

**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)



This presentation does not contain any proprietary, confidential, or otherwise restricted information

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



Quad Chart Overview

Timeline

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

Project Goal

Develop and demonstrate a gas fermentation system integrated with CO₂ electrolysis to convert CO₂ to isopropanol, leveraging arginine as a nitrogen and energy source.

End of Project Milestone

0.65 g/L/h continuous isopropanol production from CO₂ at >37% carbon efficiency, with Arginine as the sole nitrogen source.

	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

Funding Mechanism

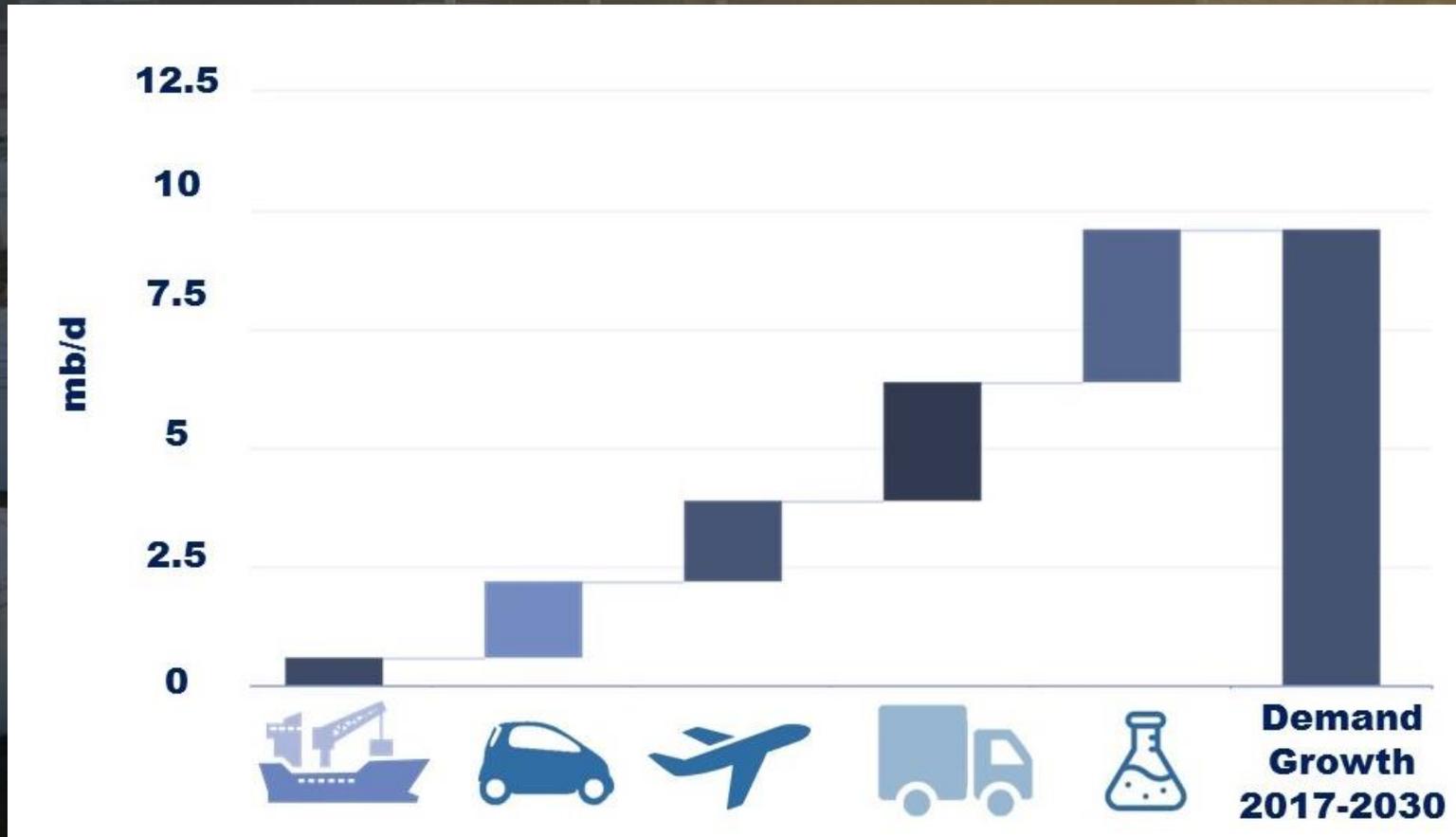
BioEnergy Engineering for Products Synthesis, FOA-0001916, topic area 5 – Rewiring Carbon Utilization. 2018. Award number DE-EE0008500.

Project Overview



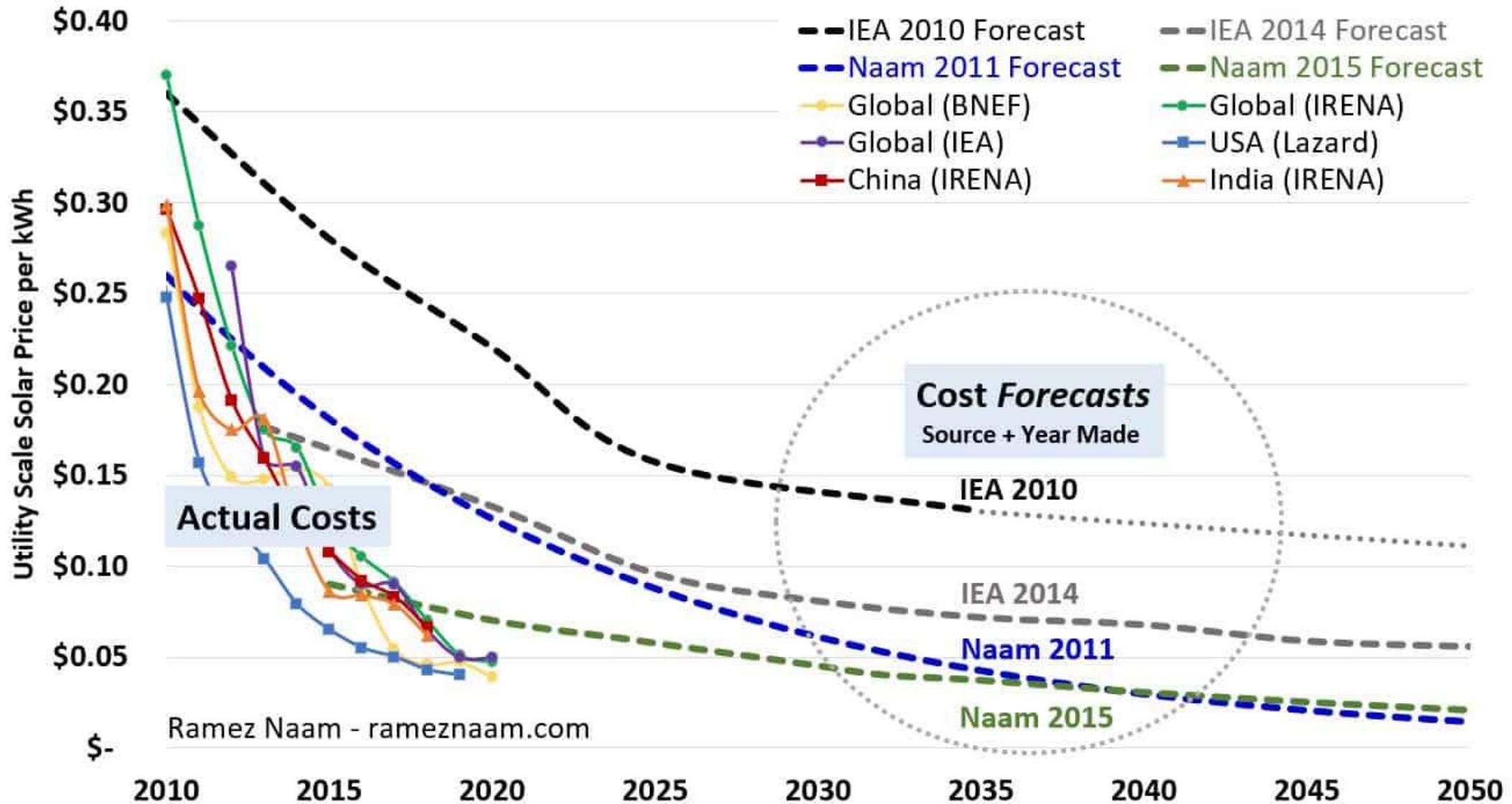
Chemicals for Everyday Products **need Carbon**

Oil Demand Growth by Sector, 2017-2030



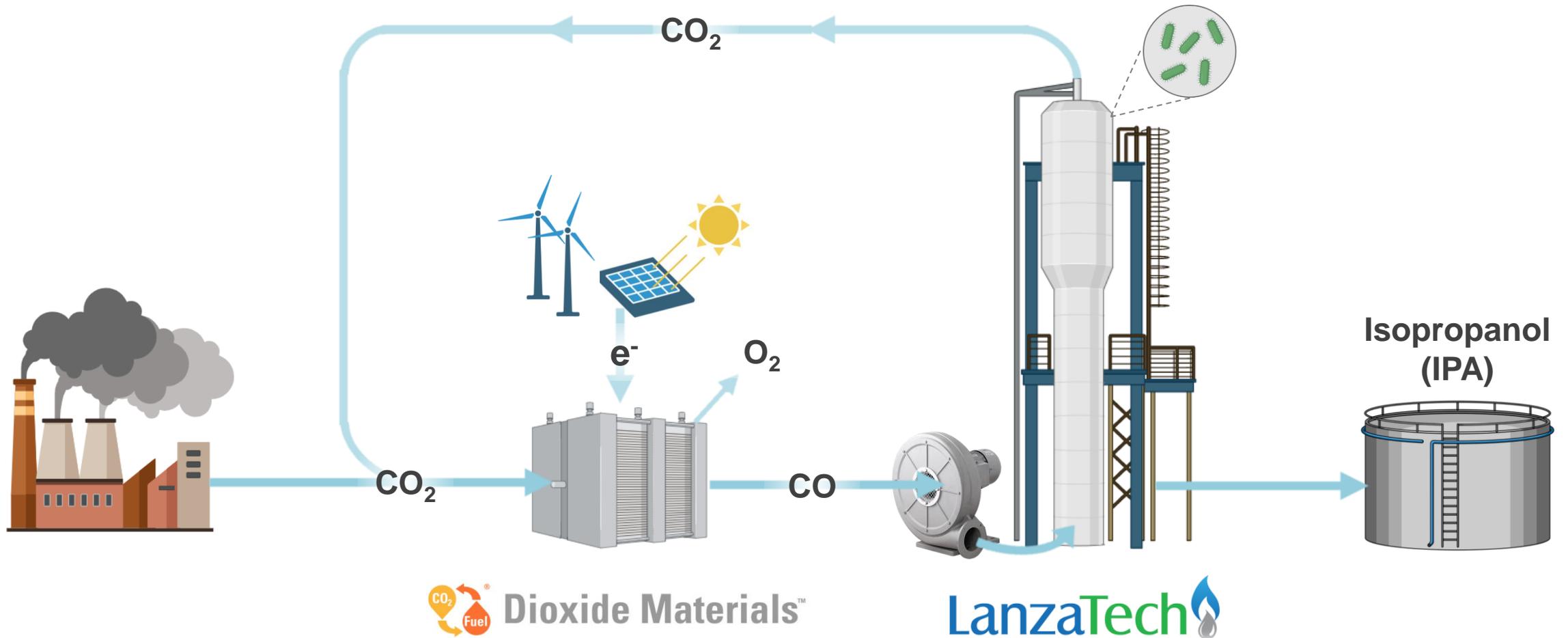
Petrochemicals will be the largest driver of global oil demand over the coming decades

Solar Costs Are Decades Ahead of Forecasts



CO₂ Electrolysis + Gas Fermentation: a CO₂ to chemicals platform

- Anion Exchange Membrane (AEM) based CO₂ electrolysis
 - High energy efficiency achievable at room temperature
- Acetogen yield improvement through advanced metabolic engineering and arginine supplementation.



1 – Management

Team



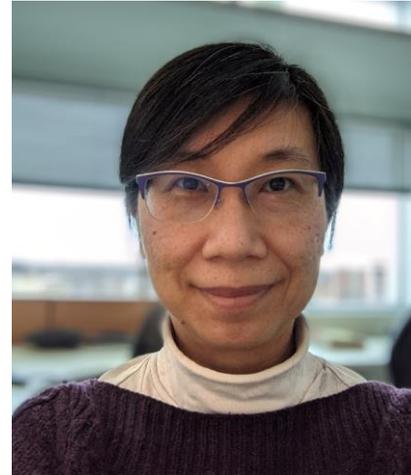
LanzaTech
Christophe Mihalcea



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 CO₂ 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 CO₂ crossover

2. Strain engineering for microbial yield improvements

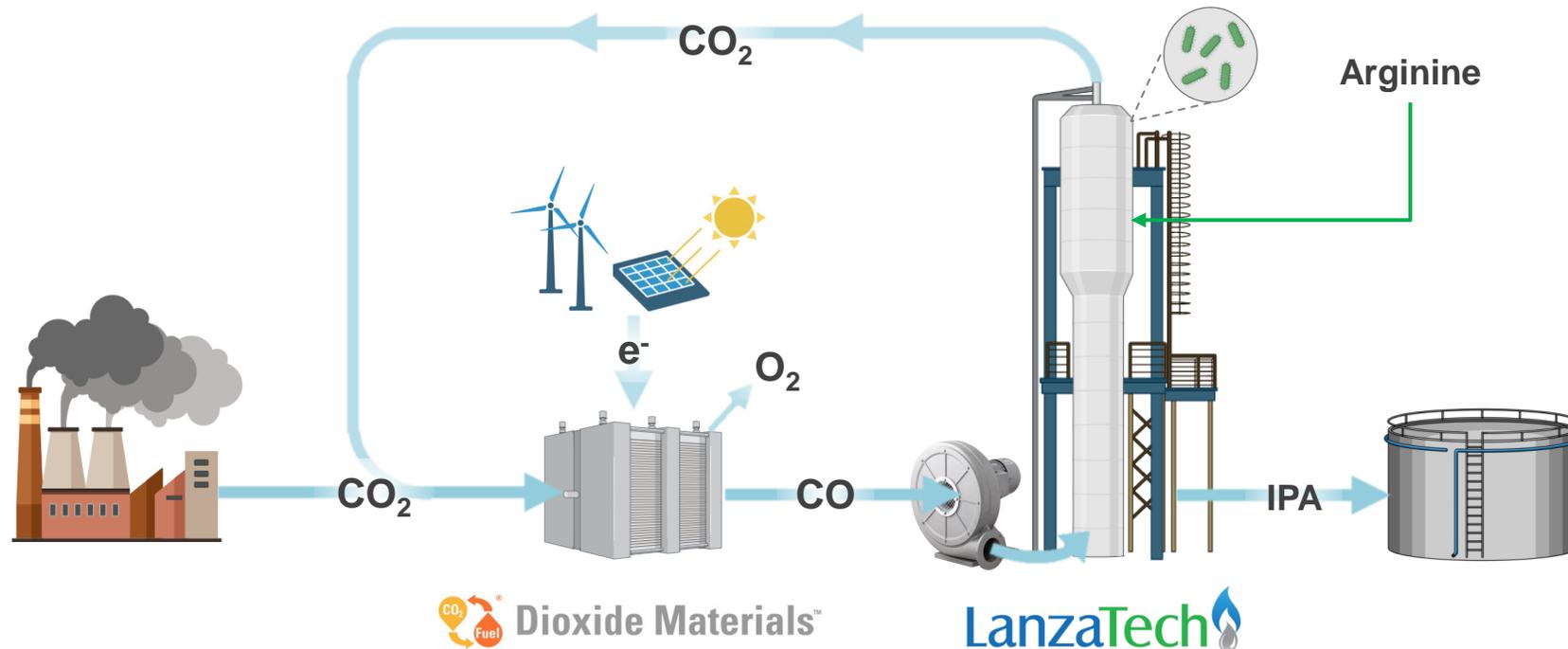
Synthetic pathway for arginine co-utilization to increase yield

3. Fermentation studies and electrolyzer integration

Optimizing IPA production and carbon efficiency

4. TEA / LCA

Feedback on economic and carbon impact



Aim 1: Electrolyzer Enhancements



Fuel Cell based 2018 version



**New materials
New flow field**

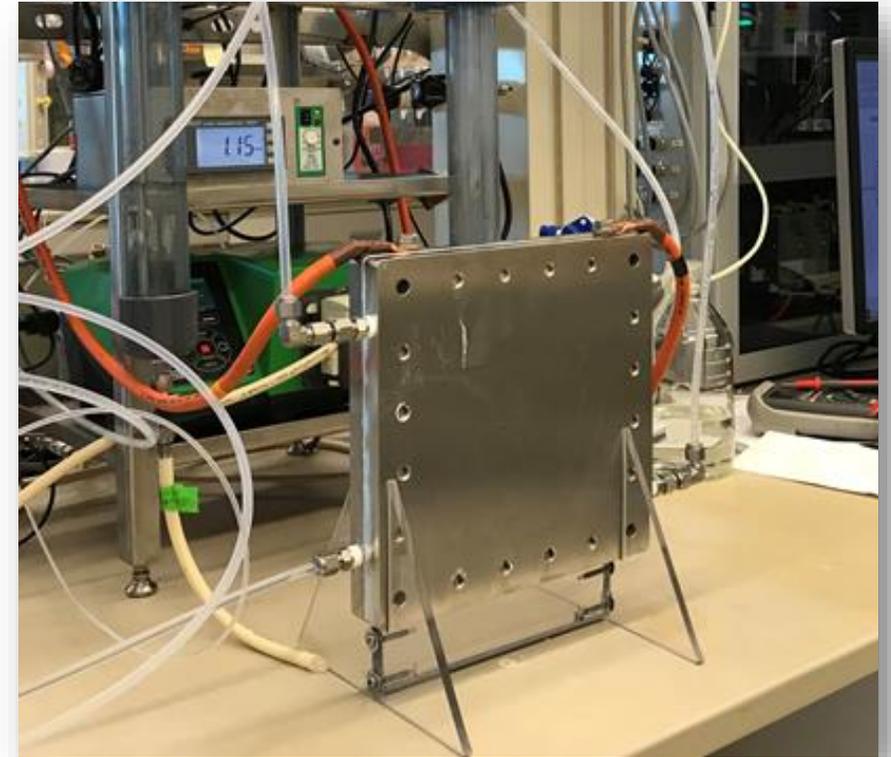


**Pressure drop reduced:
30 psi → 2 psi**

Better energy efficiency

Lower CO₂ crossover

Custom made 2020 version



Aim 2: Microbial Yield Improvement

- 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

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ELSEVIER

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

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ARTICLE INFO

ABSTRACT

Keywords: Acetogen, feedstock, Clostridium autoethanogenum, Syngas, Genomic-scale modelling, Arginine catabolism, RNA-sequencing

Acetogen feedstock, Clostridium autoethanogenum, Syngas, Genomic-scale modelling, Arginine catabolism, RNA-sequencing, preference heterotrophic growth, 1 (u–4 h) during an abetaloid (AD) put which rec l-janglish demonstr or enhan

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

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(84) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, FR, GB, GR, GT, HK, HU, IL, IN, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LI, LR, LS, LU, LV, MA, MK, MN, MU, MW, MY, NZ, NL, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SI, SK, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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

(57) Abstract: The invention provides methods for improving efficiency of fermentation by arginine supplementation, and genetically modified bacteria for use therein. More particularly, the invention provides methods for (i) increasing the production ATP as a source product with arginine supplementation, (ii) increasing utilization of arginine by a C1-fixing bacterium, and (iii) providing C1-fixing bacterium with optimized arginine deiminase pathways.

Fig. 1

2016-08-08

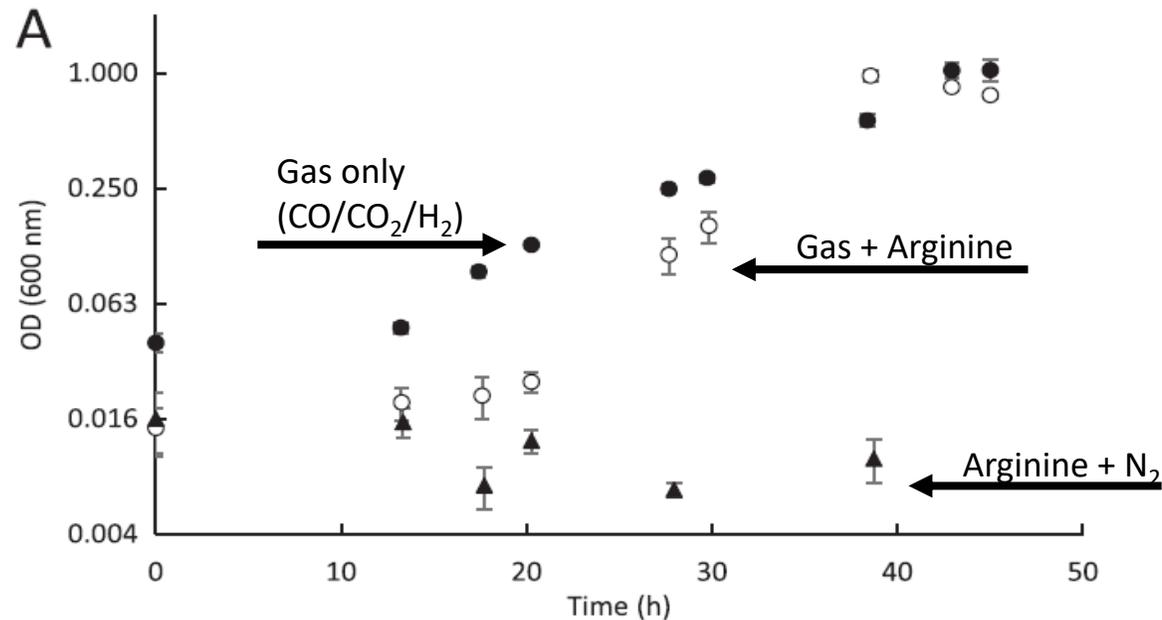
WO 2017/096324 A1

1. Introduction

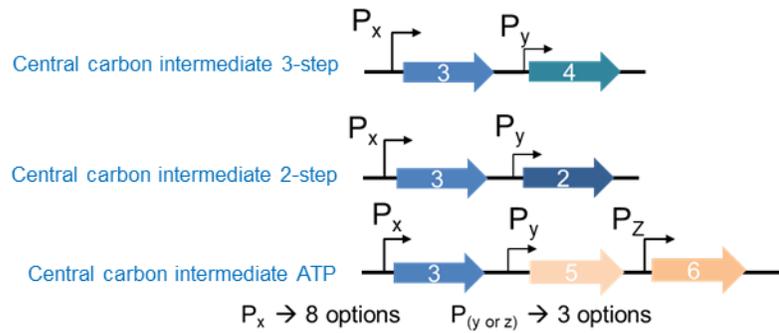
Gas fermentation has emerged as an attractive renewable and sustainable production of fuels and abundant, inexpensive and non-food based feedstock waste gases, syngas (CO/CO₂ and H₂) and methan Miller, 2015; Daniell et al., 2012; Driwe and Litzmann et al., 2011; Latif et al., 2014; Lew et al., 2016b). Pro and chemicals via gas fermentation does not compete and food resources, in contrast to using farmed suga Furthermore, gas fermentation offers high product capturing carbon that would otherwise be released into Syngas fermentation is particularly interesting as CO as

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Available online 23 April 2017
1096-7176/© 2017 International Metabolic Engineering Society. Pn



Aim 2: Microbial Yield Improvement: Ornithine utilization

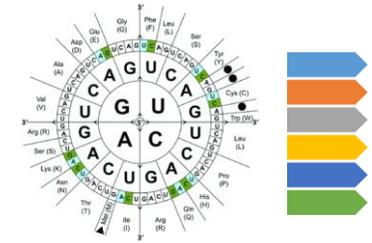


Pathway and Gene Candidate Identification

Recipient	Gene 1	pDN2	Gene 2	pDN4	Gene 3
pMTL8315_16_PwL...	Gene A	>DN2-PwI Bsal D...	Gene A	DN4-PwI Bsal D...	Gene A
pMTL8225_16_Pfe...	Gene B	pDN2-Pfer Bsal ..	Gene B	DN4-Pfer Bsal ..	Gene B
pMTL8225_16_Pfo...	Gene C	>DN2-Ppfor Bsal...	Gene C	DN4-Ppfor Bsal...	Gene C
pMTL8225_16_Ppt...			Gene D		Gene D
pMTL8225_16_PwL...			Gene E		Gene E
pMTL8315_16_Pfe...					Gene F
pMTL8315_16_Pfo...					
pMTL8315_16_Ppt...					

teselagen
BIOTECHNOLOGY

Pathway Design



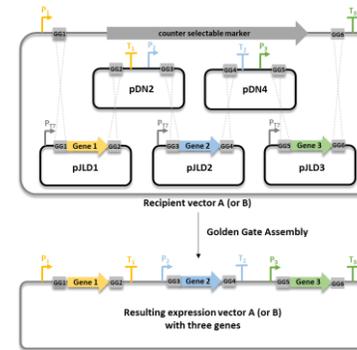
Codon Adaptation



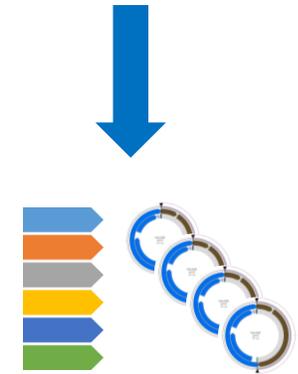
Strain Screening



Strain Generation



Library Assembly



Gene Synthesis/
Pre-Cloning

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

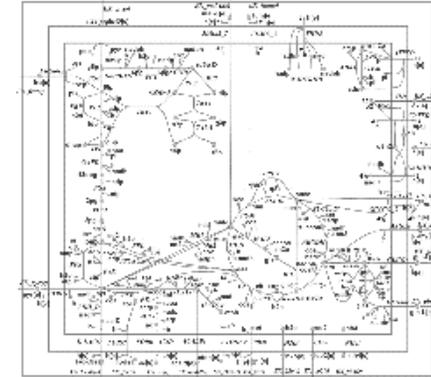
- Flux balance analysis and minimization of metabolic adjustment to predict phenotypes
- Strength Pareto Evolutionary Algorithm 2 (SPEA2) and minimal cut set (MCS) analysis to characterize design landscape ([Maia 2015](#), [Kamp 2014](#))

Definitions

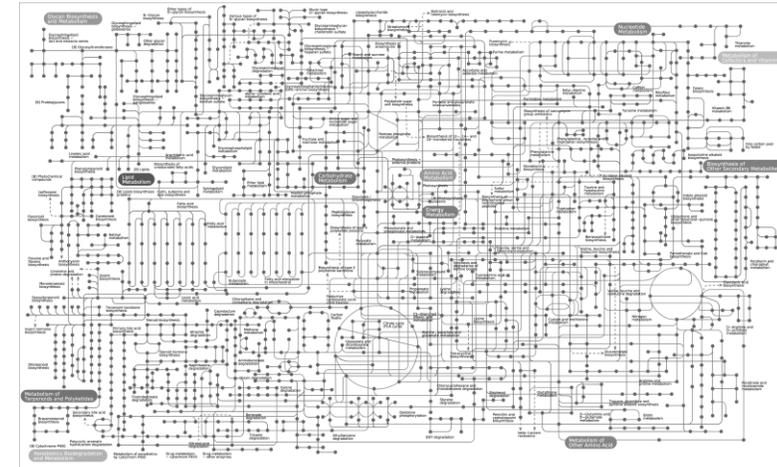
High H_2 = 5:1 H_2 :CO uptake

CO = 0:1 H_2 :CO uptake

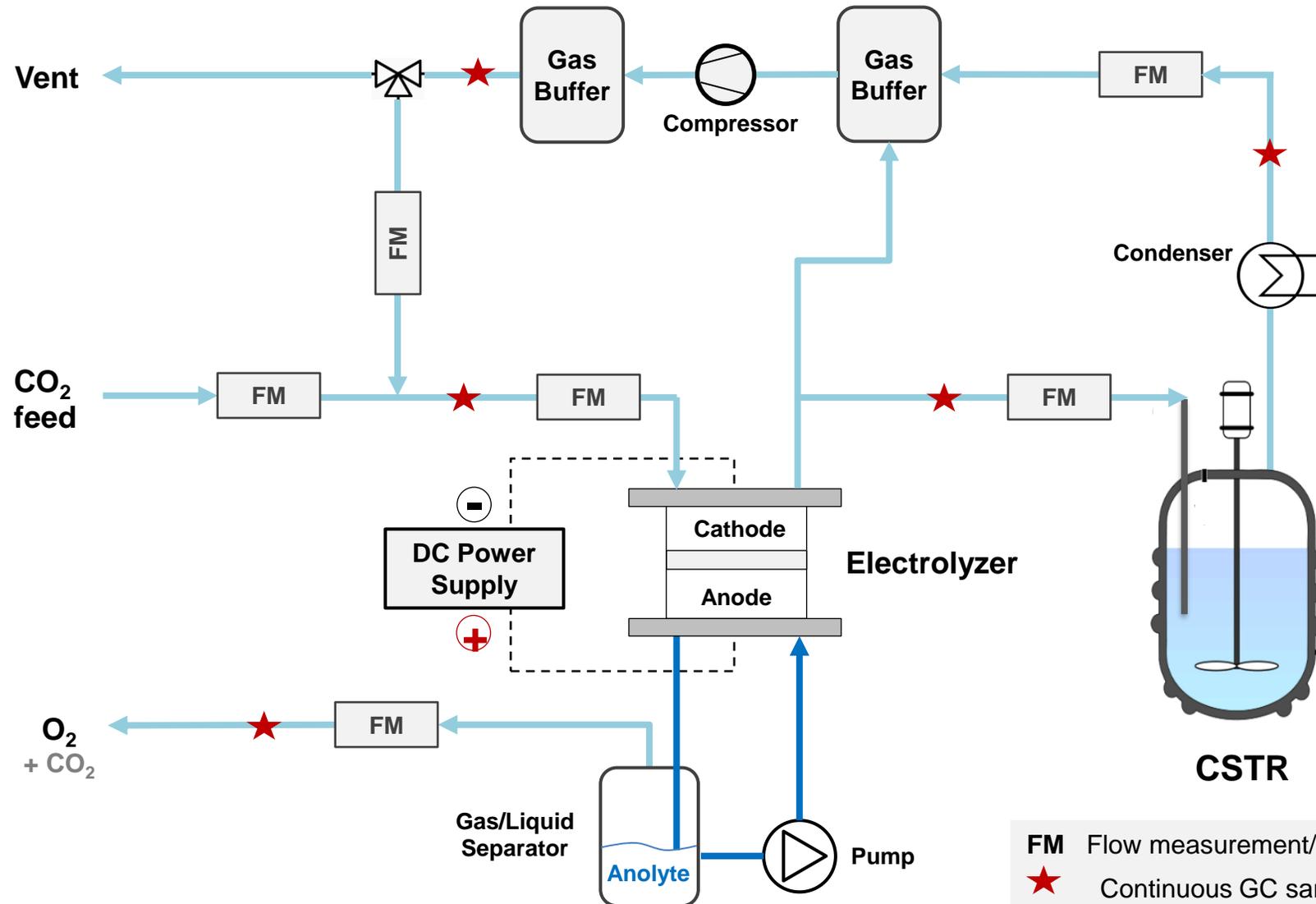
Example core metabolism



Example genome-scale metabolism



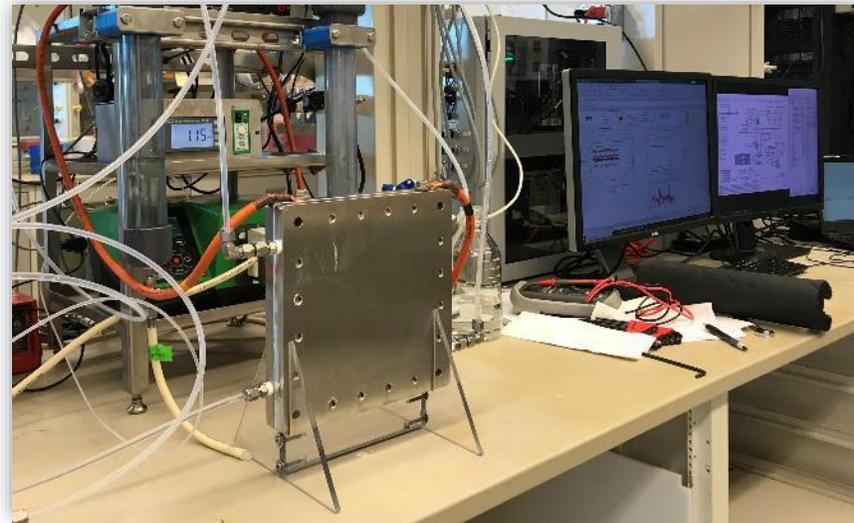
Aim 3: Electrolyzer Integration



- 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

FM Flow measurement/control
★ Continuous GC sample point

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

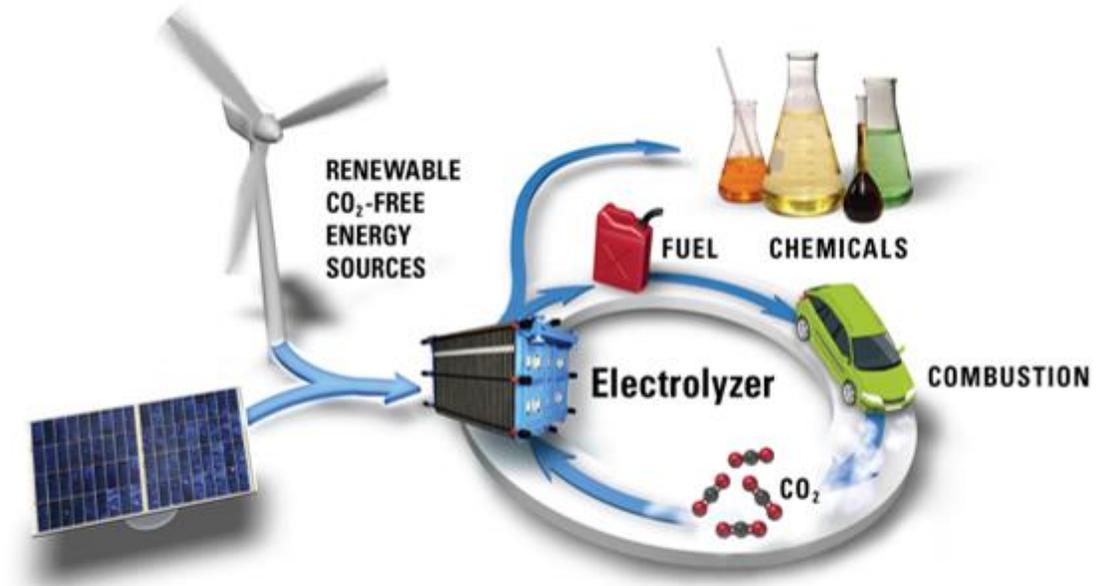
Key Performance Parameters	Metric	BP1 ✓ (go/no-go)	BP2 (go/no-go)	BP3 (final target)
Carbon Efficiency to products	%	28	33	37
Electricity use (Electrolyzer efficiency)	kWh / Nm ³ CO	9.57	8.72	7.97
% N feed as Arginine	mmol / g biomass	0	25	100

3 – Impact

Ensure U.S. Leads in Sustainable Energy Technology

We will reduce CO₂ 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 CO₂ stream
- Optimize microbial process conditions to maximize both growth and productivity to inform and enable future predictive understanding of microbial metabolism in gas fermentation

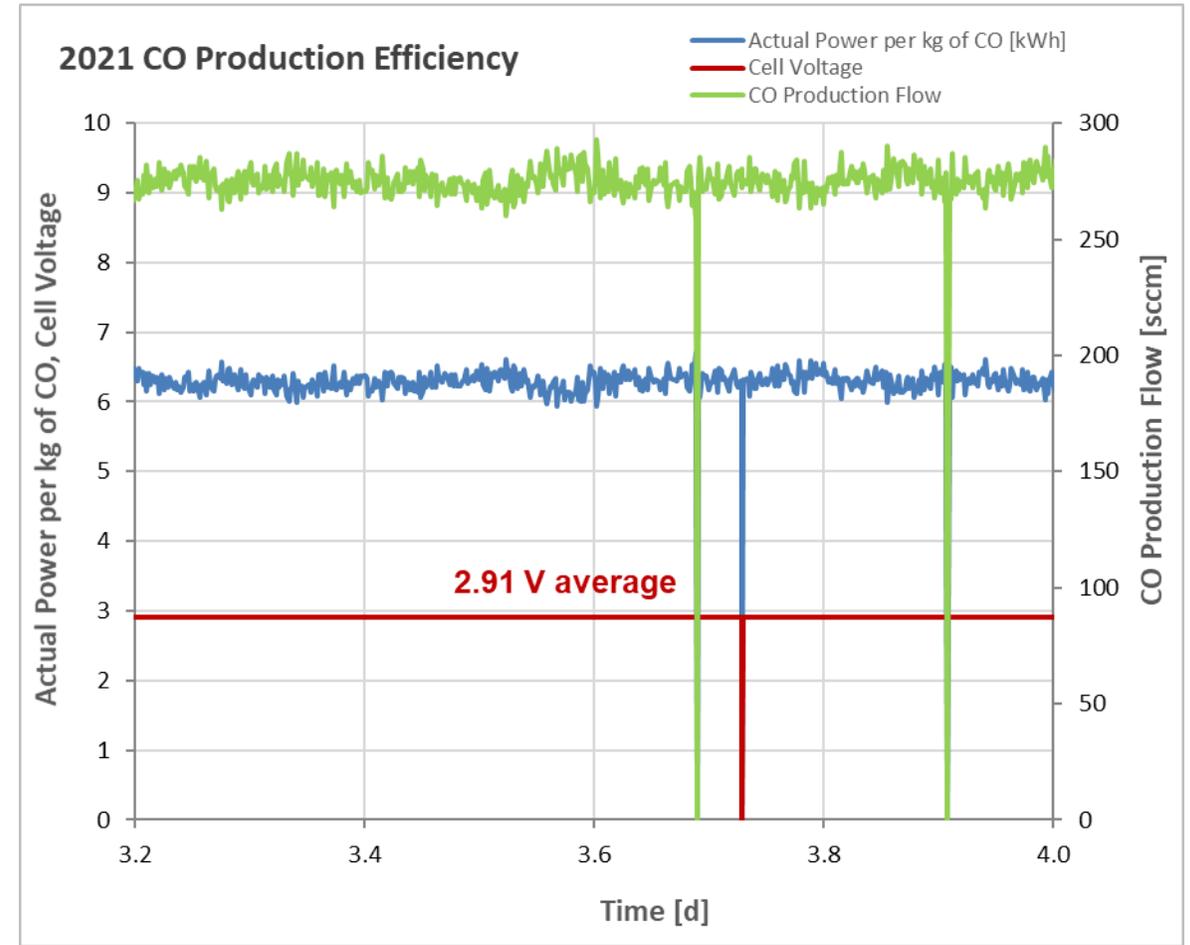
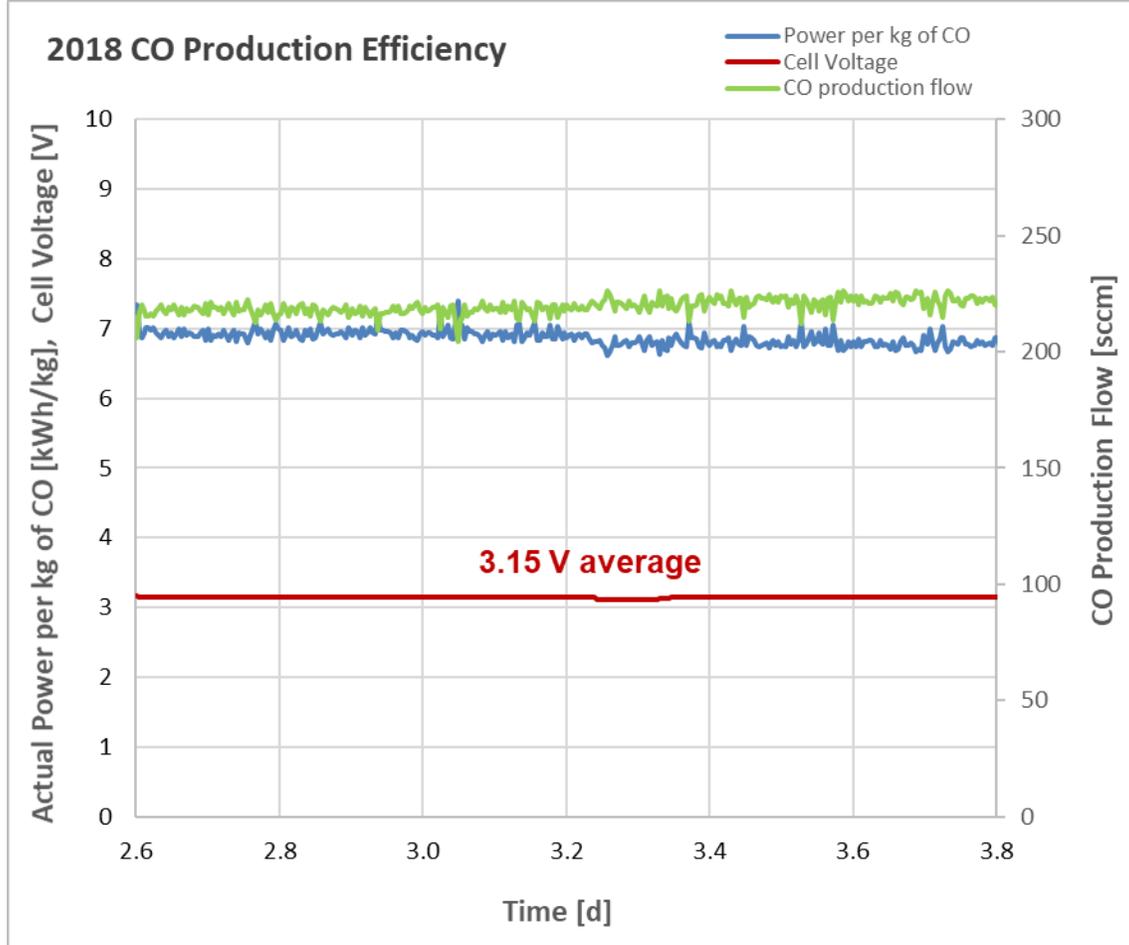


Creating a Carbon Smart World

- 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 CO₂ 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

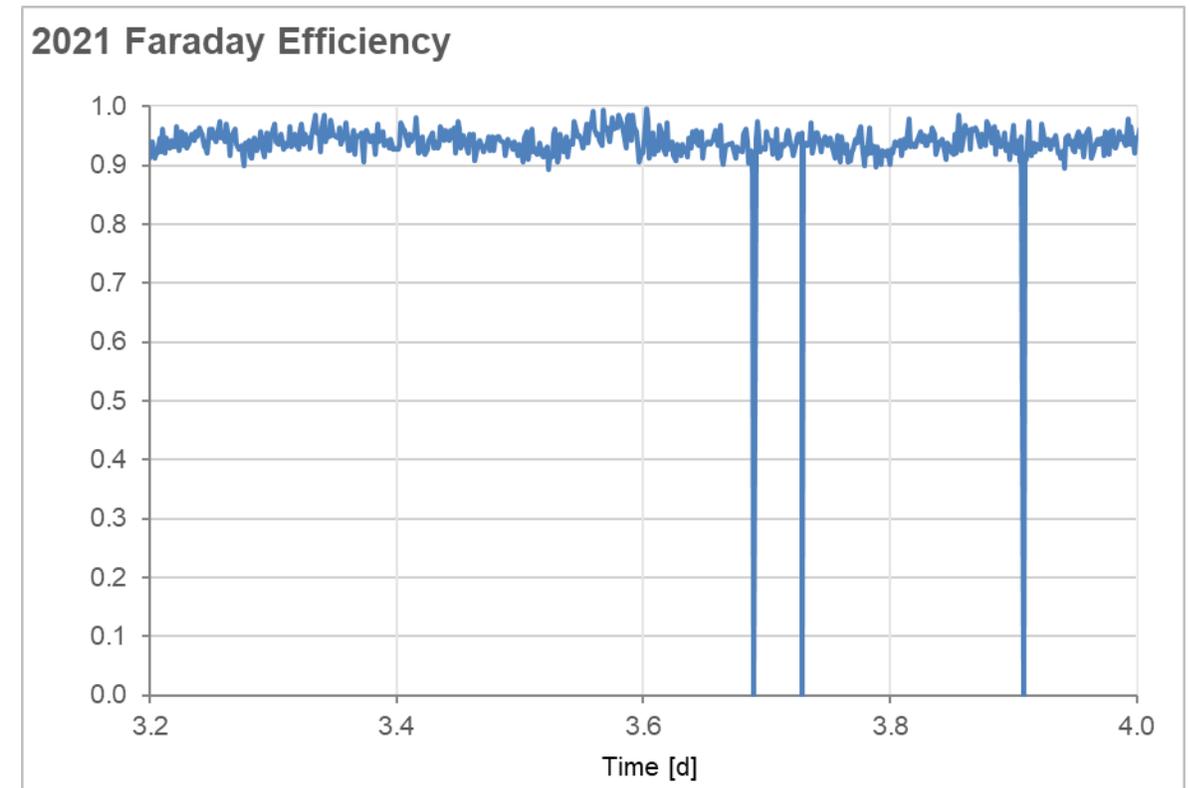
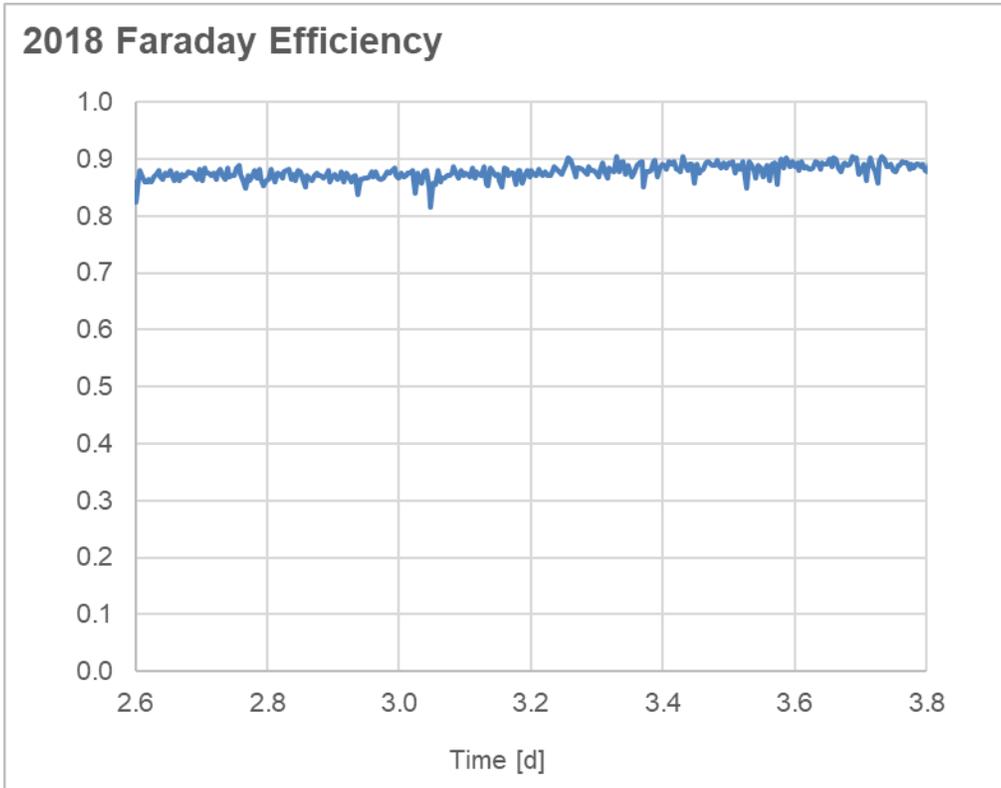
Aim 1: Electrolyzer enhancement results



→ 2021 cell is ~8% more energy efficient

Aim 1: Electrolyzer enhancement results

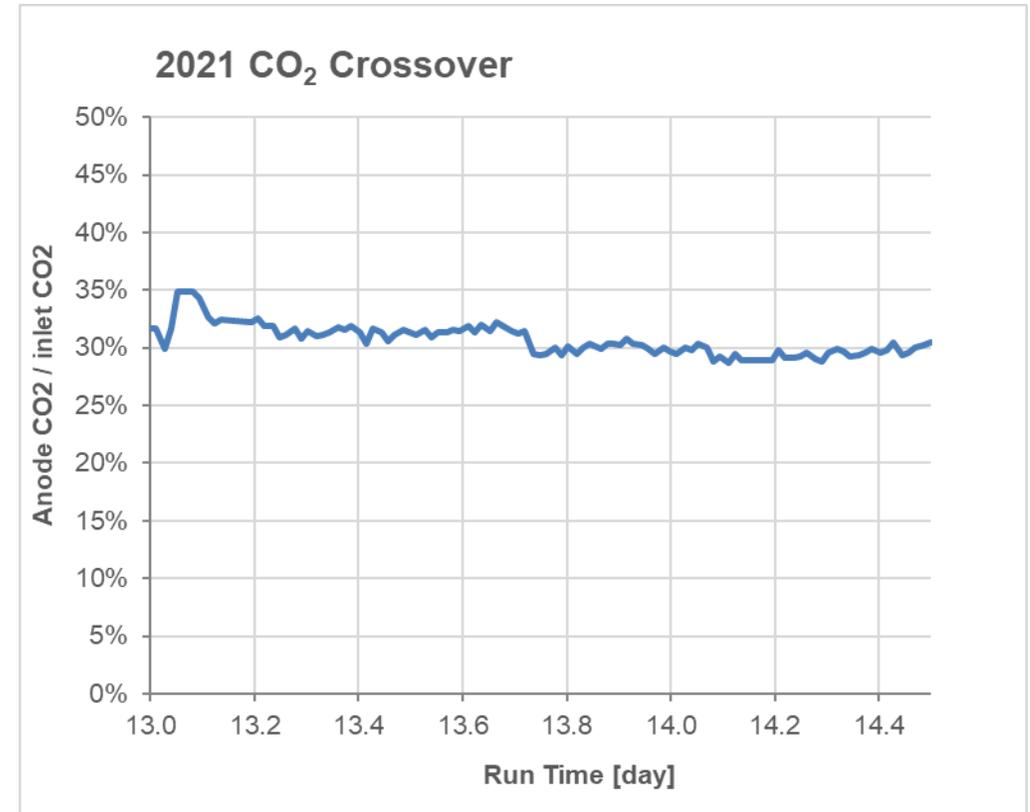
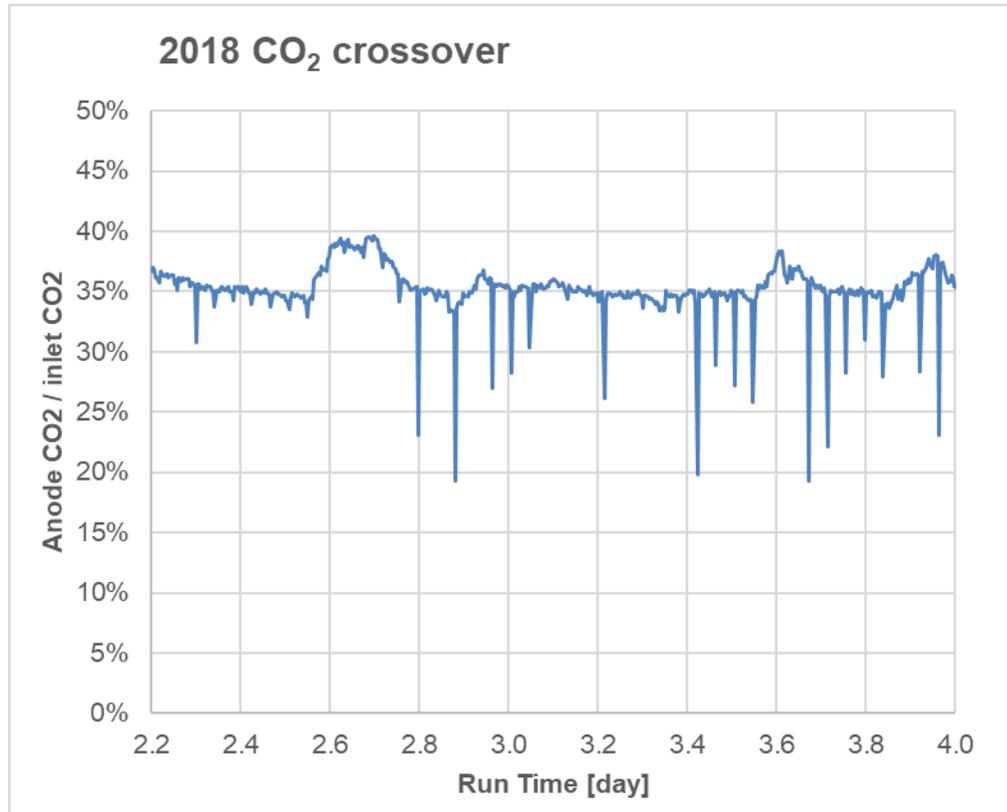
Faraday Efficiency = Charge per equivalent of CO (theoretical / measured)



→ 2021 cell achieves 95% Faraday Efficiency

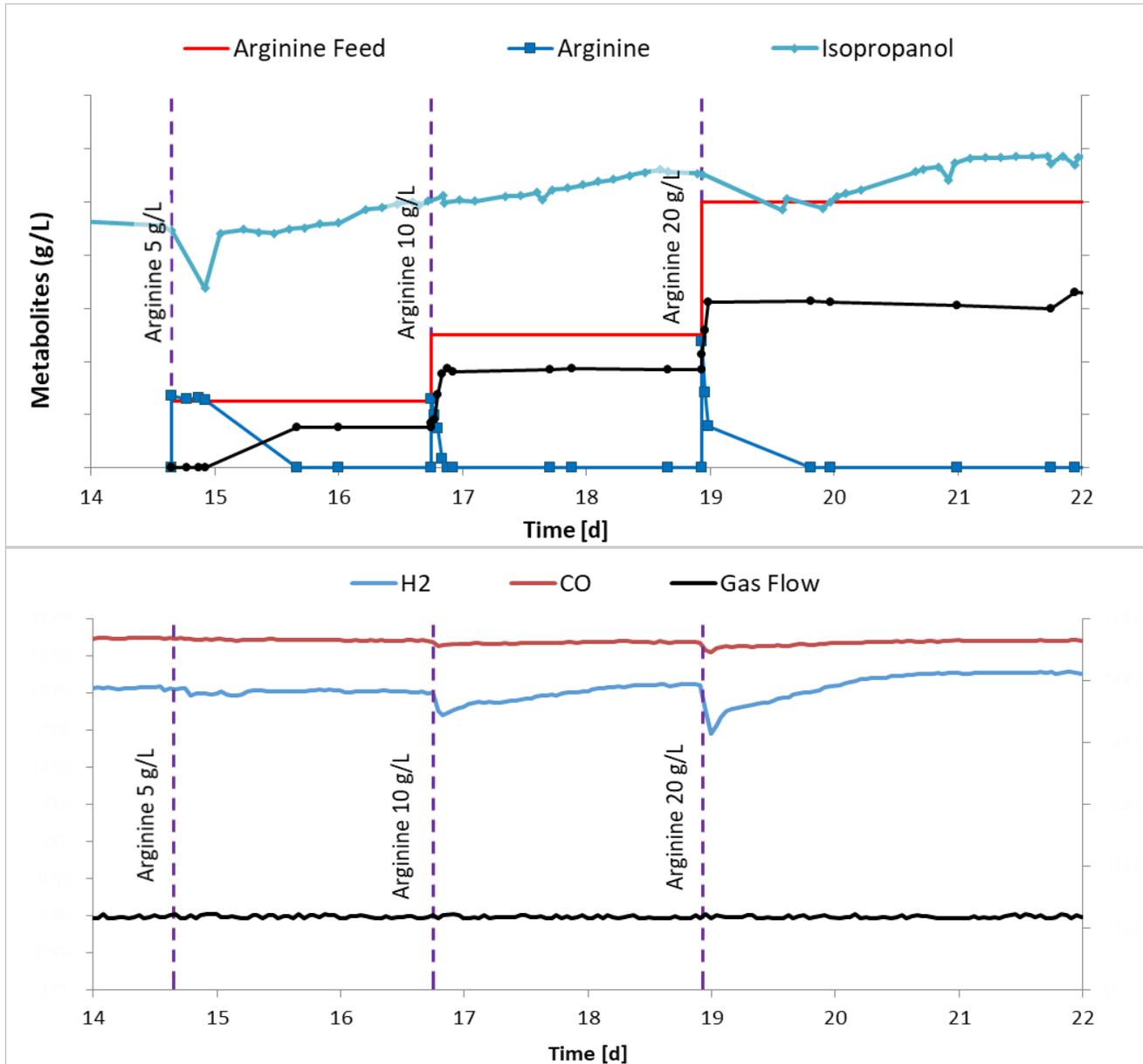
Aim 1: Electrolyzer enhancement results

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



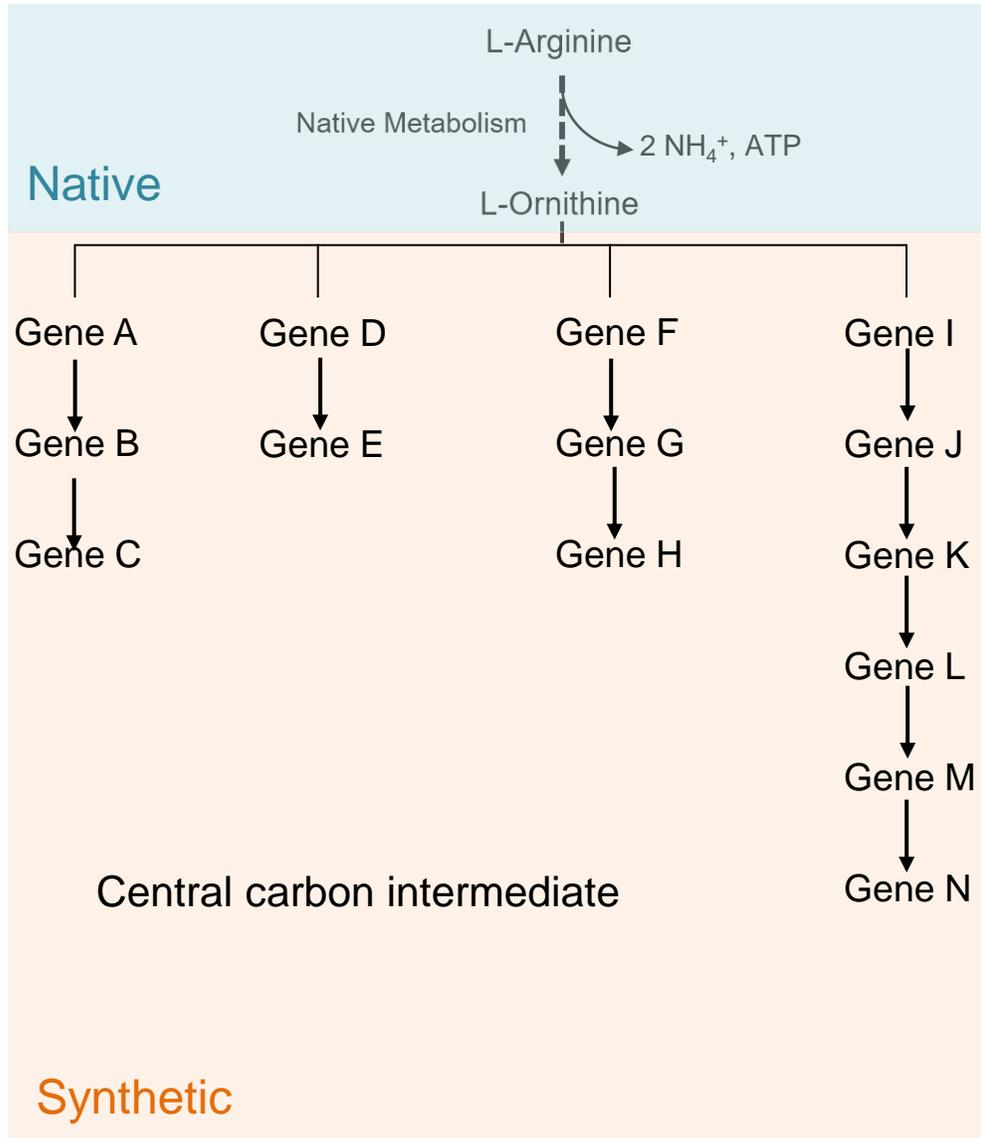
→ 2021 cell reduces CO_2 crossover

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



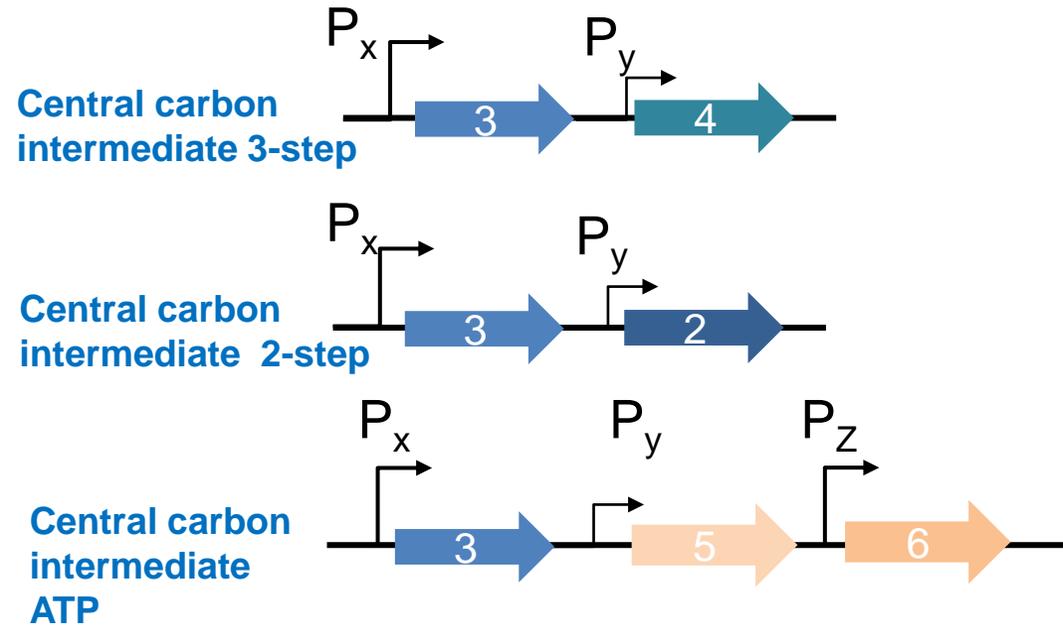
- Arginine was fed into continuous gas fermentation at increasing rates.
- Both CO and arginine uptake happened simultaneously.
- A slight increase in IPA selectivity was observed at higher dose of arginine supplement.

Aim 2: Arginine metabolism and Ornithine assimilation pathways



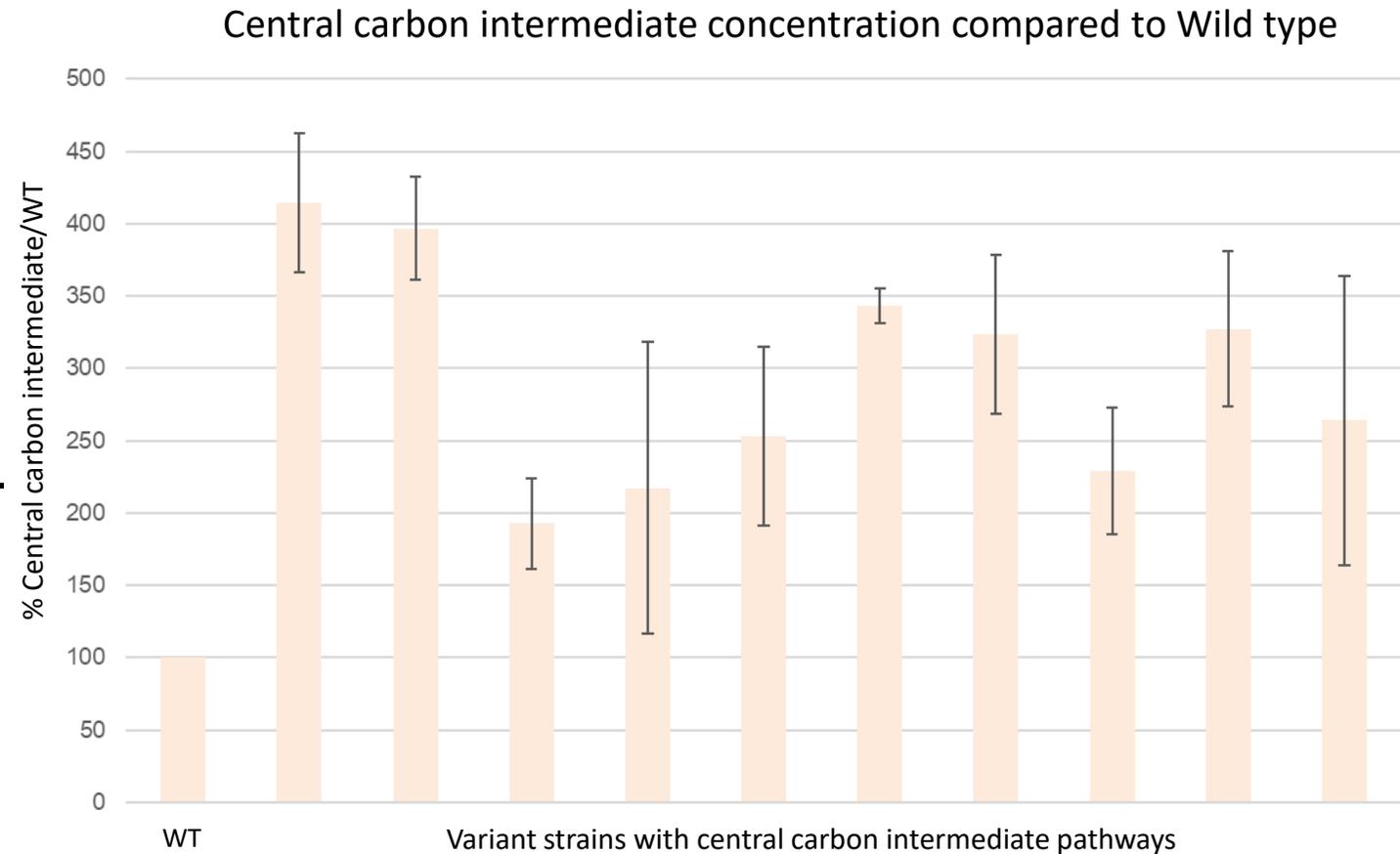
- Total of possible 24,192 unique pathway combinations, exceeding the Milestone 4.2.1 - Identify 100 pathway gene combinations for conversion of ornithine to central metabolism.
- Assembled over 384 unique combinations to date.
- Screening is on going.

Aim 2: Library Assembly Examples and Results to date



$P_x \rightarrow 8$ options

$P_{(y \text{ or } z)} \rightarrow 3$ options



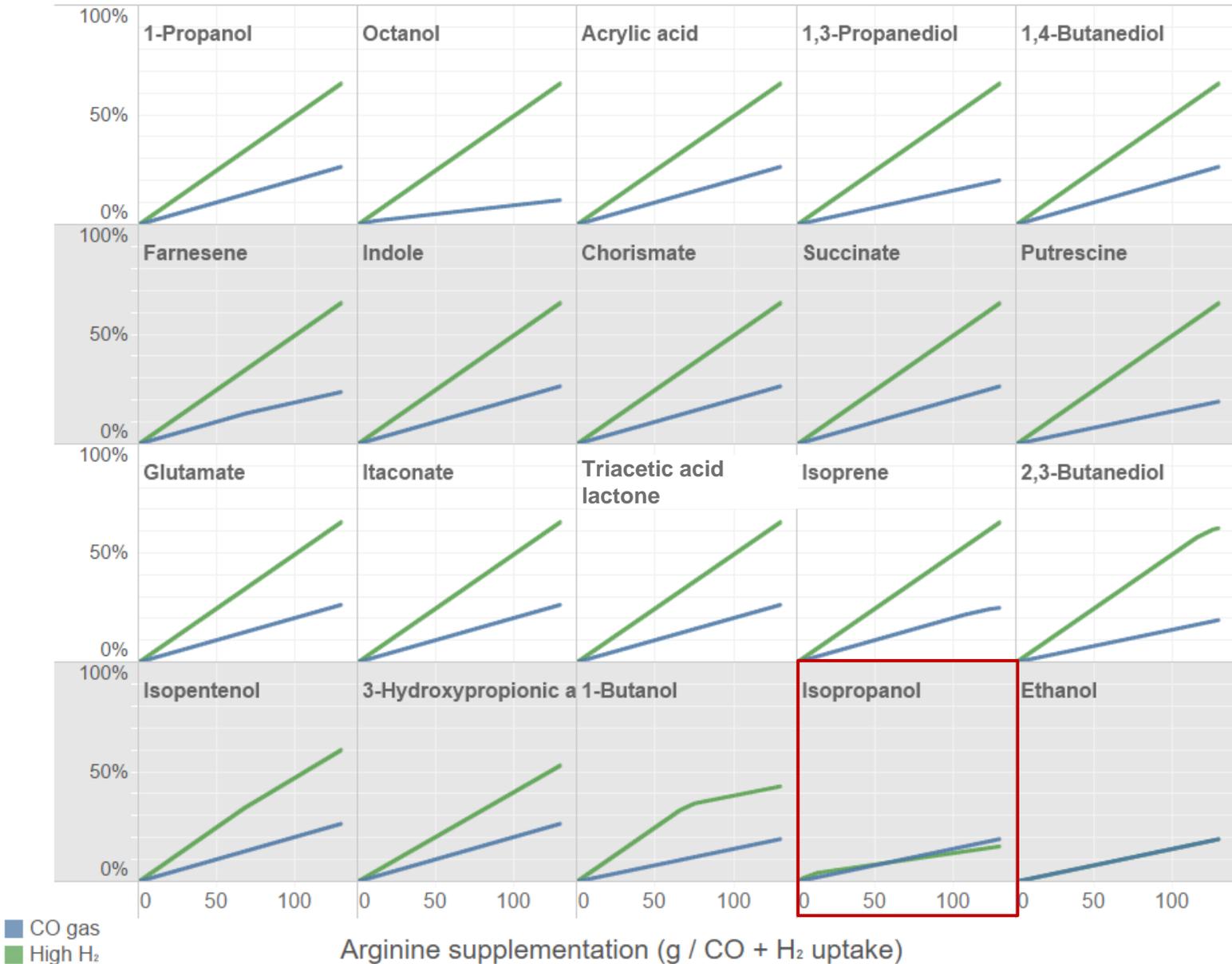
Increased levels of central intermediates observed upon introduction of developed pathways in context of arginine feeding

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.

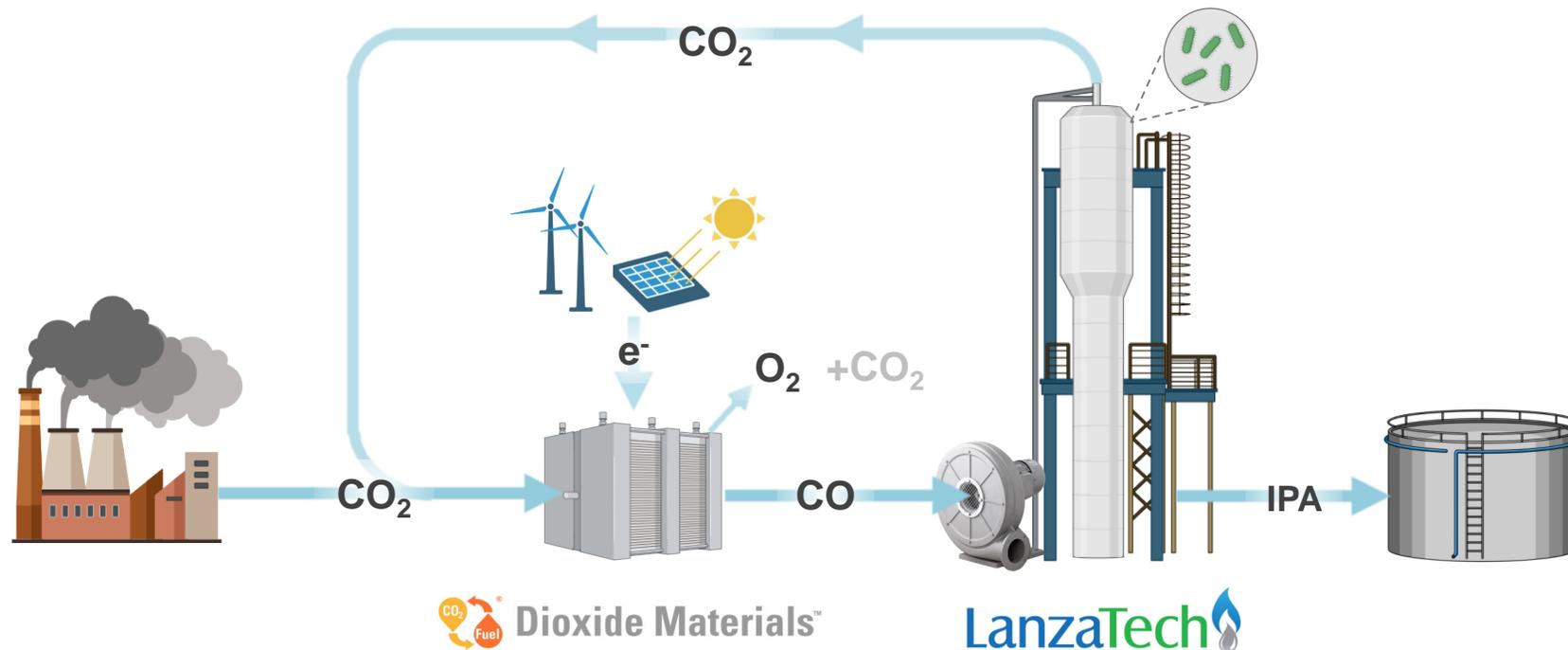
Modelling confirms arginine supplementation increases yield for a range of tested target molecules.



Summary

Efficient CO₂ electrolysis and deeper understanding of acetogen metabolism: building blocks for a circular carbon economy utilizing renewable energy

- Improved CO₂ electrolyzer design increased energy efficiency and reduced CO₂ crossover
 - CO₂ 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