A national laboratory of the U.S. Department of Energy
Office of Energy Efficiency & Renewable Energy

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Innovation for Our Energy Future

Biomass Characterization Using NIR Spectroscopy:
1. Introduction to NREL Methods
2. Correlation of NDF/ADF with Compositional Data

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February 13th, 2008

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Talk Outline

Part 1

- Traditional Methods of Biomass Compositional Analysis
- NREL NIR Methods

Part 2

- Using NIR Methods: Comparing NDF/ADF with Compositional Data

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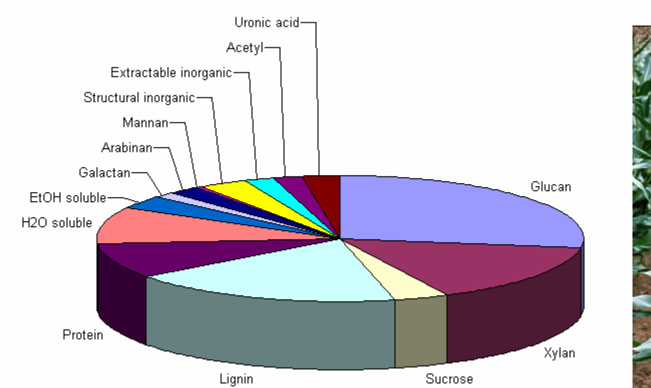
- A U.S. Department of Energy National Laboratory
- Only national laboratory ***dedicated*** to renewable energy and energy efficiency R&D
  - Fundamental ***science*** to ***technology*** solutions
  - ***Collaboration*** with industry and university partners
- Current staff of 1100 and budget of $300 million/yr
- Visit us at: www.nrel.gov

Part 1 - Biomass Analysis at NREL

- Biomass Analysis Expertise
  - Analytical chemistry
  - Natural products chemistry
  - Biochemistry
  - Chemometrics

Develop standard methods for analysis of biomass feedstocks and process intermediates

Corn Stover Composition



- 14 constituents
- ~2 week, $2000 per sample analysis
- Ongoing research to improve wet chemical methods for more accurate results and further breakdown

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Laboratory Analytical Procedures - LAPs

- Available at http://www.nrel.gov/biomass/analytical_procedures.html
- Biomass Analysis LAPs
  - Sample prep
  - Extraction
  - Total solids
  - Carbohydrates and lignin
  - Inorganics
  - Protein
  - Starch
  - Liquid fraction of pretreated slurry analysis
  - SSF (solids) and fermentation (liquors)

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History

- NREL LAPs are based on the Uppsala Method for the analysis of dietary fiber.
  - Summative analysis
  - Micro method
    - Furda, I; *Simultaneous analysis of soluble and insoluble dietary fiber,* The Analysis of Dietary Fiber in Food, W.P. T. James and O. Theander(Eds), Marcel Dekker, New York, 1981, pp.163 –172.
  - Used for Validation of NIST biomass Standard Reference Materials in International Energy Agency sponsored Round Robin
    - Milne, T. A.; Chum, H. L.; Agblevor, F. A.; Johnson, D. K.; *Standardized Analytical Methods,* Proceedings of International Energy Agency Bioenergy Agreement Seminar, Vol 2(1-6), April, 1992, (341-366).

NIST Standards

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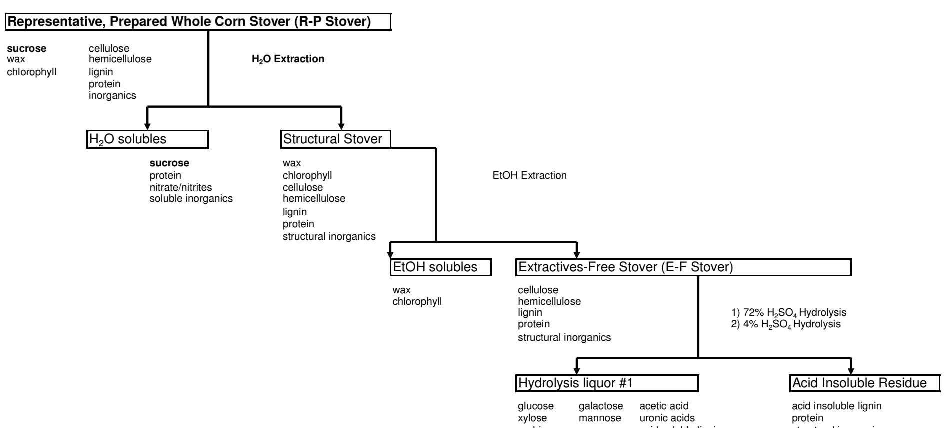
Flow Chart of Biomass Stover Feedstock Analysis



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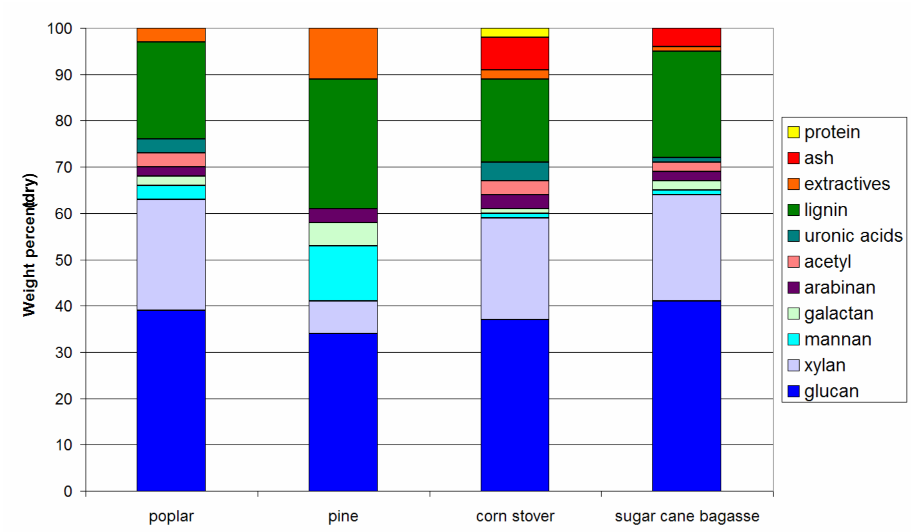
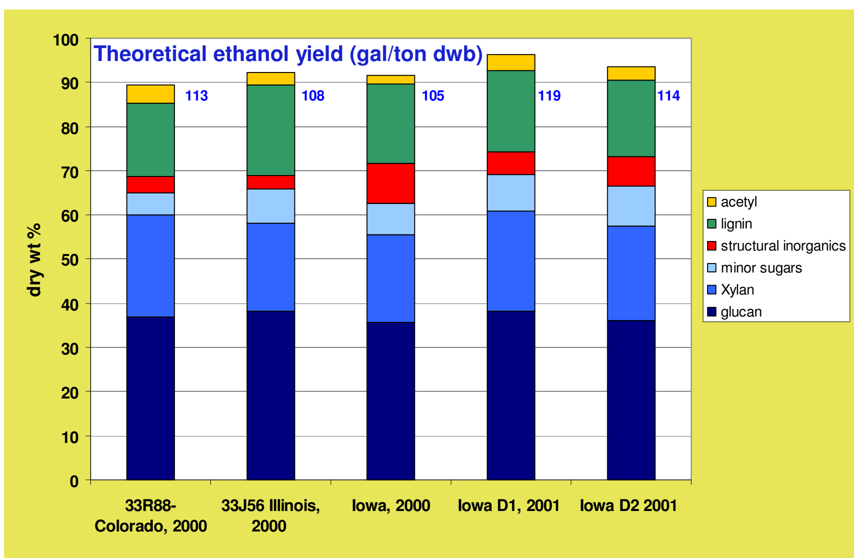
Feedstock Comparison

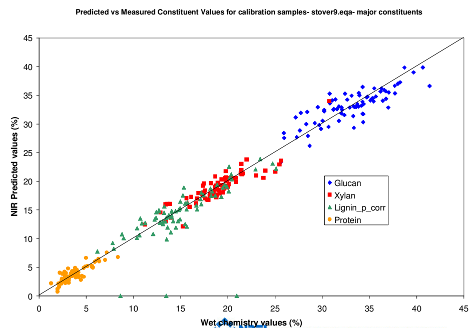


Variability Within a Feedstock (Corn stover)



Rapid Biomass Analysis – NIR Spectroscopy

- Wet chemical methods are used to calibrate rapid analysis methods
- Retain precision and accuracy of calibration methods
- Fast/Less labor intensive
- Inexpensive for routine samples



- **Corn Stover Feedstock Equation:**
  - 77 samples in the calibration
  - Predicts 14 constituents (4 shown) with average mass closure 94.1% and std. dev. 5.0 % (dwb)

Current NIR /PLS Biomass Methods

- Feedstock
  - Corn stover
  - Hardwood
  - Softwood
- Pretreated process intermediates
  - Wood and Stover solid fraction (washed, dried, and milled)
  - Wood and Stover hydrolysate liquid fraction
- Other
  - % Solids (moisture analysis) for corn stover feedstock

Corn Stover Feedstock Method stover9

- 77 samples analyzed with newest methods
- Average mass closure- 100.0 ± 3.8%
- Implemented in WinISI® and Vision®

Constituent	Min	Max
EtOH_sol	1.5	6.7
Sucrose	0.1	13.6
H2O_ext_oth	3.7	21.9
Glucan* C-6	**25.7**	**40.7**
Xylan* C-5	**11.2**	**30.8**
Galactan	0.5	2.4
Arabinan	0.9	6.1
Mannan	0.2	1.4
Lignin*	**6.2**	**25.1**
Struct_inorg	0.3	13.5
Ext_inorg	0.0	7.5
Protein	1.3	8.4
Acetyl	0.7	4.2

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Applications of Corn Stover Feedstock Method

– NREL/DOE Program - Thousands of samples predicted, equivalent to millions of dollars in wet chemical analysis

– Genetic diversity study
  - 2100 samples
    – Wet chemistry= 2,100 * $2,000/sample = $4.2MM
    – NIR rapid analysis = 2,100 * $10/sample = $21K

– Other collaborations
  - Idaho National Laboratory
  - Industry (Abengoa, Broin, Cargill, DuPont)
  - Universities
    – University of Wisconsin
    – University of Kentucky
    – Michigan State University
    – USDA ARS, Lincoln, NE
    – University of Nebraska
    – Iowa State University

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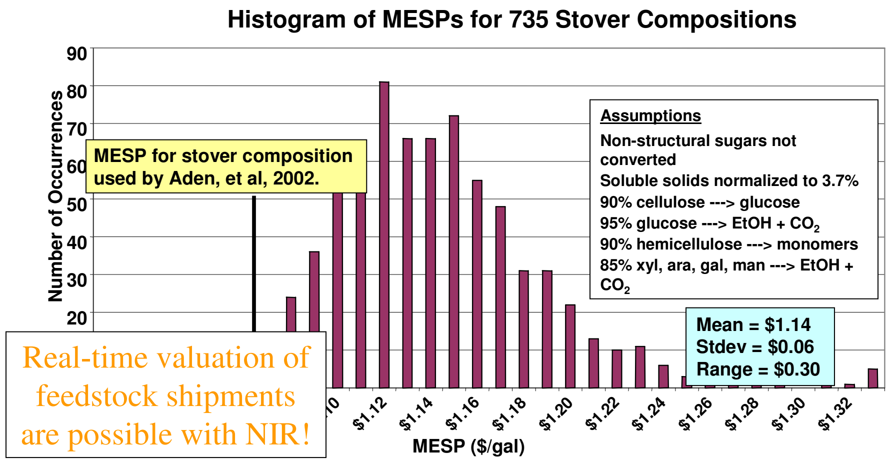
Impact of Stover Composition On Economics of Ethanol Production



Pretreated Corn Stover Methods

- A slurry contains solid and liquid fractions of acid pretreated corn stover
- Methods
  - Solids; washed, dried, and milled
    - Robust method developed over six years
    - 117 samples
    - Average mass closure 102.8 $\pm$ 3.6%
    - Implemented in WinISI® and Vision®
  - Liquids; separated and filtered
    - Preliminary and experimental
    - 24 samples
    - Implemented in Vision®

Corn Stover Feedstock Model (SNV-detrend-1D)

Constituent	Mean	Max	Min	# Samples	PCs	SECV	1-VR	SEC
Ethanol Extractives	4.2	2.0	10.7	68	7	1.4	0.41	0.97
Sucrose	[illegible]	[illegible]	[illegible]	[illegible]	5	0.9	0.92	0.71
Water Extractives	1[illegible]	[illegible]	[illegible]	[illegible]	3	2.6	0.50	2.17
Glucan	3[illegible]	[illegible]	[illegible]	[illegible]	7	1.5	0.79	1.09
Xylan	18.4	13.5	21.5	68	7	0.9	0.76	0.58
Galactan	1.5	0.5	2.9	69	3	0.3	0.38	0.29
Arabinan	2.6	0.9	4.1	69	3	0.4	0.46	0.33
Mannan	0.4	0.2	1.4	69	3	0.2	0.20	0.16
Lignin	14.1	8.6	24.7	68	6	1.4	0.73	1.02
Structural Inorganics	3.4	0.8	8.8	66	7	1.2	0.70	0.88
Extractable Inorganics	2.6	0.4	7.5	69	8	1.2	0.39	0.74
Protein	3.4	1.3	7.0	69	8	0.5	0.78	0.34
Acetyl	2.2	0.7	3.4	69	5	0.3	0.69	0.24

66% of total

19% of total

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Effect of Spectral Pretreatment on Glucose Model Predictions

Pretreatment	PCs	SECV	1-VR	SEC
SNV, detrend, 1st derivative	7	1.66	0.75	1.22
None	10	1.81	0.70	1.36
MSC	9	1.78	0.71	1.39
MSC + 1st derivative	7	1.60	0.77	1.16
SNV	10	1.82	0.70	1.35
SNV + 1st derivative	7	1.62	0.76	1.17
1st derivative	9	1.65	0.75	1.05

None are statistically significantly "better" (P=0.05)

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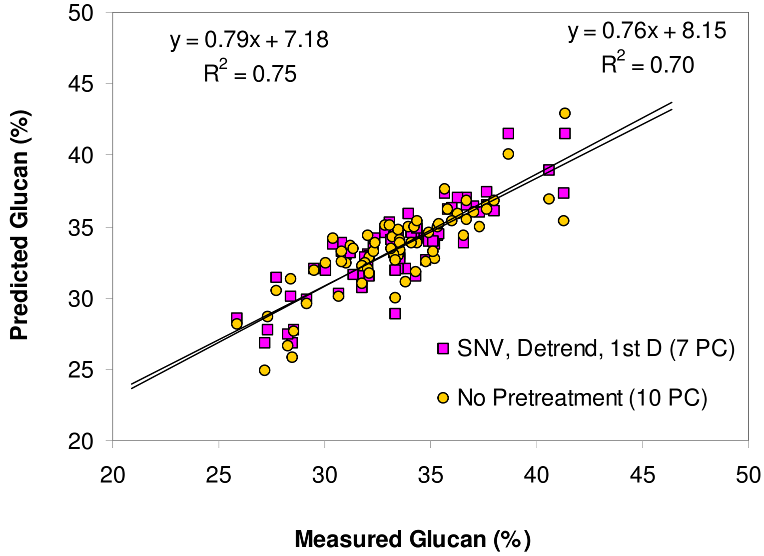
Effect of Spectral Pretreatment on Model Accuracy



Conclusions – Part 1

- NREL has implemented analysis procedures for the compositional analysis of biomass feedstocks
- We have developed NIR calibration models for a variety of potential biomass feedstocks
- Future work will focus on
  - New Feedstocks - Dedicated Energy Crops
  - Corn Stover process intermediates
  - Widespread distribution of models

Part 2 - Correlating NDF/ADF with Compositional Data

- NDF= “Neutral Detergent Fiber”
- ADF= “Acid Detergent Fiber”
- Techniques for rating the nutritive value of forages
- Surfactant extraction of forages (w/ & w/o acid) followed by weighing
- Forages are valued ($$) according to moisture (DM), crude protein, NDF/ADF

NDF/ADF Measures

- Extractions followed by gravimetric determination of “what’s left behind”
- ADF is a more severe extraction than NDF, so NDF #s are higher
- Literature suggests rough correlations to structural carbohydrates
- NDF ~ cellulose + hemicellulose + lignin
- NDF-ADF ~ hemicellulose

NIR in Forage Analysis

- There are 50+ forage laboratories in the US who perform these analyses (~$50/per)
- NIR calibrations exist for many forages
- http://www.uwex.edu/ces/forage/NIRS/home-page.htm
- So, NIR is an accepted technique for forage analysis, and lots of historical data for NDF/ADF, protein, and DM exist

What about these Historical Data?

- What if we could establish a correlation between forage analysis data (NDF/ADF) and current compositional analysis data (glucan, xylan)?
- Could we use these correlations to predict compositional data *a priori* from historical forage data sets?
- This may represent a “shortcut” to future feedstock variability studies!

The UW Corn Stover Data Set

- NREL researchers had established an informal collaboration with Prof. Jim Coors, Department of Agronomy, University of Wisconsin
- NREL agreed to provide compositional analysis predictions of corn stover samples milled and scanned by UW researchers
- NREL would get NDF/ADF data for 2 harvest years (05, 06)

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Examination of the Dataset

- Goal: develop correlations between NDF/ADF predictions (UW predictions) and compositional data (NREL predictions)
- Note: all of the data to be presented are NIR predictions; no wet chemistry data!
- ~100 total samples predicted using both models; this analysis excludes the bmr hybrids which had higher extractives content & therefore lower structural carbohydrates

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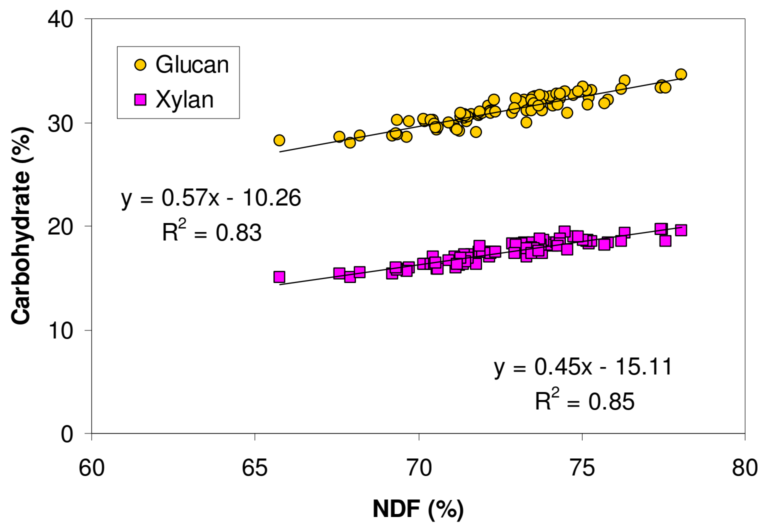
Carbohydrates (%) vs. NDF (%)



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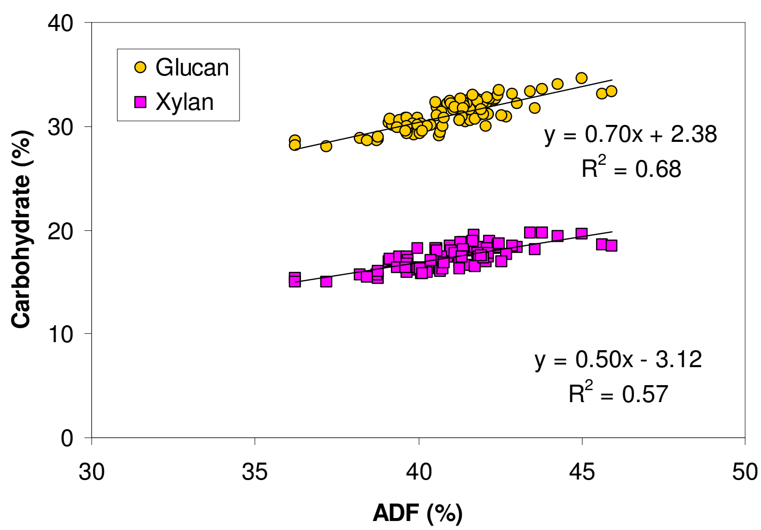
Carbohydrates (%) vs. ADF (%)



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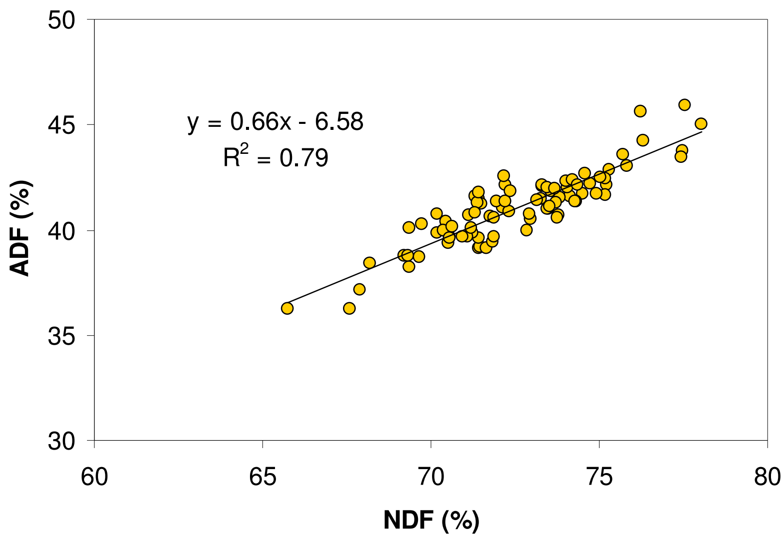
NDF & ADF are Correlated



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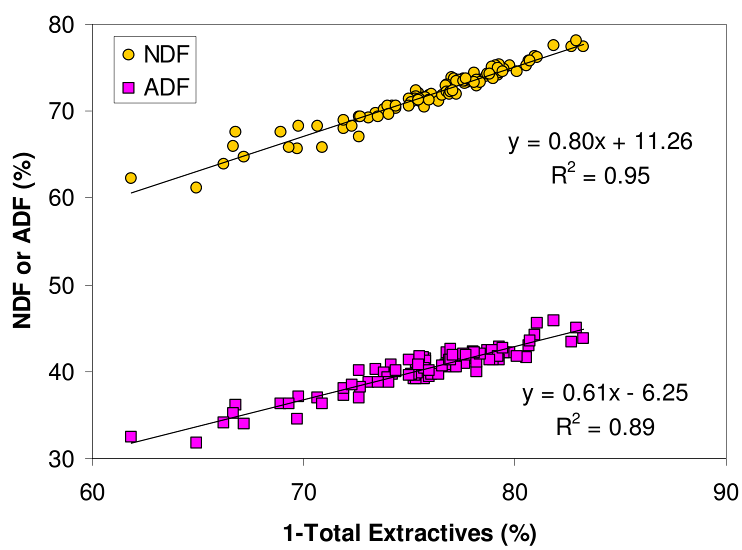
NDF/ADF vs. Total Extractives



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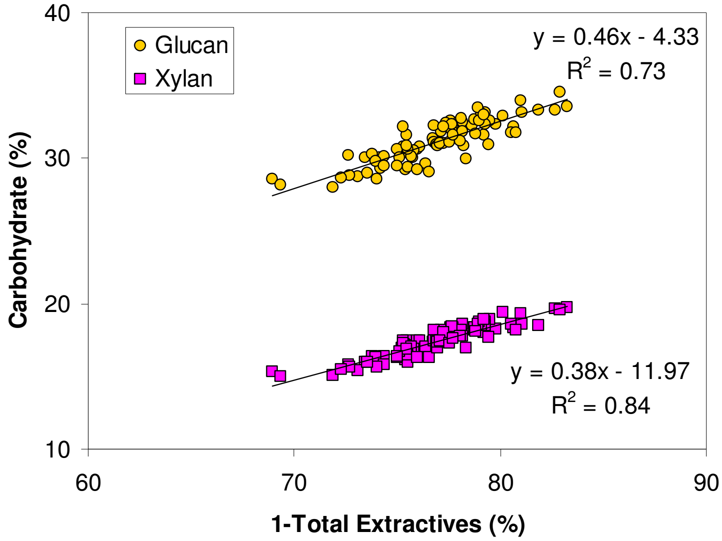
Carbohydrates vs. 1-Total Extractives



Results So Far

- NDF is highly correlated (R>0.9) to glucan and xylan
- ADF is highly correlated (R>0.7) with both glucan and xylan
- NDF and ADF are both highly correlated with total extractives
- Glucan and xylan are both highly correlated with total extractives
- Is there anything not correlated with anything? What does this mean?

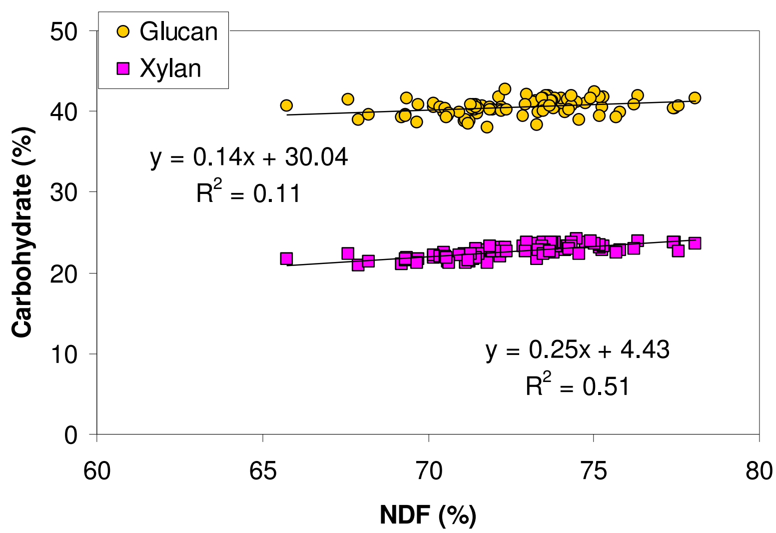
Results So Far (2)

- Not surprising that the three different biomass extraction techniques are correlated; they are supposed to do similar things!
- So, we can predict the glucan and xylan content of a batch corn stover if we know its NDF or ADF values (preferably NDF)
- The correlation between total extractives and glucan and xylose is real, but is an artifact of the ratio closure; everything sums to ~100%
- But what is REALLY going on here?

What Does This Mean?

- Is the correlation between NDF/ADF and "Total Extractives" driving the correlation between NDF/ADF and carbohydrates?
- What if we look at the carbohydrates on an extractives-free basis?

Extractives-Free Carbohydrates vs. NDF%



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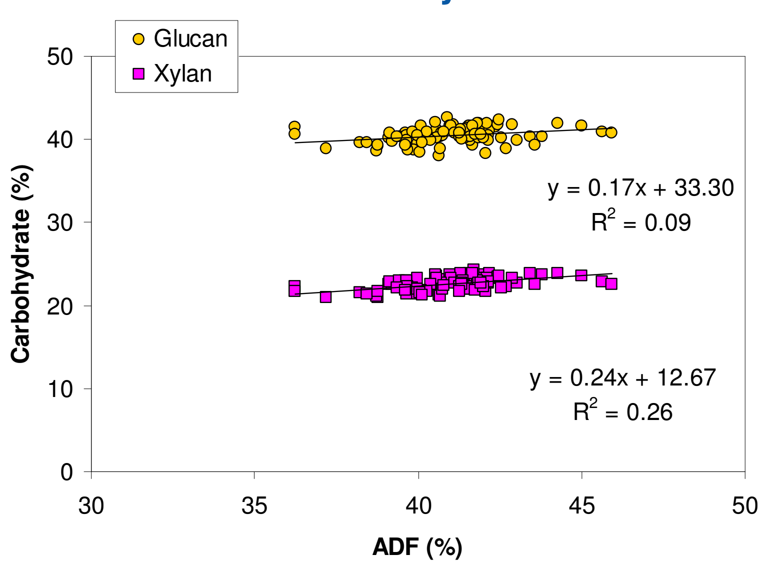
Extractives-Free Carbohydrates vs. ADF%



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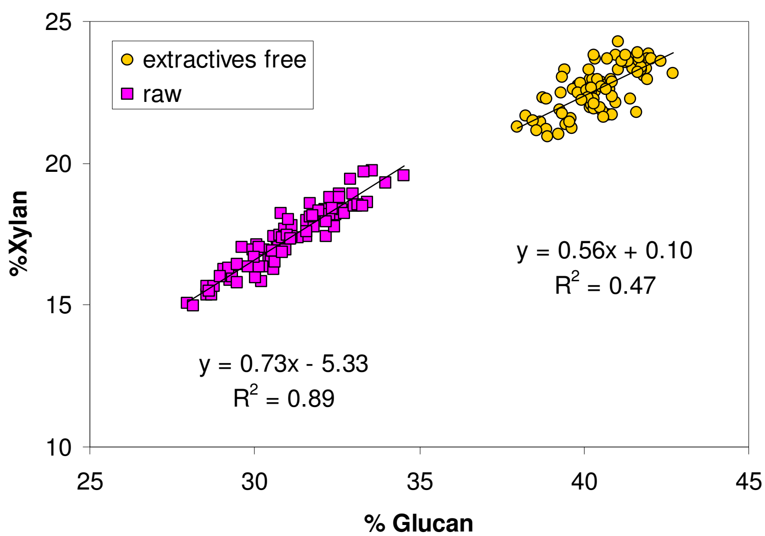
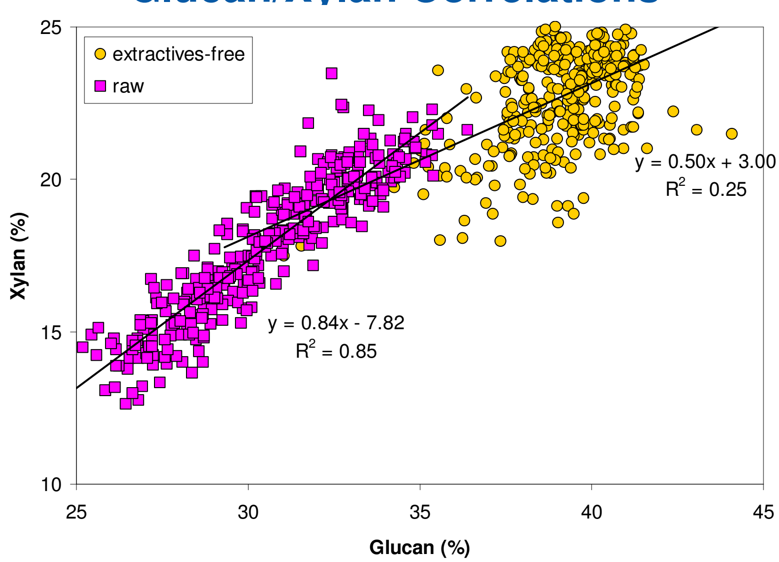
Glucan/Xylan Correlations



Glucan/Xylan Correlations



What Does this Mean?

- NDF/ADF data tells us very little about the structural carbohydrates in biomass, at least directly
- Why should they? They are basically extractive techniques!
- But what does this say about carbohydrate variability in corn stover?

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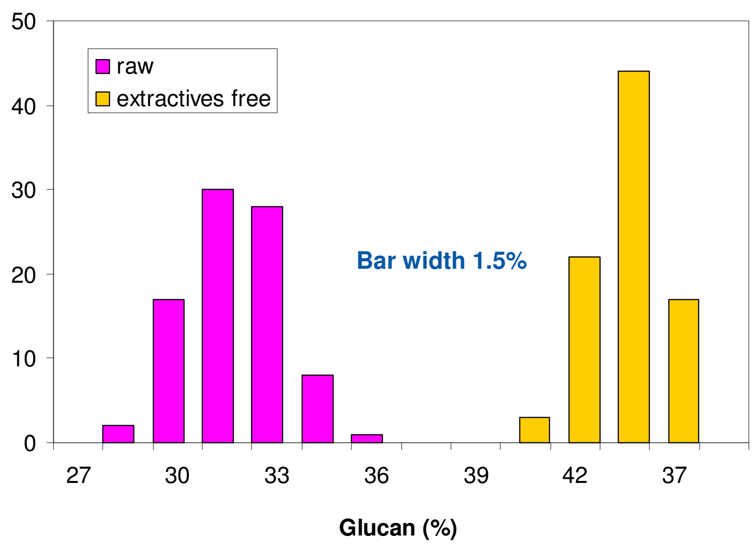
Histogram of Glucan Composition



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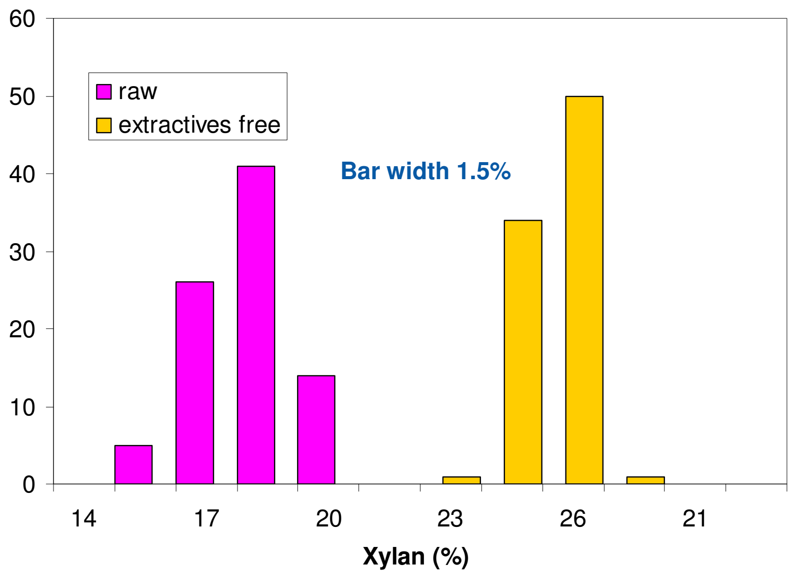
Histogram of Xylan Composition



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Data Experiment: Can We Create Synthetic Variance?

- Assume that all samples have the same glucan/xylan composition on an extractives-free basis; all variability comes from extractives!
- Calculate the mean value of glucan and xylan on an "extractives free" basis

$$Mean\ Glucan_{EF} = \frac{1}{N}\sum \frac{Glucan_i^{DW}}{1 - Extractives_i}$$

- Convert this to a "dry weight" basis using the actual total extractives content of each sample

$$Glucan_i^{SYN} = Mean\ Glucan_{EF} \cdot (1 - Extractives_i)$$

- See what the correlation looks like…

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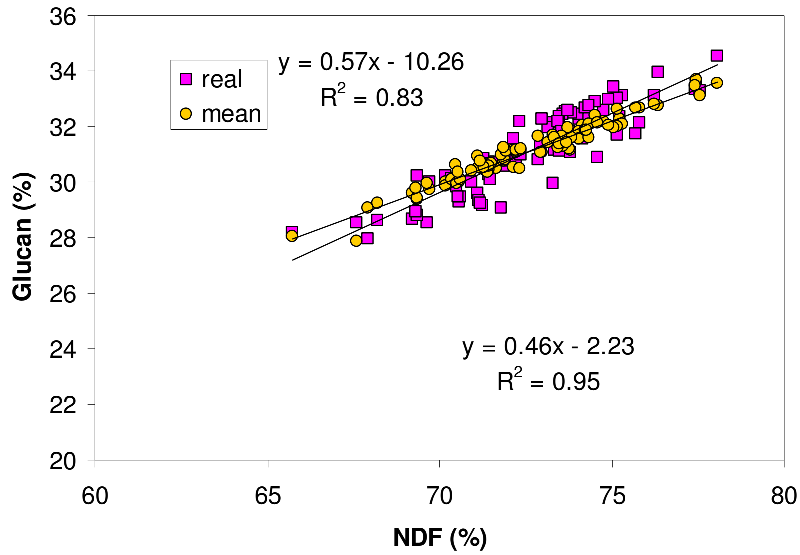
"Synthetic" Correlation slightly better!



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Conclusions – Part 2

- For the Coors dataset, NDF/ADF data are very highly correlated with glucan, xylan, and total extractives
- Any of the three compositional data can be predicted from the ADF/NDF data
- These correlations are driven to a large extent (~75%) by the extractives content
- We can learn little about structural carbohydrates from NDF/ADF data

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The Bigger Questions

- How "good" should NIR Models be?
  - Valuing feedstocks
  - Process control
  - Automated laboratory systems

- How can NREL best work with the larger community to develop, distribute, & support models?