DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Production of Bioproducts from Electrochemically-Generated C1 intermediates

March 11, 2021
CO2 Utilization

Jason Bromley (Co-PI, presenter)
Michael Köpke (Co-PI)

LanzaTech

Acknowledgements

Sponsor:

U.S. Department of Energy

Office of Energy Efficiency & Renewable Energy

Bioenergy Technologies Office

DOE Project Officer: Ian Rowe

Project Monitor: Ben Simon

LanzaTech

Dioxide Materials™ The CO_2 Recycling Company™

Argonne NATIONAL LABORATORY

Quad Chart Overview

Timeline
- Start: October 01, 2018
- End: December 31, 2021

	FY20 Costed	Total Award
DOE Funding	(10/01/2019 – 9/30/2020)	$545,481
Project Cost Share		$157,623

Project Partners*
- Dioxide Materials
- Argonne National Lab

Project Goal
Develop and demonstrate a gas fermentation system integrated with CO_2 electrolysis to convert CO_2 to isopropanol, leveraging arginine as a nitrogen and energy source.

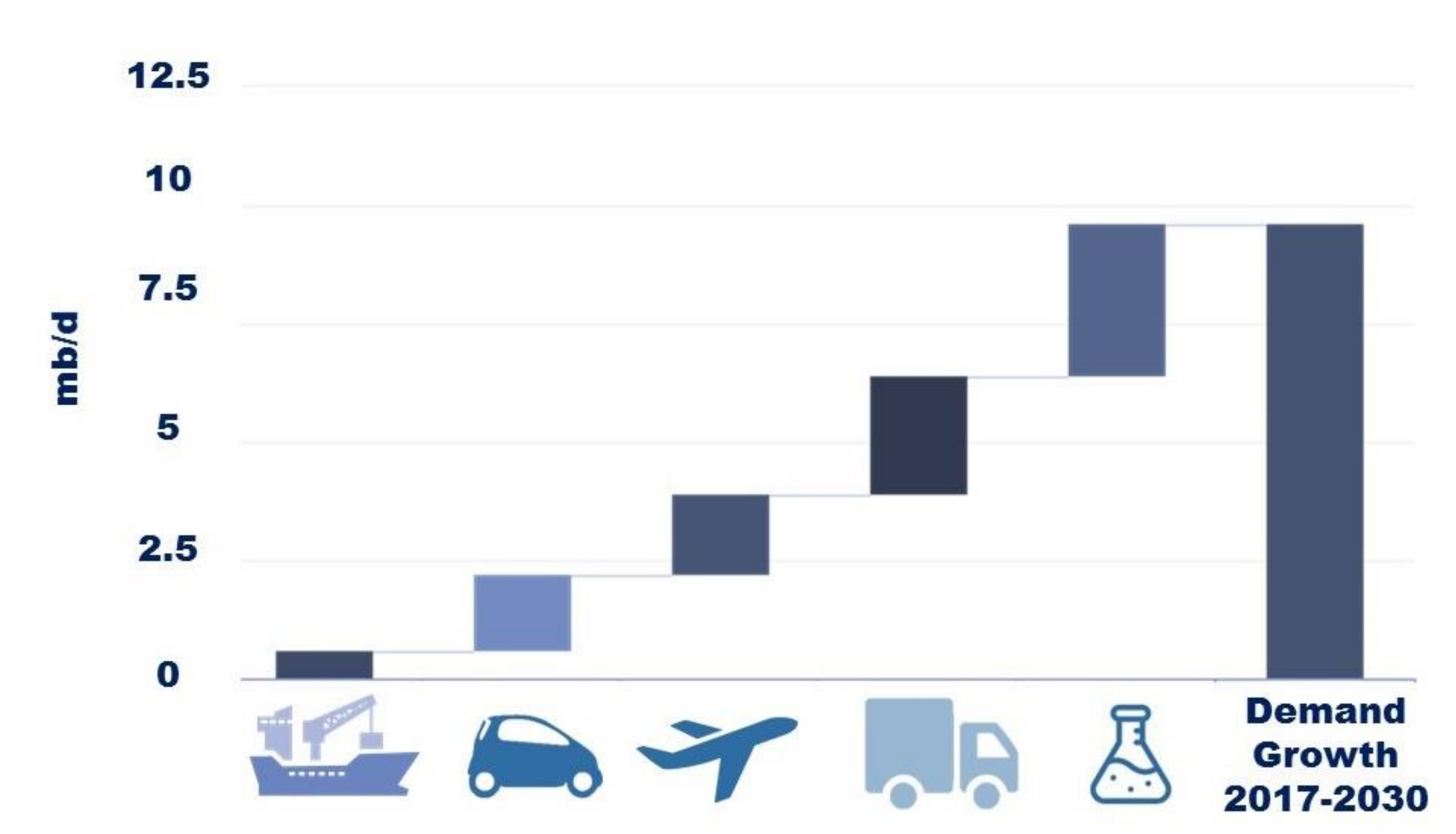
End of Project Milestone
0.65 g/L/h continuous isopropanol production from CO_2 at >37% carbon efficiency, with Arginine as the sole nitrogen source.

Funding Mechanism
BioEnergy Engineering for Products Synthesis, FOA-0001916, topic area 5 – Rewiring Carbon Utilization. 2018. Award number DE-EE0008500.

Project Overview

Chemicals for Everyday Products need Carbon

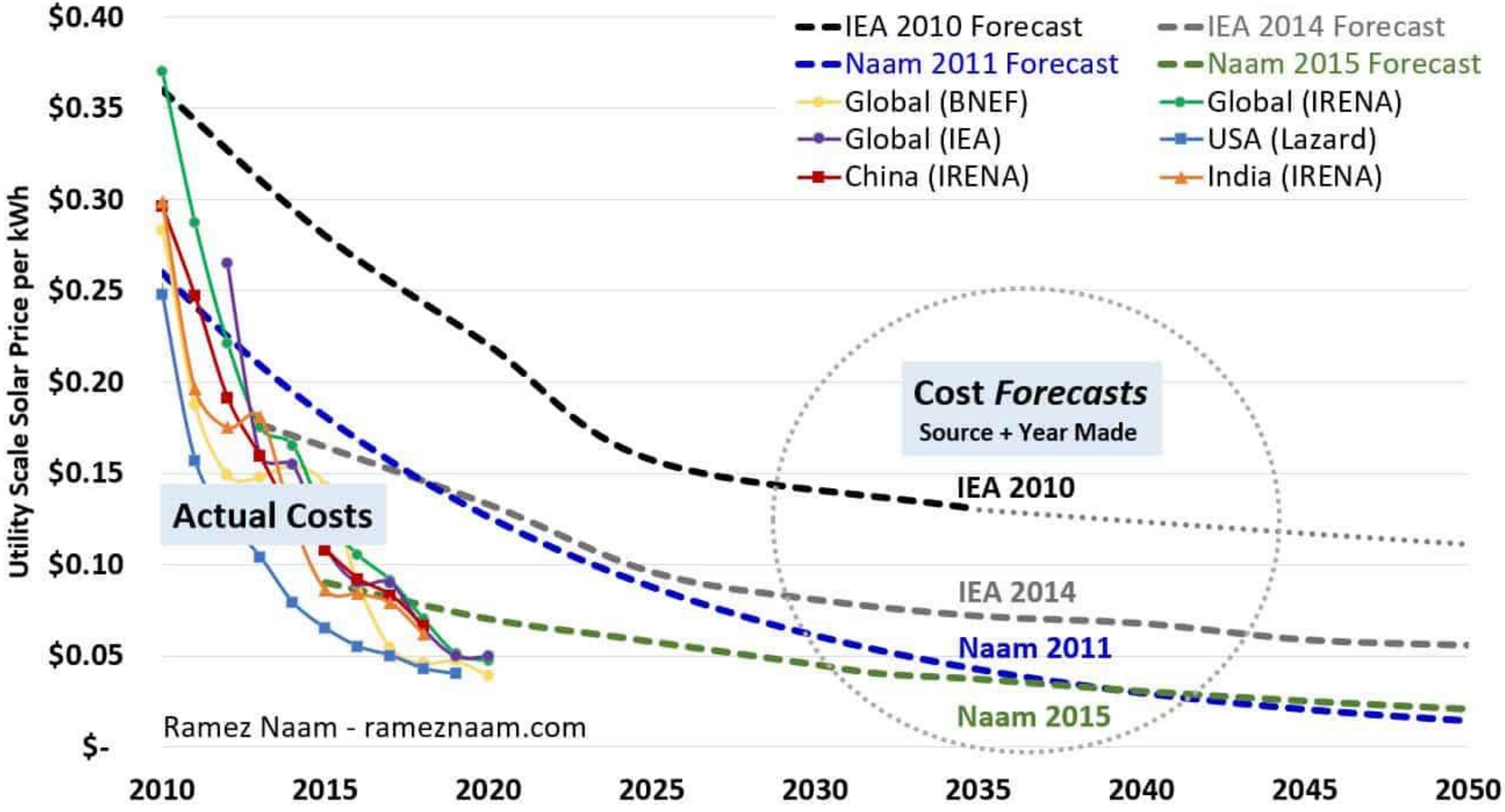
Oil Demand Growth by Sector, 2017-2030



Petrochemicals will be the largest driver of global oil demand over the coming decades

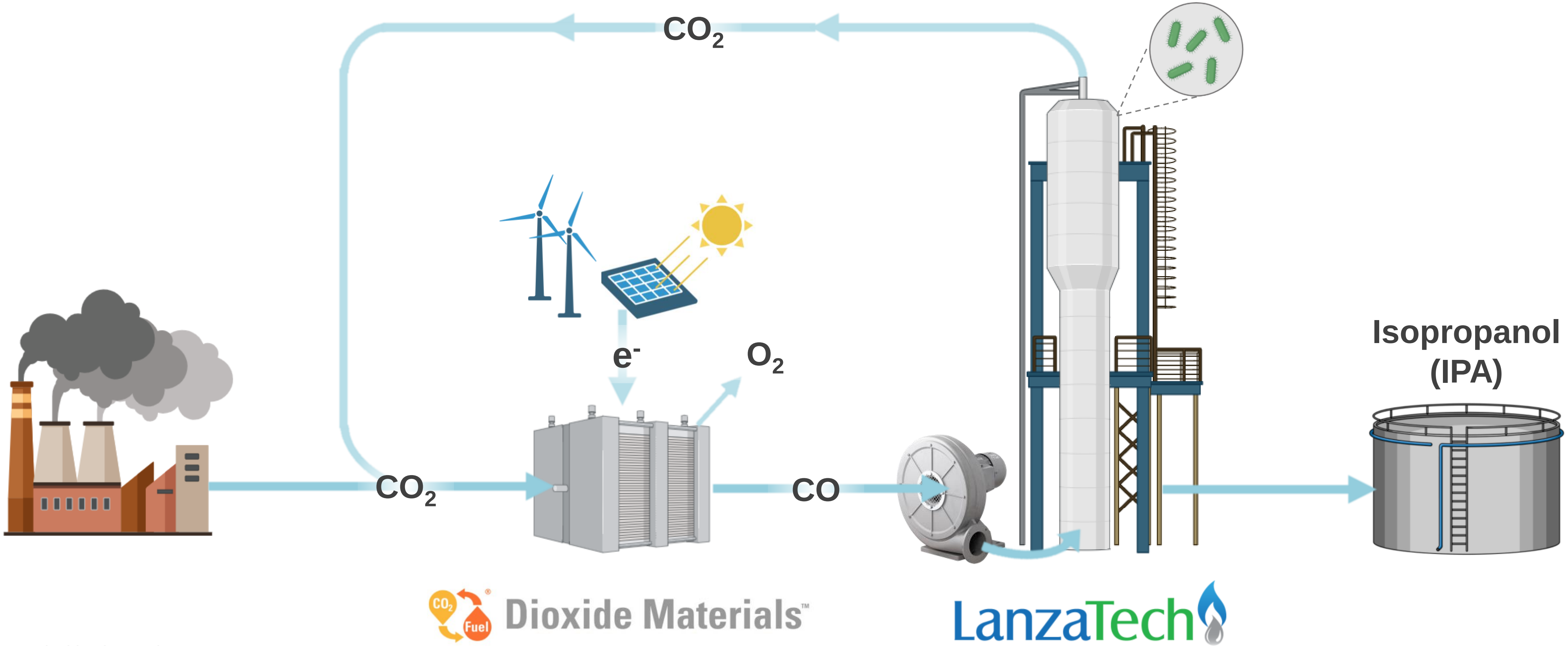
https://www.iea.org/petrochemicals/

Solar Costs Are Decades Ahead of Forecasts



CO_2 Electrolysis + Gas Fermentation: a CO_2 to chemicals platform

- Anion Exchange Membrane (AEM) based CO_2 electrolysis
  - High energy efficiency achievable at room temperature
- Acetogen yield improvement through advanced metabolic engineering and arginine supplementation.



images created with BioRender.com

1 – Management

Team

LanzaTech
Christophe Mihalcea

LanzaTech
Jason Bromley, **Co-PI**
Process Integration

LanzaTech
Michael Köpke, **Co-PI**
Synthetic Biology

LanzaTech
Ching Leang
Strain Development

Dioxide Materials
Richard Masel
Electrolyzer Enhancements

Building on a track record of success to ensure efficient communication within the team and reporting to DOE/BETO

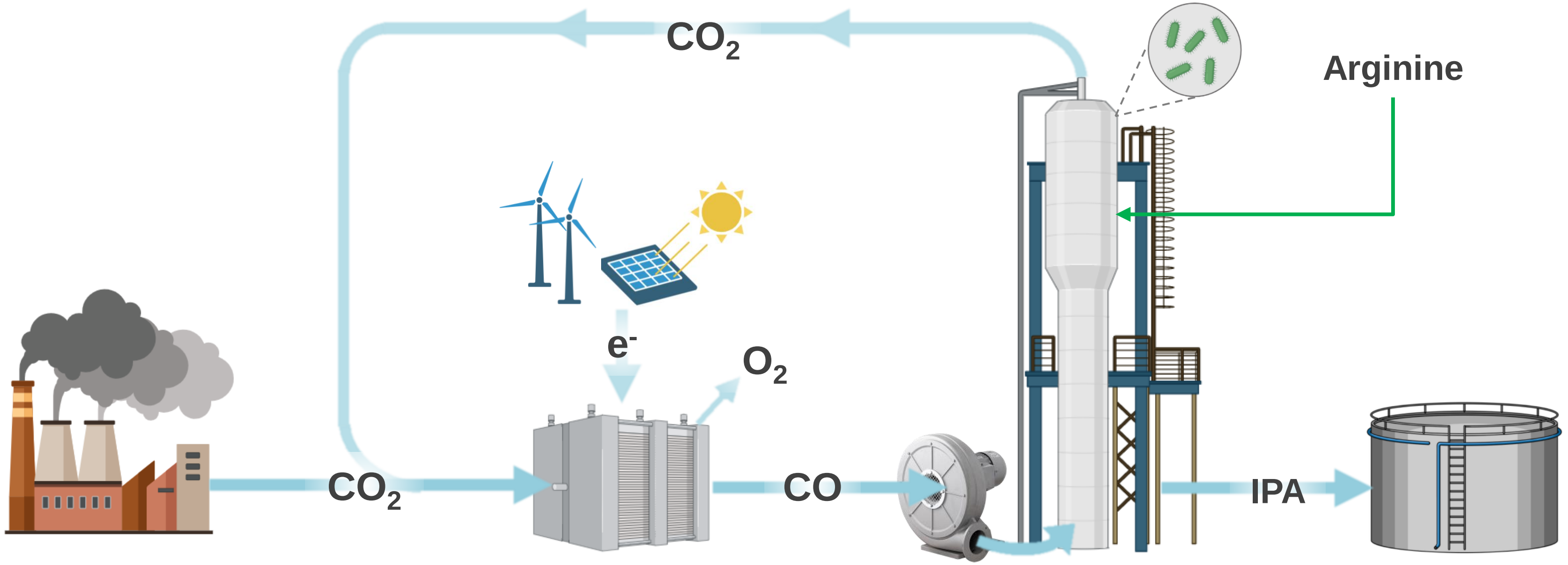
- LanzaTech and Dioxide Materials have created a successful mechanism for technical coordination, data sharing, and integration through the collaborative effort to integrate CO_2 electrolysis with gas fermentation. DE-SC0018540
- Bi-weekly meetings review the teams progress and any matters requiring action such as schedule, milestones, and deliverables.
  - This process helps identify, address, and mitigate emerging risks.
  - Roy Bertola (LanzaTech) leads project management: maintaining schedule and budget, communication and coordination with project partners, organization project meetings

	Budget Period	**1**	**2**					**3**				
Task	**Quarter**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Task Timeline												
1,7,13	Verification											
3,9	Electrolyzer Improvements											
4,10	Microbial Yield Improvements											
5,11	Fermentation Studies											
6,12	TEA & LCA											
2,8,14	Project Management											

2 – Approach

To Achieve our Vision:

1. **Electrolyzer enhancements**
   Improve efficiency, stability and CO_2 crossover
2. **Strain engineering for microbial yield improvements**
   Synthetic pathway for arginine co-utilization to increase yield
3. **Fermentation studies and electrolyzer integration**
   Optimizing IPA production and carbon efficiency
4. **TEA / LCA**
   Feedback on economic and carbon impact



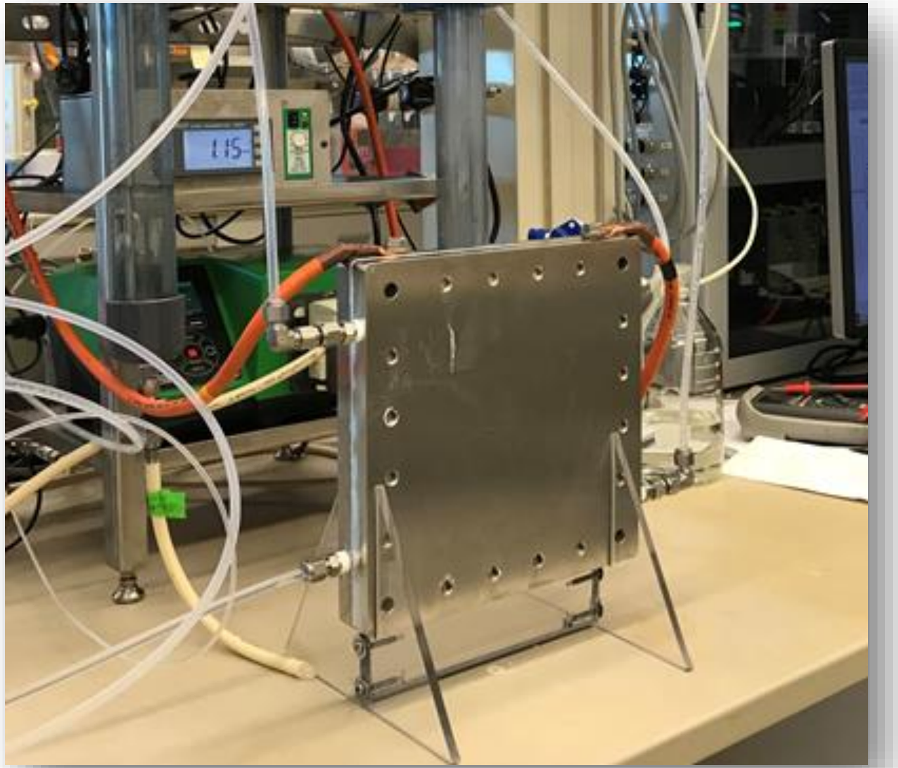
images created with BioRender.com

Aim 1: Electrolyzer Enhancements

Dioxide Materials
The CO_2 Recycling Company™

Fuel Cell based 2018 version

Custom made 2020 version



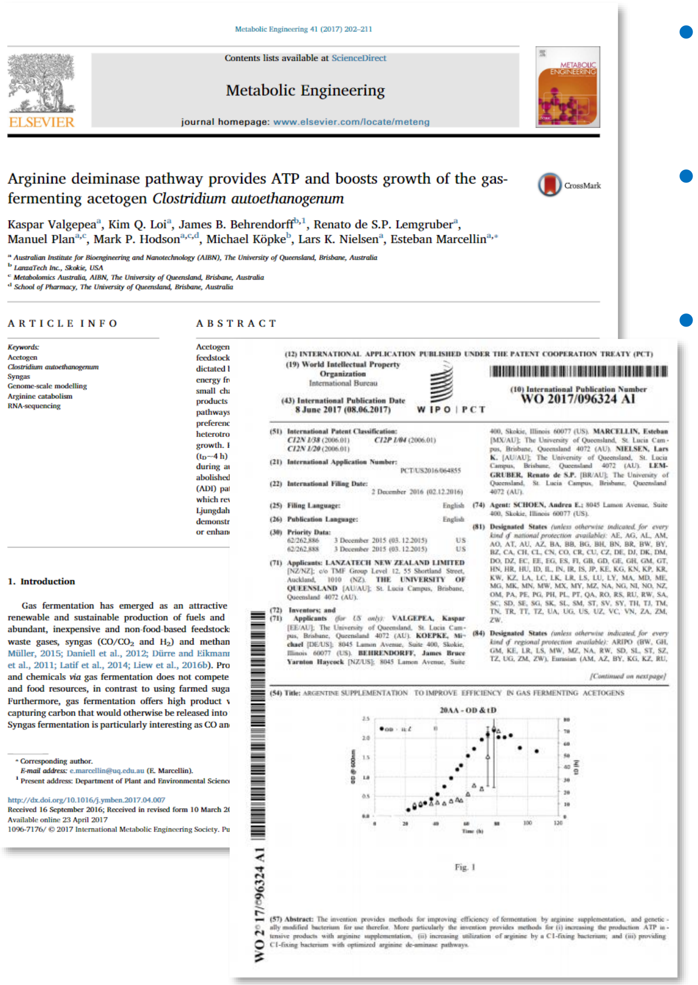
New materials
New flow field

Pressure drop reduced:
30 psi → 2 psi

Better energy efficiency

Lower CO_2 crossover

Aim 2: Microbial Yield Improvement


Metabolic Engineering 41 (2017) 202–211

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/meteng

Arginine deiminase pathway provides ATP and boosts growth of the gas-fermenting acetogen *Clostridium autoethanogenum*

Kaspar Valgepea, Kim Q. Loi, James B. Behrendorff, Renato de S.P. Lemgruber, Manuel Plan, Mark P. Hodson, Michael Köpke, Lars K. Nielsen, Esteban Marcellin

ARTICLE INFO

ABSTRACT

1. Introduction

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

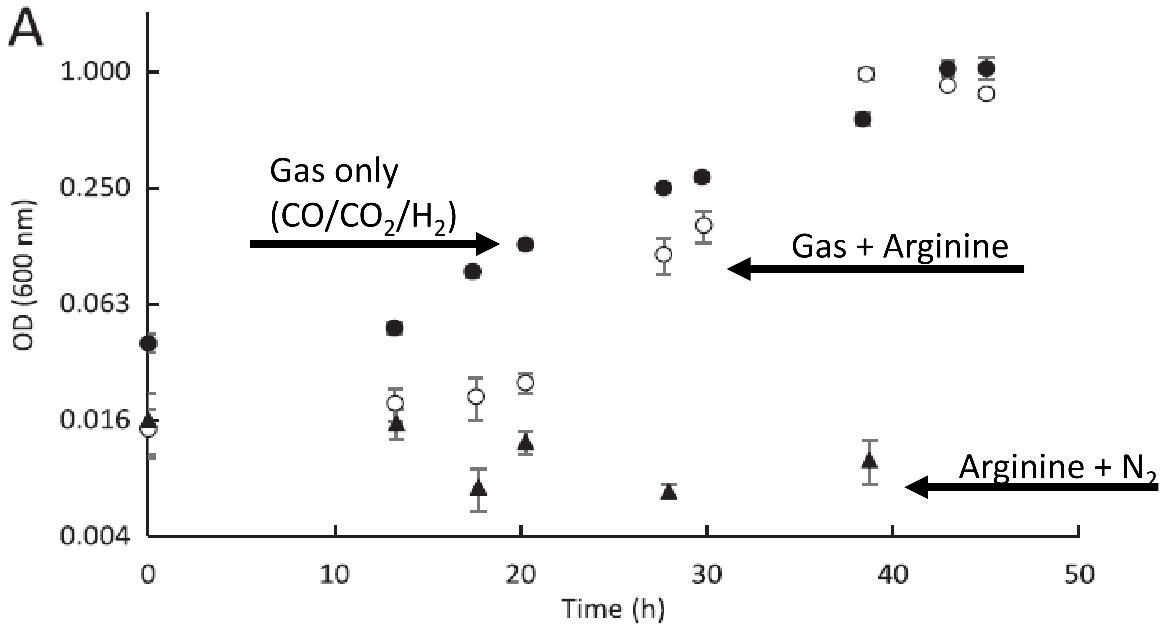
(19) World Intellectual Property Organization International Bureau

(43) International Publication Date 8 June 2017 (08.06.2017)

(10) International Publication Number WO 2017/096324 A1

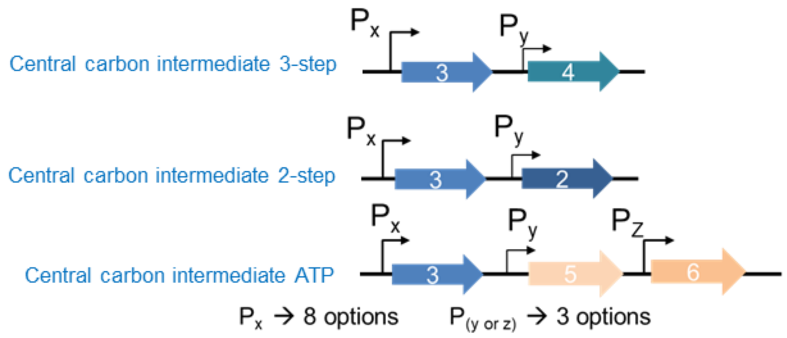
(54) Title: ARGININE SUPPLEMENTATION TO IMPROVE EFFICIENCY IN GAS FERMENTING ACETOGENS

- L-Arginine is an amino acid that is preferentially consumed by *C. autoethanogenum*
- Arginine could replace ammonia as the nitrogen source at competitive cost on a per mol N basis
- In 2015 we found that arginine supplementation
  - Improves growth rates and product yields in bottles.
  - Leads to **ornithine** accumulation

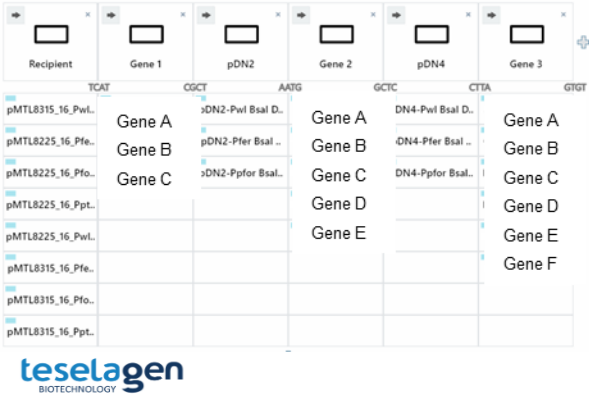


http://doi.org/10.1016/j.ymben.2017.04.007
https://patents.google.com/patent/WO2017096324A1/en

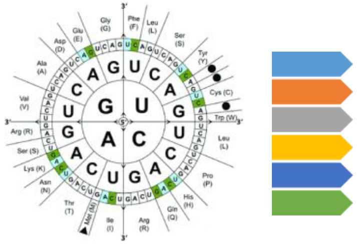
Aim 2: Microbial Yield Improvement: Ornithine utilization



Pathway and Gene Candidate Identification

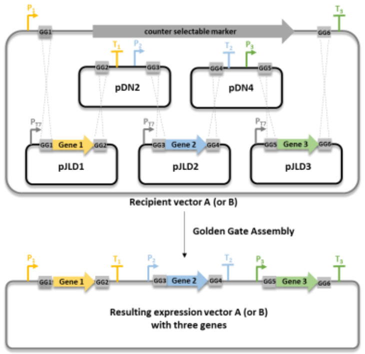


Pathway Design



Codon Adaptation

Gene Synthesis/ Pre-Cloning



Library Assembly

Strain Generation

Strain Screening

Aim 2: Simulations using genome-scale models and flux balance analysis

Models used
- Full genome-scale model (GEM)
- Structurally simplified GEM
- Core metabolism model

Maximum yields (ATP and product)
- Flux balance analysis with shadow price analysis
- Manually inspected each predicted fluxome to verify biological plausibility.

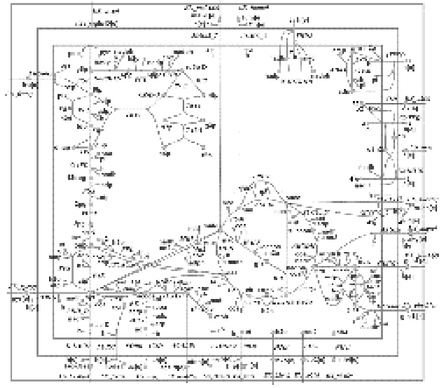
Design landscape assessment
- Flux balance analysis and minimization of metabolic adjustment to predict phenotypes
- Strength Pareto Evolutionary Algorithm 2 (SPEA2) and minimal cut set (MCS) analysis to characterize design landscape (Maia 2015, Kamp 2014)

Definitions

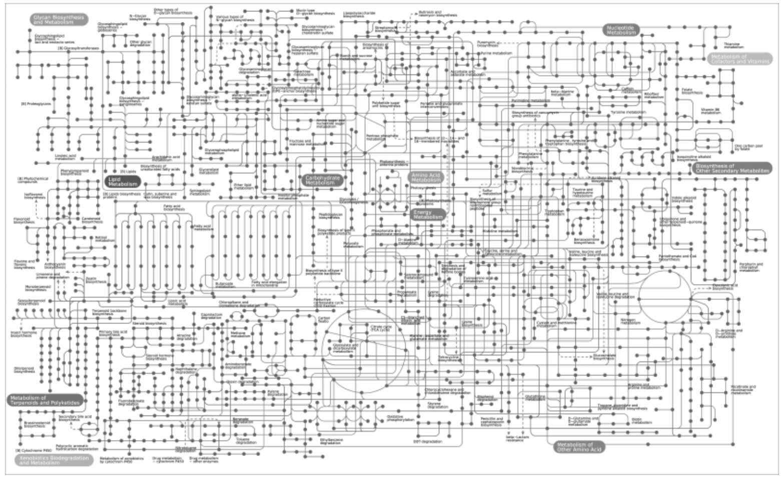
High H_2 = 5:1 H_2:CO uptake

CO = 0:1 H_2:CO uptake

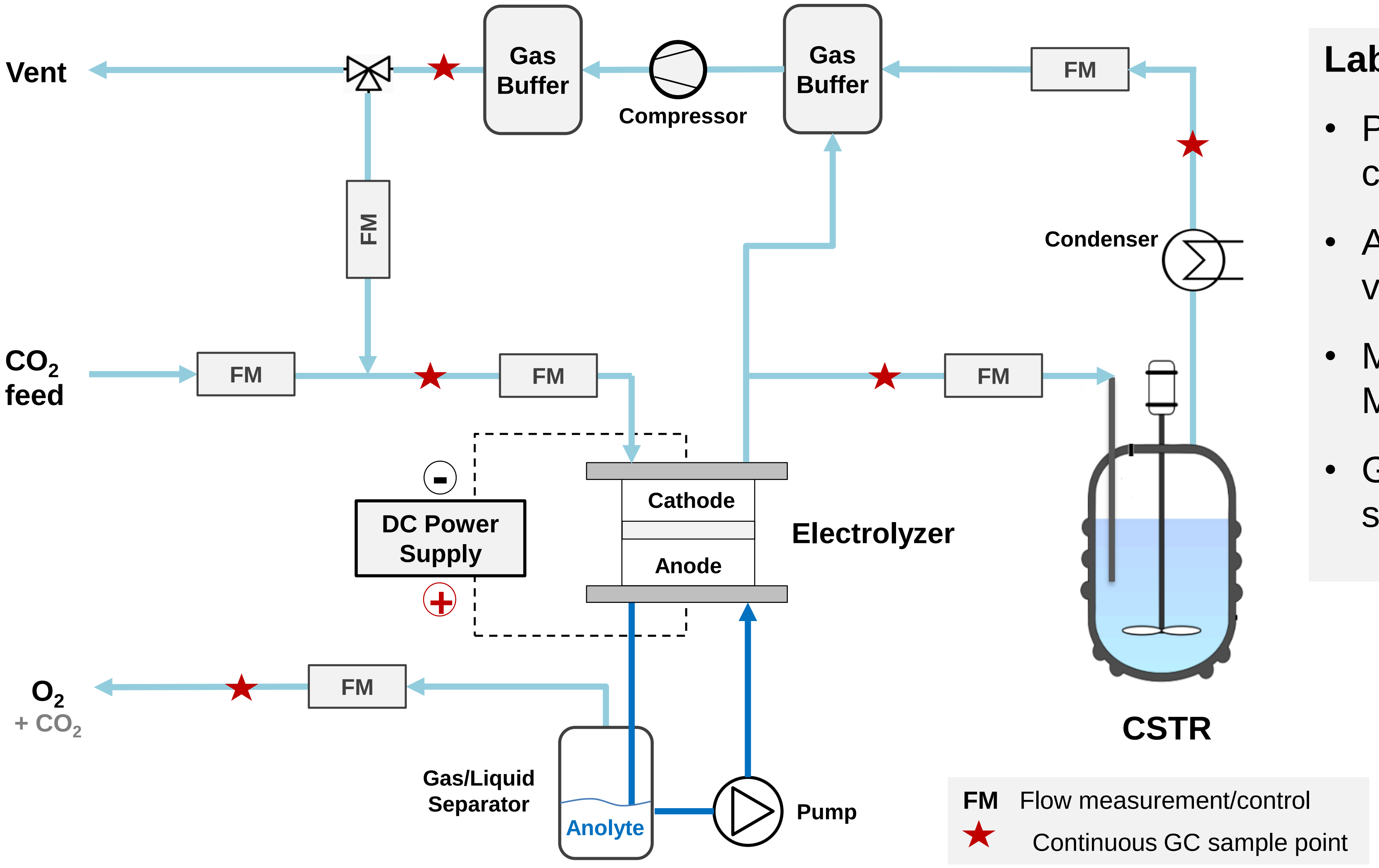
Example core metabolism



Example genome-scale metabolism



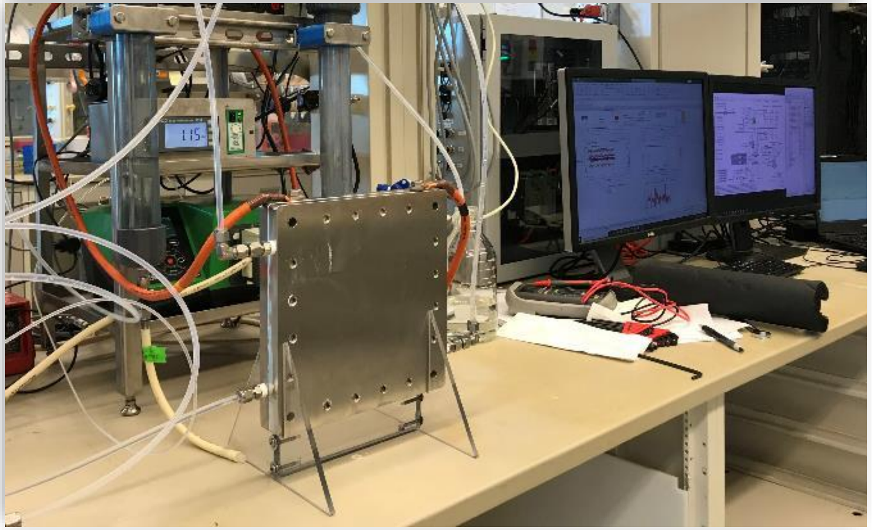
Aim 3: Electrolyzer Integration



Lab Scale Challenges:

- Pressure and gas recycle control
- Accurate gas flow metering with varying composition
- Moisture damages GC and MFCs
- GC sampling requires significant flows

Aim 3: Electrolyzer Integration



Approach:

- Integrated unit with sophisticated software control
- Condenser and gas cleanup on fermenter outlet
- Measurement redundancy (pressure, flow, composition)
- Low-flow GC minimizes sample requirements
- GC results used to adjust thermal mass flow controller calibration in real time

Metrics, Targets, and Decision Points

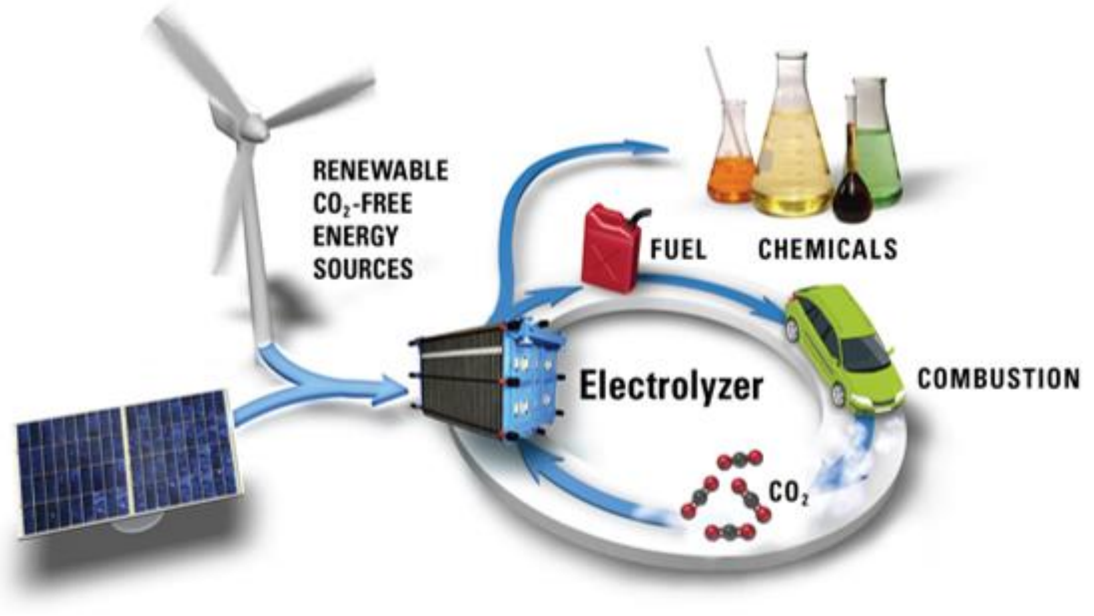
Key Performance Parameters	Metric	BP1 ✓ (go/no-go)	BP2 (go/no-go)	BP3 (final target)
Carbon Efficiency to products	%	28	33	37
Electricity use (Electrolyzer efficiency)	kWh / Nm^3 CO	9.57	8.72	7.97
% N feed as Arginine	mmol / g biomass	0	25	100

3 – Impact

Ensure U.S. Leads in Sustainable Energy Technology

We will reduce CO_2 to CO via electrolysis for gas fermentation to generate isopropanol, to enable:

- Biorefineries with increased carbon utilization of non-fossil resources for sustainable chemical production
- Increased demand and integration of renewable electricity for carbon negative production



Necessary Innovations

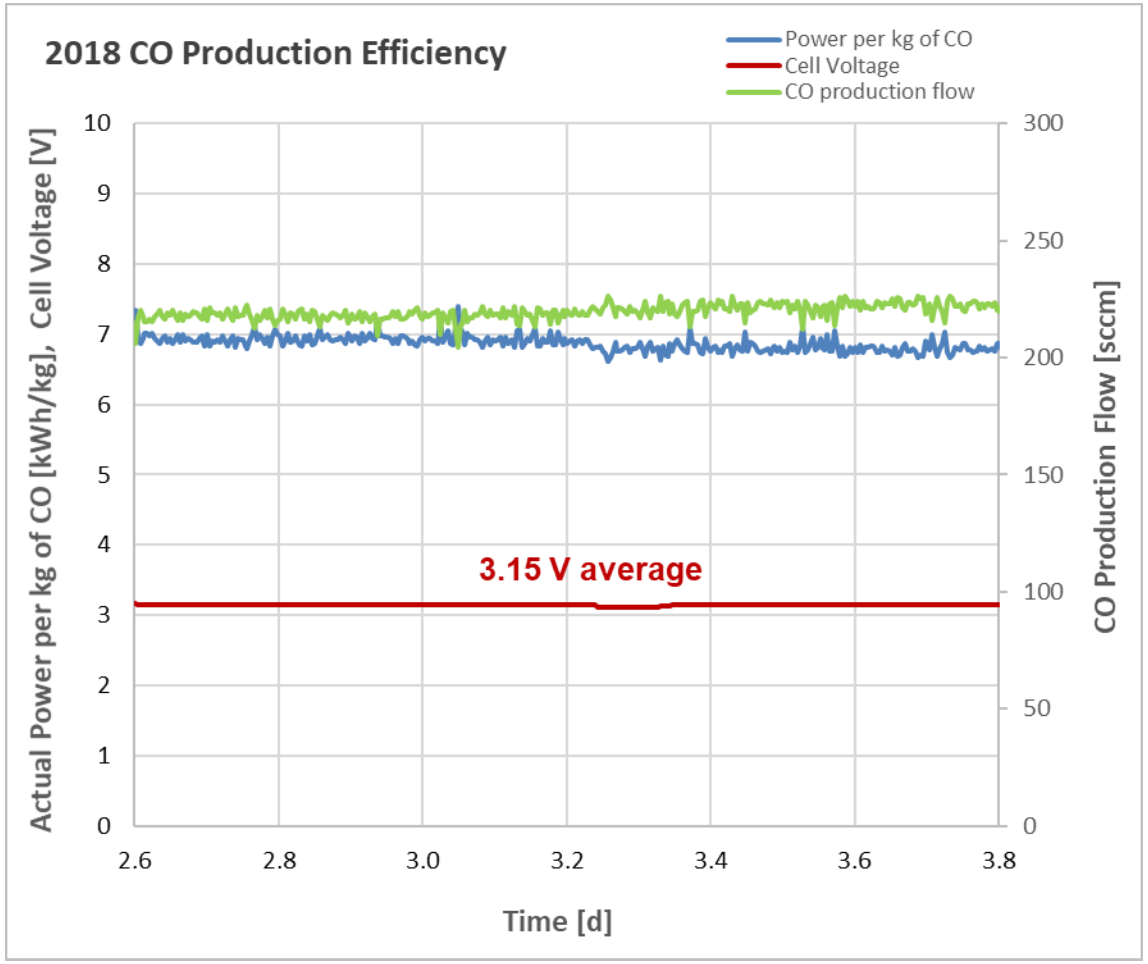
- Innovations to electrolyzer membrane, durability, and scalability are needed to achieve a minimum of 37% conversion of the CO_2 stream
- Optimize microbial process conditions to maximize both growth and productivity to inform and enable future predictive understanding of microbial metabolism in gas fermentation

Creating a Carbon Smart World

- Realize the potential for carbon utilization through improved understanding of component sensitivities and lifetimes.
- Develop integrated approaches necessary to demonstrate combined conversion to a building block chemical.
- Enhance the bioenergy value proposition by converting CO_2 to a value-added chemical
- Expanding stakeholder engagement and collaboration through the integration of different technologies
- Bolster development of end-use markets through LanzaTech's existing business relationships.

4 – Progress and Outcomes

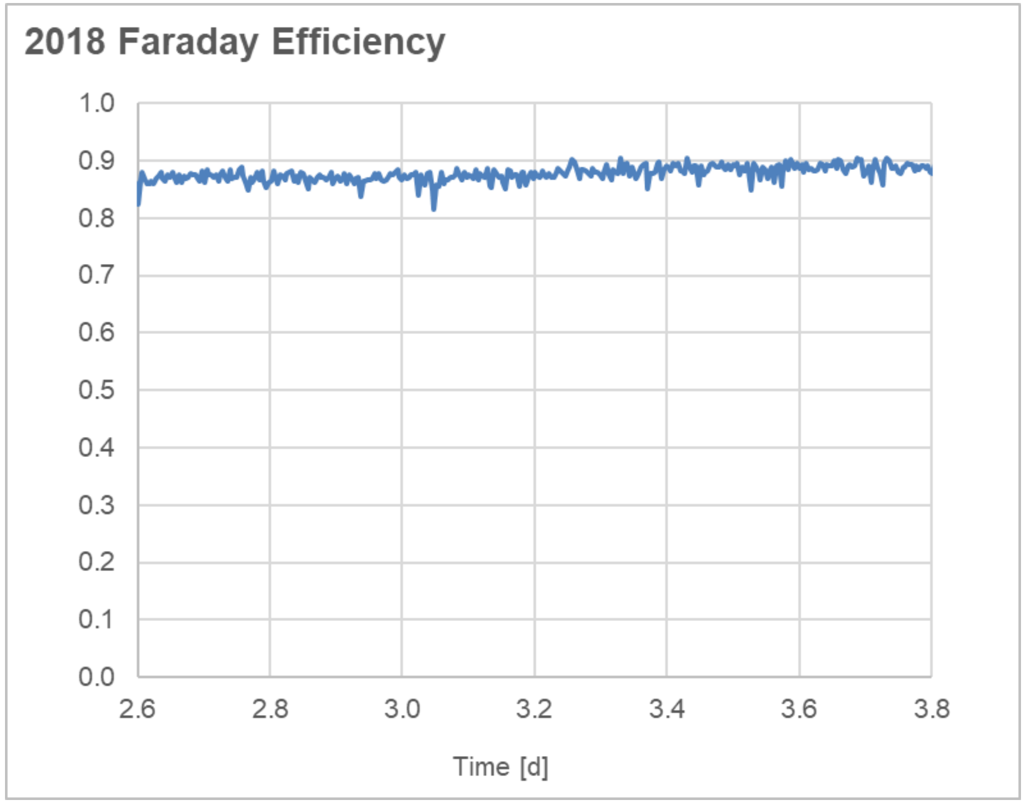
Aim 1: Electrolyzer enhancement results

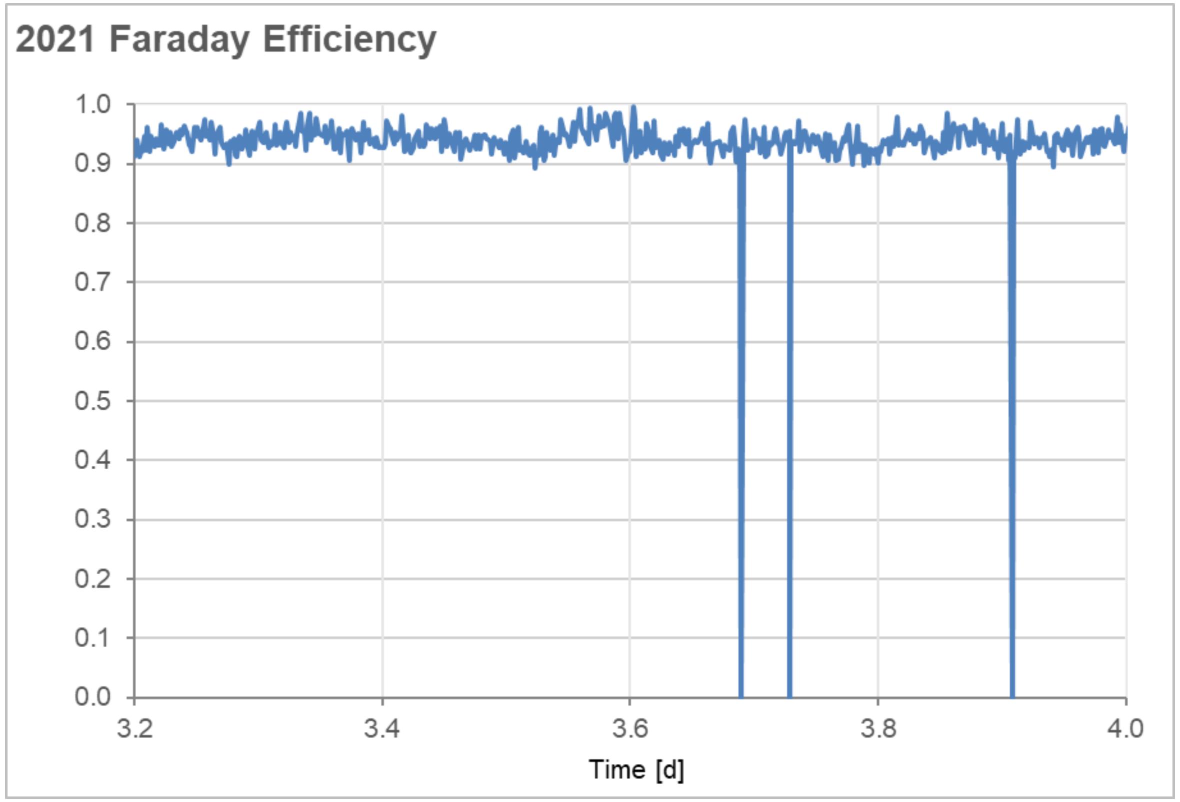


→ 2021 cell is ~8% more energy efficient

Aim 1: Electrolyzer enhancement results

Faraday Efficiency = Charge per equivalent of CO (theoretical / measured)

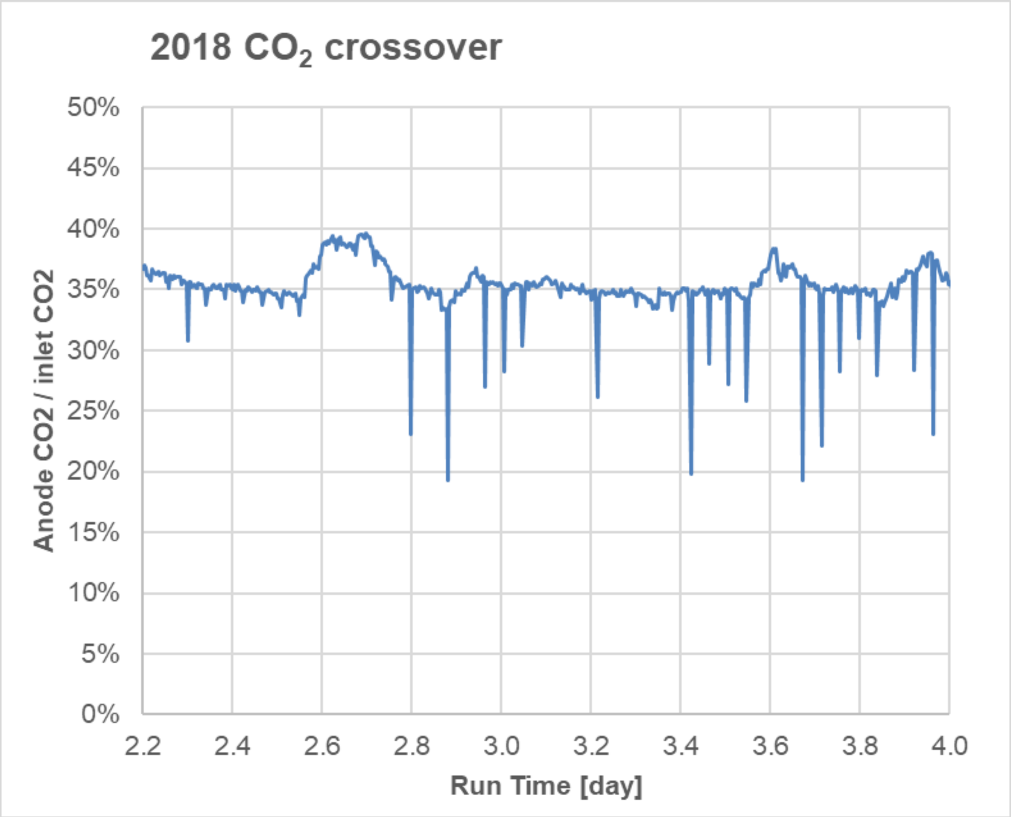


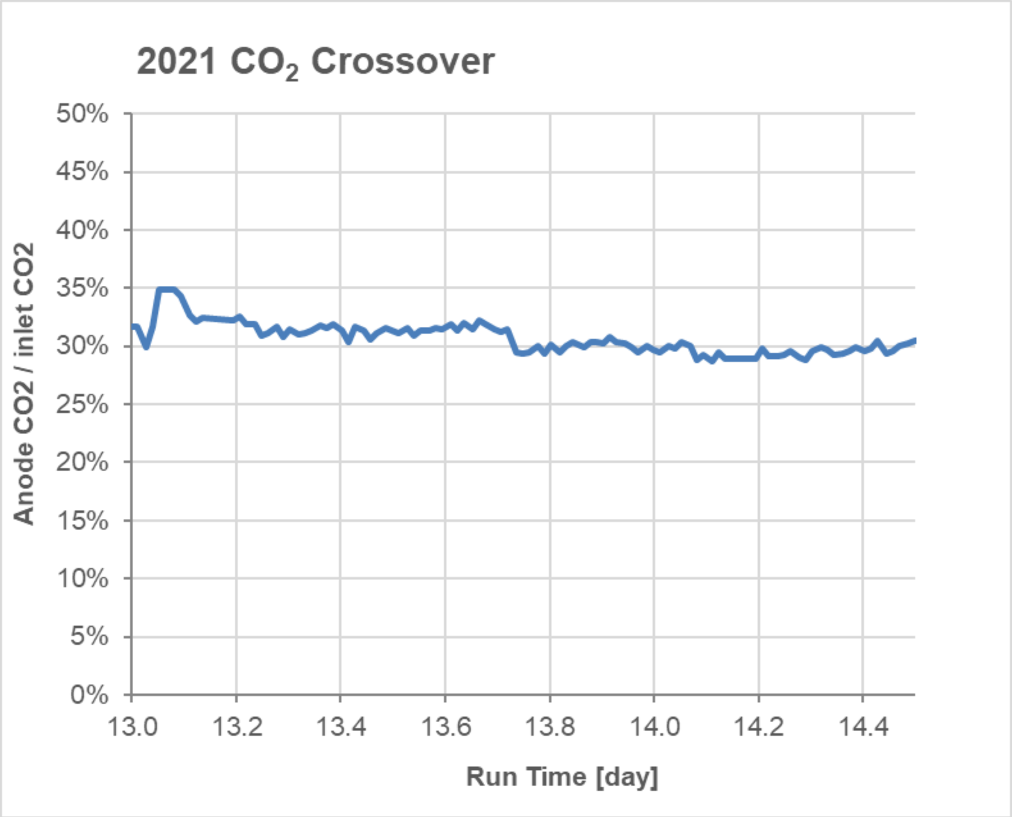


→ 2021 cell achieves 95% Faraday Efficiency

Aim 1: Electrolyzer enhancement results

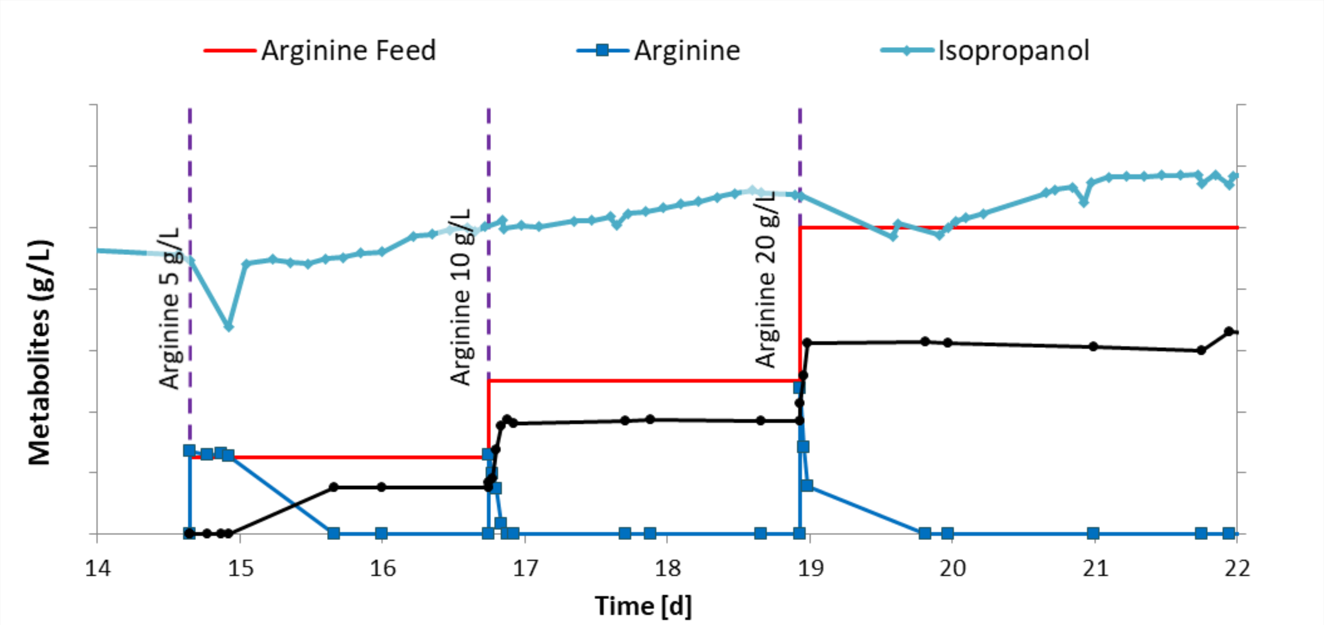
CO_2 crossover = Molar Carbon flow (anode out / cathode out)

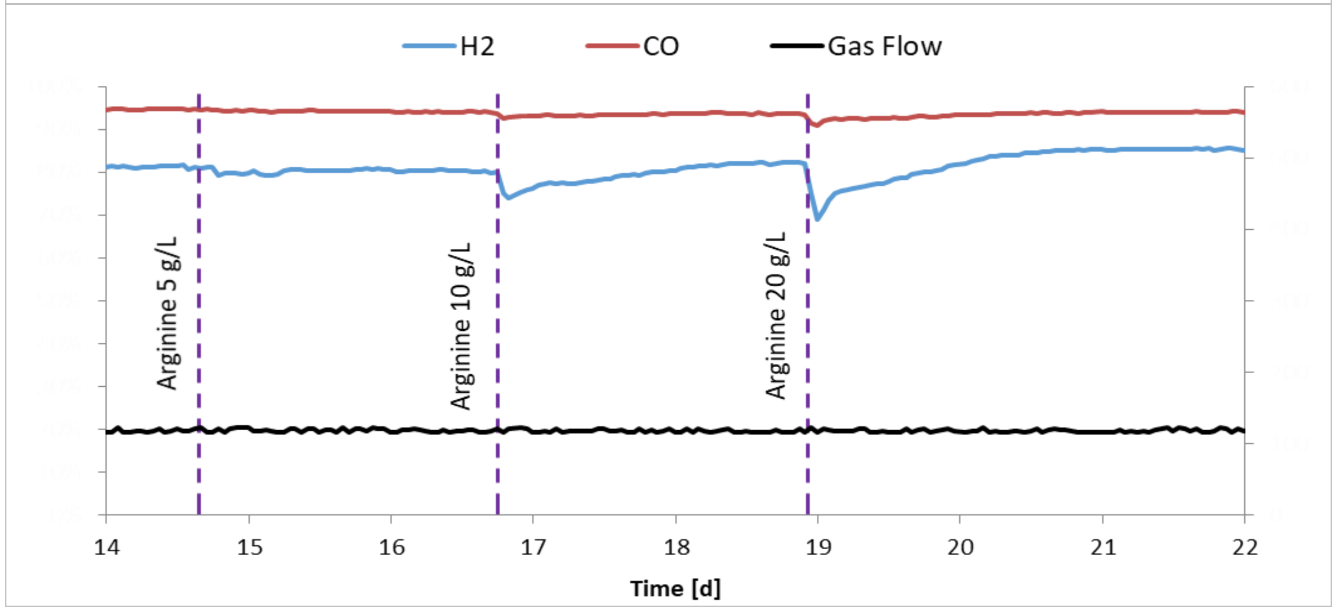




→ 2021 cell reduces CO_2 crossover

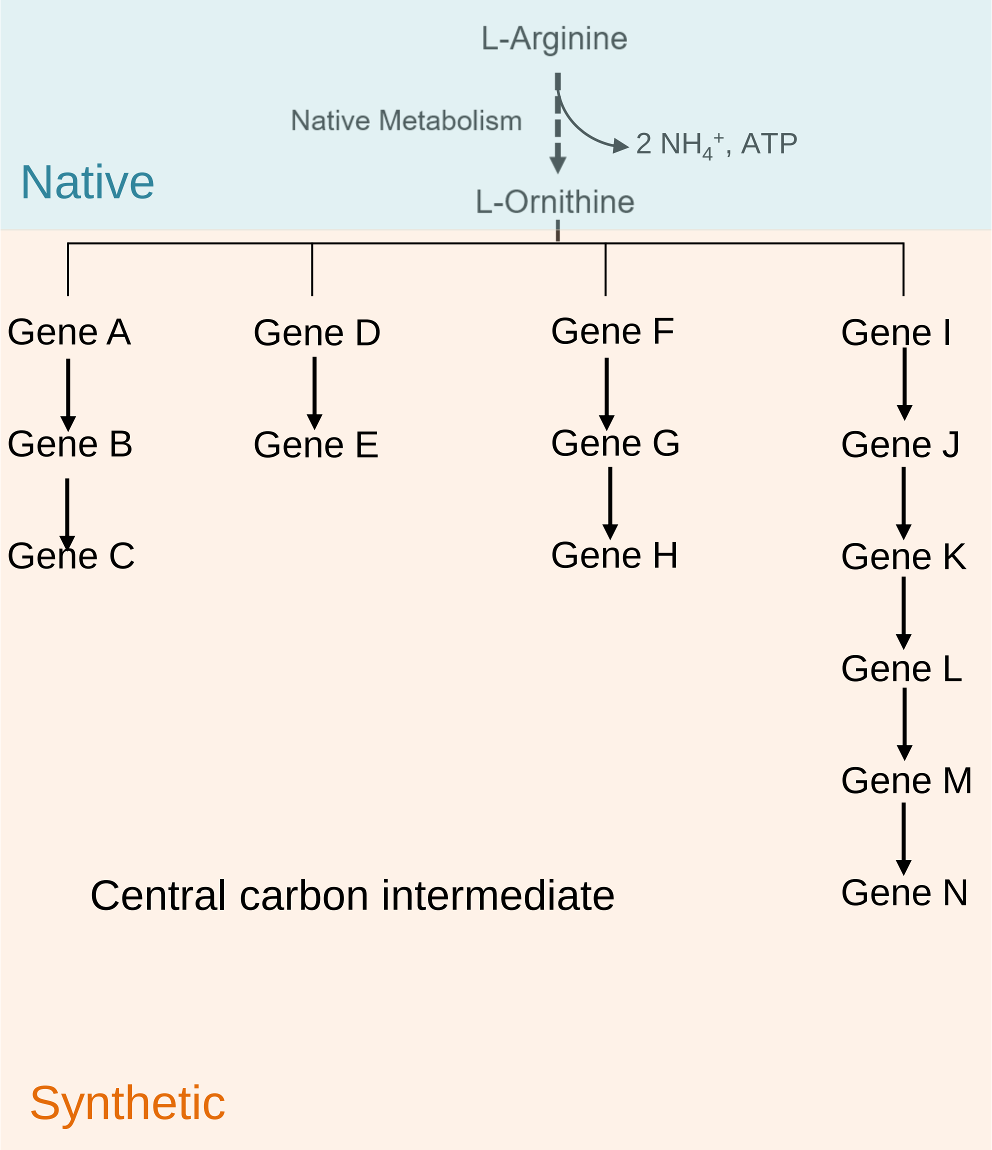
Aim 2: Arginine and CO co-utilization demonstrated in IPA production strain





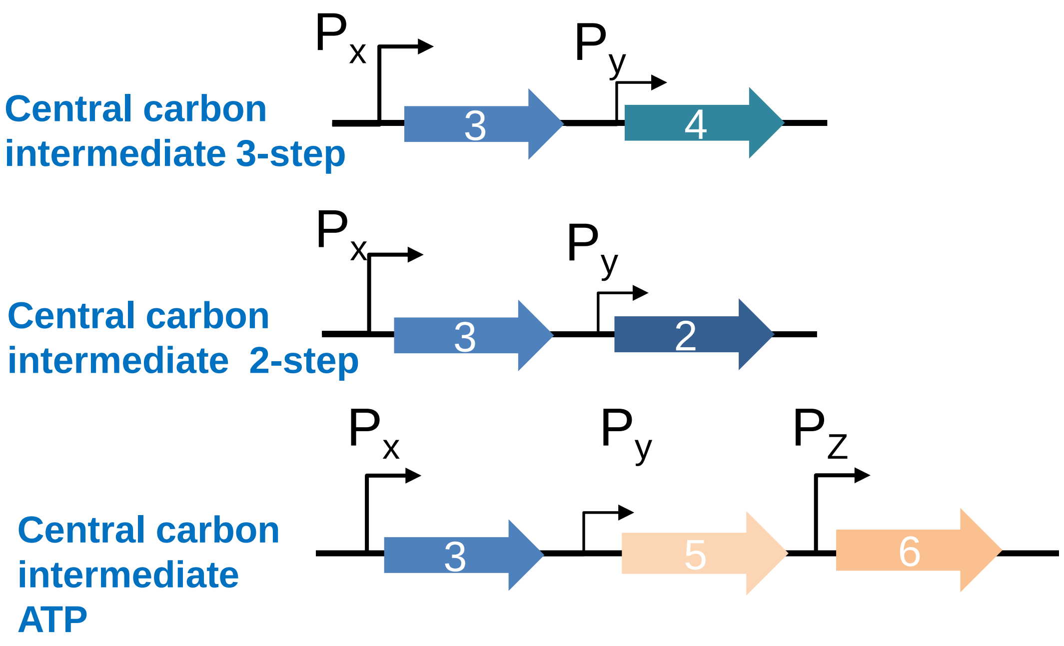
- Arginine was fed into continuous gas fermentation at increasing rates.
- Both CO and arginine uptake happened simultaneously.
- A slight increase in IPA selectivity was observed at higher dose of arginine supplement.

Aim 2: Arginine metabolism and Ornithine assimilation pathways



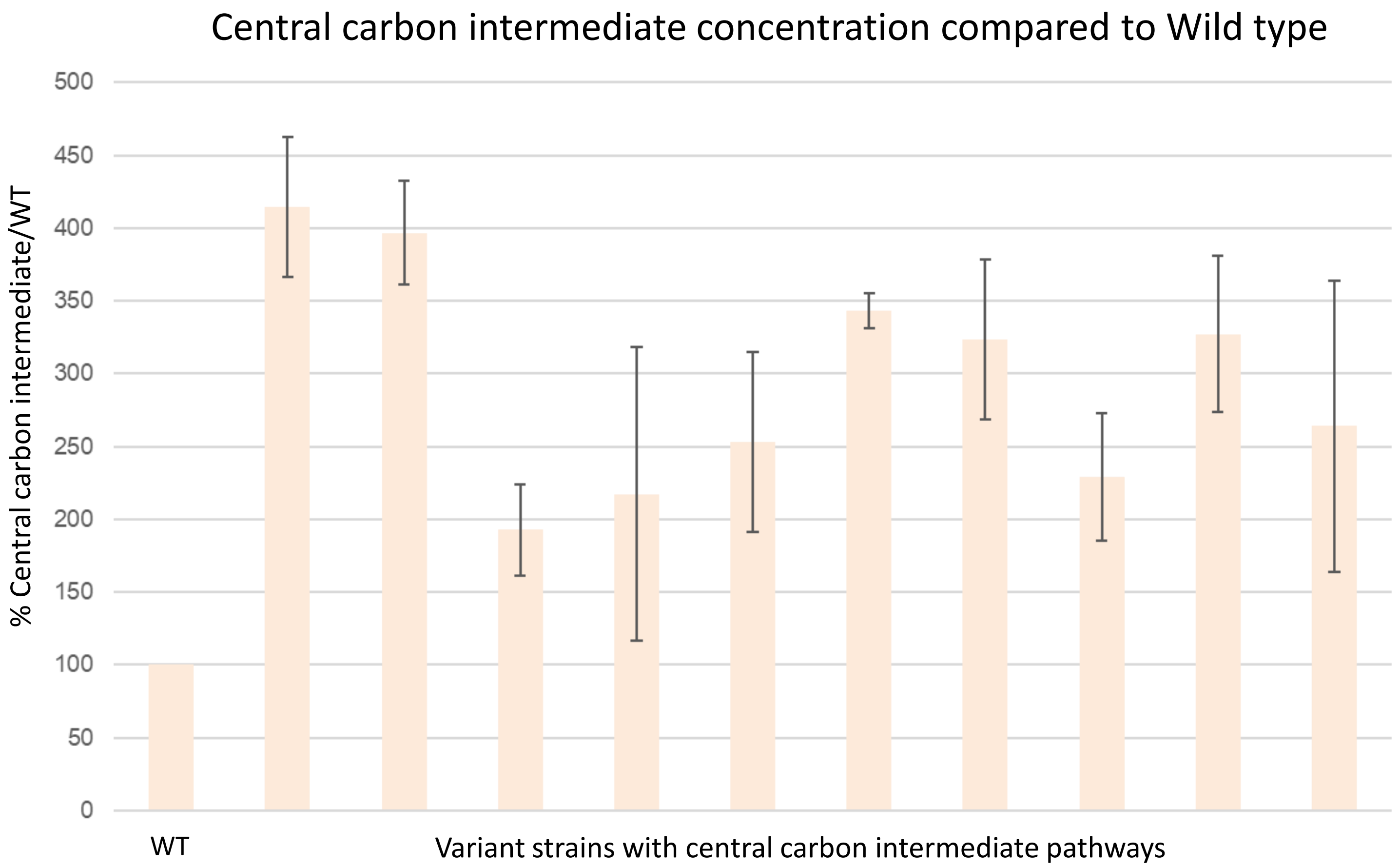
- Total of possible 24,192 unique pathway combinations, exceeding the Milestone 4.2.1 - Identify 100 pathway gene combinations for conversion of ornithine to central metabolism.
- Assembled over 384 unique combinations to date.
- Screening is on going.

Aim 2: Library Assembly Examples and Results to date



P_x → 8 options

$P_{(y\ or\ z)}$ → 3 options



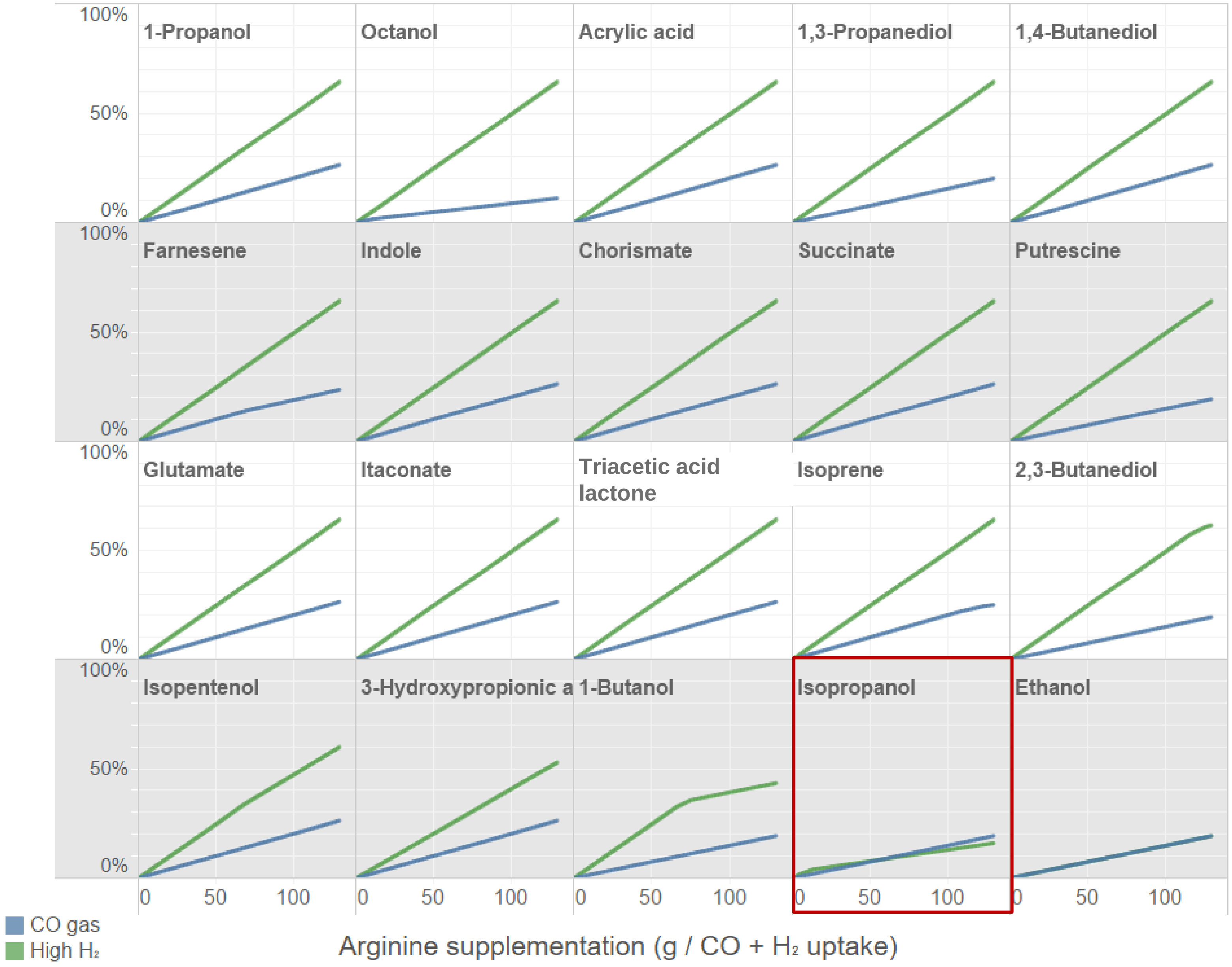
Increased levels of central intermediates observed upon introduction of developed pathways in context of arginine feeding

Yield increase vs arginine supplementation for 20 different chemicals

The impact of arginine supplementation on the predicted maximum yields for 20 different *C. autoethanogenum* chemical production strains.

Calculated using flux balance analysis.

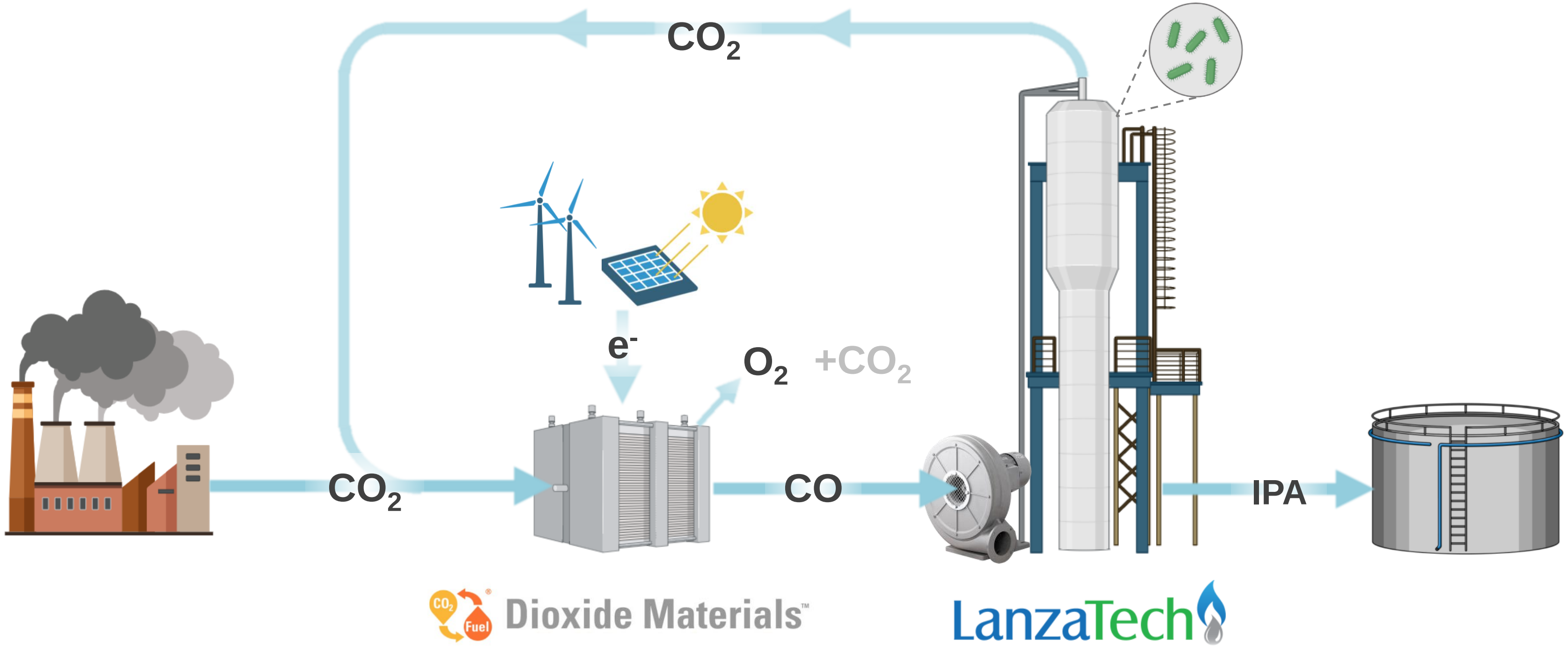
Modelling confirms arginine supplementation increases yield for a range of tested target molecules.



Summary

Efficient CO_2 electrolysis and deeper understanding of acetogen metabolism: building blocks for a circular carbon economy utilizing renewable energy

- Improved CO_2 electrolyzer design increased energy efficiency and reduced CO_2 crossover
  - CO_2 crossover remains the greatest carbon yield loss
- Co-utilization of Arginine and CO proven, integration pathways identified, and several strains built
  - Modelling highlights other products and gas mixes that would have greater benefit from arginine
- Integrated electrolyzer, gas fermentation and recycle unit built to prove out recycle concept
  - Fermentation studies ongoing



Questions?

Publications, Patents, Presentations, Awards, and Commercialization

- TEA and LCA support stakeholder and potential customer engagement.
- LanzaTech Business Development is maintaining and growing relationships with many potential customers.
- There are no publications, patents, or awards associated with this project yet