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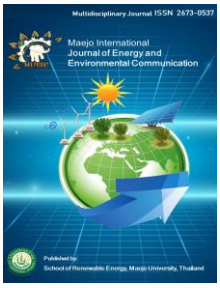

ARTICLE

The possibility of aquatic weeds serving as a source of feedstock for bioethanol production: A review

Phuong Thi Vu[1], Rameshprabu Ramaraj[1], Prakash Bhuyar[2], Yuwalee Unpaprom[3,*]

[1] School of Renewable Energy, Maejo University, Chiang Mai 50290, Thailand
[2] International College, Maejo University, Chiang Mai 50290, Thailand
[3] Program in Biotechnology, Maejo University, Chiang Mai 50290, Thailand

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ABSTRACT

Anaerobic digestion is recognized as an attractive option for the effective management and treatment of lignocellulosic biomass as well as waste recovery of resources for bioethanol production. Long enough testing has been done on bioethanol production using lignocellulosic biomass. This helps to reduce stress and global energy problems. Global wide has a variety of environmental impacts due to its use of fossil fuels. Bioethanol might be produced in Asian locations from many types of biomasses, including agricultural waste, forest waste, and wood biomass. This would be an environmentally friendly process. Unfortunately, there is very little research into the production of ethanol from rice field weeds. This makes it difficult to develop bioethanol production. This review is aimed at developing bioethanol production and the trend towards organic products that began nowadays. Unwanted weed growth is a major problem in rice cultivation. This review demonstrated the waste-to-energy aspect of the bioethanol production process using two weeds, gooseweed (*Sphenoclea zeylanica*), and small-flowered nutsedge (*Cyperus difformis*).

1. Introduction

Due to the rapid growth in the human population, there is a high demand for fuel production. This has made it a critical issue all over the globe (Junluthin et al., 2021). Even though the majority of current energy sources are from fossil sources, their reserves will run out in the next 40-50 years. The 60% of the world's fossil fuel consumption is by the transportation sector, which contributes to massive environmental pollution (Khunchit et al., 2020). The current energy use and development patterns cannot be sustained in the long term (Wannapokin et al., 2017). These conventional fuels have had severe environmental impacts worldwide, both from their long-term exploitation and prolonged application (Pimpimol et al., 2020; Saengsawang et al., 2020).

The rapid rise in CO_2 emissions from industrial activities and transport has caused significant climate change. It is clear that climate change could have an adverse effect on agricultural activities, which directly affect food supply, because of the dependence of agriculture on the weather (Nong et al., 2022a). Global issues such as energy security and the environment have increased the demand for a greener energy source. Many types of green energy can be used to decrease dependence on traditional hydrocarbon deposits (Nong et al., 2022b). These green energy sources are renewable and long-lasting. They can be derived from biomass, wind, hydro and wave. Except for biofuel, the technology to use this green energy is well-developed (Wannapokin et al., 2018).

* Corresponding author.
E-mail *address: yuwaleeun@gmail.com ; yuwalee@mju.ac.th*

Biofuels include bioethanol, biogas, biodiesel, biohydrogen, biobutanol, etc (Ramaraj & Dussadee, 2015; Saetang & Tipnee, 2021). Biomass can be made from it via both chemical and biological processes (Khammee et al., 2022). With the massive promotion of industrial-scale production in the European Union and the United States, biofuel has received a lot of attention. Many countries, such as the USA, Brazil, and China, have pledged to support biofuel programs in an effort to reduce dependence on fossil fuels (Nong et al., 2022c). According to the International Energy Agency, biomass is the most important source of renewable liquid fuels for vehicle and air transportation. According to the International Energy Agency, the use of biomass fuels for transportation purposes will increase from 2% in 2012 up to 20% by 2040. If we look back at the past, it is clear that ethanol has been widely used in the transportation sector in the United States and Europe since the early 1900s as an alternative fuel. Bioethanol was first used in the internal combustion engines of internal combustion engines (IGEs) by France and Germany in 1984. Brazil has been using bioethanol since 1925. However, due to the high cost of production, ethanol was not well-known by the market and government.

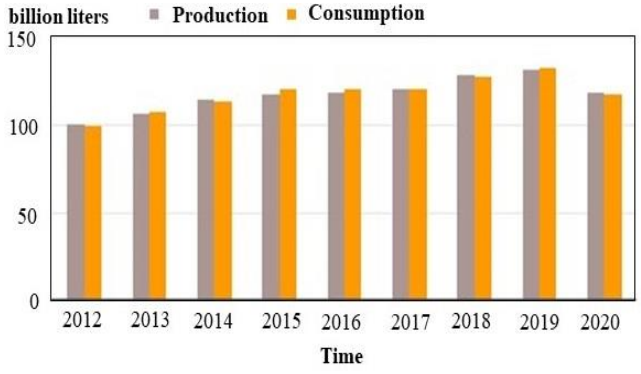

Figure 1 World ethanol production and consumption (OECD-FAO, 2021)

World ethanol production and consumption have shown in Figure 1. Global bioethanol production reached 100 billion liters in 2016, mainly due to the United States and Brazil. The United States is the world's largest producer of bioethanol from corn, with Brazil second from sugarcane. However, these crops cannot meet alternative energy bioethanol production demand. To meet increasing demand, it is necessary to find new materials and improve existing processes in order to increase ethanol yield (Ramaraj et al., 2021). The inset plot shows global annual production volume of bioethanol and biodiesel. Different symbols represent different world regions (Azadi et al., 2017).

The global market for ethanol is even more concentrated than the global biodiesel market, with the top two ethanol manufacturers accounting for 74% of the industry's total production (Figure 2). The amount of ethanol that was produced in the United States (59,809 million liters), Brazil (36,238 million liters), China (10,500 million liters), the European Union (6,370 million liters), India (3073 million liters), and the rest of the globe combined was 30,733 million liters (13,360 million liters). Today, ethanol is mainly produced from sugar- or starch-based feedstocks (Ramaraj & Unpaprom, 2019). Thailand, a country blessed with many plants and lands is on its way to producing biofuels from edible sources (sugarcane or cassava), to meet the nation's high-demand strategies (2015-2036). The target of the Thai government is to increase bioethanol yield to 11.3 billion liters/day in 2036. This is important in light of global fuel demand. However, it also raises serious concerns about food production. Therefore, the interest in producing ethanol from second-generation lignocellulosic biomass has increased recently (Khammee et al., 2021).

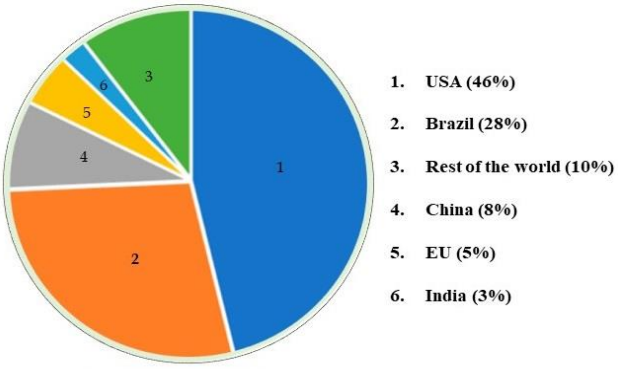

Figure 2 The contribution of ethanol production (OECD-FAO, 2021).

Even though second-generation bioethanol is not as beneficial as the first, its potential for national production is enormous due to feedstock availability. Bioethanol made from non-edible and residue crops should be double the price of conventional bioethanol to increase demand and reduce conflict between fuel and food (Figure 3). This pathway requires more work from engineers and researchers to overcome the capital and operating cost bottlenecks that prevent large-scale development (Sophanodorn et al., 2022a). The United States is home to the first-ever cellulosic-ethanol plant. It was established in 2014 and has since been used for bioethanol production.

The common and widespread herbaceous weeds of wetland rice are Gooseweed (*Sphenoclea Zeylanica* Gaertn), and Small-flowered nusedge (*Cyperus difformis*). The family Sphenocleaceae includes gooseweed, while small-flowered nutsedge belongs to the family Cyperaceae (Carter and al., 2014). It is found in the Eastern Hemisphere, including Thailand, Viet Nam and Indonesia. These two species are a problem non-woody plants on transplanted wetland rice fields because they prefer wetland and water bodies. Holm and his colleagues (1977) deemed them to be the worst weeds in the world. Ghosh & Ganguly (1993) claims that dominant gooseweed and other species caused a 32-50% loss of rice yield in India due to nutrient and living-space competition with rice. This weed can be removed by farmers using a variety of methods, including chemical and biological, which take a lot of time and effort and don't provide any economic benefits (Mabbayad & Watson, 1995). Gooseweed and small-flowered nutsedge are promising materials for bioethanol production. In this review, the helpfulness of the pretreatment parameter that affects lignin degradation and yield of reducing sugar is the topic of discussion. Additionally, conduct research on the feasibility of manufacturing bioethanol from gooseweed and small-flowered nutsedge. In addition, research should be done to determine whether or not it is possible for termite colonies to digest gooseweed and small-flowered nutsedge. This investigation has demonstrated that these noxious weeds can be utilized in the production of bioethanol, an important commodity.

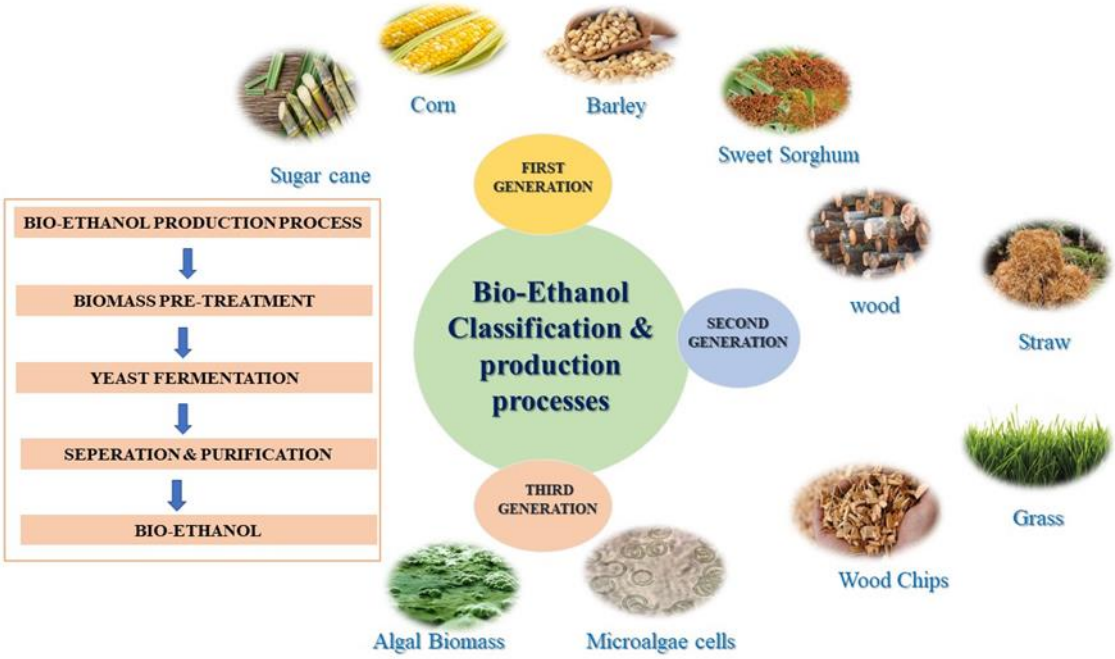

Figure 1 Classification of bioethanol generations and production process

2. Characteristics of bioethanol

Figure 4 shows the three-dimensional structure of ethanol compound, which is composed of 6 hydrogen, 2 carbon and 1 oxygen. Table 1 lists the chemical and physical characteristics that make ethanol a promising fuel for the transportation sector. Ethanol is safer and more environmentally friendly than gasoline in terms of its octane value, range of flame flammability limit concentration volume, flashpoint, and ignition temperature (Sophanodorn et al., 2022b). The ethanol's higher octane numbers allow it to burn at a lower compression ratio and for a shorter time. This results in less engine knock. It also has a higher flash point, which makes it safer to work at ambient temperatures. Due to the oxygen in the ethanol molecules, the combustion efficiency for ethanol is 15% higher than gasoline. Bioethanol, unlike petroleum fuel, is more toxic and readily biodegradable. It also produces less air-bone pollutants than petroleum fuel (John et. al., 2011). When ethanol is mixed with it, ethanol can improve the performance of gasoline.

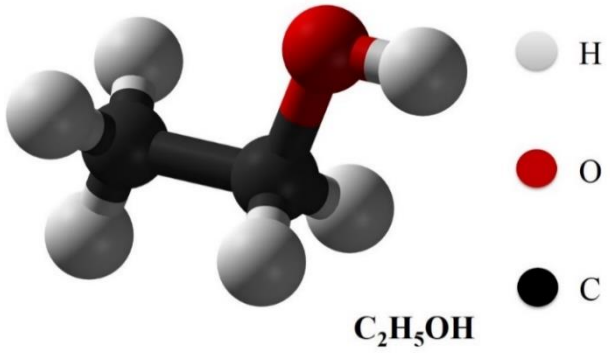

Figure 4 Ethanol molecule in 3D

Table 1 A comparison of ethanol and gasoline

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

3. Lignocellulosic biomass

Recently, lignocellulosic materials' potential as viable feedstock for ethanol production has attracted attention due to its vast availability and low cost. Forestry wastes, agricultural residues (sugarcane bagasse, corn stover, etc.), aquatic plants, herbs, and energy crops (poplar, switch grass, giant red, elephant grass, etc.) are all examples of lignocellulosic materials (Pantawong, et al. 2015). Cellulose makes about 30–50% of lignocellulosic biomass, while hemicellulose contributes 15–35%, and lignin accounts for 10–20%. These polymers form hydrogen bonds and van der Waals interactions to form inflexible matrices (Figure 5). The cellulose chains are the primary factor that has a significant favorable effect

on both sugar production and ethanol yield. Conversely, the presence of hemicellulose and lignin weakens hydrolysis activities and reduces sugar production. Therefore, it is crucial to know the features of biomass to create an appropriate pretreatment that improves the hydrolysis process.

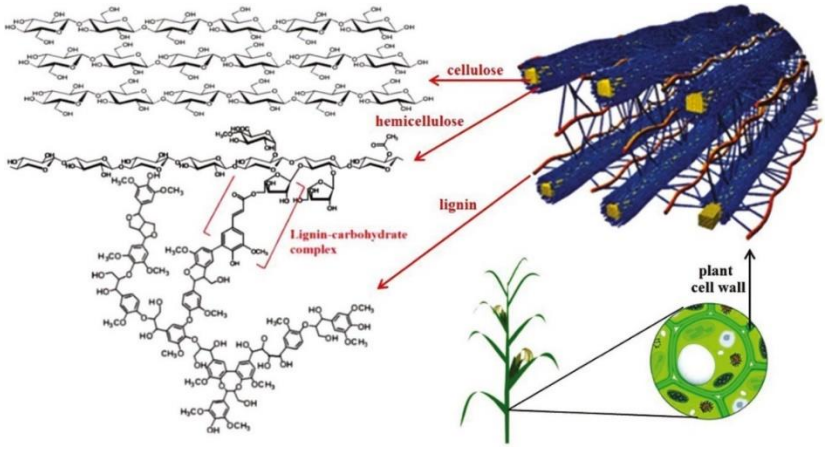

Figure 5 Structure of lignocellulosic materials (Volynets et al., 2017).

3.1 Cellulose

Cellulose, which is 1.5 x 10 12 tons, is the most common organic compound. Cellulose is made up of many D-glucose molecules that are linked by β (1→4)- glycosidic and hydrogen bonds. There is the potential for many thousand glucose units to exist in a single chain of cellulose, which can subsequently take on either crystalline or amorphous forms (Figure 6). Crystalline cellulose is resistant to hydrolysis by chemicals or enzymes that can convert amorphous cellulose into monosaccharides. Hydrolysis is the process in which cellulose is broken down into simpler sugars. Cellulose is resistant to being degraded by bacteria in any way. Because of this, a pretreatment technique to weaken it is required before the enzymatic hydrolysis can even begin to take place. The results of this reaction are a more straightforward molecule of D-glucose, as well as the structural component cellobiose. Crystallized cellulose has been shown to have yields and enzymatic hydrolysis rates that are more than one hundred times higher than amorphous cellulose.

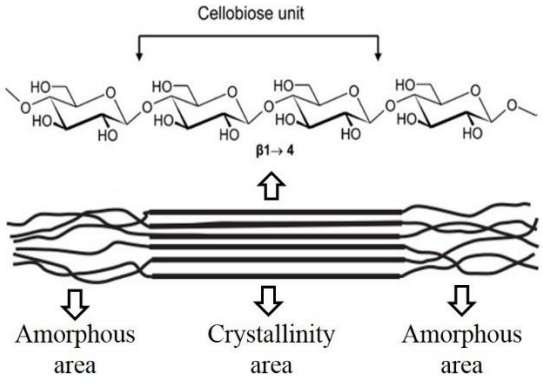

Figure 6 Cellulose's molecular structure and three-dimensional form

This has been proved through research. If there is a 10% increase in crystalline cellulose, the enzymatic hydrolysis rates will decrease by around 40% (Hall et al., 2010). It is necessary to undergo pretreatment to lessen cellulose's crystallization and make it more accessible to hydrolysis agents. In point of fact, temperatures of up to 320 °C and pressures of up to 25 megapascals may be required for water to make the transition from crystalline to amorphous (MPa) (Deguchi et al., 2006).

3.2 Hemicellulose

Hydrogen bonds are responsible for the strong attachment of hemicelluloses, which are cell wall polysaccharides, to cellulose microfibrils. It is composed of pentoses, such as β-D-xylose and α -L-arabinose, as well as hexoses, such as β-D-mannose, β -D-glucose, and α-D-galactose (Girio et al., 2010). A trace quantity of uronic acid and other sugars, such as a-L rhamnose or a-L fucose, can also be found in the compound. However, the most abundant form of hemicellulose that can be discovered in secondary cell walls is xylan. Xylan is responsible for up to half of the biomass that is found in grasses and cereals.

3.3 Lignin

The lignin content is between 3-30% (Demirbas 2005; Van Tran et al., 2020). In fact, lignin can be burned to produce steam or power, or it can undergo enzymatic polymerization to produce mono-aromatic chemicals such as gallic and ferrulic acids. The creation of phenolic compounds will be made possible as a result of this. A higher enzyme loading was required for the adsorption lignin to cellulase (Unpaprom et al., 2021). This is because binding creates an inefficient enzyme attachment that limits the availability of cellulose to cellulase. Cellulolytic enzymes are also significantly deactivated by phenolic compounds that result from the degradation of litinin. Enzymatic hydrolysis can therefore be affected. As studies have shown that lignin, once extracted from biofuel processes, can be an energy source self-sustaining to maintain financial solvency of bio refineries, it could be beneficial to preserve the lignin.

4. The production processes for ethanol

In most cases, the process of converting lignocellulosic biomass into bioethanol involves a series of consecutive phases, which are referred to as pretreatment, hydrolysis, fermentation, and

distillation respectively. They are able to be designed in a variety of various ways, each of which has the potential to both improve the working conditions and lower the overall production costs of each stage (Whangchai et al., 2021). Researchers have recently developed a number of processes, including simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), separate hydrolysis and co-fermentation (SHF), separate hydrolysis and co-fermentation (SHCF), separate hydrolysis and co-fermentation (SHF), and separate hydrolysis and co-fermentation (SHCF) (CBP). The steps involved in each procedure are illustrated in Figure 7. In the process known as co-fermentation (CF), a microbe contained within a fermenter is responsible for the fermentation of both xylose and glucose. The following table provides an overview of the benefits and drawbacks associated with each production route: (Table 2).

4.1 Pretreatment method

Pretreatment upstream operations involve mainly thermochemical and physical processes that disrupt the recalcitrant biomass material (Manmai et al., 2019a). Pretreatment technology today produces by-products that have a lower ethanol productivity. This makes it economically impossible to produce bioethanol from lignocellulosic material (Manmai et al., 2020). The process of turning lignocellulosic biofuel into ethanol starts with the destruction of cell walls and the release of starch or sugars from the plants. The combination of lignin and hemicellulose can be difficult to break down into simple sugars. Pretreatment is necessary because of this.

Researches have shown that hydrolysis efficiency is affected by the surface area, lignin and crystallinity of the cellulose polymer (Manmai et al., 2021). Pretreatment is designed to overcome the physical barriers that prevent biomass from being able to be metabolized. It can use any combination of biological, chemical, or biological methods. Pretreatment can alter the structure of the cell walls by using chemical or physical agents (Manmai et al., 2019b). Pretreatment can cause the breakdown of the lignin layer, the degrading of hemicellulose to monomers, and the easy exposure to enzymes that convert the cellulose chains into simple sugars. Pretreatment reduces the pressure at saccharification by altering the crystallinity of the cellulose matrix. Many researchers have demonstrated that pretreatment can alter the structure of cells (Manmai et al., 2021). Dilute acid or hydrothermal pretreatment can reveal the molecular weights and structure of lignin (Behera, et al. 2014).

It is imperative that the application of an appropriate pretreatment should be based, in large part, on the properties of the feedstock. In general, the pretreatment process can begin with a physical material's size reduction method that increases the accessible surface of materials (Manmai et al., 2020). Subsequently, harsh conditions (high temperature or pressure and strong chemical) can be used to completely break the linkage of the cell wall (Mejica et al., 2022). This is done to ensure that the materials can be processed. The increase in the surface area that is accessible results in an increase in the efficiency of the pretreatment and hydrolysis processes. An efficient pretreatment is essential for achieving optimal success with hydrolysis, since it cuts down on the creation of chemicals that hinder the process.

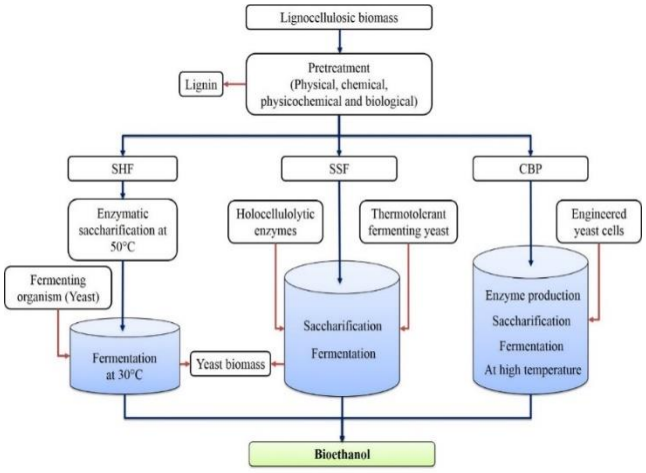

Figure 7 Modified schematic diagram of bioethanol production through different routes (Choudhary et al., 2016)

Table 2 The pros and cons of different bioethanol production routes

Types	Advantages	Disadvantages	Reference
SHF (SHCF)	The hydrolysis and fermentation processes can be completed at optimal condition of pH and temperature.	An accumulation of glucose concentration can impede a costly Hydrolysis process.	(Taherzadeh and Niklasson, 2004)
SSF (SSCF)	Good value Excellent ethanol production rate Rapid processing time decrease the detrimental effect of manufactured glucose on hydrolysis	Fermentation and saccharification have different optimum temperatures.	(Baeyens et al., 2015)
CBP	Reduce inhibitors, and operation cost	Suitable microorganism strains for commercial purposes are not yet available	(Fan, 2014)

4.1.1 Physical pretreatment

When it comes to pretreatment of lignocellulosic feedstock, the physical processes require just the use of mechanical methods, together with high temperatures and pressures, and do not involve any chemical reagents. The results of a comparison research showed that using maize stover with a particle size between 53 and 75 um delivers outcomes that are 1.5 times more beneficial than using substrate with a higher particle size (Zeng et al., 2007). The method involves a significant amount of financial investment and a high level of energy expenditure as its primary drawbacks.

4.1.2 Additional physicochemical treatment

Behera and his colleagues (2014) emphasized the significance of lignocellulosic feedstock undergoing chemical pretreatment prior to the synthesis of bioethanol, and they provided a list of various chemical pretreatment methods that are appropriate for use on an industrial scale. On the basis of life cycle assessment studies, recent research has evaluated and compared the processes of biochemical and thermochemical conversion (Ramaraj & Unpaprom, 2016). They came to the conclusion that even though the two methods produce an equal amount of alcohol and are equally efficient with energy, biochemical conversion is thought to have better environmental performance in the short run compared to thermochemical conversion. This is because biochemical conversion uses less energy.

4.1.3 Acid pretreatment

Acid pretreatment is a promising method to industrially produce bioethanol from lignocellulosic material. High concentrations of acid may cause cell wall damage, even though the lignocellulosic structure is highly deformed. This could make it possible for downstream microorganisms to become inactive (Whangchai et al., 2021). High heat and acidity in the pretreatment cause the sugars produced by hydrolysis to be broken down into furfural (from the breakdown of pentoses like xylose and arabinose) and 5-hydroxymethilfurfural (or HMF) (degradation of hexoses: glucose, mannose, and galactose). Even though furfural can polymerize or formic acid, HMF yields chemically comparable amounts of levulinic and formic acids at the molecular level (Dagnino, 2013). A concentration of around 10g/L of acid acetic can be achieved using dilute acid pretreatments of agricultural residues, as well as herbaceous or hardwoods (Larsson et al., 1999). The hydrolysis of the acetyl group produces acetic acid.This is a component in the hemicellulosic portion and is available as a substituent for xylose monomers in both the solid phase and oligomers.

4.1.4 Alkaline pretreatment

Sodium hydroxide, potassium hydroxide, lime, ammonium aqueous solution, and other alkaline reagents can be used to treat lignocellulosic biomass, and other similar substances rather than treating it with concentrated or diluted acids while subjecting it to high temperatures or pressures (Mejica et al., 2022). It's possible that the reaction between the lignocellulosic biomass and the alkaline reagent will go on for a very long time—anywhere from one hour to many days (Ramaraj & Unpaprom, 2019). Even if it is not required to use more energy, having a longer retention period is one of the most significant drawbacks of this alkaline pretreatment that has been proven.

4.1.5 Steam explosion

High-pressure saturated steam was the cause of the explosion caused by the steam. The pressure is then rapidly dropped, putting the feedstock in the path of an explosive decompression that opens up the structure of the biomass and makes the enzymes more accessible. The pressure is often in the range of 1.1 to 2.3 MPa, while the temperature frequently varies between 160 and 220 °C. The utilization of steam explosion as a method for the generation of ethanol from a variety of lignocellulosic materials has proven to be fruitful (Nguyen et al., 202b). During the pretreatment process, the biomass is heated up by the condensation of steam, which ultimately results in the formation of microporous structures that are filled with hot liquid. The weak acidity of water brings the pH down to a range between 3 and 4, which is the starting point for the depolymerization of hemicellulose (Sophanodorn et al., 2022b). This kind of pretreatment is, however, relatively analogous to the diluted and concentrated acid methods, both of which encourage the development of inhibitor compounds for hydrolysis and fermentation.

4.1.6 Liquid hot water pretreatment

The pretreatment of liquids using hot liquids is a hydrothermal technique that does not include the use of any catalysts or chemicals. At temperatures between 160 and 240 °C, the application of pressure is required to keep water in its liquid condition. The solubilization and breakdown of the hemicellulose by liquid hot water is the primary action of liquid hot water, which also makes the cellulose more accessible (Khunchit et al., 2020). The pH range can be regulated anywhere from 4 to 7, allowing for more precise regulation of the inhibitor production process.

4.1.7 Biological pretreatment

Biological pretreatment generally uses microorganisms capable of degrading lignin or hemicellulose. A variety of microbes can be used to remove lignin, hemocellulose, and/or cellulose. The termite gut, a similar microorganism, serves as their digestive system. It has microorganisms in its gut that can degrade lignocellulosic structure (Vu et al., 2018). The microorganisms play a key role in degrading lignocellulosic biomacromolecules, which contain lignin or hemicellulose. They use a variety enzymes to break down polymer chains and make simpler molecules. Termites can digest lignocellulosic material such as wood and grass (Ramarajv& Dussadee, 2015). Higher termites have a significant advantage in that they can degrade lignocellulosic biomass. Because it requires very little energy, this pretreatment is extremely popular. Other advantages of biological pretreatment include the absence of chemical pretreatments and mild conditions.

4.1.8 Acidic hydrolysis

There are two types of acidic hydrolysis: concentrated and dilute. Concentrated acid hydrolysis occurs at lower temperatures using high acid concentrations. Because pentose sugars are more quickly degradable than hexose sugars, acid hydrolysis of lignocellulosic biomass is performed in two stages. This produces a large number of inhibitors. Acid hydrolysis has several drawbacks that limit its use in industry. First, it is difficult to control the degradation of sugars into by-products. Large amounts of acid can contaminate the environment (Khunchit et al., 2020). Dilute acid is corrosive to machinery, but it is less problematic at high levels of acid. Finally, acid hydrolysis has the disadvantage of increasing production costs due to acid recovery and recycling difficulty.

4.1.9 Saccharification

After the pretreatment stage, the feedstock is subject to hydrolysis. This allows it to be converted into fermentable sugar for use in the next phase of bioethanol production. The efficiency of hydrolysis depends on several factors (Nasution et al., 2022). This category includes: The amount and quality of cellulose, hemicellulose and lignin, as well as the crystallinity, porosity, and the crystallinity of the cell walls, all of these characteristics are important (Manmai et al., 2020). A plant's cell wall is protected by hemicellulose and lignin. This barrier stops enzymes from accessing the cellulose chains within the cell wall. The rate at which hydrolysis occurs can also be affected by the structure of cellulose (Jayabalan, 2019). This may be determined using terms like crystallinity or amorphous. As a result of the formation of crystallinity in the cellulose chains, hydrolysis occurs at a slower pace. The two main types of hydrolysis used most often are acidic hydrolysis or enzymatic.

4.1.10 Enzymatic hydrolysis

Because of its higher yields and selectivity, enzyme hydrolysis is preferred over chemical processes, as well as the lower operating costs and milder conditions than chemical processes. Cellulase enzymes can hydrolyze cellulose (Nguyen et al., 2020). These enzymes work together to hydrolyze cellulose to cellobiose or glucose. However, hemicellulose is more difficult to break down than cellulose because of its complex structure, which necessitates more enzymes (Manmai et al., 2020). Endoxylanase, exoxylanase, b-xylosidase, a-arabinofuranosidase, a-glucuronidase, acetyl xylan esterase, and ferulic acid esterase are all part of the multi-enzyme system responsible for xylan hydrolysis. A brown liquid is produced when the majority of the sample's solid components are hydrolyzed.

4.1.11 Fermentation

Bioethanol production largely depends on yeast, a microorganism that ferments various sugars into ethanol (Nguyen et al., 2022a). Bioethanol production relies on yeast's capacity to convert six-carbon molecules, such as glucose, into two-carbon components, such as ethanol, without converting to the end oxidation result, carbon dioxide. Properties such as high ethanol yield (>90% theoretical yield), tolerance (>40.0 mg/L), high ethanol production (>1.0 g/L/h), growth on simple media and undiluted fermentation broths, and resistance to inhibitors and retard contaminants from growing conditions make them ideal for use in industrial plants (Manmai et al., 2020). It has been claimed that yeast strains including *Pichia stitis*, *S. cerevisiae*, and *Kluyveromyces fagilis* are particularly effective at fermenting a wide range of carbohydrates into ethanol (Lewandowska, et al. 2016). Temperature, sugar concentration, pH, fermentation pace, inoculum size, and agitation rate are all variables that impact bioethanol production. There is an optimum temperature range of 20–35 oC for fermentation. The S. cells are now at large. Since heat may be transferred from the particle surface to the interior of the cells, the optimal temperature for *S. cerevisiae* is 30ºC, but for cells it is slightly higher (Liu & Shen 2008).

Ethanol can be produced effectively in yeast cells. This is because they are more resistant to external factors and have a better cell cycle cost, contamination risk, dilution rate, and susceptibility. Because of their proximity, cells in the medium can hinder the production of products and the uptake of substrates. Most of the issues that arise in free-cell systems are mitigated by the immobilization procedure (Nguyen et al., 2022b). There is no discernible difference in the amount of ethanol that can be produced by yeast cells vs free yeast cells. Z. Mobilise is the most widely used microorganism, but both Z. Mobilise and *S. cerevisiae* cannot ferment pentose sugars. P. stipitis is known for their ability to convert pentose glucose (xylose) into pentose sugar. These bacteria are not efficient with high-caring handling (Dev et al., 2019). They are fragile and easily damaged by an acidic environment, inhibitors, and excessive amounts of ethanol. During the fermentation of glucose, *S. cerevisiae* contains two genes that are responsible for catalyzing both the reduction of acetaldehyde to ethanol and the reverse conversion of ethanol into acetaldehyde. Both reactions are necessary for the production of ethanol.

4.1.12 Distillation

Fractional distillation is the most popular and straightforward method among the several distillation processes. This is due to the fact that in addition to other components, such as water, alcohol, and lignin, the fermenter also contains a variety of volatile chemicals. Unconverted hydrocarbons are also present in the fermenter. Since the boiling point of ethanol is lower than that of water (78.3 °C at 1.13 atmospheres), ethanol will turn into steam before water does. The boiling point of water is 100 °C at 1.013 atmospheres, while the boiling point of ethanol is 78.3 °C at 1.13 atmospheres. The system will often be divided into two columns on a regular basis. The first column is able to remove the dissolved carbon dioxide, and the majority of the ethanol that makes up the product's water comes in at between 37 and 40%. The ethanol, on the other hand, can be concentrated in the second column to a composition that is virtually totally azeotropic (approximately 92.4% ethanol). After going through this procedure, the ethanol concentration will be at its maximum allowable level, which is 96 percent by weight (Cardona & Sanchez, 2007). In order to complete the further dehydration that is necessary to bring the overall percentage up to 99.5 %, molecular sieve adsorption in the vapor phase is required.

This traditional method of purification offers a significant advantage in the form of high ethanol recoveries, but it also has a major drawback: more energy consumption at lower ethanol fractions. Alternative technologies are needed to reduce energy consumption and increase ethanol recovery. Recent research has reported on advanced distillation technologies that are energy-efficient, economically efficient, and high ethanol recovery. Pervaporative separation, membrane distillation, liquid/liquid extraction, and steam/gas stripping are some of the techniques that fall under this category. In addition to that, one of these methods is known as membrane distillation. The process of evaporating liquid through the use of a hydrophobic membrane is what is meant to be understood when one refers to membrane distillation. The difference in vapor pressure that exists between the two locations is taken into account while determining it. The direction that this process goes in is determined both by the temperature of the feed input and the chemical composition of it. Pervaporation distillation, which is another method of membrane distillation with a similar purpose, is the process of separating liquid mixtures by partial vaporization via solid membranes such as (non-) porous membranes and vapor permeation. This method of membrane distillation is used to separate liquid mixtures (Kiss 2014).

5. Gooseweed and small-flowered nutsedge weed step by step approach in bioethanol production

According to Vu et al. (2017; 2018), organic rice fields on the campus of Maejo University Sansai in Chiang Mai, Thailand, were used to cultivate Good weed and small-flowered nutedge. After that, the fresh samples were transported to Maejo University's Energy Center Research for further analysis. After that, they were rinsed in running water to remove any remaining muck and filth. The samples were dried for one day on a drying rack that was placed directly in the sunlight. After being exposed to the sun, the samples were dried further in a hot oven at a temperature of 50 °C for one day. After that, the powder was reduced to a finer consistency using a sieve with a 1.0 mm opening (Figure 9). Almost immediately after that, the powdered materials were utilized in various experiments.

The sum of the biomass yields of all the plants growing in a given area is referred to as the total biomass yield. Gooseweed and small-flowered nutsedge are two types of plants that thrive in locations with little to no movement, such as the rice fields surrounding Maejo University in Chiang Mai, Thailand (18°53'36.3"N 99°01'14.4"E). Figure 10 depicts the random placement of a one meter by one meter quadrat in a field of rice. In the middle of a rice field, two plants were planted there at random. We utilized the data to calculate the density (plants per square meter) and the biomass yield (kg per hectare).

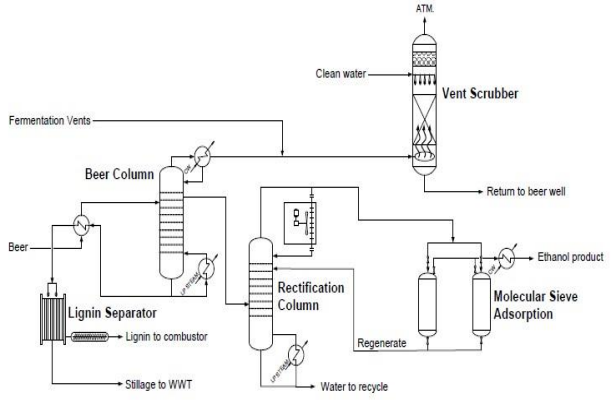

Figure 8 Simplified flow diagram of the separation process. (Humbird et al., 2011)

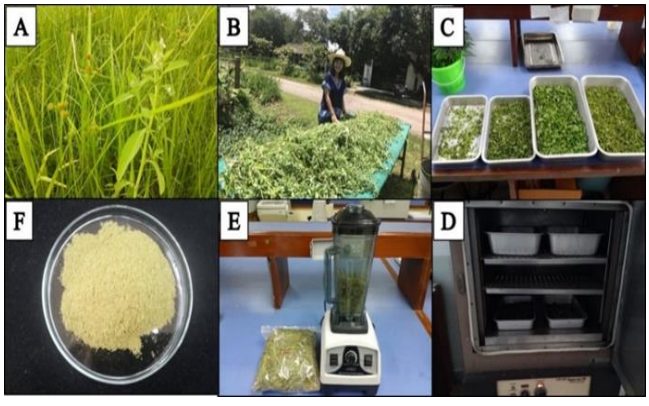

Figure 9 Gooseweed and small-flowered nutsedge in a rice field (A); Sunlight drying (B); Hot air drying (C, D); Powdering process (E, F) (adopted from Vu et al., 2017).

The chemical preparation was modified from what was described in the previous research. Figure 8 represents simplified flow diagram of the separation process The powdered samples were left out in the open for between 24 and 72 hours in order to be treated with sodium hydroxide and hydrogen peroxide. We employed pretreatment to investigate how the concentration of the chemical, the amount of time it took for the reaction, and the proportion of solid samples to chemical solution impacted the amount of sugar that was produced. According to Vu et al. (2018), the hydrolysis procedure was carried out with the help of a cellulase enzyme that was purchased commercially. The supplier's enzymes have a pH of 4, beta-glucosidase activity of 5,77 units/g, and enzyme activity of 2398 units/g. Cellulase at a concentration of 2 % (v/v) and hydrochloric acid were used to bring the pH of conical flasks containing 200 milliliters of pretreatment sample to 5. The mixture was swirled at 150 revolutions per minute while it was held at a temperature of 50 °C for 24, 48, 72, and 72 hours. When calculating the total sugar level and the rate of decrease in sugar, each time period was considered. The formula (Eq3) that was used to obtain the hydrolysis efficiency is as follows:

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

Figure 10 Sample counting and collection within a 1m x 1m quadrat (Vu et al., 2017)

The yeast strain *Saccharomyces cerevisiae* TISTR 5020 was grown on liquid YPD media that had been autoclaved (heated to 120 °C for fifteen minutes) and rotated at a rate of 150 revolutions per hour for an entire day (10 g l-1 yeast extract, 20 g l-1 peptone, 20 g l-1 dextrose). After that, the broth was placed into centrifuge tubes and spun at a temperature of 4 °C and 7000 rpm for 10 minutes in order to separate the yeast cells from the media. After adding an amount of sodium alginate that was equivalent to 2%, the yeast cell pellet was fully mixed after the addition of the sodium alginate. A syringe was used to inject the material into a flask that already contained 150 milliliters of calcium chloride with a concentration of 0.05 millimolar. Immobilizing the yeast cells in the final step of the process involved first cleaning them with distilled water that had been autoclaved and then keeping them in the refrigerator at a temperature of 4 °C. The cell count of *S. cerevisiae* that was actively developing was measured with a hemocytometer, and the outcome was found to be 2.5 x 107 cells per milliliter.

Batch fermenters were utilized throughout the entirety of the

fermentation process in the research that was conducted by Vu et al. (2017; 2018). A hydrolysate solution that had a pH of 5.6 was added to a fermenter that had a working volume of 100 mL, and the mixture was fermented with *S. cerevisiae* beads that contained yeast. The mixture was maintained in an incubator at a temperature of 35 °C for a time period that ranged from three to nine days (Thangavelu et al., 2014). After 3, 5, 7, and 9 days, aliquots of fermented samples, each containing a volume of 50 milliliters, were collected from the fermenter and run through an ebulliometer to determine the amount of ethanol that was present (Dujardin-Salleron, Alcohol Burner, France). The following equation (Eq4) is the one that was used to compute the amount of ethanol that was produced:

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

Where, E represents the concentration of ethanol in g/L and G represents the concentration of reducing sugar in g/L.

The samples used in Vu et al. (2017) research were completely new to ethanol production. Both small-flowered nutsedge and gooseweed were pre-tested for starch using iodine solutions. This method is used to identify potential feedstocks for bioethanol production. It reduces the time and costs of research. Starch can be found in both weeds (Vu et al. 2017;2018). These two weeds could have been used as raw materials because of their high starch content and abundant numbers.

Table 3 contains the results of proximate and compositional analyses conducted on gooseweed and small-glowered nutsedge. The moisture content of both samples used in this study was quite low when compared to the moisture content of other aquatic plants, such as *Impereta cylindrical*, *Eragrostis airoides*, *Typha angustifolia*, *Arundinella khasiana*, and *Echinochloa stagnina*, amongst others. This finding was determined by comparing the moisture content of the two samples to the moisture content of other aquatic plants. The percentages of these other aquatic plants' phosphorus content were as follows: 8.55%, 8.28%, 13.95%, 10.37 %, and 10.27%, respectively (Singh et al., 2017). Because it affects how biomass is stored, handled, fed, and turned into something new, moisture is an essential component that must be taken into account.

The physicochemical characteristics of the biomass have an effect on the way in which it is handled, stored, and moved, while the composition of the biomass has an effect on the efficiency with which it is transformed into energy. Materials that have a high moisture content can be dealt with by bio-conversion, whereas the solid and gas 'conversion process favors materials that have a low moisture content (less than 15%). Materials with a low moisture content are typically preferred by the solid and gas 'conversion process. In addition, the material in question needs to have a high percentage of volatile matter and a low percentage of ash in order to be taken seriously as a potential feedstock for the synthesis of bioethanol. These two characteristics are essential for the viability of the feedstock. Volatile matter values of goosweed and small-flowered nutsedge resulted in similar values with other potential lignocellulosic biomass such as wheat straw, flax straw, timothy grass, pinewood, and barley straw, with a range of 77.9 – 82.4 %. These values were determined by comparing the goosweed and small-flowered nutsedge to the other potential lignocellulosic biomass. It was discovered that these values are equivalent to the values of other potential forms of lignocellulosic biomass.

When it comes to assessing whether or not lignocellulosic biomass can be turned into bioethanol that can be sold for a profit, the amount of cellulose and hemicellulose that is already present is the single most critical component. In a different meaning, cellulose chains are polysaccharides that are composed of a considerable lot of fermentable sugars (D-glucose), whereas hemicellulose is composed of both pentose and hexose sugars. Cellulose chains are found in plant cell walls and are a major component of plant cell walls. Hemicellulose is a component of the cell walls of plants (Ravindran and Jaiswal, 2016). According to the information presented in the table that follows, the levels of cellulose, hemicellulose, and lignin that are present in geese weed are all noticeably lower compared to the levels that are present in small-flowered nutsedge. In comparison to water hyacinth, which had percentages of cellulose, hemicellulose, and lignin of 18.3 %, 23.3%, and 17.7%, respectively, this plant had percentages of lignin that were 17.7% (Gao et al., 2013).

Studies on promising materials (edible, lignocellulosic, and algal biomass) have been conducted, and lignocellulosic biomass research for the manufacture of biofuel accounts for 40% of the overall share of those studies (Azadi et al., 2017). The study was carried out in rice areas where these two weed plants were the most prevalent. The average density of goose weed was 59 plants per square meter, and the density of small-flowered nutsedge was 38 plants per square meter. Because weeds and rice plants compete for nutrients and other critical elements, a high density of these plants can reduce the amount of rice that can be harvested from a given area. The rice yield generated by gooseweed was 207 kg/ha, while the rice yield produced by small-flowered nutsedge was 201 kg/ha. Rice yields varied depending on the season (these plants prefer wetland to arid land), as well as the type of rice cultivation (Vu et al., 2017). For instance, in organic rice fields, gooseweed and small-flowered nutsedge thrive in far greater numbers and abundance when compared to conventional rice fields that make use of chemical fertilizers.

Table 3 Gooseweed and small-flowered nutsedge proximate analyses and compositions

Parameters	**Gooseweed**	**Small-flowered nutsedge**
Physical analysis (%)		
Total solid	93.94 ± 0.12	94.39 ± 0.22
Moisture	6.06 ± 0.12	5.61 ± 0.22
Fixed carbon	1.77 ± 0.10	2.72 ± 0.05
Volatile matter	83.12 ± 0.06	82.42 ± 0.17

Ash	9.5 ± 0.09	9.25 ± 0.09
Compositions (%)		
Cellulose	13.69 ± 0.23	22.05 ± 0.11
Hemicellulose	11.44 ± 0.41	30.2 ± 1.06
Lignin	2.51 ± 0.17	2.78 ± 0.09

Because of its principal effect on the stiff structure of lignocellulose, the pretreatment stage is critical for converting lignocellulosic biomass to bioethanol (Vu et al., 2017;2018). When compared to untreated materials, both pretreatments (biological and chemical) improved the release of reducing sugars. This implies that pretreatment has a favorable influence on biomass structures. Cellulose is resistant to biodegradation and must be digested in an initial pretreatment step into its constituent cellobiose units and simpler D-Glucose units before it can be converted biochemically. In order to hydrolyze lignocellulosic biomass using enzyme, a suitable pretreatment that may efficiently disrupt connected lignin and crystalline cellulose must be used (Taherzadeh and Niklasson, 2004). Both samples went through the identical bioethanol manufacturing process, from pretreatment to distillation.

Acid pretreatment (at concentrations of 5 and 10% H_2SO_4) and physical pretreatment (using an autoclave) have been demonstrated to be less effective (Menegol et al., 2014). According to other research findings, alkaline pretreatments, such as those involving NaOH and H_2O_2, make the enzymatic hydrolysis process more effective. Higher reducing sugar yields came from samples treated with alkaline, resulting in a lower lignin content and an enhanced cellulose content. The author also observed this on elephant grass (Menegol et al., 2014). A maximum decreasing sugar content of 10.8 grams per one hundred grams of water hyacinth was achieved after pretreatment with NaOH and H_2O_2, which was then followed by cellulase hydrolysis (Mishima et al., 2006). Xia et al. (2013) obtained a maximum reduction sugar yield of 48.3/100 g of water hyacinth after subjecting it to treatment with 1% H_2SO_4 at 140 °C for 15 minutes and then hydrolyzing it with cellulase enzyme.

To understand the differences in the structure of the biomass both before and after pretreatment, powder of raw and pretreated gooseweed and small-flowered. In addition, the fibers tend to bundle together, which makes it more difficult for cellulase to reach the cellulose, and the cell wall of untreated samples tends to be thicker than the cell wall of pretreated samples. Following the application of the pretreatment, it appears that the fibers in both of the samples were not fractured or otherwise disturbed in any way. The surface structure of the alkaline-treated samples tended to be smooth, which resulted in the exposure of more fiber bundles. As a consequence, the accessibility of fiber bundles to cellulase could be improved. Some minor debris that was on the fiber surface was removed, and the surface structure also tended to be smooth. On the other hand, when examined more closely, it was found that the surface of the individual threads had been severely distorted (Vu et al. 2017;2018). During the pretreatment, sodium hydroxide might have removed some of the hemicellulose and lignin from the material. This is one probable explanation. Due to the fact that the fibers did not sustain any significant damage throughout the pretreatment process, it is possible to draw the conclusion that no inhibitor chemicals were formed. This lends credence to the findings of the compositional study, which suggested that gooseweed and small-flowered nutsedge may be made up of a greater number of soluble components, such as protein and soluble carbohydrates, and a lower percentage of fibers. Pretreatment of biomass with alkaline peroxide was demonstrated to be an effective method for delignification when compared to treatment with diluted sulfuric acid and hot water (Abraham et al., 2013).

By hydrolyzing the big polysaccharides to fermentable sugars, saccharification/hydrolysis aims to reduce the degree of polymerization of cellulose (Vu et al., 2017; 2018). The most notable benefits of enzymatic hydrolysis are its higher sugar output than acid hydrolysis, its ability to perform at lower temperatures and pressures, and the absence of corrosion problems (Dwivedi et al., 2009). It took Vu et al. (2017, 2018) 72 hours of enzymatic hydrolysis to determine the optimal period for this process (Table 4). Since chemical pretreatment yielded better results than biological pretreatment, the enzymatic hydrolysis step was investigated after chemical pretreatment rather than before. The average levels of sugar before and after hydrolysis exhibit some variation. After 24 hours of hydrolysis, there are notable shifts in the samples' relative sugar concentrations. Sugar levels might be decreased, but degradation of polysaccharide did not appear to continue. Overall, the samples utilized in the investigations by Vu et al. (2017;2018) underwent enzymatic hydrolysis successfully within 24 hours. The number of sugar monomers in solution is represented by the degree of polymerization (DP). In other words, there is unmistakable proof of enzyme activity in the reduction of DP, demonstrating the breaking down of large sugar chains into smaller ones. Optimal hydrolysis performance for goose weed and small-flowered nutsedge is around 50% and 47%, respectively. This study confirms the findings of previously published works (Takagi et al., 2012; Das et al., 2016).

Table 4 Total sugar and reducing sugar after hydrolysis

Sugar (g/g)	**0 hour**	**24 hours**	**48 hours**	**72 hours**
Gooseweed				
Total sugar	0.144 ± 0.004^a	0.143 ± 0.007^a	0.125 ± 0.005^a	0.125 ± 0.004^a
Reducing sugar	0.029 ± 0.001^a	0.073 ± 0.006^b	0.068 ± 0.002^b	0.071 ± 0.002^b

DP	5.0	1.9	1.9	1.8
Small-flowered nutsedge				
Total sugar	0.199 ± 0.003^{a}	0.196 ± 0.006^{ab}	0.188 ± 0.003^{b}	0.195 ± 0.004^{ab}
Reducing sugar	0.020 ± 0.000^{a}	0.094 ± 0.001^{b}	0.079 ± 0.000^{c}	0.089 ± 0.002^{d}
DP	9.8	2.1	2.4	2.2

Gooseweed ethanol is produced via fermentation, a biological process that utilizes the yeast *S. cerevisiae*'s inherent fondness for sugar as a carbon source. Within 3, 5, 7, and 9 days, the range for ethanol content was recorded as 0 g/L to 11.84 g/L. By day five of fermentation, the ethanol concentration had reached its peak of 11.84 g/L, and it has since steadily been falling. Estimates were made of sugar reduction during fermentation in order to monitor yeast sugar consumption. After three days, there was a marked drop in the amount of decreasing sugar, which thereafter fluctuated slightly. Glycerol, a byproduct of fermentation, may contribute to a decrease in bioethanol concentration after day 5 (Ahn et al., 2012). Gooseweed and small-flowered nutsedge both produced higher concentrations of ethanol than the water hyacinth used in the aforementioned study (9.61 g/L; Takagi et al., 2012; He et al., 2015). There are two types of sugar in the reducing sugar mix, hexoses and pentoses, but only hexoses can be fermented by the yeast *S. cerevisiae*. As a result, the depletion of the sugar substrate may have halted the fermentation process. During the fermentation of glucose, acetaldehyde is reduced to ethanol via two genes in the yeast *S. cerevisiae*, and ethanol is converted back into acetaldehyde via a third gene. This clarifies why bioethanol concentration drops off when maximum ethanol concentration is reached. This study's greatest ethanol concentration was comparable to that of others that used different types of lignocellulosic biomass as inputs (Table 5).

Table 5 The comparison of this study's ethanol content with that of previous studies

Feedstocks	**Pretreatment**	**Ethanol**	**References**
Water hyacinth	Conc. H_2SO_4	9.61 g/l	Takagi et al., 2012
Wetland plants	$NaOH/H_2O_2$	1.491 g/l	He et al., 2014
Water hyacinth	$H_2O_2/NaOH$	0.16 g/g biomass	Yan et al., 2015
Water hyacinth	H_2SO_4	13.6 g/l	Das et al., 2016
Gooseweed	$NaOH/H_2O_2$	11.84 g/l	Vu et al., 2017
Small-flowered nutsedge	$NaOH/H_2O_2$	12.36 g/l	Vu et al., 2017

After conducting testing on a smaller scale, a straightforward mass balance was derived for the production of bioethanol from gooseweed and small-flowered nutsedge. When 10 grams of dried samples were utilized, gooseweed produced 1.184 g of ethanol, whereas small-flowered nutsedge produced 1.236g of ethanol. One ton of dried materials can yield anywhere from 118 to 124 kgs of ethanol when it is fermented. The findings of Vu et al. (2017; 2018) research are in line with those obtained from studies involving various types of lignocellulosic biomass, such as fresh sweet sorghum (91.9 kg ethanol) (Li et al., 2013). However, this amount is far lower than the 382 kg of ethanol that can be produced from paper sludge (Prasetyo et al., 2011).

6. Significance of research on gooseweeds and small-flowered nutsedges

There are a lot of lignocellulosic plants in places like Viet Nam, Indonesia, Malaysia, and Thailand, which can be used to make bioethanol. There are a lot of plants that are high in lignocellulose that can be used to make bioethanol. Each year, the amount of bioethanol made around the world has grown in response to demand. People in Thailand are becoming more interested in ethanol as a fuel alternative to gasoline and other fossil fuels. This study is based on very little research, and the two weeds it talks about are not being looked into as biofuels yet. Thailand had about 10,800 hectares of rice land in 2014 (Vu et al., 2017; 2018). These weeds can hurt rice fields and must be killed with herbicides because they compete with rice for important nutrients. The results of the study will be very important for finding new materials for bioethanol of the second generation. It will also make farmers more likely to use chemicals to get rid of them instead of doing it by hand. Second, biological pretreatment is becoming more important because it uses less energy and is better for the environment. The cost of pretreatment and saccharification can be cut down by treating samples with termites first. The study will focus on simple termite tests to find out how lignocellulosic biofuels affect the amount of ethanol produced (Vu et al., 2017; 2018). The study also looks at how much it would cost to build a plant. By looking into how these weeds could be used to make bioethanol, not only are new materials found, but rural economies are also helped.

7. Conclusion and Future Perspectives

The goal of this review is to see if new lignocellulosic biomass

could be used as a good feedstock for making bioethanol.This review paper presents a green method for delignifying small-flowered nutsedges and gooseweed to make bioethanol. This research review contains the main ideas such as field surveys to collect, analyze, and calculate the biomass yield of small-flowered nutsedge and gooseweed. The biochemical pathways that led to bioethanol were pretreatment, enzymematic hydrolysis and fermentation. Fractional distillation was also used. Pretreatment conditions were also optimized by response surface modeling. Saccharification demonstrated the use of cellulase enzyme. The hydrolysate samples obtained were fermented worty with robust yeast *Saccharomyces cerevisiae* (TISTR 5020). The use of pentose and amylase enzymes may help to speed up hydrolysis, as hemicellulose was discovered to be an important fraction of lignocellulosic biomass and starch.

This study shows that delignification is cost-effective and quick. Pretreatment of aquatic weeds with ligninolytic extract was successful. Considering the short pretreatment period, absence of inhibitory chemicals, and low cost of bioethanol mass production, pretreatments may be a feasible, simple, and environmentally-friendly strategy for aquatic weeds. Monophasic methods for biofuel generation from rice straw could include pretreatment, saccharification, hydrolysis and fermentation. These methods improve saccharification efficiency and are less expensive. They also reduce enzyme inhibition. The lignin-based biorefinery, which can be used to valorize aquatic weed biomass, can add a new dimension of sustainability. It increases carbon efficiency and manages overall capital costs. You can make fuels, medicines, polymers, and other materials from lignin-derived monolignols or phenols. Unit operations and associated policies are essential for successful integration of 2G technology.

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