In-Cell and External Hydrolysis of Biomass for Carbohydrate Determinations Using Automated Solvent Extraction

Terri Christison, Richard Carlson, Lipika Basumallick, Linda Lopez
Thermo Fisher Scientific, Sunnyvale, CA, USA

Overview

Purpose: Demonstrate a complete workflow to evaluate alternative fuel samples from sample preparation to analytical determinations of carbohydrates.

Methods: Sample preparation included in-cell hydrolysis using the Thermo Scientific™ Dionex™ ASE™ 350 Automated Solvent Extractor system and traditional hydrolysis. Carbohydrates were determined using High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD).

Results: The extraction time, temperature, and hydrolysis conditions were optimized to obtain the highest extraction with the least amount of degradation by the sugar.

Introduction

Renewed interest in alternative fuel sources, including sources of ethanol, have been driven by increased worldwide fuel demand, limited petroleum resources, and increased public demand for renewable energy. Ethanol is a desirable alternative fuel source because it is renewable, and has lower emissions than petroleum products when burned. In the U.S., corn is the primary sugar feedstock of fermentation processes used to produce ethanol. However, corn is also an important food source for both people and livestock, raising concerns about increased food costs and effects on food supplies. To determine the feasibility of replacing corn as a fuel source, scientists are researching the available carbohydrate content (biomass) during fermentation of non-food vegetation, such as corn stalks (corn stover) and switchgrass.

Here we demonstrate the whole solution for carbohydrate determinations in biomass, from sample preparation to analysis, using accelerated solvent extraction to conduct acid-hydrolysis and extraction of carbohydrates, which is followed by instrumental analysis and data management.

Methods

Sample Preparation Equipment and Reagents

- Dionex ASE 350 Accelerated Solvent Extractor system
- Thermo Scientific™ Dionex™ Dionium™ extraction cells, 66 mL
- Collection Bottles, 250 mL
- Glass Fiber Filters
- Ottawa Sand (Fisher Scientific)
- Standard Laboratory Grinder or Mill
- HPLC Grade Water (Fisher Scientific)
- H_2SO_4, 8 M (Sigma-Aldrich)

Sample Preparation Conditions

Extract the samples into 250 mL sample collection bottles according to the conditions below.

In-Cell Hydrolysis	
Sample:	1 to 10 g of ground sample mixed with Ottawa sand
Pressure:	1500 psi
Temperature:	140–150 °C$^+$
Solvent:	0.1 M Sulfuric Acid in HPLC water
Static Time:	5 min
Static Cycles:	3
Flush:	10 %
Purge:	120 s
Total Run Time:	~20 min

$^+$ 150 °C is the optimum temperature for corn stover. Lower temperatures are recommended for other grasses.

Pre-Cell Hydrolysis

These samples were hydrolyzed in beakers and then extracted by accelerated solvent extraction using the following conditions.

Pre-Cell Hydrolysis	
Sample:	Hydrolyze 2 g of sample using 0.5% sulfuric acid at 200 °C prior to accelerated solvent extraction
Pressure:	1500 psi
Temperature:	100 °C
Solvent:	HPLC water
Static Time:	7 min
Static Cycles:	3
Flush:	50 %
Purge:	120 s

Gravimetric

For percent extraction, evaporate the extractions to dryness. Record extraction weight as a percentage of the original weight.

Ion Chromatography Instrumentation

Thermo Scientific Dionex ICS-3000, ICS-5000 or ICS-5000$^+$ system including:

- SP Single or DP Dual Pump
- EG Eluent Generator Module
- DC Detector/Chromatography Compartment
- High-Pressure Injection Valve, 4-port
- ED Electrochemical Detector
- Gold on PTFE Disposable Working Electrode
- pH, Ag/AgCl Reference Electrode
- PTFE gasket, 0.015” thick
- Thermo Scientific Dionex AS-AP Autosampler

Conditions	
Columns:	Thermo Scientific™ Dionex™ CarboPac™ SA10 guard, separation
Eluent:	1 mM KOH
Eluent Source:	Thermo Scientific Dionex EGC III KOH Cartridge with Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column
Flow Rate:	1.5 mL/min
Inj. Volume:	0.4 or 2.5 µL
Column Temp.:	45 °C
Cell Temp.:	30 °C
Detection:	PAD, Four-Potential Waveform
Working Electrode:	Gold on PTFE
RE Mode:	Ag/AgCl
Post Column:	None or 200 mM NaOH @ 0.4 mL/min

Pre-hydrolyzed samples were centrifuged at 15,000 rpm for 10 min to remove particulates.

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software

Results

Definitions

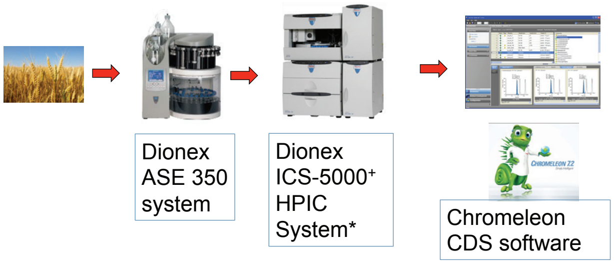
Corn stover includes the non-commercial parts of the corn plant: cob, stalk, leaves, and husk.

Switchgrass is a wild grass native to American plains.

Workflow Diagram

Figure 1 shows the total workflow for carbohydrates in biomass.

FIGURE 1. Total Workflow from Sample Preparation to Analysis and Data Management.



Sample Extraction Using In-Cell Hydrolysis

The extraction experiments showed higher gravimetric results at 190 °C than at 150 °C (Table 1). However, HPLC analysis showed that the 190 °C temperature caused a breakdown of the sugars (not shown).

Sample Extraction of Pre-Hydrolyzed Switchgrass

Switchgrass hydrolyzed with 0.2% sulfuric acid had higher gravimetric results (Table 2) but lower glucose extractions (not shown).

Tables 1–2. Percent Extractables.

Table 1. Corn Stover Using In-Cell Hydrolysis[1]

Extraction Temperature	% Total Extractables
150 °C	48.7
190 °C	87.3

Table 2. Pre-hydrolyzed Switchgrass[1]

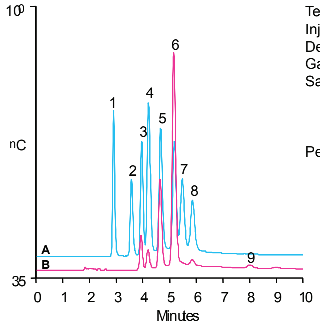
Acid Hydrolysis (%)	% Total Extractables
0.2% H_2SO_4	76.76
0.5% H_2SO_4	72.39

Biofuel Sugars by HPAE-PAD

Acid-treated biomass samples have high concentrations of sugars such as xylose, glucose, and galactose which may cause carryover. To minimize carryover, it is recommended to add a syringe flush of 500 µL deionized water between samples and a 100 mM KOH column wash for 20 min is recommended. This method was optimized for high carbohydrate samples such as biomass samples by reducing the sample injection to 0.4 µL onto a high-capacity Dionex CarboPac SA10 column and reducing the flow path through the ED cell with the thicker gasket (0.015” thick).

Figure 2 shows the separation and detection of 0.5 mg/mL of eight biofuel sugars using a 0.4 µL sample injection.

FIGURE 2. Biofuel Sugars (A) and Acid-Hydrolyzed Corn Stover Sample (B) on the Dionex CarboPac SA10 column.[2]



Column: Dionex CarboPac SA10 with guard (4 × 250 mm)
Eluent (EG): 1 mM KOH
Flow Rate: 1.5 mL/min
Temperature: 45 °C
Inj. Volume: 0.4 µL
Detection: PAD (Au on PTFE)
Gasket: 0.015” thick
Samples: A. Standard mixture
B. Acid-hydrolyzed corn stover (100-fold dilution)

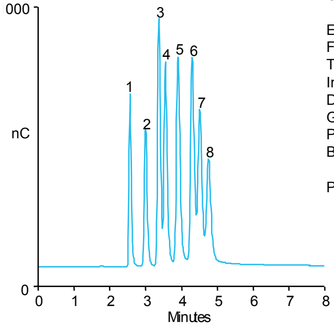
Peaks:	A	B
1. Fucose	0.5	—
2. Sucrose	0.5	—
3. Arabinose	0.5	<0.5
4. Galactose	0.5	<0.5
5. Glucose	0.5	<0.5
6. Xylose	0.5	0.8
7. Mannose	0.5	—
8. Fructose	0.5	—

Table 3. Biofuel Sugar Recoveries in Corn Stover Acid Hydrolysate (n = 3 days).[2]

Analytes	Amount Added (mg/mL)	Amount Detected (mg/mL)	Recovery (%)	Recovery (%RSD)
Fucose	1.01	0.81	80.5	6.1
Sucrose	0.87	0.61	69.6	2.3
Arabinose	0.90	0.87	96.7	1.5
Galactose	1.01	0.98	98.0	10.1
Glucose	1.00	0.98	99.2	6.6
Xylose	0.59	1.33	112.7	5.2
Mannose	0.99	0.86	87.3	3.2
Fructose	1.02	1.08	108.5	1.0

Figure 3 shows the separation and detection of 1 mg/mL biofuel sugars using a 2.5 µL sample injection with a post-column base addition to increase the linear range.

FIGURE 3. Separation of Biofuel Sugars on the Dionex CarboPac SA10 Column.[2]



Columns: Dionex CarboPac SA10 with guard (4 × 250 mm)
Eluent (EG): 1 mM KOH
Flow Rate: 1.5 mL/min
Temperature: 45 °C
Inj. Volume: 2.5 µL
Detection: PAD, (Gold on PTFE)
Gasket: 0.015“ thick
Postcolumn Base: 200 mM Sodium hydroxide at 0.4 mL/min

Peaks:	
1. Fucose	0.5 mg/mL
2. Sucrose	1.0
3. Arabinose	1.0
4. Galactose	1.0
5. Glucose	1.0
6. Xylose	1.0
7. Mannose	1.0
8. Fructose	1.0

Conclusion

- Carbohydrate analysis is important to determine the glucose content of various biomass samples.
- The optimum accelerated solvent extraction temperature for corn stover is 150 °C. Switchgrass should be extracted at a lower temperature.
- The Dionex ASE 350 system provides automated in-cell hydrolysis.
- HPAE-PAD was optimized for high carbohydrate samples by reducing the injection volume and the flow path, and adding base post-column.

References

1. Carlson, R.; Knowles, D.; Richter, B. AN 363, Using Accelerated Solvent Extraction in Alternative Fuel Research, AN70489_E 01/13S.Thermo Fisher Scientific, Sunnyvale, CA, USA, 2013.
2. Basumallick, L. Rohrer, J. Application 282, Rapid and Sensitive Determination of Biofuel Sugars by Ion Chromatography, AN282_E 04/12S LPN2876, 01/13S. Thermo Fisher Scientific, Sunnyvale, CA, USA, 2012.

Acknowledgements

National Renewable Energy Laboratory (NREL) in Golden, Co., developed the accelerated solvent extraction method to extract corn stover. Dr. Doug Raynie from South Dakota State University, Dept. of Chemistry and Biochemistry provided the switchgrass data.

www.thermofisher.com

Australia	+61 3 9757 4486
Austria	+43 1 333 50 34 0
Belgium	+32 53 73 42 41
Brazil	+55 11 3731 5140
China	+852 2428 3282
Denmark	+45 70 23 62 60
France	+33 1 60 92 48 00
Germany	+49 6126 991 0
India	+91 22 6742 9494
Italy	+39 02 51 62 1267
Japan	+81 6 6885 1213
Korea	+82 2 3420 8600
Netherlands	+31 76 579 55 55
Singapore	+65 6289 1190
Sweden	+46 8 473 3380
Switzerland	+41 62 205 9966
Taiwan	+886 2 8751 6655
UK/Ireland	+44 1442 233555
USA and Canada	+847 295 7500

Thermo
SCIENTIFIC
Part of Thermo Fisher Scientific