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Evaluation of bioethanol production from rice field weed biomass

Phuong Thi Vu, Yuwalee Unpaprom, Rameshprabu Ramaraj

Abstract

Bioethanol has attracted more attention as a clean-burning fuel that can benefit both environment and energy sector. Gooseweed and small-flowered nutsedge are abundant in rice fields in form of weeds and considered as a major agricultural problem. Thus, this paper aims to evaluate the possibility of ethanol production from these two weeds by calculating the theoretical ethanol yield from its reducing sugars and cellulose content. Experiment was conducted in rice fields in Chiang Mai province, Thailand and 207 kg/ha and 201 kg/ha biomass yield was obtained from gooseweed and small-flowered nutsedge plants. The theoretical ethanol yield of gooseweed and small-flowered nutsedge were 160 L/Mg and 223 L/Mg, respectively that suggest utilizing these materials as promising feedstocks for bioethanol production.

Keywords gooseweed, small-flowered nutsedge, theoretical ethanol yield

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Introduction

With the rapid development of population, additional energy has been needed in order to meet the growing demand of the world. Fossil fuels are the main source of energy all over the world. However, the use of fossil fuels has been associated with a lot of environmental issues which affects the whole biosphere and its inhabitants [1-2]. Another downside of using non-renewable energy is its limited supply. Especially nowadays, due to its high consumption, it is approaching their natural limits and it takes a considerable long time to be created. Thus, in order to meet the demand of energy as well as to control the quality of environment, biofuels should be considered as a feasible option. Biofuels has already been investigated around the world and continuously being utilized for the enhancement of global energy security. It can be used as an alternative source of energy for various purposes such as engine fuels, cooking, heating, electricity generating, etc. [4].

Most biofuels such as bioethanol, biogas, biodiesel, and biohydrogen are made from biomass and waste that helps to reduce the pressure on the environment [4]. Among different kinds of biofuel, bioethanol has drawn much widespread attention due to its promise of providing a clean transport fuel [8]. Even though its energy content is approximately same as gasoline, bioethanol has higher octane number (106-110) than gasoline which makes it an antiknock fuel [5-9]. Hence, it is often blended with gasoline or diesel with appropriate ratios in order to create new mixtures to reduce the harmful gas emission and increase the engine performance [10]. USA and Brazil are two top leading countries in bioethanol production from edible sources (corn and sugarcane) with 56.1 and 28.2 billion liters bioethanol production in 2015, respectively [11].

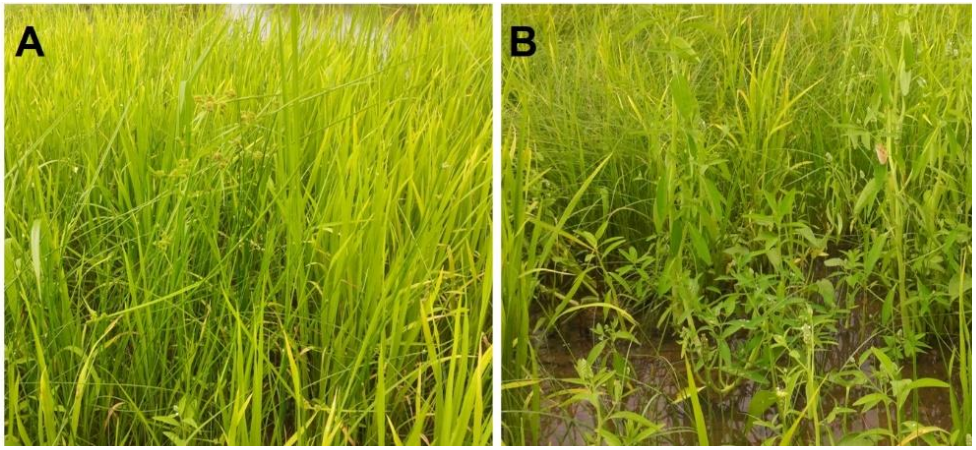

Figure 1. Small-flowered nutsedge (A); Gooseweed (B) in the rice field at Maejo University, Chiang Mai, Thailand

However, using edible biomass for bioethanol production has led to an argument of "food versus fuel" [12]. More lands and other sources such as water, fertilizers, and labors are needed to grow crops for energy [13-14]. Thus, lignocellulosic biomass, so-called second generation of bioethanol, has been preferred due to its abundance, low price, and worldwide distribution [15].

Gooseweed and small-flowered nutsedge both are short life-cycle plants and dominant in wet land areas. In general, they are considered as a problem in the rice field, as they compete with nutrients, water source, sunlight, etc. (Figure 1). Thus, in order to reduce the loss of rice yield, these materials are often taken out manually by farmers or chemical method which causes harmful effect on human health and increases the cost of labor. Hence, although being an invaluable waste, the feasibility of bioethanol production from these two materials should be investigated by calculating the theoretical ethanol yield.

Methodology

Material collection and preparation

Both gooseweed and small-flowered nutsedge were collected from the rice field at Maejo University, Nong Han, Sansai, Chiang Mai, Thailand (18º 53' 37.4"N; 99º 01'13.4"E). The two materials were firstly washed with tap water to remove dirt and mud. They were then chopped into 1-2 cm long pieces and dried in hot air oven at 50ºC for 3 days. Size reduction was carried out by high-speed blender (Otto BE-127, Thailand) (Figure 2, 3). Dried powder after blending was passed through a 1mm mesh sieve and stored in a desiccator for further experiment.

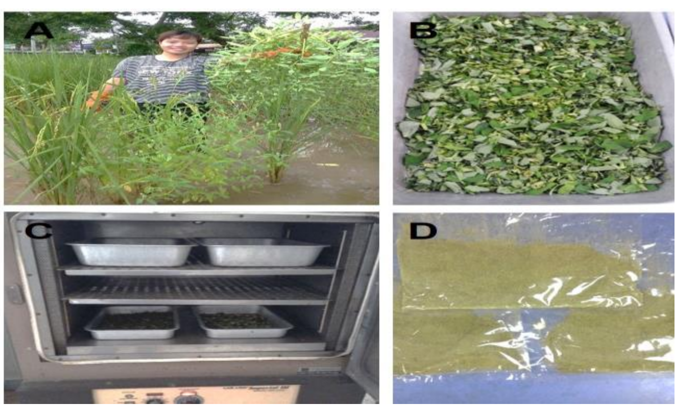

Figure 2. Gooseweed: (A) Sample collection; (B) Chopping; (C) Drying in hot air oven; (D) Powdered samples

Figure 3. Small-flowered nutsedge: (A) Sample collection; (B) Chopping; (C) Drying in hot air oven; (D) Powdered samples

Biomass yield

Biomass yield was calculated by the total mass of plants within a given unit of environment area. Since both gooseweed and small-flowered nutsedge grew in the stagnant area, especially in the rice fields located in Maejo University, Chiang Mai, Thailand (18°53'36.3"N 99°01'14.4"E). A 1 x 1m quadrat was placed in rice field randomly (Figure 4). The two plants were counted, collected and weighted as fresh samples followed by drying in hot air oven until it reached constant weight. The recorded data was used to calculate density (plant/m^2) and biomass yield (kg/ha).

Figure 4. 1 X 1 m quadrat in the rice field

Biochemical analysis

Reducing sugar was determined by HPLC with following description. Sugars of liquid phase by pre-treatment were analyzed by high performance liquid chromatography (HPLC) (condition: mobile phase-5 mM H_2SO_4; flow rate-0.7 mL/min; temperature of column: 60°C; Hi-Plex H column). The amount of cellulose, hemicellulose, and lignin was calculated using the method of fiber analysis reported by Van Soest [18].

Ethanol estimation procedure

For lignocellulosic biomass, cellulose, a main part of plant cell wall which is formed of many β (1→4) linked D-glucose units, is an important source of sugar for bioethanol production [15]. Besides, soluble reducing sugars or simple sugars such as monosaccharides (glucose, arabinose, fructose, etc.) that are found outside the cell wall are another source of fermentation substrate. Hence, it can be assumed that sugars from cellulose chains and soluble reducing sugars could be totally converted into bioethanol. As a result, a theoretical ethanol yield could be estimated from amount of cellulose and soluble reducing sugars present in the samples [19, 20]. The conversion of cellulose and reducing sugar into bioethanol were performed according to the below mentioned chemical equations (Eq1, Eq2, and Eq3). By using a balanced chemical equation where total mass of reactants and total mass of products are equal, so-called stoichiometry, theoretical bioethanol yield can be calculated as the below equations (Eq4, Eq5, and Eq6) [14, 19, 21, 22].

Ethanol density: 0.789 g/mL

Cellulose $(C_6H_{10}O_5)_n + nH_2O \rightarrow 2nC_2H_5OH + 2nCO_2$ (Eq1)

Hexose $C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$ (Eq2)

Pentose $3C_5H_{10}O_5 \rightarrow 5C_2H_5OH + 5CO_2$ (Eq3) [23]

Ethanol from cellulose (TEC) in 1 g of dry biomass

TEC (g) = cellulose (g) * 0.57 (Eq4)

Ethanol from reducing sugar (TER) in 1 g of dry biomass

TER (g) = Reducing sugar (g) * 0.51 (Eq5)

Ethanol yield from biomass (TEB)

TEB (L/Mg) = (TEC + TER)*1267 (Eq6)

Results and Discussion

Characteristics of gooseweed

Gooseweed is a kind of tropical weed that grows invasively in damp land, especially in lowland rice field. Table 1 shows the basic classification of gooseweed. Its life cycle is coincident with rice plants and it is often dominant in rice field [24]. The appearance of this plant may cause many unexpected consequences for rice production due to the competition of essential nutrients with rice plants.

Table 1. Taxonomy of gooseweed

Classification	Gooseweed
Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Campanulales
Family	Campanulaceae
Scientific Name	*Sphenoclea zeylanica* Gartn.

For these reasons, this plant had been recognized as one of the worst weeds in the world by Holm et al. [25]. A full description about dispersal, ecology, and morphology of gooseweed was reported by Carter et al. [26], since gooseweed had been considered as contaminant of rice feed in North America. In addition, reducing sugars including fructose, xylose, arabinose, and glucose were 19.02 mg/g dry biomass, 3.23 mg/g dry biomass, 2.72 mg/g dry biomass, and 3.63 mg/g dry biomass, respectively (Figure 5). Abundance in quantity of this material can be a big advantage comparing to sugar/ starch-feedstock for bioethanol production [23].

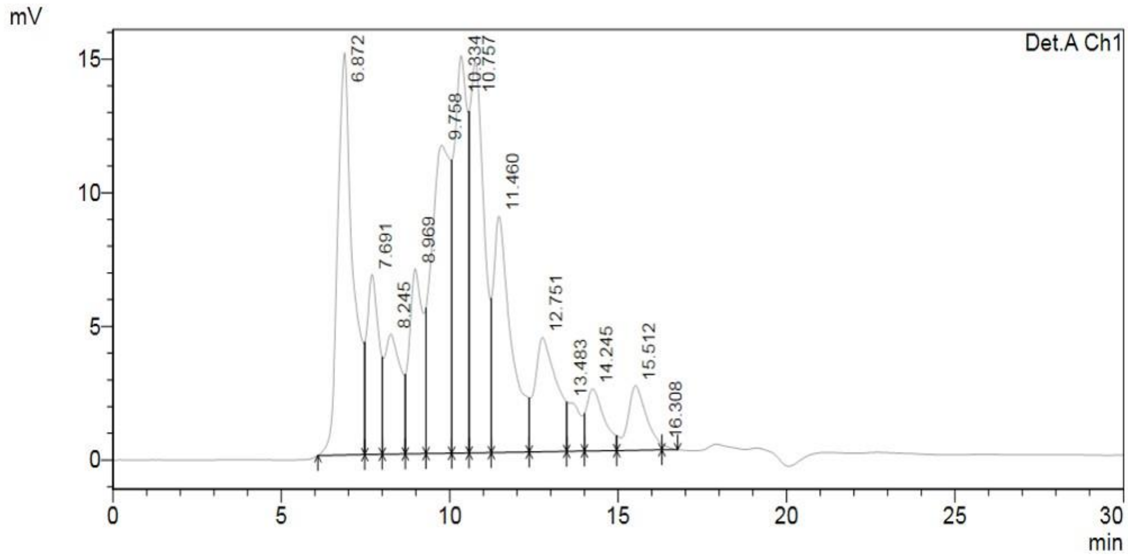

Figure 5. The peaks of sugars from gooseweed released after pretreatment (mobile phase–5 mM H_2SO_4; flow rate-0.7 mL/min; temperature of column: 60°C; Hi-Plex H column) [30]

Characteristic of small-flowered nutsedge

Small-flowered nutsedge, named *Cyperus difformis* L (Table 2), is listed in the Holm's list of the world's worst weeds [25]. It is worldwide distributed and grows in several parts of Thailand [5]. It is an invasive plant which grows on wetland and highly considered as a problematic weed in rice fields that is found anywhere at the bank of water bodies, in the field with crops plant, and its resourceful nature makes it easy to cultivate [28-30]. Though this material can be a good substrate for bioethanol fermentation, very few studies have been done on this comparatively new material [30].

Table 2. Taxonomy of small-flowered nutsedge

Classification	Gooseweed
Kingdom	Plantae
Phylum	Tracheophyta
Class	Liliopsida
Order	Cyperales
Family	Cyperaceae
Scientific Name	*Cyperus difformis* L..

The quality and quantity of sugars were analyzed by HPLC after pre-treatment with 1% NaOH and 1% H_2O_2 (Figure 6). The reducing sugar present in small-flowered nutsedge included 12.1 mg/ g dry biomass, 4.7 mg/g dry biomass, 2.02 mg/g dry biomass, and 1.2 mg/g dry biomass of fructose, glucose, xylose, and arabinose were respectively (Figure 6).

Biomass yield

The research was conducted in rice fields in which these two weed plants were dominant. The average density of gooseweed and small-flowered nutsedge were 59 plants/m2 and 38 plant/m2, respectively. High density of these plants causes the loss of rice yield due to the competition of nutrients and other essential elements between weeds and rice plants [31-35]. Region with gooseweed showed 207 kg/ha rice yield, while small-flowered nutsedge produced 201 kg/ha rice yield. Yields varied with season, types of rice plant, and the method of growing rice.

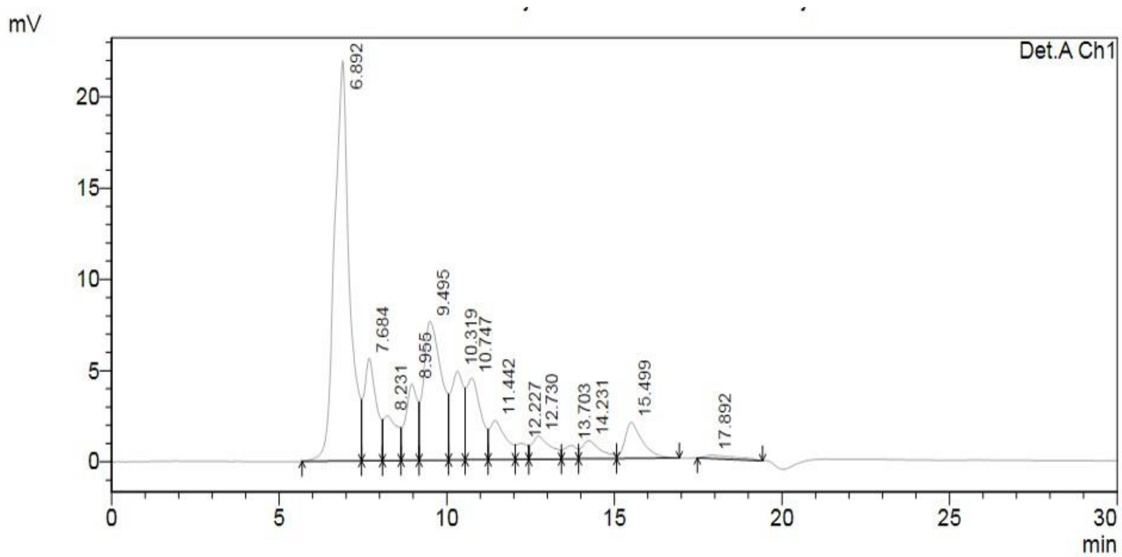

Figure 6. The peaks of sugars from small-flowered nutsedge released after pretreatment (mobile phase–5 mM H_2SO_4; flow rate-0.7 mL/min; temperature of column: 60°C; Hi-Plex H column).

Ethanol yield estimation

Table 3 shows cellulose, reducing sugar contents and theoretical ethanol yield of gooseweed and small-flowered nutsedge.

Table 3. Cellulose, reducing sugar content and theoretical ethanol yield of gooseweed and small-flowered nutsedge

Plant	Cellulose (g) *	Reducing sugar (g) *	TEC (g) *	TER (g) *	TEB (L/ Mg)**
Small-flowered nutsedge	0.22 ± 0.001	0.100 ± 0.001	0.125	0.051	223.5
Gooseweed	0.137 ± 0.003	0.096 ± 0.0	0.078	0.049	160.9

*Performed as g per 1 g dry biomass.
*Reducing sugar: glucose, fructose, xylose, and arabinose.
** Theoretical ethanol yield (L) per Mg (Ton) of dry biomass.

The components of plant such as cellulose, hemicellulose, lignin, and soluble carbohydrate could be different due to season, environment condition, and age of plant [19]. The average theoretical ethanol yield from gooseweed and small-flowered nutsedge were 160 L/Mg and 223.5 L/Mg, respectively.

Conclusion

The yield of gooseweed and small-flowered nutsedge in the rice field were 207 kg/ha and 201 kg/ha, respectively. Several types of sugars were founded such as glucose, fructose, xylose, and arabinose in both materials. Gooseweed and small-flowered nutsedge contained 14% and 22% cellulose, respectively. Gooseweed and small-flowered nutsedge are almost untapped biomass feedstock for bioethanol production via fermentation. The theoretical ethanol yield of gooseweed and small-flowered nutsedge were 160 L/ Mg and 223 L/Mg respectively. The feasibility of bioethanol production from these two materials should be investigated in future by performing other required laboratory experiments.

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