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Steam explosion and fermentation of sugar beets from Southern Florida and the Midwestern United States☆

Christina Dorado[a,*], Randall G. Cameron[a], Kevin Cooper[b]

[a] U.S. Department of Agriculture, Agricultural Research Service, U.S. Horticultural Research Laboratory, Ft. Pierce, FL 34945, United States
[b] Advanced Technology, Indian River State College, 3209 Virginia Ave., Ft. Pierce, FL 34981, United States

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ABSTRACT

Sugar beets have recently gained interest for cultivation in southern Florida for their economic potential as cattle feed, a feedstock for ethanol production and their use to improve the quality of water via soil nutrient accumulation. Sugar beets grown in southern Florida, Minnesota and Nebraska were subjected to steam explosion. Analysis of soluble and insoluble sugars as well as pectin from raw and steam exploded sugar beet were completed. Fermentations of raw and steam exploded sugar beets, with and without enzymes, were conducted. There was no significant difference for ethanol production in the fermentation of steam exploded sugar beets with and without enzymes indicating that addition of enzymes are not necessary for fermentation. Pilot scale fermentations of the steam exploded sugar beet gave 6.7–9.7% ethanol by volume. Raw, milled sugar beet were limed and pressed. The press cake and press liquor were analyzed for dry weight and sugar content.

1. Introduction

Based on the 2015 State Agriculture Overview for Florida, oranges had the most harvested acres among agricultural crops in the state followed by sugarcane (USDA-NAS, 2015). Sugarcane production in Florida was initiated when the U.S. ceased importation of the crop from Cuba in 1960 and is now the largest sugarcane producing region in the U.S. (USDA-ERS, 2016). Another major sugar crop grown in the U.S. is the sugar beet (USDA-ERS, 2016). While sugar beet production is concentrated mostly in the northern states of Oregon, Washington, Idaho, Montana, Wyoming, Colorado, Nebraska, North Dakota, Minnesota, and Michigan it can be also found as far south as Brawley, California (Sugar Produced Magazine, 2015). Sugar beets have been grown primarily in the Midwest for the production of table sugar and have recently gained interest for planting and harvesting in other areas of the U.S. for use as cattle feed (American Famer, 2015; Lardy, 2016; Rust and Buskirk, 2016), ethanol production (Panella, 2010; Akbas and Stark, 2016; Westbury, 2016; Cox, 2016) and phytoremediation (Cox, 2016; Wiszniewska et al., 2016; Liu et al., 2015; Kohler et al., 2014; Sun et al., 2007). Recently scientists at the University of Florida's Everglades Research & Education Center have embarked on research involving the cultivation of sugar beets in Florida (Westbury, 2016). One possible use for sugar beets in Florida would be to combat the algae blooms that plague the Treasure Coast of eastern Florida during the hot summer months and are suspected to be partly caused by run off from agricultural land (Lindsay, 2016). Sugar beets could serve as a barrier crop to reduce the amount of nutrient run-off that ends up in the waterways (Cox, 2016) and thus prevent the proliferation of algae blooms. Another possible use for sugar beets in Florida could be as cattle feed. Florida registered a total of 1.69 million cattle and calves for 2016 (USDA-NAS, 2016a) with $410 million spent on animal feed in Florida in 2015 (USDA-NAS, 2016b). Supplementing cattle feed with sugar beets can reduce the overall input cost to the farmer as sugar beets can be produced with less water and produce more dry matter tons per acre than corn (American Famer, 2015). While there is some production of sugar beet in Florida for use as cattle feed, sugar beets are also being considered as a possible feedstock for ethanol production and for extending the use of sugar mills past the end of the sugar cane harvesting season (Salisbury, 2015). There has also been work on utilizing the waste from sugar beets for the production of methane to off-set the energy use for sugar extraction (Pullammanappallil, 2016; Bevill, 2008). The sugar industry generates $20 billion a year in economic activity across the U.S. alone (ASA, 2016) and the use of sugar for the production of ethanol in the state of Florida is economically enticing considering that in 2013, which is the most recent data available, Florida consumed 18.9 million barrels of ethanol and

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* Corresponding author.
E-mail address: christina.dorado@ars.usda.gov (C. Dorado).

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produced none (USEIA, 2014). With an already established infrastructure for sugar cane milling (ASA, 2016) and ethanol production from agricultural crops and residues (Ethanol Producer Magazine, 2015) pursuing sugar beets as an energy crop could be economically favorable.

Another potential value added product from sugar beets is pectin. Pectin is a complex polysaccharide composed largely of galacturonic acid (GalA) (Cameron et al., 2008). It contains two major regions, a homogalacturonan region (HG), a linear polymer of α-(1,4) linked GalA, and a rhamnogalacturonan region (RG I) which is a linear copolymer with a repeating dimer of GalAα-(1 → 2) Rha (Coenen et al., 2007). Sugar beet pectin has a high amount of acetyl esterification at O2 or O3 of GalA in the HG region or O3 in RG I (Remoroza et al., 2014). These acetyl esters prevent sugar beet pectin from gelling which sets it apart from pectin derived from other sources such as citrus pectin (Williams et al., 2005). Sugar beet pectin also has a higher proportion of RG I regions and phenolic esters (ferulic acid) attached to galactose and arabinose in RG I side chains (Guillon et al., 1989). Sugar beet pectin has been reported to have emulsifying and anti-cancer activities (Maxwell et al., 2016; Schmidt et al., 2015; Drusch, 2007) which are not dependent on maintaining high molecular weight. In fact, depolymerized pectin was reported to have better emulsifying properties than higher molecular weight pectins (Leroux et al., 2003). The unique structure and properties of sugar beet pectin can be exploited for use in pharmaceuticals, nutraceuticals, cosmetics and functional foods making it a desirable value added product from sugar beets.

The U.S. Horticultural Research Laboratory located in Ft. Pierce, FL has conducted research utilizing a continuous pilot scale steam explosion system for the fragmentation of citrus processing waste for the extraction of valuable components, such as pectic hydrocolloids and phenolic compounds, and for fermentation to ethanol (Widmer et al., 2011, 2010; Stewart et al., 2013; Zhou et al., 2008; Grohman et al., 2013; Cameron et al., 2016, 2017). Recently, this pilot scale system was utilized for fragmentation followed by fermentation of sugar beets that were grown and harvested in southern Florida, Minnesota and Nebraska for an initial investigation of sugar beets for the production of ethanol, pectin and cattle feed. Here we discuss the experiments leading up to the pilot scale steam explosion of sugar beets followed by fermentation including a preliminary experiment that utilized a static steam explosion system. Analysis of the steam exploded sugar beets included isolation and characterization of pectic materials and quantification and qualification of sugars. Various conditions for fermentation were tested using the steam exploded sugar beet including enzyme type and loading for optimization of ethanol yields. The ethanol yield from steam exploded material was also compared to that which was produced from physically homogenized and enzymatically hydrolyzed material. Milled and steam exploded sugar beets were also subjected to conventional liming and pressing procedures to determine if this could serve as a method for increased sugar isolation from the sugar beets and for the production of cattle feed.

2. Materials and methods

2.1. Materials

Florida sugar beets (SB) were obtained from a farm located at the University of Florida Everglade's Research and Education Center in Belle Glade, FL and from farms in Clewiston, FL and were of the variety BTS AC221 (BetaSeed, Bloomington, MN). Minnesota SB were obtained from a farm in Moorhead, MN and were of the Red River Commercial Variety (BetaSeed, Bloomington, MN). Nebraksa SB were from a farm within 60 miles of Scottsbluff, Nebraska. SB were submerged in an initial water bath and brushed to remove excess soil and were then rinsed in a second clean water bath and allowed to air dry. Clean and dry SB were stored at 4 °C until the next day. SB were removed from cold storage and cut by hand to approximately 3 cm cubes and then further size reduced using a Fitz-Mill Model D-S6 (W. J. Fitzpatrick Company, Chicago, U.S.A.). Size reduced SB were stored in sealed plastic 5 gallon buckets at 4 °C until they were ready to use.

2.2. Static steam treatment of SB

Size reduced SB was subjected to steam explosion using a static bench scale system (Widmer et al., 2010; Grohman et al., 2013). Approximately 600 g of size reduced SB was placed in the vertical pipe reactor. The SB was exposed to steam at approximately 50 psi (≈ 150 °C) of pressure for approximately 2 min The pressure was released using a manual pressure relief valve and the fragmented SB was expelled and collected in a plastic bag. The samples in the plastics bags were sealed and then stored at − 20 °C.

2.3. Continuous pilot scale steam treatment of SB

Size reduced SB was subjected to steam explosion using a pilot scale continuous system (Widmer et al., 2011; Stewart et al., 2013). The SB was fed into a hopper and transported to a holding tube using a high solids pump. The SB was exposed to steam at approximately 50 psi (≈ 150 °C) of pressure by a jet cooker of a length designed to allow for 1–3 min of contact time. Temperature and pressure were monitored and maintained using a back pressure relief valve located at the end of the holding tube. The SB was then vented by opening the back pressure relief valve into a flash tank at atmospheric pressure which led to further fragmentation of the SB. The resulting mash was then pumped into re-sealable plastic bags and stored in sealed plastic 5 gallon buckets at − 20 °C.

2.4. Carbohydrate composition of raw and steam treated SB

Carbohydrate composition of SB was determined by hydrolysis of finely ground raw or treated SB in 50 mmol L^{-1} sodium acetate buffer, pH 4.8 using an excess of pectinase (Pectinex Ultra SPL), cellulase (Celluclast 1.5 L) and β-glucosidase (Novozyme 188) enzymes for 24 h at 45 °C. Soluble sugars and polysaccharides (e.g. pectic hydrocolloids) were determined by extracting weighed amounts of homogenized (Polytron homogenizer, ModelPT 10/35, Brinkman Instruments, Switzerland) raw or treated SB with fourfold excess of deionized water and removing insoluble solids by filtration using a 0.45 μm GD/X Nylon syringe filter. Soluble sugars were determined by direct high performance ion exchange chromatography (HPIEC) analysis of the clarified extracts (Widmer, 2011). Soluble polysaccharides were determined by hydrolysis of the same extracts adjusted to 50 mol L^{-1} sodium acetate buffer, pH 4.8 using an excess of pectinase (Pectinase Ultra SPL, 6 μL mL^{-1} of solution) supplemented with cellulase (GC 220, 1 μL mL^{-1} of solution) for 24 h at 45 °C followed by sugar determination using HPIEC (Widmer, 2011). Error estimates for standards used in this method were discussed previously (Grohman et al., 2013). Total dry matter (TDM) contents of samples were determined by drying according to the modified AOAC method 934.01 (Widmer, 2011; AOAC, 1990). Insoluble and soluble solids content was determined by filtration and drying as described (Widmer et al., 2010).

2.5. Bench scale fermentation of raw and steam treated SB

Pilot Scale-A and Pilot Scale-C steam treated sugar beet samples were subjected to bench scale fermentation with and without enzyme. Glucose was purchased from Acros Organics (ThermoFisher Scientific, Waltham, MA, USA). Peptone and yeast extract were purchased from Fisher Scientific (ThermoFisher Scientific, Waltham, MA, USA). *Saccharomyces cerevisiae* was used in the form of Fleischmann's Instant Dry Yeast Hi-Active (Product #2139, Fleishmann Co., St. Louis, MO, USA). Cellic CTec2 (CTec2) and Novozyme 188 (N188) were obtained from Novozymes A/S (Bagsvaerd, Denmark) and the pectinases