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Research paper

Corn stover ethanol yield as affected by grain yield, Bt trait, and environment

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ABSTRACT

Literature values for glucose release from corn stover are highly variable which would likely result in tremendous variability in bio-refinery ethanol yield from corn stover feedstock. A relatively recent change in United States corn genetics is the inclusion of the *Bacillus thuringiensis* (Bt) trait, which now accounts for three-fourths of all US planted corn acreage. The objective of this study was to evaluate the effect of corn grain yield, *inclusion of the* Bt trait, and location environment on corn stover quality for subsequent ethanol conversion. Two hybrid pairs (each having a Bt and non-Bt near-isoline) were analyzed giving a total of 4 hybrids. In 2010 and 2011, field plots were located in Michigan at four latitudinal differing locations in four replicated plots at each location. Stover composition and enzymatic digestibility was analyzed and estimated ethanol yield (g g^{-1}) was calculated based on hydrolyzable glucan and xylan levels. Analysis showed that there were no significant differences in total glucose or xylose levels nor in enzymatically hydrolyzable glucan and xylan concentrations between Bt corn stover and the non-Bt stover isolines. Regression analyses between corn grain yield (Mg ha^{-1}) and corn stover ethanol yield (g g^{-1}) showed an inverse relationship indicative of a photosynthate source-sink relationship. Nevertheless, the quantity of stover produced was found to be more critical than the quality of stover produced in maximizing potential stover ethanol yield on a land area basis.

1. Introduction

Corn stover, composed of stalks, leaves and cobs after grain is harvested, has been identified as the most abundant agricultural crop residue available in the Midwest and it is expected to be a primary source of bioethanol cellulosic feedstock with the production of about 200 million Mg per year [1–3]. Technology for producing biofuels (such as ethanol, butanol, or various hydrocarbons) and biobased chemicals from lignocellulosic material is experiencing significant advances in an effort to meet global energy and chemical needs [4,5]. Examples of lignocellulosic biomass materials considered as feedstocks for bioethanol production include crop residues such as corn stover and wheat straw, woody residues from forest thinning and paper production, cool- and warm-season grasses such as switchgrass and fescue, and crops such as sorghum [6]. Lignocellulosic ethanol is predicted to have a favorable greenhouse gas profile, alleviate dependence on foreign oil, compensate for decreasing worldwide petroleum reserves, and provide an economic boost to rural communities [7]. The importance of cellulosic biomass as a renewable energy resource has increased with the anticipated shortage of fossil reserves and increased air pollution [8].

Total stover biomass produced in corn is generally at a 1:1 mass ratio with grain production [9,10]. However, the amount of corn stover available to harvest is dependent on many factors including: total biomass produced; weather and field conditions at harvest; harvest equipment shortcomings; and, the grower's management bias for retaining some stover biomass in the field for

Abbreviations: Bt, *Bacillus thuringiensis*; AFEX, Ammonia Fiber Expansion; NREL, National Renewable Energy Laboratory; EtOH, Ethanol; ASE, Accelerated Solvent Extractor; SRS, Sugar Recovery Standards; ABSL, Acetyl Bromide Soluble Lignin; SHF, Separate Hydrolysis and Fermentation.

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environmental and agronomic reasons [11]. The composition of corn stover has been shown to be highly variable. Factors such as harvest year, environment, and variety result in glucose, lignin, or xylose values ranging up to 10% on an absolute basis [12]. Location and genotype could also play significant roles in the biomass composition and ethanol yield of corn stover. A recent publication from Banerjee et al. [13], reported differences in corn stover glucose release ranging from 64 to 95%. These significant differences in fermentable glucose levels would likely result in tremendous variability in bio-refinery ethanol yield from corn stover feedstock.

Currently, 76% of corn stover produced in the U.S. is derived from plants containing a Bt transgene [14]. The Bt transgene originates from *Bacillus thuringiensis,* a naturally occurring soil bacterium that produces proteins (Bt) toxic to specific target insects. Plants genetically modified to produce the *Bt protein* have a built-in larvicidal toxin that kills lepidopteran pests, especially the European corn borer (*Ostrinia nubilalis*), a major pest in Europe and North America that can reduce yields of corn by 3–7% per borer, [15]. Work published by Saxena and Stotzky in 2001 [16] showed that the lignin content, an anti-quality agent for ethanol production was significantly higher (33–97% higher) for Bt lines compared to their respective non-Bt isoline.

The objective of this study was to evaluate the effect of corn grain yield, Bt trait, and location environment on corn stover quality for subsequent ethanol conversion.

2. Materials and methods

2.1. Sample collection

Corn was grown on four farms located in Ingham, Menominee, Mason, and Saginaw counties all located in Michigan in 2010 and 2011. The latitude and longitude of the research locations within Ingham, Mason, Menominee and Saginaw counties were 42*38′08.31″N and 84*13′32.37″W; 43*57′40.37″N and 86*09′28.92″W; 45*27′01.27″N and 87*35′33.03″W and 43*07′55.66″N and 83*58′04.65″W respectively. Two hybrid pairs, one Bt and one near-isoline relative were analyzed giving a total of 4 hybrids with the specific hybrid pairs grown at each respective location (Table 1). The field plots were managed consistent with the Michigan State University, Corn Hybrid Testing Program [17] and information on the soils present at each research location is shown in Table 2. Soil samples were taken at time of planting and soil conditions were observed and testing was completed by the Michigan State University soil and plant nutrient laboratory. Fertilizers (Nitrogen, Phosphorous and Potassium) were applied in all the four locations at different application rates (Table 3). Hybrids were planted from mid-May to early June and harvested from mid-October to early November depending upon location (Table 3). Previous crop planted in 2010 at Ingham was soybean, carrots at Mason, alfalfa in Menominee, and corn in Saginaw. In 2011 previous crops were soybeans for Ingham and Saginaw locations and corn for Mason and Menominee. At each location, the entire corn plants in the two 1 m sections taken from the center two rows of a 4 row plot for each selected hybrid were hand harvested, cut approximately 15 cm above ground level, bagged, and labeled by individual plot. Stover and cob fractions were dried at 65 °C with forced air and ground to 1 mm and grain was air dried at room temperature. The experiment was replicated four times for every treatment at each location. The ears from the plants were removed and the grain from the cob was shelled. Wet and dry weights were recorded from all three fractions for each plot (stover, cob, and grain). The grain was discarded after recording dry matter yields. Stover and cob fractions were all ground through a 1 mm sieve and stored in labeled quart size zip-lock bags. The dried ground samples were stored at 4 °C previous to analytical processing. The total number of stover biomass samples collected for this study was 104 combining both the 2010 and 2011 years.

2.2. Composition analysis

Composition of the untreated corn stover samples was determined by following the procedures developed by the National Renewable Energy Laboratory (NREL) for determination of extractives [18] and structural carbohydrates [19]. Analysis was performed in triplicate for each sample. Ground sample (1 g) was used for total oil extraction by accelerated solvent extractor ASE 200 (Dionex, Idstein, Germany) equipped with an 11 ml stainless steel extractor cell. The following conditions were set on the ASE system: preheat for 6 min, heat for 6 min, oven temperature at 105 °C, static time for 10 min, flush volume 70%, and purge time for 60 S, 2 static cycles, and extraction pressure at 1000psi. After process, extraction solvent hexane was evaporated by purging oxygen free compressed nitrogen (AGA gas) above the surface. Moisture content analysis was performed to determine the dry weight of the corn stover using an A & D Moisture analyzer, MX-50 (Milpita, CA). Polysaccharide content assays were run in triplicates in 100 ml autoclavable high-pressure tube (Chemglass Life Science Inc., Vineland, NJ). Extracted corn stover (0.3 g) and 37 ml 72% (w/w) sulfuric acid were added in to the tube and after continuous shaking for 1 h in a shaker at 32 °C, 84 ml distilled water was added and then the tubes were autoclaved at 121 °C for 1 h. Filtered hydrolyzate samples were run on HPLC to analyze the sugars (as described in the HPLC analysis section of this paper) along with the sugar recovery standards (For 100 mL solution, 0.4 g glucose, 0.2 g xylose, and 0.08 g arabinose is added). All samples were analyzed in triplicate to ensure the quality of the composition levels.

Lignocellulosic isolate from each sample was weighed out into

Table 1
Hybrid name, Bt traits, location, and year grown, for the corn stover analyzed in the experiment.

Isoline	RM[e]	Bt trait	Location (County, MI)	
			2010	2011
Nutech 2A 804	104	No	Ingham, Saginaw, Menominee	Ingham, Saginaw, Mason, Menominee
Nutech 5N 804 (GT[a]/CB[b]/LL[c]/RW[d])	104	Cry1AB, mCRY3	Ingham, Saginaw, Menominee	Ingham, Saginaw, Mason, Menominee
Bayside 4090	90	No	Mason, Menominee	Ingham, Saginaw, Mason, Menominee
Bayside 3090 (GT[a]/CB[b]/LL[c])	90	Cry1AB	Mason, Menominee	Ingham, Saginaw, Mason, Menominee

RM: Relative Maturity.
[a] GT: Glyphosate Tolerant.
[b] CB: Corn Borer control.
[c] LL: Liberty Link (Glufosinate tolerance).
[d] RW: Root Worm control.