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Evaluation of ethanol production from sugar and lignocellulosic part of energy cane

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ABSTRACT

Energy cane is considered an ideal energy crop because it produces readily fermentable sugars and high yields of lignocellulosic biomass. Four improved clones of the energy cane series "TByEFC" (Tiphuyae and Banyang Energy and Forage-cane Clone), which was developed at the Kasetsart University Kamphaeng Saen campus in Thailand, were evaluated in terms of their potential ethanol yields from both sugar juice and bagasse. Juice from TByEFC05-1558 and TByEFC04-1208 displayed the highest total sugar production, ranging from 171.03–179.72 g/L, and reached the maximum ethanol production, ranging from 78.02–82.56 g/L, without any nutrient supplementation at 37 °C using *Saccharomyces cerevisiae* ND48. All four energy cane clones showed a maximum ethanol concentration from bagasse in the range of 9.11–10.68 g/L. The combination of agronomic productivity and ethanol production yield indicated that the highest ethanol yield from juice was reached with TByEFC04-1155 (2697.00 kg/ha), while the highest ethanol yield from bagasse was reached with TByEFC09-0098 (1695.45 kg/ha). Among the tested clones, TByEFC04-1155 was shown to be useful for combined ethanol production from both juice and bagasse, yielding 3923.48 kg/ha. This energy cane clone combines good agronomic features for ethanol production from sugar substances and lignocellulosic biomass, making it an attractive energy crop for the ethanol industry.

1. Introduction

With the depletion of limited fossil fuel stocks, countries worldwide have been compelled to search for new energy sources to substitute for petroleum following the "fossil fuel crisis" in the 1970s. In Thailand, ethanol plays an important role in renewable energy for transport according to government policy. Ethanol has been used as a substitute for conventional gasoline by blending ethanol with gasoline (called gasohol), i.e., E10, E20 and E80. E10 gasohol, made by blending 10% ethanol and 90% octane 95 gasoline, was introduced into the market in 2004; E20 and E85 were introduced in 2008. Due to government promotion strategies, the total ethanol consumption in Thailand has increased from 1.2 million liters per day (M L/day) in 2010–3.2 M L/day in 2014 (AEDP, 2015). The Thai renewable energy policy has set a target to increase ethanol production to 11.3 M L/day by 2022. Currently, sugar cane molasses and cassava starch are the main raw materials for ethanol production in Thailand; however, these feedstocks cannot sufficiently meet the policy production targets. In addition, sugar cane molasses can be used as a raw material for several fermentation industries, which can lead to an increase in the cost performance of ethanol. Therefore, diverse feedstock sources are required for ethanol production.

Currently, with the growing need for alternative sources of energy, lignocellulosic biomass is a natural, abundant and renewable resource that is a promising feedstock for achieving the Thai government's energy policy goals. The government of the USA considers the use of biomass for ethanol production and electricity generation as important for reducing the use of fossil fuels, and the European Union has a plan to supply 20% of its total energy needs with biomass energy by 2020 (Matsuoka et al., 2012). Thailand is a tropical, agriculture-based country where a great effort has been applied to develop sugar cane as a potential energy crop through sugar cane breeding programs. "Energy cane" results from effectively selecting sugar cane that has a higher fiber yield than sugar yield, making it more vigorous and rustic. These characteristics bring a series of economic and environmental advantages (Carvalho-Netto et al., 2014; Kim and Day, 2011). Energy

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cane is considered an ideal energy crop because it produces readily fermentable sugar juice and a high yield of lignocellulosic biomass. After the juice extraction process, the bagasse can be used either as a feedstock for cellulosic ethanol production or as a raw material for thermal energy in electricity plants. Sugar cane breeders in the Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen campus, Thailand, have attempted to breed the energy cane series "TByEFC" (Tiphuyae and Banyang Energy and Forage-cane Clone) by crossing sugar cane (*Saccharum* spp.) with wild cane (*S. spontaneum*). Compared with sugar cane, the energy cane series TByEFC contains a higher fiber content, has better tolerance to insects and diseases and requires less fertilizer and less water input. In addition, the crop cycle of the energy cane series TByEFC is 8–10 months, shorter than that of sugar cane, which requires a harvesting period of 12 months (Chatwachirawong et al., 2009).

Much of the research to date has focused on the lignocellulosic part of energy cane as a bioethanol feedstock. Pretreatment methods and optimizations have been reported for maximum enzymatic hydrolysis and ethanol yield from energy cane bagasse (Aita et al., 2011; Qiu et al., 2012, 2013; Oladi and Aita, 2017). However, for economic ethanol production, the juice, fiber and cane yields per cultivated area are important parameters that are associated with ethanol production. In this current study, four improved TByEFC energy cane clones (with higher biomass, juice and total sugar contents) and one commercial sugar cane variety, LK92-11 (reference material), were evaluated for their efficiency in ethanol production from both the juice and bagasse. This study is the first to report an evaluation of ethanol production from the juice and bagasse of energy cane at the laboratory scale in combination with their agronomic productivity. Additionally, the chemical compositions of the energy cane juice and bagasse are also reported.

2. Materials and methods

2.1. Raw materials and preparation of samples

The experiment was conducted from October 2014 to March 2016 on silt loam soil texture at the Field Crops Building, Kasetsart University, Kamphaeng Saen campus, Thailand. A randomized complete block design with 4 replications was used. Four energy cane clones (TByEFC04-1155, TByEFC04-1208, TByEFC05-1558, and TByEFC09-0098) and a commercial sugar cane variety (LK92-11, used as a reference material) were harvested 10 months after planting. Each plot consisted of 5 adjacent 1.50-m-wide rows (8 m long) with a 1.50-m alley between each plot. The plants in the 3 middle rows were manually cut at the ground level and topped at their natural break point for yield determination. At the same time, 10 sampling cane stalks were crushed twice in a small local roller press immediately after being cut. The juice was filtered through cheesecloth and kept at −20 °C for further studies. The cane yield was calculated from the millable stalk weight of the 3 middle rows. The juice volume was used to calculate the juice yield. The sugar yield was calculated from juice volume and total sugar content (sucrose, glucose and fructose), whereas the fiber yield was calculated from the cane yield and fiber content.

2.2. Physical and chemical composition determination of the energy cane juices

The energy cane juices were measured for total soluble solids (TSS, °Brix) using a refractometer (N.O.W., Japan). Sugars in the juices were quantified using a High Performance Liquid Chromatography (HPLC) system (Water 600E, Waters Inc., USA) with a refractive index detector and a sugar pak I column at 85 °C. The mobile phase was deionized water at 0.5 mL/min (Senatham et al., 2016). Free amino nitrogen (FAN) was determined using the ninhydrin official method with glycine as the standard (AOAC, 1980). The total phenolic content in the juices was determined with the Folin-Ciocalteu reagent using gallic acid as the standard (Vinson et al., 2001).

2.3. Fermentation of the energy cane juice

Saccharomyces cerevisiae ND48 cells (with high ethanol production from sucrose, unpublished data) pre-cultivated overnight in YPD broth (2% glucose, 1% yeast extract and 2% peptone) were inoculated into energy cane juice medium without nutrient supplementation and pH adjusted to 5.0 with 0.1 N NaOH. The initial cell concentration was adjusted to a cell density of 5×10^5 cells/mL. Fermentation was performed on a rotary shaker at 100 rpm and 37 °C for 72 h. Samples were withdrawn every 12 h to determine the cell density at 600 nm (OD_{600}) using UV–vis spectrophotometer (GENESYS™ 10S UV–vis, Thermo Scientific, USA). The concentrations of ethanol and residual sugar were monitored using HPLC.

2.4. Chemical composition determination of energy cane bagasse

The energy cane bagasse chemical composition was determined using a slight modification of the standard procedure developed by the National Renewable Energy Laboratory (NREL), as detailed in Sluiter et al. (2008). In brief, 2–10 g of a milled, dried bagasse sample was extracted with 95% ethanol for 12 h using a Soxhlet apparatus. A sample of 0.3 g of dried extractive-free material (moisture below 10%) was treated with 3 mL of 72% H_2SO_4 at 30 °C for 60 min. The acid was diluted to 4% H_2SO_4 by adding 84 mL of deionized water, and the mixture was then autoclaved at 121 °C for 60 min. After cooling to room temperature, the mixture was filtered, and the solid fraction was rinsed with warm deionized water until a neutral pH was recorded, followed by oven drying at 105 °C to a constant weight. The material was then heated in a furnace set at 575 °C for 24 h. The weight difference before and after incineration was considered to be the acid insoluble lignin in the bagasse. The soluble lignin-in-liquid fraction was determined from absorbance measurements at a wavelength of 280 nm using UV–vis spectrophotometer. The sugar concentrations were analyzed using HPLC with a sugar pak I column and refractive index detector as described above. Furfural and 5-hydroxymethyl furfural (HMF) were eluted with 20% acetonitrile in deionized water (80%) at a flow rate of 1.1 mL/min. Acetic acid was eluted with 1% acetonitrile in 0.05 M KH_2PO_4 (99%) at a flow rate of 1.0 mL/min (Senatham et al., 2016).

The concentrations of glucose, cellobiose and HMF were used for determination of the cellulose content, while the concentrations of xylose, arabinose, glucuronic acid, acetic acid and furfural were used for determination of the hemicellulose content. The cellulose content and hemicellulose content were calculated using the formulas used by the NREL (Sluiter et al., 2008).

2.5. Preparation of energy cane bagasse hemicellulosic hydrolysate using dilute-acid hydrolysis

The milled bagasse was dried at 60 °C for 24 h. The dried bagasse in a solid-liquid ratio of 1:10 was soaked in 1% H_2SO_4 for 30 min at ambient temperature and then autoclaved at 121 °C for 30 min. The hydrolysate was filtered and neutralized with CaO to pH 5.5. The precipitate that formed was removed using centrifugation and filtration (Senatham et al., 2016). Detoxification of energy cane bagasse hemicellulosic hydrolysate was performed with slightly modified method described by Branco et al. (2011). The neutralized hemicellulosic hydrolysate was treated with 2% (w/v) activated charcoal with stirring at 150 rpm for 1 h at 30 °C. The solids were then removed using vacuum filtration.

2.6. Fermentation of the energy cane bagasse hemicellulosic hydrolysate

Scheffersomyces shehatae TTC79 (Senatham et al., 2016) cells pre-