

Investigation of Macrophyte for Bioproduct Potential: A Review

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Abstract: Overpopulation of macrophyte in drainage ditch can lead to a problem in maintenance and reduce the efficiency of the system. Therefore, this study aims to review the feasibility of *Eleocharis dulcis* (*E. dulcis*) for bioproduct potential as a sustainable use of macrophyte. A sustainable approach in the production of the product is crucial to overcome the risks and impacts to the environment. The selection of macrophyte as feedstock for bioethanol can lead to solve the needs of renewable sources of fuels for energy generation applications. In conclusion, *E. dulcis* had the potential for bioproduct and the benefits of macrophyte as bioproducts is impactable not only for the drainage ditch problem but also for other environmental problems.

Keywords: *E. dulcis*, , Sustainable, Bioproduct

1. Introduction

Parit Raja is one of the towns located in the district of Batu Pahat, Johor that equipped with open-drainage ditch as its irrigation system. The role of a drainage ditch is to direct the surface flow to the catchment area [1] and the drainage system should not be restricted by any means and be well maintained to avoid flooding [2]. However, the overgrowth of macrophyte in the drainage leads to difficulty for maintenance and reduce the efficiency of the system. According to Department of Drainage and Irrigation Malaysia (DID), disrupted drainage water flow due to the high paced growth of macrophyte was a prolonged problem and needed to be overcome [3]. Therefore, the production of bioproduct can be seen as a sustainable use of macrophyte.

A bioproduct is product produce for commercial purposes and from biomass which include biological materials (from agriculture, forestry, marine sources), waste biological and micro-biological waste, by-products from processing and wood-based products [4]. The benefits of bioproducts are not just on the environmental aspect but also on the health and economic, which also can be a source of income for the local community [4]. Hence, the production of bioproduct from macrophytes can be

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benefited to the local villagers of Parit Raja and towards its nature environment in the future. The aim of this research is to review *E. dulcis* potential for bioproduct production.

In Malaysia, bioproducts production can be seen heavily lean towards biochemical and bioenergy, especially in biofuel and in 2005, the National Biofuel Policy (NPB) was formulated. The policy was expected to reduce the dependency on fossil fuels, make new demand for palm oil, utilise local resources for biofuels and mitigate climate change by reducing GHG emissions. Furthermore, according to the Fifth-Fuel Policy under the Eight Malaysia Plan (2001-2005), biomass was identified, as fuel resources after coal, hydro, gas and petroleum. It can be concluded that production of bioproduct helps increases the economic and highly supported by the government. Therefore, the focused bioproduct for this study was the conversion of bioethanol as bioenergy using the overpopulated macrophyte as biomass.

2. *Eleocharis Dulcis*

E. dulcis or commonly referred to as Chinese water chestnut is a perennial aquatic sedge, growing to a height of 1.5m [5]. In Malaysia, *E. dulcis* is more familiarise as 'purun' plant [6] and in the east coast states of Peninsular Malaysia, its known to be a dominant weed that grows in marshes and black water rivers, water bodies that usually acidic [7]. *E. dulcis* also found in irrigation and drainage ditches that often lead to blockages that need to mechanically destroy by heavy machinery. It has a wide range of *E. dulcis* usage from food sources to soil improvement. According to CABI (2020), the tuber of *E. dulcis* is used in local dishes as vegetables that either cooked or eaten raw. In a certain part of China, it is cultivated and developed to have a bigger size and taste sweeter than those produced by wild plants [5]. Meanwhile, Sulawesi used the stems for making mats and bags.

Every part of *E. dulcis* has a distinctive feature that could be used or benefited. The stem is usually used for traditional handicraft while tuber is more known as a food source and medicine. *E. dulcis* juice containing puchiin antibiotic that was effective against *Aerobacter aerogenes*, *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) [8]. A mature plant which after 6 to 8 weeks will have the daughter plants grow around the parent plant [9]. Meanwhile, the single terminal spikelet was an important feature to differentiate *Eleocharis sp.* within the sedge family and the characteristic of the stems helps distinguished *E. dulcis* from other *Eleocharis sp.* [5]

3. Bioethanol as Bioenergy

Bioethanol is an alternative to fossil fuels that have the potential for energy security and had less impact on environment safety [6]. It is a sustainable and renewable source that used in various industries as a chemical feedstock, fuel and even as a solvent [7]. Commonly, bioethanol is obtained from organic compounds and agro-waste such as corn, cassava, rice, sugarcane and sweet potato[8]. However, continuous development on this area had improved the production of bioethanol from various sources. Table 1 shows review on the sources in generating bioethanol from previous studies. The first-generation of bioethanol production is from sugar and starch crops which had been commercialised, producing about 50 billion litres annually [9]. However, the production of first-generation bioethanol had its limits due to the concerning impacts on biodiversity and food price [9]. It was alleged that if the production increase, food prices will also increase because of the limited feedstock. Therefore, to solve the shortcomings of the first-generation, studies and production of second-generation bioethanol was available. Weedy such as macrophyte plants are amongst the sustainable feedstock of lignocellulosic biomass for bioethanol conversion [10].

Advantage of second-generation bioethanol is the abundant and inexpensive non-food materials that do not compete with food supply. Despite that, the conversion of these types of biomass is complex and depend on new technologies for its production [11]. Meanwhile, the third generation of bioethanol, algae was utilised due to the high growth rate and can be easily cultivated in a various water environment [12]. Furthermore, algae have high lipid and carbohydrate contents which seen as assuring alternative feedstock [13] even if the process is difficult and needed various methods (flocculation, floatation, etc.) for producing the algae biomass [14]. In this study, the second generation of bioethanol was further discussed as lignocellulosic biomass can address issues rises with first generation bioethanol

and compared to third generation, feedstocks from lignocellulosic biomass are abundance and the availability is not limited while production of algae biomass are difficult.

Table 1: Review on the sources in generating bioethanol

Generation of bioethanol	Feedstock	Advantages	Disadvantages	References
First generation: Sugar & starch	Vegetables oil, corn, sugarcane	Improve domestic energy security and economic	Limited feedstock (food vs fuel) and impacts on biodiversity.	[9]
Second generation: Lignocellulosic biomass	White wood chips, agricultural and forest residues, municipal solid waste	Inexpensive biomass and do not compete with food supply	Complex conversion process	[11]
Third generation: Algae	Microalgae and Macroalgae (seaweed)	High production rate	Difficult process design and limited investments.	[12]

3.1 Composition of Lignocellulosic Biomass

The yield of ethanol and conversion efficiency depends on the types of biomass that require high cellulose and hemicellulose content and low lignin content. Table 5 shows the composition of different lignocellulosic biomass from previous studies.

Table 2: Composition of different lignocellulosic biomass

Lignocellulosic Biomass	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	References
Switchgrass	31	24	18	[15]
Napier grass	47	31	22	[16]
<i>Gmelina arborea</i>	23	-	23.3	[17]
<i>Salvadora oleoides</i>	24	-	21.8	
<i>E. dulcis</i>	24.7	37.19	7.82	[18]
	21.80	19.74	28.04	[19]
	19.71	20.82	35.20	
<i>Imperata cylindrica</i>	44.4	31.1	6.7	
<i>Cyperus cyperoides</i>	29.7	24.6	10.9	
<i>Scoparia dulcis</i>	36.5	19.1	6.6	[20]
<i>Eragrotis amabilis</i>	39.7	29.6	7.2	
<i>Typha angustifolia</i>	47.1	16.9	10.0	

Fibre content (cellulose, hemicellulose and lignin) of *E. dulcis* and others biomass from previous studies were in range content of lignocellulosic biomass (cellulose; 9-80%, hemicellulose; 10- 50%, lignin; 5-35%) [21] as shown in Table 2, proved could be a promising carbohydrates source and feedstocks for bioproducts. Based on Table 2, *E. dulcis* had a lower value of cellulose (24.7%) compared to others and relatively high value of hemicellulose (37.19%) which can still be regarded as potential lignocellulosic biomass. Meanwhile, by having a low lignin value (7.82%), *E. dulcis* plant is suitable for bioprocessing without having to reduce the lignin content by manipulated the plants genetic. Lignin composition is a major obstacle in biomass pre-treatment, but an excessive reduction of lignin can also affect the sugar recovery efficiency [22].

In bioethanol production, the source of C6 and C5 sugars for bacterial fermentation are from cellulose and hemicellulose. Therefore, estimation of ethanol yields can be made using the value of cellulose and hemicellulose to investigate the potential for conversion of bioproduct. Table 3 shows the estimation of theoretical ethanol yields of *E. dulcis* using the value in Table 2. The correlation between cellulose, hemicellulose and ethanol yields can also be observed. A high yield of ethanol depends on high amount of cellulose and hemicellulose. The highest obtained value was 451.35 L/Ton while the lowest was 295.00 L/ton. However, in a real process of production, the efficiency of ethanol yields depends on several other factors that is discuss in later sub-sections. Overall, the potential of bioethanol conversion depends on the major compositions of lignocellulosic biomass which is cellulose, hemicellulose and lignin.

Table 3: Estimation of theoretical ethanol yield

Lignocellulosic Biomass	Cellulose (%)	Hemi-cellulose (%)	EtOH TY (L/Ton)
<i>E. dulcis</i>	24.7	37.19	451.35
	21.80	19.74	302.09
	19.71	20.82	295.00

3.3 Factors Affecting on Processing Lignocellulose to Biethanol.

There are severals factors to be considered in the process of converting to bioethanol. This study had review the process involved. Conversion of bioethanol from lignocellulosic biomass can either be through thermochemical or biochemical [23]. The common method for bioethanol production is the biochemical and it depends to its efficiency of biomass conversion. The major components of the biochemical method are pre-treatment, hydrolysis, fermentation and separation/ purification of product. In each process step, there factors affecting the effieciency conversion of lignocellulose to bioethanol.

3.3.1 Pre-treatment Process

Pre-treatment is aimed for cellulose to easily access the enzymes which transform carbohydrate polymers into fermentable sugars by disrupting recalcitrant structures of cellulosic biomass. Any type of pre-treatment should improve glucose yield to prevent degradation [24]. However, there are optimum pre-treatment conditions studies lead to have better glucose yield as shown in Table 4

Table 4: Ideal pre-treatment conditions of different pre-treatments

Pre-treatment	Pre-treatment Condition	Glucose yield (%)	Feedstock	Researchers
Dilute Acid	162°C, 9.8min, 0.8% H ₂ SO ₄	75	Corn stover	[25]
Hydrothermal Lime	100°C, 15min, 0.1g Ca (OH) ₂ /g of dry biomass	87	Coastal Bermuda grass	[26]
NaOH	121°C, 60min, 0.1% NaOH	90	Wheat straw	[27]
NH₃	69°C, 60min, 20% aqueous NH ₃	60	Rapeseed straw	[28]
Phosphoric Acid	50°C, 30min, 85% aqueous H ₃ PO ₄	96	Corn stover	[29]

It can be noted that factors affecting the glucose yield in pre-treatment process are temperature, time and the concentration or amount of materials use as pre-treatment method. Based on the previous study of glucose yield using dilute acid, the use of 0.8% of H₂SO₄ at 162°C and for 9.8 minutes incubation time was set to be the optimum condition for maximum glucose yield (75%) for corn stover. Meanwhile, the use of phosphoric acid as the pre-treatment method with the optimum conditions of 85% aqueous H₃PO₄ at 50°C for 30 minutes. Usually, the addition of dilute or concentrated acid in pre-treatment acid is about 0.2 % (w/w) to 2.5 % (w/w) to the biomass. Pre-treatment using dilute acid can be performed at high temperature (T > 160 °C) for low solid loading in a continuous mode while for

high solid loading is at a lower temperature ($T < 160\text{ }^{\circ}\text{C}$) in batch mode. Therefore, different pre-treatment method has their ideal conditions for efficient conversion of sugar from various feedstock.

3.3.2 Hydrolysis

The acid hydrolysis process uses in the previous study is 2.5 % (w/w) of H_2SO_4 at 120°C for 1hour that produce 1.6 mg/mg of glucose. The degradation of cellulose and hemicellulose using acid hydrolysis happened at high pressure and temperature for a short incubation time [30]. However, as indicated in Table 7, the produced glucose is low and decomposition of glucose will occur with a high chance of development unwanted by-products [30]. The severe conditions of this method cause higher operating costs and required neutralization of downstream.

Meanwhile, for the hydrolysis of the same feedstock using enzyme can occur at 50°C and pH 4.8 to produce glucose of 16mg/ml (Table 7). This suggests that enzymatic hydrolysis performed under milder conditions. The suggested conditions are usually 40°C to 50°C and pH value range from 4.5 to 5. Additionally, enzymatic hydrolysis is more environmental- friendly but use high-priced enzyme and longer hydrolysis period. However, for a long run, Horn & Eijsink (2010) [31] considered enzymatic hydrolysis as the factor for economically feasible production of ethanol.

Table 5: Hydrolysis condition of acid and enzyme hydrolysis process [32]

Hydrolysis	Hydrolysis Condition	Glucose	Feedstock
Acid (H_2SO_4)	120°C , 1hour, 2.5 % (w/w) H_2SO_4	1.6 mg/mg	<i>C. calothyrsus</i>
Enzyme (ONOZUKA R-10)	50°C , pH 4.8, 70 hours, 0.05M sodium nitrate buffer	16 mg/ml	

3.3.3 Fermentation Process

Table 8 shows the ethanol yield with respect to several parameters such as time, temperature and pH. Changes in pH value effect the fermentation reaction. Table 8a shows that the percentage of ethanol yield varies according to the pH. The optimum percentage of ethanol yield (55%) was at slightly acidic condition, pH value of 4.5. Furthermore, as the value of pH rises, the ethanol yield decreases. It can be concluded that the ideal condition for yeast growth and production was slightly acidic.

Temperature also has impacts on fermentation reaction. As displayed in Table 8b, the optimum temperature for fermentation reaction using yeast was 30°C . At 30°C , the yeast cells are structurally sound and can reproduce healthily and efficiently [33]. Therefore, a lower temperature was the ideal condition for bioethanol fermentation rather than high temperature.

Table 8c presented the ethanol yield with respect to different time interval for reaction temperature of 30°C and pH value of 4.5. The ethanol yield increase until the 72 hr. After passing the 72hr marks of fermentation, ethanol yield decreases which means that the growth rate of yeast started to slow down due to the depletion of glucose and ethanol was used as a source of carbon for the growth of yeast [33]. Hence, it is found that 72hr was the time taken for maximum sugar conversion for reaction temperature of 30°C and 4.5 value of pH.

4. Conclusion

It is demonstrated from the previous research that macrophyte has potential as source of bioproduct. Therefore, the use of macrophyte for bioproduct can solve the problem of macrophyte overpopulated especially in village drainage ditch. The composition of *E. dulcis* grass as lignocellulosic biomass also proven from previous study. It is expected that the *E. dulcis* can produce bioethanol, with several factors need to be considered in the process of pre-treatment, hydrolysis, and the fermentation.

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