Steam Explosion and Fermentation of Sugar Beets from Southern Florida and Minnesota

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ABSTRACT

Sugar beets grown in southern Florida and Minnesota were subjected to steam explosion using a continuous steam explosion pilot scale system. Analyses of soluble and insoluble sugars 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 percent ethanol by volume.

Keywords: sugar beet, ethanol, fermentation, steam explosion, enzyme

1 INTRODUCTION

Sugar beets have been grown primarily in the Midwestern United States for the production of table sugar and have recently gained interest for planting and harvesting in other areas of the U.S. for ethanol production. Ethanol from sugar beets has great economic potential in Florida where 18.9 billion barrels of fuel ethanol were consumed in 2013 but none was produced in the state [1]. Sugar beets, therefore, have the potential to serve as a new source of income to farmers in the state of Florida based on their use as a feedstock for ethanol production. A pilot scale steam explosion system [2,3] was utilized for fragmentation followed by fermentation of sugar beets that were grown and harvested in southern Florida and Minnesota. Here we discuss these experiments including quantification and qualification of sugars. Various conditions for subsequent fermentation were tested including enzyme type and loading for optimization of ethanol yields.

2 EXPERIMENTAL

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). 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 ^{o}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 ^{o}C until they were ready to use.

2.2 Continuous Pilot Scale Steam Treatment of SB

Size reduced SB was subjected to steam explosion using a pilot scale continuous system [2,3]. 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 ^{o}C) of pressure by a jet cooker through a hot holding tube of a length designed to allow for 1-3 minutes 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 ^{o}C.

2.3 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 1.5L) and β-glucosidase (Novozyme 188) enzymes for 24 h at 45 ^{o}C. Soluble sugars and polysaccharides (e.g. pectin) were determined by extracting weighed amounts of homogenized (Polytron homogenizer, ModelPT 10/35, Brinkman Instruments, Switzerland) raw or treated SB with fourfold

Table 2. Soluble Sugars (%dw)[a]

Pretreatment	Raw	Pilot Scale-A	Raw	Pilot Scale-B	Raw	Pilot Scale-C
Rhamnose	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Arabinose	0.00 ±0.00	0.10 ±0.01	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Galactose	0.37 ±0.01	0.26 ±0.01	0.32 ±0.00	0.31 ±0.01	0.30 ±0.02	0.27 ±0.01
Glucose	0.39 ±0.03	15.95 ±0.22	0.83 ±0.01	8.75 ±0.03	0.39 ± 0.04	7.47 ±0.07
Xylose	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Fructose	0.21 ±0.01	16.97 ±0.31	0.89 ±0.01	8.87 ±0.07	0.18 ±0.02	7.04 ±0.07
Sucrose	81.12 ±0.61	50.12 ±2.17	74.62 ±1.87	55.06 ±1.37	65.23 ± 1.96	61.00 ±0.56
Cellobiose	0.25 ±0.08	0.18 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Galacturonic Acid	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00
Total Sugars	82.33 ±0.60	83.57 ±2.69	76.65 ±1.89	72.99 ±1.42	66.10 ±1.98	75.78 ±0.69

[a] Averages and standard deviations calculated from duplicate experiments with duplicate analysis are given

Table 1. Ethanol Production from Raw and Steam Treated Sugar Beets[a]

			Ethanol (% of Dry Weight)[b] Fermentation Time and Conditions			
State/Month	Pretreatment	Dry Weight (wt%)	24 hr	24 hr SSF	48 hr	48 hr SSF
FL/Apr	Raw	17.38 ±0.11	---	45.04	---	49.10
	Pilot Scale-A	16.80 ±0.48	33.19	35.23	32.64	34.70
FL/May	Raw	18.26 ±0.27	ND	38.05[c]	ND	41.34[c]
	Pilot Scale-B	16.03 ±0.11	ND	35.75[c]	ND	36.89[c]
MN/Sep	Raw	21.23 ±0.22	ND	36.80	ND	39.63
	Pilot Scale-C	17.64 ±0.19	35.44	38.28	35.23	38.08

[a] Averages and standard deviations calculated from duplicate experiments with duplicate analysis are given unless otherwise noted

[b] Ethanol as a % of the dry weight was calculated from ethanol (%w/v) and the dry weight (wt%) of the raw or treated sugar beet

[c] Fermentation experiments were conducted on 100 mg samples

ND=No Data

--- Not enough liquid could be collected to create a sample for analysis after fermentation due to insufficient breakdown of sugar beet in the absence of enzymes

excess of deionized water and removing insoluble solids by filtration using a 0.45µm GD/X Nylon syringe filter. Soluble sugars were determined by direct high performance ion exchange chromatography (HPIEC) analysis of the clarified extracts [4]. Soluble sugars 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 ºC followed by sugar determination using HPIEC [4]. Error estimates for standards used in this method were discussed previously [5]. Total dry matter (dw) contents of samples were determined by drying according to the modified AOAC method 934.0139,40. Insoluble and soluble solids content was determined by filtration and drying as described [6].

2.4 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 (SSF) 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 Rapidase PNS (PNS) and Rapidase PAC (PAC) were purchased from DSM Food Specialties (Delft, Netherlands). Streptomycin was purchased from MP Biomedicals (Santa Ana, CA, USA) and Neomycin was purchased from Sigma-Aldrich (St. Louis, MO, USA). For experiments where 50 g of SB were subjected to fermentation, 1.3 mL of a solution of 15:2:2:2:3 (by mass) of DI Water:glucose:peptone:yeast extract:Saccharomyces Cerevisiae and 100 µL of a Neomycin and Streptomycin aqueous solution (5:5:1 Neomycin:Streptomycin:Water by mass) were added to raw or steam treated SB in a 250 mL polypropylene wide mouth bottles closed by a rubber stopper and vented by plastic check valve (pp 1/4–3/8", Bell Air Products, Pequannech, NJ). In simultaneous saccharification and fermentation (SSF) experiments 90µL PAC, 90 µL PNS, 45 µL CTec2 and 45 µL N188 were added. For experiments where 100 g of SB were subjected to fermentation, 1.3 mL of a 10:1:1:1:2 (by mass) solution of DI Water:glucose:peptone:yeast extract:Saccharomyces Cerevisiae and 100 µL of the same Neomycin and Streptomycin aqueous solution described earlier were added to raw or steam treated SB. For SSF experiments 144 µL PAC, 144 µL PNS, 72 µL CTec2 and 72 µL N188 were added. The stoppered bottles were rotated in an incubator at 37 ºC, and sampled after 24 and 48h.

2.5 Pilot Scale Fermenation of Continuous Pilot Scale Steam Treated SB

An initial fermentation was completed at Indian River State College (IRSC) in Fort Pierce, Florida. Ice water was added to the 5 gallon buckets containing re-sealable bags with fresh continuous pilot scale steam treated SB produced from Pilot Scale-B. After sufficient cool down, 45-50 gallons of treated SB were transferred to a 50 gallon stainless steel fermenter along with 135 grams of Alcotec Turbo Yeast Pure 48 (Chesterfield, UK). The yeast and SB solution began to bubble within 30 minutes of adding the yeast. A catch container was placed beneath the fermenter to catch any mash that may have over flowed from the fermenter. Approximately 48-72 hours after initiation of fermentation, the bubbles began to slow down. One bubble per minute was used as an indication that the fermentation was complete. The fermented mash was then drained into a 50 gallon still fitted with a six stage distillation column with 2 heat exchangers, and heated to approximately 180ºF. Distilled ethanol was collected in a stainless steel drum mini keg. As distillation of ethanol began to slow the temperature was increased incrementally not to exceed 211ºF. Once the ethanol stopped flowing the distillation was complete and 5 gallons of 176 proof ethanol as determined by hydrometer measurement were obtained.

A second fermentation was completed in a similar manner at IRSC. After sufficient cool down, 25-30 gallons of treated SB produced from Pilot Scale-C were transferred to a 50 gallon stainless steel fermenter along with 135 grams of Alcotec Turbo Yeast Pure 48 (Chesterfield, UK). The yeast and SB solution began to bubble within 30 minutes of adding the yeast. Approximately 96 hours after initiation of fermentation, the bubbles began to slow down. This fermentation took longer than the previous fermentation because less material was used initially leading to minimized interaction of the yeast and the sugar beet. One bubble per minute was used as an indication that the fermentation was complete. The fermented mash was then drained into a 50 gallon still fitted with a six stage distillation column with 2 heat exchangers, and heated to approximately 180ºF-200ºF. Distilled ethanol was collected in a stainless steel drum mini keg. As distillation of ethanol began to slow the temperature was increased incrementally not to exceed 211ºF. Once the ethanol stopped flowing the distillation was complete and 2 gallons of 180 proof ethanol as determined by hydrometer measurement were obtained.

Table 3. Total Composition (%dw)[a]

Pretreatment	Raw	Pilot Scale-A	Raw	Pilot Scale-B	Raw	Pilot Scale-C
Rhamnose	0.14±0.02	0.21±0.01	0.02±0.02	0.17±0.01	0.00 ±0.00	0.00 ±0.00
Arabinose	5.46±0.77	5.92±0.14	4.32±0.29	5.49±0.15	4.14 ±0.39	4.55 ±0.08
Galactose	1.29±0.00	1.03±0.18	0.57±0.04	0.85±0.02	0.67 ±0.04	0.70 ±0.01
Glucose	42.30±0.21	39.85±1.30	39.20±1.39	39.50±0.63	43.47 ±0.15	42.75 ±0.38
Xylose	0.24±0.04	0.16±0.01	0.11±0.00	0.14±0.01	0.00 ±0.00	0.00 ±0.00
Fructose	37.30±1.02	36.18±0.92	39.14±0.52	36.38±0.46	38.80 ±0.89	34.53 ±1.65
Sucrose	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00 ±0.00	0.00 ±0.00
Cellobiose	0.28±0.19	0.16±0.09	0.27±0.02	0.29±0.02	0.11 ±0.04	0.09 ±0.04
Galacturonic Acid	3.96±0.07	2.28±0.11	3.60±0.18	2.76±0.06	3.31 ±0.16	2.86 ±0.07
Total Sugars	90.98±2.15	85.79±2.55	87.23±0.39	85.57±1.26	90.50 ±0.68	85.48 ±2.03

[a] Averages and standard deviations calculated from duplicate experiments with duplicate analysis are given

Table 4. Comparison of Ethanol Yields for Fermentation of Steam Treated Sugar Beet With and Without Enzyme[a]

	Ethanol (%w/v) Fermentation Time and Conditions						Ethanol (% of Dry Weight)[b] Fermentation Time and Conditions					
	24 hr	24 hr w/ Enzyme	48 hr	48 hr w/ Enzyme	t-test		24 hr	24 hr w/ Enzyme	t-test	48 hr	48 hr w/ Enzyme	t-test
Pilot Scale-A Fermentation-1	5.41 ±0.01	5.93 ±0.01	5.59 ±0.00	5.73 ±0.01	0.08		32.11	35.15	0.01	33.12	34.01	0.07
Pilot Scale-A Fermentation-2	5.73 ±0.00	5.91 ±0.00	5.38 ±0.00	5.92 ±0.00			34.01	35.04		31.92	35.13	
Pilot Scale-C Fermentation-1	6.21 ±0.03	6.80 ±0.00	6.22 ±0.01	6.78 ±0.02			35.21	38.53		35.28	38.45	
Pilot Scale-C Fermentation-2	6.29 ±0.01	6.71 ±0.00	6.21 ±0.00	6.65 ±0.01			35.66	38.03		35.18	37.70	

[a] Fermentation experiments were conducted on 50 mg samples

[b] Ethanol as a % of the dry weight was calculated from ethanol (%w/v) and the dry weight (wt%) of the treated sugar beet

3 RESULTS

In order to determine if the SB could serve as a viable feedstock for ethanol production via fermentation, the raw and stream treated SB was subjected to fermentation and SSF. The amount of ethanol (%w/v) produced after 24 hours and 48 hours of fermentation or SSF are reported in Table 1. The amount of ethanol produced based on the dry weight of the sugar beet was calculated so that the water introduced to the SB by steam treatment would not affect the result (Table 1). The amount of ethanol produced from raw SB compared to steam treated SB was greater in all cases except the 24 hour SSF of Raw SB and Pilot Scale-C SB. This is due to the degradation of fermentable sugars during the steam

treatment process. This is evident in Table 2 where sucrose has been converted to its components, fructose and glucose, for the steam treated SB and in Table 3 where the compositonal sugars show that there is less total sugars in the steam treated SB compared to their Raw counterparts. Steam treatment of the SB inevitably led to the condensation of water in the resulting steam exploded material giving slightly lower dry matter values compared to the corresponding raw values. The ethanol produced (% of dry weight) from the fermentation with (SSF) and without enzyme of two of the pilot scale steam pretreated SB samples were compared and subjected to a t-test (Table 4). The use of enzymes for the SSF of raw and steam treated SB, however, did not significantly affect the yield of ethanol when compared to the fermentation of SB in the absence of enzymes. This indicates that steam explosion alone is an effective tool for the pretreatment of SB for its subsequent fermentation to ethanol. This will also reduce the overall cost of the process since further enzymatic degradation of steam exploded material is not necessary as it does not impact the yields significantly. The fermentation of Pilot Scale-B steam treated SB was capable of producing 8.8-9.7% ethanol by volume and the fermentation of Pilot Scale-C steam treated SB produced 6.7-8.0% ethanol by volume. It was suspected that the reduced ethanol production was attributed to the reduced initial volume of steam exploded SB used for fermentation (25-30 gallons in the latter compared to 45-50 gallons in the former) which led to reduced interaction of the yeast with the pretreated SB due to minimized circulation in the 50 gallon fermentation vessel. In future work we intend to optimize the continuous pilot scale steam explosion process and the pilot scale fermentation process to maximize the yield of ethanol by testing various conditions on both systems.

4 CONCLUSION

Steam treatment of SB via a continuous pilot scale steam explosion system proved to be an effective method for pretreatment of the SB for fermentation. The use of enzymes did not have a significant effect on the fermentation of raw or steam treated SB, making the overall process more cost effective. Pilot scale fermentation of the steam treated SB can produce upto 9.7% ethanol by volume. The results of this work will be helpful to those interested in scaling up the conversion of SB to ethanol in southern Florida.

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6 DISCLAIMER

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