Accelerated Solvent Extraction for Alternative Fuel Research

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Introduction

As global interest in alternate fuel sources increases, many laboratories are researching effective ways to test, develop, and produce fuel from renewable energy sources. One option is the production of alcohol from biomass, called bioalcohol. Alcohol produced from biomass has several benefits of fossil fuels since it is produced from crops, a renewable energy source, and causes little environmental pollution when burned. As bioalcohol is produced from biomass via sugar fermentation, it is necessary to research which types of plants produce the best yields of usable sugars.

Thermo Scientific™ Dionex™ ASE™ 150/350 Accelerated Solvent Extractor systems provide a fast and efficient way to extract various biomass samples for sugar analysis to determine sample viability for alcohol production. The Dionex ASE 150 and 350 Accelerated Solvent Extractor systems with the pH-hardened pathways provide additional benefits to the method by allowing pre-hydrolyzed biomass samples or those with acidified solvents to be extracted. This allows samples to be hydrolyzed in the extraction cell. This poster describes methods for the extraction of sugars from biomass using acidified solvents (in-cell hydrolysis) and extracting pre-hydrolyzed samples[1].

Equipment

- Dionex ASE 350 Accelerated Solvent Extractor (P/N 083114 [120 V] or 083146 [240 V])
- Thermo Scientific™ Dionium™ Extraction Cells 66 mL (P/N 068102)
- Collection Bottles 250 mL (P/N 056284)
- Glass Fiber Filters (P/N 056781)
- Ottawa Sand (Fisher Scientific)
- Standard Laboratory Evaporation System
- Standard Laboratory Grinder or Mill

Reagents

- HPLC Grade Water (Fisher Scientific)
- H_2SO_4 8 M (Sigma Aldrich)

Sample Preparation

Grind raw sample material using a standard laboratory grinder or mill.

Acidified Solvent Extraction (In-cell Hydrolysis)

Weigh approximately 1.0–10.0 g of ground sample into a tared 66 mL Dionium extraction cell containing a glass fiber filter. Fill any cell void with Ottawa Sand to reduce the amount of solvent that is required for the extraction. Weigh the appropriate number of 250 mL collection vials and place them onto the Dionex ASE 150/350 Accelerated Solvent Extractor system.

Pre-Hydrolyzed Samples

Weigh approximately 2 g of sample and hydrolyze using 0.5% H_2SO_4 at 200 ºC. Transfer the acidified sample mixture to a 66 mL Dionium extraction cell containing a glass fiber filter. Weigh the appropriate number of 250 mL collection bottles and place them onto the Dionex ASE 150/350 Accelerated Solvent Extractor system.

Extraction Conditions

	Method 1 (Acidified Solvent)	Method 2 (Pre-hydrolyzed Samples)
Pressure	1500 psi*	1500 psi*
Temperature	140-150 ºC[a]	100 ºC
Solvent	0.1 M H_2SO_4 solution with HPLC water	HPLC water
Static Time	5 min	7 min
Static Cycles	3	3
Flush	10%	50%
Purge	120 s	120 s

* Pressure studies indicate that 1500 psi is the optimum extraction pressure for all accelerated solvent extraction technique applications.

[a] Tests concluded that an extraction temperature of no higher than 150 ºC was optimal for the corn stover matrices. Other biomass sample matrices may vary and could require an extraction temperature lower than 150 ºC.

FIGURE 1. Dionex ASE 150 / 350 Accelerated Solvent Extractor System.

Extraction

Place the extraction cells containing the samples onto the Dionex ASE 150/350 Accelerated Solvent Extractor cell tray. Create an extraction method based on the desired method listed above. Once the extractions are complete, prepare the extracts according to the analytical method needed. For gravimetric determination, evaporate the solvent to dryness using a standard laboratory sample evaporation system and weigh the collections vials to determine % residue (weight of evaporated vial – tared weight) / sample weight × 100.

If sample is to be analyzed chromatographically for sugar content, the appropriate volume adjustment with eluent should be made before injection into the chromatographic system.

Results and Discussion

Acidified Solvent Extraction (In-cell Hydrolysis)

Corn stover samples were extracted for the quantifiable determination of sugars used for ethanol production. These samples consist of the leaves and stalks of the maize plants left in the field after harvest and include the residue stalk, the leaf, husk, and cob (Figure 2). Two different extraction temperatures were examined for determining the best method (Table 1). Results show that the samples extracted at 190 ºC produced more total extractables. However, HPLC analysis showed that this higher temperature caused a breakdown of the sugars. The higher temperature was too aggressive and caused an extraction of unwanted coextractables resulting in the increased final weight. Analysis of the extracts at 150 ºC showed no breakdown of sugars and no increase in unwanted coextractables (Figures 3 and 4).

TABLE 1. Corn stover extracted with acidified solvent.

Extraction Temperature	%Total Extractables
150 ºC	48.7
190 ºC	87.3

Pre-Hydrolyzed Samples

Two different concentrations of acidic solvents were examined for hydrolysis of switch grass to determine the optimal concentrations for recovery of glucose (Table 2). The HPLC analysis showed that for samples pretreated with 0.2% H_2SO_4, xylose was the predominant sugar present (Figure 5). Samples pretreated with 0.5% H_2SO_4 had a significant increase in glucose levels (Figure 6). Table 3 lists the HPLC conditions.

TABLE 2. Pre-hydrolyzed switch grass samples extracted with HPLC-grade water.

Acidic Solvent Concentration	% Total Extractables
0.2% H_2SO_4	76.76
0.5% H_2SO_4	72.39

FIGURE 2. Corn stover prior to extraction on the Dionex ASE 350 system.

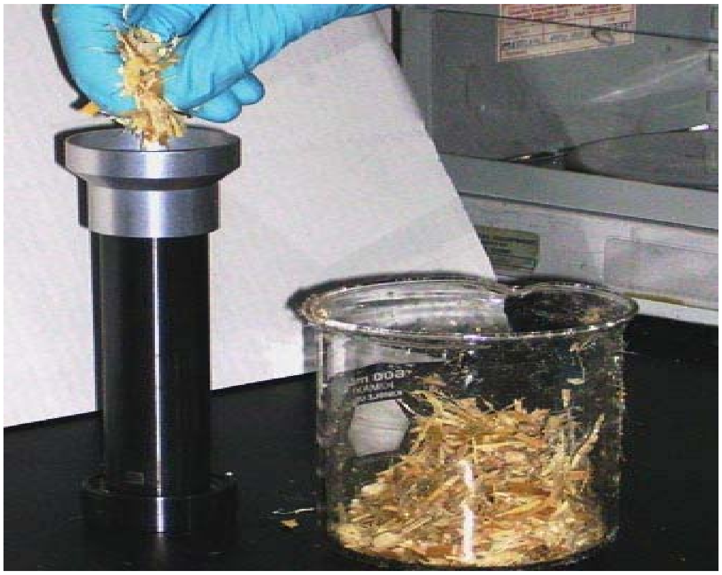

FIGURE 3. Dionex ASE 350 system extracts at 150°C with 0.1 M H_2SO_4.

FIGURE 4. Dionex ASE 350 system extracts at 190°C with 0.1 M H_2SO_4.

FIGURE 5. HPLC characterization of switch grass pretreated with 0.2% H_2SO_4.

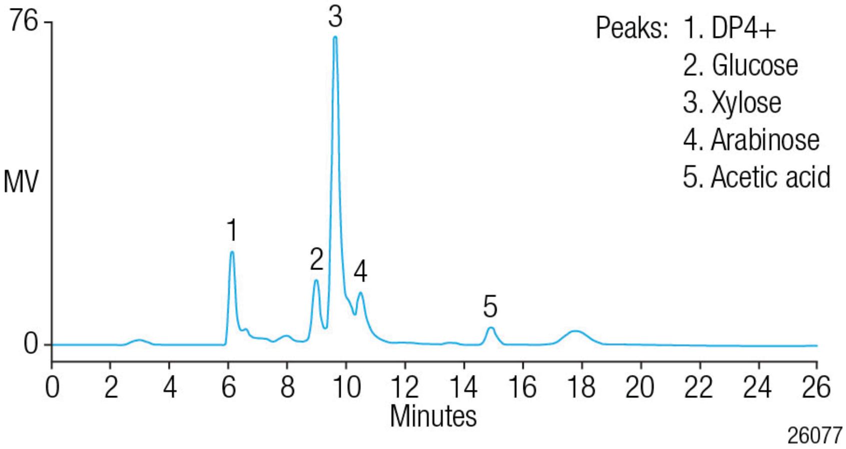

FIGURE 6. HPLC characterization of switch grass pretreated with 0.5% H_2SO_4.

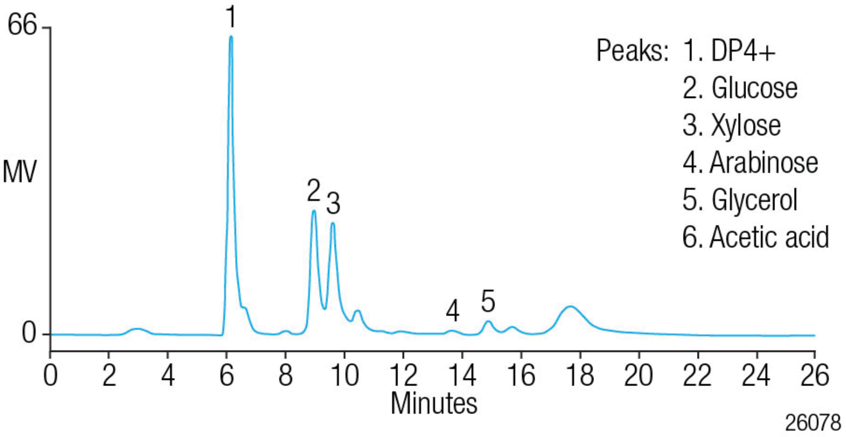

TABLE 3. HPLC conditions for Figures 4 and 5.

Parameter	Conditions
Column	Commercial Size Exclusion / Ligand Exchange
Flow Rate	0.6 mL/min
Temperature	65 ºC
Mobile Phase	0.0005 N H_2SO_4
Detector	Refractive Index
Run Time	26 min
Post Run Time	10 min

Conclusions

- The pH-hardened pathways of the Dionex ASE 150 and Dionex ASE 350 Accelerated Solvent Extractor systems allow the extraction of samples that have been pre-treated with acid, and also allows in-cell hydrolysis with acidified solvents.
- The feature, along with the rapid extraction time and minimal solvent usage, provides laboratories with a way to rapidly quantify biomass samples for biofuels research.
- The accelerated solvent extraction method requires substantially less time than by the Soxhlet extraction method, permitting a fully automated extraction process that can be performed in minutes for fast and easy extraction with low solvent consumption.

Acknowledgements

The accelerated solvent extraction method for extraction of corn stover samples was developed by the National Renewable Energy Laboratory (NREL) in Golden, CO.

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References

1. Raynie, D. Innovations in Sample Preparation poster presented at the Minnesota Chromatography Forum, Minneapolis, MN, May 7, 2008.

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