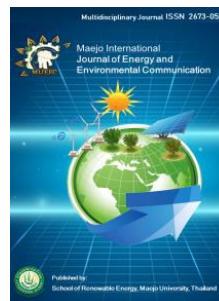




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## ARTICLE

### Efficient conversion of night-blooming giant water lily into bioethanol and biogas

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#### ABSTRACT

This study aimed to characterize and evaluate an inedible giant water lily as a bioenergy feedstock. The conversion of giant water lily organics to bioenergy can produce renewable energy. Composition study indicated that giant water lily is an excellent feedstock for bioethanol and biogas production. Fermentation effluent wastes from anaerobic digestion were transformed directly into ethanol using an alkali pretreatment. Under mild operating conditions, alkaline pretreatment with NaOH enhanced ethanol and biogas output. Anaerobic digestion of giant water lily yielded a methane content of 62.44% digestion with cow dung inoculum. The highest ever achieved was an ethanol yield of 4.82 g/L of digested effluent after only 24 hours of fermentation. The pretreated materials were then enzymatically hydrolyzed, fermented to ethanol. Furthermore, co-digestion in biogas plants may be economically advantageous for biorefineries because the by-products (digestate) are obtained within the biorefinery itself and are acceptable for external feedstocks for ethanol fermentation.

## 1. Introduction

Concerns about the security of oil supplies are growing, as are concerned about the harmful impacts of fossil fuels on the environment, particularly in terms of greenhouse gas emissions. This has increased society's demand to develop more environmentally friendly energy sources (Bhuyar et al. 2021; Khammee et al. 2021a). Even though more integration is meant to be good for the environment, the data reveal that not all impact categories have seen these benefits and that the results depend on the allocation techniques, energy system, and assumptions utilized. As a result of growing concerns about global warming and energy use, renewable energy has gained appeal in recent decades (Unpaprom et al., 2019; Nithin et al., 2020). As a result, energy authorities have shown an interest in seeing the growth of many

types of renewable energy technology, including solar, geothermal, wind, and biomass, among others. Because of its availability and renewability, biomass (for example, wood, agricultural, and municipal wastes) has a great potential for use as a renewable energy source, and it is expected to play a considerable part in global energy supply in the near future (Unpaprom et al., 2015; Ramaraj and Dussadee, 2015).

This means that lowering greenhouse gas emissions while diversifying fuel supplies, boosting self-sufficiency, and developing the economy is becoming more critical. On the other side, biofuels have come under fire owing to growth. As a result, lowering greenhouse gas emissions while diversifying fuel supplies, becoming self-sufficient, and developing the economy is becoming more critical. Biofuels, on the other hand, has drawn a great deal of criticism due to the rise (Unpaprom et al., 2017;

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Saengsawang et al., 2020; Mejica et al., 2021; Unpaprom et al., 2021).

Biofuels are widely discussed, as are whether they compete with food and how much energy they demand. Publications in science compare different energy systems based on assumptions, boundaries, and technologies. Studies on energy efficiency of various goods vary greatly. Many inputs and outputs are used in biofuel production, which some say is harmful to the environment. So biofuel producers must think about how to use resources efficiently while improving environmental performance. To do so, industrial ecology, specifically industrial symbiosis, can be used. Bioenergy from renewable resources can now replace fossil fuels (Bhuyar et al., 2021). However, in order to meet the growing need for bioenergy, a varied range of raw resources must be considered. Bioenergy can be made from lignocellulosic cellulose, the most common organic material on the planet because it is the most common organic material on the earth.

Agricultural and industrial operations and biomass derived from the aquatic environment generate a significant amount of lignocellulosic waste each year, which is then recycled. Unfortunately, they are frequently misused and, in some cases, dumped into the environment without being treated, causing major environmental pollution issues. A green economy has been promoted throughout the past several decades, with energy generation from various biomass feedstocks such as bioethanol, biogas, biodiesel, crude bio-oil, and syngas being some of the most important popular (Wannapokin et al., 2018). Bioethanol and biogas, in particular, have gotten much attention lately because they are the most frequently used biofuels for energy generation and transportation. Many organic wastes are now being used to make biogas, whereas corn, sugarcane molasses, and cassava are the most commonly used crops to produce bioethanol (Sophanodorn et al., 2020).

#### Nomenclature and Abbreviation

AD	Aerobic digestion
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon dioxide
O <sub>2</sub>	Oxygen
H <sub>2</sub> S	Hydrogen sulfide
HMF	5-hydroxymethylfurfural
LCC	Lignin–carbohydrate complexes
NaOH	Sodium hydroxide

The manufacture of biofuel from these crops is not viable to meet the huge increase in energy use due to the dispute over food vs fuel, resulting in food shortages and price increases. So recent breakthroughs in research and development have been extended to include additional feedstocks such as agricultural wastes, waste biomass, microalgae and seaweeds, among other things. Moreover, due to their rapid growth and high carbohydrate content, aquatic organisms have been proposed as a possible feedstock for biofuel generation, among other reasons.

Aquatic plant biomass is also included on the list for biofuel production because, in addition to being utilized for biofuel production, aquatic weeds have the ability to reduce environmental issues. For example, the night-blooming giant water lily (*Victoria amazonica*) is a species of aquatic weed that can be found in tropical places, particularly in Southeast Asia, and blooms at night. It is a beautiful aquatic macrophyte with enormous floating leaves and massive entomophilous flowers that attract many insects (Glimn-Lacy and Kaufman, 2006). Giant water lilies have the potential to be a biofuel feedstock due to their rapid growth and high carbohydrate content.

Furthermore, the plant was simple to harvest, allowing the biomass to be used for a variety of purposes. Giant water lilies are lignocellulosic biomass, and they can be used to make biogas and bioethanol. While making biogas/ethanol from sugars and starch is easier, making biogas/ethanol from lignocellulose has extra technological obstacles, such as the necessity for pretreatment. Cellulose and hemicellulose are linked together by lignin in lignocellulosic compounds. Cellulose and hemicellulose are polymers made up of long chains of sugar monomers that can be transformed to ethanol by microbial fermentation after pretreatment and hydrolysis. Indeed, biomass pretreatment with various chemical, physical, and biological approaches could boost biofuel outputs from the giant water lily. Pretreatment, hydrolysis, fermentation, and separation are the four steps involved in producing renewable energy from lignocellulosic biomass.

Pretreatment and hydrolysis are the operational cost bottlenecks of the entire process. Therefore, the refractory structures of cellulose fibrils should be changed, and inhibitors of hydrolysis and fermentation should be removed using a suitable pretreatment procedure to improve hydrolysis efficiency. Pretreatment typically involves the use of diluted strong inorganic acid or alkaline, such as sulfuric acid or sodium hydroxide. Unlike organic acid pretreatment, harsh inorganic acid pretreatment produces inhibitory by-products, such as 5-hydroxymethylfurfural (HMF) and furfural, which hinder enzymatic hydrolysis, fermentation, and microorganism development in downstream processes. The goal of pretreatment for bioethanol production is to free up the lignocellulosic structure to allow for enzymatic hydrolysis. As a result, the monomeric sugars in cellulose and hemicellulose are liberated during enzymatic hydrolysis and become accessible for conversion into ethanol. *Saccharomyces cerevisiae*, or regular baker's yeast, is the most often utilized microbe for ethanol synthesis. In this study, we used night-blooming giant water lilies to convert biogas and bioethanol efficiently.

## 2. Material and methods

### 2.1 Collection and preparation of giant water lily

The plant sample was obtained from a local garden pond in MaeTang, Chiang Mai Province, Thailand. Scientist Dr. Yuwalee Unpaprom, Program in Biotechnology, Faculty of Science Maejo University, identified it as *Victoria amazonica* (often known as giant water lily). Plants were first rinsed with a 3% NaOCl solution to remove algal, bacterial, and fungal contaminants. Then,

proximate analysis was performed on the whole plant, which included leaves, petioles, roots, and rhizomes, all of which were tested in triplicate.

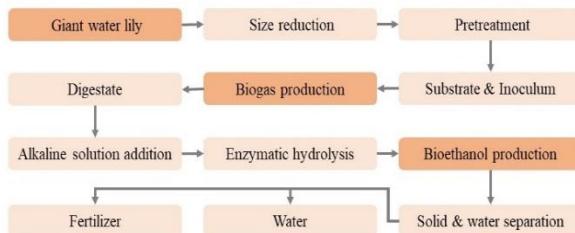


Figure 1 Giant water lily utilization potentials of AD process for biogas and bioethanol production

## 2.2 Inoculum preparation, experimental process, and biogas production

Cow dung from a nearby dairy farm was used to make inoculum for anaerobic digesters. The homogenized slurry was passed through a 7-mm sieve to remove larger fragments after being mixed with water in a 3:7 ratio. The mixture will be poured into an anaerobic digester with a gas collection bag, subsequently filled with gas. The inoculum was then stored at room temperature for 14 days, during which time it began to produce biogas and became less gaseous, making removal easier.

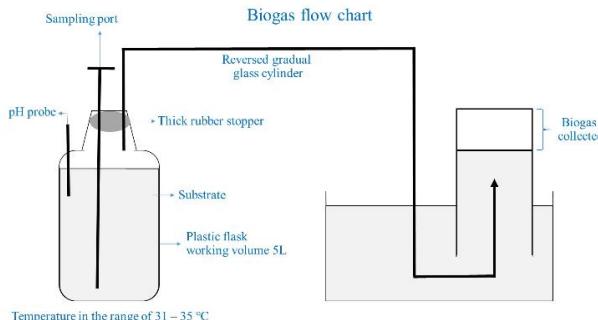


Figure 2 Laboratory scale biogas production system

Figure 1 shows the potential of giant water lilies to be used in an anaerobic digestion (AD) technique for biogas and bioethanol production. Biogas measurement, digested analysis, and related analytical methods were adopted from Chuanchai and Ramaraj (2018). 2% NaOH pretreated biomass and inoculum were used as the substrate for anaerobic digestion. For 35 days, all 6 L reactors with a 5 L operating volume were run simultaneously (Figure 2). A portable gas analyzer was used to determine the concentrations of methane ( $\text{CH}_4$ ) and other gases such as carbon dioxide ( $\text{CO}_2$ ), hydrogen sulfide ( $\text{H}_2\text{S}$ ), and oxygen ( $\text{O}_2$ ) in biogas produced (BIO5000, UK). The volume of biogas produced was monitored every three days, and the biogas composition was analyzed.

## 2.3 Fermentation of giant water lily biomass for bioethanol production

Laboratory scale bioethanol production system demonstrated in Figure 3 Alkaline pretreatment of giant water lily biomass converted the biomass to glucose, which was then fermented to ethanol. As fermentation was carried out using yeast strain

*Saccharomyces cerevisiae*, which could only ferment glucose to ethanol, alkaline pretreatment was used to convert plant starch to glucose. For this purpose, 2% NaOH was used to hydrolyze feedstock. Hydrolysis was carried out for 24 hrs at room temperature. After pretreatment, the hydrolysate fermentable sugars concentration was measured through the DNS method. Fermentation of plant-derived fermentable sugars with *Saccharomyces cerevisiae*. After pretreatment, the hydrolysate pH was adjusted to 4.5. Enzymatic ethanol measurement was used with an ebulliometer to determine the ethanol content after fermentation, according to the manufacturer's recommendations (Vu et al., 2018).

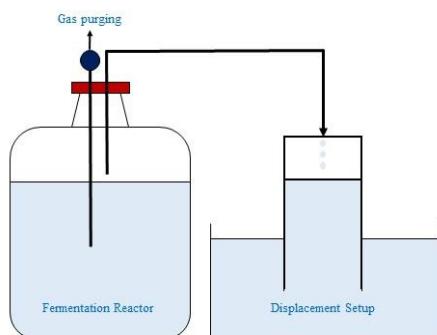


Figure 3 Laboratory scale bioethanol production system

## 2.4 Statistical analysis

The experiments were carried out in triplicate, and the average findings were presented and discussed.

## 3. Results and discussion

### 3.1. Giant water lily adaptation and characteristics

*Victoria amazonica* is a flowering plant that belongs to the Nymphaeaceae family. Its natural habitats include Central Africa, tropical South America, and South-East Asia. *V. amazonica* is a rhizomatous aquatic, strongly aculeate, herbaceous perennial with a short, thick, tuberous rhizome that is brown on the outside and white on the inside, turning purple when cut; the rhizome contains numerous cylindric adventitious roots that abound in air spaces (Lim, 2016). The *V. amazonica* is called a giant water lily, which has enormous leaves that can reach 3 m in diameter and float on the water's surface on a 7–8-m-long submerged stalk. It is the largest waterlily in the world. The growing nature of the giant water lily is displayed in Figure 4. Giant water lily is found in the shallow waters of the river basin, such as oxbow lakes and bayous (Kunii et al., 2006). The flowers open after the sun sets in their natural habitat and can take up to 48 hours to unfold fully. These blossoms can grow to be 40 cm in diameter. Each plant will produce flowers throughout the growing season, and they have co-evolved with a kind of scarab beetle from the genus *Cyclocephala* to improve pollination. A single patch's buds will all open simultaneously, producing a delightful perfume as they do so. At this stage, the flower petals are white, and beetles are drawn to both the flower's color and aroma.



Figure 4 Growing nature of the giant water lily



Figure 5 Giant water lily underside leaf ribs

By midnight, the flower had closed, the odor had faded, and the beetle had entered the carpillary appendages. The Para carpels protect the stamens, and the flower shuts for the day. The beetle is imprisoned in a hole made of spongy, starchy tissue. The plant produces anthocyanins, which cause the petals to turn from white to a reddish pink, indicating pollination. The stamens slide inward as the beetle munches within the flower, and the anthers drop pollen on the stamens (Kunii et al., 1993). The blooms will have opened enough by the second day's evening for the beetle to exit, and it will be covered with pollen as it does so. Insects will then go in search of a newly opened huge water lily and cross-pollinate it. Giant water lily underside leaf ribs were explained in Figure 5. The leaves of giant waterlilies are extremely adaptable (Glimm-Lacy and Kaufman, 2006). The leaves float because air is trapped between the ribs on the underside of the leaves. A spider-web of tall supporting ribs with a thickness of less than 1 cm and a height of up to 7.5 cm covers their underside. They create a plethora of pockets beneath the leaf that trap air and give the leaves their incredible buoyancy.

### 3.2 Biogas production

Biochemical processes are made up of chemical reactions that are carried out by microorganisms and/or enzymes that turn fermentable substrates into fuel or other high-value products. There are two types of biochemical processes that are the most common,

according to Ramaraj et al. (2016b). These are fermentation and anaerobic digestion. There must be enough oxygen for organic matter to be fermented, so it is called "fermentation." The first uses microorganisms and/or enzymes to break down a food source that can be turned into something else that can be used. Anaerobic digestion breaks down organic matter into biogas, which are mainly made up of methane and carbon dioxide (Ramaraj and Unpaprom, 2016; Nong et al., 2020b). This process, which takes place in an oxygen-free environment, has been used to break down a wide range of biodegradable materials, including food waste (Kaewdiew et al., 2019). Aquatic biomass plants are primarily composed of lignocellulose as their primary constituent.

Lignocellulose concentration of young aquatic biomass plants varies significantly depending on their age (Chuanchai et al., 2019). In giant water lily biomass plants, lignocellulose is the primary structural component of the cell wall. It is a linear polysaccharide consisting of D-pyran glucopyranose anhydride (1-5) joined by a glycosidic bond at the  $\beta$  (1-4') position. It is a type of polysaccharide that is extensively branched, with its primary chain consisting of one or more sugar-based components. Hemicellulose is found in a variety of plant tissues. In its most basic form, lignin is a complex amorphous polymer made up of phenylpropanoid structural units linked together by ether bonds and carbon-carbon bonds (Nong et al., 2020c). In this step, biogas is produced by the anaerobic digestion of organic biomass, which is known as fermentation (Nong et al., 2020d). Aquatic biomass that has been pretreated is appropriate for use in typically stirred biogas digesters, where it can be used to substitute other input materials such as water hyacinth. The technical feasibility of utilizing aquatic biomass in this manner has been demonstrated in our project as well as in previous research. The economic feasibility of the approach is what we are primarily concerned with within this case. After looking at the final phase in the aquatic biomass supply chain, we'll go into the strategies we use to accomplish this.

Because the complex and rigid structure of cellulosic, hemicellulosic, and lignocellulosic biomass prevents efficient hydrolysis, pretreatment of a substrate is required to get better biogas and methane production (Ramaraj et al., 2016c). According to Zhang et al. (2014), direct microbial enzymatic hydrolysis of lignocellulose yields less than 20% glucose from the cellulosic fraction, and methane yields rarely exceed 60%, in fact the raw giant water lily biomass with cow manure inoculum methane yield was reached 49.58%, so pretreatment of the substrate is required to make the holocelluloses more accessible to microbial attack and to improve hydrolysis (Ramaraj et al., 2016d). The biodegradability of the lignocellulosic substrate is nevertheless hampered by the cleaving of lignin-carbohydrate complexes (LCC) linkages and the removal of lignin. When LCC bonds are cleaved, cellulose and hemicelluloses are significantly easier to hydrolyze and ferment using enzymes (Monlau et al., 2013). Chemical pretreatment is one of the most extensively studied pretreatment options; it was initially developed for the delignification of cellulosic materials in the paper industry (Ramaraj et al., 2016c; Wannapokin et al., 2017). Sodium hydroxide, perchloric or peracetic acid, organic solvent, acid hydrolysis with sulfur or formic acid, and ammonium

fiber explosion are frequently used (Van Tran et al., 2019; Nong et al., 2020a; Van Tran et al., 2020). In this study, we utilized 2 % of sodium hydroxide (2% NaOH). In Raw and NaOH pretreated substrates daily biogas production amount was shown in Figure 6. For 35 days of fermentation, the total biogas produced by giant water lily (raw) anaerobic fermentation was 6,924.67 mL, while the total biogas produced by pretreated giant water lily anaerobic

fermentation was 14,748 mL. The study's findings revealed that pretreatment is critical and can result in a two-fold increase in biogas yield. The findings of the comparison of giant water lily feedstock with other feedstocks revealed that the latter had a possibly greater yield (Table 1) and was relevant for large-scale biogas generation in the future.

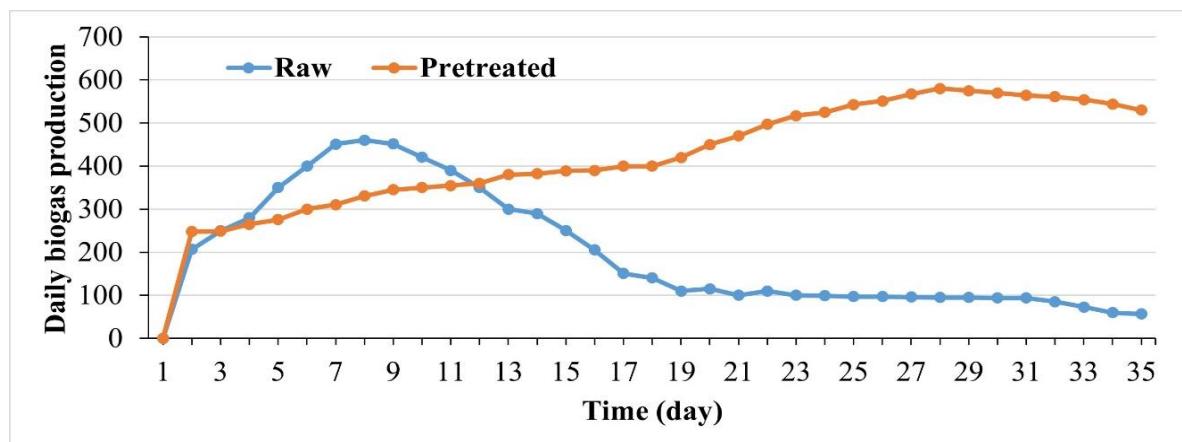


Figure 6 Biogas production (raw & pretreated sample)

Table 1 Biogas composition, yield of fresh and dry biomass cattail with inoculum

Substrate	CH <sub>4</sub> %	CO <sub>2</sub> %	O <sub>2</sub> %	H <sub>2</sub> S (ppm)	Reference
Giant water lily (Raw)	49.58	47.21	0.1	211	
Giant water lily (Pretreated)	62.44	37.4	0.21	393	This study
Fresh cattail	61	34.8	0.1	26	Gotore et al., 2021
Dry cattail	57	33	1.09	35	
Cafeteria kitchen wastes	62.2	37.4	-	1359.3	Minza et al., 2021
Sorghum silage with slurry	56.96	41.6	-	83.3	
Corn silage, crushed potatoes with slurry	52.95	47	-	210.1	Kažimírová et al., 2018
Maize silage	54.77	41.96	0.375	289.65	
Maize silage and grass haylage	53.97	42.64	0.30	182.65	
Maize silage, grass haylage and rye grain	54.37	42.49	0.38	175.47	Herout et al., 2011
<i>Chlorella sp.</i>	58	42	-	438	Mann et al., 2009

### 3.3 Disposal of residues and bioethanol production

Bioethanol, mostly made from sugars and starch-rich materials, is thought to be one of the best ways to replace a lot of the liquid fuels made from petroleum (Khammee et al., 2021b). It can be used in gasoline mixtures with up to 10% alcohol without changing the engine. Vehicles that can run on various fuels are known as flex-fuel vehicles (or fuel blends). Gasoline and many alternative fuels, such as pure ethanol and ethanol-gasoline mixes, are utilized most. Flex-fuel vehicles can run on up to 85% alcohol in gasoline mixtures without having to change the engine. It is also possible to use 100% bioethanol in engines that have been specially designed. Recently, bioethanol has been found to be one of the best bio-based raw materials for the chemical industry. It comes in second only to ethanol in terms of potential. In recent research, bioethanol has been found to be the world's most popular biofuel for cars and trucks. There are at least four steps in its biochemical production:

hydrolysis, fermentation, distillation, and dehydration, as well as other steps (Vu et al., 2017). During the hydrolysis of complex molecules into simple compounds, the biodegradation of biomass starts, and these simple compounds can be used for the fermentation process right away. Some microorganisms convert sugar into alcohol, lactic acid, or other products, depending on how the process is run and what raw materials are used.

The widespread use of AD has resulted in a steady increase in the amount of solid digestate that must be disposed of in a separate facility. The material remaining after AD of a biodegradable feedstock is called digestate. Though it can serve as a valuable fertilizer, the marketing of digestate is in its infancy and fermentation residues often have to be disposed of at considerable cost. These costs are included in the last step of our calculation model. They can be higher than a process using standard materials

such as maize silage, given the lower dry matter content of aquatic biomass, which leads to a higher throughput of material and thus higher quantities of residues. Solid digestate is a by-product of the AD process that contains indigestible food that has not been digested and processes mediators and dead microorganisms. Disposal costs vary greatly depending on the region where the disposal site is located (Ngoie et al., 2020). There is tremendous potential for growth in the anaerobic digestate effluent, which comprises a diverse collection of bacteria, nutrients, water, and other organic components. One of the possibilities is to use recycled water to produce bioethanol instead of freshwater, eliminating the need for additional fertilizers (Gao and Li, 2011).

To appropriately manage anaerobic wastes and produce competitive biomass for biofuel production, using digestate as a bioethanol production substrate seems reasonable. A substrate for bioethanol fermentation has been researched using digestate. Anaerobic wastes were handled in a manner that ensured the supply of biomass for biofuel generation was not compromised. The combination anaerobic digestion/fermentation method requires more investigation to understand its strengths and weaknesses (Tasic et al., 2021). Inversely, reversing the method works well. As a substrate for bioethanol synthesis, digestate (giant water lily and dairy inoculum) was used in this investigation. Pretreatment affects the total efficiency of bioconversion. This pretreatment method was among the first to be employed due to its many advantages. Enzymatic hydrolysis of the pretreated substrate is critical in the bioethanol manufacturing process. Microbial enzyme cellulase is employed to speed things up.

The cellulase enzyme accelerates the hydrolysis of cellulose and breaks it down into simple sugars. The saccharification procedure identifies the most effective 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 responsive. As a result, commercial cellulase was used. The ethanol estimation and total sugar (TS) and reducing sugar (RS) estimation were done every 24 h of fermentation for 5 days; TS and RS data were not shown in this document. To find the maximum ethanol yield, the fermentation process was performed for 5 days. When the fermentation was completed in 24 hours, the highest ethanol output of 4.82 0.12 g/L was achieved. The substitution of digestate for pure nutrients, which are expensive and time-consuming, could increase the economic feasibility of bioprocessing. In addition to autoclaving, another digestate processing technique is heat treatment, which involves heating the digestate to 121 degrees Celsius for 15 minutes. This technique is similar to heat treatment, generally used in bioprocesses to sterilize refined nutrients and synthetic medium (as a fertilizer).

#### 4. Conclusion

Biotechnological and biochemical conversion pathways might be considered when designing energy-driven biorefineries. However, in order to determine the optimal way for upgrading aquatic biomass (lignocellulosic material) and to identify the energy and environmental requirements for these processes in various conditions, energy and environmental aspects. Aquatic

biomass is identified as a potential feedstock to obtain bioenergy. The AD of giant water lily and the addition of cow manure slurry (inoculum) enhanced the anaerobic process, resulting in methane generation, while the cow manure supplement provided the most consistent anaerobic digestion. The anaerobic digestion effluent can be utilized to substitute freshwater in bioethanol production while also increasing bioethanol concentration. The study describes the future challenge of freshwater use for bioethanol production and proposes an environmentally benign method for boosting the cost-effectiveness of the biorefinery industry. Consequently, the future success of digestate valorization schemes will be determined by their technological and environmental efficiency, as well as their cost-effectiveness.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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