DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Upscaling of non-recyclable plastic waste into CarbonSmart™ monomers

April 5th, 2023
Plastics Deconstruction and Redesign

Shivani Garg (on behalf of Ching Leang)
LanzaTech Global

Project Overview

Monoethylene Glycol (MEG) is a vital ingredient

Uses*

- *55% in polyester fibers*
- *25% in PET packaging and bottles*
- *Also used in coolants, antifreeze, heat transfer, solvents, paints, etc.*

Global demand*

- *28 million tonnes (2015)*

lululemon athletica

ZARA

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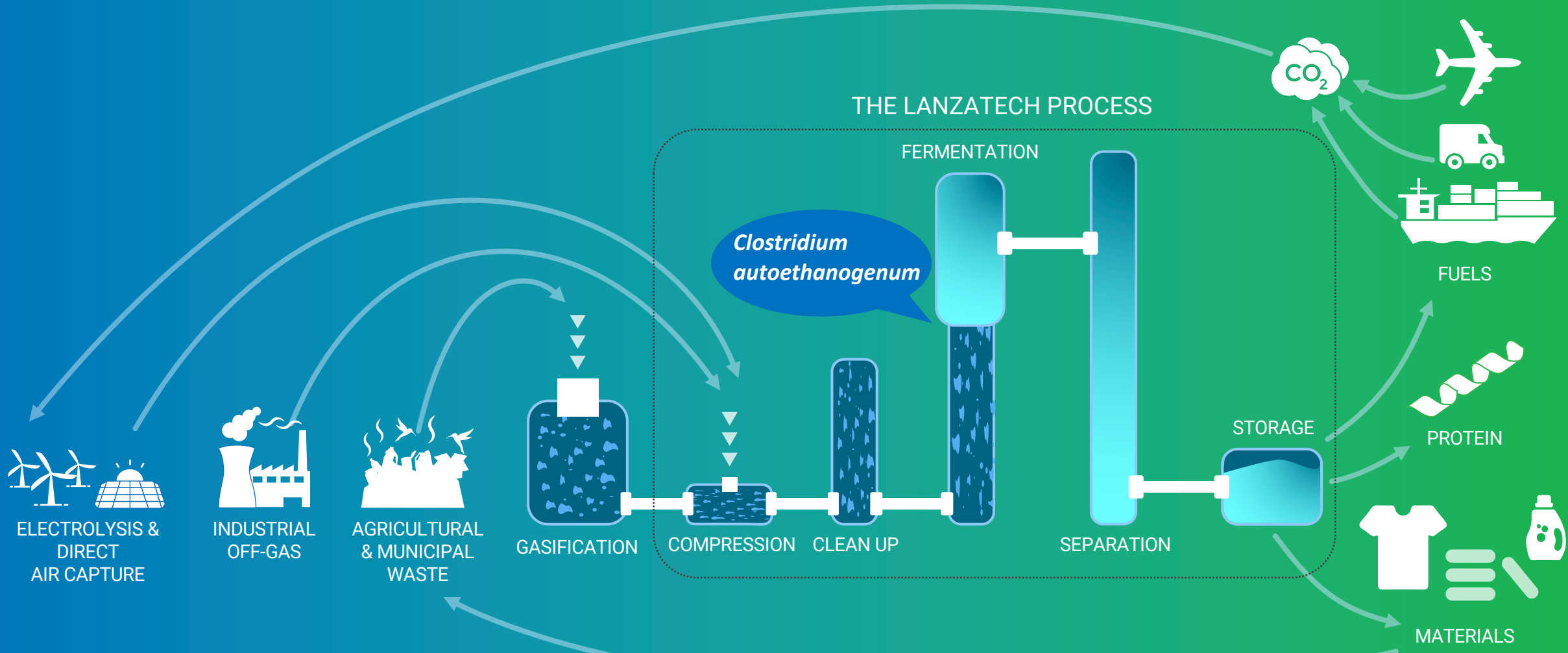
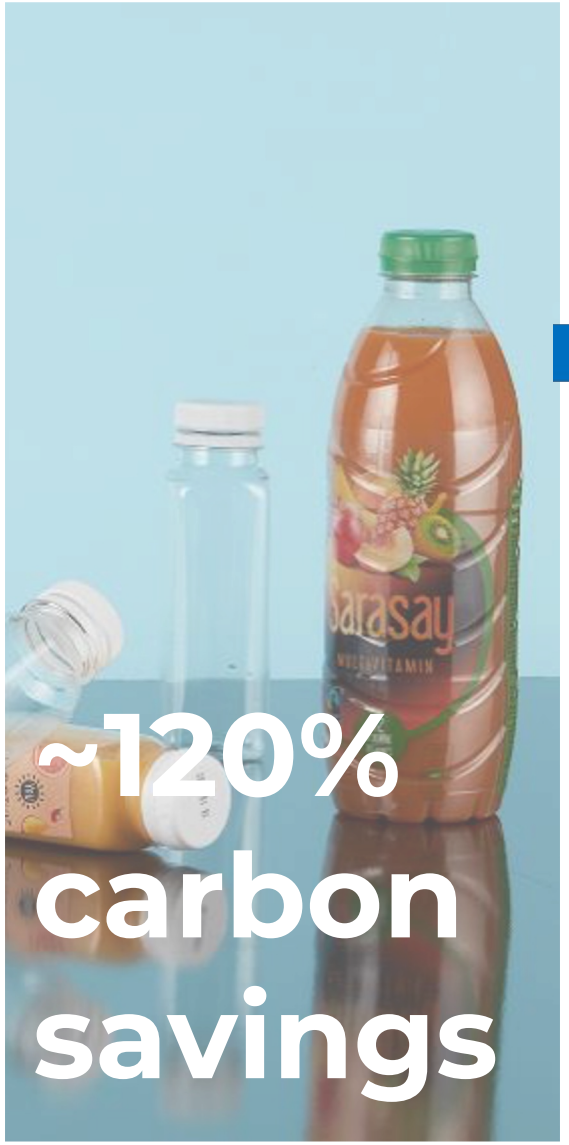
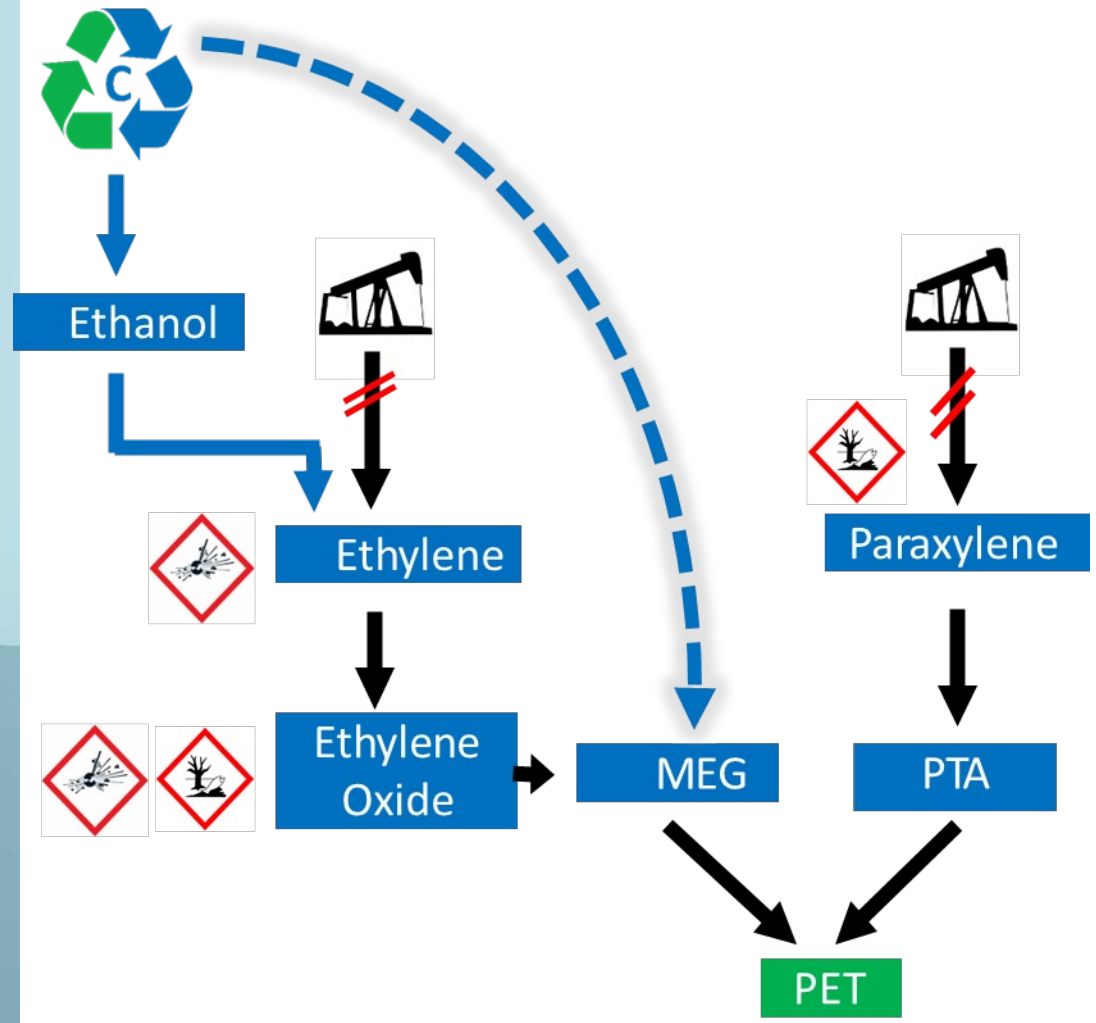
* https://www.shell.com/business-customers/chemicals/factsheets-speeches-and-articles/factsheets/mono-ethylene-glycol.html#:~:text=Mono%2Dethylene%20glycol%20%2D%20or%20MEG,kitchenware%2C%20engine%20coolants%20and%20antifreeze.

The LanzaTech Process



Traditional Route vs Proposed Route





- Mono ethylene glycol (MEG) is a chemical used in the manufacture of PET fibers and plastics used widely for packaging
- Ethanol can be used to produce MEG, however the conversion requires extra steps, energy, and chemical processing
- LanzaTech has developed the tools to engineer our biocatalyst to produce products other than ethanol from the same waste resources
- This project seeks to engineer the LanzaTech biocatalyst for direct production of MEG to enable more sustainable and economic manufacture of MEG.

1 – Approach (Management)

Project Team-LanzaTech

Principal Investigator
Dr. Sean Simpson

Co-founder of LanzaTech

Principal Investigator
Dr. Ching Leang

Director, Strain Engineering, Synthetic Biology

Project Manager
Dr. Anthony Goering

Project Manager, Synthetic Biology

TEA Lead
Dr. Robert Conrado

VP, Engineering Design & Development

Automation Lead
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Biofoundry Manager, Synthetic Biology

LCA Lead
Dr. Christina Canter

Senior LCA Engineer, Design Development

Technical Lead
Dr. Michael Köpke

VP, Synthetic Biology

Gasification Lead
Dr. Taylor Schulz

Senior R&D Engineer, Analytics and Catalysis

Modeling Lead
Dr. Rupert Norman

Modeler, Computational Biology

Enzymology Lead
Dr. Ryan Tappel

Enzymology Manager, Synthetic Biology

Fermentation Lead
Dr. Steve Brown

Director, Strain Development

Project Team-InEnTec

Gasification Expert
Jeff Surma

President and CEO of InEnTec

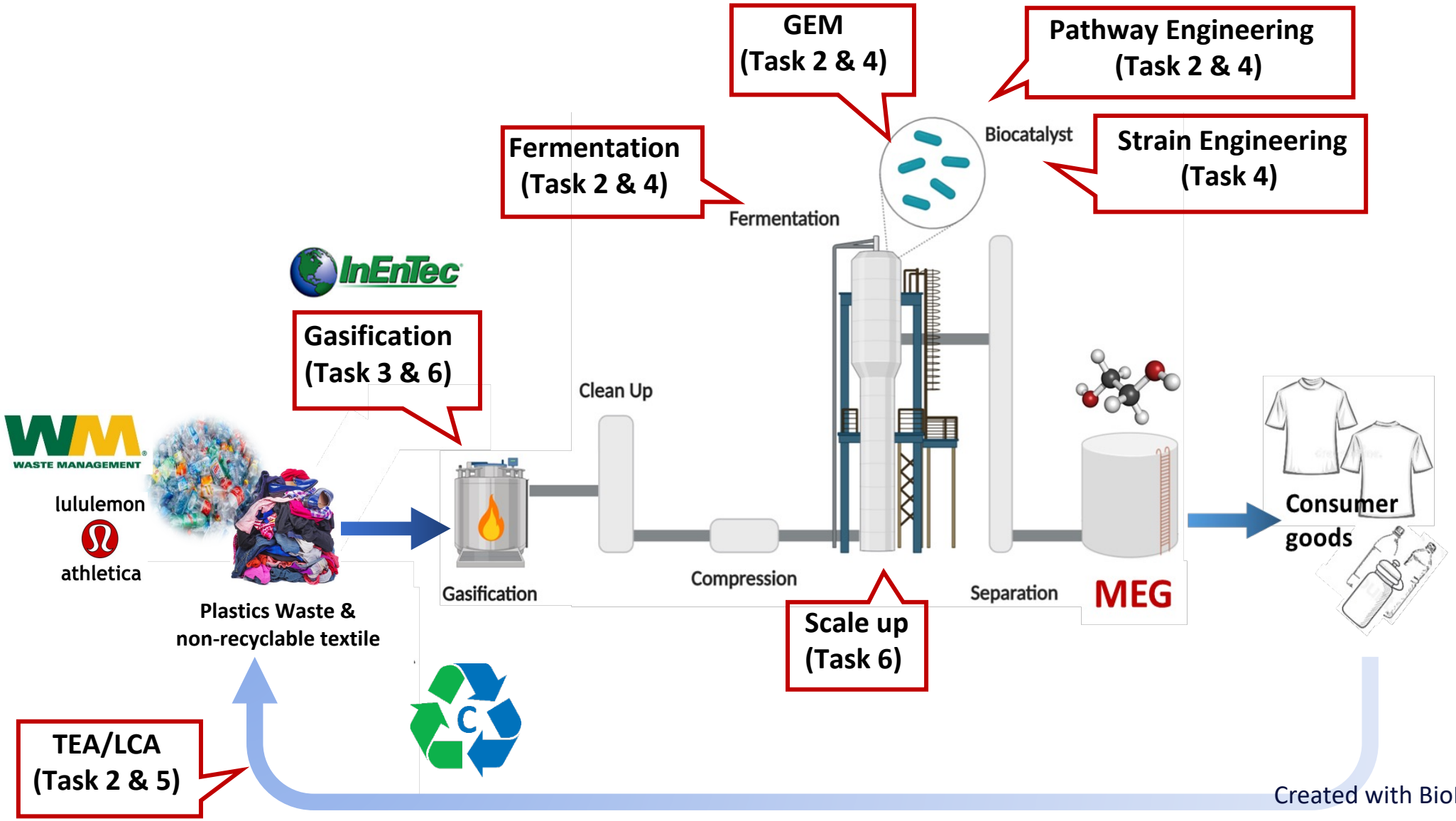
Gasification Expert
Dr. Jijun Xu

Director of Technology

INENTEC®

1 – Approach (Technical)

Approach Overview



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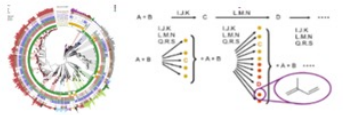
Strain Engineering

Discovery

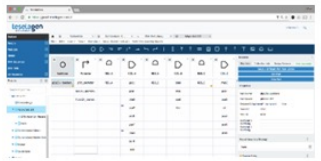
- *Sequence/Knowledgebase*
- *Retrobiosynthesis*



JGI · Northwestern Center for Synthetic Biology · U.S. Department of Energy

Computer-Aided Design

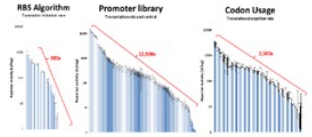
- *BioCAD*



teselagen BIOTECHNOLOGY

Genetic Parts

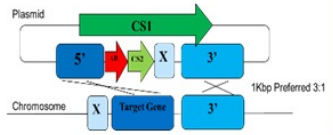
- *Reporters, Markers*
- *Promoters, Terminators*
- *RBS, Codon Usage algorithms*



PennState · U.S. Department of Energy

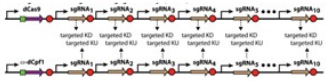
Genetic System

- *DNA transfer*
- *Modular plasmids*
- *Homologous recombination, CRISPR*



Advanced Toolbox

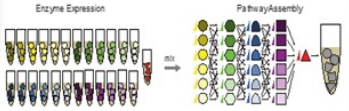
- *Multiplexing*
- *Genome-wide*
- *Genetic circuits*



PennState · U.S. Department of Energy

Rapid Prototyping

- *Cell-free protein synthesis*

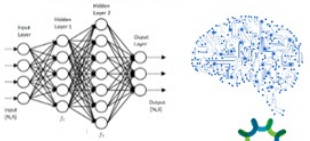


Northwestern Center for Synthetic Biology · U.S. Department of Energy

State of Art Strain Engineering Platform developed

AI

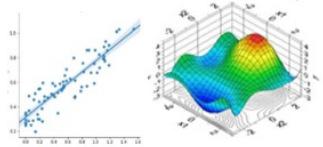
- *Machine Learning*



teselagen BIOTECHNOLOGY · Agile BioFoundry · Argonne National Laboratory · U.S. Department of Energy

Modelling

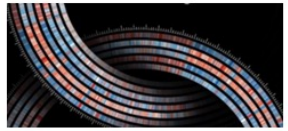
- *Genome-scale*
- *Kinetic*
- *Technoeconomic*



Northwestern Center for Synthetic Biology · U.S. Department of Energy

Systems Biology

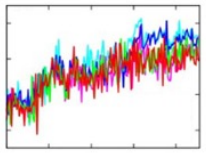
- *Multi-Omics*
- *Enzymology*



Oak Ridge National Laboratory · U.S. Department of Energy

Automated Strain Evolution

- *Automated Strain Evolution*



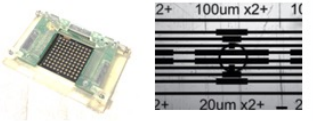
Automated Strain Engineering

- *Anaerobic Biofoundry*

U.S. Department of Energy

Miniaturization

- *Microfluidics*



JBEI Joint BioEnergy Institute · U.S. Department of Energy

Genome Scale Modeling

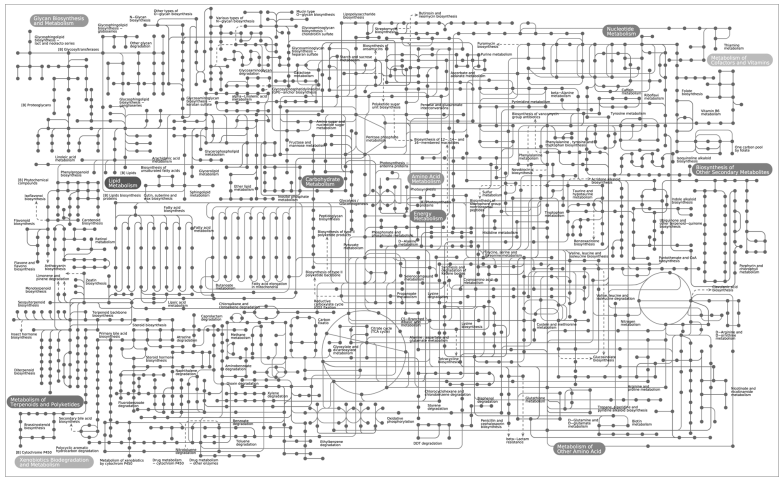
Models used
- Full genome-scale model (GEM)
- Structurally simplified GEM
- Core metabolism model

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

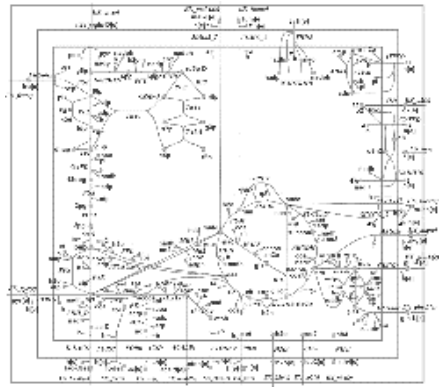
Design landscape assessment
- Flux balance analysis and minimization of metabolic adjustment to predict phenotypes
- Strength Pareto Evolutionary Algorithm 2 (SPEA2) and minimal cut set (MCS) analysis to characterize design landscape (Maia 2015, Kamp 2014)

Inform strain designs; Accurately predicts gas profile, growth, and product formation across the full spectrum of gas mixes.

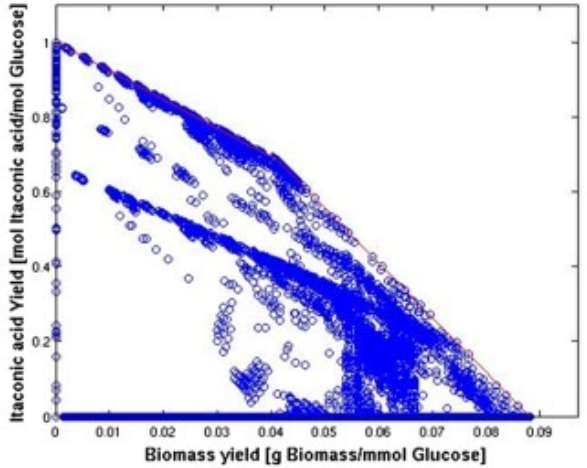
Genome-scale metabolism



Core metabolism

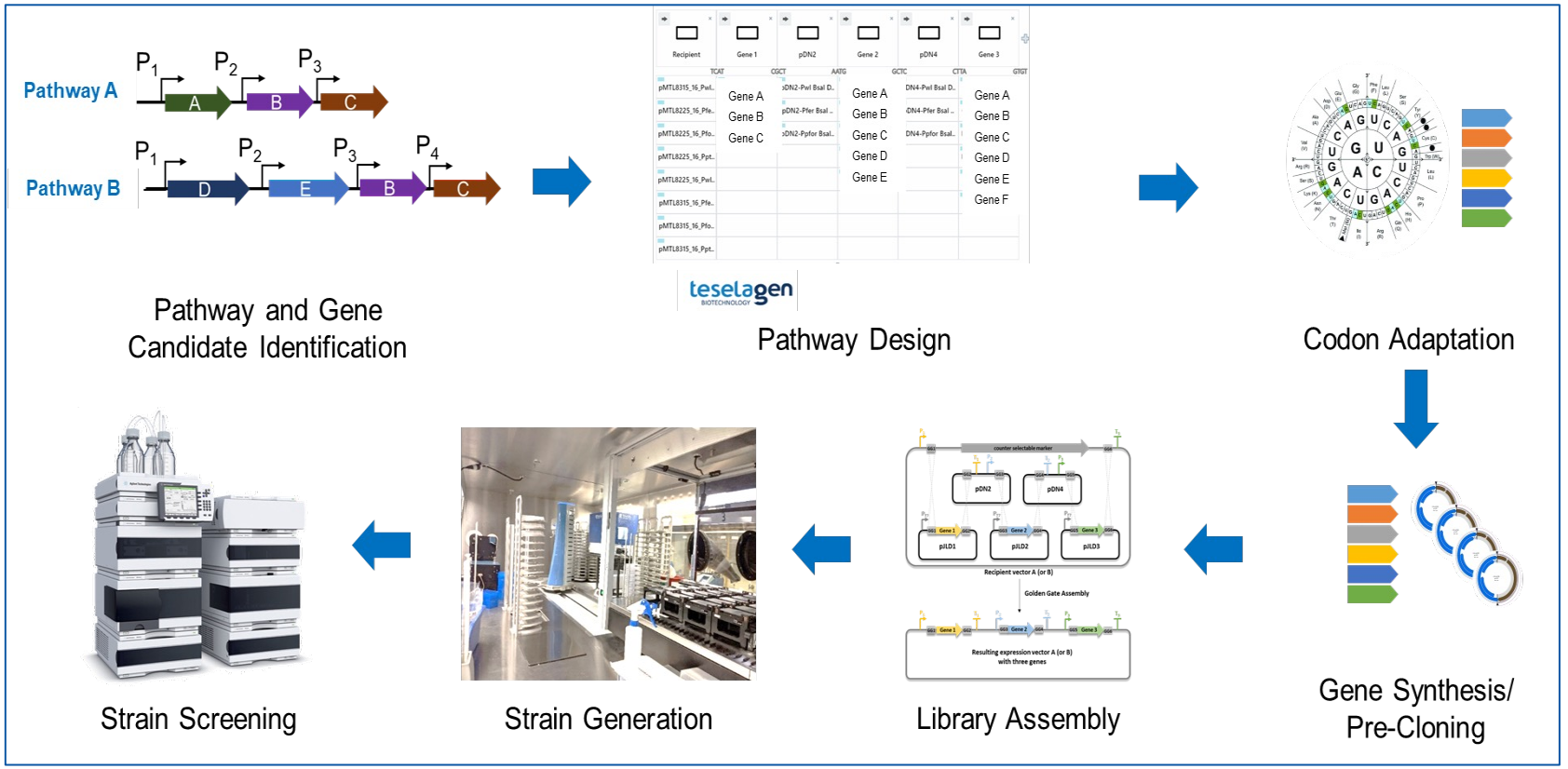


Design landscape assessment



Pathway Engineering

CO, CO_2, H_2 → Wood-Ljungdahl pathway → Acetyl-CoA → Pyruvate → Central metabolism → Glyoxylate → glycolate → glycolaldehyde → **MEG**



- **Random libraries approach combined with automated Biofoundry system to screen for thousands of strains in short time.**
- **Ensure covering all design landscapes and testing all possibilities of pathway combinations.**

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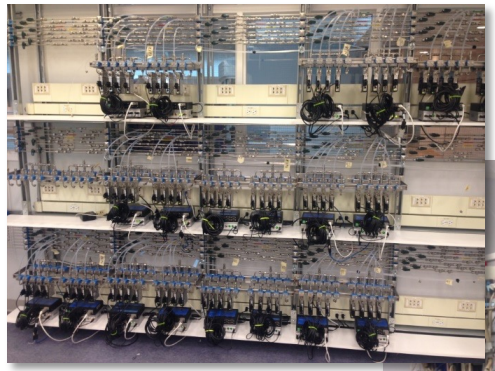
Process Development

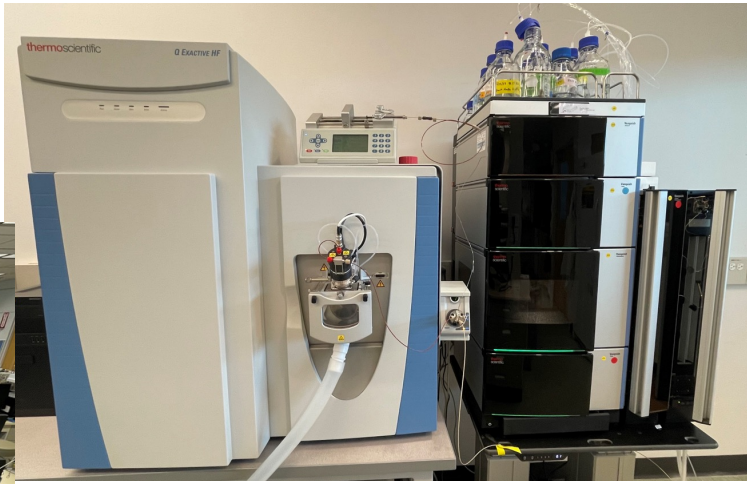
State of Art
Gas Fermentation Facilities

- Total gas composition flexibility
- On-line analytics (gas, biomass, metabolites)
- Multiple reactors configurations
- Over 100 dedicated gas fermentation reactors



State of Art
Analytics and Metabolomic Facilities

- Metabolomics (including energy related metabolites)
- Over 150 quantitative compounds and semi-targeted compounds library of more than 600 additional compounds.



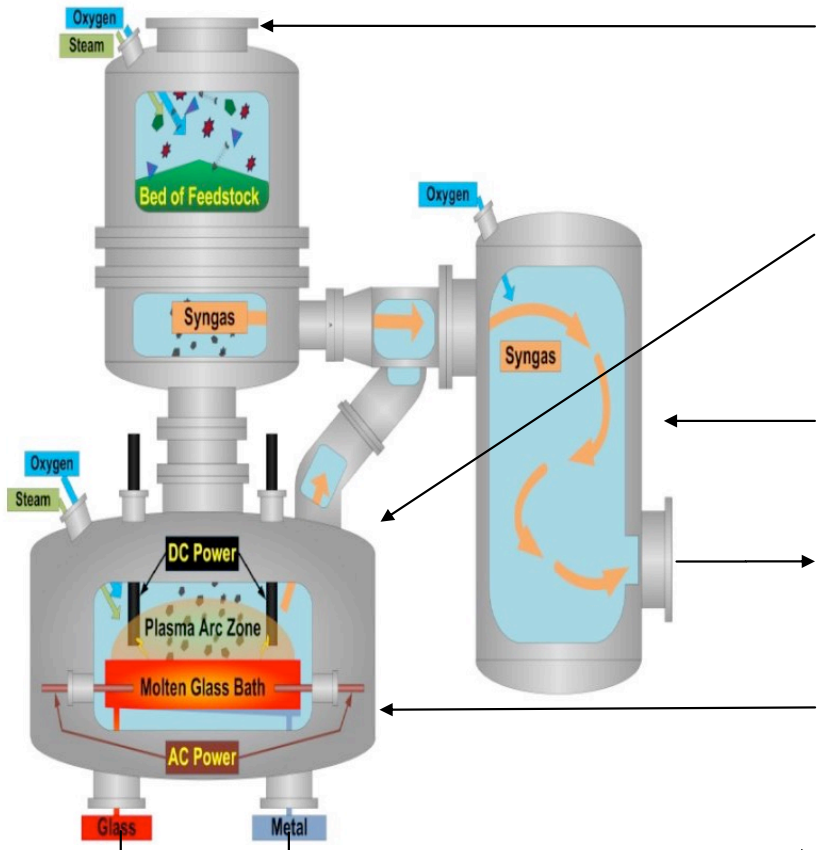
Gasification Partner

InEnTec State-of-the-Art Gasification Facility

- Municipal solid waste (MSW) is difficult to gasify
- Composition of MSW from same facility can vary widely
- MSW can contain halogens, metals, and other compounds not generally found in biomass
- Syngas from MSW may need more purification prior to use
- InEnTec chosen as MSW-gasification partner
- Founded in 1995 in Richland, WA

InEnTec Plasma-Enhanced Melter® Technology

- Plasma Enhanced Melter® (PEM®) system
  - Reduced handling and disposal costs
  - Lower carbon footprint
  - Robust, scalable technology capable of processing any waste material
  - Near 100% recycle of waste into reusable energy and industrial products



Down-draft gasifier（800°C - 1,200°C）
First stage reaction of waste with oxygen and steam at temperature. Down draft design produces a cleaner, hydrogen rich raw syngas product. Active grate to control transfer of material to PEM.

Plasma Enhanced Melter（1,200°C - 1,400°C）
Char and ash materials pass to the PEM chamber where plasma arc gasifies remaining materials (nearly 100% recovery of organic fraction in syngas phase). Arc temp is 10,000 °C, Immediate area around arc is 5,000 °C.

TRC（1,100°C - 1,400°C）
Additional time at temperature allows reactions to reach equilibrium and fully destroy tars and oils resulting in the cleaner raw syngas.

Syngas
Standard industrial technologies used to clean and condition raw syngas in preparation for use in selected back-end technology.

Molten glass bath（1,100°C - 1,500°C）
Inorganic fraction is entrained in the molten glass bath（Joule heated via submerged AC electrodes).

Vitrified Glass and Metal
Inorganic waste fraction is recovered as metal ingots and/or vitrified glass slag.

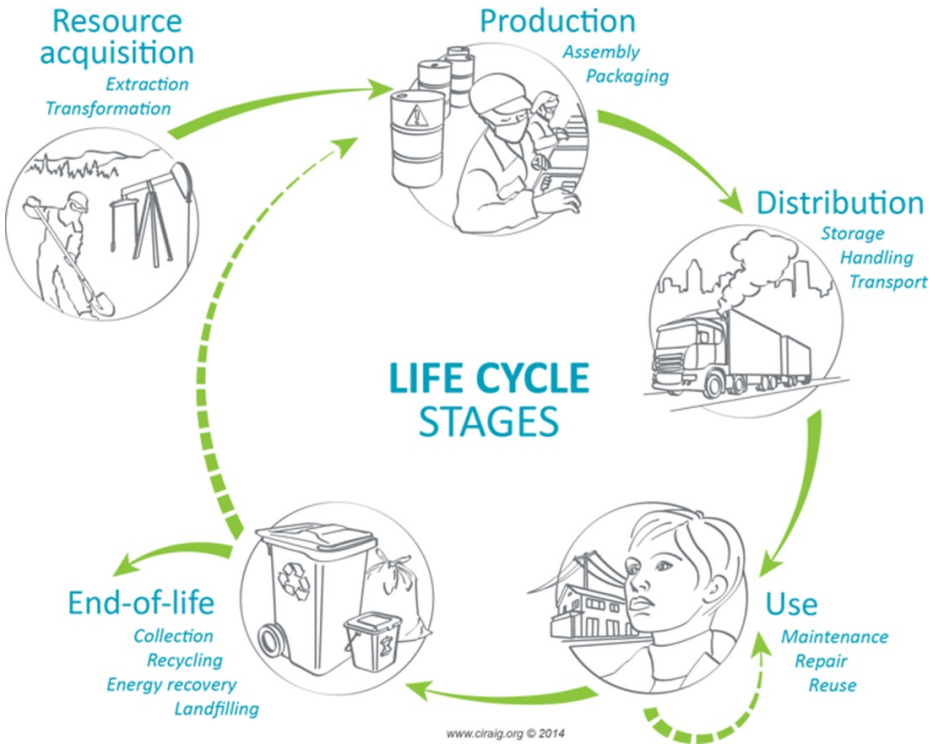
Economic Assessment

TEA

- A team of TEA specialists for LanzaTech's commercial and new technology.
- Work jointly to guide strain and process development to move down the cost curve as quickly as possible.

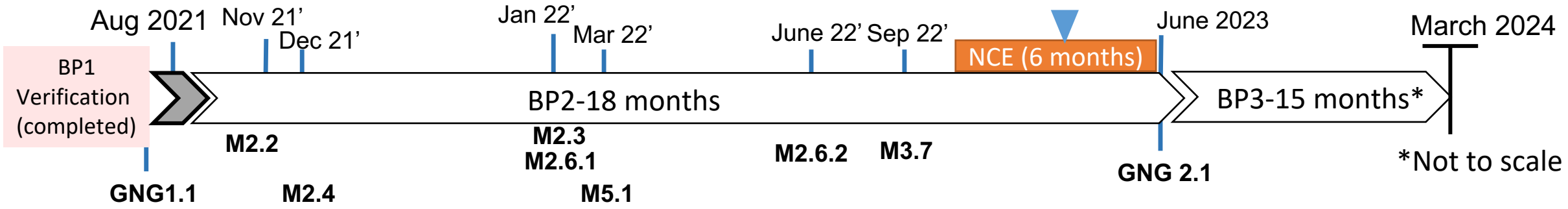
LCA

- Life cycle analysis from cradle-to-gate to determine LanzaTech process GHG emissions reductions compared to other conventional processes.



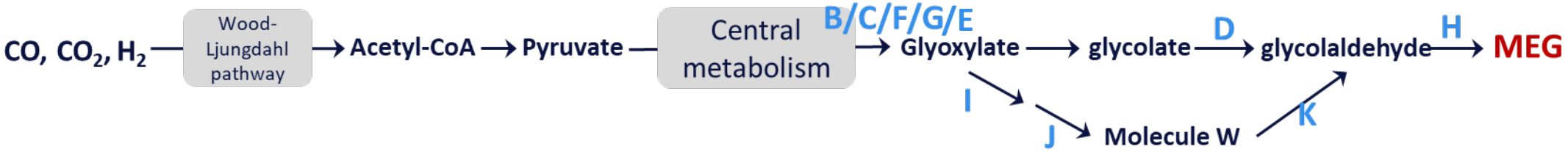
2 – Progress and Outcomes

Milestones

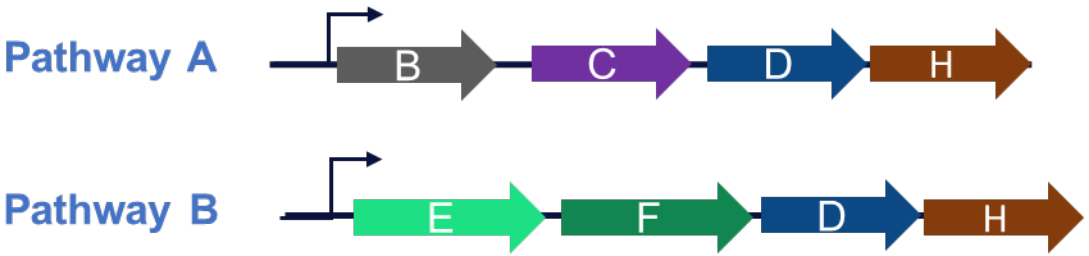


Progress summary (BP1+BP2 only)		
Task/Milestones		**Status**
1.0 Initial Verification that the reported state of art is reproduceable		Completed
2.0 MEG Pathway Engineering		
2.2	Library design and construction	In Progress (94%)
2.3	Decarboxylase variants screening	Completed
2.4	Biosensor evaluation	In Progress
2.6.1	Library Validation: screening at least 5000 MEG pathway strains	In Progress (43%)
2.6.2	Library Validation: Validate results and sequencing top 1000 performers and bottom 100 performers	In Progress (43%)
3.0 Gasification R&D		
3.7	Gasification of Mixed MRF residuals and/or sorted plastic film residues	In Progress (80%)
5.0 TEA/LCA		
5.1	Preliminary TEA and LCA	Completed
GNG 2.1	Increase MEG titer 500X	In Progress (80%)

Building a Biocatalyst for MEG Production



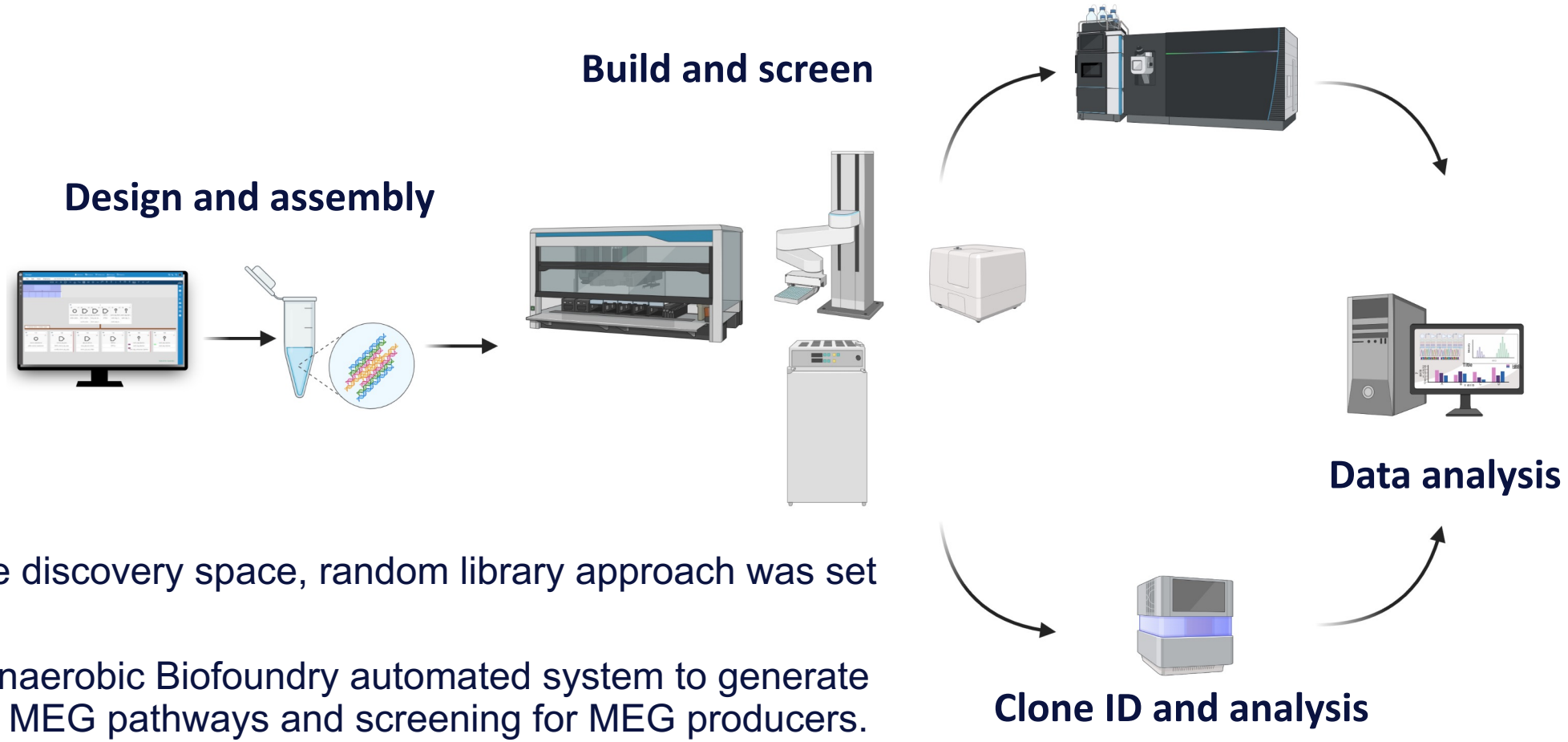
- No known MEG production microbes in nature.
- Synthetic pathways identified via literature searches and modeling
- At least two pathways identified that will take carbon from C1 gas substrates to MEG directly.



Multi-Pronged Engineering Processes

- Pathway Engineering: Random and targeted library
- Enzyme screening: cell free and *in vivo*
- Intermediate feeding to identify bottleneck steps

Pathway Engineering



- To cover all possible discovery space, random library approach was set up.
- Using world's first anaerobic Biofoundry automated system to generate strains with random MEG pathways and screening for MEG producers.

Overcome challenge: Improve strain generation efficiency and reduce screening time

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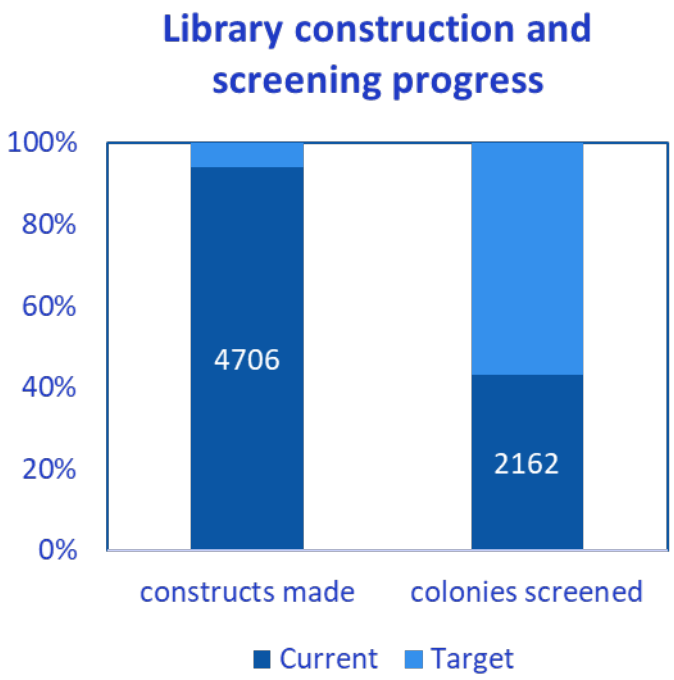
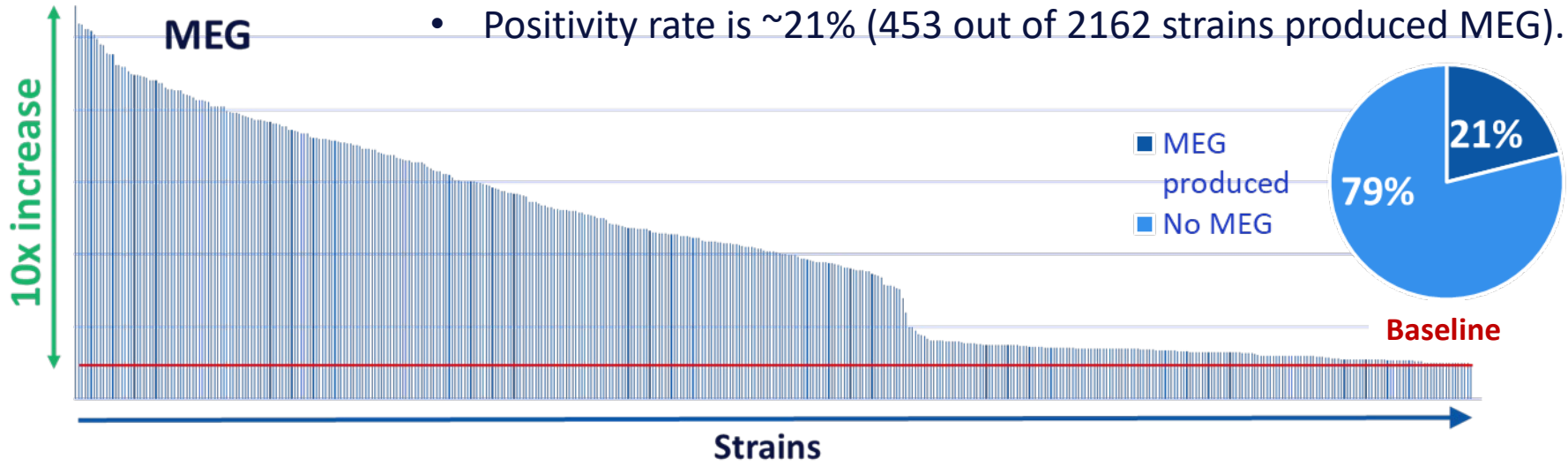
Pathway Engineering



- Total of 94% target clones designed and constructed.
- Total of ~43% target strains screened
- Positivity rate is ~21% (453 out of 2162 strains produced MEG).

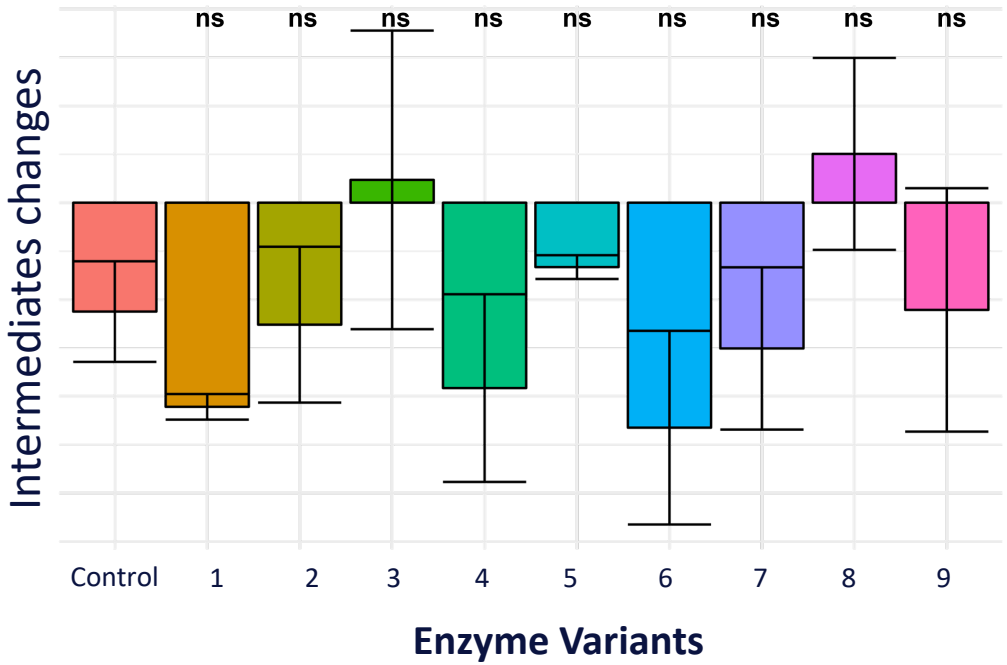


- Identified MEG producers with ~10X increase in titer compared to the control strain.
- Random libraries screening proved to be a powerful tool to screen for pathway variants and identify best gene combinations.

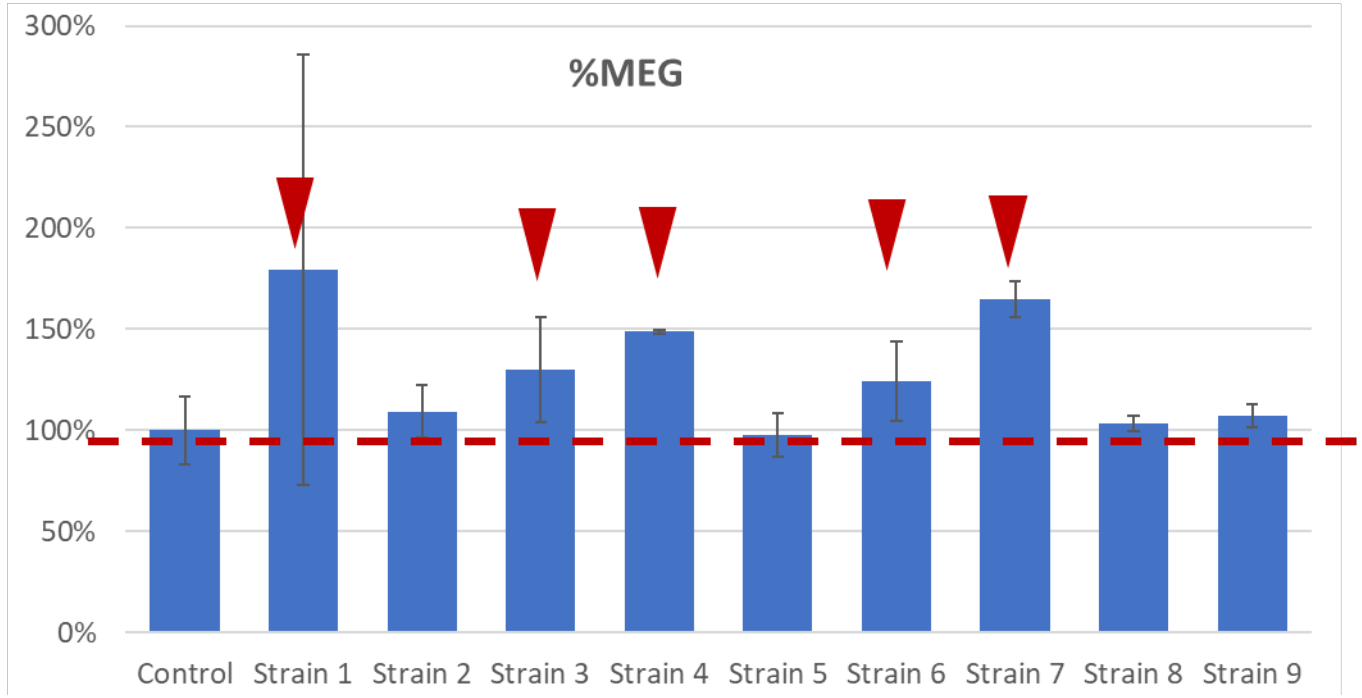
Overcome Challenge: Multiple QC steps implemented throughout the process to ensure complete pathway assembly and strain generation.

Enzyme Screening

Cell Free Prototyping



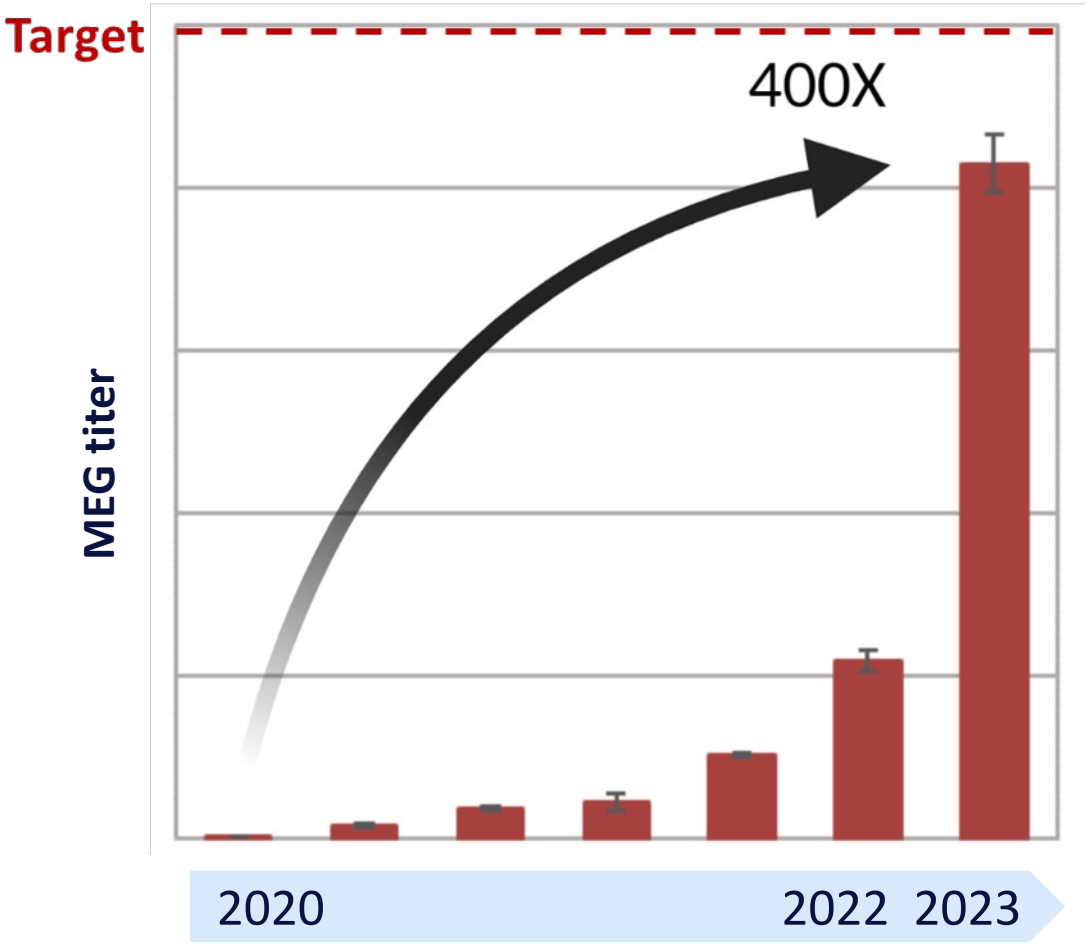
In vivo Screening



- Nine different enzyme variants were screening by both cell free prototyping or in vivo assays.
- Due to instability of the intermediate molecule, cell free prototyping results were not conclusive.
- Instead, in vivo screening successfully identified several enzyme variants that work better than the control.

Overcome Challenge: ***Different screening tools were applied in order to identify best enzyme variants***

MEG production increased 400X with multi-pronged engineering approaches



- To identify bottlenecks and/or ensure pathway gene expressions are sufficient, intermediate feeding experiments were carried out.
- Over half dozen intermediates were fed to MEG producing strains one by one and compare MEG production to those without intermediates feeding.
- Combined with enzyme screening, intermediate feeding, pathway libraries screening, MEG titer increased 400X compared to at the start of the project.

Overcome Challenge: Major bottleneck steps identified, titer increased 400X compared to at the start of the project.

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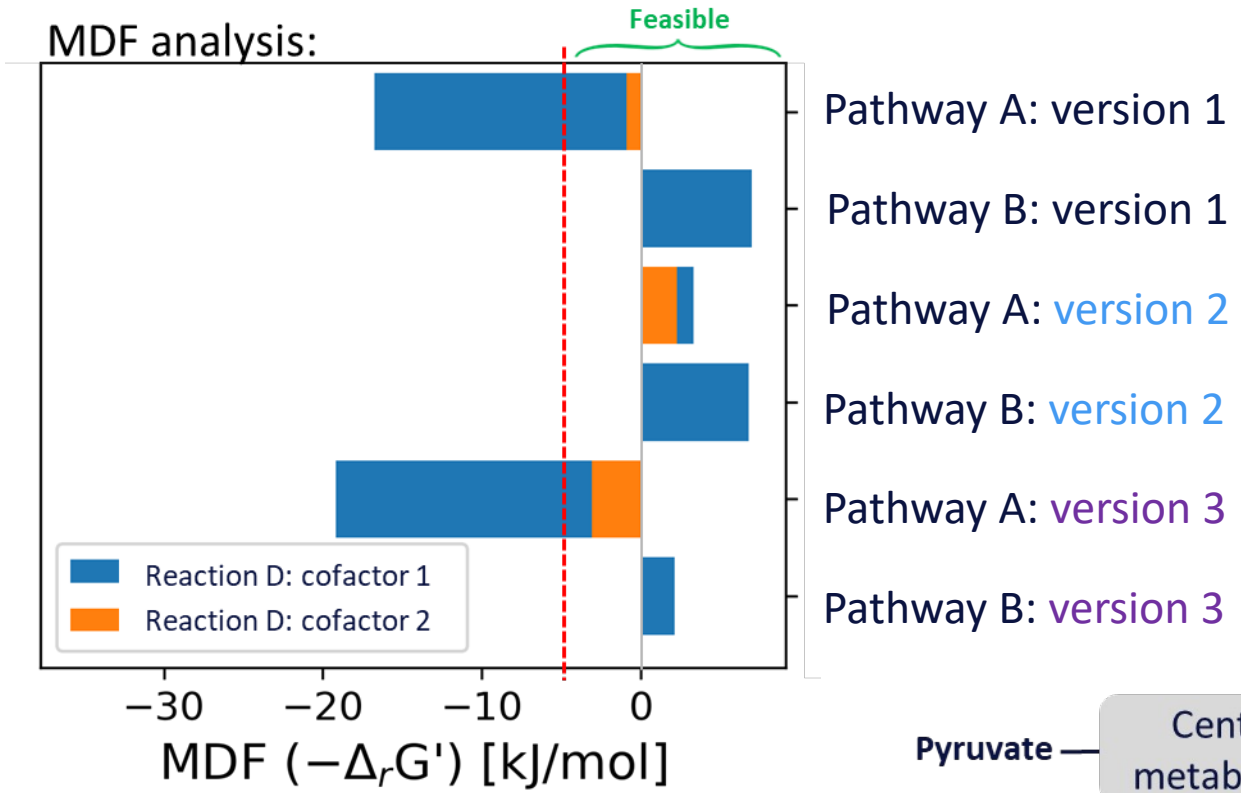
Modeling

Analysis of MEG pathway thermodynamics

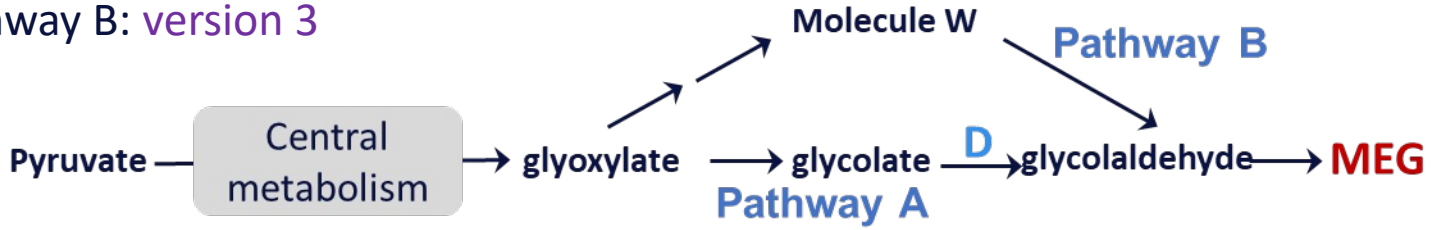
We performed Max-min Thermodynamic Driving Force (MDF)[1] analysis of MEG pathways to identify the most promising candidates. MDF analysis finds the maximum reaction driving force ($-\Delta_r G$ kJ/mol) that can be achieved by every reaction in a pathway simultaneously. MDF << 0 kJ/mol indicates infeasibility.

References:
1. Noor, Bar-Even et al. (2014). *PLOS Comput. Biol.*



Key results:

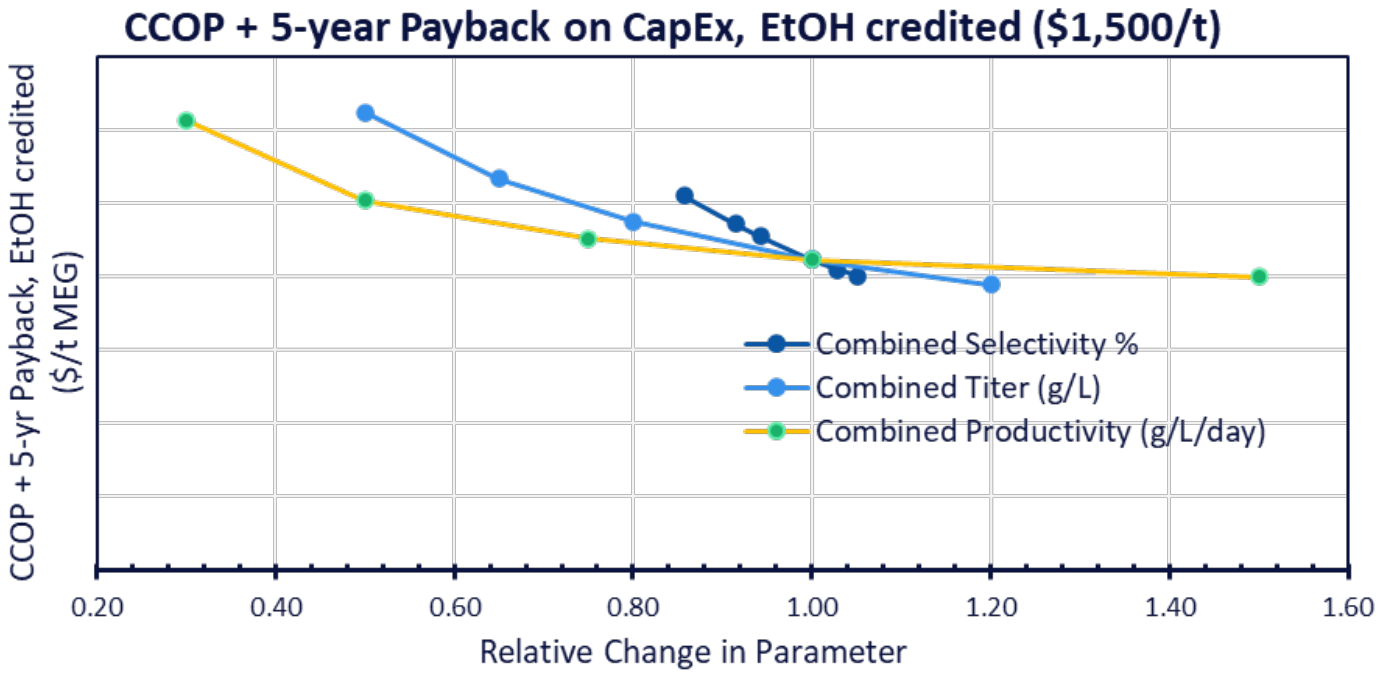
- The MDF values for Pathway A indicate that feasibility depends on **cofactor dependence** of Reaction D.
- The thermodynamically favorability is improved by addition of Pathway B.
- Version 2 is the most thermodynamically favorable version for Pathway A, while version 1 is the best choice for Pathway B.



Technoeconomic Analysis

MEG Sensitivity Analysis – Production Cost Sensitivity

- Plastic/MRF residuals gasified Syngas (CO + H2 > 60%) to co-produce MEG and ethanol, US location basis:
- Cost basis is:
  - Cash cost of production (CCOP) = variable + fixed operating costs with no capital repayment
  - 5-yr payback on CapEx = required operating margin such that cumulative operating margin over 5-years equals the capital investment
  - Ethanol Credit = Ethanol sales at a fixed price, with remaining costs allocated entirely to MEG
- Sensitivity Analysis shows variation of a single parameter from the midpoint.
  - Ranges: -15% to +5% of midpoint combined selectivity, -70% to +50% of midpoint productivity, and -50% to +50% of midpoint titer

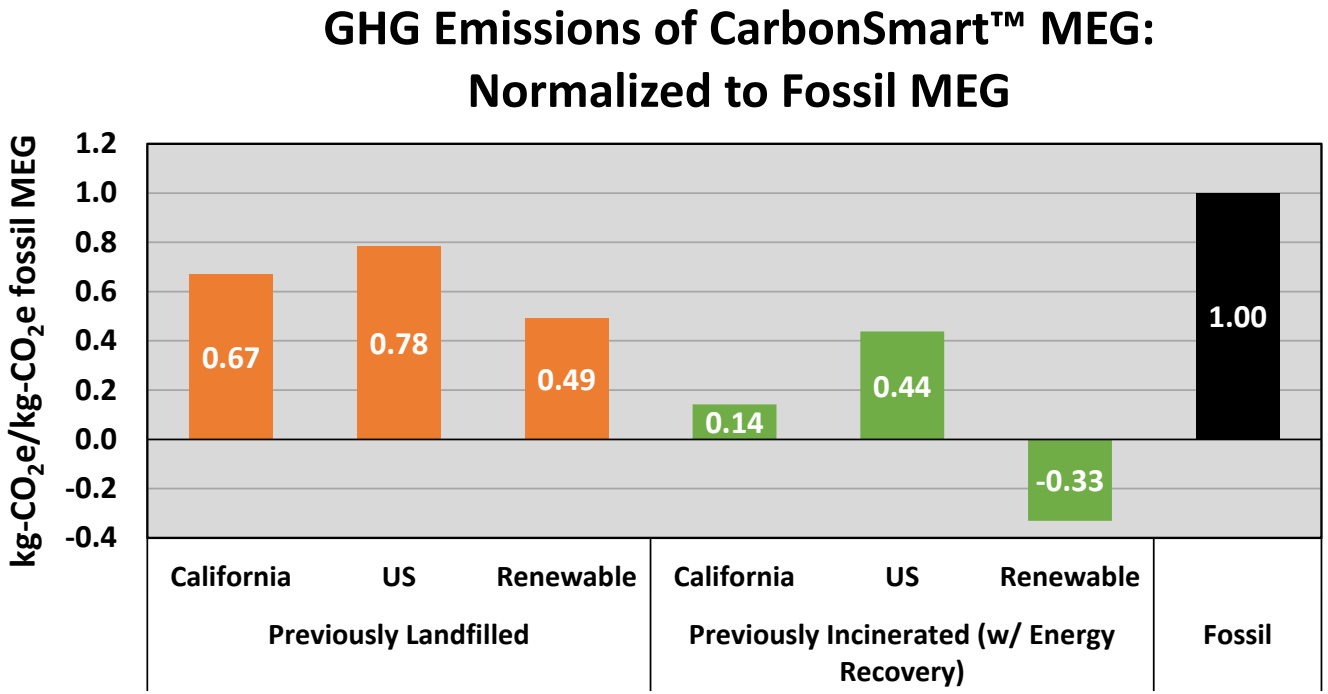


Sensitivity analysis suggested that selectivity is the most important factor in reducing CCOP. (The steeper the line, the bigger the impact of a smaller improvement)

Life Cycle Analysis (LCA)

- LCA evaluates two previous use scenarios for NRP (non-recyclable plastics): disposal in a landfill or incineration with energy recovery (i.e., power production)

- CarbonSmart MEG produced from NRP is estimated to provide a 20% to 130% savings compared to fossil-derived MEG, with greatest savings from using previously incinerated NRP and renewable power.



MSW Collection, Analysis, and Gasification Update

- WM performed augmented audits of waste streams to assess Materials Recovery Facility (MRF) waste stream variability.
- WM provided samples from multiple MRFs to demonstrate waste stream variability
- MRF Residue shown below

Contents As Received

Shredded

- WM samples were analyzed by standard methods (ultimate, proximate, etc.)
- Metals and halogens were also measured
- InEnTec demonstrated proprietary model of syngas composition from solids
- Based on analysis, InEnTec has predicted a syngas suitable for fermentation
- Predicted syngas composition from MSW sources contains >60% $CO+H_2$

Impact

- *Mixed and contaminated waste plastics represent a significant percentage of potentially recyclable MSW residues that are currently landfilled due to a lack of technoeconomically-viable processes for material recycle/upcycle.*
- *LanzaTech is developing technologies to convert these waste plastic residues to MEG, an important chemical in the production of polyethylene terephthalate (PET), a plastic polymer used in common, single use consumer goods, such as drinks bottles, food package as well as polyester fiber in clothing.*
- *MEG produced from non-recyclable plastic wastes, offers a sustainable, cost-effective alternative to fossil-derived MEG, to meet the needs of end users in the chemical and consumer products industries seeking to defossilize.*

    - *Provide a sustainable alternative to petrochemical MEG production.*
    - *Provide up to 130% GHG emission savings over petrochemical method*
    - *Feedstock is derived from non-recyclable plastic waste, does not require land and water usage, does not compete with food resources.*
    - *The MEG biocatalyst developed in this project can be directly dropped in any of the existing LanzaTech commercial plants and start to produce MEG in a short notice.*

Summary

Overview:

- *To develop a novel microbial process to produce monoethylene glycol (MEG) directly from non-recyclable, mixed waste plastic at high yields and efficiency.*

Approach:

- *Interdisciplinary approach of strain & pathway engineering, process development, scale up and economic modelling. State-of-art Clostridium Synthetic Biology, gas fermentation process development and multi-omics combined with integrated models.*

Technical Accomplishments/Progress/Results:

- *Multi-pronged engineering approaches successfully identified pathway bottlenecks and have improved MEG production 400X.*
- *Enzyme variants identified via in vivo assays.*
- *Intermediate feeding experiments identified bottlenecks for MEG production.*
- *InEnTec demonstrated fermentable syngas from multiple MSW feedstocks*

Relevance:

- *MEG is important in production of polyethylene terephthalate (PET), a plastic polymer used in common, single use consumer goods, such as drinks bottles, food package as well as polyester fiber in clothing. MEG produced from the LanzaTech process, using the feedstocks derived from non-recyclable plastic wastes, the project offers a sustainable, cost-effective alternative to meet the needs of end users in the chemical and consumer products industries seeking to "green" their supply chains.*

Summary

Future work:

- *Based on the learnings from past 18 months, the team will focus on integrating the best enzyme variant and MEG producing pathway to C. autoethanogenum chromosome.*
- *Removing by-products formation by deleting gene(s) from the chromosome.*
- *Further process optimization will be carried out for the MEG production strain to identify best run conditions for MEG production.*

Thank you!

Technology Manager: Kathryn Peretti
Project Monitor: Eric Peterson

Quad Chart Overview

Timeline

- *Project start date June 2021*
- *Project end date March 2024*

	FY22 Costed	Total Award
DOE Funding	*$712,125* *(10/01/2021 – 9/30/2022)*	***$2,700,456***
Project Cost Share *	$305,223	$1,890,001

Project Goal

This project is to develop a novel microbial process for the production of ethylene glycol (MEG) directly from non-recyclable plastic syngas at high yields and efficiencies.

End of Project Milestone

Demonstrate MEG production from gasified non-recyclable mixed-plastic waste at lab-pilot scale for a minimum of 3 days

Funding Mechanism

FOA: DE-FOA-0002245
Topic area 2
2020

TRL at Project Start: 0
TRL at Project End: 7

Project Partners*

- InEnTec
- Lululemon Athletica
- Waste Management

Additional Slides

Presentations

Conference	Date	Title	Presenter
Society for Industrial Microbiology and Biotechnology (SIMB) annual meeting	Aug 7-10, 2022	Carbon-negative production of engineered products by gas fermentation at industrial pilot scale	Ching Leang
Society for Industrial Microbiology and Biotechnology (SIMB) annual meeting	Aug 7-10, 2022	Stepping on the gas towards a circular economy: engineering gas fermenting organisms for CarbonSmart Biomanufacturing	Ching Leang
ICIS recycled polymers conference-North America	Dec 6-7, 2022	Upcycling non-recyclable plastic waste through carbon capture technology-Innovating for a CarbonSmart Future	Ching Leang

Responses to Previous Reviewers' Comments

- NA