Bio-Manufacturing Solutions Workshop
February 10th 2021

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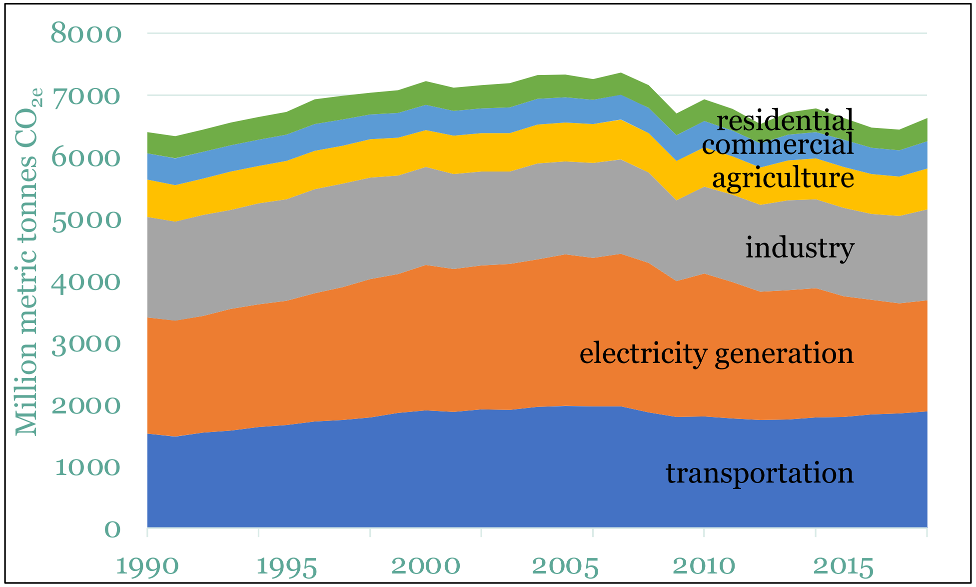
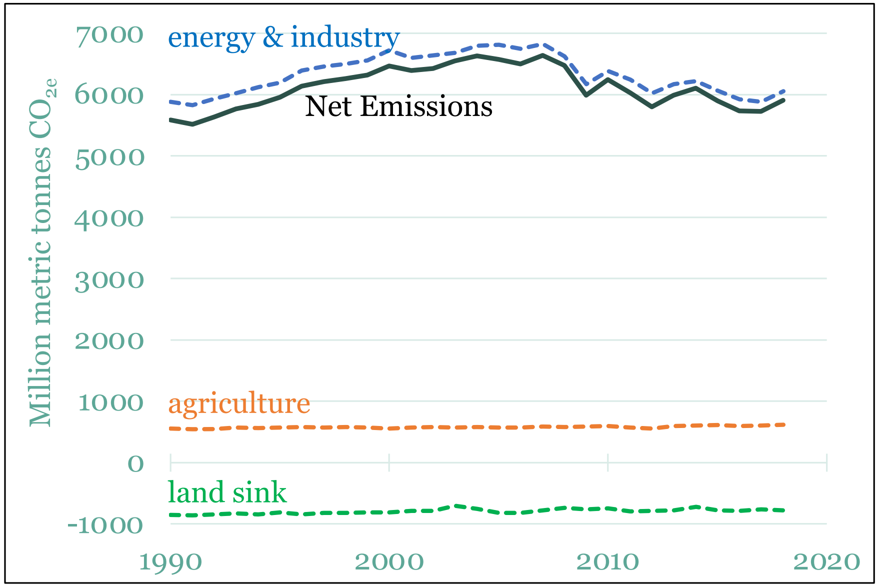
Energy sector and transportation fuels remain a prominent factor contributing to GHG emissions





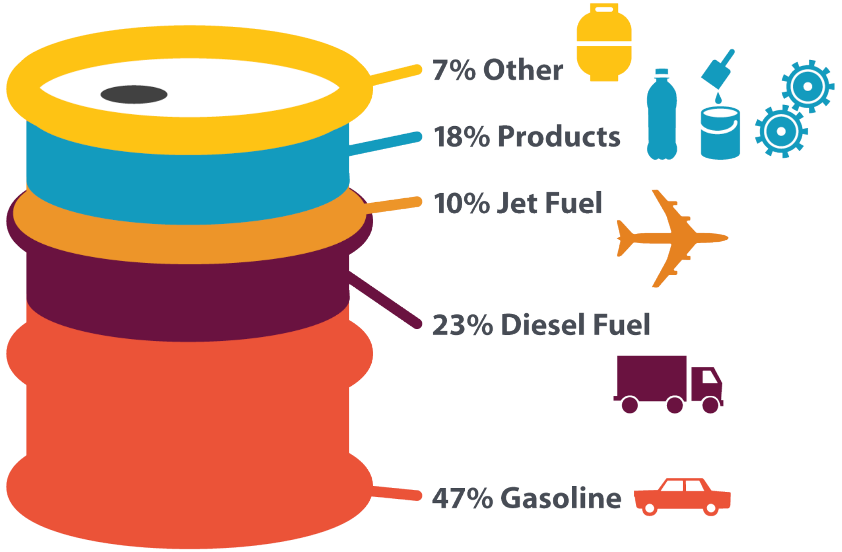
Princeton_NZA_Interim_Report_15_Dec_2020_FINAL

EPA GHG Inventory

Total global emissions rate is ~40 $GtCO_2$/year. (Majumdar and Deutsch, *Joule* 2018)

Energy sector and transportation fuels remain a prominent factor contributing to GHG emissions

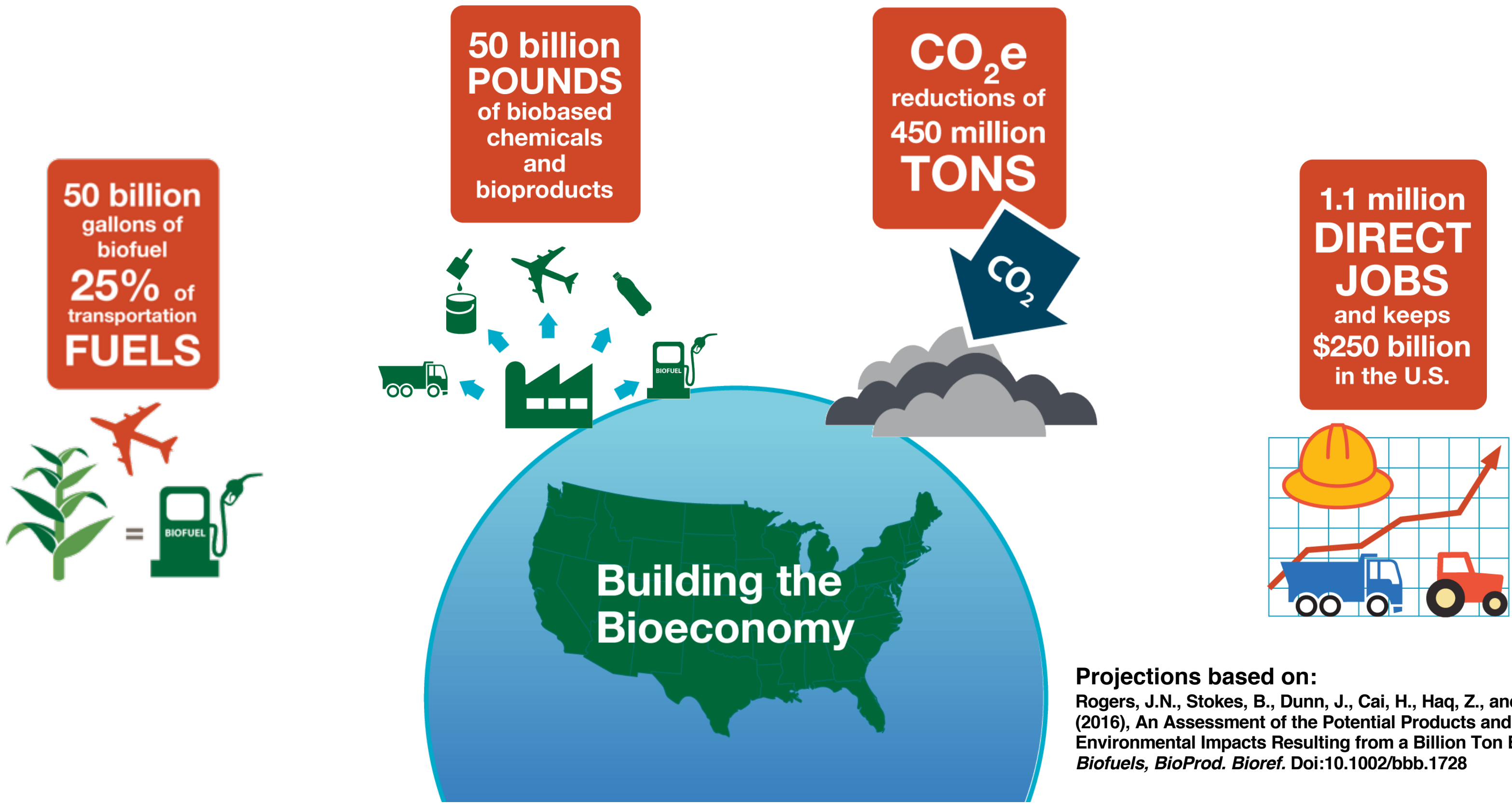
In the U.S. petroleum is the primary source for transportation fuels and chemicals



Source: U.S. Department of Energy

Sustainably-grown biomass could be a source for a significant fraction of these fuels

A billion dry tons of sustainable biomass has the potential to..



Projections based on:

Rogers, J.N., Stokes, B., Dunn, J., Cai, H., Haq, Z., and Baumes, H. (2016), An Assessment of the Potential Products and Economic and Environmental Impacts Resulting from a Billion Ton Bioeconomy. *Biofuels, BioProd. Bioref.* Doi:10.1002/bbb.1728

The US. Department of Energy has funded Four **Bioenergy Research Centers**

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The BRC mission is to develop the science, technology, and knowledgebase necessary to enable sustainable, cost-effective production of advanced biofuels and bioproducts from nonfood plant biomass in support of a new biobased economy.

- Sustainability
- Feedstock development
- Deconstruction
- Conversion

GREAT LAKES BIOENERGY RESEARCH CENTER

cbi THE CENTER FOR BIOENERGY INNOVATION

CABBI CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

JBEI Joint BioEnergy Institute

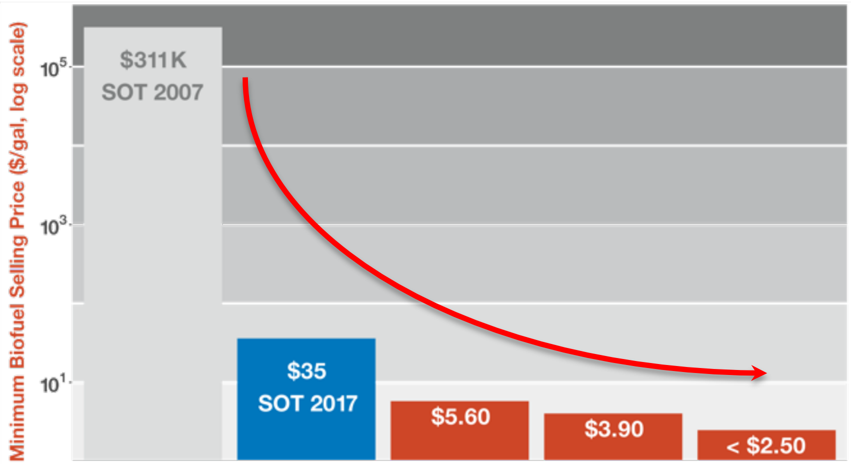
Total funding going back to 2007 > 1B

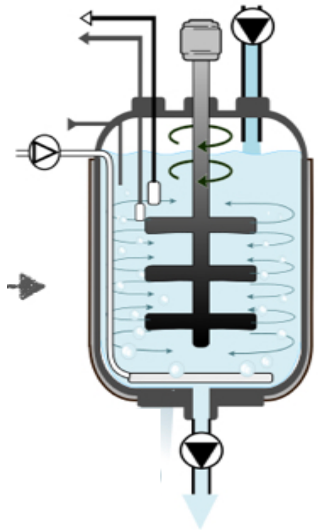
JBEI's basic science provides

- **Engineered bioenergy crops** with low susceptibility to disease and drought that can be readily deconstructed into sugar and aromatic intermediates

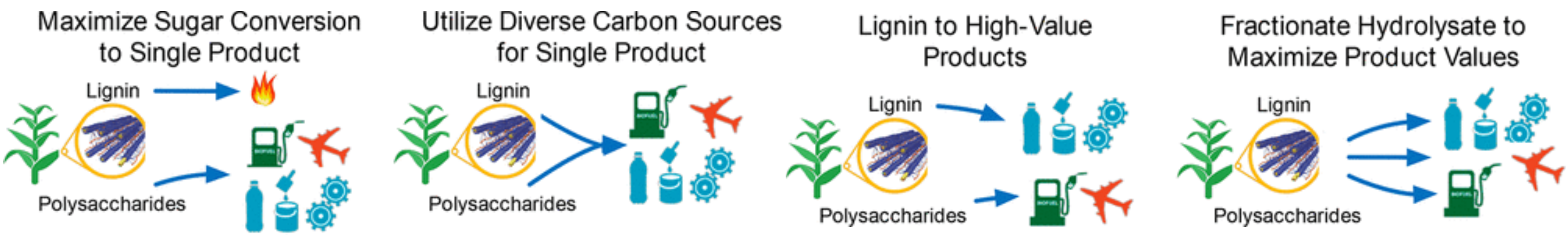
- An **feedstock agnostic deconstruction process** using ionic liquids that liberates ≥90% of sugars and lignin-derived intermediates

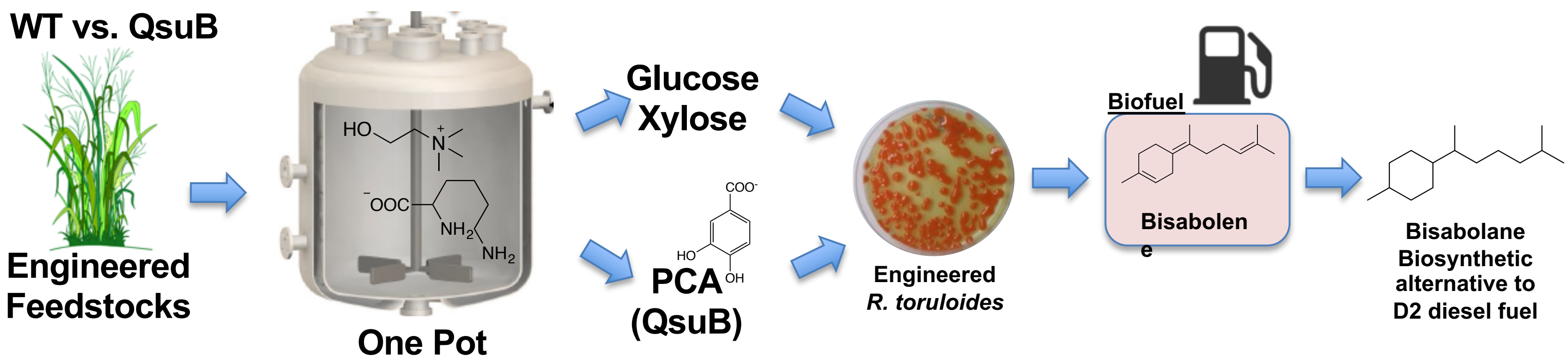
- Engineered microorganisms that **simultaneously utilize the sugars and lignin-derived intermediates** to produce targets at industrially relevant titers, rates, and yields (TRY)





The goal is to provide many options



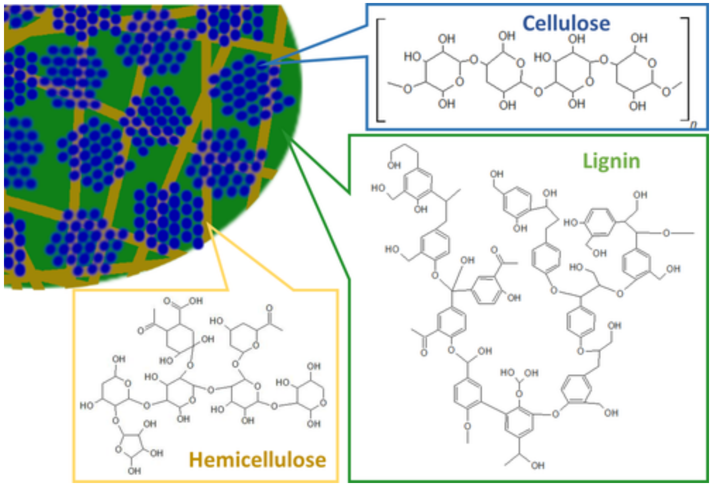


Baral et al ACS Sustainable Chem. Eng. 2019
Rodriguez et al. Biores. Technol 2019

Abundant sustainable and renewable Feedstocks vary geographically

Plant-based biomass can vary considerably and require different deconstruction and thus different downstream conversion host

Lignocellulosic biomass is sugars (2/3) and aromatics (1/3)



Dahmen et al 2018 GCB-Bioenergy

Cultivated Lignocellulosic biomass is not the only carbon feedstock

Gas feedstocks

Ag Waste/ forage

Municipal solid waste

Other waste streams

We need conversion systems for all of these available feedstocks

Considerations for host microbe selection ..

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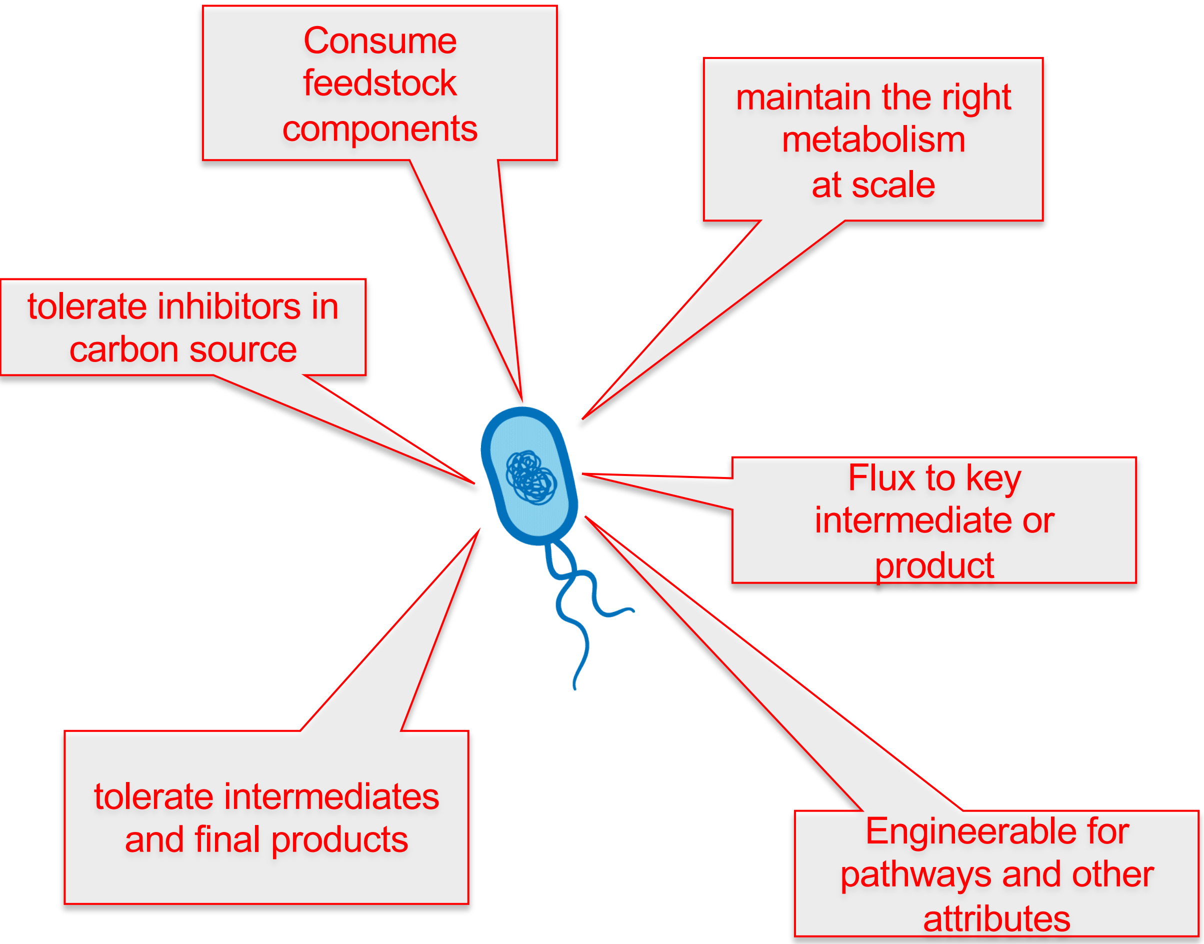
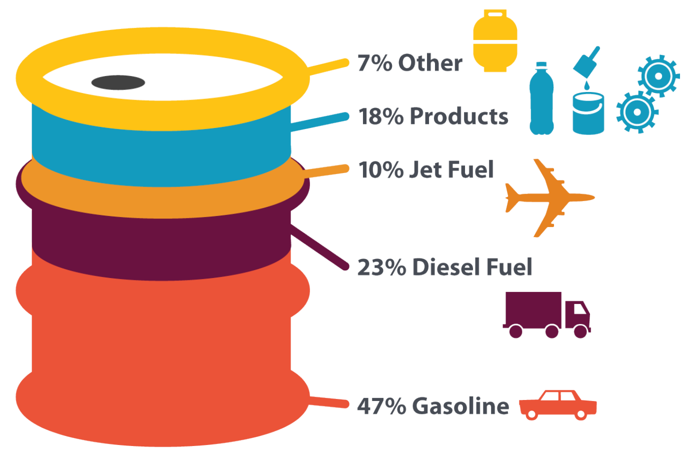


Biomanufacturing can target a range of products



Are all target bioproducts amenable to all hosts?

No..

Bioproduct case study: Need for sustainable materials for dyes and pigments

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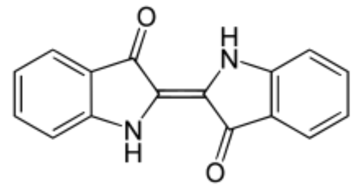
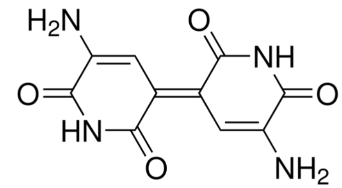
http://www.tejidosroyo.com/



Indigo



Indigoidine

Is this pigment really the best candidate to reduce GHG emissions?? Both TEA and LCA are required

Common industrial yeast would not be the ideal candidate for this compound

Molar yields using Glucose	Different industrial hosts used as production platforms				
	P. putida	*C. glutamicum*	*E.coli*	*R. toruloides*	*S. cerevisiae*
Indigoidine	0.54	0.4	0.4	0.5	0.079
Glutamine	1.14	1	1.14	1.12	0.48
Biomass	0.098	0.092	0.088	0.075	0.029
Genome-scale metabolic model	iJN1462[1]	iCW773[2]	iML1515[3]	iRhto1108C[4]	iMM904[5]
ATPM	0.92	-	6.86	1.012	1

ATPM – ATP maintenance

Genome-scale models used in this analyses:

[1]Nogales *et al.* (2020). *Environ. Microbiol. 22*(1), 255–269.
[2]Zhang *et al.* (2017). *Biotechnol. Biofuels*, *10*(1), 1–16.
[3]Monk *et al.* (2017). *Nat. Biotechnol.*, *35*(10), 904–908.
[4]Dinh *et al.* (2019). *Metabol. Eng. Commun.*, *9*, e00101.
[5] Mo *et al.* (2009). BMC Syst. Biol. 3: 37.

Banerjee, Eng et al. Nat Commun 11, 5385 (2020).

Microbes engineered and optimized to produce high levels of Indigoidine

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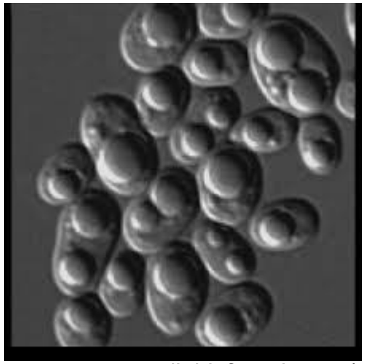
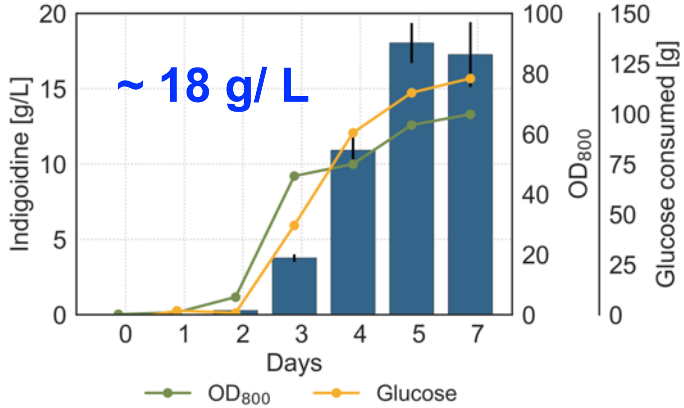
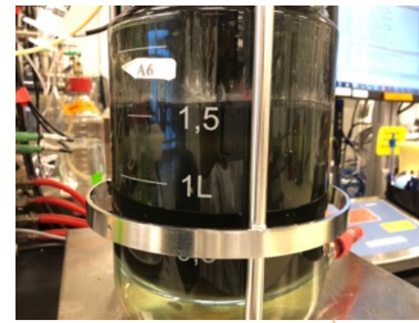
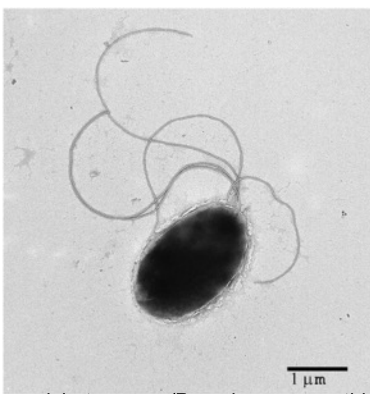


agilebiofoundry.org/

R. toruloides is a oleaginous yeast

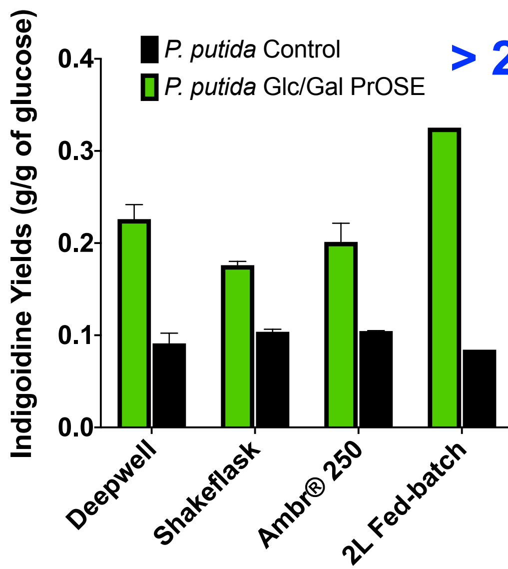






alchetron.com/Pseudomonas-putida

P. putida is a soil bacterium



> 25 g/ L

Engineered for scale.

ABPDU
ADVANCED BIOFUELS AND BIOPRODUCTS PROCESS DEVELOPMENT UNIT

Team Science
Long term goals
Need partners

BERKELEY LAB | U.S. DEPARTMENT OF ENERGY

US Patent Appl. Ser. No. 62/980,054, 21-Feb-2020
16/417,499 (2019/04/20)

Banerjee, Eng et al. Nat Commun 2020
Wehrs, et. al., Green Chemistry 2019

Leveraging synergies between BER and EERE

ABPDU

ADVANCED BIOFUELS AND BIOPRODUCTS PROCESS DEVELOPMENT UNIT

Agile BioFoundry

ABPDU understands and solves Scale-up Challenges

BioSciences

Supported by DOE EERE from the BioEnergy Technologies Office (BETO)

ADVANCED BIOFUELS AND BIOPRODUCTS PROCESS DEVELOPMENT UNIT

Worked with over 60 companies

Supporting Industry in Raising Non-Dilutive Funds

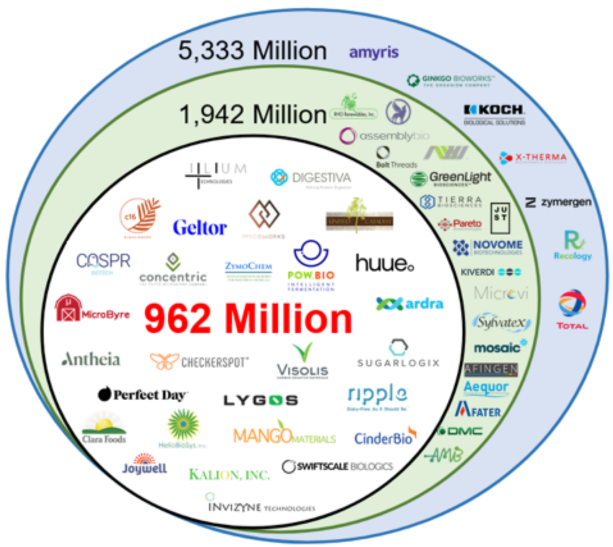
SBIR · STTR America's Seed Fund

NIH National Institutes of Health

USDA

DARPA

NSF



Securing Private Financing

DOE Agile BioFoundry

BioSciences

- **Goal**: Enable biorefineries to achieve 50% reductions in time to bioprocess scale-up as compared to the current average of around 10 years by establishing a distributed Agile BioFoundry to productionize synthetic biology

- **Outcomes**: Development and deployment of technologies enabling commercially relevant biomanufacturing of a wide range of bioproducts by both new and established industrial hosts

- **Relevance**: $20M/year public infrastructure investment that increases U.S. industrial competitiveness and enables opportunities for private sector growth and jobs

- **Risks**: Past learnings do not transfer well across target molecules and microbial hosts. Experiment data sets are of insufficient quality/quantity/consistency to learn from

Barriers – these are interrelated topics

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The barriers that limit biomanufacturing's contributions to climate change mitigation include:

•Lack of focus on and support for this objective among bioscientists and -engineers
•Mismatch between organisms used in laboratories and those best-suited to biomanufacturing
•Difficulty and cost of using the most abundant and sustainable feedstocks
•Inefficiency of scaled-up processes and high cost of separation and purification
•Inability to predict results of scaling-up, leading to variation in output
•Insufficient systems for data analysis and integration
•Lack of domestic intermediate-scale facilities for process development and optimization
•Lack of funding for demonstration and early commercial production facilities
•Poor technology transfer and lack of standardized process recipe tools
•Weakness of end-use markets to stimulate sufficient private investments in innovation
•Potential conflicts over the future of agriculture

Next steps.. (no silver bullets)

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- Renewable Jet fuels is a key piece in GHG reductions.
  - Careful selection (TEA, LCA) of non-fuel targets can help offset GHG emissions in meaningful ways
- Development of the process needs to consider
  - Diverse Feedstocks and starting material
  - Selecting the host to match the feedstock and product
  - Starting with Scalability and separations in mind
- Federal funds support many valuable efforts – DOE, USDA and others but this interdisciplinary problem requires cross/ inter-agency work
- For successful tech transfer
  - The pieces need to come together – team science
  - End to end optimizations take time – long term funding
  - Big team long term projects may need different set of incentives and metrics to measure success, than what is currently in place
- More such workshops that initiate/ continue dialogue on what barriers still exist. With Scientists, engineers, economists – we may also need social science folks.