PET Upcycling

March 5th, 2019
Technology Session Review Area:
Biochemical Conversion
PIs: Gregg T. Beckham, Adam Guss
National Renewable Energy Laboratory
and Oak Ridge National Laboratory
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 (β-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%

**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

<table>
<thead>
<tr>
<th>Total Costs Pre FY17</th>
<th>FY17 Costs</th>
<th>FY18 Costs</th>
<th>Total Plan Funding (FY19-Project End Date)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOE funded</td>
<td>--</td>
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<td>$300k</td>
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**Objective**
Develop efficient, cost-effective strategies that harness biological and catalytic systems for PET depolymerization and conversion to an exemplary value-added compound β-ketoadipate.

**End of Project Goal**
50% yield of β-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 β-ketoadipate at 6.25 g/L/day rate.

**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
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, β-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)

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

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

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

Can biological recycling strategies help you?
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 β-ketoadipate
• β-ketoadipate can be used for advantaged bioproducts (e.g., improvednylons)
• 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 β-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 β-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

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*Yoshida et al., Science 2016*

*Masai et al.,*
Technical accomplishments: Q1 Milestone

Demonstrate PETase and MHETase secretion in *P. putida* through fluorescent protein labeling
Integrate and test TPA transport and catabolic genes into *P. putida* and baseline initial TPA catabolic rates

**Shake flask experiment**

- TPA (mg/L) vs. Time (h)
- OD_{600} vs. Time (h)

- **Comamonas Sp. E6**
- **P. putida:TPA**
**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 reclaimer. See figure 7 for detail on total flake produced by US reclaimer from bottles.*

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The New Plastics Economy, Ellen Macarthur Foundation (2016)
Business Insider, Sailors for the Sea (2012)
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
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

1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate
protocatechuate
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

**Bioscreen C**

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
- β-ketoadipate from PET imparts performance advantages
- Conducting bioreactor cultivations from 0.5-10 L scale

- **End-of-project milestone:** 50% yield of β-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 β-ketoadipate at 6.25 g/L/day rate.

<table>
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<tr>
<th>Polymer</th>
<th>T&lt;sub&gt;g&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;m&lt;/sub&gt; (°C)</th>
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<tbody>
<tr>
<td>BKA-Nylon</td>
<td>130</td>
<td>-</td>
</tr>
<tr>
<td>Adipic-Nylon</td>
<td>60</td>
<td>260</td>
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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., β-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 β-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 β-ketoadipate at 6.25 g/L/day rate.
Acknowledgements

BETO: Jay Fitzgerald

Contributors
- Adam Guss (co-PI, ORNL)
- Brenna Black
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- Japheth Gado
- Lahiru Jayakody
- Thom Mand
- William Michener
- Nicholas Rorrer
- Caralyn Szostkiewicz

Collaborators
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- Steve Dunkle, Shaw Floors
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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

<table>
<thead>
<tr>
<th>Quarter</th>
<th>Period</th>
<th>Task Description</th>
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<tbody>
<tr>
<td>Q1</td>
<td>FY19</td>
<td>Demonstrate PETase and MHETase secretion in <em>P. putida</em> KT2440 or MFL185 through GFP and RFP (or mCherry) labeling, respectively, via at least two sets of signal peptides.</td>
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<tr>
<td>Q2</td>
<td>QPM</td>
<td>Integrate and test at least two cassettes of TPA transport and catabolic genes into <em>P. putida</em> and baseline initial TPA catabolic rates. Initiate evolution if needed to improve TPA catabolic rates. Knockout the genes necessary to accumulate β-ketoadipic acid production.</td>
</tr>
<tr>
<td>Q3</td>
<td>QPM</td>
<td>Measure toxicity of BHET, MHET, TPA, and EG to <em>P. putida</em> as a precursor to design fed-batch cultivation strategies.</td>
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<tr>
<td>Q4</td>
<td>Annual</td>
<td>Produce at least 30% yield of β-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.</td>
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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