Biomass and Bioenergy 90 (2016) 42–49

Contents lists available at ScienceDirect

Biomass and Bioenergy

journal homepage: http://www.elsevier.com/locate/biombioe

Research paper

Second-generation bioethanol of hydrothermally pretreated stover biomass from maize genotypes

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ARTICLE INFO

Article history:
Received 4 February 2015
Received in revised form
9 February 2016
Accepted 23 March 2016
Available online 6 April 2016

Keywords:
Genetic variation
Corn breeding
Autohydrolysis
Enzymatic saccharification
Phenotyping

ABSTRACT

Twelve maize genotypes, were agronomically evaluated and their stover hydrothermally pretreated in a temperature range of 210–225 °C to assess the effects of genotype and pretreatment severity on stover recalcitrance toward bioethanol conversion. Maize genotypes exhibited significant variation for biomass yield and all agronomic evaluated, while among all cell wall constituents measured in the unpretreated stover, only ash content showed differences among genotypes. The pretreatment severities assayed impacted most stover compositional traits, and the glucose recovered after enzymatic hydrolysis displayed a similar profile among genotypes with similar genetic background. Harsher pretreatment conditions maximized the potential cellulosic bioethanol production (208–239 L/t), while the mildest maximized the bioethanol from the hemicellulosic hydrolysates (137–175 L/t). Consequently, when both pentose and hexose sugars were considered, the total potential bioethanol produced at the lowest and highest pretreatment temperatures was similar in all genotypes (292–358 L/t), indicating that the lowest temperature (210 °C) was the optimal among all assayed. Importantly, the ranking of genotypes for bioethanol yield (L/ha) closely resembled the ranking for stover yield (t/ha), indicating that breeding for biomass yield would increase the bioethanol production per hectare regardless of the manufacturing process. Similarly, the genetic regulation of corn stover moisture is possible and relevant for efficient energy production as biomass moisture has a potential impact on stover transportation, storage and processing requirements. Overall, these results indicate that local landrace populations are important genetic resources to improve cultivated crops, and that simultaneous breeding for production of grain and stover bioethanol is possible in corn.

1. Introduction

Bioethanol manufactured from sugars or starch found in food crops is the most common biofuel used commercially today. These so-called first generation biofuels are limited because they cannot be produced in a large scale without threatening food supplies. In fact, if emissions from production and transport are taken into account, the life cycle assessment from first generation biofuels frequently approaches those of traditional fossil fuels [1]. Lignocellulosic biomass is an abundant and renewable feedstock source that can be converted to liquid transportation fuels and other chemicals via fermentation. Cellulosic bioethanol is a promising near-term technological alternative to reduce transportation sector greenhouse gas emissions, and it can supply a larger proportion of current fuel supply affordably, sustainably, and with the added benefit that it is not used for food purposes.

In particular, maize (*Zea mays* L.) stover has been considered as a potential source of feedstock for the production of lignocellulosic bioethanol because of its abundance, relative proximity to existing grain bioethanol production plants, and its amenability to conventional harvesting practices [2–4]. The collection of crop residues, such as maize stover, as a co-product of grain allows the simultaneous production of food and energy, providing greater reductions in greenhouse gas emissions than dedicated energy

List of abbreviations: EU, European Union; US, United States of America; HPLC, High performance liquid chromatography; T_{MAX}, Maximum temperature; LSR, Liquid to solid ratio; ESR, Enzyme to substrate ratio; IU, International Units; FPU, Filter Paper Units for enzymatic activities; S_0, Pretreatment severity; T_{MAX}, Maximum temperature; T, temperature; HMF, hydroxymethylfurfural; Gluco-o, xylo-o, arabino-o, acetyl-o, glucose, xylose, arabinose and acetyl oligomers, respectively; CGC, Cellulose-to-glucose conversion; KL, Klason lignin.

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http://dx.doi.org/10.1016/j.biombioe.2016.03.029
0961-9534/

crops because a change in land use is not necessary [5]. In contrast to starchy raw materials employed in the manufacture of first-generation bioethanol, the lignocellulosic biomass consists primarily of cell wall material made up of cellulose, hemicellulose and lignin polymers, and it is recalcitrant to bioconversion of its polysaccharides into fermentable sugars. Current biomass fermentation processes for fuels and chemicals have a relatively high cost primarily due to this recalcitrance, which in turn has limited commercialization of biomass bioethanol [6,7].

The conversion of lignocellulosic biomass to bioethanol is a three step process that involves pretreatment followed by polysaccharide hydrolysis to simple sugars followed by sugar fermentation to ethanol [8]. The pretreatment is one of the most expensive steps [9], and it serves to reduce biomass recalcitrance by altering the cell wall structure so that the polysaccharide fractions (mainly cellulose) can become more accessible to enzymatic hydrolysis. Numerous pretreatment approaches including physical, chemical, and biological techniques have been developed to reduce recalcitrance and improve sugar yield of cellulosic biomass. The pretreatment with hot compressed water (hydrothermal processing or autohydrolysis) allows the solubilization of hemicelluloses into mono- and oligo-polysaccharides and has limited requirements of energy and generation of wastes [10,11]. In addition, the solid fraction is enriched in cellulose and lignin and presents high accessibility to enzymatic hydrolysis.

To achieve sustainable energy production, it is necessary to overcome the chemical and structural properties of biomass that inhibit its deconstruction, while maintaining excellent agronomic performance. Several studies have found genetic variation for maize stover composition and glucose release [12,13], suggesting that plant breeding would be an effective tool for increasing bioethanol production. However, the pretreatment conditions evaluated in previous experiments have been considered fixed and are different from those that can be used in large-scale industrial production. How genetic variation and ranking of varieties with potential use for biofuel production is affected by different pretreatment conditions is largely unknown in most bioenergy crops.

The current work aims to study the genetic variation in the main agronomic traits and the stover biomass yield and quality of a representative sample of several maize varieties. Stover samples were pretreated in aqueous media under non-isothermal conditions to reach maximal temperatures in the range 210–225 °C in order to evaluate the effect of pretreatment severity on the fractionation of the stover samples, the susceptibility toward enzymatic hydrolysis and the potential bioethanol production.

2. Materials and methods

2.1. Plant materials and experimental design

Three commercial hybrids and nine local populations of maize (Table 1) that represent a wide range of the genetic base for commercial hybrid development were grown at two locations in western Spain in 2011. The hybrids evaluated were B73 × Mo17, of historical importance, and PR34613 and PR36B08B, currently commercialized by Pioneer. The local populations evaluated were three US-Corn Belt Dent varieties: Minnesota No. 13, BS17 (originating from the Iowa Stiff Stalk Synthetic-BSSS) and BSL(S)C6 (originating from Lancaster). Most inbreds used in temperate breeding derive from these three varieties [14–16]. In addition, six European Flint varieties were evaluated: Posada de Llanera, Lazcano, and Aranga from Atlantic regions, and Rastrojero, Vejer, and Faro from Mediterranean regions.

The experimental design at both locations was a randomized complete block with three replicates. Each plot had two rows spaced 0.80 m apart, and each row consisted of 25 two-kernel hills spaced 0.18 m apart. After thinning to one plant per hill, plant density was approximately 60,000 plants/ha. Cultivation operations, fertilization, and weed control were carried out according to local practices and crop requirements. After the vegetative development stage, the following data for main agronomic traits were collected in each plot: female flowering (silking) date measured as the number of days from planting to 50% of plants showing silks, plant height (cm), percentage of stalk lodging or prostrate plants with the stem broken below the main ear, percentage of grain moisture at harvest, and dry grain and cob yield (t/ha). Ten representative plants of each plot were chosen after removing the ears, and the vegetative fraction was chopped with a yard waste chipper and weighed to estimate the stover biomass yield (t/ha). The stover samples were consisted of stalks, leaf blades, leaf sheaths, husk leaves, and ear shanks. Two stover subsamples of approximately 400 g were obtained from each field replicate. One subsample was dried for five days at 100 °C in order to correct the biomass yield to a dry matter basis. The other subsample was frozen, lyophilized, ground in a knife mill, and reground in a cyclone-type mill to pass a 0.5-mm screen. A representative sample composed by 100 g from each field replicate at each location was homogenized in a single lot and stored in a dark and dry place until used for compositional analysis.

2.2. Analysis of the raw corn stover

The raw corn stover was analyzed for extractives and moisture, ashes and quantitative hydrolysis according to TAPPI T204 cm-97 [17], TAPPI T211 om-93 [18], and TAPPI T249 cm-85 [19], respectively. The liquid phase from the latter assay was analyzed by HPLC for sugars and acetic acid, using a Refractive Index detector and a BioRad Aminex HPX-87H column (300 × 7.8 mm), eluted with 0.01 M H_2SO_4 at a flow rate of 0.6 mL/min. The concentration of glucose, xylose, arabinose and acetic acid were used to calculate the sample contents of glucan, xylan, arabinosyl substituents and acetyl groups. Although mannose and galactose content in corn stover is relatively low (<3%), it is important to note that these sugars will elute together with xylose in this column. The Klason lignin was determined from the solid residue obtained in the quantitative acid hydrolysis step after correction for ashes. Analyses were carried out in triplicate.

2.3. Autohydrolysis of corn stover and analysis of the resulting liquid phase and spent solids

Corn stover samples and water were mixed at a liquid to solid ratio (LSR) of 8 kg of water/kg of oven-dry raw corn stover in a Parr reactor (Parr Instruments Company) of 0.6 L of internal volume following the standard heating temperature-time profile [20] to reach the target temperature (denoted T_{MAX} = 210, 215, 220 and 225 °C). Then the medium was immediately cooled by flowing water through an internal stainless steel loop. The harness of the autohydrolysis treatments was expressed in terms of "severity" (S_0) [21] and calculated using Equation (1) (see Calculations section).

An aliquot of autohydrolysis liquors was filtered through 0.45 μm membranes and used for direct HPLC determination of glucose, xylose, arabinose, acetic acid, hydroxymethylfurfural (HMF) and furfural. A second aliquot was subjected to quantitative posthydrolysis with 4% w/w sulfuric acid at 121 °C for 40 min, filtered through 0.45 μm membranes, and analyzed by HPLC as indicated above. The increase in the concentration of sugars and acetic acid caused by posthydrolysis provided a measure of the oligomers and their degree of substitution by acetyl groups. Gluco-, xylo-, arabino- and acetyl-oligomers (gluco-o, xylo-o, arabino-o,