DOE Bioenergy Technologies Office (BETO) 2023 Continuous Enzymatic Hydrolysis Development (CEHD)

April 7, 2023

Biochemical Conversion and Lignin Utilization

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National Renewable Energy Laboratory

Overview – Product inhibition reduces productivity of batch hydrolysis

Goal

- Reduce the capex and opex of batch conventional enzyme hydrolysis EH and post hydrolysis solid-liquid separation using continuous EH (CEH) technology.
- Process risk reduction in an era of changing fermentation modalities.

Industrial diafiltration cartridge header

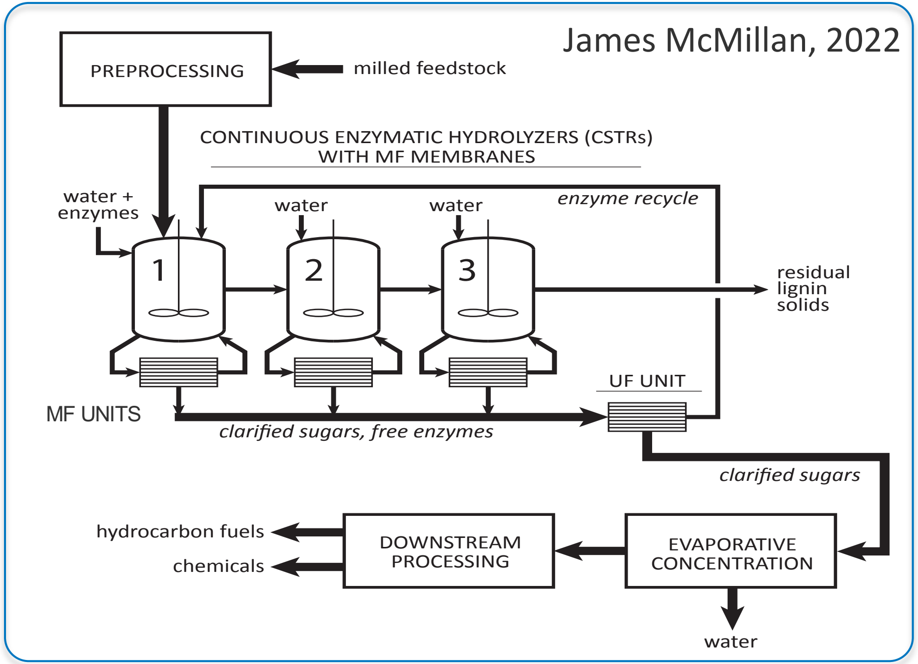
Questions to be Answered

- Can CEH serve as a disruptive improvement to SAF production via intermediates (ethanol, BDO, etc) from biomass sugars?
- Can the enhanced performance from CEH offset the cost of its deployment?
- Can CEH enable a nascent cellulosic sugar production technology model?

Overview

History

- Project in third year (initiated 2020)
- M. Himmel on-boarded as PI in September 2022.
- Using commercial enzyme and on-site produced *T. reesei* broth.
- Reestablishing critical parameters at bench scale.
- Will soon resume PDU skid work.

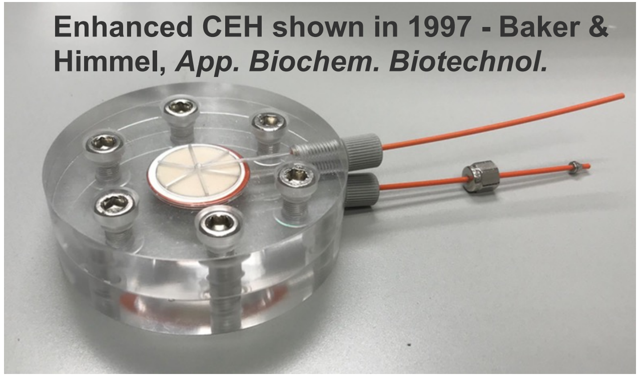


Relevance to BETO

- Enhance corn stover DMR solids>sugars (sugars>ETOH>SAF) process intensity.
- Significantly increase enzyme efficacy and lifetime in process (reduce catalyst production and use associated costs).

1. Approach – Project & Management plans

- We demonstrate that CEH has the potential to advance the state of the art of lignocellulose deconstruction to sugars which supports BETO's corn stover to sugars; as well as the corn stover to ethanol to SAF goals.



- Our revised Management Plan (for year 3):
  - Retain existing project engineers/subcontractor and add biochemists familiar with cellulase functionality (i.e., same budget).
  - Reconfigured Project year 3 final milestone which generates a TEA based on preliminary small scale CEH skid data and company feedback.
  - Revised Risk Assessment Plan (see next slides)
  - Identified **new** stake-holders/lines of communication are being established.
  - Identified Diversity, Equity, and Inclusion Plan (see next slides).

1. Approach – Stake Holders & DEI

State Holders

- Company interest in the CEH project is critical for generating reliable TEA:
  - Novozymes – Michael Burns, Head of Americas, Biomass
  - SAFFIRE Renewables – Mark Yancey, CTO
  - CeraMem LLC/ALSYS Group – Kevin Donahue, BD Manager
  - Subcontract for process rheology – Prof. Joe Samaniuk, CSM

Diversity, Equity, and Inclusion

- We will work with CSM to attract students from the Multicultural Engineering Program and CU Boulder's Environmental Engineering Program called IDEA (Inclusion, Diversity and Excellence in Academics).
- We will seek a minority/woman owned industrial partners and vendor for fabrication/repair of 30-Liter pre-saccharification tank.

1. Approach – Technology & Challenges

Technical Approach

- Develop CEH based on commercial UF/MF systems to greatly enhance process intensity, enzyme performance, and enzyme lifetime in the CS>sugars>EtOH>SAF process.
- Confirm CEH assumptions with rigorous new scale bench studies.
- For TEA, collect UF performance data using leading enzyme formulation and PDU CEH skid.

Commercial RO: ~500,000 L/h

Potential Challenges

- Unknown ability of commercial ceramic membranes/housings/pumps to process relevant slurries (10% w/w and greater) of pretreated biomass.
- Unknown lifetimes of membrane compartmentalized hydrolytic enzymes.

1. Approach - Risks for CEH

Risk	Status	Target Completion Date	Severity	Mitigation/Resolution	Outcome
CTec3HS performance is not improved by end-product removal (Novozymes product bulletin)		12/30/2022	High	For Novozymes CtTec3 HS, showed that CEH is significantly superior to EH batch	
Commercial UF/MF systems are incompatible with DMR slurries		9/30/2023	High	Results with PDU skid will be predictive of full commercial scale to a point (core/shell clearance)	Initial results in years 1-2 suggest **this is not the case.**
For enzyme recycle, particle to particle transfer not sufficient					

1. Approach – FY2023 (final year) Milestones

- **Discuss Go/No-Go decision points and why they are critical to the project**.

FY22Q2 GNG – Implement a virtual engineering (VE) approach for rapid model-based evaluation of cost impacts of CEH design and operational parameters to enable in silico optimization. (Joint with BPA (WBS 2.1.0.100) and Virtual Engineering (WBS 3.1.1.006)

- Inconclusive. Delayed indefinitely.

FY23Q1 GNG – Determine if commercial enzyme formulations are end product inhibition relieved as stated in product bulletin. Go supports continuation in FY23.

- Project is Go! UF processing greatly exceeded the performance of both on-site produced *T. reesei* and commercial enzyme preparations.

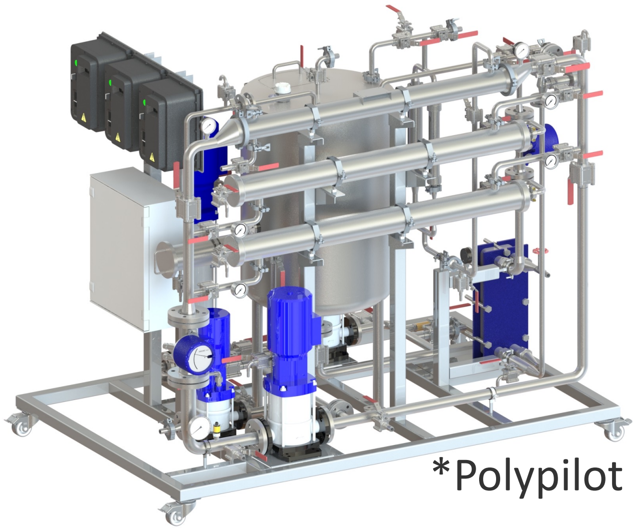
1. Approach - Historical CEH Project Milestones

Milestone Name/Description/Criteria	End Date	Type
Q1. Consolidate and curate at least three CEH operational datasets by data-mining all previous experimental runs done on industrially-relevant equipment. Transmit datasets to virtual engineering (VE) project to support upcoming Go/No-Go joint milestone.	12/31/2021	Quarterly *Delivered*
Q3. Perform additional characterization experiments on reactor vessel agitation, recirculation pump energy requirements, membrane performance and/or other equipment or process dataset identified necessary by VE to refine models. Supply new data to VE project.	6/30/2022	Quarterly *Delivered with Q4*
Q4. Update CEH State of Technology (≥72 hours pseudo steady-state operation at ≥ 8.5% insoluble solids and ≤ 12 mg/g enzyme loading) using best available enzyme(s) and DMR feedstock. Quantify TEA impact of experimentally-measured technical improvements to CEH single stage system performance. Recommend strategies for further cost reduction.	9/30/2022	End of Yr 2 Annual SMART *Partially Delivered*
Q4. Demonstrate that CEH offers cost incentives for replacing SHF in ethanol production or Separate hydrolysis (SH) for the “sugar depot.”	9/30/2023	End of 3-Yr Project

1. Approach - Commercial UF with Ceramic Membranes is Commonplace

Commercial scale potential

NREL mini skid: (1x120 cm) ~ 0.5 m^2 tubular monochannel =~25 L/h
ALSYS PolyPilot skid: (3x120 cm) ~ 1.5 m^2 tubular multichannel = ~500 L/h
Commercial UF: (48x330 cm) ~72 m^2 tubular multichannel >1000 L/h
Commercial RO: (400 plus) multichannel membranes ~500,000 L/h



*Polypilot 200

Tangshan UF system for SWRO pre-treatment. UF membranes housed in the T-Rack system, treating 110,000 m^3/day of seawater.

- **Pall Corporation**
- **ALSYS Group***
- **Sanborn Technologies**
- **AquaPore**
- **Porex**

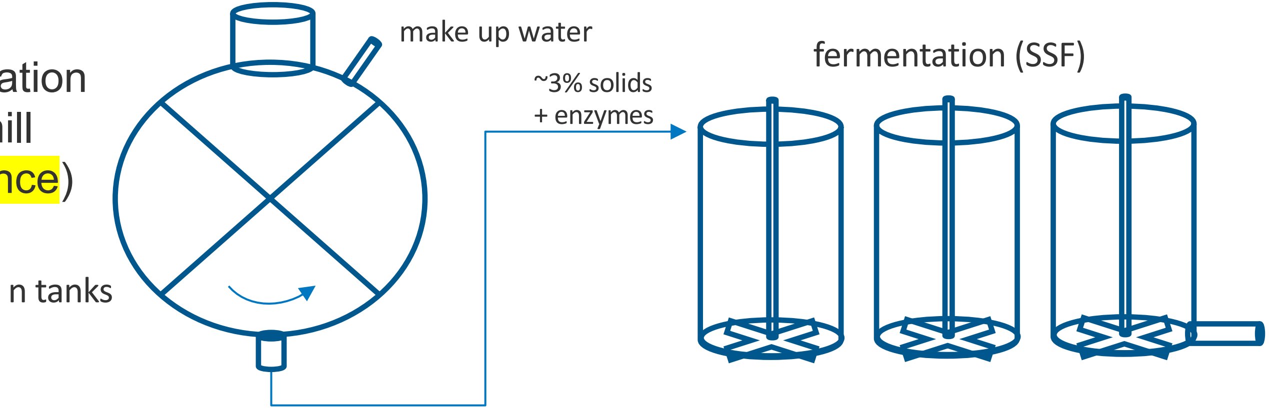
1. Approach – CEH Preserves Valuable Enzymes*

NREL FY22 SOT is Batch Saccharification*

***Traditional enzyme hydrolysis process – hybrid SHF or SSF. No enzyme recycle feasible.**

*20% DMR solids + 10 mg Ctec Htec/ gram glucan; 84% glucan and 94% xylan conversion

Enzyme Saccharification (EH) pug mill (6-d residence)

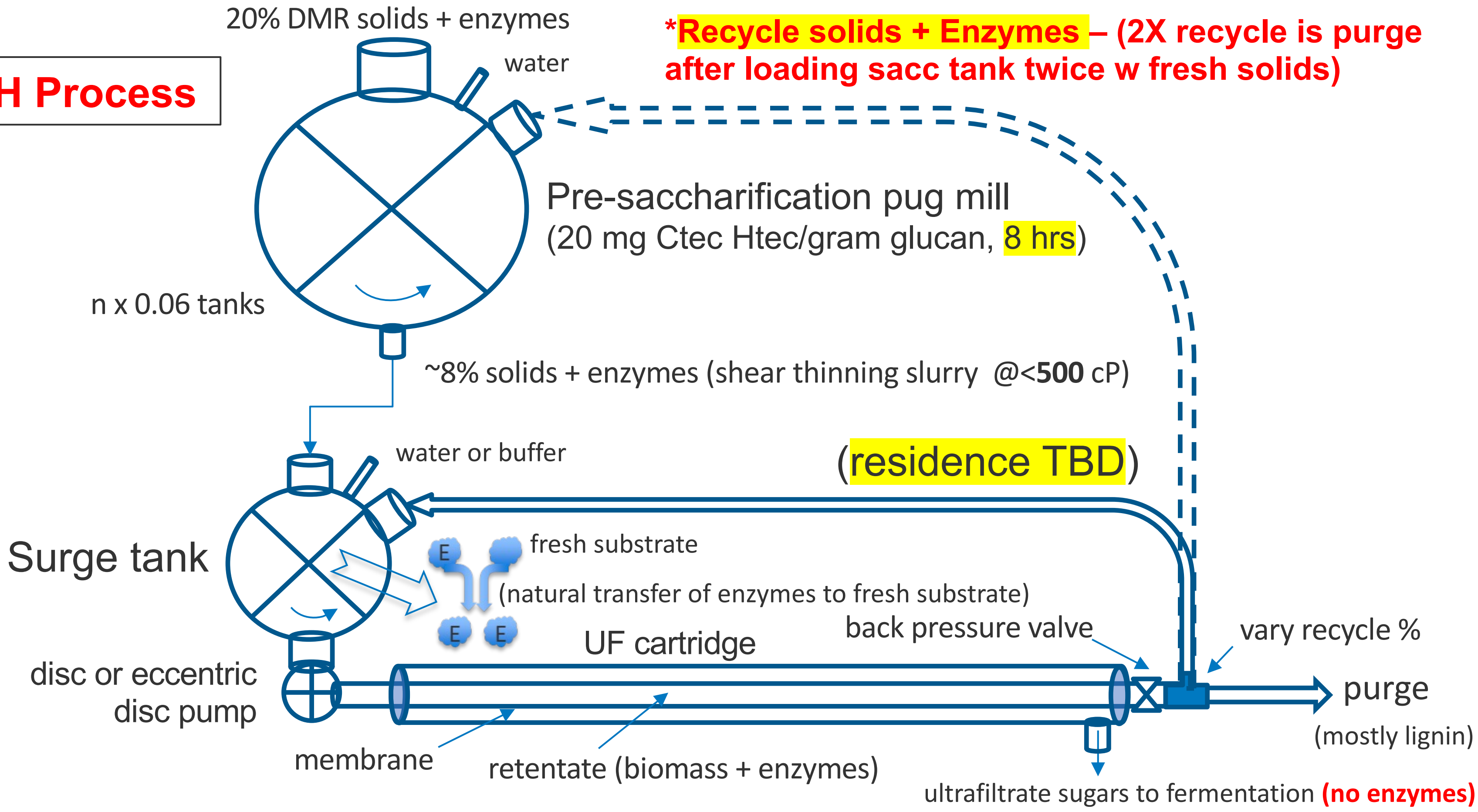


enzymes lost in fermentation residue and distillation bottoms

1. Approach – CEH Preserves Valuable Enzymes*

CEH Process

***Recycle solids + Enzymes – (2X recycle is purge after loading sacc tank twice w fresh solids)**



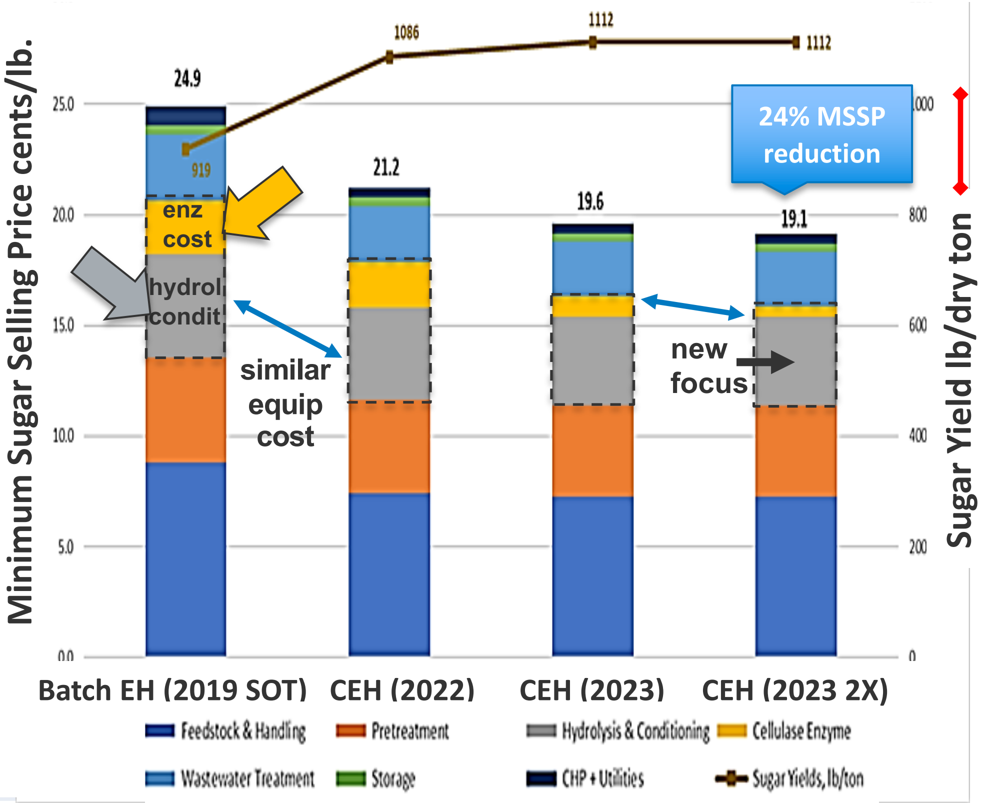
1. Approach – Preliminary Economics & Assumptions*

Bench scale CEH preliminary assessment shows a ~24% reduction in MSSP relative to Batch

MSSP, $/lb	Batch EH (2019 SOT)	CEH (2022 update)	CEH (2023 update)	CEH (2023 update + 2X cycling)
Feedstock & Handling	$0.088	$0.074	$0.073	$0.073
Pretreatment	$0.048	$0.042	$0.041	$0.041
Hydrolysis & Conditioning	$0.047	$0.042	$0.040	$0.040
Cellulase Enzyme OSPTr	$0.024	$0.021	$0.009	$0.005
Wastewater Treatment	$0.030	$0.025	$0.025	$0.025
Storage	$0.004	$0.004	$0.004	$0.004
Boiler/Turbogenerator	$0.003	-$0.001	-$0.001	-$0.001
Utilities	$0.006	$0.006	$0.005	$0.005
Total	$0.249	$0.212	$0.196	$0.196

MSSP, cents/lb	Batch EH (2019 SOT)	CEH (2022 update)	CEH (2023 update)	CEH (2023 update + 2X cycling)
Feedstock & Handling	8.8	7.4	7.3	7.3
Pretreatment	4.8	4.2	4.1	4.1
Hydrolysis & Conditioning	4.7	4.2	4.0	4.0
Cellulase Enzyme	2.4	2.1	0.9	0.5
Wastewater Treatment	3.0	2.5	2.5	2.5
Storage	0.4	0.4	0.4	0.4
CHP + Utilities	0.9	0.4	0.4	0.4
Total	24.9	21.2	19.6	19.1
Sugar Yields, lb/ton	919	1086	1112	1112

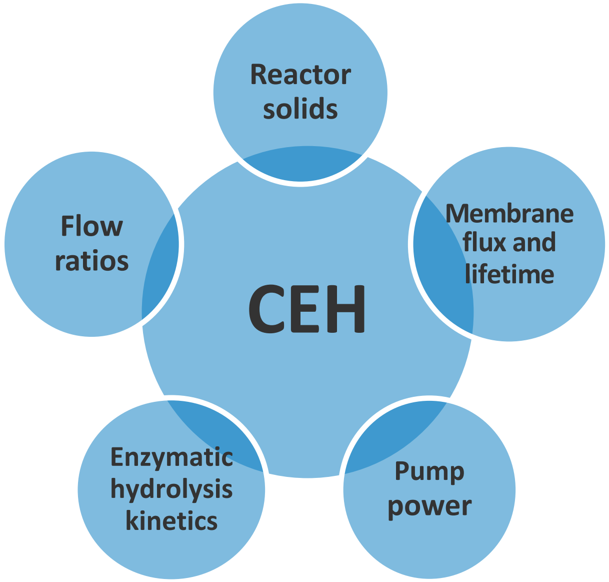
***2022 NREL SOT - BDO-lignin burn (conversion-only costs excluding feedstock contributions) 2016 dollars case.**



2. Progress and Outcomes - Overview

Progressive R&D followed Platform models :

- Yr1 focused on building 30-L CEH skid (Porex 1.2 m UF membrane) and testing dilute acid (DA) solids
- Yr2 extended the skid system to testing DMR solids which illustrated the need for a pre-saccharification stage to reduce feed viscosity.
- Yr3 will focus on collecting performance data for DMR solids using the PDU pug mill and CEH skid for final TEA.



TEA based on bench scale data

- TEA has already shown potential of CEH to lower MSSP over EH from bench scale data.

First scale up studies - CEH skid with 1.2 m Porex membrane

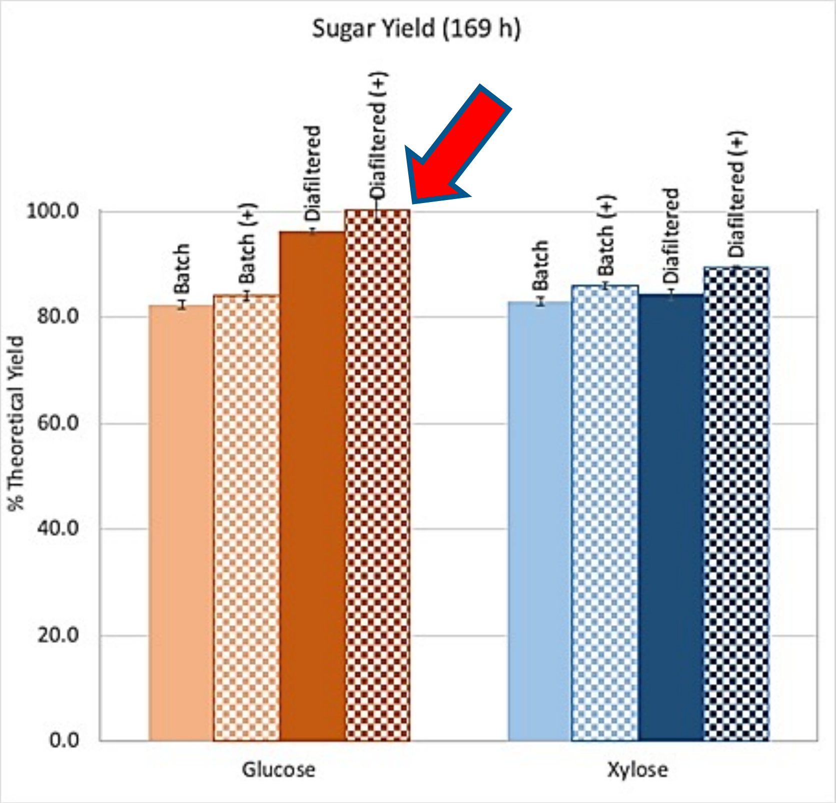
- Dilute acid (DA) and deacetylation & mechanical refining (DMR) solids tested.
- Plan to use CEH skid plus 1900-L pug mill to conduct system performance testing

2. Progress and Outcomes - Bench Scale for FY23Q1 MS

- 10% (w/v) DMR Stover, 40 mL, pH 4.8, 50°C, in air
- Diafiltered daily with Centricon tubes - then fresh buffer added back
- New lower enzyme loading - **5 mg CTec3HS/g glucan**
- +/- enzyme prep low mw added back (contain LPMO mediators)

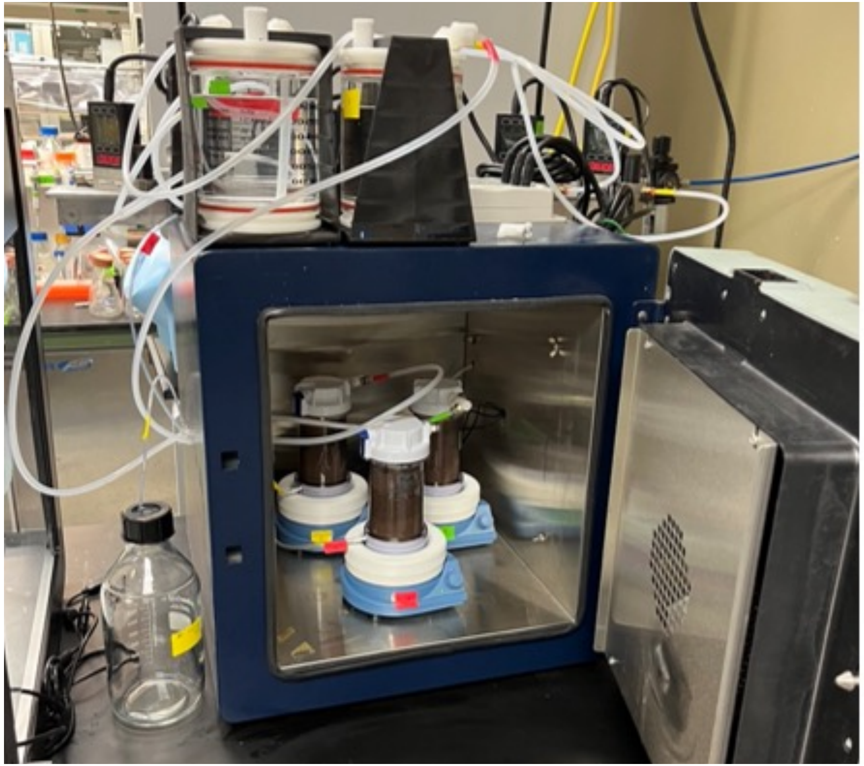
169 h	Glucose	Stdev	Xylose	Stdev	%increase glc	%increase xyl
Batch	82.3	0.7	82.9	0.7	-	-
Batch (+)	84.2	0.9	85.9	0.5	-	-
Diafiltered	96.2	0.5	84.3	1.2	16.8	1.6
Diafiltered (+)	100.3	2.2	89.4	0.2	19.1	4.0

✓ **CEH increased yield of glucan by ~20% with lower enzyme loading!**



2. Progress and Outcomes – New Bench Scale

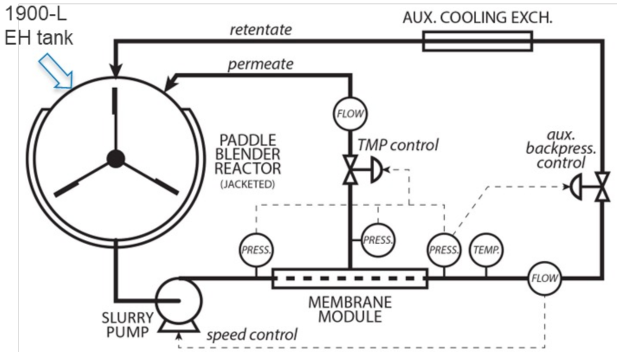
Now using larger Bench scale CEH with Stirred Cells (15% solids, 200 mL)

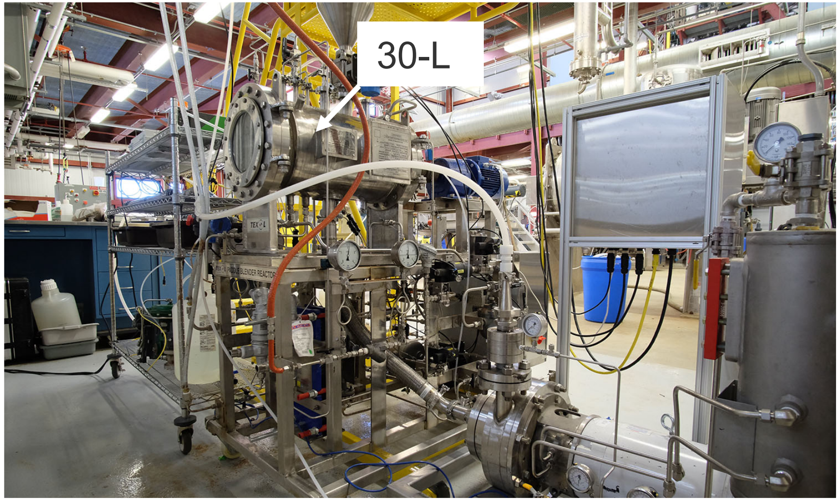


2. Progress and Outcomes, PDU CEH skid System

FY21 Assembled the PDU CEH skid

- Single-stage UF system using a 30-L surge tank, one Porex 1.2 m, 0.05 μm **PVDF** membrane, and an integrated pumped external recycle loop.
- CEH skid system was fed pre-saccharified DMR slurry (8% w/v) transferred from the 1900-L horizontal paddle blender.
- Novel vessel level measurement method (gas law) implemented and validated.

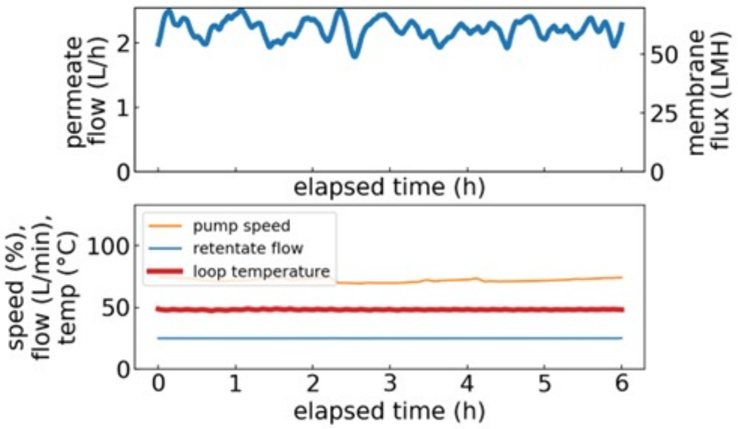


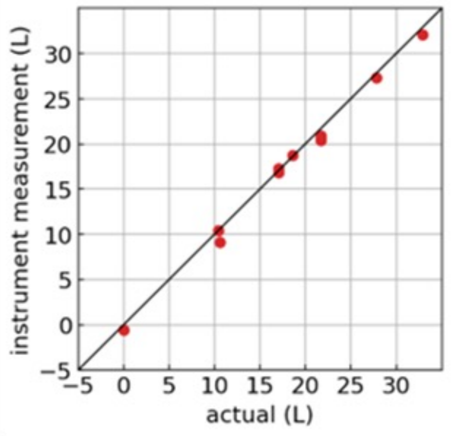


2. Progress and Outcomes - Initial Operation of CEH skid

FY 21 CEH reactor-membrane qualification for DA solids

- Understand key performance sensitivities and interactions.
- Demonstrate extended pseudo steady state performance.
- Preliminary performance data collected to qualify system.
- Initial qualification testing showed ability to operate at ≥ 8.5% IS using dilute acid (DA) pretreated corn stover.





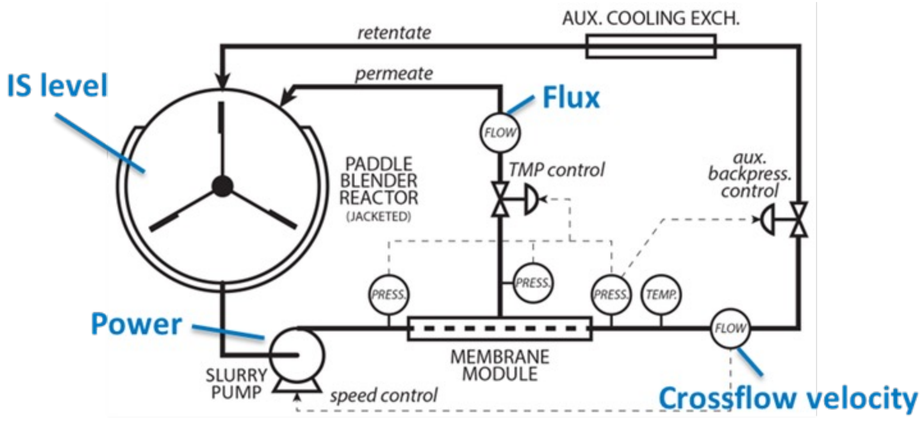
2. Progress and Outcomes, DA and DMR Feedstocks

DMR feedstocks present more challenges rheologically than dilute acid corn feedstocks

- **Max. pumpable DMR EH slurry insoluble solids (IS) level is <10%** depending on extent of enzymatic hydrolysis; **vs. ≥ 10%** for DA substrate.
- Extent of conversion significantly influences performance.
- This was the rationale for adding the 1900-L pre-saccharification blender to deliver ~8% solids to the CEH skid.

✓ Next, evaluate a range of **ceramic** membrane pore sizes (KleanSep 50 & 150 kDa; 0.03 & 0.1 μm).

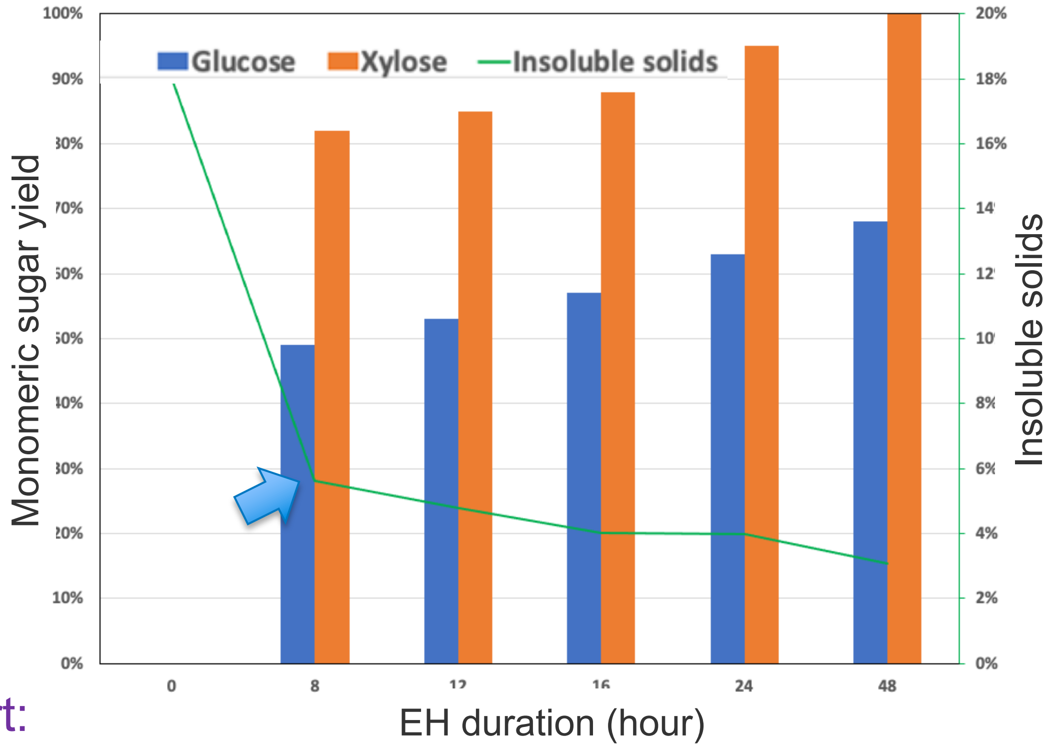
	DMR PCS Feedstock Slurry	DMR PCS EH Slurry	
Recirc. loop flowrate (LPM)	30	25	30
Maximum Steady State IS (%)	5.1	12.6	8.3
Avg. Permeate Flux (LMH/bar)	188	46	25



2. Progress and Outcomes - Fy22 Q4 Milestone

The performance of the CEH is affected by the slurry properties which are a function of the extent of saccharification.

- ➔ Performed pilot scale batch EH (1900-L pug mill) to obtain DMR hydrolysate slurries sampled at **8**,12,16, 24, and 48 hrs.
- ➔ Enzymes deactivated and slurries transferred to CEH skid.
- ➔ Testing revealed some useful UF performance parameters and functionality problems (enzyme loss).



Take-away lessons from Q4 report:

- As extent of conversion increases, pump power decreases.
- As the insoluble solids decrease, the membrane performance increases.
- ✓ Need membranes better suited to cellulase hydrolysis (i.e., ceramic and smaller pores)

Quad Chart Overview

Timeline
- Project start date – October FY2021
- Project end date – October FY2023

	FY22 Costed	Total Award
DOE Funding	(10/01/2021–9/30/2022)	$550,000 $550,000 $550,000
Project Cost Share*		0

TRL at Project Start: TR2-3

TRL at Project End (FY26): TRL5 (ready for commercial pilot scale testing)

Project Goal
The drawback of SHF technology is the negative impact on saccharification elicited by build up of free sugars. Moreover, the new enzyme formulations from Novozymes, now the sole supplier of commercial Gen2 enzymes in North America, are no longer rated for SSF. Membrane retention of these enzymes during hydrolysis has the potential to greatly decrease cost by enhancing enzyme kinetics and their process lifetime (reduce enzyme replacement).

End of Project Milestone
Demonstrate that CEH offers cost incentives >20% for 1) replacing the current batch saccharification SHF ethanol production process and 2) replacing separate hydrolysis for nascent "sugar depot" concepts.

Project Partners
- SAFFiRE Renewables – DMR solids – Mark Yancey
- CSM Subcontract – rheology - Joe Samaniuk
- Novozymes – commercial enzymes – Michael Burns
- Alsys – commercial scale UF – Kevin Donohue

3. Impact of CEH – MSSP and MFSP

CEH is a transformative route to clarified biomass sugars and uncontaminated (upgradable) lignin

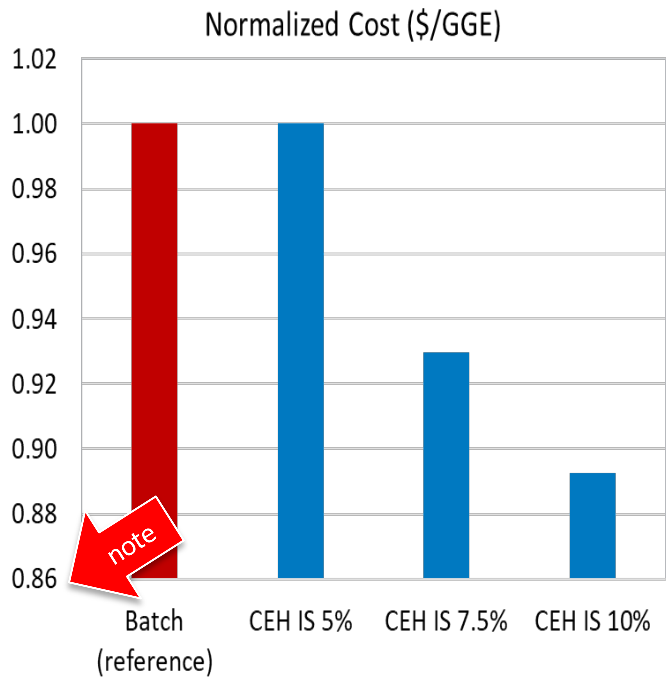
TEA from bench scale studies using CTec3HS predict large cost savings in sugar model over batch due to higher conversion yields & lower enzyme production cost:

- MSSP Batch EH = 24.9 cents/lb – current base case
- MSSP CEH FY22 = 21.2 cents/lb
- MSSP CEH FY23 = 19.6 cents/lb
- MSSP CEH FY23 2x = 19.1 cents/lb (recycle beyond 2x is very credible)
- This MSSP reduction of 5.8 cents/lb corresponds to a GGE* reduction of $1.45 of $6.24/GGE for the BDO-lignin burn (conversion-only costs excluding feedstock contributions) case in the 2022 NREL SOT.

*gallon gasoline equivalent

3. Impact - Key Take-Aways for Commercial Implication – CEH versus EH

- Largely similar CAPEX for batch EH and CEH
  - CEH requires different equipment. EH requires more blender (pug) mills and CEH requires UF units.
- Largely similar OPEX
  - CEH requires more electricity for evaporation to concentrate the more dilute sugars produced.
  - EH requires greater enzyme usage.
- Higher electricity costs of CEH are offset by eliminating (problematic) polyelectrolyte flocculant used for EH.
- Batch EH TEA is dominated by equipment, enzymes, and post hydrolysis solid-liquid separation (flocculation) for lignin separation.
- CEH TEA dominated by equipment, evaporation, and higher power requirements.



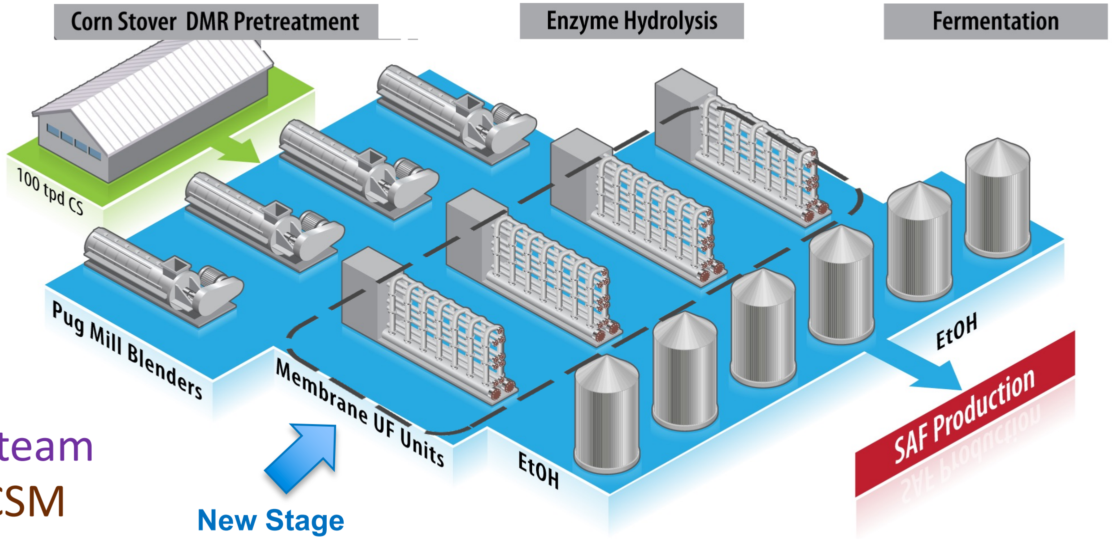
Summary

Project goal: Develop CEH technology to improve cost, efficiency, and manufacturability of biomass sugar-lignin platform

1. **Management:** Change in leadership beginning FY23 (year 3). Team includes chemical and mechanical engineers and now, biochemists. Several companies have expressed an interest in our progress. *Some milestones will be renegotiated*.
2. **Approach:** Innovative application of process intensification using CEH to replace EH in sugar production. Taking a step back to collect bench data and returning to small pilot plant scale using improved 30-L CEH skid fed from 1900-L presacc blender.
3. **Impact:** CEH can be transformational. We showed that 100% conversion of glc and xyl is possible and that the critical lignin S/L separation is "built in." This performance must now be achieved and sustained at pilot plant scale.
4. **Progress & Outcomes:** Bench scale CEH data suggests very favorable TEA (i.e., ~24% reduction in MSSP). Refocused FY23 work to install better membranes in the CEH skid to generate data for the FY23 TEA.

Thank You and Q&A

Beau Hoffman, DOE HQ
Zia Abdullah, NREL LRM
Mike Guarnieri, NREL PL
Yudong Li, CEH team
Steve Decker, CEH team
Roman Brunecky, CEH team
Dave Sievers (NREL)
Ryan Davis, NREL Process A&S team
Joe Samaniuk, subcontractor, CSM



This work was authored in part by the National Renewable Energy Laboratory, operated by Alliance for Sustainable Energy, LLC, for the U.S. Department of Energy (DOE) under Contract No. DE-AC36-08GO28308. Funding provided by U.S. Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office. The views expressed in the article do not necessarily represent the views of the DOE or the U.S. Government. The U.S. Government retains and the publisher, by accepting the article for publication, acknowledges that the U.S. Government retains a nonexclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this work, or allow others to do so, for U.S. Government purposes.

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Additional Slides

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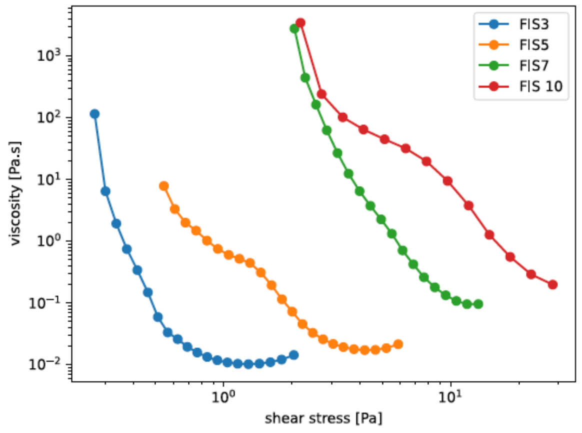
We Are Developing A Broad Variety Of Technologies To Produce Intermediates, and To Upgrade the Intermediates to SAF – Zia Abdullah

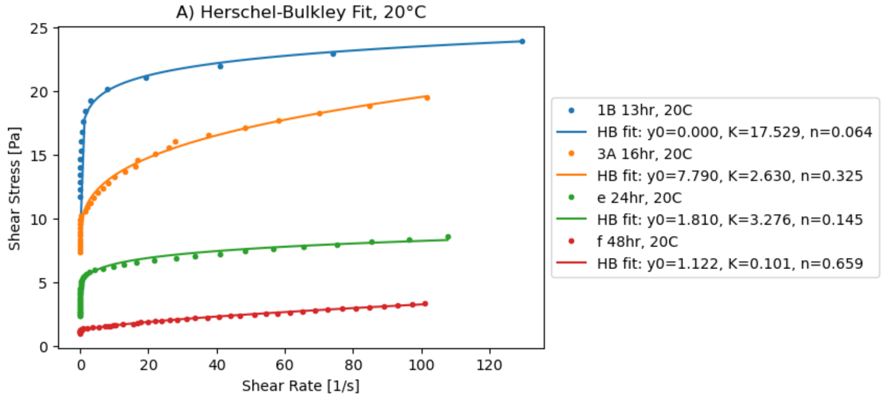
Value Chain From Biomass to SAF and Chemicals May Involve Multiple Business Partners

4. Progress and Outcomes, CSM Rheology, J. Samaniuk

The viscosity/rheology of slurry impact the transportation and handling designs and operations. We performed rheology measurements for slurries with different extent of saccharification.

- Enzymatic hydrolysate is a shear thinning slurry. The higher the transfer speed the lower the viscosity of the slurry.
- Higher saccharification extent reduces slurry viscosity significantly which means better handling performance
- Lower insoluble solids content reduces slurry viscosity significantly





FY22 Milestones

	Milestone Description	Criteria	End Date	Type
✓Q1	Develop ≥ 3 three CEH operational datasets by data-mining previous experiments done on industrially-relevant equipment.	Provide datasets mined from previous experimental runs using industrially-relevant/scalable equipment (reactor, pump, membrane) to Virtual Engineering (VE) project to support Q2 Go/No-Go joint milestone.	12/31/2021	Quarterly Progress Measure
✓Q2	Implement a virtual engineering (VE) approach for rapid model-based evaluation of cost impacts of CEH design and operational parameters to enable in silico optimization. (Joint with BPA (WBS 2.1.0.100) and Virtual Engineering (WBS 3.1.1.006)	Integrate enzymatic hydrolysis (python), unit operations (python or ASPEN), and fuels-coproducts biorefinery (ASPEN) models to enable automated / more facile evaluation of CEH design and operation cost impacts. Identify critical CEH design parameters and evaluate at least 10 realistic variations of CEH design and operating conditions in an automated fashion (≤ 5% deviation from traditional costing approach for at least 1 case).	3/31/2022	Go/No-go
✓Q3	Perform additional characterization experiments as identified necessary by VE / Q2 milestone to refine models. Supply new data to VE project.	Improved process dataset focus guided by VE to further characterize CEH performance = f(reactor vessel agitation, recirculation pump energy requirements, membrane performance, etc.)	6/30/2022 Experiments In progress; delivery in Q4.	Quarterly Progress Measure
? Q4	Update CEH State of Technology (≥72 hours pseudo steady-state operation at ≥ 8.5% insoluble solids and ≤ 12 mg/g enzyme loading) using best available enzyme(s) and DMR feedstock.	Quantify TEA impact of experimentally-measured technical improvements to CEH single stage system performance. Recommend strategies for further cost reduction.	9/30/2022	End of Year 2 Annual SMART

Responses to Previous Reviewers' Comments

Q: "The PIs are targeting slurries with 10% solids. To provide a benchmark, it would be useful if the PIs could present industrial analogies that carry out selective, continuous transformations at high solids levels to get a sense of the challenge."

A: Examples include conventional cement pumps and mortar pumps used for 3D printing dwellings. These are extreme applications, far more rigorous than pumping 10% biomass. We agree that further proof-of-concept CEH operation over longer times and at higher insoluble solids levels is necessary, and we have planned and are already performing experiments on this using an industrially relevant (mini-pilot) CEH equipment. Scale is the key and operating such small-scale equipment is challenging.

Q: "Information on whether the restrictions of DMR material negatively influence the potential for success of the overall process would be helpful."

A: The rheology of DMR slurries does indeed differ from dilute acid slurries, but we have observed experimentally only a tendency of the former to flow less consistently at transitions to small piping (i.e.,1 to 1-1/2-inch ID). The performance of positive displacement pumps seems unchanged for DMR slurries below about 12% solids, but as always, particle size, viscosity, and dissolved solids titers all contribute.

Q: "This project uses a normalized metric of the MSSP instead of the MFSP that is used in other projects within the BETO portfolio. The change in metrics makes it difficult to compare."

A: This was true in FY21, but the FY22 SOT builds more on the sugar model (MSSP). We see sugars as an outstanding intermediate from which industry can generate a host of fuels, including SAF. In any case, we will stress MFSP more in the future.

Publications, Patents, Presentations, Awards, and Commercialization

- J. O. Baker, T. B. Vinzant, C. I. Ehrman, W. S. Adney, M. E. Himmel, Use of a new membrane-reactor saccharification assay to evaluate the performance of celluloses under simulated SSF conditions : effect on enzyme quality of growing *Trichoderma reesei* in the presence of targeted lignocellulosic substrate**.** **Appl Biochem Biotechnol** 63-65, 585-595 (1997).
- J. J. Stickel, B. Adhikari, D. A. Sievers, J. Pellegrino, Continuous enzymatic hydrolysis of lignocellulosic biomass in a membrane-reactor system. **J Chemical Technol & Biotechnol** 93, 2181-2190 (2018).
- "Kinetics Modeling for Design of Continuous Enzymatic Hydrolysis," James J. Lischeske, Nicholas Grundl, David Sievers, James D. McMillan, Jonathan Stickel, **Symposium on Biotechnology for Fuels and Chemicals**, 4/27–5/2, 2019; NREL/PO-5100-73788,
- J. J. Lischeske, J. J. Stickel, "A two-phase substrate model for enzymatic hydrolysis of lignocellulose: application to batch and continuous reactors". **Biotechnol Biofuels** 12, 299 (2019).
- "Process Intensification and Scale-up of a Continuous, Enzymatic Hydrolysis and Separation Process," David A. Sievers, James J. Lischeske, and James D. McMillan, **Symposium on Biomaterials, Fuels, and Chemicals**, Industrial Microbiology Biotechnology, 4/26–28, 2021; NREL/PO-5100-79587.

Describe the status of any technology transfer or commercialization efforts.

- SAFFiRE Renewables will provide samples of DMR slurry when operational (letter of interest available)
- CeraMem LLC - ALSYS Group is providing cost and use scenarios for UF/MF systems for the TEA
- U.S. provisional patent application No. 63/074,846 NREL ROI No. 20-39 for novel nonintrusive vessel level measurement was filed on September 4, 2020, United States Patent & Trademark Office.