# POTENTIAL EVALUATION OF BIOETHANOL PRODUCTION FROM AQUATIC WEED ELEPHANT EAR PLANT

MARTHA MARLEN TREJO PEREZ

MASTER OF ENGINEERING IN RENEWABLE ENERGY ENGINEERING

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF ENGINEERING
IN RENEWABLE ENERGY ENGINEERING
ACADEMIC ADMINISTRATION AND DEVELOPMENT MAEJO UNIVERSITY
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## บทคัดย่อ

ไบโอเอทานอลเป็นหนึ่งในพลังงานทางเลือกที่ได้รับความสนใจเป็นอย่างมากสำหรับการเลือกใช้พลังงานในอนาคตเนื่องจากเป็นการใช้พลังงานที่ไม่ทำให้เกิดก๊าซคาร์บอนไดออกไซด์และยังสามารถที่จะผลิตเป็นแหล่งพลังงานหมุนเวียนเพื่อทดแทนการใช้เชื้อเพลิงฟอสซิลยกตัวอย่างเช่นวัสดุชีวมวลจำพวกลิกโนเซลลูโลสในการศึกษานี้ได้นำต้นบอนทั้งแบบสดและแห้งมาผลิตไบโอเอทานอลโดยประเมินผลกระทบในช่วงเวลาต่างๆ (0, 15และ 30นาที)จากการรวมตัวกันของการระเบิดด้วยไอน้ำและแคลเซียมออกไซด์(CaO)ที่ได้รับจากเถ้าลอยในอัตราส่วน 0%, 10%และ 20%เลือกสภาวะที่เหมาะสมที่สุดเพื่อดำเนินการหมัก และกลั่นโดยหลังจาก 24ชั่วโมงต้นบอนที่นำมานั้นมีความเข้มข้นของเอทานอลสูงถึง 2.7±0.82 g/Lเมื่อเทียบกับปริมาณความเข้มข้นของเอทานอลในตัวอย่างสดที่ได้คือ 1.21±0.12 g/Lทำให้บ่งบอกถึงประสิทธิภาพการหมักถึง 72%และการใช้น้ำตาลถึง 60%ในการใช้วิธีการหมักโดยใช้อุณหภูมิ 50°C, 60°Cและ 70°Cพบว่าเมื่อให้ความร้อนที่อุณหภูมิ 60°Cเอทานอลถูกนำกลับคืนมาโดยได้ผลผลิตสูงสุดโดยคิดเป็นร้อยละ 9 ในส่วนของแบบจำลองจลนศาสตร์ที่พัฒนาขึ้นสำหรับการหมักเพื่อการอธิบายขั้นตอนและกระบวนการที่ระกับความเชื่อมั่น $R^2$>0.95และศักยภาพการผลิตเอทานอลของต้นบอนสูงสุด (pm)ถึง 2.4 g/Lทำให้ต้นบอนเป็นพืชที่มีศักยภาพอย่างมากในการผลิตเอทานอล

คำสำคัญ : ต้นบอน, วัชพืชน้ำ, ไบโอเอทานอล, เถ้าลอย

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| **Title** | POTENTIAL EVALUATION OF BIOETHANOL PRODUCTION FROM AQUATIC WEED ELEPHANT EAR PLANT |
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## ABSTRACT


Bioethanol is perceived as one of the most encouraging next-generation transportation fuels due to its decarbonization and ability to be produced using renewable energy sources like lignocellulosic biomass. Fresh and dry elephant ear plant was used as a biomass source to produce bioethanol. The synthesis of ethanol from dried elephant ear plant was investigated in this study. The effects of a combination of steam explosions at different times (0 min, 15 min, and 30 min) and CaO obtained from fly ash at different ratios 0%, 10% and 20% was evaluated. The most optimal circumstances were selected in order to proceed with fermentation, which was then followed by distillation. After 24h, dry elephant ear plant presented a higher ethanol concentration reaching 2.7±0.82 g/L compared with the fresh sample 1.21±0.12 g/L, indicating a fermentation efficiency of 72% and a sugar consumption of 60%. By utilizing a simple distillation method at three different temperatures 50°C, 60°C, and 70°C in the heater, ethanol was recovered with the higher yield obtained at 60°C was over 9%. Finally, the kinetic model developed for the fermentation accurately describes the process with a confidence level of $R^2$>0.95, and a potential maximum ethanol production ($p_m$) of 2.4 g/L as the result of the fermentation. The elephant ear plant has the potential to be a value-laden plant in the production of bioethanol.


Keywords : Bioethanol, Flying ash, Aquatic weeds, Elephant ear plant

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Martha Marlen Trejo Perez

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# CHAPTER 1
# INTRODUCTION

## 1.1 Background

The globally energy sector main concern is the increasing energy demand (Karmakar and Halder, 2019; Yilmaz and Atmanli, 2017). New technologies for social-economical interactions as well as the rapid urbanization and industrial expansion make energy vital in the daily life of all people (Cruz et al., 2018). The world economy is heavily dependent on fossil fuels such as oil, coal, and natural gas, the major commercial energy and non-renewable sources. The worldwide consumption of fossil fuels intensified the emission of greenhouse gas released to the atmosphere and all the climate changes promoted by global warning (Cunha et al., 2020; Ramachandra and Hebbale, 2020). Therefore, current environmental problems caused for the use fossil fuel and new approaches to generate sustainable carbon neutral renewable energy sources have taken importance. In this context, biofuels are an emerging alternative to liquid fuels due their also high energy content but significantly less $CO_2$ emissions associated with their use. Bioethanol is a potential alternative fuel due to its properties in comparison with gasoline such as higher flame speed, higher heats of vaporization, and higher-octane number which makes it an antiknock fuel, are some of the main reasons to encourage its production (Gavahian et al., 2019; Lee et al., 2021; Vu et al., 2017).

According to the International Energy Agency (IEA), in 2019 globally fuel ethanol production reached 115 billion L. However, Covid-19 crisis causes global bioethanol production to drop 15% in 2020, the first contraction in biofuel output in two decades. Even thought, biofuels are expected to meet around 5.4% of road transport energy demand in 2025, rising from just under 4.8% in 2019. In 2023-25, bioethanol average output is anticipated to be 119 billion L, with Brazil, China, and India the key growth markets over this period (IEA, 2019, 2020). Meanwhile in Thailand, with the cost reduction of variable energy, conventional Thai power

generation starts giving way to alternative sources. During 2023-25, average bioethanol yearly production in Thailand of 2.4 billion liters is expected (Figure 1).

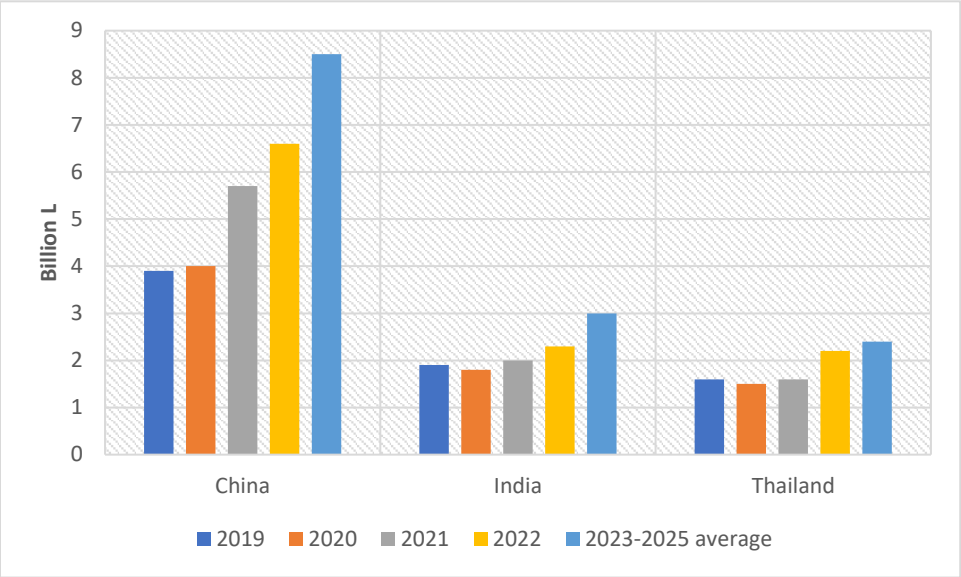



**Figure 1** Ethanol production overview for key Asian markets, 2019-2025. Source: IEA, Renewables 2020.

Bioethanol can be produced from several different biomass sources. It was the first biofuel produced from food-based crops, or first-generation bioethanol, that involves feedstocks like sucrose from sugarcane in Brazil or starch, mainly from corn, in the USA (Devarapalli and Atiyeh, 2015; Duden et al., 2021; Kumar, 2011). Despite first-generation bioethanol is being produced commercially in several countries, the use of edible biomass encountered resistance due the limited stock and due to the food versus fuel argument. There has been a great effort in exploring alternatives feedstocks for second-generation bioethanol production based on lignocellulosic biomass. The complex and recalcitrant structure of lignocellulosic biomass is composed of cellulose, hemicellulose, and lignin including also water is small amount and some trace amounts of protein, minerals, and other components that are also present in the raw material. Lignocellulosic biomass is usually referred to non-edible crops, agriculture and forestry residues, aquatic plants, and it is

considered one of the most abundant renewable biomass sources on earth (Phukoetphim et al., 2017; Sharma et al., 2020).

The Araceae family of plants, which contains over 1800 know species, has been described as the most common cause of symptomatic plant ingestion in some countries. Most species in the family contain raphine (calcium oxalate) crystals which are needle-shaped and arranged in compact bundles (Frohne and Pfänder, 1997; Krenzelok and Jacobsen, 1997). Upon chewing of the plant, the crystals are ejected from specialized explosive ejector cells (idioblasts) and may become lodged in the lining of the mouth, tongue and throat leading to local inflammatory reactions which include burning, irritation, and edema of the buccal cavity, hypersalivation, and aphonia (Kuballa et al., 1981; Wiese et al., 1996). Elephant ear plant, is a member of the Arum family (Araceae), is a tuberous, stemless, frost-tender aquatic and semi-aquatic herbaceous specie. The plant is a perennial capable of producing large (60 cm length and 35 cm width) leaves on 1-2.5 m petioles (Weber, 2017) that emanate from an upright corm. Under ideal growing conditions, a single elephant ear plant can grow 2.4 m tall with a similar spread in width. Reproduction of the elephant ear is mostly vegetative, rarely by seed, and occurs when whole corms divide in winter or early spring (Atkins and Williamson, 2008; Kikuta et al., 1938). Only a portion of the crown and petiole is needed to stablish a new plant. The aim of this study is to use elephant ear plant, a hazardous plant also considered an invasive species, as a font of nod-edible lignocellulosic biomass for bioethanol production. This study main aim is to use elephant ear plant to determinate the proper pretreatment and fermentation techniques through experimentation and optimization of the time and enzyme hydrolysis for the enhancement and improvement of bioethanol yield.

**1.2 Research objectives**

1. To explore the potential of bioethanol production from elephant ear plant.
2. To examine the effect of physicochemical pretreatment methods on lignocellulosic components degradation.
3. To evaluate the energy efficiency by applying the kinetic model for bioethanol production using elephant ear plant.

## 1.3 Scope of research

1. This study will use elephant ear plant as a feedstock for bioethanol production.
2. The mathematical model of response surface methodology (RSM) will be used to optimize the time and enzyme hydrolysis for bioethanol production.
3. Compositions and characterization of lignocellulosic elephant ear plant will be analyzed.
4. Determination of the best physicochemical pretreatment methods for bioethanol output from elephant ear plant.
5. Compare the physicochemical pretreatment methods for elephant ear plant biomass degradation.

## 1.4 Significance of the research

1. Utilization of the available lignocellulosic residues from elephant ear plant for bioethanol production.
2. The suitable method bioethanol production from lignocellulosic from elephant ear plant.
3. This study will add value to lignocellulosic residues from elephant ear plant using it as feedstock for bioethanol production.
4. The result of this study will contribute to enhance the possible lignocellulosic feedstocks used for bioethanol production.

# CHAPTER 2
# LITERATURE REVIEW

Bioethanol represents one of the most promising biofuels, exhibiting several advantages, such as high-octane number, low cetane number high heat of vaporization and, most importantly, reduction of greenhouse gas emissions. A variety of biomass feedstock have been explored for ethanol production including sucrose rich crops such as sugarcane and sugar beet, starch-rich crops such as maize and grain sorghum, and lignocellulosic materials such as woody biomass, herbaceous perennials, and various wastes (Faraco, 2013).

In the United States, the Department of Energy has set a goal of 60 billion gallons of renewable fuels per year to be produced by 2030. In the European Union there is a mandatory target to substitute 10% of transportation fuels with renewable fuels by 2020. Production of ethanol from corn starch in United States has almost reached its full capacity. Moreover, ethanol production from this edible feedstock poses concerns about competition with food and feed supplies. The only sustainable alternative substrate for ethanol production is lignocellulosic biomass. Conversion of lignocellulosic biomass is emerging as one of the most important technologies for sustainable production of renewable fuels and chemicals due to its widespread availability, large quantity, non-competitiveness with food supply, potential as platform for green chemicals, and high mitigation effects on GHG emissions (Watanabe, 2013).

## 2.1 Chemistry of ethanol

Ethanol is a clear colorless, volatile, and flammable liquid that is made by the fermentation of different biological materials. Ethanol is also called ethyl alcohol or grain alcohol. It has a characteristic, agreeable odor. In dilute aqueous solution, it has a somewhat sweet flavor, but in more concentrated solutions, it has a burning taste (Bajpai, 2020). Ethanol is an alcohol, a group of chemical compounds whose molecules contain a hydroxyl group, –OH, bonded to a carbon atom showed at Figure 2.

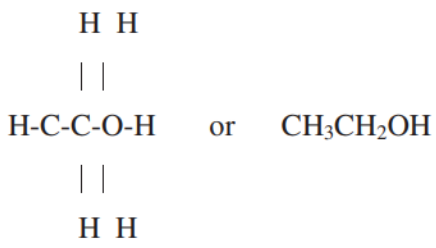



**Figure 2** Structural formula and condensed structural formula of ethanol.

Ethanol melts at −114.1°C, boils at 78.5°C, and has a density of 0.789 g/mL at 20°C. Its low freezing point has made it useful as the fluid in thermometers for temperatures below −40 °C, the freezing point of mercury, and for other low-temperature purposes, such as for antifreeze in automobile radiators (Table 1). The molecular weight is 46.07 g/mol. One gallon of 190 proof ethanol weighs 6.8 pounds. Ethanol has no basic or acidic properties. When burned, ethanol produces a pale blue flame with no residue and considerable energy, making it an ideal fuel. Ethanol mixes readily with water and with most organic solvents. It is also useful as a solvent and as an ingredient when making many other substances including perfumes, paints, lacquer, and explosives. The flash point of ethanol is the lowest temperature (i.e., 12.8 °C) where enough fluid can evaporate to form an ignitable concentration of vapor and characterizes the temperature at which ethanol becomes flammable in air. The ignition point of ethanol is the minimum temperature at which it is able to burn independently (i.e., 425°C). Ethanol has a high-octane rating (99), which is a measure of a fuel's resistance to preignition, meaning that internal combustion engines using ethanol can have a high compression ratio giving a higher power output per cycle. Regular petrol (gasoline) has an average octane rating of 88. Ethanol's higher-octane rating increases resistance to engine knocking, but vehicles running on pure ethanol have fuel consumption (miles per gallon or kilometers per liter) 10–20% less than petrol (but with no loss in engine performance/acceleration) (Bajpai, 2007; Bajpai, 2021; Walker, 2010).

**Table 1** Physicochemical properties of ethanol.

| **Property** | |
|---|---|
| Molecular formula | $C_2H_5OH$ |
| Molecular mass | 46.07 g/mol |
| Appearance | Colorless liquid (between −117 and 78°C) |
| Water solubility | Miscible |
| Density | 0.789 kg/l |
| Boiling temperature | 78.5°C (173°F) |
| Freezing point | −117 °C |
| Flash point | 12.8 °C (lowest temperature of ignition) |
| Ignition temperature | 425 °C |
| Explosion limits | Lower 3.5 % v/v; upper 19 % v/v |
| Vapor pressure | 38 °C 50 mmHg |
| Higher heating value (at 20°C) | 29,800 kJ/kg |
| Lower heating value (at 20°C) | 21,090 kJ/L |
| Specific heat, Kcal/Kg | 60 °C |
| Acidity (pKa) | 15.9 |
| Viscosity | 1.200 mPa·s (20°C) |
| Refractive index (nD) | 1.36 (25°C) |
| Octane number | 99 |
| Carbon (wt) | 52.1 % |
| Hydrogen (wt) | 13.1 % |
| Oxygen (wt) | 34.7 % |
| C/H ratio | 4 |

**2.2 Types of ethanol**

Ethanol can be produced in two forms hydrous and anhydrous. Hydrous ethanol is usually produced by distillation from biomass fermentation, and it contains some water residue. It is suitable for use as neat spark ignition fuel in warm climates such as that in Brazil. A further process of dehydration is required to produce anhydrous ethanol (100% ethanol) for blending with petrol. Anhydrous ethanol can be used as an automotive fuel by itself or can be mixed with petrol in various proportions to form a petrol/ethanol blend. Anhydrous ethanol is typically blended up to 10% by volume in petrol, known as E10, for use in unmodified engines. Historically, the US has supported the use of E10 blends, and more recently, Europe has adopted E10 blends. Certain materials in vehicles commonly used with petrol fuel are incompatible with alcohols, and varying degrees of modification are required depending on the percentage blend of ethanol with petrol. For this reason, in the European Union (EU), all member states are required to ensure that fuel grade E5 is available in the market as a protection grade for older vehicles that are not compatible to run on E10 (Bajpai, 2013; Chandel et al., 2007; Hahn-Hägerdal et al., 2006).

**2.3 Feedstock for bioethanol**

The search for alternative and renewable energy sources attracts the researchers to face challenges like energy crisis, rising fuel prices, and harmful environmental emissions by fossil fuels (Kulkarni and Ghanegaonkar, 2019). From various alternative energy resources, bioethanol is the most promising resource because of its biological and renewable origins, normally derived from energy. Various feedstocks, such as sugar, starch, and lignocelluloses, have been employed for bioethanol production. Biomass is considered carbon neutral as the carbon dioxide released during its conversion is still part of the carbon cycle. The use of biomass helps to reduce carbon dioxide emissions and minimize negative impacts on the environment. Physical attributes (i.e. moisture, particle size, and density), rheological properties (i.e. elastic and cohesive), and chemical characteristics (i.e.

proximate, ultimate, and energy properties) of raw biomass limit its use at a scale necessary for biofuels applications (Tumuluru et al., 2016).

**2.4 Economic importance of biomass**

The use of bio-based renewable resources holds great potential value for industries in many sectors, including energy, organic chemicals, polymers, fabrics, and healthcare products. In general, a bio-based economy offers many benefits and opportunities such as new areas of economic growth and development for the many regions that have plentiful biomass resources, creation of new innovative business sectors and entrepreneurial skills, improved energy security via reducing dependence on nonrenewable resources, enhanced economic and environmental linkages between the agricultural sector, and a more prosperous and sustainable industrial sector. These also further help in the reduction of greenhouse gas emissions, improved health by reducing exposure to harmful substances through substitution of natural bio-based materials for chemical and synthetic materials as well as job creation and rural development (Amaniampong et al., 2020).

**2.5 Bioethanol conversion**

In practice, a variety of different conversion pathways and upgrading routes have been implemented to convert biomass into bioethanol (Gaurav et al., 2017). There are two main categories of conversion technologies for bioethanol production from lignocellulosic biomass: biochemical and thermochemical. Grassy biomass with high ash content is typically more favored by biochemical conversion, because biochemical conversion is strongly dependent on cellulose and hemicellulose content, while the low ash and high lignin content of woody biomass make it more suitable for thermochemical processes (Li et al., 2016). While biochemical conversion requires that the biomass is first grinded into particles. Then, the lignocellulosic structure needs to be broken down into chemical fractions that include cellulose, hemicellulose, and lignin polymer fractions, using a suitable pretreatment method (Figure 3). The pretreatment before hydrolysis is necessary for lignocellulosic biomass in order to alter cellulose structures for enzyme accessibility. This is unlike for sugar

and starch-based biomass, which only requires extraction and hydrolysis to get fermentable sugars (Morales et al., 2021).

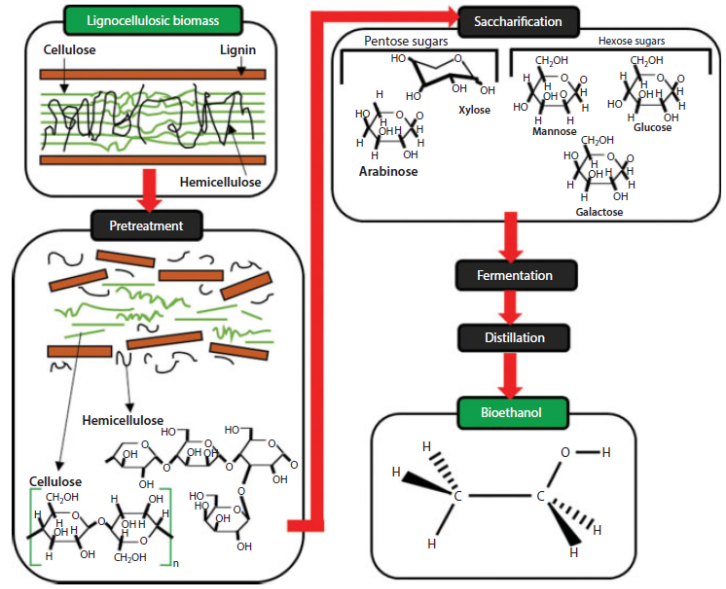



**Figure 3** Overview of the key modifications and products attained at various stages of lignocellulosic biomass to bioethanol processing.

## 2.6 Lignocellulosic biomass

Lignocellulosic biomass is the most abundant organic material on earth, and various studies have determined that enough of such materials could be collected from waste streams and future dedicated crop plantations to produce enough bioethanol to have a major impact on petroleum consumption for transportation sector. Bioethanol production from lignocellulosic biomass materials typically has lower life-cycle greenhouse gas (GHG) emissions and lower risks to compete with food security than bioethanol production from food and feed crops. Lignocellulosic biomass consists of three major components: cellulose (40-60%), hemicellulose (20-40%) and lignin (10-25%). It also contains minute quantity of pectin, protein, extractives, and ash. The quantity of the components varies from one species of plant to another, depending on their age and growth stage (Padella et al., 2019; Su et al., 2020; Zabed et al., 2017).

However, the use of lignocellulosic materials presents some challenges in biofuels (Mosier et al., 2005). The complex polymeric structure of lignocellulosic biomass makes it difficult for microorganisms to access the fermentable sugars. This implies that an initial pretreatment process is needed, prior to the fermentation process (Ayeni, 2013; Gierer, 1997). Generally, no particular method of pretreatment is absolutely suitable for all lignocelluloses. Each pretreatment is specific depending on choices and have their own advantages and disadvantages. An efficient methodology must meet the requirements so as to effectively break the lignocellulosic structure, have reduced crystallinity, had minimum inhibitory compounds, and had low operational costs (Ayeni et al., 2020).

2.6.1 Elephant ear plant

One of the major factors used to evaluate the feasibility of biomass for the production of bioethanol is the reserve and easiness of supply (Lebeau et al., 2007). Thus, elephant ear plant can be an option as a new lignocellulosic biomass source for bioethanol production. Elephant ear is the common name for a group of tropical perennial plants grown for their large, heart-shaped leaves. Most of these herbaceous species in the arum or aroid family (Araceae) that are offered as ornamentals belong to the genera Colocasia, Alocasia, and Xanthosoma, although there are others that have similar appearance and growth habits. The first two genera are native to tropical southern Asia, Indonesia, Malaysia, New Guinea, parts of Australia, or the Pacific Islands. This species can form mature plants from corms within 14-20 weeks. Once established, mature plants can produce large amount of foliage in the first 6-9 months, and may also produce up to 10 or more corms within 10 months. Elephant ear plant is a fast-growing herb that can become invasive in tropical and subtropical regions of the world. Plants produce underground corms and stems which can produce new plants very quickly. In addition, corms may remain dormant in very heavy shade and resprout when a light gap is formed. In consequence, the probability of invasion of this species, especially in areas near to cultivated fields, remains high (Cha-um et al., 2019; Prajapati et al., 2011).

## 2.7 Pretreatment

Among other factors, the type of pretreatment can have an important role in affecting the overall system performances of bioethanol production (Maurya et al., 2015; Talebnia et al., 2010; Tomás-Pejó et al., 2011). Several types of materials are found to be suitable for the production of biofuels. However, it is not always possible to transfer the results of pretreatment from one type of biomass material to another. Furthermore, one technology that is effective for a particular type of biomass material might not be suitable for another material (Bajpai, 2016).

A pretreatment step is necessary for the enzymatic hydrolysis process. It is able to remove the lignin layer and to decrystallize cellulose so that the hydrolysis enzymes can easily access to the biopolymers. The pretreatment is critical step in the cellulosic bioethanol technology because it affects the quality and the cost of the carbohydrates containing streams (Binod et al., 2013; Kumar et al., 2009). Different methods of pretreatment had been employed to promote the conversion of lignocellulosic substrate to value-added products. Majorly, all these pretreatment types are grouped into chemical, physical, biological, and physicochemical methods (Table 2) (Alvira et al., 2010).

All of the pretreatment methods can lead to a high yield of glucose from cellulose as long as suitable feedstock and sufficient enzyme activities are used in hydrolysis. It is not the enzymatic accessibility that actually matters in the overall cost of biomass processing. However, the other factors such as enzyme dosing, total recovery of sugars (especially hemicellulose sugars), equipment, and energy cost, and so forth, can vary dramatically among the different types of pretreatment technologies and will result in different overall process economics.

Also, it is obvious that the solid substrates obtained from different pretreatment methods vary greatly in composition and properties, which shows that the optimal enzyme recipes could be very different for each of the substrates. An in-depth understanding of the substrates and how they affect the enzyme functions is very important (Bajpai, 2016).

**Table 2** Methods for biomass lignocellulosic pretreatment.

| Pretreatment process | Advantages | Limitations and Disadvantages |
|---|---|---|
| Mechanical pretreatment | Minimizes cellulose crystallinity and increases surface area | Power utilization usually more than ingrained substrate energy; needs to be combined with other treatment |
| Steam explosion | Increase of allowable surface area; higher substrate digestibility; depolymerization of lignin; solubilization of hemicellulose | Destruction of a part of the xylan fraction; partial rupture of the lignin-carbohydrate matrix; formation of compounds inhibitory to microorganisms |
| Ammonia fiber explosion (AFEX) | Low formation of inhibitors; increase of accessible surface area | Not suitable for substrates with high content of lignin; expensive plant and ammonia |
| CO2 explosion | No toxicity; easy recovery; expansion of accessible surface area; efficient hydrolysis of hemicellulose | High cost of plant; does not modify lignin or hemicelluloses |
| Liquid hot water (LHW) pretreatment | Enhanced substrate edibility; low formation of inhibitors; inexpensive plant | High energetic requirements; high water input |
| Chemical process | Hydrolyzes hemicellulose to xylose and other sugar alters lignin structure | Equipment corrosion: formation of toxic substances; residual salts in biomass |
| Biological process | Degrades lignin and hemicelluloses: low energy requirements | Slow hydrolysis rates; long time is required |

## 2.8 Hydrolysis

Hydrolysis is the process in which polymers of cellulose and hemicellulose are hydrolyzed into their constituent fermentable reducing sugars. The most prevalent sugar monomers produced are the hexose sugars: glucose, galactose, and mannose; and the pentose sugars: xylose and arabinose (Figure 4). Hydrolysis is commonly achieved via chemical or enzymatic methods (Binod et al., 2011). Chemical methods include concentrated acid hydrolysis (CAH) and dilute acid hydrolysis (DAH); these methods are also considered as effective pretreatments to be used in conjunction with other hydrolysis procedures. However, the corrosive nature of acids is detrimental to the reactors, causing corrosion of equipment, inhibitor formation, slurry requires neutralization, and in order to be cost effective, the acids must be recovered and recycled (Chaturvedi and Verma, 2013; Sun and Cheng, 2002). Enzymatic approaches to the hydrolysis of lignocellulose are more environmentally assured, operate under milder conditions (40–50 °C), and encompass less corrosion issues.

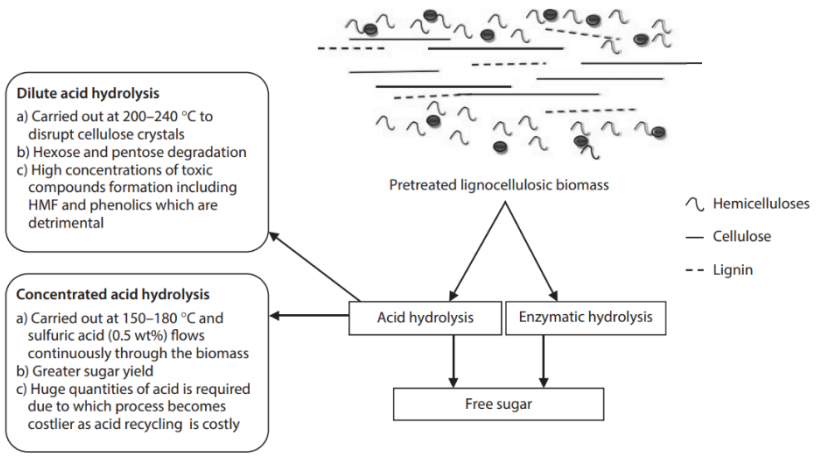



**Figure 4** Hydrolysis process for lignocellulosic material.

### 2.8.1 Cellulase

Cellulases are proteins that have been conventionally divided into three major groups: endoglucanase, which attacks low crystallinity regions in the cellulose fibers by endoaction, creating free chain-ends; exoglucanases or cellobiohydrolases

which hydrolyze the 1, 4-glycocidyl linkages to form cellobiose; and β-glucosidase which converts cello-oligosaccharides and disaccharide cellobiose into glucose residues. In addition to the three major groups of cellulose enzymes, there are also a number of other enzymes that attack hemicelluloses, such as glucoronide, acetylesterase, xylanase, β-xylosidase, galactomannase and glucomannase. These enzymes work together synergistically to attack cellulose and hemicellulose. Cellulases are produced by various bacteria and fungi that can have cellulolytic mechanisms significantly different.

Cellulases are naturally synthesized by a wide range of fungi, bacteria, and plants, the most extensively documented and industrially utilized of these being the fungus *Trichoderma reesei* (Menon and Rao, 2012). Hemicellulose is a collective term for an array of enzymes which can be categorized into two main groups: depolymerizing enzymes responsible for backbone cleavage and enzymes responsible for the removal of substituents causing hindrances to depolymerizing catalytic proteins. Ultimately, cellulases and hemicelluloses catalyze the degradation of cellulose and hemicellulose into both hexose and pentose sugars (Figure 5).

The cellulases enzyme system is a mixture of endo-β-glucanase, exo-β-glucanase and β-glucosidase. Cellulase acts on cellulose in the following manner: endo-β-glucanase acts randomly inside the cellulose chain, exo-β-glucanase acts on the external end of the cellulose chain and β-glucosidase degrade cellobiose into glucose or free monomeric sugar (Figure 5). Individual enzymes are not capable of degrading the cellulose chain to a monomeric unit, hence synergistic action leads to a proper saccharification (Kuila et al., 2016).

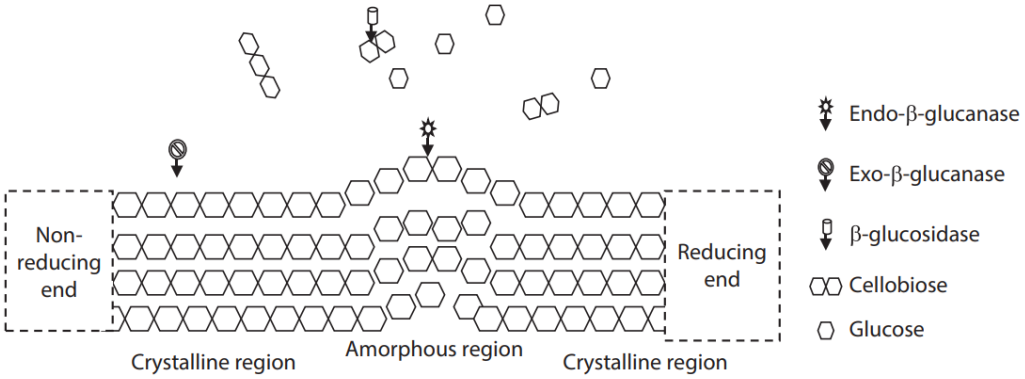



**Figure 5** Schematic representation of cellulase mediated hydrolysis.

Major synergism has been noticed firstly between endo and exo-β-glucanase and secondly between exo-β-glucanases which act from both reducing and nonreducing end. β-glucosidase overcomes catabolic repression by preventing accumulation of cellobiose (Kuila et al., 2016).

2.8.1 Factors affecting the cellulase mediated hydrolysis

Adsorption of cellulase enzymes onto the surface of the cellulose consists of primarily three steps:

1. Bioconversion of cellulose to fermentable sugars
2. Desorption of cellulase
3. The governing factors for these steps are mainly substrate concentration, enzyme dosage and reaction conditions.

At low substrate concentration the reducing sugar yield and reaction rates are increased but at high substrate concentration the reducing sugar yield and reaction rates are decreased. At high substrate concentration the decrease in the reducing sugar yield and reaction rates are due to end product inhibition of cellulase enzyme (Mojović et al., 2006). High enzyme dosage enhances the reducing sugar yield but at the same time significantly increases the processing cost. Therefore, selection of optimum parameters such as temperature, pH, and incubation time at low enzyme dosage can be one approach to overcome the issues (Kuila et al., 2016). Lignin has also an adverse effect on cellulases. It affects the whole process by nonproductive

adsorption and irreversible binding of enzymes which limits the accessibility of cellulose to cellulase (Kuila et al., 2016).

**2.9 Alcohol fermentation**

Ethanol fermentation using the hydrolysate, obtained after the hydrolysis of biomass, that contains large number of fermentable sugars, is the last step in lignocellulosic bioethanol production process. Fermentation is the term used to describe any process for the production of a product by means of the mass culture of a microorganism. In simple way, it is a chemical change brought on by the action of microorganisms (Todaro and Vogel, 2014). The two key components in the fermentation process are the microorganism and substrate. The major characteristics of an organism to be used in ethanol production are the ability to give a high yield of ethanol, to produce it with a high productivity and to withstand high ethanol concentration. In addition, the organism should possess the ability to utilize multiple sugars as well as that to tolerate inhibitors that are usually present in the hydrolysate obtained after pretreatment and enzymatic hydrolysis. It should also possess the ability to tolerate temperature and low pH, in order to minimize the risk of contamination. There are a limited number of microorganisms which ferment carbohydrates, mainly pentose sugars or hexose sugars, into alcohols. Yeast is the most commonly and widely used microorganism for commercial ethanol production due to its some special characteristics such as fast growth rates, efficient glucose repression, efficient ethanol production, and a tolerance for environmental stresses, like high ethanol concentration and low oxygen levels (Parekh and Wayman, 1986).

In addition to yeast, there are a limited number of microorganisms that ferment carbohydrates, mainly pentose sugars or hexose sugars, into alcohols, under various fermentation conditions (Table 3) (Binod et al., 2013).

**Table 3** Bacteria and fungi that can produce ethanol.

| Bacterial strains | Fungal species |
|---|---|
| *Clostridium acetobutylicum* | *Aspergillus oryzae* |
| *Klebsiella pneumoniae* | *Endomyces lactis* |
| *Leuconostoc mesenteroides* | *Kloeckera sp.* |
| *Sarcina ventriculi* | *Kluyreromyees fragilis* |
| *Zymomonas mobilis* | *Mucor sp.* |
| | *Neurospora crassa* |
| | *Rhizopus sp.* |
| | *Saccharomyces beticus* |
| | *S. cerevisiae* |
| | *S. elltpsoideus* |
| | *S. oviformis* |
| | *S. saki,Torula sp.* |
| | *Trichosporium cutaneum* |

**2.10 Advances process for bioethanol production**

Despite the advances in the lignocellulose-based process for ethanol production, further improvements are needed in our basic understanding and engineering applications to make ethanol competitive with gasoline and to enable economical production sufficient for the current energy demand (Figure 6) (Wyman, 1996). Notwithstanding the advances in the lignocellulose-based process schemes for ethanol production, considerable improvements are needed in basic research and engineering to make ethanol a viable competitor to gasoline and to produce quantities that are sufficient to meet the country's current energy demands. An increased emphasis on innovative approaches for ethanol production would help explore avenues for increasing the competitiveness of ethanol as a transportation fuel.

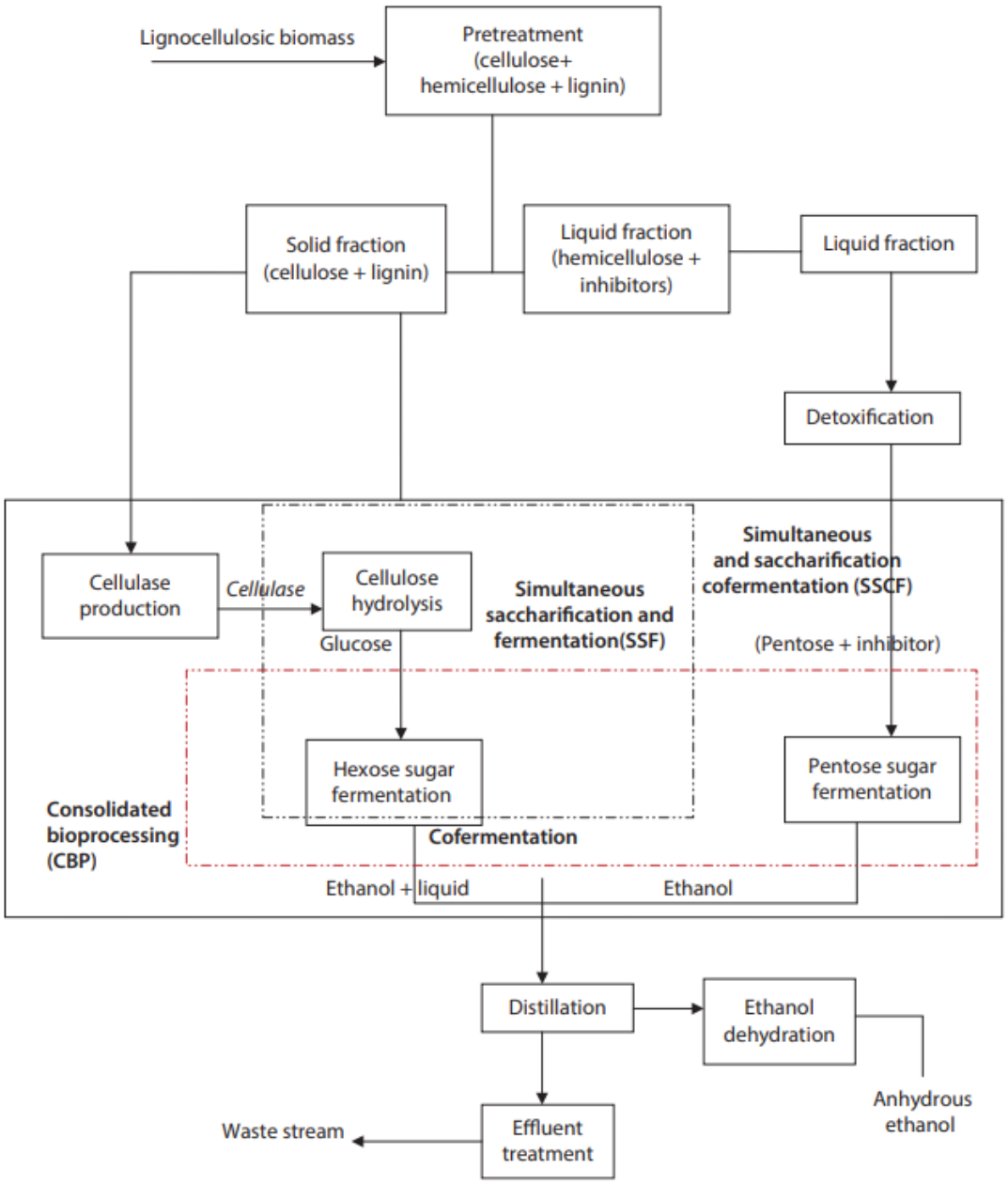



**Figure 6** Generic block diagram of bioethanol production from lignocellulosic biomass showing possibilities of various reaction- reaction integrations.

2.10.1 Separate hydrolysis and fermentation (SHF)

Chemical or enzymatic hydrolysis performed separately from fermentation step in SHF (Chandel et al., 2007; Sree et al., 2000; Wingren et al., 2003). To produce cellulosic ethanol on a pilot scale, typically it involves treatment of milled or grinded biomass with hot acid resulting into hydrolysis of cellulose, hemicellulose,

and other polysaccharides which cause disruption of the association of lignin with the carbohydrate (Menon and Rao, 2012; Vohra et al., 2014). The hydrolysate is then subjected to neutralization and separated from the insoluble and solid fraction. It is then fermented to produce alcohol. The insoluble fraction is then kept for treatment with glycosidase and cellulase to release glucose sugar which is again fermented for ethanol production. Lignin, in the form of residual insoluble material, is burnt for energy generation for the overall process (Huber et al., 2006; Vohra et al., 2014). Some developments of plants are in the process to modify lignin which can be readily hydrolyzed, or chemical catalysts or enzymes improvement for lignin hydrolysis can result in lignin use as a plastic component or as a liquid fuel fermentation feedstock production. Typically, the fermentation process generates a nutrient-enriched microbial cell mass which can be used as fertilizer after inactivation, and mineral nutrients can be recycled to the land (Somerville, 2007; Vohra et al., 2014). SHF is the most extensively tested configuration. Pentose fermentation is carried out in an independent unit. In SHF, joint liquids that flow from both reactors after sugar release first enters into the glucose fermentation bioreactor. Leaving the unconverted xylose behind, the mixture is then distilled to remove the pure ethanol. In the second reactor, xylose fermentation takes place and the same procedure follows. Each step can be carried out at optimum condition which is main advantage of SHF (Balat and Balat, 2009; Cardona and Sánchez, 2007; Vohra et al., 2014), but it has proved to be very costly.

2.10.2 Simultaneous saccharification and fermentation (SSF)

Saccharification and fermentation are both carried out in a single reactor simultaneously which saves overall costs, reduces inhibitor formation, and increases the hydrolysis rate of the process (Foust et al., 2009; Vohra et al., 2014). However, the process conditions for optimization of enzymes used for saccharification and the microorganisms for fermentation at the same time is the most critical issue of this method (Chiaramonti, 2007; Vohra et al., 2014). The key point which should be considered for this process is that the sugar should be converted rapidly into ethanol after its formation following saccharification so that its accumulation is diminished.

Considering that sugars are more inhibitive than ethanol for the conversion process, compared to SHF, SSF can reach a higher ethanol formation rate and yield (Brethauer and Wyman, 2010; Vohra et al., 2014). As no separate hydrolysis reactors are needed, SSF offers an easy operation and requires less instruments than SHF. In addition, the ethanol presence in both leads to less vulnerability of the action of undesired microorganisms to the reaction mixture. Yet, SSF has the disadvantage of difficulty in controlling process parameters as optimum conditions for saccharification and fermentation are different. Furthermore, a very high number of exogenous enzymes are needed for this process (Taylor et al., 2009; Vohra et al., 2014). The most well-suited temperature for hydrolysis using cellulolytic enzymes is around 50 °C, whereas most of the fermenting microorganisms have an optimum temperature between 28 °C and 37 °C for ethanol fermentation. Even through protein engineering, it is difficult to reduce the optimum temperature of cellulases. temperature of cellulases. High-temperature fermentation is highly desired for SSF due to which thermotolerant yeast strains have been screened for alcohol fermentation (Hasunuma and Kondo, 2012a, 2012b; Vohra et al., 2014).

2.10.3 Simultaneous saccharification and co-fermentation (SSCF)

SSCF is subjected to the complete assimilation of all the sugars which are released during the pretreatment and hydrolysis of lignocellulosic biomass. Using mixed culture of yeasts which can ferment both pentose and hexose sugars has been proposed, but hexose utilizing microbes grow faster compared to pentose utilizing microbes; therefore, the conversion of hexose to ethanol is more elevated (Cardona and Sánchez, 2007; Vohra et al., 2014).

A single microbe is capable of assimilating both pentose and hexose sugars in an optimal way and can also be used to produce a high sugar conversion and ethanol yield (Banerjee et al., 2010). Although these microbes exist, high conversion can only be reached through the genetic modification of these organisms which are already adapted to the ethanolic fermentation (Cardona and Sánchez, 2007; Vohra et al., 2014).

2.10.4 Consolidated bioprocessing (CBP) or direct microbial conversion (DMC)

Ethanol and all the enzymes required for its production are formed in a single bioreactor by a single microbial community (Carere et al., 2008; Vohra et al., 2014). Reaction-reaction integration for the biomass transformation into ethanol is the consolidated bioprocessing (CBP) or direct microbial conversion (DMC) (Figure 6). The only difference between CBP and other technologies like SSF for ethanol production is that a single microbial community is used to carry out both cellulases production and fermentation. All three steps; cellulase enzyme production, hydrolysis of cellulose, and fermentation are carried out in a single reactor and a single step. Zero capital or operation costs are required for enzyme production, which is an additional advantage (Lynd et al., 2005; Vohra et al., 2014). Also, part of the substrate does not diverge for cellulase production. Additionally, the enzymatic and fermentation processes are fully compatible (Cardona and Sánchez, 2007; Vohra et al., 2014). Thermophilic cellulolytic bacteria which are anaerobic have been examined extensively as potential ethanol producers. Some popular strains of these bacteria are *Clostridium thermosaccharolyticum*, *Clostridium thermohydrosulfuricum*, *Thermoanaerobium brockii*, *Thermoanaerobacter ethanolicus*, and *Thermoanaerobacter mathranii*. They can directly use a variety of inexpensive feedstocks and can withstand extreme temperatures, which makes it more beneficial. However, low alcohol tolerance (<2%, v/v) is a major limitation to their industrial application for ethanol production (Balat, 2011; Carere et al., 2008; Vohra et al., 2014). Procurement or production of cellulase enzyme contributes significantly to the enzymatic hydrolysis process overall cost. DMC cannot be considered the leading potential process alternative because of the non-availability of a robust organism to produce cellulases or some other cell wall degrading enzyme with high yield ethanol.

A generic block diagram for bioethanol production from lignocellulosic biomass showing possibilities of various reaction- reaction integrations (SHF, SSF, SSCF and DMC) is presented in Figure 6.

**2.10 Mass and energy balance**

Mass and energy balance (MEB) analyses are the first steps in the calculations for an engineering process. They are useful tools for chemical, mechanical, energy, and environment engineers. Engineers will have a better understanding of the principles of thermodynamics when they have a good perception of MEB. MEB is at the roots of the important issues such as process design and system optimization (Ashrafizadeh and Tan, 2018).

The law of conversation of mass states that “matter is neither created nor destroyed and just converted from one form to another.” Nowadays, energy is one of the few critical challenges that human beings are facing. Current human civilization is industrialized that heavily depends on energy. Energy is needed almost everywhere in our daily lives. The counterpart of energy is environment. Energy production and consumption come with environmental pollution and likely climate change (Ashrafizadeh and Tan, 2018).

Material and energy balances for fermentation processes are developed based on the facts that the heat of reaction per electron transferred to oxygen for a wide variety of organic molecules, the number of available electrons per carbon atom in biomass, and the weight fraction carbon in biomass are relatively constant. Mass–energy balance equations are developed which relate the biomass energetic yield coefficient to sets of variables which may be determined experimentally. Organic substrate consumption, biomass production, oxygen consumption, carbon dioxide production, heat evolution, and nitrogen consumption are considered as measured variables. Application of the balances using direct and indirect methods of yield coefficient estimation is illustrated using experimental results from the literature. Product formation is included in the balance equations and the effect of product formation on biomass yield estimates is examined (Erickson et al., 2000).

**2.11 Kinetic models for bioethanol fermentation**

In systems where (bio)chemical reactions take place, kinetic modeling and simulation refer to mathematical description of changes in properties of the system of interest, for instance, concentrations of metabolites, proteins, or other cellular components, and reaction fluxes in the case of biological system with respect to time (Lee, 2013). Kinetic modelling is considered as one of the most crucial steps in developing fermentation processes for large scale application. These process models define the production process under different input conditions which can help improve the product yield, productivity and reduce undesirable by-products. This will reduce costs and increase the product quality. Logistic models are employed to describe the changes in microbial cell growth as a function of growth rate, initial and maximum biomass concentration, and time (Phukoetphim et al., 2017). Microbial growth kinetics is described by a logistic equation which is a common unstructured growth model. The logistic model is the differential form (equation 1) and integrated form (equation 2) represents the exponential and stationary phases of growth. This logistics model illustrates the relationship of biomass ($X$) to initial cell concentration ($X_0$), maximum cell concentration ($X_{max}$) and maximum specific growth rate ($\mu_{max}$) at specific times ($t$) during the exponential and stationary phases of yeast growth.

$$\frac{dX}{dt} = \mu_{max}\left(1 - \frac{X}{X_{max}}\right)X \qquad \text{Equation 1}$$

$$X = \frac{X_0 e^{\mu max \cdot t}}{\left[\left(\frac{X_0}{X_{max}}\right)(1 - e^{\mu max \cdot t})\right]} \qquad \text{Equation 2}$$

Product formation kinetics with the yield coefficient ($Y_{P/S}$) is described by the following equation:

$$\frac{dp}{dt} = Y_{P/S}\,\frac{dX}{dt} \qquad \text{Equation 3}$$

In a batch process, substrate consumption kinetics with the yield coefficient ($Y_{X/S}$) and maintenance coefficient ($m$) is described by the following equations:

$$-\frac{dS}{dt} = \frac{1}{Y_{X/S}}\frac{dX}{dt} + mX \qquad \text{Equation 4}$$

$$S = S_0 - \frac{1}{Y_{\frac{X}{S}}}\left[\frac{X_0 e^{\mu max \cdot t}}{X_0}\right] - \frac{X_m m}{\mu_{max}} \ln \frac{X_m - X_0 + X_0 e^{\mu max \cdot t}}{X_m} \qquad \text{Equation 5}$$

Monod model is generally used to describe the growth of the cells. Excess substrate concentration that includes a substrate and product inhibition is described as follows:

$$\mu = \frac{\mu_{max} S}{K_S + S + \frac{s^2}{K_I}} \frac{K_P}{K_P + P} \qquad \text{Equation 6}$$

Where $S$ is substrate concentration (g/L), $S_0$ initial substrate concentration (g/L), $X_m$ the maximum biomass concentration which is identical to carrying capacity, $K_S$ saturation constant, $K_I$ inhibition parameter for sugar, $K_P$ a constant representing the inhibition effect due the product, $P$ ethanol concentration (g/L).

**2.12 The modified Gompertz model**

The model relates to bioethanol concentration (P) to the potential maximum bioethanol concentration ($P_m$), maximum bioethanol production rate ($r_{pm}$) and the lag time ($t_L$) as follows:

$$P = P_m \cdot e^{\{-e[r_{pm} \cdot e^1] \cdot (t_L - t) + 1\}} \qquad \text{Equation 7}$$

Where P is bioethanol concentration (g/L), $P_m$ is potential maximum bioethanol concentration (g/L), $r_{pm}$ is maximum bioethanol production rate (g/L/h) and $t_L$ is the time from the beginning of fermentation to exponential bioethanol (h).

**2.13 Ethanol recovery**

The broth recovered from fermentation is a solution composed of water and ethanol, however, ethanol composes only 5–12 wt.% and so product purification is an essential process to increase ethanol concentration to a wt.% acceptable for use as a biofuel (Morales et al., 2021). The differing boiling points of water (100 °C) and ethanol (78.37 °C) allows distillation to be utilized as a means of refinement as when the fermented broth is heated in a distillation column the substances take their gaseous forms. Ethanol and water form an azeotropic solution causing co-distillation at 95.6 wt.% ethanol at 78.15 °C and so cannot be separated sufficiently by a simple conventional distillation. A three-step process is therefore required for adequate ethanol purification involving distillation, rectification, and dehydration (Canilha et al., 2012). Distillation and rectification produce a solution with an ethanol concentration of ~92.4 wt.%. This solution then undergoes dehydration, often by azeotropic distillation, extractive distillation, liquid-liquid extraction, adsorption, or membrane pervaporation. The final ethanol product has an ethanol concentration of 95–96 wt.%, limited by the formation of the water-ethanol (Kumar et al., 2013; Waldron, 2010).

2.13.1 The existing extractive distillation sequences

Extractive distillation is used to separate azeotropic mixtures by adding a solvent in the same column where the feed is introduced, usually called the extractive column. Another distillation column is necessary to recover the solvent that is recycled back to the extractive column. When the bioethanol process is considered, due to the dilution of the feed, the extractive column is preceded by a pre-concentration distillation column used to approach the azeotropic composition. The corresponding configuration is reported in Figure 7, and is composed of three columns. It is possible to notice that this sequence has been developed following

the heuristic rule that suggests removal of the mass separation agent in the separator immediately after the one into which it is introduced (Seader and Westerberg, 1977). This sequence has been studied extensively in the literature for its optimal design (Kiss and Ignat, 2013; Vázquez-Ojeda et al., 2013). The possibility to use a partial condenser in the pre-concentrator column in order to have a vapor feed in the extractive column together with recycling between the solvent recovery column distillate and the pre-concentration column, have been widely studied (Seader et al., 1997; Seader and Westerberg, 1977; Taylor and Wankat, 2005).

More recently it was proposed a configuration with a post-fractionator after the solvent recovery column. The principle used to develop this configuration derived from the equilibrium diagram for the ethanol–water system. The authors noticed that below 21% mol ethanol, the relative volatility of the system without the solvent is higher than the system with the solvent. This concentration value was set as the feed composition to the post-fractionator (Li and Bai, 2012).

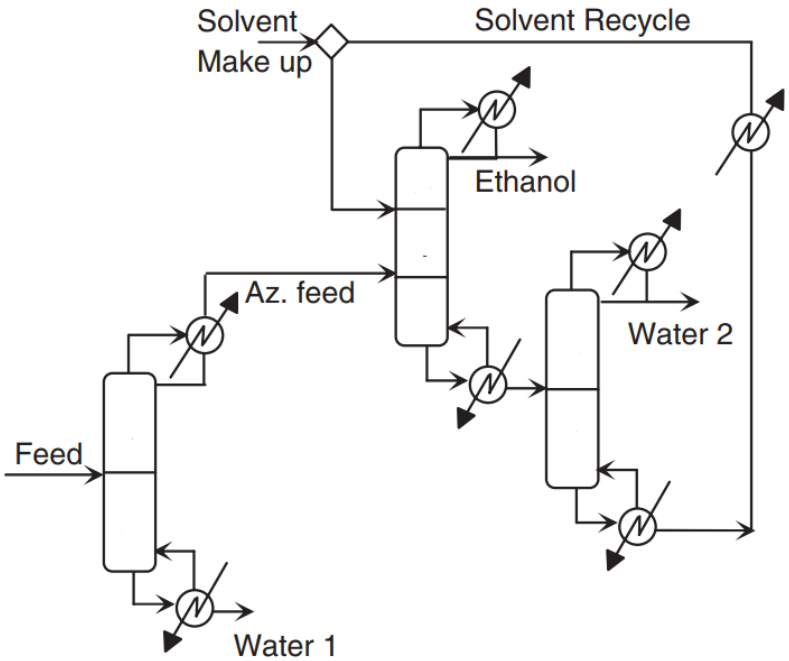



**Figure 7** Classical extractive distillation sequence.

**2.14 Response surface methodology**

Response surface methodology (RSM) consists f a group of mathematical and statistical techniques used in the development of an adequate functional relationship between a response of interest, y, and a number of associated control (or input) variables denoted by x1, x2... xk. In general, such a relationship is unknown but can be approximated by a low-degree polynomial model of the form (Khuri and Mukhopadhyay, 2010):

$$y = f'(x)\beta + \epsilon$$ Equation 8

Response surface design methodology is often used to refine models after you have determined important factors using screening designs or factorial designs; especially if you suspect curvature in the response surface.

The difference between a response surface equation and the equation for a factorial design is the addition of the squared (or quadratic) terms that lets you model curvature in the response, making them useful for:

- Understanding or mapping a region of a response surface. Response surface equations model how changes in variables affect a response of interest.
- Finding the levels of variables that optimize a response.
- Selecting the operating conditions to meet specifications.

This methodology was introduced by Box and Wilson, is a collection of mathematical and statistical techniques whose purpose is to analyze, by an empirical model, problems. More concretely, the objectives of the RSM are the following (Sarabia et al., 2020):

- To generate knowledge in the experimental domain of interest.
- To reliably estimate the experimental variability (pure error).

- To guarantee the adequacy between the proposed model and the experimental data (to make it easy to detect the lack of fit).
- To predict the observed response, as exactly and precisely as possible, in points within the experimental domain where no experiments were done.
- To propose sequential strategies to carry out the experimentation with different alternatives according to the results obtained.
- To maintain a high efficiency with respect to economical cost, time, and any other practical limitations.
- To make the identification of outlier data easy.
- To make the decision making possible under uncertainty conditions, reducing the ambiguity.

2.14.1 Types of response surface designs

There is a large number of experimental designs in the literature. Some of them come from theoretical studies and are consequences of the optimality criteria. Others have been generated to solve concrete problems (Figure 8) (Witek-Krowiak et al., 2014). Researchers can easily get access to the software that provides simple and clear use of these methods.

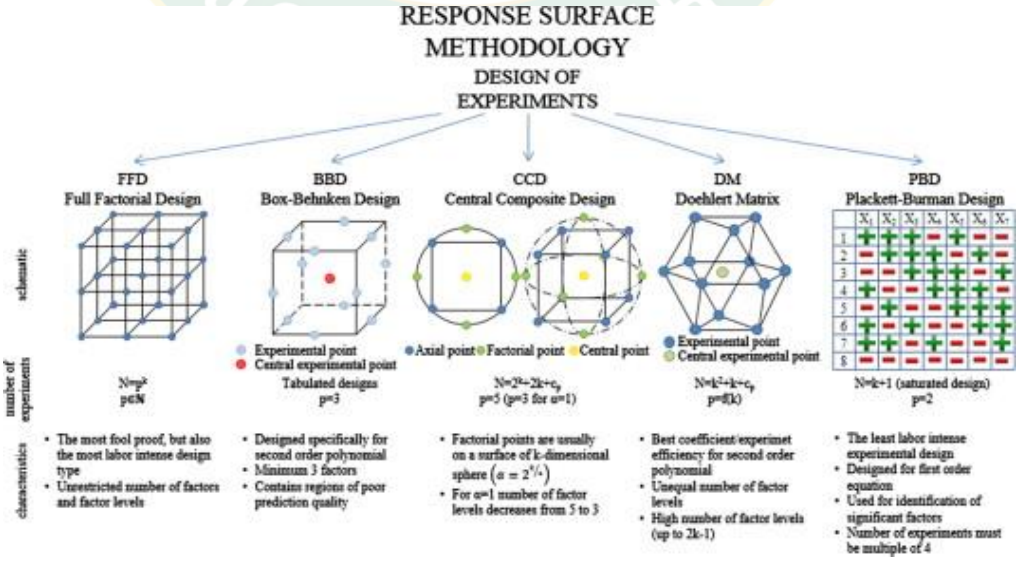



**Figure 8** Basic model designs used in RSM.

The most popular programs for RSM studies are Design Expert (Stat-Ease, Inc.), Minitab (Minitab Inc.), Statistica (StatSoft), JMP (SAS) and Matlab (MathWorks).

2.14.1.1 Full factorial design (FFD)

A common experimental design is the full factorial design, where all input parameters are set at two levels. FFD includes all possible combinations of variables with multiple levels. The full factorial design allows to determine the main and low-order interaction effects with great flexibility and efficiency. However (Anderson-Cook, 2004), the application of this design may pose greater problems with fitting second- or higher-order polynomial models. The second-order model can significantly improve the optimization process, especially in the case of three level factorial designs, by estimate higher-order interactions between factors. For this purpose, (Box and Wilson, 1992) have developed a central composite design (CCD).

2.14.1.2 Central composite design (CCD)

The central composite design yields as much information as the 3n full factorial design, however this methodology requires a smaller number of experimental runs than FFD. Additionally, CCD provides high quality predictions of linear and quadratic interaction effects of parameters affecting the process.

The CCD contains the full factorial or fractional factorial design at two levels (2n), center points (cp), which corresponds to the middle level of the factors, and axial points (2n), which in turn depends on specific properties desired for the design and the number of parameters related (Myers et al., 2016). Depending upon where the axial points are located, the CCD can be divided into three types: CCC (circumscribed central composite), CCI (inscribed central composite) and CCF (face-centered composite). In the selection of the right type of CCD it is the most important to compare the region of operability with the region of interest.

2.14.1.3 Box–Behnken design (BB)

(Box and Behnken, 1960) developed a 3-level incomplete factorial design as an alternative to the labor extensive full factorial design. To accurately describe linear, quadratic and interaction effects, second order polynomial has to be used in the modelling. Box and Behnken created this design to minimize the number of experiments, specifically in quadratic model fitting. Experiment matrices are built by means of two-level factorial designs (+1, -1) with incomplete block designs. The final matrix is completed with several replications of the central point, what improves precision. There are no experimental points in this design, where all factors have extreme values. This feature might be beneficial in experiments where undesired phenomena might occur in extreme conditions. The BB is slightly more labor efficient than the CCD and much more labor efficient than the FFD. The BB has only two significant restrictions: the number of experimental factors has to be equal or higher than three and the BB should not be used for fitting other equations than second order polynomial.

2.14.1.4 Doehlert design (D)

The Doehlert Matrix or the Uniform Shell Design is an experimental design method created on the basis of a simplex. In the first step, a k-dimensional regular simplex is created, which has one apex in the central point (Doehlert, 1970).

In the next step, the simplex points are subtracted from each other yielding the Doehlert Matrix as a result. The greatest advantage of this type of design is its flexibility. The Doehlert Matrix is fully sequential. Due to the simplex-based architecture the -factor D can be upgraded to (k+1)-factor by adding a few experimental points. Another feature of the Uniform Shell Design is the unequal number of experimental levels. In sequential modelling more levels can be applied to the most significant factor.

2.14.1.5 Plackett–Burman design (PB)

The Plackett–Burman design has been developed as a short-cut method for determining main factor effects for multiple factor systems (Plackett and Burman, 1946). This design requires only N = k + 1 experiments. This type of design is called ‘‘saturated design’’ because the number of experiments is equal to the number of parameters in the first order RSM model, and the degree of freedom of such a design is equal to zero. A high degree of the experiment number reduction imposes some modelling constrains. Due to design saturation, it is impossible to use a second order polynomial and this design gives no information on interaction effects. Secondly, due to the specific methodology of the experimental matrix design, the number of experiments must be a multiple of 4. However, this restriction can be avoided, when dummy factors are used. Dummy variables can be later used in standard deviation calculation.

# CHAPTER 3:
# MATERIAL AND METHODS

## 3.1 Sample collection

Fresh elephant ear plant was collected at Maejo University installations (18°53'46.5"N 99°01'05.5"E). Leaves and stalks were brought to the laboratory and rinsed thoroughly with tap water to eliminate contaminants. The sample was then sliced into tiny pieces (1–2 cm) and dried for three days using a solar drier. Finally, a mechanical blender was used to grind the dried elephant ear plant (PHILIPS Blender 600W Model HR2118/02). The powder was stored for further experiments (Figure 9).


**Figure 9** Wetland for sample collection (a, and elephant ear leaves and stalk (b.

## 3.2 Sample composition analysis

An analysis of the raw material was carried out in order to get further information about the composition of elephant ear plant using the procedures outlined in Table 4.

In order to characterize the elephant ear plant, parameters were measured of its moisture content (mc %), pH, total sugars (TS), reducing sugars (RS), and energy value (E). Three duplicates of each test were created.

**Table 4** Physicochemical parameters.

| Parameter | Equipment or method |
|---|---|
| Total solids | APHA 2015 |
| Volatile solids | |
| Ash content | |
| Moisture | |
| pH | pH meter |
| Alkaline | Titration method |
| Total sugar | Spectrophotometer |
| Reducing sugar | Spectrophotometer |

3.2.1 Moisture content

The hot air oven technique was used to determine the amount of moisture present. A fresh elephant ear plant sample was sliced into little pieces (1 to 2 cm in size) and mixed till it reached the consistency of a paste using a mechanical blender until the desired result was achieved (PHILIPS Blender 600W Model HR2118/02) and 5 g was used to determine moisture content. The sample was heated in a forced air oven at 130±5°C for 2 h (Miah et al., 2002). The moisture content of the wet base was determined using the following equation:

$$mc\% = \left[1 - \left(\frac{dry\ sample\ (g)}{wet\ sample\ (g)}\right)\right] \times 100 \qquad \text{Equation 9}$$

3.2.2 pH determination

The pH was determined in both the wet and dry samples. A total of 20 g of sample was weighted and transferred to a 50 mL beaker, along which 20 mL of distilled water was added, the suspension was covered, and the mixture was constantly agitated for 5 min. In order to enable most of the suspended clay to settle out of the solution, the suspension was allowed to stand for about 1 hour before being filtered or centrifuged off the aqueous phase in order to test the pH.

The pH of the supernatant was determined with the use of a potentiometer (Apera PH700 Benchtop) (USEPA, 2004).

3.2.3 Sugars content

Sugar concentrations were determined with the use of spectrometry by using a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan). The quantification of total sugars was carried out following the phenol-sulfuric acid method, while the estimation of reducing sugars was done by the 3,5-dinitrosalicylic acid (DNS) method (Dubois et al., 1956; Miller, 1959).

3.7.4 Degree of polymerization

Based on how the original cellulose fiber was obtained and treated, the degree of polymerization (DP) of the cellulose might vary significantly (Blanco et al., 2018). The number of monomer units in a polymer is defined as the density of the polymer (Zuckerkandl et al., 2012). The degree of polymerization of a polymer is proportional to the length of its chain (the number of monomer units in the chain). Calculated as the ratio of the molecular weight of a polymer to the molecular weight of the repeat unit, it is an important factor in polymer design. The two most common forms of DP utilized for measuring the DP are the number average DP and the weight average DP. Higher DP is desired in order to get superior mechanical characteristics (Reyhani et al., 2018).

$$DP = \frac{S_P}{S_M} \qquad \text{Equation 10}$$

Where; $S_p$ is the average molecular weight of the polymer, and $S_m$ is the repeating unit or monomer.

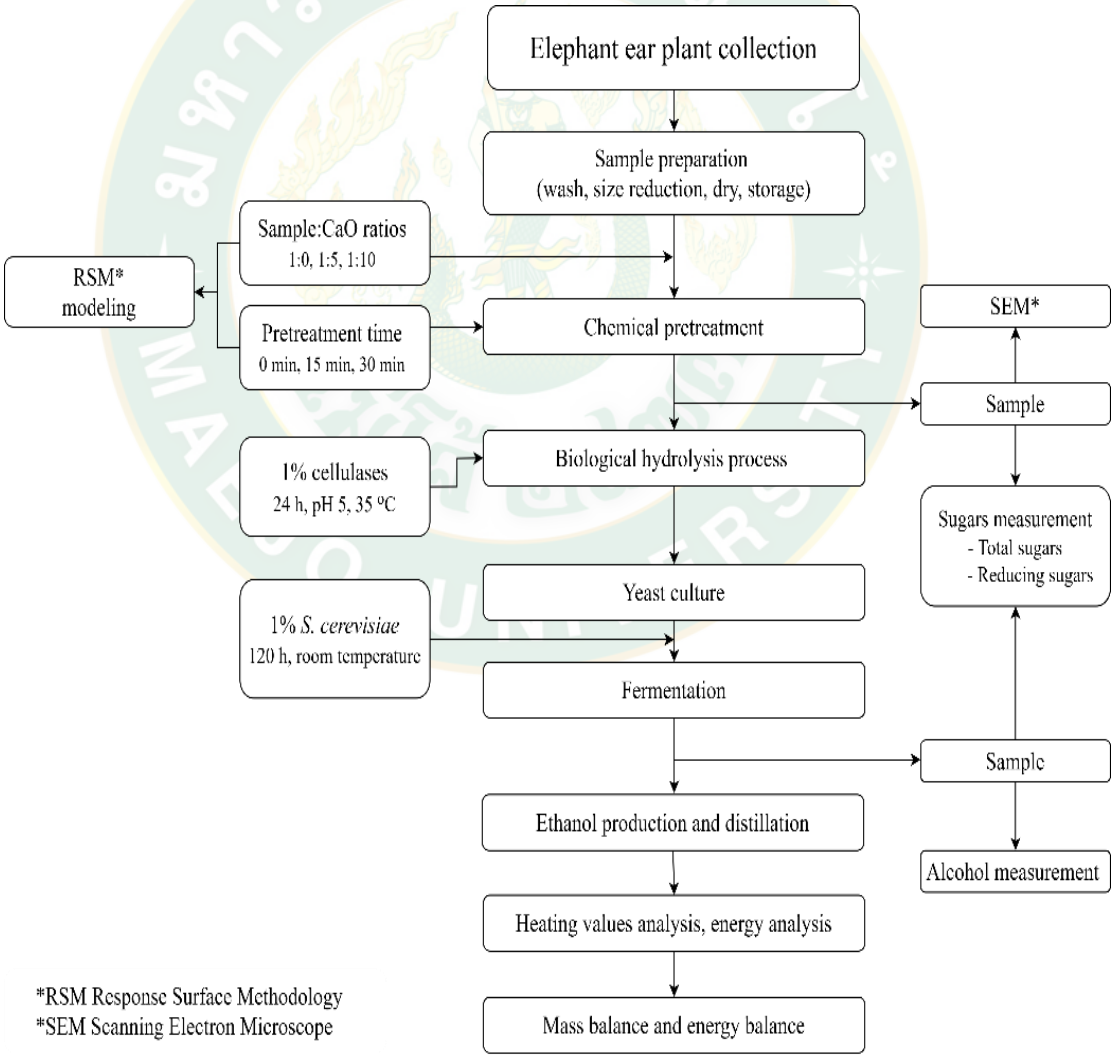
3.2.5 Energy value

The estimation of the energy value was calculated according to the Atwater factor 17 kJ/g (4.0 kcal/g) for carbohydrate content (Atwater and Woods, 1896). The approach is based on the temperatures of combustion of protein, fat, and carbohydrate, which are then adjusted to account for losses in microbial digestion,

absorption, and urine excretion throughout the course of the experiment. It employs a single factor for each of the energy-producing substrates (protein, fat, and glucose), independent of where the substrate is located in the body's fat stores (Southgate and Durnin, 1970).

### 3.3 Material preparation

Figure 10 depicts the methodology through which the tests are carried out. Using tap water, the elephant ear plant was washed thoroughly to eliminate all of the undesirable contaminants from the stem and leaves obtained.



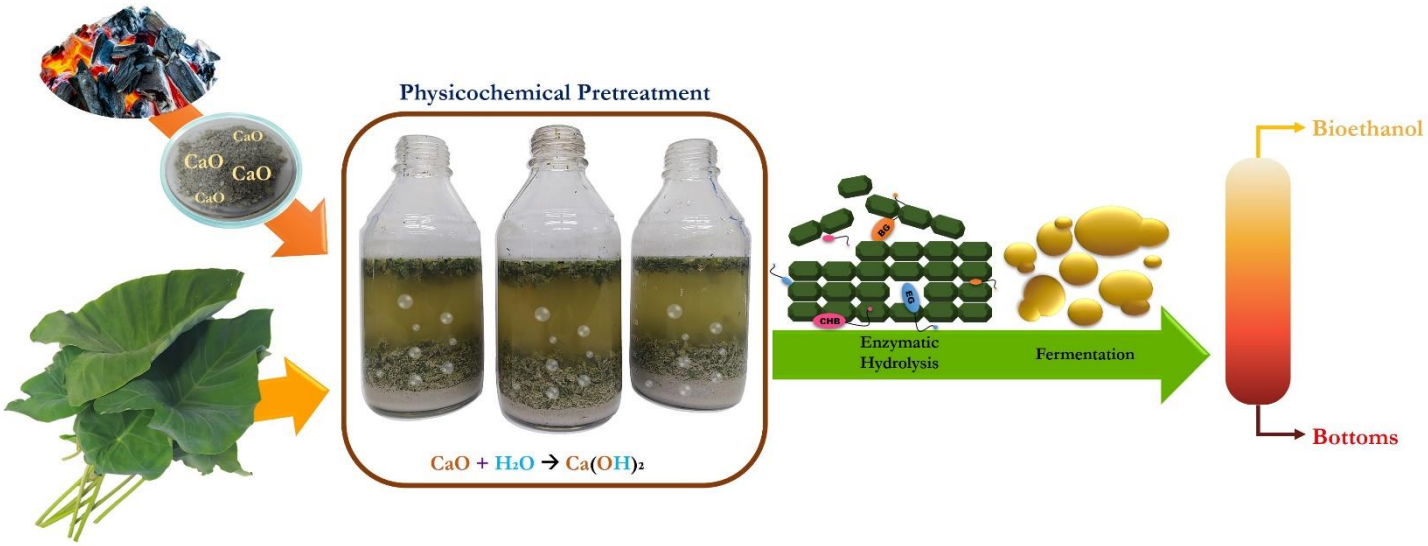



**Figure 10** Methodology for bioethanol production flowchart.

Elephant ear plant was cut into tiny pieces (1 to 2 cm), and half of it was homogenized in a blender to make a paste (Figure 11), which was used for fresh sample trials. The second half of the sample will be dried, pulverized, and preserved for future research purposes (Figure 11).

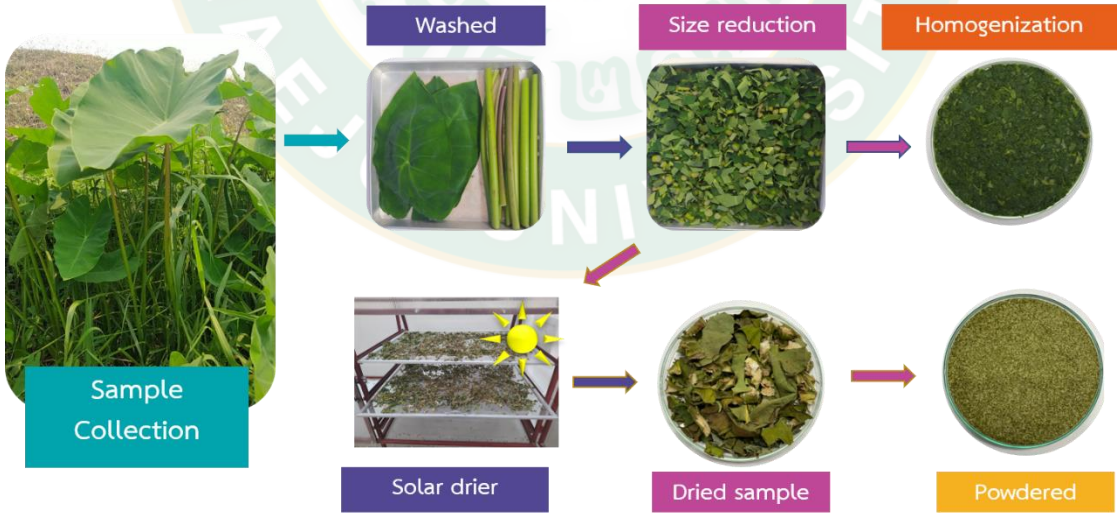



**Figure 11** Preparation of material. (a) Fresh sample homogenization, (b) dry sample powdered

Fresh and dry material was subjected to pretreatment, hydrolysis, fermentation, and bioethanol recovery procedures before being processed.

The sample preparation was carried out in the manner seen in Figure 11. It was necessary to weigh a specimen of elephant ear plant before adding various ratios of ash solution to the sample in order for it to go through physical pretreatment at different periods. To soften the materials, an autoclave was utilized at 121°C and 15psi. Experiments was conducted in duplicate to ensure accuracy. Particle size is reduced by physical and chemical preparation, and the cell wall is broken down, resulting in improved hemicellulose hydrolysis.

### 3.4 Pretreatment of the sample

3.4.1 Chemical pretreatment

Fly ash was used as source of CaO for the alkaline pretreatment. A solution was prepared by mixing 200g of flying ash with 1L of distilled water. The ash solution was mixed at different ratios (0%, 10%, and 20%) with 5g of elephant ear plant powder and 10g for the fresh sample (Figure 12).

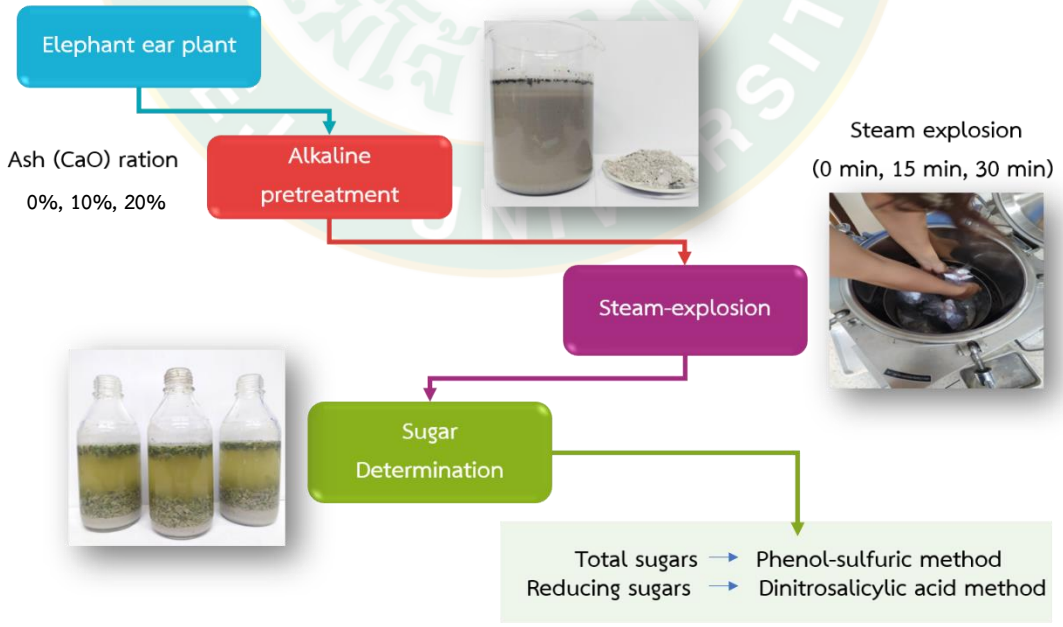



**Figure 12** Sample pretreatment process.

3.4.2 Physical pretreatment

Then, the mixture was under steam explosion at different times of exposure (0 min, 15 min, and 30 min) using autoclave apparatus. Experiments were done by triplicate to conduct the experimental arrangements described in Figure 13, and the combination with the higher fermentable sugar was chosen to continue with hydrolysis step.

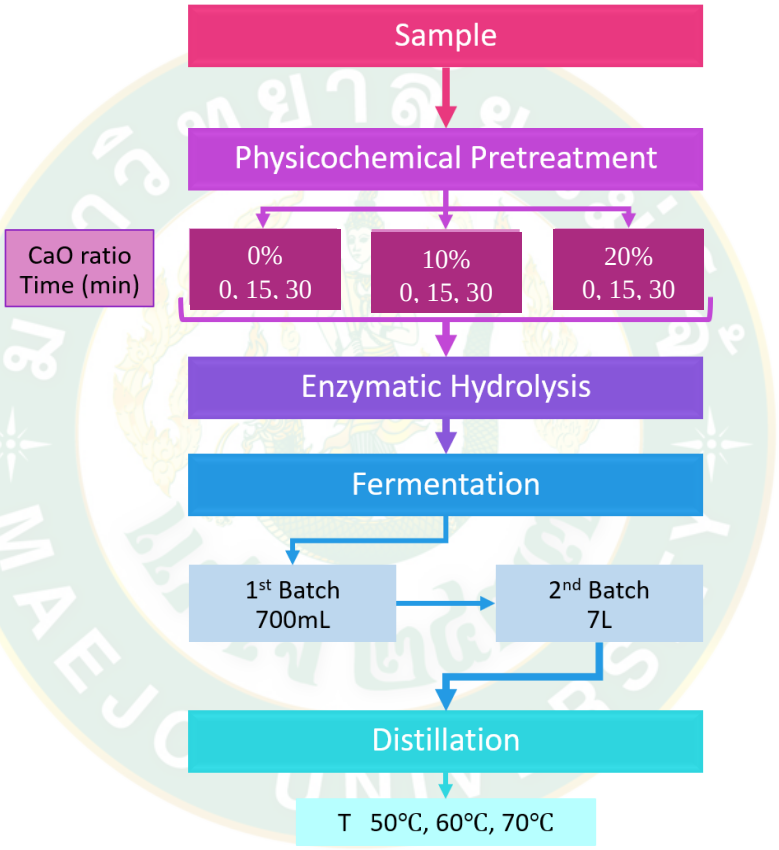



**Figure 13** Sample pretreatment flowchart.

**3.5 Enzymatic hydrolysis**

For the experiments perform, *Cellulase* enzyme will be used in hydrolysis pretreatment at 1% at 50 °C for 24 hours (Figure 14). By the end of hydrolysis process, total sugar and reduced sugar in the broth will be measured by spectrophotometer.

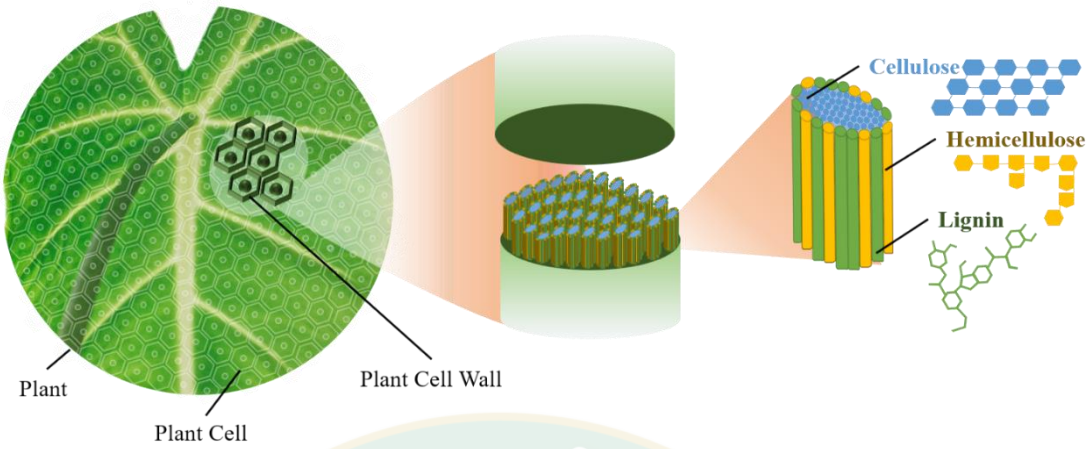



**Figure 14** Basic structure of plant tissues.

The batch fermentation step will be done by inoculating the samples with *S. cerevisiae* at 1% and kept in room temperature in the absence of oxygen for 120 hours and controlling the pH at 5.6. The bioethanol concentration of each sample will be measured by ebulliometer after 24 hours (Figure 15).

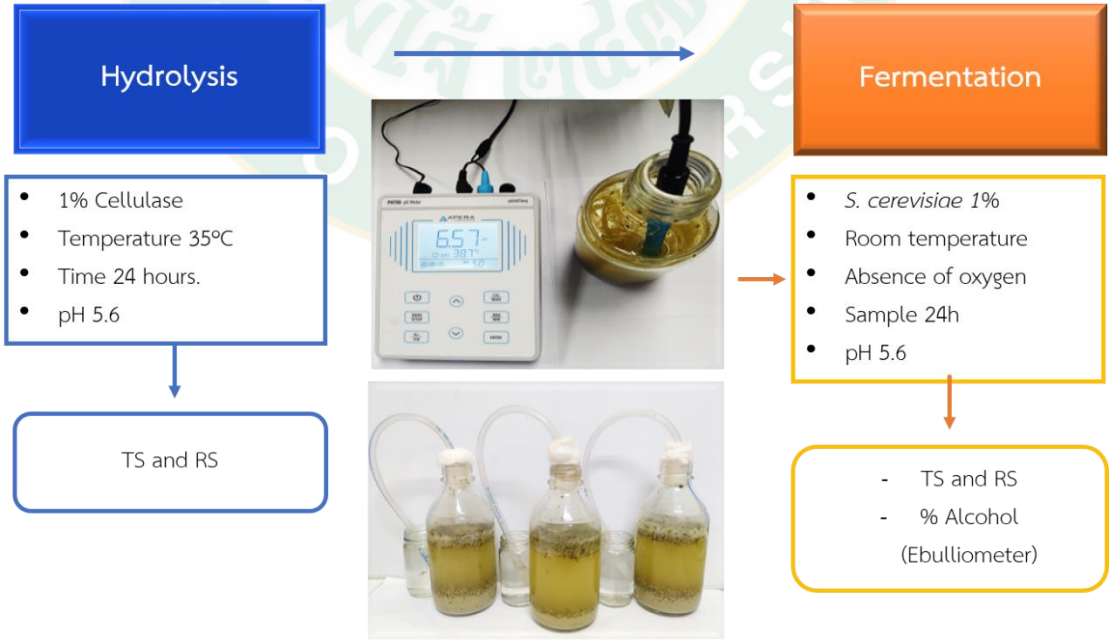



**Figure 15** Preparation and pretreatment of the sample flowchart.

Enzymatic hydrolysis is a step in the lignocellulosic biomass conversion technique that includes the use of enzymes to depolymerize the biomass before it was used for energy production. A common use for the saccharide components that are released is as fermentation feedstock (Modenbach and Nokes, 2013). Cellulases enzymes are widely used to perform the hydrolysis of lignocellulosic biomass. The combination of cellulase as well as suitable IL-cellulases system, appear promising for the effective activation and hydrolysis of native biomass to generate bioenergy (Wang et al., 2011).

After pretreatment, the pH of the combined solution was adjusted at 5.0 and the samples were inoculated with 1% commercial cellulase for the hydrolysis process, afterwards, the solution was kept in an incubator at 35°C for 24 h to perform the hydrolysis process (Figure 16).

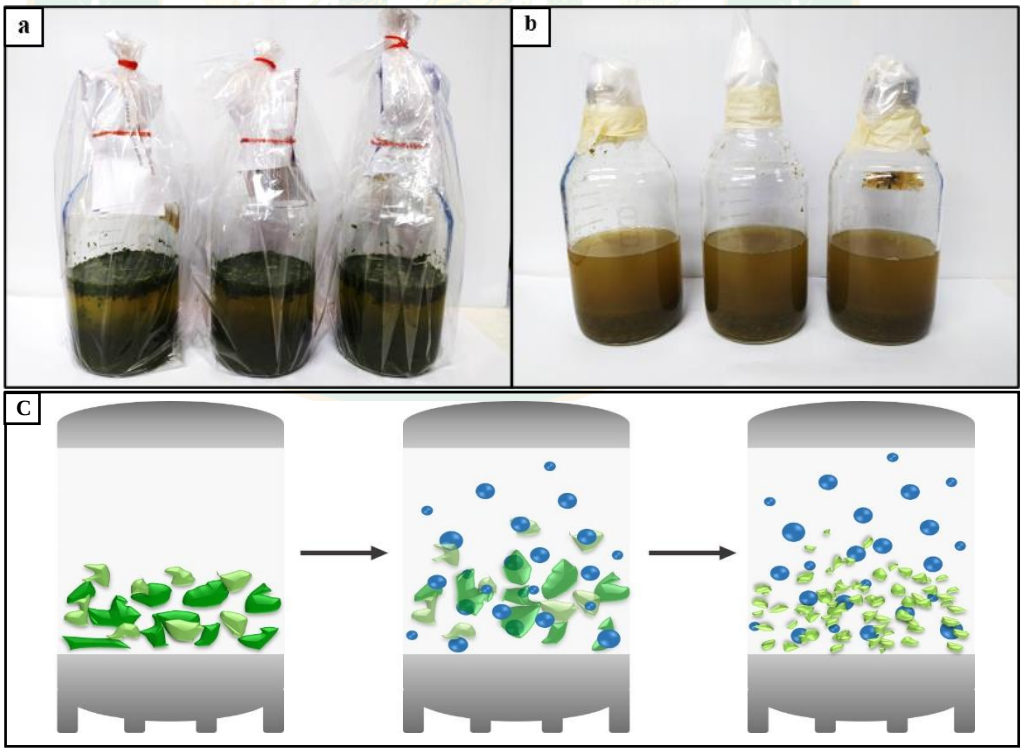



**Figure 16** Samples prepared for steam-explosion pretreatment (a, and hydrolysis (b. and c) steam-explosion pretreatment effect representation.

**3.6 Batch fermentation**

For the batch fermentation step, the hydrolysate with the highest content of reducing sugars was selected. The pH of the hydrolysate was measured and adjusted in the range of 5–5.5 before being inoculated with 1% of *S. Cerevisiae*. Fermentation was carried out by triplicates for 5 days and maintained at room temperature (30±5°C).

A 60 mL sample was taken every 24 h throughout the fermentation process and the resulting values for alcohol, total sugar, and reducing sugar were calculated to track the reaction.

3.6.1 Alcohol determination

The ebulliometer method was used to compare the boiling point of a particular amount of distiller water with the boiling point of a specified volume of broth in order to measure the ethanol production. Ebulliometer is a simple instrument for estimating the boiling point of pure substances or mixtures. They have been used to evaluate the alcohol content of wines for more than a century to quantify the amount of alcohol present in a beverage (Cottrell, 1919; Howell & Byrne, 2014). Equations 11 and 12 were used to estimate the bioethanol yield over total sugar consumption and % sugar utilization (Srimachai et al., 2015).

$$Y_{P/S} = \frac{P_f - P_0}{S_0 - S_f} \qquad \text{Equation 11}$$

$$\%S_c = \left(1 - \frac{S_f}{S_0}\right) \cdot 100 \qquad \text{Equation 12}$$

Where $Y_{P/S}$ is the bioethanol yield, $P_f$ and $P_0$ are the final and initial bioethanol concentration (g/L), $S_f$ and $S_0$ are the final and initial sugar concentration (g/L), and $\%S_c$ is the percentage of sugar consumption.

3.6.2 Ethanol characterization

Several characteristics of the bioethanol produced following the distillation process were investigated, including those listed in Table 5. The calculations were carried out utilizing analytical techniques and the information gathered during the distillation process (volume, weight, temperature).

**Table 5** Parameter evaluated for the obtained bioethanol.

| Parameter | Unit |
|---|---|
| Distilled volume | ml |
| Alcohol content | vol% |
| Density | $Kg/m^3$ |
| Specific gravity | |
| Moisture | % |

**3.7 Bioethanol recovery**

Following fermentation, bioethanol will be recovered using a simple distillation process. Distillation is the process of heating a liquid in order to produce vapor, which is then collected and separated from the original liquid after it is cooled. It is based on the fact that the components have varying boiling points or volatility values (2 or 3°C). When the temperature in the distillation flask is well monitored, it is feasible to achieve a pretty good separation between different components of a mixture or to help in the purification of the mixture. When the temperature reaches roughly 78.37°C, ethanol begins to evaporate. As the distillation process advances, the concentration of the component with the lowest boiling point will gradually drop. Eventually, the temperature within the device will begin to fluctuate, indicating that a pure chemical is no longer being distilled. The temperature will continue to rise until it reaches the boiling point of the next-lowest-

boiling compound, at which point it will stabilize. It is possible that the equipment used for distillation is referred to as a distillation apparatus (Figure 17).

The vapor was collected and condensed with the use of a cold-water circulation system that circulated around the column. The ethanol-containing distillate was collected in a conical flask located at the opposite end of the column for further processing.

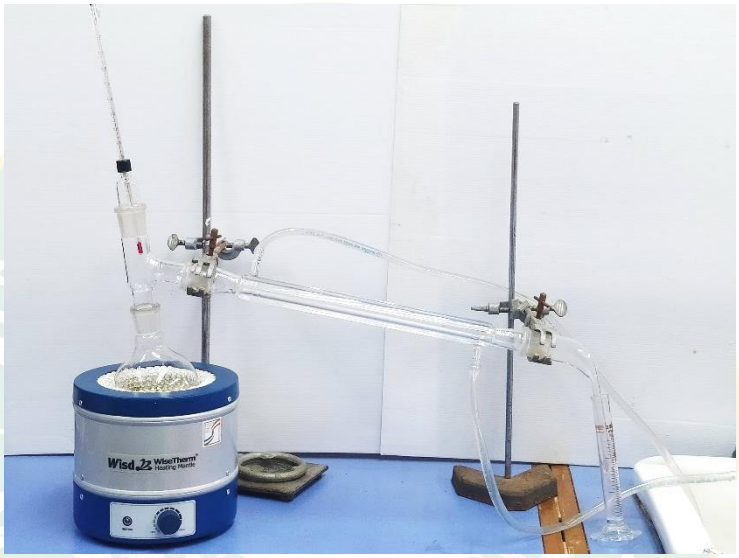


**Figure 17** Simple distillation apparatus.

A column of cold water was circulated around the column to collect and condense the vapor that had gathered. It was collected in a conical flask at the opposite end of the column, where the ethanol was recovered from the distillate. The ethanol produced during the fermentation process was recovered using a simple distillation process. The simple distiller apparatus was used to distillate 1L of broth at three different temperatures 50°C, 60 °C, and 70 °C.

**3.8 Calorimetric analysis**

3.8.1 Specific heat

The specific heat is the amount of heat needed per unit mass to raise 1ºC in temperature. When it comes to heat and temperature change, the connection is often described in the manner given below, where c is the specific heat of a substance (Equation 13).

$$Q = c \cdot m \cdot (\theta_f - \theta_0) \qquad \text{Equation 13}$$

Where *Q* refers to the heat energy in Joules (J), *m* is the mass of the substance in kilogram (kg), *c* is the specific heat in joules per kilogram (J/kg•k), $\theta_0$ and $\theta_f$ is the difference between the initial and final temperature in kelvins (K).

3.8.2 Heat capacity

Calorific value of a fuel refers to the quantity of heat released by a fuel's full combustion in a combustion chamber. For solid and liquid fuels, calorific value is expressed in kJ/kg, whereas for gaseous fuels, it is expressed as kJ/$m^3$ where $m^3$ is the average cubic meter measured at NTP conditions, i.e., at 0°C temperature and 760 mm Hg barometric pressure (1.01325 bar). Fuel is made up of combustible elements such as carbon, hydrogen, carbon monoxide, hydrocarbons, sulfur, and other elements.

A fuel calorimeter is a piece of equipment that is used to determine the calorific value of a fuel source (Figure 18). It is the transport of heat from combustion of a particular weight of fuel to water and the vessel that is the fundamental principle of calorimeters. By comparing the heat given out by the fuel to the heat taken in by the water and the container as the temperature of water and container rises, the calorific value of fuel can be estimated with the increase in temperature of water and container.

To know the heat taken by the container, the water equivalent of the container should be known. In this method of determining the calorific value of the fuel, the following conditions should be satisfied:

I. The combustion of the fuel must be complete
II. (The heat must be entirely transferred to the water
III. Cooling losses from the calorimeter must be corrected
IV. The rise of water temperature after must be correctly determined because the mass of the fuel is mini compared with the quantity of the water heated.

The equation used to calculate the heat value is shown in Equation 14:

$$P = 4185.5\frac{Q}{m}\ J/kg \qquad \text{Equation 14}$$

Where m is the fuel mass (kg), Q is the specific heat (J), 4185.5 is the distilled water specific heat (J/kgK).

**Figure 18** Calorimeter apparatus.

**3.9 Kinetic model**

When it comes to any fermentation process, a kinetic model may be used to explain the generation of the fermentation product in terms of time. Throughout the fermentation process, the kinetics of the reaction were followed by an increase in alcohol concentration and a decrease in sugar concentration. The graph that was obtained was utilized to calculate the optimal moment at which the reaction produced the maximum concentration of ethanol, at which time the broth could be distilled to extract the ethanol.

The modified Gompertz model predicted the amount of fermentation ethanol produced as a function of the fermentation period, the maximum product productivity, and the maximum prospective product output. The modified Gompertz model is described in Equation 15 (Bailey and Ollis, 1994)

$$P = P_m.e^{\left\{-e^{\left[\frac{r_m \cdot e^1}{P_m}\right]\cdot(t_L - t)+1}\right\}} \qquad \text{Equation 15}$$

Where $P_m$ was the potential maximum ethanol production (g/L), $r_m$ was the maximum ethanol productivity (g/L), and $t_L$ was the time from the beginning of fermentation to exponential ethanol production (h).

This equation was used in the present experiment to describe the change in ethanol concentration during fermentation, and it was chosen because of its success in prior investigations (Ginkel et al., 2001, Mu et al., 2006, Dodić et al., 2012; Phukoetphim et al., 2017) in modeling ethanol production using the modified Gompertz model. The ethanol concentration was calculated as a function of the fermentation period, the maximum product productivity, and the projected maximum product output using this equation.

### 3.10 Energy analysis

Energy analysis is a traditional method of studying the way energy is utilized in an activity including the physical or chemical processing of materials, as well as the transmission and/or conversion of energy, and it is still widely used today. When evaluating the performance of a system, energy analysis is often utilized. It may be used to analyze energy/fuel consumption and energy efficiency, and it can also be used to offer information on the amount of energy input and output of a system. The energy analysis for this research will be based on the energy used by processes such as feedstock preparation, pretreatment, hydrolysis, fermentation, and distillation, and will be based on this information.

Equation 16 was used to determine the total energy output and input from the bioethanol synthesis from elephant ear plant for the purpose of calculating the energy balance.

$$Input\ enegy = Output\ energy \qquad \text{Equation 16}$$

### 3.11 Mass balance

During the bioethanol manufacturing process, which included sample preparation, gelatinization, liquefaction, hydrolysis, fermentation, and distillation, data for the mass and energy balances were obtained. Figure 19 displays the bioethanol production process.

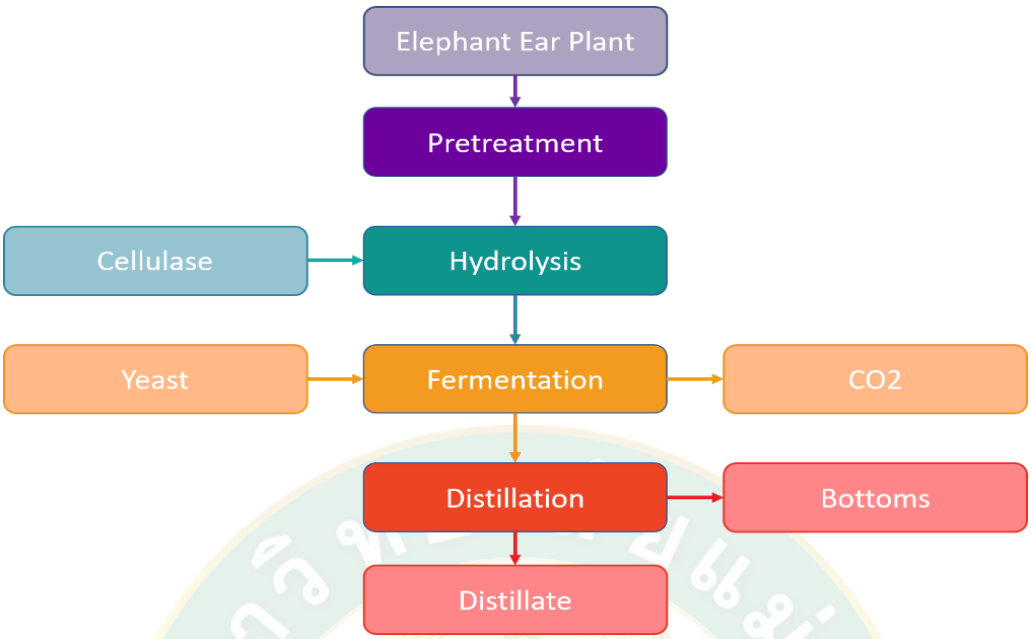



**Figure 19** The block flow diagram of bioethanol production from mass balance.

### 3.12 Economic analysis

This study was conducted to assess the effectiveness of the economic foundation of pretreatment procedures that were developed in this work. The study also included an economic analysis of the production of bioethanol from elephant ear plant. The entire amount of expenses (including both capital expenditures and operational costs) was calculated. When calculating capital costs, it is necessary to consider items like as equipment, facilities, and other utilities that were not influenced by how much product was produced.

### 3.13 Statistical analysis

The mean and standard error of the mean from triplicate observations are presented. There were statistically significant variations between the means. All statistical analyses were conducted with the help of the Statgraphics Centurion 19.

When the p-value for a correlation was less than 0.05 ($p<0.05$), it was considered to be significant.

# CHAPTER 4:
# RESULTS AND DISCUSSION

## 4.1 Feedstock characterization

Elephant's ear is a hardy perennial plant that thrives in wet or damp environments like wetlands, riverbanks, or moist open places. It will also thrive in recovering former grassland or badly disturbed shrubland and woodland. This plant has a lengthy life span and may outcompete other species by smothering wet places. It is drought resistant once established, and since it is harmful to livestock, it may take over grazed areas. It has the potential to spread and become a serious environmental weed (Serviss et al., 2000).

Most Araceae species contain insoluble calcium oxalate, which is poisonous because to the physical discomfort produced by needle-shaped crystals in the leaves (Tagwireyi and Ball, 2010). When the plant is eaten, the crystals are discharged from the idioblast cells and get caught in the mouth, tongue, or throat lining. This results in local inflammatory reactions such as discomfort, irritation, and edema of the buccal cavity, excessive salivation, and aphonia (inability to speak) (Miyamoto et al., 2021). According to Du Thanh et al. (2017) after the analysis of the leaves of seven different Colocasia esculenta cultivars contains in average 635.2±92.4 mg/100 g wet basis of total oxalate, with the lowest and highest value reported as 433.8±7.9 and 856.1±7.7 mg/100 g wet basis respectively.

Table 6 displays the findings of the physicochemical examination of elephant ear plant samples taken from both fresh and dried forms. It was found that moisture content in the elephant ear plant was 89.74%, with a dry matter percentage of the 10.26%. The total sugars content comparison showed an increment in the dry sample (3.394±0.129 g/L) in contrast with the fresh sample (1.132±0.086 g/L). This difference is the main factor for the energy value difference from the fresh and dry samples resulted in 4.536±0.031 and 12.825±0.514 kcal/5 g sample, respectively. Furthermore, the reducing sugars content increased from 0.907±0.005 g/L in the fresh sample to 2.633±0.039 g/L from the dry sample.

**Table 6** Elephant ear plant composition.

| Parameter | Elephant Ear Plant | |
|---|---|---|
| Moisture content (%) | 89.74 | |
| Dry matter (%) | 10.26 | |
| | **Fresh** | **Dry** |
| TS (g/L) | 1.012±0.086 | 3.394±0.129 |
| RS (g/L) | 0.707±0.005 | 2.633±0.039 |
| pH measured in water at 30±5 °C | 5.01±0.015 | 5.27±0.101 |
| Energy value (kcal/5 g sample) | 4.536±0.031 | 12.825±0.514 |

### 4.2 Influence of pretreatment on lignocellulosic biomass degradation

In this study, ash as a source of CaO was investigated at three different ratios (0%, 10%, 20%) as a chemical pretreatment of fresh elephant ear plant. Kumar, eat al., (2017), mention that CaO can provide a certain alkalinity as calcium hydroxide ($Ca(OH)_2$) while reacting with water (A. K. Kumar & Sharma, 2017) (Kumar & Sharma, 2017). The combination was then subjected to processing with hydrothermal and steam explosions. Before and after the hydrolysis stage, samples were tested for total sugar and reducing sugar (mg/mL) concentrations to ensure that the goals were met.

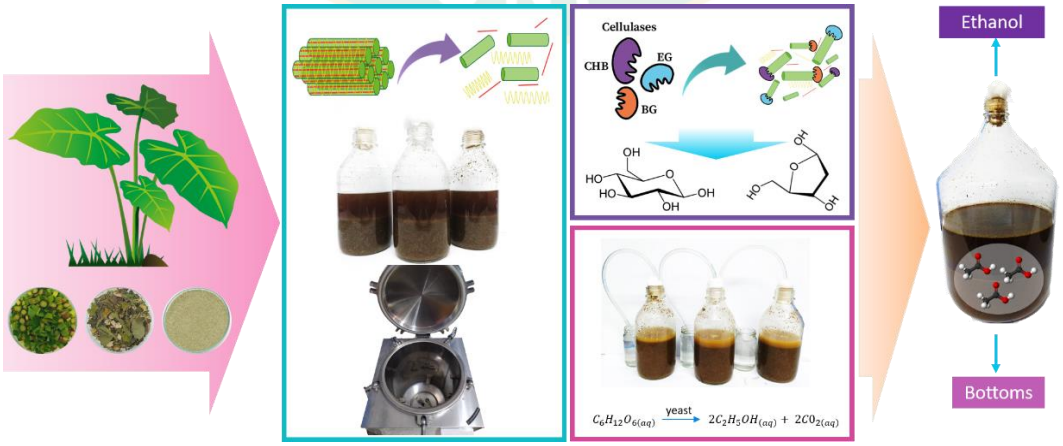



**Figure 20** Physicochemical pretreatment for elephant ear plant.

Figure 20 shows the results obtained of total sugar and reducing sugar at three different CaO ratios. The concentration of total sugar archived after the physicochemical pretreatment were 2.22 ± 0.10 mg/mL, 1.60 ± 0.02 mg/mL, and 1.11 ± 0.03 mg/mL respectively. On the other hand, the reducing sugar concentration obtained were 1.90 ± 0.12 mg/mL, 1.37 ± 0.07 mg/mL, 0.97 ± 0.01 mg/mL showed in Figure 21.

Biomass pretreatment reduces lignin and hemicelluloses, improving cellulose hydrolysis substantially (Whangchai et al., 2021). Reducing sugars or simple sugars such as glucose, xylose, and arabinose are degraded from the glycosidic bond rupture of polymers to allow rapid and efficient carbohydrate hydrolysis to fermentable sugars (Nguyen et al., 2020). It is necessary to explore the extraction of sugars from aquatic weeds in order to obtain the most cost-effective bioethanol production method (Sindhu et al., 2016).

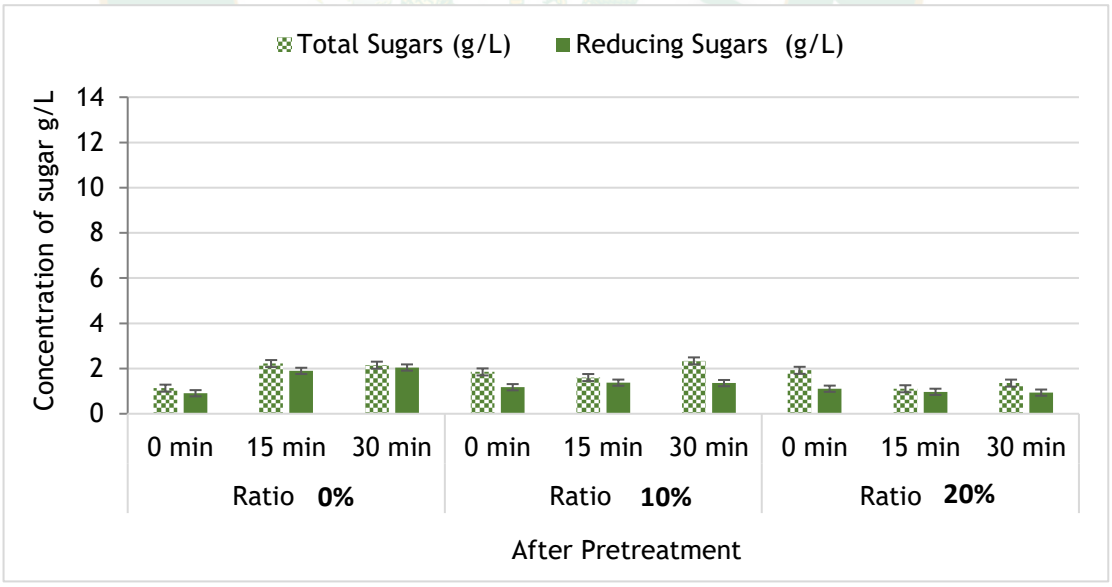



**Figure 21** Sugars content accumulated after steam explosion pretreatment fresh basis.

The results from the physicochemical pretreatment from dry elephant ear plant is showed in Figure 22. It can be observed that the sugar concentration increased accordingly to the exposure time of steam explosion pretreatment when the CaO ratio is 0%, with the higher concentration for total and reducing sugars of 4.991±0.029 and 3.685±0.021 g/L, respectively. This represents an improvement compared with the results reported from fresh elephant ear plant at the same conditions with a total sugar and reducing sugars content of 1.088 and 0.895 g/L respectively (Trejo et al., 2021).

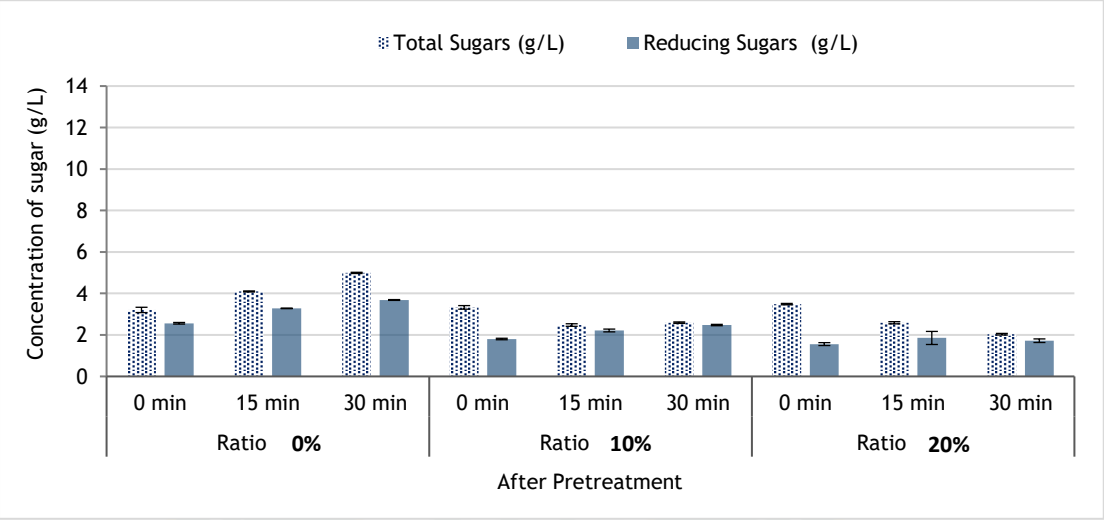



**Figure 22** Sugars content accumulated after steam explosion pretreatment dry basis.

This could be attributed to the calcium oxalate reduction as reported from Perez-Pimienta et al (2016) were the presence of low levels of calcium oxalate in agave bagasse showed a positive effect on pretreatment performance improving sugar production and faster enzymatic hydrolysis. The content of calcium oxalate observed to be reduced in the recovered product as a function of the sample pretreatment temperature (Perez-Pimienta et al., 2015).

At the opposite, the results obtained from the experiments using CaO ratio 10% and 20% showed a lower sugar content. Alkaline pretreatment with CaO is

beneficial since it improves the opening of cellulosic fibers, but it does not degrade sugars at this stage, just makes the material vulnerable to enzymatic degradation (Alvira et al., 2010; Amezcua-Allieri et all., 2017).

### 4.3 Effect of hydrolysis process for fermentable sugar generation

Meanwhile, the concentration of total sugar after enzyme hydrolysis step 3.63 ± 0.05 mg/mL, 6.51 ± 0.027 mg/mL, 6.43 ± 0.16 mg/mL (Figure 3). While for reducing sugars, 1:5 ratio (5.41 ± 0.11 mg/mL) presented the highest concentration of reducing sugars compared with 0% y 20% (3.56 ± 0.03 mg/mL and 5.30 ± 0.11 mg/mL, respectively). In a previous study using fresh elephant ear plant under hydrothermal and steam explosion treatment for 15 min, and enzymatic hydrolysis for 24 h, the highest total sugar and reducing sugar were 1.130 ± 0.04 mg/mL and 0.907 ± 0.03 mg/mL respectively (Trejo et al., 2021). As a result, in this work using a CaO ratio of 10% and after 15 minutes of pretreatment (hydrothermal and steam explosion) and 24 h of hydrolysis, 10%  ratio had a highest fermentable sugars concentration, what represents an improvement in the method.

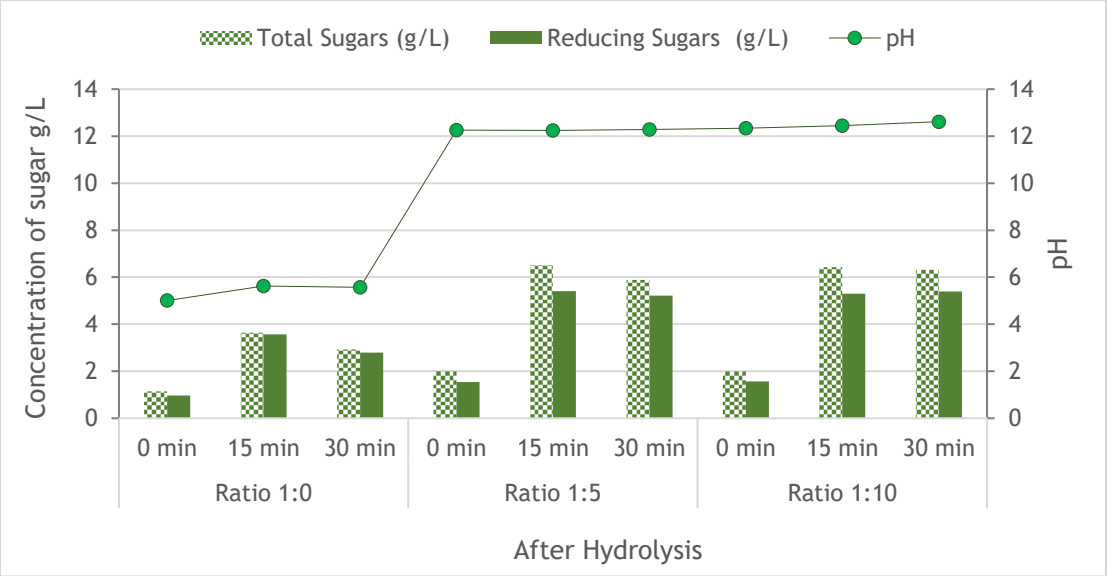



**Figure 23** Sugars content in fresh sample accumulated after enzymatic hydrolysis.

The diverse nature of aquatic weed biomass makes it difficult for successful biofuel extraction and conversion. The saccharification procedure identifies the most efficient pretreatment for releasing polysaccharides by breaking the cross-linkage bond of lignin barriers. When it comes to breaking down cellulose into glucose, cellulase is more sensitive than other enzymes (Ramaraj et al., 2019; Vu et al., 2018). Low content of results calcium oxalate in more free accessible area to enzymes that could react on the cellulose. The results from the hydrolysis process are displayed in Figure 23. Following the pretreatment behavior, the sugar concentration was higher for the samples pretreated with a CaO ratio of 0%. The total sugar and reducing sugars accumulation were 6.382±0.076 and 6.019±0.019 g/L, respectively. In a study carried out by Fernandez et al. (2015), *Cynara cardunculus* was pretreated by using steam explosion for producing bioethanol, the results showed partial solubilization of hemicellulose and improved the accessibility of residual polysaccharides towards enzymatic hydrolysis.

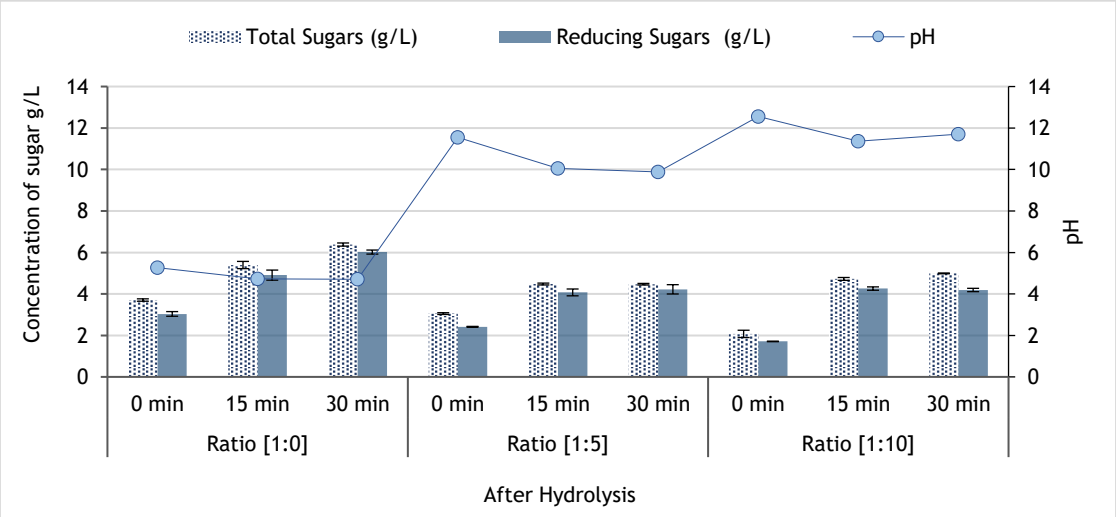



**Figure 24** Sugars content in dry sample accumulated after enzymatic hydrolysis.

After 24h of hydrolysis, the pH was measured in the samples (Figure 24), it was found that pH value using CaO were all above 10, and for the ones with 0 min

of steam explosion the pH reached 12 and 13 for the CaO ratio 10% and 20%. The low sugar releasing obtained using CaO at the rations could be explained due the low enzymatic activity during the hydrolysis produced for the high pH value. According to previous studies, cellulases are active at the pH range of 6.0 to 7.0 from (Akiba et al., 1995). Irfan et al (2012) found the optimum pH for endoglucanase activity at 7.5 and stable at pH 6.5 to 9.5. Increasing or decreasing pH beyond this resulted in decline in enzyme activity as was reported by El-Sersy et al. (2010) that cellulase enzyme production decreased about 50% at pH 9 from *S. ruber*, proving that any change in pH caused changes in the enzyme active site.

**4.4 Enhancement of ethanol production**

Aquatic weeds are rich in cellulose, hemicellulose, starch with low lignin content. When compared to other lignocellulosic biomass, they are easy to hydrolyze into fermentable sugars and create an efficient and cost-effective biofuel source (Kaur et al., 2018). Although aquatic weeds are used in on-site wastewater treatment, they provide both wastewater treatment and biofuel generation (Mehariya et al, 2021).

Following the best conditions obtained from the physicochemical pretreatment enzymatic hydrolysis, the fermentation process was settled with a broth prepared using a CaO ratio of 0% and 30 min of steam explosion. The broth was inoculated with 1% of commercial yeast and kept 5 days at room temperature (30±5ºC). Figure 25 and 26 displays the time curse for the sugars and ethanol content during the fermentation process.

Both, fresh and dry elephant ear plant were under fermentation. The conditions for the pretreatment conditions of the fresh sample were 15 min of steam explosion and a CaO ratio of 10%. Meanwhile, for the dry sample was 30 min of steam explosion with CaO ration of 0%.

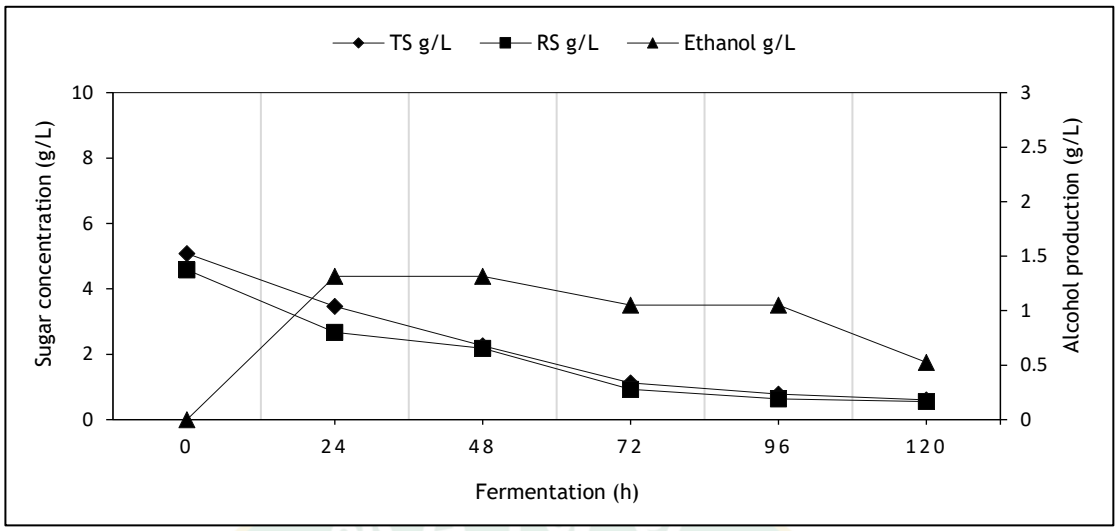



**Figure 25** Time course of the concentration of sugars and ethanol in the fermentation process of fresh sample.

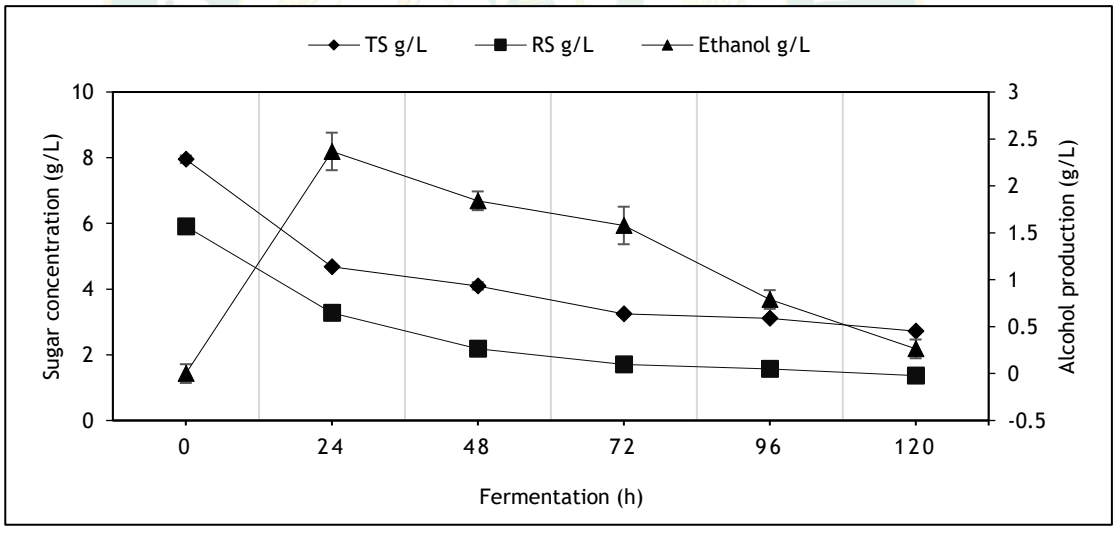



**Figure 26** Time course of the concentration of sugars and ethanol in the fermentation process of dry sample.

Fermentation produces ethanol and carbon dioxide as its final products. Under ideal conditions, when the liberated cellulose and hemicellulose are completely hydrolyzed and all sugars are converted to alcohol, the estimated potential for ethanol generation from the reducing sugars in the hydrolysate mixture

was calculated. The theoretical potential of bioethanol production was computed under ideal conditions, with the maximum bioethanol concentration obtained of 2.76 ±0.06 mg/mL after 15 min of hydrothermal and steam explosion pretreatment and a CaO ratio of 10%. Zhang, et al., (2018) reported a final ethanol concentration of 1.40 mg/mL from water hyacinth using *P. chrysosporium* for a microbial-diluted acid pretreatment followed by a fermentation by *S. cerevisiae* (Zhang et al., n.d.). Another aquatic plant that has been studied for bioethanol production is *salvinia molesta*. Abdullahi et al. (2016) reported 2 mg/mL of bioethanol production from *salvinia molesta* using acid hydrolysis and steam explosion as pretreatment from 15 min, and *S. cerevisiae* and *S. carlsbergensis* for fermentation step (Abdullahi et al., 2016).

**4.5 Ethanol distillation**

The distillation of ethanol formed during fermentation from ethanol-water solution will lead finally to production of hydrous (azeotropic) ethanol (theoretical maximum achievable 95.5% wt. ethanol and 4.5% water). To remove the remaining water, special processes are required to reach anhydrous ethanol, that include: chemical dehydration process, dehydration by vacuum distillation process, azeotropic distillation process, extractive distillation processes, membrane processes, adsorption processes, and diffusion distillation process.

To perform the ethanol recovery by simple distillation, a 7L batch of most were prepared from both, fresh and dry sample, under the best conditions reported in the previous stages and hydrolysis were settled for 24h at 35±5ºC. According to the previous fermentation results, 5L from were withdraw from the reactor after 24 h. The sample were then filtered and stored to stop the reaction until the distillation process. The remained 2L were kept under the fermentation conditions to follow the ethanol production and sugar content for the 4 days left.

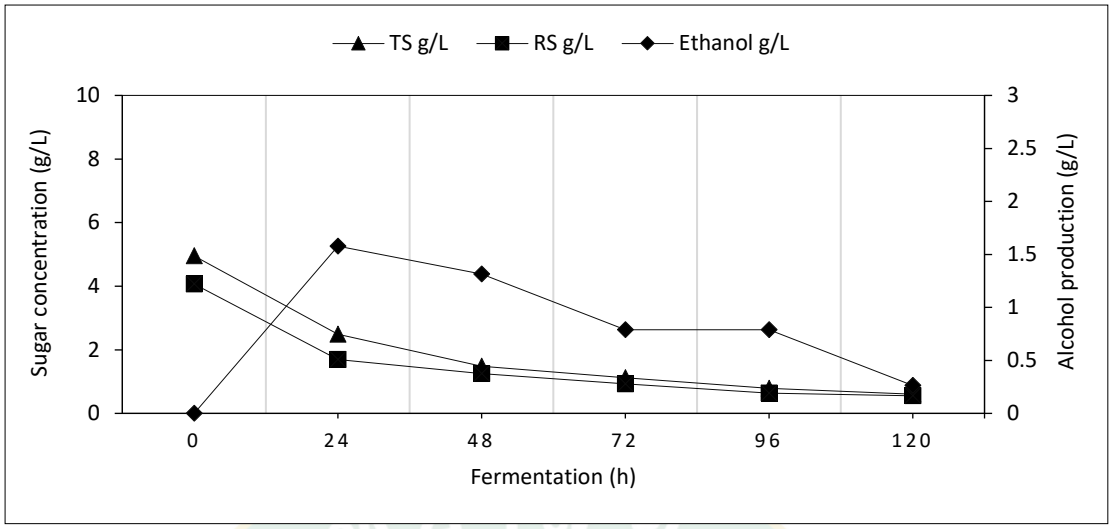



**Figure 27** Sugars and ethanol concentration from the 7L batch from fresh sample.

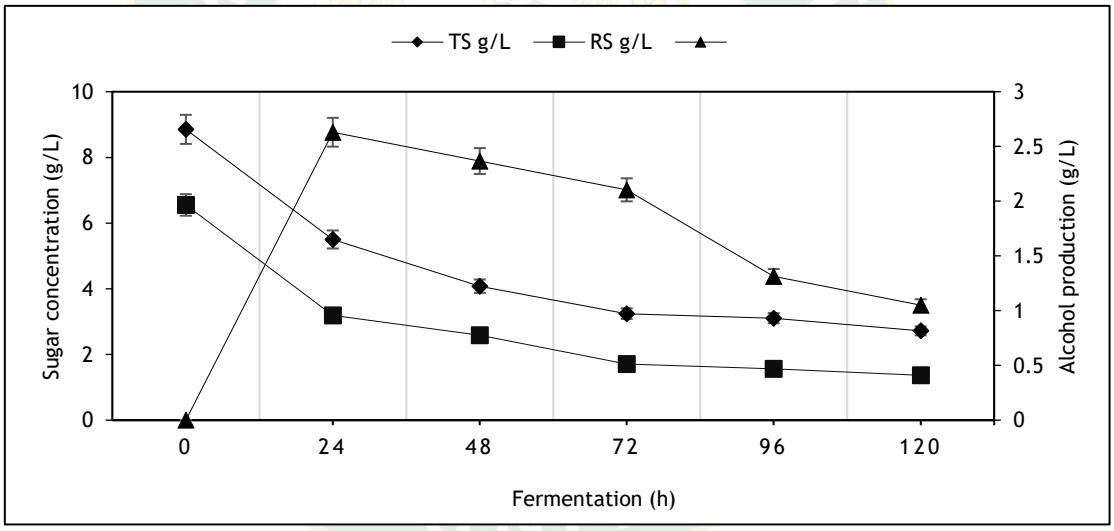



**Figure 28** Sugars and ethanol concentration from the 7L batch from dry sample.

In order to evaluate the amount of ethanol that can be recovered by simple distillation, samples of fermented broth were distilled at different temperatures 50°C, 60 °C, and 70 °C. Temperatures were chosen lower than the temperature of the ethanol evaporation (77 - 78°C). Figure 27 and 28 shows the fermentation process for the 7L batch prepared. The fermentation efficiency was estimated at

71.82% with a sugar consumption of 59.48%. The distillation process was carried out after 24h of fermentation with an ethanol concentration of 2.631±0.821 g/L.

**4.6 Ethanol Characterization**

The comparison of characterization of the bioethanol obtained after the distillation is shown in Table 7, which was compared with bioethanol quality standards (Hanum et al., 2013). The density was determined by the volumetry method. The %ethanol in mL/ mL of distilled was calculated in the base of the total distilled volume.

**Table 7** Evaluation of the ethanol obtained after the double distillation.

| Parameter | | Fresh | Dry | Hanum et al., 2013 | Sulaiman et al., 2021 | ASTM D4806 |
|---|---|---|---|---|---|---|
| Distilled | g/L | 13.6 | 7.84 | - | - | - |
| | mL | 16 | 9 | - | - | - |
| Density | g/L | 0.975 | 0.961 | 0.966 | 0.460 | Max. 0.8215 |
| Specific gravity | | 0.975 | 0.962 | 0.966 | 0.460 | Max. 0.8215 |
| pH | | 6.5 | 7.3 | - | - | 6.5 - 9 |
| Calorific Value | kcal/kg | 309 | 736 | 167 | 3702 | Max. 5000 |
| Ethanol | mL | 1.65 | 2.33 | - | - | - |
| | % | 2.41 | 13.59 | 18.99 | 24.8 | Min. 92.1 |
| Moisture | mL | 12.24 | 7.01 | - | - | - |
| | % | 98 | 87 | - | 76 | Max. 2 |

Based on the results, the ethanol content of bioethanol made from fresh and dry elephant ear plant were 2.41% and 13.59%. This indicates that the product does not satisfy the internationally recognized requirement of 94.1% bioethanol. This mismatch could be attributed to the fact that the distillation process has not been

repeated (Gil et al., 2008). The distillation procedure that is used will have an impact on the findings of the ethanol content test results. To obtain the desired ethanol concentration, it is necessary a series of continuous distillation process (Madson, 2003).

The water content test was carried out by dividing the original weight of the fermented product by the final weight after distillation, which resulted in the starting weight being divided by the final weight. The fresh and dry samples obtained in this investigation had water contents of 98 % and 87 %, respectively, that are similar to the obtained for Sulaiman et al., (2021). The results of this investigation reveal that the water concentration of bioethanol does not fulfill the quality standards for bioethanol, which call for a maximum water content of 2%. The reason for this is because the ethanol produced is not completely pure due to the fact that it is blended with water (Luo and Kiss, 2015). Considering that the distillation procedure used was a normal distillation process, the ethanol produced from elephant ear plant with yeast variants includes a significant amount of water. The lower the heat of combustion, the greater the amount of water in the mixture (Speight, 2019).

The calorific value of this study's data is impacted by specific gravity and density. Because fuel density is projected to strongly effect fuel use, higher densities are likely to increase consumption or waste (Sayyed et al., 2022). This suggests that a low density yields a high specific gravity and a low calorific value, indicating high grade bioethanol from bananas. Conversely, a high density produces an specific gravity and a low heating value, resulting in bad quality.

### 4.7 Mass balance

The mass balance for the distillation process at the different temperatures is presented is Table 8. The volume of ethanol present in the distilled sample at 70°C was 1.03±0.196 mL, the higher volume compared with the 0.21±0.127 and 0.84±0.243 mL obtained at 50°C and 60°C, respectively. However, in terms of ethanol

yield, the percentage obtained at 60℃ represents the higher value in the contrast with the 4.208 at 50℃ and 7.890 at 70℃.

**Table 8** Comparison of ethanol recovered by distillation at different temperatures.

| | Fresh | | |
|---|---|---|---|
| Temperature (℃) | **50** | **60** | **70** |
| Distilled Vol. (mL/1000 mL) | 7 | 14 | 16 |
| Ethanol mL | 0.21±0.012 | 0.33±0.247 | 0.87±0.235 |
| Ethanol yield (%) | 12 | 20 | 52 |
| Water (mL) | 6.80±1.16 | 13.67±2.59 | 15.13±1.625 |
| Bottoms Vol. (mL/1000 mL) | 993 | 986 | 984 |
| Ethanol (mL) | 1.47±0.524 | 1.33±0.412 | 0.801±0.213 |
| Water (mL) | 991 | 985 | 983 |
| | **Dry** | | |
| Temperature (℃) | **50** | **60** | **70** |
| Distilled Vol. (mL/1000 mL) | 5 | 9 | 13 |
| Ethanol mL | 0.21±0.127 | 0.84±0.243 | 1.03±0.196 |
| Ethanol yield (%) | 10.22 | 40.87 | 49.81 |
| Water (mL) | 4.79±0.275 | 8.16±0.079 | 11.97±0.321 |
| Bottoms Vol. (mL/1000 mL) | 995 | 991 | 987 |
| Ethanol (mL) | 2.051±0.263 | 1.105±0.629 | 0.828±0.563 |
| Water (mL) | 992 | 989 | 986 |

The efficiency of the fermentation stage was 73.13% from the reducing sugars concentration determination before and after the 48 h of fermentation before distillation (Table 9). Meanwhile, for the highest ethanol concentration (11.066 g/L), the sugar consumption rate was estimated at 59.66% and an ethanol yield of 0.63 g of ethanol/ g of substrate. Besides, taking 1.976 g/L at standard temperature and pressure (1 atm and 273 K) for the $(CO_2)_g$ was estimated stoichiometrically in 23.05

g/L. For the double distillation, the distillation of the dry sample presented an efficiency of 73.17% of g of ethanol/ L of broth distillate, higher than 63.42% estimated for the fresh sample. In general, the dry sample had a greater bioethanol production efficiency than the fresh sample. Since the ethanol concentration is higher, this has a direct influence on ethanol recovery.

**Table 9** Evaluation of the efficiency per stage for bioethanol production and distillation.

| Stage | | Fresh | Dry |
|---|---|---|---|
| Fermentation efficiency (%) | %EF | 63.42 | 73.17 |
| Sugar consumption (%) | %SC | 68.19 | 59.66 |
| Ethanol yield (g of ethanol/ g of substrate) | Y P/S | 0.47 | 0.63 |
| Distillation efficiency (g of ethanol/ L broth distillate) | %EF | 5.03 | 10.68 |

Table 10 illustrate the literature survey of various plant weeds utilized for bioethanol production with different pretreatment and hydrolysis protocols. It was reported that after dilute acid pretreatment, hemicellulose disintegrates, and xylose is released into solution, whereas alkaline pretreatment preserves a portion of hemicellulose while removing most of the lignin component (Aswathy et al., 2010; Lin et al., 2016). The combination microbial-chemical method could significantly boost the generation of reducing sugars in water hyacinth hydrolysates compared to a single MB method (Zhang et al., 2018). However, as with other cellulosic bioethanol feedstocks, such as herbaceous grasses and agriculture or forestry residues, aquatic and semi-aquatic plants require a pretreatment step, followed by a hydrolysis and fermentation process as a general method for bioethanol production (Isarankura-Na-Ayudhya et al., 2007; Taherzadeh & Karimi, 2008; Whangchai et al., 2021).

**Table 10** Comparison of various pretreatment utilized to produce bioethanol.

| Feedstock | Methodology | Ethanol | Refence |
|---|---|---|---|
| Water hyacinth (*E. crassipes*) Dry base | Alkali pretreatment 5% NaOH, furnace 10min at 150°C<br>Enzymatic hydrolysis by cellulase and xylanase for 60h at 50°C<br>Fermentation by *Pichia Stipites*. | 3.193 mg/mL | Kumari et al. (2014) |
| Water hyacinth *(E. crassipes*) Fresh base | Fermentation by Malt and Barley for 7 days at 30°C. | 1.019 mg/L | Rezania et al. (2014) |
| *Salvinia* sp. Dry base | Acid hydrolysis with 10% of $H_2SO_4$, steam explosion for 15min.<br>Fermentation by *S. cerevisiae* and *S. carlsbergensis* for 3 weeks at 30°C. | 2 mg/mL | Muhammad et al. (2016) |
| *Azolla* sp. | Hydrolysis by diluted acid and cellulase enzyme under steam explosion.<br>Fermentation by *S. cerevisiae* after 48h. | 3.990 mg/mL | Sharafi et al. (2013) |
| Elephant ear plant | Steam explosion pretreatment for 15min.<br>Hydrolysis was conducted by cellulases for 24 h at 35°C.<br>Fermentation by *S. cerevisiae* for 5 days at room temperature (30 ±5°C). | 1.130 mg/mL | This study |

**4.8 Energy balance**

Aquatic weeds are fast growing and invasive in nature. These characteristics of aquatic weeds need to be given proper attention when grown for their potential application for production of biofuel and other products (Bayrakci et al., 2014). While aquatic weed has demonstrated significant potential for biofuel production and other purposes, there are still obstacles that must be overcome before it can be successfully implemented to benefit the environment and humankind.

The energy balance and the cost for the energy consumption per stage for the overall bioethanol generation from dry elephant ear plant is shown in Table 11. As the solar dewatering of the sample did not need any energy input, it was excluded from the energy analysis. As can be observed, hydrolysis represents the mayor energy input with 45.60kWh. thus, hydrolysis also represents he main inversion with 4.469USD. Removing the hydrolysis process from the process, leaves an energy input of 1.050±0.002kWh and a cost expense of 0.103±0.001USD, that still above the energy output calculated in 0.856±0.040 kWh valuated in 0.084±0.002USD.

**Table 11** Energy balance per stage.

| Stage | Equipment | W | kW | h | kWh | kWh (USD)* |
|---|---|---|---|---|---|---|
| Sample preparation | Blender | 600 | 0.60 | 0.1 | 0.06 | 0.006 |
| Physical pretreatment | Autoclave | 2500 | 2.50 | 0.3 | 0.75 | 0.074 |
| Hydrolysis | Oven | 1900 | 1.90 | 24 | 45.60 | 4.469 |
| Distillation | Heater | 240 | 0.24 | 1 | 0.24 | 0.024 |
| Energy Input | | | | | 46.65 | 4.572 |
| Energy Output (Fresh) | | | | | 0.360±0.001 | 0.035±0.012 |
| Energy Output (Dry) | | | | | 0.856±0.040 | 0.084±0.002 |

*1฿ Thai Baht = 0.030 USD

The difficulties associated with producing aquatic weed biofuels on a scale up may include harvesting, drying, transporting, and developing a cost-effective conversion technology (Xu et al., 2013; Jambo et al., 2016).

The energy balance analysis of bioethanol production indicates that the hydrolysis process consumes the majority of energy, which is also due to the long period of incubation. Reduced energy consumption during hydrolysis is possible when less heating is required, however, it is important to maintain optimum incubation temperature during biological pretreatment since long incubation time due to low delignification rate is one of the major barriers for large scale application of biological pretreatment (Isroi et al., 2011). Aquatic weed biomass can include up to 90% water, which might impact the process of biofuel conversion (Alam et al, 2021). Efficient and cost-effective dewatering technologies should be studied to facilitate the downstream process of aquatic weed biofuel production (Chen et al., 2015; Jeevanandam et al., 2020).

4.8.1 Tecno-economic analysis

Feedstock, capital, and operational and maintenance costs are the four key categories of ethanol manufacturing costs and benefits from by-products - The price of feedstock Location, seasons, local supply-demand factors, and transportation all affect feedstock prices. Operating and maintenance costs are two market price variables that can influence choosing a feedstock type for ethanol production. Labor, energy, electricity, materials (e.g. enzymes, yeasts, etc.), repairs and maintenance, taxes, insurance fees, and administrative expenses are all part of the operation and maintenance costs. Capital expenditures, the initial costs of all necessary production equipment and their installation, are a capital investment. The capital costs include charges for pipe, instrumentation, insulation, foundations, and site preparation. Land, buildings, and waste treatment facilities are all included in these costs. The cost for each phase in the ethanol production from elephant ear plant is disclosed in Table

10. During the use of the oven for the hydrolysis step (24 h at 35 ºC), the highest energy cost is produced with 4.469USD per batch. Meanwhile, using a solar drier oven reduces the usage of energy to eliminate the moisture from fresh elephant ear plant. Even though the cost of ethanol was calculated in 0.084 USD/L, it is a first approach to the bioethanol obtention from second-generation starch feedstock.

**4.9 Ethanol heat power**

As part of the characterization, the obtained bioethanol was under equality determination by calorimetry evaluation to obtain the heat power. To calculate the heat values, the method reported by Rapin and Jacquard (1997) was used to determine the specific heat. Meanwhile, the equation used for Kates and Luck (2003) was used for the heat power. The results are reported by triplicate in Table 12.

**Table 12** Heat values obtained for the heat power determination.

| Sample | Specific heat J/kg ºC | Heat capacity Q (J) | Heat power (MJ/kg) |
|---|---|---|---|
| 1 | 227.78 | 2.87 | 1.31 |
| 2 | 210.74 | 3.03 | 1.28 |
| 3 | 219.26 | 2.95 | 1.30 |
| Fresh | 219.26±4.92 | 2.95±2.95 | 1.30±1.30 |
| 1 | 540.97 | 6.81 | 3.11 |
| 2 | 500.50 | 7.2 | 3.04 |
| 3 | 520.73 | 7.00 | 3.081 |
| Dry | 520.73±11.68 | 7.00±2.11 | 3.08±0.93 |

The heat power of the ethanol obtained from the elephant ear plant was estimated at 1.30±1.30 MJ/kg for fresh and 3.08±0.93 MJ/kg for dry sample, under the

range of 16.6 to 21.2 MJ/kg reported for Charles (2004) for different feedstocks. The calorific value of the fresh and dry elephant ear plant material came close to the bioethanol quality standards. The ASTM D4806 sets a maximum calorific value of 20.92 MJ/kg for bioethanol.

According to the findings of the calorific value of bioethanol produced from elephant ear plant using a simple distillation technique, the calorific value is still relatively low (1.30 and 3.08 MJ/kg, respectively), but it is near to the standard value for bioethanol quality. Because of the findings of this research, the calorific value acquired is greater than the calorific value generated Hanum et al., (2013) in durian seeds, which is 0.699 MJ/kg.

**4.10 Energy engineering aspects of maximum ethanol production**

4.10.1 Kinetics model

For optimizing the conversion of lignocellulosic biomass into sugar, it is necessary to understand the principles of sugar production and how all of the components that influence sugar production interact with one another. Aside from the fermentation conditions, it is also important to understand the fermentation kinetics in order to understand the metabolism of yeast throughout the bioethanol fermentation process. In order to suggest the biochemical pathways that would result in the most efficient bioethanol generation and yeast growth, many mathematical models, including the Monod, logistic, Contois, and Tessier, have been examined (Ahmad et al, 2011; Rorke at al., 20177). Aside from that, the information gathered might be valuable in the development and design of a system for large-scale manufacturing.

In order to do this, it is necessary to compare experimental and predicted data together in order to identify difficulties related with the lignocellulosic ethanol process. Additional knowledge of cell development and product generation dynamics will result in considerable improvements in process design as well as

production yield (Almquist et al., 2014). The kinetics of bioethanol production during fermentation of fresh and dry elephant ear plant is shown in Figure 29 and 30.

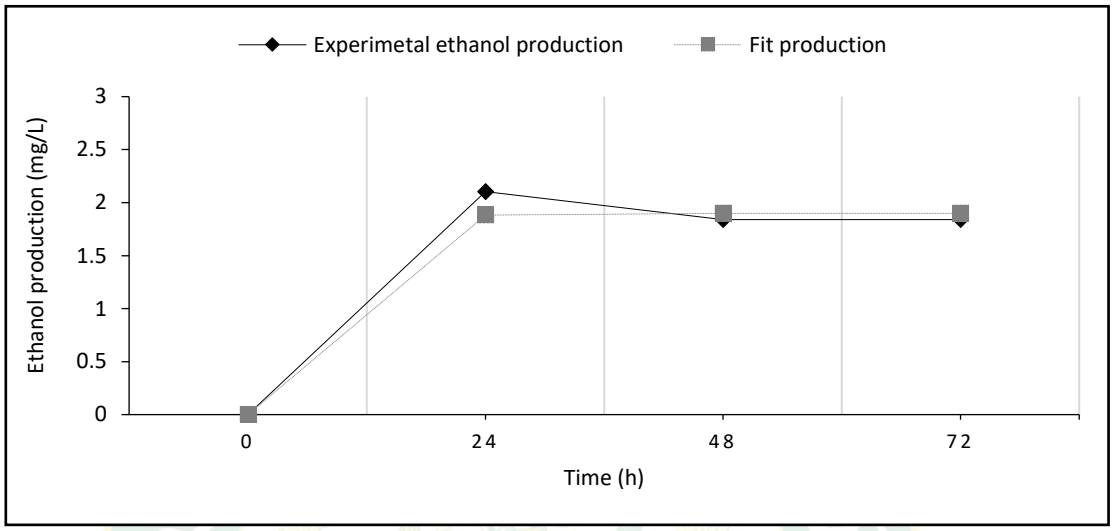



**Figure 29** Product kinetics results of experimental values for fresh sample.

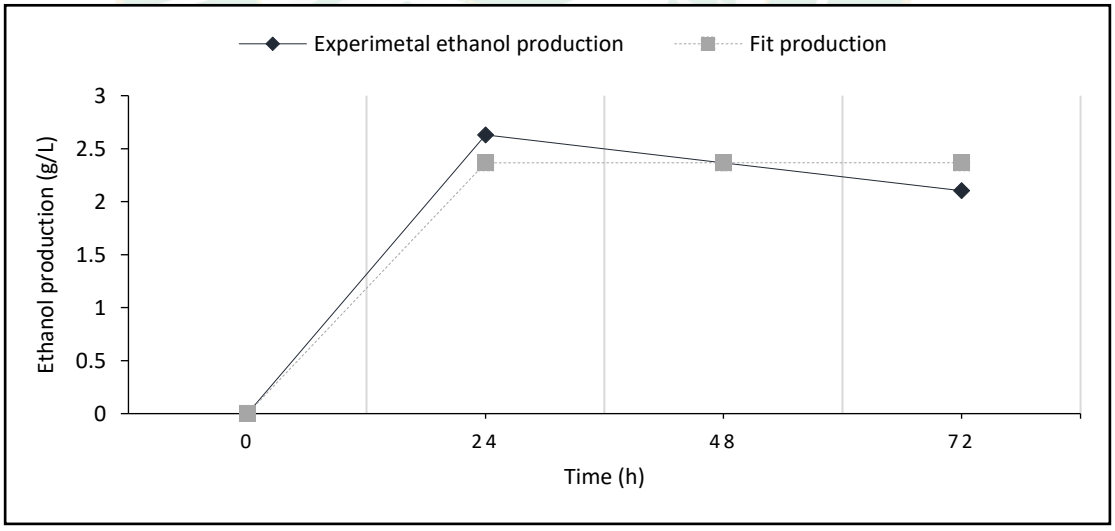



**Figure 30** Product kinetics results of experimental values for dry sample.

Experiments were carried out at a pH range of 5 to 5.5 and room temperature (30±5 ℃) using 1% of commercial yeast. The production of bioethanol started after 7 h (Table 4) from the period of inoculation increased slightly when the microorganism was in the phase of exponential growth. Because the organism displayed lag phase during this fermentation time period, it is possible that the delay in ethanol generation was caused by incorrect absorption of the substrate by the organism during this fermentation time period. During the fermentation process, the bioethanol content increased and reached a maximum at around 24 h. As the organism progressed through the stationary growth phase, the rate of production steadily decreased beyond 30 h.

**Table 13** Values obtained from the modified Gompertz model.

| Kinetics parameters | Fresh | Dry |
|---|---|---|
| pm (g/L) | 1.27 | 2.367 |
| rpm (g/L*h) | 0.64 | 0.475 |
| tL (h) | 11 | 7.834 |
| $R^2$ | 0.974 | 0.968 |
| Error | 0.123 | 0.069 |
| SSR | 0.262 | 0.138 |

Table 13 content the kinetic parameters calculated by using the Modified Gompertz model. The maximum bioethanol production rate (rpm) value indicates that 0.475 g/L of ethanol was produced every hour. The model describes the process with an accuracy of 0.968 indicated for the correlation factor. Sarto et al. (2019) published a study in which they investigated the kinetics of water hyacinth biomass pretreatment using a power-law model based on the first-order model. They demonstrated that the first-order model can be used to correctly calculate the rate

constant of the majority of pretreatment processes, which may be useful in the future in order to maximize the efficiency of the pretreatment process.

In comparison to previous research, the lag time (tL) for bioethanol synthesis the lowest was obtained using te dry sample (7.8 h). As a consequence, the yeast cells needed less time to adjust to the fermentation medium compared with the fresh sample, resulting in bioethanol production at the start of the fermentation process. Wang et al. (2013) and Jugwanth et al. (2019) both showed low lag periods of 0 h and 0.97 h, respectively, in their research. Rorke and Gueguim Kana (2017) and Chohan et al. (2020), on the other hand, found much larger lag periods of 6.31 h and 4.658 h, respectively. The kinetic data from this investigation shows significant advances in our understanding of the potential of lignocellulosic bioethanol production from elephant ear plant.

4.10.2 Ethanol production process scale up

Apart from sugarcane (in Brazil), corn grain (in USA), tapioca starch and sugarcane molasses (in Thailand), weeds, like elephant ear plant is a promising large-scale energy feedstock because its stalks contain a large amount of fermentable sugar, and it can be cultivated at nearly all temperatures including tropical climate areas. Table 14 displays a evaluation from the ethanol yield (g/g) and fermentation efficiency (%) obtained in this study compared with the reported by Pace et al., (2000). The results demonstrate a not significant difference ($p<5$), which represents a suitable condition to develop a large scale the process. Process expansion requires the generation of kinetic models that are typically useful for engineering applications as part of the overall process scaling process, as well as the energy and mass balance that provide the information required to the feedstocks and products projection. Never the less, the techno-economic balance will demonstrate the profit level expected from the process.

**Table 14** Ethanol scale-up performance using different feedstock.

| Feedstock | Volume (L) | Ethanol yield of biomass | Ethanol | | Ref. |
|---|---|---|---|---|---|
| | | | g/ton | L/ton | |
| *Lemna minor* | 0.25 | 0.218 g /g of biomass | 872 | 1105 | Gusain and Suthar, (2017) |
| *Pistia stratiotes* | 0.25 | 0.215 g /g of biomass | 860 | 1090 | |
| *Eichhornia sp.* | 0.1 | 0.14 – 0.17 g /g of biomass | 1400-1700 | 1774-2155 | Mishima et al., 2008 |
| *Water lettuce* | 0.5 | 0.15 – 0.16 g /g of biomass | 300-320 | 380-406 | |
| Water hyacinth | 0.25 | 0.4 g/g of biomass | 1600 | 2028 | Cheng et al. (2014) |
| Duckweed | 0.3 | 0.485 g/g of biomass | 1617 | 2049 | Aswathy et al. (2010) |
| Sunflower stalks | 1 | 0.439 g /g of biomass | 4390 | 5560 | Sharma et al., 2002 |
| | 15 | 0.437 g /g of biomass | 2900 | 3700 | |
| Elephant ear plant | 0.7 | 0.56 g /g fresh of biomass | 800 | 1014 | This study |
| | 7 | 0.67 g /g of biomass | 9600 | 1210 | |

Preliminary process designs of industrial-scale ethanol fermentation plants were made employing the aforementioned modes of operation: batch, continuous,

continuous with cell recycle, and vacuum with cell recycle (Wang et al, 2011). The process design studies employed the aforementioned laboratory fermentation kinetics. Each design assumes optimal fermentation temperature, pH, and oxygen tension. Perfect laboratory conditions are unlikely in industrial settings. Although the absolute cost calculations may be unrealistic, designs based on laboratory data should offer fair comparisons between alternative processing systems. This is particularly true of the fermentation substrate (Cysewski and Wilke., 1978).

A study by Cotana et al. 2015 obtained an ethanol yield of 0.165 g/g from *Phragmites australis* after pretreatment with steam explosion method. However, there are limited studies in literature that have explored the potential of aquatic weeds for biofuel production except for a few reports on *Eichhornia sp.* and duckweed. Our results of ethanol production are also comparable to other lignocellulosic materials being used for bioethanol production. An ethanol yield of 0.172 g/g and 0.24 g/g biomass from rice straw and corn stover has been recorded in two different studies (Wi et al., 20013; Saha and Cotta, 2014). Ramadoss et al., 20165 achieved an ethanol yield of 0.18 g/g biomass from sugarcane bagasse subsequent to hydrogen peroxide treatment. Process expansion requires the generation of kinetic models that are typically useful for engineering applications as part of the overall process scaling process, as well as the energy and mass balance that provide the information required to the feedstocks and products projection.

Designing cost-effective methods for ethanol production requires selecting the best feedstocks and defining a process configuration that converts raw materials into a finished product that meets certain requirements. Process engineering for ethanol production comprises developing new creative process designs to reduce ethanol production costs. So, before going into industrial manufacturing, ethanol production should be scaled up to check the findings (Cardona et al., 2007).

# CHAPTER 5:
# SUMMARY, CONCLUSION, AND RECOMMENDATION

The results of this study shown that the application of steam explosion pretreatment can effectively improve the fermentable sugar content in dried elephant ear plant. The batch assays were evaluated comparatively via the modified Gompertz-model based on the important fermentation parameters that characterizing the process, with a resulting value of pm 2.367 g/L and rpm 0.475 g/L*h, the model can predict the process with a confidence of $R^2$>0.95. Furthermore, the use of dry elephant ear plant as a bioenergy feedstock for bioethanol production may be a potential alternative. These results provide a better understanding on how to improve the cost, productivity, and environmental outlook of future scale-up procedures, which are all critical considerations.

The elephant ear plant, which is considered invasive, can be utilized to produce bioethanol. The physical pretreatment technique (hydrothermal and steam explosion) was used to improve cellulose enzyme accessibility and produce high sugar concentrations from fresh elephant ear plants successfully. The results revealed that the chemical composition differed across treatments. After 15 min of hydrothermal and steam explosion pretreatment, the maximum fermentable sugar concentration in the hydrolysate utilizing ash as a source of CaO in a ratio of [5:1] was 5.41 ±0.11 mg/mL, with a potential generation of ethanol of 2.76 ±0.06 mg/mL. As a result, the elephant ear plant has the potential to be an efficient bioethanol feedstock

Physical pretreatment (steam-explosion) was successfully employed to increase cellulose enzyme accessibility and produce high sugar concentrations from fresh elephant ear plant for bioethanol production. Sugar concentrations differed between treatments, according to the findings. After 15 min of steam-explosion pretreatment, the maximum fermentable sugar concentration in the hydrolysate was

4.320±0.011 mg/mL. The maximum ethanol concentration 1.841±0.263 mg/mL was reached after 24 h with a fermentation efficiency of 83.56%. Besides, the ethanol yield was estimated at 0.31 g of ethanol/ g of substrate with a sugar consumption rate of 68.28%. As a conclusion, the elephant ear plant can be a promising bioethanol feedstock.

Further experimentation is necessary to demonstrate the capacity to enhance the ethanol yield obtained at lower temperatures, which could result in a reduction in the energy required for the distillation process, which would have a direct effect on cost reduction.

Despite the performance of the dry sample, when it comes to be part of a scale up process, the best option is to pass from the harvested fresh sample instead of set to dry and storage the dry powder. Dry and storage stock ca be an option when the fresh material is not available thought the year, but since the Elephant ear plant can be cultivated during the seasons. For this reason, the fresh sample match better for the scale up, an also do not really presents a significant discrepancy compared with the dry one.

To prove the all data collected during this work, it is necessary to scale up the process and maintain the data to project the whole performance during the hydrolysis and batch fermentation.

Additional changes to the distillation process need to be applied with the goal of boosting the energy efficiency of bioethanol purification.

# APPENDICES

# APPENDIX A FIT STATISTICS

I. Steam-Explosion Pretreatment

**Table 15** Total and reducing sugars released after pretreatment for fresh sample.

| **Total sugars** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **TS g/L** | **Error** |
| | 0 | 1.118 | 1.145 | 1.132 | 0.086 | 1.132 | 0.008 |
| 1:0 | 15 | 2.079 | 2.171 | 2.408 | 0.013 | 2.219 | 0.098 |
| | 30 | 2.224 | 2.092 | 2.132 | 0.005 | 2.149 | 0.039 |
| | 0 | 1.868 | 1.697 | 1.987 | 0.011 | 1.851 | 0.084 |
| 1:5 | 15 | 1.566 | 1.645 | 1.592 | 0.003 | 1.601 | 0.023 |
| | 30 | 2.408 | 2.289 | 2.316 | 0.178 | 2.338 | 0.036 |
| | 0 | 1.868 | 1.934 | 1.961 | 0.004 | 1.921 | 0.027 |
| 1:10 | 15 | 1.066 | 1.158 | 1.092 | 0.013 | 1.105 | 0.027 |
| | 30 | 1.342 | 1.197 | 1.526 | 0.084 | 1.355 | 0.095 |
| **Reducing Sugars** | | | | | | | |
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **RS g/L** | **Error** |
| | 0 | 0.933 | 0.944 | 0.844 | 0.005 | 0.907 | 0.032 |
| 1:0 | 15 | 1.911 | 2.100 | 1.678 | 0.019 | 1.896 | 0.122 |
| | 30 | 2.044 | 2.078 | 2.011 | 0.003 | 2.044 | 0.019 |
| | 0 | 0.989 | 1.400 | 1.133 | 0.019 | 1.174 | 0.120 |
| 1:5 | 15 | 1.289 | 1.511 | 1.322 | 0.011 | 1.374 | 0.069 |
| | 30 | 1.944 | 1.133 | 0.989 | 0.046 | 1.356 | 0.297 |
| | 0 | 1.167 | 0.989 | 1.167 | 0.009 | 1.107 | 0.059 |
| 1:10 | 15 | 0.978 | 0.944 | 0.989 | 0.002 | 0.970 | 0.013 |
| | 30 | 0.967 | 0.822 | 1.011 | 0.009 | 0.933 | 0.057 |

**Table 16** Total and reducing sugars released after hydrolysis for fresh sample.

| Total Sugars | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **TS g/L** | **Error** |
| | 0 | 1.171 | 1.118 | 1.145 | 0.002 | 1.145 | 0.015 |
| 1:0 | 15 | 3.553 | 3.618 | 3.724 | 0.008 | 3.632 | 0.050 |
| | 30 | 2.987 | 2.908 | 2.882 | 0.005 | 2.925 | 0.032 |
| | 0 | 2.053 | 1.987 | 2.026 | 0.003 | 2.022 | 0.019 |
| 1:5 | 15 | 6.382 | 7.039 | 6.118 | 0.007 | 6.513 | 0.274 |
| | 30 | 6.053 | 5.724 | 5.855 | 0.005 | 5.877 | 0.096 |
| | 0 | 2.039 | 1.947 | 2.013 | 0.004 | 2.000 | 0.027 |
| 1:10 | 15 | 6.645 | 6.118 | 6.513 | 0.004 | 6.425 | 0.158 |
| | 30 | 6.513 | 6.118 | 6.316 | 0.003 | 6.316 | 0.114 |
| **Reducing Sugars** | | | | | | | |
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **RS g/L** | **Error** |
| | 0 | 0.833 | 0.989 | 1.089 | 0.012 | 0.970 | 0.074 |
| 1:0 | 15 | 3.500 | 3.611 | 3.578 | 0.005 | 3.563 | 0.033 |
| | 30 | 2.867 | 2.744 | 2.778 | 0.006 | 2.796 | 0.036 |
| | 0 | 1.533 | 1.578 | 1.500 | 0.004 | 1.537 | 0.023 |
| 1:5 | 15 | 5.222 | 5.389 | 5.611 | 0.004 | 5.407 | 0.113 |
| | 30 | 4.833 | 5.444 | 5.389 | 0.006 | 5.222 | 0.195 |
| | 0 | 1.511 | 1.611 | 1.567 | 0.005 | 1.563 | 0.029 |
| 1:10 | 15 | 5.500 | 5.111 | 5.278 | 0.004 | 5.296 | 0.113 |
| | 30 | 5.222 | 5.500 | 5.444 | 0.003 | 5.389 | 0.085 |

**Table 17** Total and reducing sugars released after pretreatment for dry sample.

| Total Sugars | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **TS g/L** | **Error** |
| | 0 | 3.395 | 3.263 | 2.961 | 0.244 | 3.206 | 0.129 |
| 1:0 | 15 | 4.079 | 4.145 | 4.066 | 0.003 | 4.096 | 0.024 |
| | 30 | 4.934 | 5.013 | 5.026 | 0.004 | 4.991 | 0.029 |
| | 0 | 3.500 | 3.224 | 3.263 | 0.011 | 3.329 | 0.086 |
| 1:5 | 15 | 2.579 | 2.368 | 2.487 | 0.008 | 2.478 | 0.061 |
| | 30 | 2.645 | 2.605 | 2.526 | 0.197 | 2.592 | 0.035 |
| | 0 | 3.526 | 3.421 | 3.513 | 0.004 | 3.487 | 0.033 |
| 1:10 | 15 | 2.618 | 2.658 | 2.487 | 0.005 | 2.588 | 0.052 |
| | 30 | 2.092 | 2.053 | 1.961 | 0.197 | 2.035 | 0.039 |
| **Reducing Sugars** | | | | | | | |
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **RS g/L** | **Error** |
| | 0 | 2.633 | 2.556 | 2.500 | 0.006 | 2.563 | 0.039 |
| 1:0 | 15 | 3.300 | 3.256 | 3.289 | 0.002 | 3.281 | 0.013 |
| | 30 | 3.678 | 3.656 | 3.722 | 0.003 | 3.685 | 0.020 |
| | 0 | 1.867 | 1.744 | 1.800 | 0.006 | 1.804 | 0.035 |
| 1:5 | 15 | 2.122 | 2.344 | 2.178 | 0.010 | 2.215 | 0.067 |
| | 30 | 2.533 | 2.422 | 2.478 | 0.005 | 2.478 | 0.032 |
| | 0 | 1.422 | 1.644 | 1.611 | 0.011 | 1.559 | 0.069 |
| 1:10 | 15 | 1.389 | 1.722 | 2.456 | 0.049 | 1.856 | 0.315 |
| | 30 | 1.556 | 1.844 | 1.767 | 0.013 | 1.722 | 0.086 |

**Table 18** Total and reducing sugars released after hydrolysis for dry sample.

| Total Sugars (After Hydrolysis) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **TS g/L** | **Error** |
| | 0 | 3.618 | 3.684 | 3.816 | 0.002 | 3.706 | 0.058 |
| 1:0 | 15 | 5.132 | 5.329 | 5.724 | 0.012 | 5.395 | 0.174 |
| | 30 | 6.382 | 6.250 | 6.513 | 0.004 | 6.382 | 0.076 |
| | 0 | 3.066 | 2.974 | 3.118 | 0.006 | 3.053 | 0.042 |
| 1:5 | 15 | 4.447 | 4.566 | 4.421 | 0.006 | 4.478 | 0.045 |
| | 30 | 4.500 | 4.395 | 4.513 | 0.005 | 4.469 | 0.037 |
| | 0 | 1.947 | 1.855 | 2.421 | 0.009 | 2.075 | 0.175 |
| 1:10 | 15 | 4.618 | 4.697 | 4.855 | 0.007 | 4.724 | 0.070 |
| | 30 | 4.961 | 5.026 | 4.987 | 0.003 | 4.991 | 0.019 |
| **Reducing Sugars** | | | | | | | |
| **Ratio** | **time (min)** | **R1** | **R2** | **R3** | **SD** | **RS g/L** | **Error** |
| | 0 | 3.056 | 2.833 | 3.222 | 0.006 | 3.037 | 0.113 |
| 1:0 | 15 | 4.722 | 4.611 | 5.389 | 0.008 | 4.907 | 0.243 |
| | 30 | 6.056 | 6.167 | 5.833 | 0.003 | 6.019 | 0.098 |
| | 0 | 2.456 | 2.400 | 2.389 | 0.003 | 2.415 | 0.021 |
| 1:5 | 15 | 4.000 | 4.389 | 3.833 | 0.005 | 4.074 | 0.165 |
| | 30 | 4.389 | 3.778 | 4.500 | 0.007 | 4.222 | 0.225 |
| | 0 | 1.711 | 1.733 | 1.689 | 0.002 | 1.711 | 0.013 |
| 1:10 | 15 | 4.278 | 4.389 | 4.111 | 0.003 | 4.259 | 0.081 |
| | 30 | 4.333 | 4.183 | 4.056 | 0.003 | 4.191 | 0.080 |

**Table 19** Total and reducing sugars during fermentation (700 mL) for dry sample.

| **Total Sugar** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **ABS** | | | **g/L** | | | | | |
| | **A1** | **A2** | **A3** | **R1** | **R2** | **R3** | **Error** | **SD** | **TS g/L** |
| Pretreatment | 0.489 | 0.469 | 0.498 | 6.520 | 6.253 | 6.640 | 0.114 | 0.015 | 6.471 |
| Hydrolysis | 0.596 | 0.598 | 0.595 | 7.947 | 7.973 | 7.933 | 0.012 | 0.002 | 7.951 |
| 24 | 0.364 | 0.353 | 0.335 | 4.853 | 4.707 | 4.467 | 0.113 | 0.015 | 4.676 |
| 48 | 0.304 | 0.316 | 0.302 | 4.053 | 4.213 | 4.027 | 0.058 | 0.008 | 4.098 |
| 72 | 0.244 | 0.246 | 0.240 | 3.253 | 3.280 | 3.200 | 0.024 | 0.003 | 3.244 |
| 96 | 0.232 | 0.234 | 0.233 | 3.093 | 3.120 | 3.107 | 0.008 | 0.001 | 3.107 |
| 120 | 0.201 | 0.207 | 0.204 | 2.680 | 2.760 | 2.720 | 0.023 | 0.003 | 2.720 |
| 144 | 0.190 | 0.195 | 0.188 | 2.533 | 2.600 | 2.507 | 0.028 | 0.004 | 2.547 |
| **Reducing Sugar** | | | | | | | | | |
| | **ABS** | | | **g/L** | | | | | |
| | **A1** | **A2** | **A3** | **R1** | **R2** | **R3** | **Error** | **SD** | **RS g/L** |
| Pretreatment | 0.382 | 0.384 | 0.372 | 3.820 | 3.840 | 3.720 | 0.037 | 0.006 | 3.793 |
| Hydrolysis | 0.598 | 0.586 | 0.589 | 5.980 | 5.860 | 5.890 | 0.036 | 0.006 | 5.910 |
| 24 | 0.329 | 0.337 | 0.317 | 3.290 | 3.370 | 3.170 | 0.058 | 0.010 | 3.277 |
| 48 | 0.218 | 0.213 | 0.225 | 2.180 | 2.130 | 2.250 | 0.035 | 0.006 | 2.187 |
| 72 | 0.169 | 0.177 | 0.167 | 1.690 | 1.770 | 1.670 | 0.031 | 0.005 | 1.710 |
| 96 | 0.157 | 0.162 | 0.151 | 1.570 | 1.620 | 1.510 | 0.032 | 0.006 | 1.567 |
| 120 | 0.130 | 0.141 | 0.139 | 1.300 | 1.410 | 1.390 | 0.034 | 0.006 | 1.367 |
| 144 | 0.098 | 0.096 | 0.101 | 0.980 | 0.960 | 1.010 | 0.015 | 0.003 | 0.983 |

**Table 20** Total and reducing sugars during fermentation (7L) for dry sample.

| **Total Sugar** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **ABS** | | | **g/L** | | | | | |
| | **A1** | **A2** | **A3** | **R1** | **R2** | **R3** | **Error** | **SD** | **TS g/L** |
| Pretreatment | 0.472 | 0.514 | 0.469 | 6.293 | 6.853 | 6.253 | 0.194 | 0.025 | 6.467 |
| Hydrolysis | 0.684 | 0.642 | 0.666 | 9.120 | 8.560 | 8.880 | 0.162 | 0.021 | 8.853 |
| 24 | 0.398 | 0.392 | 0.448 | 5.307 | 5.227 | 5.973 | 0.237 | 0.031 | 5.502 |
| 48 | 0.298 | 0.312 | 0.308 | 3.973 | 4.160 | 4.107 | 0.056 | 0.007 | 4.080 |
| 72 | 0.244 | 0.246 | 0.240 | 3.253 | 3.280 | 3.200 | 0.024 | 0.003 | 3.244 |
| 96 | 0.232 | 0.234 | 0.233 | 3.093 | 3.120 | 3.107 | 0.008 | 0.001 | 3.107 |
| 120 | 0.201 | 0.207 | 0.204 | 2.680 | 2.760 | 2.720 | 0.023 | 0.003 | 2.720 |
| 144 | 0.190 | 0.195 | 0.188 | 2.533 | 2.600 | 2.507 | 0.028 | 0.004 | 2.547 |
| **Reducing Sugar** | | | | | | | | | |
| | **ABS** | | | **g/L** | | | | | |
| | **A1** | **A2** | **A3** | **R1** | **R2** | **R3** | **Error** | **SD** | **RS g/L** |
| Pretreatment | 0.443 | 0.444 | 0.398 | 4.430 | 4.440 | 3.980 | 0.152 | 0.026 | 4.283 |
| Hydrolysis | 0.628 | 0.625 | 0.713 | 6.280 | 6.250 | 7.130 | 0.288 | 0.050 | 6.553 |
| 24 | 0.336 | 0.305 | 0.317 | 3.360 | 3.050 | 3.170 | 0.090 | 0.016 | 3.193 |
| 48 | 0.260 | 0.245 | 0.272 | 2.600 | 2.450 | 2.720 | 0.078 | 0.014 | 2.590 |
| 72 | 0.169 | 0.177 | 0.167 | 1.690 | 1.770 | 1.670 | 0.031 | 0.005 | 1.710 |
| 96 | 0.157 | 0.162 | 0.151 | 1.570 | 1.620 | 1.510 | 0.032 | 0.006 | 1.567 |
| 120 | 0.130 | 0.141 | 0.139 | 1.300 | 1.410 | 1.390 | 0.034 | 0.006 | 1.367 |
| 144 | 0.098 | 0.096 | 0.101 | 0.980 | 0.960 | 1.010 | 0.015 | 0.003 | 0.983 |

**Table 21** Ethanol production from dry sample (700 mL).

| | % | | | g/L | | | | | Ethanol g/L | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | A1 | A2 | A3 | R1 | R2 | R3 | Error | SD | Predicted | Real |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0 | 0 | 0 |
| 24 | 0.3 | 0.3 | 0.3 | 2.367 | 2.367 | 2.367 | 0.2 | 0.000 | 3.014 | 2.367 |
| 48 | 0.2 | 0.3 | 0.2 | 1.578 | 2.367 | 1.578 | 0.1 | 0.058 | 1.671 | 1.841 |
| 72 | 0.2 | 0.2 | 0.2 | 1.578 | 1.578 | 1.578 | 0.2 | 0.000 | 1.115 | 1.578 |
| 96 | 0.2 | 0 | 0.1 | 1.578 | 0 | 0.789 | 0.1 | 0.100 | 0.872 | 0.789 |
| 120 | 0.1 | 0 | 0 | 0.789 | 0 | 0 | 0.1 | 0.058 | 0.799 | 0.263 |
| 144 | 0.1 | 0 | 0 | 0.789 | 0 | 0 | 0.1 | 0.058 | 0.697 | 0.263 |

**Table 22** Ethanol production from dry sample (7L).

| | % | | | g/L | | | | | Ethanol g/L | |
|---|---|---|---|---|---|---|---|---|---|---|
| Time | A1 | A2 | A3 | R1 | R2 | R3 | Error | SD | Predicted | Real |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 24 | 0.4 | 0.3 | 0.3 | 3.156 | 2.367 | 2.367 | 0.263 | 0.058 | 3.342 | 2.63 |
| 48 | 0.3 | 0.3 | 0.3 | 2.367 | 2.367 | 2.367 | 0 | 0.000 | 1.629 | 2.367 |
| 72 | 0.3 | 0.3 | 0.2 | 2.367 | 2.367 | 1.578 | 0.263 | 0.058 | 1.321 | 2.104 |
| 96 | 0.2 | 0.2 | 0.1 | 1.578 | 1.578 | 0.789 | 0.263 | 0.058 | 0.872 | 1.315 |
| 120 | 0.1 | 0.2 | 0.1 | 0.789 | 1.578 | 0.789 | 0.263 | 0.058 | 0.799 | 1.052 |
| 144 | 0.1 | 0 | 0 | 0.789 | 0 | 0 | 0.263 | 0.058 | 0.697 | 0.263 |

# APPENDIX B PUBLICATIONS

## Environment, Development and Sustainability

A Multidisciplinary Approach to the Theory and Practice of Sustainable Development

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Environment, Development and Sustainability is an international, multidisciplinary journal covering all aspects of the environmental impacts of socio-economic development. Concerned with the complex interactions between development and environment, its purpose is to seek ways and means for achieving sustainability in all human activities aimed at such development. Coverage includes interactions among society, development and environment, and their implications for sustainable development; technical, economic, ethical and philosophical aspects of sustainable development; local, regional and global sustainability and their practical implementation; development and application of indicators of sustainability; development, verification, implementation and monitoring of policies for sustainable development; sustainable use of land, water, energy and biological resources in development; impacts of agriculture and forestry activities on soil and aquatic ecosystems and biodiversity, and much more. See Aims and Scope for more details. — show all

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| | | |
|---|---|---|
| **3.219 (2020)** Impact factor | **75 days** Submission to first decision | **567,774 (2020)** Downloads |
| **3.118 (2020)** Five year impact factor | | |

Environment, Development and Sustainability
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# Advancement of fermentable sugars from fresh elephant ear plant weed for efficient bioethanol production

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**Abstract**
Bioethanol is considered one of the most promising next-generation automotive fuels, as it is carbon neutral and can be produced from renewable resources, like lignocellulosic materials. The present research investigation aimed to utilize the elephant ear plant, a hazardous plant (weed) also considered an invasive species, as a font of non-edible lignocellulosic biomass for bioethanol production. The freshly collected elephant ear plant (leaves and stalk) was chopped into small pieces (1–2 cm) and then homogenized to a paste using a mechanical grinder. The sample pretreatment was done by flying ash for three different time durations ($T1 = 0$ min, $T2 = 15$ min, and $T3 = 30$ min) with 3 replications. All treatment samples were measured for total sugar and reducing sugar content. The concentration of reducing sugar archived was $T1 = 0.771 \pm 0.1$ mg/mL, $T2 = 0.907 \pm 0.032$ mg/mL, and $T3 = 0.895 \pm 0.039$ mg/mL, respectively. The results revealed that the chemical composition was different among treatments. The hydrolysis was performed using cellulase enzymes at 35 °C for the hydrolysis process. The hydrolysate was inoculated with 1% of *S. cerevisiae* and maintained at room temperature without oxygen for 120 h. Bioethanol concentration was measured by using an ebulliometer. The efficient ethanol percentage was $1.052 \pm 0.03$ mg/mL achieved after the fermentation. Therefore, the elephant ear plant invasive weed could be an efficient feedstock plant for future bioethanol production.

**Keywords** Elephant ear plant · Total sugar · Reducing sugar · Hydrolysis · Fermentation

## 1 Introduction

Globally, derived fossil fuels are the primary energy source, especially in the transportation sector (Bhuyar et al., 2021; Ramaraj et al., 2021a, b). Consequently, the greenhouse gases released into the atmosphere have increased 1.4 per cent per year on average, according

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to the UNEP (United Nations, 2020), contributing to environmental pollution and global warming. Therefore, the search for new energy alternatives, environmentally friendly and derived from renewable sources, has become crucial. New technologies for social-economical interactions and rapid urbanization, and industrial expansion make energy vital in the daily life of all people (Cruz et al., 2018). The world economy is heavily dependent on fossil fuels such as oil, coal, natural gas, primary commercial energy, and non-renewable sources (Ramaraj et al., 2016). The worldwide consumption of fossil fuels intensified greenhouse gas emissions released to the atmosphere and all the climate changes promoted by global warming (Dussadee et al., 2014; Ramaraj & Dussadee, 2015). In this context, biofuels are an emerging alternative to liquid fuels due to their high energy content and significantly less $CO_2$ emissions associated with their use (Dussadee et al., 2016). Bioethanol is a potential alternative fuel due to its properties in comparison with gasoline, such as higher flame speed, higher heats of vaporization, and higher-octane number, which makes it an antiknock fuel, are some of the main reasons to encourage its production (Gavahian et al., 2019; Vu et al., 2017).

According to the International Energy Agency (IEA), in 2019, globally fuel ethanol production reached 115 billion L. However, the COVID-19 crisis causes global bioethanol production to drop 15% in 2020, the first contraction in biofuel output in two decades. Despite the fact that biofuels are predicted to meet around 5.4% of road transport energy demand in 2025, and it was up from just under 4.8% in 2019. Bioethanol output is expected to reach 119 billion liters in 2023–25, with Brazil, China, and India serving as key growth areas (IEA, 2019). Meanwhile, in Thailand, conventional Thai power generation starts giving alternative sources with the cost reduction of variable energy. As a result, during 2023–25, the average bioethanol yearly production in Thailand of 2.4 billion litres is expected.

Bioethanol can be produced from several different biomass sources (Manmai et al., 2019, 2020a, b; Nguyen et al., 2020). The first biofuel produced from food-based crops, or first-generation bioethanol, involves feedstocks like sucrose from sugarcane in Brazil or starch, mainly from corn, in the USA (Duden et al., 2021; Kumar, 2011). However, even though first-generation bioethanol is being produced commercially in several countries, edible biomass encountered resistance due to the limited stock and the food versus fuel argument. Therefore, there has been a great effort in exploring alternatives feedstocks for second-generation bioethanol production based on lignocellulosic biomass. Lignocellulosic biomass is usually referred to as non-edible crops, agriculture, forestry residues, aquatic plants, and it is considered one of the most abundant renewable biomass sources on earth (Bhuyar et al., 2020; Khammee et al., 2021). The complex and recalcitrant structure of lignocellulosic biomass comprises cellulose, hemicellulose, and lignin, including water in small amounts and some trace amounts of protein, minerals, and other components of raw material (Khammee et al., 2019; Nong et al., 2020; Unpaprom et al., 2021; Van Tran et al., 2020). Lignocellulosic biomass is usually referred to as non-edible crops, agriculture and forestry residues, aquatic plants, and it is considered one of the most abundant renewable biomass sources on earth (Phukoetphim et al., 2017; Ramaraj et al., 2021a, b; Sharma et al., 2020).

The Araceae family of plants, which contains over 1800 know species, has been described as the most common cause of symptomatic plant ingestion in some countries (Atkins & Williamson, 2008). Most species in the family contain raphine (calcium oxalate) crystals which are needle-shaped and arranged in compact bundles (Frohne & Pfänder, 1997; Krenzelok & Jacobsen, 1997). Upon chewing of the plant, the crystals are ejected from specialized explosive ejector cells (idioblasts). As a result, they may become lodged

in the lining of the mouth, tongue, and throat leading to local inflammatory reactions, including burning, irritation, and oedema of the buccal cavity, hypersalivation, and aphonia (Kuballa et al., 1981; Wiese et al., 1996). The elephant ear plant, a member of the Arum family (Araceae), is a tuberous, stemless, frost-tender aquatic and semi-aquatic herbaceous species. The plant is a perennial capable of producing considerable (60 cm length and 35 cm width) leaves on 1–2.5 m petioles (Weber, 2017) that emanate from a good corm. Under ideal growing conditions, a single elephant ear plant can grow 2.4 m tall with a similar spread in width. Reproduction of the elephant ear is primarily vegetative, rarely by seed, and occurs when whole corms divide in winter or early spring (Atkins & Williamson, 2008; Kikuta et al., 1938). Thus, only a portion of the crown and petiole is needed to establish a new plant. The invasive weed utilization for bioenergy generation is the novel approach towards renewable energy. The present investigation aimed to use the elephant ear plant, a hazardous plant also considered an invasive species, as a font of non-edible lignocellulosic biomass for bioethanol production. The bioethanol production was done, followed by pretreatment and hydrolysis techniques. The alcohol determination was done by ebulliometer.

## 2 Materials and methods

### 2.1 Plant collection and sample preparation

The elephant ear plant is a common weed that grows near the water bodies like canals, lakes, puddles, and rivers. Samples of elephant ear plants were collected from Maejo University located at Sansai-Phrao Road, Nongharn, Sansai District, Chiang Mai, 50,290 Thailand, and transferred to the Faculty of Science's laboratory. Collected samples (leaves and kernel) were washed with tap water to remove impurities and chopped into small pieces (1–2 cm) and then homogenized to a paste using a mechanical grinder (PHILIPS Blender 600 W Model HR2118/02).

### 2.2 Pretreatment and hydrolysis

In the pretreatment step, a total of 50 g of the homogenized fresh elephant ear plant was taken in a 1000 mL graduated bottle mixed with 500 mL of distiller water; this mixture was undergone autoclaving apparatus at 121 °C, 15 psi, at different time durations ($T1 = 0$ min, $T2 = 15$ min, and $T3 = 30$ min). After pretreatment, the pH of the combined solution was adjusted at $5.0 \pm 0.3$, and the samples were inoculated with 1% commercial cellulase (Union Science, Pvt. Ltd., Chiang Mai, Thailand) for the hydrolysis process. Afterwards, the solution was kept in an incubator at 35 °C for 24 h to perform the hydrolysis. Figure 1 shows the elephant ear plant (leaves and stalk) collected and homogenized and the mixture before and after the hydrolysis step.

### 2.3 Fermentation

After physicochemical hydrolysis, fermentation was performed. The fermentation protocol was followed as described by Khammee et al. (2020). The pH of the hydrolysate solution was adjusted at $5.6 \pm 0.3$ before being inoculated with 1% (wt./v) of *Saccharomyces*

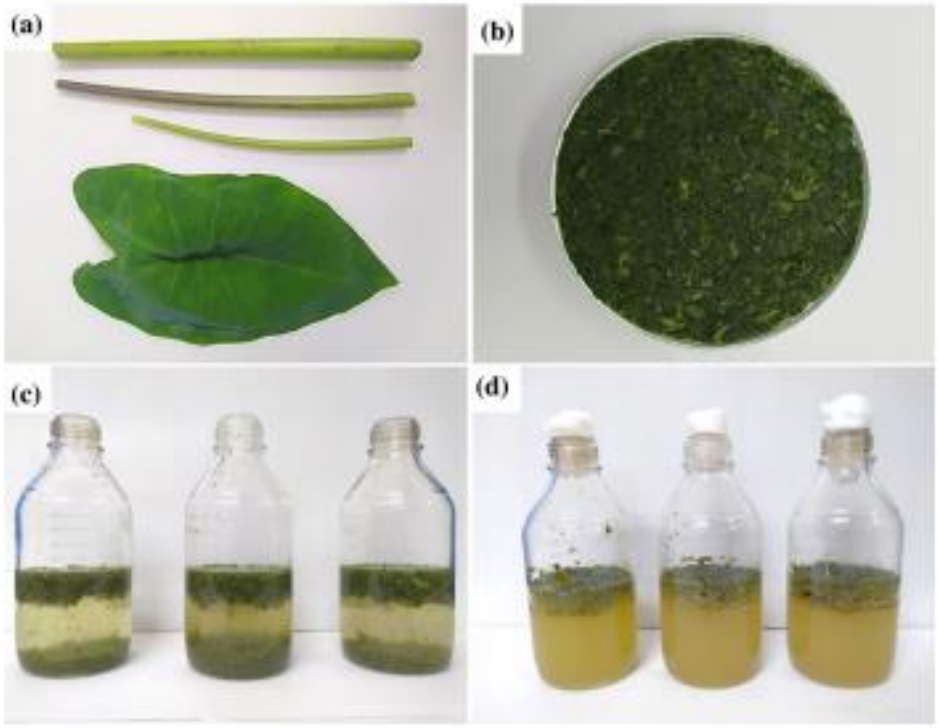


**Fig. 1** **a** Elephant ear plant collected, **b** elephant ear plant homogenized and **c** mixed with water to proceed with the pretreatment, and **d** mixture after hydrolysis process

*cerevisiae*. The fermented mixture was kept at room temperature in the absence of oxygen for 120 h. The fermentation was carried out for 5 days and monitored by withdrawing 80 mL of the sample every 24 h for sugars and alcohol measurement. The alcohol measurement was carried out by using an ebulliometer.

### 2.4 Total and reducing sugar assay

A UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) was used to estimate sugars concentration through spectrometry. Total sugars and reducing sugars were determined by the phenol sulfuric acid method and the dinitrosalicylic acid (DNS) method (Dubois et al., 1956; Miller, 1959; Saengsawang et al., 2020). For total sugar determination, 500 µL of phenol solution (5% w/v) was added to 500 µL of the sample. The mixture was homogenized and followed by the addition of 2.5 mL of the con. sulfuric acid ($H_2SO_4$ at 98%). The sample was submerged in water for 10 min and then homogenized using a vortex, and the absorbance was read at 540 nm using distilled water as control. Meanwhile, 500 µL of DNS reagent was added to 500 µL of the sample for reducing sugars. The mixture was put in a boiling water bath for 10 min. Then, it was cooled, and 4 mL of distiller water was added. The absorbance was read at 540 nm using distilled water as control. All the procedures for reducing sugars determination were done under dark conditions due to the photosensitive nature of the DNS reagent. For both total sugars and reducing sugars, a

standard curve was generated using standard D-Glucose solution to derive the concentration of an unknown sample in mg/mL.

### 2.5 Alcohol determination

Ethanol content measurement was carried out using an ebulliometer (Dujardin-Salleron, Alcohol Burner, France). Ebulliometer is based on the principle that the boiling point of an alcoholic mixture is depressed compared to the boiling point of water due to the alcohol content in the alcoholic mixture (Olson, 1989). Alcohol analysis was performed by using the ebulliometer chamber which was filled with 50 mL of sample and boiled until a steady temperature. The resulting distiller water boiling point was used to compare the ebulliometer disc provided with the apparatus.

### 2.6 Statistical analysis

Statistical analysis was performed using Statgraphics Centurion 19. For the present study, three replicates for all experiments were conducted. Data were shown as mean ± SE from triplicate. A significant difference was examined at the level of $p < 0.05$.

## 3 Results and discussion

### 3.1 Physical pretreatment and enzymatic hydrolysis

In this study, hydrothermal and steam explosion pretreatment to fresh elephant ear plants was investigated for three different time durations ($T1 = 0$ min, $T2 = 15$ min, and $T3 = 30$ min). The pretreatment with the highest total sugar concentration was selected to perform the hydrolysis step. The hydrolysis process converts total sugars to reducing (fermentable) sugar by breaking the polysaccharides into monosaccharides. The highest fermentable sugar content sample was utilized for the fermentation process. Samples were analyzed before and after the pretreatment and hydrolysis step to study the changes in total sugar and reduce sugar (mg/mL). Figure 2 representing the pictorial representation of the pretreatment process.

Figures 3a and b displayed total sugar results and reduced sugar at three different time durations. The concentration of reducing sugar archived was $T1 = 0.771 \pm 0.1$ mg/mL, $T2 = 0.907 \pm 0.032$ mg/mL, and $T3 = 0.895 \pm 0.039$ mg/mL, respectively. Meanwhile, the concentration of fermentable sugar after enzyme hydrolysis procedure was $T1 = 0.838 \pm 0.033$ mg/mL, $T2 = 1.130 \pm 0.042$ mg/mL, $T3 = 1.067 \pm 0.013$ mg/mL as shows Fig. 2. $T2$ presented the highest concentration of reducing sugars compared with $T1$ and $T3$.

Results revealed that the pretreatment of 15 min steam explosion results in higher fermentable sugars. Therefore, the $T2$ condition was selected to perform the fermentation procedure. Table 1 illustrating the literature survey of various plant weeds utilized for bioethanol production with different pretreatment and hydrolysis protocols. It was reported that after dilute acid pretreatment, hemicellulose disintegrates, and xylose is released into solution, whereas alkaline pretreatment preserves a portion of hemicellulose while removing most of the lignin component (Aswathy et al., 2010; Lin et al., 2016). The combination microbial-chemical method could significantly boost the generation of reducing sugars in

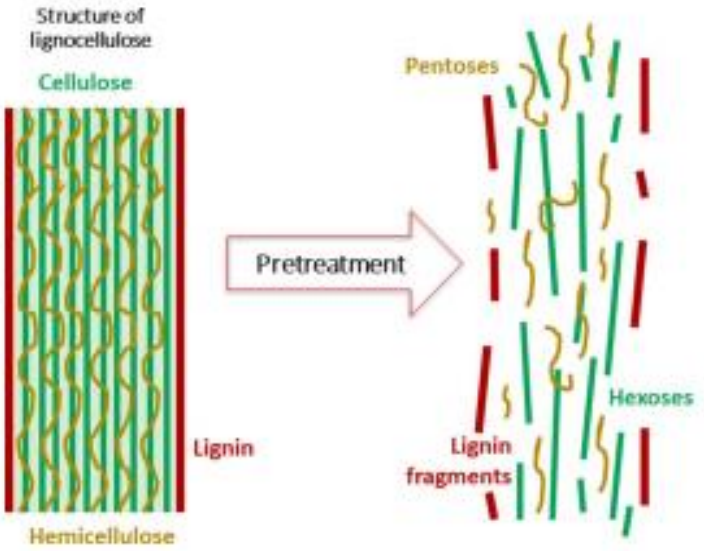


**Fig. 2** Pictorial representation of pretreatment

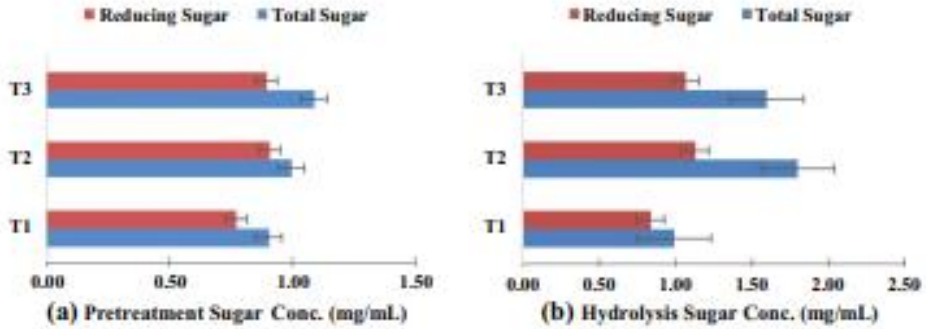


**Fig. 3** The concentration of sugar at three different pretreatment times and after the enzyme hydrolysis step **a** pretreatment and **b** after hydrolysis

water hyacinth hydrolysates compared to a single MB method (Zhang et al., 2018). However, as with other cellulosic bioethanol feedstocks, such as herbaceous grasses and agriculture or forestry residues, aquatic and semi-aquatic plants require a pretreatment step, followed by a hydrolysis and fermentation process as a general method for bioethanol production (Isarankura-Na-Ayudhya et al., 2007; Taherzadeh & Karimi, 2008; Whangchai et al., 2021).

### 3.2 Bioethanol production

The hydrolysate mixture obtained from T2 was undergone a fermentation process using 1% wt./v of *S. cerevisiae* (dry yeast). The bioethanol production was monitored for 5 days at room temperature, and a sample was withdrawn each 24 h to record the bioethanol concentration. Results are shown in Fig. 4. After 48 h, the fermentable sugars were

**Table 1** Comparison of various pretreatment utilized to produce bioethanol

| Feedstock | Methodology | Ethanol yield | Refences |
|---|---|---|---|
| Water hyacinth | Fermentation by Malt and Barley (5%, 10% and equal %) for 7 days at 30 °C | 1.019 mg/L | Rezania et al. (2014) |
| Pistia stratiotes | Physical pretreatment by milling the sample to 0.2–2 mm size<br>Fermentation by *S. cerevisiae* for 7 days | 0.205 mg/mL | Sunil anic et al. (2015) |
| Alternanthera sessilis | Acid hydrolysis by $H_2SO_4$<br>Fermentation by *S. cerevisiae* for 7 days | 0.387 mg/mL | |
| Parthenium hysterophorus | Steam explosion pretreatment for 15 min<br>Enzymatic hydrolysis by *A spergillus niger* for 24 h at 40 °C<br>Fermentation by *S. cerevisiae* for 96 h days a 30 °C | 0.219 mg/mL | Gupta and et al. (2017) |
| Elephant ear plant | Steam explosion pretreatment for 15 min<br>Hydrolysis was conducted by cellulases for 24 h at 35 °C<br>Fermentation by *S. cerevisiae* for 5 days at room temperature (30 ± 5 °C) | 1.130 mg/mL | This study |

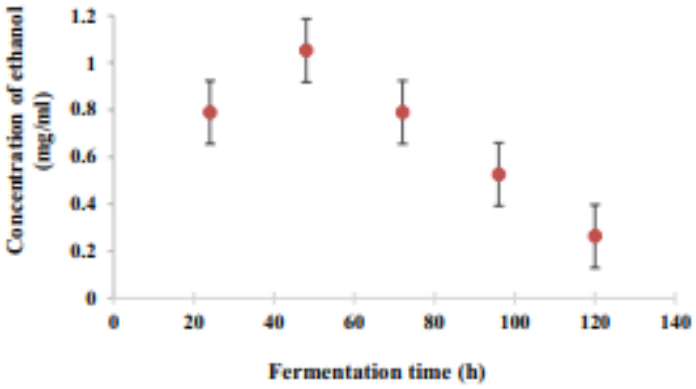


**Fig. 4** Bioethanol production from fresh elephant ear plant from 120 h

recorded as $0.826 \pm 0.02$, at which the highest bioethanol concentration was reached at $1.052 \pm 0.03$ mg/mL. As the sugars were exhausted gradually, the ethanol production was reduced, as displayed in Fig. 4.

The fermenting microorganisms play an essential role in bioethanol production from lignocellulosic biomasses by fermenting a wide range of sugars to ethanol (Dien et al., 2003). Compared to other types of microorganisms, yeast is the most common microbe employed in ethanol production. *S. cerevisiae* is the widely used fermenting yeast due to its high ethanol productivity, high ethanol tolerance, and the ability to ferment a wide range of sugars (Azhar et al., 2017). In addition, the recombinant microorganisms would improve ethanol production from aquatic plants with high hemicellulose content, which can be transformed into a mixture of pentoses and hexoses by saccharification processes (Mishima et al., 2008).

Over recent years, biotechnological advances in the production of bioethanol from aquatic plants have been demonstrated. The use of aquatic and semi-aquatic plants as a renewable energy source presents advantages, such as an absence of competition against food crops for arable land (Mishima et al., 2006, 2008). The elephant ear plant is a lignocellulose source that possesses a rapid growth rate, with a minimal fertilizer needed, and does not compete for arable land, which is attributed to an ideal biofuel feedstock (Low et al., 2011; Miranda et al., 2016). Compared to wood and other lignocellulosic biomass (agro- and forest residue), aquatic and semi-aquatic weeds can be readily hydrolyzed to fermentable sugars and provide an efficient and cost-effective feedstock for renewable energy production, like biofuels. While minimizing the economic and ecological damage caused by their rapid undesired growth can be impressively utilized for the enhanced bioenergy generation (Borah et al., 2016; Rather & Bhagat, 2021).

## 4 Conclusions

The elephant ear plant, a member of the Arum family (Araceae), is an emergent aquatic and semi-aquatic herbaceous species. The elephant ear plant, considered an invasive species, can be used to produce bioethanol. The physical pretreatment process (hydrothermal and steam explosion) was applied with significant success to enhance the accessibility of

enzyme and the high sugar concentration achieved. The results revealed that the chemical composition differed across treatments. The steam explosion for 15 min (*T2*) is ideal and resulted in the enhanced fermentable sugars. The fermentation was initiated by infecting the hydrolysate with 1% *S. cerevisiae* and maintained at room temperature without oxygen for 120 h. The efficient ethanol percentage was $1.052 \pm 0.03$ mg/mL achieved after the fermentation. The 48 h of fermentation is an ideal period to produce enhanced ethanol. Thus, the elephant ear plant has the potential to be an efficient feedstock plant for bioethanol production.

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# Study of the influence of different pretreatment conditions on the release of sugar from dried elephant ear plant

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**Abstract.** The synthesis of ethanol from dried elephant ear plant was investigated in this study. The effects of a combination of steam explosions at different times (0 minutes, 15 minutes, and 30 minutes) and CaO obtained from fly ash at different ratios [0:1, [5:1,] and [10:1] on the formation of CaO was evaluated. The most favorable conditions were chosen to proceed with fermentation followed by distillation. After 24 h, the ethanol concentration reached its highest level of 2.6310.821 g/L, indicating a fermentation efficiency of 71.82 % and a sugar consumption of 59.48 %. By utilizing a simple distillation method with a temperature of 60°C in the heater, ethanol was recovered with a yield of 9.351 %. Finally, the kinetic model developed for the fermentation accurately describes the process with a confidence level of R2>0.95, and a potential maximum ethanol production ($p_m$) of 2.367 g/L as the result of the fermentation.

**Keywords:** Elephant ear plant, Enzymatic hydrolysis, Total sugars, Reducing sugars, Bioethanol

**Graphical Abstract.**


## 1 Introduction

The world's rising issues are irreversible depletion of nonrenewable resources such as fossil fuels (Bhuyar et al., 2021). The accumulation of hazardous emissions from cars contributes to the greenhouse effect and global warming (Ramaraj et al., 2021). The world is in critical need of alternate kinds of energy that pose little or no environmental risk. Weed plants, due to their lignocellulosic content, prove to be an essential feedstock material (Trejo et al., 2021). Lignocellulosic biomass can serve as a replacement for traditional energy sources because of its sustainable physiognomies and its economic position in the face of rising energy demand (Khammee et al., 2021). The lignocellulosic source can save the world from the impending energy problem through several conversion methods (Gavahian et al., 2019; Vu et al., 2017).

Biofuel has garnered considerable attention in the research world because of its potential to usher in a new era of biofuel use (Saengsawang et al., 2020). Biofuel generation from renewable sources

can help to minimize reliance on fossil fuels while also promoting environmental and economic sustainability (Bhuyar et al., 2020). Biofuels are non-toxic, biodegradable, and produce less greenhouse gas emissions than traditional fossil fuels when used in combustion engines (Borah et al., 2016). The Renewable Energy Directive defines biofuel as "liquid or gaseous fuel for transportation derived from biomass". Biofuels are mostly bioethanol and biodiesel. Bioethanol is an alcohol-based fuel produced by fermenting and distilling starch-, sugar-, and lignocellulose-based materials (Cunha et al., 2020). In contrast, biodiesel is typically a mixture of fatty acid alkyl monoesters produced by chemical transesterification of triglycerides from vegetable oils and fats with similar structures to Petro diesel (Cruz et al., 2018).

Biofuel can be classified into three generations based on the feedstock: first, second, and third. Even though first-generation bioethanol is being produced commercially in several countries, edible biomass has experienced resistance due to scarcity and the food vs fuel debate (Bhuyar et al., 2020; Whangchai et al., 2021). As a result, much effort has been spent researching alternate feedstocks for second-generation bioethanol production based on lignocellulosic biomass (Phukoetphim et al., 2017). Non-edible crops, agriculture, forestry wastes, and aquatic plants are examples of lignocellulosic biomass, one of the most abundant renewable biomass sources on the planet (Sharma et al., 2020). Non-edible crops, agricultural and forestry leftovers, and aquatic plants are examples of lignocellulosic biomass, which is regarded as one of the most plentiful renewable biomass sources on the planet (Ramaraj et al., 2021).

The common name for a group of tropical perennial plants grown for their big, heart-shaped leaves is "elephant ears." The majority of these beautiful herbaceous species in the arum or aroid family

(Araceae) belong to the genera Colocasia, Alocasia, and Xanthosoma, while others have similar looks growing habits (Atkins & Williamson, 2008). The leaves are edible; however, they (and all plant sections) contain needle-like crystals of calcium oxalate that irritate the skin; thus, they must be boiled first (Frohne & Pfänder, 1997; Krenzelok & Jacobsen, 1997). Thus, they may become trapped within a person's oral cavity, resulting in inflammation of the buccal cavity and hypersalivation and an inability to speak (Kuballa et al., 1981; Wiese et al., 1996). There are two types of elephant ears: a tuberous, stemless, frost-sensitive aquatic or semi-aquatic herbaceous species known as Araceae (Arum). A single elephant ear plant can reach 2.4 m in height and spread out to a similar width in perfect conditions (Trejo et al., 2021). The utilization of invasive weeds for bioenergy generation represents an innovative method for developing renewable energy.

The current experiment sought to determine whether the elephant ear plant, a potentially dangerous plant that is also considered an invasive species, may be used as a source of non-edible lignocellulosic biomass for use in bioethanol manufacturing. The experiment aimed to bioethanol production by applying pretreatment and hydrolysis procedures. The secondly the fermentation was employed for efficient bioethanol generation followed by distillation by Soxhlet apparatus. The finally the economic survey was carried out to prove the effectivity of the ethanol production.

## 2 Material and Methods

### *2.1 Sample collection and preparation*

Fresh elephant ear plant was collected at Maejo University installations (18°53'46.5"N 99°01'05.5"E). Leaves and stalk were taken to the laboratory and washed with tap water to remove the impurities. Then, the sample was chopped into small pieces (1 to 2 cm) and dried using a solar dryer for three days. Finally, the dried elephant ear plant was pulverized by using a mechanical

blender (PHILIPS Blender 600W Model HR2118/02). The powder was stored for further experiments.

*2.2 Sample characterization*

Elephant ear plant was under characterization by measuring moisture content (mc%), pH, total sugars (TS), reducing sugars (RS), and energy value. All the test were prepared by triplicate.

*2.2.1 Moisture content*

Moisture content was determined by the hot air oven method. Fresh elephant ear plant sample was chopped into small pieces (1 to 2 cm) and blended until a paste consistency using a mechanical blender (PHILIPS Blender 600W Model HR2118/02) and 5 g was used to determine moisture content. The sample was heated in a forced air oven at 130±5 °C for 2 h (Miah et al., 2002). Wet basis moisture content was measured using the following equation:

$$mc\% = \left[1 - \left(\frac{dry\ sample\ (g)}{wet\ sample\ (g)}\right)\right] \times 100 \quad (1)$$

*2.2.2 pH determination*

Wet and dry sample were measured for pH. For the analysis, 20 g of sample were weighted and transferred to a 50-mL beaker, 20 mL of distilled water were added, the suspension was covered, and continuously stirred for 5 min. The suspension was left to stand for about 1 h to allow most of the suspended clay to settle out from the suspension or filter or centrifuge off the aqueous phase for pH measurement. The supernatant was measure for pH using a potentiometer (Apera PH700 Benchtop) (USEPA, 2004).

110

111 *2.2.3 Sugars content*

112 Spectrometry was utilized to determine sugar concentrations using a UV-Spectrophotometer
113 detector DV-8000 (Drawell, Osaka, Japan). The quantification of total sugars was carried out
114 following the phenol-sulfuric acid method, while the estimation of reducing sugars was done by
115 the 3,5-dinitrosalicylic acid (DNS) method (Dubois et al., 1956; Miller, 1959).

116

117 *2.2.4 Energy value*

118 The estimation of the energy value was calculated according to the Atwater factor 17 kJ/g (4.0
119 kcal/g) for carbohydrate content (Atwater and Woods, 1896). The system is based on the heats of
120 combustion of protein, fat and carbohydrate, which are corrected for losses in digestion, absorption
121 and urinary excretion of urea. It uses a single factor for each of the energy-yielding substrates
122 (protein, fat, carbohydrate), regardless of the source in which it is found (Southgate and Durnin,
123 1970).

124

125 *2.3 Physicochemical pretreatment*

126 Fly ash was used as source of CaO for the alkaline pretreatment. A solution was prepared by
127 mixing 200g of flying ash with 1L of distilled water. The ash solution was mixed at different ratios
128 (0:1, 5:1, and 10:1) with 5g of elephant ear plant powder. Then, the mixture was under steam
129 explosion at different times of exposure (0 min, 15 min, and 30 min) using autoclave apparatus.
130 Experiments were done by triplicate to conduct the experimental arrangements described in Figure
131 1, and the combination with the higher fermentable sugar was chosen to continue with hydrolysis
132 step.

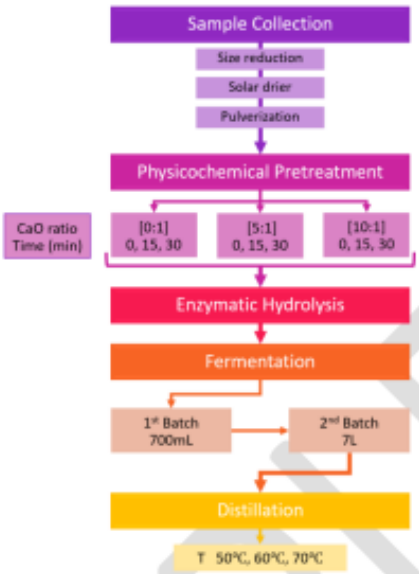



**Figure 1** Methodology flowchart.

*2.4 Enzymatic hydrolysis and fermentation*

Taking the best conditions after the pretreatment step, 700 mL of hydrolysate was prepared using 35 g of dry sample. After pretreatment, the mixture was measured for sugar content, pH and modified in a range of 4.9 to 5.10 before being infected with 1% commercial cellulases and kept in an incubator at 35±5 °C for 24h. The pH of the hydrolysate was modified in a range of 5 to 5.5 to set the fermentation process using 1% of commercial yeast. The fermentation process was stablished for 6 days at room temperature 30±5 °C, a sample was withdrawer every 24h to measure sugars content and alcohol content. Ethanol content was carried out using an ebulliometer (Dujardin-Salleron, Alcohol Burner, France). Based on the decreasing on the sugars content and the ethanol production during the fermentation process, the distillation was settled.

147 *2.5 Ethanol recovery*

148 To proceed with the ethanol recovery, a batch of 6 L of broth was prepared using 300g of dry
149 sample. Data was collected during the process to perform mass and energy balance. The ethanol
150 generated during the fermentation step was recovered by simple distillation. the simple distiller
151 apparatus was used to distillate 1L of broth at three different temperatures 50°C, 60°C, and 70°C.
152
153 *2.5.1 Kinetics model*
154 This equation was employed in the current investigation to explain the change in ethanol
155 concentration during fermentation, based on the success of previous studies (Ginkel et al., 2001,
156 Mu et al., 2006, Dodić et al., 2012; Phukoetphim et al., 2017) in modeling ethanol production
157 using the modified Gompertz model. This model gave ethanol content as a function of the
158 fermentation time, the maximum product productivity, and the potential maximum product
159 production. The modified Gompertz model is described in Equation 2 (Bailey and Ollis, 1994).
160

161 $$P = p_m \cdot e^{\left\{-e^{\left[\frac{r_{pm}\cdot e^1}{P_m}\right]\cdot(t_L - t)+1}\right\}} \quad (2)$$

162
163 Where $p_m$ was the potential maximum ethanol production (g/L), $r_{pm}$ was the maximum ethanol
164 productivity (g/L), and lag time ($t_L$) was the time from the beginning of fermentation to exponential
165 ethanol production (h).
166 *2.7 Data analysis*
167 All of the experiments in this study were replicated three times. The data were presented as a mean
168 standard deviation from three replicates and a significant difference was examined at the level of

169 $p < 0.05$. Data of Physicochemical analysis of the samples were expressed as mean of three
170 replicates ± standard error (SE).
171
172 **3 Results and Discussion**
173 *3.1 Physicochemical analysis*
174 Insoluble calcium oxalate is found in the majority of Araceae species, which causes toxicity due
175 to physical irritation caused by needle-shaped crystals (Tagwireyi and Ball, 2010). The crystals
176 are expelled from the idioblast cells when the plant is chewed, and become trapped in the mouth,
177 tongue, or throat lining, this leads to local inflammatory responses including pain, irritation, and
178 edema of the buccal cavity, excessive salivation, and aphonia (Miyamoto et al., 2021). According
179 to Du Thanh et al. (2017) after the analysis of the leaves of seven different *Colocasia esculenta*
180 cultivars contains in average 635.2±92.4 mg/100 g wet basis of total oxalate, with the lowest and
181 highest value reported as 433.8±7.9 and 856.1±7.7 mg/100 g wet basis respectively.
182 Table 1 shows the results from the physicochemical analysis from both, fresh and dry elephant ear
183 plant. It was found that moisture content in the elephant ear plant was 89.74%, with a dry matter
184 percentage of the 10.26%. The total sugars content comparison showed an increment in the dry
185 sample (3.394±0.129 g/L) in contrast with the fresh sample (1.132±0.086 g/L). This difference is
186 the main factor for the energy value difference from the fresh and dry samples resulted in
187 4.536±0.031 and 12.825±0.514 kcal/5 g sample, respectively.
188 Furthermore, the reducing sugars content increased from 0.907±0.005 g/L in the fresh sample to
189 2.633±0.039 g/L from the dry sample.
190
191 **Table 1** Elephant ear plant composition.

192

| Parameter | Elephant Ear Plant | |
|---|---|---|
| Moisture content (%) | 89.74 | |
| Dry matter (%) | 10.26 | |
| | **Fresh** | **Dry** |
| TS (g/L) | 1.012±0.086 | 3.394±0.129 |
| RS (g/L) | 0.707±0.005 | 2.633±0.039 |
| pH measured in water at 30±5 ºC | 5.01±0.015 | 5.27±0.101 |
| Energy value (kcal/5 g sample) | 4.536±0.031 | 12.825±0.514 |

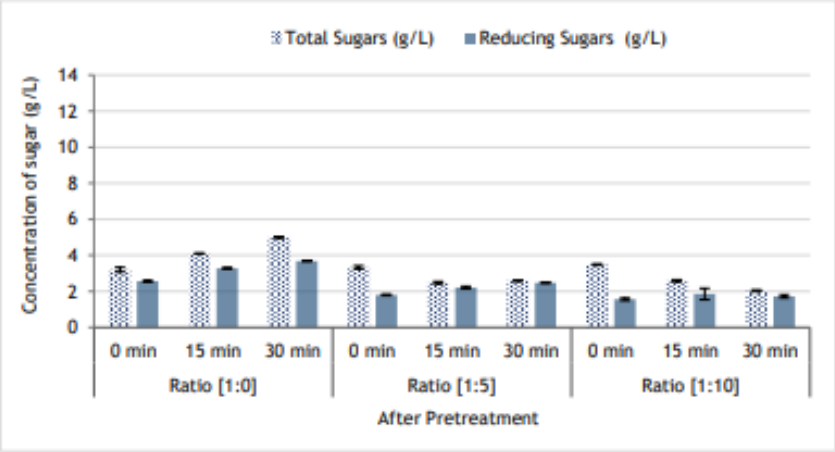
193

194 *3.2 Effect of physicochemical pretreatment in sugars content*

195 Biomass pretreatment reduces lignin and hemicelluloses, improving cellulose hydrolysis
196 substantially (Whangchai et al., 2021). Reducing sugars or simple sugars such as glucose, xylose,
197 and arabinose are degraded from the glycosidic bond rupture of polymers to allow rapid and
198 efficient carbohydrate hydrolysis to fermentable sugars (Nguyen et al., 2020). It is necessary to
199 explore the extraction of sugars from aquatic weeds in order to obtain the most cost-effective
200 bioethanol production method (Sindhu et al., 2016).

201 The results from the physicochemical pretreatment from dry elephant ear plant is showed in Figure
202 2. It can be observed that the sugar concentration increased accordingly to the exposure time of
203 steam explosion pretreatment when the CaO ratio is [1:0], with the higher concentration for total
204 and reducing sugars of 4.991±0.029 and 3.685±0.021 g/L, respectively. This represents an
205 improvement compared with the results reported from fresh elephant ear plant at the same
206 conditions with a total sugar and reducing sugars content of 1.088 and 0.895 g/L respectively
207 (Trejo et al., 2021).

208




209

210 **Figure 2** Sugars content accumulated after steam explosion pretreatment.

211

212 This could be attributed to the calcium oxalate reduction as reported from Perez-Pimienta et al
213 (2016) were the presence of low levels of calcium oxalate in agave bagasse showed a positive
214 effect on pretreatment performance improving sugar production and faster enzymatic hydrolysis.
215 The content of calcium oxalate observed to be reduced in the recovered product as a function of
216 the sample pretreatment temperature (Perez-Pimienta et al., 2015).
217 At the opposite, the results obtained from the experiments using CaO ratio [1:5] and [10:1] showed
218 a lower sugar content. Alkaline pretreatment with CaO is beneficial since it improves the opening
219 of cellulosic fibers, but it does not degrade sugars at this stage, just makes the material vulnerable
220 to enzymatic degradation (Alvira et al., 2010; Amezcua-Allieri et all., 2017).

221

222 *3.3 Effect of hydrolysis in sugar content*

223 The diverse nature of aquatic weed biomass makes it difficult for successful biofuel extraction and
224 conversion. The saccharification procedure identifies the most efficient pretreatment for releasing

polysaccharides by breaking the cross-linkage bond of lignin barriers. When it comes to breaking down cellulose into glucose, cellulase is more sensitive than other enzymes (Ramaraj et al., 2019; Vu et al., 2018). Low content of results calcium oxalate in more free accessible area to enzymes that could react on the cellulose.

The results from the hydrolysis process are displayed in Figure 3. Following the pretreatment behavior, the sugar concentration was higher for the samples pretreated with a CaO ratio of [0:1]. The total sugar and reducing sugars accumulation were 6.382±0.076 and 6.019±0.019 g/L, respectively. In a study carried out by Fernandez et al. (2015), *Cynara cardunculus* was pretreated by using steam explosion for producing bioethanol, the results showed partial solubilization of hemicellulose and improved the accessibility of residual polysaccharides towards enzymatic hydrolysis.

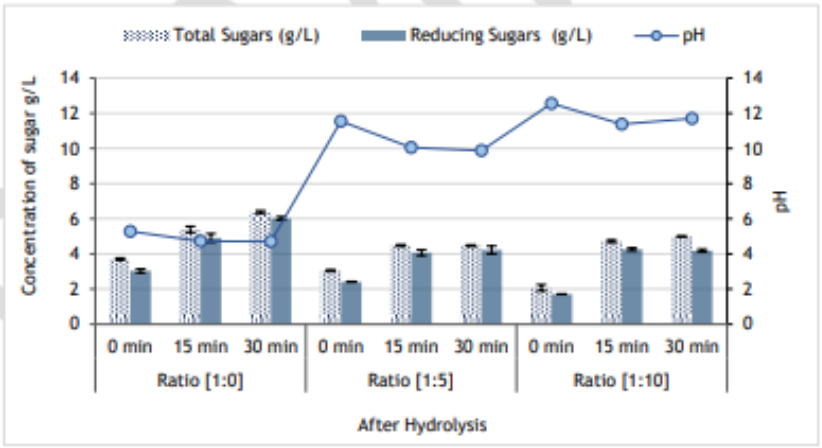



**Figure 3** Sugars content accumulated after enzymatic hydrolysis.

240 After 24h of hydrolysis, the pH was measured in the samples (Figure 3), it was found that pH value
241 using CaO were all above 10, and for the ones with 0 min of steam explosion the pH reached 12
242 and 13 for the CaO ratio [5:1] and [10:1]. The low sugar releasing obtained using CaO at the rations
243 could be explained due the low enzymatic activity during the hydrolysis produced for the high pH
244 value. According to previous studies, cellulases are active at the pH range of 6.0 to 7.0 from (Akiba
245 et al., 1995). Irfan et al (2012) found the optimum pH for endoglucanase activity at 7.5 and stable
246 at pH 6.5 to 9.5. Increasing or decreasing pH beyond this resulted in decline in enzyme activity as
247 was reported by El-Sersy et al. (2010) that cellulase enzyme production decreased about 50% at
248 pH 9 from *S. rub*er, proving that any change in pH caused changes in the enzyme active site.
249
250 *3.4 Enhancement of ethanol production*
251 Aquatic weeds are rich in cellulose, hemicellulose, starch with low lignin content. When compared
252 to other lignocellulosic biomass, they are easy to hydrolyze into fermentable sugars and create an
253 efficient and cost-effective biofuel source (Kaur et al., 2018). Although aquatic weeds are used in
254 on-site wastewater treatment, they provide both wastewater treatment and biofuel generation
255 (Mehariya et al, 2021).
256 Following the best conditions obtained from the physhycohemical pretreatment enzymatic
257 hydrolysis, the fermentation proces was settled with a broth prepared using a CaO ratio of [0:1]
258 and 30 min of stema explosion. The broth was innoculated with 1% of comercial yeast and kept 5
259 days at room temperture (30±5°C). Figure 4 displays the time curse for the suagars and ethanol
260 content during the fermentation process.

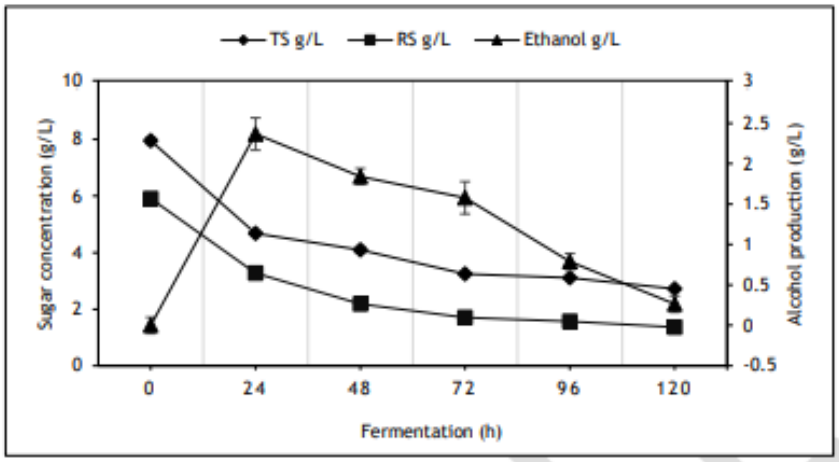



**Figure 4** Time course of the concentration of sugars and ethanol in the fermentation process.

Ethanol production reached the higher concentration after 24h of fermentation, with a volume of 2.351±0.691 g/L. The sugar content remains constant after 48h, whereas the ethanol concentration gradually decreases. Temperature, incubation length, agitation, inoculum size, and substrate concentration are all factors that impact ethanol production. The concentration of the substrate has an effect on ethanol production; the greater the substrate (not more than 15%), the higher the ethanol output (Rodrigues et al., 2005).

*3.5 Ethanol distillation*

The distillation of ethanol formed during fermentation from ethanol-water solution will lead finally to production of hydrous (azeotropic) ethanol (theoretical maximum achievable 95.5% wt. ethanol and 4.5% water). To remove the remaining water, special processes are required to reach anhydrous ethanol, that include: chemical dehydration process, dehydration by vacuum distillation

process, azeotropic distillation process, extractive distillation processes, membrane processes, adsorption processes, and diffusion distillation process.

In order to evaluate the amount of ethanol that can be recovered by simple distillation, samples of fermented broth were distilled at different temperatures 50°C, 60°C, and 70°C. Temperatures were chosen lower than the temperature of the ethanol evaporation (77 - 78 °C). Figure 5 shows the fermentation process for the 7L batch prepared. The fermentation efficiency was estimated at 71.82% with a sugar consumption of 59.48%. The distillation process was carried out after 24h of fermentation with an ethanol concentration of 2.631±0.821 g/L.

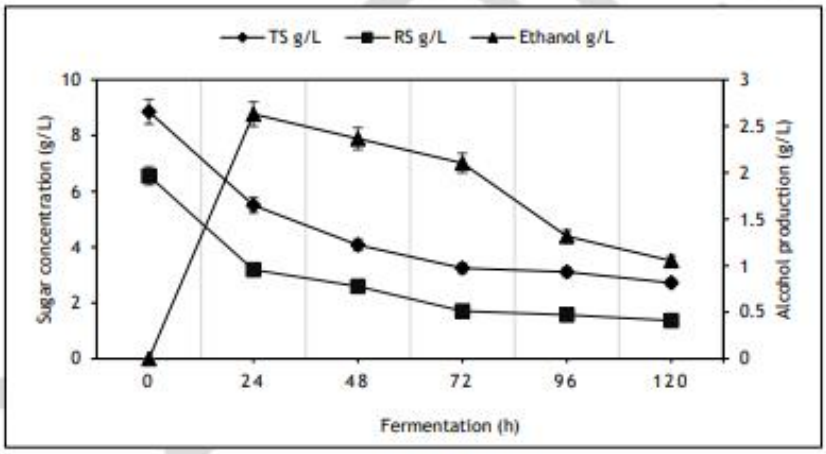



**Figure 5** Sugars and ethanol concentration from the 7L batch.

The mass balance for the distillation process at the different temperatures is presented is table 2. The volume of ethanol present in the distilled sample at 70°C was 1.03±0.196 mL, the higher volume compared with the 0.21±0.127 and 0.84±0.243 mL obtained at 50°C and 60°C,

293 respectively. However, in terms of ethanol yield, the percentage obtained at 60°C represents the
294 higher value in the contrast with the 4.208 at 50°C and 7.890 at 70°C.
295 Further experimentation is necessary to demonstrate the capacity to enhance the ethanol yield
296 obtained at lower temperatures, which could result in a reduction in the energy required for the
297 distillation process, which would have a direct effect on cost reduction.
298
299 **Table 2** Comparison of ethanol recovered by distillation at different temperatures.
300

| | Temperature (°C) | | |
|---|---|---|---|
| | 50 | 60 | 70 |
| Distilled Vol. (mL/1000 mL) | 5 | 9 | 13 |
| Ethanol mL | 0.21±0.127 | 0.84±0.243 | 1.03±0.196 |
| Ethanol yield (%) | 4.208 | 9.351 | 7.890 |
| Water (mL) | 4.79±0.275 | 8.16±0.079 | 11.97±0.321 |
| Bottoms Vol. (mL/1000 mL) | 995 | 991 | 987 |
| Ethanol (mL) | 2.051±0.263 | 1.105±0.629 | 0.828±0.563 |
| Water (mL) | 992.949 | 989.895 | 986.172 |

301
302 *3.6 Energy consumption*
303 Aquatic weeds are fast growing and invasive in nature. These characteristics of aquatic weeds need
304 to be given proper attention when grown for their potential application for production of biofuel
305 and other products (Bayrakci et al., 2014). While aquatic weed has demonstrated significant
306 potential for biofuel production and other purposes, there are still obstacles that must be overcome
307 before it can be successfully implemented to benefit the environment and humankind.
308 The energy balance and the cost for the energy consumption per stage for the overall bioethanol
309 generation from dry elephant ear plant is shown in Table 3. As the solar dewatering of the sample

did not need any energy input, it was excluded from the energy analysis. As can be observed, hydrolysis represents the mayor energy input with 45.60kWh. thus, hydrolysis also represents he main inversion with 4.469USD. Removing the hydrolysis process from the process, leaves an energy input of 1.050±0.002kWh and a cost expense of 0.103±0.001USD, that still above the energy output calculated in 0.856±0.040 kWh valuated in 0.084±0.002USD.

**Table 3** Energy balance per stage.

| Stage | Equipment | W | kW | h | kWh | kWh (USD)* |
|---|---|---|---|---|---|---|
| Sample preparation | Blender | 600 | 0.60 | 0.1 | 0.06 | 0.006 |
| Physical pretreatment | Autoclave | 2500 | 2.50 | 0.3 | 0.75 | 0.074 |
| Hydrolysis | Oven | 1900 | 1.90 | 24 | 45.60 | 4.469 |
| Distillation | Heater | 240 | 0.24 | 1 | 0.24 | 0.024 |
| Energy Input | | | | | 46.65 | 4.572 |
| Energy Output | | | | | 0.856±0.040 | 0.084±0.002 |

*1฿ Thai Baht = 0.030 USD

The difficulties associated with producing aquatic weed biofuels on a scale up may include harvesting, drying, transporting, and developing a cost-effective conversion technology (Xu et al., 2013; Jambo et al., 2016).

The energy balance analysis of bioethanol production indicates that the hydrolysis process consumes the majority of energy, which is also due to the long period of incubation. Reduced energy consumption during hydrolysis is possible when less heating is required, however, it is important to maintain optimum incubation temperature during biological pretreatment since long

incubation time due to low delignification rate is one of the major barriers for large scale application of biological pretreatment (Isroi et al., 2011).

Aquatic weed biomass can include up to 90% water, which might impact the process of biofuel conversion (Alam et al, 2021). Efficient and cost-effective dewatering technologies should be studied to facilitate the downstream process of aquatic weed biofuel production (Chen et al., 2015; Jeevanandam et al., 2020).

*3.7 Maximum ethanol production*

For optimizing the conversion of lignocellulosic biomass into sugar, it is necessary to understand the principles of sugar production and how all of the components that influence sugar production interact with one another. In order to do this, it is necessary to compare experimental and predicted data together in order to identify difficulties related with the lignocellulosic ethanol process. Additional knowledge of cell development and product generation dynamics will result in considerable improvements in process design as well as production yield (Almquist et al., 2014). The kinetics of bioethanol production during fermentation of dry elephant ear plant is shown in Figure 6.

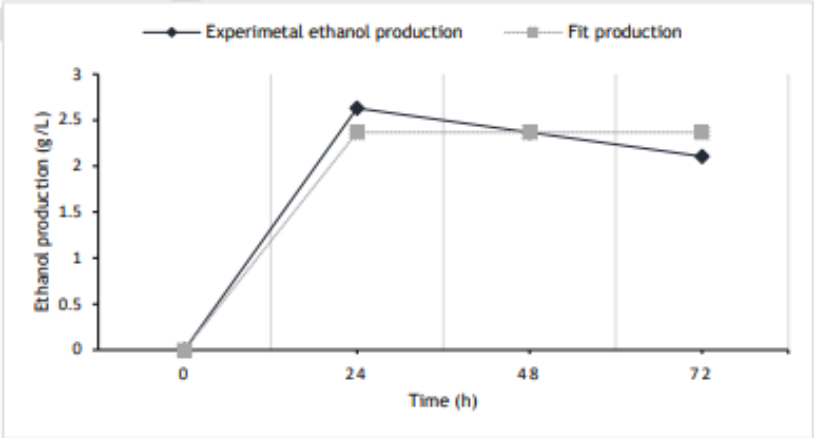


**Figure 6** Product kinetics results of experimental values with predicted values.

Experiments were carried out at a pH range of 5 to 5.5 and room temperature (30±5 °C) using 1% of commercial yeast. The production of bioethanol started after 7 h (Table 4) from the period of inoculation increased slightly when the microorganism was in the phase of exponential growth. Because the organism displayed lag phase during this fermentation time period, it is possible that the delay in ethanol generation was caused by incorrect absorption of the substrate by the organism during this fermentation time period. During the fermentation process, the bioethanol content increased and reached a maximum at around 24 h. As the organism progressed through the stationary growth phase, the rate of production steadily decreased beyond 30 h.

**Table 4** Values obtained from the modified Gompertz model.

| Kinetics parameters | Value |
|---|---|
| $p_m$ (g/L) | 2.367 |
| $r_{pm}$ (g/L*h) | 0.475 |
| $t_L$ (h) | 7.834 |
| $R^2$ | 0.968 |
| Error | 0.069 |
| SSR | 0.138 |

Table 4 content the kinetic parameters calculated by using the Modified Gompertz model. The maximum bioethanol production rate ($r_{pm}$) value indicates that 0.475 g/L of ethanol was produced every hour. The model describes the process with an accuracy of 0.968 indicated for the correlation

361 factor. Sarto et al. (2019) published a study in which they investigated the kinetics of water
362 hyacinth biomass pretreatment using a power-law model based on the first-order model. They
363 demonstrated that the first-order model can be used to correctly calculate the rate constant of the
364 majority of pretreatment processes, which may be useful in the future in order to maximize the
365 efficiency of the pretreatment process.
366
367 **Conclusions**
368 The results of this study shown that the application of steam explosion pretreatment can effectively
369 improve the fermentable sugar content in dried elephant ear plant. The batch assays were evaluated
370 comparatively via the modified Gompertz-model based on the important fermentation parameters
371 that characterizing the process, with a resulting value of $p_m$ 2.367 g/L and $r_{pm}$ 0.475 g/L*h, the
372 model can predict the process with a confidence of $R_2$>0.95. Furthermore, the use of dry elephant
373 ear plant as a bioenergy feedstock for bioethanol production may be a potential alternative. These
374 results provide a better understanding on how to improve the cost, productivity, and environmental
375 outlook of future scale-up procedures, which are all critical considerations.
376
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APPENDIX C CONFERENCE PAPERS

3rd Symposium on Industrial Science And Technology (SISTEC 2021)
Faculty of Industrial Sciences and Technology
Universiti Malaysia Pahang
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**Paper Tittle:** Enhancement Of Fermentable Sugars From Fresh Elephant Ear Plant For Bioethanol Production Using Ash As A Source Of CaO

**Participant:** Miss. Martha Marlen Trejo Perez

Dear Prof./Dr./Sir/Mdm.,

**NOTIFICATION OF FULL PAPER ACCEPTANCE**

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3. Based on the publication committee, your paper portrays the good topic and findings which suitable for SISTEC2021 publication in Scopus-Indexed Proceeding. Please refer further comment sending to you for any improvements needed and submitted again to the platform provided.

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Universiti Malaysia PAHANG | 3rd SYMPOSIUM ON INDUSTRIAL SCIENCE AND TECHNOLOGY 2021 (SISTEC 2021)

# Enhancement of fermentable sugars from fresh elephant ear plant for bioethanol production using ash as a source of CaO

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**Abstract.** Bioethanol production from non-crop-based lignocellulosic material is gaining popularity across the world. Lignocellulosic materials are the most abundant renewable organic resources on the planet (200 billion tons per year) and can be converted to ethanol. Aquatic weeds have a rapid rate of reproduction and are high in cellulose and hemicellulose with low lignin content, making them a promising next-generation biofuel crop. In this work, the use of the elephant ear plant as a source of lignocellulosic feedstock for bioethanol production was studied. The experimental part included a physicochemical pretreatment using ash as a source of CaO in three different ratios: [1:0], [1:5], and [1:10], followed by hydrothermal and steam explosion treatment for 15 min. All treatment samples were measured for total sugar and reducing sugar content. The results showed that the fermentable sugars content was different among treatments. Enzyme-treated elephant ear plants had higher total sugars (6.51 ±0.27 mg/mL) than untreated samples (1.60 ±0.02 mg/mL). Moreover, the enzyme-treated elephant ear plant had a higher reducing sugar content than the untreated (5.40 ±0.11 mg/mL and 1.37 ±0.06 mg/mL, respectively). The ethanol potential for the fermentable sugars in the hydrolysate mixture was theoretically estimated. The highest efficient ethanol potential obtained was 2.75 ±0.06 mg/mL after 15 min under thermochemical pretreatment, with an ash ratio of [5:1] and 1% of cellulose for the hydrolysis step. The elephant ear plant has the potential to be a value-laden plant in the production of bioethanol.

## INTRODUCTION

Energy resources that are currently available (such as fossil fuels) are finite and are being depleted at an alarming rate throughout the planet [1, 2, 3]. Biofuels are often described as solid, liquid, or gaseous fuels derived from plant biomass or biodegradable portions of plant-derived products [4, 5]. Biofuels are projected to provide about 5.4% of road transport energy demand in 2025, according to the International Energy Agency (IEA) increasing from slightly under 4.8% in 2019. In the meanwhile, worldwide fuel ethanol output approached 115 billion L in 2019. Average output is expected to reach 119 billion L in 2023-2025 [6]. Because biofuels are made from plant-derived polysaccharides (mainly starch, cellulose, hemicellulose), atmospheric $CO_2$ levels do not rise when they are burned, a notion known as carbon neutrality [7, 8]. Thus, the utilization of biofuels in place of fossil fuels is an effective way to combat global climate change by reducing greenhouse gas (GHG) emissions and decreasing the dependence on limited sources of fossil fuels [9, 10]. There is a rising effort to characterize qualitative and quantitative biofuel characteristics of biomass feedstock in order to discover a suitable biomass feedstock for biofuel generation. Due to the serious environmental and human food security issues linked with first-generation biofuels, research has turned to utilize non-edible feedstock such as lignocellulosic biomass or algal biomass rather than carbohydrates-rich food crops [11, 12]. Weeds found in aquatic habitats have the potential to be used as a lignocellulosic feedstock for biofuel production [13]. Lignocellulosic biomass is made up of a complex combination of cellulose, hemicellulose, and lignin,

regardless of which plant it originates from. Hemicellulose, after cellulose, is the portion of the plant cell wall with the greatest potential for bioethanol synthesis [14, 15]. The bioconversion of lignocellulosic to ethanol involves three steps: (a) thermochemical and enzymatic depolymerization of structural polysaccharides into fermentable sugars, (b) fermentation of these sugars into ethanol, and (c) ethanol recovery.

The critical parameters for selecting plants for fuel ethanol production include cell wall composition, growth rate, suitability for growth in different geographical regions, and resource use efficiencies [16]. Pretreatment is necessary to change the biomass's macro- and microscopic size and structure, as well as its sub-microscopic chemical composition so that the carbohydrate fraction may be hydrolyzed quickly and with higher yields [17]. Pretreatment involves the use of acids, alkalis, and organic solvents. According to Gu, (2015), alkaline pretreatment could effectively degrade hemicellulose into soluble oligomers and monomeric sugars [18]. Secondary materials, like CaO from ash, might be identified as viable options for alkaline pretreatment, providing significant economic and environmental benefits over pure chemicals [19, 20]. The liberated cellulose and hemicellulose molecules in the processed biomass are subsequently chemically or enzymatically degraded into soluble sugars, which are then transformed to bioethanol during microbial fermentation.

Biofuels are expected to investigate the potential of aquatic weeds as biofuel feedstock because the characteristics of an ideal biofuel feedstock (fast growth with little fertilizer and water requirements) are similar to those of conventional weeds and because production will be on a large scale. Aquatic weeds are invasive plants that inflict significant economic and ecological harm once they enter an aquatic habitat. Their rapid growth rate, diverse routes of spread, and worldwide dispersion have the potential to have significant ecological and economic implications [21].

The common name for a group of tropical perennial aquatic and semi-aquatic weed is elephant ears plants, for their enormous, heart-shaped leaves. The elephant ear plant is a common sight along the shorelines of bodies of water, as well as in marshes, canals, and along stream banks. Dense populations develop large stands as a result of vegetative growth, altering the vegetational structure and dynamics of riparian plant communities [22, 23].

The aim of this study was to evaluate the potential of fresh elephant ear plants, an emerging aquatic and semi-aquatic weed, as a possible feedstock for bioethanol production. To enhance the reducing sugar concentration, a physicochemical pretreatment was carried out using CaO followed for hydrothermal and steam explosion treatment. Furthermore, the theorical bioethanol production was estimated using the highest reducing sugar obtained from the experiments.

## MATERIALS AND METHODS

### Sample Collection and Material Preparation

Elephant ear plant samples were collected near water sources from Maejo University, located at Sansai-Phrao Road, Nongharn, Sansai District, Chiang Mai, 50290 Thailand and sent to the Faculty of Science's laboratory. The leaves and stalk collected were rinsed with tap water to eliminate contaminants, cut into small pieces (1 to 2 cm), and then homogenized to a paste using a mechanical grinder (PHILIPS Blender 600W Model HR2118/02).

### Pretreatment and Hydrolysis

The use of CaO for alkaline pretreatment on lignocellulosic biomass (i.e., wheat straw, sunflower stalks, and algae) have demonstrated a significant increase in biomass degradation to be converted to reducing sugars [24, 25]. In this study, for the pretreatment stage, 50 g of homogenized fresh elephant ear plant was combined with 500 mL of a solution of ash as a source of CaO in the ratios of [0:1], [5:1], and [10:1] in a 1000 mL graduated bottle. The mixture was undergone hydrothermal and steam explosion treatment using autoclaving apparatus at 121 °C, 15 psi for 15 min. The pH of the mixed solution was adjusted to 5 after pretreatment, and the samples were infected with 1% commercial cellulase for the hydrolysis procedure. The solution was then placed in an incubator at 35 °C for 24 h to complete the

hydrolysis process. Figure 1 shows the elephant ear plant past (leaves and stalk) before and during the hydrolysis step.

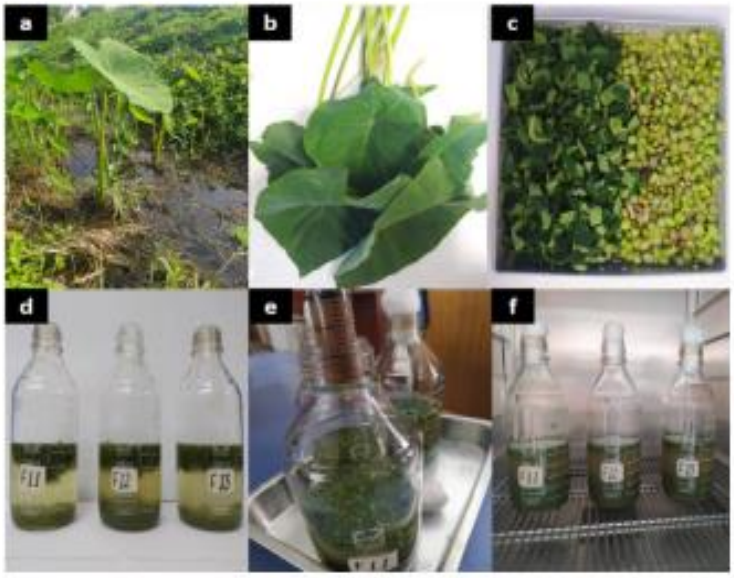


**FIGURE 1.** (a) Elephant ear plant in the local canals, (b) elephant ear plant collected and (c) size reduction, (d) sample weighted and mixed with CaO solution, (e) inoculation with cellulose, and (f) samples settled to hydrolysis process.

## Sugar Analysis

Spectrometry was utilized to determine sugar concentrations using a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan). The phenol sulfuric acid technique and the dinitrosalicylic acid (DNS) method were used to quantify total sugars and reducing sugars [26, 27]. 500 L of phenol solution (5%w/v) was added to 500 µL of the sample for total sugar measurement. After homogenizing the mixture, 2.5 mL of concentrated sulfuric acid was added ($H_2SO_4$ at 98%). Using distiller water as control, the absorbance was read at 540nm. Meanwhile, 500 µL of DNS reagent was added to 500 µL of the sample for reducing sugars. For 15 min, the mixture was immersed in boiling water. The mixture was then chilled before adding 4 mL of distiller water. Using distiller water as a control, the absorbance was measured at 540nm. Since the DNS reagent is photosensitive, the entire method for determining reducing sugars was carried out under dark conditions. A standard curve was created using standard D-Glucose solution to determine the concentration of an unknown sample in mg/mL for both total and reducing sugars.

## Ethanol Determination

The ethanol potential of a material is based on the total amount of reducing sugars contained. In this work, reducing sugars were determined using the spectrometry method.

| | Glucose | | Ethanol | | Carbon Dioxide |
|---|---|---|---|---|---|
| Chemical equation | $C_6H_{12}O_6$ | → | $C_2H_6O$ | + | $CO_2$ |
| Balanced equation | $C_6H_{12}O_6$ | → | $2C_2H_6O$ | + | $2CO_2$ |
| Molecular weight | 180 g/mol | | 92 g /mol | | 88 g /mol |

**FIGURE 2.** Fermentation reaction's stoichiometry.

The stoichiometry of the fermentation reaction (Fig. 2) was used to generate Eq. 1 and calculate the ethanol potential under ideal conditions from the fresh elephant ear plant.

$$E_P = RS_H * (0.51) \quad (1)$$

Where; $E_p$ is the ethanol potential in (g/L), ($RS_H$) is the reducing sugar content determined after the enzymatic hydrolysis in g, and 0.51 is the stoichiometry factor determined by the fermentation reaction balance.

### Statistical Analysis

Statgraphics Centurion 19 (© 2021 Statgraphics Technologies, Inc.) was used to perform the statistical analysis. All of the experiments in this study were replicated three times. The data were presented as a mean standard deviation from three replicates and a significant difference was examined at the level of $p < 0.05$.

## RESULTS AND DISCUSSION

### Physicochemical Pretreatment and Enzymatic Hydrolysis

In this study, ash as a source of CaO was investigated at three different times (R1 = [0:1], R2 = [5:1], and R3 = [10:1]) as a chemical pretreatment of fresh elephant ear plant. Kumar, eat al., (2017), mention that CaO can provide a certain alkalinity as calcium hydroxide ($Ca(OH)_2$) while reacting with water [28]. Then, the mixture was under hydrothermal and steam explosion pretreatment. To follow total sugar and reducing sugar (mg/mL) concentration, samples were analyzed before and after the hydrolysis step. Figure 3 shows the results obtained of total sugar and reducing sugar at three different CaO ratios. The concentration of total sugar archived after the physicochemical pretreatment were R1= 2.22 ± 0.10 mg/mL, R2 = 1.60 ± 0.02 mg/mL, and R3 = 1.11 ± 0.03 mg/mL respectively. On the other hand, the reducing sugar concentration obtained were R1= 1.90 ± 0.12 mg/mL, R2 = 1.37 ± 0.07 mg/mL, R3 = 0.97 ± 0.01 mg/mL showed in Fig. 3.

Meanwhile, the concentration of total sugar after enzyme hydrolysis step R1= 3.63 ± 0.05 mg/mL, R2 = 6.51 ± 0.027 mg/mL, R3 = 6.43 ± 0.16 mg/mL (Fig. 3). While for reducing sugars, R2 (5.41 ± 0.11 mg/mL) presented the highest concentration of reducing sugars compared with R1 y R3 (3.56 ± 0.03 mg/mL and 5.30 ± 0.11 mg/mL, respectively). In a previous study using fresh elephant ear plant under hydrothermal and steam explosion treatment for 15 min, and enzymatic hydrolysis for 24 h, the highest total sugar and reducing sugar were 1.130 ± 0.04 mg/mL and 0.907 ± 0.03 mg/mL respectively [29]. As a result, in this work using a CaO ratio of [5:1] and after 15 minutes of pretreatment (hydrothermal and steam explosion) and 24 h of hydrolysis, R2 had a highest fermentable sugars concentration, what represents an improvement in the method.

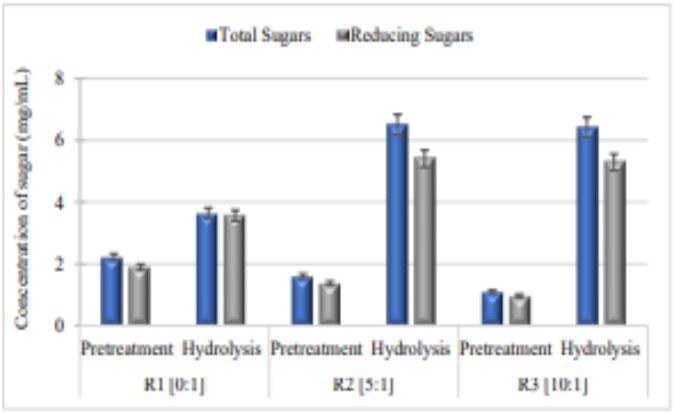


**Figure 3.** The concentration of sugar at three different ratios of ash (CaO) and after enzyme hydrolysis step.

## Bioethanol Production

Fermentation produces ethanol and carbon dioxide as its final products. Under ideal conditions, when the liberated cellulose and hemicellulose are completely hydrolyzed and all sugars are converted to alcohol, the estimated potential for ethanol generation from the reducing sugars in the hydrolysate mixture was calculated. The theoretical potential of bioethanol production was computed under ideal conditions, with the maximum bioethanol concentration obtained of 2.76 ±0.06 mg/mL after 15 min of hydrothermal and steam explosion pretreatment and a CaO ratio of [5:1]. Zhang, et al., (2018) reported a final ethanol concentration of 1.40 mg/mL from water hyacinth using *P. chrysosporium* for a microbial-diluted acid pretreatment followed by a fermentation by *S. cerevisiae* [30]. Another aquatic plant that has been studied for bioethanol production is *salvinia molesta*. Abdullahi et al. (2016) reported 2 mg/mL of bioethanol production from *salvinia molesta* using acid hydrolysis and steam explosion as pretreatment from 15 min, and *S. cerevisiae* and *S. carlsbergensis* for fermentation step [31].

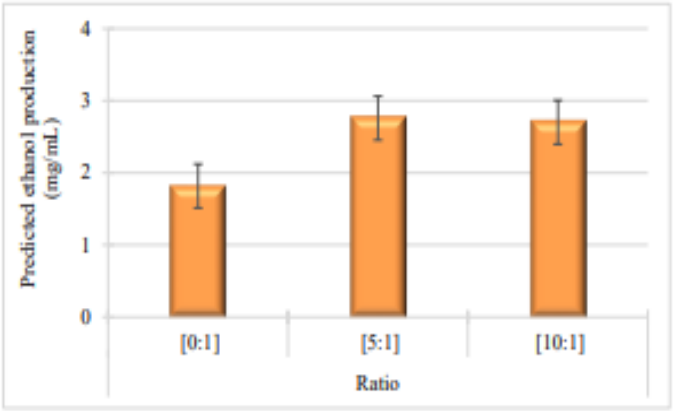

**FIGURE 4.** Estimation of bioethanol production based on the concentration of reducing sugars in the hydrolysate mixture.

## CONCLUSIONS

The elephant ear plant, which is considered invasive, can be utilized to produce bioethanol. The physical pretreatment technique (hydrothermal and steam explosion) was used to improve cellulose enzyme accessibility and produce high sugar concentrations from fresh elephant ear plants successfully. The results revealed that the chemical composition differed across treatments. After 15 min of hydrothermal and steam explosion pretreatment, the maximum fermentable sugar concentration in the hydrolysate utilizing ash as a source of CaO in a ratio of [5:1] was 5.41 ±0.11 mg/mL, with a potential generation of ethanol (under ideal conditions) of 2.76 ±0.06 mg/mL. As a result, the elephant ear plant has the potential to be an efficient bioethanol feedstock.

## ACKNOWLEDGMENTS

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(Use the Microsoft Word template style: *Heading 1*) or (Use Times New Roman Font: 12 pt, Bold, ALL CAPS, Centered)

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**The 3rd International Conference on Renewable Energy, Sustainable Environmental and Agricultural Technologies**

**Virtual (Online) mode conference**
**Venue: Maejo University, Chiang Mai, Thailand)**
**Date: 22th – 23th, December 2021**

## i-RESEAT 2021 ACCEPTANCE LETTER

21 December 2021

**Paper No.: i-RESEAT-2021-SET-244**

**Title:** Effect off steam-explosion treatment in the enhancement of fermentable sugar from elephant ear plant for bioethanol production

**Authors:** Michael Benjamin, Devaraj Manoj, K Theyagarajan, Duraisamy Saravanakumar, Sellappan Senthilkumar*

Dear Dr. R. Ramaraj and authors,

Verification of abstract acceptance for the 3rd International Conference on Renewable Energy, Sustainable Environmental and Agri-Technological Innovation

We are pleased to inform you that your abstract entitled "**Effect off steam-explosion treatment in the enhancement of fermentable sugar from elephant ear plant for bioethanol production** " has been accepted and received for **Poster presentation** at the "i-RESEAT-2021" which will take place on December 22 – 23, 2021. The event starts at 9.00 am, please refer to the attached schedule/conference website.

The venue and time of your presentation is announced in the following schedule. For more information about the event please refer to our website via the following link: https://i-reseat.mju.ac.th/ .

You may now complete the **payment for registration** by bank procedure on following page. We sincerely hope that you will participate in the i-RESEAT-2021 successfully. Thank you.

Yours Sincerely,

**Dr. RAMESHPRABU RAMARAJ**

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# Effect off steam-explosion treatment in the enhancement of fermentable sugar from elephant ear plant for bioethanol production

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**Abstract.** Alternative energy sources are becoming increasingly important across the world. It is feasible to reduce greenhouse gas emissions, avoid pollution, and enhance domestic energy and energy system production by utilizing renewable energy sources. Among the potential alternative energy sources; bioethanol is the most widely utilized biofuel in the transportation industry, and it has a long history as an alternative fuel. The feasible use of steam-explosion as a pretreatment for elephant ear plant as a biomass feedstock for bioethanol production was investigated in this study. The pretreatment was evaluated at three different times (0, 15, and 30 min). After pretreatment, the samples were inoculated with 1% of celluloses enzymes to proceed with the hydrolysis for 24 h. The hydrolysate with the highest fermentable sugar concentration was fermented using 1% of S. cerevisiae. The result showed that the higher reducing sugar concentration was obtained after 30 min under steam-explosion pretreatment with 1.943 ±0.023 mg/mL. However, a higher concentration of reducing sugar after hydrolysis was found after 15 min of steam explosion followed by 24 h of hydrolysis with 3.153 ±0.066 mg/mL. The hydrolysate was settled for fermentation during 5 days at room temperature (35 ±5 ℃), the alcohol measurement showed that the higher bioethanol rate of 1.315 mg/mL was reached after 24 hours of fermentation.

**Keywords:** Elephant Ear Plant, Steam-Explosion, Bioethanol, Biomass, Reducing Sugars

## 1. Introduction

In the highly progressive world of economic globalization and rapid energy depletion, more than the desire for alternative forms of energy, there is a need to find a more reliable, sustainable, and renewable source of energy that does not have any adverse effects on the environment (Manmai et al., 2022; Souvannasouk et al., 2021). The efforts to find new energy alternatives to cover the current energy demand have led to renewable energies such as solar cells, hydrogen, and biofuels. Biofuels appear as a renewable energy source since biomass is put up (Ma'arof et al., 2021; Bhuyar et al., 2021). Biomass is defined as all non-fossil material of biological origin. Second-generation biofuels present the advantages of a variety and abundance of lignocellulosic sources as a feedstock for biofuels production (Khammee et al., 2021a). In addition, the fact that bioethanol has better antiknock qualities than gasoline, such as higher flame speed, higher vaporization heat, and a higher-octane number, makes it a promising alternative fuel (Saengsawang et al., 2020). According to the International Energy Agency (IEA), biofuels are expected to account for 5.4 % of road transport energy consumption in 2025, up from 4.8 % in 2019. Meanwhile, in 2019, global fuel ethanol production was estimated at 115 billion liters. Moreover, the 119 billion L of average output is predicted by 2023-2025 (IEA 2021).

Biofuels are manufactured from plant polysaccharides (primarily starch, cellulose, and hemicellulose) that $CO_2$ levels in the atmosphere do not rise when burned; this is known as carbon neutrality (Unpaprom et al., 2021). Because of this, biofuels can be used in place of fossil fuels to combat global climate change by reducing greenhouse gas (GHG) emissions and lessening the dependence on limited sources of fossil fuels (Nguyen et al., 2020a,b). Biomass feedstock is increasingly being characterized to discover a suitable biomass feedstock for biofuel generation in qualitative and quantitative biofuel

characteristics (Khammee et al., 2021b). However, First-generation biofuels pose significant environmental and human food security concerns; hence researchers are turning to non-edible feedstocks such as lignocellulosic biomass or algal biomass instead. For biofuel production, the lignocellulosic feedstock can be obtained from aquatic weeds (Vu et al., 2008; Ramaraj et al., 2021). What matters is that the lignocellulosic biomass is made up of cellulose, hemicellulose, and other plant constituents. After cellulose, hemicellulose has tremendous potential for bioethanol synthesis in the plant cell wall (Figure 1). Depolymerization of structural polysaccharides into fermentable sugars, fermentation of these sugars into ethanol, and ethanol recovery are all processes in the bioconversion of lignocellulosic to ethanol (Nguyen et al., 2021; Whangchai et al., 2021).

The selection of fuel ethanol plants is heavily influenced by cell wall composition, growth rate, geographical adaptability, and resource efficiency. Microbial fermentation then converts the cellulose and hemicellulose molecules extracted from the processed biomass into bioethanol, which can then be used to produce biofuels (Nguyen et al., 2020c,d; Bautista et al., 2022). Large-scale production of biofuels is predicted to lead to an investigation of aquatic weeds as a biofuel feedstock because of their rapid growth and low fertilization and water requirements (Vu et al., 2017). Species of aquatic weeds that invade aquatic habitats can cause significant economic and ecological damage. Invasive weed species contain a large number of lignocellulosic biomasses (Ramaraj and Unpaprom, 2019), but eventually, these weeds become a threat to the environment. In terms of ecological and economic consequences, it is possible that their fast expansion, a wide range of dispersal routes, and global dispersion would cause significant harm. Elephant ears plants get their name from the heart-shaped leaves on tropical, aquatic, and semi-aquatic weeds. Elephant ear plants can grow in marshes, canals, and along stream banks worldwide (Trejo et al., 2021). Large stands form in dense populations due to vegetative development, affecting the structure and dynamics of riparian plant communities. In this study, fresh elephant ear plants, and aquatic and semi-aquatic weeds, were evaluated as a viable feedstock for bioethanol synthesis. Steam-explosion, a physical pretreatment, was used to increase the reducing sugar concentration. Experiments yielded the highest reducing sugar, which was used to estimate theoretical bioethanol production.

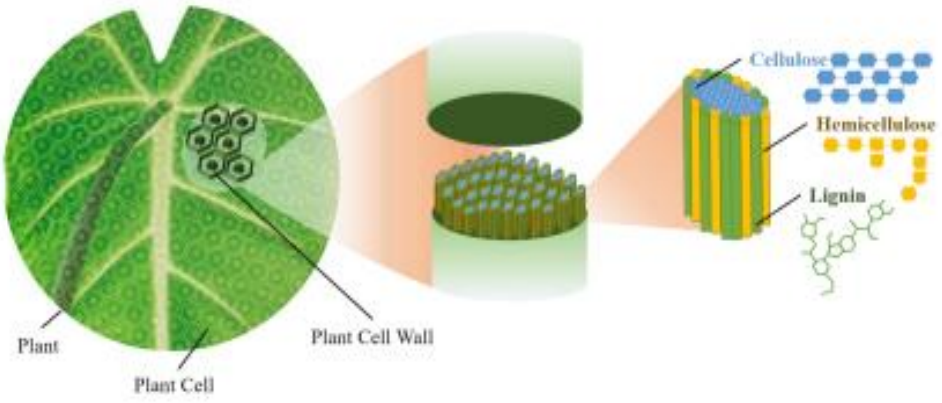


**Figure 1** Basic structure of plant tissues.

## 2. Material and Methods

### 2.1 Sample collection and preparation

The elephant ear plant, also commonly referred to by its botanical name Colocasia, is a tropical plant. It is naturally a swamp plant; it will grow a resilient and strong root system even when fully submerged

under water. The elephant ear plant is a common weed found near bodies of water such as canals, lakes, puddles, and rivers. Elephant ear plants were sampled from a wetland area on the Maejo University campus in Chiang Mai, Thailand (18.895902912837297, 99.01827891274498) (Figure 1a), and transferred to the Faculty of Science's laboratory. The leaves and stalks were collected and rinsed with tap water to remove impurities, then chopped into small pieces (1 to 2 cm) and homogenized to a paste using a grinder (PHILIPS Blender 600W Model HR2118/02) (Figure 2b).


**Figure 2** Wetland for sample collection (a, and elephant ear leaves and stalk (b.

### 2.2 Steam-explosion pretreatment

Many studies have looked at steam explosion pretreatment to improve the enzymatic digestibility of lignocellulosic biomass, and currently, it is the widest pretreatment method in industrial biorefineries (Druzhinina et al., 2016). For the pretreatment procedure, a total of 50 g of the homogenized fresh elephant ear plant was taken in a 1000 mL graduated bottle mixed with 500 mL of distiller water, and this mixture was undergone autoclaving apparatus at 121 °C, 15 psi, at different times; 0 min, 15 min, and 30 min (Figure 3a). Sugar analysis was conducted before and after the steam-explosion process to evaluate the pretreatment performance at different times of exposure.

### 2.3 Enzymatic hydrolysis

Enzymatic hydrolysis is a process in the lignocellulosic biomass conversion method that involves enzymes to depolymerize the biomass.

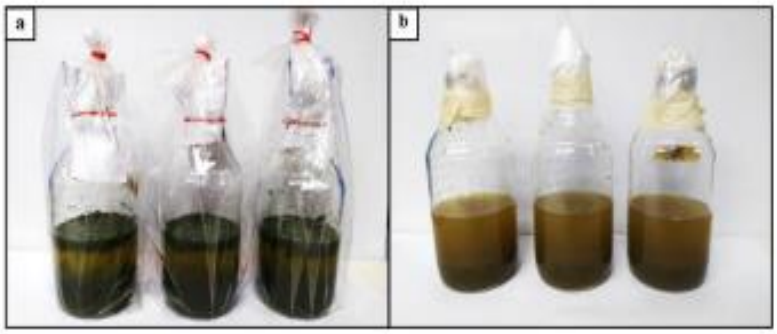


**Figure 3** Samples prepared for steam-explosion pretreatment (a, and hydrolysis (b.

The saccharide components released are often used as fermentation feedstock (Modenbach and Nokes, 2013). After pretreatment, the pH of the combined solution was adjusted at 5.0 and the samples were inoculated with 1% commercial cellulase for the hydrolysis process. Afterward, the solution was kept in an incubator at 35 °C for 24 h to perform the hydrolysis process (Figure 3b).

### 2.4 Fermentation

Fermentation was carried out after enzymatic hydrolysis. The pH of the hydrolysate solution was adjusted at 5.6 before being inoculated with 1% (wt./v) of commercial yeast. The fermented mixture was maintained at room temperature in the absence of oxygen for 120 h, with 80 mL of sample extracted every 24 hours to measure sugars and alcohol.

The fermentation efficiency was calculated using Equation 1 (Bermejo et al., 2021).

$$\%FE = \left(\frac{C_f}{C_p}\right) \cdot 100 \quad (1)$$

Where %FE is the fermentation efficiency in %, $C_f$ is the final bioethanol concentration (g/L), and $C_p$ is the maximum predicted bioethanol concentration (g/L).

### 2.5. Alcohol measurement

The ebulliometer technique was used to compare the boiling point of a given volume of distiller water with a known volume of broth to determine ethanol production. An ebulliometer is a simple instrument used to evaluate the alcohol concentration of a sample by measuring the boiling point of pure substances or mixtures (Cottrell, 1919; Howell and Byrne, 2014). The bioethanol yield ($Y_{p/s}$) over total sugar consumption and percent sugar utilization ($\%S_c$) were calculated using Equations 2 and 3 (Srimachai et al., 2015).

$$Y_{P/S} = \frac{C_f - C_0}{S_0 - S_f} \quad (2)$$

$$\%S_c = \left(1 - \frac{S_f}{S_0}\right) \cdot 100 \quad (3)$$

Where $Y_{P/S}$ is the bioethanol yield, $C_f$ and $C_0$ are the final and initial bioethanol concentration (g/L), $S_f$ and $S_0$ are the final and initial sugar concentration (g/L), and %Sc is the percentage of sugar consumption.

### 2.6. Sugar analysis

Spectrometry was utilized to quantify sugar concentrations using a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan). The phenol-sulfuric acid method and the Dinitrosalicylic acid (DNS) method were used to determine total sugars and reducing sugars, respectively (Dubois et al., 1956; Miller, 1959). A standard curve was produced using standard D-Glucose solution to determine the concentration of an unknown sample in mg/mL for both total and reducing sugars.

### 2.7. Data analysis

All of the experiments in this study were replicated three times. The data was presented as a mean, standard deviation from three replicates. Statgraphics Centurion 19 was used to do the statistical analysis. At the $p<0.05$ level, a significant difference was assessed.

## 3. Results and Discussion

### 3.1. Effect of steam-explosion pretreatment on the sugar formation

Steam-explosion was studied as a pretreatment of fresh elephant ear plant at different times (0 min, 15 min, and 30 min) in this study. Figure 4 illustrates the findings of total sugars and reducing sugars after steam-explosion pretreatment at three different times of exposure and after enzymatic hydrolysis.

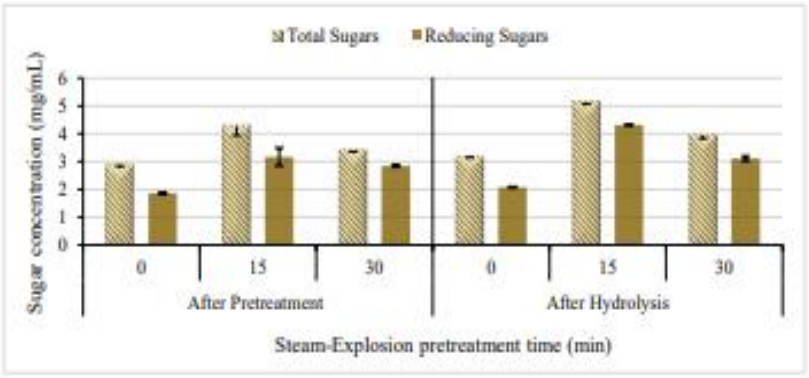


**Figure 4** Sugar content at different times of steam-explosion pretreatment and after enzymatic hydrolysis.

**Table 1** Bioethanol concentration during fermentation process.

| | Bioethanol mg/mL | | | | | | |
|---|---|---|---|---|---|---|---|
| Time (h) | $EP_1$ | $EP_2$ | $EP_3$ | Error | SD | Real | Predicted |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 24 | 1.578 | 2.367 | 1.578 | 0.263 | 0.058 | 1.841 | 2.203 |
| 48 | 0.789 | 1.578 | 1.578 | 0.263 | 0.058 | 1.315 | 0.699 |
| 72 | 0.789 | 1.578 | 1.578 | 0.263 | 0.058 | 1.315 | 0.598 |
| 96 | 1.578 | 0.789 | 0.789 | 0.263 | 0.058 | 1.052 | 0.572 |
| 120 | 0.789 | 0.789 | 0.789 | 0.000 | 0.000 | 0.789 | 0.538 |

It can be observed that the content of sugars after 15 and 30 min of pretreatment are similar (2.842±0.030 mg/mL and 3.167±0.035 mg/mL, respectively). However, after the enzymatic hydrolysis process, the hydrolysate exposed to steam-explosion for 15 min achieved the higher reducing sugars content with 4.320±0.011 mg/mL, compared with the obtained at 0 min and 30 min (2.077±0.059 mg/mL 3.111±0.023 mg/mL, respectively). For this reason, it was chosen to carry out the fermentation process. The steam explosion pretreatment procedure eliminated a significant amount of the hemicellulose fraction from the final product. The steam explosion pretreatment had a minor effect on the relative percentage of lignin in the samples, which was marginally lower than before (Hu et al., 2013). As suggested by authors under very mild pretreatment conditions, hemicelluloses were the most seriously impacted biomass components after steam explosion, which explains the high concentration of sugars in the water-soluble (Pitarelo et al., 2012).

### 3.2. Fermentation efficiency

Fermentation efficiency is a measure of how much alcohol was produced in a given amount of time compared to the quantity that could theoretically be produced. Table 1 displays the predicted ethanol production and real data collected during the fermentation process using commercial yeast. The efficiency of the fermentation stage (%FE) at the higher ethanol concentration (1.841±0.263 mm/mL) was 83.56% from the concentration of the reducing sugar estimated after 24 h of fermentation. Furthermore, as proven by Andrietta et al. (2012), when the byproducts technique was used to measure fermentation efficiency, it was not sufficiently robust to detect differences in the process induced by variables that had a significant impact on fermentation. Therefore, this methodology should be advantageous based on mass balances and the discounting of byproduct generation from a theoretical efficiency of 100% because the computed efficiency will never surpass this maximum value, which should be beneficial.

### 3.3. Bioethanol production

Bioethanol production from fresh elephant ear plants by fermentation using commercial yeast is presented in Figure 5. The fermentation process showed efficient ethanol production for the initial 24 h, which later became stationary to 48 h, and finally declined to 72 h. The higher ethanol content reached was 1.841±0.263 mg/mL after 24 h of fermentation. Besides, taking 1.841±0.264 mg/mL at standard temperature and pressure (1 atm and 273 K), the production of carbon dioxide $(CO_2)_g$ was estimated stoichiometrically in 4.611±0.123 mg/mL. Microorganisms that ferment a wide spectrum of carbohydrates into ethanol are critical to generating bioethanol from biomasses such as wood and lignocellulosic wastes (Dien et al., 2003). Compared to other microorganisms, yeast is the most frequently used in ethanol manufacturing. As a result of its high ethanol production and tolerance to ethanol, *S. cerevisiae* is the most utilized yeast for the fermentation of carbohydrates (Azhar et al., 2017). Recombinant microbes could also boost ethanol production from aquatic plants with high hemicellulose content, which can be converted into a mixture of pentoses and hexose by saccharification methods (Mishima et al., 2008).

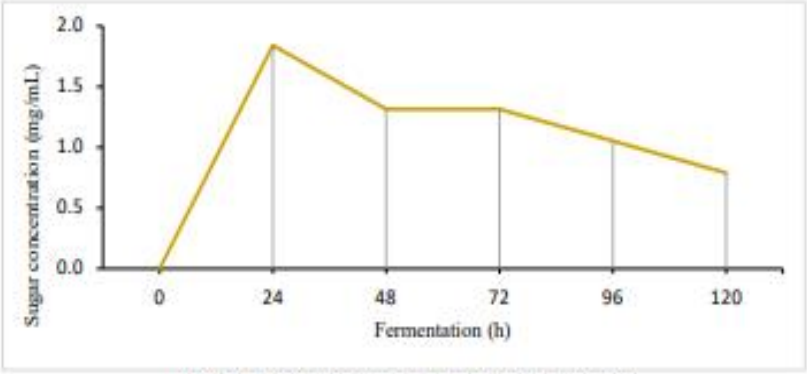


**Figure 5** Ethanol content during the fermentation stage.

### 3.4. Substrate utilization

In the fuel ethanol industry, yield is defined as the volume units of ethanol obtained via fermentation from a mass unit of the substrate (Soto et al., 2005). Table 2 resumes the data for the sugar content during the bioethanol production; for the highest ethanol concentration obtained in the present study (1.841±0.264 mg/mL), the ethanol yield ($Y_{p/s}$) was estimated at 0.31 g of ethanol/ g of substrate. It was shown that the expression of the two Calvin cycle enzymes in batch cultures reduced glycerol formation by 60% and

enhanced ethanol output on galactose by 8%. However, the biomass production on galactose in anaerobic batch cultures was not higher than in chemostat cultures, despite the predictions that it would be. Even while the galactose excess circumstances used in this batch cultivation may have imposed a slight metabolic load, the elevated expression levels of PRK may still result in an overall improvement in yield (Guadalupe-Medina et al., 2013).

**Table 2** Sugars content during the bioethanol production process.

| | | | Total Sugars mg/mL | | | | |
|---|---|---|---|---|---|---|---|
| | | | $TS_1$ | $TS_2$ | $TS_3$ | Error | SD |
| After Pretreatment | | | 4.213 | 4.227 | 4.347 | 0.042 | 0.006 |
| After Hydrolysis | | | 5.160 | 5.213 | 4.920 | 0.090 | 0.012 |
| | Fermentation | (h) | | | | | |
| | | 24 | 2.467 | 2.907 | 2.733 | 0.128 | 0.017 |
| | | 48 | 2.293 | 2.840 | 2.627 | 0.159 | 0.021 |
| | | 72 | 2.253 | 2.680 | 2.547 | 0.126 | 0.016 |
| | | 96 | 2.013 | 2.600 | 2.427 | 0.174 | 0.023 |
| | | 120 | 1.840 | 2.493 | 2.373 | 0.201 | 0.026 |
| | | | Reducing Sugars mg/mL | | | | |
| | | | $RS_1$ | $RS_2$ | $RS_3$ | Error | SD |
| After Pretreatment | | | 3.210 | 3.110 | 3.180 | 0.030 | 0.005 |
| After Hydrolysis | | | 4.320 | 4.360 | 4.280 | 0.023 | 0.004 |
| | Fermentation | (h) | | | | | |
| | | 24 | 1.470 | 1.390 | 1.250 | 0.064 | 0.011 |
| | | 48 | 1.210 | 1.170 | 1.140 | 0.020 | 0.004 |
| | | 72 | 1.190 | 1.070 | 1.102 | 0.036 | 0.006 |
| | | 96 | 1.050 | 1.020 | 1.097 | 0.022 | 0.004 |
| | | 120 | 0.830 | 0.920 | 1.010 | 0.052 | 0.009 |

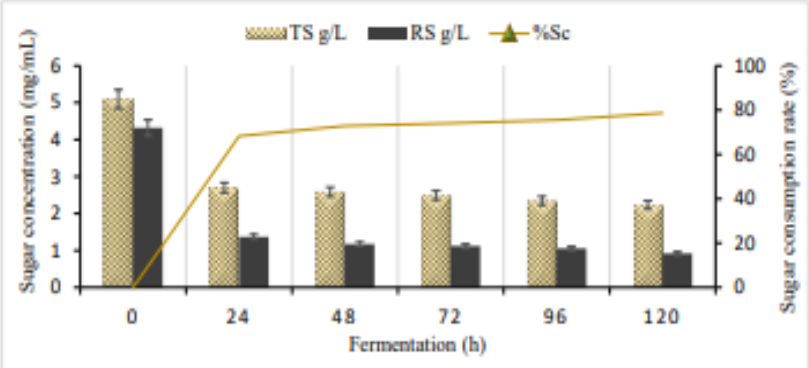


**Figure 6** Sugars means during the fermentation process.

Based on the initial fermentable sugar content, the percentage of sugar consumed during the fermentation process is denominated sugar consumption rate (%$S_c$) (Pătrașcu et al., 2009). It can be observed in Figure 6 that average values of the sugar consumption rate (%$S_c$) after 24 h of fermentation were estimated at 68.28%. Sugars are mainly transformed into ethanol, but a minor amount is also changed into other by-products such as glycerol and some flavor compounds in a well-run fermentation process. Fermentation is complete as soon as alcohol content reaches 5% to 7%, or the sugars are entirely used. The distillery may employ alcohol content, final gravity, or °Brix to monitor and appraise the completion of the fermentation process (Jacques et al., 2003; Mangwanda et al., 2021).

## 5. Conclusion

To enhance cellulose enzyme accessibility and produce high sugar concentrations from fresh elephant ear plants for bioethanol generation, a physical pretreatment (steam explosion) was successfully applied. According to the findings, there were differences in sugar concentrations between treatments. After 15 min of steam-explosion pretreatment, the maximum fermentable sugar concentration in the hydrolysate was 4.320 mg/mL. The maximum ethanol concentration of 1.841mg/mL was reached after 24 h with a fermentation efficiency (%FE) of 83.56%. Besides, the ethanol yield ($Y_{p/s}$) was estimated at 0.31 g of ethanol/ g of the substrate with a sugar consumption rate (%Sc) of 68.28%. Consequently, the elephant ear plant has the potential to produce bioethanol and, as a result, may serve as an excellent feedstock for the bioethanol production process itself.

## Acknowledgements

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# APPENDIX D CONFERENCES' CERTIFICATES

I. International conference on Energy and Environment (ICEE 2021)

Jyothi Engineering College

NAAC Accredited College with NBA Accredited Programmes*

Approved by AICTE & affiliated to APJ Abdul Kalam Technological University

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International Conference on Energy and Environment
(ICEE 2021)
April 09-10, 2021.

CERTIFICATE

This is to certify that the following paper has been presented and published in the International Conference on Energy and Environment (ICEE 2021 – Virtual Conference) during April 09-10, 2021 organized by Jyothi Engineering College, Thrissur, Kerala, India in association with Indian Society for Technical Education (ISTE).

**Title of the Paper :** Production of Bioethanol from Elephant Ear Plant by Hydrolysis Process

**Author(s) :** Marlen Trejo, Yuwalee Unpaprom, Rameshprabu Ramaraj

**Presented by :** Marlen Trejo

Rev. Fr. Roy Vadakkan
Chairman ICEE 2021

Dr. Sunny Joseph Kalayathankal
General Chair ICEE 2021

Dr. Deepanraj B.
Organizing Secretary ICEE 2021

II. 3rd Symposium on Industrial Science and Technology (SSTEC 2021)

SisteC 2021
3rd Symposium on Industrial Science & Technology

# *Certificate of Participation*

*Hereby expresses sincere gratitude and appreciation to*
**MISS. MARLEN TREJO**

*Has participated in the*
**3rd SYMPOSIUM ON INDUSTRIAL SCIENCE AND TECHNOLOGY**
**(SISTEC 2021)**
*as Presenter*

*on* **25th - 26th AUGUST, 2021**

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III. International Conference on Challenges and Progress in Biosciences and Engineering (ICCPBE 2021)

**INTERNATIONAL CONFERENCE ON CHALLENGES AND PROGRESS IN BIOSCIENCES AND ENGINEERING – (ICCPBE 2021)**

October 28th & 29th 2021

**CERTIFICATE**

This is to certify that **Ms. Marlen Trejo** of **Maejo University** has presented an Oral presentation entitled "Optimization and Kinetics Model of Bioethanol Production from Elephant Ear Plant in Batch Fermentation" in the **International Conference on Challenges and Progress in Biosciences and Engineering – (ICCPBE 2021)** organized by **Bharath Institute of Higher Education and Research (BIHER), India** and **MAHSA University, Malaysia** held on 28th and 29th October 2021.

**Prof. Dr. S. Suresh Kumar**
Pro Vice-Chancellor (Grants and Publications)
BIHER

**Prof. Dr. Rusli Bin Nordin**
Dean, FOMBN, MAHSA University

**Dr. P. Velmurugan**
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IV. 2021 University Consortium Graduate Forum

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No. 1, Section 4, Roosevelt Road, Taipei City 10617, Taiwan

## CERTIFICATE OF AWARD

**This is to certify that**

## Marlen Trejo

as **THE SPEAKER**

on the sub-theme ***Smart Agriculture***

**2021 UNIVERSITY CONSORTIUM GRADUATE FORUM**

**"Agricultural Adaptation Strategies for Coping with Climate Change"**

co-organized by

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VI. 3rd International Conference on Renewable Energy, Sustainable Environmental and


Agri-Technological Innovation (i-RESEAT)

VII. The Best Presenter Award at - 3rd International Conference on Renewable Energy, Sustainable Environmental and Agri-Technological Innovation (i-RESEAT)

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