DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Production of Bioproducts from Electrochemically-Generated C1 Intermediates

7 April 2023
Carbon Dioxide Utilization

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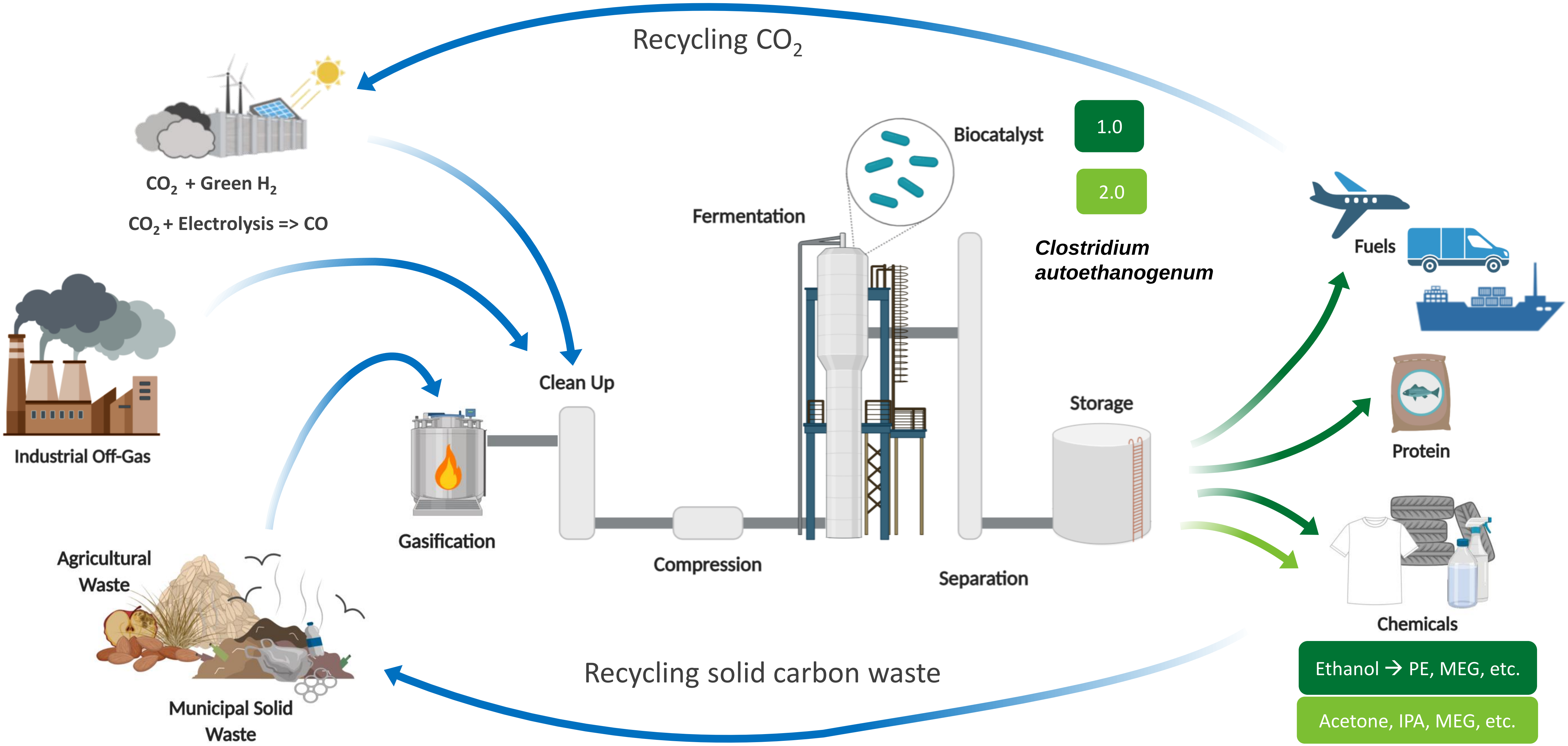
Gas Fermentation



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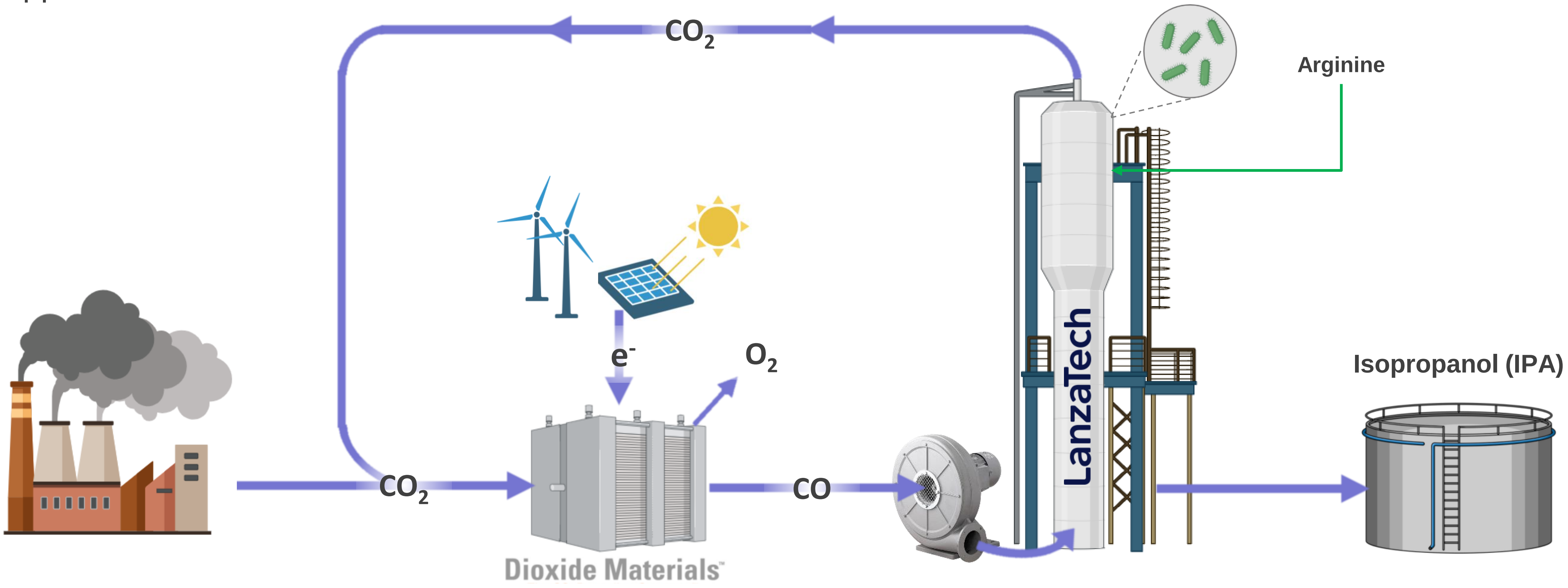
Project Overview

CO_2 Electrolysis + Gas Fermentation: a CO_2 to Chemicals Platform

- Room Temperature CO_2 electrolysis (Anion Exchange Membrane based technology)
- Gas fermentation yield improvement through process development, metabolic engineering and arginine supplementation.



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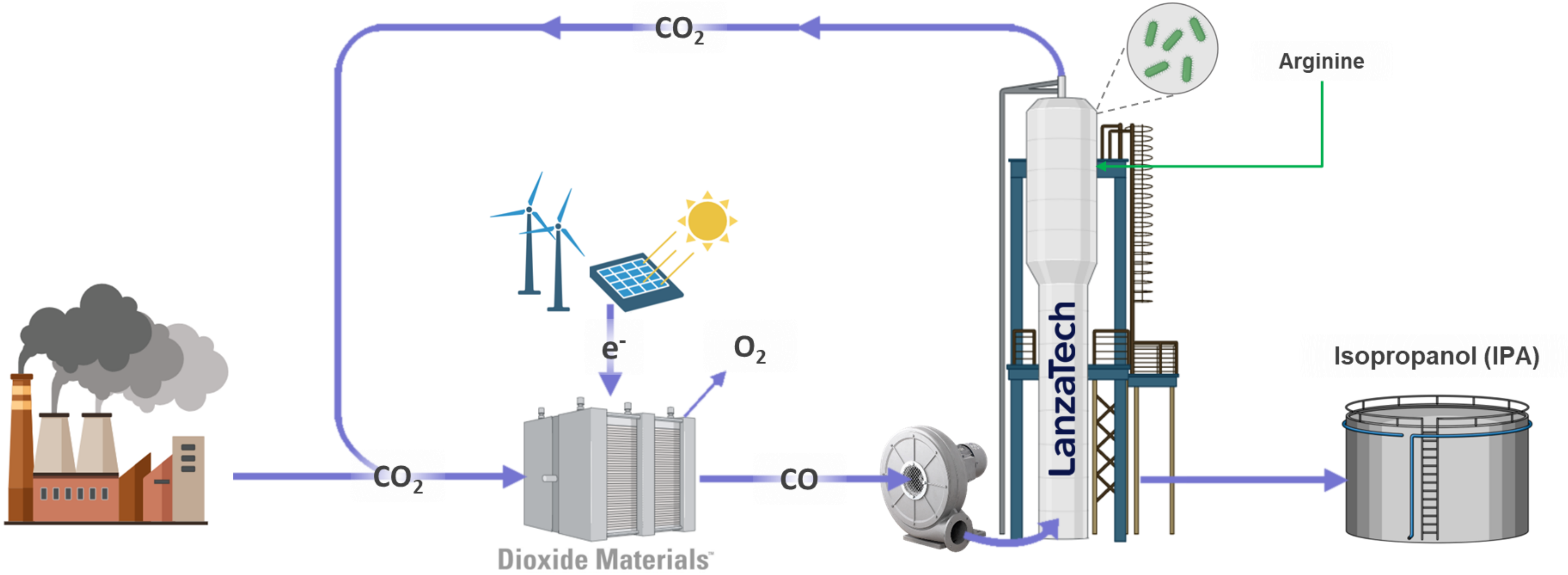
1 – Approach

A. **Electrolyzer enhancements**
Improve efficiency, stability and CO_2 crossover

B. **Microbial yield improvements**
Synthetic pathway for arginine co-utilization to increase yield

C. **Fermentation studies and electrolyzer integration**
Optimizing IPA production and carbon efficiency

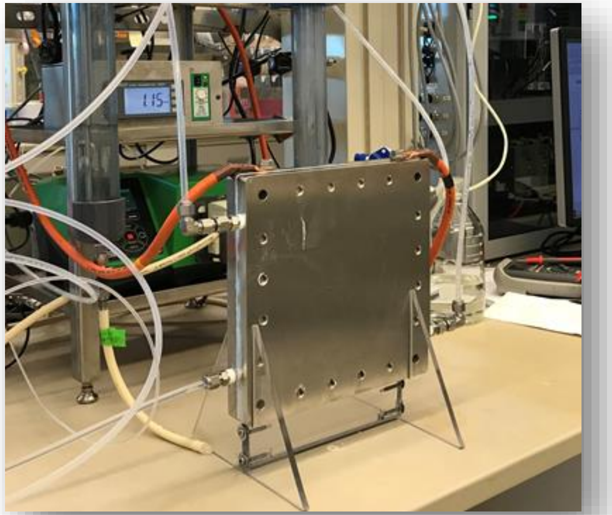
D. **TEA / LCA**
Feedback on economic and carbon impact



A: Electrolyzer Enhancements

Dioxide Materials
The CO_2 Recycling Company™

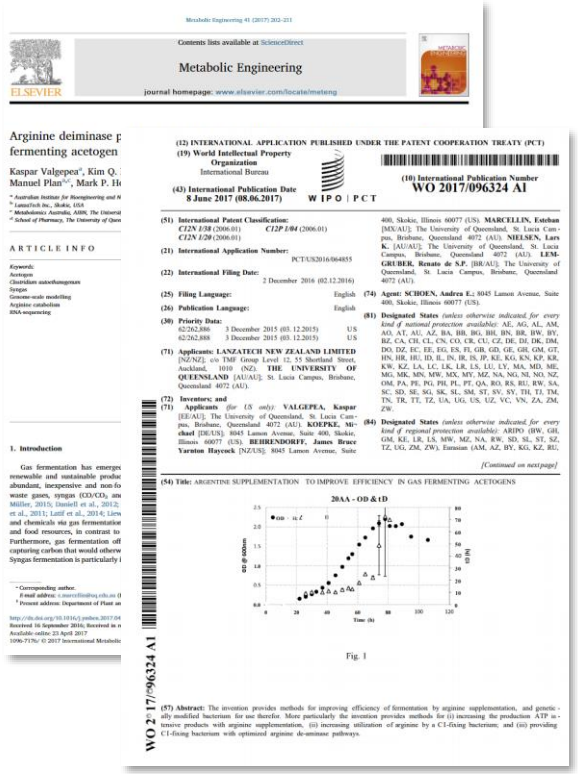
- **Design new 250 cm^2 electrochemical cell**
- **Develop improved membranes**
- **Supply cell and membranes to LanzaTech**



Challenges / Changes to Approach:

- Achieved energy efficiency targets early → shift to improving membrane robustness in BP3
- CO_2 crossover found to be inherent to technology, difficult to reduce without impacting energy efficiency
- Based on feedback from intermediate verification, cell temperature and relative humidity instrumentation was installed for BP3 testing.

B: Microbial Yield Improvement


Metabolic Engineering

Arginine deiminase p… fermenting acetogen…

(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)

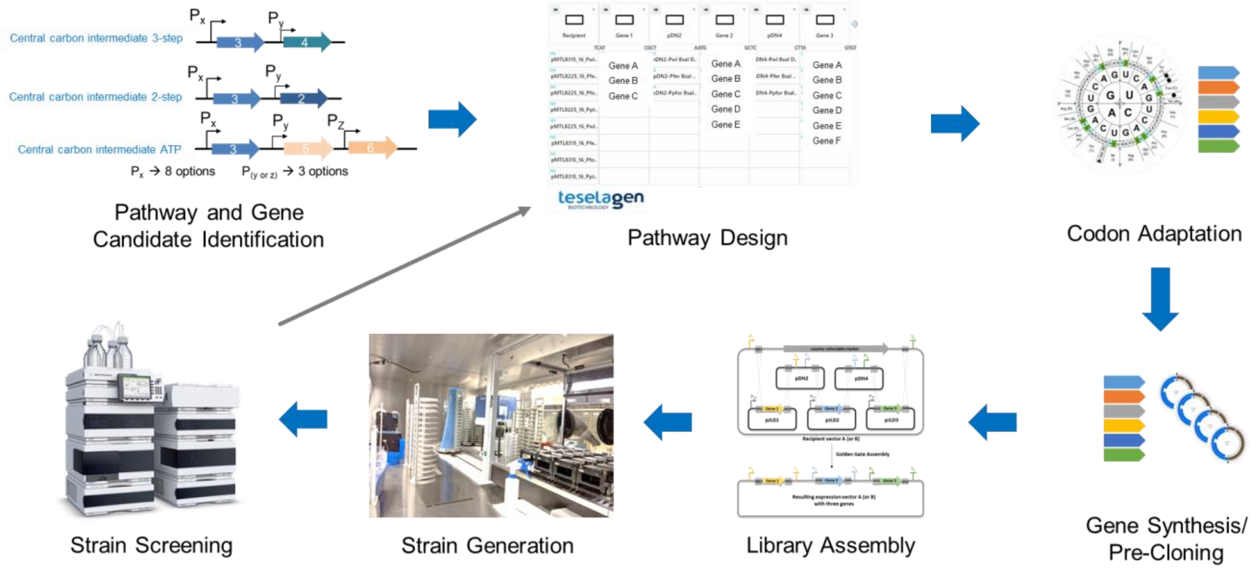
(10) International Publication Number WO 2017/096324 A1

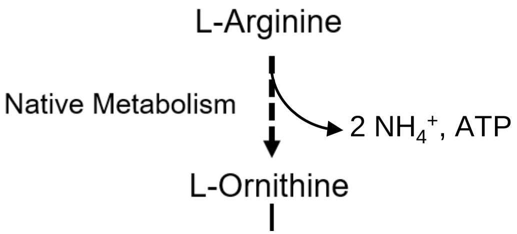
(54) Title: ARGININE SUPPLEMENTATION TO IMPROVE EFFICIENCY IN GAS FERMENTING ACETOGENS

Fig. 1

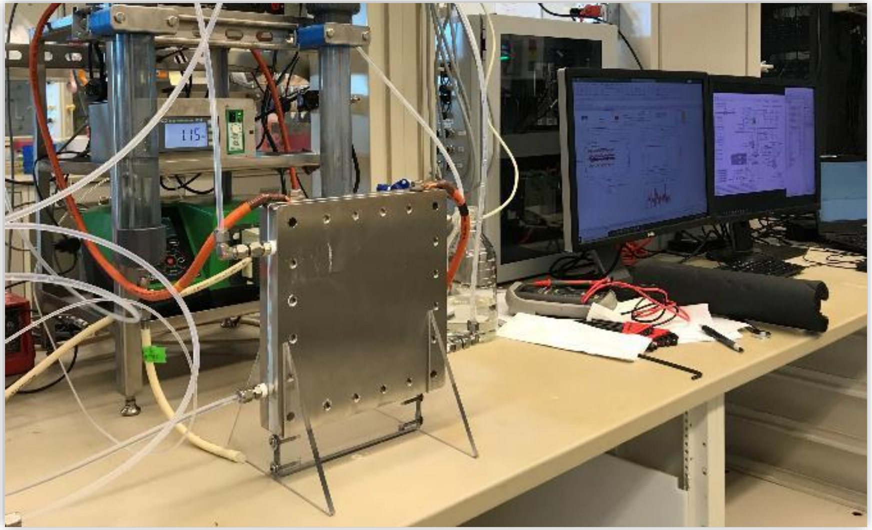
- L-Arginine is an amino acid that is preferentially consumed by *C. autoethanogenum*
- Arginine could replace ammonia as the nitrogen source while yielding extra ATP
- Native metabolism of arginine results in ornithine accumulation
- Strain development needed to utilize ornithine

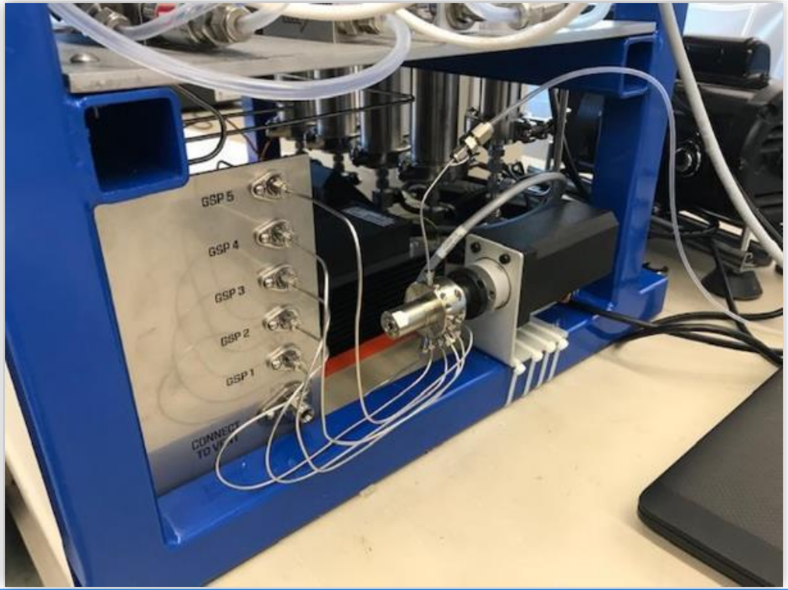
http://doi.org/10.1016/j.ymben.2017.04.007
https://patents.google.com/patent/WO2017096324A1/en





C: Electrolyzer Integration

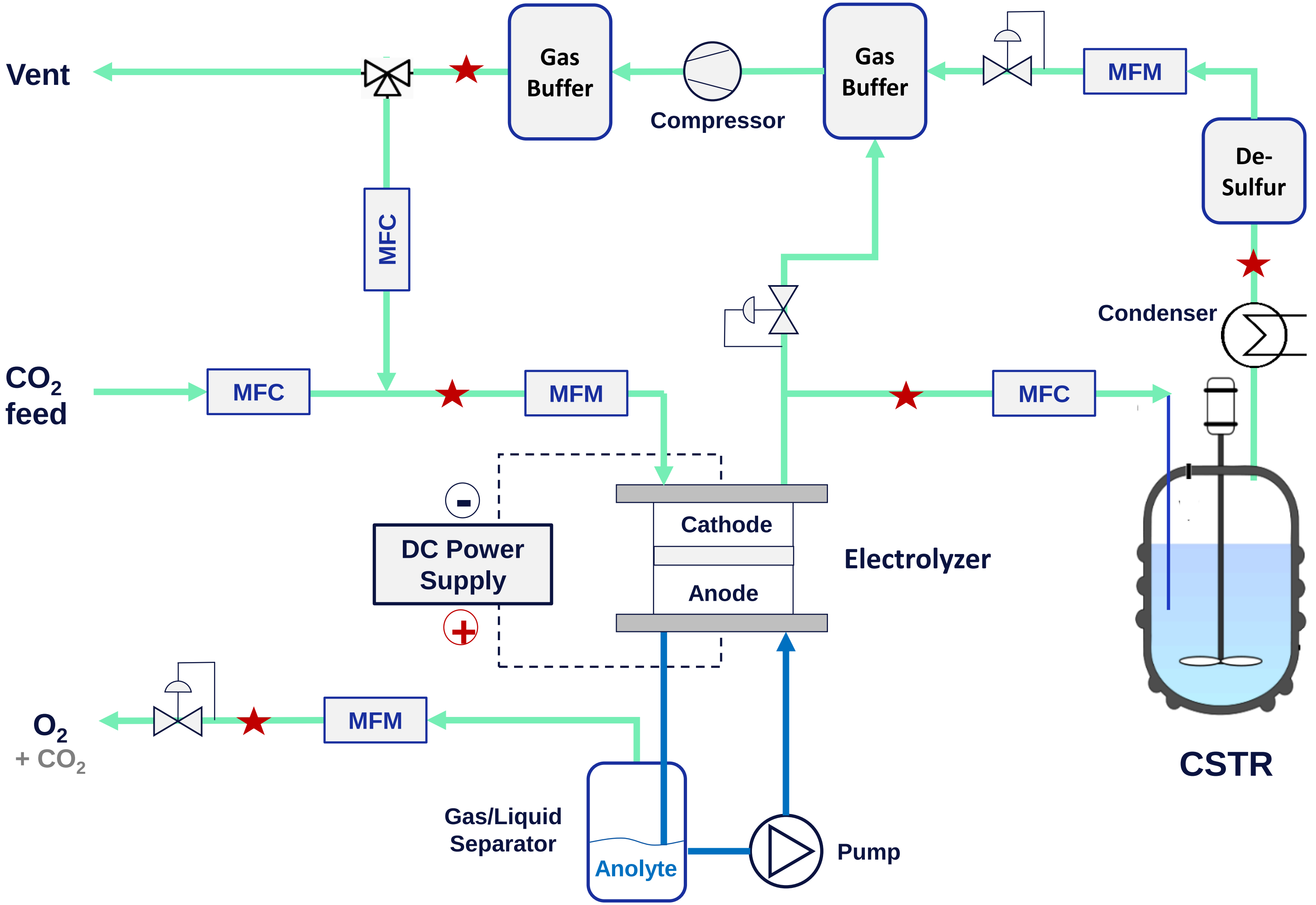




Approach:

- PLC-controlled integration unit
- 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

C: Electrolyzer Integration

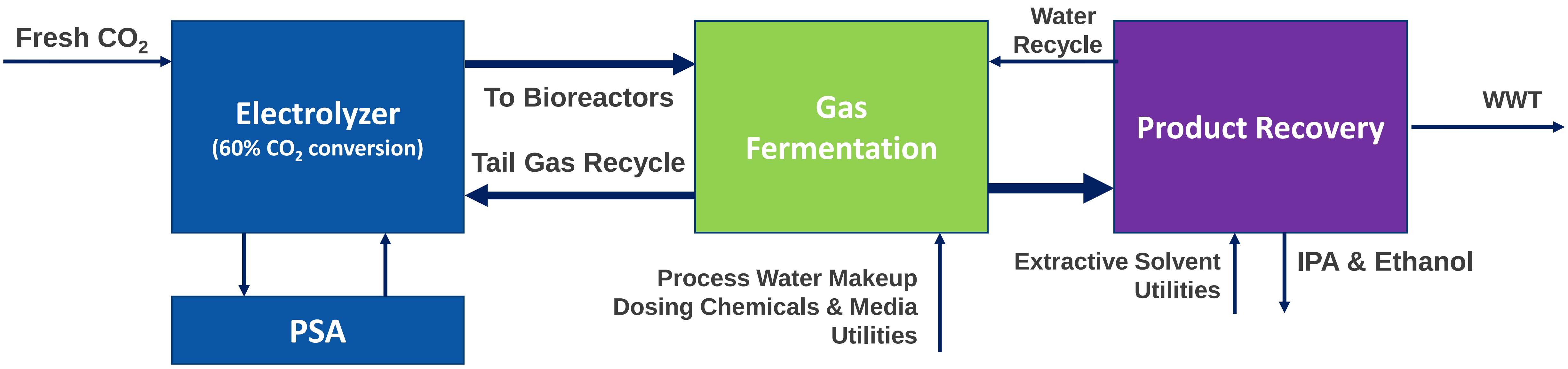


Lab Scale Challenges:

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

MFM Mass Flow Meter
MFC Mass Flow Controller
★ Continuous GC sample point

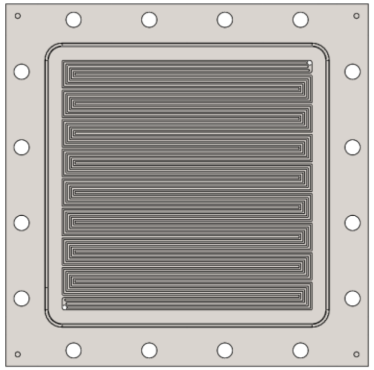
D: TEA & LCA

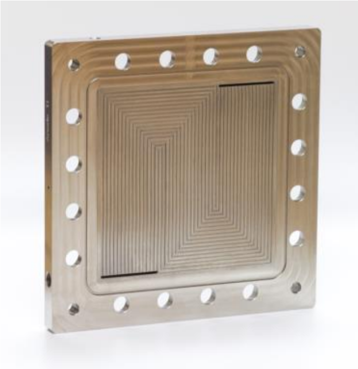


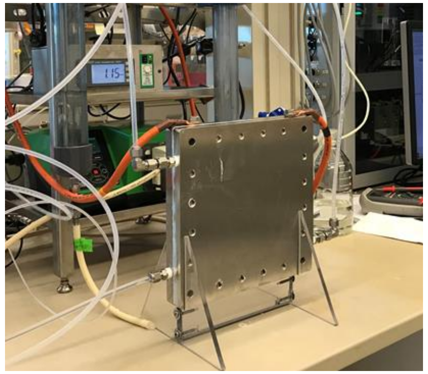
- TEA is performed with LanzaTech's technoeconomic model, which sizes process equipment based on mass and energy balances, and estimates capital and operating expenses.
  - Key inputs are fermentation productivity and yield.
- Major flow-scheme change after initial testing and integration of electrolyzer reveals CO2 crossover as unavoidable – addition of CO2 concentration and recycle from anode gas stream using PSA.
- Life cycle analysis (LCA) is performed by partners ANL after transferring TEA information.

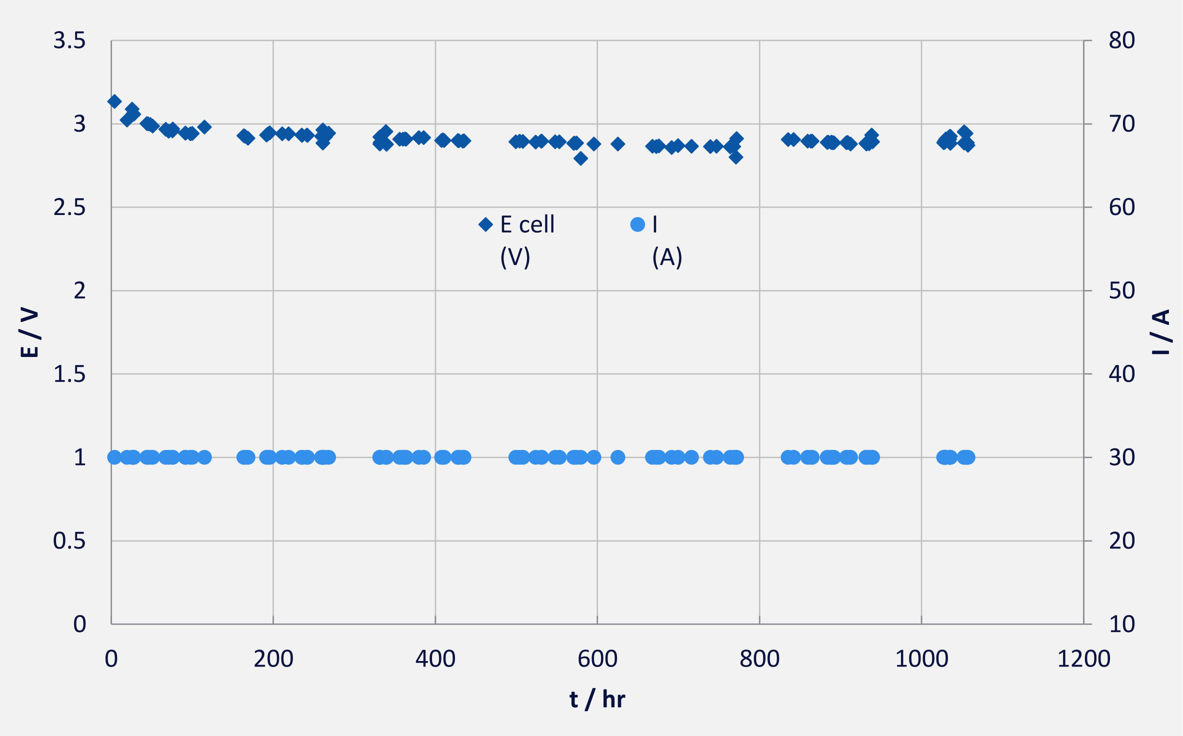
Argonne NATIONAL LABORATORY

A: Electrolyzer Enhancements







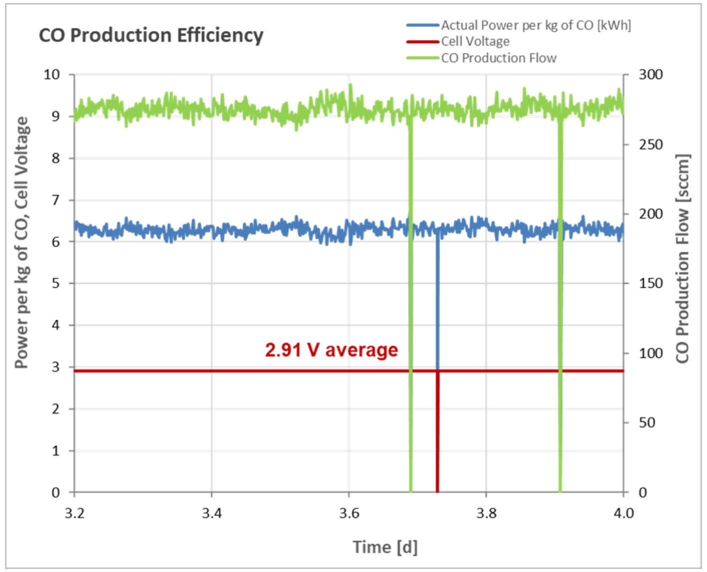


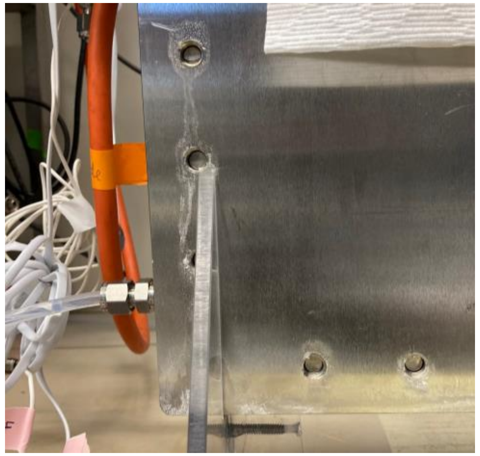
- **Improved 250 cm² cell developed**
  - Better flow distribution & bubble removal
  - Lower pressure drop
- **Performance Demonstrated**
  - 1000 hour longevity and 7.8 kWh / Nm^3_{CO} efficiency
- **Membrane reliability/robustness challenges**
  - Issues with supplier quality through COVID: impurities, carbon paper, and dust lead to performance degradation
  - Membrane strengthening and variations in production improved robustness and efficiency but did not exceed initial results
- **Cells, membranes and catalyst layers delivered to LanzaTech.**

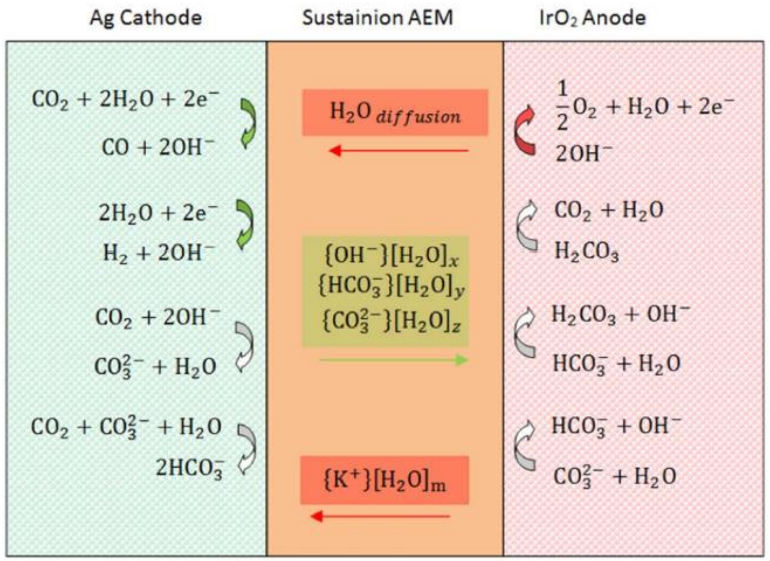
A: Electrolyzer Enhancements

LanzaTech operation of the electrolyzer:

- 7.8 kWh / Nm^3_{CO} efficiency corroborated
- Cell assembly and operation challenges (pics)
- Cell temperature and anolyte concentration are key parameters
- Tradeoff between efficiency, current density, & outlet CO conc (CO_2 flow)
- CO_2 crossover to anode – compounding effect with recycle
  - Mitigation through operation limited – requires external equipment on anode gas stream (PSA)

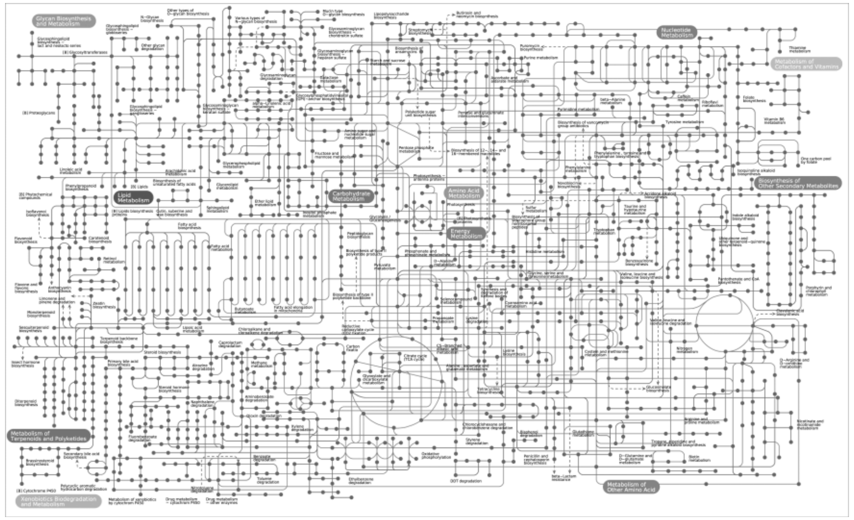






Zengcai Liu et al 2018 J. Electrochem. Soc. 165 J3371

B: Microbial Yield Improvement

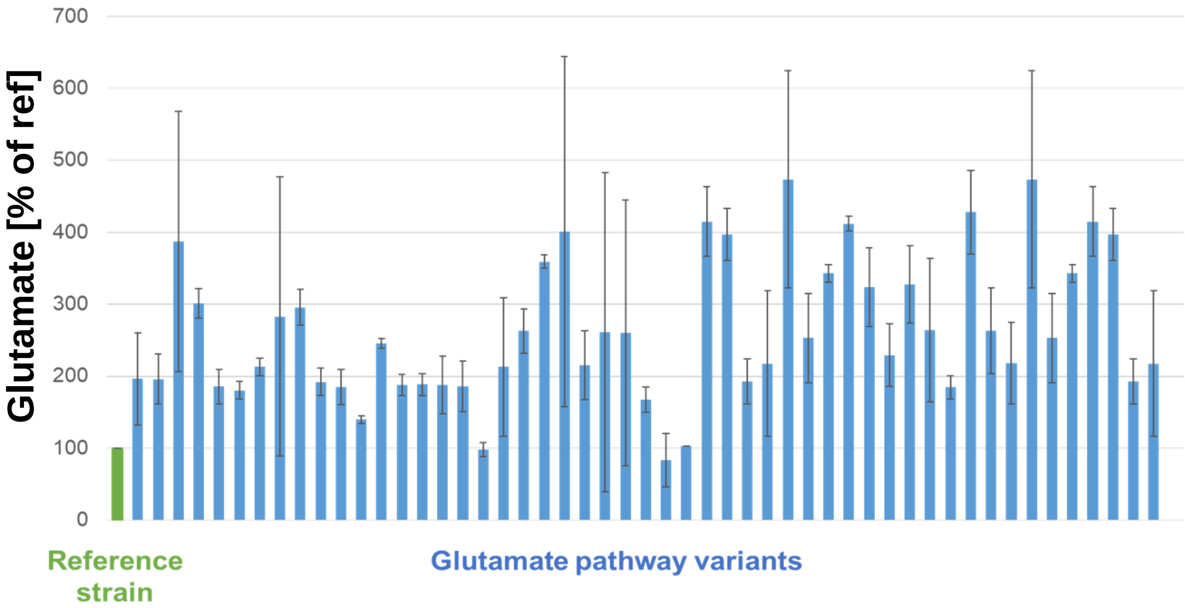


Modeling arginine as a supplement

- In-house genome scale model and flux balance analysis with shadow price analysis
- Modelling confirms arginine supplementation increases yield for a range of tested target molecules.
    - Greatest % benefit for high ATP cost products

Rerouting Ornithine to Central Metabolism

- 4 major pathways to glutamate or alanine investigated
- 24000+ unique pathway combinations identified
- 50+ strains built and tested (focus to glutamate)
    - Use of the anaerobic biofoundry
- Arginine transport challenge into cell identified



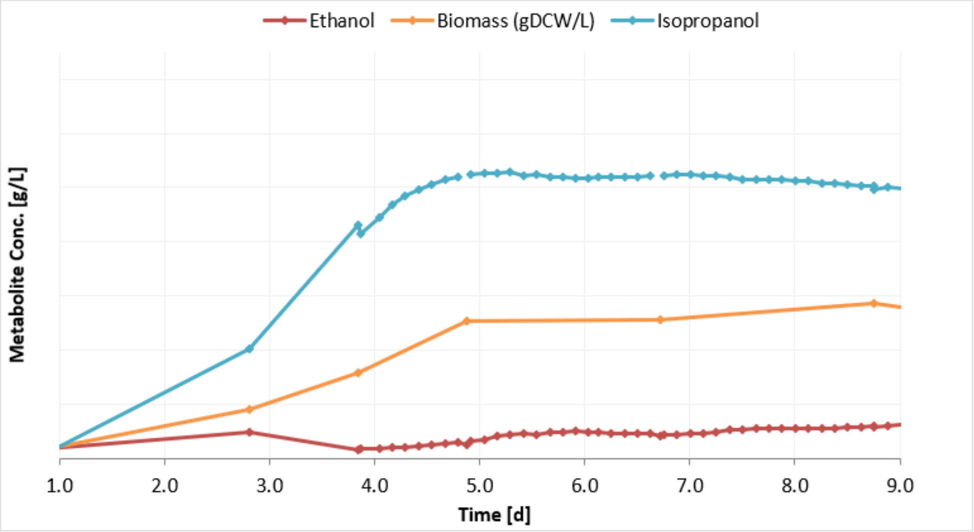
Arginine as a NH_3 replacement

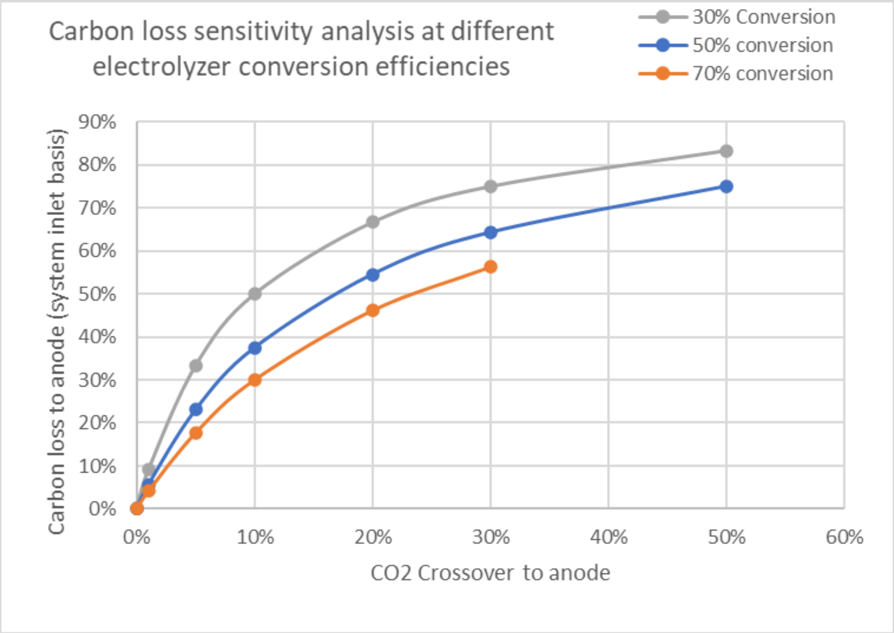
- Attempts to run fermentation without NH_3 unsuccessful

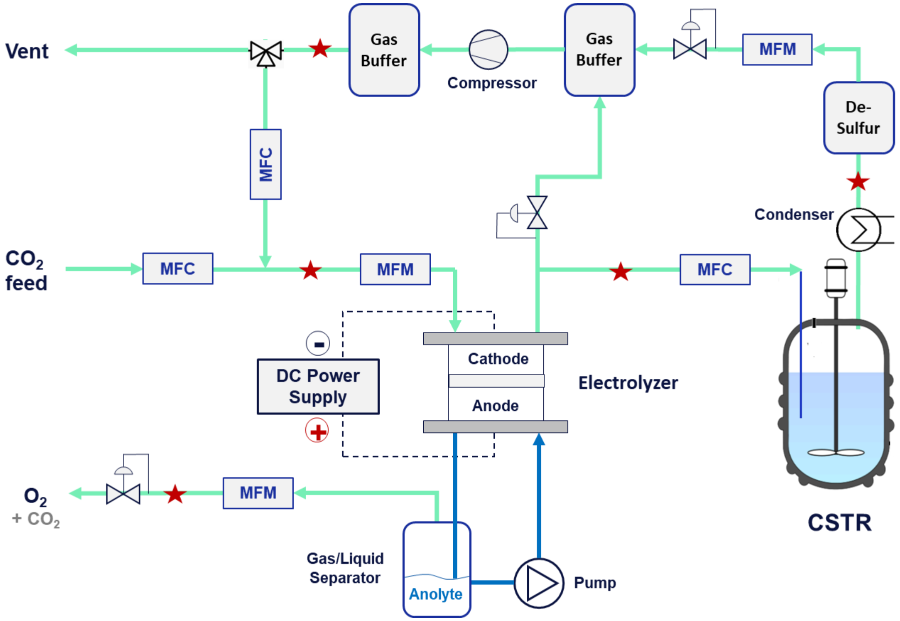
C: Fermentation - Electrolyzer Integration

Integration with full gas recycle successful at lab scale

- Final Isopropanol productivity and titer targets reached
- Mass and carbon balance closure difficult
  - High system complexity and small scale: GC sampling, leaks, flow measurement error
  - Suggest pilot scale requirement for future integration
- CO_2 crossover limits carbon yield:







D: TEA & LCA

TEA

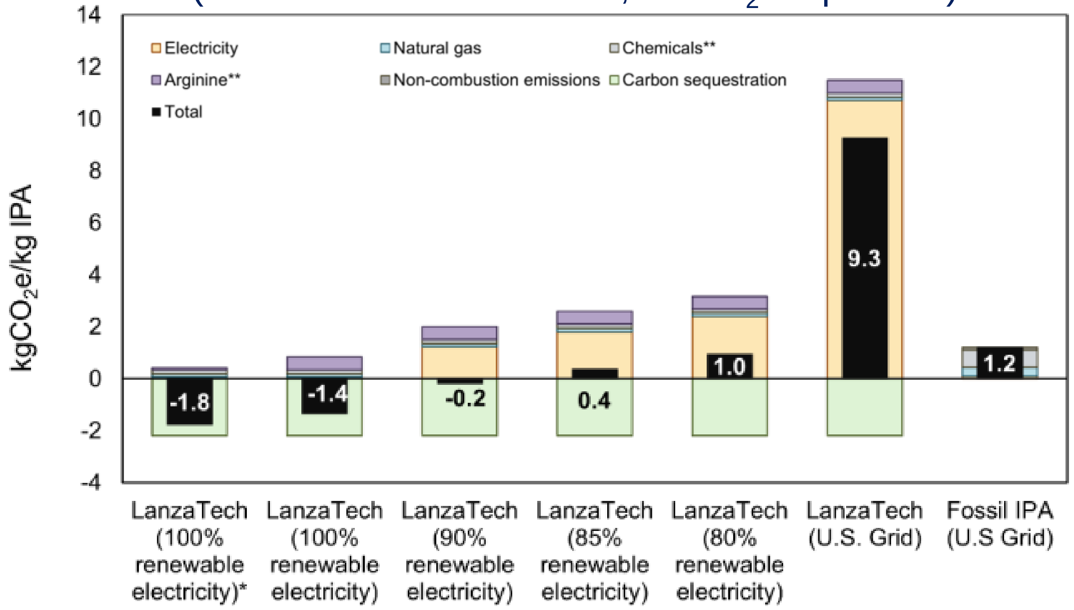
- Final capital cost reduced by 37% compared to baseline due to higher IPA productivity and titer achieved
  - ~3/4 of the capital cost in fermentation and electrolysis.
- Operating cost dominated by electricity – $0.01 to $0.02 /kWh required to be competitive with fossil IPA.
- Arginine would need to be < $1 /kg to make economic sense at current IPA prices.
  Higher value and higher ATP-requiring products might still benefit

LCA

- LCA carbon intensity dominated by electricity generation.
  - Process has lower GHG emissions compared to fossil IPA at 80%+ renewable electricity.
  - Negative GHG emissions at 90%+ renewable e-.
- Process ideally co-located with a renewable electricity generator.

GHG emissions

(market allocation method, with O_2 co-product)



Final Project Results

Key Performance Parameters	Metric	BP1 (go/no-go)	BP2 (go/no-go)	BP3 (final target)	Final Results
Carbon Efficiency to IPA (Net carbon yield)	%	28	33	37	2 to 8 * 40 †
Electricity use (Electrolyzer efficiency)	kWh / Nm^3 CO	9.57	8.72	7.97	7.81
IPA Productivity	g/L.h	Baseline	111%	120%	198%
IPA Titer	g/L	Baseline	100%	100%	119%
Arginine utilization - Ammonia loading - Arginine loading (mmol fed per g biomass produced)	mmol Ammonia / g_{CDW} biomass	>10	10	0	0 ‡
	mmol Arginine / g_{CDW} biomass	0	3.3	3.3	~10

* Directly measured in lab setup

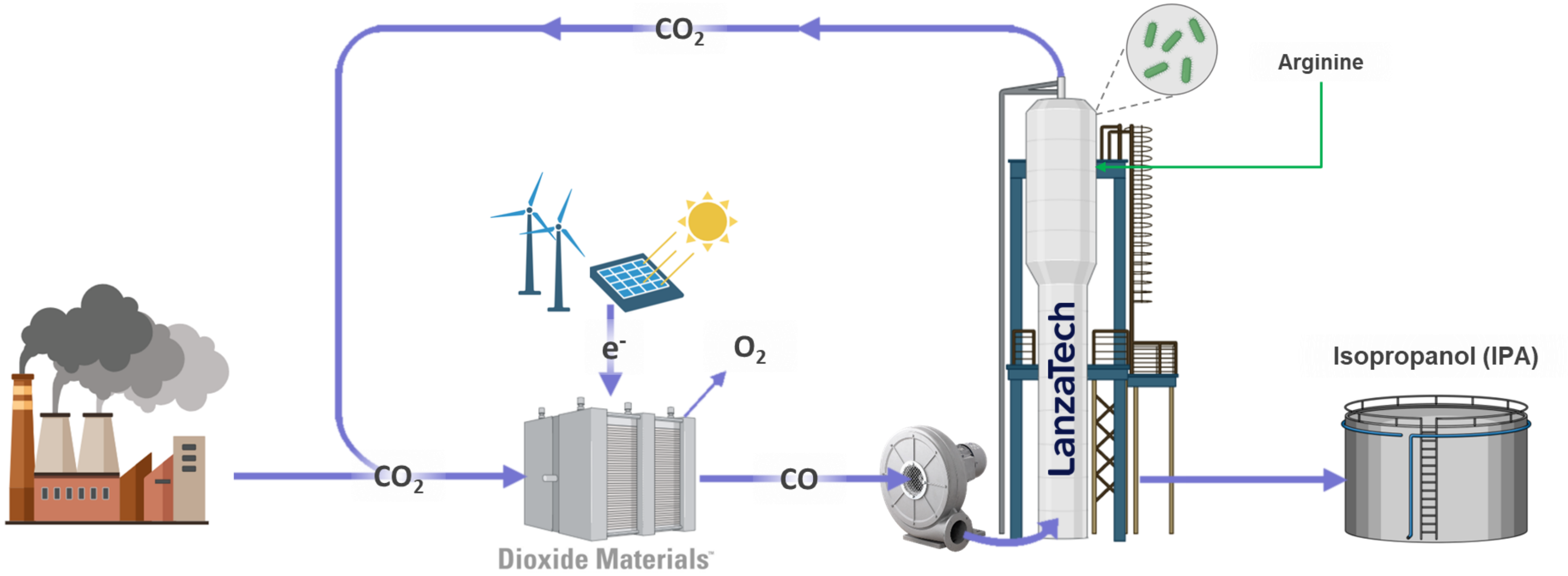
† According to a commercial basis design with tail gas recycle and PSA recovery of anode CO_2

‡ Causes fermentation decline

3 – Impact

Developed a platform CO_2 + electricity to a platform chemical

- Advanced the state-of-the-art for:
  - Low temperature CO_2 electrolysis through anion exchange membranes
  - Synthetic biology and modelling tools for *Clostridium autoethanogenum*
  - Arginine as a nitrogen, carbon and energy source for *Clostridium autoethanogenum*
  - Gas fermentation to produce isopropanol (non-native product)



Gas Fermentation Stoichiometry

(Ethanol example)

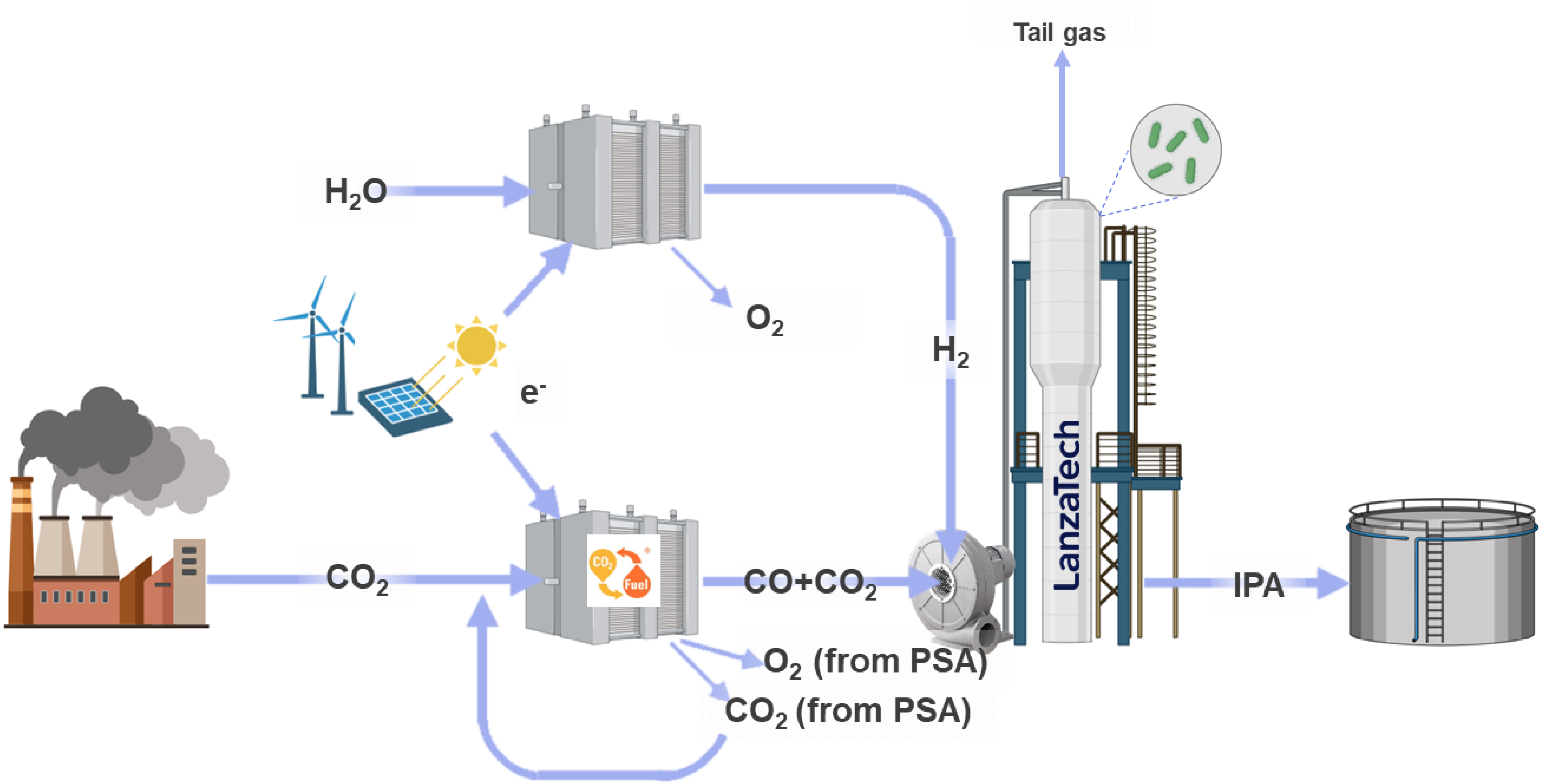
		H_2:CO Ratio	Carbon Efficiency
CO	$6\ CO + 3\ H_2O \rightarrow$ <u>EtOH</u> $+ 4\ CO_2$	0:1	**33.3%**
$CO + H_2$	$3\ H_2 + 3\ CO \rightarrow$ <u>EtOH</u> $+ CO_2$	1:1	**66.7%**
$CO + H_2$	$4\ H_2 + 2\ CO \rightarrow$ <u>EtOH</u> $+ H_2O$	2:1	**100%**
$CO+H_2+CO_2$	$5\ H_2 + 1\ CO + 1\ CO_2 \rightarrow$ <u>EtOH</u> $+ 2\ H_2O$	5:1	**100%**

Carbon efficiency improves with increased H_2 uptake

3 – Impact

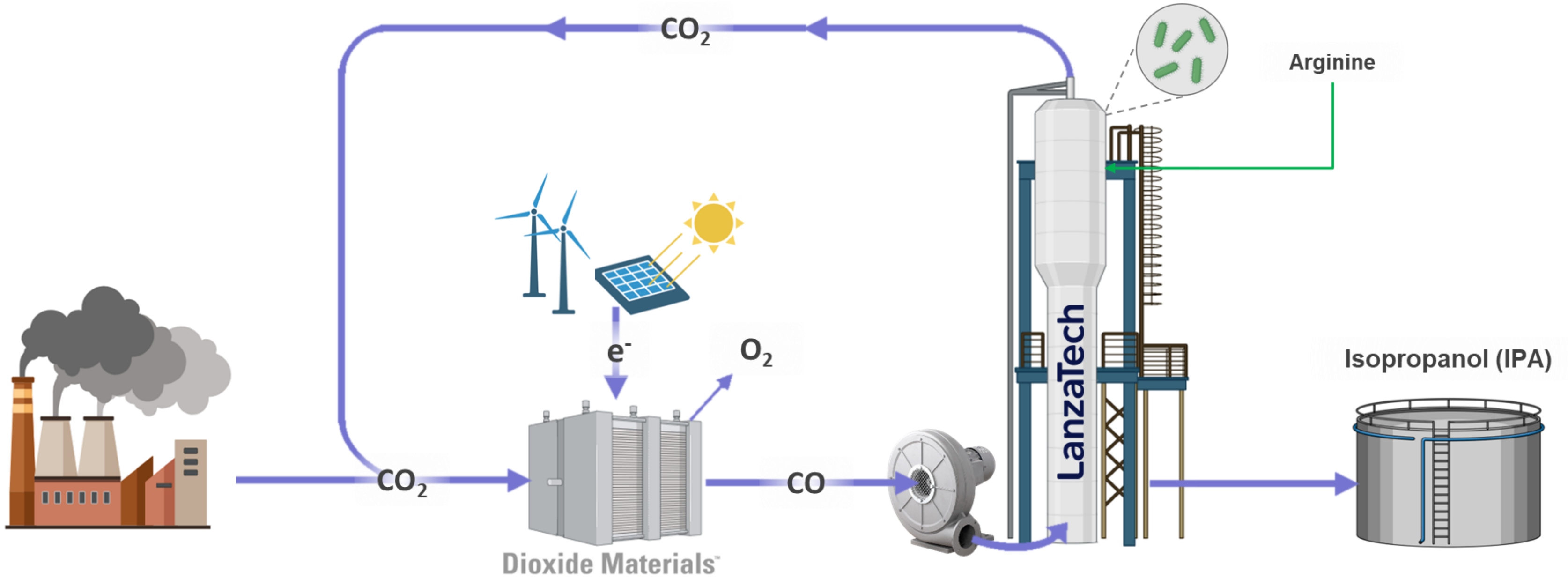
Next steps (outside project scope):

Utilizing H_2 from water electrolysis for more efficient conversion of green electricity and minimizing CO_2 loss



Summary

- Gas fermentation can be paired with CO_2 electrolysis to create a platform for carbon negative chemicals from CO_2. Low cost, renewable electricity is a key enabler for the technology.
- Anion-exchange-membrane based CO_2 electrolyzers can achieve high efficiency at room temperature but requires further development to scale into a robust industrial process. Crossover of CO_2 to the anode stream is a hurdle to carbon efficiency but can be mitigated using known technology (PSA) with minor to moderate cost.
- Arginine co-feeding boosts productivity in *C. autoethanogenum*, but challenges stemming from cell transport limit effectiveness.



Quad Chart Overview

Timeline
- Start: October 01, 2018
- End: September 16, 2022 (COVID delay)

	FY22 Costed	Total Award
DOE Funding	*$5,355.04*	*$1,350,000.00*
Project Cost Share *	$1,525.50	$384,576.00

TRL at Project Start: 2
TRL at Project End: 4 - 5

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 Partners*
- Dioxide Materials
- Argonne National Lab

Additional Slides

Responses to Previous Reviewer Comments

From 2021 Peer review:

- The benefit of arginine over ammonia needs more justification.
  This idea was based on preliminary data at the start of the project, however subsequent work including modelling and economic analysis ultimately proved that arginine is not a benefit over ammonia for production of short chain alcohols like IPA, and there is additionally a significant barrier to full utilization of arginine due to energy transport costs into the cell.
  Arginine may still be interesting for high ATP requirement, high value products.
- Lacks discussion on TEA, including design choices and scalability.
  While details on the TEA include confidential information, the key outcomes are discussed
- It is unclear how close the team is to achieving a gas fermentation that meets the target metrics for IPA productivity. No results were presented for an integrated system.
  The fully integrated system was run including gas recycling, however the electrolyzer and mass flow controllers had low reliability, so most fermentation runs were completed either without gas recycling, or on a synthetic mix generated from pure gases.
  Equivalence in biological performance was shown between electrolyzer gas and synthetic gas.

From Intermediate validation:

- Measure humidity and temperature of the electrolyzer in operation for additional insight.
  Subsequent tests proved this true. Temperature is especially important to performance and future work with these electrolyzers should include temperature control (not just relying on passive heating from current flow). Water saturated conditions inside the cell is also important, achieved with the liquid anolyte setup.

LanzaTech