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Dilute-acid pretreatment of distillers' grains and corn fiber

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ABSTRACT

Distillers' grains and corn fiber are the coproducts of the dry grind and wet corn milling industries, respectively. Availability of distillers' grains and corn fiber at the ethanol plant and their high levels of lignocellulosic material make them attractive feedstock for conversion to ethanol. In this study, dilute sulfuric acid hydrolysis for the conversion of distillers' grains and corn fiber to monomeric sugars and the formation of furfural were investigated. The extent of solubilization of biomass beyond monomeric sugars was also monitored. Biomass loadings in the range of 5–20 wt.% at 5% intervals, acid concentrations in the range of 0.5–1.5 vol.% at 0.5% intervals, and temperatures of 120 and 140 °C were studied. The highest yields of monomeric sugars were observed when the least amount of biomass loading was pretreated with the highest concentration of sulfuric acid and when the temperature was 140 °C. For the majority of the cases under consideration, the most effective period of hydrolysis appeared to be during the initial 20–30 min of the reaction. Formation of furfural during the course of hydrolysis was significantly lower at 120 °C and also lower for the distillers' grains samples compared with the corn fiber samples. The total amount of the solubilized matter during the hydrolysis was significantly higher than the amount of the monomeric sugars. Analyses according to standard procedure were performed to quantify moisture, oil, carbohydrates, and ash in distillers' grains and corn fiber samples. The total carbohydrate content of distillers' grains and corn fiber were 57.7 ± 2.0 and 77.0 ± 1.0 wt.%, respectively. The presented results will provide a foundation for the suitability of the pretreated distillers' grains and corn fiber for enzymatic hydrolysis step.

1. Introduction

One of the more promising alternative fuels to replace or supplement gasoline is ethanol. The US production of ethanol was 9.0 billion gallons in 2008 (Renewable Fuels Association, 2009), nevertheless, more than 95% of the current production of ethanol is produced from corn and other starch rich grains. For the ethanol industry to realize its goal of more than 10 billion gallons production per year, it needs to rely on a more sustainable and inexpensive feedstock. Lignocellulosic biomass resources, such as agricultural residues, food-processing wastes, wood, municipal solid wastes, and wastes from the pulp and paper industry are plentiful and have the potential to be the feedstock for the production of ethanol.

Among the many potential lignocellulosic biomass resources, distillers' grains and corn fiber are of particular interest as they are coproducts of the dry grind and wet corn milling industries, respectively. The availability of distillers' grains and corn fiber at the ethanol plant and their lower value compared with corn make these products attractive feedstocks for conversion to ethanol. On a dry mass basis, distillers' grains and corn fiber make up about 10–12% of the corn kernel with about 50% and 70% lignocellulosic material and unutilized starch in distillers' grains and corn fiber, respectively (Rasco et al., 1987; Saha and Bothast, 1999).

Dilute-acid-catalyzed pretreatment of oligosaccharides from a variety of lignocellulosic substrates has been reported in the past (see e.g., Wayman, 1986; Torget et al., 1991; Zhu et al., 2004; Kim et al., 2001; Um et al., 2003). These studies have resulted in a range of conditions which can readily facilitate the formation of monomeric sugars. A major concern raised in these studies is the degradation of monomeric sugars and formation of product which can inhibit the fermentation of the hydrolyzate. Recent research has explored hot water pretreatment (LHW) and ammonia fiber expansion pretreatment (AFEX) methods prior to the enzymatic hydrolysis of distillers' grains (Ezeji and Blaschek, 2008; Kim et al., 2008a,b; Ladisch et al., 2008). Both pretreatment methods were effective in enhancing the digestibility of the distillers' grains and minimizing the formation of monomeric sugars prior to the enzymatic hydrolysis step. For example, Kim et al. (2008a), have reported 100% of the theoretical ethanol yields for both LHW and AFEX pretreated wet distillers' grains.

Researchers have also explored the potential for the utilization of corn fiber as a lignocellulosic substrate. Weil et al. (1998)

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pretreated corn fiber using liquid water at temperatures between 220 and 260 °C and 5.0–7.0 pH for 50–60 min. Enzymatic hydrolysis of the prepared substrate showed significant improvement compared with untreated corn fiber. Macdonald et al. (1983) reported a pretreatment method using an alkali such as NaOH and corn stover as a model feedstock. Enzymatic hydrolysis of the prepared substrate at 150 °C and 2% NaOH concentration showed significant improvement compared to the untreated corn stover. Moniruzzaman et al. (1997) optimized the conditions necessary for pretreatment of corn fiber using ammonia fiber explosion method. The best results were obtained at 90 °C with ammonia and dry corn fiber ratio of 1:1, and a residence time of 30 min where more than 80% of the theoretical sugar yield was obtained during enzymatic hydrolysis of the pretreated corn fiber.

In this study, dilute sulfuric acid hydrolysis of distillers' grains and corn fiber was investigated. The effects of temperature, solid loading, acid concentration and reaction time were studied. The results provide a range of experimental conditions for the pretreatment of distillers' grains and corn fiber and examine the suitability of these pretreated substrates for enzymatic hydrolysis step. Analyses according to standard procedure were also performed to quantify moisture, oil, carbohydrates (starch, cellulose, hemicellulose, lignin), and ash in distillers' grains and corn fiber samples.

2. Methods

2.1. Materials

The distillers' grains samples were collected from Abengoa Bioenergy (dry grind, York, NE). The distillers' grains samples did not contain the condensed distillers' solubles (syrup). This material will be referred to as DDG in the manuscript. Corn fiber samples were collected from Cargill (wet milling, Blair, NE). The collected samples were kept in a refrigerator (4 °C) before using. Carbohydrates: D(+)Glucose; D(+)Xylose; D(+)Arabinose; D(+)Cellobiose; D(+)Galactose); and furfural were purchased from Sigma Chemical Company (St. Louis, MO). Sulfuric acid (98%) and sodium hydroxide (10 N) were purchased from Fisher Scientific (Pittsburg, PA).

2.2. Equipment

The apparatus used for the pretreatment reactions was a 450 mL bench-top high pressure reactor with a detachable head (Parr 4562, Parr Instrument Company, Moline, IL). A 2.5 in. ID glass liner (Parr 762HC2) was used to prevent corrosion in the reaction vessel. The reactor was equipped with a turbine impeller with a magnetic drive and a 1/12 hp variable speed motor. An electric heating mantle and an internal water cooling loop maintain the desired temperature throughout each run via a PID controller (Parr 4843). One of the reactor valve ports was connected to a 3 hp metering pump (Pulsatron series E plus, Pulsafeeder-Standard Products Operation, Punta Gorda, FL), which provided acid to the reactor bomb. The pump settings provided 30 mL of acid into the bomb in about 20 s.

2.3. Composition determination procedures

Analyses according to standard procedures were performed to determine the compositions of the DDG and corn fiber samples.

2.3.1. Moisture

The NREL procedure described by Sluiter (2005a) was used. A convection oven was used to dry the samples to constant weight. The percent moisture content was based on:

$$\%\ \text{Moisture} = \left(1 - \frac{\text{wt}_{\text{dry sample}}}{\text{wt}_{\text{wet sample}}}\right) \times 100$$

2.3.2. Oil

The Randal/Soxtec/Submersion method (Thiex et al., 2003) was used. Hexane was used for the extraction. The percent oil was based on:

$$\%\ \text{Oil} = \frac{\text{wt}_{\text{extracted oil}}}{\text{wt}_{\text{sample}}} \times 100$$

2.3.3. Starch

The NREL procedure described by Sluiter (2005b) was used. The oil-free samples were completely hydrolyzed using α-amylase and amyloglucosidase. A standard starch sample was also hydrolyzed as reference. The hydrolyzed samples were then centrifuged and filtered before HPLC analysis. The amount of starch hydrolyzed in the standard sample (R) and the starch sample were based on:

$$\%\ R_{\text{starch}} = \frac{\text{conc.}_{\text{glucose in starch standard -measured by HPLC}} \times \text{vol}_{\text{solution}}}{\text{wt}_{\text{starch}}} \times 100$$

$$\%\ \text{Starch} = \frac{\text{conc.}_{\text{glucose in sample - measured by HPLC}} \times \frac{\text{vol}_{\text{solution}}}{\text{wt}_{\text{sample}} \times 1.11}}{\%\ R_{\text{starch}}} \times 100$$

where, "1.11" is the correction factor for hydrolysis of starch into glucose.

2.3.4. Structural carbohydrates

The NREL procedure described by Sluiter (2007) was used. Carbohydrates in the samples were quantified as glucose, xylose, galactose and arabionse. Oil-free and de-starched samples were hydrolyzed with concentrated sulfuric acid. Degradation losses were compensated for with a set of sugar recovery standards (SRS). SRS sugar concentrations were chosen to closely resemble the concentration of sugars in the biomass samples. % R of SRS and carbohydrates were based on:

$$\%\ R_{\text{sugar } i} = \frac{\text{conc.}_{\text{sugar } i \text{ in SRS - after hydrolysis - measured by HPLC}}}{\text{conc.}_{\text{sugar } i \text{ in SRS - before hydrolysis - measurerd by HPLC}}} \times 100$$

$$\%\ \text{Carbohydrate}_{\text{sugar } i} = \frac{\text{conc.}_{\text{sugar } i \text{ in sample as measured by HPLC}} \times \text{CF} \times \text{vol}_{\text{solution}}}{\%\ R_{\text{sugar } i} \times \text{wt}_{\text{sample}}} \times 100$$

where, CF is the anhydrous correction factor for the concentration of polymeric sugars from the corresponding concentration of monomeric sugars. The value of CF was 0.9 for glucose and galactose and 0.88 for xylose and arabinose.

2.3.5. Lignin

The NREL procedure described by Sluiter (2007) was used. The dilute-acid pretreated samples were separated into an insoluble fraction which contained the acid insoluble lignin, and the liquid fraction which contained the acid soluble lignin. The insoluble fraction was ashed and the acid insoluble lignin was based on:

$$\%\ \text{Acid insoluble lignin} = \frac{\text{wt}_{\text{residue}} - \text{wt}_{\text{ash}}}{\text{wt}_{\text{sample}}} \times 100$$

The acid soluble lignin was measured on a UV–Visible spectrophotometer (Genensys™ 10, Thermo Electron Corporation, Milford, MA) at 320 nm using the hydrolysis liquor aliquot. Deionized water was used as background.

$$\%\ \text{Acid soluble lignin} = \frac{\text{Absorbance}_{320\ \text{nm}} \times \text{vol}_{\text{solution}}}{\text{Absorptivity} \times \text{wt}_{\text{sample}}} \times 100$$

Absorptivity was 30 L/g/cm.

2.3.6. Ash

The NREL procedure described by Sluiter (2005c) was used. The amount of ash in the biomass was based on:

$$\% \text{ Ash} = \frac{\text{wt}_{\text{ash}}}{\text{wt}_{\text{sample}}} \times 100$$

2.3.7. Crude protein

Total crude protein content was determined by Dumas method performed on the 'Elementar Rapid N' (Sparks, 1996). This analysis was carried out at the Soil & Plant Analytical Laboratory of the University of Nebraska-Lincoln.

2.4. Pretreatment procedures

Biomass was grinded in a grinder (Mr. Coffee IDS55, Cleveland, OH) and then passed through a 0.5 mm sieve (Ferrero Corp, Chicago, IL). Prior to its use, the grinded biomass was dried in a convection oven at 65 °C. The reactor was then charged with a specific amount of dried and ground biomass and 170 mL of deionized water. The procedure was started by the stirring of the substrate at a constant speed of 500 rpm, while it was heated to the desired temperature. As the desired temperature was reached, 30 mL of sulfuric acid solution was pumped into the reactor. The timing of the reaction started immediately after the complete charge of the acid solution into the reactor. Pretreatment reactions were carried out by varying conditions for temperature (120, 140 °C), biomass loading (5, 10, 15, 20 wt/vol.%, mass of biomass to the total volume of the initial hydrolysis substrate), and sulfuric acid concentration (0.5, 1.0, 1.5 vol.%, volume of 98% acid to the total volume of the dilution water).

2.5. Sampling and analysis

Samples were about 15 mL each and were taken at 0, 5, 10, 15, 20, 30, 45, and 60 min. The initial concentration of sugars in the substrates was taken as the 0 min sample concentration. The sampling port was back flushed with compressed air prior to each sampling to ensue samples to be a uniform representation of the material inside the reactor. To ensure the uniformity of samples compared with the remainder of the material inside of the reactor, a separate experiment was performed and the solid content of the samples were measured. The highest deviation for the solid content of the samples was observed at 20% biomass loading and was less than 0.5 wt.% which confirmed the uniformity of the samples.

The withdrawn samples were immediately placed in an ice-water bath and their pH were adjusted to 7 by drop wise addition of a 10 N solution of sodium hydroxide. Samples were then centrifuged at 10,000 rpm for 15 min (Multi Ventilated 8464, Thermo Electron Corporation, Milford, MA). The separated liquid phase was then passed through a 0.2 μm cellulose acetate syringe filter (Toyo Roshi Kaisha, Japan).

The HPLC analysis quantified the concentration of monomeric sugars (glucose, xylose, galactose, and arabinose) and furfural in the pretreated liquor. A Waters HPLC Alliance system (2695, Waters Corporation, Milford, MA) was used for the chromatography work, and a Waters Empower software was used for the analysis of data. The HPLC was equipped with an ion-exchange column (Aminex® HPX-87P 300 × 8.7 mm, Bio-Rad Laboratories, Hercules, CA), a guard column (Micro-guard de-ashing 30 × 4.6 mm, Bio-Rad Laboratories) and a RI detector (2414, Waters Corporation). The column temperature was maintained at 85 °C and the RI detector was held at 30 °C. Purified deionized water (Simplicity™, Millipore Inc., Burlington, MA) was used as the mobile phase at a flow rate of 0.6 mL/min. Sample volumes were 20 μL. The elution times were about 7–9 min for hemicellulose oligomers and dextrin, 10 min for cellobiose, 11 to 6 min for monomeric sugars (glucose, xylose, galactose, and arabinose) and 43 min for furfural. The total run time for this method was 50 min. Calibration of the HPLC method was carried out for glucose, xylose, galactose, arabinose, cellobiose, and furfural. Experiments for 20 and 30 min of reaction time and experiments for 15.0% of biomass loading and 1% acid concentration were all performed in duplicates to determine the precision and repeatability of the experiments. Results of the quantitative experimental analysis of these replicates yielded a standard deviation of less than 3.5%.

In order to determine the total amount of the solubilized matter in the pretreated liquor, about 2.5 g of the centrifuged liquor was dried in a convection oven (Quincy Lab 20GC, Chicago, IL) at 65 °C.

3. Result and discussion

3.1. Distillers' grains and corn fiber composition

Analyses according to standard procedures were performed to quantify moisture, oil, carbohydrates (starch, cellulose, hemicellulose, lignin), and ash in DDG and corn fiber samples. Results are summarized in Table 1. As is presented in this table, the total carbohydrate content of DDG and corn fiber was 57.7 ± 2.0 and 77.0 ± 1.0 wt.%, respectively. The experimental results were consistent with the previously reported compositions for these materials (Kim et al., 2008c; Grohmann and Bothast, 1997).

3.2. Dilute-acid hydrolysis

Variations in temperature, sulfuric acid concentration, biomass loading, and reaction time were investigated. Based on the initial composition of the substrates (Table 1), yields for the formation of monomeric sugars are presented in Tables 2–5. Formation of furfural (mg/mL) is summarized in Tables 6–9. The experimental results confirmed an increasing trend in the formation of monomeric sugars as a function of time. The highest yields of monomeric sugars were observed at the lower substrate loadings (5 and 10 wt.%), higher concentrations of sulfuric acid (1.0 and 1.5 vol.%) and when the temperature was 140 °C. For most of the cases under consideration, the most effective period for the hydrolysis appeared to be during the initial 30 min of the reaction.

3.2.1. Effect of temperature

Experimental results for the hydrolysis of DDG are presented in Tables 2 and 3. For the case for which the hydrolysis temperature was 120 °C, the formation of sugars started to slow down after the initial 30 min of the reaction, however, continued to increase and the maximum yields were reached at the end of the reaction period (1 h). At the hydrolysis temperature of 140 °C the maximum amount of sugars was recorded during the first 20–30 min of the reaction and further increases in the reaction time resulted in little increase or a decrease in the total amount of the formed sugars. The decreasing trend in the formation of monomeric sugars at 140 °C and acidic condition may be due to the thermal decomposition of monomeric sugars to organic compounds such as formaldehyde, carbon monoxide, furfural (2-furaldehyde), hydroxymethylfufural (5-hydroxymethyl-2-furaldehyde), acetic acid, levulinic acid (4-oxopentanoic acid), lactic acid, and other organic compounds (Larsson et al., 1999). In this study, the formation of furfural was monitored and was assumed to be indicative of the trend in the formation of other organic compounds. The decreasing trend in the formation of monomeric sugars at 140 °C and at reaction times beyond 30 min correlated well with the formation of

Table 1
Composition of DDG and corn fiber.

Components (%, dry basis)	DDG	WS[d]	Corn fiber	DDGS[e]	Corn fiber[f]
Moisture	5.8 ± 0.5	84.2 ± 0.1	5.3 ± 0.1	11.2 ± 0.0	–
Crude oil	9.5 ± 1.9	10.9 ± 0.4	4.5 ± 0.2	11.6 ± 0.1	2.5 ± 0.2
Carbohydrates	57.7 ± 2.0	59.3 ± 1.9	77.0 ± 1.0	53.5	77.5 ± 5.0
Starch[a]	6.2 ± 0.4	13.2 ± 0.3	17.7 ± 0.2		19.7 ± 0.9
Cellulose[b]	17.0 ± 0.3	14.5 ± 0.3	13.0 ± 0.2		17.5 ± 1.0
Hemicellulose[c]	25.8 ± 0.5	23.9 ± 0.9	38.8 ± 0.4		32.5 ± 0.4
Xylose	11.7 ± 0.3	10.7 ± 0.3	20.3 ± 0.4		17.6 ± 1.8
Galactose	2.7 ± 0.1	2.6 ± 0.2	4.4 ± 0.1		3.6 ± 0.3
Arabinose	11.4 ± 0.3	10.7 ± 0.5	14.1 ± 0.1		11.3 ± 1.5
Lignin	8.7 ± 1.8	7.7 ± 0.4	7.5 ± 0.3		7.8 ± 0.7
Crude protein	30.3 ± 0.8	24.0 ± 0.6	9.9 ± 0.5	27.3	11.0 ± 0.5
Ash	1.0 ± 0.1	4.5 ± 0.2	0.6 ± 0.1	4.5	0.6 ± 0.1
Unknown	1.5	1.3	8.0	3.1	8.5[g]

[a] As a source of glucose from residual starch including residual glucose.
[b] As a source of glucose from cellulosic part.
[c] As a source of xylose, galactose, and arabinose.
[d] Whole stillage.
[e] Kim et al. (2008c).
[f] Grohmann and Bothast (1997).
[g] Acetyl groups included.

Table 2
Total yield of monomeric sugars in the dilute-acid pretreatment of DDG at 120 °C [g sugar/100 g carbohydrates].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	5.0	4.6	4.3	4.6	5.7	4.9	4.4	4.7	6.5	5.3	4.6	4.8
5	12.6	9.4	11.4	9.6	16.7	15.0	14.7	10.2	19.8	18.6	17.4	17.2
10	17.3	13.7	14.8	14.1	23.6	20.1	19.1	18.8	28.3	26.7	23.5	22.6
15	19.9	16.1	16.3	15.2	28.5	23.7	22.6	21.9	33.3	30.9	28.4	27.0
20	21.6	17.3	18.0	16.3	32.4	26.0	26.1	24.5	35.2	32.8	30.7	29.9
30	25.7	19.5	20.1	18.4	37.7	31.5	29.6	27.7	38.3	34.7	33.4	32.7
45	30.5	21.3	22.3	20.4	39.8	35.5	32.4	30.6	39.0	38.5	34.2	33.3
60	32.1	23.6	23.8	21.7	43.9	37.2	34.1	32.3	38.8	38.8	34.1	33.9

Table 3
Total yield of monomeric sugars in the dilute-acid pretreatment of DDG at 140 °C [g sugar/100 g carbohydrates].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	11.9	9.9	9.9	8.5	11.7	10.2	10.4	9.2	12.7	10.7	9.9	9.9
5	32.8	23.8	27.9	26.5	42.2	31.5	30.8	27.0	44.3	41.3	39.4	34.9
10	41.4	31.6	37.7	32.5	48.8	41.2	40.1	34.4	47.1	46.0	44.8	42.0
15	49.0	36.6	40.8	35.1	50.7	44.7	42.5	37.5	46.9	46.7	44.9	44.0
20	54.0	40.2	40.9	36.8	51.1	46.8	42.4	39.4	46.9	46.0	45.6	43.3
30	55.9	43.8	42.2	36.7	49.6	46.9	43.2	39.8	46.3	45.1	44.0	43.9
45	57.0	45.3	40.1	35.6	49.0	46.8	41.5	38.7	43.8	42.7	42.8	41.6
60	56.8	45.4	39.0	34.5	47.2	48.3	40.2	36.8	41.1	40.5	40.5	39.5

Table 4
Total yield of monomeric sugars in the dilute-acid pretreatment of corn fiber at 120 °C [g sugar/100 g carbohydrates].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	5.4	5.2	5.1	5.3	5.5	6.2	5.6	5.6	6.5	6.1	6.0	5.8
5	17.8	21.5	17.3	13.2	22.8	21.2	20.0	19.7	26.3	26.3	24.1	23.5
10	21.7	27.6	21.6	21.4	29.9	30.0	29.8	28.8	39.6	38.8	36.6	35.2
15	27.8	29.4	24.8	25.4	37.7	37.0	36.8	35.7	49.9	46.4	44.9	44.0
20	32.4	30.8	26.1	28.1	45.4	41.6	41.3	40.5	53.2	50.1	48.8	47.6
30	41.4	36.5	31.7	31.4	53.6	49.1	48.6	47.0	59.0	54.7	54.5	53.8
45	50.5	44.0	39.1	36.9	59.4	56.0	52.9	52.1	61.1	57.3	56.4	55.4
60	57.2	51.2	43.7	40.8	66.1	60.8	57.4	54.3	61.1	59.8	57.1	56.9

Table 5
Total yield of monomeric sugars in the dilute-acid pretreatment of corn fiber at 140 °C [g sugar/100 g carbohydrates].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	7.1	5.9	5.4	6.3	6.0	5.4	6.6	6.1	8.2	7.5	6.4	6.9
5	42.2	37.6	30.9	27.7	51.9	51.5	45.9	42.1	59.6	56.2	46.6	44.4
10	56.2	51.8	45.4	40.6	62.5	60.8	58.1	49.6	62.2	62.1	58.5	51.4
15	61.0	57.0	55.9	47.6	61.7	63.3	59.8	52.7	63.1	63.0	59.8	53.4
20	60.4	59.4	59.1	53.5	64.2	63.2	60.7	54.0	61.4	62.1	60.2	53.7
30	61.4	60.8	60.4	55.6	63.5	63.5	60.2	55.7	61.9	59.7	59.4	54.3
45	58.7	60.7	61.2	55.4	60.5	61.2	60.2	55.2	57.0	56.5	57.4	52.4
60	56.1	59.6	60.6	55.7	55.9	61.3	60.4	55.7	53.9	53.1	54.6	51.5

Table 6
Furfural production in the dilute-acid pretreatment of DDG at 120 °C [mg/mL].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.1
45	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.0	0.1	0.1	0.1
60	0.0	0.0	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.2

Table 7
Furfural production in the dilute-acid pretreatment of DDG at 140 °C [mg/mL].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.1	0.1	0.3	0.2	0.1
10	0.0	0.1	0.0	0.0	0.1	0.3	0.2	0.3	0.1	0.4	0.4	0.3
15	0.1	0.1	0.1	0.3	0.2	0.6	0.4	0.4	0.4	0.6	0.9	0.6
20	0.1	0.3	0.2	0.5	0.3	0.8	0.6	0.7	0.5	0.8	1.0	1.0
30	0.2	0.4	0.4	0.7	0.4	1.0	1.0	1.1	0.6	1.1	1.5	1.5
45	0.3	0.6	0.8	1.1	0.6	1.2	1.4	2.1	0.8	1.4	2.2	2.4
60	0.5	0.8	1.3	1.6	0.8	1.5	1.9	2.6	1.0	1.6	2.6	3.0

Table 8
Furfural production in the dilute-acid pretreatment of corn fiber at 120 °C [mg/mL].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.1	0.1
15	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.1	0.1
20	0.0	0.0	0.0	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.2	0.2
30	0.0	0.1	0.1	0.2	0.1	0.1	0.2	0.2	0.1	0.2	0.3	0.3
45	0.1	0.1	0.1	0.3	0.1	0.2	0.3	0.3	0.2	0.2	0.4	0.4
60	0.1	0.1	0.2	0.3	0.1	0.2	0.4	0.4	0.2	0.3	0.5	0.5

furfural at this temperature (Table 7). The formation of furfural at 120 °C (Table 6) followed a similar behavior as for 140 °C but was much lower in concentration. The highest amount furfural was recorded at 3.0 mg/mL (Table 7) at 140 °C and 20 wt.% DDG loading. At 120 °C and otherwise identical conditions the furfural formation was measured at 0.15 mg/mL (Table 6). Presence of furfural and other decomposed organic compounds is not desirable in the hydrolyzate products as they are know to be inhibitory to the fermentation of the hydrolyzate to ethanol (Nilvebrant et al., 2003).

Experimental results for the pretreatment of corn fiber are presented in Tables 4, 5, 8 and 9. As these tables reveal, the formation of sugars followed a similar trend to the hydrolysis of DDG. Similarly, a decreasing trend in the formation of sugars was observed at 140 °C which may also be attributed to the formation of furfural and other inhibitory compounds (Table 9). The highest amount furfural was recorded at 3.8 mg/mL (Table 9) at 140 °C and 20% corn fiber loading. At 120 °C and otherwise identical conditions the furfural formation was measured at 0.5 mg/mL (Table 8).

Table 9
Furfural production in the dilute-acid pretreatment of corn fiber at 140 °C [mg/mL].

Acid conc. (vol.%)	0.5				1.0				1.5			
Solid loading (wt.%)	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0	5.0	10.0	15.0	20.0
Reaction time (min)												
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.3	0.2	0.6
10	0.1	0.1	0.1	0.2	0.1	0.2	0.4	0.6	0.3	0.3	0.6	1.0
15	0.1	0.1	0.2	0.5	0.2	0.5	0.6	0.8	0.5	0.8	1.0	1.4
20	0.2	0.2	0.4	1.0	0.4	0.7	0.9	1.2	0.7	1.2	1.3	1.9
30	0.3	0.5	0.8	1.4	0.5	1.1	1.6	1.8	1.0	1.6	1.9	2.5
45	0.5	0.9	1.2	2.0	0.8	1.4	2.4	2.4	1.5	2.1	2.8	3.2
60	0.7	1.2	1.5	2.4	1.2	1.7	2.9	3.1	2.0	2.6	3.5	3.8

3.2.2. Effect of biomass and acid concentrations

Biomass loadings in the range of 5–20 wt.% at 5% intervals and acid loadings in the range of 0.5–1.5 vol.% at 0.5% intervals were examined. Examination of Tables 2–5 confirms an increasing trend in yields as the acid concentration was increased. The effect of acid concentration was more profound when acid concentration was increased from 0.5% to 1.0% compared to a change from 1.0% to 1.5%. For example, on the average yields of the monomeric sugars for the hydrolysis of 15 and 20 wt.% corn fiber loading at 120 °C showed an increase of approximately 10% when the acid concentration was increased from 0.5% to 1.0% (Tables 2 and 4), whereas, the yields increased by an average of about 5% when the acid concentration was increased from 1.0% to 1.5% and under otherwise identical conditions. To a lesser extent, this pattern was also observed at 140 °C but mostly within the initial 20–30 min of the reaction as the yields were fully reached during this period (Table 5). Higher acid concentrations also resulted in faster rate of completion of the reaction at 140 °C. As shown in Table 5, maximum yields were reached after 15–20 min at 1.5% acid concentration compared to 30–45 min at 0.5% acid concentration. On the whole, the acid concentration and temperature may be viewed to maintain parallel effects throughout the hydrolysis reaction. Temperature appeared to be the controlling factor at 140 °C and acid concentration assumes a controlling role at 120 °C. For the hydrolysis of corn fiber at 140 °C, maximum yields were reached early in the reaction (20–30 min) at about 62–64%, while at the lower temperature of 120 °C, comparable yields were only realized at the higher end of the acid concentration rang. At the maximum yield points, however, the formation of furfural were significantly higher at 140 °C compared with the reactions at 120 °C.

Examination of hydrolysis of DDG (Tables 2 and 3) for the effect of acid concentration and solid loading presents a similar behavior as with the corn fiber with two primary exceptions. Unlike corn fiber where a significant difference in the yield of monomeric sugars was observed when acid concentration was increased from 0.5% to 1.0% compared to a change from 1.0% to 1.5%, the DDG samples showed a similar rate increases in both incremental changes in the acid concentration. This may be attributed to a relatively large fraction of starch in corn fiber which is easily hydrolyzed and also the more refractory nature of the lignocellulosic material in DDG which may be due to the higher concentration of protein and oil in this substrate. Similar to the corn fiber samples, the formation of furfural (Tables 6 and 7) correlated well with the biomass loading and was much higher at 140 °C. However, the formation of furfural with DDG was lower than the corn fiber samples and reached a maximum of 3.0 mg/mL (Table 7) and 0.2 mg/mL (Table 6), compared with 3.8 mg/mL (Table 8) and 0.5 mg/mL (Table 9) for corn fiber, at 140 and 120 °C, respectively.

3.2.3. Biomass solubilization

The solubilized material in the liquid fraction of the hydrolyzate contained monomeric and oligomeric carbohydrates, acid soluble lignin, glycerol, oil, soluble proteins and minerals. The presence of noncarbohydrates in the liquid fraction of the hydrolyzate may have a limiting effect on the solubility of monomeric sugars and ultimately on the concentration of the ethanol product. Solubility measurements were carried for all the pretreatment samples shown in Tables 2–5. The trend of change for the solubilization of DDG and corn fiber closely followed the formation of monomeric sugars in the hydrolyzate. A representative sample of the solubility data for DDG is shown in Fig. 1. This figure summarizes the solubility results at four levels of DDG, 1.0% acid concentration, 20 min of reaction time, and at 120 and 140 °C. The trend lines for the formation of monomeric sugars are also included in this figure. In general, the incremental increases in the solubility of DDG appeared to be smaller than the incremental increases in the biomass loading with the trend also getting smaller at higher biomass loadings. For example, at the hydrolysis temperature of 140 °C, when the DDG loading was increased from 5% to 10% the solubility was increased from 42.3 to 69.2 mg/mL of hydrolyzate (63% increase in solubility), whereas, when the loading was increased from 15% to 20% the solubility was increased from 88.6 to 97.6 mg/mL of hydrolyzate (31% increase in solubility). The increase in the formation of monomeric sugars was more consistent with the incremental increases in the DDG loading. This observation suggests some limitations to the solubilization of DDG as the ratio of solid to solvent is increased which appear to be more significant for the total solute than for the monomeric sugar components. Monomeric sugars constituted to about 47% of the total solutes at 20% DDG loading compared to about 33% at 5%. High concentration of oil and protein in DDG may be responsible in limiting the overall solubility of this substrate at larger loading levels.

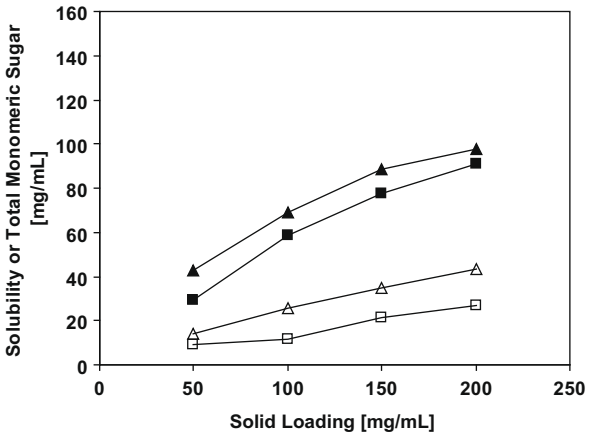

Fig. 1. Solubilization of biomass in the dilute-acid pretreatment of DDG at 1.0% of acid concentration and 20 min of reaction time. (■) Solubility at 120 °C, (▲) solubility at 140 °C, (□) total monomeric sugar at 120 °C, (Δ) total monomeric sugar at 140 °C.

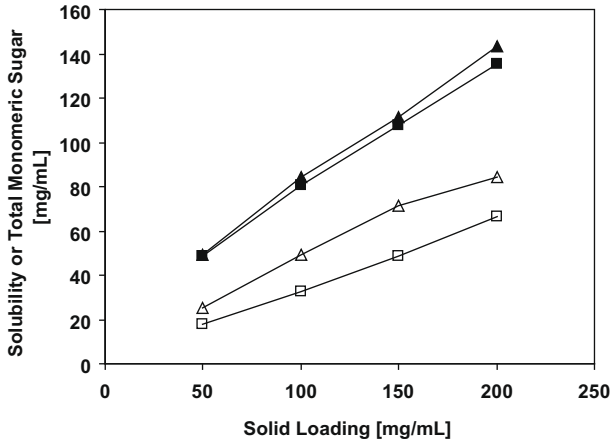

Fig. 2. Solubilization of biomass in the dilute-acid pretreatment of corn fiber at 1.0% of acid concentration and 20 min of reaction time. (■) Solubility at 120 °C, (▲) solubility at 140 °C, (□) total monomeric sugar at 120 °C, (△) total monomeric sugar at 140 °C.

Data for the solubilization of corn fiber at four loading levels, 1.0% acid concentration, 20 min of reaction time, and at 120 and 140 °C is presented in Fig. 2. As shown in this figure, the change in temperature from 120 to 140 °C had very little effect on the solubilization of corn fiber. The incremental increases in the biomass loading correlated linearly with the changes in the solubilization and no significant limitations to their solubility were observed. In most cases studied, the total amount of monomeric sugars contributed to more than 50% of the solute, which may be attributed to the much lower concentration of protein and oil in corn fiber.

3.2.4. Total sugars versus individual sugars

The formation of individual sugars and its correlation with the total amount of the monomeric sugars was examined. The purpose of this assessment was to demonstrate the contribution of individual sugars to the total amount of the sugars and to verify the linearity of this correlation with respect to the reaction parameter. Data for the composition of the individual sugars as a function of time is presented in Tables 10 and 11. In general, most of the arabinose and galactose appeared to be formed early in the hydrolysis reaction. This caused the percent contribution of these sugars in the total amount of the sugars to actually decrease in time as the formation of xylose and glucose followed a monotonically increasing path throughout the reaction. Early formation of arabinose and galactose is expected as these sugars and their oligomers contribute to about 80% of the side chains of heteroxylans backbone of the hemicellulose structure and are cleaved faster than the backbone itself (Saha, 2003). This behavior was more distinct at the lower temperature of 120 °C and lower acid concentration of 0.5%.

Table 10
Monomeric sugar production in the dilute-acid pretreatment of DDG at 1.0% acid concentration and 10.0% biomass loading [mg/mL].

	120 °C					140 °C				
	Glu	Xyl	Gal	Ara	Total	Glu	Xyl	Gal	Ara	Total
Reaction time (min)										
0	0.2	0.7	0.1	1.6	2.7	0.5	1.1	0.2	3.9	5.6
5	0.7	2.4	0.3	4.9	8.3	1.9	7.3	1.1	7.0	17.3
10	0.8	4.3	0.6	5.3	11.1	3.7	9.7	1.5	7.7	22.7
15	1.2	5.7	0.8	5.4	13.1	4.6	10.3	1.7	8.0	24.6
20	1.7	6.2	1.0	5.5	14.3	5.0	10.6	1.8	8.3	25.8
30	2.3	7.6	1.3	6.2	17.3	5.2	10.4	1.8	8.3	25.8
45	3.3	8.6	1.5	6.2	19.6	5.3	10.1	1.9	8.4	25.8
60	3.7	9.2	1.5	6.1	20.5	5.3	11.0	1.9	8.3	26.6

Abbreviations used: Glu, Glucose; Xyl, Xylose; Gal, Galactose; Ara, Arabinose.

Table 11
Monomeric sugar production in the dilute-acid pretreatment of corn fiber at 1.0% acid concentration and 10.0% biomass loading [mg/mL].

	120 °C					140 °C				
	Glu	Xyl	Gal	Ara	Total	Glu	Xyl	Gal	Ara	Total
Reaction time (min)										
0	0.7	0.6	0.0	3.5	4.8	0.5	0.6	0.1	3.0	4.2
5	1.7	5.1	0.9	8.8	16.6	9.4	16.2	2.8	11.7	40.2
10	3.4	9.0	1.5	9.5	23.4	12.4	19.4	3.3	12.3	47.5
15	5.6	11.9	1.8	9.6	28.9	13.1	20.1	3.5	12.6	49.4
20	7.2	13.5	2.0	9.8	32.5	13.2	19.9	3.5	12.7	49.4
30	9.9	15.5	2.4	10.5	38.4	13.4	19.7	3.6	12.8	49.6
45	12.0	17.4	2.8	11.6	43.7	13.1	18.6	3.5	12.6	47.8
60	13.3	18.7	3.1	12.4	47.5	13.5	18.3	3.5	12.6	47.9

Abbreviations used: Glu, Glucose; Xyl, Xylose; Gal, Galactose; Ara, Arabinose.

4. Conclusions

Lignocellulosic biomass resources such as agricultural residues and wastes from the corn milling facilities are plentiful and have the potential to be utilized in the production of ethanol. Availability of DDG and corn fiber at the ethanol plant and their high levels of lignocellulosic material make them attractive feedstock for conversion to ethanol. In this study, dilute sulfuric acid hydrolysis of DDG and corn fiber to monomeric sugars was investigated. For both substrates, the highest yield of monomeric sugars was observed when the smallest amount of biomass loading (5 wt.%) was pretreated with the highest concentration of sulfuric acid (1.5 vol.%) and when the temperature was 140 °C. For most of the cases under consideration, the most effective period for the hydrolysis appeared to be during the initial 20–30 min of the reaction. Formation of furfural during the course of hydrolysis was significantly lower at 120 °C and also lower for the DDG samples compared with the corn fiber samples. The total amount of the solubilized matter during the hydrolysis was significantly higher than the amount of the monomeric sugars. The total carbohydrate content of DDG and corn fiber were determined to be 57.7 ± 2.0 and 77.0 ± 1.0 wt.%, respectively.

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