



Analytical Approaches for Characterization of Lignocellulosic Biomass Cell Walls to Develop Optimized Feedstocks for the Bioderived Value Chain

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Liz Ware

Researcher/Scientist

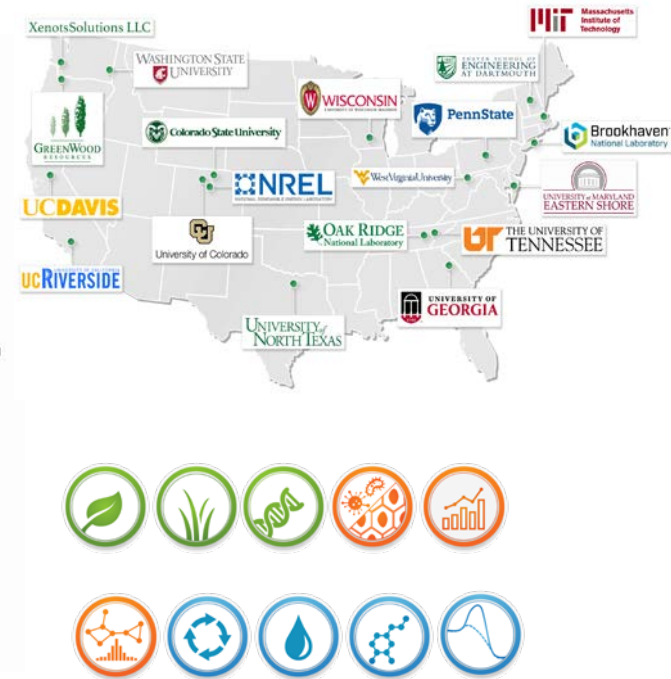
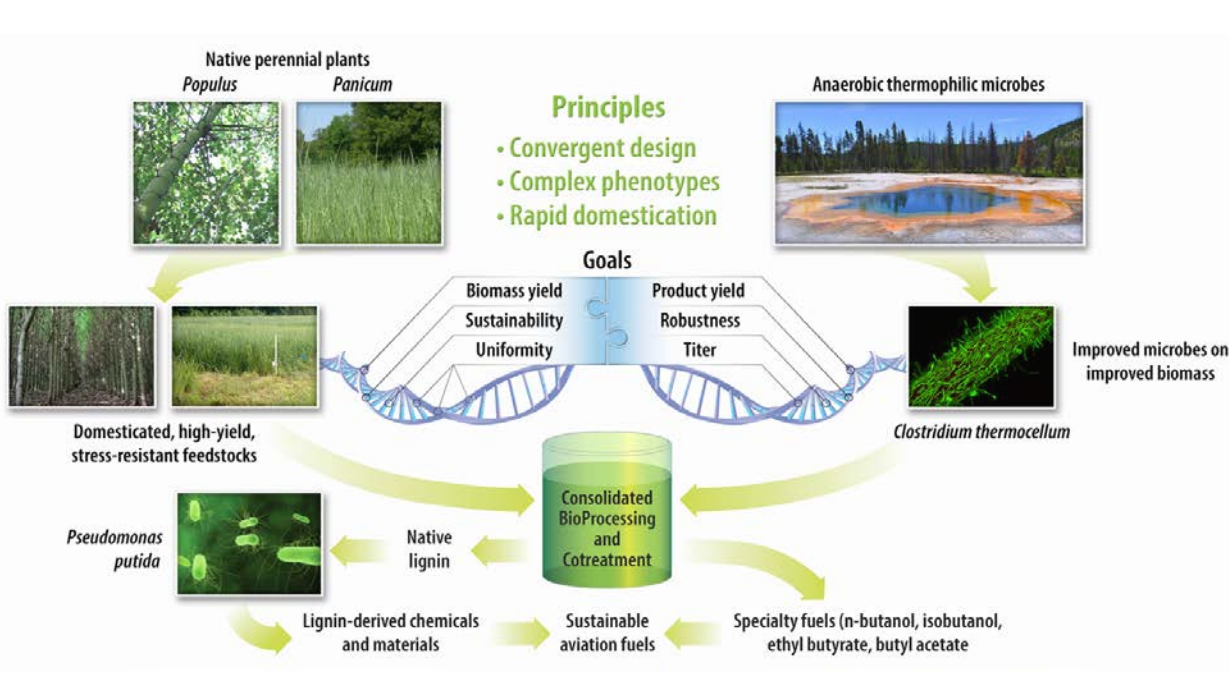
- B.S. Chemistry, Concord University, WV
- Ph.D. Chemistry, Univ. of Kentucky, Center for Applied Energy Research
- NREL – postdoctoral research, staff
- PI in Center for Bioenergy Innovation
 - DEIA Task Force Chair
 - Data Management
- Outreach – DOE Science Bowl, others

Human

- Hiking, yoga, cooking, drawing
- Married, 2 kids, cat, turtle

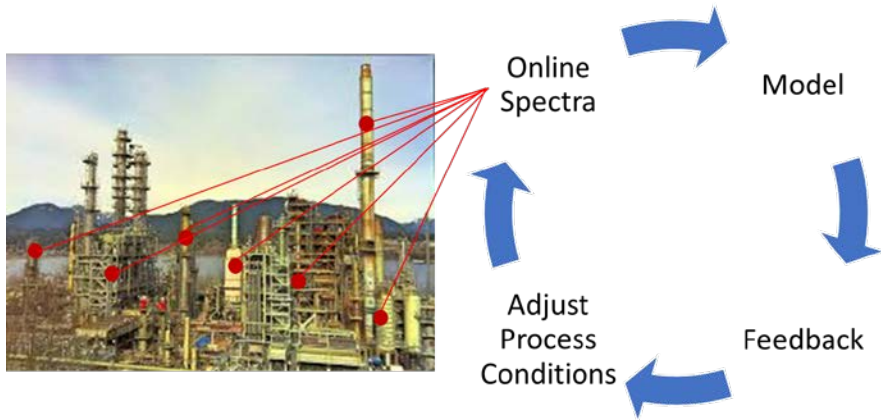


Center for Bioenergy Innovation (CBI)



DOE Office of Science Bioenergy Research Center (cbi.ornl.gov)

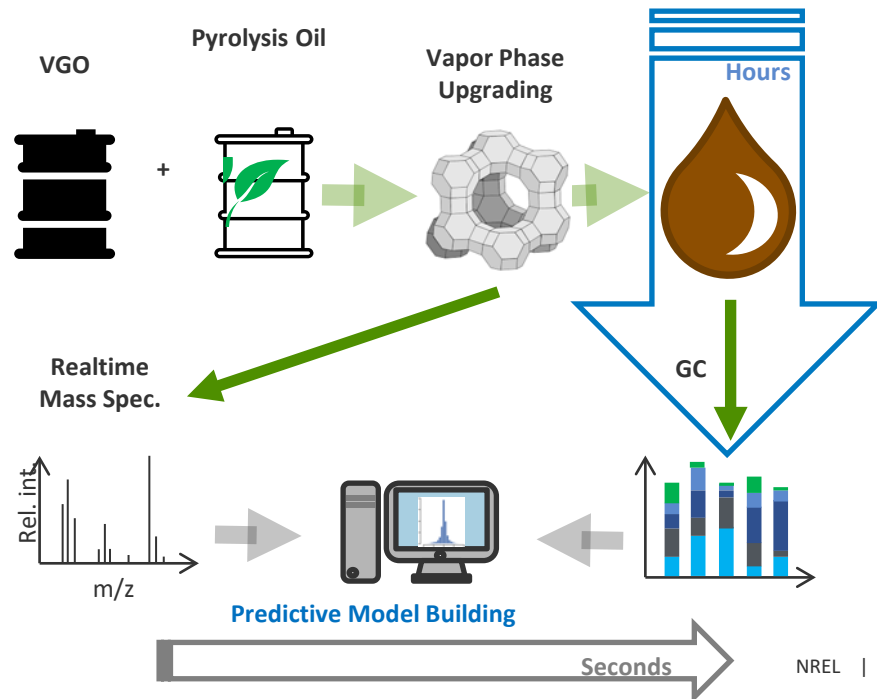
DOE Bioenergy Technologies Office: Systems Development & Integration Office: Process Monitoring and Performance



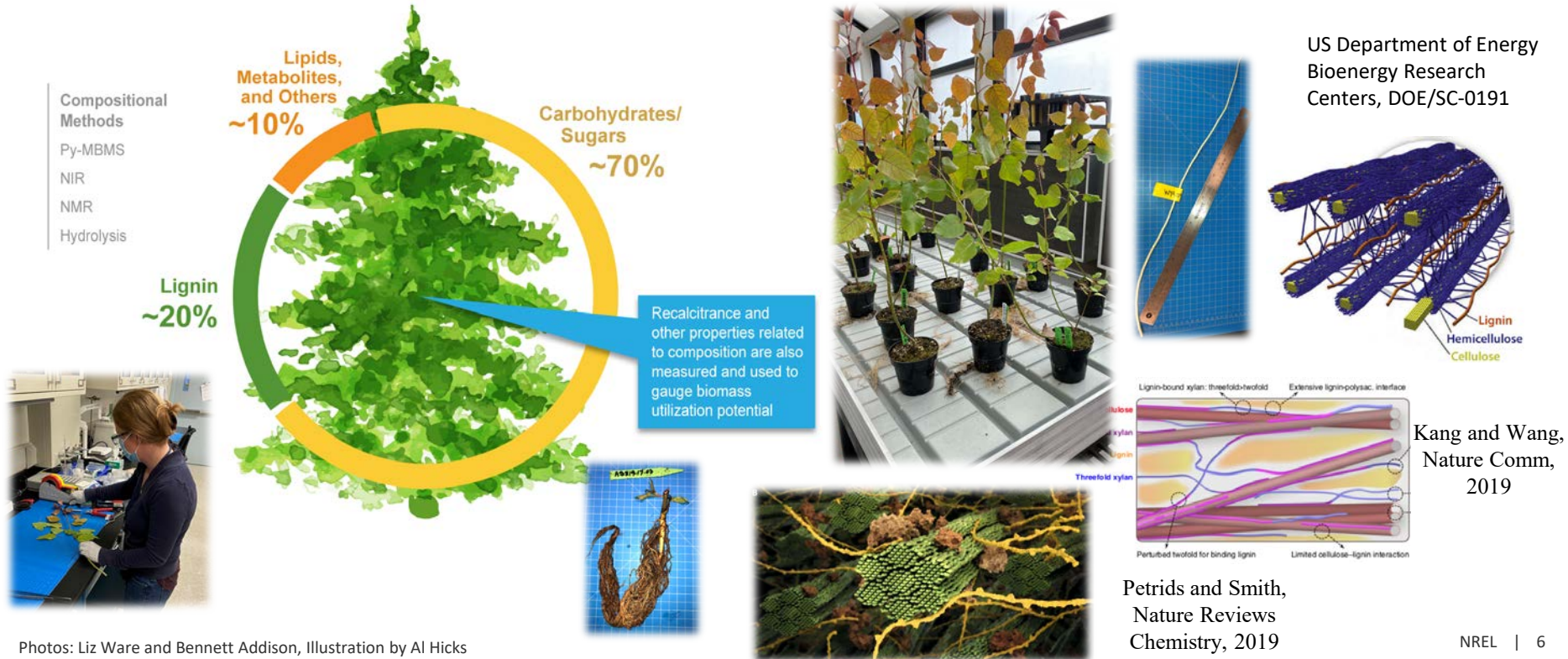
Benchmark: **gas chromatography-based** analysis of **condensed** product

Opportunity to obtain faster
(seconds to minutes vs. hours)
feedback on product composition
that is desired by refiners

Analytical Tool: **On-line**, slip stream
vapor phase **mass spectrometry**

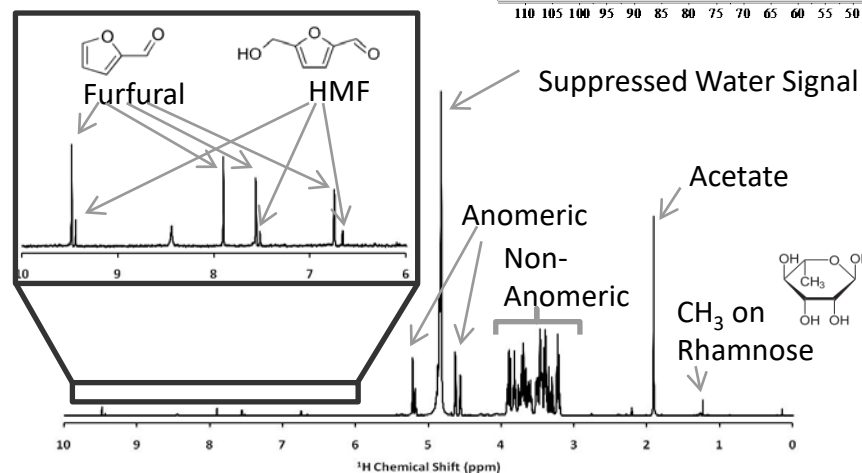
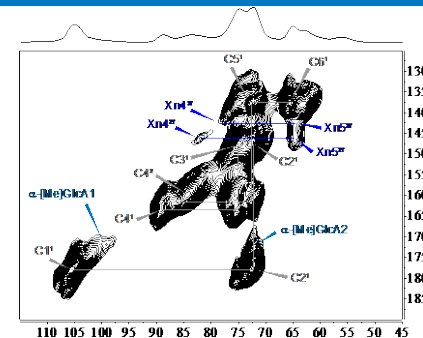


Lignocellulosic Biomass



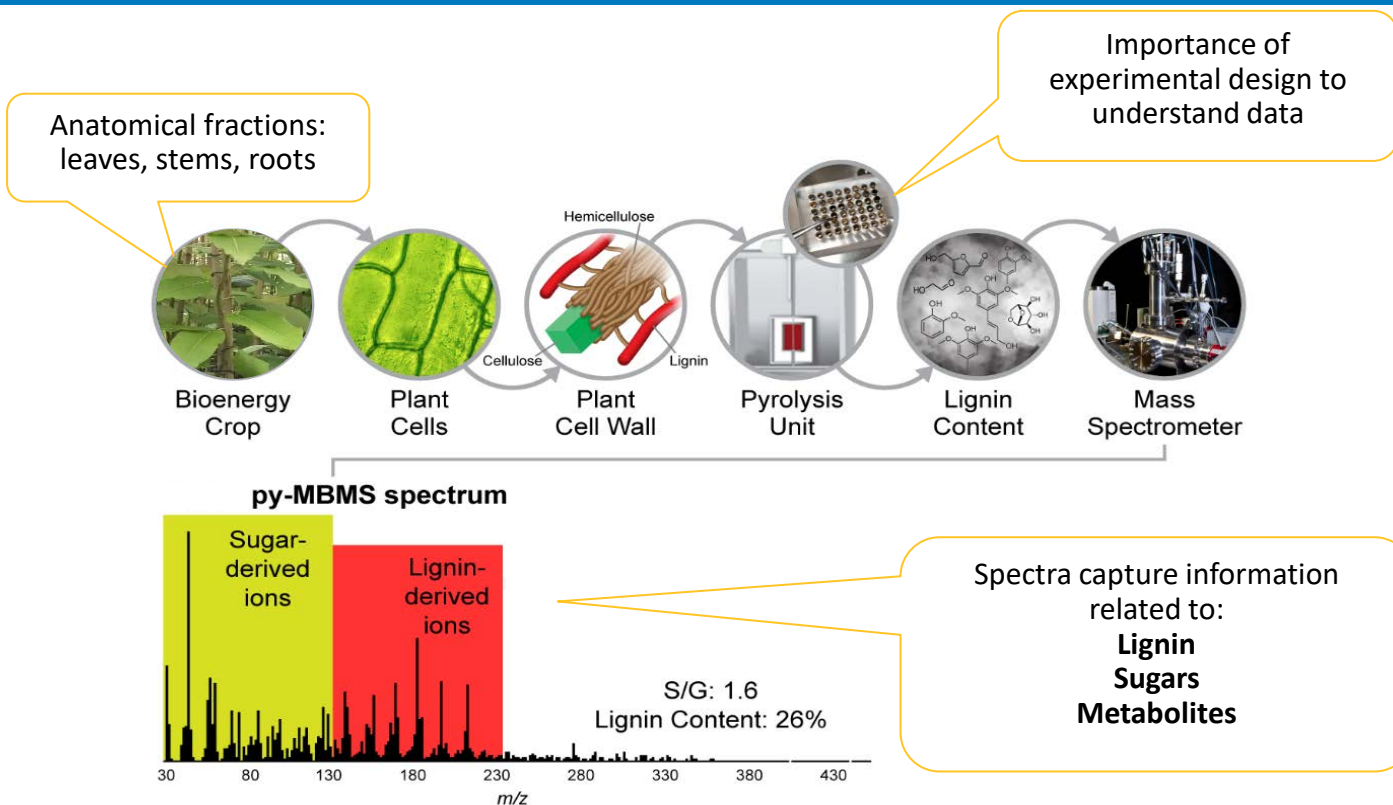
Analytical Methods for Studying Biomass - NMR

- Can analyze whole biomass, fractions and components (sugars, metabolites, lignin, etc.)
- Liquid/gel and solid state
- Various nuclei (^1H , ^{13}C , ^{15}N , etc.)
- Single and multidimensional
- Various throughput, sample prep



<https://www2.chem.wisc.edu/~cic/nmr/bruk-framestext.htm>

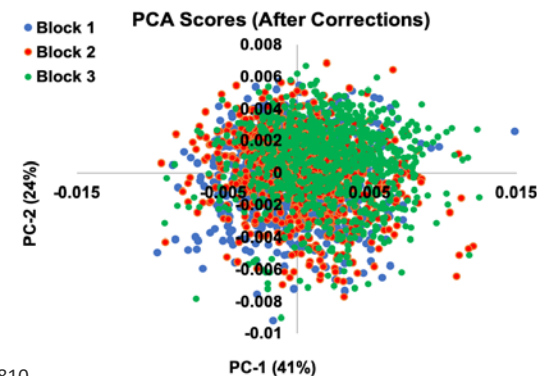
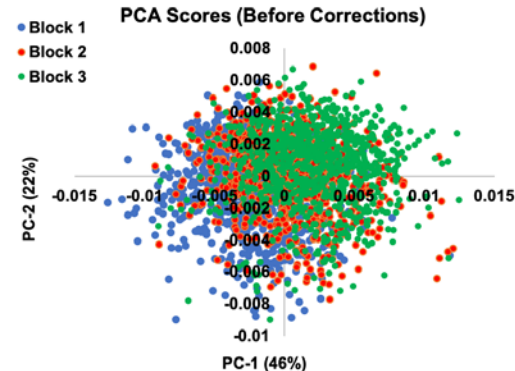
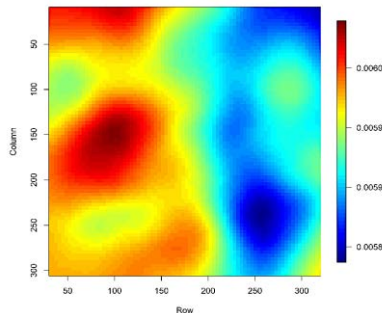
Analytical Methods – Pyrolysis-Molecular Beam Mass Spectrometry



High-throughput Analyses: Py-MBMS for *Populus* Wood Compositional Heritability

- To design and breed optimal Poplar (composition, growth and other phenotypes) we need to measure phenotypes across large population of genotypes and pedigrees, assign genes (genome-wide association studies) and determine heritability of genotypes
- We need to understand and account for genotype x environment (GxE) interactions at various scales, understand phenotypic plasticity

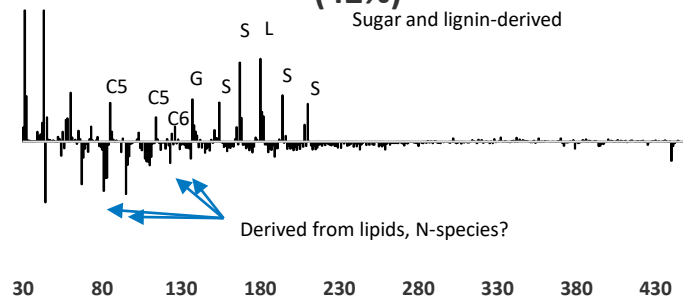
<i>Populus</i> family analysis (7x7, n= 2721)	Heritability (H^2)
Lignin content	0.56
S/G ratio	0.81
Glucose content	0.32
Xylose content	0.34



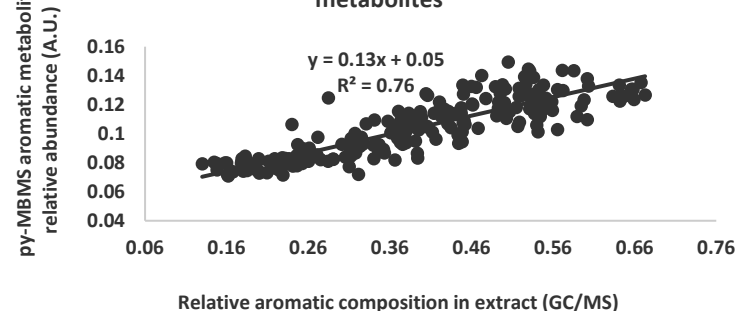
Py-MBMS for Other Applications

- Analysis of roots and leaves in *Populus*
 - Identify source of variance in composition
 - Aromatic metabolites, lipophilic polymers (suberin, cutin, etc.) for insights on resilience in response to biotic/abiotic stress
 - GWAS on ion features

GWAS *Populus* roots PC-1 Loadings
(42%)



Py-MBMS simplified ion method to predict aromatic metabolites



Kalluri, U.C., Harman-Ware, A.E., *et al.* Variation in root chemistry offers potential for optimizing above-below-ground plant systems *submitted to Nature Plants*

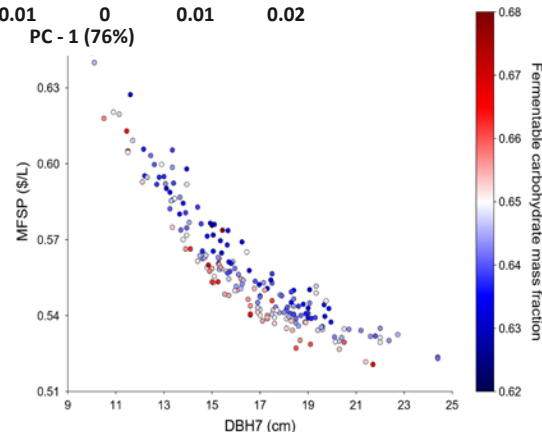
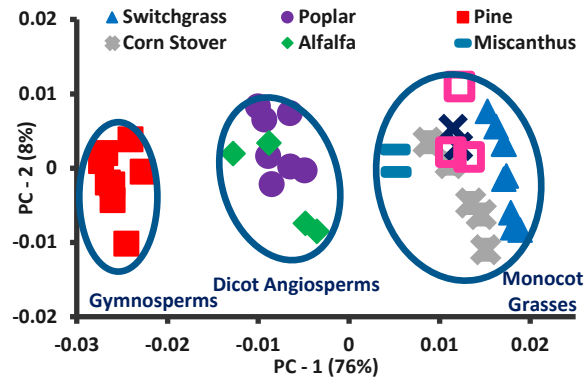
Harman-Ware, A.E., *et al.* Rapid screening of secondary aromatic metabolites in *Populus trichocarpa* leaves

[Doi:10.21203/rs.3.rs-96304/v1](https://doi.org/10.21203/rs.3.rs-96304/v1)

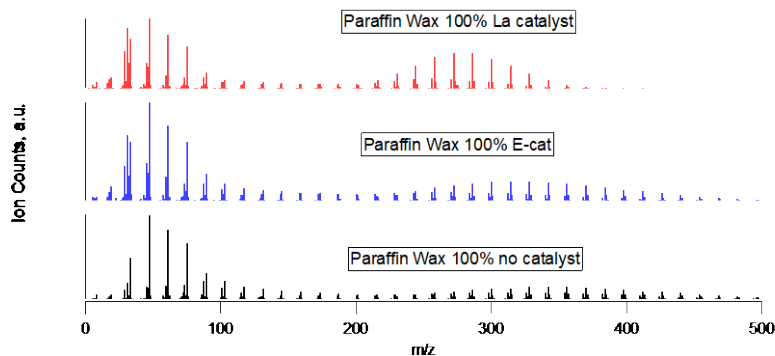
NMR for Moderate Throughput Sugar Composition Analyses – Contribution to TEA

Renee.Happs@nrel.gov

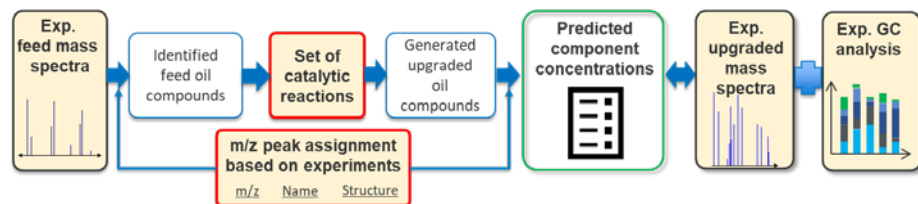
- Microscale 2-stage acid hydrolysis followed by ^1H NMR analysis of hydrolysate
- Construct models using NMR spectra and HPLC data, predict sugar composition in GWAS population, perform technoeconomic analyses
- “...ethanol yield per land area per year and minimum fuel selling price were most strongly impacted by tree size, when considering the largest 25% of trees, size and carbohydrate content were nearly identical influences on minimal fuel selling price, highlighting the need to focus on both size and carbohydrate content in selecting economically optimal feedstocks”
- Also use data for GWAS to assign genes related to sugar composition!



High-throughput screening to build on-line product prediction models (PMP)

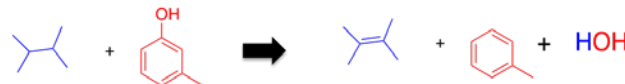


Identified co-processing reactions from literature and historic data and incorporated into new neural network model for co-processing



Example SMARTS pattern of hydrodeoxygenation (HDO) via hydrogen transfer from alkane (VGO) to phenolics (bio-oil) during co-processing:

$[C:1]-[C:2]. [c:3]1:[c:4]:[c:5]:[c:6]:[c:7](-[O:9]):[c:8]:1 >> [C:1]=[C:2]. [c:3]1:[c:4]:[c:5]:[c:6]:[c:7]:[c:8]:1. [O:9]$

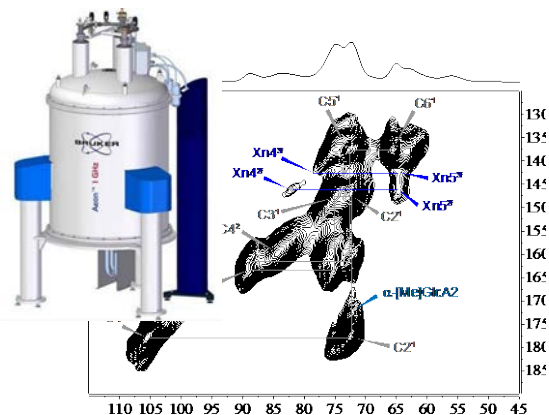


Catalysts supplied by Johnson Matthey

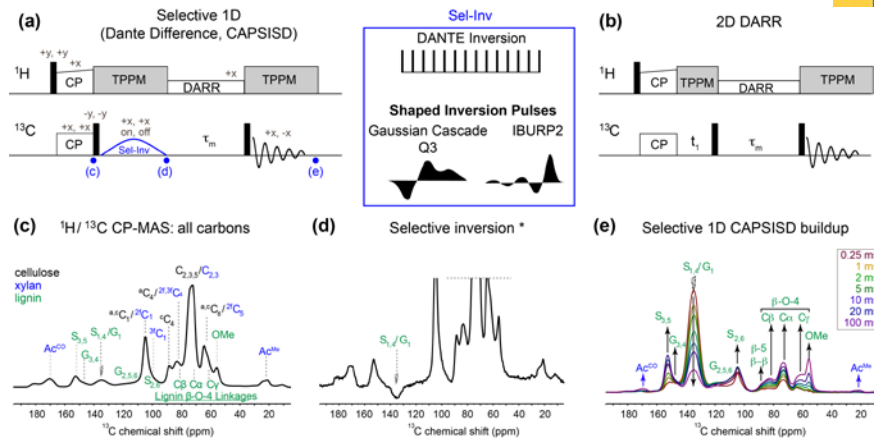
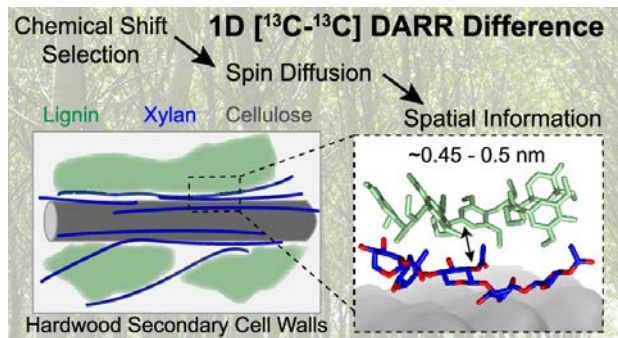
Compound	E-cat	La Catalyst
1-hexene	aromatics	aromatics
Benzene	no upgrading	no upgrading
Tetralin	no upgrading	no upgrading
2,4-dimethylphenol	alkylated products	alkylated products
Toluene	no upgrading	no upgrading
ethylbenzene	no upgrading	cracking products
cyclohexane	aromatics, olefins	aromatics, olefins
ethylcyclohexane	aromatics	no upgrading
n-hexane	no upgrading	no upgrading
2,3-dimethylbutane	no upgrading	no upgrading
furfuryl alcohol	aromatics, cracking products	aromatics
Guaiacol	cracking products	cracking products, aromatics
isoeugenol	cracking products, olefins	cracking products
naphthalene	no upgrading	no upgrading
1-methylnaphthalene	alkylated products, cracking products	no upgrading
phenanthrene	no upgrading	no upgrading
Glycerol	cracking products	cracking products
Palmitic acid	cracking products	cracking products
Paraffin wax (85%)	cracking products, aromatics	cracking products, aromatics
Paraffin wax (100%)	cracking products, aromatics	cracking products, aromatics



Bennett.Addison@nrel.gov



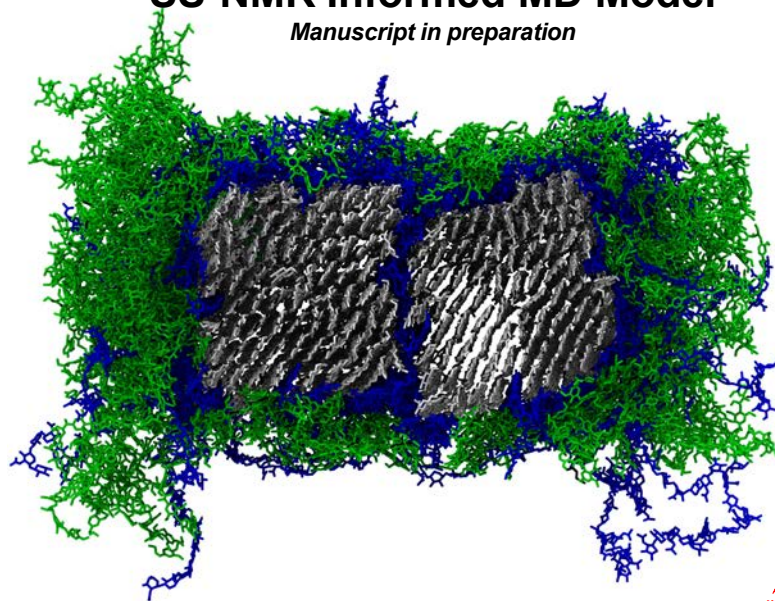
Biochemistry 54, no. 14 (2015): 2335–45.
Nature Communications 7, no. 1 (2016): 13902.
Nature Plants 3, no. 11 (2017): 859–65.
Nature Communications 10, no. 1 (2019): 4978.
Nature Communications 10, no. 1 (2019): 347.
J. Phys Chem B 124, no. 44 (2020): 9870–9883.



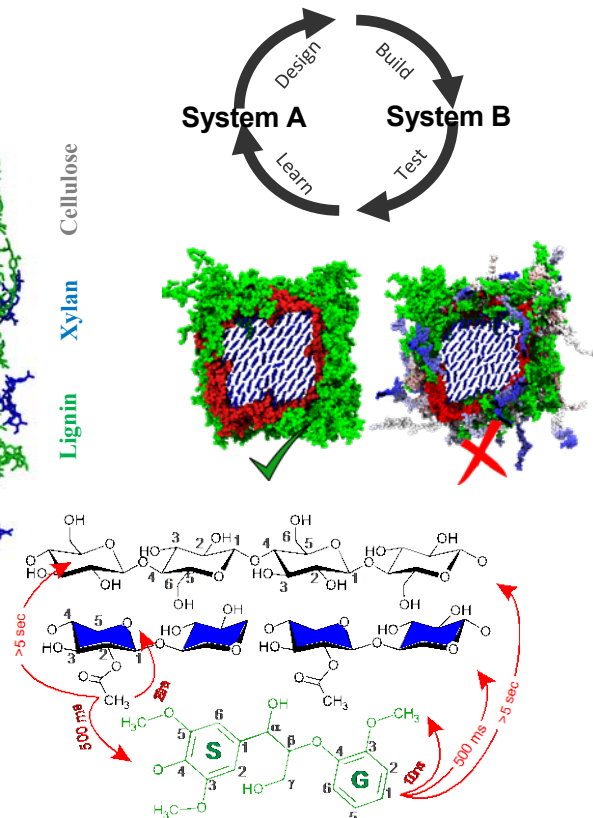
Secondary Cell Wall Architecture - Models

SS-NMR Informed MD Model

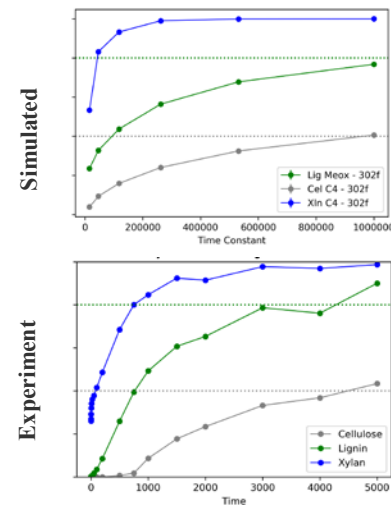
Manuscript in preparation



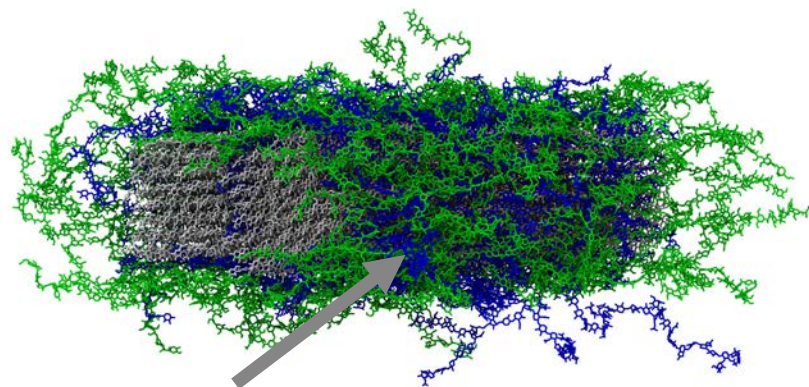
Example Observable:
Inter-polymer distance
estimates from ssNMR



Compare experiment to simulation (Polymer-Polymer Contacts)

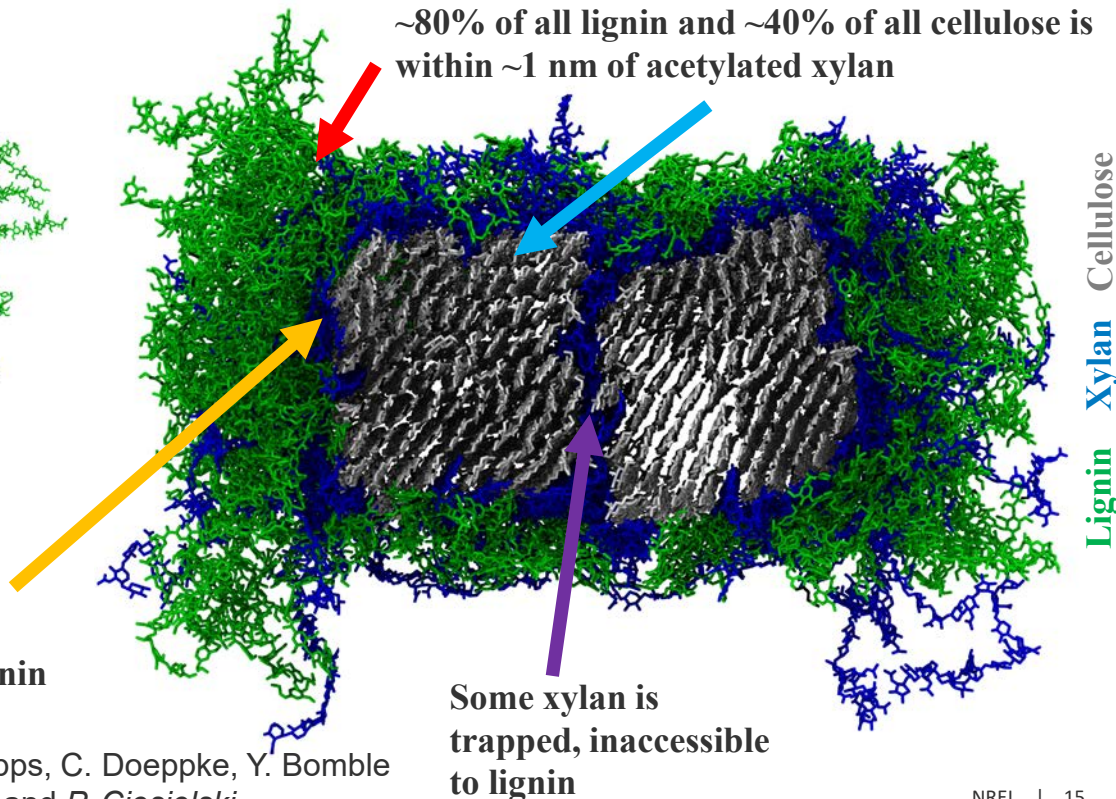


Secondary Cell Wall Architecture - Models



Lignin and xylan are nano-heterogeneously mixed such that large isolated lignin globules (>1-2 nm) are minimal

xylan is “in the middle” between cellulose and lignin, and primarily adopts a linearized 2-fold conformation so that acetyl decorations face lignin



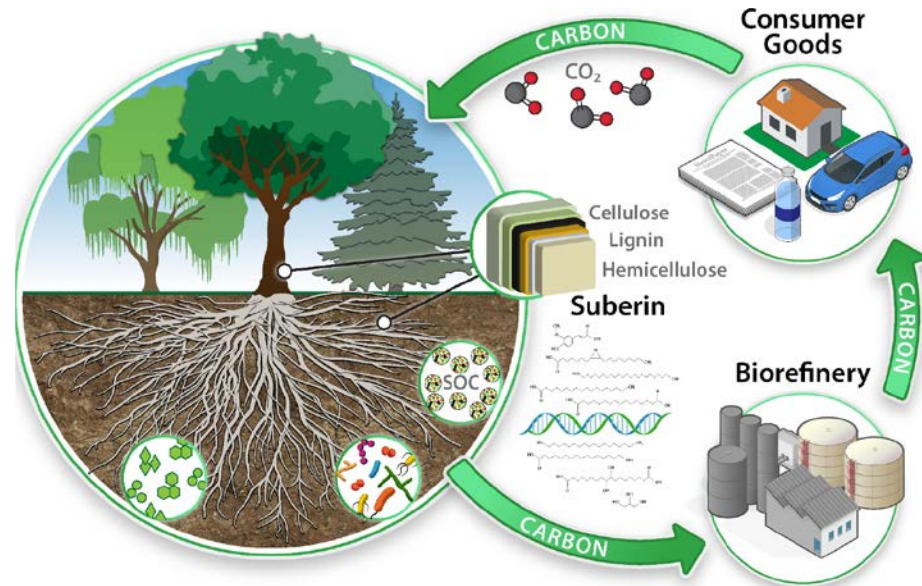
Experimental: Bennett Addison, L. Ware, R. Happs, C. Doeppke, Y. Bomble
Modeling: L. Bu, V. Bharadwaj, M. Crowley, and P. Ciesielski

Future Directions

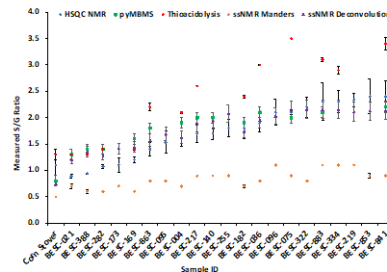
- Suberin
- Roots
- C sequestration
- Cell wall adhesion
- In-field analyses
- Sustainable aviation fuels
- More models



Photo credit: Udaya Kalluri



Further Reading



- Harman-Ware AE, Happs RM, Macaya-Sanz D, Doeppke C, Muchero W and DiFazio SP (2022) Abundance of Major Cell Wall Components in Natural Variants and Pedigrees of *Populus trichocarpa*. *Front. Plant Sci.* 13:757810. doi: 10.3389/fpls.2022.757810
- Anne K. Starace, David D. Lee, Kristen T. Hietala, Yeonjoon Kim, Seonah Kim, Anne E. Harman-Ware, and Daniel L. Carpenter, *ACS Sustainable Chemistry & Engineering* **2022** 10 (1), 234-244, DOI: 10.1021/acssuschemeng.1c05916
- Stefano Dell’Orco, Steven M. Rowland, Anne E. Harman-Ware, Daniel Carpenter, Thomas Foust, Earl D. Christensen & Calvin Mukarakate (2022) Advanced spectrometric methods for characterizing bio-oils to enable refineries to reduce fuel carbon intensity during co-processing, *Applied Spectroscopy Reviews*, 57:1, 77-87, DOI: [10.1080/05704928.2021.1920030](https://doi.org/10.1080/05704928.2021.1920030)
- Harman-Ware, A.E., Sparks, S., Addison, B. *et al.* Importance of suberin biopolymer in plant function, contributions to soil organic carbon and in the production of bio-derived energy and materials. *Biotechnol Biofuels* **14**, 75 (2021). <https://doi.org/10.1186/s13068-021-01892-3>
- Nag A, Gerritsen A, Doeppke C, Harman-Ware AE. Machine Learning-Based Classification of Lignocellulosic Biomass from Pyrolysis-Molecular Beam Mass Spectrometry Data. *International Journal of Molecular Sciences*. 2021; 22(8):4107. <https://doi.org/10.3390/ijms22084107>
- Happs, R.M., Addison, B., Doeppke, C. *et al.* Comparison of methodologies used to determine aromatic lignin unit ratios in lignocellulosic biomass. *Biotechnol Biofuels* **14**, 58 (2021). <https://doi.org/10.1186/s13068-021-01897-y>
- Harman-Ware, A.E., Macaya-Sanz, D., Abeyaratne, C.R. *et al.* Accurate determination of genotypic variance of cell wall characteristics of a *Populus trichocarpa* pedigree using high-throughput pyrolysis-molecular beam mass spectrometry. *Biotechnol Biofuels* **14**, 59 (2021). <https://doi.org/10.1186/s13068-021-01908-y>
- Bennett Addison, Dillan Stengel, Vivek S. Bharadwaj, Renee M. Happs, Crissa Doeppke, Tuo Wang, Yannick J. Bomble, Gregory P. Holland, and Anne E. Harman-Ware, *The Journal of Physical Chemistry B* **2020** 124 (44), 9870-9883, DOI: 10.1021/acs.jpcc.0c07759
- Ahlawat YK, Nookaraju A, Harman-Ware AE, Doeppke C, Biswal AK and Joshi CP (2021) Genetic Modification of KNAT7 Transcription Factor Expression Enhances Saccharification and Reduces Recalcitrance of Woody Biomass in Poplars. *Front. Plant Sci.* 12:762067. doi: 10.3389/fpls.2021.762067
- Ohlsson, J.A., Harman-Ware, A.E., Sandgren, M. *et al.* Biomass Recalcitrance in Willow Under Two Biological Conversion Paradigms: Enzymatic Hydrolysis and Anaerobic Digestion. *Bioenerg. Res.* **13**, 260–270 (2020). <https://doi.org/10.1007/s12155-019-10079-6>
- Happs, Renee M., Harman-Ware, Anne E., Ben, Haoxi, and Ferrell III, Jack R. *Determination of Carbon Functional Groups in Pyrolysis Bio-Oils using ¹³C NMR. Laboratory Analytical Procedure (LAP), Issue Date: October 7, 2021.* United States: N. p., 2021. Web. doi:10.2172/1825871.

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Brian Davison

Stan Martin

Katrien Devos

Udaya Kalluri

Tom Pendergast

Mitra Mazarei

Tom Juenger

Questions? Thank you!

www.nrel.gov

NREL/PR-2800-81764

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