BioHydrogen (BioH2) Consortium to Advance Fermentative H_2 Production

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National Renewable Energy Laboratory
DOE Project Award/AOP #: HFTO.2.4.0.516
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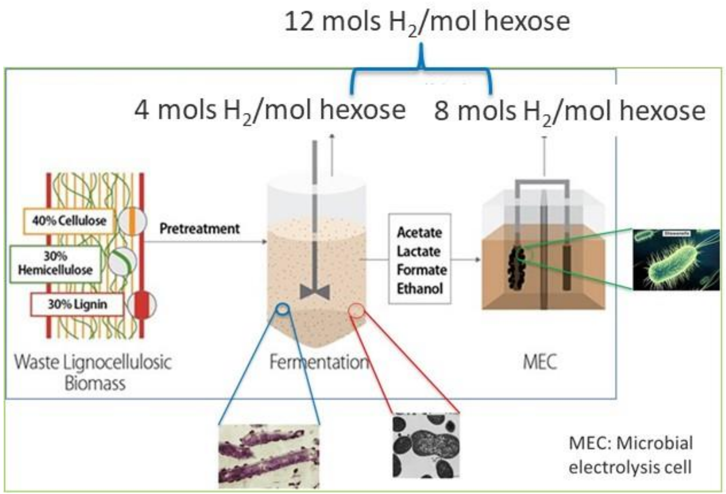
DOE Hydrogen Program
2021 Annual Merit Review and Peer Evaluation Meeting

Project ID: P179

Project Goal



MEC: Microbial electrolysis cell

Overall Objective: Develop a high-solids microbial fermentation technology to convert renewable lignocellulosic biomass resources into H_2 and integrate microbial electrolysis cell (MEC) to meet DOE H_2 production cost goal of \$2/kg-H_2.

Current Project Year Objectives (Oct 2020 – March 2021)

Task 1. Strain Development and Improvement (NREL Lead)

- Lower feedstock cost and improve hemicellulose (5-carbon sugars) conversion to H_2 via genetic engineering of *Clostridium thermocellum* which is capable of direct cellulose fermentation for H_2 production.

Task 2. High-solids Bioreactor Development (LBNL Lead)

- Evaluate scale-up effects for *C. thermocellum bioreactor fermentations,* determine relative impacts of fed-batch vs batch cultivation methods, onboard the NREL 19-9 strain and compare H_2 production rates with pretreated biomass vs avicel feedstock

Task 3. Microbial Electrolysis Cell (PNNL Lead)

- Evaluate flow-through MEC process performance on actual fermentation effluent provided by LBNL using *Geobacter-Shewanella* co-culture that efficiently oxidizes fermentation by-products for increased H_2 production and yield.

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

- Design a conceptual, large-scale system to integrate the dark fermentation (DF) and MEC for bioH_2 production. Model the overall system with Aspen Plus, evaluate economics with H2A model, analyzes CO_2 emissions with GREET model.

Overview

Timeline and Budget

- Project start date: 10/1/2018
- FY20 DOE funding: $1.13M
- FY21 planned DOE funding:

	FY19	FY20	FY21
NREL	$485K	$600K	$450K
LBNL	$200K	$200K	$150K
PNNL	$200K	$200K	$200K
ANL	$200K	$125K	$125K
Total	$1.08M	$1.13M	$925K

- Total DOE funds received to date*$3.1M

*for the consortium since the project started

Barriers

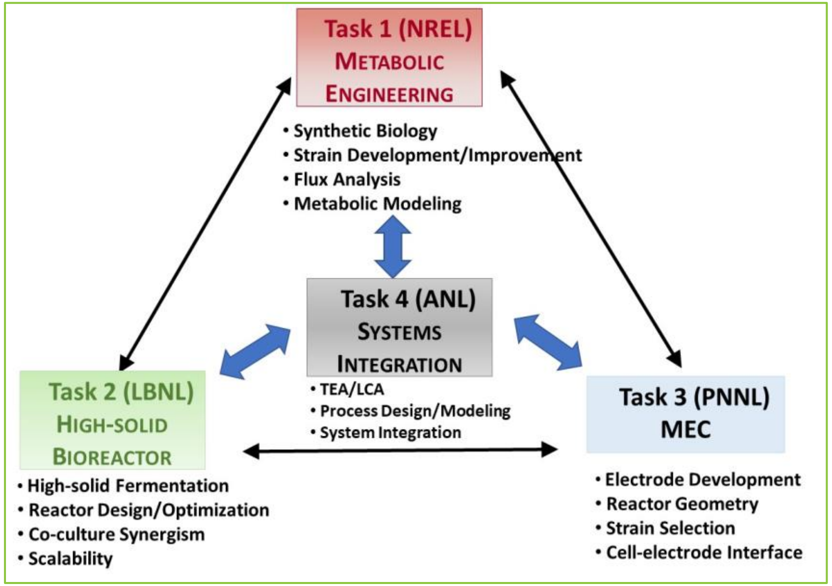
- H_2 molar yield (AX)
- Feedstock cost (AY)
- System engineering (AZ)

Partners

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

Relevance – BioH2 Consortium & Synergies

Rationale: We assembled a highly productive and collaborative team of scientists from **four** National Labs whose research accomplishments and expertise lay down a strong foundation in addressing knowledge gaps and technical barriers for long-term success toward meeting the HFTO H_2 production cost goal (\$2/kg H_2).

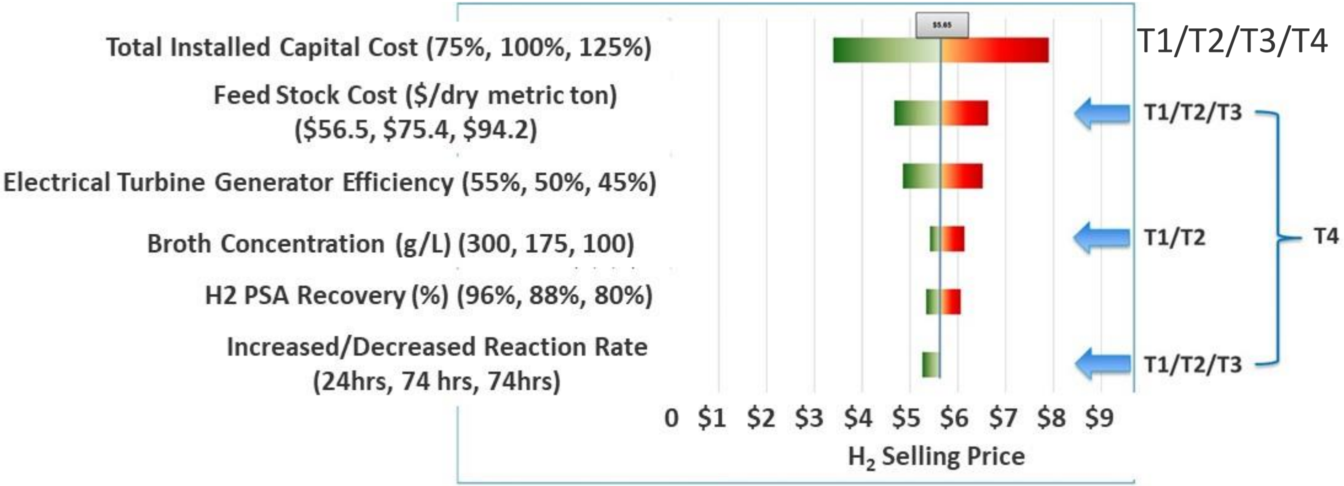


Task 1 (NREL)
METABOLIC ENGINEERING
- Synthetic Biology
- Strain Development/Improvement
- Flux Analysis
- Metabolic Modeling

Task 4 (ANL)
SYSTEMS INTEGRATION
- TEA/LCA
- Process Design/Modeling
- System Integration

Task 2 (LBNL)
HIGH-SOLID BIOREACTOR
- High-solid Fermentation
- Reactor Design/Optimization
- Co-culture Synergism
- Scalability

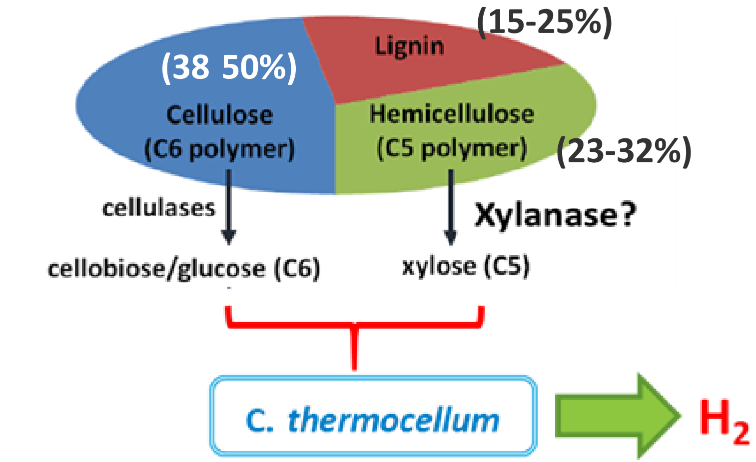
Task 3 (PNNL)
MEC
- Electrode Development
- Reactor Geometry
- Strain Selection
- Cell-electrode Interface



Approach: Task 1. Strain improvement (NREL)

Approach: Via further targeted genetic engineering post adaptive laboratory evolution (FY19-21), we continue to improve hemicellulose (five-carbon xylose polymer) utilization. Cellulose-hemicellulose co-utilization will lower the cost of biomass feedstock.

Engineer Cellulose-Degrading Microbe to Co-metabolize C5 Sugars



*C. thermocellum (Δ**hpt**)* utilizes cellulose (C6), but not hemicellulose (C5 sugars)

1926 – 2016

NREL genetically modified strain (***xylAB***) to enable C5 sugar (xylose) co-utilization

2017 – 2018

2018 - 2019

NREL evolved strains (created strain ***19-9***) for improved growth and H_2 production rate on **hemicellulose (HC) sugars**

2020 – 2021

Co-utilizing hemi-/cellulose for H_2 production

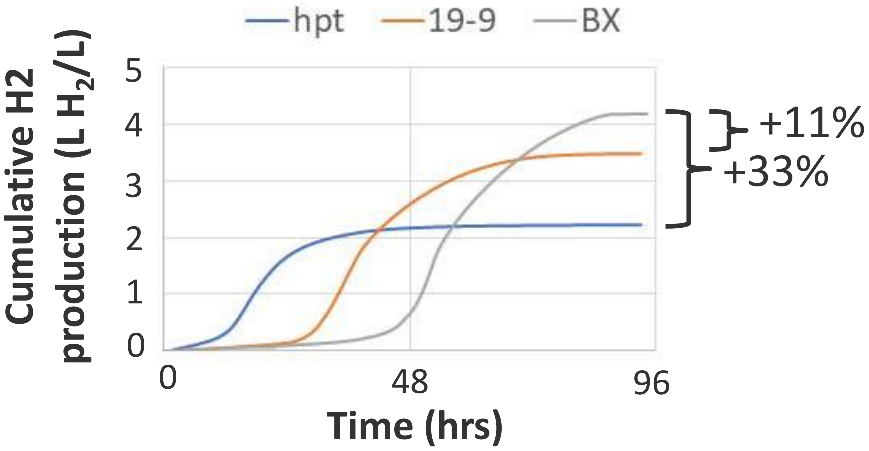
Enabled the co-utilization of hemi-/cellulose (**BX**)

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

Task 1. Accomplishments: 11% increase in H_2 production rate from current baseline (39% increase total) via better hemicellulose utilization (NREL)

FY21 Go-No-Go Enhance Xylan Utilization	10% increase in H_2 production rate in an engineered strain expressing a heterologous β-xylosidase enzyme using DMR fermentation by 19-9 as the baseline (2.75 L H_2/L/day). This will be a 35% increase over the non-engineered Δ*hpt* strain baseline (2.2 L H_2/L/day).	March 2021	**Complete**

- Fermentation of 88.4 g/L real deacetylated and mechanically refined (DMR) biomass with **30 g/L as cellulose** in 500 mL bioreactor



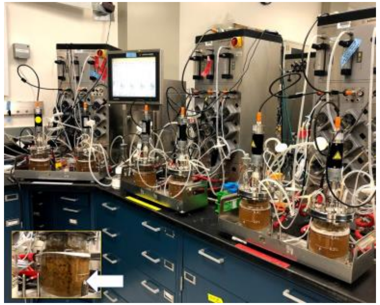
- 3 strains compared for H_2 production:
  - **Δ*hpt*** : minimally engineered
  - ***19-9***: engineered and evolved in lab
  - ***β-xylosidase*** (**BX**): 19-9 further expresses an enzyme to breakdown polymers of hemicellulose sugars into sugar monomers

Strain	Avg. H_2 production rate (mL L^{-1} d^{-1})
hpt	2210.75 ± 25%
19-9	2746.24 ± 10%
BX	**3075.77 ± 5%**

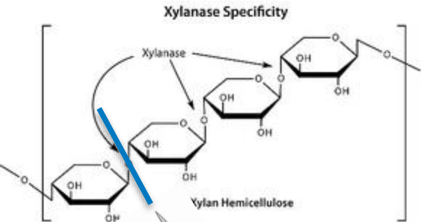
39% ↑

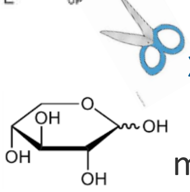
11% ↑

Task 1. Accomplishments: 89% improvement in hemicellulose derived xylo-oligomeric sugars depolymerization, leading to 11% higher total H_2 production



- All three strains are capable of solubilize glucan and xylan
- Both Δ*hpt* and *19-9* accumulated soluble oligomeric hexose and pentose sugars, but the engineered strain BX (*19-9* expressing β-xylosidase) depolymerizes majority of the xylo-oligomeric sugars

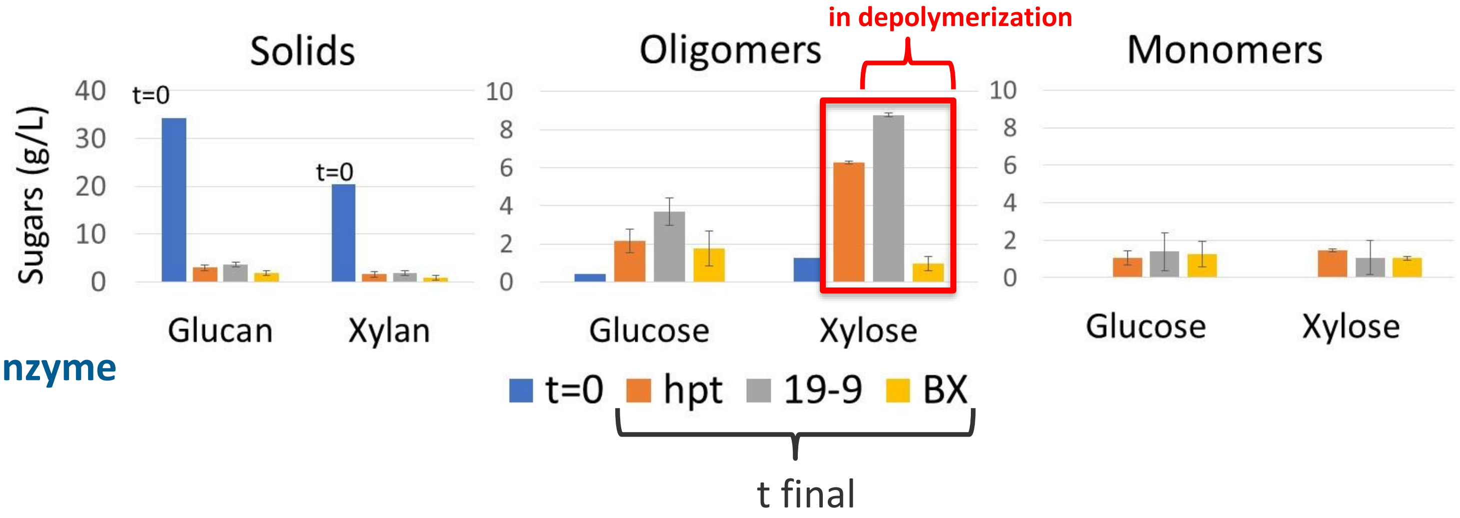




xylosidase enzyme

monomeric

89% improvement in depolymerization



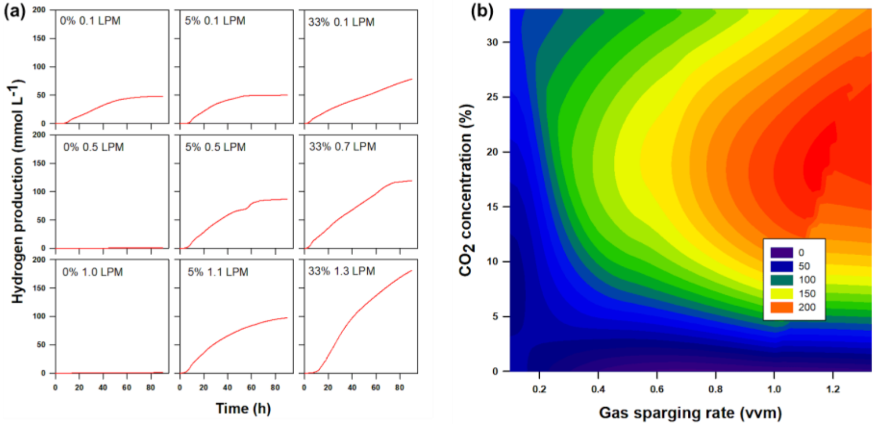
Data from fermentation of 88.4 g/L real DMR biomass with **30 g/L as cellulose** in 500 mL bioreactor

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

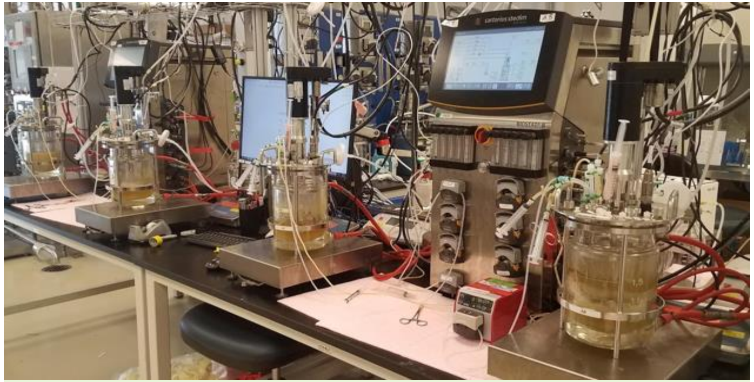
Approach: Leverage bioreactor design and operating conditions to optimize substrate availability, inorganic carbon supply, and gas removal for enhanced H_2 production at high solids loading

- Optimize bioreactor parameters for *C. thermocellum,* both wild type and engineered strains, under high solids conditions (targeting 175 g/L biomass)
- Co-optimize mixing conditions, gas sparging conditions, and fed-batch operation to enhance gas-liquid mass transfer for high-viscosity fermentations

Combined effect of N_2 sparging and CO_2 supply on H_2 production



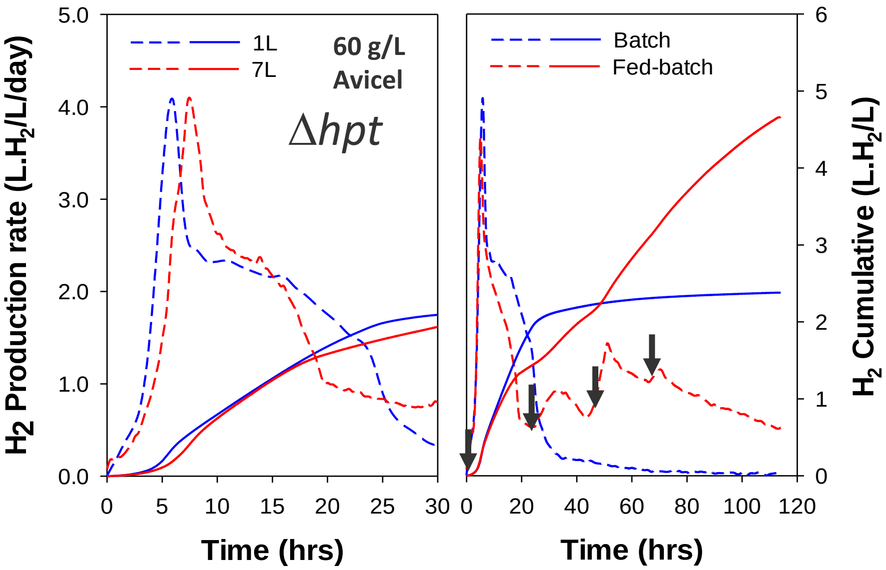
Design of experiments approach for co-optimization of H_2 removal and CO_2 supply



ABPDU fermentation suite: 4 x 2L bioreactors, process mass spectrometer

Task 2. Accomplishments and Progress: Evaluated scale-up, fed-batch operation, and comparison of biomass and avicel production by 19-9 and Δ*hpt* (baseline) strains (LBNL)

FY21 Milestones	Q1: Scale H_2 production from Avicel to >7 L; Determine H_2 production at > 45 g/L Avicel in 7 L reactor volume. Q2: Compare H_2 production from Avicel and DMR corn stover with *C. thermocellum* strain *19-9* from NREL; Measure H_2 production at 45 g/L Avicel and 100 g/L DMR corn stover (45 g/L cellulose loading)	12/2020 3/2021	**Complete**



***Solid line: cumulative (L·H_2/L)**
Dashed line: rate (L·H_2/L/day)

Arrow: added 15g/L avicel

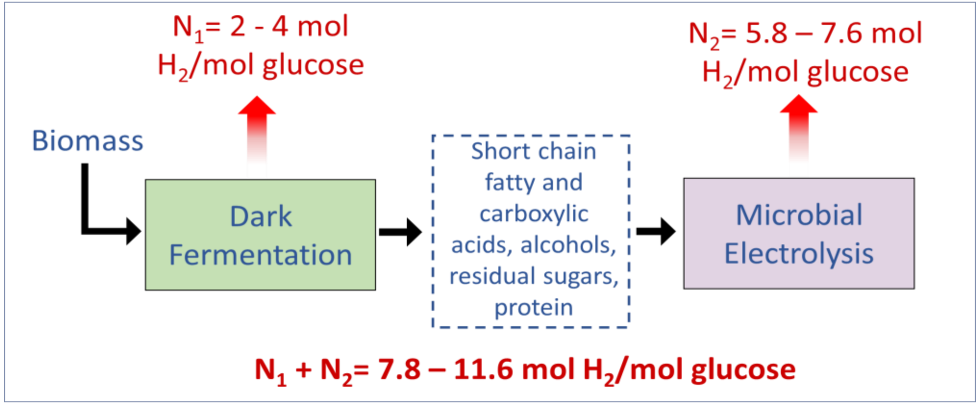
- Successful scale-up from 1 L to 7 L fermentation
- Fed-batch operation doubles H_2 production in Δ*hpt* to 4.66 L·H_2/L at 60 g/L avicel loading
- Fermentations initiated with NREL strain ***19-9***: 10% improvement in H_2 production with 100 g/L DMR

			Max H_2 production (L·H_2/L/day)	**Total H_2 titer (70h) (L·H_2/L)**	
DMR (100 g/L)	**19-9**	Batch	**2.94 ± 9%**	**2.91**	**(≈ 9.9%▲)**
		Fed-Batch	**3.29 ± 5%**	**2.82**	
	Δ*hpt*	Batch	**3.14 ± 15%**	**2.65**	
Avicel (45 g/L)	**19-9**	Batch	**1.45 ± 6%**	**1.45**	**(≈ 39.1%▼)**
		Fed-Batch	**2.64 ± 11%**	**1.37**	
	Δ*hpt*	Batch	**4.01 ± 27%**	**2.38**	

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 defined exoelectrogenic microbes and co-cultures

- Assemble robust exo-electrogenic co-cultures with broad metabolic capacity to increase H_2 production from fermentation effluent. Previous approaches employed undefined enrichments from environmental samples that cannot be controlled and stably maintained for optimal H_2 output
- 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.



N_1= 2 - 4 mol H_2/mol glucose

N_2= 5.8 – 7.6 mol H_2/mol glucose

Biomass

Dark Fermentation

Short chain fatty and carboxylic acids, alcohols, residual sugars, protein

Microbial Electrolysis

N_1 + N_2= 7.8 – 11.6 mol H_2/mol glucose

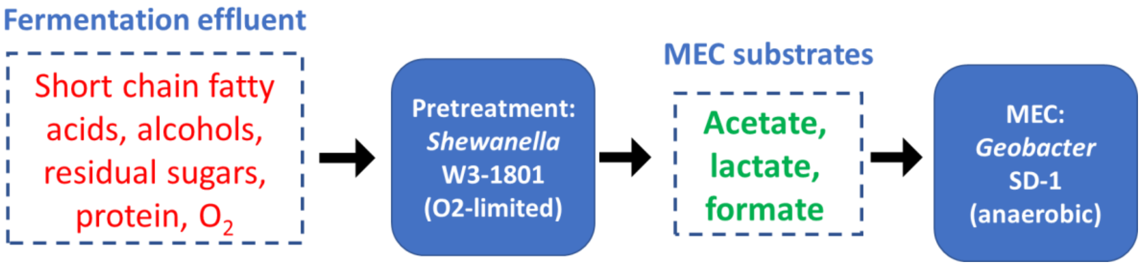
H-type microbial electrolysis cell for fermentation effluent conversion to H_2

Task 3: Accomplishments and Progress: Sustained Current Production (PNNL)

FY21 Q1 Milestones	Demonstrate continuous (>24 hours) BES operation on Avicel-derived effluent; design a flow-through MEC (FT-MEC) system with integrated biological pretreatment to maximize carbon conversion efficiencies and H_2 yield	12/2020	**Complete**

Key results

- Reproducibly operated MECs for **> 200 hs** (Avicel)
- Achieved 2.5 A/m^2 current density on Avicel effluent
- Designed pre-treatment process enabling flow-through (FT MEC) for increased effluent utilization and H_2 yields



Fermentation effluent

Short chain fatty acids, alcohols, residual sugars, protein, O_2

→

Pretreatment: *Shewanella* W3-1801 (O2-limited)

→

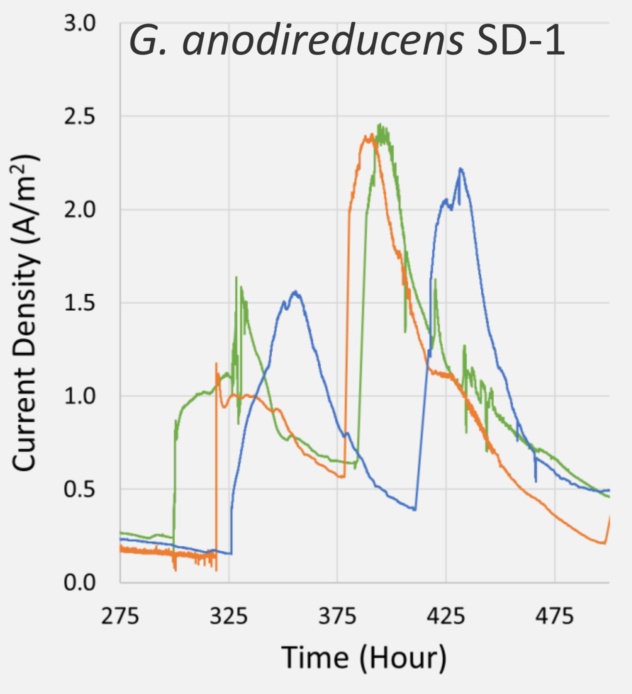
MEC substrates

Acetate, lactate, formate

→

MEC: *Geobacter* SD-1 (anaerobic)

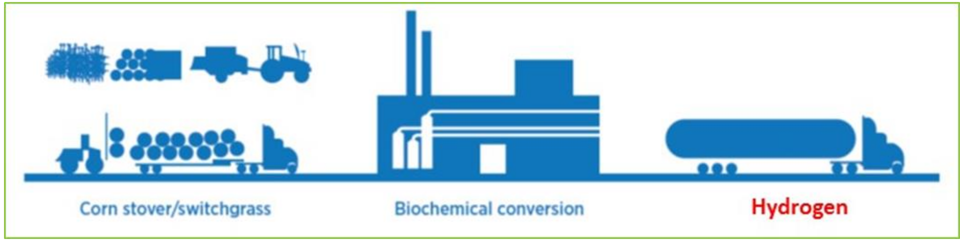
Two-stage flow through MEC conversion process



Approach

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

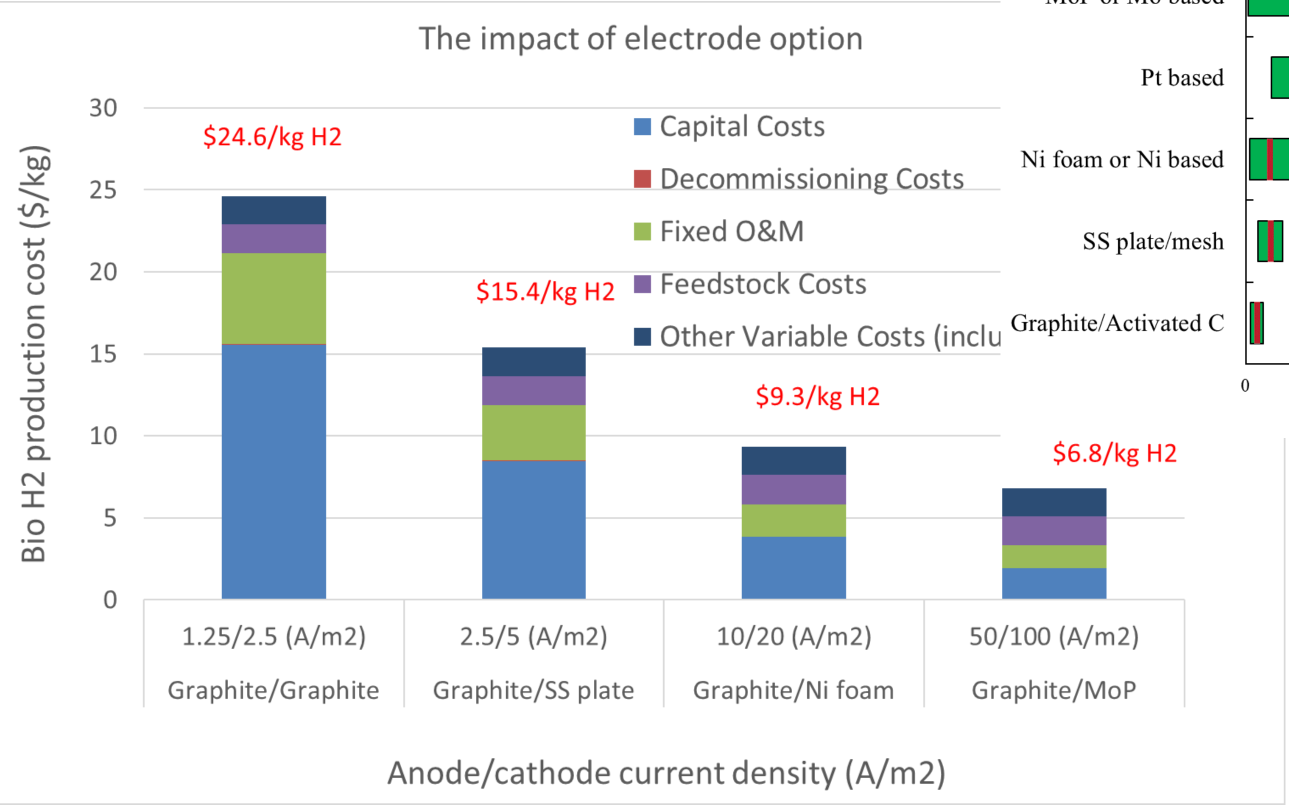
Approach: Use TEA/LCA to set research targets and guide research directions by addressing system engineering challenges to achieve cost targets

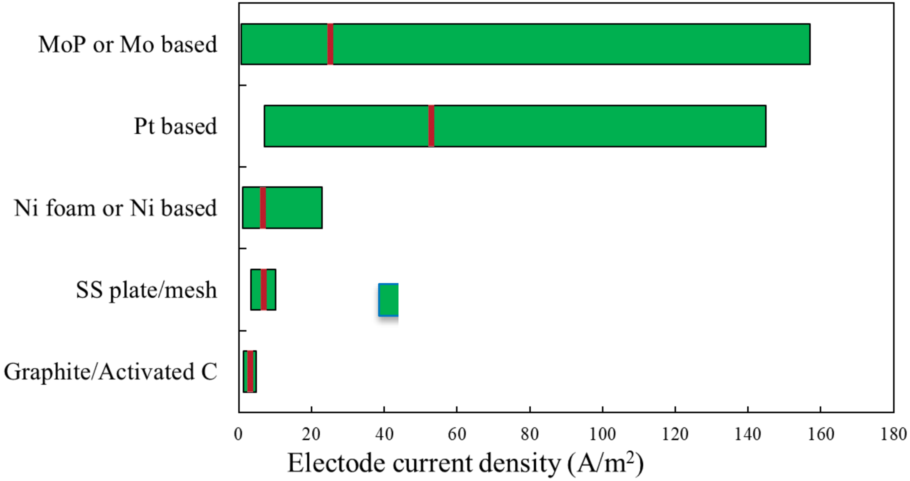


- Engineering process modeling in Aspen-Plus
- Capital cost of components
- Feedstock and material costs
- H_2 collection and onsite compression/storage needs
- Incorporate design and operation parameters into TEA model, conduct sensitivity analyses to above parameters
- Develop LCA model for production process, mass and energy balance to calculate energy use and emission associated with H_2 production and all process input (feedstock, materials, electricity, process heat, etc.)
- TEA/LCA set research targets and guide future research directions

Task 4 Accomplishments: The direction of reducing MEC cost

FY21 Q2 milestone: Evaluate MEC cost with various electrode options. (Complete)





- The key to reduce MEC cost is increasing current density and reducing electrode cost

- Current density highly depends on electrode material

Accomplishments and Progress: Responses to Previous Year Reviewers' Comments

None - a new start in FY19. A poster was presented in 2019 AMR but not reviewed

AMR slides were submitted in FY20 but not presented due to COVID-19

Collaboration and Coordination

- **Task 1 (Strain Development and Improvement)**
  - Dr. Katherine Chou (PI) and team develop and test strains to improve H_2 production and send the strains to LBNL for testing in high solids fermentation for Task 2.
  - NREL assumes the leadership of setting & coordinating directions & efforts between labs.
  - NREL leverages BETO investment in biomass pretreatment and Office of Science BER investments in understanding C*. thermocellum*. Collaboration with UCLA (BER support) laid the foundation to supply CO_2 for improved bacterial culturing.
- **Task 2 (High-solids Bioreactor Development)**

  Drs. Eric Sundstrom and Steve Singer (LBNL) develop and optimize bioreactors for high solid loadings and supply fermentation effluent to PNNL for Task 3.

- **Task 3 (Microbial Electrolysis Cell)**

  Dr. Alex Beliaev and the PNNL team are optimizing MEC-driven effluent conversion to address the H_2 molar yield, biological system performance and capital cost barriers

- **Task 4 (System Integration, TEA and LCA)**

  Dr. Amgad Elgowainy will use TEA/LCA to set research targets and guide research directions, working closely with all the tasks.

Remaining Challenges and Barriers

Task 1 Strain Development and Improvement (NREL)

- Continue to reduce biomass feedstock cost by minimizing pretreatments, and enable stable co-utilization of both cellulose and hemicellulose components of biomass
- Further improving the H_2 rate, yield, and productivity in bioreactor

Task 2. High-solid Bioreactor Development (LBNL)

- High solid-substrate loading (175 g/L) is needed to lower H_2 selling price.
  - Continue to assess impeller designs in concert with gas sparging characteristics to improve high viscosity mixing. Will continue to test and optimize fed-batch fermentation.

Task 3. Microbial Electrolysis Cell (PNNL)

- Maximize effluent carbon utilization efficiency using FT-MEC for improved H_2 molar yield, increase current density and reduce electrode costs using high-performance cathode materials

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

- Upon the establishment of the large scale MEC design, the MEC cost is estimated by using literature reported data (that is suitable for large scale MEC), such as high current density and usage of high performing electrodes. These assumptions need to be validated in experimental runs by testing high performing electrodes to achieve high current density and harmonize assumptions.

Proposed Future Work

Task 1 (NREL)

- Improve the engineered strain's (BX) stability by integrating the genes into bacterial genome in co-fermenting DMR real biomass, which will further reduce the fermentation lag time and improve overall H_2 productivity (FY21).
- Identify rate-limiting mechanisms to degrade hemicellulose (FY21).

Task 2 (LBNL)

- Evaluate higher solids loading with new impellers, including stacked conditions co-evaluating current best practices, including combined CO_2/nitrogen sparging and fed-batch operation (FY21)

Task 3 (PNNL)

- Enable flow-through MEC operation through biological effluent pretreatment to increase concentration of electrogenic substrates (acetate, lactate) and eliminate inhibitors (proteins, alcohols, O_2). Test high-performance cathode materials to increase current density & H_2 yield (FY21)

Task 4 (ANL):

- Continue to evaluate and incorporate inputs from project team, and update the Aspen Plus, H2A and GREET models based on progress from experimental work. Update electrode cost information by periodically research. Will investigate the economic benefit of high purity CO_2 sales and tax credit by CO_2 sequestration (45Q) (FY21/22).

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

Summary

Task 1 (NREL)

- **Meeting Go/No-Go Milestone: 11% increase in H_2 production rate from current baseline (39% increase total) via better hemicellulose utilization** in bioreactor loaded with 88.4 g/L real biomass (30 g/L as cellulose)
- Targeted expression of β-xylosidase enzyme improved the deconstruction of xylo-oligomeric sugar by 89% using the most current baseline strain

Task 2 (LBNL)

- Doubled H_2 production rate at 60 g/L avicel loading via shift to fed-batch operation
- Successful scale-up from 1L to 7L, NREL strain 19-9 successfully onboarded with 100 g/L DMR

Task 3 (PNNL)

- Achieved continuous MEC operation (>200 hs) on high-load fermentation effluent (Avicel)
- Obtained 2.5 A/m^2 current densities on high-load fermentation effluent (Avicel)

Task 4 (ANL)

- Obtained a preliminary large scale MEC design with various electrode options and calculated H_2 cost accordingly
- Provided recommendations to collaborators to test high performing electrodes
- Obtained life cycle analysis results of bioH_2 production from the integrated system

Thank You

www.nrel.gov

Publication Number

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Technical Back-Up Slides

(Include this “divider” slide if you are including back up technical slides [maximum of five]. These back up technical slides will be available for your presentation and will be included in Web PDF files released to the public.)

Technology Transfer Activities

Technology-to-market or technology transfer plan or strategy

- Co-localize biohydrogen refinery to the source of feedstock and expand the use of H_2 to current biorefinery

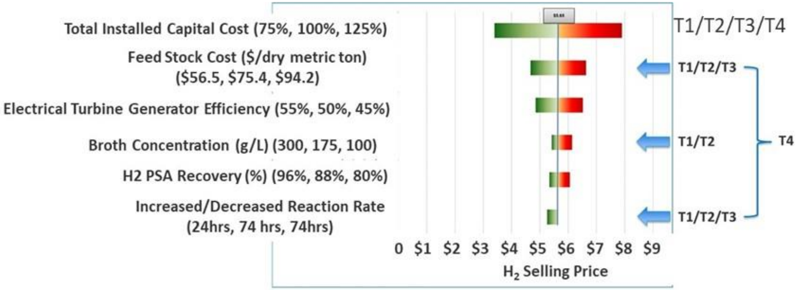
Plans for future funding

- Pursue opportunities to collaborate with industry for potential future funding support.
- Expansion of feedstock portfolio beyond terrestrial biomass to potentially include waste
- Network with biofuels industry to expand the use of H_2.
- Advocate the advantages of "green" H_2 rather than fossil-fuel derived H_2.

Patents, licensing

- A patent application is accepted by USPTO on a genetic device developed by NREL team to enable "tunable gene regulatory control in thermophilic bacteria."
- A Record of Invention (ROI-14-70) is filed for developing the proprietary genetic tools tailored for *C. thermocellum*.
- A second ROI-15-42 has been filed for generating xylose-metabolizing strain, leading to enhanced biomass utilization.

Progress toward DOE Targets or Milestones



The BioH2 consortium is setup to directly address technical barriers:

- –Feedstock Cost (AY): by fully utilizing all sugars in biomass derived from both cellulose and hemicellulose;
- –MEC current density and capital cost

Addressed Barrier/ Technical Parameter	2019 status at project start	2021 Target	2021 Status by Q2
Feedstock cost (AY)/ Utilization of biomass (**NREL**)	Utilize mainly cellulose to produce H_2	Ferment both cellulose and hemicellulose for H_2 production	Co-utilize cellulose & hemicellulose, which led to 33% increase in H_2 production per unit of real DMR biomass by weight (FY21 Q2 G/NG milestone completed)
H_2 productivity (Dark Fermentation) (**NREL**)	2.2 L H_2/L /day	3.0 L H_2/L /day	3.1 L H_2/L /day (FY21 Q2 Go/No-Go milestone completed)
High solids fermentation (**LBNL**)	30 g/L crystalline cellulose as model substrate	Ferment 67.5 g/L as cellulose in 150 g/L real DMR biomass (Q3-4)	Ferment 45 g/L as cellulose in 100 g/L real DMR biomass (FY21 Q2)
MEC Current Density (**PNNL**)	4 A/m^2 (simulated effluent, acetate)	5-10 A/m^2 (actual Avicel effluent, Q3-4)	2.5 A/m^2 (actual Avicel effluent, high solid load fermentation, FY21 Q1-2)
H_2 production cost (**ANL**)	$58/kg-$H_2$ (for Dark Fermentation only)	$9-15/kg-$H_2$*	$15-38/kg-$H_2$*

***Assumptions: Lower bound of H_2 cost assumes the use of Zirfon as separator/diaphragm, while the higher bound assumes the use of Nafion as membrane**

Special Recognitions and Awards

- NREL accomplishment in improving H_2 production by 190% by a *C. thermocellum* strain engineered to co-ferment Avicel and Xylan is selected as one of the top 10 NREL accomplishments by the HFTO program in FY20 PEMP (Performance Evaluation and Measurement Plan)
- Dr. Katherine Chou (Project PI) is nominated and selected to be a US Representative for International Energy Agency Hydrogen Implementing Agreement (IEA H2) Task 34: Biological Hydrogen For Energy and Environment. This organization pursues collaborative hydrogen R&D and information exchange among its member countries.

Publications and Presentations

Publications

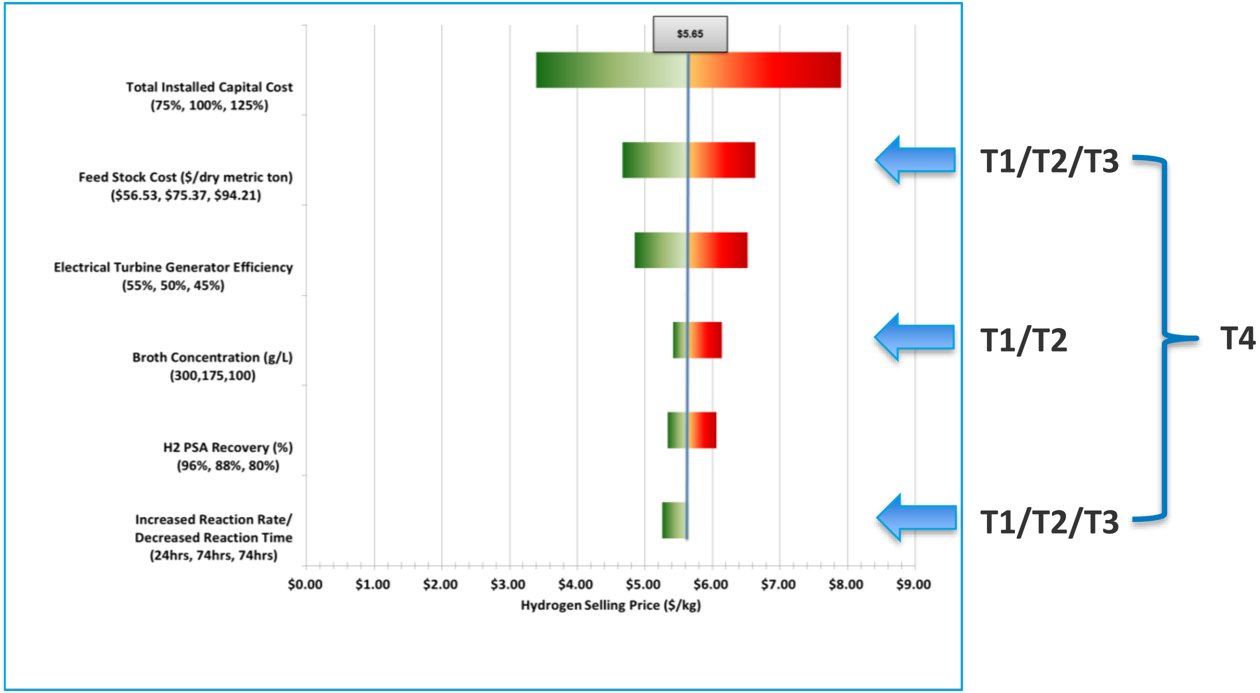
- ***Developing riboswitch-mediated gene regulatory controls in thermophilic bacteria***. Marcano, J. G., J. Lo, A. Nag, **P. C. Maness, K. C. Chou***. ACS Synthetic Biology. 2019, 8, 4, 633-640. DOI: 10. 1021/acssynbio.8b00487
- ***Integrated thermodynamic analysis of electron bifurcating [FeFe]-hydrogenase to inform anaerobic metabolism and H_2 production***. Jay, Z., Hunt, K.A., **Chou, K.J.** Schut,[3]G.J., **Maness, P.C.,** Adams, M.W.W., Carlson, R.C. 2020. BBA Bioenergetics. 2020 Jan 1;1861(1):148087. DOI: 10.1016/j.bbabio.2019.148087
- ***Transcriptomic analysis of a Clostridium thermocellum strain engineered to utilize xylose: responses to xylose versus cellobiose feeding***. Rangel, A.E.T., Croft, T.J., Barrios, A.G., Reyes, L.H., Maness, P.C.*, Chou, K.J.* Scientific Reports, in review.
- ***Renewable Hydrogen from Biomass Fermentation***. **Chou, K.J., Magnusson, L.R.,** Seibert, M., **Maness, Pin-Ching.** A book chapter for *Encyclopedia of Biological Chemistry*. Manuscript accepted and in print by Elsevier, Aug. 2021

Presentations

- Maness P.C., "**BioHydrogen Consortium to Advance Fermentative H_2 Production**," 2019 HFTO AMR. April 30, 2019.
- Chou, K. J., "**Engineering a cellulolytic and thermophilic bacterium *Clostridium thermocellum* for biofuel production**," Invited Presentation at UCLA Chemistry and Biochemistry Departmental Seminar. Jan. 10, 2020
- Chou, K.J., "**Discovery and Genetic Engineering of a Thermophilic bacterium Clostridium thermocellum for Consolidated BioProcessing**", Invite Virtual Presentation at Boise State Chemistry and Biochemistry Departmental Seminar: Nov. 10, 2020

Relevance: Research Directions are guided by a Cost Analysis from Strategic Analysis, Inc.

Tornado chart showing parameter sensitivities for the future central fermentation case (2025 goal), which guides research direction.



Case Study	Low Value ($/kg H_2)	Baseline ($/kg H_2)	High Value ($/kg H_2)
Current Case (2014)	$48.49	**$58.53**	$68.57
Future Case(2025)	$3.39	**$5.65**	$7.90

Task 4 Accomplishments: Develop TEA Framework

FY21 Q2 Milestone	**Evaluate MEC cost with various electrode options.**	3/2021	**Complete**

- The integrated system was modeled by using Aspen Plus to simulate a conceptual bio-H_2 facility with the capacity of 50 tonne/day. MEC unit was modeled using an equilibrium reactor.
- The process modeling results (e.g., flow rate of various streams) were incorporated in the H2A model framework to size all equipment and calculate capital cost. The equipment cost was obtained via literature search and vendor quotes.
- The MEC process is identified as the major cost driver for the overall system. Given the absence of scaled MEC, we designed the large scale MEC relying on MEC fundamentals and chemical engineering practices, and adopting design elements from PEM and industrial chloralkali electrolyzer.
- The developed methodology for MEC scale up consists of sizing several elements individually: reactor tank volume, cathode surface area, anode surface area, membrane, frames, stainless steel plates, etc.
    - ✓ The electrodes cost dominates MEC cost.
- $Electrode\ cost = \frac{I\ (A) \times Electrode\ unit\ cost\ (\frac{\$}{m2})}{I_{density}\ (\frac{A}{m2})}$, *I* is MEC current, related to plant capacity, thus fixed.

Task 4 Accomplishments: The impact of financing options

- Financing option impacts bioH_2 production cost
- Low interest rate favors low equity/debt ratio (H2A default equity share is 40%)