Research Article

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Bioethanol Production from *Ceratophyllum demersum* L. and Carbon Footprint Evaluation

Thirasit Kusolsongtawee, Thawipon Wuttilerts and Suphang Chulalaksananukul*
Department of Chemical Engineering, Faculty of Engineering, Mahidol University, Salaya, Nakhon Pathom, Thailand

Luksanaree Maneechot
Research Center for Environmental Quality Management, Kyoto University, Japan

* Corresponding author. E-mail: suphang.chu@mahidol.ac.th DOI: 10.14416/j.ijast.2018.04.002
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Abstract
The aim of this research was to find the suitable conditions for bio-ethanol production from *Ceratophyllum demersum* L., an abundant aquatic plant. The parameters affected to alcohol production were evaluated. Percentage of solid (10, 20, and 30% (w/v)), the amount of yeast (5, 10, and 15% (w/v)), pH value (4, 5, and 6) and temperature (30, 35, and 40°C) were carried out. The results elucidated that the conditions to produce the maximum bio-ethanol from *Ceratophyllum demersum* L. was applied with 10% (w/v) of solid, 10% (w/v) of yeast, controlled pH value of 6 and temperature of 30°C. The highest yield of bio-ethanol production was reached 2.92 g ethanol/L within 24 h. Moreover, the Carbon Footprint for ethanol production was calculated only from 2 steps of life cycle analysis which were the step of raw material acquisition from macro algae cultivation and manufacturing process from bio-ethanol production. Consequently, the carbon footprint for ethanol production from *Ceratophyllum demersum* L. was 77.88 kg CO_2 equivalent.

Keywords: Bio-ethanol, *Ceratophyllum demersum* L., Carbon footprint

1 Introduction

Nowadays, global warming becomes the significant problem of the world. This incidence is occured rapidly due to the increasing of greenhouse gas in the atmosphere, especially carbon dioxide from the combustion of fossil fuels and anthropogenic activities [1]. Global warming causes the increasing average temperatures, the extreme weather events, the enchancing of sea levels and ocean acidification.

The proposed guidelines for reducing the carbon dioxide in the atmosphere are the method of biological systems in carbon dioxide capture, instead of improving chemical catalyst or the expensive carbon dioxide capture processes. In the method of biological systems, there are not seriously concerns with the large area of storage and a leak from a carbon capture storage site [2]. The carbon capture and utilization of biological systems by photosynthesis, such as plants, algae, anoxygenic photosynthetic bacteria, and cyanobacteria convert the carbon into the biomass. They utilize carbon dioxide as a carbon source.

At the present, the lands of bioenergy production systems are now facing problems related to indirect carbon dioxide emission and food security due to the food crop change to the energy crop [2]–[6]. Therefore, sustainable biofuels which reduce greenhouse gas emissions and protect ecosystems are likely to require nonagricultural feedstocks, such as wastes and residues, incorporation with food production, and

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Figure 1: *Ceratophyllum demersum* L. species.

algae, as well as plants grown on low-quality land.

Algae are a group of aquatic organisms which have the capacity to conduct photosynthesis [7]. The growth rate and carbon dioxide uptake of algae is so fast. Moreover, the abundant byproducts e.g., lipid, carbohydrate, aquaculture nutrients are found in most of algae. Therefore, the algae are able to be the biofuel sources and the carbon capture and utilization.

Renewable biofuels usually relate the carbon fixation that occurs in plants or algae through the process of photosynthesis. It uses the biomass of plants, algae or the recently living organism, such as cyanobacteria [2] to produce the biofuels. This biomass can be converted to appropriate forms in three different ways: thermal conversion, chemical conversion, and biochemical conversion. This biomass conversion can result in fuel in solid, liquid, or gaseous form [8]–[9].

Ceratophyllum demersum L. species shown in Figure 1 is an abundant aquatic plant in Thailand and probably causes water pollution due to its blooming effect. According to its high growth rate and consisted of high carbohydrate content (approximately 40%), it is suitable to be an alternative feedstock for alcohol production.

In the process of bio-ethanol production, the cultivation and processing of crops are considered the energy efficiency and green house gas reduction potential [5]. Therefore, the carbon footprint evaluation is applied to investigate the amount of generated CO_2 from the process in this research.

The purpose of this research is to investigate the feasibility of bio-ethanol production from *Ceratophyllum demersum* L. species in different conditions. The parametric study is used to study the effects of each parameter in fermentation to the yield in bio-ethanol production. Besides, the study aims to apply a life cycle assessment as a framework for assessing the environmental impact in the cultivation and production.

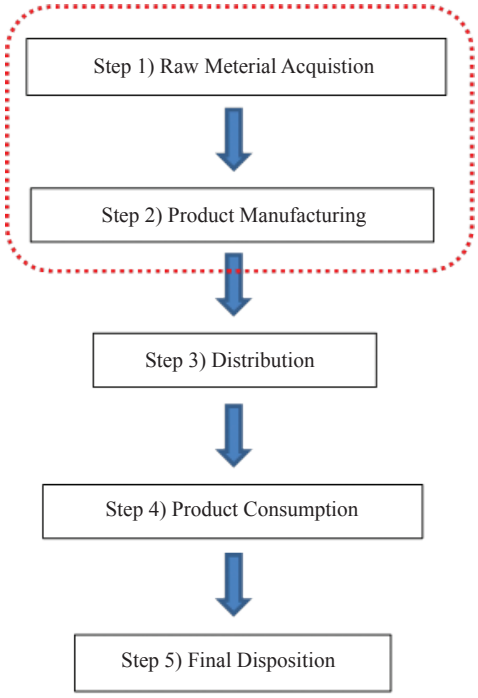

Figure 2: The flowchart of carbon footprint evaluation.

This is the first time that alcohol production from this aquatic plant is reported, including of a carbon footprint for production process is also evaluated.

2 Experimental Method

The experiments were divided into 2 parts.

Part 1 was the study of alcohol production in laboratory scale. The pH value, temperature, % solid, % yeast and fermentation time were investigated to maximize the yield of ethanol production.

Part 2 was the carbon footprint evaluation. Life cycle analysis [10] was assessed to the production process which expressed into 5 steps as raw materials acquisition, product manufacturing, distribution, product consumption, and final disposition. In this research, the evaluation of carbon footprint for ethanol production from *Ceratophyllum demersum* L. is

focused on 2 steps including raw materials acquistion and product manufacturing due to limitation in performing only in laboratory or noncommercial scale. The flowchart of carbon footprint evaluation is presented in Figure 2.

2.1 *Macro algae preparation*

The *Ceratophyllum demersum* L. was collected from Chemical Engineering Laboratory, Mahidol University. It could generally be found in Thailand and it could grow easily in a proper condition. The biomass was washed repeatedly with water in order to remove dirt and mud. Then, the *Ceratophyllum demersum* L. was cut and blended by a blender to reduce the size of biomass and increase the efficiency in fermentation.

Moreover, the chemical composition of *Ceratophyllum demersum* L. biomass was analyzed to determine the carbohydrate, lipid, and protein content. Yield of bio-ethanol was depended on the carbohydrate content.

2.2 *The fermentation process for bio-ethanol production*

The *Ceratophyllum demersum* L. was fermented to produce the bio-ethanol. The parameters affected the maximum bio-ethanol production were performed.

The experimental conditions were applied with various pH (pH 4, 5 and 6), various temperature (30, 35 and 40°C), and various yeast concentration (5, 10 and 15% (w/v)). The samples were taken at time of 24, 48, 72, 96 and 120 h, and ethanol concentration was analyzed in further by High Performance Liquid Chromatography (HPLC).

2.3 *Analysis method*

Ethanol content from the fermentation was determined using HPLC. The analysis condition was using Aminex HPX-87H column, 300×7.8 mm. (Aminex, USA); column temperature was 50 degree Celsius; mobile phase was acetonitrile and was fed with flow rate of 0.6 mL min^{-1}. The collected samples were filtered through 0.22 μm Chromafil AO filters, Macherey–Nagel (Duren, Germany) prior to injected in the column. Ethanol concentration was identified by comparison with standard solutions.

2.4 *Carbon footprint evaluation*

The carbon footprint of a bioethanol process was defined as the Greenhouse Gas (GHG) emissions associated with the life cycle of bioethanol that is calculated from cradle to grave [11]–[13]. The carbon footprint was a versatile tool that can also be related to the direct correlation with the phenomena of climate change. In fact, the carbon footprint is the part of the Life Cycle Assessment (LCA) method. As a general rule, it is expressed as the amount of carbon dioxide equivalent.

Normally, the steps of life cycle analysis evaluated the carbon footprint that was described in 5 boundary steps as mentioned before. In this research, however, the carbon footprint emphasized on raw materials acquisition step and product manufacturing step was also focused on.

The carbon footprint was calculated through the multiple of emission factor associated with the mass of product [14]. It could be expressed as in Equation (1).

$$CF = E \times M \quad (1)$$

Where CF is the carbon footprint of bioethanol from *Ceratophyllum demersum* L. (kg CO_2 equivalent), E is the emission factors (kg CO_2 equivalent/kg of product), and M is the mass of carbon dioxide transformed from the product (kg).

3 Results and Discussions

3.1 *Macro algae composition*

The chemical composition in biomass of *Ceratophyllum demersum* L. species was determined and reported in Table 1. All of chemical compositions are obtained from Association of Analytical Communities (AOAC) method.

Table 1: The chemical composition of *Ceratophyllum demersum* L.

Chemical Composition	Percentage
Total Carbohydrate	39.37
Fiber	17.87
Total Lipid	0.43
Protein	17.37
Ash	17.12
Moisture	7.84

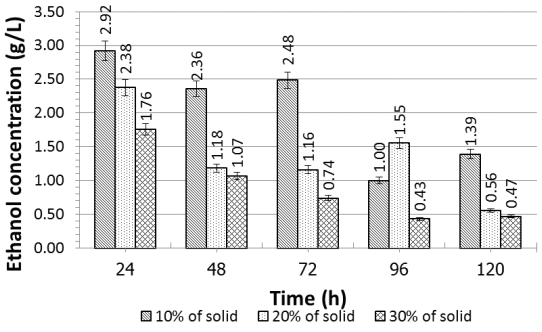

Figure 3: Alcohol production using different percentage of solid mass at 10% (w/v) of yeast, pH value 6 and 30°C.

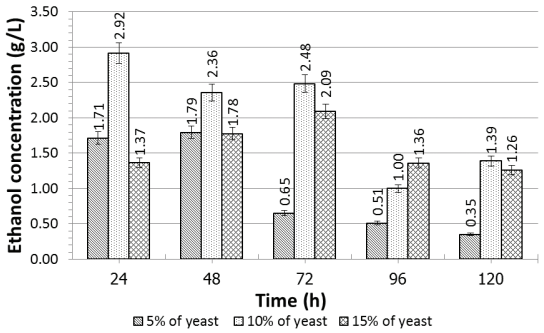

Figure 4: Alcohol production using different the amount of yeast at 10% (w/v) of solid, pH value 6 and 30°C.

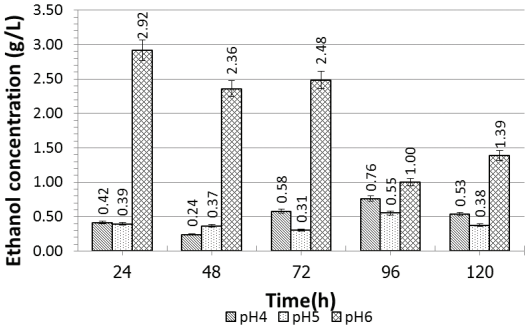

Figure 5: Alcohol production using different pH value at 10% (w/v) of solid, 10% (w/v) of yeast and 30°C.

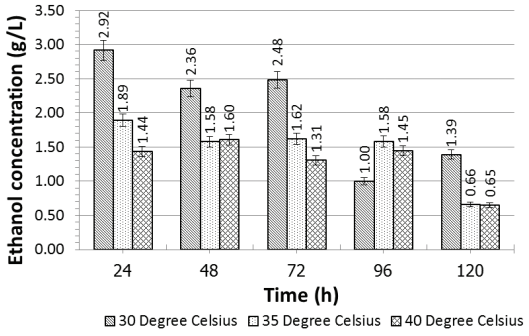

Figure 6: Alcohol production using different temperature at 10% (w/v) of solid, 10% (w/v) of yeast and pH value 6.

The total carbohydrate content is about 39.37% by weight. This is a satisfactory value for ethanol production since this implies the high sugar content that can be converted in further to be an alcohol [15].

3.2 *The fermentation process for bio-ethanol production*

The maximum bio-ethanol production from the fermentation of *Ceratophyllum demersum* L. was investigated under the studied parameters including percentage of solid, the amount of yeast, pH value, and temperature. The results were exhibited in Figures 3–6, respectively.

The fermentation with 10, 20 and 30% (w/v) of solid were all achieved maximum ethanol production within 24 h. However, it was found to get different amount of alcohol with different solid content, which were 2.92, 2.38 and 1.76 g/L of alcohol, respectively, shown in Figure 3.

As time passed, the alcohol content was decreased which may be due to the loss from the evaporation during aerobic fermentation. The maximum alcohol content was observed at 10% (w/v) of solid. However, at high concentration of solid inhibited the growth of yeast resulting to obtain low concentration of alcohol. For instance, the suitable time for fermentation of *Ceratophyllum demersum* L. was at 24 h and maximum ethanol concentration of 2.92 g/L was produced at this time conditions (10% (w/v) of biomas solid and applied with 10% of yeast, controlled pH value of 6 and temperature of 30°C).

The fermentation was studied in further to determine the effect of yeast applied to the system. The various percentage of yeast (5, 10 and 15% (w/v)) was applied. The result was shown in Figure 4.

The result found that 10% (w/v) yeast concentration was suitable for fermentation of *Ceratophyllum demersum* L. sp. and at 24 h of fermentation achieved a maximum ethanol concentration of 2.92 g/L as the same result as the previous condition of experiment.

For the study of pH value in bio-ethanol production, it was one of the interesting parameters to evaluate since the initial pH value affected to the bio-ethanol production. Many researches in bio-ethanol production from algae were maintained pH in the range of 4–6 according to pH which are generally contributed to control the contamination from other microorganism during the fermentation [16]. Figure 5 expressed the maximum ethanol production at various pH.

The maximum result achieved of 2.92 g/L of alcohol, as well, within 24 h of fermentation.

Figure 6 was the comparison between the fermentation temperature. It was found that the bio-ethanol production from fermentation of *Ceratophyllum demersum* L. sp. at 30°C was higher than that of 35 and 40°C because yeast S. cerevisiae grew well at 30°C and the mechanism of yeast was droped when the temperature increased.

From Figures 3–6, the suitable conditions for producing bio-ethanol from *Ceratophyllum demersum* L. is 10% (w/v) of solid, 10% (w/v) of yeast, controlled pH value 6 and temperature at 30°C. The achieved maximum ethanol production of 2.92 g/L was also found at this condition.

In Figures 3, 4 and 6, the ethanol concentration at 96 h did not match the description in contrast to Figure 5. So, The effects of pH value were more than the other parameters at 96 h of fermentation but this hypothesis was investigated in the future research.

3.3 *Carbon footprint evaluation*

The carbon footprint for bio-ethanol production from *Ceratophyllum demersum* L. could be able to calculate from only of raw material acquisition and product manufacturing process boundaries.

In the raw material acquisition boundary, it was calculated from the water consumption for cultivating *Ceratophyllum demersum* L., included the utilization of electricity from CO_2 regulator heater and instrument operation and the consumption of carbon dioxide for photosynthesis to be biomass of algae.

In the step of product manufacturing processs, the data of all chemicals consumption, water consumption and any waste produced from bio-ethanol production process were gathered and calculated. The result was illustrated in Figure 7.

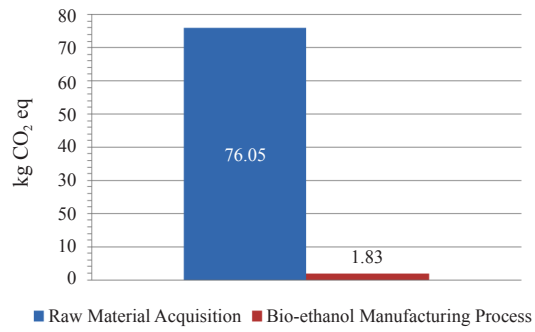

Figure 7: Carbon footprint of bio-ethanol process from *Ceratophyllum demersum* L.

Relatively, the emission factor and the mass of carbon dioxide from the utilization of electricity are the main factors to corporate in the carbon footprint evaluation. According to the maximum bio-ethanol production from the fermentation of *Ceratophyllum demersum* L., this was achieved 2.92 g/L. and the emission factors of bio-ethanol manufacturing process was 1,481.70 kg CO_2 equivalent/kg ethanol.

The result was found that the carbon footprint calculated within the boundary of the raw material acquisition was 76.05 kg CO_2 equivalent and the carbon footprint evaluated within the boundary of bio-ethanol manufacturing process was 1.83 kg CO_2 equivalent. Consequently, the total carbon footprint of bio-ethanol production from *Ceratophyllum demersum* L. was 77.88 kg CO_2 equivalent. It could be seen that the raw material acquisition boundary emited the higher carbon dioxide content than emited from the boundary of bio-ethanol manufacturing process.

4 Conclusions

Ceratophyllum demersum L. was able to be an alternative feedstock for alcohol production. The maximum achieved concentration of alcohol was 2.92 g/L at 24 h when using 10% (w/v) of solid mass and applied with 10% (w/v) of yeast. The system was controlled at pH value 6 and 30°C of fermentation.

The calculated carbon footprint for ethanol production was elucidated to be 77.88 kg CO_2 equivalent.

The carbon footprint of raw material acquisition was higher than the carbon footprint of bio-ethanol manufacturing process due to the utilization of electricity.

In fact, the result from this research does not only provide a promising alternative feed stock for alcohol production but also elucidate the important information of carbon footprint to promote the consciousness to the environmental performance.

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