Bioresource Technology 183 (2015) 70–77

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Effects of genetic variation and growing condition of prairie cordgrass on feedstock composition and ethanol yield

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HIGHLIGHTS

- Compositions showed significant differences within and between years.
- Ethanol yields of 1379–3446 kg/ha were achieved by *S. cerevisiae* SR8.
- IL-102 a high biomass yield cultivar, produced the highest ethanol yields (kg/ha).
- Ethanol yields (kg/ha) had strong correlation to biomass yields.
- Ethanol yields of prairie cordgrass were comparable to other feedstocks.

ARTICLE INFO

Article history:
Received 13 November 2014
Received in revised form 6 February 2015
Accepted 7 February 2015
Available online 13 February 2015

Keywords:
Prairie cordgrass
Genetic variation
Dilute acid pretreatment
Engineered yeast
Ethanol yields

ABSTRACT

Prairie cordgrass (*Spartina pectinata* L.) has the potential to be a feedstock for bioethanol. It is native to North America, and has extensive genetic diversity. Eleven natural populations of prairie cordgrass harvested in 2011 and 2012 were studied. Compositions of the samples showed significant differences within the same year, and between the two years. Two highest, one medium and two lowest glucan concentration samples from each year were selected to evaluate ethanol yield after dilute acid pretreatment and simultaneous saccharification and co-fermentation using *Saccharomyces cerevisiae* SR8 that can ferment both glucose and xylose. Up to 88% of theoretical ethanol yields were achieved. Our research demonstrates the potential of prairie cordgrass as a dedicated energy crop with ethanol yields of 205.0–275.6 g/kg biomass and 1748–4368 L/ha, depending on feedstock composition and biomass yield. These ethanol yields are comparable with those of switchgrass, corn stover and bagasse.

1. Introduction

Petroleum is the largest energy source in the United States, representing about 32% of total energy consumption in 2012 (EIA, 2014). About 40% of petroleum and other liquid fuels consumed in the U.S. are imported (EIA, 2014). In order to reduce petroleum and other liquid fuel dependency from abroad, biofuels are receiving attention from researchers as an alternative energy source. Considering that 93% of the petroleum consumption is used as transportation fuel (EIA, 2014), liquid forms of biofuels, such as bioethanol, have the potential to increase energy security. The Renewable Fuel Standard (RFS-2) mandates annual production of 15 and 21 billion gallons of corn ethanol and advanced biofuels, respectively, by 2022. Advanced biofuels can be further categorized as cellulosic biofuel, biomass-based diesel or undifferentiated advanced biofuel and the annual production levels of which were required to be 16, 1 and 4 billion gallons, respectively, by 2022. Corn ethanol production is close to meeting the RFS-2 mandate. In 2013, 192 corn ethanol plants were operating in the United States with annual capacity of 15.0 billion gallons with an actual corn ethanol production of 13.3 billion gallons (RFA, 2014). However, cellulosic biofuel and undifferentiated advanced biofuel production are behind schedule. Several challenges exist in cellulosic ethanol production, including feedstock supply, pretreatment and fermentation efficiency.

The U.S. Department of Agriculture (USDA) and U.S. Department of Energy (USDOE) concluded that various types of feedstock are required to meet annual production of 21 billion gallons of advanced biofuels by 2022, as mandated by the RFS-2

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http://dx.doi.org/10.1016/j.biortech.2015.02.020
0960-8524/

(USDA, 2010; USDOE, 2011). Perennial grasses have the potential to be a cellulosic biofuel feedstock along with crop residues, forestry residues and woody crops because they can grow on marginal lands, improve soil/water quality and reduce greenhouse gases (Searchinger et al., 2009). Much research has been focused on perennial grasses such as switchgrass (*Panicum virgatum* L.) and *Miscanthus x. giganteus* (Heaton et al., 2008; Schmer et al., 2008; Somerville et al., 2010). Switchgrass was identified as a model energy crop by the USDOE due to its high biomass yields and its adaptability to a wide range of soil types and climate conditions. *Miscanthus x. giganteus* is also a good candidate for cellulosic ethanol feedstock due to its high biomass yield. Recently, prairie cordgrass (*Spartina pectinata* L.) (PCG) has gained attention as a biofuel feedstock (Cybulska et al., 2010). As the most northerly distributed C4 grass, it is native to North America and features strong rhizome and root systems (Boe et al., 2009). PCG has high flood and salt tolerance, which makes it grow well on marginal lands with poorly drained or salt-affected soils that are not suitable for other grasses and food crops (Boe et al., 2009). Also, PCG can accumulate biomass for extended growing season with its cold tolerance (Long, 1983). In some cases, biomass yield of PCG is comparable to that of switchgrass (Boe and Lee, 2007; Madakadze et al., 1998). Boe and Lee (2007) compared biomass production of PCG with switchgrass, and observed that biomass production of PCG was similar to that of switchgrass during the first two years. In the third and fourth years, PCG produced a greater amount of biomass than switchgrass with a considerable variation among different populations.

Improving fermentation efficiency is one of the challenges in cellulosic ethanol production. Conventional yeast *Saccharomyces cerevisiae* cannot utilize C5 sugars, which compose one third of biomass (Wyman, 2007). In order to increase ethanol yields and achieve high-efficiency utilization of sugars in biomass, research has been focused on engineered yeasts that can consume both C5 and C6 sugars. Introduction of pentose-utilization pathways of xylose reductase (XYL1) and xylitol dehydrogenase (XYL2) from *Scheffersomyces stipitis* or other fungi enable xylose fermentation (Ho et al., 1998; Walfridsson et al., 1997). Overexpression of endogenous xylulokinase (XKS) with XYL1 and XYL2 enhances the specific rate of xylose utilization (Toivari et al., 2001). Conversion of D-xylose into D-xylulose by xylose isomerase from bacteria is also a possible pathway to convert xylose into ethanol (van Maris et al., 2007).

There have been no studies conducted on ethanol production from PCG using engineered yeast that can convert both C5 and C6 sugars. In this study, PCG was investigated as a feedstock, and engineered yeast was used to ferment sugars into ethanol. The effects of genetic variation in eleven natural populations and growing conditions (2011 and 2012 years) on feedstock compositions and ethanol yields were evaluated. Also, compositions, biomass yields and ethanol yields from PCGs and other cellulosic ethanol feedstocks were compared.

2. Methods

2.1. Sample selection

Eleven natural populations of PCG planted in a field nursery at the Energy Biosciences Institute Energy Farm in Urbana, IL (40° 6′ N, 88° 13′ W) and biomass was harvested at the end of growing season in the 2011 and 2012. Greenhouse-grown seedlings of PCG were transplanted to the farm during the spring of 2010, and were arranged in a randomized complete block design with four field replications. Populations (origin) used in this study are 9046805 (Plant Material Center of USDA-NRCS), IL-102 (Illinois), ND (North Dakota), PC09-101 (Connecticut), PC17-116 (Illinois), PC29-106 (Missouri), PC40-104 (Oklahoma), PC55-102 (Wisconsin), PCG-109 (South Dakota), PC46-105 (South Dakota), and RR (Plant Material Center of USDA-NRCS). Harvested samples were required air-drying until the moisture concentration in the samples was less than 5%. Samples were then ground by Heavy-Duty Cutting Mill (SM 2000, Retsch, Germany) using 1 mm sieve size. Ground samples were stored in sealed containers at 4 °C for further experiments.

2.2. Composition analysis of post-harvested and pretreated prairie cordgrass

Compositions of post-harvested PCGs were analyzed to determine theoretical ethanol yields. Composition analysis of post-harvested PCGs was performed following NREL protocols (website: http://www.nrel.gov/biomass/analytical_procedures.html, accessed September 2014). Water and ethanol soluble extractives were first removed from the samples via a Soxhlet method adapted from "Determination of extractives in biomass (NREL/TP-510-42619)" to prevent any interference for further analysis. Compositions of extractives-free samples were analyzed by a two-step acid hydrolysis procedure described in "Determination of structural carbohydrates and lignin in biomass (NREL/TP-510-42618)." After the two-step hydrolysis, the sample was filtered. Filtered solids were analyzed for acid-insoluble lignin (AISL) and ash concentrations by drying at 105 °C for 24 h followed by drying at 575 °C for 4 h. Filtrate was analyzed for acid-soluble lignin (ASL) by recording absorbance at 220 nm, and for sugar concentrations by HPLC (Aminex HPX-87P, Bio-Rad, Hercules, CA).

Composition analysis of pretreated sample was performed for liquid phase (soluble solids) and solid phase (insoluble solids). Monomeric sugars and inhibitor concentrations in the soluble solids were analyzed by HPLC (Aminex HPX-87H, Bio-Rad, Hercules, CA). Total sugar concentrations (monomeric and oligomeric sugars) in the soluble solids were measured by HPLC (Aminex HPX-87P) after acid hydrolysis as described in the NREL protocol, "Determination of sugars, byproducts and degradation products in liquid fraction process samples (NREL/TP-510-42623)." To obtain oligomeric sugar concentration, monomeric sugar concentration was subtracted from the measured total sugar concentration. Sugar recovery yield, which is the ratio of the amount of monomeric or oligomeric sugars released into the liquid to the corresponding carbohydrate concentrations in original samples, was calculated. Water-insoluble solids were prepared by washing the solids, adapted from the NREL protocol, "Determination of insoluble solids of pretreated biomass material (NREL/TP-510-42627)." The washing step was repeated until pH of samples reached 5–7. Compositions of water-insoluble solids were determined by the two-step acid hydrolysis method as described above.

The prepared samples to analyze carbohydrates concentrations were filtered through a 0.2 μm syringe filter into 200 μL HPLC vials. Filtered liquid was injected into ion exclusion column HPX-87H or HPX-87P. The HPX-87H column uses 5 mM sulfuric acid as eluent and operates at 50 °C, while the HPX-87P column uses water as eluent and operates at 85 °C. Carbohydrates were measured with a refractive index detector (model 2414, Waters Corporation, Milford, MA). HPLC detection limits of sugar are 0.001% (w/v).

2.3. Pretreatment

According to the PCG composition analysis, two samples of the highest glucan concentration, two samples of the lowest glucan concentration and one sample of similar glucan concentration over the two years (2011 and 2012) were chosen for pretreatment and simultaneous saccharification and co-fermentation (SSCF). Among 2011 samples, IL-102 and PC29-106 were chosen for the highest