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Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of *Sphenoclea zeylanica* for bioethanol production

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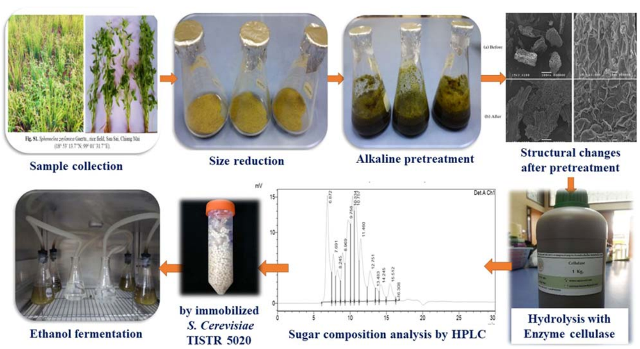
GRAPHICAL ABSTRACT



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ABSTRACT

Gooseweed (*Sphenoclea zeylanica* Gaertn.) is a pest on the rice field that has a potential to be a promising substrate for bioethanol production. Dry powdered gooseweed was firstly pretreated with 1% NaOH, following 1% H_2O_2 at variety conditions. The hydrolysis process was set at 50 °C for 24–72 h with enzyme cellulase (β-glucosidase) while the fermentation process was carried using *Saccharomyces cerevisiae* TISTR 5020 at 33 °C for nine days. The ethanol concentration was recorded for three, five, seven, and nine days using an ebulliometer. The results showed that the treatment with only 1% NaOH for 24 h has the highest sugar performance. In regard with hydrolysis, the optimum retention time was at 24 h. Lastly, the highest ethanol concentration was achieved at 11.84 g/L after five days and a rapid decreasing after seven to nine days was also observed.

1. Introduction

The use of bioethanol, a renewable fuel, has been significantly increasing all over the world due to the limitation of fossil fuels (petroleum, coal, natural gases, etc.), environmental issues (climate change, pollution, resource depletion, and so on), and its own characteristic as a high octane number fuel. In addition, bioethanol industry also has been creating a huge amount of direct and indirect jobs. Currently, bioethanol is produced mostly in U.S and Brazil which make up approximately 60% and 27% percentage of the world, respectively. By joining in the global market, Thailand contributed 322 million gallons (1%) to the total production in 2016 (Fig. 1) (RFA, 2017).

At the present, the conversion of biomass into bioethanol has been upgraded to four generations which are sugar/starch-based crops, lignocellulosic biomass, algae, and advanced bioconversion techniques. The second generation-lignocellulosic material, known as abundant,

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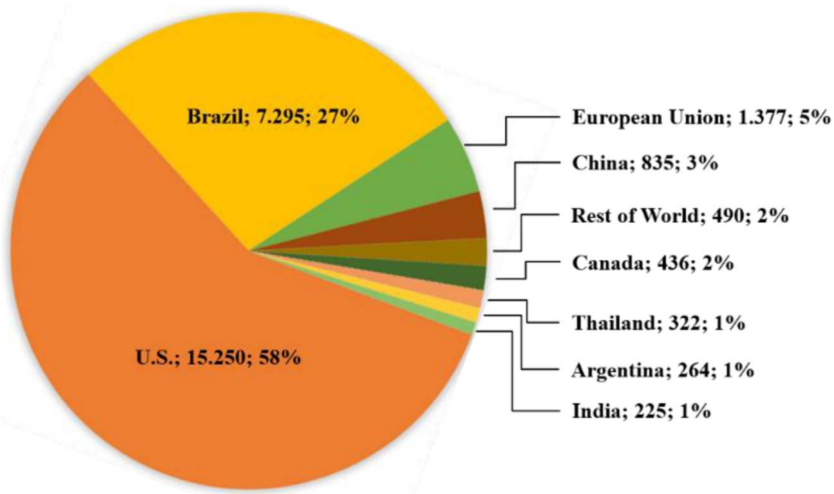

Fig. 1. Bioethanol production by country, million gallons, 2017 (RFA, 2017).

renewable, and nonedible biomass in the world, are untapped resources for bioethanol production because of the lack of researches. There are many types of feedstock that belong to these criteria such as agricultural wastes, forest residues, aquatic plants, food/beverage wastes, and other industrial wastes. Aquatic/wetland plants are considered as promising materials due to its typical characteristics, e.g. to be formed of cellulose, hemicellulose, and lignin; high speed growth; abundance, living on water surface or wetland areas; the ability of neutralize polluted water bodies, etc. The core factor of wetland plants as bioethanol feedstock is cellulose chains formed from a thousand D-glucose units which are essential substrates for bioethanol fermentation. Besides, the efficiency of conversion processes, particularly pretreatment and hydrolysis, are susceptible to biomass attributes. As a result, it is essential to study the physical characteristic and chemical compositions of new cellulosic biomass.

To be composed mainly of cellulose, hemicellulose, and lignin, the process of converting from lignocellulosic biomass into a clean-burning fuel is quite different from the first generation which is the addition of pretreatment steps (Rastogi and Shrivastava, 2017). In order to obtain as much as possible fermentable sugar through hydrolysis by enzyme or microorganisms, it is vital to make cellulose chains free of lignin and hemi-cellulose cover by pretreatment. Based on the structure of feedstock, there has been a lot of efficient pretreatment methods developed recently: physical, chemical, biological, and combination of those methods. Firstly, biomass might be milled, grinded, or blended into small pieces to increase the activated surface and porosity of the lignocelluloses. The powdered feedstock or pieces are then continuously treated at high temperature or pressure, with or without chemical, or microorganisms. At severe condition (high temperature or pressure), the architecture of feedstock is damaged and broken into separately components. On the other hands, the using of some chemical such as dilute/concentrated acid, alkaline, oxidant substances can disturb and cut the hydrogen bonds and covalent bonds between cellulose, hemicellulose, and lignin (Ravindran and Jaiswal, 2016). Biological method, nevertheless, using the metabolism of other living things such as microorganism, fungi, mold, etc. degrade the structure of biomass to simple sugar. Some other advanced methods and application are described in the Table 1.

This study focuses on alkaline/oxidant pretreatment using sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) as efficient reagent to affect biomass attribute and support hydrolysis step (Yan et al., 2015). Co-treatment with sodium hydroxide and hydrogen peroxide is considered as an environment-friendly and effective method for various types of lignocellulosic biomass including water hyacinth (Mishima et al., 2008; Yan et al., 2015), wheat straw (Barakat et al., 2014), etc. The reaction between sodium hydroxide/hydrogen peroxide and biomass requires a long retention time which is up to hours (Haghighi Mood et al., 2013). Nevertheless, dilute alkaline oxidant pretreatment does not create much inhibitors and damage equipment comparing to dilute/concentrate acid methods.

Gooseweed (*Sphenoclea zeylanica* Gaertn.) is a usual and widespread herbaceous weed of wetland rice (Holm et al., 1977) (Fig. S1). This species was placed in the family *Campanulaceae* which is known as the bellflower family including about 2400 species of non-woody plants (Berry, 2009; Mani, 2014). In regards to taxonomy, Richard and his colleagues described gooseweed in detail; stems are green, erect, hollow and often much-branched; leaves are alternate; flowers are sessile and bisexual. In addition, gooseweed is able to develop both on terrestrial and freshwater systems in tropical to warm temperature areas (Carter et al., 2014). It is native to the Eastern Hemisphere including Thailand, Viet Nam, Indonesia, etc. Since its preferred habitat is wetland and aquatic bodies, this species has been a problematic non-woody plant on

Table 1
Some pretreatment methods using for bioethanol production from lignocellulosic biomass.

Pretreatment	Describes		Reference
Physical	Disrupting the structure of biomass at high temperature, and pressure.	Microwave, pyrolysis	Klein et al. (2016) and Luque et al. (2014)
Physico-chemical	A combination of high physical condition (temperature, pressure) and chemical reaction (with or without catalyst).	Steam explosion, liquid hot water pretreatment, AFEX* process, organosolv process	Agbor et al. (2011), Kupiainen et al. (2012), Mesa et al. (2011) and Zhao et al. (2015)
Chemical	Using concentrated or dilute acid, bases, and different types of ion liquid substances which are able to dissolve cellulose and break the link of lignin to cellulose: H_2SO_4, HCl, NaOH, $Ca(OH)_2$, KOH, NH_4OH, H_2O_2, etc.	Acid/alkaline treatment, ion liquid	Bensah and Mensah (2013), Heinze et al. (2005), Hu et al., (2008), Shafiei et al. (2013) and Yan et al. (2015)
Biological	Taking advantages from microorganisms and bacteria to cut down the structure of lignocellulosic biomass.	White-rot fungi, brown-rot fungi, soft rot fungi	Hwang et al. (2008), López-Abelairas et al. (2013) and Sindhu et al. (2016)

* AFEX: Ammonia fiber explosion.