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A comparative study on normal and high sugary corn genotypes for evaluating enzyme consumption during dry-grind ethanol production

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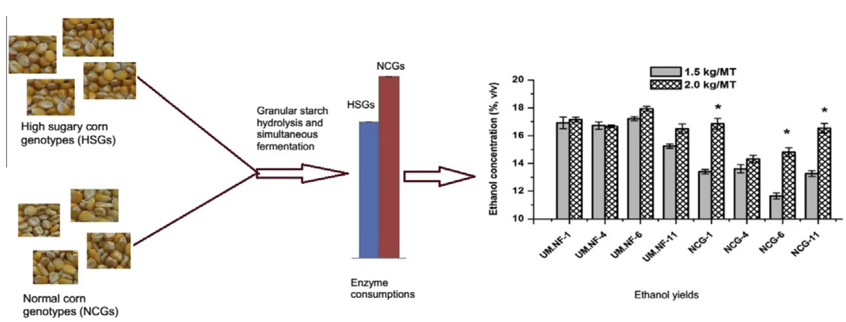
HIGHLIGHTS

- Free sugars in corn should be evaluated for cost effective ethanol production.
- We compared normal and high sugary corn genotypes (HSGs) for enzyme requirements.
- HSGs produced higher amounts of reducing sugars with lower enzyme dose.
- HSGs produced higher amounts of ethanol with lower enzyme dose.
- HSGs could be viable feedstocks for the dry-grind ethanol production.

GRAPHICAL ABSTRACT



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ABSTRACT

The necessity of amylolytic enzymes to convert starch into glucose during ethanol production is considered one of the cost increasing factors for corn ethanol. Enzyme consumption could be decreased partially 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. The present work was conducted to evaluate the effects of grain sugar on the fermentable sugar and ethanol yields as well as enzyme requirement using four high sugary corn genotypes (HSGs) and their parent field corn lines (PFCs). The reducing sugar yield in HSGs did not vary significantly above the enzyme load of 1.5 kg/MT of dry corn, while PFCs showed a range between 2.0 and 2.5 kg/MT. The average final ethanol concentrations in HSGs and PFCs ranged from 15.25% to 17.5% (v/v) and 11.66% to 13.65%, respectively with the enzyme load at 1.5 kg/MT, which reached to 16.49–17.94% in HSGs and 14.32–16.85% in PFCs as the enzyme load increased to 2.0 kg/MT. These results suggest that high sugar content in corn kernels has the potential for decreasing enzyme consumption during dry-grind ethanol production with higher yields.

1. Introduction

The growing concerns on energy security, declining in oil reserves, unstable prices of fossil fuels and global warming have boosted the demand for producing bioethanol as an alternative fuel. Three types of raw materials are being used in current bioethanol research, such as sugar crops, starchy crops and lignocellulosic biomass, which differ considerably from each other

Abbreviations: CFU, colony forming unit; GSH, granular starch hydrolysis; GSHE, granular starch hydrolyzing enzyme; GSHSF, granular starch hydrolysis and simultaneous fermentation; HSGs, high sugary corn genotypes; PFCs, parent field corn lines; RS, reducing sugars; TSS, total soluble sugars.

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with regard to the obtainment of sugar solutions before fermentation [1]. Sugar crops require only an extraction process to get fermentable sugars, whereas, starchy crops require a hydrolysis step to convert starch into glucose using amylolytic enzymes. Lignocellulosic biomass have to be pretreated before hydrolysis in order to alter cellulose structures for enzyme accessibility, which makes the process more challenging and complicated. Consequently, lignocellulosic materials are considered economically non- competitive for bioethanol production, despite the fact that they are abundant, inexpensive and substantial research has been done on lignocellulosic ethanol in recent years [2]. Henceforth, almost all commercial ethanol is produced from sugar and starchy feedstocks, and not surprisingly, the former produces cheaper ethanol than the latter [3]. However, starch based bioethanol is relatively well established and produces about 60% of the total ethanol, compared to nearly 40% from sugar sources [1].

Corn is the major feedstock in starch based bioethanol industry and its use has increased dramatically during last 15 years [4]. United States is the dominant producer of corn ethanol, which it produced in record amounts (14.3 billion gallons) in 2014, and at the same time exported roughly 825 million gallons of ethanol to 51 countries across the world [5]. Recently, it has been reported that some European countries, for example Serbia, which produces nearly 40% excess corn above its domestic need has shown interest in producing ethanol from corn [6]. Therefore, corn ethanol is gradually becoming a global biofuel. Ethanol is produced from corn either by the dry grind or wet mill method, where most of the ethanol comes from the former in current practices [7]. The conventional dry grind method involves preparation of a slurry by mixing corn flour with water, which is then cooked and liquefied at high temperature with thermostable α-amylase to breakdown starch into dextrin, followed by saccharification of the liquefied slurry to convert dextrin into glucose using glucoamylase at a relatively lower temperature, and subsequently subjected to yeast fermentation to produce ethanol from glucose [8].

There are two major challenges for producing cost-effective fuel ethanol in a conventional dry-grind industry, and these are a high energy input and consumption of costly enzymes [9], when compared to the contemporary sugar based bioethanol industry that gets fermentable sugar without any costly pretreatment process [3,10]. It has been estimated that energy requirement for conventional cooking and liquefaction is equivalent to 30–40% of the fuel value of the ethanol produced [11]. In recent years, a non-cooking method has been developed to reduce energy consumption during ethanol production that involves hydrolysis of granular starch at sub-gelatinization temperature using granular starch hydrolyzing enzymes [12]. However, a low hydrolysis rate and incomplete hydrolysis of starch at sub-gelatinized temperature due to structural heterogeneity and crystallinity of native starch has resulted in an additional challenge for cost effective ethanol production using this process [13]. To overcome these shortcomings and increase hydrolysis efficiency as well as ethanol yield, enzyme manufacturers have recommended to conduct a mild heat treatment (*e.g.* at 60 °C) prior to fermentation and to supplement the media with urea and protease [14]. Urea has been reported to increase hydrolysis rate and sugar yield [13], while protease has the potential for using high dry solid with increased fermentation efficiency as it accelerate yeast growth, substrate consumption, ethanol yield and productivity during fermentation of starch based feedstocks [15,16]. However, in spite of these process modifications, it is still necessary to use large quantity of amylolytic enzymes for converting starch to fermentable sugar. Although several efforts have been made for fermenting starch directly without adding any exogenous enzyme, by developing recombinant yeast capable of expressing amylolytic enzymes [9,17], all of these efforts are still confined to the laboratory and have not reached a satisfactory level for industrial usage [18]. Furthermore, utilization of higher amounts of dry solid in a batch is always of commercial interest, but starch concentrations in the slurries are important factors for its efficient conversion, since starch above a certain level may cause substrate inhibition of the enzymes, resulting in an incomplete conversion of starch, lower ethanol yield and increased production costs [19]. Considering the above facts, it can be assumed that increased free sugar contents in corn kernels would have the potential for decreasing enzyme consumption to a certain level by increasing sugar concentration in the mash during fermentation. Kernel sugars will be released directly without consuming any enzyme and fermented simultaneously with the product of starch hydrolysis.

Normal corn, which is currently being used for ethanol production contains poor amounts of free sugars compared to sweet corn, as the latter is characterized for higher amount of sucrose [20], glucose and fructose [21]. However, apart from a recent report on ethanol production from stover [22], sweet corn grains have not been considered for bioethanol production, possibly due to the facts that it is exclusively used as human food, requires special care during growth, and more importantly, its grain yield is lower than the yield of normal corn [23,24]. It is conceivable that high sugary corn genotypes can be developed for increased sugar content using conventional breeding between sweet corn and normal corn. The present work was carried out to study the potential of four high sugary corn genotypes (HSGs) to decrease enzyme consumptions during dry-grind ethanol production using granular starch hydrolysis and simultaneous fermentation (GSHSF) process. The results obtained were compared with the respective parent field corn lines (PFCs), which were used to develop HSGs by crossing with sweet corn composite lines.

2. Materials and methods

2.1. Corn materials

Seeds of 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. All the corn genotypes were grown in the field at the University of Malaya (3° 7′ 1″ N and 101° 39′ 12″ E) during August–November 2013 under rain-fed conditions in a randomized complete block design (RCBD) with four replications, in sandy loam soil with pH of 6.3 ± 0.21. The climatic conditions during the study are given in Table 1. Individual experimental plots consisted of 6 rows with 5 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 a mixed fertilizer with an NPK ratio of 15:15:15. Grain moisture content was monitored weekly 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 40 °C until the final moisture content reached around 15%. Subsequently, grains were removed from cobs, ground in a hammer

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

Months	Temperature (°C)	Humidity (%)	Rainfall (mm)
August, 2013	28.5	71.3	189.8
September, 2013	27.7	74.7	249.4
October, 2013	27.9	75.5	341.2
November, 2013	27.1	81.7	289.8

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