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Effects of fertilizer application and dry/wet processing of Miscanthus x giganteus on bioethanol production

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HIGHLIGHTS

- Effects of wet/dry processing of MxG on ethanol production were studied.
- 88% theoretical ethanol yield was the highest observed for the SSF process.
- Wet samples did better than dry samples for ethanol production.
- Swine manure treated MxG produced higher ethanol yields and concentrations.

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ABSTRACT

The effects of wet and dry processing of miscanthus on bioethanol production using simultaneous saccharification and fermentation (SSF) process were investigated, with wet samples showing higher ethanol yields than dry samples. Miscanthus grown with no fertilizer, with fertilizer and with swine manure were sampled for analysis. Wet-fractionation was used to separate miscanthus into solid and liquid fractions. Dilute sulfuric acid pretreatment was employed and the SSF process was performed with *saccharomyces cerevisiae* and a cocktail of enzymes at 35 °C. After pretreatment, cellulose compositions of biomass of the wet samples increased from 61.0–67.0% to 77.0–87.0%, which were higher than the compositions of dry samples. The highest theoretical ethanol yield of 88.0% was realized for wet processed pretreated miscanthus, grown with swine manure. Changes to the morphology and chemical composition of the biomass samples after pretreatment, such as crystallinity reduction, were observed using SEM and FTIR. These changes improved ethanol production.

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1. Introduction

Fuel ethanol is a major product from the biorefining process, and has been produced commercially in several countries in the world for more than two decades as an alternative fuel (Nghiem et al., 2011). Even though nowadays corn and sugar cane are still the most widely used feedstocks for commercial production of ethanol, lignocellulosic biomass feedstocks (e.g. Miscanthus x giganteus) are considered the ultimate feedstocks for ethanol production (Nghiem et al., 2011). Factors such as high yields of dry matter and the ability to grow under diverse climates and on marginal lands, are some of the features of giant miscanthus that have been touted as making the plant ideal for bioenergy purposes (Kärcher et al., 2015). However, giant miscanthus for bioenergy is a relatively new crop and a new subject of research in the United States. As such, it will take some time to determine how it will react to fertilization on various soil types. Nevertheless, some studies have already shown that perennial miscanthus requires relatively fewer fertilizer inputs to sustain growth compared to other annual C_4 grass crops (Christian et al., 2008).

The economic competitiveness of the ethanol production process depends strongly on the amount of heat and power used (Pfeffer et al., 2007). Both starch-based and carbohydrate materials, such as sorghum grain, corn, and municipal solid waste (MSW) normally require the use of an external energy source in the conventional ethanol production process (Ferrari et al., 2013). However, an energy balance can only be considered favorable if

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the energy needed to produce a biofuel unit is lower than the energy exiting the system (Ferrari et al., 2013). It is imperative to evaluate what kind of energy is being used and also minimize energy usage to make the process more energy efficient.

An important step in the bioethanol production process is sample preparation. Of first and second-generation feedstock that has high moisture content, drying of the materials before fermentation is a conventional practice in the industry. Fennel and Boldor (2014) identified increased transportation costs and biomass losses during long-term storage as some of the reasons for drying the biomass before fermentation. Even though efficiencies of fermentation processes are already fairly high, it is important to improve the energy efficiency of the ethanol production processes within the context of a biorefinery platform by developing innovations that allow for the use of lignocellulosic biomass materials that do not have to undergo physical/thermal processing such as drying. It is important to note that second-generation ethanol production has not yet reached commercial maturity and requires the investigation of different process configurations to develop efficient conversion processes to speed up commercialization (Silva Ortiz and de Oliveira, 2014).

Moreover, for commercialization purposes, it is important to also consider the issue of water balance in the ethanol production process, as distillation (dewatering) is also a high-energy consumption step in the process, with energy consumption increasing as the water to be removed increases, which can be due to high moisture content in the feedstock (Ferrari et al., 2013). One way to attain energy savings on distillation is to achieve high ethanol concentrations from the fermentation process, such as high gravity fermentation, where the initial fermentable sugar concentration is high (Bai et al., 2008). Therefore, there is a need to develop an innovative approach to improve the energy efficiency of the process and maximize ethanol yield.

Bioethanol production based on dry processing of biomass has been addressed by a number of researchers. Fennell and Boldor (2014) examined continuous microwave drying of sweet sorghum bagasse biomass for ethanol production, and found that the drying rate for microwave drying was significantly higher than conventional drying (Fennell and Boldor, 2014). An energy evaluation of drying sweet potato for ethanol production has also been performed (Ferrari et al., 2013). This study concluded generally, that the energy consumption was greater than the energetic content of the bioethanol produced when drying sweet potato biomass was involved in the process (Ferrari et al., 2013). Scordia et al. (2013) dried giant miscanthus (65 °C ± 5 °C) and milled it to a particle size smaller than 2 cm for their assessment on the effect of oxalic acid pretreatment of miscanthus biomass for ethanol production. This was done even though the moisture content at the time of harvest was only 15% (Scordia et al., 2013). Similarly, wheat straw (Saha et al., 2015), miscanthus (Cha et al., 2015), rapeseed straw (Choi et al., 2013) and elephant grass (Eliana et al., 2014), have all been subjects of recent studies which examined drying at various temperatures, milling and using for the production of ethanol through a simultaneous saccharification and fermentation process.

Although quite a number of studies have been conducted on dry processing of biomass for ethanol production using miscanthus as a feedstock, bioethanol production from wet miscanthus has not been examined thus far. Furthermore, these studies did not take into account how fertilizer (organic/inorganic) application to soils during miscanthus cultivation affected ethanol yields and concentrations. Therefore, the overall goal of this research was to evaluate the feasibility of ethanol production from miscanthus grown with different fertilizer treatments via wet processing. A comparison of ethanol yields and concentrations from dry and wet processed biomass will help to determine the effectiveness of the wet processing of miscanthus in bioethanol production. The ability to produce a high concentration of ethanol and a high theoretical ethanol yield, without drying the biomass will undoubtedly improve the energy efficiency of the process, a desirable outcome for commercial considerations.

2. Methods

2.1. Materials

Miscanthus was harvested from the North Carolina A & T State University Farm in Greensboro during July, 2015, using a Tanaka TPH 270s-pole hedge trimmer for consistent cuts. Miscanthus grown with two fertilizer treatments of NPK 17-17-17 (T1: 0 lbs/ac and T5: 280 lbs/ac) and swine manure (Tsw: 1000 lbs/ac) were used for the study. Swine manure is applied on agricultural soils as fertilizer, since it contributes to increased organic matter (OM) in soil and is active in the provision of plant nutrients (Segat et al., 2015). Commercial enzymes, cellulase (Novozymes, NS 50013), β-glucosidase (Novozymes, NS 50010), and hemicellulase (Novozymes, NS 22002) were supplied by Novozymes North America Inc. (Franklinton, North Carolina) and were used as soon as they were received. *Saccharomyces cerevisiae* (ATCC 24858) was purchased from American Type Culture Collection (ATCC) (Manassas, VA) for fermentation processes.

2.2. Sample preparation

Freshly harvested miscanthus was shredded using a DR wood chipper/shredder (14.50 Pro Manual Start, DR Power Equipment, Vergennes, Vermont), bagged, sealed and stored at 4 °C. Harvested biomass was pressed and separated into juice and solid cake, using a Carver laboratory press (#2094 cage equipment, Carver Inc., Wabash, IN) at an optimized force of 30,000 lbs for 15 min. The green juice was stored in a freezer for use in further downstream processes as part of a biorefinery platform. The pressed solid cake was divided into two portions; one taken through a dry processing method and the second, a wet processing method.

2.2.1. Dry processing method

The miscanthus biomass was dried in an isotemp oven (Fisher Scientific, USA) at a temperature of 105 °C for a period of 24 h, thereby completely removing any moisture from the solid cake. Using a rotary knife mill (Thomas Model 4 Wiley mill, Thomas Scientific, Swedesboro, NJ) the dried miscanthus was ground to particle sizes between 0.3 and 0.6 mm for further analyses and downstream processing.

2.2.2. Wet processing method

100 g of deionized water was added to 50 g of the pressed miscanthus solid cake and thoroughly mixed together. This 2:1 (water: miscanthus) cake was then homogenized using a knife mill Grindomix GM 200 (Retsch®, Verder Scientific Inc. Newtown, PA) at a speed of 9000 RPM for 2 min. Subsequently, the slurry was separated into solid and liquid fractions using a centrifuge (Centra-GP8R Centrifuge, ThermoIEC) at a rotational speed of 2600 RCF for 10 min at room temperature. The solid cake was stored in sealable containers at −4 °C for further analysis and downstream processing.

2.3. Biomass analytical procedures

Compositional analysis of the biomass was done using the laboratory analytical procedures (LAPs) of the National Renewable Energy Laboratory (NREL). The moisture content of the biomass

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