solid and gas conversion process, while high moisture content materials can be dealed with bio-conversion (Nanda et al., 2013). Besides, to be considered as a promising feedstock for bioethanol production, high volatile matter and low ash contents are highly preferred. Volatile matter values of both goosweed and small-flowered nutsedge were resulted in similar values with other potential lignocellulosic biomass such as wheat straw, flax straw, trimothy grass, pinewood, and barley straw with a range of 77.9 – 82.4%.

On the other hands, the conversion of lignocellulosic biomass into valuable bioethanol depends mostly on the cellulose and hemicellulose content. In another way, cellulose chains are polysaccharides which are composed of a lot of fermentable sugars (D-glucose) while hemicellulose is made up of both pentose and hexose sugars (Ravindran and Jaiswal, 2016). The cellulose, hemicellulose, and lignin content of gooseweed is lower than small-flowered nutsedge as reported in the below table. In comparison with water hyacinth whose cellulose, hemicellulose and lignin were 18.3%, 23.3%, and 17.7%, separately (Gao et al., 2013).

Table 4 Proximate analysis and compositions of gooseweed and small-flowered nutsedge

Parameters	Gooseweed	Small-flowered nutsedge
Physical analysis (%)		
Total solid	93.94 ± 0.12	94.39 ± 0.22
Moisture	6.06 ± 0.12	5.61 ± 0.22
Fixed carbon	1.77 ± 0.1	2.72 ± 0.05
Volatile matter	83.12 ± 0.06	82.42 ± 0.17
Ash	9.5 ± 0.09	9.25 ± 0.09
Compositions (%)		
Cellulose	13.69 ± 0.23	22.05 ± 0.11
Hemicellulose	11.44 ± 0.41	30.2 ± 1.06
Lignin	2.51 ± 0.17	2.78 0.09

Biomass yield

The research of lignocellulosic biomass for biofuel production makes up 40% of the total share of studies on promising materials (edible, lignocellulosic, and algal biomass) (Azadi et al., 2017). The research was conducted in rice fields in which these two weed plants were dominant. The average density of gooseweed and small-flowered nutsedge were 59 plants/m^2 and 38 plants/m^2, respectively. High density of these plants causes the loss of rice yield due to the competition of nutrients and other essential elements between weeds and rice plants. Gooseweed resulted 207 kg/ha rice yield, while small-flowered nutsedge produced 201 kg/ha rice yield. Yields varied with season (these plants prefers wet land than drought land) and the method of growing rice. For example, both gooseweed and small-flowered nutsedge grow abundantly and vastly in organic rice fields when compares with normal rice fields using chemical fertilizers.

Lab scale experiment of bioethanol production

Pretreatment

Pretreatment step is vital for the conversion of lignocellulosic biomass to bioethanol due to its main effect on rigid structure of lignocellulose. It was found that both pretreatments (biological and chemical) enhanced the release of reducing sugars when compared with untreated materials. This implies the positive effects of pretreatment on biomass structures. Cellulose is recalcitrant to biodegradation and needs to by hydrolyzed in an initial pretreatment step into its constituent cellobiose units and into simpler D-Glucose units in order to be liable to biochemical conversion. In order to hydrolyze lignocellulosic biomass with enzyme successfully, it is also important to apply a suitable pretreatment that can effectively disrupt linked lignin and crystalline cellulose (Taherzadeh and Niklasson, 2004). Both two samples were treated at the same bioethanol production procedure which from pretreatment to distillation as the final step.

Biological pretreatment

Using biological method as pretreatment has been widely studied recently due to its less harmful and less energy consumption than others pretreatment method. The bottlenecks of this type of method are long retention time and low sugar yield when compares with other methods. However, be considering with sustainable pretreatment way, biological method still has its potential as high efficient rout to achieve sustainable bioethanol production. Herein, termite colonies were used to digest the biomass instead of applying chemical pretreatment. The results from experiments were performed as figure 24 and 25. The rate of sugar degradation of total sugar is faster than the rate of reducing sugar degradation. In addition, it can be assumed from the control experiments that boiling for 1 hour does not have significant effects on total sugar yield. On the other hand, the results from pretreated small-flowered nutsedge are inverse. The main reason of this strange phenomenon may be caused by the loss of sugar due to the appearance of fungi that mainly live on soluble organic matters including fermentable sugar. Even though termite could lead to an

increase of total sugar and reducing sugar in gooseweed, sugar yields produced from these experiments are quite low comparing to chemical experiment.

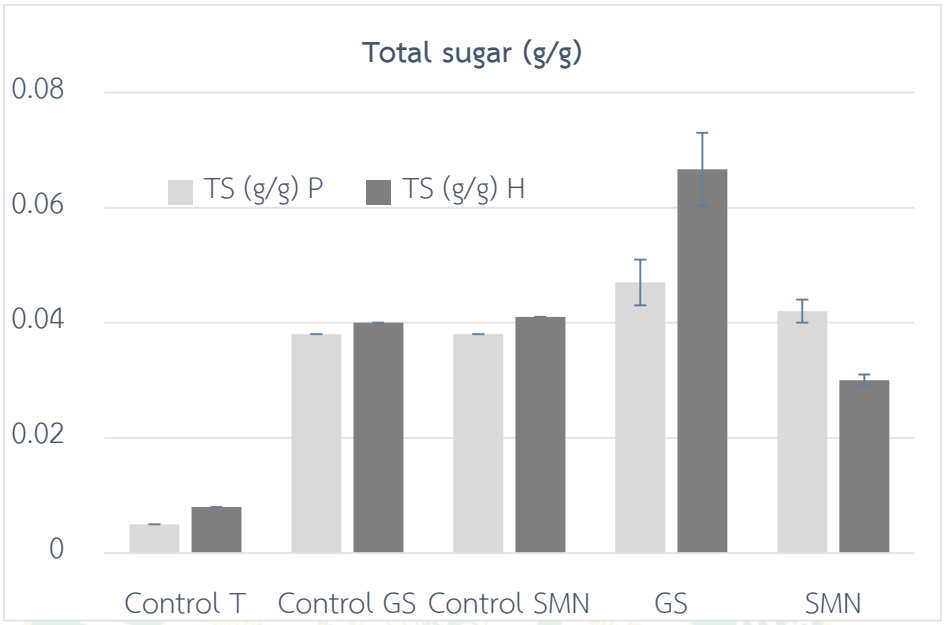


Figure 24 Total sugar production from gooseweed (GS) and small-flowered nutsedge (SMN) with biological pretreatment. Control T is the experiment of termite only

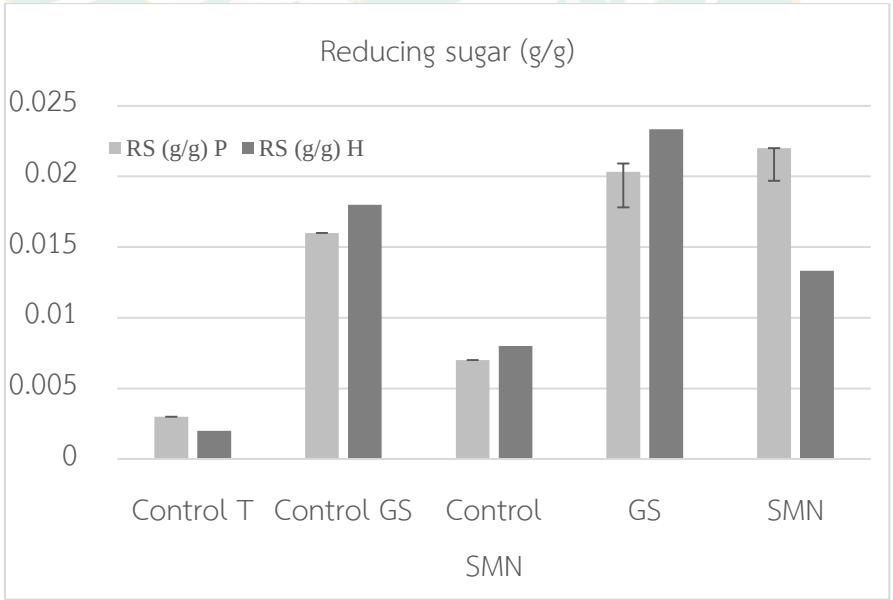


Figure 25 Reducing sugar production from gooseweed (GS) and small-flowered nutsedge (SMN) with biological pretreatment

Chemical pretreatment

For non-woody plants, pretreatment with alkaline reagents, NaOH, were proved to be more effective than acid pretreatment (5% and 10% H_2SO_4) and physical pretreatment (autoclave) (Menegol et al., 2014). Other researches also were in an agreement with the effectiveness of alkaline pretreatments using NaOH and H_2O_2 for the improvement of enzymatic hydrolysis process. The higher yields of reducing sugar from treated samples with alkaline reagent resulted from lower lignin content and higher cellulose content. This was also observed on elephant grass by (Menegol et al., 2014). Pretreatment of water hyacinth with NaOH/H_2O_2, followed by cellulase hydrolysis yielded a maximum reducing sugar of 10.8 g/100 g hyacinth (Mishima et al., 2006). Xia et al. (2013) obtained a maximum reducing sugar yield of 48.3/100 g water hyacinth when treated the biomass with 1% H_2SO_4 at 140°C for 15 min and carried out hydrolysis with cellulase enzyme.

Optimization of chemical pretreatment by Box-Behnken design

The two materials, gooseweed and small-flowered nutsedge, were investigated as a promising feedstock for bioethanol production. To explore the effect of chemical pretreatment at mild condition on total sugar, Box-Behnken design was applied to optimize the total sugar after pretreatment process with four selected independent variables (ratio of solid/liquid, NaOH concentration, H_2O_2 concentration, and time).

A second order polynomial equation was used to test the effects of independent factors on the response in order to predict the optimal condition divided into linear, quadratic, and interactive components as below. The empirical models in terms of coded factors for total sugar responses after pretreatment are given in following equations. The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

$$Y_1 = 0.0406 - 0.0412A - 0.0018B + 0.0028C - 0.0062D + 0.0040AB - 0.0195AC - 0.0085AD + 0.0073BC - 0.0013BD - 0.0023CD + 0.0886A^2 - 0.0049B^2 - 0.0057C^2 - 0.0024D^2$$

(Eq14)

Where Y_1 are the total sugar (g/g dried biomass) from gooseweed; A, B, C, and D are, respectively, the ratio of solid/liquid, NaOH concentration (%), H_2O_2 concentration (%), and time (h).

From the above Eq 14 (gooseweed), it can be implied that the increase of three factors including ratio of S/L, NaOH concentration, and time did not lead to the positive increase of total sugar as the response. The interaction of efficiency of determination also show the similar trend which means that the change of one factors did not have significant effect on the relationship of response and the other factors. In short, these effects of two parameters on response at once time can be described by figure 27 - 32. The ANOVA analysis was conducted to determine the significance of model equation and model terms and was performed as table 5. The Model F-value of 57.25 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.05 indicate model terms are significant. In this case A, AC, A^2 are significant model terms. Values greater than 0.1 indicate the model terms are not significant. Lack of fit F-value of 3.59 implies the lack of fit is not significant relative to the pure error. There is a 23.74% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit indicates the fit of model that does not be affected by pure errors when the number of factors increase. Predicted R^2 of 0.9178 is in reasonable agreement with the Adjusted R^2 of 0.9680; i.e. the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 22.878 indicates an adequate signal. This model can be used to navigate the design space. In conclusion, from the result of ANOVA analysis test, it can be concluded that the model was definitely fit to the experimental data and can be used to predict the response value. Furthermore, the figure 26 show the very high correlation between actual and predicted value which emphasizes the reliable of the model.

The relationship between the response and variables was visualized by the iso-response contour plots and three-dimensional surface plots to see the influence of the parameters. Surface and contour plots demonstrating the effects of different

process parameters, two parameters were varied at a time while keeping the third one constant at a middle level. Such as were used to understand the both linear and interaction effects of the two variables. Figures 27 - 32 show the surface response plots for optimization of the conditions for alkaline pretreatment. The curvature nature of the surfaces showed that there are significant and moderate interactions among ratio of solid/liquid, NaOH concentration, and time.

The maximum total sugar (0.187 g/g) was obtained using solid to liquid ratio of 0.05, NaOH concentration of 1.5%, H_2O_2 concentration of 1.5 for 48 h. On the other hand, the corresponding conditions for minimum total sugar (0.018 g/g) were solid to liquid ratio of 0.175, NaOH concentration of 2%, H_2O_2 concentration of 0.5% for 48 h. The optimal values of selected factors for the pretreatment condition of gooseweed is solid to liquid ratio of 0.05, NaOH concentration of 1%, H_2O_2 concentration of 1% for 48 h. The total sugar concentration was achieved from the regression equation (0.171 g/g) which is near to the experimental value (0.161 g/g ± 0.008).

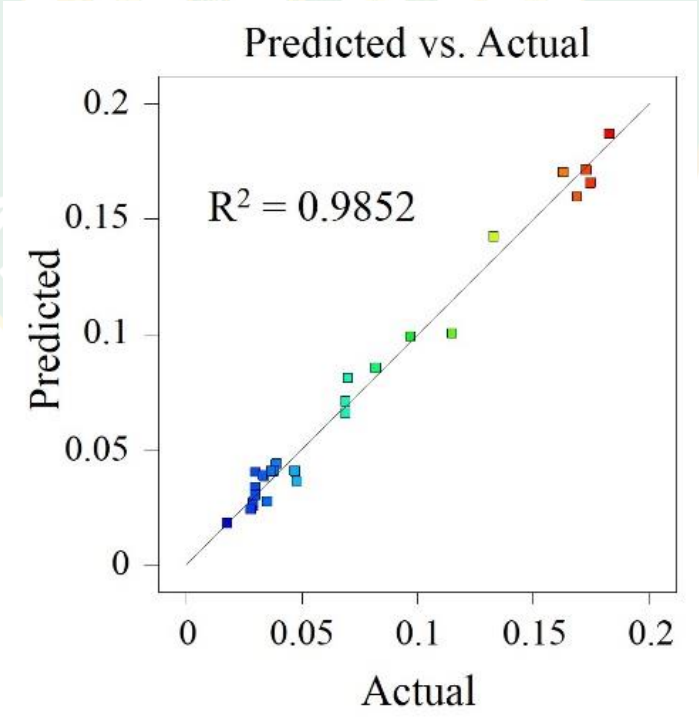


Figure 26 Experimental data plotted against RSM model predicted data of pretreatment for gooseweed

Table 5 ANOVA analysis for quadratic model from experimental design for gooseweed

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0787	14	0.0056	57.25	< 0.0001	significant
A-Ratio of S/L	0.0203	1	0.0203	207.10	< 0.0001	
B-NaOH	0.0000	1	0.0000	0.4145	0.5318	
C-H_2O_2	0.0001	1	0.0001	0.9868	0.3401	
D-Time	0.0005	1	0.0005	4.65	0.0521	
AB	0.0001	1	0.0001	0.6518	0.4352	
AC	0.0015	1	0.0015	15.49	0.0020	
AD	0.0003	1	0.0003	2.94	0.1119	
BC	0.0002	1	0.0002	2.16	0.1677	
BD	6.250E-06	1	6.250E-06	0.0636	0.8051	
CD	0.0000	1	0.0000	0.2062	0.6578	
A^2	0.0418	1	0.0418	426.16	< 0.0001	
B^2	0.0001	1	0.0001	1.32	0.2727	
C^2	0.0002	1	0.0002	1.75	0.2100	
D^2	0.0000	1	0.0000	0.3183	0.5830	
Residual	0.0012	12	0.0001			
Lack of Fit	0.0011	10	0.0001	3.59	0.2374	not significant
Pure Error	0.0001	2	0.0000			
Cor Total	0.0799	26				
Std. Dev.	0.0099					
Mean	0.0742					
C.V. %	13.36					

Adj R^2*: Adjusted R^2; Pred R^2*: Predicted R^2 ; Adeq Precision *: Adequate precision

Table 6 Experimental design, actual and predicted values for total sugar.

Run	A: Ratio of S/L	B:NaOH	C:H_2O_2	D:Time	Total sugar g/g		Residual
	-	%	%	hours	Predicted	Actual	
1	0.05	1.5	1	24	0.1656	0.1750	0.0094
2	0.3	2	1	48	0.0852	0.0820	-0.0032
3	0.3	1	1	48	0.0809	0.0700	-0.0109
4	0.175	1.5	0.5	24	0.0336	0.0300	-0.0036
5	0.175	1	1	24	0.0400	0.0300	-0.0100
6	0.05	1.5	1	72	0.1703	0.1630	-0.0073
7	0.175	2	0.5	48	0.0180	0.0179	-0.0001
8	0.175	1	1	72	0.0302	0.0300	-0.0002
9	0.05	1.5	0.5	48	0.1423	0.1330	-0.0093
10	0.175	2	1	24	0.0388	0.0330	-0.0058
11	0.3	1.5	0.5	48	0.0990	0.0970	-0.0020
12	0.05	1	1	48	0.1713	0.1730	0.0017
13	0.3	1.5	1	72	0.0709	0.0690	-0.0019
14	0.175	1.5	1	48	0.0406	0.0380	-0.0026
15	0.175	1.5	1	48	0.0406	0.0368	-0.0038
16	0.175	1	1.5	48	0.0274	0.0350	0.0076
17	0.05	1.5	1.5	48	0.1870	0.1830	-0.0040
18	0.3	1.5	1	24	0.1003	0.1150	0.0147
19	0.175	1.5	1	48	0.0406	0.0470	0.0064
20	0.05	2	1	48	0.1596	0.1690	0.0094
21	0.175	1.5	1.5	24	0.0438	0.0390	-0.0048
22	0.175	1	0.5	48	0.0363	0.0480	0.0117
23	0.3	1.5	1.5	48	0.0657	0.0690	0.0033
24	0.175	1.5	0.5	72	0.0257	0.0290	0.0033
25	0.175	2	1	72	0.0240	0.0280	0.0040
26	0.175	1.5	1.5	72	0.0269	0.0290	0.0021
27	0.175	2	1.5	48	0.0383	0.0340	-0.0043

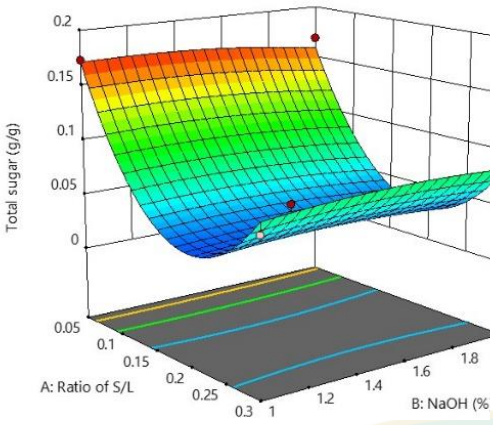


Figure 27 The effect of ratio of S/L and NaOH concentration on total sugar.

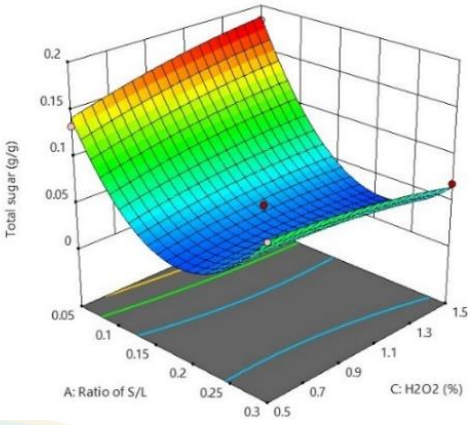


Figure 28 The effect of ratio of S/L and H_2O_2 concentration on total sugar

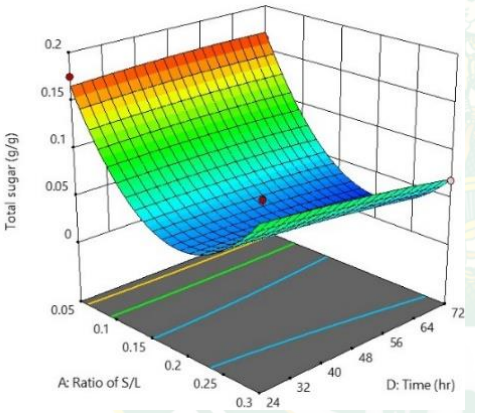


Figure 29 The effect of ratio of S/L and time on total sugar

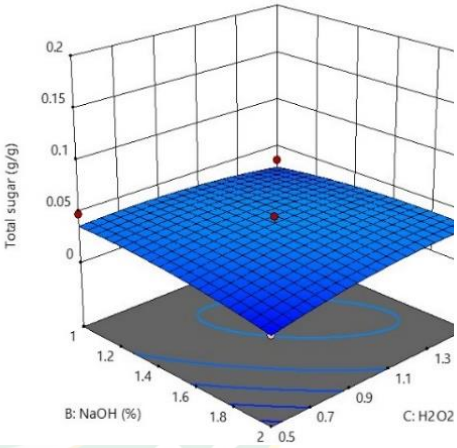


Figure 30 The effect of NaOH and H_2O_2 concentration on total sugar

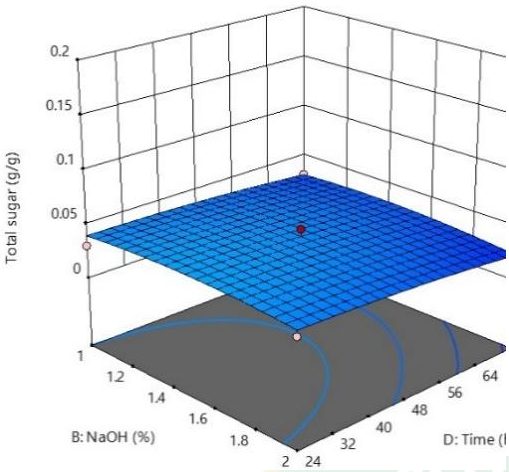


Figure 31 The effect of NaOH concentration and time on total sugar

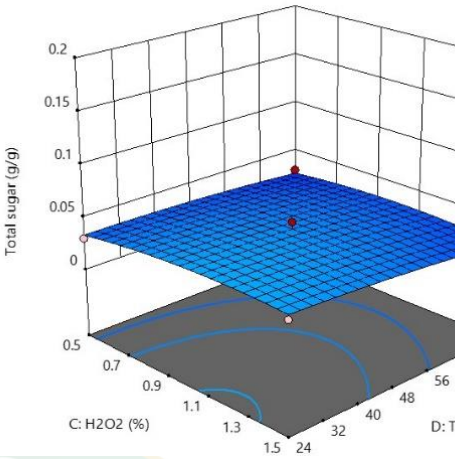


Figure 32 The effect of H_2O_2 concentration and time on total sugar

$$Y_2 = 0.0350 - 0.0626A + 0.0013B + 0.0023C + 0.0062D + 0.0023AB - 0.0002AC - 0.0047AD + 0.0060BC + 0.0038BD + 0.0003CD + 0.0768A^2 + 0.0025B^2 + 0.0052C^2 - 0.0019D^2 \quad \text{(Eq15)}$$

Where Y_2 are the total sugar (g/g dried biomass) from small-flowered nutsedge; A, B, C, and D, respectively, the ratio of solid/liquid, NaOH concentration (%), H_2O_2 concentration (%), and time (h).

In this study, the obtained data was fitted to a second-order polynomial (quadratic) model of BBD that contains main effects and interaction terms. This quadratic model could be used in theoretical prediction of sugar yield from alkaline pretreatment of small-flowered nutsedge. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients. A positive value of coefficient indicates the synergistic effect that favors the optimization, while a negative value of coefficient represents antagonistic effect that prefers an inverse relationship between factors and the responses (Mourabet et al., 2017).

With regards to Eq15 for small-flowered nutsedge, the coefficient of linear term (A: ratio of solid/liquid) has negative sign indicates that the increase value of ratio of solid/liquid leads the decrease of sugar that can be seen clearly at the Figure 34. With regards to the interaction between tested variables, the Eq15 shows that the interaction of ratio of solid/liquid with H_2O_2 concentration and time were found to be

negative which implies that the more positive of ratio of solid/liquid is, the more negative the effect of H_2O_2 concentration (time) on sugar yield (Figure 34 and 35). This trend can be found on the interaction of ratio of solid/liquid with NaOH concentration due to the very small value of coefficient (Figure 33). The coefficient of the squared term (time) is negative which means that the maximum sugar yields at the central point and it decreases when there is an increasing or decreasing the time from central point. On the other hand, the positive values of the rest squared terms (ratio of solid/liquid, NaOH concentration, and H_2O_2 concentration) indicate the minimum value of sugar observed at the central points of these parameters.

ANOVA analysis for the chemical pretreatment model has a F-value of 72.76 with low probability value ($P<0.05$) (Table 7). Furthermore, lack of fit p-value of 0.2229 implies the lack of fit is not significant relative to the pure error. The significant value for model and non-significant value of lack of fit proved the validity of the obtained quadratic model (Rawat et al., 2013; Das et al., 2015). The factors that significantly affected the responses have a confidence level above 95% which p-value less than 0.05 as show in the Table 7. While the p-value for each model term that A, D, and A^2 have significant effect on the total sugar, the other terms that B, C, AB, AC, AD, B^2, C^2, D^2 were insignificant. However, these factors could not be reduced to support hierarchy of the model because of high adjusted determination coefficient (adjusted R^2 = 0.9748). The goodness of fit of the model was evaluated by the determination of R-squared, predicted R-squared, and adjusted R-squared coefficients. The predicted R^2 of 0.9349 is in reasonable agreement with the adjusted R^2 of 0.9748; i.e. the difference is less than 0.2. Adequate precision measures the signal to noise ratio and a ratio greater than 4 is desirable (Cai et al., 2012). The ratio of 22.831 indicates an adequate signal. This model can be used to navigate the design space. Guan and Yao (2008) suggested that R^2 should be at least 0.80 for the good fit of model (Guan and Yao, 2008). In this case, value of correlation co-efficient (R^2) and adjusted co-efficient (adj. R^2) are 0.9884 and 0.9748, respectively which illustrate that the fit of RSM model is significant and can be used to predict the optimal setting up. The fit of predicted and actual value are performed in figure 33 and table 8 shows the results of actual and predicted values. 3D-response surface obtained by the analysis of the experimental

data of BBD, demonstrate a correlation between the two variables in same time, while managing the third variable at fixed level.

The optimal values of selected factors for the pretreatment condition of small-flowered nutsedge is solid to liquid ratio of 0.05, NaOH concentration of 1%, H_2O_2 concentration of 1% for 72 h. The total sugar concentration was achieved 0.183 g/g from the regression equation which is near to the experimental value (0.194 g/g ± 0.003).

Table 7 ANOVA analysis of model for optimization of pretreatment for small-flowered nutsedge

Source	Sum of Squares	df	Mean Square	F-value	p-value	Conclusion
Model	0.0863	14	0.0062	72.76	< 0.0001	significant
A-Ratio of S/L	0.0470	1	0.0470	554.80	< 0.0001	
B-NaOH	0.0000	1	0.0000	0.2518	0.6249	
C-H_2O_2	0.0001	1	0.0001	0.7712	0.3971	
D-Time	0.0005	1	0.0005	5.53	0.0366	
AB	0.0000	1	0.0000	0.2390	0.6337	
AC	2.500E-07	1	2.500E-07	0.0030	0.9576	
AD	0.0001	1	0.0001	1.07	0.3224	
BC	0.0001	1	0.0001	1.70	0.2168	
BD	0.0001	1	0.0001	0.6640	0.4310	
CD	2.500E-07	1	2.500E-07	0.0030	0.9576	
A^2	0.0315	1	0.0315	371.65	< 0.0001	
B^2	0.0000	1	0.0000	0.3805	0.5489	
C^2	0.0001	1	0.0001	1.71	0.2158	
D^2	0.0000	1	0.0000	0.2313	0.6392	
Residual	0.0010	12	0.0001			
Lack of Fit	0.0010	10	0.0001	3.87	0.2229	not significant
Pure Error	0.0001	2	0.0000			

Cor Total	0.0873	26
Std. Dev.	0.0092	
Mean	0.0717	
C.V. %	12.84	

Adj R^2*: Adjusted R^2; Pred R^2*: Predicted R^2 ; Adeq Precision *: Adequate precision

Table 8 Experimental designed runs with actual and predicted values of total sugar

Run	A:Ratio of S/L	B:NaOH	C:H_2O_2	D:Time	Total sugar g/g		Residual
	-	%	%	hours	Predicted	Actual	
1	0.05	1	1	48	0.1778	0.174	-0.0038
2	0.175	1	1.5	48	0.0377	0.040	0.0023
3	0.175	1.5	0.5	24	0.0300	0.029	-0.0010
4	0.3	1.5	0.5	48	0.0524	0.049	-0.0034
5	0.175	1	0.5	48	0.0450	0.049	0.0040
6	0.175	1	1	72	0.0367	0.035	-0.0017
7	0.3	1	1	48	0.0481	0.049	0.0009
8	0.05	1.5	0.5	48	0.1770	0.174	-0.0030
9	0.05	1.5	1	24	0.1615	0.167	0.0055
10	0.175	1.5	1	48	0.0350	0.030	-0.0050
11	0.05	2	1	48	0.1760	0.162	-0.0140
12	0.175	2	0.5	48	0.0357	0.045	0.0093
13	0.3	1.5	1	24	0.0458	0.047	0.0012
14	0.175	2	1	24	0.0269	0.030	0.0031
15	0.175	1.5	1	48	0.0350	0.035	0.0000
16	0.175	1.5	1.5	72	0.0471	0.035	-0.0121
17	0.175	1.5	1	48	0.0350	0.040	0.0050
18	0.3	1.5	1.5	48	0.0565	0.061	0.0045
19	0.175	1	1	24	0.0317	0.030	-0.0017
20	0.175	2	1.5	48	0.0523	0.060	0.0077

21	0.3	1.5	1	72	0.0488	0.055	0.0062
22	0.05	1.5	1	72	0.1835	0.194	0.0105
23	0.3	2	1	48	0.0553	0.046	-0.0093
24	0.05	1.5	1.5	48	0.1822	0.187	0.0048
25	0.175	1.5	1.5	24	0.0341	0.027	-0.0071
26	0.175	2	1	72	0.0469	0.050	0.0031
27	0.175	1.5	0.5	72	0.0420	0.036	-0.0060

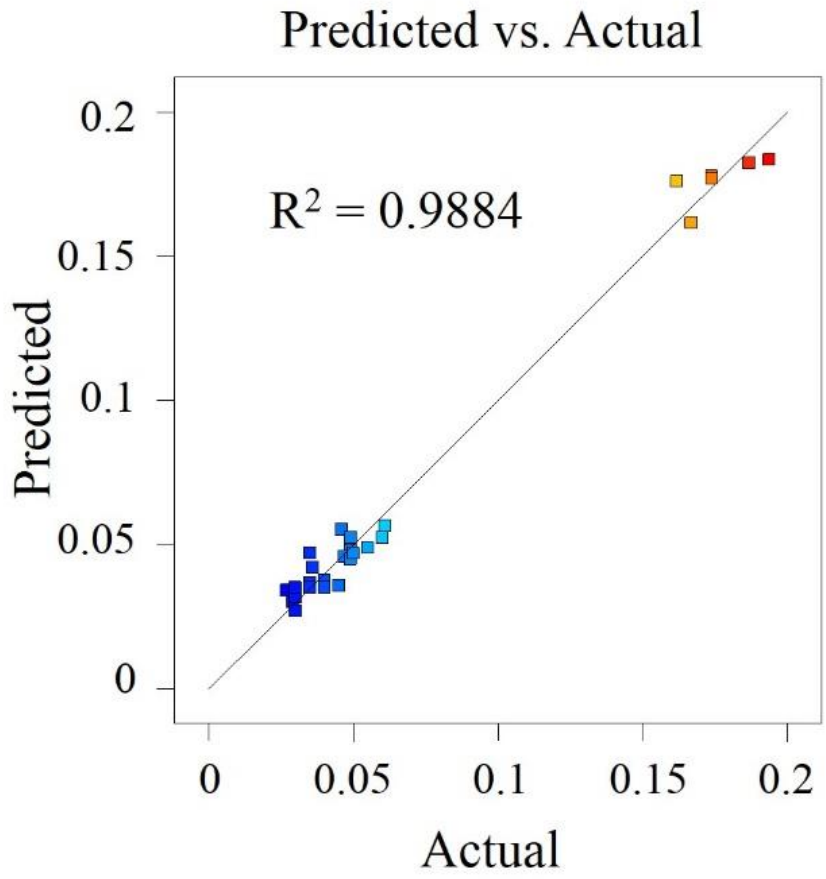


Figure 33 Experimental data plotted against RSM model predicted data of pretreatment for small-flowered nutsedge

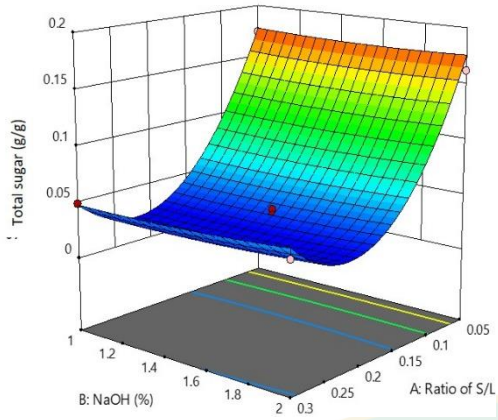


Figure 34 The effect of NaOH concentration and ratio of S/L on sugar yield

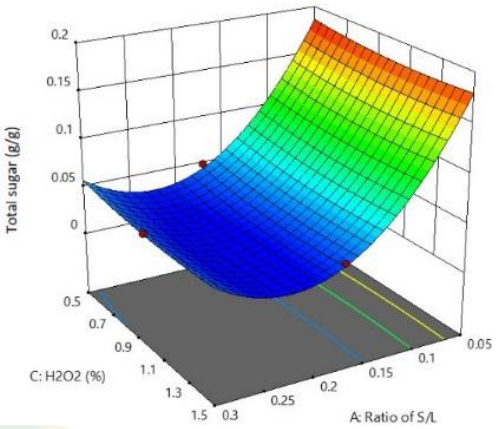


Figure 35 The effect of H_2O_2 concentration and ratio of S/L on sugar yield

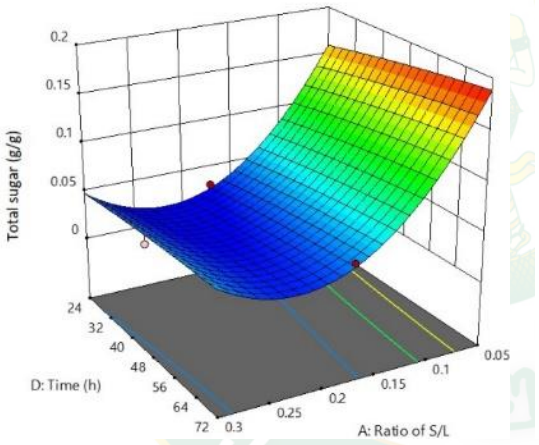


Figure 36 The effect of time and ratio of S/L on sugar yield

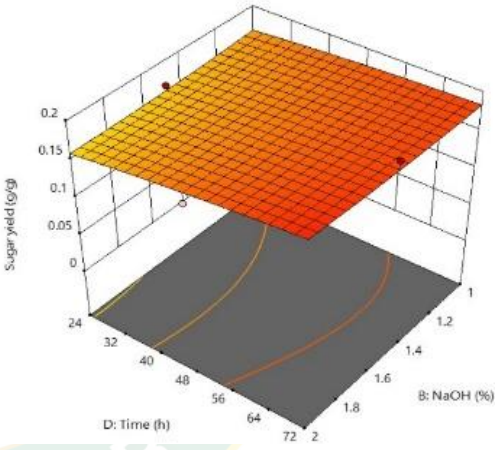


Figure 37 The effect of time and NaOH concentration on sugar yield

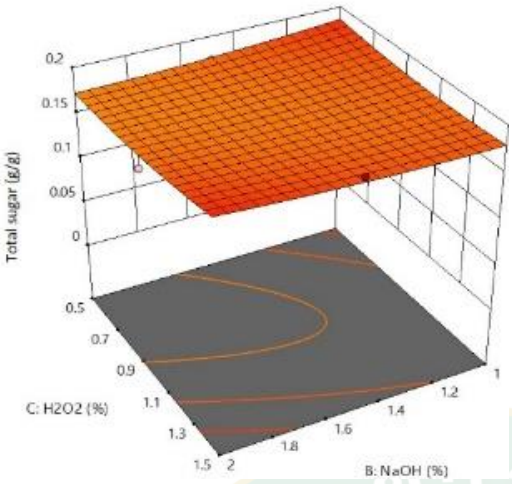


Figure 38 The effect of H_2O_2 and NaOH concentration on sugar yield

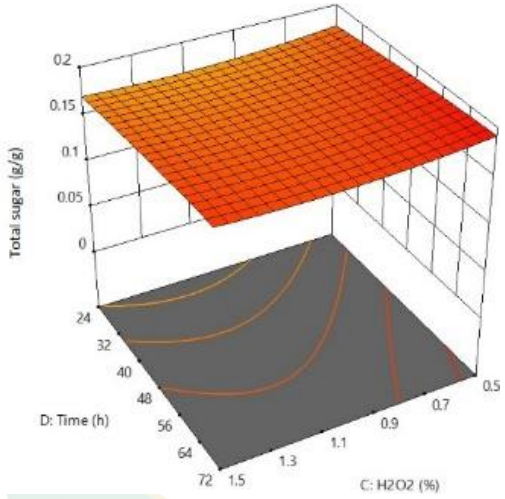


Figure 39 The effect of time and H_2O_2 concentration on sugar yield

Scanning electron microscope

Scanning electron microscope (SEM) is one of the powerful tools for investigating the structural transformation of lignocellulosic materials at micro and Nano scale (Amiri and Karimi, 2015; Karimi and Taherzadeh, 2016). In order to understand the changes of biomass structure before and after pretreatment, powder of raw and pretreated gooseweed and small-flowered nutsedge were scanned under SEM machine at 200 and 1000 magnification.

The SEM picture of untreated and alkaline pretreated gooseweed and small-flowered nutsedge are performed as figure 40 and 41. It can be seen that the surface of raw samples was covered completely with many deposits and makes hard to see the fiber clearly. In addition, the fiber arranges in bundles which impeded the accessibility of cellulase to cellulose and the cell wall of untreated samples is thicker than the pretreated sample. After pretreatment, the fibers of both samples seem to be intact rather than being broken or otherwise disrupted. Some minor debris on the fiber surface was removed, and the surface structure of the alkaline-treated samples tended to be smooth, resulting in the exposure of more fiber bundles; thus, the accessibility of fiber bundles to cellulase could be improved. However, upon closer observation, the surface of the individual fibers had been deformed drastically. A

possible reason was the partial removal of hemicellulose and lignin by sodium hydroxide during the pretreatment. Being without any severe damage on the fibers, it can be concluded that there are no inhibitor compounds were produced during pretreatment process. This support the results from compositional analysis that gooseweed and small-flowered nutsedge might be composed of many soluble components such as protein and soluble sugars and less fibers. Alkaline peroxide pretreatment was proved as an efficient tool for delignification on biomass comparing to diluted sulfuric acid, hot water (Abraham et al., 2013).

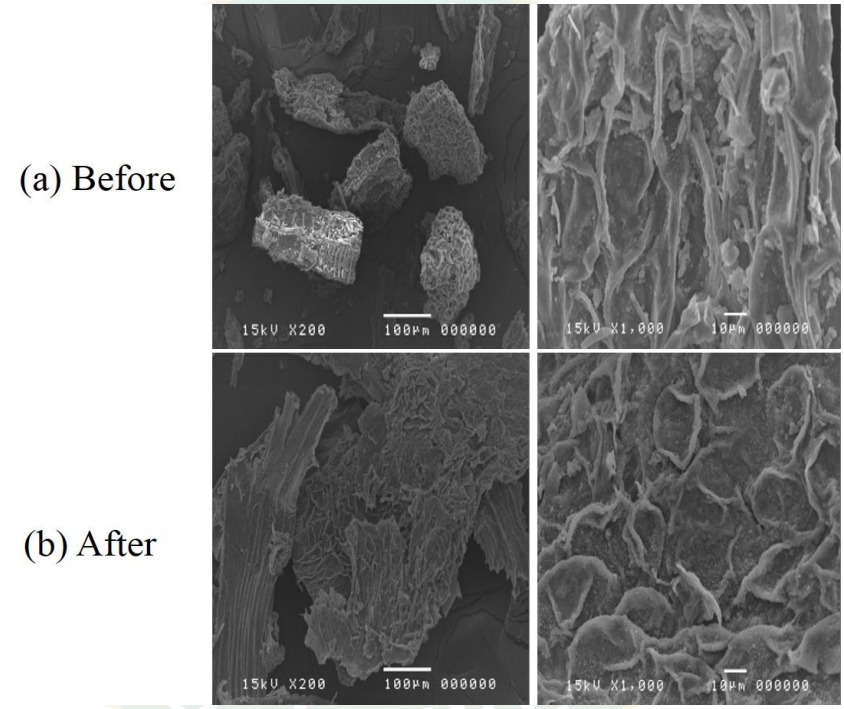


Figure 40 SEM of gooseweed before (a) and after (b) alkaline pretreatment

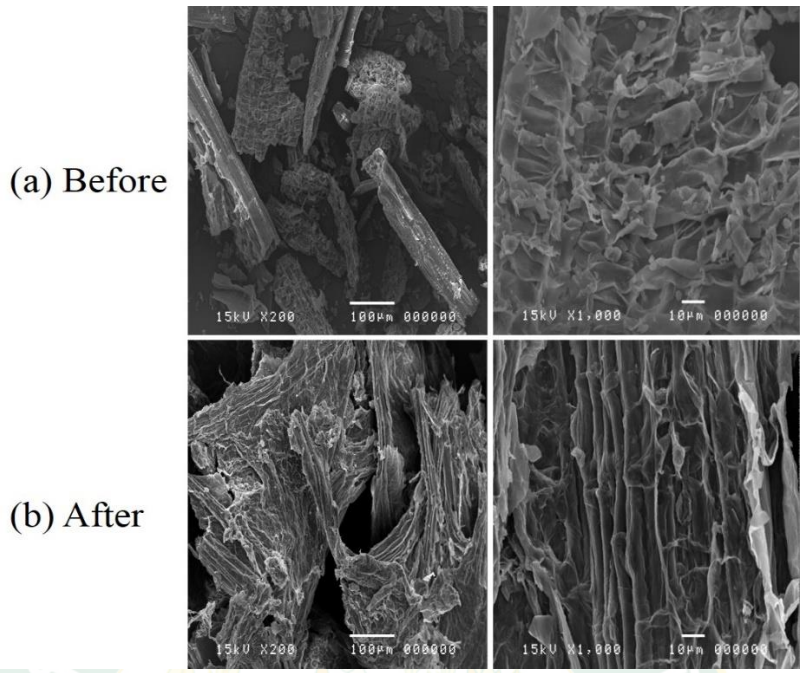


Figure 41 SEM of small-flowered nutsedge before (a) and after (b) alkaline pretreatment

Enzymatic hydrolysis

The main goal of saccharification/hydrolysis is to decrease the degree of polymerization of cellulose by hydrolyzing the large polysaccharides to fermentable sugars. Enzymatic hydrolysis is preferred method because of its highlight advantages such as higher sugar yield when compares with acid hydrolysis, carrying out at milder temperature and pressure, and no corrosion issues (Dwivedi et al., 2009).

Table 9 Total sugar and reducing sugar after hydrolysis

Sugar (g/g)	0 hour	24 hours	48 hours	72 hours
		Gooseweed		
Total sugar	0.144 ± 0.004[a]	0.143 ± 0.007[a]	0.125 ± 0.005[a]	0.125 ± 0.004[a]
Reducing sugar	0.029 ± 0.001[a]	0.073 ± 0.006[b]	0.068 ± 0.002[b]	0.071 ± 0.002[b]
DP	5.0	1.9	1.9	1.8

	Small-flowered nutsedge			
Total sugar	0.199 ± 0.003[a]	0.196 ± 0.006 [ab]	0.188 ± 0.003[b]	0.195 ± 0.004[ab]
Reducing sugar	0.020 ± 0.000[a]	0.094 ± 0.001[b]	0.079 ± 0.000[c]	0.089 ± 0.002[d]
DP	9.8	2.1	2.4	2.2

DP: degree of polymerization

Standard deviation was less than 10%. Means with the same letter at the same are not significantly different ($p < 0.05$). The test was based on Tukey test at the 95% confidence interval.

This study carried out enzymatic hydrolysis process for totally 72 hours in order to find out the most suitable time level for this process (Table 9). Besides, enzymatic hydrolysis step was studied after chemical pretreatment instead of biological pretreatment since chemical pretreatment reulted the higher results than biological method. It can be seen that the means of total sugar before and after hydrolysis are slightly fluctuated. In contrast, there are significant changes of reducing sugar amount for both samples after 24 hours of hydrolysis. However, the degradation of polysaccharide seemed to be stopped because reducing sugar level did not become different meaningfully. In conclusion, enzymatic hydrolysis process could be occurred perfectly within 24 hours for the used samples in this study. Degree of polymerization (DP) presents the number of monomer of sugar presented in solution. In other words, the reduction of DP show a very clear evidence of ezyme activities on breaking down the big sugar chains into smaller chains. The hydrolsys efficiency of gooseweed and small-flowered nutsedge could be reach the maximum of 50% and 47%, respectively. The outcome of this study is agreed with others previous papers (Takagi et al., 2012; Das et al., 2016).

Fermentation

In regards to gooseweed, fermentation is a biological process that use the natural preference for sugar as a carbon source by *S. cerevisiae* to convert to ethanol. Ethanol concentration within three, five, seven, and nine days was recorded as Figure

40 and the range is 0 – 11.84 g/L. The maximum ethanol concentration obtained was 11.84 g/L within five days of fermentation and declined rapidly after that. However, according to the figure 42 and the standard deviation bar, there is no significantly difference of ethanol concentration between three and five days. Reducing sugar during the fermentation was estimated in the meantime to observe the sugar consumption of yeast. It is clearly observed by the amount of reducing sugar dramatically decreased after three days and slightly fluctuated then. With regards to small-flowered nutsedge, ethanol concentration within 3, 5, 7, and 9 days was recorded as Figure 5 and the range is 0 - 12.36 g/L. The maximum ethanol concentration obtained was 12.36 g/L within 5 days of fermentation and declined rapidly after that (Figure 43). The reduction of bioethanol after 5 days of fermentation can be a results of the formation of glycerol as a byproduct (Ahn et al., 2012). The achieved ethanol from gooseweed and small-flowered nutsedge are higher than water hyacinth which was 9.61 g/L in previous literatures (Takagi et al., 2012) and 1.491 g/l (He et al., 2015). Even though the highest ethanol concentration was reached at different fermentation time, the trend after the fifth day of fermentation of both two samples are quite similar as describes in the figure 42 and 43. While ethanol concentration after fifth days of fermentation reduced, the amount of reducing sugar kept stable. The reducing sugar includes both hexoses and pentose sugar but the yeast *S. cerevisiae* can only ferment hexoses sugar. Thus, this can be a reason for the stopping fermentation process as the sugar substrate was run out. Generally, the yeast *S. cerevisiae* contain two genes that catalyze not only the reduction of acetaldehyde to ethanol during the fermentation of glucose, but also the reverse action of ethanol into acetaldehyde (Bennetzen and Hall, 1982). This explains the reduction of bioethanol concentration after reaching the highest ethanol concentration. The highest ethanol concentration obtained in this research was similar with some of previous studies that used other lignocellulosic biomass as raw materials (Table 10).

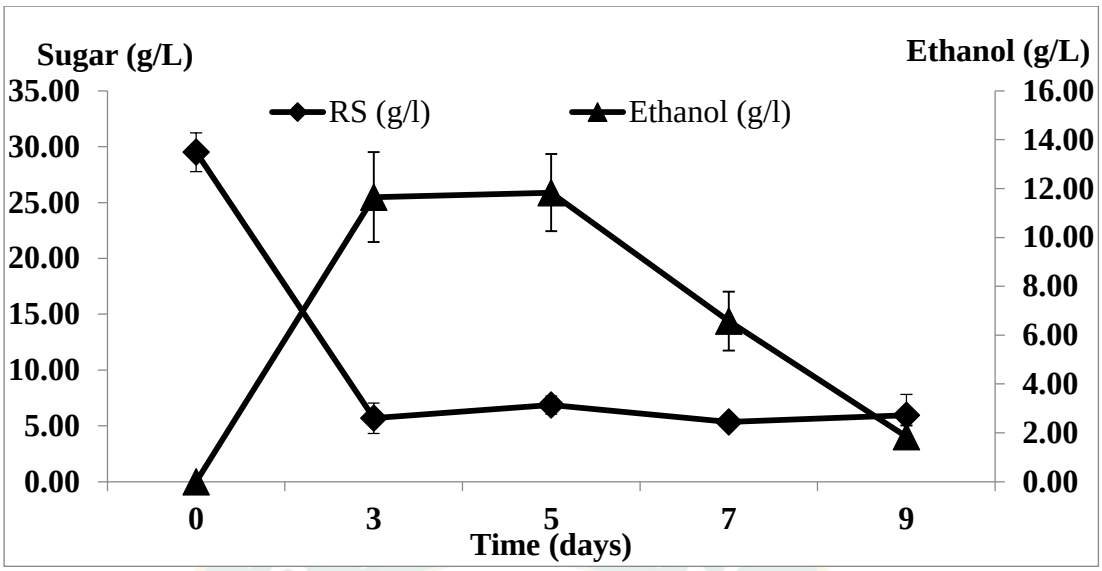


Figure 42 Ethanol and sugar concentration during fermentation of gooseweed

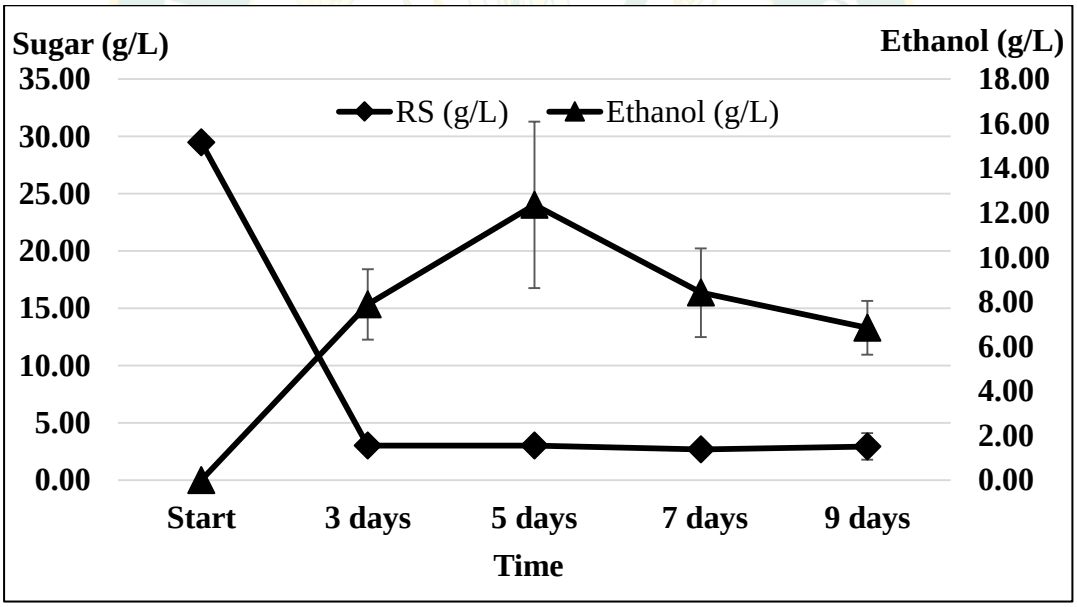


Figure 43 Ethanol and sugar concentration during fermentation of small-flowered nutsedge

Table 10 The comparison of ethanol concentration from this study with other researches

Material	Pretreatment	Ethanol	References
Water hyacinth	Conc. H_2SO_4	9.61 g/l	Toshiyuki et al., 2012
Wetland plants	$NaOH/H_2O_2$	1.491 g/l	He et al., 2014
Water hyacinth	$H_2O_2/NaOH$	0.16 g/g biomass	Yan et al., 2015
Water hyacinth	Conc.* H_2SO_4	13.6 g/l	Das et al., 2016
Gooseweed	$NaOH/H_2O_2$	11.84 g/l	This study
Small-flowered nutsedge	$NaOH/H_2O_2$	12.36 g/l	This study

Conc.: concentrated

Based on the lab scale experiments, a simple mass balance for bioethanol production from gooseweed and small-flowered nutsedge was estimated (Figure 44). When 10 g of dried samples were used, amount of 1.184 g ethanol (gooseweed) and 1.236 g ethanol (small-flowered nutsedge) were obtained. From the chart, with the use of 1 ton dried materials, around 118-124 kg ethanol can be obtained. The results from this research are in agreement with other lignocellulosic biomass such as fresh sweet sorghum (91.9 kg ethanol) (Li et al., 2013). However it is lower than that of paper sludge with a yield of 382 kg ethanol (Prasetyo et al., 2011).

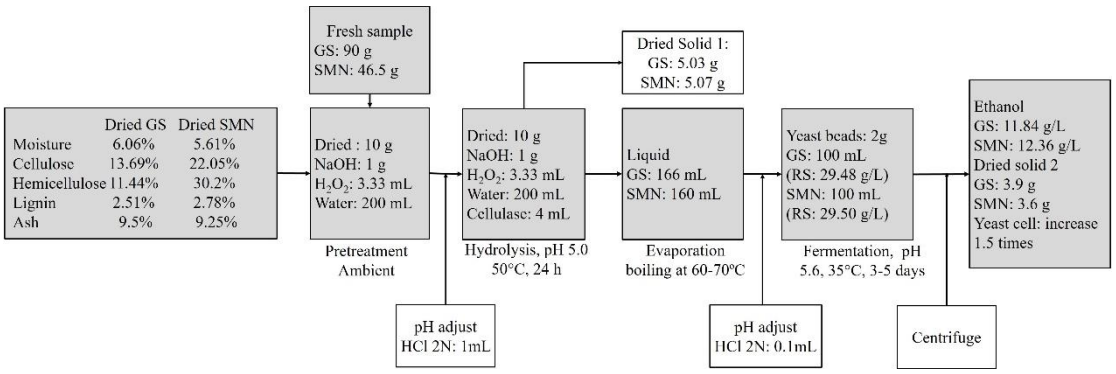


Figure 44. Mass balance of bioethanol production from aquatic weeds as lab scale; GS: Gooseweed; SMN: Small-flowered nutsedge

Scale up for bioethanol production

Scale up experiments were conducted with the batch fermentation and followed the process used in lab scale. A pretreatment with NaOH/ H_2O2 were conducted at the same ratio of solid into liquid, time, concentration which already optimized using response methodology. In general, refined ethanol is considered as an oxygenate and an octane enhancer when blend with gasoline in different ratios in order to produce a greener liquid fuel. The refined ethanol gained in the large scale have the higher heating value (kJ/kg) of 12.61 (gooseweed) and 25.31 (small-flowered nutsedge).

Economic analysis

The year of 2017 had been predicted the amount of ethanol consumption which might reach 1.4 billion liters due to the growing demand for E20 and E85. June 19 2017, ethanol price was 28 US cents/gallon (33.8B/USD). The purpose of this economic analysis is to demonstrate the possibility of cellulosic ethanol for a cost-competitive on its own market. The economic analysis in this study assume the capacity of the project is 5000 L/day and located in rural area which brings benefit about material source as well as cheap land and installation cost. It was assumed that the bioethanol yield in this scale up model was similar with lab experiments so that the amount of chemicals was also scale up from lab scale experiments. Most of the price was referred on the largest trade website (Alibaba). The amount of chemical

materials was estimated based on the lab scale experiments. In this work, we listed only the main factors for building a community-scale of lignocellulosic-ethanol factory (Table 11). The capital and operational cost was estimated and described in the table 11 which was mainly follow the procedure from National Renewable Energy Lab (Humbird et al., 2011). The cost per unit of ethanol estimated from gooseweed and small-flowered nutsedge in the research is lower than that one from corn stover (2.25$, 2012) reported by Humbird (2011). The main reason for the difference is that the cost of available feedstock was assumed as zero in this project.

While the cost of the first generation of bioethanol mostly depends on feedstock, the cost of second generation counts on process cost (pretreatment, enzymatic hydrolysis, and distillation). According to economic evaluations, the main contributors to the overall cost of producing ethanol from biomass are the raw material (30-40%), the capital investment (30-45%), cellulase enzymes (10-20%). The price of enzyme is quite high so that most of ethanol plants have their own on-site enzymatic production. Tao and Aden performed a survey of economic models of existing biofuels and pointed out that pretreatment and saccharification processes are regarded the two most expensive processing steps in the bioethanol production from lignocellulosic biomass (Tao et al., 2014). Based on the analysis, it is noted that enzyme cost is critical cost contributors to the new development bioethanol production from gooseweed and small-flowered nutsedge. There is a consideration of on-site and off-site enzyme production. Applying sustainable process such as reuse water and increasing the quality of by-product such as fertilizer, lignin residue to increase the profit as well as reduce the operation cost. According to (Gnansounou and Dauriat, 2011), the biomass conversion cost made up the largest portion of the total bioethanol production cost from lignocellulosic feedstock. Creation of direct and indirect job in rural area is another benefit of this project. The leftover residue can be used to produce electricity for the plant to make it self-sufficient, or to provide electric power back to the grid.

Table 11 Capital and operation cost of a project of bioethanol factory with a capacity of 5000 L/day bioethanol (95%)

Capital cost	Amount	Purchased cost (USD)	Installed cost (USD)
Feed handling (grass grinder)	2	1000	3,400
Pretreatment (tank, storage)	32	24000	72,000
Distillation (2 columns, 1 cooling columns)	3	15000	63,000
Utilities (pumps)	40	980	2,254
Additional piping 4.5% of Total equipment cost (Humbird et al., 2011)			1844.1
Warehouse 4.0% of Total equipment cost (Humbird et al., 2011)			1639.2
Home office % construction fee 20% of Total installed cost (Humbird et al., 2011)			28130.8
Other cost (start-up, permits, etc.) 10% of Total installed cost (Humbird et al., 2011)			14065.4
Total capital investment cost			186,334
Operational expenses			
Feedstocks	-	-	-
Sodium hydroxide/hydrogen peroxide (kg)	1667	0.3	500
Cellulase (kg)	1667	1	1,667
Hydrochloric acid (L)	3542	0.15	531
Yeast dried powder (kg)	29	2	29
Calcium chloride 0.05M (kg)	32	0.12	1.92
Sodium alginate 2% (kg)	29	13	188.5
Water (m^3)	166,667	0.3	50
Electricity (kWh)	229.6	0.13	30
Fixed cost (labor and *maintenance cost) (Humbird et al., 2011)	-	-	2,107

Total operating cost			5,104
Annual cost (1 year = 365 days)			
Annual net expenses			1,863,108
Annual net Income			2,208,250
Income			345,142
Cost per unit of ethanol (USD/L)			1.23

CHAPTER 5
SUMMARY

This research investigates the potential of new lignocellulosic biomass as promising feedstock for bioethanol production. The research covers the mains ideas as the following:

1. Field surveys for searching, collecting, basic analyzing and calculating the biomass yield of gooseweed and small-flowered nutsedge.
2. Bioethanol was produced as the biochemical pathways which includes these important steps: pretreatment, enzymatic hydrolysis, fermentation, and fractional distillation.
3. Response surface modelling was also applied to optimize the condition of alkaline-peroxide pretreatment.
4. A science application of taking advantage of termite as biological pretreatment.
5. The process of saccharification was carried out with the utilize of cellulase enzyme at 50^{o}C for 24 hours. The obtained hydrolysate samples were fermented using *Saccharomyces cerevisiae* TISTR 5020 at 35^{o}C.
6. Economic analysis of a scale-up models was completed for a small capacity of 5000 L/da with a result of the cost per unit of ethanol (1.23 USD/L)
7. The gained bioethanol was tested the heating value with bomb calorimeter.

Suggestion and recommendation.

1. The use of NaOH/ H_2O_2 with mild condition for long time (ambient temperature and pressure) can reduce energy consumption during bioethanol production. However, there is a suggestion of shorter retention time by increasing temperature.
2. As it was found that hemicellulose is the other important fraction of lignocellulosic biomass and starch also is available in these two new

materials, a use of amylase and pentose enzyme may enhance hydrolysis process.

REFERENCES

Abraham, R. E., Barrow, C. J. & Puri, M. 2013. Relationship to reducing sugar production and scanning electron microscope structure to pretreated hemp hurd biomass (Cannabis sativa). **Biomass and Bioenergy**, 58(180-187.

Aditiya, H. B., Mahlia, T. M. I., Chong, W. T., Nur, H. & Sebayang, A. H. 2016. Second generation bioethanol production: A critical review. **Renewable and Sustainable Energy Reviews**, 66(631-653.

Ahn, D. J., Kim, S. K. & Yun, H. S. 2012. Optimization of pretreatment and saccharification for the production of bioethanol from water hyacinth by Saccharomyces cerevisiae. **Bioprocess and biosystems engineering**, 35(1-2), 35-41.

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

Amiri, H. & Karimi, K. 2015. Improvement of acetone, butanol, and ethanol production from woody biomass using organosolv pretreatment. **Bioprocess and biosystems engineering**, 38(10), 1959-1972.

Antony, J. 2014. **Design of experiments for engineers and scientists**. Elsevier.

Avci, A., Saha, B. C., Dien, B. S., Kennedy, G. J. & Cotta, M. A. 2013. Response surface optimization of corn stover pretreatment using dilute phosphoric acid for enzymatic hydrolysis and ethanol production. **Bioresource technology**, 130(603-612.

Ayeni, A., Hymore, F., Mudliar, S., Deshmukh, S., Satpute, D., Omoleye, J. & Pandey, R. 2013. Hydrogen peroxide and lime based oxidative pretreatment of wood waste to enhance enzymatic hydrolysis for a biorefinery: Process parameters optimization using response surface methodology. **Fuel**, 106(187-194.

Azadi, P., Malina, R., Barrett, S. R. H. & Kraft, M. 2017. The evolution of the biofuel science. **Renewable and Sustainable Energy Reviews**, 76(1479-1484.

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.

Baboukani, B. S., Vossoughi, M. & Alemzadeh, I. 2012. Optimisation of dilute-acid pretreatment conditions for enhancement sugar recovery and enzymatic hydrolysis of wheat straw. **Biosystems engineering**, 111(2), 166-174.

Baeyens, J., Kang, Q., Appels, L., Dewil, R., Lv, Y. & Tan, T. 2015. Challenges and opportunities in improving the production of bio-ethanol. **Progress in Energy and Combustion Science**, 47(60-88.

Balat, M. & Balat, H. 2009. Recent trends in global production and utilization of bio-ethanol fuel. **Applied Energy**, 86(11), 2273-2282.

Behera, S., Arora, R., Nandhagopal, N. & Kumar, S. 2014. Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. **Renewable and Sustainable Energy Reviews**, 36(91-106.

Bennetzen, J. & Hall, B. 1982. The primary structure of the Saccharomyces cerevisiae gene for alcohol dehydrogenase. **Journal of Biological Chemistry**, 257(6), 3018-3025.

Bienkowski, P., Ladisch, M., Narayan, R., Tsao, G. & Eckert, R. 1987. Correlation of glucose (dextrose) degradation at 90 to 190 C in 0.4 to 20% acid. **Chemical Engineering Communications**, 51(1-6), 179-192.

Birol, F. 2014. International Energy Agency. **World Energy Outlook 2014**, 2014(

Box, G. E. P. & Behnken, D. W. 1960. Some New Three Level Designs for the Study of Quantitative Variables. **Technometrics**, 2(4), 455-475.

Cai, B.-Y., Ge, J.-P., Ling, H.-Z., Cheng, K.-K. & Ping, W.-X. 2012. Statistical optimization of dilute sulfuric acid pretreatment of corncob for xylose recovery and ethanol production. **biomass and bioenergy**, 36(250-257.

Cai, J., He, Y., Yu, X., Banks, S. W., Yang, Y., Zhang, X., Yu, Y., Liu, R. & Bridgwater, A. V. 2017. Review of physicochemical properties and analytical characterization of lignocellulosic biomass. **Renewable and Sustainable Energy Reviews**, 76(309-322.

Cardona, C. A. & Sánchez, Ó. J. 2007. Fuel ethanol production: process design trends and integration opportunities. **Bioresource technology**, 98(12), 2415-2457.

Carter, R., Jones, J. C. & Goddard, R. H. 2014. Sphenoclea zeylanica (Sphenocleaceae) in North America—Dispersal, Ecology, and Morphology. **Castanea**, 79(1), 33-50.

Cavazzuti, M. 2012. **Optimization methods: from theory to design scientific and technological aspects in mechanics**. Springer Science & Business Media.

Chiaramonti, D., Prussi, M., Ferrero, S., Oriani, L., Ottonello, P., Torre, P. & Cherchi, F. 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method. **Biomass and Bioenergy**, 46(25-35.

Choudhary, J., Singh, S. & Nain, L. 2016. Thermotolerant fermenting yeasts for simultaneous saccharification fermentation of lignocellulosic biomass. **Electronic Journal of Biotechnology**, 21(82-92.

Cowling, E. B. 1975. **Physical and chemical constraints in the hydrolysis of cellulose and lignocellulosic materials**.

Dagnino, E., Chamorro, E., Romano, S., Felissia, F. & Area, M. 2013. Optimization of the acid pretreatment of rice hulls to obtain fermentable sugars for bioethanol production. **Industrial Crops and Products**, 42(363-368.

Das, A., Ghosh, P., Paul, T., Ghosh, U., Pati, B. R. & Mondal, K. C. 2016. Production of bioethanol as useful biofuel through the bioconversion of water hyacinth (Eichhornia crassipes). **3 Biotech**, 6(1), 70.

Das, S., Bhattacharya, A., Haldar, S., Ganguly, A., Gu, S., Ting, Y. & Chatterjee, P. 2015. Optimization of enzymatic saccharification of water hyacinth biomass for bio-ethanol: Comparison between artificial neural network and response surface methodology. **Sustainable Materials and Technologies**, 3(17-28.

Deguchi, S., Tsujii, K. & Horikoshi, K. 2006. Cooking cellulose in hot and compressed water. **Chemical Communications**, 31), 3293-3295.

Demirbas, A. 2005. Bioethanol from cellulosic materials: a renewable motor fuel from biomass. **Energy sources**, 27(4), 327-337.

Demirbas, A. & Karslioglu, S. 2007. Biodiesel Production Facilities from Vegetable Oils and Animal Fats. **Energy Sources, Part A: Recovery, Utilization, and Environmental Effects**, 29(2), 133-141.

Dien, B. S., Cotta, M. A. & Jeffries, T. W. 2003. Bacteria engineered for fuel ethanol production: current status. **Applied Microbiology and Biotechnology**, 63(3), 258-266.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. 1956. Colorimetric Method for Determination of Sugars and Related Substances. **Analytical Chemistry**, 28(3), 350-356.

Dwivedi, P., Alavalapati, J. R. & Lal, P. 2009. Cellulosic ethanol production in the United States: Conversion technologies, current production status, economics, and emerging developments. **Energy for Sustainable Development**, 13(3), 174-182.

Ebringerová, A., Hromádková, Z. & Heinze, T. 2005. Hemicellulose. **Advances in Polymer Science** 186(67.

Fan, Z. (2014). Chapter 7 - Consolidated Bioprocessing for Ethanol Production A2 - Qureshi, Nasib. In D. B. Hodge และA. A. Vertès (Eds.), **Biorefineries** (pp. 141-160). Amsterdam: Elsevier.

Gao, J., Chen, L., Yan, Z. & Wang, L. 2013. Effect of ionic liquid pretreatment on the composition, structure and biogas production of water hyacinth (Eichhornia crassipes). **Bioresource Technology**, 132(361-364.

Gírio, F. M., Fonseca, C., Carvalheiro, F., Duarte, L. C., Marques, S. & Bogel-Łukasik, R. 2010. Hemicelluloses for fuel ethanol: A review. **Bioresource Technology**, 101(13), 4775-4800.

Gnansounou, E. & Dauriat, A. (2011). Chapter 6 - Technoeconomic Analysis of Lignocellulosic Ethanol. In **Biofuels** (pp. 123-148). Amsterdam: Academic Press.

Gryta, M., Morawski, A. W. & Tomaszewska, M. 2000. Ethanol production in membrane distillation bioreactor. **Catalysis Today**, 56(1), 159-165.

Guan, X. & Yao, H. 2008. Optimization of Viscozyme L-assisted extraction of oat bran protein using response surface methodology. **Food Chemistry**, 106(1), 345-351.

Guo, M., Song, W. & Buhain, J. 2015. Bioenergy and biofuels: History, status, and perspective. **Renewable and Sustainable Energy Reviews**, 42(Supplement C), 712-725.

Hall, M., Bansal, P., Lee, J. H., Realff, M. J. & Bommarius, A. S. 2010. Cellulose crystallinity – a key predictor of the enzymatic hydrolysis rate. **FEBS Journal**, 277(6), 1571-1582.

He, M.-x., Hu, Q., Zhu, Q., Pan, K. & Li, Q. 2015. The feasibility of using constructed wetlands plants to produce bioethanol. **Environmental Progress & Sustainable Energy**, 34(1), 276-281.

Hendriks, A. T. W. M. & Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. **Bioresource Technology**, 100(1), 10-18.

Holm, L. G., Plucknett, D. L., Pancho, J. V. & Herberger, J. P. 1977. **The world's worst weeds**. University Press.

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A., Schoen, P., Lukas, J., Olthof, B., Worley, M., Sexton, D. & Dudgeon, D. (2011). Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. In **NREL Technical Report NREL/TP-5100-47764**.

Jeoh, T., Ishizawa, C. I., Davis, M. F., Himmel, M. E., Adney, W. S. & Johnson, D. K. 2007. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. **Biotechnology and Bioengineering**, 98(1), 112-122.

John, R. P., Anisha, G. S., Nampoothiri, K. M. & Pandey, A. 2011. Micro and macroalgal biomass: A renewable source for bioethanol. **Bioresource Technology**, 102(1), 186-193.

Karimi, K. & Taherzadeh, M. J. 2016. A critical review of analytical methods in pretreatment of lignocelluloses: Composition, imaging, and crystallinity. **Bioresource Technology**, 200(1008-1018.

Kazi, F. K., Fortman, J. A., Anex, R. P., Hsu, D. D., Aden, A., Dutta, A. & Kothandaraman, G. 2010. Techno-economic comparison of process technologies for biochemical ethanol production from corn stover. **Fuel**, 89(S20-S28.

Kim, T. H., Taylor, F. & Hicks, K. B. 2008. Bioethanol production from barley hull using SAA (soaking in aqueous ammonia) pretreatment. **Bioresource Technology**, 99(13), 5694-5702.

Kiss, A. A. 2014. Distillation technology–still young and full of breakthrough opportunities. **Journal of Chemical Technology and Biotechnology**, 89(4), 479-498.

Klemm, D., Heublein, B., Fink, H.-P. & Bohn, A. 2005. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. **Angewandte Chemie International Edition**, 44(22), 3358-3393.

Kumar, A., Singh, L. & Ghosh, S. 2009. Bioconversion of lignocellulosic fraction of water-hyacinth (Eichhornia crassipes) hemicellulose acid hydrolysate to ethanol by Pichia stipitis. **Bioresource Technology**, 100(13), 3293-3297.

Küüt, A. 2013. **Characteristics of Bioethanol Fuel Obtained From LignocelluloseI Biomass in Internal Combustion Reciprocating Engines With Spark-and Compressioignition.** Eesti Maaülikool.

Larsson, S., Palmqvist, E., Hahn-Hägerdal, B., Tengborg, C., Stenberg, K., Zacchi, G. & Nilvebrant, N.-O. 1999. The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. **Enzyme and Microbial Technology**, 24(3), 151-159.

Lewandowska, M., Szymańska, K., Kordala, N., Dąbrowska, A., Bednarski, W. & Juszczuk, A. 2016. Evaluation of Mucor indicus and Saccharomyces cerevisiae capability to ferment hydrolysates of rape straw and Miscanthus giganteus as affected by the pretreatment method. **Bioresource Technology**, 212(262-270.

Li, J., Li, S., Han, B., Yu, M., Li, G. & Jiang, Y. 2013. A novel cost-effective technology to convert sucrose and homocelluloses in sweet sorghum stalks into ethanol. **Biotechnology for biofuels**, 6(1), 174.

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.

Liu, R. & Shen, F. 2008. Impacts of main factors on bioethanol fermentation from stalk juice of sweet sorghum by immobilized Saccharomyces cerevisiae (CICC 1308). **Bioresource Technology**, 99(4), 847-854.

Liu, Z.-H. & Chen, H.-Z. 2015. Xylose production from corn stover biomass by steam explosion combined with enzymatic digestibility. **Bioresource technology**, 193(345-356.

Mabbayad, M. O. & Watson, A. K. 1995. Biological control of gooseweed (Sphenoclea zeylanica Gaertn.) with an Alternaria sp. **Crop Protection**, 14(5), 429-433.

McNeil, M., Darvill, A. G., Fry, S. C. & Albersheim, P. 1984. Structure and function of the primary cell walls of plants. **Annual review of biochemistry**, 53(1), 625-663.

Menegol, D., Luisi Scholl, A., Claudete Fontana, R., Pinheiro Dillon, A. J. & Camassola, M. 2014. Potential of a Penicillium echinulatum enzymatic complex produced in either submerged or solid-state cultures for enzymatic hydrolysis of elephant grass. **Fuel**, 133(232-240.

Miller, G. L. 1959. Use of Dinitrosalicylic acid reagent for determination of reducing sugar. **Anal Chem**, 31(

Mishima, D., Kuniki, M., Sei, K., Soda, S., Ike, M. & Fujita, M. 2008. Ethanol production from candidate energy crops: Water hyacinth (Eichhornia crassipes) and water lettuce (Pistia stratiotes L.). **Bioresource Technology**, 99(7), 2495-2500.

Mishima, D., Tateda, M., Ike, M. & Fujita, M. 2006. Comparative study on chemical pretreatments to accelerate enzymatic hydrolysis of aquatic macrophyte biomass used in water purification processes. **Bioresource Technology**, 97(16), 2166-2172.

Montgomery, D. & Runger, G. (2002). **Applied Probability and Statistics to Engineering**: McGraw Hill, Mexico.

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.

Mourabet, M., El Rhilassi, A., El Boujaady, H., Bennani-Ziatni, M. & Taitai, A. 2017. Use of response surface methodology for optimization of fluoride adsorption in an aqueous solution by Brushite. **Arabian Journal of Chemistry**, 10(S3292-S3302.

Mu, D., Seager, T., Rao, P. S. & Zhao, F. 2010. Comparative life cycle assessment of lignocellulosic ethanol production: biochemical versus thermochemical conversion. **Environmental management**, 46(4), 565-578.

Murphy, C. W. & Kendall, A. 2015. Life cycle analysis of biochemical cellulosic ethanol under multiple scenarios. **Gcb Bioenergy**, 7(5), 1019-1033.

Myers, R. H., Montgomery, D. C. & Anderson-Cook, C. M. 2016. **Response surface methodology: process and product optimization using designed experiments**. John Wiley & Sons.

Nanda, S., Mohanty, P., Pant, K., Naik, S., Kizinski, J. & Dalai, A. 2013. Characterization of North American lignocellulosic biomass and biochars in term of their candidacy for alternate renewable fuels. **Bioenergy Resource**, 6), 663-677.

NREL. 2008. **Determination of Ash in Biomass: Laboratory Analytical Procedure**.

Ooshima, H., Burns, D. S. & Converse, A. O. 1990. Adsorption of cellulase from Trichoderma reesei on cellulose and lignacious residue in wood pretreated by dilute sulfuric acid with explosive decompression. **Biotechnology and Bioengineering**, 36(5), 446-452.

Patel, M., Zhang, X. & Kumar, A. 2016. Techno-economic and life cycle assessment on lignocellulosic biomass thermochemical conversion technologies: A review. **Renewable and Sustainable Energy Reviews**, 53(1486-1499.

Piccolo, C. & Bezzo, F. 2009. A techno-economic comparison between two technologies for bioethanol production from lignocellulose. **Biomass and Bioenergy**, 33(3), 478-491.

Poornejad, N., Karimi, K. & Behzad, T. 2013. Improvement of saccharification and ethanol production from rice straw by NMMO and [BMIM][OAc] pretreatments. **Industrial Crops and Products**, 41(408-413.

Popp, D., Hascic, I. & Medhi, N. 2011. Technology and the diffusion of renewable energy. **Energy Economics**, 33(4), 648-662.

Pothiraj, C., Arumugam, R. & Gobinath, M. 2014. Sustaining ethanol production from lime pretreated water hyacinth biomass using mono and co-cultures of isolated fungal strains with Pichia stipitis. **Bioresources and Bioprocessing**, 1(1), 27.

Prasetyo, J., Naruse, K., Kato, T., Boonchird, C., Harashima, S. & Park, E. Y. 2011. Bioconversion of paper sludge to biofuel by simultaneous saccharification and fermentation using a cellulase of paper sludge origin and thermotolerant Saccharomyces cerevisiae TJ14. **Biotechnology for biofuels**, 4(1), 35.

Pu, Y., Hu, F., Huang, F., Davison, B. H. & Ragauskas, A. J. 2013. Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. **Biotechnology for biofuels**, 6(1), 15.

Ragauskas, A. J., Williams, C. K., Davison, B. H., Britovsek, G., Cairney, J., Eckert, C. A., Frederick, W. J., Hallett, J. P., Leak, D. J. & Liotta, C. L. 2006. The path forward for biofuels and biomaterials. **science**, 311(5760), 484-489.

Ravindran, R. & Jaiswal, A. K. 2016. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. **Bioresource Technology**, 199(92-102.

Rawat, R., Kumbhar, B. K. & Tewari, L. 2013. Optimization of alkali pretreatment for bioconversion of poplar (Populus deltoides) biomass into fermentable sugars using response surface methodology. **Industrial Crops and Products**, 44(220-226.

Rentizelas, A. A., Tolis, A. J. & Tatsiopoulos, I. P. 2009. Logistics issues of biomass: The storage problem and the multi-biomass supply chain. **Renewable and Sustainable Energy Reviews**, 13(4), 887-894.

RFA. (2017). **Ethanol industry outlook 2017: Building partnerships and growing markets**. Document Number)

Ruangmee, A. & Sangwichien, C. 2013. Response surface optimization of enzymatic hydrolysis of narrow-leaf cattail for bioethanol production. **Energy Conversion and Management**, 73(381-388.

Sadhukhan, J., Ng, K. S. & Hernandez, E. M. 2014. **Biorefineries and chemical processes: design, integration and sustainability analysis**. John Wiley & Sons.

Saini, J. K., Anurag, R. K., Arya, A., Kumbhar, B. & Tewari, L. 2013. Optimization of saccharification of sweet sorghum bagasse using response surface methodology. **Industrial Crops and Products**, 44(211-219.

Saini, J. K., Saini, R. & Tewari, L. 2015. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. **3 Biotech**, 5(4), 337-353.

Singh, Y. D., Mahanta, P. & Bora, U. 2017. Comprehensive characterization of lignocellulosic biomass through proximate, ultimate and compositional analysis for bioenergy production. **Renewable Energy**, 103(490-500.

Solomon, B. D., Barnes, J. R. & Halvorsen, K. E. 2007. Grain and cellulosic ethanol: History, economics, and energy policy. **Biomass and Bioenergy**, 31(6), 416-425.

Subhedar, P. B. & Gogate, P. R. 2014. Alkaline and ultrasound assisted alkaline pretreatment for intensification of delignification process from sustainable raw-material. **Ultrasonics sonochemistry**, 21(1), 216-225.

Sun, J., Ding, S.-Y. & Doran-Peterson, J. (2014). CHAPTER 1 Biomass and its Biorefinery: Novel Approaches from Nature-Inspired Strategies and Technology. In **Biological Conversion of Biomass for Fuels and Chemicals: Explorations from Natural Utilization Systems** (pp. 1-13): The Royal Society of Chemistry.

Swain, M. R., Kar, S., Sahoo, A. K. & Ray, R. C. 2007. Ethanol fermentation of mahula (Madhuca latifolia L.) flowers using free and immobilized yeast Saccharomyces cerevisiae. **Microbiological Research**, 162(2), 93-98.

Taherzadeh, M. J. & Niklasson, C. (2004). Ethanol from Lignocellulosic Materials: Pretreatment, Acid and Enzymatic Hydrolyses, and Fermentation. In **Lignocellulose Biodegradation** (Vol. 889, pp. 49-68): American Chemical Society.

Takagi, T., Uchida, M., Matsushima, R., Ishida, M. & Urano, N. 2012. Efficient bioethanol production from water hyacinth Eichhornia crassipes by both preparation of the saccharified solution and selection of fermenting yeasts. **Fisheries science**, 78(4), 905-910.

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.

Thangavelu, S. K., Ahmed, A. S. & Ani, F. N. 2014. Bioethanol production from sago pith waste using microwave hydrothermal hydrolysis accelerated by carbon dioxide. **Applied Energy**, 128(Supplement C), 277-283.

Tilman, D., Socolow, R., Foley, J. A., Hill, J., Larson, E., Lynd, L., Pacala, S., Reilly, J., Searchinger, T. & Somerville, C. 2009. Beneficial biofuels—the food, energy, and environment trilemma. **Science**, 325(5938), 270-271.

Twidell, J. & Weir, T. 2015. **Renewable energy resources**. 2nd. Routledge.

Van Soest, P. J., Robertson, J. B. & Lewis, B. A. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. **Journal of Dairy Science**, 74(10), 3583-3597.

Vohra, M., Manwar, J., Manmode, R., Padgilwar, S. & Patil, S. 2014. Bioethanol production: Feedstock and current technologies. **Journal of Environmental Chemical Engineering**, 2(1), 573-584.

Volynets, B., Ein-Mozaffari, F. & Dahman, Y. (2017). **Biomass processing into ethanol: pretreatment, enzymatic hydrolysis, fermentation, rheology, and mixing**, *Green Processing and Synthesis* (Vol. 6, pp. 1).

Wedges, S. 2004. Solving the Climate Problem for the Next 50 Years with Current Technologies Pacala, S.; Socolow, R. **Science (Washington, DC, United States)**, 305(5686), 968-972.

Wei, P., Cheng, L.-H., Zhang, L., Xu, X.-H., Chen, H.-l. & Gao, C.-j. 2014. A review of membrane technology for bioethanol production. **Renewable and Sustainable Energy Reviews**, 30(388-400.

Witek-Krowiak, A., Chojnacka, K., Podstawczyk, D., Dawiec, A. & Pokomeda, K. 2014. Application of response surface methodology and artificial neural network methods in modelling and optimization of biosorption process. **Bioresource technology**, 160(150-160.

Xia, A., Cheng, J., Song, W., Yu, C., Zhou, J. & Cen, K. 2013. Enhancing enzymatic saccharification of water hyacinth through microwave heating with dilute acid pretreatment for biomass energy utilization. **Energy**, 61(158-166.

Yan, J., Wei, Z., Wang, Q., He, M., Li, S. & Irbis, C. 2015. Bioethanol production from sodium hydroxide/hydrogen peroxide-pretreated water hyacinth via

simultaneous saccharification and fermentation with a newly isolated thermotolerant Kluyveromyces marxianu strain. **Bioresource Technology**, 193(103-109.

Yang, B., Dai, Z., Ding, S.-Y. & Wyman, C. E. 2011. Enzymatic hydrolysis of cellulosic biomass. **Biofuels**, 2(4), 421-449.

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(11.

Zeng, M., Mosier, N. S., Huang, C. P., Sherman, D. M. & Ladisch, M. R. 2007. Microscopic examination of changes of plant cell structure in corn stover due to hot water pretreatment and enzymatic hydrolysis. **Biotechnology and bioengineering**, 97(2), 265-278.

Appendix

APPENDIX A

Total sugar determination by phenol sulfuric method (DuBois et al., 1956)

❖ Reagents

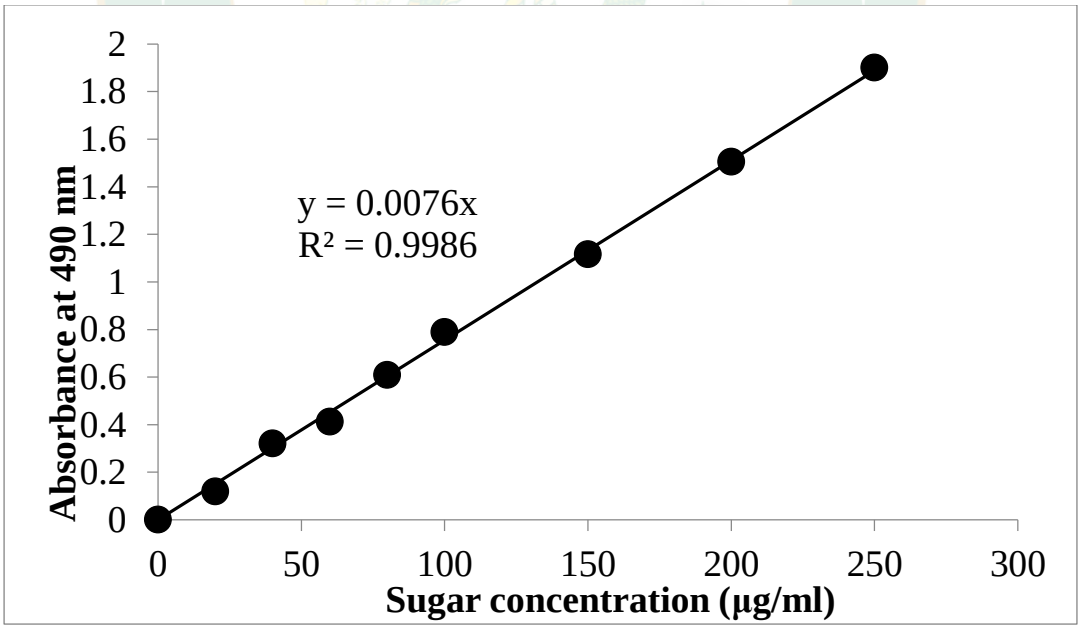
- Sulfuric acid (H_2SO_4) concentrated 98% (v/v)
- Phenol 5% (w/v)

❖ Standard glucose preparation

0.1g of glucose was dissolved in distilled water in a 100 mL volumetric flask to get 1000 µg/ml glucose solution.

❖ Procedure

Standard curve of sugar was prepared using the serial concentration of glucose solution (0-250 µg/mL) in distilled water. The 500 µL of each concentration was transferred to test tubes and added with 500 µL of 5% phenol solution. The mixtures were homogenized by vortex and subsequently stand for 10 min. The absorbance (490 nm) of the reaction mixture was measured. The relation between A490 and glucose was plotted.



1 Standard curve of total sugar by phenol sulfuric method using glucose as standard

APPENDIX B

Reducing sugar determination by DNS method (Miller, 1959)

- Preparation of DNS solution

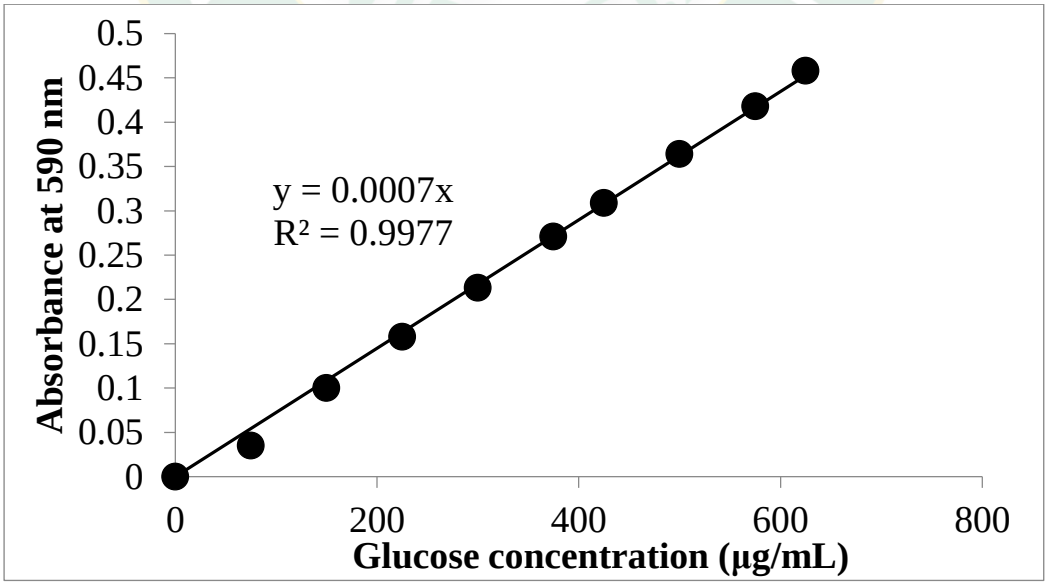
- Dissolving 5 g of 3, 5 Dinitrosalicylic acid in 100 mL of NaOH 2N.
- Adding 150 g of sodium potassium tartrate and stir until completely dissolve.
- Adjusting the volume up to 500 mL.

- Preparation of glucose solution

0.1g of glucose was dissolved in distilled water in a 100 mL volumetric flask to get 1000 µg/ml glucose solution

- Procedure

Standard curve preparation of reducing sugar was prepared using serial concentration of glucose (1000 µg/mL) in distilled water. The 500 µL of each concentration was filled into test tube and added with 500 µL of DNS solution and subsequently boiled for 15 min. After that, cooling and addition with 4.0 mL of distilled water was performed. The absorbance at 540 nm was measured. The relation between glucose concentration and A540 was plotted.



2 Standard curve of reducing sugar by phenol sulfuric method using glucose as standard

APPENDIX C

Determination of ethanol

Ethanol content in this study was measured with an Alcohol Ebulliometer (LDS Model 360, France) which was designed to estimate the boiling point of different types of liquids. The principle is based on the comparison of the boiling point of pure water (distilled water) and the boiling point of wine. Since ethanol has the boiling point at 78.3°C, the boiling point of ethanol-mixture are lower than the one of pure water. The different temperature of pure water and ethanol-mixture are converted into ethanol percentage via an Ebulliometer disc provided by supplier.

Firstly, 20 mL of distilled water was poured into the boiling chamber and inserted the thermometer. Then, the condenser chamber was filled with cold water, lighted the alcohol lamp, and placed it under the instrument. Finally, observing and recording the mercury level when it is stable (do not fluctuate) (Figure 3). With regard to measuring the boiling point of sample, the mixture from fermenter was firstly centrifuged using the Benchtop centrifuge (Universal 320, USA) to eliminate the impact of suspended solids. The procedure is similar except 50 mL of sample used instead of 20 mL. Ethanol content was calculated by using the special Ebulliometer disc in which the boiling point of pure water was set at zero,

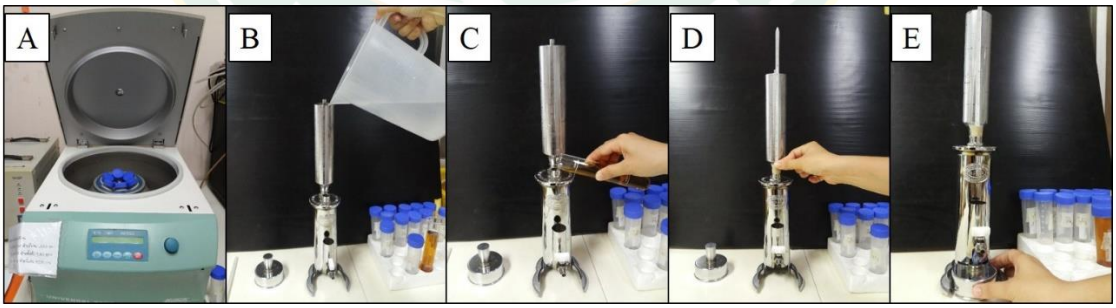


Figure 3 Centrifuge samples (A); Filling condenser with cold water (B); Pouring solution into boiling chamber (C), Inserting thermometer (D), Lighting the alcohol lamp and placing under instrument (E)

APPENDIX D

Table 12 Price reference

Items	Price	Installation factors	Reference
Grass grinder	1000 USD/machine	1.7	https://www.alibaba.com/product-detail/New-design-model-poultry-use-chaff_60396255887.html?spm=a2700.7724838.2017115.35.3a7d12efLc6P8O
Distillation colums	5000 USD/column	2.4	https://www.alibaba.com/product-detail/stainless-steel-ethanol-and-methanol-distiller_60653401551.html?spm=a2700.7724838.2017115.285.21222c51lQO0qH
Pretreatment reactor system	1500 USD/tank	1.5	https://www.alibaba.com/product-detail/Promotional-chemical-water-storage-tank-100000_60678721933.html?spm=a2700.7724838.2017115.24.4c025fb3IdFkDt&s=p
Pump, stailess steel	24.5 USD / piece	2.3	https://www.alibaba.com/product-detail/QW126A-WATER-PUMP-FROM-QIANGWEI_60591426014.html?spm=a2700.7724838.2017115.1.5d3e5fa5Dbga6R
Tank, storage, stailess steel	1500 USD/tank	1.8	https://www.alibaba.com/product-detail/Promotional-chemical-water-storage-tank-100000_60678721933.html?spm=a2700.7724838.2017115.24.4c025fb3IdFkDt&s=p
Cooling tower	10000 USD/set	-	https://www.alibaba.com/product-detail/cooling-tower_60149820167.html?spm=a2700.7724838.2017115.2.65a2124fW1IY8a

NaOH	300 USD/metric ton	0.3 USD/kg	https://www.alibaba.com/trade/search?fsb=y&IndexArea=product_en&CatId=&SearchText=sodium+hydroxide
HCl	90 USD/metric ton	0.15 USD/L	https://www.alibaba.com/product-detail/Hydrochloric-acid-HCL-30-33-_50006591753.html?spm=a2700.7724838.2017115.25.21222c51lQO0qH
Cellulase enzyme	1 USD/kg	-	https://www.alibaba.com/product-detail/Excellent-Food-Feed-Industrial-Cellulase-enzyme_60357472565.html?spm=a2700.7724838.2017121.49.3cae550arsMgdQ
Ethanol	1.05 USD/L	--	https://www.alibaba.com/product-detail/Pure-Ethanol-95-96-99-5-_50033535078.html?spm=a2700.7724838.2017115.80.3d652386v1bfcn
Fertilizer	150 USD/ton	-	https://www.alibaba.com/product-detail/Organic-fertilizer-powder_144456778.html?spm=a2700.7724857.main07.37.2e662736cWJUGV
1USD = 31.31 Thai Baht (31/01/2018)			

APPENDIX E

PUBLICATION

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Biotechnological Application Of Sustainable Bio-ethanol Production From Aquatic Biomass, Gooseweed

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Abstract: Bioethanol is one of the most promising and clean-burning fuel with high octane number and energy content compared to gasoline. This eco-flammable liquid is often blended with gasoline for transportation purposes in order to reduce emission of harmful gases. The main route to produce bioethanol is through bioconversion of biomass which includes edible sources such as sugar/starch-based foods, and non-edible sources like cellulose biomass, algae, and modified energy crops, etc. The second generation of bioethanol called lignocellulosic feedstock has drawn much attention due to the availability of the material all over the world. Among a various material, gooseweed (*Sphenoclea zeylanica* Gaertner) is recognized not only as one of major weed but also considered one of the most invasive weed inThailand. This species interferes with rice crops by taking nutrients, space, and sunlight. Total sugar and reducing sugar after pretreatment was 12.82 g/L and 4.46 g/L, respectively. Moreover, the amount of reducing sugar increased 64% after enzymatic hydrolysis. Finally, was reached 10.02 g/L after three days with *Saccharomyces cerevisias* TISTR 5020. In conclusion, gooseweed is a promising feedstock for bioethanol production.

Key words: *Sphenoclea zeylanica* Gaertner, bioethanol, lignocellulosic biomass, *Saccharomyces cerevisias*.

1. Introduction

Generally, bioethanol had been used as an eco-friendly fuel due to its high octane number and energy content compared to gasoline (Baeyens et al., 2015). Currently, high pure concentrated bioethanol is blended with gasoline or petrol to produce green fuels for transportation purposes. However, the production of bioethanol in the world is limited by feedstock sources as edible plants such as sugar cane, sugar beet, maize, sweet sorghum, etc. used for the main materials (Zabed et al., 2017). Although these edible biomasses have ahigh sugar content, the core disadvantages of them are the threat of food demand, seasonal cultivation, and geographical distribution. For ethanol industries, it is extremely important to maintain a constant and reliable raw sources to reduce transportation, storage, and operation cost. Therefore, the second generation of bioethanol (so-called lignocellulosic biomass) which is abundant and global spreading had been drawn much attention from all over the world (Limayem and Ricke, 2012).

Lignocellulosic biomass can be grouped into energy crops, aquatic plants, agricultural wastes, forestry residues, and organic fraction of municipal solid wastes (Zabed et al., 2017). Unlike sugar-based feedstock, most of lignocellulosic materials do not release fermentable sugars directly as juice. In another way, most of simple sugars (glucose, fructose, etc.) are linked to cellulose, hemicellulose, and lignin which are cell wall compounds. Thus, the conversion of these sources into bioethanol has to undergo at least four main steps: pretreatment, hydrolysis, fermentation, and purification (Aditiya et al., 2016). There are many different pretreatment ways which includes mechanical, physical, chemical, biological, and the combination of these above methods (Rastogi and Shrivastava, 2017). Suitable pretreatment methods, depending in the characteristic of the sample are chosen in order to reach the maximum sugar yield and minimum toxic substances. Sodium hydroxide (NaOH) was proved to have strong effects on the linkage of biomass structure so that lignin can be removed. The used of NaOH at mild condition also does not produce inhibitory fermentation like diluted/ concentrated acid (Bensah and Mensah, 2013; Yan et al., 2015; Carvalho et al., 2016; Akhtar et al., 2017). Due to the pretreatment, a variety of sugars can be access easily by enzyme and split to simple sugars through hydrolysis process. The process is continued by yeast fermentation and the final products (ethanol and CO_2) are release. The above described process is separate hydrolysis and fermentation (SHF) system.

Gooseweed (*Sphenoclea zeylanica* Gaertner) is a harmful weed which are commonly found on rice fields as well as any wetland area on earth. In Thailand and other tropical counties like Viet Nam, and Indonesia, it is observed that this weed interferes and compete nutrient with rice (Noda et al., 1986). There are many suggested ways including physical, chemical, and biological methods to eliminate this weeds (Mabbayad and Watson, 1995). Consequently, this plant becomes an agricultural waste. A simple experiment was carried to check plant morphology and starch content (iodine method). The result showed that this plant might be a good substrate for bioethanol production. For those reasons, gooseweed was chosen to be investigated the feasibility of producing ethanol via SHF process.

2. Material and methods

The methodology is illustrated in Fig. 1. This study applied separate hydrolysis and fermentation (SHF) process for bioethanol production.

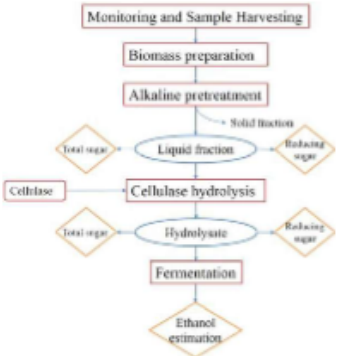

Fig. 1. Flow chart of methodology

2.1. Raw material

Gooseweed from fields in Chiang Mai, Thailand was harvested in September and October, 2016. The collected materials were firstly washed by tap water to remove visible contaminants like mud, soil and chopped into 2 -3 cm. The raw samples were allowed to be air-dried for three days and in hot air oven overnight in order to achieve a moisture content of no more than 10%. Finally, these raw sample were blended by a blender (OTTO BE – 127, Thailand) to a specific particle-size which past 1 mm mesh sieve and stored in a desiccator for further experimental (Fig. 2).

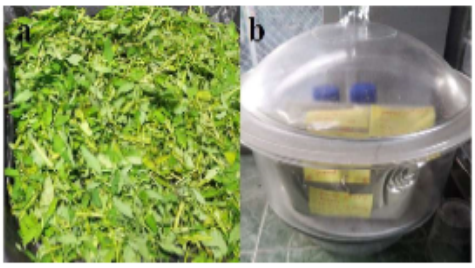

Fig. 2. Sample preparation: Air-drying sample (a), storing in a desiccator (b).

2.2. Pretreatment and hydrolysis

Alkaline pretreatment was performed by soaking 10 g of powdered sample with 1% NaOH (w/v) to reach a biomass loading concentration of 10% (w/v) in 250 mL flasks. The experiment was carried out at ambient temperature for 24 hours which allowed to use a shaker (Excellar E24, Canada) at 150 rpm to mix properly. After reaction, solid and liquid fractions were separated by one layer cloth. Total and reducing sugar in the liquid fraction were estimated by following phenol – sulfuric (Dubois et al., 1956) and dinitrosalicylic (DNS) colorimetric method (Miller, 1959), respectively. D-Glucose (Merck, USA) in different concentration was used as standard substance for those above method.

The pH of liquid fraction was adjusted to 5.0 (PCSTestr 35, Singapore) by HCl 1 M before hydrolysis (Singhania et al., 2013). Sugars in the liquid fraction was hydrolyzed into fermentable sugars by a cellulase enzyme provided by Union Science Company (Chiang Mai, Thailand). The enzyme specification stated by supplier was 2398 units/g, beta glucosidase 577 units/g, and optimal pH 4. Generally, 2% of cellulase enzyme (v/v) was added in liquid fraction and the process was incubated at 50°C in an incubated shaker (150 rpm) for 24 hours. In order to check the efficiency of hydrolysis step, total sugar and reducing sugar were determined followed after 24 hours. In addition, pH of hydrolysate solution was also rechecked. All the experiment as well as analysis test were performed in triplicate.

2.3. Lab-scale fermentation set up

The collected hydrolysate was fermented to ethanol through bioprocess of immobilized yeast *Saccharomyces cerevisia* TISTR 5020. The yeast strain was firstly cultured in inoculum culture medium containing 10 g/L yeast extract, 20 g/L peptone, and 20 g/L D-glucose for 24 hours. Immobilized bead was produced by following Williams and Munnecke (1981) (Williams and Munnecke, 1981). For fermentation, 2% of immobilized bead (w/v) was added into 100 mL hydrolysate whose pH was 5.4 and incubated at 30°C ± 3 for three days. In order to measure ethanol concentration, a manual Ebulliometer was used (Fig. 3). The method is based on the difference of boiling point of pure water (distilled water) from the boiling point of the mixture of water and ethanol. A calculation disk was used to calculate ethanol concentration as percentage unit.

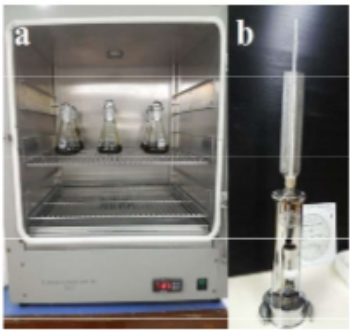

Fig. 3. Batch bioethanol experiment (a), Ebulliometer (b).

3 Results and discussion

Unlike edible source, non-edible sources had been considered as a promising feedstock for biofuels production which includes bioethanol (Aditiya et al., 2016). However, the sugar contents in these type of biomass can't be used directly by yeast or bacteria due to their complex structure. This leads to the compulsory requirement of pretreatment and hydrolysis steps whose functions produces fermentable sugars. Sodium hydroxide is a strong base so that it has tough effects on the link of biomass structure and split it into separated compounds such as lignin, hemicellulose, and cellulose.

In this study, the obtained total sugar and reducing sugar after pretreatment were 12.8 g/L and 4.5 g/L, respectively. The recorded pH after pretreatment with 1% NaOH was 10.14 – 10.33. Even though the amount of reducing sugar was quite low after pretreatment, cellulase was supported to increase reducing sugar yield by break big sugar molecules to simple ones. As a result, reducing sugar reached 7.3 g/L after hydrolysis process. As the material of this research is quite new and has not been studied before, the results from this were compared with other aquatic lignocellulosic biomass such as water hyacinth, water leccute, etc. (Table 1). The concentration of chemical and the condition of pretreatment are important elements to disturb biomass structure and dissolve sugar. More concentrated chemical together with more harsh condition such as high temperature and pressure, more reducing sugar is released. Nevertheless, along with fermentable sugar, some inhibitory substances like furfural, 5-hydroxymethylfurfural, and phenol are also produce. Those inhibitors interferes the activity of yeast and other microorganism which leads to the decrease of ethanol yield (Xia et al., 2013; Kang et al., 2014; Zabed et al., 2017). Though the amount of fermentable sugar in this study is less than other mentioned literatures, the comparison of other aspects like energy input, the formation of inhibitors, concentration of chemical, neutralization leads the treatment of diluted NaOH at mild condition and enzymatic hydrolysis are more suitable for non-woody biomass, particularly gooseweed.

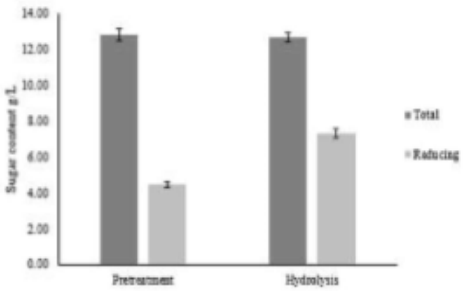

Fig. 4. Sugar content after pretreatment and hydrolysis.

Table 1. Comparison of reducing sugar obtained from different lignocellulosic biomass (water hyacinth and gooseweed).

Material	Pretreatment	Reducing sugar (g/L)	Reference
WH	2 % NaOH at 121°C, 15 psi pressure, 40 min, and enzymatic hydrolysis	13.5	(Singh and Bishnoi, 2013)
WH	Microwave heating with 1% H_2SO_4 and enzymatic hydrolysis	9.6	(Xia et al., 2013)
WH	3% NaOH/1.5% H_2O_2, 30°C	9.7	(Yan et al., 2015)
GS	1% NaOH at mild condition and enzymatic hydrolysis	7.3	This study

WH water hyacinth; GS gooseweed

During fermentation, immobilized *S. cerevisia* beads converted simple sugars to ethanol and carbon dioxide. The technology of using immobilized beads had been investigated and developed long time ago (Williams and Munnecke, 1981). This method takes advantage of free cell by ease storage, usage, and high recovery rate. *S. cerevisia* yeast strain is a common, wide available, and high efficient in transforming sugar to ethanol. An ethanol concentration of about 10.02 g/L was achieved after three days of fermentation. To compare with some previous literatures, ethanol concentration from fermentation of water hyacinth were 4.3 g/L, 6.2 g/L, and 9.8 g/L by monoculture *S. cerevisia, S. stipites,* and co-culture, respectively (Singh and Bishnoi, 2013). Another research of water hyacinth conducted by Yan et al., (2015), the maximum ethanol concentration was reached 7.34 g/L by using simultaneous saccharification and fermentation (SSF) system along with *Kluyveromyces marxianu* strain the maximum.

4. Conclusion

The conversion of a very new material gooseweed to bioethanol had been investigated in this study. Alkaline pretreatment at mild condition (ambient temperature and pressure) once again was proved as an efficient method for treating lignocellulosic biomass, particularly non-woody material. The using of commercial cellulase for the hydrolysis of large sugar molecules resulted approximately 64% of reducing sugar increasing within 24 hours. Fermentation process was supported by a well-known brewer yeast *S. cerevisia* and obtained ethanol was 10.02 g/L. To sum up, data from fermentation indicates that gooseweed biomass can be an alternative and promising feedstock for bioethanol production. Moreover, further research is needed to optimize each process and achieve the maximum sugar and ethanol yield but minimum inhibitors and energy consumption.

References

Aditiya, H. B., T. M. I. Mahlia, W. T. Chong, H. Nur and A. H. Sebayang (2016). Second Generation Bioethanol Production: A critical review, Renewable and Sustainable Energy Reviews, 66, 631-653.

Akhtar, N., D. Goyal and A. Goyal (2017). Characterization of Microwave-alkali-acid Pre-treated Rice Straw for Optimization of Ethanol Production via Simultaneous Saccharification and Fermentation (SSF), Energy Conversion and Management, 141, 133-144.

Baeyens, J., Q. Kang, L. Appels, R. Dewil, Y. Lv and T. Tan (2015). Challenges and Opportunities in Improving the Production of Bio-ethanol, Progress in Energy and Combustion Science, 47, 60-88.

Bensah, E. C., and M. Mensah (2013), Chemical Pretreatment Methods for the Production of Cellulosic Ethanol: Technologies and Innovations, International Journal of Chemical Engineering, 2013, 21.

Carvalho, D. M. d., O. Sevastyanova, J. H. d. Queiroz and J. L. Colodette (2016). Cold Alkaline Extraction as a Pretreatment for Bioethanol Production from Eucalyptus, Sugarcane Bagasse and Sugarcane Straw, Energy Conversion and Management, 124, 315-324.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers and F. Smith (1956). Colorimetric Method for Determination of Sugars and Related Substances, Analytical Chemistry, 28(3), 350-356.

Kang, Q., L. Appels, T. Tan and R. Dewil (2014), Bioethanol from Lignocellulosic Biomass: Current Findings Determine Research Priorities, The Scientific World Journal, 2014.

Limayem, A., and S. C. Ricke (2012), Lignocellulosic biomass for bioethanol production: Current perspectives, potential issues and future prospects, Progress in Energy and Combustion Science, 38(4), 449-467.

Mabbayad, M. O. and A. K. Watson (1995). Biological Control of Gooseweed (*Sphenoclea zeylanica* Gaertn.) with an *Alternaria* sp, Crop Protection, 14(5), 429-433.

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar, Analytical Chemistry, 31(3), 426-428.

Noda, K., N. W. S. R. I. Project and K. K. Jigyōdan (1986). Major Weeds in Thailand: Illustrated by Color, 142 pp., National Weed Science Research Institute Project.

Rastogi, M. and S. Shrivastava (2017). Recent Advances in Second Generation Bioethanol Production: An Insight to Pretreatment, Saccharification and Fermentation Processes, Renewable and Sustainable Energy Reviews, 80, 330-340.

Singh, A. and N. R. Bishnoi (2013). Comparative Study of Various Pretreatment Techniques for Ethanol Production from Water hyacinth, Industrial Crops and Products, 44, 283-289.

Singhania, R. R., A. K. Patel, R. K. Sukumaran, C. Larroche and A. Pandey (2013). Role and Significance of Beta-glucosidases in the Hydrolysis of Cellulose for Bioethanol Production, Bioresource Technology, 127, 500-507.

Williams, D. and D. M. Munnecke (1981). The Production of Ethanol by Immobilized Yeast Cells, Biotechnology and Bioengineering, 23(8), 1813-1825.

Xia, A., J. Cheng, W. Song, C. Yu, J. Zhou and K. Cen (2013). Enhancing Enzymatic Saccharification of Water

Hyacinth through Microwave Heating with Dilute Acid Pretreatment for Biomass Energy Utilization, Energy, 61, 158-166.

Yan, J., Z. Wei, Q. Wang, M. He, S. Li and C. Irbis (2015). Bioethanol Production from Sodium Hydroxide/Hydrogen Peroxide-pretreated Water Hyacinth via Simultaneous Saccharification and Fermentation with a Newly Isolated Thermotolerant *Kluyveromyces marxianu* strain, Bioresource Technology, 193, 103-109.

Zabed, H., J. N. Sahu, A. Suely, A. N. Boyce and G. Faruq (2017). Bioethanol Production from Renewable Sources: Current Perspectives and Technological Progress, Renewable and Sustainable Energy Reviews, 71, 475-501.

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Life Sciences Research

Research Article

Evaluation of bioethanol production from rice field weed biomass

Phuong Thi Vu, Yuwalee Unpaprom, Rameshprabu Ramaraj

Abstract

Bioethanol has attracted more attention as a clean-burning fuel that can benefit both environment and energy sector. Gooseweed and small-flowered nutsedge are abundant in rice fields in form of weeds and considered as a major agricultural problem. Thus, this paper aims to evaluate the possibility of ethanol production from these two weeds by calculating the theoretical ethanol yield from its reducing sugars and cellulose content. Experiment was conducted in rice fields in Chiang Mai province, Thailand and 207 kg/ha and 201 kg/ha biomass yield was obtained from gooseweed and small-flowered nutsedge plants. The theoretical ethanol yield of gooseweed and small-flowered nutsedge were 160 L/Mg and 223 L/Mg, respectively that suggest utilizing these materials as promising feedstocks for bioethanol production.

Keywords gooseweed, small-flowered nutsedge, theoretical ethanol yield

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Introduction

With the rapid development of population, additional energy has been needed in order to meet the growing demand of the world. Fossil fuels are the main source of energy all over the world. However, the use of fossil fuels has been associated with a lot of environmental issues which affects the whole biosphere and its inhabitants [1-2]. Another downside of using non-renewable energy is its limited supply. Especially nowadays, due to its high consumption, it is approaching their natural limits and it takes a considerable long time to be created. Thus, in order to meet the demand of energy as well as to control the quality of environment, biofuels should be considered as a feasible option. Biofuels has already been investigated around the world and continuously being utilized for the enhancement of global energy security. It can be used as an alternative source of energy for various purposes such as engine fuels, cooking, heating, electricity generating, etc. [4].

Most biofuels such as bioethanol, biogas, biodiesel, and biohydrogen are made from biomass and waste that helps to reduce the pressure on the environment [4]. Among different kinds of biofuel, bioethanol has drawn much widespread attention due to its promise of providing a clean transport fuel [8]. Even though its energy content is approximately same as gasoline, bioethanol has higher octane number (106-110) than gasoline which makes it an antiknock fuel [5-9]. Hence, it is often blended with gasoline or diesel with appropriate ratios in order to create new mixtures to reduce the harmful gas emission and increase the engine performance [10]. USA and Brazil are two top leading countries in bioethanol production from edible sources (corn and sugarcane) with 56.1 and 28.2 billion liters bioethanol production in 2015, respectively [11].

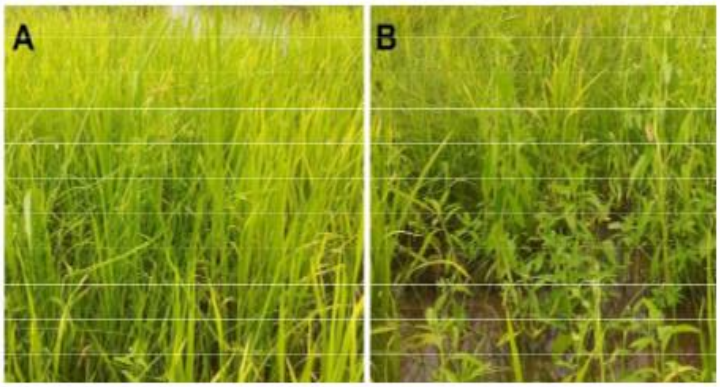

Figure 1. Small-flowered nutsedge (A); Gooseweed (B) in the rice field at Maejo University, Chiang Mai, Thailand

However, using edible biomass for bioethanol production has led to an argument of "food versus fuel" [12]. More lands and other sources such as water, fertilizers, and labors are needed to grow crops for energy [13-14]. Thus, lignocellulosic biomass, so-called second generation of bioethanol, has been preferred due to its abundance, low price, and worldwide distribution [15].

Gooseweed and small-flowered nutsedge both are short life-cycle plants and dominant in wet land areas. In general, they are considered as a problem in the rice field, as they compete with nutrients, water source, sunlight, etc. (Figure 1). Thus, in order to reduce the loss of rice yield, these materials are often taken out manually by farmers or chemical method which causes harmful effect on human health and increases the cost of labor. Hence, although being an invaluable waste, the feasibility of bioethanol production from these two materials should be investigated by calculating the theoretical ethanol yield.

Methodology

Material collection and preparation

Both gooseweed and small-flowered nutsedge were collected from the rice field at Maejo University, Nong Han, Sansai, Chiang Mai, Thailand (18° 53' 37.4"N; 99° 01'13.4"E). The two materials were firstly washed with tap water to remove dirt and mud. They were then chopped into 1-2 cm long pieces and dried in hot air oven at 50°C for 3 days. Size reduction was carried out by high-speed blender (Otto BE-127, Thailand) (Figure 2, 3). Dried powder after blending was passed through a 1mm mesh sieve and stored in a desiccator for further experiment.

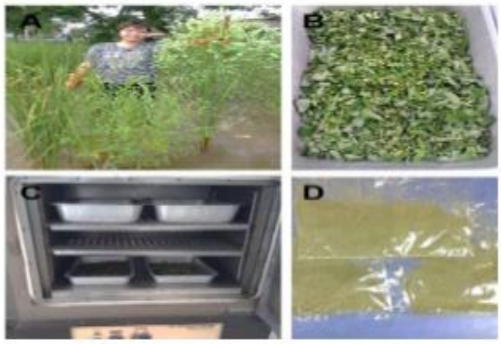

Figure 2. Gooseweed: (A) Sample collection; (B) Chopping; (C) Drying in hot air oven; (D) Powdered samples

Figure 3. Small-flowered nutsedge: (A) Sample collection; (B) Chopping; (C) Drying in hot air oven; (D) Powdered samples

Biomass yield

Biomass yield was calculated by the total mass of plants within a given unit of environment area. Since both gooseweed and small-flowered nutsedge grew in the stagnant area, especially in the rice fields located in Maejo University, Chiang Mai, Thailand (18°53'36.3"N 99°01'14.4"E). A 1 x 1m quadrat was placed in rice field randomly (Figure 4). The two plants were counted, collected and weighted as fresh samples followed by drying in hot air oven until it reached constant weight. The recorded data was used to calculate density (plant/m^2) and biomass yield (kg/ha).

Figure 4. 1 X 1 m quadrat in the rice field

Biochemical analysis

Reducing sugar was determined by HPLC with following description. Sugars of liquid phase by pre-treatment were analyzed by high performance liquid chromatography (HPLC) (condition: mobile phase-5 mM H_2SO_4; flow rate-0.7 mL/min; temperature of column: 60°C; Hi-Plex H column). The amount of cellulose, hemicellulose, and lignin was calculated using the method of fiber analysis reported by Van Soest [18].

Ethanol estimation procedure

For lignocellulosic biomass, cellulose, a main part of plant cell wall which is formed of many β (1→4) linked D-glucose units, is an important source of sugar for bioethanol production [15]. Besides, soluble reducing sugars or simple sugars such as monosaccharides (glucose, arabinose, fructose, etc.) that are found outside the cell wall are another source of fermentation substrate. Hence, it can be assumed that sugars from cellulose chains and soluble reducing sugars could be totally converted into bioethanol. As a result, a theoretical ethanol yield could be estimated from amount of cellulose and soluble reducing sugars present in the samples [19, 20]. The conversion of cellulose and reducing sugar into bioethanol were performed according to the below mentioned chemical equations (Eq1, Eq2, and Eq3). By using a balanced chemical equation where total mass of reactants and total mass of products are equal, so-called stoichiometry, theoretical bioethanol yield can be calculated as the below equations (Eq4, Eq5, and Eq6) [14, 19, 21, 22].

Ethanol density: 0.789 g/mL

$(C_6H_{10}O_5)_n + nH_2O \rightarrow 2nC_2H_5OH + 2nCO_2$ (Eq1)

$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ (Eq2)

$3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$ (Eq3) [23]

Ethanol from cellulose (TEC) in 1 g of dry biomass

TEC (g) = cellulose (g) * 0.57 (Eq4)

Ethanol from reducing sugar (TER) in 1 g of dry biomass

TER (g) = Reducing sugar (g) * 0.51 (Eq5)

Ethanol yield from biomass (TEB)

TEB (L/Mg) = (TEC + TER)*1267 (Eq6)

Results and Discussion

Characteristics of gooseweed

Gooseweed is a kind of tropical weed that grows invasively in damp land, especially in lowland rice field. Table 1 shows the basic classification of gooseweed. Its life cycle is coincident with rice plants and it is often dominant in rice field [24]. The appearance of this plant may cause many unexpected consequences for rice production due to the competition of essential nutrients with rice plants.

Table 1. Taxonomy of gooseweed

Classification	Gooseweed
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Campanulales
Family	Campanulaceae
Scientific Name	*Sphenoclea zeylanica* Gartn.

For these reasons, this plant had been recognized as one of the worst weeds in the world by Holm et al. [25]. A full description about dispersal, ecology, and morphology of gooseweed was reported by Carter et al. [26], since gooseweed had been considered as contaminant of rice feed in North America. In addition, reducing sugars including fructose, xylose, arabinose, and glucose were 19.02 mg/g dry biomass, 3.23 mg/g dry biomass, 2.72 mg/g dry biomass, and 3.63 mg/g dry biomass, respectively (Figure 5). Abundance in

quantity of this material can be a big advantage comparing to sugar/ starch-feedstock for bioethanol production [23].

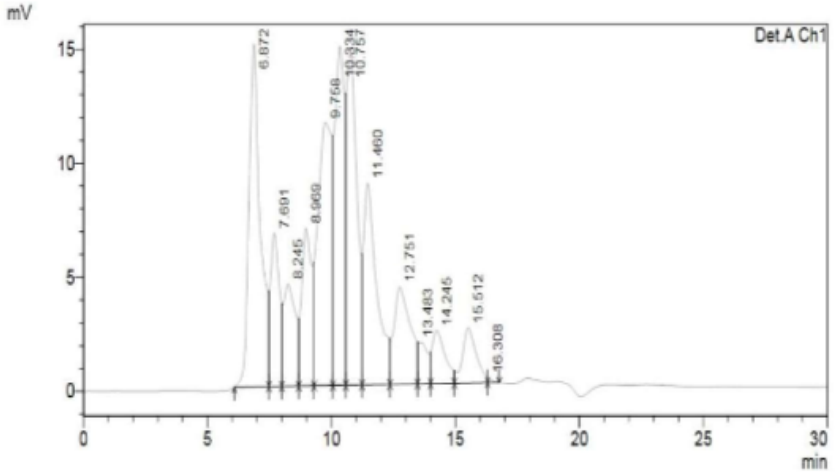

Figure 5. The peaks of sugars from gooseweed released after pretreatment (mobile phase–5 mM H_2SO_4; flow rate-0.7 mL/min; temperature of column: 60°C; Hi-Plex H column) [30]

Characteristic of small-flowered nutsedge

Small-flowered nutsedge, named *Cyperus difformis* L (Table 2), is listed in the Holm's list of the world's worst weeds [25]. It is worldwide distributed and grows in several parts of Thailand [5]. It is an invasive plant which grows on wetland and highly considered as a problematic weed in rice fields that is found anywhere at the bank of water bodies, in the field with crops plant, and its resourceful nature makes it easy to cultivate [28-30]. Though this material can be a good substrate for bioethanol fermentation, very few studies have been done on this comparatively new material [30].

Table 2. Taxonomy of small-flowered nutsedge

Classification	Gooseweed
Kingdom	Plantae
Phylum	Tracheophyta
Class	Liliopsida
Order	Cyperales
Family	Cyperaceae
Scientific Name	*Cyperus difformis* L..

The quality and quantity of sugars were analyzed by HPLC after pre-treatment with 1% NaOH and 1% H_2O_2 (Figure 6). The reducing sugar present in small-flowered nutsedge included 12.1 mg/ g dry biomass, 4.7 mg/g dry biomass, 2.02 mg/g dry biomass, and 1.2 mg/g dry biomass of fructose, glucose, xylose, and arabinose were respectively (Figure 6).

Biomass yield

The research was conducted in rice fields in which these two weed plants were dominant. The average density of gooseweed and small-flowered nutsedge were 59 plants/m2 and 38 plant/m2, respectively. High density of these plants causes the loss of rice yield due to the competition of nutrients and other essential

elements between weeds and rice plants [31-35]. Region with gooseweed showed 207 kg/ha rice yield, while small-flowered nutsedge produced 201 kg/ha rice yield. Yields varied with season, types of rice plant, and the method of growing rice.

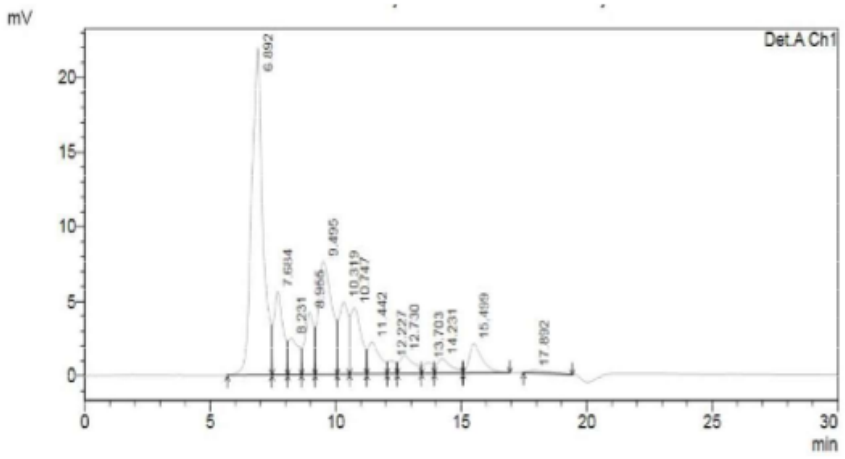

Figure 6. The peaks of sugars from small-flowered nutsedge released after pretreatment (mobile phase–5 mM H_2SO_4; flow rate-0.7 mL/min; temperature of column: 60°C; Hi-Plex H column).

Ethanol yield estimation

Table 3 shows cellulose, reducing sugar contents and theoretical ethanol yield of gooseweed and small-flowered nutsedge.

Table 3. Cellulose, reducing sugar content and theoretical ethanol yield of gooseweed and small-flowered nutsedge

Plant	Cellulose (g) *	Reducing sugar (g) *	TEC (g) *	TER (g) *	TEB (L/ Mg)**
Small-flowered nutsedge	0.22 ± 0.001	0.100 ± 0.001	0.125	0.051	223.5
Gooseweed	0.137 ± 0.003	0.096 ± 0.0	0.078	0.049	160.9

*Performed as g per 1 g dry biomass.
*Reducing sugar: glucose, fructose, xylose, and arabinose.
** Theoretical ethanol yield (L) per Mg (Ton) of dry biomass.

The components of plant such as cellulose, hemicellulose, lignin, and soluble carbohydrate could be different due to season, environment condition, and age of plant [19]. The average theoretical ethanol yield from gooseweed and small-flowered nutsedge were 160 L/Mg and 223.5 L/Mg, respectively.

Conclusion

The yield of gooseweed and small-flowered nutsedge in the rice field were 207 kg/ha and 201 kg/ha, respectively. Several types of sugars were founded such as glucose, fructose, xylose, and arabinose in both materials. Gooseweed and small-flowered nutsedge contained 14% and 22% cellulose, respectively. Gooseweed and small-flowered nutsedge are almost untapped biomass feedstock for bioethanol production

via fermentation. The theoretical ethanol yield of gooseweed and small-flowered nutsedge were 160 L/ Mg and 223 L/Mg respectively. The feasibility of bioethanol production from these two materials should be investigated in future by performing other required laboratory experiments.

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References

[1] Y. J. Kaufman, R. S. Fraser and R. L. Mahoney **(1991)**. Fossil fuel and biomass burning effect on climate-heating or cooling. J. Climate, **4:** 578-588.
[2] R. Wilson **(1980)**. Health effects of fossil fuel burning: assessment and mitigation. Ballinger Publishing Company. ISBN: 9780884107149, https://books.google.com.tr/books?id=B6iEAAAAIAAJ
[3] M. F. Demirbas **(2011)**. Biofuels from algae for sustainable development. Appl. Energy, **88:** 3473-3480.
[4] R. Strzalka, D. Schneider and U. Eicker **(2017)**. Current status of bioenergy technologies in Germany. Renew. Sust. Energ. Rev., **72:** 801-820.
[5] H. Zabed, J. N. Sahu, A. Suelya, A. N. Boycea and G. Faruq **(2017)**. Bioethanol production from renewable sources: Current perspectives and technological progress. Renew. Sust. Energ. Rev., **71:** 475-501.
[6] K. Smith **(2013)**. Biofuels, air pollution, and health: a global review. Springer Science & Business Media.
[7] RFA **(2017)**. Ethanol industry outlook, Building partnerships and growing markets, in Washington, DC, February 2017.
[8] Y. N. Guragain, J. De Coninck, F. Husson, A. Durand and S. K. Rakshita **(2011)**. Comparison of some new pretreatment methods for second generation bioethanol production from wheat straw and water hyacinth. Bioresour. Technol., **102:** 4416-4424.
[9] P. S. Nigam and A. Singh **(2011)**. Production of liquid biofuels from renewable resources. Prog. Energy Combust. Sci., **37:** 52-68.
[10] J. Baeyens, Q. Kang, L. Appels, R. Dewil, Y. Lv and T. Tan **(2015)**. Challenges and opportunities in improving the production of bio-ethanol. Prog. Energy Combust. Sci., **47:** 60-88.
[11] H. Chen and X. Fu **(2016)**. Industrial technologies for bioethanol production from lignocellulosic biomass. Renew. Sust. Energy Rev., **57:** 468-478.
[12] L. R. Brown **(1980)**. Food or fuel. Worldwatch paper, **35**: p.43.
[13] G. Koçar and N. Civaş **(2013)**. An overview of biofuels from energy crops: Current status and future prospects. Renew. Sust. Energy Rev., **28:** 900-916.
[14] M. Chen, L. Xia and P. Xue **(2007)**. Enzymatic hydrolysis of corncob and ethanol production from cellulosic hydrolysate. Int. Biodeterior. Biodegradation, **59:** 85-89.
[15] H. B. Aditiya, T. M. I. Mahlia, W. T. Chong, H. Nur and A. H. Sebayang **(2016)**. Second generation bioethanol production: A critical review. Renew. Sust. Energy Rev., **66:** 631-653.
[16] P. Alvira, E. Tomás-Pejó, M. Ballesteros and M. J. Negro **(2010)**. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour. Technol., **101:** 4851-4861.
[17] S. H. Mood, A. H. Golfeshan, M. Tabatabaei, G. S. Jouzani, G. H. Najafi and M. Gholami et al. **(2013)**. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew. Sust. Energy Rev., **27:** 77-93.
[18] J. Yan, Z. Wei, Q. Wang, M. He, S. Li and C. Irbis **(2015)**. Bioethanol production from sodium hydroxide/hydrogen peroxide-pretreated water hyacinth via simultaneous saccharification and fermentation with a newly isolated thermotolerant *Kluyveromyces marxianu* strain. Bioresour. Technol., **193:** 103-109.

[19] C. Toquero and S. Bolado **(2014)**. Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing. Bioresour. Technol., **157:** 68-76.
[20] Y. Zheng, Z. Pan and R. Zhang **(2009)**. Overview of biomass pretreatment for cellulosic ethanol production. Int. J. Agri. & Biol. Eng., **2:** 51-68.
[21] P. J. Van Soest, J. B. Robertson and B. A. Lewis **(1991)**. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci., **74:** 3583-3597.
[22] K. P. Vogel, B. S. Dien, H. G. Jung, M. D. Casler, S. D. Masterson and R. B. Mitchell **(2011)**. Quantifying actual and theoretical ethanol yields for switchgrass strains using NIRS analyses. BioEnergy Res., **4:** 96-110.
[23] K. J .Ptasinski **(2016)**. Efficiency of biomass energy: An exergy approach to biofuels, power, and biorefineries. John Wiley & Sons, ISBN: 9781119118169.
[24] S. K. Thangavelu, A. S. Ahmed and F. N. Ani **(2014)**. Bioethanol production from sago pith waste using microwave hydrothermal hydrolysis accelerated by carbon dioxide. Appl. Energy, **128:** 277-283.
[25] B. C. Sah, N. N.Nichols, N. Qureshi, G. J. Kennedy, L. B. Iten and M. A. Cotta **(2015)**. Pilot scale conversion of wheat straw to ethanol via simultaneous saccharification and fermentation. Bioresour. Technol., **175:** 17-22.
[26] 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.
[27] K. Saito, K. Azoma and J. Rodenburg **(2010)**. Plant characteristics associated with weed competitiveness of rice under upland and lowland conditions in West Africa. Field Crops Res., **116:** 308-317.
[28] L. G. Holm, D. L. Plucknett, J. V. Pancho and J. P. Herberger **(1977)**. The world's worst weeds. University Press.
[29] R. Carter, J. C. Jones and R. H. Goddard **(2014)**. *Sphenoclea zeylanica* (Sphenocleaceae) in North America-Dispersal, ecology, and morphology. Castanea, **79:** 33-50.
[30] C. Bryant **(2010)**. Third Berkeley Conference on Bioeconomy.
[31] O. Ueno and T. Takeda **(1992)**. Photosynthesis pathways, ecological characteristics, and the geographical distribution of the Cyperaceae in Japan. Oecologia, **89:** 195-203.
[32] B. Sanders **(1994)**. The life cycle and ecology of *Cyperus difformis* (rice weed) in temperate Australia: a review. Aust. J. Exp. Agr., **34:** 1031-1038.
[33] R. Vishwakarma and R. Banerjee **(2016)**. Enhancement of sugar content of *Cyperus* sp. through cellulolytic enzymes for bioethanol generation. Lignocellulose, **5:** 94-105.
[34] B. Chauhan and D. Johnson **(2009)**. Ecological studies on *Cyperus difformis*, *Cyperus iria* and *Fimbristylis miliacea*: three troublesome annual sedge weeds of rice. Ann. Appl. Biol., **155:** 103-112.
[35] M. O. Mabbayad and A. K. Watson **(1995)**. Biological control of gooseweed (*Sphenoclea zeylanica* Gaertn.) with an *Alternaria* sp. Crop Prot., **14:** 429-433.

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Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production

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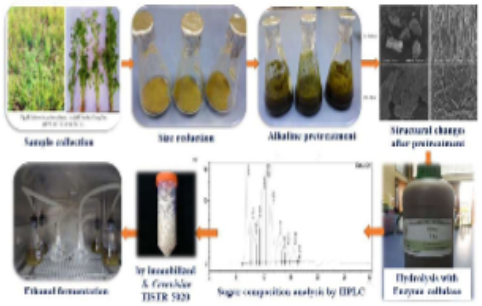
GRAPHICAL ABSTRACT



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ABSTRACT

Gooseweed (*Sphenoclea zeylanica* Gaertn.) is a pest on the rice field that has a potential to be a promising substrate for bioethanol production. Dry powdered gooseweed was firstly pretreated with 1% NaOH, following 1% H_2O_2 at variety conditions. The hydrolysis process was set at 50 °C for 24–72 h with enzyme cellulase (β-glucosidase) while the fermentation process was carried using *Saccharomyces cerevisiae* TISTR 5020 at 33 °C for nine days. The ethanol concentration was recorded for three, five, seven, and nine days using an ebulliometer. The results showed that the treatment with only 1% NaOH for 24 h has the highest sugar performance. In regard with hydrolysis, the optimum retention time was at 24 h. Lastly, the highest ethanol concentration was achieved at 11.84 g/L after five days and a rapid decreasing after seven to nine days was also observed.

1. Introduction

The use of bioethanol, a renewable fuel, has been significantly increasing all over the world due to the limitation of fossil fuels (petroleum, coal, natural gases, etc.), environmental issues (climate change, pollution, resource depletion, and so on), and its own characteristic as a high octane number fuel. In addition, bioethanol industry also has been creating a huge amount of direct and indirect jobs. Currently, bioethanol is produced mostly in U.S and Brazil which make up approximately 60% and 27% percentage of the world, respectively. By joining in the global market, Thailand contributed 322 million gallons (1%) to the total production in 2016 (Fig. 1) (RFA, 2017).

At the present, the conversion of biomass into bioethanol has been upgraded to four generations which are sugar/starch-based crops, lignocellulosic biomass, algae, and advanced bioconversion techniques. The second generation-lignocellulosic material, known as abundant,

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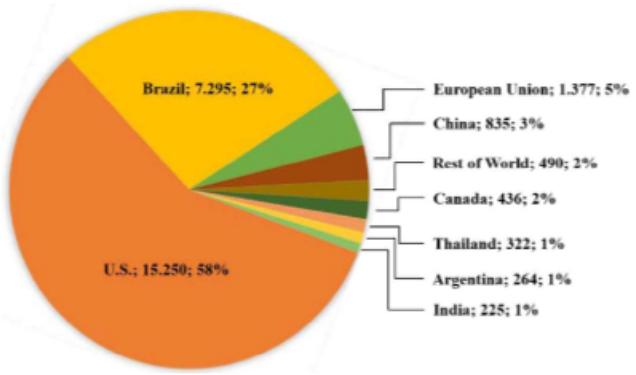

Fig. 1. Bioethanol production by country, million gallons, 2017 (RFA, 2017).

renewable, and nonedible biomass in the world, are untapped resources for bioethanol production because of the lack of researches. There are many types of feedstock that belong to these criteria such as agricultural wastes, forest residues, aquatic plants, food/beverage wastes, and other industrial wastes. Aquatic/wetland plants are considered as promising materials due to its typical characteristics, e.g. to be formed of cellulose, hemicellulose, and lignin; high speed growth; abundance, living on water surface or wetland areas; the ability of neutralize polluted water bodies, etc. The core factor of wetland plants as bioethanol feedstock is cellulose chains formed from a thousand D-glucose units which are essential substrates for bioethanol fermentation. Besides, the efficiency of conversion processes, particularly pretreatment and hydrolysis, are susceptible to biomass attributes. As a result, it is essential to study the physical characteristic and chemical compositions of new cellulosic biomass.

To be composed mainly of cellulose, hemicellulose, and lignin, the process of converting from lignocellulosic biomass into a clean-burning fuel is quite different from the first generation which is the addition of pretreatment steps (Rastogi and Shrivastava, 2017). In order to obtain as much as possible fermentable sugar through hydrolysis by enzyme or microorganisms, it is vital to make cellulose chains free of lignin and hemi-cellulose cover by pretreatment. Based on the structure of feedstock, there has been a lot of efficient pretreatment methods developed recently: physical, chemical, biological, and combination of those methods. Firstly, biomass might be milled, grinded, or blended into small pieces to increase the activated surface and porosity of the lignocelluloses. The powdered feedstock or pieces are then continuously treated at high temperature or pressure, with or without chemical, or microorganisms. At severe condition (high temperature or pressure), the architecture of feedstock is damaged and broken into separately components. On the other hands, the using of some chemical such as dilute/concentrated acid, alkaline, oxidant substances can disturb and cut the hydrogen bonds and covalent bonds between cellulose, hemicellulose, and lignin (Ravindran and Jaiswal, 2016). Biological method, nevertheless, using the metabolism of other living things such as microorganism, fungi, mold, etc. degrade the structure of biomass to simple sugar. Some other advanced methods and application are described in the Table 1.

This study focuses on alkaline/oxidant pretreatment using sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) as efficient reagent to affect biomass attribute and support hydrolysis step (Yan et al., 2015). Co-treatment with sodium hydroxide and hydrogen peroxide is considered as an environment-friendly and effective method for various types of lignocellulosic biomass including water hyacinth (Mishima et al., 2008; Yan et al., 2015), wheat straw (Barakat et al., 2014), etc. The reaction between sodium hydroxide/hydrogen peroxide and biomass requires a long retention time which is up to hours (Haghighi Mood et al., 2013). Nevertheless, dilute alkaline oxidant pretreatment does not create much inhibitors and damage equipment comparing to dilute/concentrate acid methods.

Gooseweed (*Sphenoclea zeylanica* Gaertn.) is a usual and widespread herbaceous weed of wetland rice (Holm et al., 1977) (Fig. S1). This species was placed in the family *Camparulaceae* which is known as the bellflower family including about 2400 species of non-woody plants (Berry, 2009; Mani, 2014). In regards to taxonomy, Richard and his colleagues described gooseweed in detail; stems are green, erect, hollow and often much-branched; leaves are alternate; flowers are sessile and bisexual. In addition, gooseweed is able to develop both on terrestrial and freshwater systems in tropical to warm temperature areas (Carter et al., 2014). It is native to the Eastern Hemisphere including Thailand, Viet Nam, Indonesia, etc. Since its preferred habitat is wetland and aquatic bodies, this species has been a problematic non-woody plant on

Table 1
Some pretreatment methods using for bioethanol production from lignocellulosic biomass.

Pretreatment	Describes		Reference
Physical	Disrupting the structure of biomass at high temperature, and pressure.	Microwave, pyrolysis	Klein et al. (2016) and Luque et al. (2014)
Physico-chemical	A combination of high physical condition (temperature, pressure) and chemical reaction (with or without catalyst).	Steam explosion, liquid hot water pretreatment, AFEX* process, organosolv process	Agbor et al. (2011), Kupiainen et al. (2012), Mesa et al. (2011) and Zhao et al. (2015)
Chemical	Using concentrated or dilute acid, bases, and different types of ion liquid substances which are able to dissolve cellulose and break the link of lignin to cellulose: H_2SO_4, HCl, NaOH, $Ca(OH)_2$, KOH, NH_4OH, H_2O_2, etc.	Acid/alkaline treatment, ion liquid	Bensah and Mensah (2013), Heinze et al. (2005), Hu et al., (2008), Shafiei et al. (2013) and Yan et al. (2015)
Biological	Taking advantages from microorganisms and bacteria to cut down the structure of lignocellulosic biomass.	White-rot fungi, brown-rot fungi, soft rot fungi	Hwang et al. (2008), López-Abelairas et al. (2013) and Sindhu et al. (2016)

* AFEX: Ammonia fiber explosion.

wetland transplanted rice field and was recognized as one of the worst weed in the world by Holm et al. (1977). According to Ghosh and Ganguly (1993), dominant gooseweed and other sedges caused 32–50% yield loss in rice field in India because of nutrient and living space competition with rice. Thus, farmers remove this weed by manual, chemical, and biological methods (Mabbayad and Watson, 1995). For these reason, this agricultural weed waste biomass was choosing to explore the feasibility of bioethanol conversion.

2. Materials and methods

2.1. Material collection and preparation

Gooseweed grown in the wetland of rice fields, was obtained at San Sai, Chiang Mai, Thailand (18° 53′ 13.7″N; 99° 01′ 31.7″E) during September to October 2016. The samples were transferred to the lab of Energy Center Research, Maejo University and removed sand, mud, and other contaminants by tap water manually. Fresh samples were then placed under sunlight at ambient temperature around 38–40 °C for 3 days. The sun dried samples were continually dried at 50 °C in the hot air oven overnight. Finally, desiccated materials were ground to powder that could pass through 1 mm mesh by a high speed blender (Otto BE, Thailand) and were stored in a desiccator until further analysis.

2.2. Physical analysis

Moisture content (%) was determined by drying at 105 ± 3 °C for 4 h (Singh et al., 2017). The moisture content of sample was estimated by percentage of mass loss at 105 °C. Ash content (%) was estimated using muffle furnace at 575 °C for 4 h (NREL, 2008). Moisture, total solids (TS) and ash content were calculated as weight percentage using Eqs. (1)–(3). For estimation of volatile matter (VM), the crucibles and sample were kept in a muffle furnace at 925 °C for 7 min (Singh et al., 2017). The percentage of volatile solid was the difference in weight loss at 925 °C. The calculation of fixed carbon (FC) and VM were followed Eqs. (4) and (5).

Crucibles and sample in above mention were allowed to cool in a desiccator and recorded the weight using an analytical balance with 4 digits (Ohaus, USA).

$$\%\text{Total solid(TS)} = \frac{\text{Weight}_{\text{oven sample and crucible}} - \text{Weight}_{\text{crucible}}}{\text{Weight}_{\text{initial sample}}} \times 100 \quad (1)$$

$$\%\text{Moisture} = 100 - \%\text{TS} \quad (2)$$

$$\%\text{Ash} = \frac{\text{Weight}_{\text{ash}}}{\text{Weight}_{\text{initial sample}}} \times 100 \quad (3)$$

$$\%\text{VM} = \frac{\text{Weight}_{\text{initial sample}} - \text{Weight}_{\text{oven sample}}}{\text{Weight}_{\text{initial sample}}} \times 100 \quad (4)$$

$$\%\text{FC} = \%\text{TS} - (\%\text{VM} + \%\text{ash}) \quad (5)$$

2.3. Compositional analysis of material

Sugars and acetic acid concentration of liquid phase from pretreatment were analyze by high performance liquid chromatography (HPLC) (condition: mobile phase–5 mM H_2SO_4; flow rate-0.7 mL/min; temperature of column: 60 °C; Hi-Plex H column). The compositions of sample were determined following method from Van Soest et al. (1991) in faculty of Animal Science, Maejo University. The percentage of cellulose, hemicellulose, and lignin are calculated from neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL). Firstly, 1.0 g of milled sample was reacted with 100 mL NDF detergent solution and 0.5 g of sodium sulfite. Since detergent dissolved soluble matters, the left residue was only cell wall which is cellulose, hemicellulose, and lignin. The mixture was boiled in a reflux system for two hours. Then, the crucibles and samples were washed three times with hot water and acetone by a cold extraction unit (FT 121 Fibertec™, Denmark). These above samples and crucibles were continuously added ADF detergent solution and boiled by a hot extraction unit (FT 122 Fibertec™, Denmark) for 1.30 h. For ADL, the crucibles and residues from ADF were treated by 72% H_2SO_4 for 3 h. After reaction with reagents in above experiments, the sample and crucibles were washed with boiled water, distilled water, acetone and dried at 105 ± 3 °C for 4 h, kept in desiccator for cooling and weighted out by analytical balance 4 digits (Ohaus, USA). The percentage of NDF, ADF, and ADL were calculated using Eqs. (6)–(8).

$$\%\text{NDF} = \frac{\text{Weight}_{\text{NDF and crucible}} - \text{Weight}_{\text{crucible}}}{\text{Weight}_{\text{initial sample}}} \times 100 \quad (6)$$

$$\%\text{ADF} = \frac{\text{Weight}_{\text{ADF and crucible}} - \text{Weight}_{\text{crucible}}}{\text{Weight}_{\text{initial sample}}} \times 100 \quad (7)$$

$$\%\text{ADL} = \frac{\text{Weight}_{\text{ADF and crucible}} - \text{Weight}_{\text{crucible}}}{\text{Weight}_{\text{initial sample}}} \times 100 \quad (8)$$

Eqs. (9)–(11) show the calculation of lignin, cellulose, and hemicellulose percentage:

$$\%\text{Lignin} = \%\text{ADL} \quad (9)$$

$$\%\text{Cellulose} = \%\text{ADF} - \%\text{ADL} \quad (10)$$

$$\%\text{Hemicellulose} = \%\text{NDF} - \%\text{ADF} \quad (11)$$

2.4. Alkaline-oxidation pretreatment

For lignocellulosic materials, pretreatment step is required to make cellulose more accessible for saccharification. In this study, the pretreatment method was adopted and modified from Mishima, 2006 and 2008 (Mishima et al.,. 2006, 2008; Yan et al., 2015). To summarize, powdered samples were treated with sodium hydroxide (1%) and hydrogen peroxide (1%) at different temperatures (99 °C and ambient) and time ranges (1.5 h, 24 h, 48 h, and 96 h). After pretreatment step, the solution was obtained by filtering through one layer of cloth in order to remove large particles. The filtrate was then checked for total sugar and reducing sugar by using phenol – sulfuric procedure (Dubois et al., 1956) and DNS method (Miller, 1959), respectively. The standard curves were built with D-glucose (Merck, USA).

2.5. Hydrolysis by cellulase

Hydrolysis process was carried out with commercial cellulase enzyme with 2398 units/g, β-glucosidase 577 units/g, and pH 4 supplied by Union Science Company, Chiang Mai, Thailand. Conical flasks containing 200 mL pretreated sample were adjusted to pH 5 by addition of hydrochloric acid and added 2% (v/v) of cellulase. The mixture was kept at 50 °C and agitated at 150 rpm for 24, 48, and 72 h. The small amount of sample was taken out at each period of time to measure total sugar and reducing sugar following the mentioned methods.

2.6. Immobilized yeast preparation

A yeast strain, *S. cerevisiae* TISTR 5020, was obtained from Faculty of Science, Maejo University, Chiang Mai, Thailand. This yeast was cultivated in autoclaved (120 °C for 15 min) liquid included yeast extract, peptone, and dextrose which so-called YPD (Yeast Extract-Peptone-Dextrose) media (10 g l^{-1} yeast extract, 20 g l^{-1} peptone, 20 g l^{-1} dextrose) at 150 rpm for 24 h. Then the broth was transferred into centrifuge tubes and centrifuged (7000 rpm, 4 °C, 10 min) to separate yeast cells and medium. A same volume of sodium alginate 2%

Table 2
Total sugar and reducing sugar performance after pretreatment of gooseweed.

Reagents	T° (°C)	Time (hours)	Total sugar (g/g dry biomass)	Reducing sugar (g/g dry biomass)
NaOH 1%	Ambient	24	0.162 ± 0.004[b]	0.032 ± 0.001[b]
NaOH 1%	Ambient	48	0.129 ± 0.002[a]	0.023 ± 0.000[a]
NaOH 1%, H_2O_2 1%	99	1.30	0.171 ± 0.010[b]	0.022 ± 0.000[a]
NaOH 1%, H_2O_2 1%	Ambient	24	0.157 ± 0.016[b]	0.035 ± 0.001[b]
NaOH 1%, H_2O_2 1%	Ambient	48	0.168 ± 0.003[b]	0.024 ± 0.001[a]
NaOH 1%, H_2O_2 1%	Ambient	96	0.161 ± 0.008[b]	0.022 ± 0.001[a]

Standard deviation was less than 10%. Means with the same letter at the same row are not significantly different ($p < 0.05$). The test was based on Tukey's B test at the 95% confidence interval.
° T: temperature.

was added to the yeast cell pellet and mixed properly. A syringe was used to drop the mixture into a flask of 150 mL calcium chloride 0.05 M. Finally, immobilized yeast cells were washed with autoclaved distilled water and kept in fridge at 4 °C for further using.

2.7. Fermentation

For fermentation, hydrolysate solution which was adjusted to pH 5.6, was fermented with 2% (w/v) of immobilized yeast *S. cerevisiae* beads in 200 mL working volume fermenter. The mixture was incubated at 33 °C from three to nine days. The temperature fluctuated due to the high ambient temperature during April in Thailand. Aliquots of fermented samples (50 mL) were collected in the fermenter after 3, 5, 7, and 9 days to measure the percentage of ethanol by using Ebulliometer (Dujardin-Salleron, Alcohol Burner, France). The principle of this method is based on the different boiling points of pure water (distilled water) from water-alcohol solutions. The sample solution should be centrifuged in order to be free of suspended solid before measure temperature with Ebulliometer. A calculating dial is used to determine the percentage of ethanol by comparing those two temperatures. Moreover, total sugar and reducing sugar were also determined in these periods of time to test the degradation.

2.8. Statistical analyses

The data obtained was expressed as mean ± standard deviation (SD). The statistical analyses were performed using one-way analysis of variance (ANOVA), IBM SPSS statistical package version 22.0 (IBM Corp., New York, USA). Differences between means were paralleled by Least Significant Difference (LSD) and Tukey's B. The statistical significances were reached when $p < 0.05$.

3. Results and discussion

3.1. Physical and compositional analysis

Essential properties of gooseweed were studied and reported to estimate their potential as a promising materials for bioethanol production. The physicochemical properties influences to handling, storage, and transportation facilities while the compositions of biomass effects on conversion efficiency of feedstock into energy (Cai et al., 2017). The quantity of water in feedstock is represented as moisture content which performed as a percentage of the air-dried biomass weight. Dried gooseweed contents 6.06% of water implying the good drying process and storage condition as mentioned above. Other parameters like TS, VM, FC, and ash reported 93.94%, 83.12%, 1.77%, and 9.95%, respectively.

The percentage of NDF, ADF, and ADL performed on air-dried biomass were 27.65%, 16.20%, and 2.51%, respectively. NDF solution and boiling conditions were able to dissolve and digestible cell contents such as sugar, starch, protein, pectin, etc. from biomass and leave the fibrous residues (Van Soest et al., 1991). The low NDF value obtained in this study indicates that more than 80% of gooseweed biomass was formed by soluble substances. Nevertheless, the cell wall fraction contents having 27.65% of air-dried gooseweed including cellulose (13.69%), hemicellulose (11.44%), and lignin (2.51%). Thus, alkaline pretreatment at mild condition on gooseweed biomass might be a suitable method to cope with lignin barrier and dissolve starch and sugar.

3.2. Gooseweed pretreated with $NaOH/H_2O_2$

Previous studies have proved the efficiency of sodium hydroxide/hydrogen peroxide ($NaOH/H_2O_2$) on delignification, decreased crystallinity of biomass and released high sugar yield (Eliana et al., 2014; Toquero and Bolado, 2014; Yan et al., 2015). Since gooseweed is mainly formed by digestible compounds, pretreatment with alkaline solution effectively dissolve starch, sugar, and other soluble compound which was also reported previously (de Souza et al., 2013; Sharma et al., 2013).

In this study, the amount of total sugar and reducing sugar of gooseweed treated with $NaOH/H_2O_2$ at different mild conditions shown in Table 2. In regards to total sugar, Turkey's B test indicated that there is no significant difference of total sugar in five treatments. It ranges from 0.129 to 0.171 g/g dry biomass. The lowest sugar obtained is 0.129 ± 0.002 g/g dry biomass when treated gooseweed with 1% NaOH for 48 h at ambient temperature. It is observed that increasing retention time did not make any improvement on release of sugar. This similar trend was recorded previous paper (Yan et al., 2015). Moreover, the combination of physicochemical method by setting the high temperature (99 °C) together with sodium hydroxide released an equal amount of sugar comparing to the other treatments. In this study, the presence of hydrogen peroxide did not make any difference for total sugar yield. Consequently, due to the results, environmental, economical, and operational aspects, pretreatment of gooseweed with 1% NaOH for 24 h at ambient temperature was applied for further process.

3.3. Sugars analysis of liquid pretreated gooseweed by HPLC

The liquid after pretreatment was analyzed and identified sugar contents by HPLC machine. The results show that gooseweed mainly contains pentose sugars like fructose (19.02 mg/g biomass), xylose (3.23 mg/g dry biomass), and arabinose (2.72 mg/g dry biomass) (Table 3). This species also includes di/tri saccharide such as maltotriose, cellobiose which can be hydrolyzed to digestible sugars. In addition, glucose found in the liquid was 3.63 mg/g dry biomass. Acetic acid founded was 1.37 mg/g dry biomass (0.5 g/L) which was quite low to be considered as severe inhibitor for hydrolysis and fermentation (Toquero and Bolado, 2014). Since most of alkaline solution disturb and

Table 3
Different types of sugar presented in the liquid after pretreatment with 1% NaOH at ambient temperature for 24 h.

Retention time	Components	mg/g dry biomass
6.892	Inulin	5.11
7.684	Maltotriose	1.40
8.231	Cellobiose	1.18
8.955	Citric acid	1.90
9.495	Glucose	3.63
10.319	Xylose	3.23
10.747	Fructose	19.02
11.442	Arabinose	2.72
15.499	Acetic acid	1.37

Table 4
Sugar yield after hydrolysis by cellulase.

Parameter	Hydrolysis			
(g/g dry biomass)	0 h	24 h	48 h	72 h
Total sugar	0.144 ± ± 0.004[b]	0.143 ± 0.007[b]	0.125 ± 0.005[a]	0.125 ± 0.004[a]
Reducing sugar	0.029 ± 0.001[a]	0.073 ± 0.006[b]	0.068 ± 0.002[b]	0.071 ± 0.002[b]
DP	5.0	1.9	1.9	1.8

Standard deviation was less than 10%. DP degree of polymerization (performed by total sugar divided by reducing sugar). Means with the same letter at the same row are not significantly different ($p < 0.05$). The test was based on Tukey's B test at the 95% confidence interval.

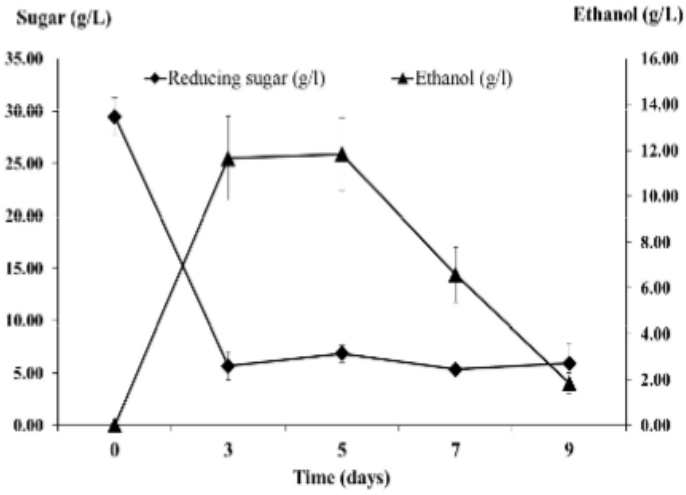

Fig. 2. Ethanol concentration and reducing sugar obtained through fermentation process for total nine days (pH 5.6; immobilized *S. cerevisiae* 2% (w/v); 33 °C).

break down the linkage lignocellulosic biomass but do not degrade hemicellulose like acid pretreatment, furfural and other inhibitory from hemicellulose degradation were not considered in this study. The variety of presented sugars in liquid fraction after alkaline pretreatment indicates sodium hydroxide is able to absorb many soluble substances at mild conditions.

3.4. Hydrolysis

Enzyme hydrolysis is the next essential step that required for the conversion of biomass into bioethanol. The main goal of this process is to decrease the degree of polymerization of cellulose by hydrolyzing the large polysaccharides to simple sugars which yeast can use for producing bioethanol. Table 4 shows the results of total sugar and reducing sugar from hydrolysis process at different times. Turkey's B test indicates that the amount of total sugar is significantly different between the first 24 h and after 48–72 h. It ranges from 0.125 to 0.144 g/g dry biomass. On the other hand, the means of reducing sugar were significantly different after 24 h. The highest reducing sugar 0.073 g/g dry biomass was achieved within 24 h and stable then. As a result, hydrolysis reaction could be occurred perfectly within 24 h (Das et al., 2016; Takagi et al., 2012).

3.5. Ethanol production

The bioconversion of lignocellulosic biomass into bioethanol can be variety in many specific approaches: separate hydrolysis and fermentation (SHF) (Koradiya et al., 2016), simultaneous saccharification and fermentation (SSF) (Boakye-Boaten et al., 2016), simultaneous saccharification and co-fermentation (SSCF) (Yu et al., 2017), and consolidated bioprocessing (CBP) (Rastogi and Shrivastava, 2017). In this study, SHF approach was applied because this process allows to optimize both hydrolysis and fermentation conditions which is very vital for a new material (Rastogi and Shrivastava, 2017). A yeast strain *S. cerevisiae* has been used to transform reducing sugars (hexose sugars: glucose, galactose, mannose, etc.) to ethanol by their own specific metabolism (Zabed et al., 2017).

Fermentation is a biological process that use the natural preference for sugar as a carbon source by *S. cerevisiae* to convert to ethanol. This yeast strain is effective and worldwide distribution for beer and bioethanol industry due to its high tolerance to the inhibitors, effectively ethanol production and widely available comparing to other yeast trains and bacteria (Behera et al., 2010, Lewandowska et al., 2016). Ethanol concentration within three, five, seven, and nine days was recorded as Fig. 2 and the range is 0–11.84 g/L. The maximum ethanol concentration obtained was 11.84 g/L within five days of fermentation and declined rapidly after that (Fig. 2). Fig. 2 exhibited that the standard deviation bar, there is no significantly difference of ethanol concentration between three and five days which is suitable for the change of reducing sugar. It is clearly observed by the amount of reducing sugar dramatically decreased after three days and slightly fluctuated then. The significant ethanol concentration from gooseweed in this study is higher than water hyacinth in previous literatures which was 9.61 g/L (Das et al., 2016) and 1.491 g/L (He et al., 2015). This results indicate that gooseweed biomass can be used to produce bioethanol, as well as a value-added product according to "waste to wealth and waste to energy" concepts.

4. Conclusions

Gooseweed, a harmful weed, is an untapped, renewable and low cost biomass for biofuel. However, it has never been investigated as an efficient feedstock for bioethanol production. The results showed that high soluble sugars were achieved after pretreatment with 1% NaOH for 24 h at mild condition. In addition, enzyme cellulase was able to break and produce reducing sugar up to 50% of total sugar within 24 h. The highest ethanol achieved was 11.84 g/L within five days by fermentation with immobilized *S. cerevisiae* TISTR 5020. Briefly, gooseweed can be considered as a potential feedstock for bioethanol production. However, further studies are necessary to improve pretreatment and hydrolysis process.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.09.012.

References

Agbor, V.B., Cicek, N., Sparling, R., Berlin, A., Levin, D.B., 2011. Biomass pretreatment: fundamentals toward application. Biotechnol. Adv. 29, 675–685.

Barakat, A., Chuetor, S., Monlau, F., Solhy, A., Rouau, X., 2014. Eco-friendly dry chemo-mechanical pretreatments of lignocellulosic biomass: impact on energy and yield of the enzymatic hydrolysis. Appl. Energy 113, 97–105.

Behera, S., Mohanty, R.C., Ray, R.C., 2010. Comparative study of bio-ethanol production from mahula (*Madhuca latifolia* L.) flowers by *Saccharomyces cerevisiae* and *Zymomanas mobilis*. Appl. Energy 87, 2352–2355.

Bensah, E.C., Mensah, M., 2013. Chemical pretreatment methods for the production of cellulosic ethanol: Technologies and innovations. Int. J. Chem. Eng. 2013, 21.

Berry, P.E., 2009. Campanulaceae. Encyclopædia Britannica, inc. [accessed: 2017 june 16], https://www.britannica.com/plant/Campanulaceae.

Boakye-Boaten, N.A., Xiu, S., Shahbazi, A., Wang, L., Li, R., Mims, M., Schimmel, K., 2016. Effects of fertilizer application and dry/wet processing of *Miscanthus x giganteus* on bioethanol production. Bioresour. Technol. 204, 98–105.

Cai, J., He, Y., Yu, X., Banks, S.W., Yang, Y., Zhang, X., Yu, Y., Liu, R., Bridgwater, A.V., 2017. Review of physicochemical properties and analytical characterization of lignocellulosic biomass. Renew. Sustain. Energy Rev. 76, 309–322.

Carter, R., Jones, J.C., Goddard, R.H., 2014. *Sphenoclea zeylanica* (Sphenocleaceae) in north America—dispersal, ecology, and morphology. Castanea 79, 33–50.

Das, A., Ghosh, P., Paul, T., Ghosh, U., Pati, B.R., Mondal, K.C., 2016. Production of bioethanol as useful biofuel through the bioconversion of water hyacinth (*Eichhornia crassipes*). 3. Biotech 6, 70.

de Souza, A.P., Leite, D.C.C., Pattathil, S., Hahn, M.G., Buckeridge, M.S., 2013. Composition and structure of sugarcane cell wall polysaccharides: implications for second-generation bioethanol production. Bioenergy Res. 6, 564–579.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Eliana, C., Jorge, R., Juan, P., Luis, R., 2014. Effects of the pretreatment method on enzymatic hydrolysis and ethanol fermentability of the cellulosic fraction from elephant grass. Fuel 118, 41–47.

Ghosh, D.C., Ganguly, S., 1993. Cultural Factors Affecting Weed Infestation and Crop Productivity in Wetland Transplanted Rice. ISWS, Hisar, pp. 30–33.

Haghighi Mood, S., Hossein Golfeshan, A., Tabatabaei, M., Salehi Jouzani, G., Najafi, G.H., Gholami, M., Ardjmand, M., 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renew. Sustain. Energy Rev. 27, 77–93.

He, M.X., Hu, Q., Zhu, Q., Pan, K., Li, Q., 2015. The feasibility of using constructed wetlands plants to produce bioethanol. Environ. Prog. Sustain. Energy 34, 276–281.

Heinze, T., Schwikal, K., Barthel, S., 2005. Ionic liquids as reaction medium in cellulose functionalization. Macromol. Biosci. 5, 520–525.

Holm, L.G., Plucknett, D.L., Pancho, J.V., Herberger, J.P., 1977. The World's Worst Weeds. The University Press of Hawaii, Honolulu.

Hu, Z., Wang, Y., Wen, Z., 2008. Alkali (NaOH) pretreatment of switchgrass by radio frequency-based dielectric heating. Appl. Biochem. Biotechnol. 148, 71–81.

Hwang, S.S., Lee, S.J., Kim, H.K., Ka, J.O., Kim, K.J., Song, H.G., 2008. Biodegradation and saccharification of wood chips of *Pinus strobus* and *Liriodendron tulipifera* by white rot fungi. J. Microbiol. Biotechnol. 18, 1819–1826.

Klein, M., Griess, O., Pulidindi, I.N., Perkas, N., Gedanken, A., 2016. Bioethanol production from *Ficus religiosa* leaves using microwave irradiation. J. Environ. Manage. 177, 20–25.

Koradiya, M., Duggirala, S., Tipre, D., Dave, S., 2016. Pretreatment optimization of sorghum pioneer biomass for bioethanol production and its scale-up. Bioresour. Technol. 199, 142–147.

Kupiainen, L., Ahola, J., Tanskanen, J., 2012. Hydrolysis of organosolv wheat pulp in formic acid at high temperature for glucose production. Bioresour. Technol. 116, 29–35.

Lewandowska, M., Szymańska, K., Kordala, N., Dąbrowska, A., Bednarski, W., Juszczuk, A., 2016. Evaluation of *Mucor indicus* and *Saccharomyces cerevisiae* capability to ferment hydrolysates of rape straw and *Miscanthus giganteus* as affected by the pretreatment method. Bioresour. Technol. 212, 262–270.

López-Abelairas, M., Álvarez Pallín, M., Salvachúa, D., Lú-Chau, T., Martínez, M.J., Lema, J.M., 2013. Optimisation of the biological pretreatment of wheat straw with white-rot fungi for ethanol production. Bioprocess Biosyst. Eng. 36, 1251–1260.

Luque, L., Westerhof, R., Van Rossum, G., Oudenhoven, S., Kersten, S., Berruti, F., Rehmann, L., 2014. Pyrolysis based bio-refinery for the production of bioethanol from demineralized ligno-cellulosic biomass. Bioresour. Technol. 161, 20–28.

Mabbayad, M.O., Watson, A.K., 1995. Biological control of gooseweed (*Sphenoclea zeylanica* Gaertn.) with an *Alternaria* sp. Crop Prot. 14, 429–433.

Mani, S., 2014. Sphenoclea zeylanica. [accessed: 2017 june 16]. http://dx.doi.org/10.2305/IUCN.UK.2014-2.RLTS.T164176A63303037.en.

Mesa, L., González, E., Cara, C., Gonzalez, M., Castro, E., Mussatto, S.I., 2011. The effect of organosolv pretreatment variables on enzymatic hydrolysis of sugarcane bagasse. Chem. Eng. J. 168, 1157–1162.

Miller, G.L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31, 426–428.

Mishima, D., Kuniki, M., Sei, K., Soda, S., Ike, M., Fujita, M., 2008. Ethanol production from candidate energy crops: Water hyacinth (*Eichhornia crassipes*) and water lettuce (*Pistia stratiotes* L.). Bioresour. Technol. 99, 2495–2500.

Mishima, D., Tateda, M., Ike, M., Fujita, M., 2006. Comparative study on chemical pretreatments to accelerate enzymatic hydrolysis of aquatic macrophyte biomass used in water purification processes. Bioresour. Technol. 97, 2166–2172.

NREL, 2008. Determination of ash in biomass: Laboratory analytical procedure.

Rastogi, M., Shrivastava, S., 2017. Recent advances in second generation bioethanol production: An insight to pretreatment, saccharification and fermentation processes. Renew. Sustain. Energy Rev. 80, 330–340.

Ravindran, R., Jaiswal, A.K., 2016. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. Bioresour. Technol. 199, 92–102.

RFA, 2017. Ethanol industry outlook 2017: Building partnerships and growing markets, Washington, DC.

Shafiei, M., Zilouei, H., Zamani, A., Taherzadeh, M.J., Karimi, K., 2013. Enhancement of ethanol production from spruce wood chips by ionic liquid pretreatment. Appl. Energy 102, 163–169.

Sharma, R., Palled, V., Sharma-Shivappa, R.R., Osborne, J., 2013. Potential of potassium hydroxide pretreatment of switchgrass for fermentable sugar production. Appl. Biochem. Biotechnol. 169, 761–772.

Sindhu, R., Binod, P., Pandey, A., 2016. Biological pretreatment of lignocellulosic biomass – an overview. Bioresour. Technol. 199, 76–82.

Singh, Y.D., Mahanta, P., Bora, U., 2017. Comprehensive characterization of lignocellulosic biomass through proximate, ultimate and compositional analysis for bioenergy production. Renew. Energy 103, 490–500.

Takagi, T., Uchida, M., Matsushima, R., Ishida, M., Urano, N., 2012. Efficient bioethanol production from water hyacinth *Eichhornia crassipes* by both preparation of the saccharified solution and selection of fermenting yeasts. Fish. Sci. 78, 905–910.

Toquero, C., Bolado, S., 2014. Effect of four pretreatments on enzymatic hydrolysis and ethanol fermentation of wheat straw. Influence of inhibitors and washing. Bioresour. Technol. 157, 68–76.

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Yan, J., Wei, Z., Wang, Q., He, M., Li, S., Irbis, C., 2015. Bioethanol production from sodium hydroxide/hydrogen peroxide-pretreated water hyacinth via simultaneous saccharification and fermentation with a newly isolated thermotolerant *Kluyveromyces marxianu* strain. Bioresour. Technol. 193, 103–109.

Yu, H., Guo, J., Chen, Y., Fu, G., Li, B., Guo, X., Xiao, D., 2017. Efficient utilization of hemicellulose and cellulose in alkali liquor-pretreated corncob for bioethanol production at high solid loading by *Spathaspora passalidarum* U1–58. Bioresour. Technol. 232, 168–175.

Zabed, H., Sahu, J.N., Suely, A., Boyce, A.N., Faruq, G., 2017. Bioethanol production from renewable sources: current perspectives and technological progress. Renew. Sustain. Energy Rev. 71, 475–501.

Zhao, X., Moates, G.K., Elliston, A., Wilson, D.R., Coleman, M.J., Waldron, K.W., 2015. Simultaneous saccharification and fermentation of steam exploded duckweed: improvement of the ethanol yield by increasing yeast titre. Bioresour. Technol. 194, 263–269.

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