NREL Transforming ENERGY

Pacific Northwest NATIONAL LABORATORY

BERKELEY LAB

Argonne NATIONAL LABORATORY

BioHydrogen (BioH2) Consortium to Advance Fermentative H_2 Production

Katherine Chou (PI/Presenter)
National Renewable Energy Laboratory
DOE Project Award/AOP #: HFTO.2.4.0.516
June 8, 2023

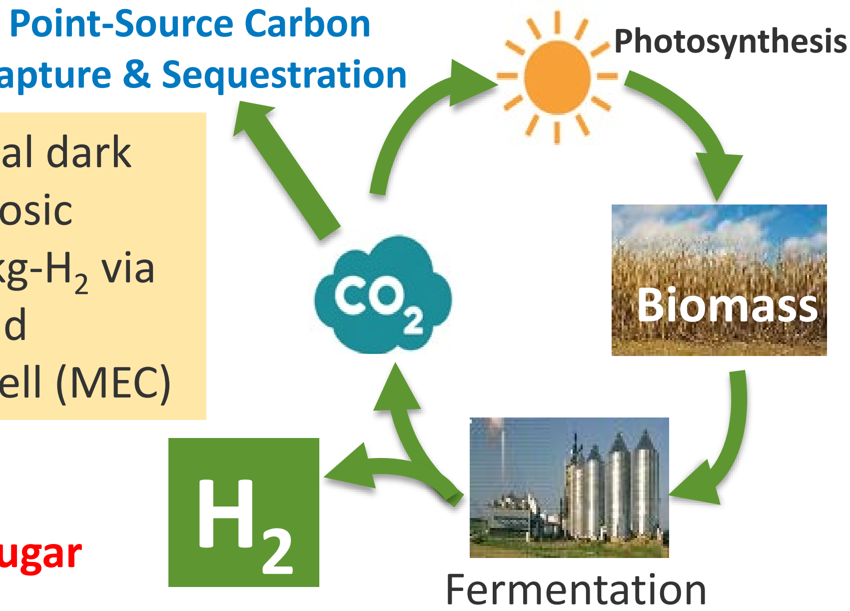
BioH2 Consortium

DOE Hydrogen Program
2023 Annual Merit Review and Peer Evaluation Meeting

Project ID #: P179

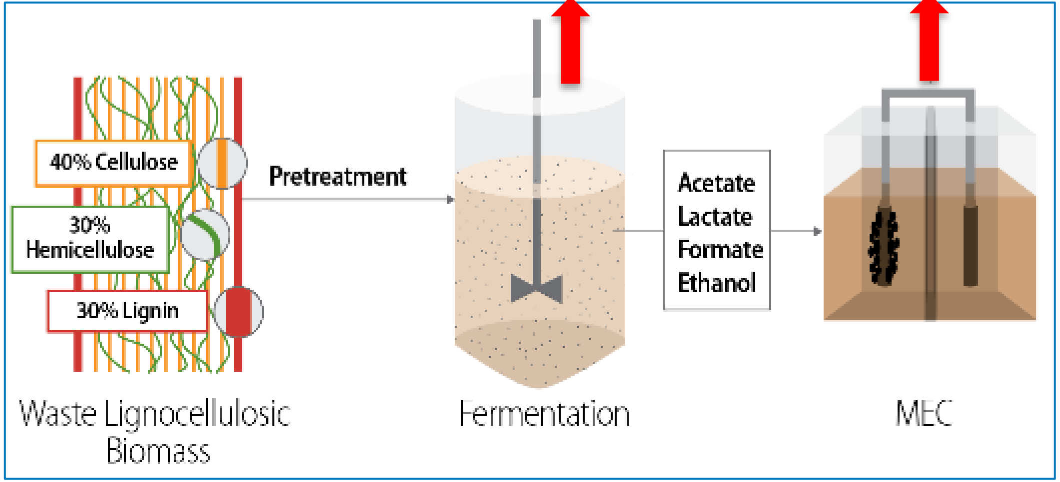
Project Goal

Overall Objective: Develop a carbon-neutral, microbial dark fermentation technology to convert waste lignocellulosic biomass into H_2 with a production cost less than \$2/kg-H_2 via strain engineering, bioprocess design for scale-up, and integrating fermentation with microbial electrolysis cell (MEC)


Point-Source Carbon Capture & Sequestration

12 mol H_2/mol sugar

4 mol H_2/mol hexose sugar **8 mols H_2/mol sugar**



Successful Outcomes:

- **Decentralized, green H_2 production with decarbonization potential**
- **Monetize organic wastes for H_2 production**
- **Support rural & developing economies**

Overview

Timeline and Budget

- Project start date: 10/1/2018
- FY22 DOE funding: $675K
- FY23 planned DOE funding: $1.13M
- Total DOE funds received to-date *$4.5M

*Dollars received by the consortium since project start

	FY19	FY20	FY21	FY22	FY23
NREL	$485K	$600K	$600K	$300K	$250K
LBNL	$200K	$200K	$150K	$150K	$100K
PNNL	$200K	$200K	$200K	$150K	$100K
ANL	$200K	$125K	$125K	$ 75K	$100K
Total	$1.08M	$1.13M	$1.08M	$675K	$550K

Barriers

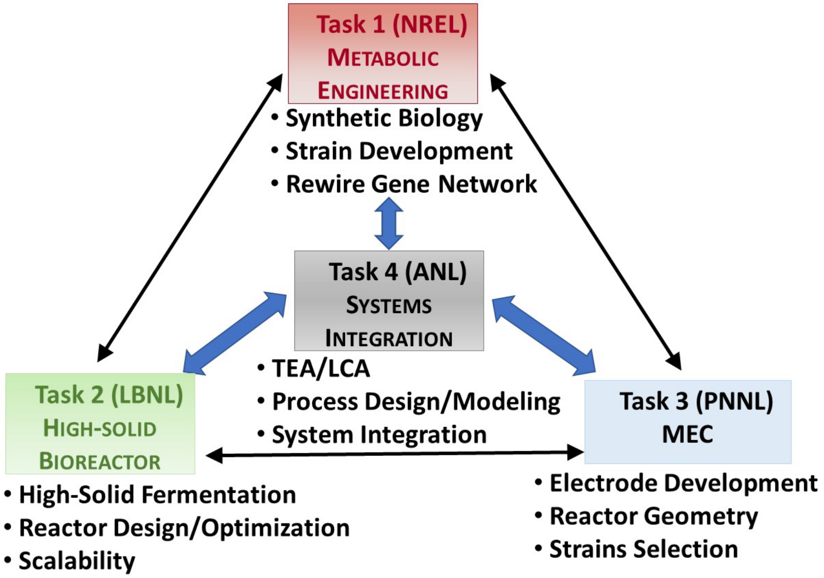
- Capital cost
- Feedstock cost (AY)
- H_2 molar yield (AX)
- System engineering (AZ)

Partners

- Project lead: Dr. Katherine Chou (PI, NREL)
- Co-PIs: Drs. Eric Sundstrom (LBNL), Alex Beliaev (PNNL), Amgad Elgowainy (ANL)
- Lawrence Berkeley National Lab (LBNL), Pacific Northwest National Lab (PNNL), Argonne National Lab (ANL)

Relevance/Potential Impact

A collaborative team of scientists from **four** National Labs whose expertise builds a strong foundation in addressing knowledge gaps and technical barriers for long-term success toward meeting the H_2 production cost goal (< \$2/kg H_2).



Task 1 (NREL)
METABOLIC ENGINEERING

- Synthetic Biology
- Strain Development
- Rewire Gene Network

Task 4 (ANL)
SYSTEMS INTEGRATION

- TEA/LCA
- Process Design/Modeling
- System Integration

Task 2 (LBNL)
HIGH-SOLID BIOREACTOR

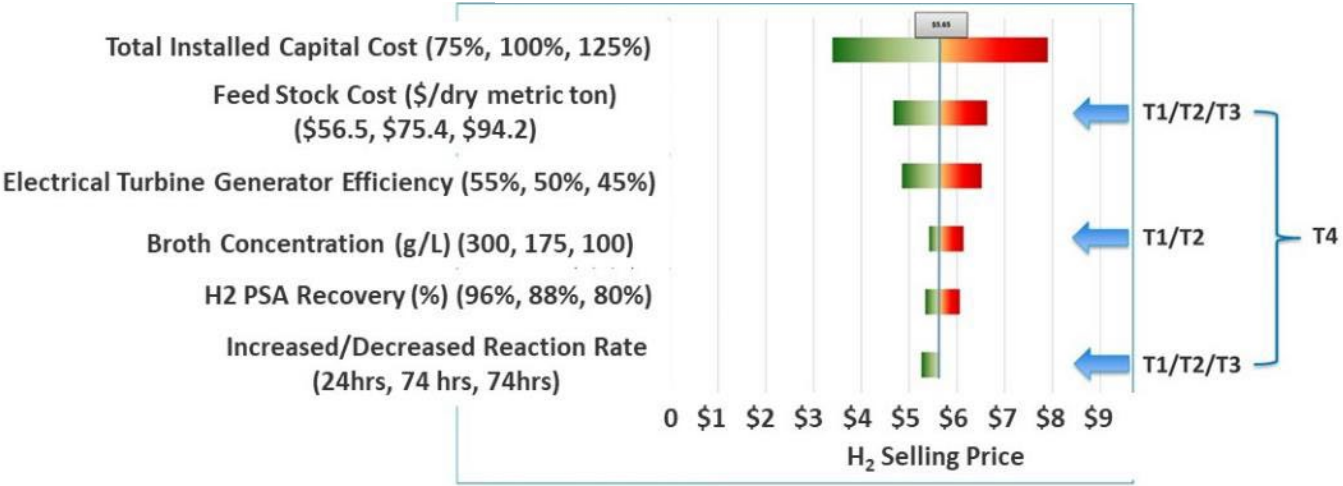
- High-Solid Fermentation
- Reactor Design/Optimization
- Scalability

Task 3 (PNNL)
MEC

- Electrode Development
- Reactor Geometry
- Strains Selection

R&D is prioritized to lower capital & feedstock costs

- Maximize H_2 yield
- Reducing bioreactor footprint
  - high loadings of biomass
- Efficient biomass deconstruction, utilization, & conversion

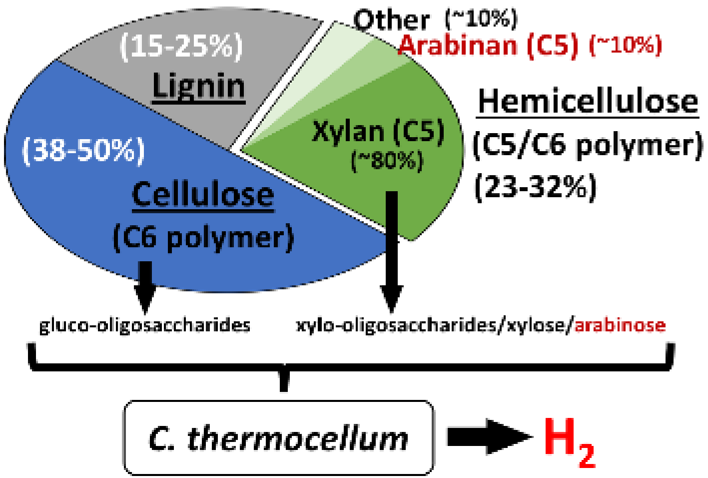


Approach: Task 1. Improve biomass utilization and conversion (i.e., H_2 Yield) via *Clostridium thermocellum* strain development (NREL)

FY23

- Engineered strains can co-utilize cellulose (glucose) and hemicellulose (xylose, arabinose)
- Improve H_2 molar yield (per mole of glucose).
- Reduce feedstock preprocessing costs by using minimally pre-treated corn stover.

Engineer Cellulose-Degrading Microbe to Co-metabolize C5 Sugars



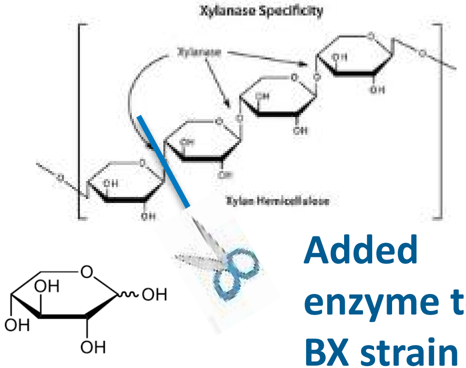
1926 – 2016	*C. thermocellum (Δ**hpt**)* utilizes cellulose (C6), but not hemicellulose (C5 sugars)
2017 – 2018	NREL genetically modified strain (***xylAB***) to co-utilize C5 sugar (xylose)
2018 – 2019	NREL evolved strains (created strain ***19-9***) for improved growth and **H_2 production rate on hemicellulose sugars**
2020 – 2021	Enabled the co-utilization of hemi-/cellulose (**BX**)
2021 – 2022	Genetically modified *19-9* to co-utilize arabinose

Ferment all the sugars to H_2 in <u>one</u> bioreactor: lowering both feedstock and reactor costs.

Task 1 Accomplishments & Progress: H_2 is produced from <u>simultaneous co-utilization of cellulose & xylan</u> (NREL, FY22 Q4)

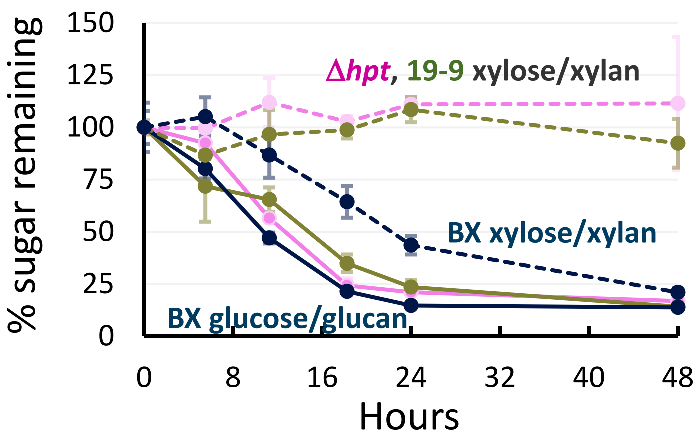
Feeding cellulose (2.5 g/L) and xylan (2.5 g/L) from corn core

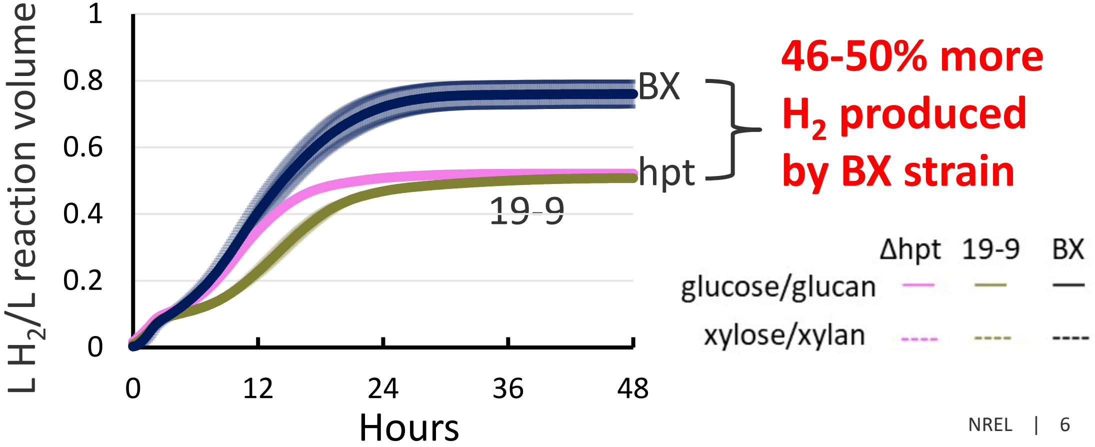
- All strains consumed ~85% of the cellulose
- Only BX strain consumed 79% of the xylan, and co-ferment cellulose/xylan simultaneously. All xylan remained for ***19-9*** and **Δ*hpt*** (no added enzymes).
  - BX strain: breaks xylan into monomeric xylose (pentose)
  - 19-9 strain: uses monomeric xylose but not xylan (polymers of pentose)
  - Δ*hpt* strain: utilize only cellulose



Added enzyme to BX strain

Combined oligomeric & monomeric sugars





46-50% more H_2 produced by BX strain

Task 1 Accomplishments/Progress: Increased H_2 molar yield by 82% and identified iron as a limiting growth nutrient in supporting H_2 production (NREL FY23 Q1)

Two strains (Δ*hpt* vs. Δ*Rnf*) and 7 growth nutrients were assessed for their impact on H_2 production.

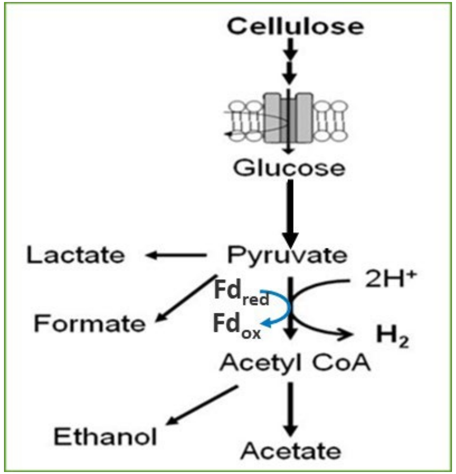
- Design of Experiment approach reduces the number of experiments required to identify factors impacting H_2 production.

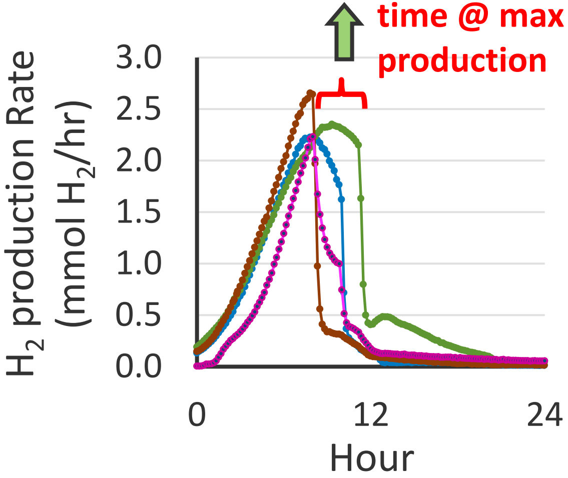
Rnf mutant, produces H_2 at higher yields, conserves the key electron carrier (ferredoxin, Fd) to produce H_2

High H_2 yield → low feedstock & bioreactor costs

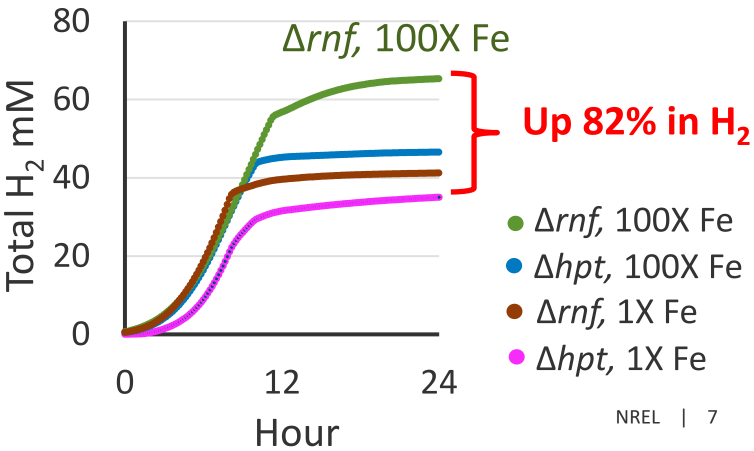
- Iron – a limiting factor for H_2 production
- ΔRnf strain at higher iron levels (>20 mg/L, 100X) <u>increased the H_2 molar yield by 82%</u>
- The period of max H_2 production rate was prolonged by the high iron level for either strain.

RNF: $\mathbf{Fd_{red}} + H^+ + \text{ion} + NAD^+ \dashrightarrow Fd_{ox} + NADH + \Delta\mu\text{ion}$



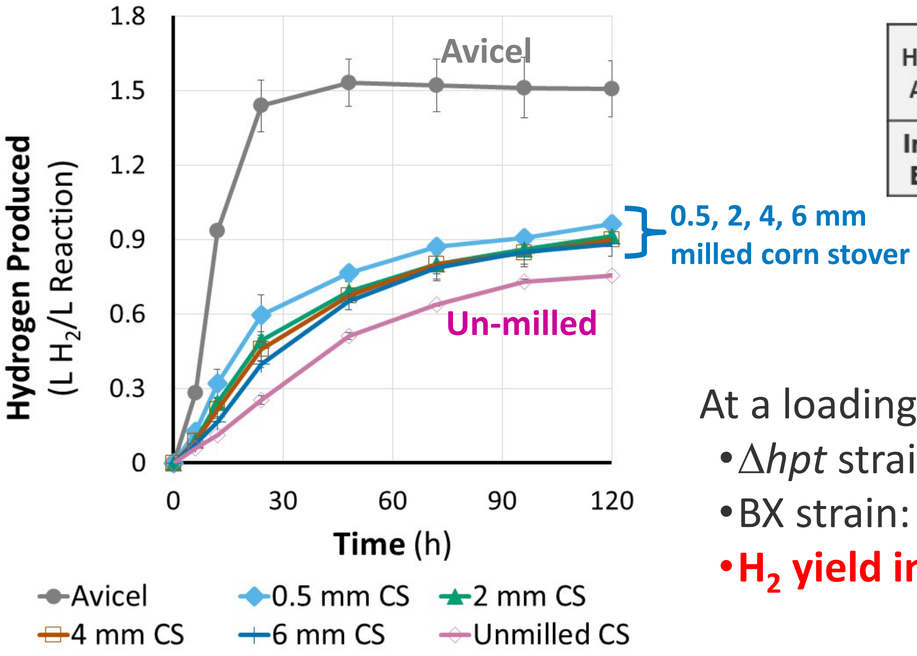


pH controlled, 5 g/L cellobiose



Task 1. Accomplishments: Demonstrate up to 36% increase in H_2 yield from fermenting untreated, milled biomass by an engineered BX strain (NREL FY23 Q2)

FY23 Go-No-Go (Q2)	Status
Using H_2 production by the minimally engineered strain (Δ*hpt*) previously shown in FY22 as the baseline, demonstrate at least 20% increase in total H_2 production by a more advanced strain (BX) generated at NREL using untreated and milled corn stover as the feedstock .	complete



		Unmilled	6 mm	4 mm	2 mm	0.5 mm	DMR	Avicel
H_2 Yield Relative to Avicel Control (%)	Δ*hpt*	37	46	44	45	48	**86**	100
	BX	50	58	60	60	64	**123**	100
Increase in H_2 Yield by Engineered Strain (%)		**35**	**27**	**36**	**34**	**33**	**43**	-

- **Reduced feedstock preprocessing steps**
- **Lower cost associated with feedstock**

At a loading of 4 g/L as cellulose (10 g/L milled corn stover, CS)

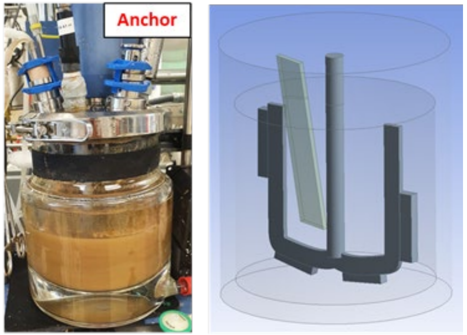
- Δ*hpt* strain: H_2 yield was 37-48% of the refine cellulose Avicel
- BX strain: H_2 yield was **50-64%** of the Avicel
- **H_2 yield increased 27 – 36% by BX,** exceeding the 20% target

Approach: Task 2. High-Solids Bioreactor Development (LBNL)

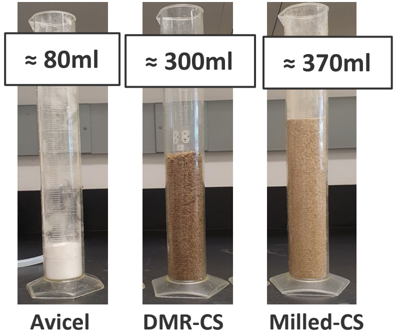
Optimize H_2 production under high solids conditions to achieve 1 L H_2/L/day (FY23) in continuous operation, while transitioning from DMR-pretreated to unpretreated milled corn stover biomass

- Lignin is removed in DMR (deacetylated/mechanically refined) corn stover (CS), NaOH-treated
- Compare major metabolites in untreated & milled corn stover vs. DMR corn stover

Customized bioreactors with anchor impeller & flow breaker

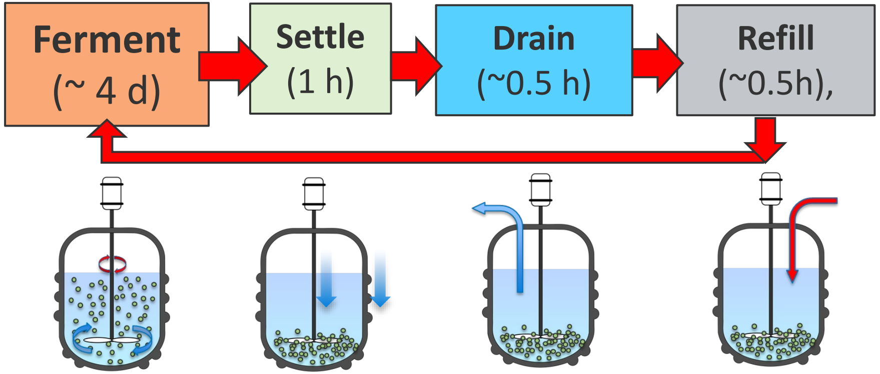


Less pre-processed biomass (bearing 30g as cellulose) occupies greater volume



ABPDU fermentation suite is equipped with Rushton and anchor impeller bioreactors, process mass spectrometer, 50 L scale-up reactor, customized high-solids mixing geometry

Continuous Fed-Batch Fermentation through "F-S-D-RF"



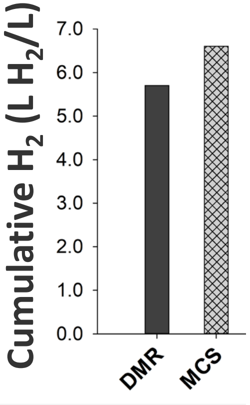
Ferment (~ 4 d) → **Settle** (1 h) → **Drain** (~0.5 h) → **Refill** (~0.5h),

Continuous bioprocessing to maximize biomass throughput for milled and DMR-pretreated CS

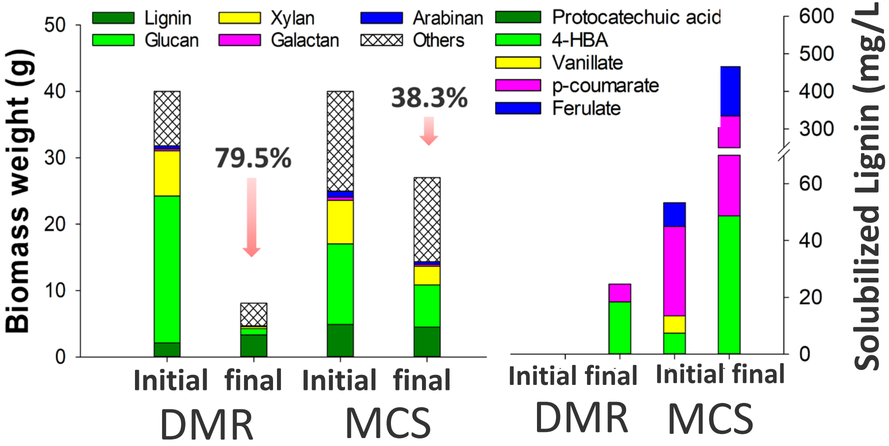
Task 2. Accomplishments/Progress: A continuous operation using DMR demonstrated long-term operation and produced 24 L H_2/L, a 220% increase (LBNL)

- Successfully transitioned from DMR pretreated corn stover (DMR CS) to milled corn stover (MCS) using the high solids anchor impeller system (FY22 Q4)
- **Nearly 80% of DMR CS was solubilized (80% glucan and 80% xylan solubilization)**. For MCS, 38% of biomass was solubilized with glucan and xylan utilization of 52 % and 61 %, respectively.
- Significantly higher quantities of lignin-derived aromatics are detected in MCS supernatant.

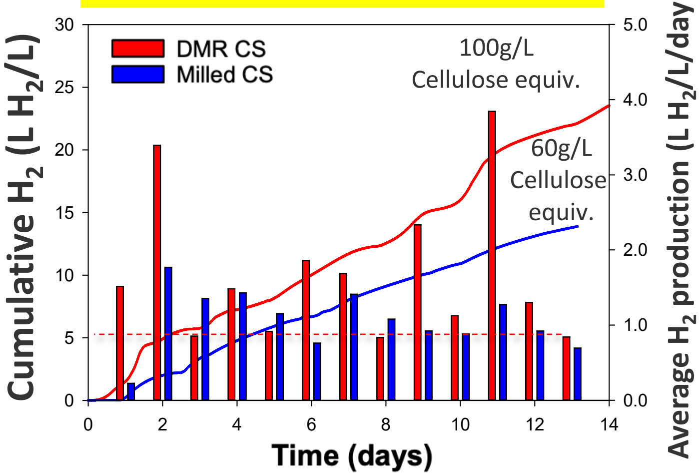
- A continuous fill-settle-decant-refill process enables long-term operation without accumulation of toxic organic acids and lignin decomposition products.
- (FY23 Q2) Total H_2 production from **13 days of operation with DMR biomass exceeds 24.9 L/L – a 220% increase from the FY22 AMR value of 7.78 L/L**



❖ Biomass solubilization: batch operation



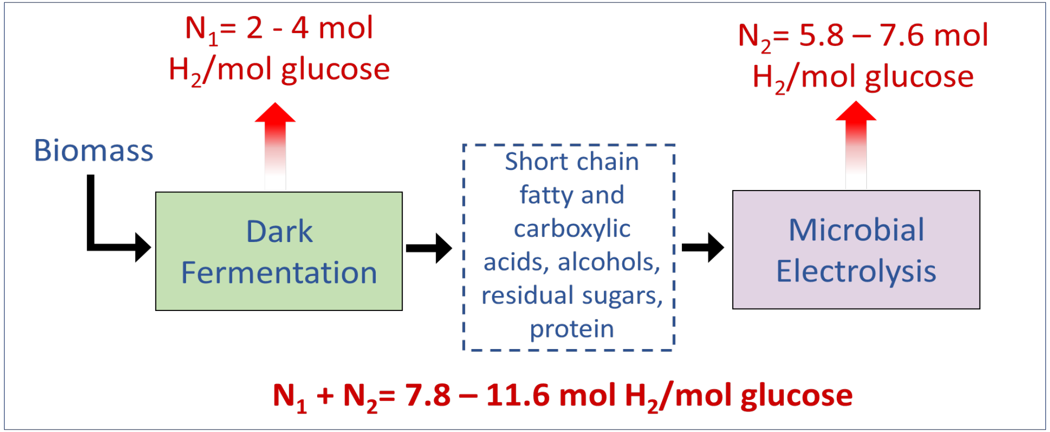
❖ Continuous bioreactor operation



Approach: Task 3. Microbial Electrolysis Cell (PNNL)

Approach: Design MEC process integrated with dark fermentation (Tasks 1 & 2) for conversion of the fermentation effluent to H_2 using robust exo-electrogenic microbes & consortia

- Deploy robust and controllable exo-electrogenic consortia with broad metabolic capacity to increase H_2 production from fermentation effluent
- Rationally design continuous MEC process for conversion of lignocellulosic fermentation effluent (e.g., organic acids, alcohols, proteins, sugars) to H_2 with increased efficiencies and productivities.

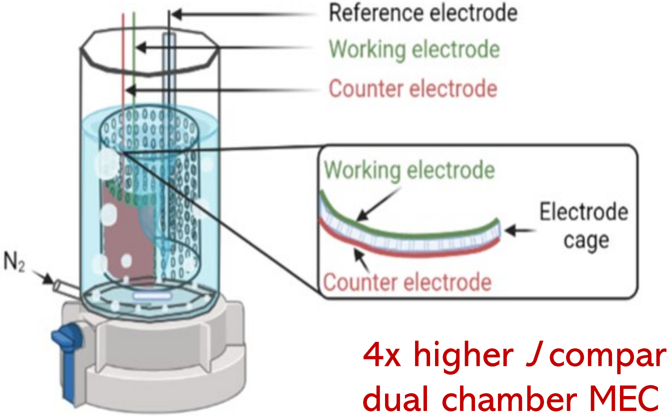


Process flow diagram of the integrated fermentation-MEC process for H_2 production from waste biomass

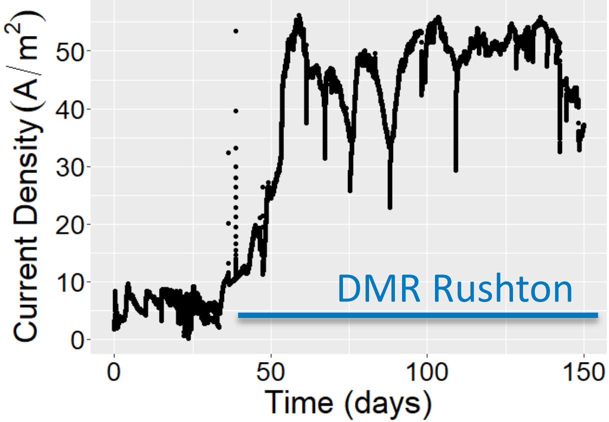
H_2 production in single-chamber MEC's using effluent from high-solid loading DMR fermentation

Task 3 Accomplishments and Progress: Achieved sustainable MEC operation at 40-55 A/m^2 over a 100-day period using DMR fermentation effluent (PNNL)

FY22 Q4 Milestone: Optimize the performance of single-chamber MEC using DMR effluent from high- solid load fermentation to achieve ≥ 30 A/m^2 and ~4 L H_2 / L reactor volume/day	Complete Sept 2022



4x higher *J* compared to dual chamber MEC



- New 3D-printed MECs were designed for: a) improved mass transfer; b) decreased charge transfer & uncompensated resistance; and c) lower OPEX (elimination of PEM)
- MECs were inoculcated with anaerobic granules from WWTP
- The consortia biofilms demonstrated increased robustness, metabolic versatility and quicker start times *vs.* defined species

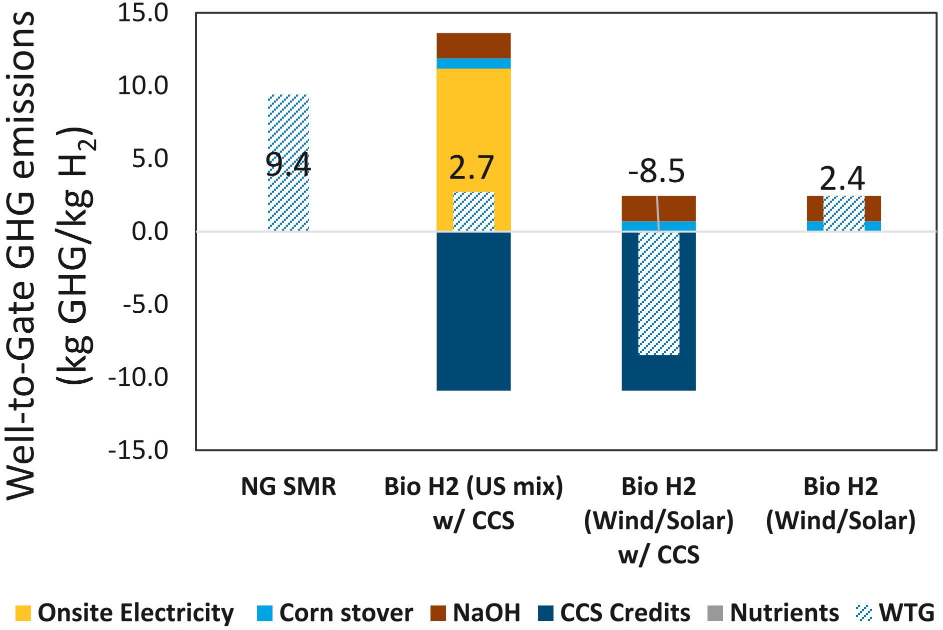
- MEC optimization efforts resulted in the ability to continuously operate in fed-batch mode (>100 days)
- Sustained current densities > 40 A/m^2 were obtained on effluent from high-solid loading DMR fermentation process
- H_2 production rates reached ~4 L/L reactor volume/day at peak current densities

Ongoing efforts: *(i) improving J (A/m^2) and Y_{H_2} from milled biomass effluent & (ii) characterization of anodic biofilm enriched consortium to enable rational design and control*

Approach: Task 4. System Integration, Techno-economic Analysis (TEA) and Life Cycle Analysis (LCA, ANL)

Approach: Use TEA (Aspen Plus) and LCA (GREET) to set research targets, guide research directions and suggest system design to achieve cost targets and reduce life cycle greenhouse gas emission.

- Electricity use for Bio H_2 production is 23.9 kWh/kg, less than PEM water electrolyzer (55.5 kwh/kg).
- Tax credit provided by Inflation Reduction Act (IRA 45 V) has a significant impact on H_2 production cost, emphasizing the essential role LCA plays.
- With grid electricity use and with CCS, Bio H_2 has GHG emissions of 2.7 kg CO_2 /kg H_2, potentially qualifying for IRA tax credit of \$0.6/kg H_2. Cleaner grid electricity will reduce the GHG greatly.
- With renewable wind/solar electricity and CCS, net well-to-gate GHG emissions for BioH2 is -8.5 kg CO_2/kg H_2, potentially qualifying for an IRA tax credit of \$3.0/kg H_2.
- Alternatively, CCS can potentially qualify for \$0.93/kg H_2 given 10.9 kg high-purity CO_2 can be produced and sequestered for 45Q tax credit (\$85/MT CO_2)

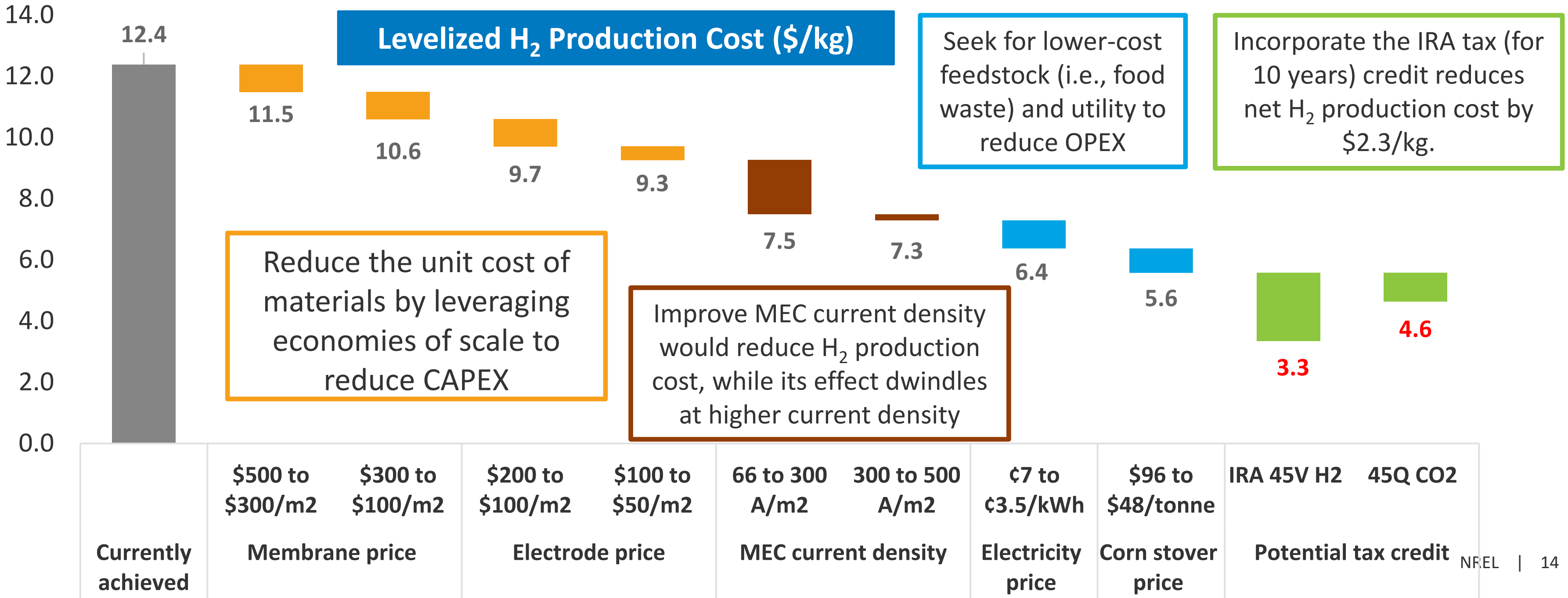


CCS: Carbon Capture & Sequestration (CCS)
WTG: Well-to-gate

Task 4 Accomplishments & Progress: Identified bio-H_2 cost reduction opportunities through material cost reduction and tax credit

FY22 Q4 Milestone: Update LCA results based on the new MEC current density by using various electrode materials, as well as on other updated energy consumption information. For the integrated system, the leading CO_2 emission source is electricity consumption, which is in turn dominated by MEC energy use.	**Sept 2022, Complete**

Base case: anode/cathode: carbon cloth ($200/m^2); membrane: Nafion ($500/m^2); current density: 66 A/m^2



Reduce the unit cost of materials by leveraging economies of scale to reduce CAPEX

Improve MEC current density would reduce H_2 production cost, while its effect dwindles at higher current density

Seek for lower-cost feedstock (i.e., food waste) and utility to reduce OPEX

Incorporate the IRA tax (for 10 years) credit reduces net H_2 production cost by $2.3/kg.

Accomplishments and Progress: Responses to Previous Year Reviewers' Comments (addressed via emails)

Reviewer Comments: This project was not reviewed at AMR in FY22. Below is our email response to questions derived from a review meeting in FY21.

I believe in previous quarters, PNNL has indicated that a component of the DMR fermentation effluent is poisoning or restricting their microbial colony, and have continued their reactor design using avicel. The TEA reports on the promise of the fermentation + MEC system to be able to produce H_2 at a cost of \$5.60 to \$6.60 per kg H_2, without the sale of the CO_2. Is that assuming 175 g/L loading? There is a considerable amount of effort is needed for the MEC to accept the fermentation effluent and achieving a current density of 100 A/m^2. Is it possible for you to consider the steps that would be needed to move away from the DMR and use the mill biomass while meeting the same (or improved) H_2 yield as you prepare your writeup for future scope options? Would the effluent then expected to be compatible with the MEC reactor, or negatively impact the solid loading work? How would the mill biomass as a feedstock affect the predicted cost of hydrogen?

Responses to Reviewers Comments:

For your first question, yes, 175 g/L loading was part of the assumption made for H_2 production at \$5.6 to \$6.6/kg H_2 for the combined (fermentation + MEC) system. This number (175 g/L) first came from a TEA model developed by Strategic Analysis (SA). This TEA model was for dark fermentation alone and concluded that the solid loading needs to be 175 g/L to be economical. That said, as we continue to improve the state of the technology of (fermentation + MEC) and analyze the system as an integrated technology and not stand-alone technologies, it could be beneficial to revisit this assumption and the integrated system's sensitivity to this particular target loading at a later point.

One big incentive for us to use milled biomass and not DMR biomass is the \$1 saved from the cost of \$5.6 – 6.6/kg H_2. Based on ANL's TEA model, \$1 of that 5.6 – 6.6/kg H_2 comes from the cost of NaOH used to pre-treat and remove acetate and lignin from the milled biomass.

As you probably saw too, even if we can demonstrate successful fermentative H_2 production directly from milled biomass in FY22, we would expect additional benefits and certain challenges associated with milled biomass, which we would need to address beyond FY22.

One benefit is that we actually attain more acetate from biomass for MEC. Currently the acetate in the fermentation effluent is sourced from the breakdown of hexose and pentose sugars (cellulose/hemicellulose) by the bacteria, so the acetate removed during chemical pretreatment to attain DMR biomass will be retained and that provides additional acetate to MEC.

Some of the challenges of milled biomass could be toxicity from lignin on fermentation and/or MEC, or other unforeseen issues to resolve. We would likely need to continuously assess what the issues may arise and identify potential remedies. We would also need to take into considerations the cost of the proposed remedies in comparison to the \$1 saved. Currently lignin is burned for energy production in our TEA model, which is commonly the case across what people do in practice with lignin, as it's not readily used by microbes. I believe BETO is investing in R&D to valorize lignin, and we can see what we could leverage from their investment for H_2 production as needed.

Before we know milled-biomass is feasible, NREL can set one milestone in FY22 to assess the feasibility of H_2 production from fermenting milled-biomass in lower loading and smaller scale pilot experiments. In the meanwhile, PNNL and LBNL continue to use DMR biomass in FY22 as the feedstock to optimize their systems. LBNL can continue with co-optimizing fed-batch fermentation with wall-scraping impeller at high solids fermentation. PNNL can re-assess and troubleshoot DMR effluent on their MEC system, optimize their MEC parameters using DMR biomass to establish baseline, and continue to improve current density. After all, DMR remains an excellent feedstock to fall back on (and for establishing baseline and parameter optimization) if milled biomass creates unsolvable problems.

Collaboration and Coordination

- **Task 1. Strain Development and Improvement (NREL)**
  - NREL takes the lead on setting direction and coordinating efforts between participating labs.
  - Develop and test strains to improve H_2 production and send the strains to LBNL for testing in high solids fermentation.
  - NREL leverages BETO investment in biomass pretreatment and Office of Science BER investments (UCLA, Oak Ridge National lab) in understanding C*. thermocellum* physiology and cellular/gene regulation.
- **Task 2. High-solids Bioreactor Development (LBNL)**
  - Develop and optimize bioreactors for high solid loadings and supply fermentation effluent to PNNL.
  - Receives modified strains from NREL for testing.
- **Task 3. Microbial Electrolysis Cell (PNNL)**
  - Collaborate with Washington State University – bioelectrical system design
  - Optimizing fermentation-MEC integration with NREL/LBNL and improves the H_2 molar yield
- **Task 4. System Integration, TEA and LCA (ANL)**

  Develop and use TEA/LCA to set research targets and guide research directions, working closely with all other tasks to lower production costs

Remaining Challenges and Barriers

Tasks 1. Strain Development and Improvement (NREL)

- H_2 yield is lower at high loadings – further strain engineering is required to maximize yield
- Partial solubilization/utilization of cellulose/hemicellulose at high solids loading

Task 2. High-solid Bioreactor Development (LBNL)

- Overall conversion efficiency declines at high solids loading due to bulk viscosity
- Nitrogen gas is currently used for H_2 removal and ensure anaerobic conditions. Full deployment will require an alternative to avoid costly gas separations.

Task 3. Microbial Electrolysis Cell (PNNL)

- Improve conversion efficiencies and H_2 molar yield on milled biomass effluent
- Improve electron transfer in electrogenic biofilms and at microbe-electrode interface

Task 4. System Integration, TEA and LCA (ANL)

Identify cost-advantaged feedstocks and factors contributing to lower H_2 production cost

Proposed Future Work

Note: Any proposed future work is subject to change based on funding levels.

Task 1. Strain Development and Improvement (NREL)

- Identify key chemical bonds in biomass to break/deconstruct so more sugars (arabinose, xylose, glucose) are accessible for utilization and H_2 production
- Improve biomass deconstruction, utilization, and H_2 yield at higher loadings

Task 2. High-solid Bioreactor Development (LBNL)

- Optimize the continuous fed-batch process conditions (e.g., residence times at different stage, gas sparging/H_2 removal, impeller geometry) at higher solids loading and larger scales.
- Leverage analytical techniques to identify hydrolyzed biomass components and their impact on fermentation, which informs strain engineering/process design toward better biomass deconstruction.

Task 3. Microbial Electrolysis Cell (PNNL)

- Optimization of milled biomass effluent conversion to achieve higher H_2 production rates
- Characterization of anodic biofilm enriched consortium to enable rational design and control

Task 4. System Integration, TEA and LCA (ANL)

- Identify cost advantaged solid and liquid waste streams as feedstocks to provide revenues to offset H_2 production costs

Summary

Task 1. Strain Development and Improvement (NREL)

- **Increased H_2 molar yield by up to 82%** and identified iron as a limiting growth nutrient in supporting H_2 production
- **Demonstrated simultaneous co-utilization of cellulose and xylan using an engineered (BX) strain**
- Demonstrated **up to 36% increase in H_2 yield from fermenting untreated, milled biomass** by an engineered strain compared to the baseline generated via a non-engineered strain, exceeding the Go/No-Go Milestone

Task 2. High-solid Bioreactor Development (LBNL)

- Successfully transitioned from DMR-pretreated biomass to milled corn stover, achieving >80% solubilization of DMR biomass carbohydrates and a >10x increase in solubilized lignin-derived aromatics.
- Enhanced H_2 production and process longevity at high solids loading by shifting to continuous operation, increasing H_2 production by 220% from the FY22 AMR, to **a maximum of 24.8 L H_2 / L in fed-batch operation with DMR-pretreated biomass, and 13.9 L H_2 / L with milled corn stover**

Task 3. Microbial Electrolysis Cell (PNNL)

- New single-chamber design significantly improves MEC performance (higher current density, improved process robustness, decreased resistance)
- Process improvements resulted in **continuous (>100 days) MEC operation at high current densities (at 40-55 A/m^2)** on DMR effluent

Task 4. System Integration, TEA and LCA (ANL)

- **Identified bio-H_2 cost reduction opportunities through material cost reduction and tax credit.**

NREL
Katherine Chou, Ph.D.
Trevor Croft, Ph.D.
Eric Schaedig
Skyler Hebdon, Ph.D.
Pin-Ching Maness
Lauren Magnusson
Wei Xiong, Ph.D.
Emily Miller

LBNL
Steve Singer, Ph.D.
Eric Sundstrum, Ph.D.
Dylan Song, Ph.D.

PNNL
Alex Beliaev, Ph.D.
Eric Hill, Ph.D.
Washington State Univ.
Haluk Beneyal, Ph.D.

ANL
Amgad Elgowainy, Ph.D.
Pingping Sun, Ph.D.
Xinyu Liu, Ph.D.
Arna Ganguly

Thank You

www.nrel.gov

Publication Number

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 Hydrogen and Fuel Cell 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.