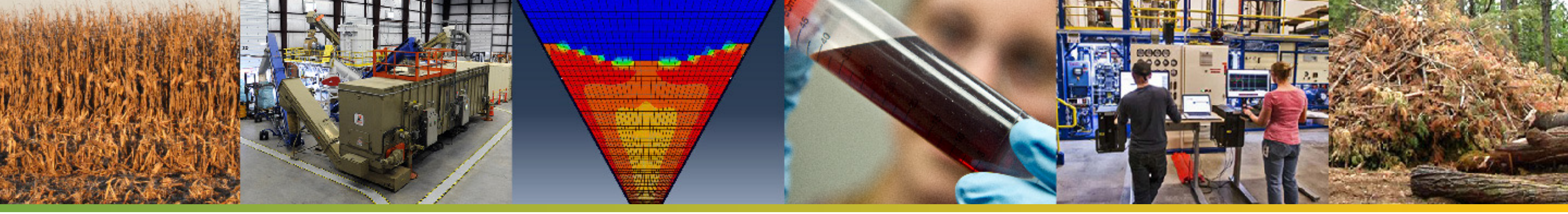

DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

FCIC Task 7 – Low-Temperature Conversion

April 6, 2023
Feedstock-Conversion Interface Consortium (FCIC)

Lead: Philip Laible[1] and Co-Lead: James Gardner[2]

[1]Argonne National Laboratory

[2]Lawrence Berkeley National Laboratory

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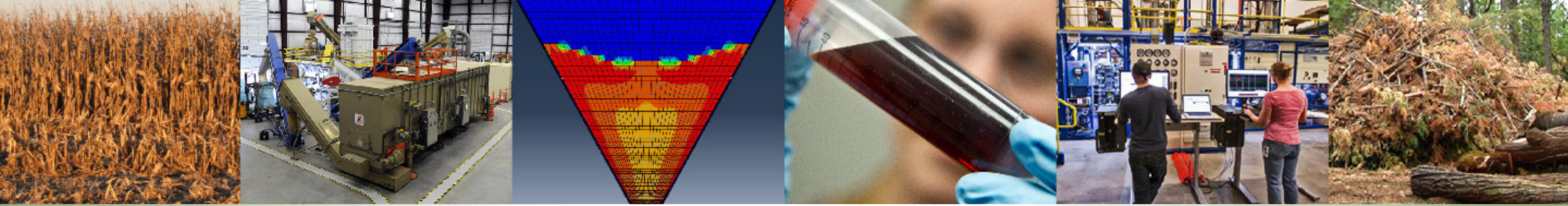
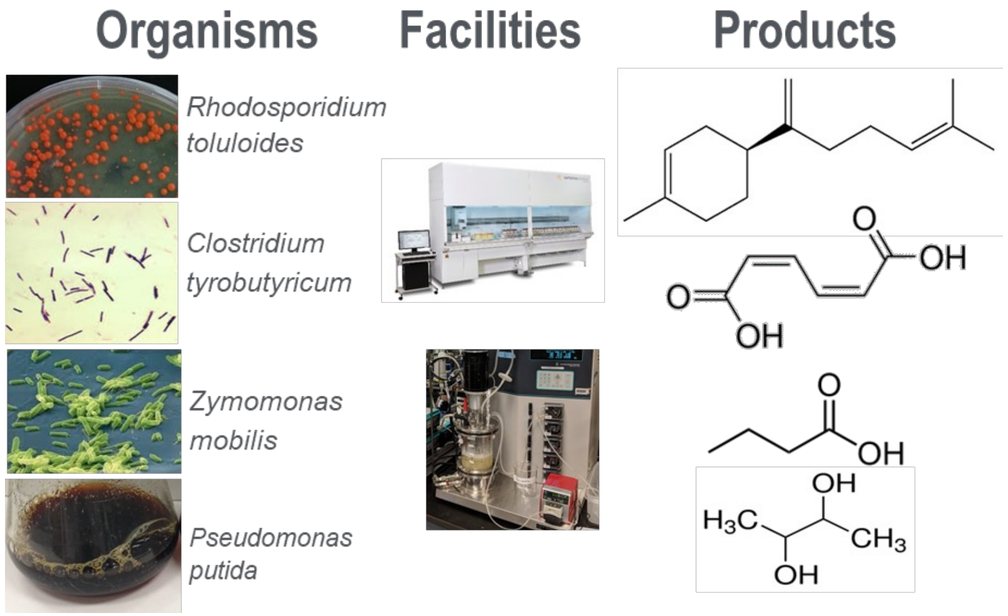


Project Overview

Task 7 Project Overview

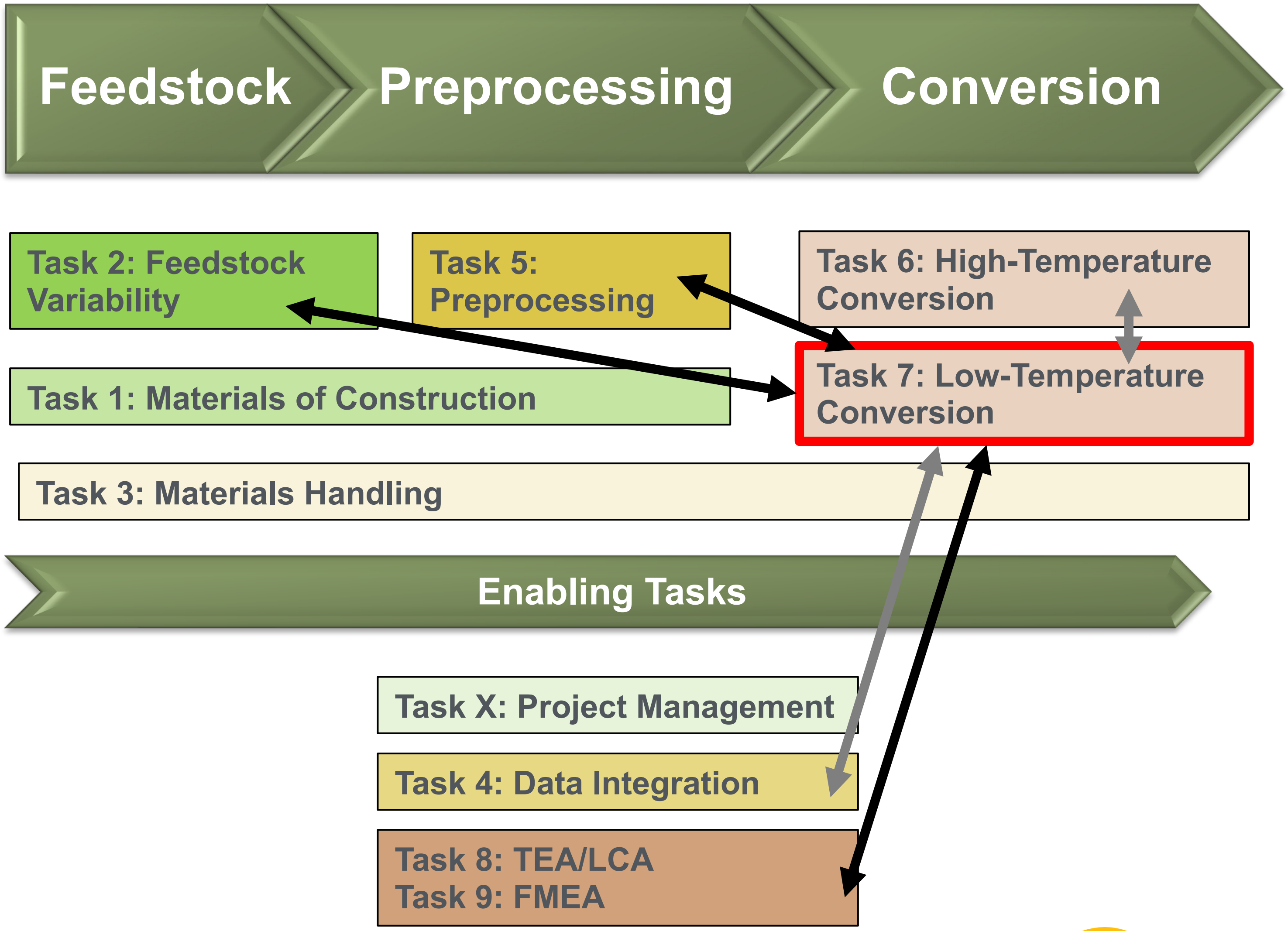
Goals: Determine the effects of biomass feedstock variability on the low-temperature conversion processes (both sugar and lignin pathways) and develop tools to mitigate the risks posed by this variability.

- **Current limitations:** No systematic information is available presently that relates feedstock variability to low-T conversion performance across the entire supply chain (with economic and environmental viability considerations).
- **Relevance:** Meets BETO objectives by developing actionable findings and useful predictions for industrial partners that will enable sustainable production of aviation fuels from low-temperature processes from variable feedstocks, including MSW.



- **Risks and Mitigation Strategies:**
  - Comprehensive survey of existing feedstock variability impossible to approach experimentally (downselection critical but may mislead); mitigation by harnessing diverse consortium expertise and capabilities and knowledge from experienced industrial and academic partners
  - Variability of sourced feedstocks may prove insignificant for sugar or lignin utilization leading to product formation; mitigate or minimize by deployment of standardized protocols used in pretreatment/deconstruction.

FCIC Task Organization



Task X: Project Management: Provide scientific leadership and organizational project management

Task 1: Materials of Construction: Specify materials that do not wear, or break at unacceptable rates

Task 2: Feedstock Variability: Quantify & understand the sources of biomass resource and feedstock variability

Task 3: Materials Handling: Develop tools that enable continuous, steady, trouble free feed into reactors

Task 4: Data Integration: Ensure the data generated in the FCIC are curated and stored – FAIR guidelines

Task 5: Preprocessing: Enable well-defined and homogeneous feedstock from variable biomass resources

Task 6 & 7: Conversion (High- & Low-Temp Pathways): Produce intermediates for further processing

Task 8:Crosscutting Analyses TEA/LCA: Valuation of intermediate streams & quantify variability impact

Task 9:Failure Mode & Effects Analysis (FMEA): Standardized approach for assessing attribute criticality

Task 7 QbD Summary

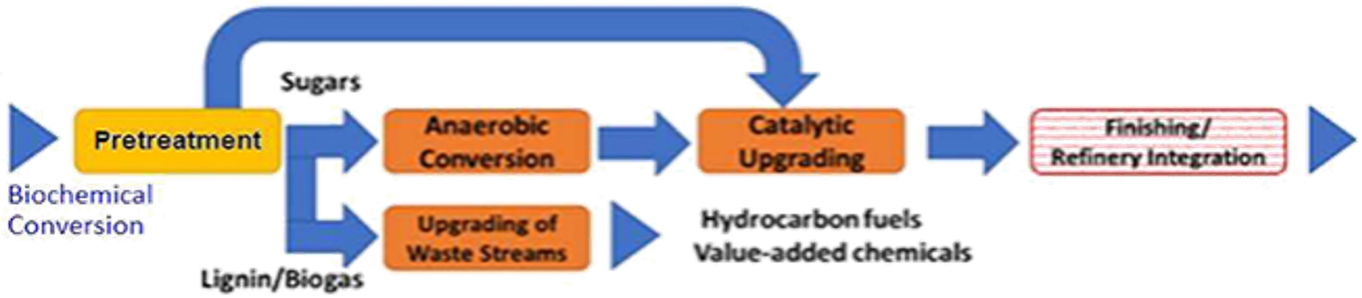
Unit Operation	CMA	CPP	CQA
Pretreatment	Anatomical fraction distribution	NaOH loading; duration and temperature of deacetylation	Sodium and/or acid counter ion levels in black liquor Lignin monomers in sugar streams
Deconstruction	Density of corn stover anatomical fractions	Duration and sieves needed in knife mill or crumbler	Size of particles heading into enzyme hydrolysis
Sugar Conversion	Lignin species left over from enzyme hydrolysis	Duration and rates of bioconversion step	Primarily, yields and purity of final product heading to down-stream processing
Lignin Conversion	Sodium, acid counter ions and inorganic species levels	Vary complexity or media additions to counter criticalities from pretreatment and feedstock variability	Primarily, yields and purity of final product heading to downstream processing

1 – Integration of Low Temperature Conversion

Required frequent, productive intra- and inter-consortium (and SOT) interactions:

- Feedstock Variability (materials selection / characterization)
- Data Integration (data storage and harmonized inputs to modeling)
- Preprocessing (preprocessing optimization)
- Crosscutting Analyses (economic and life-cycle sensitivities)
- Agile BioFoundry (host tolerances and engineering needs for economic bioconversion)
- Separations Consortium (Feedstock streams cleanup; final product purity)

Biochemical Conversion Pathway



BETO Research Consortia Coverage

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BIOPROCESSING SEPARATIONS CONSORTIUM

Agile BioFoundry

BIOPROCESSING SEPARATIONS CONSORTIUM

The Task 7 Team

Funding, diversity, and collaboration within low temperature conversion – interdisciplinary approaches spanning six national laboratories

Argonne NATIONAL LABORATORY

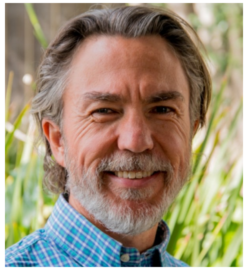
- Philip Laible
- Peter Larsen
- Gyorgy Babnigg
- Nicholas Dylla
- Alex Hertel





BERKELEY LAB

- James Gardner
- Akash Narani
- Deepti Tanjore
- Onyinye Okonkwo



NREL NATIONAL RENEWABLE ENERGY LABORATORY

- Ed Wolfrum
- Jeff Linger
- Davinia Salvachua
- Ilona Ruhl
- Robert Nelson
- Xiaowen Chen
- Jake Kruger
- Rui Katahira
- Ryan Davis
- Bryon Donohue




Sandia National Laboratories

- Bioproduct analytical team

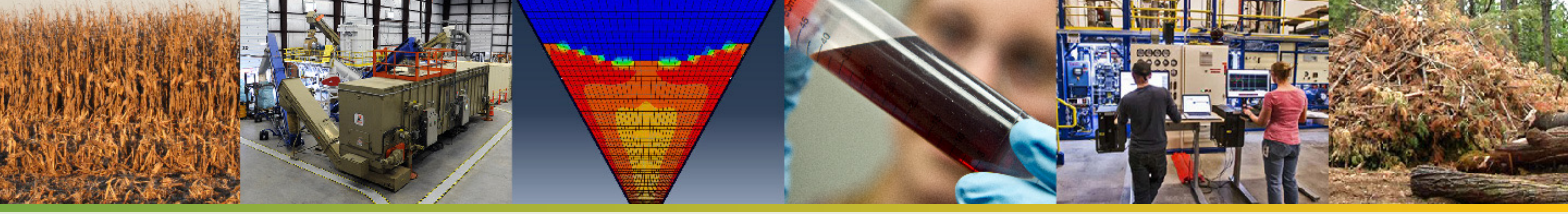
Pacific Northwest NATIONAL LABORATORY

- Jim Collett

INL Idaho National Laboratory

- Vicki Thompson
- Jordan Klinger
- Ling Ding

Thank you!



1 – Approach

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1 – Task 7 Approach: *Complementary experimental and modeling approaches*

Technical Approach: Coordinate experimental/modeling efforts across three laboratories to:

- Use standardized deconstruction strategies to produce sugar and lignin streams,
- Investigate the effects of feedstock variability on performance of biological (and chemical) conversion processes,
- Integrate experimental data with metabolic capacities and regulation to ascertain a first-principles understanding of the underlying biochemistry that is influenced by feedstocks; and
- Develop tools required by industry to predict the performance of downstream microbial (and chemical) conversion processes with variable inputs.

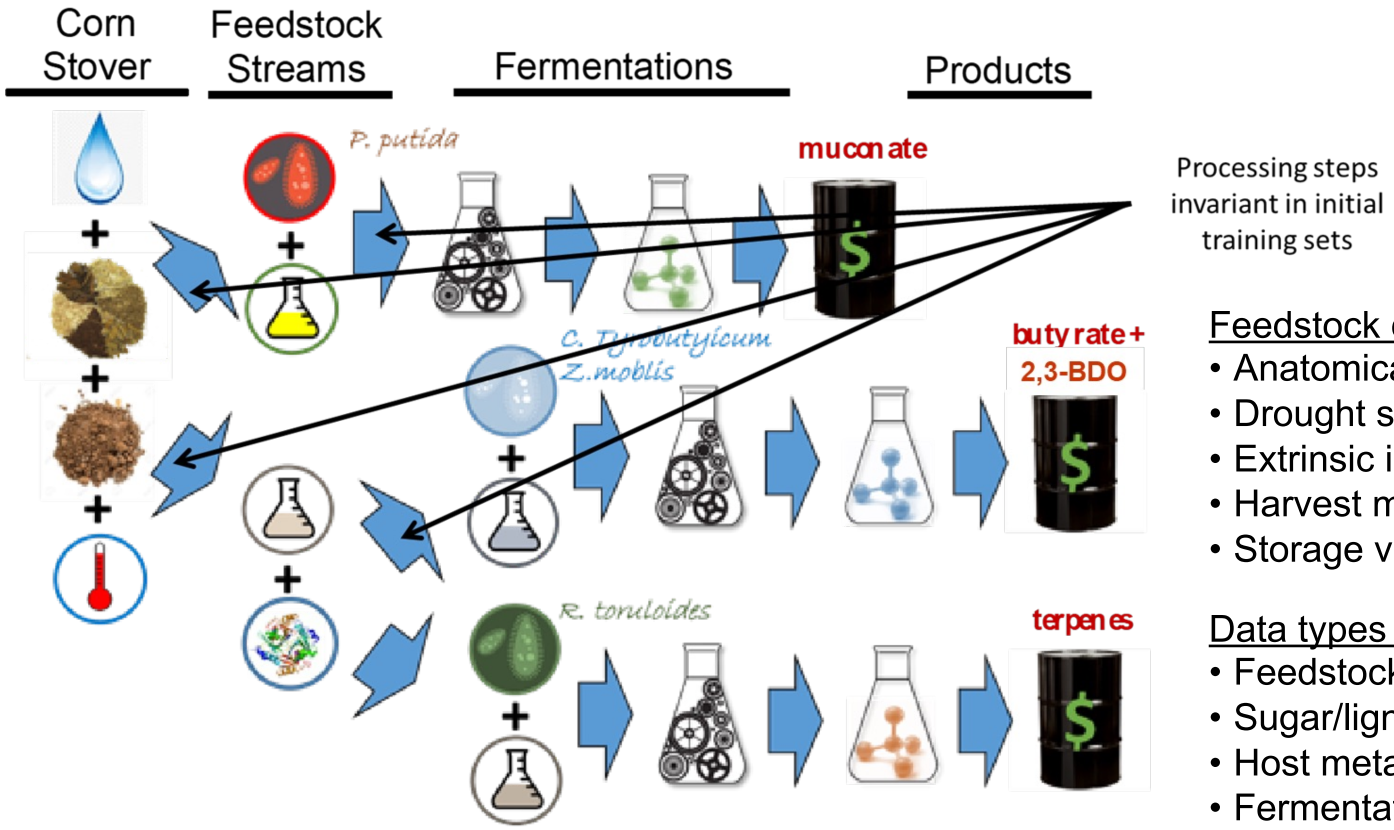
Challenges: Standardized protocols can mask influences of feedstock variability; microorganisms have different growth requirements and attribute tolerances; and difficulties exist in setting up controlled experiments that address the true extent of variability that is expected to be encountered.

Metrics: Generation of feedstock streams from corn stover stored or harvested in various conditions or fractionated anatomically. Performance variability of converting such streams four biocatalysts (e.g., titers, rates, and yields). Trained AI approaches use diverse biocatalyst performance (4 strains, 4 products) to validate AI tools by predicting performance of new organisms on variable sugar and lignin streams (match at 80%).

1 – Task 7 Approach: Complementary experimental and modeling approaches

Coordinated efforts needed for maximized learnings

- Determine operating ranges of key attributes in sugar and lignin streams from variable corn stover feedstocks that can be tolerated by a diverse set of organisms and processes
- Train machine-learning tools with metabolic models, no-cost literature information, and new experimental findings to develop predictive tools to shield future low temperature conversion processes from variability impacts



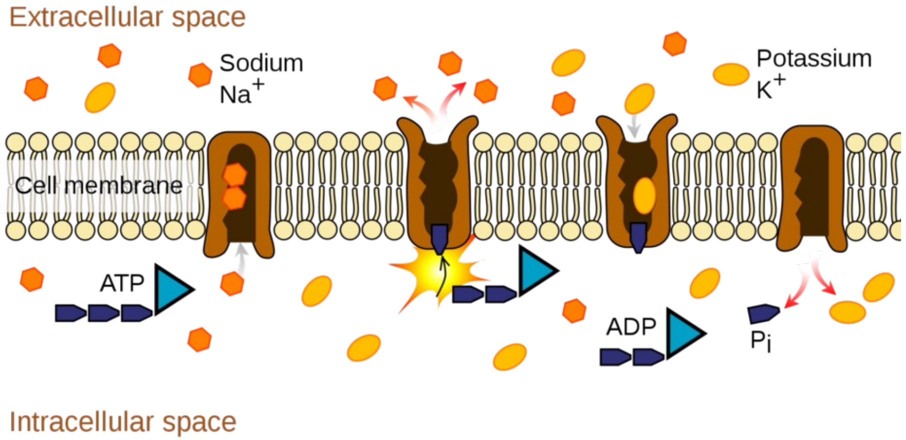
Feedstock campaigns pursued
- Anatomical fractions
- Drought series
- Extrinsic inorganic content
- Harvest methodology
- Storage variability

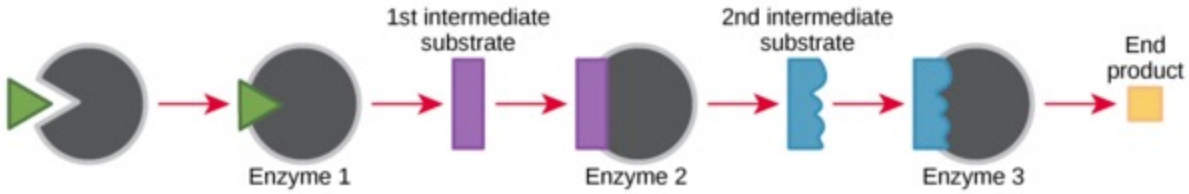
Data types used
- Feedstock history/composition
- Sugar/lignin stream composition
- Host metabolism
- Fermentation impacts (TRYs, tolerance ranges)
- Scaled operational assessments

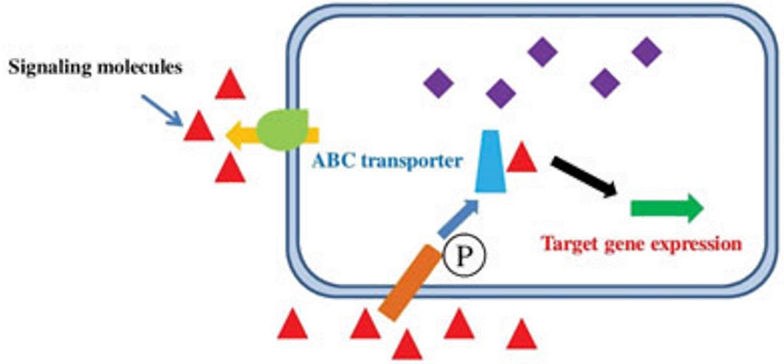
Expanding sets of data of quality and quantity now available for model training

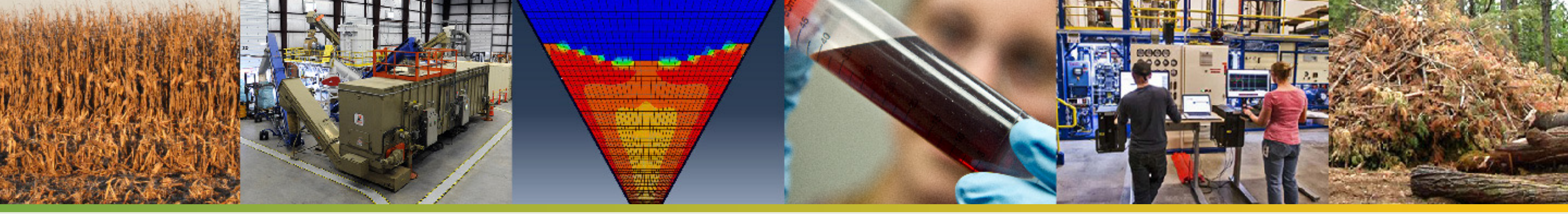
1 – Task 7 Approach: *Types of cellular interferences observed/ expected with low temperature processes*

- Cellular transport systems
- Metabolic pathway interference
- Sensing/Regulatory control
  - organism may be blind to attributes as lacking appropriate sensors; or alternatively hypersensitive to attributes
- If utilizing continuous processes, mismatches in ratios of utilization of vs. abundance in feedstock streams









2 – Progress and Outcomes

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2 – Survey of MAs to determine criticality

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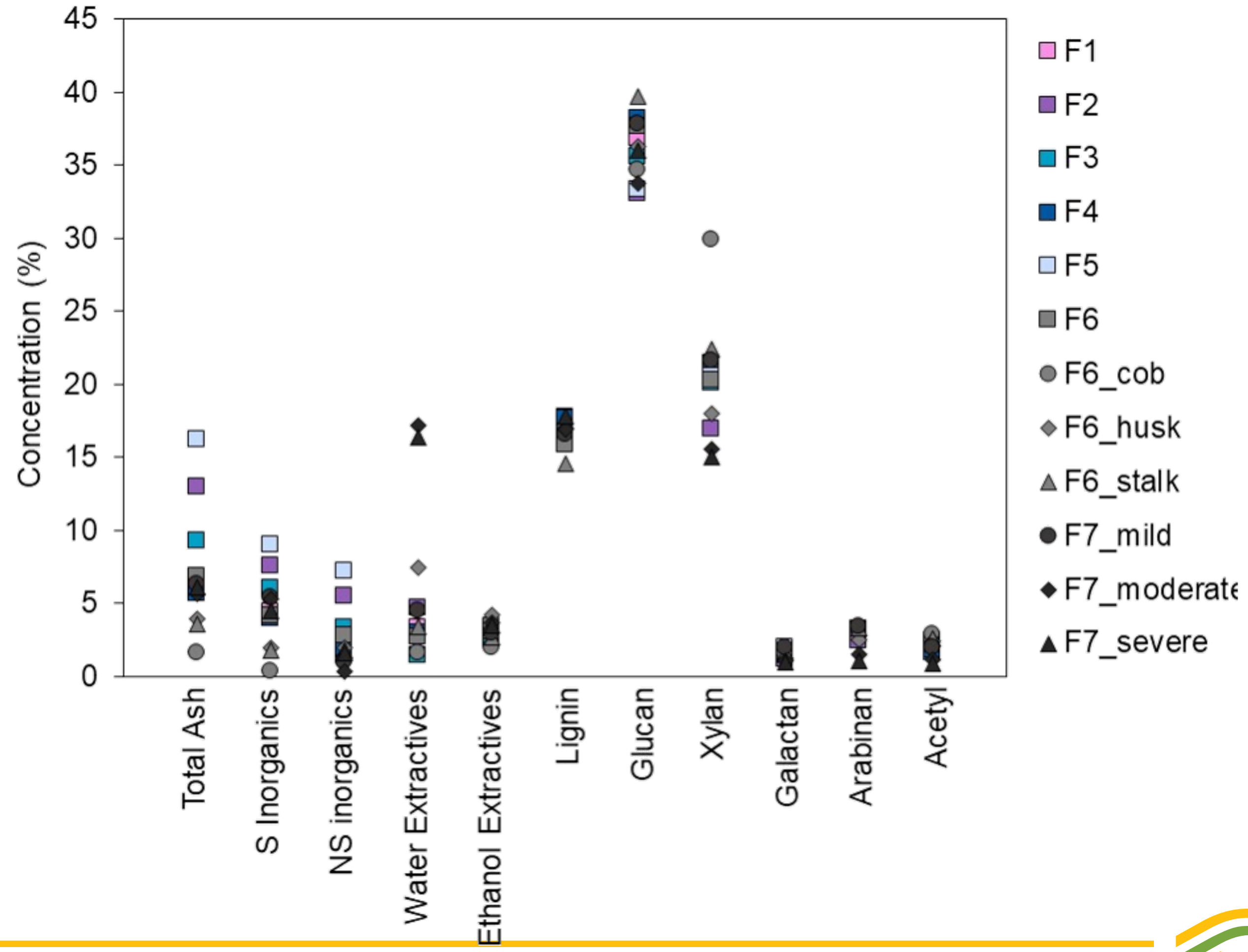
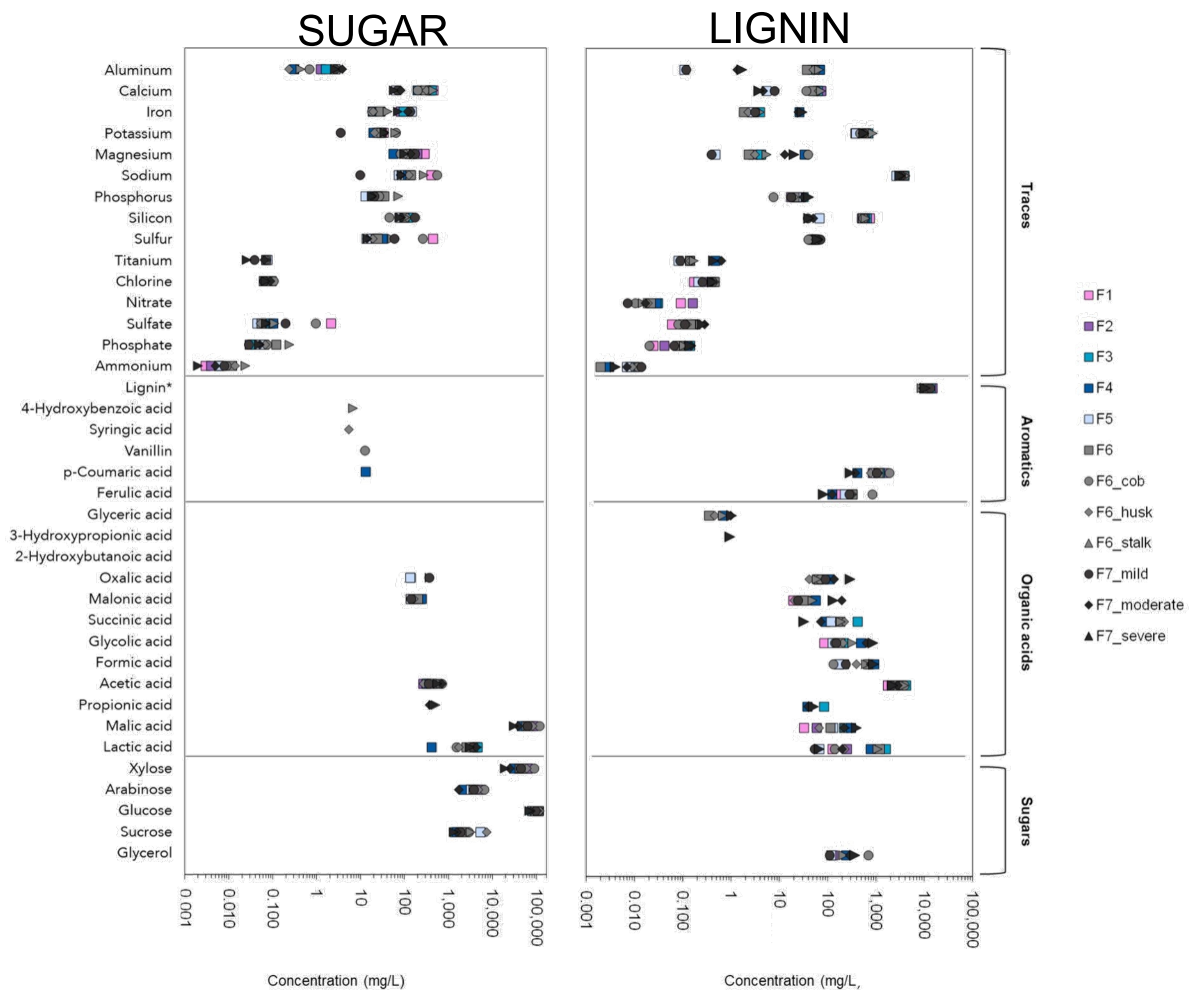
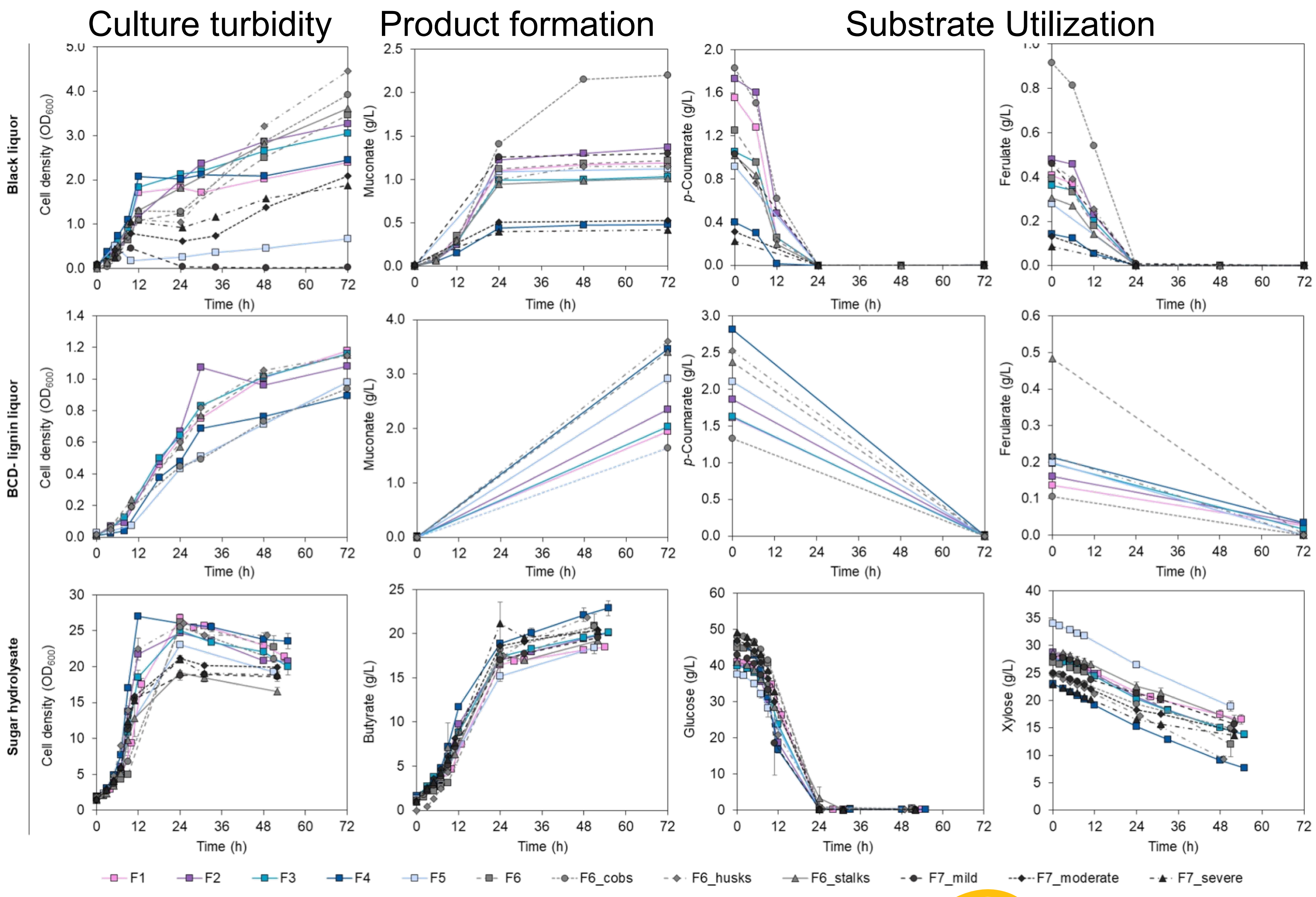
Examples of Materials Attributes Variability of Raw Feedstocks



Translation to (critical) materials attributes in sugar and lignin streams

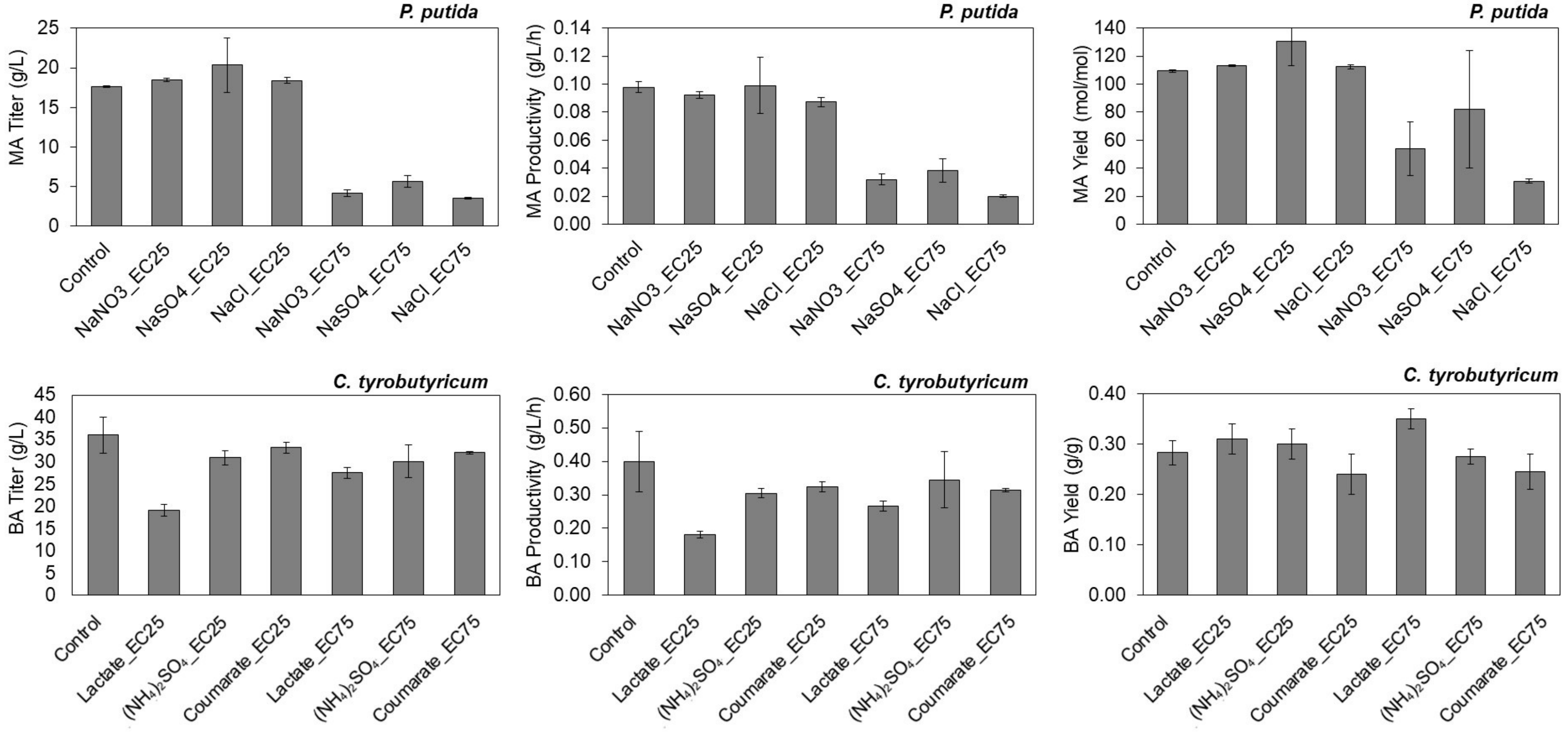


2 – Effects of (C)MAs on low temperature performance



- The extent of the impacts of variable materials attributes on the performance of low temperature processes (gauged by titers, rates, and yields) are used to determine criticality
- Criticality determination sets the stage for follow-up experiments that determine allowed operating ranges versus known concentration in feedstock streams expected to be used in biomanufacturing in the future

2 – Scaled evaluations used for crosscutting analyses



Economic analyses and environmental assessments required bench and/or pilot bioreactor campaigns to enable data required for comparative metrics at production scales

2 – Growing interaction networks for differential impacts of feedstock variation on LowT processes

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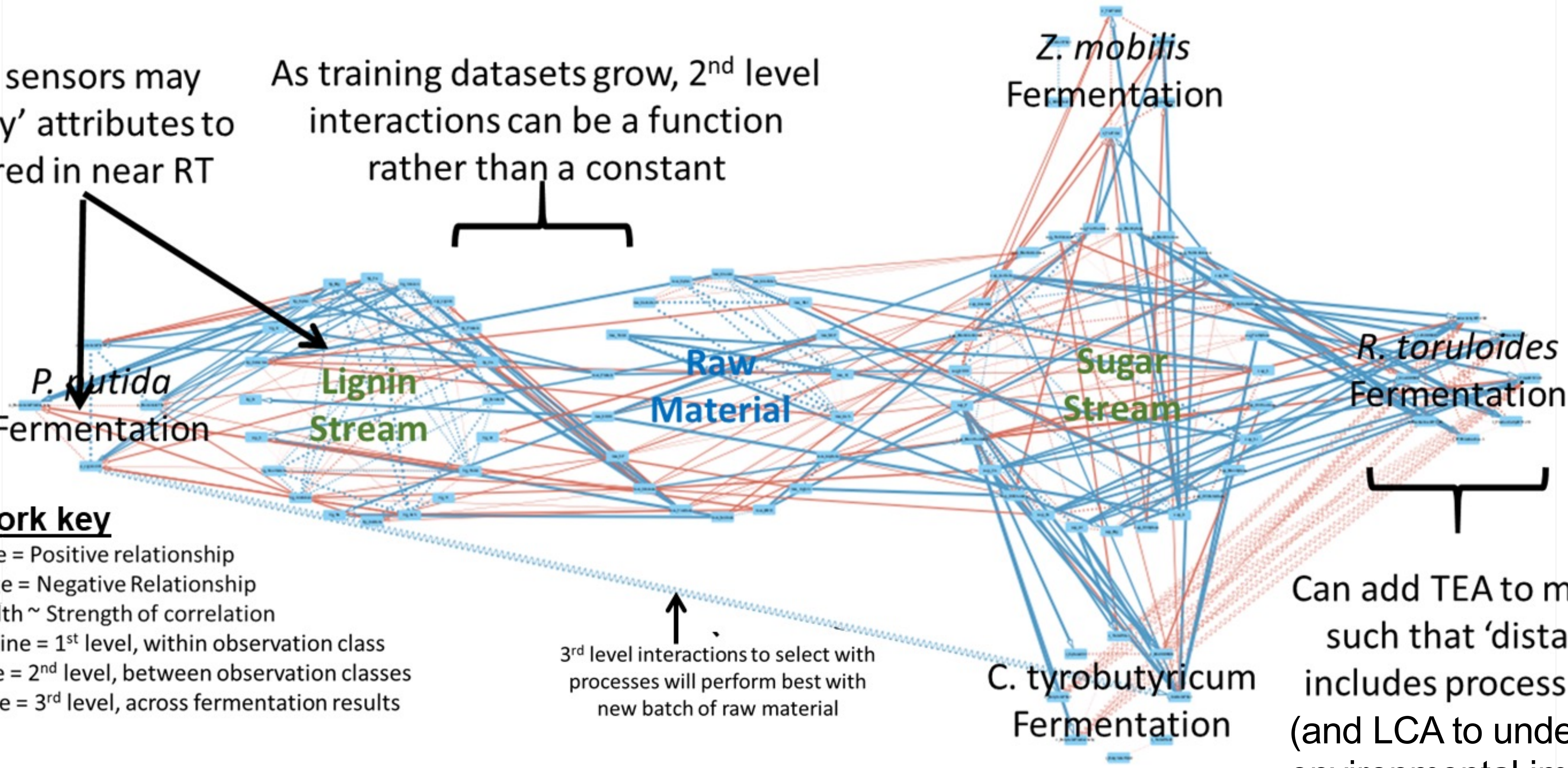
Advanced sensors may allow for ‘key’ attributes to be monitored in near RT

As training datasets grow, 2nd level interactions can be a function rather than a constant



Network key

Red edge = Positive relationship
Blue edge = Negative Relationship
Line Width ~ Strength of correlation
Dashed line = 1st level, within observation class
Solid line = 2nd level, between observation classes
Wavy line = 3rd level, across fermentation results

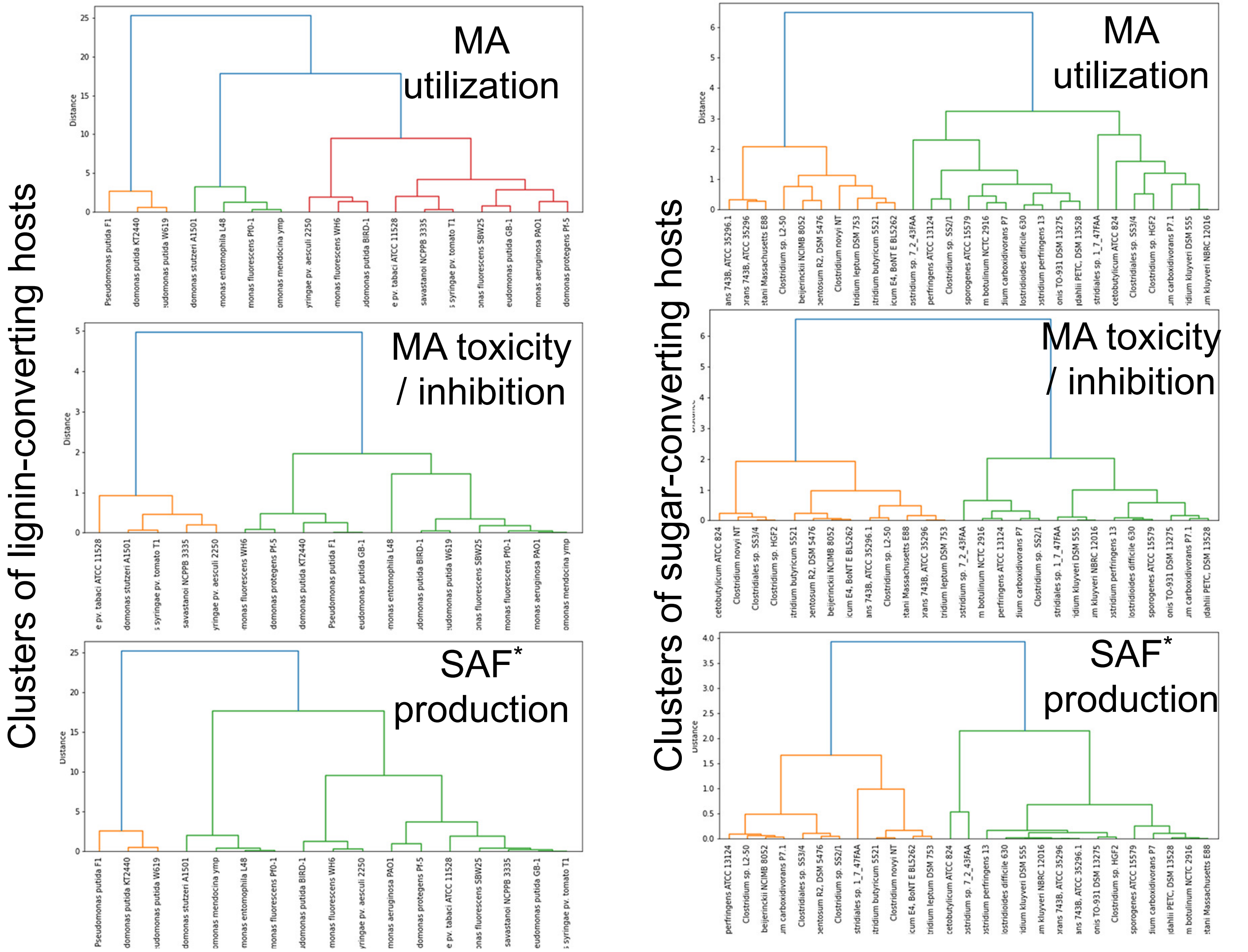
3rd level interactions to select with processes will perform best with new batch of raw material

Can add TEA to models, such that ‘distance’ includes process costs (and LCA to understand environmental impacts)

2 – Towards predictions and tool utilization

Metabolic clustering / model predictions:

- Metabolic reconstructions have been compared for over 3000 organisms, separately for sugar and lignin conversion to bioproducts serving SAF market.
- Expected similarities/differences known for CMA susceptibility from experimental teams. Validation and reinforcement underway.



*referring to SAF or SAF-precursor production potential

2 – Drought stress does not significantly impact LowT conversion performances

Knowledge

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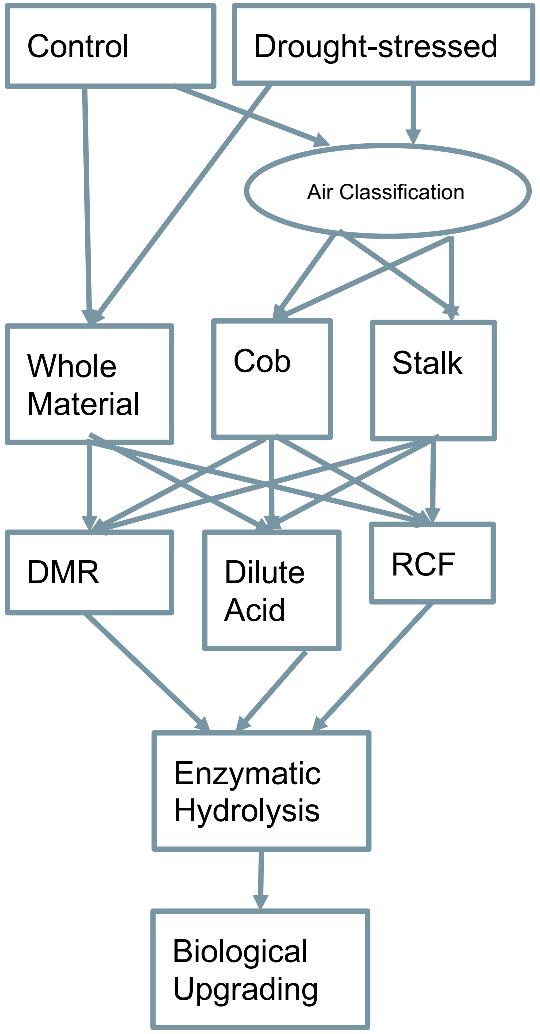
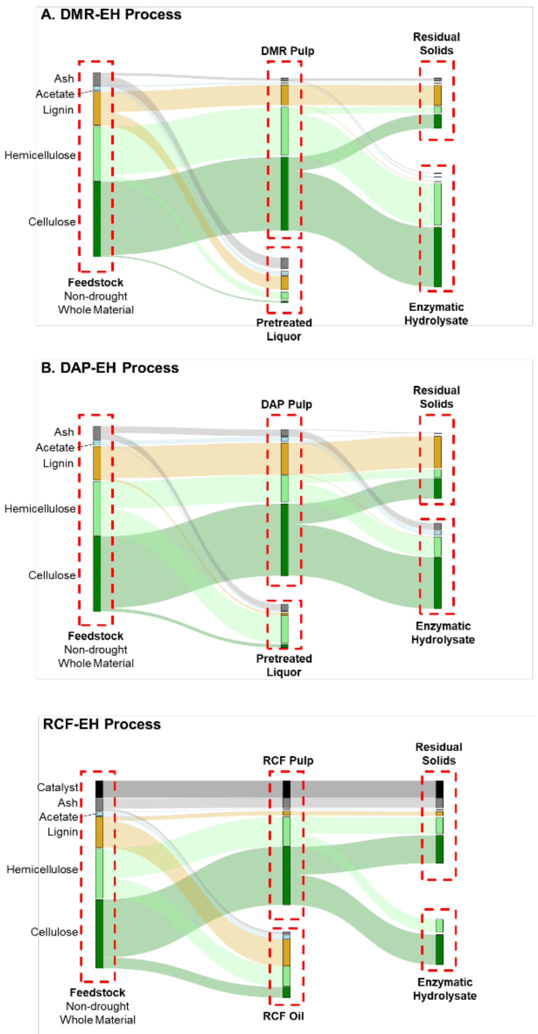




Current Knowledge Gap

- The performance of pretreatment, deconstruction, and enzymatic hydrolysis unit operations on drought-stressed (DS) corn stover compared to stover from non-drought (ND) years is unknown.

Achievement

- We have compared DMR, dilute acid, and Reductive Catalytic Fractionation (RCF) pretreatment approaches across air-classified anatomic fractions of control (ND) and DS corn stover, including whole material, cobs, stalks, and leaves/husks.
- We showed the that DS corn stover (in bulk or as fractionated by air classification) performs similarly to ND corn stover with respect to biomass digestibility, while variation between the anatomical fractions is similar in both DS and ND stover.

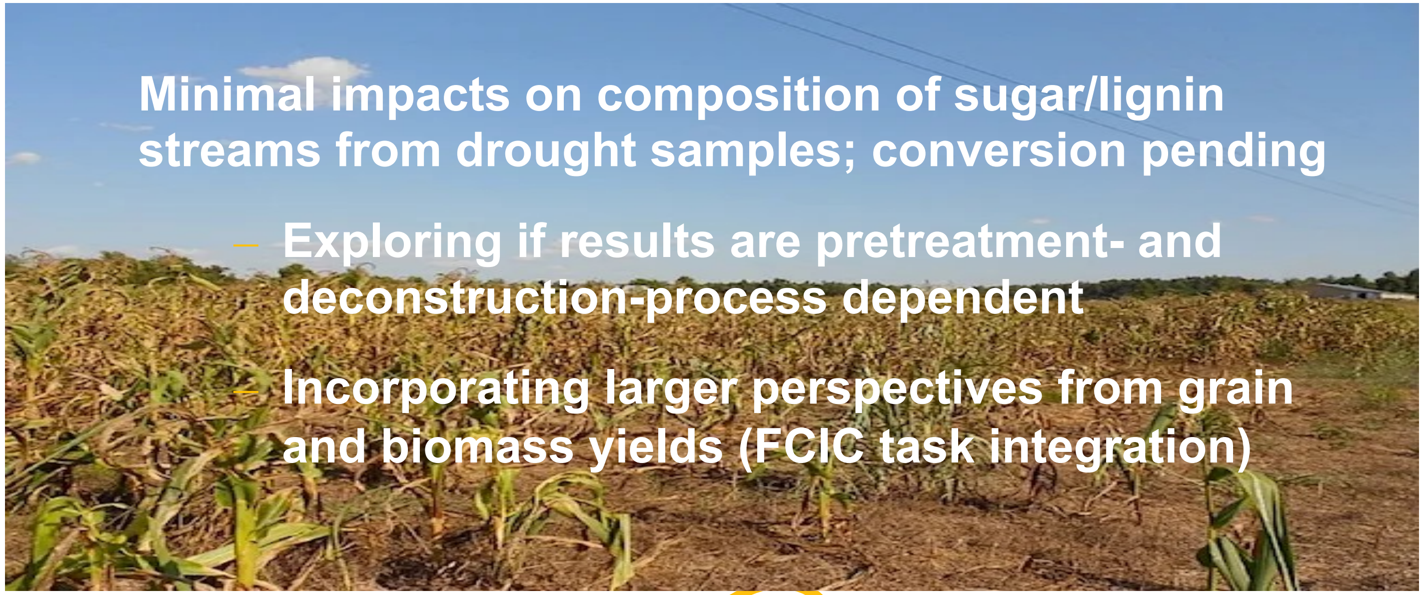
Relevance

- While the total stover yield from a plot of land may vary widely between drought and non-drought years, the performance of the DS and ND stover through common pretreatment processes and enzymatic hydrolysis does not appear to be substantially different for either unclassified or air-classified material.
- We have shown that what was hypothesized to be a critical source of feedstock variability has relatively minor impacts on low temperature conversion, and thus de-risked a significant source of uncertainty for biorefineries.

2 – Expansion into additional sources of feedstock variability, beyond corn from drought conditions

Complementary experimental/modeling approach greatly aided by miniaturization efforts

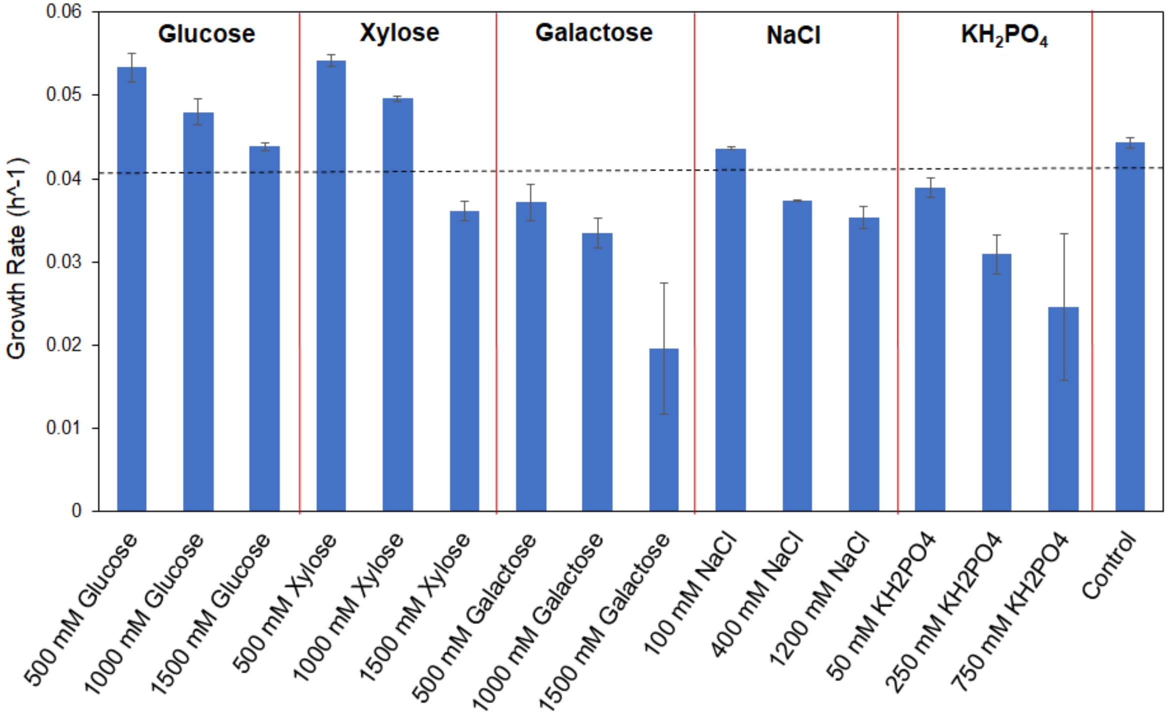
- Increased numbers of feedstock samples to be studied
- Reduced/minimized barriers for study of larger campaigns with reduced acquisition times and costs
- Pretreatment and deconstruction protocols can be more easily optimized and/or standardized
- Additional pretreatment and deconstruction processes can be explored
- Low temperature processes can be studied using plate-based and/or miniaturized bioreactor instruments
- Independent, additive, and/or synergistic effects in mixtures of (C)MAs can be identified for first time



2 – Towards industry-forward validation of initial predictions

Leveraging rapid characterization platforms, tests have begun with engineered biocatalysts or alternative hosts (outside initial training set), in the presence of CMAs and CMA mixtures.

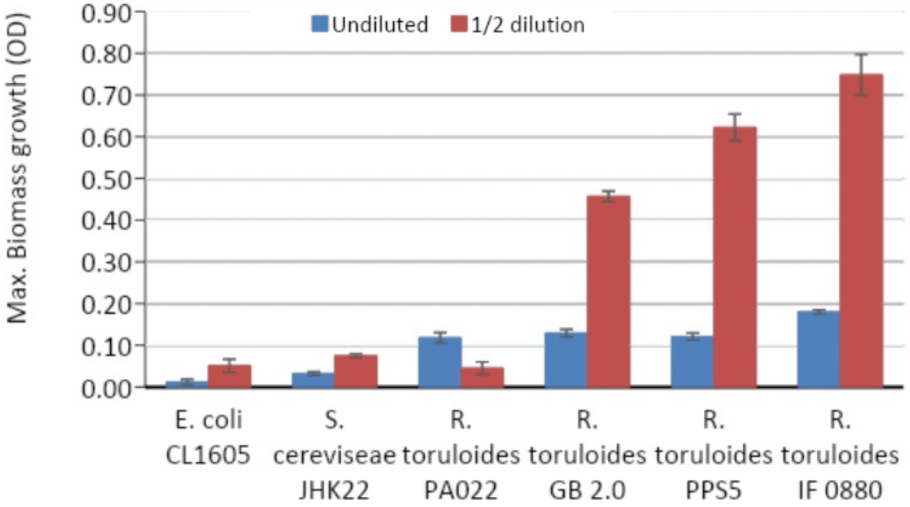
Initial CMA Testing



DMR concentrate sugar/acid composition

Component	mM
Glucose	1484.85
Xylose	1055.34
Galactose	23.62
Arabinose	105.25
Lactic Acid	72.93
Acetic Acid	12.4

Biocatalyst Test results

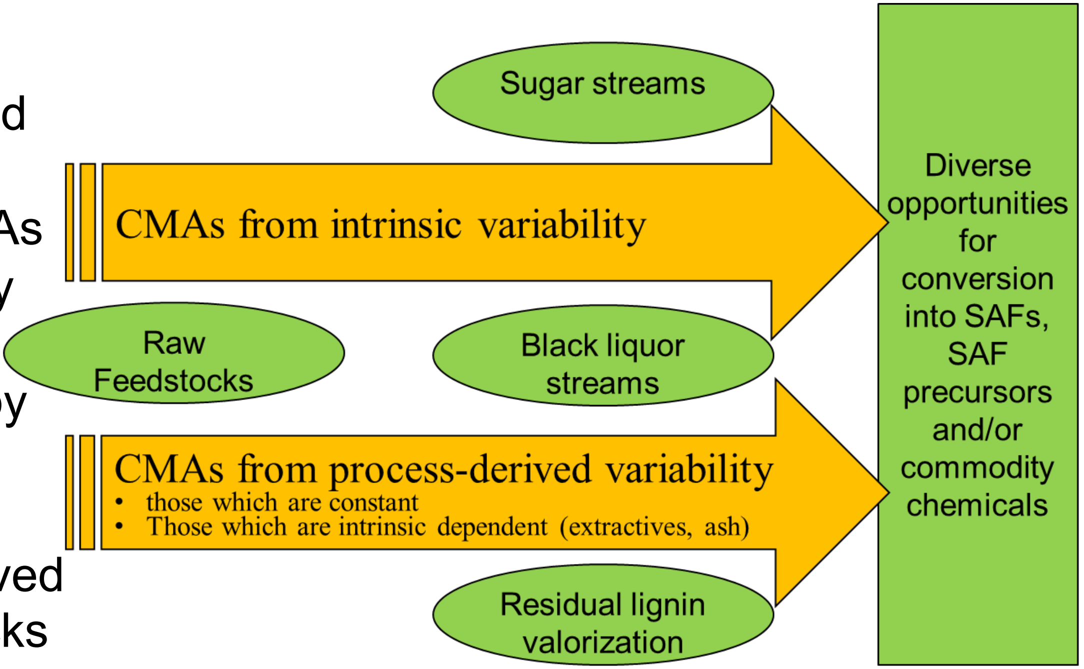


2 – Towards an industry-forward, public report on QbD evaluations and criticalities

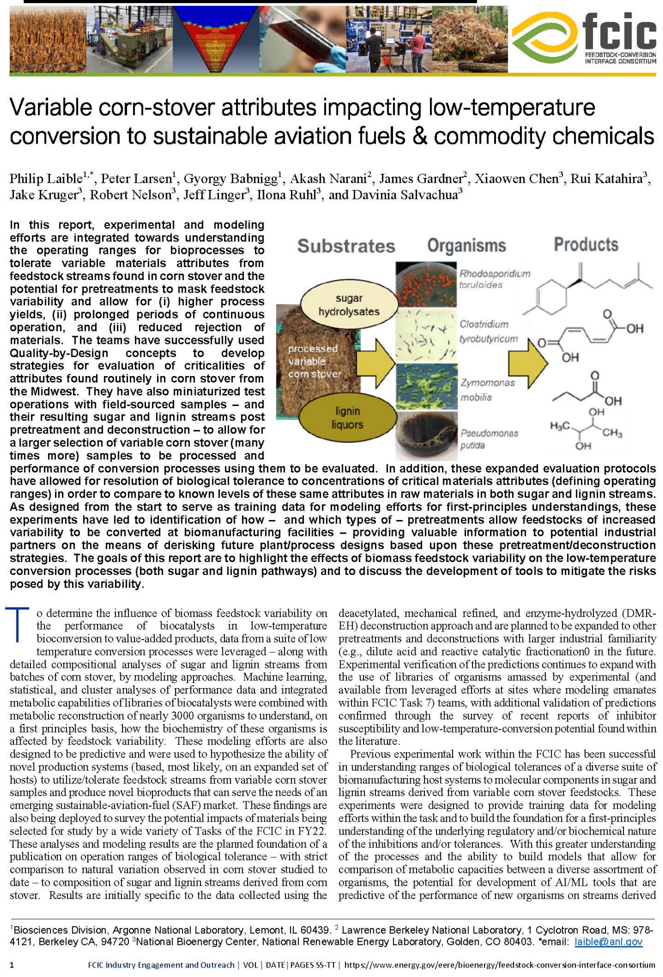
Working outline:

- Ranges of MAs observed in raw materials
- MA and PP criticalities
- Intrinsic versus Process-Derived criticalities
- Allowed operating ranges of MAs
- What criticalities are masked by processes?
- What criticalities are exposed by processes?
- Assessment of criticalities uncovered versus range observed in corn-stover-specific feedstocks studied by the FCIC to date
- How this easily translates to study of MSW from low-temperature-conversion perspective

Emphasized concepts:



Working front page:



Variable corn-stover attributes impacting low-temperature conversion to sustainable aviation fuels & commodity chemicals

Philip Laible[1,*], Peter Larsen[1], Gyorgy Babnigg[1], Akash Narani[2], James Gardner[2], Xiaowen Chen[3], Rui Katahira[3], Jake Kruger[3], Robert Nelson[3], Jeff Linger[3], Ilona Ruhl[3], and Davinia Salvachua[3]

In this report, experimental and modeling efforts are integrated towards understanding the operating ranges for bioprocesses to tolerate variable materials attributes from feedstock streams found in corn stover and the potential for pretreatments to mask feedstock variability and allow for (i) higher process yields, (ii) prolonged periods of continuous operation, and (iii) reduced rejection of materials. The teams have successfully used Quality-by-Design concepts to develop strategies for evaluation of criticalities of attributes found routinely in corn stover from the Midwest. They have also miniaturized test operations with field-sourced samples – and their resulting sugar and lignin streams post pretreatment and deconstruction – to allow for a larger selection of variable corn stover (many times more) samples to be processed and performance of conversion processes using them to be evaluated. In addition, these expanded evaluation protocols have allowed for resolution of biological tolerance to concentrations of critical materials attributes (defining operating ranges) in order to compare to known levels of these same attributes in raw materials in both sugar and lignin streams. As designed from the start to serve as training data for modeling efforts for first-principles understandings, these experiments have led to identification of how – and which types of – pretreatments allow feedstocks of increased variability to be converted at biomanufacturing facilities – providing valuable information to potential industrial partners on the means of derisking future plant/process designs based upon these pretreatment/deconstruction strategies. The goals of this report are to highlight the effects of biomass feedstock variability on the low-temperature conversion processes (both sugar and lignin pathways) and to discuss the development of tools to mitigate the risks posed by this variability.

To determine the influence of biomass feedstock variability on the performance of biocatalysts in low-temperature bioconversion to value-added products, data from a suite of low temperature conversion processes were leveraged – along with detailed compositional analyses of sugar and lignin streams from batches of corn stover, by modeling approaches. Machine learning, statistical, and cluster analyses of performance data and integrated metabolic capabilities of libraries of biocatalysts were combined with metabolic reconstruction of nearly 3000 organisms to understand, on a first principles basis, how the biochemistry of these organisms is affected by feedstock variability. These modeling efforts are also designed to be predictive and were used to hypothesize the ability of novel production systems (based, most likely, on an expanded set of hosts) to utilize/tolerate feedstock streams from variable corn stover samples and produce novel bioproducts that can serve the needs of an emerging sustainable-aviation-fuel (SAF) market. These findings are also being deployed to survey the potential impacts of materials being selected for study by a wide variety of Tasks of the FCIC in FY22. These analyses and modeling results are the planned foundation of a publication on operation ranges of biological tolerance – with strict comparison to natural variation observed in corn stover studied to date – to composition of sugar and lignin streams derived from corn stover. Results are initially specific to the data collected using the deacetylated, mechanical refined, and enzyme-hydrolyzed (DMR-EH) deconstruction approach and are planned to be expanded to other pretreatments and deconstructions with larger industrial familiarity (e.g., dilute acid and reactive catalytic fractionation0 in the future. Experimental verification of the predictions continues to expand with the use of libraries of organisms amassed by experimental (and available from leveraged efforts at sites where modeling emanates within FCIC Task 7) teams, with additional validation of predictions confirmed through the survey of recent reports of inhibitor susceptibility and low-temperature-conversion potential found within the literature.

Previous experimental work within the FCIC has been successful in understanding ranges of biological tolerances of a diverse suite of biomanufacturing host systems to molecular components in sugar and lignin streams derived from variable corn stover feedstocks. These experiments were designed to provide training data for modeling efforts within the task and to build the foundation for a first-principles understanding of the underlying regulatory and/or biochemical nature of the inhibitions and/or tolerances. With this greater understanding of the processes and the ability to build models that allow for comparison of metabolic capacities between a diverse assortment of organisms, the potential for development of AI/ML tools that are predictive of the performance of new organisms on streams derived

[1]Biosciences Division, Argonne National Laboratory, Lemont, IL 60439. [2] Lawrence Berkeley National Laboratory, 1 Cyclotron Road, MS: 978-4121, Berkeley CA, 94720 [3]National Bioenergy Center, National Renewable Energy Laboratory, Golden, CO 80403. *email: laible@anl.gov

1 FCIC Industry Engagement and Outreach | VOL | DATE | PAGES SS-TT | https://www.energy.gov/eere/bioenergy/feedstock-conversion-interface-consortium

2 – Milestones and other decision points

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Milestone Name/Description	Criteria	End Date
Task 7 FY23 Q1: Compare MSW pretreatment/deconstruction approaches and generate lists of MAs to be experimentally assessed for criticality	Compare and contrast pretreatment and deconstruction processes on pure MSW streams to assess yields and MA→CMA ranked orderings and operating ranges.	12/30/2022
Task 7 FY23 Q3: Experimental validation of model predictions	Use engineered processes from BETO SOT projects, consortia, or CRADA projects to validate > 5 model predictions.	6/30/2023
Task 7 FY23 Q4 : Conversion efficiency of MSW streams	Characterize/convert streams from ≥ 3 MSW samples to outline future research plans for low-temperature conversion of MSW streams/blends in FY24 and beyond.	9/30/2023
Task 7 FY24 Q4 (ANL): Low Temperature Conversion End-of-Project	In bioreactor-cultivation conditions, validate modeled performance of an industry-sourced/recommended, SAF-precursor-producing strain on 2 or more sugar or lignin streams derived from 2 or more variable corn stover samples (with prediction/experiment agreement > 80%).	09/30/2024

✓

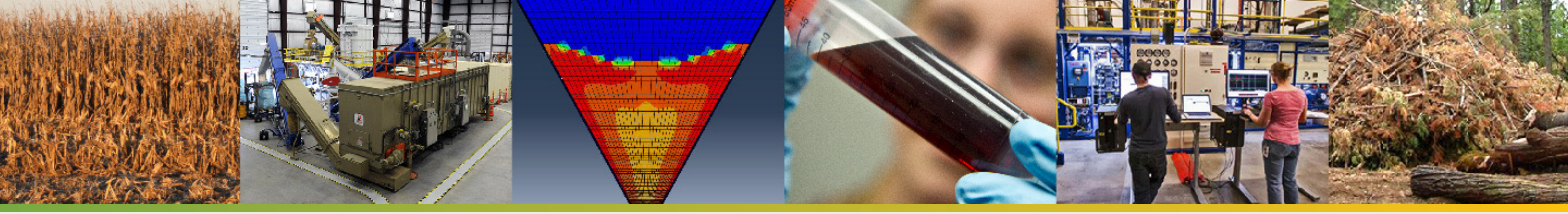
Underway

Underway

Go/No-Go Decision

Description	Criteria	Date
Report – in a public, industrial-facing disclosure – on raw material, sugar stream, and/or lignin stream CMAs as having detrimental operating ranges within or outside measured intrinsic and process-derived variability.	From larger lists of raw material, sugar stream, and lignin stream MAs, identify > 20 CMAs with potentially detrimental operating ranges with data planned to be stored long term in LabKey for general dissemination and for use in the start of future modeling activities.	03/31/23

Underway



3 – Impact

3 – Impact

- The success of the low temperature conversion effort of the FCIC involves frequent interactions with the biomanufacturing community commercializing low-temperature processes that utilize lignocellulosic feedstocks. We benefit from frequent interactions of this community at the Advanced BioProcessing Demonstration Unit at LBNL and are able to offer users access to sugar and lignin hydrolysates for testing in conversion strategies.
- Coordinated efforts within the low temperature conversion team and interactions with industry focus on two high-impact, readily-published, FCIC-webpage-highlightable activities: (i) experiments and modeling efforts will be centered on understanding operating ranges for bioprocesses to tolerate variable MAs in feedstock streams relevant to MA ranges found in corn stover expected at throats of conversion plants and (ii) understanding the types of pretreatments that potentially mask feedstock variability and allow for higher process yields and prolonged periods of continuous operation and reduced rejection of materials.
- The knowledge and tools generated by this task enable sustained high levels of production in low-temperature conversion processes through first-principles understanding of materials attributes that negatively influence predictable performance (e.g., inhibitory effects) that can be used to guide changes to process parameters downstream to minimize impacts.
- Ultimately, these results encompass valuable information that provides potential industrial partners with the means of derisking future plant/process designs based upon these pretreatment/deconstruction strategies.

3 – Summary

Management: This is a highly dynamic and interactive interdisciplinary research team that works in a distributed experimental setting to understand how LowT conversion processes are impacted by feedstock variability. Frequent meetings and nested experimental activities require constant interaction for effective coordination and milestone coverage.

Technical Approach: Generate streams from variable corn stover feedstocks; study conversion impacts of soluble lignin, sugar, and residual lignin streams on conversion performance of a wide variety of microorganisms; combine experimental results, metabolic and regulatory models, and genomics repositories to understand impacts of materials attributes and LowT process parameters on a first-principles basis; use trained, machine learning models to predict performance of new conversion hosts on varied feedstocks.

Impact: Knowledge and tools that mitigate the risks posed by feedstock variability on the performance of LowT conversion processes – minimizing impacts of CMAs upstream, of CPPs in conversion, and CQAs downstream via first-principles understanding of criticalities that facilitate performance predictability for future LowT processes.

Achievements: Criticalities and operating ranges now known for larger numbers of feedstock, sugar stream, and lignin stream MAs; conversion performance tested experimentally; predictive models generated that are in various stages of validation and reinforcement; public report on attributes that impact LowT conversion prepared; DEI outreach expanding through presentations to MSIs and farming communities in FY23 and beyond.

3 – Quad Chart Overview

Timeline
- *October 1, 2021*
- *September 30, 2024*

	FY22 Costed	Total Award
DOE Funding	*(10/01/2021 – 9/30/2022)* *$1070K*	*(negotiated total federal share)* *$3210K*
Project Cost Share *	NA	NA

TRL at Project Start: 2
TRL at Project End: 5

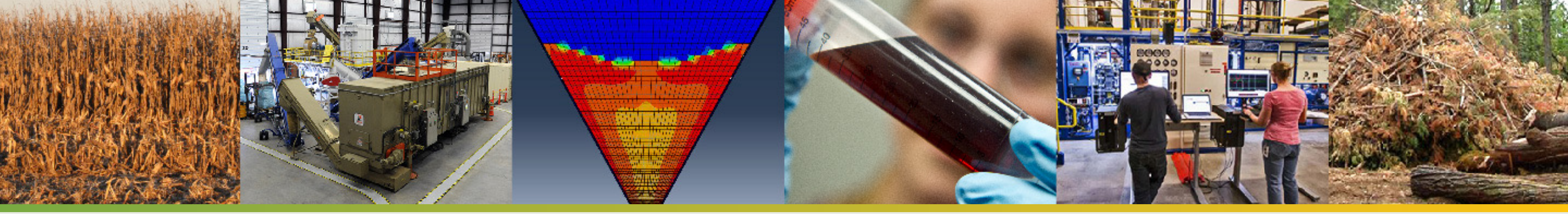
Project Goal
The objectives of Task 7 are to determine the effects of biomass feedstock variability on the low-temperature conversion processes (both sugar and lignin pathways) and to develop tools to mitigate the risks posed by this variability.

End of Project Milestone
In bioreactor-cultivation conditions, validate modeled performance of an industry-sourced/recommended, SAF-precursor-producing strain on 2 or more sugar or lignin streams derived from 2 or more variable corn stover samples using a down-selected pretreatment process (with prediction/experiment agreement > 80%).

Funding Mechanism
2021 Lab Call – FCIC Merit Review

Project Partners*
- ANL (18%; $190K)
- LBNL (21%; $230K
- NREL (61%; $650K

*Only fill out if applicable.



Additional Slides

FEEDSTOCK-CONVERSION INTERFACE CONSORTIUM

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Responses to Previous Reviewers' Comments

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- Universally, the reviewers would have liked to have more information justifying the use of the DMR process in Task 7. Why is it used by the FCIC?

DMR is a low-pressure, low-severity pretreatment technology that produces very digestible material after enzymatic hydrolysis (EH). BETO has invested heavily in R&D on the DMR process. Our Task within the FCIC is specifically looking at the impact of feedstock variability on the performance of DMR/EH derived streams (lignin and sugar hydrolysates). While we are working with a single pretreatment chemistry (DMR/EH) we are looking for universal effects that may result from many other pretreatment choices (like weak acids, organosolv, ionic liquids, etc). Thus, we expect our results will be informative for other fractionation processes that share common attributes in their streams. In the future as our research evolves, we can consider the benefits of expanding our research thrust into hydrolysates arising from different pretreatment methods.

- What will the LowT team do if there is not enough variability in the feedstocks explored?

Yes, a risk in this task is that there is not enough variability in the feedstocks to generate significant differences in the biological conversion processes. If that is indeed the case, we have the opportunity to additionally evaluate the effect of various critical process parameters that change the properties of the streams (e.g., increased washing steps during the pretreatment process). The goal of changing the washing and pretreatment strategies was to understand the effect of critical process parameters (CPPs) on bioconversion processes in addition to the identification of critical material attributes (CMAs) from feedstocks. This approach ties process optimization to our ability to understand the effects of feedstock variability on low-temperature conversion processes. We apologize for not being more detailed in our discussion of this approach.

- Why were these organisms selected initially for study?

Regarding the selection of organisms, these microbes are utilized and developed in other projects in the BETO portfolio for the upgrading of sugars and lignin to biofuel and bioproduct precursors. Most importantly, these organisms are included in techno-economic analyses to report the State of Technology (SOT) to BETO every year towards decreasing the mean-fuel-selling price. Examples of this SOT work include *Pseudomonas putida* in 'Biological Lignin Valorization', *Clostridium tyrobutyricum* in 'Biological Upgrading of Sugars' and *Rhodosporidium toruloides* in the Agile BioFoundry.

Publications, Patents, Presentations, Awards, and Commercialization

- Public report on “Variable corn-stover attributes impacting low-temperature conversion to sustainable aviation fuels & commodity 3chemicals”, Philip Laible, Peter Larsen, Gyorgy Babnigg, Akash Narani, James Gardner, Xiaowen Chen, Rui Katahira, Jake Kruger, Robert Nelson, Jeff Linger, Ilona Ruhl, and Davinia Salvachua, available as of March 31, 2023

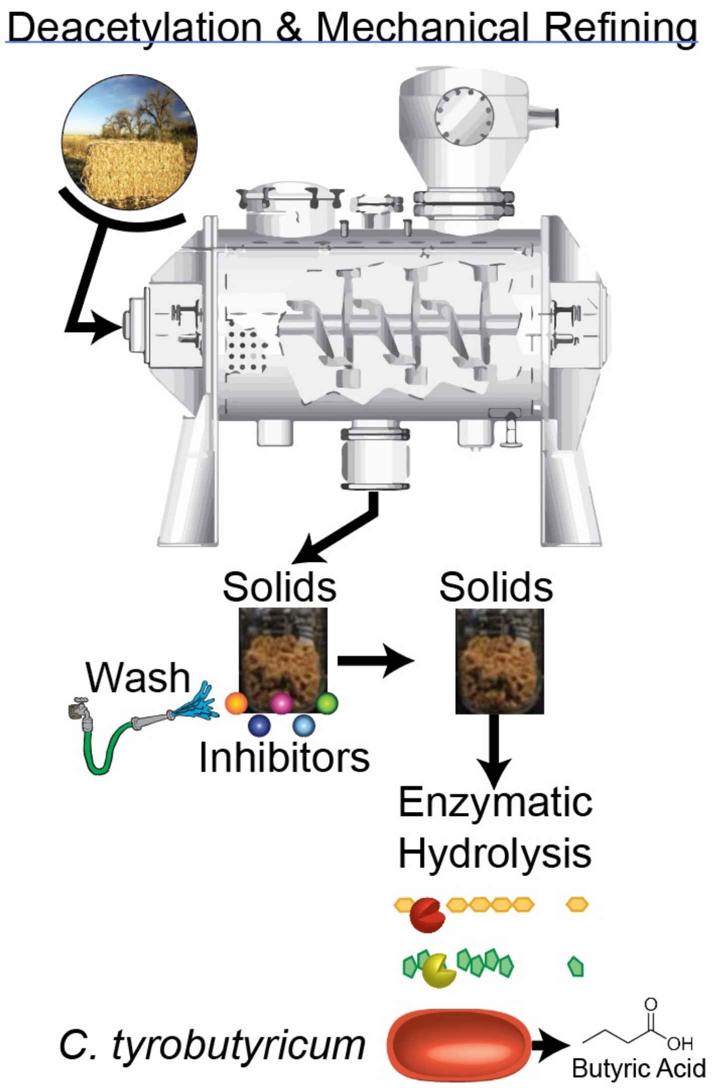
- Presented invited talk at an AICHE annual meeting session on “Feedstock Conversion Interface Consortium – Understanding Feedstock Variability to Enable Next Generation Biorefineries” entitled “Mitigating Risks of Feedstock Variability on Low-Temperature Conversion Processes” by Philip Laible on November 14, 2022 in Phoenix, AZ.

- Metabolic models and Jupyter notebooks of data wrangling and analysis methodologies available on public GitHub repositories.

- Discussions held and/or presentations given by James Gardner (Task 7 Co-Lead) to more than 15 prospective industrial partners on the potential use of sugar and lignin streams from variable corn stover in their respective bioprocesses, facilitated by interactions available at the Advanced BioProcessing Demonstration Unit at LBNL.

Mitigation of top inhibitory compounds for effective biological conversion of sugars

Knowledge



Current Knowledge Gap

- Public data regarding the impact of feedstock variability on the performance of organisms in fermentation processes using sugar streams derived from DMR pretreated biomass is unknown/underrepresented.

Achievement

- We have systematically evaluated 25 Material Attributes to identify those which are critically inhibitory to *Clostridium tyrobutyricum.*
- The top five most critical MAs were: Sodium, Ferulate, Coumarate, Ammonium Sulfate, and Lactic Acid.
- Importantly, inhibitory effects of all identified inhibitory compounds can be mitigated by washing the solids following Deacetylation and Mechanical Refining (DMR).

Relevance

- These results demonstrate that while inhibitory compounds exist in sugar streams derived from the DMR process, they can be readily mitigated by a simple wash step prior to enzymatic hydrolysis (EH).

Robust low-temperature processes readily convert degraded corn stover

Knowledge

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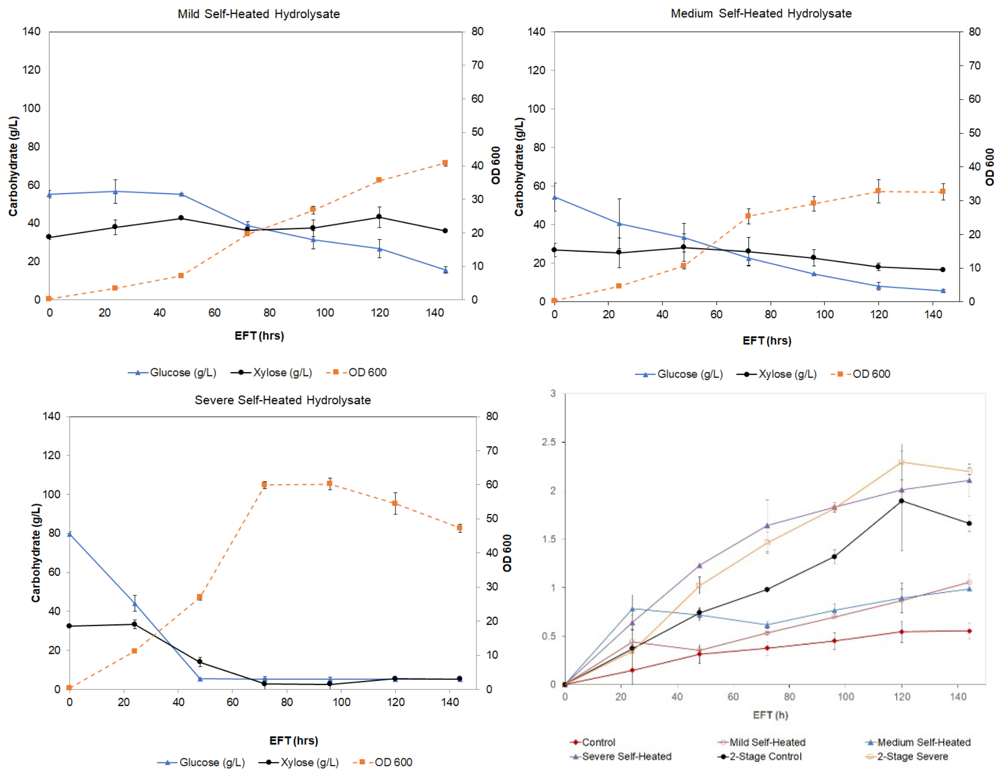
Versatile yeast strains transform sugar streams from degraded corn stover into sustainable aviation fuels efficiently.



Manuscript in preparation.

Current Knowledge Gap

Economic and environmentally-friendly bioconversion to sustainable aviation fuels (SAFs) require a series of assumptions, including the overall performance consistency of the low-temperature process itself. The influence of biomass degradation during storage (many processes involving self-heating) is unknown and ripe for controlled investigation.

Achievement

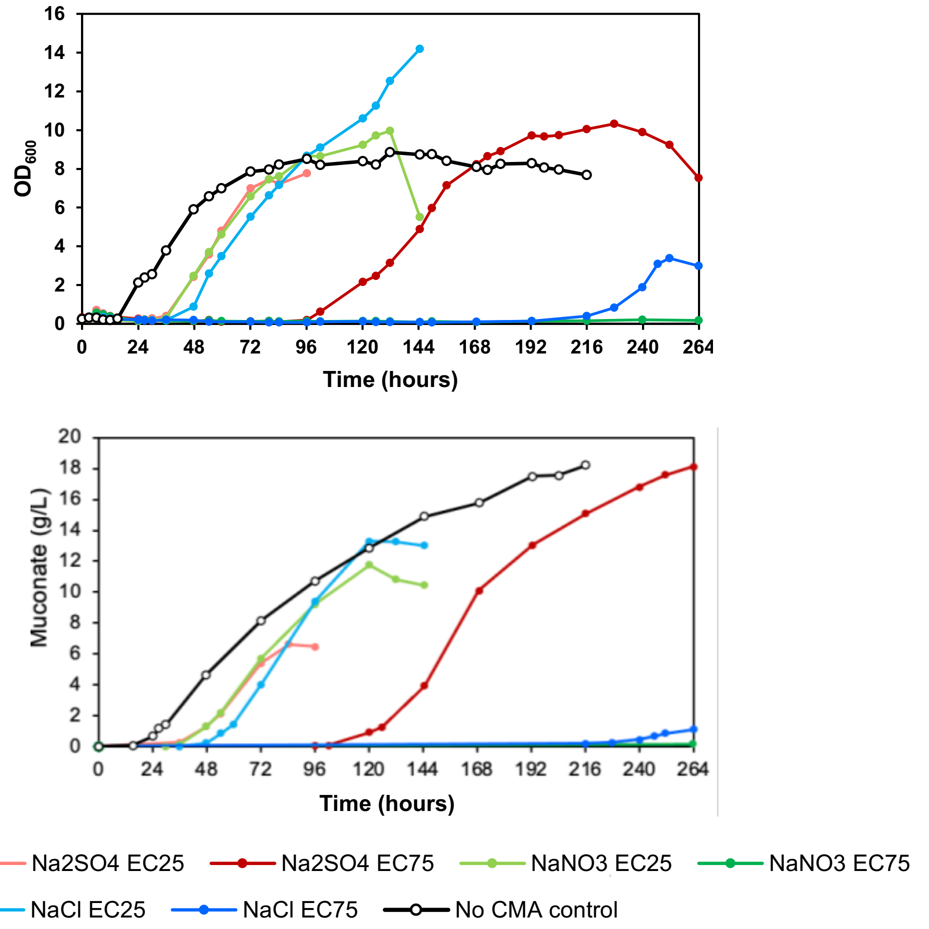
Corn stover bales which had undergone varying degrees of degradation that involved bioinduced self heating were pretreated, deconstructed, and hydrolyzed via a standardized process involving single- (NaOH) or two-stage (NaOH & Na_2CO_3) deacetylation. Sugar streams were then converted into bisabolene (a C15 molecule planned for use in SAF blends) via strains of *Rhodosporidium* (*R.*) *toruloides* where robust – perhaps even markedly improved – performance was observed, most notably for rates of growth and SAF production (increases also observed for titers and yields).

Industry Impact

This work augments the power to deploy non-standard hosts, such as *R. toruloides*, in the conversion of low-quality feedstocks into useful fuels and chemicals (e.g., bisabolene). The success with such processes aid in derisking opportunities for biocatalyst performance degradation when utilizing feedstock streams derived from corn stover stored suboptimally.

CMAs for P. putida *become inhibitory only in concentrated lignin streams*

Knowledge



***P. putida* CJ781 cultivations in bioreactor, conducted in fed-batch mode, in the presence of three CMAs at two different concentrations that inhibit growth rates by 25% and 75% (EC25 or EC75).** (Top) Bacterial growth measured as optical density at 600 nm (OD_{600}) and (bottom) muconate production over time.

Current Knowledge Gap

- Public data regarding the impact of lignin stream variability (derived from the deacetylation of various corn stover sources) on engineered muconate-producing *Pseudomonas (P.) putida* are not available.

Achievement

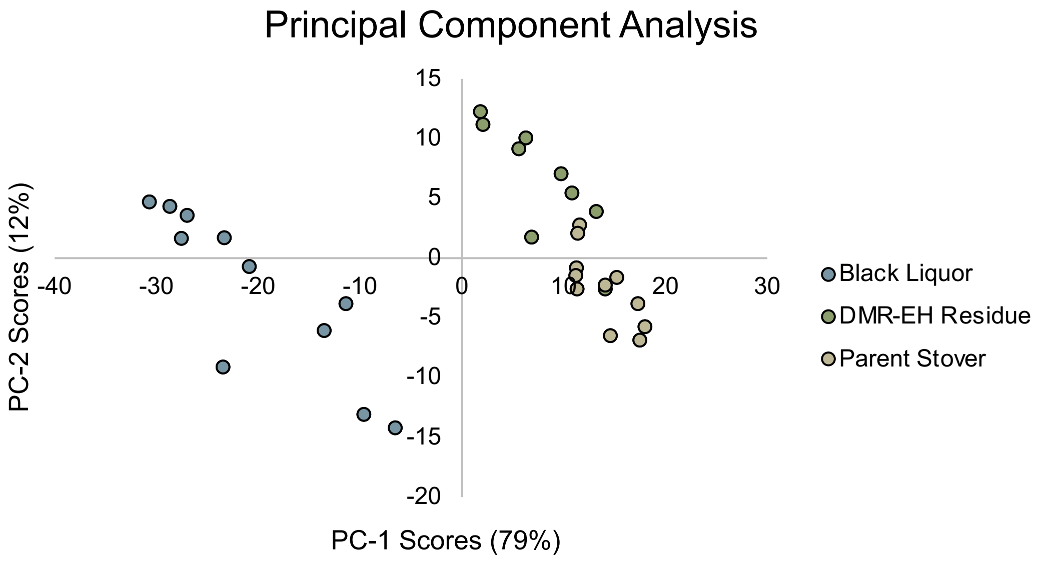
- We have identified the top three critical material attributes (CMAs) in black liquor that affect *P. putida* performance negatively. Of these three CMAs, sodium sulfate is the least inhibitory for muconate production, followed by sodium chloride and sodium nitrate. However, considering their concentrations in black liquor, sodium salts become inhibitory only if the process includes a concentration step.
- We showed that the combination of the top five CMAs did not produce any synergistic effects on *P. putida* performance, only additive effects.

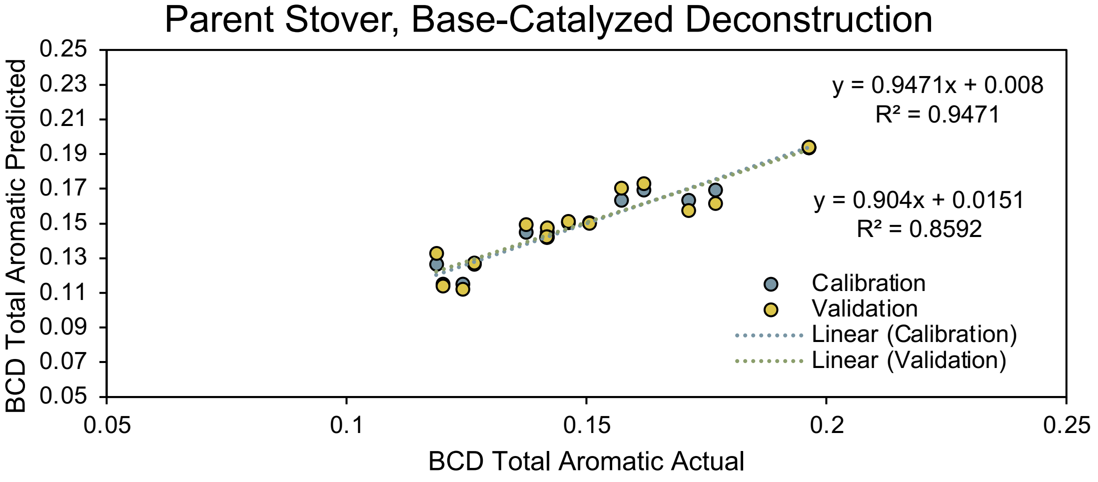
Relevance

- These results are relevant to (1) design of adequate process configurations that decrease sodium accumulation or remove salts from black liquor and (2) optimize the biocatalyst via engineering or evolution approaches to tolerate higher salt concentrations.
- The fact that we have not identified synergistic effects when combining CMAs is a positive result for the generation of predictive models.

Lignin monomer yields predictable from fast, inexpensive ATR-FTIR spectroscopy

Knowledge





Current Knowledge Gap

- The value of a corn stover feedstock to a biorefinery is heavily dependent on the valorization potential of the lignin fraction (especially lignin depolymerization yields), but there is currently no way to predict this potential prior to conversion.

Achievement

- Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopy can be used to distinguish between different qualities of corn stover feedstocks and lignin fractions from the DMR-EH process.
- When combined with principal-component analysis and partial least squares regression, ATR-FTIR can predict monomer yields during lignin depolymerization by base-catalyzed deconstruction.

Relevance

- The potential for lignin valorization when feedstocks are variable is a critical risk factor for biorefinery economics. We have shown that a fast, inexpensive spectroscopic technique can mitigate this risk by allowing biorefineries to estimate reliably how much lignin-derived value they can expect to extract from a feedstock at the point of sale.

Towards first-principle insights through layered modeling approaches

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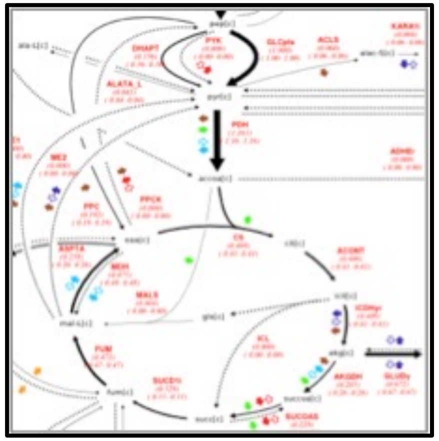
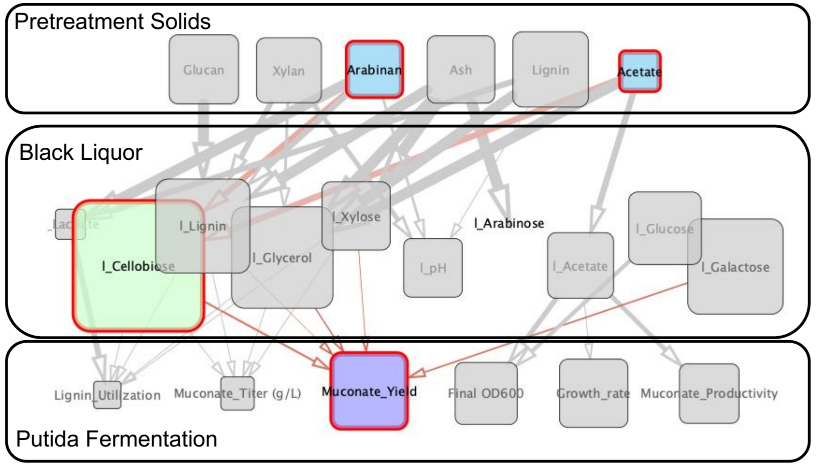
Initial biological processes deployed in studies were chosen as the systems were understood well enough to provide molecular insights into variations in fermentation strains

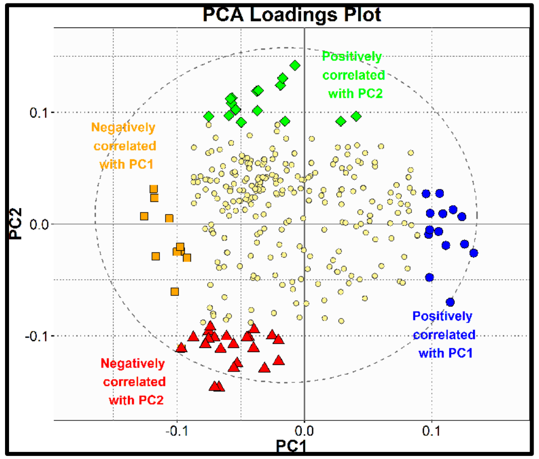
Literature-informed metabolic models



Causal networks for experimental data highlights CMAs at different stages in lowT processes



Propose molecular mechanisms of observed CMA interferences



Layered system of models with varied depth and datatypes is well-positioned for rapid reinforced learning and advancements and generalization to additional feedstocks, strains, and bioproducts.