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Evaluation of high sugar containing corn genotypes as viable feedstocks for decreasing enzyme consumption during dry-grind ethanol production

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ABSTRACT

Requirement of costly enzymes (α-amylase and glucoamylase) for converting starch into glucose before fermentation is considered one of the cost increasing factors for corn ethanol. Enzyme consumptions can possibly be reduced during dry-grind ethanol production by increasing free sugar contents in corn kernels that will be released and fermented simultaneously with the product of starch hydrolysis, producing an additional amount of ethanol without consuming any enzyme. A comparative study was conducted to evaluate the effect of kernel sugars on enzyme requirement and yields of both hydrolysis and fermentation, using four high sugary corn genotypes (HSGs) and their parent field corns (PFCs). Enzymatic hydrolysis of the genotypes with four enzyme loads (1, 2, 3 and 4 kg MT^{-1} of dry corn) showed that HSGs produced higher proportions of reducing sugars (RS) in all conditions than PFCs did. Sufficient amounts of RS were produced by HSGs utilizing an enzyme load of 3 kg MT^{-1}, whereas, PFCs consumed 4 kg MT^{-1} for their best yields. Likewise, HSGs produced higher concentrations of ethanol consuming lower amount of enzymes during fermentation. Therefore, HSGs could be considered as potential feedstocks for enhancing ethanol yield and reducing enzyme consumptions during dry-grind ethanol production.

1. Introduction

Rapid increase in fuel consumption rate against limited reserves, unstable prices of petroleum oils and alarming increase in green house gas emissions from the fossil fuels have raised the global concerns, and boosted the demand for alternative fuels. Bioethanol, in this aspect is the most promising biofuel, which is produced by converting carbohydrates obtained from biomass. Corn is the major feedstok in current bioethanol industry and its use has been increased dramatically in recent years. United States is the dominant producer of corn ethanol, which produced a record amount of ethanol from this feedstock (14.3 billion gallons) last year, and exported nearly 825 million gallons of ethanol to 51 countries across the world [1]. Therefore, corn ethanol acts as a leading biofuel around the globe. Ethanol is produced from corn either by the dry-grind or wet mill method, where, the former is more attractive due to lower capital investments and suitablity for producing ethanol on a small scale [2]. In a conventional dry-grind process, whole corn is ground, mixed with water to make a slurry and cooked; starch is hydrolyzed to convert it into glucose and subsequently subjected to microbial fermentation [3].

In spite of industrial maturity, corn ethanol is still facing some challenges including raw material and production costs due to consumption of costly enzymes for converting starch into glucose, compared to the contemporary sugar based bioethanol industry that gets fermentable sugar without any costly pretreatment process [4]. Efforts should be made to improve corn quality for addressing these challenges. Over last several years, substantial modifications have been made on technical and technological aspects of ethanol production process [5]. Nevertheless, attempts to improve corn quality are rather limited, even though quality parameters such as corn composition affect the dry-grind ethanol yield [6], which can be done by developing new varieties with increased amount of carbohydrates [7], and high starchy corn varieties has been suggested for enhanced ethanol yield [8]. However, increased grain starch will not

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Table 1
Weather conditions during the study (monthly mean).

Months	Temperature (°C)	Humidity (%)	Rainfall (mm)
Sep, 2012	28.4	75.3	141.0
Oct, 2012	27.7	79.9	459.0
Nov, 2012	27.2	83.7	684.0
Dec, 2012	27.1	83.5	455.2

Source: Department of Metrology, Ministry of Science, Technology and Innovation, Malaysia.

necessarily produce enhanced ethanol for several reasons. Firstly, ethanol yield from starch is affected by some factors such as its amylose content and physicochemical properties [9], and consequently, no significant correlation has been reported between quantity of starch and ethanol yield [10,11]. Secondly, starch to ethanol conversion in the current dry-grind ethanol process is even incomplete, representing inefficiency of the process as well as economic loss of the desired product [12]. Moreover, higher amount of starch will eventually increase enzyme consumption, in addition to the fact that starch can act as substrate inhibitor above a certain level, decreasing hydrolysis efficiency [13]. Instead, efforts can be made to increase the amount of free sugars in the corn grains that will be released and fermented simultaneously with the product of starch hydrolysis, producing an additional amount of ethanol without consuming any enzyme.

Field corn, which is currently being used for ethanol production contains poor amount of sugar, compared to sweet corn that contains higher amount of sucrose [14], glucose and fructose [15]. However, regardless of a recent report to produce ethanol from sweet corn stover [16], its grains have not been considered for bioethanol production, possibly due to the facts that it is exclusively used as human food, requires special cares during growth, and most importantly, its grain yield is lower than the field corn [17,18]. Alternatively, it is conceivable that transferring the genetic characters from sweet corn to field corn using conventional breeding or genetic engineering will produce new genotypes with altered kernel compositions, particularly with increased amount of free sugars and desired grain yield. It can be hypothesized that high sugary genotypes (HSGs) would have the potential to decrease enzyme consumptions during dry grind ethanol production with higher proportions of fermentable sugars as well as ethanol due to a) containing higher amount of kernel sugars that would be available without any hydrolysis process, and b) containing relatively lower amount of kernel starch (as replaced by free sugars), which would be hydrolyzed more efficiently than that of PFCs under the same initial solid concentration avoiding the possible substrate inhibition on the enzymes that may occur above a certain level of starch [13].

Present work has been carried out to study four HSGs, obtained from the crossing of field corn with sweet corn composite lines, as attempts to produce higher amount of ethanol and decreasing enzyme consumptions during dry-grind ethanol process. The results were also compared with the respective parent field corns (PFCs).

2. Materials and methods

2.1. Corn materials

Four HSGs (UM.NF-1, UM.NF-4, UM.NF-6 and UM.NF-11), and their PFCs, named as PFC-1, PFC-4, PFC-6 and PFC-11, respectively, were collected from Dr. Golam Faruq, Institute of Biological Sciences, University of Malaya and grown in the field at the University of Malaya (3°7′1″N and 101°39′12″E) during September–December 2012 under rain-fed condition following randomized complete block design (RCBD) with four replications. The soil type of the field was sandy loam and pH was 6.8. The climatic conditions are given in Table 1. Individual experimental plots consisted of 6 rows with 20 plants in each row. The row to row distance was 0.75 m, plant to plant distance was 0.50 m, and plot to plot distance was 2 m. Similar crop management practices were followed for all the HSGs and PFCs. The plots were fertilized two times with the mixed fertilizer NPKS at the ratio of 15:15:15:18. Grain moisture content was monitored in the field on a weekly basis with a hand held moisture tester. Ears were hand harvested after physiological maturity at the moisture level of 25% and dried in an oven at 37 °C until a final moisture content of the grains reached to around 15%. Grains obtained from each plot were weighed and converted to t ha^{-1}. Subsequently, grains were removed from the cobs, ground in a hammer mill, sieved to different particle sizes (0.5, 1.0, 2.0 and 3.0 mm) and preserved at 4 °C until analyses were carried out.

2.2. Enzymes and microorganism

Two enzymes, SPEZYME® FRED (an α-amylase) and OPTIDEX® L-400 (a glucoamylase) used in this study were the kind gifts from DuPont Industrial Biosciences (DuPont Genencor Science, Palo Alto, CA). *Saccharomyces cerevisae* (ATCC 96581) was obtained from ATCC (Manassas, VA, USA), and was maintained on YPD agar slant consisting of yeast extract (10 g L^{-1}), peptone (20 g L^{-1}), dextrose (20 g L^{-1}), agar (15 g L^{-1}) and distilled water (up to 1 L). Prior to use as inoculum, the yeast cells were aerobically grown in a 250 ml Erlenmeyer flask in a shaking incubator (200 rpm) at 30 °C for 48 h in YPD broth supplemented with KH_2PO_4 (2 g L^{-1}), $(NH_4)_2SO_4$ (1 g L^{-1}) and $MgSO_4 \bullet 7H_2O$ (0.4 g L^{-1}). Subsequently, growth medium was centrifuged at 5000 rpm for 15 min to separate the cells and a yeast suspension (cell density $\sim 1 \times 10^8$ CFU ml^{-1}) was prepared in fresh YPD broth.

2.3. Determination of kernel carbohydrate profile

Starch and total sugar were determined by the method described in Rose et al. [19]. Individual sugar such as sucrose, fructose and glucose contents were estimated using the methods described by Finley et al. [20], Johnson et al. [21], and Dubowski [22], respectively. In order to determine starch composition, it was isolated from the corn samples using the method described by Sandhu et al. [23], and amylose in the isolated starch was determined by the colorimetric method [24]. A standard curve was prepared using 2 – 12 mg pure potato amylose (Sigma; Product No. A0512).

2.4. Two-step enzymatic hydrolysis

2.4.1. Liquefaction

In a 50 ml falcon tube, 20 ml of corn slurry was prepared with a solid concentration of 250 g L^{-1} by mixing appropriate amount of corn flour (taking into account its moisture content) with distilled water. The pH was adjusted to 6.0 with 1.0 N HCl or 5.0 N NaOH. Thereafter, appropriate amount of SPEZYME® FRED was added to the slurry. The tubes were placed in a thermostated water bath at 90 °C for a specified period with vigorous shaking in the first 5 min and then every 15 min. Samples were then withdrawn, centrifuged at 5000 rpm for 15 min and supernatants were analyzed for reducing sugars (RS). Effect of particle sizes of the corn flour on RS yield was studied by preparing slurries with four different particle sizes (0.5, 1.0, 2.0 and 3.0 mm). The SPEZYME® FRED concentration was 3 kg MT^{-1} dry corn and incubation time was 2 h. Effect of enzyme concentrations on RS yield was studied using four SPEZYME® FRED loads such as 0.025, 0.05, 0.075 and 0.1%, v/v, which were equivalent to 1, 2, 3 and 4 kg MT^{-1} dry corn, respectively. Corn flour used for preparing the slurries was 1.0 mm size and incubation time was 2 h. Effect of incubation time was studied using corn flour with 1.0 mm particle size and SPEZYME® FRED concentration 3 kg MT^{-1} dry corn. Samples were withdrawn and studied for RS after 0, 0.5, 1, 2 and 3 h.