NREL Transforming ENERGY

PET Upcycling

March 5th, 2019

Technology Session Review Area: Biochemical Conversion

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National Renewable Energy Laboratory and Oak Ridge National Laboratory

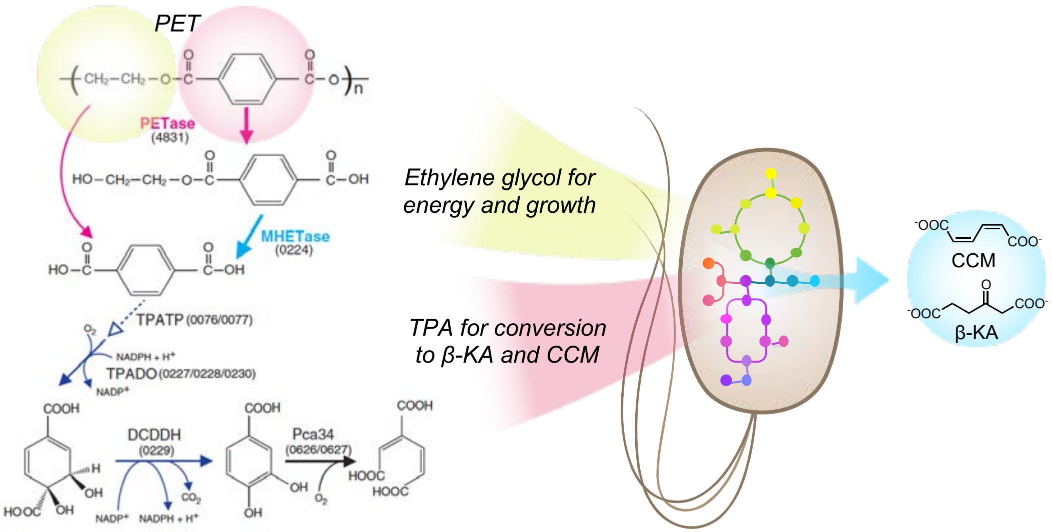
Goal statement

Goal: Develop cost-effective biological methods to upcycle PET

- Focus on polyethylene terephthalate (PET) that typically is landfilled, namely fibers in clothing and carpets, with bottles as a secondary substrate
- Employ biological and chemo-catalytic strategies for PET breakdown and upcycling

Outcome: 50% yield of a co-product (ß-ketoadipate) from reclaimed PET fibers

- Task 1: Consolidated Bioprocessing-like strategy
- Task 2: Hybrid catalytic and biological strategy via a Separate Hydrolysis and Fermentation-like strategy



Relevance: Plastics are still mostly landfilled or become environmental waste

- Can leverage decades of investment by BETO in interfacial biocatalysis, process development, metabolic engineering, chemical catalysis to enable the Circular Materials Economy for plastics
- Upcycling could enable industry at the interface of the bioeconomy and recycling

Adapted from Yoshida *et al.*, *Science* 2016

Quad chart overview

Timeline

- Start date: October 2018
- End date: September 2020
- Percent complete: 25%

	Total Costs Pre FY17	FY17 Costs	FY18 Costs	Total Plan Funding (FY19-Project End Date)
DOE funded	--	--	$300k	$300k

Partners:

BETO Projects: Biochemical Processing Modeling and Simulation, PET Upcycling – ORNL

Companies: IBM, Shaw Floors

Universities: Montana State University, University of Portsmouth, University of Kentucky, University of Georgia, Jülich, RWTH Aachen, University of Dublin

Barriers addressed

- **Ct-D Advanced Bioprocess Development**
  - Working on enzyme and microbe development for breaking down and converting solid plastics
- **Ct-B Efficient Preprocessing and Pretreatment**
  - Examining how to pretreat plastics prior to biological conversion

Objective

Develop efficient, cost-effective strategies that harness biological and catalytic systems for PET depolymerization and conversion to an exemplary value-added compound ß-ketoadipate.

End of Project Goal

50% yield of ß-ketoadipate from either a hybrid biological-catalytic strategy or a wholly biological strategy using reclaimed PET fibers from an industrially relevant stream. 80% PET biodegradation in ≤ 2 weeks or 80% conversion of BHET to ß-ketoadipate at 6.25 g/L/day rate.

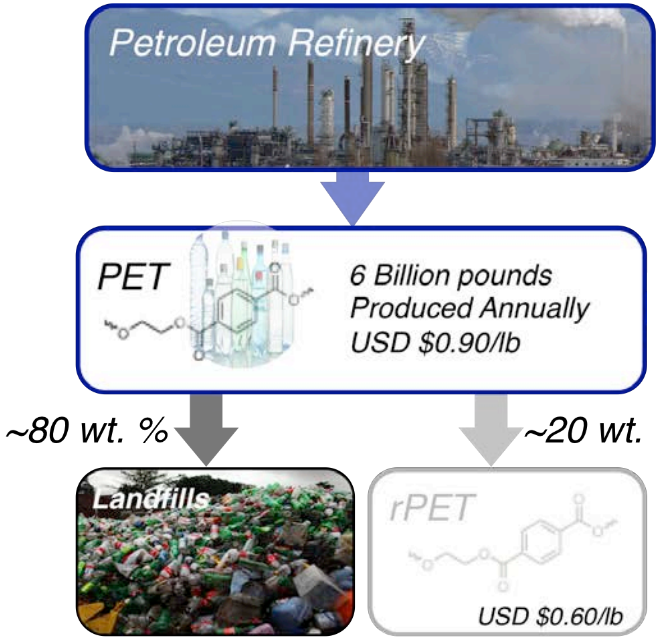
Project overview

History: New start seed project in FY19 for 2 years

- Public and scientific awareness of plastics pollution is mounting
- Biological and chemical PET breakdown has long been studied: Mostly PET-to-PET recycling
- Terephthalic acid and ethylene glycol catabolic pathways reported

Context: PET recycling today is downcycling

- PET among top 5 most abundantly manufactured plastics
- Very similar problem to biomass conversion: heterogeneous, polymer substrate that is recalcitrant to enzymatic and catalytic breakdown



Project Goals:

- Engineer efficient terephthalic acid and ethylene glycol catabolism into a base microbe to convert aromatic carbon to value-added product, ß-ketoadipic acid
- Engineer PETase enzyme secretion into same host for extracellular PET breakdown
- Employ catalyzed glycolysis process (with IBM) to produce PET monomers
- Conduct bioprocess development to demonstrate feasibility of two processes on fibers

Management approach

Team composition and structure:
- Metabolic engineering (Adam Guss, Lahiru Jayakody)
- Polymer characterization (Nicholas Rorrer)
- Microscopy (Bryon Donohoe)
- Interfacial biocatalysis and process development (G. Beckham)

Project Interfacing:
- Project meetings with ORNL once a month
- Meet with BETO Tech. Manager once a month
- Interface with industry and academic groups on plastics biodegradation

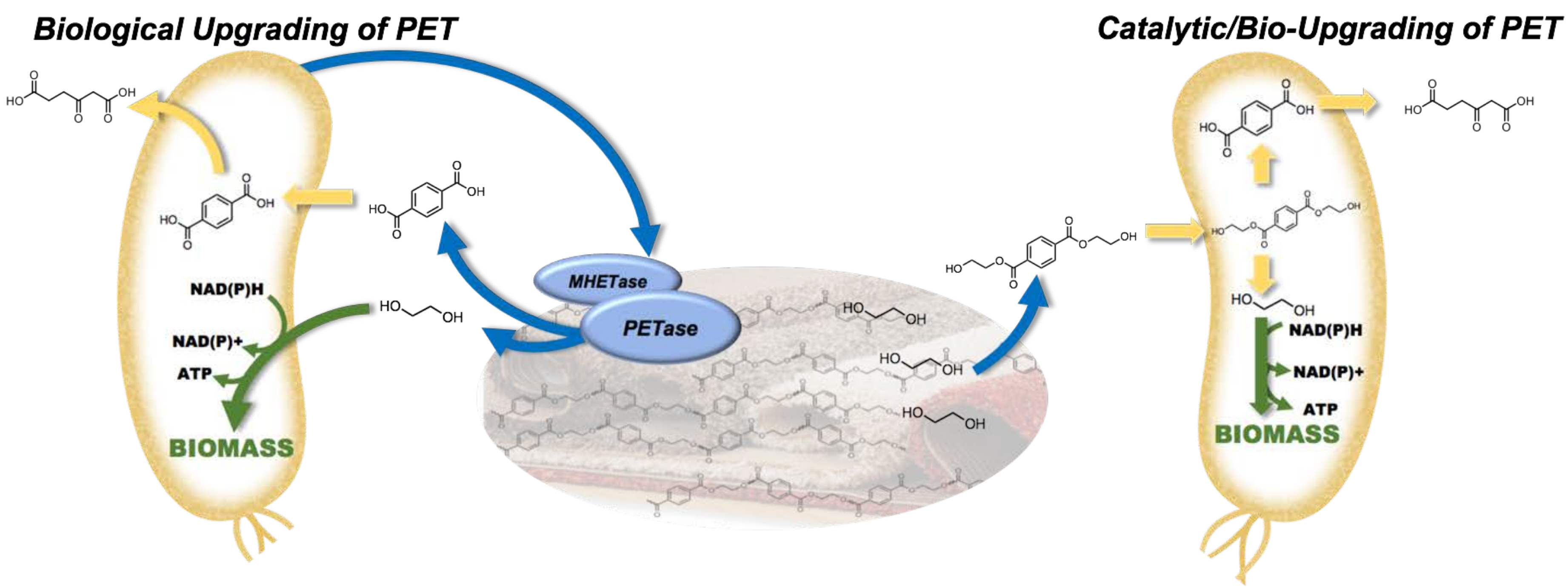
Milestones:
- Building strains, strain deployment, process development for yield targets on PET fibers
- Work with IBM on catalyzed glycolysis efforts

Industry engagement:
- Conduct “customer discovery” interviews
- Review what recycling and chemical companies will consider for new processes on waste plastic

Technical approach: Task breakdown

Two tasks

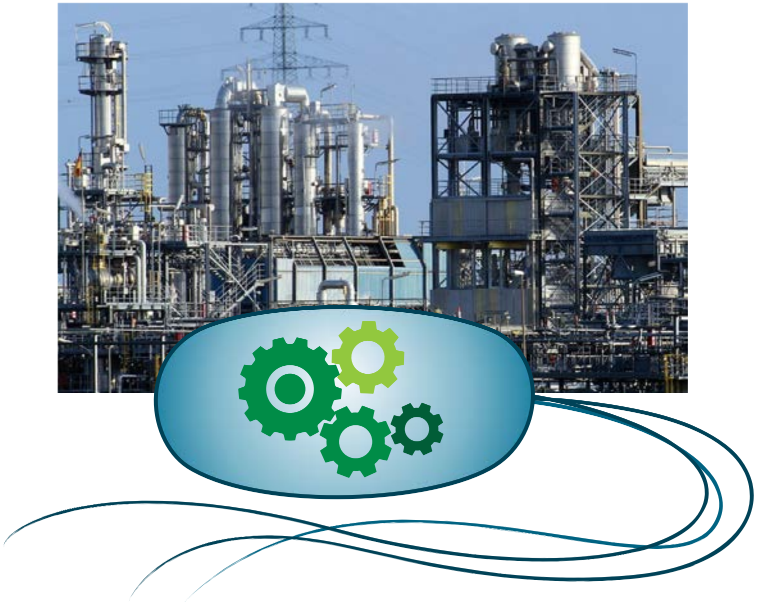
- Task 1: Solely biological approach to PET conversion (Consolidated Bioprocessing)
- Task 2: Hybrid biological and catalytic approach to PET conversion (Separate Hydrolysis and Fermentation)



Technical approach: Biology and substrate

Approach (biology):

- *Pseudomonas putida* KT2440 as a chassis organism (aromatic-catabolic bacterium)
- Genomic integration of all genes
- Leverage novel tools for gene integration from ORNL
- Convert terephthalic acid to ß-ketoadipate
- ß-ketoadipate can be used for advantaged bioproducts (e.g., improved nylons)
- Use ethylene glycol as a carbon/energy source



Approach (substrate):

- Use reclaimed PET fibers from Shaw Floors and other industry partners

Challenges:

- Genomic integration of heterologous genes
- PET conversion across substrates

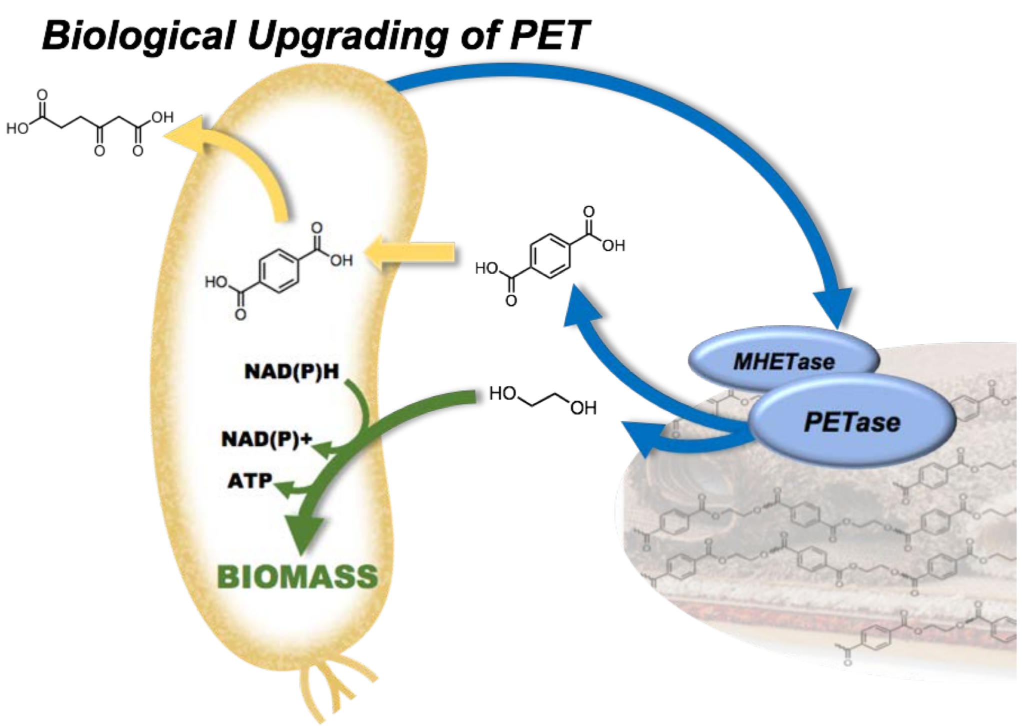
Technical approach: Task 1

Task 1: Employ a wholly biological approach to break down and upcycle PET (Consolidated Bioprocessing)

Critical Success Factors:
- Efficient biological breakdown of PET on a timescale commensurate with bioprocessing
- Efficient and sufficient enzyme secretion by an engineered microbe

Approach:
- Use *Ideonella sakaiensis* PETase and MHETase enzymes to break down PET extracellularly
- Catabolize terephthalic acid to ß-ketoadipate via known pathway
- Use ethylene glycol as the carbon and energy source

Challenges:
- High yield PET breakdown from secreted PETase/MHETase
- Balance of depolymerization and catabolic module expression



Yoshida *et al.*, *Science* 2016

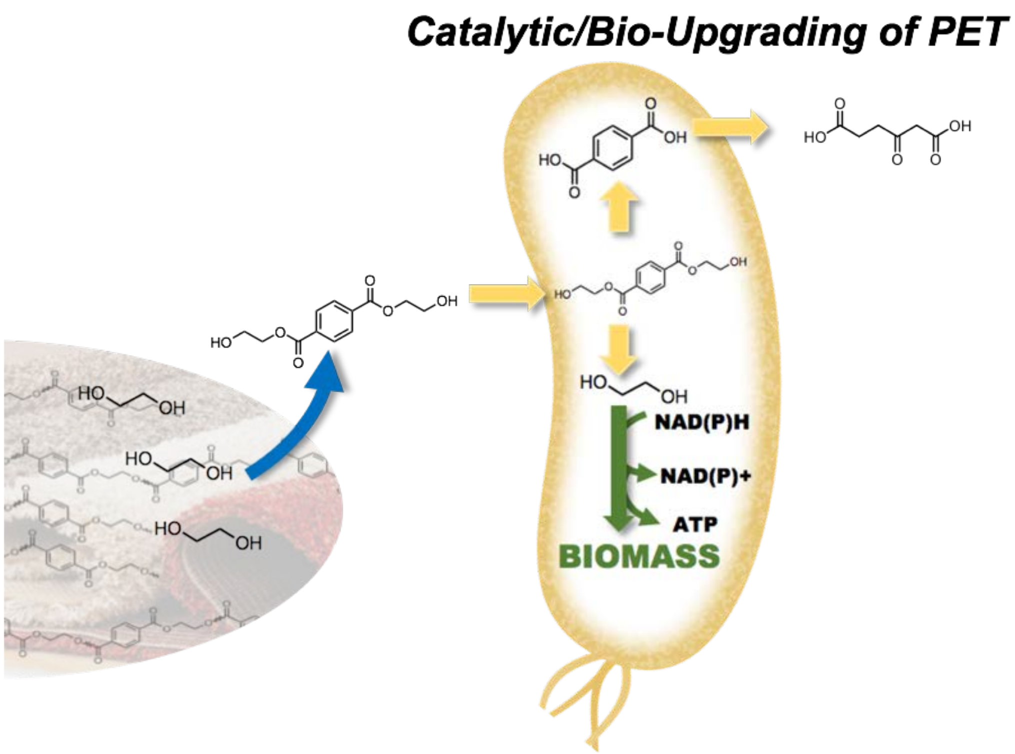
Technical approach: Task 2

Task 2: Employ a hybrid biological-catalytic approach to upcycle PET

Critical Success Factors:
- Economics of using catalytic step with excess ethylene glycol
- High titer, rate, and yield with xenobiotic compound

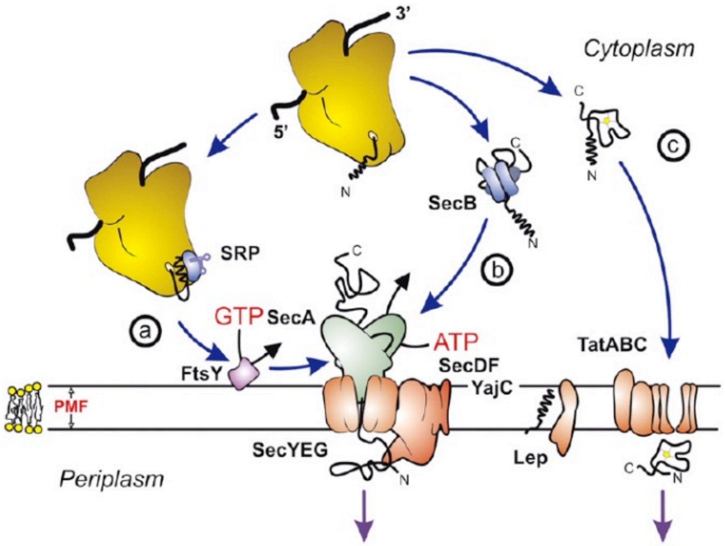
Approach:
- Catalyzed glycolysis process from IBM that employs volatile alkylamine with ethylene glycol to produce BHET
- Engineer *P. putida* to catabolize BHET to ß-ketoadipate with *intracellular* PETase and MHETase
- Bioprocess development and evolution for titer, rate, yield
- Prospecting for improved pathways

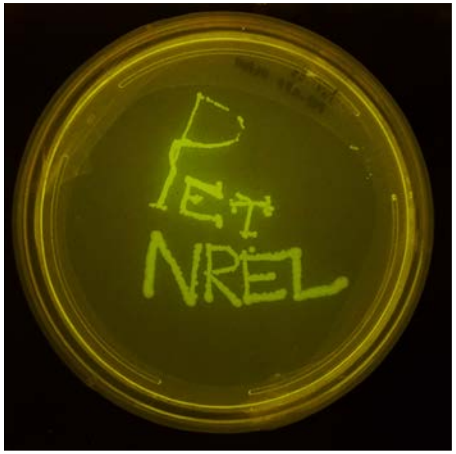
Challenges:
- BHET transport across cell membrane
- PETase enzyme with optimal activity on BHET

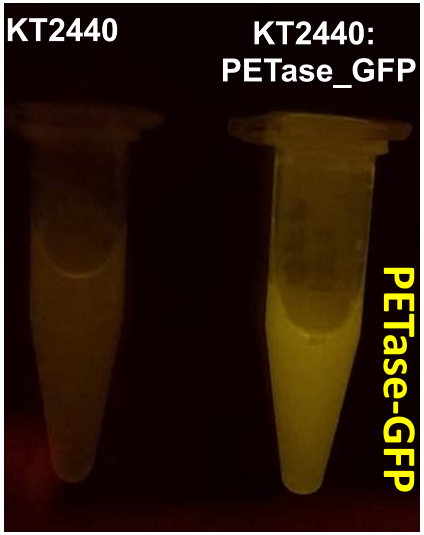


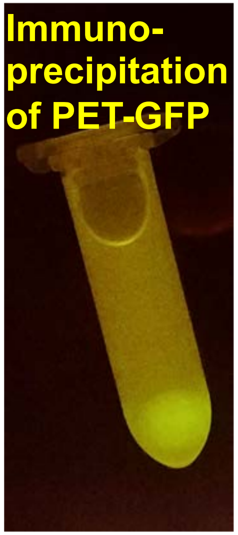
Yoshida *et al., Science* 2016
Masai *et al.,*

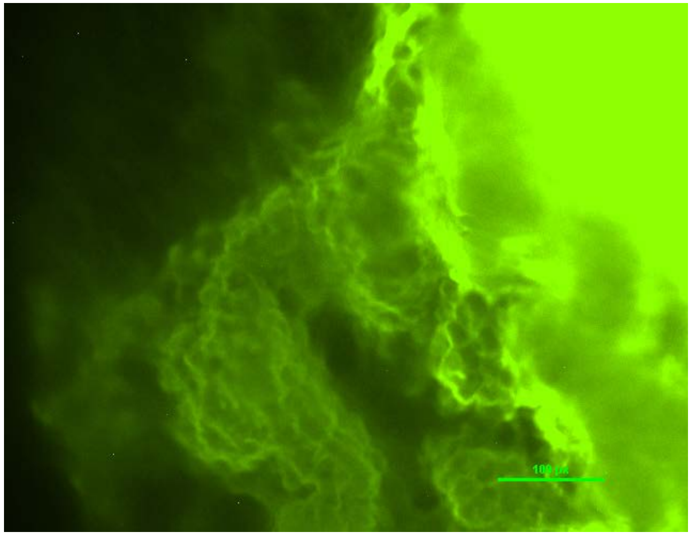
Demonstrate PETase and MHETase secretion in *P. putida* through fluorescent protein labeling





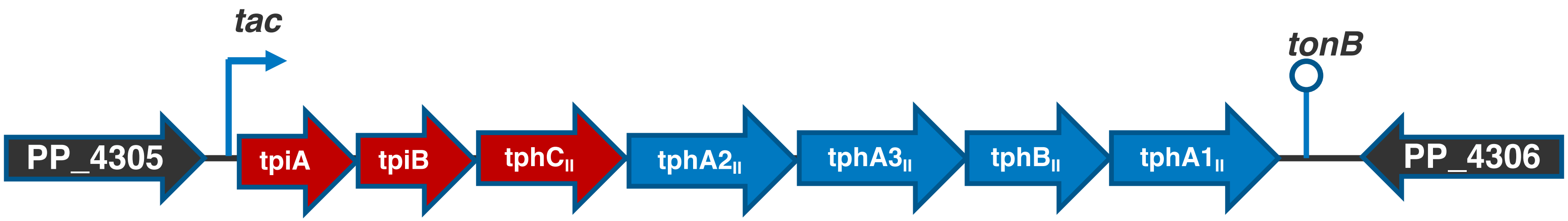




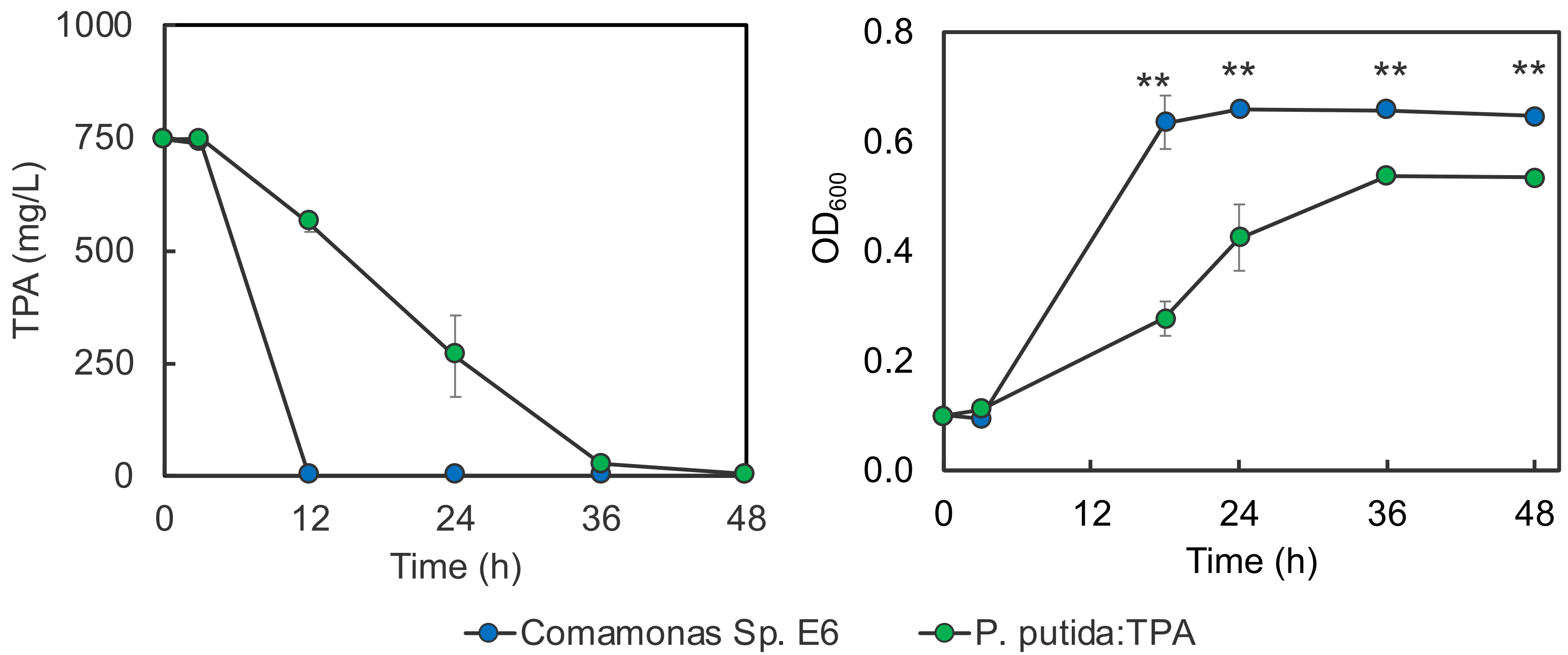


Technical accomplishments: Q2 Milestone

Integrate and test TPA transport and catabolic genes into *P. putida* and baseline initial TPA catabolic rates



Shake flask experiment

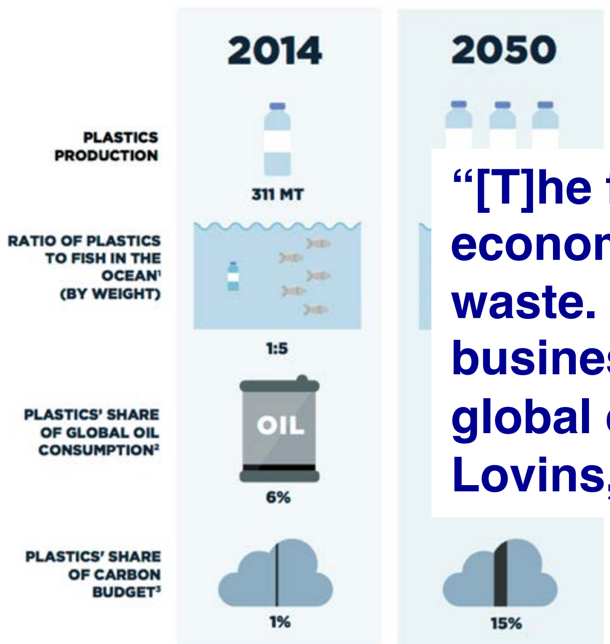


Relevance

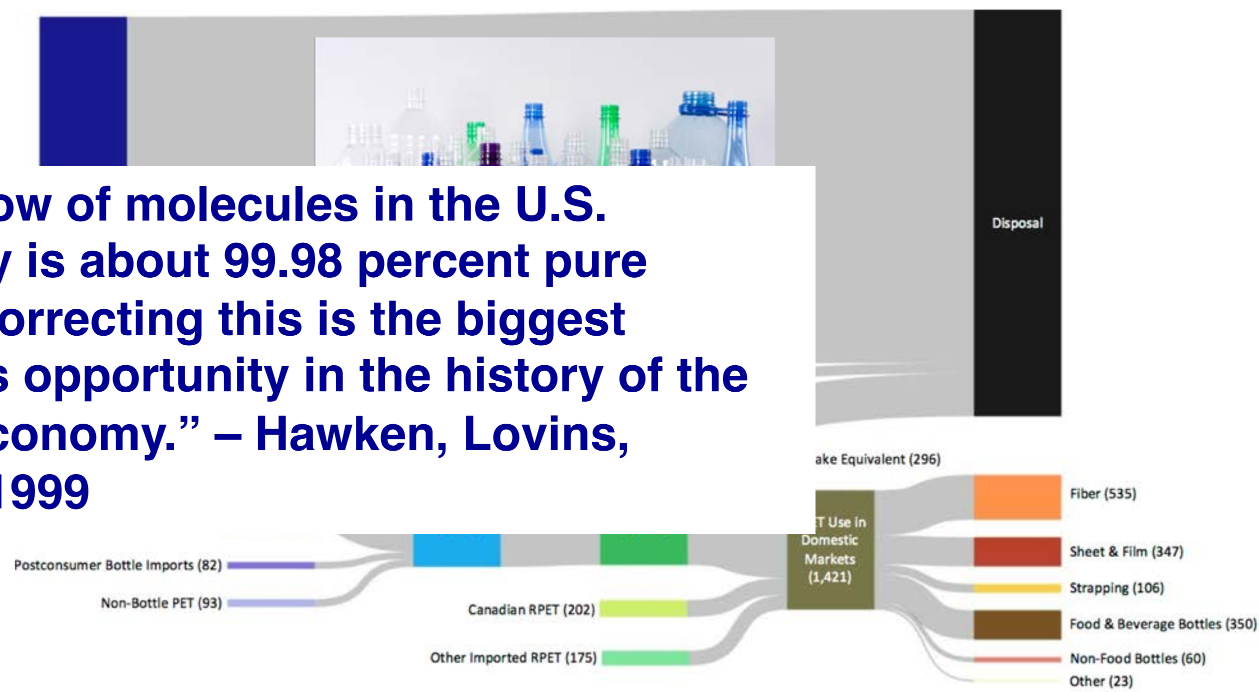
Goal: Develop bio-based strategies to *up-cycle* PET to value-added compounds

Why is this project important and what is the relevance to BETO and bioenergy goals?

- Plastics are causing an environmental crisis; upcycling can enable greater reclamation
- Bioenergy R&D can leverage massive investment to solve this very similar problem
- Can enable expansion of bioenergy R&D into new, societally critical directions



"[T]he flow of molecules in the U.S. economy is about 99.98 percent pure waste. Correcting this is the biggest business opportunity in the history of the global economy." – Hawken, Lovins, Lovins, 1999



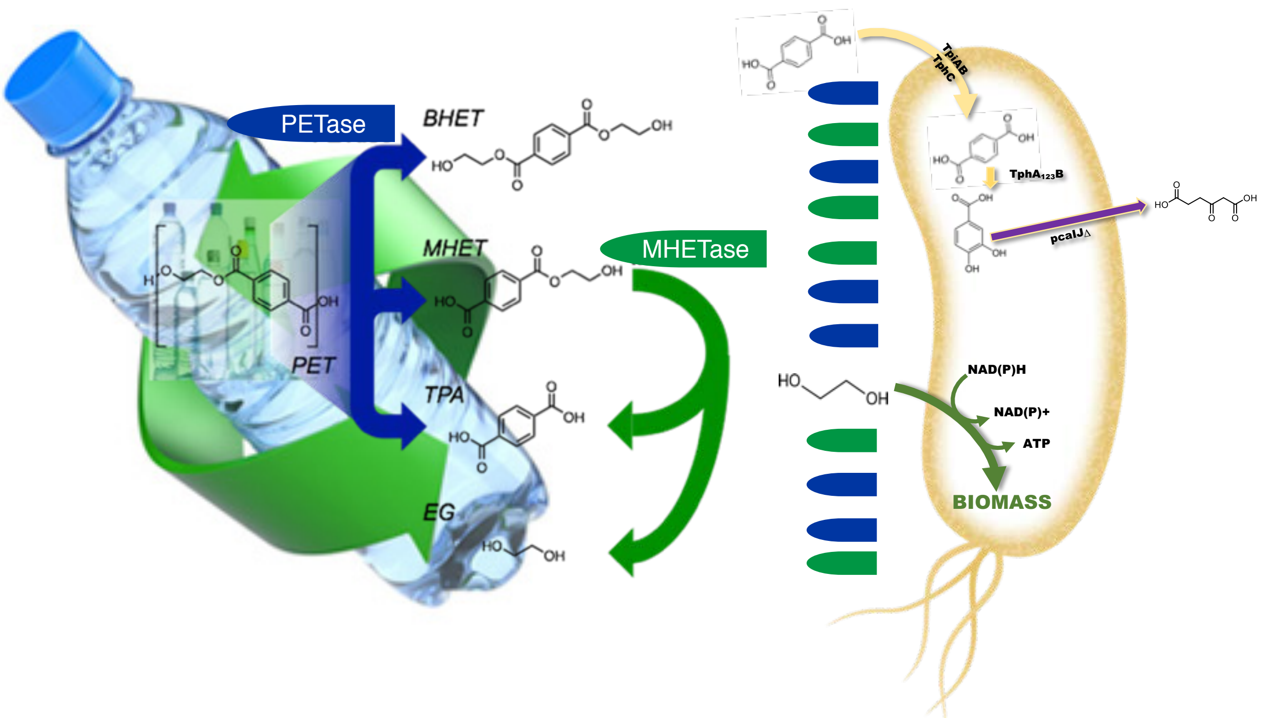
* This total represents all clean flake sold into end markets by US reclaimers. See figure 7 for detail on total flake produced by US reclaimers from bottles.

The New Plastics Economy, Ellen Macarthur Foundation (2016)
Report on Postconsumer PET Container Recycling, NAPCOR (2015)
Business Insider, Sailors for the Sea (2012)

Relevance

How does this project advance the State of Technology?

- Plastics recycling today is almost universally down-cycling
- Chemical recycling of PET today is mostly bottle-to-bottle: economics are challenging
- Bio-based solutions (enzymes, microbes, chemical catalysts) towards upcycling can offer a new strategy to advance beyond the State of Technology
- Can ultimately enable new bio-based products from waste plastics



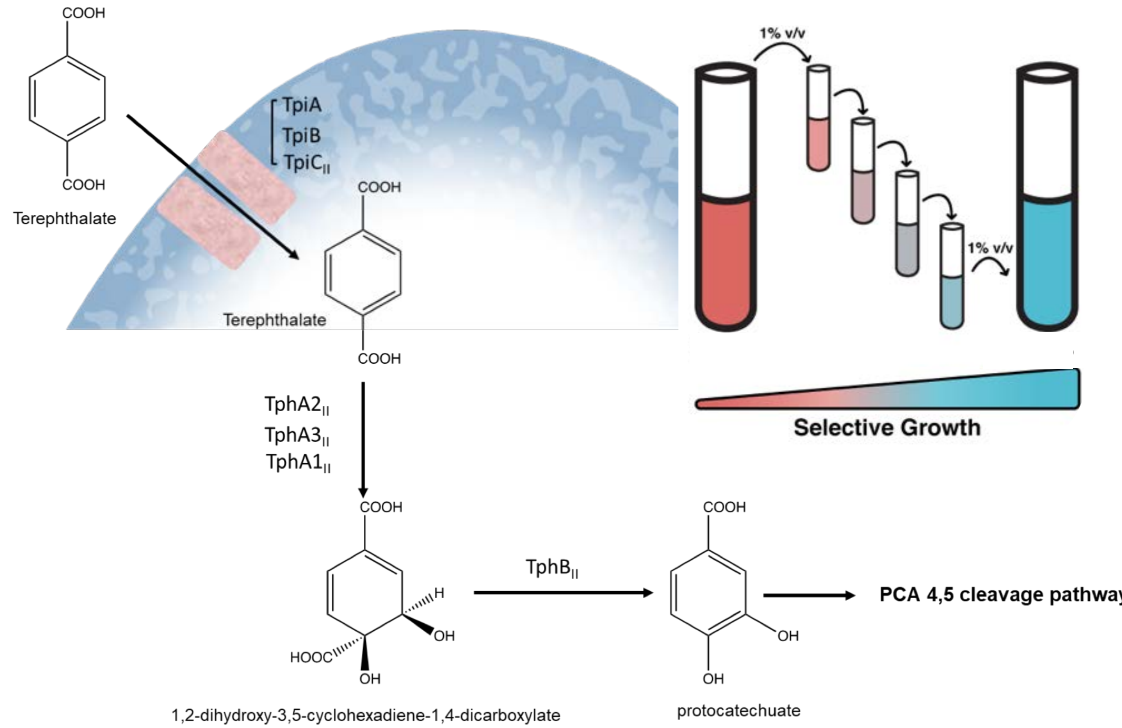
Technology transfer activities

- Filing IP applications on evolved enzymes, new pathways, all strains
- Work with IBM, Shaw Floors, et al.
- Publishing findings in peer-reviewed journals
- In talks with industrial partners for new products
- Energy I-Corps proposal in FY19/FY20

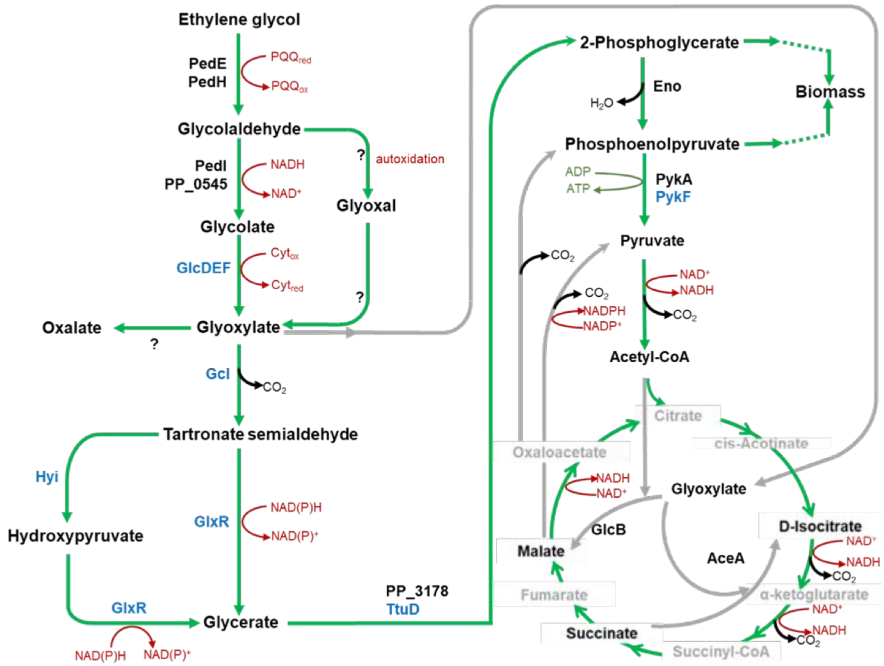
Future work: Further improvement to terephthalic acid catabolism

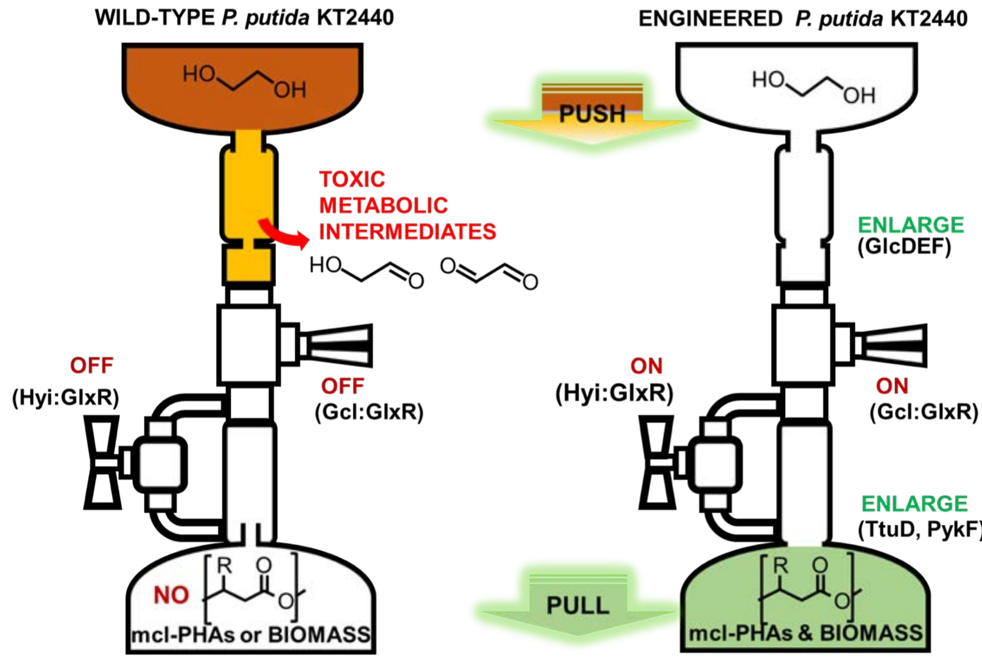
Terephthalic acid catabolism is a key driver of titer, rate, yield of any targeted product

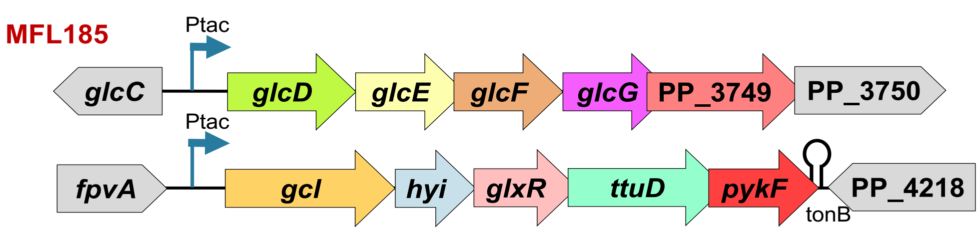
- Currently adapting pathway from *Comomonas* sp. E6 (Masai *et al.*)
- Conducting adaptive laboratory evolution for TPA catabolic pathway in *P. putida*
- Prospecting for improved TPA and BHET catabolism pathways from natural sources
- Upcoming milestone



Future work: Incorporate ethylene glycol catabolism







Known pathway for EG metabolism

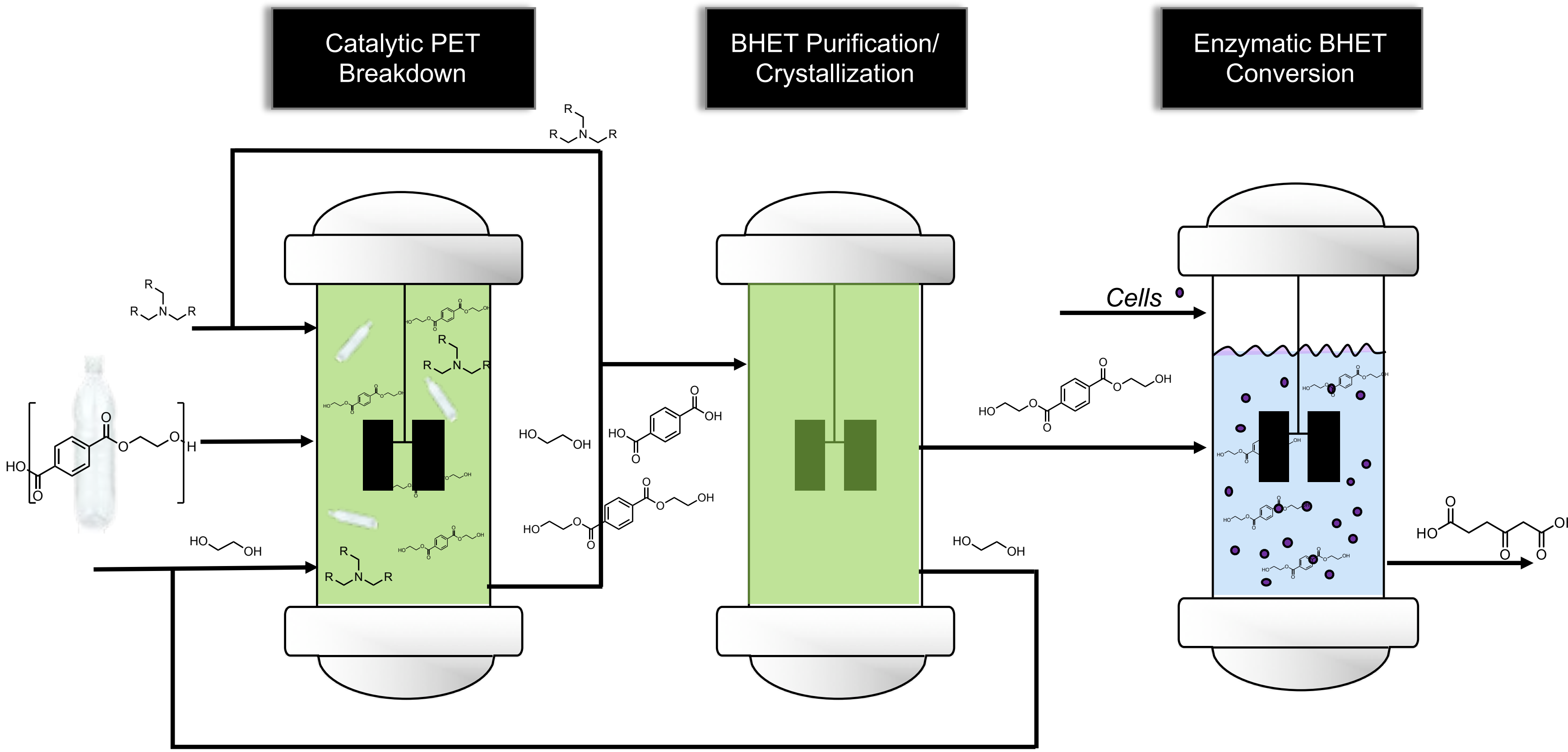
- Incorporating EG pathway in KT2440 into engineered PET degrading strains for both tasks
- Upcoming milestone

Franden, Jayakody *et al.* *Metabolic Engineering* 2018

Future work: Catalyzed glycolysis of PET

Task 2 is leveraging innovations from IBM on catalyzed glycolysis

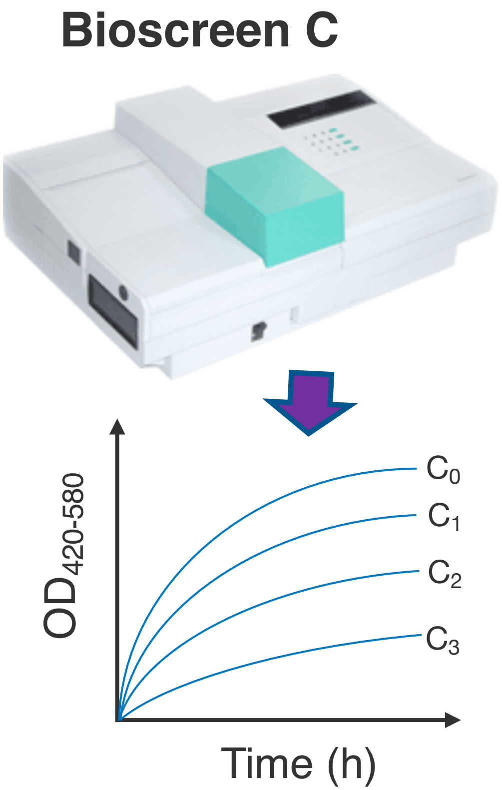
- Employing process on multiple, industrially-relevant PET-rich substrates
- Conducting parameter sweeps to understand catalytic chemistry
- Using synthetic BHET currently as a substrate for bioprocess development

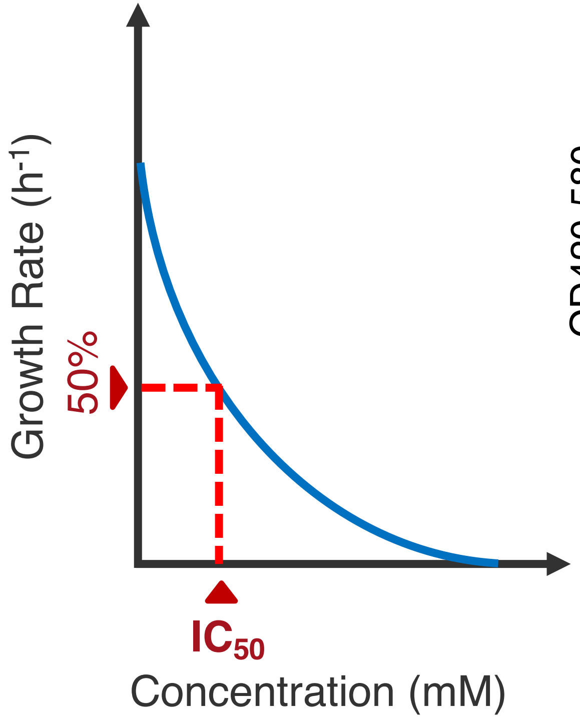


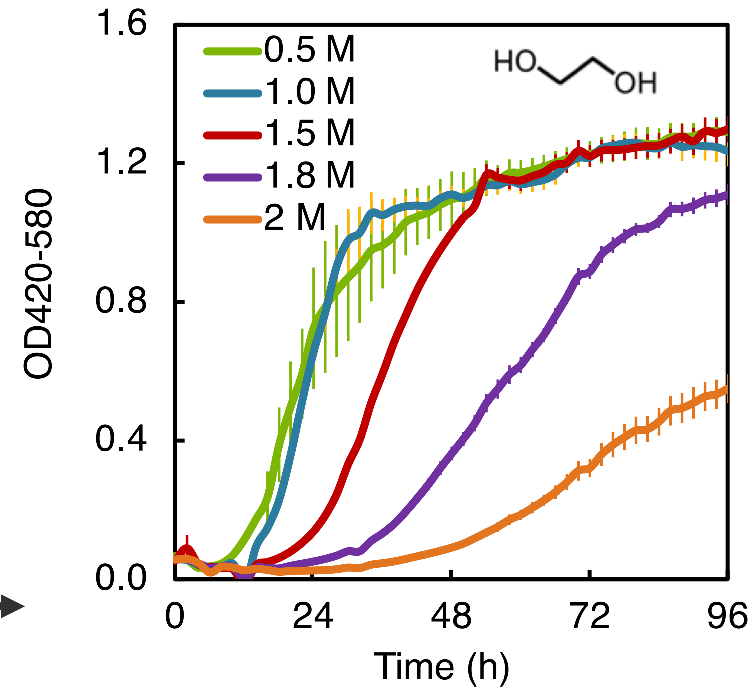
Future work: Toxicity measurements ongoing

Both tasks will need to know substrate toxicity limits for microbe (upcoming milestone)

- Measuring IC_{50} values currently for TPA, BHET, MHET, EG
- Ethylene glycol catabolism produces well-known toxic compounds (e.g., glycolaldehyde)
- For Task 2, these data will be critical for bioprocess development





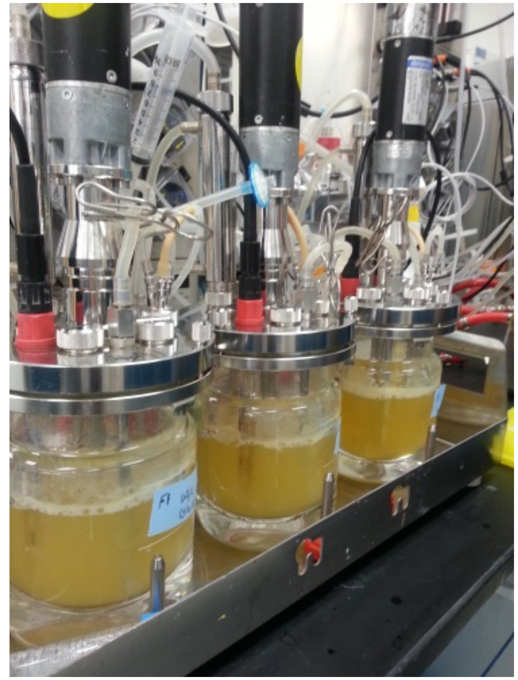


IC_{50} of EG on wild-type ~ 0.02 M
IC_{50} of EG on MFL185 ~ 2 M

Future work: Bioprocess development activities

Leveraging expertise from other BETO projects in bioprocess development for *P. putida*

- Task 2 variables include titer, rate, yield, catabolic rate of BHET
- Solid BHET due to limited solubility
- Using model substrates, will shift to BHET from catalytic process for demonstrations
- ß-ketoadipate from PET imparts performance advantages
- Conducting bioreactor cultivations from 0.5-10 L scale
- **End-of-project milestone:** 50% yield of ß-ketoadipate from a hybrid biological-catalytic strategy or a biological strategy using PET fibers. 80% PET biodegradation in ≤ 2 weeks or 80% conversion of BHET to ß-ketoadipate at 6.25 g/L/day rate.



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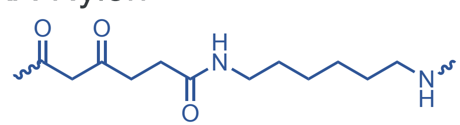
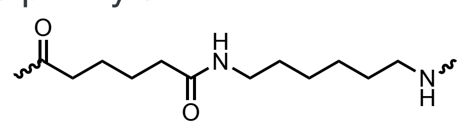
BKA-Nylon 	130	-
Adipic-Nylon 	60	260

Franden, Jayakody *et al. Metabolic Engineering* 2018

Summary

Overview

– Develop cost-effective, bio-based strategies to upcycle PET to higher-value molecules

Approach (two strategies)

– Wholly biological methods to convert PET to exemplary product (e.g., ß-ketoadipate) via Consolidated Bioprocessing-like strategy
– Hybrid biological-catalytic methods to the same product via catalyzed glycolysis and microbial conversion of glycolysis product

Technical accomplishments

– Demonstrated initial approach for PETase/MHETase secretion and preliminary TPA catabolism via Q1 and Q2 milestones

Relevance

– Plastics are causing an environmental crisis; upcycling could offer an opportunity to increase reclamation rates
– Plastics recycling today is down-cycling
– This project sits at the intersection of bioeconomy technology development and recycling industry

Future work

– Evaluate two approaches in parallel, finalize project in 1.5 years, and write forward-looking report.
– 50% yield of ß-ketoadipate from either a hybrid biological-catalytic strategy or a wholly biological strategy using reclaimed PET fibers from an industrially relevant stream. 80% PET biodegradation in ≤ 2 weeks or 80% conversion of BHET to ß-ketoadipate at 6.25 g/L/day rate.

Acknowledgements

BETO: Jay Fitzgerald

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BIOENERGY TECHNOLOGIES OFFICE

Contributors

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- Brenna Black
- Rita Clare
- Bryon Donohoe
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- Caralyn Szostkiewicz

Collaborators

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- Christina Payne, University of Kentucky
- Nick Wierckx, Jülich
- Lars Blank, RTWH Aachen University

Collaborators on BETO projects

- Michael Crowley, Biochemical Process Modeling and Simulation

Milestones

FY19		
Q1	QPM	Demonstrate PETase and MHETase secretion in P. putida KT2440 or MFL185 through G - Demonstrate PETase and MHETase secretion in P. putida KT2440 or MFL185 through GFP and RFP (or mCherry) labeling, respectively, via at least two sets of signal peptides
Q2	QPM	Integrate and test at least two cassettes of TPA transport and catabolic genes into P. putida and baseline initial TPA catabolic rates. Initiate evolution if needed to improve TPA catabolic rates. Knockout the genes necessary to accumulate ß-ketoadipic acid production.
Q3	QPM	Measure toxicity of BHET, MHET, TPA, and EG to P. putida as a precursor to design - Measure toxicity of BHET, MHET, TPA, and EG to P. putida as a precursor to design fed-batch cultivation strategies.
Q4	Annual	Produce at least 30% yield of ß–ketoadipate from either PET coupons or fibers via secreted PETase and MHETase based on liberated monomers (Task 1), or from BHET (Task 2). Employ either shake flasks or 0.5-L bioreactors.

Publications

Harry P. Austin, Mark D. Allen, Bryon S. Donohoe, Nicholas A. Rorrer, Rodrigo Silveira, Fiona Kearns, Benjamin Pollard, Graham Dominick, Ramona Duman, Kamel El Omari, Vitaliy Mykhaylyk, Armin Wagner, William E. Michener, Antonella Amore, Munir S. Skaf, Michael F. Crowley, Alan W. Thorne, Christopher W. Johnson, H. Lee Woodcock*, John E. McGeehan*, and Gregg T. Beckham*****, "Structural evolution and characterization of a plastic-degrading aromatic polyesterase", ***PNAS*** (2018) 115, 4350-4357.

Presentations

- Challenges and opportunities in plastics upcycling, 2019 Polymer Upcycling Workshop, UCSB, January 24th, 2019
- Hybrid biological and catalytic processes to manufacture and recycle plastics, USC, January 14th, 2019
- Hybrid biological and catalytic processes to manufacture and recycle plastics, Princeton University, November 28th, 2018
- Opportunities and challenges in plastics upcycling, ABLC Global, November 8, 2018
- Hybrid biological and catalytic processes to manufacture and recycle plastics, IBM Almaden, September 12th, 2018
- Enzymatic and microbial conversion of waste plastics, 9th International Congress on Biocatalysis, August 28th, 2018
- Deconstructing plants and plastics with novel enzymes and microbes, The Novo Nordisk Center for Biosustainability, DTU, August 24th, 2018
- Biocatalytic conversion of waste plastics, Green Chemistry Gordon Research Conference, July 31st, 2018
- Challenges and opportunities in plastics upcycling, USDA-DOE Summit on Realizing the Circular Carbon Economy, July 25th, 2018
- Hybrid biological and catalytic processes to manufacture and recycle plastics, University of British Columbia, June 20th, 2018
- Hybrid biological and catalytic processes to manufacture and recycle plastics, MIT, April 27th, 2018
- Interfacial biocatalysis: Engineering and understanding enzymes that break down plants and plastics, University of Wyoming, April 23rd, 2018
- Biological and chemical conversion of lignocellulose and plastics, Penn State, January 18th, 2018
- Deconstructing plants and plastics with novel enzymes and microbes, Thermal Biology Institute, Montana State University, October 23, 2017
- Deconstructing plants and plastics with novel enzymes and microbes, University of Portsmouth, October 11, 2017