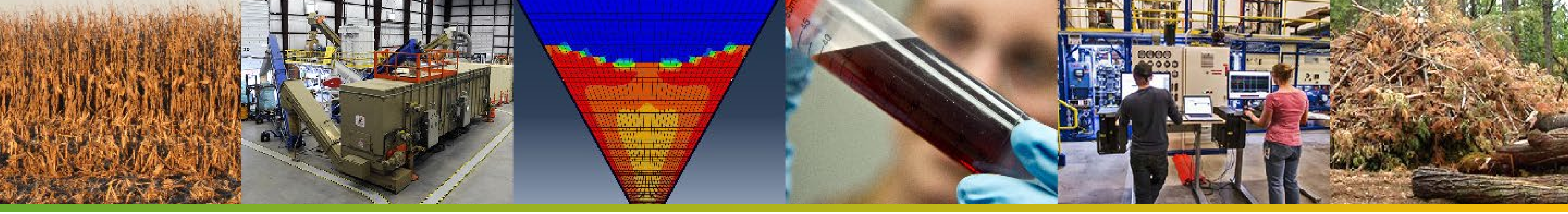


DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Task 7 – Low Temperature Conversion

March 16, 2021
Feedstock Conversion

Philip Laible, Argonne National Laboratory
Akash Narani, Lawrence Berkeley National Laboratory

FEEDSTOCK-CONVERSION INTERFACE CONSORTIUM

FCIC Task Organization

Feedstock → Preprocessing → Conversion

- Task 2: Feedstock Variability
- Task 5: Preprocessing
- Task 6: Conversion High-Temp
- Task 7: Conversion Low-Temp
- Task 1: Materials of Construction
- Task 3: Materials Handling

Enabling Tasks

- Task X: Project Management
- Task 4: Data Integration
- Task 8: TEA/LCA

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

Task 1: Materials of Construction: Specify materials that do not corrode, 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

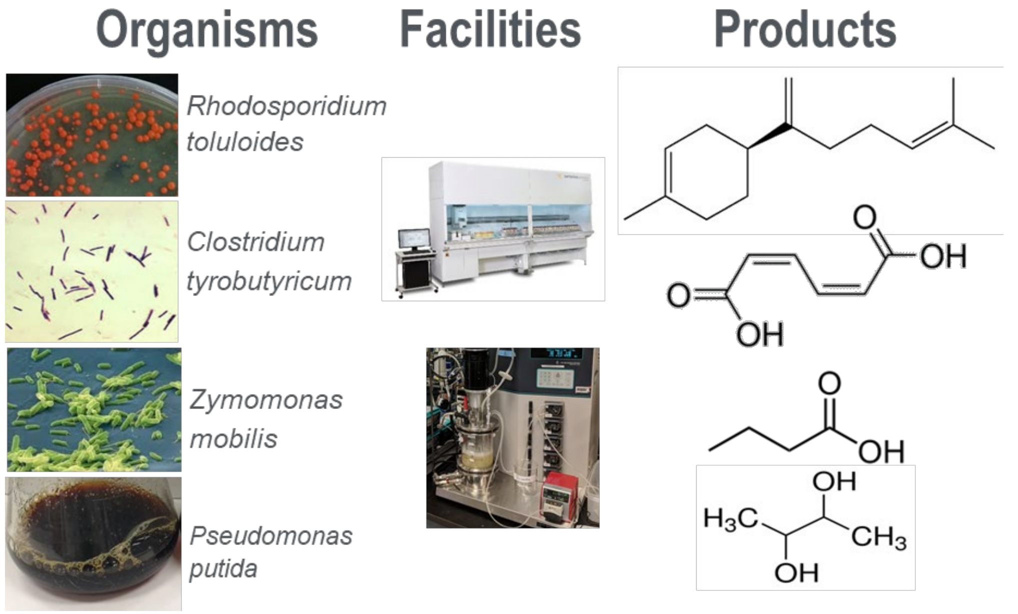
Task 6 & 7: Conversion (High- & Low-Temp Pathways): Produce homogeneous intermediates to convert into market-ready products

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

Project Overview

Objective: Determine the effects of biomass feedstock variability on the low-temperature conversion process chain (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:** This work will decrease the risk associated with feedstock variability in biorefineries, allow the ranges of efficient bioconversion operations to be defined, and develop tools to permit conversion strategies to tolerate wide ranges of materials as inputs.



- **Risks:** Comprehensive survey of existing feedstock variability impossible to approach experimentally (downselection critical but may mislead); variability of sourced feedstocks may prove insignificant on sugar or lignin utilization or product formation; the impacts of feedstock variability may be minimized by standardized protocols used in deconstruction.

1 – Management

Subtask	Lead(s)	Major Responsibilities
7.1 Real-time Monitoring of Deacetylation	Ed Wolfrum	This subtask is currently inactive.
7.2 Impacts of Feedstock Variability on Biological Sugar Conversion	Akash Narani and Jeff Linger	Understand systematically the effects of feedstock variability on sugar-stream conversion
7.3 Impacts of Feedstock Variability on Biological Lignin Conversion	Davinia Salvachua	Understand systematically the effects of feedstock variability on lignin-stream conversion
7.4 Modeling Impacts of Feedstock Variability on Low-Temperature Pathways	Phil Laible	Establish an AI framework to predict the impacts of variable feedstocks on different Low-T pathways
7.5 DMR Materials Preparation	Xiaowen Chen	Produce and characterize sugar and lignin streams
7.6 Chemical Lignin Conversion	Jake Krueger and Rui Katahira	Understand systematically the effects of feedstock variability on chemical depolymerization techniques

Risks: Understand and push the boundaries of feedstock variability studied in the FCIC. Source sufficient supply of characterized feedstock materials for experiments (invoke academic community). Organizing public genomic / metabolic data to pivot organisms and processes studied. Collect data of quality and quantity to train AI models.

Communication strategy: Shared staffing with upstream and downstream tasks. Monthly intra-task meetings are used to keep research teams coordinated and milestones on track. In addition, inter-task meetings with Feedstock Variability and/or Preprocessing coordinate materials flow and research foci. Bi-annual meetings keep Task 7 coordinated with the larger goals of the FCIC.

2 – Approach

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

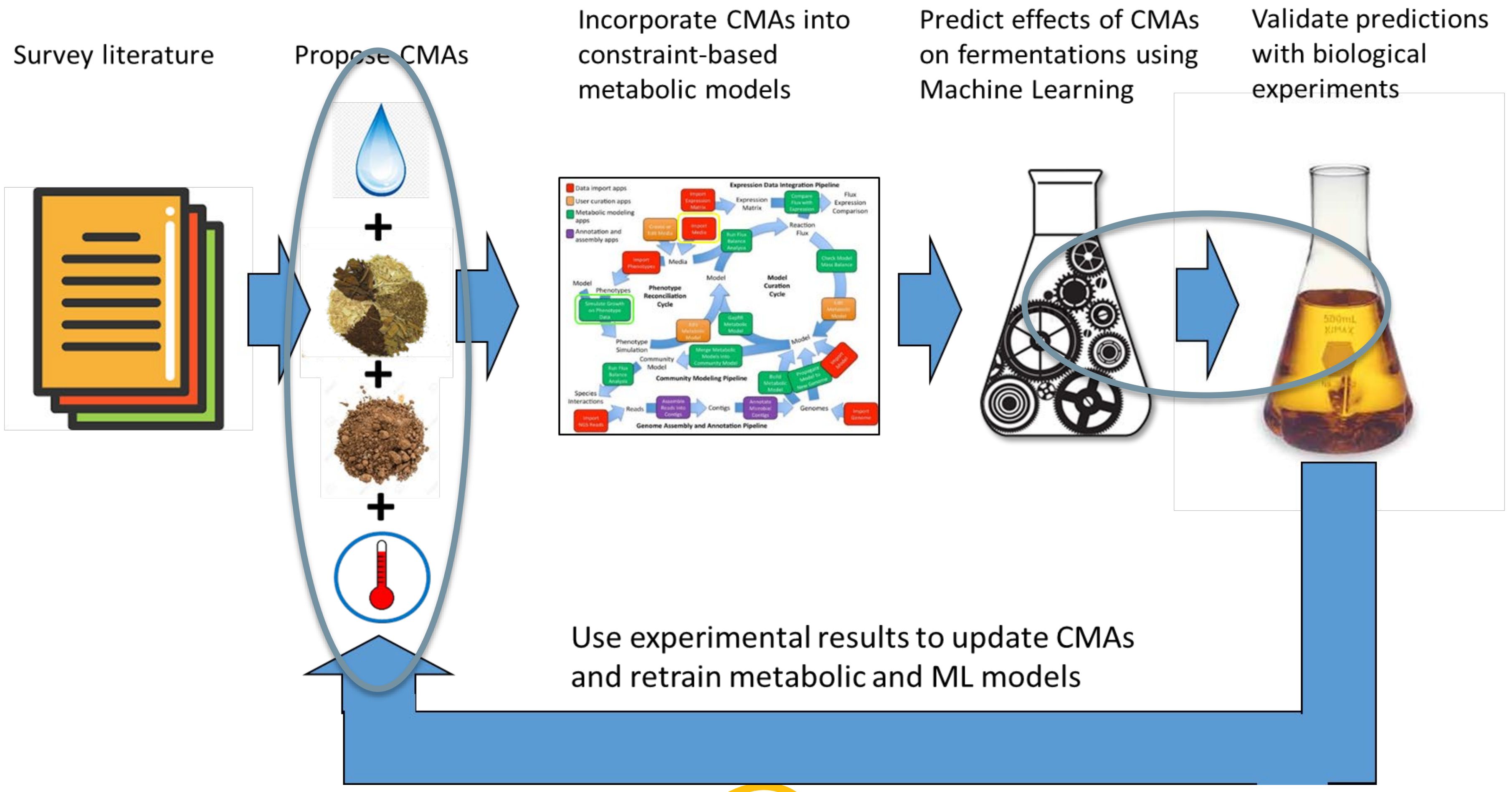
- Use standardized deconstruction strategies to produce sugar and lignin streams,
- Investigate the effects of feedstock variability on performance of biological (and chemical) conversion processes,
- Integrate experimental data with metabolic capacities and regulation to ascertain a first-principles 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%).

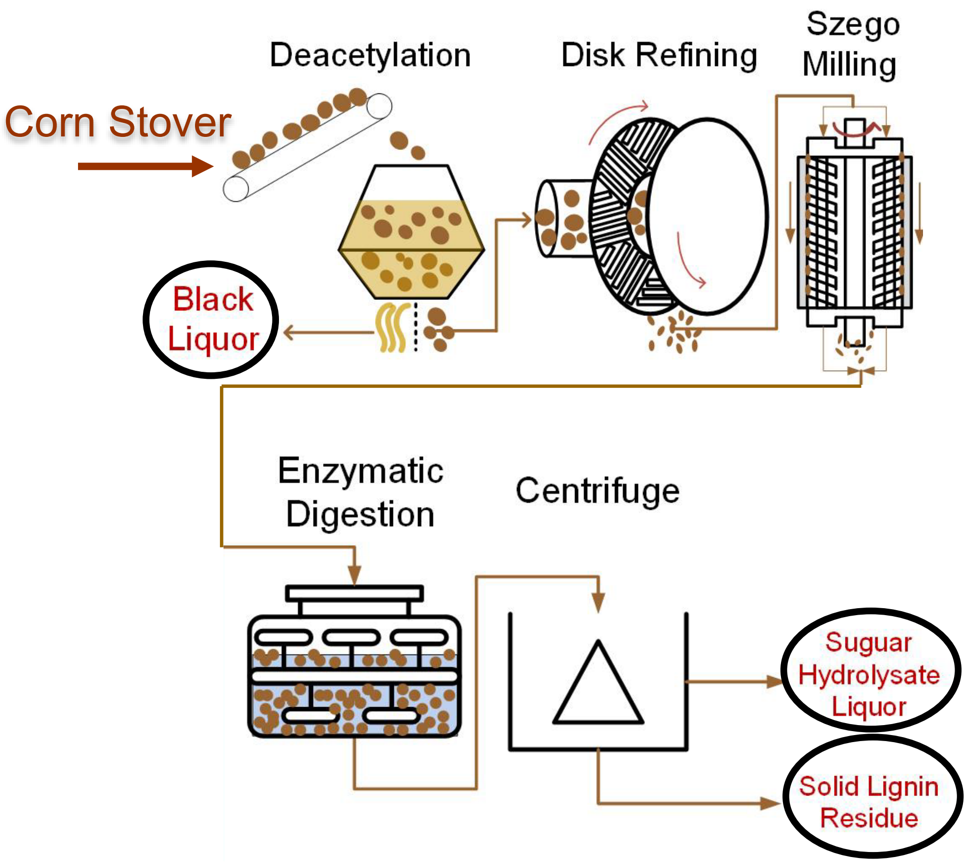
2 – Approach

Use AI tools trained on literature and experimental data of diverse fermentation systems (titer/rate/yield) to enable prediction of performance of a new organism on variable sugar and lignin streams



2 – Approach

Generate Feedstock Streams (DMR process)



Diverse Conversion Organisms

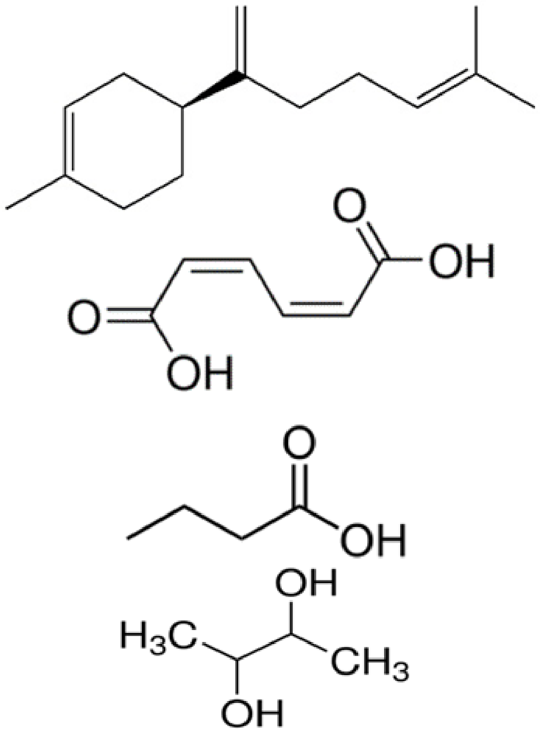
Rhodosporidium toluloides

Clostridium tyrobutyricum

Zymomonas mobilis

Pseudomonas putida

Assorted Products



3 – Impact

Impact: For greatest impact of the research by Task 7, this interdisciplinary team is uncovering knowledge and developing tools that minimize the impact of such variability. As a result, the sequential cascade of low-temperature processes can intelligently operate by understanding critical attributes of materials passed downstream and by adjustments to process parameters that allow for tolerance from upstream complications.

Dissemination: The work of the Task 7 team will not only be disseminated to the biomanufacturing community through a series of high impact publications but through important interactions with other members of the DOE/BETO community:

- Synthetic biology efforts of the Agile BioFoundry
- Recovery and feedstock-stream-cleanup challenges of the Separations Consortium
- Interactions – both direct and indirect – with the industrial community working to commercialize low-temperature processes that utilize lignocellulosic feedstock streams

LYGOS MYCOWORKS ZYMOCHEM

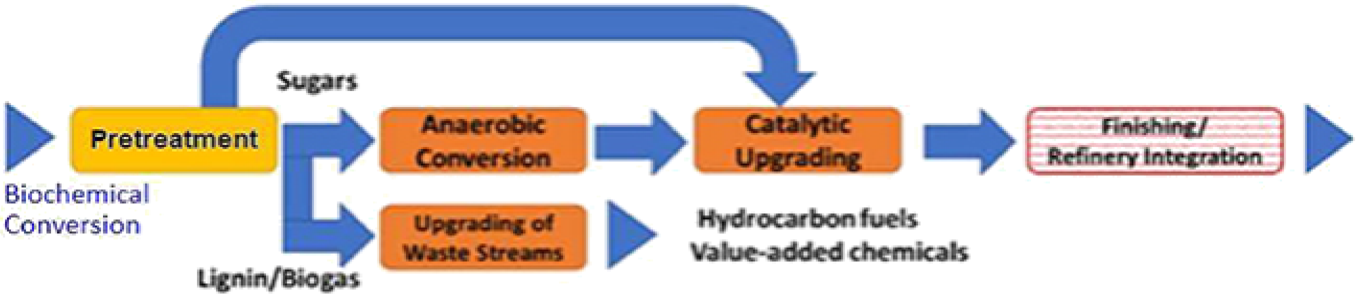
Example of interactions: hydrolysate utilization discussions by Akash Narani with industry at the Advanced Demonstration Unit at LBL.

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)

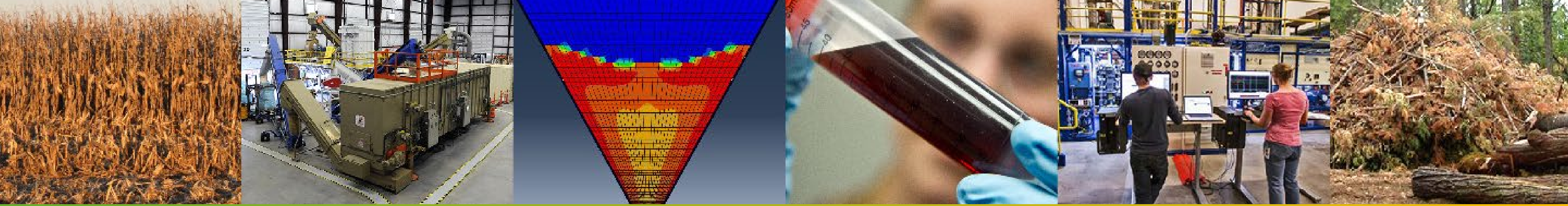
Biochemical Conversion Pathway



BETO Research Consortia Coverage

fcic FEEDSTOCK-CONVERSION INTERFACE CONSORTIUM U.S. DEPARTMENT OF ENERGY → Agile BioFoundry → BIOPROCESSING SEPARATIONS CONSORTIUM

BIOPROCESSING SEPARATIONS CONSORTIUM



4 – Progress and Outcomes

Standardized deconstruction conditions produce suboptimal results for recalcitrant anatomical fractions of corn stover

Knowledge

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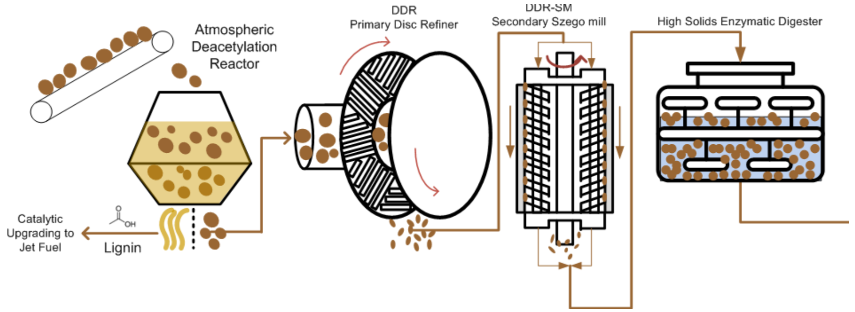
Leaves and husks of corn stover are more readily deconstructed as compared to stalks, and knowledge of differences between anatomical fractions will drive separation economics and optimized processes.



Deacetylated Materials	% Ash	% Lignin	% Glucan	% Xylan	% Galactan	% Arabinan	% Acetate	Glu/Xyl ratio
Cob	2.45	9.53	43.74	34.45	1.58	4.08	0.25	1.27
Husk	9.39	13.10	42.90	22.30	1.65	4.03	1.68	1.92
Stalk	5.39	17.71	47.62	20.62	1.22	2.91	0.68	2.31
Whole Stover	7.66	13.28	47.71	22.15	1.42	3.54	0.64	2.15

Current Knowledge Gap

- The systematic deconstruction of anatomical fractions of corn stover had never been approached with standardized conditions and sample characterization methods

Achievement

- Three distinct anatomical fractions of corn stover (cobs, husks and leaves, and stalks) were deconstructed by standard conditions with differences in sugar and lignin streams exhaustively characterized and compared to parent materials containing all fractions in proportion.

Relevance

- For the successful use of lignocellulosic materials in biocatalytic conversion, harvesting and deconstruction methods must be co-optimized for development of economically viable processes.
- This work underscores the recalcitrant nature of the bulk of corn stover materials and serves as an example of where the development of standardized methods for bulk material could prove limiting as harvesting methods advance.
- These results have process, economic, and environmental considerations for many steps along the feedstock-stream supply chain (including harvesting).

Feedstock variability influences titers, rates, and yields of bioconversion processes

Knowledge

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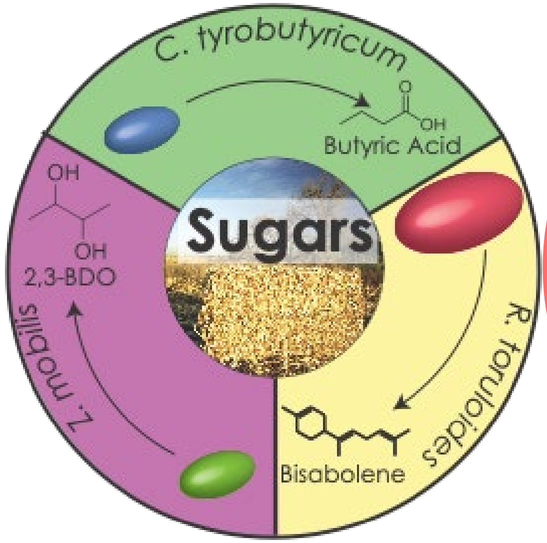
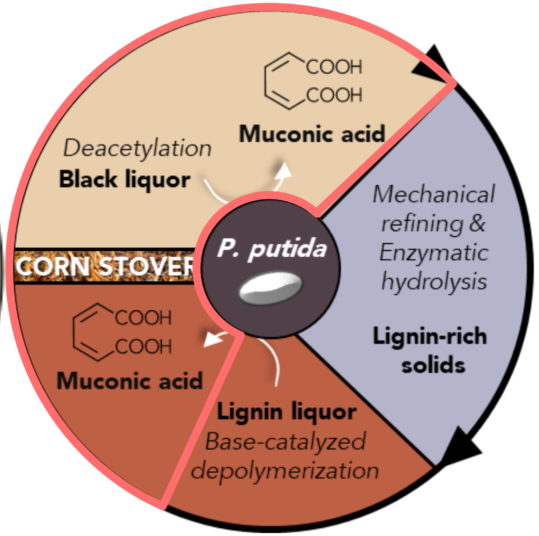
Variability in the quality of raw corn stover materials impacted the titers, rates, and yields of conversion of sugar and lignin streams to bioproducts by a wide array of microorganisms.



Sugar conversion:
High ash had least impact; Storage may have opposite effects for sugar and lignin



Lignin conversion:
Production affected greatest by high ash, with evidence for consumption of oligomeric lignin

Current Knowledge Gap

- Public data regarding the impact of feedstock variability on fermentation organism performance using sugar and lignin streams derived from DMR pretreated biomass

Achievement

- Significant changes (> 15% level) in biocatalytic productivity and substrate utilization were uncovered for feedstocks of varying quality.
- Strikingly, conversion performance was impacted for both the sugar- and lignin-converting organisms, with differential process effects.

Relevance

- These results are the first of their kind to determine, in a controlled manner, the effects of feedstock variability on the biological conversion performance of multiple streams arising from DMR pretreatment.
- Previously, with limited data, biorefineries would be forced to accept the performance risk of varying feedstocks or invest substantially to generate scientific data and understanding that are not shared publicly.
- These results will allow the FCIC to develop tools to mitigate the risks posed by this variability.

Depolymerization of residual lignin is heavily influenced by feedstock variability

Knowledge

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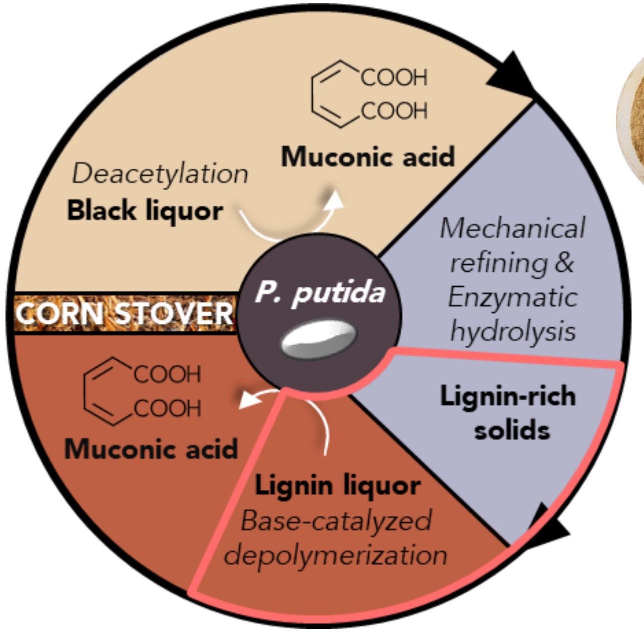
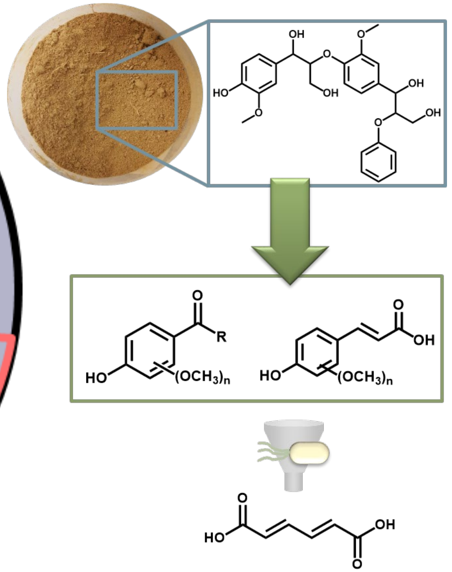
Yields of monomers from depolymerization processes used on residual lignin varied significantly depending upon the ash and moisture content of the raw corn stover utilized.





Current Knowledge Gap

- Insoluble residues from mechanical and hydrolytic deconstruction processes are not as readily available for biological upgrading.
- The effects of feedstock variability on alkaline oxidation and base-catalyzed deconstruction of these residues was previously unknown.

Achievement

- Variability in the raw materials used in standardized deconstruction of corn stover was found to impact aromatic monomer yields derived from chemical depolymerization of residual lignin significantly.

Relevance

- Valorization of lignin is critical to biorefinery economic viability, and a key step in many lignin valorization strategies is depolymerization of polymeric lignin to aromatic monomers for biocatalytic conversion or for direct sale.
- The effects of variability on depolymerization need to be understood in order to optimize this carbon recovery strategy.

Critical materials attributes identified through modeling efforts

Knowledge

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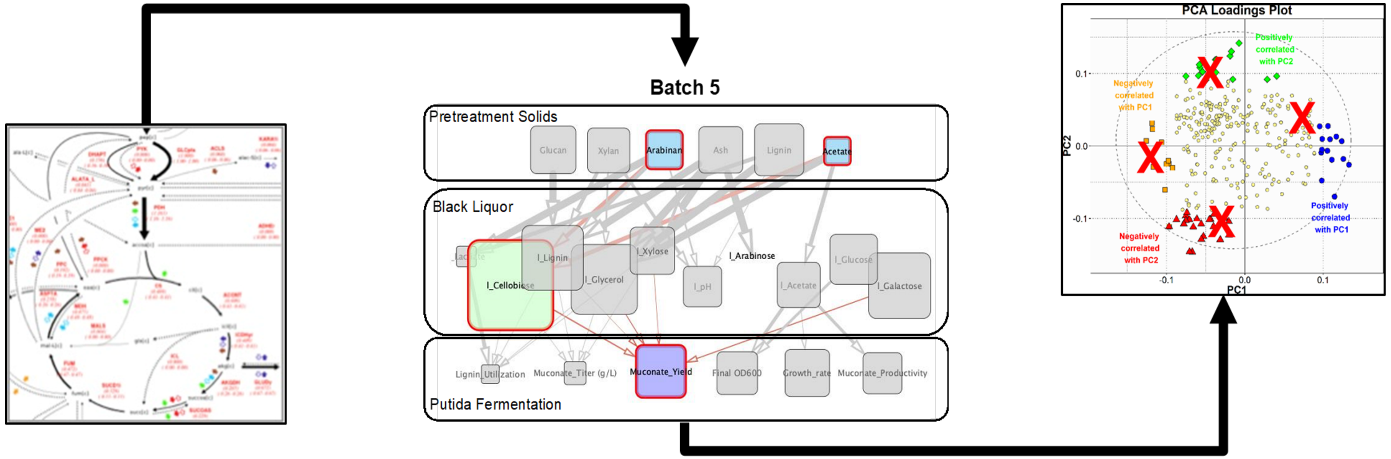
Machine-learning analysis combined no-cost literature information and results obtained by the experiment teams identified materials/quality attributes and process parameters that impact low temperature conversion.



Literature-informed metabolic models plus experimental data

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

Map experimental data to model predictions to propose specific process improvements

Current Knowledge Gap

- Differences surrounding the biological conversion of various fractions of biomass into products are not well described or understood
- No clear path forward exists for computational analysis and predictive modeling to assist with identifying impactful attributes and parameters.

Achievement

- A system of models has been constructed and trained on existing and new experimental data.
- Attributes and parameters identified as critical for one bioprocess may not be a critical for another; more drastically, a MA that increases production of a bioproduct from one process may decrease it in another.
- An opportunity to understand the genetic basis for influences that characteristics of raw materials and feedstock streams exert – whether similarly or differentially – on the biocatalytic processes.

Relevance

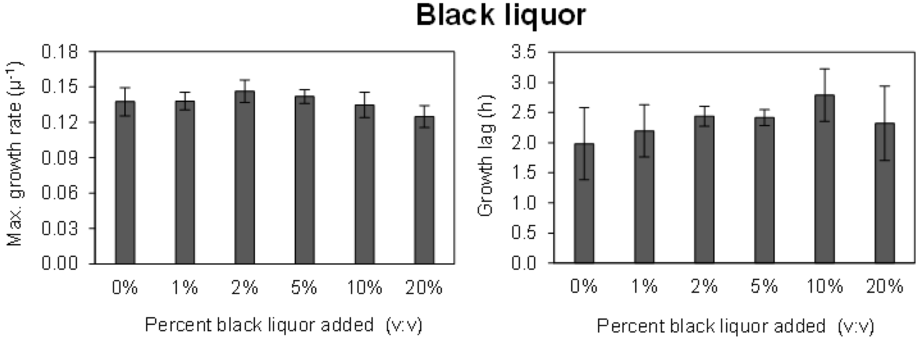
- Results derived from experiments guided by this work will substantially reduce the risk associated with feedstock variability on the biological lignin conversion unit operation.

Standardized processes may be too conservative and mask variability

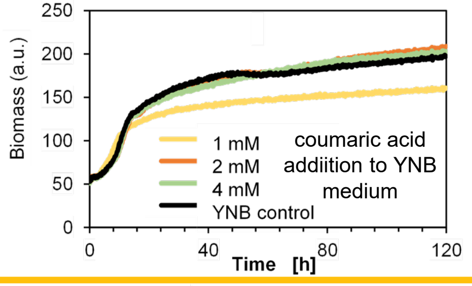
Knowledge

Black liquor contamination in sugar streams derived from the DMR-EH process can be tolerated. Washings in DMR process can be reduced with economic and environmental savings.

Clostridium tyrobutyricum



Rhodosporidium toruloides



Current Knowledge Gap

- Not all process parameters involved in producing lignin and sugar streams that have negative effects on downstream conversion are known.
- Process influences are organism specific; a first-principles understanding of pairing of organisms and negative impacts are outstanding.

Achievement

- Supplementation of up to 20% black liquor was tolerated by *C. tyrobutyricum* with no significant drop-off in biocatalytic performance.
- Coumaric acid additions of up to 4 mM had no effect on the growth rate of *Rhodosporidium toruloides*.

Relevance

- The number of wash steps can be reduced in cleaning sugar streams from the DMR process; black liquor carryover is not a critical materials attribute for *C. tyrobutyricum* or *R. toruloides*.
- Work with cross-cutting analysis teams will identify economic and environmental savings with reduced washings of material following deacetylation.

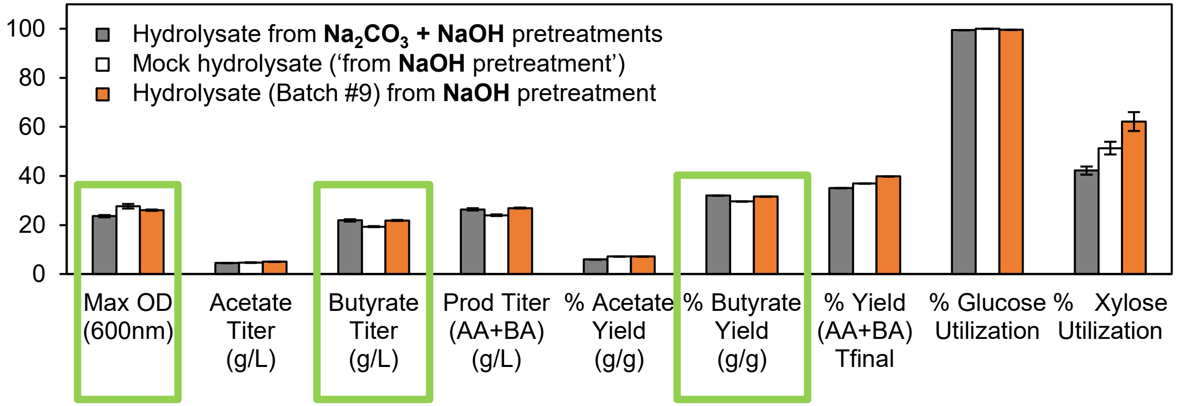
Deconstruction processes modifications: no impact on Low Temperature Conversion

Knowledge

The use of sodium carbonate as a base for deacetylation was shown to have limited impact on the performance of *Clostridial* species in conversion of sugars derived from the DMR process.

Clostridium tyrobutyricum-based conversion

Pretreatment strategy



Current Knowledge Gap

- Sodium carbonate has been proposed as a partial substitute for sodium hydroxide for pretreatment due to TEA and LCA considerations
- The use of Na_2CO_3 has proven effective on the upstream side, but the effect of the use of this base on downstream processes has not, to date, been examined.

Achievement

- Hydrolysate generated from Na_2CO_3-mediated deacetylated material was utilized as efficiently by *C. tyrobutyricum* as traditional NaOH-mediated deacetylated material.

Relevance

- The base used in deacetylation process does not appear critical/impactful for low temperature processes utilizing sugar streams (at least for some bacteria).
- Work with cross-cutting analysis teams will identify economic and environmental savings with reduced use of NaOH in the deacetylation steps.

Counter-ion selection for neutralization of alkaline processes is found not critical

Knowledge

P. putida can survive in salt concentrations exceeding those typically found in black liquor generated by deacetylation conditions; extractive interactions may prove impactful.

Current Knowledge Gap

- Excess salts resulting from deacetylation and neutralization steps comprise potentially critical MAs in black liquor.
- General effects of salts and interacting extractives have not been studied systematically across the entire supply chain (with economic and environmental viability considerations).

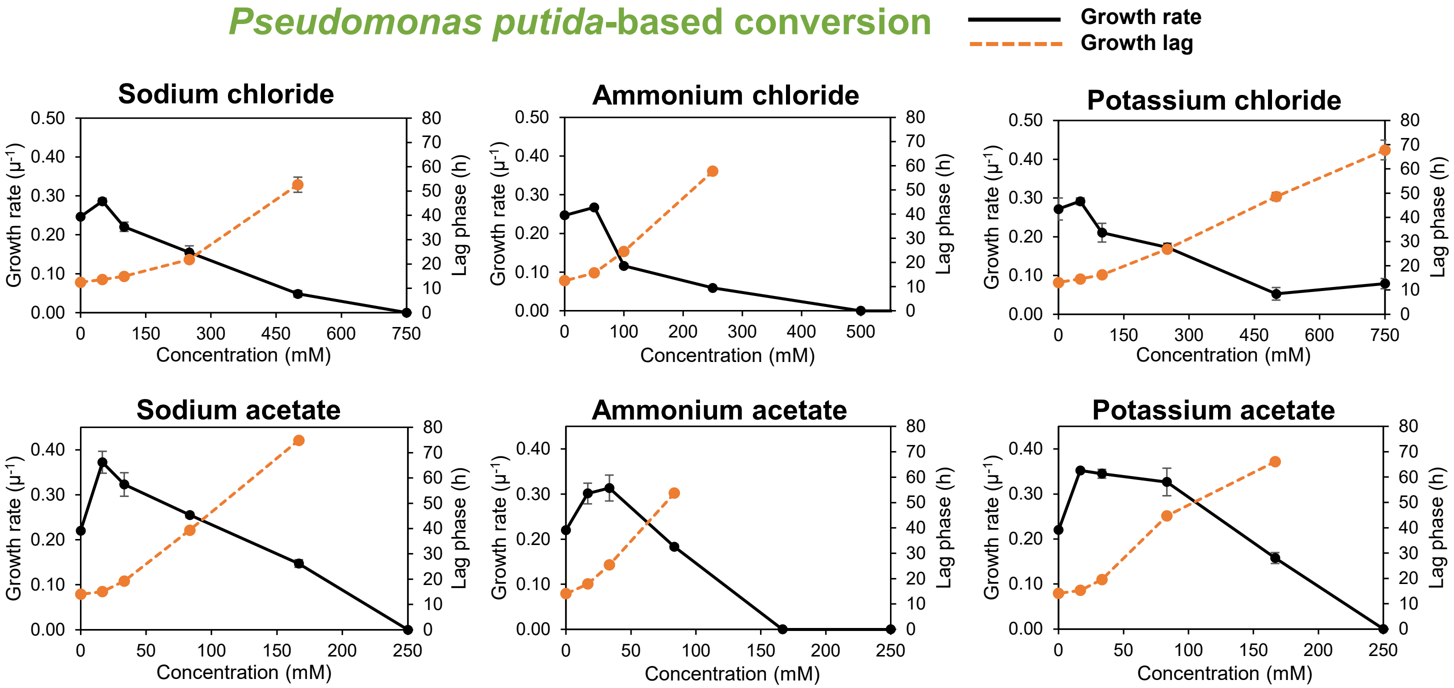
Achievement

- Salt toxicity begins at levels above those found in black liquor from typical deacetylation conditions.
- Acetate tolerated but not when pH-adjusted with ammonium hydroxide.

Relevance

- Counterions of acids and bases used in deacetylation do not appear impactful for low temperature processes utilizing lignin streams.
- Acetate in black liquor may interact negatively with some variable extractives, like ammonium.
- Results comprise important inputs into economic and environmental savings in choosing caustic agents for deacetylation.

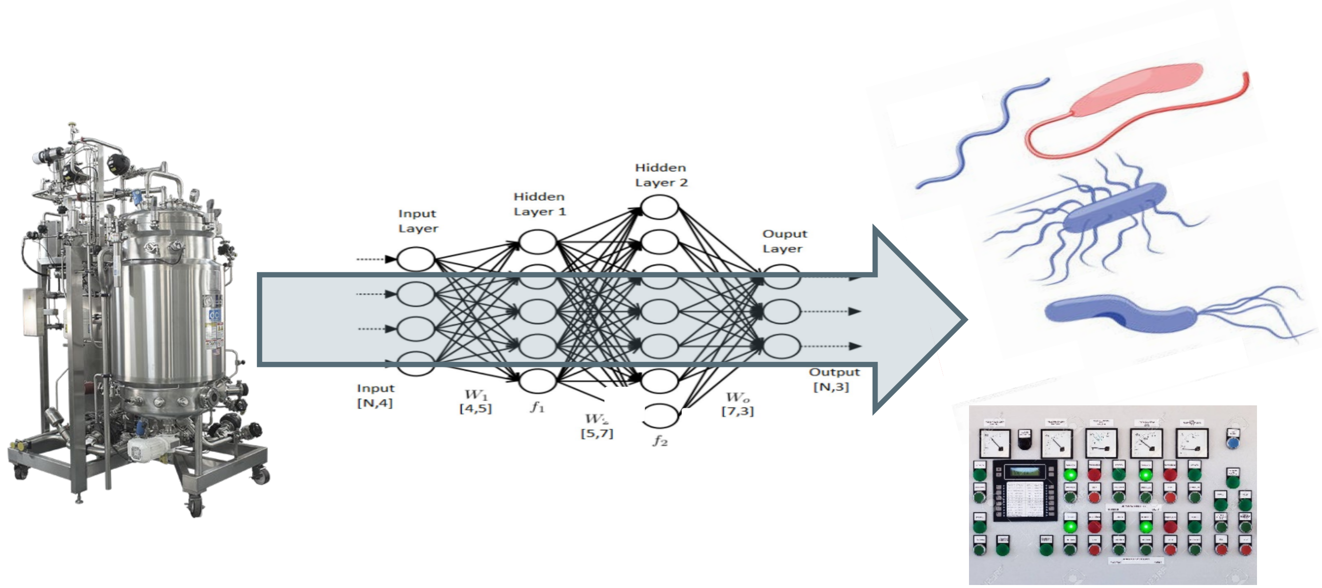
***Pseudomonas putida*-based conversion**



Train models with performance data to develop predictions for new LT processes

Plans

An opportunity exists to combine systematic performance data with metabolic/regulatory works and genomic information to build AI tools predictive of the performance of new organisms on variable feedstocks.



Current Knowledge Gap

- Performance of biocatalysts used in low temperature conversion processes on variable feedstocks streams can only be determined empirically
- Tolerance for variation in impactful materials attributes or process parameters not linked to underlying biochemistries

Planned Experiments

- Characterize performance of low temperature processes on additional sugar and lignin streams from corn stover of increased variability.
- Understand operating ranges of impactful material attributes and process parameters.
- Assemble and cluster future host organisms for tolerance to MAs and processes for variable feedstock streams
- Predict performance of new organisms on variable sugar and lignin feedstocks.

Planned outcomes

- Development and validation of an artificial-intelligence tool able to predict the performance of new organisms on variable sugar and lignin streams.

Summary

Management: This is a highly dynamic and interactive interdisciplinary research team that works in a distributive 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 low-T conversion processes – minimizing variability upstream in CPPs downstream via first-principles understanding of CMAs that facilitates performance predictability for future low-temperature processes.

Progress: Soluble lignin, sugar, and residual lignin streams generated/characterized from variable corn stover feedstocks; conversion performance tested experimentally; models generated; residual lignin salvaged through chemical depolymerization campaigns.

Quad Chart Overview- FCIC, Task 7

Timeline

- 10/1/2018* - 9/30/2021

	FY20	Active Project
DOE Funding	$1140K	FY19- $1350K FY20- $1140K FY21- $1140K Total- $3630K

Project Partners (N/A)

Barriers addressed

19-Ft-E

19-Ct-A

19-Ft-G

* Low Temperature Conversion (Task 7) activities of the FCIC were born of the FCIC restructuring in early FY19. As such, Task 7 activities started in earnest on February 15, 2019.

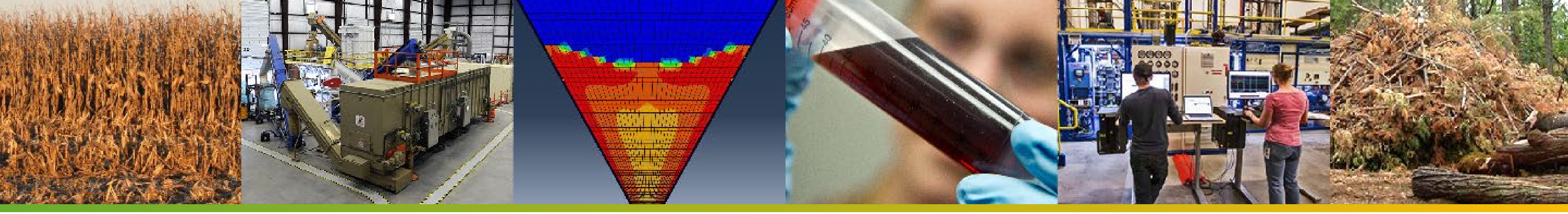
Project Goal

Determine the effects of biomass feedstock variability on the low-temperature conversion process chain (both sugar and lignin pathways) and develop knowledge and tools to mitigate the risks posed by this variability, facilitating performance predictability for future low-temperature processes

End of Project Milestone

Use data encompassing the parameters of feedstock variability (input from Task 2) correlated with 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%).

Funding Mechanism (N/A)



Thank you

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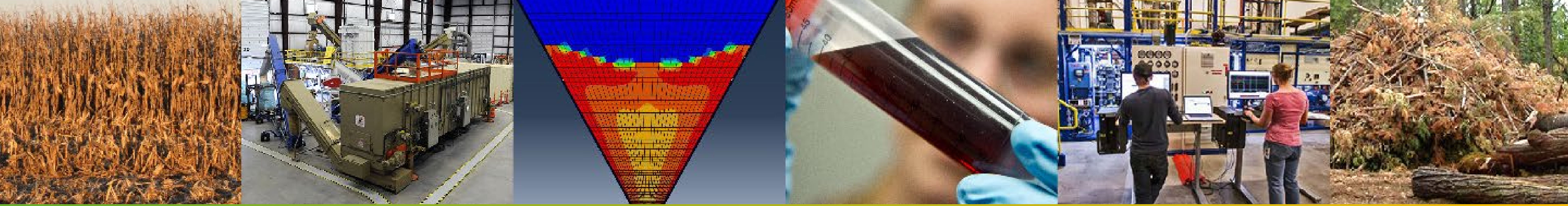

Additional Slides

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Responses to Previous Reviewers' Comments

- This project is a new start. As such, we have no responses to comments and questions from past reviews.

Publications, Patents, Presentations, Awards, and Commercialization

- This task within the FCIC is a new start.
- Four manuscripts describing intra- and inter-task work from our first 1.5 years of operation are in preparation:
  - Combined experimental and modeling approaches to predict performance of other organisms on sugar and lignin streams resulting from variable corn stover samples (coordinating publication with Feedstock Variability, Preprocessing, and Crosscutting Analyses teams)
  - Efficiency of conversion of streams from various anatomical fractions of corn stover
  - Kinetics of the corn stover deacetylation processes
  - Integrated chemical and biological upgrading of lignin species
- Technology transfer (and/or commercialization efforts) will be linked with techno-economic and life-cycle analyses. Process modifications leading to improvements will be protected/published in coordination with Crosscutting Analyses and Preprocessing (and potentially Feedstock Variability) teams. Model dissemination and public utilization will heavily leverage open-source-tools-distribution rules and plans being set forth at a higher level by the consortium and with input from other FCIC modeling efforts and the Data Integration task.