

Bio-Optimized Technologies to keep Thermoplastics out of Landfills and the Environment



## DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

BOTTLE 4 – Upcycling

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Technology Session Review Area: Plastics Deconstruction and Redesign

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Office of ENERGY EFFICIENCY & RENEWABLE ENERGY

BIOENERGY TECHNOLOGIES OFFICE ADVANCED MATERIALS & MANUFACTURING TECHNOLOGIES OFFICE

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### Upcycling is a "bridging task" in BOTTLE

- Work focuses on conversion of plastics-derived intermediates to synthesize monomers for recyclable-by-design polymers
- Upcycling aligns with DOE's Strategy for Plastics Innovation

### Upcycling of plastics-derived and bio-derived substrates

• Substrates primarily from oxidation of waste plastics and biobased inputs (sugars, aromatics from lignin, etc.)

### Upcycling approaches

- Discovery of new pathways for xenobiotic substrates
- Metabolic engineering
- Bioconversion to target molecules



## Approach



- Pathway discovery integrates with Deconstruction and allows us to identify novel metabolic pathways for microbial consumption of plastic deconstruction products
- Metabolic engineering allows us to create microbes that convert deconstruction products and synthesize monomers for "recyclable-by-design" (RBD) polymers, integrating with Redesign



## **Risks and mitigation strategies**



### **Risks:**

- Metabolic pathways for deconstruction products may not exist or may be challenging to find
- Rates of consumption or production are too slow
- Lack model organisms or established genetic tools at 70°C

### Mitigation:

- Bioprospect for pathways in diverse and extreme environments
- ✓ Evolve or engineer new enzymatic functions
- ✓ Combinatorial screening of diverse enzyme orthologs
- Evolutionary approaches to improve pathway flux
- ✓ Deep expertise in chassis domestication and genetic tool development, including in extremophiles





## Poly(ethylene terephthalate) upcycling to $\beta$ -ketoadipate



**Goal:** Enable upcycling of chemocatalytically deconstructed poly(ethylene terephthalate) (i.e., ethylene glycol, terephthalate, BHET, and MHET) to  $\beta$ -ketoadipate, a precursor for a performance-advantaged nylon-6,6 **Methods:** Engineered metabolic pathways for polymer degradation products into *Pseudomonas putida* **Outcome:** Demonstrated bioconversion of BHET to  $\beta$ -ketoadipate at 15.1 g/L, 0.76 mol/mol, and 0.16 g/L/h in bioreactors, and proof-of-concept bioconversion of glycolyzed poly(ethylene terephthalate) to  $\beta$ -ketoadipate



Werner et al. Metabolic Engineering 2021

## Progress: Upcycling of deconstructed, mixed plastics

Goal: Engineer *P. putida* to convert oxidatively deconstructed mixed plastics (HDPE, PS, PET) to exemplary products, polyhydroxyalkanoates or β-ketoadipate

Method: Engineer metabolic pathways into P. putida for catabolism of terephthalate and  $C_6$ - $C_{18}$ dicarboxylates



## Biologically upcycled deconstructed mixed plastics



Outcome 1: Engineered *P. putida* co-utilizes acetate, dicarboxylic acids (HDPE), benzoate (PS), and terephthalate (PET) Outcome 2: Engineered *P. putida* converts mixed deconstructed plastic substrates into PHAs or β-ketoadipate



**Impact:** Bioconversion overcomes the challenge of chemical heterogeneity present in mixed plastic streams by conversion of mixed substrates into a single product that is tunable, providing flexibility in substrate and product

Future work: Expand the biological funnel to include deconstruction products from additional polymers (e.g., PP), and expand the product slate to include monomers for recyclable-by-design polymers

## Discovered new metabolic pathways for co-monomers

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**Goal:** Identify environmental isolates that grow on plastic deconstruction products, including co-monomers (IPA, CHDM, and CHDC) and elucidate metabolic pathways

Methods: Use systems biology to identify responsible genes and pathways, and engineer into production strain Example: Identified, DNA sequenced, and applied systems biology to enriched organisms to discover new pathways for IPA utilization



## Built a combinatorial library for isophthalate utilization



Goal: Engineer *P. putida* to grow on isophthalate, a compound present in plastic waste streams

Methods: Built a combinatorial library of newly discovered dioxygenase, dehydrogenase, and transporter genes for isophthalate utilization





Methods: Created library with 170X coverage with all members represented, tracked abundance of each gene in the population, and grew population on IPA to enrich for the fastest grower

**Outcome:** Identified the best combination of genes for growth on isophthalate in *P. putida* that included genes from three different organisms



### Impact:

- Decreased time to identify optimal solution for heterologous pathway engineering by >10-fold
  - Identified optimal combination of isophthalate transporter, dehydrogenase, and dioxygenase in *P. putida* in a <u>single experiment</u>
  - Would ordinarily require ~<u>months</u>

**Future work:** Employ approach for engineering additional pathways for funneling of deconstruction products

## Consolidated bioprocessing of PET



Goal: Develop high-temperature bioconversion approaches for consolidated deconstruction and bioconversion of PET near the  $T_g$ , where enzymatic PET deconstruction is most efficient

Method: Combine secretion of thermophilic PETase, thermophilic metabolism of ethylene glycol and terephthalate, and a product formation pathway, where each function ~70°C

Key milestone: Deliver a thermophile that secretes a PET hydrolase and converts EG and TPA at a product yield  $\geq$ 70% and  $\geq$ 80% conversion in 4 days



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Goal: Identify organisms from the environment that grow on terephthalate and ethylene glycol <u>at T ~70°C</u>

Methods: Use systems biology to identify genes and pathways in isolates from natural environments, and move pathways into a thermophilic organism



## Engineered thermophilic PETase, TPA, and EG activity





**Impact:** Enabled genetic engineering in a thermophile that can be used for consolidated bioprocessing at or near the poly(ethylene terephthalate) glass transition temperature for more efficient bioconversion and upcycling

## Computational redesign of terephthalate dioxygenase



**Goal:** Improve Rieske dioxygenase/reductase pairs:

 Limitations of current system: Limited turnovers (300), melting temperature (50°C), and expression

**Outcome:** Protein designs showed 3-fold improvement in expression

**Ongoing:** Experimental characterization ongoing to determine  $T_m$  and optimal T





Impact: Engineered TPA dioxygenases offer another route to thermophilic TPA utilization

## **Bioproduction of recyclable-by-design monomers**

**Goal:** Engineer bioconversion of diverse feedstocks to building blocks for recyclable-by-design polymers **Outcome:** Genetic modifications resulted in an engineered strain of *P. putida* that converts glucose to a mixture of  $C_8 - C_{12}$  length 3-hydroxyacids, rather than the native polyhydroxyalkanoates, at a titer of > 1 g/L

 3-Hydroxyacid production has been demonstrated from glucose, terephthalate, and intermediates from deconstructed mixed plastics

**Impact:** Metabolic engineering for conversion of deconstructed mixed plastics to 3-hydroxyacids enables conversion of today's plastics into monomers for recyclable-by-design polymers

**Future work:** Scale-up production of 3-hydroxyacids, and explore production of additional monomers for recyclable-by-design polymers





## Impact

## Hybrid processes for conversion of incumbent plastics...

...to performance-advantaged bioproducts, and recyclable-by-design monomers

### Enabled by cutting-edge science



Pathway discovery Enables carbon-efficient

conversion

### New genetic tools Catalyze development of

bioconversion chassis for different substrates



## Thermophilic

bioconversion

Towards a step-change in rate & extent of depolymerization

In preparation

Amy A. Cuthbertson, Clarissa L. Lincoln, Joel Miscall, Lisa Stanley, David T. Moore, Brenna A. Black, Nicholas A. Rorrer, Gregg T. Beckham, Characterization of polymer properties and identification of additives in commercial research plastics, in preparation.

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## Patents and patent applications

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- Base-mediated method for the recycling of epoxy resin-carbon fiber composites, 22-130: U.S. provisional patent application 63/418,874
- Renewable bio-advantaged plasticizer generated by reductive cross coupling of lignin-derived aromatics, 22-124: U.S. provisional patent application 63/379,217
- Process for sequential acetolysis-autoxidation of plastic streams, 22-107: U.S. provisional patent application 63/383,293
- Methods and systems for dye removal from polymer textiles, 22-106: U.S. provisional patent application 63/384,137
- Biodegradable elastomeric thermosets from microbially-produced polyhydroxyalkanoates, 19-104: U.S. provisional patent application 63/386,011
- Light-driven C-C bond cleavage enabled by polyoxometalate photocatalysts, 21-95: U.S. provisional patent application forthcoming

- Hydrogenolysis of Polyethylene and Polypropylene into Propane over Cobalt-Based Catalysts, 22-81: U.S. provisional patent application 63/340,322
- Catalysts for Depolymerizing Plastics, 20-22: 17/370,244
- Plastic Degrading Fusion Proteins and Methods of Using the Same, 20-86: PCT/US21/31610
- Polymer Degrading Enzymes, 21-88: PCT/US22/25624.
- Dissolution Purification and Recovery for Polymeric Recycling, 22-16: 63/307,676
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- Upcycling Mixed Waste Plastic Through Chemical Depolymerization and Biological Funneling, 20-123: PCT/US21/63725.
- Genetically engineered Pseudomonas strains capable of metabolizing ethylene glycol, 17-26: 11,021,721
- Engineered Pseudomonas for the Deconstruction of Polymers, 18-76: 17/055,626
- Microorganisms Engineered for Muconate Production, 20-48: 17/184,580

## Patents and patent applications

- Polymers from bio-derived dicarboxylic acids (BKA to nylon), 17-48: 10,662,289
- Polymers and methods of making the same (PET formulated with adipic/muconic acids), 17-55A: 17/205,232
- Monomers, Polymers and Methods of Making the Same (Bio-plastic ABS), 18-69: 16/583,471
- Bio-derived biphenyl compounds (Polycarbonates), 18-81: 16/791,873
- Bioderived monomers as replacements in petroleum-based polymers and copolymers (novel bio-based plasticizers), 19-38: 16/790,093
- Conversion of dicarboxylic acids to monomers and plasticizers, 19-41A: 16/995,338
- Bio-derived Epoxide Triazine Networks and Methods of Making the Same, 20-26: 17/324,222
- Bio-derived Epoxy-Anhydride Thermoset Polymers for Wind Turbine Blades and Anti-Static Coatings, 20-59: 17/494,514
- Plastic waste derived polymers and resins and methods of making the same (PET upcycled to 3D printing materials). 20-37: 17/371,421
- Mixed Waste Plastics Compatibilizers for Asphalt (filed by ASU), 21-53: 63/148,423

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- Bioderived Benzoxazines, 20-130: 17/690,131
- Novel Routes to Bis-furan Diacids, Dialcohols and Diamines, US 9840485
- Improved Industrial Production of Isotactic Polylactides (PLA), US 10174161
- Chemically Recyclable Polymers to Combat Single-Use Plastics, PCT Patent Pending: WO 2021/113325
- Synthesis of Crystalline Polymers from Cyclic Diolides, US Utility Patent Pending: US 2019/0211144
- Novel Compounds and Methods for Upgrading Biomass to Produce Premium Biofuels, US Utility Patent: US 9469626 B2, US Utility Patent: US 9828354 B2
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Development of non-model microbes as chassis organisms for bioconversion. Presented at the AIChE Annual Meeting, November 2022.

Redesigning Polymers to Leverage a Circular Economy, Chemical Engineering, Purdue University, November 2022.

Bio-based Polymers with Performance & Recyclability Advantages, Braskem, virtual seminar, November 2022.

Design Principles and Chemocatalytic Methods for Circular Polymers and Biodegradable Plastics, BASF Lecture in Organic Chemistry, November 2022.

Developments in Advanced Recycling, TA Instruments Webinar, October 2022.

Design of Polyolefin-like Polyesters with Closed-loop Lifecycles, ACS WRM Polymer Symposium, October 2022.

Adopting a sustainable plastics supply chain, RISE 2022, September 2022.

Redesigning plastics to be recyclable-by-design, RISE 2022, September 2022.

Advances in lignin and plastics conversion, VITO, September 2022.

Decoding the mechanism of autoxidation deconstruction reaction of plastics by in-situ simultaneous SAXS and WAXS," XVIII International Small-Angle Scattering Conference (SAS2022), September 2022.

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Using redesigned iron catalysts to bring aromatic subunits to a common intermediate, SIMB 2022, August 2022

Techno-economic analysis and life cycle assessment for catalytic fast pyrolysis of mixed plastic waste, BioEnergy TRP Meeting, National Renewable Energy Laboratory, August 2022.

Bio-based, recyclable-by-design polymers, ACS National Meeting, August 2022

Techno-Economic analysis and life cycle assessment of mixed waste plastics via pyrolysis and gasification, ACS Fall Conference, August 2022.

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Bio-based acrylic plastics with performance and recyclability advantages, ACS Symposium: Green Polymer Chemistry and Sustainability, ACS National Meeting, August 2022.

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Design principles and chemocatalytic methods for intrinsically circular polymers and biodegradable plastics, ACS Presidential Event: Series-Enabling Circular Economy via Polymer Molecular Recycling, ACS National Meeting, August 2022.

Techno-economic, life-cycle, and socioeconomic impact analysis of enzymatic recycling of poly(ethylene terephthalate), ACS Fall Conference, August 2022.

Kinetic Monte Carlo-based tool to unravel solvolysis chemistry of step-growth polymers, National Meeting of the American Chemical Society, August 2022.

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High throughput test tools for industrially relevant microbial chassis, SIMB 2022, August 2022.

Circular polymers and biodegradable plastics, Circular Polymers and Biodegradable Plastics International Research Training Group, University of Muenster, July 2022.

Engineering P450s to alleviate a bottleneck to lignin demethylation, Intl. Conference on Porphyrins and Phthalocyanines, July 2022.

Difficult to recycle plastics, Sustainable Packaging Coalition Engage Meeting, July 2022.

Selective chemical recycling of mixed plastics waste, Polymer Physics Gordon Research Conference, July 2022.

Plastics recycling and upcycling in the BOTTLE Consortium, NASEM Committee on Repurposing Plastic Waste, July 2022.

Developing strategies for polymer redesign and recycling using reaction pathway analysis, Gordon Research Conference on Polymer Physics, July 2022.

Multi-Material Flexible Packaging Coalition SPC, February 2022.

Development of chemical recycling approaches for plastic waste (via webinar), BASF, March 18th, 2022

Development of chemical recycling approaches for plastic waste, Enzyclic Consortium (via webinar), January 2022

Development of chemical recycling approaches for plastic waste, UIUC, December 2021

Design Principles and Synthetic Methodologies for Circular Polymers with Intrinsic Recyclability and Tunable Properties, Pacifichem Conference, December 2021

New building blocks for performance-advantaged renewable and recyclable polymers, Pacifichem (via webinar), December 2021

Discovery and characterization of PET degrading enzymes, University of Rochester microplastics workgroup seminar series, December 2021.

Design Principles and Synthetic Methodologies for Intrinsically Circular Polymers and Biodegradable Plastics, Columbia University, November 2021

Selective Hydrogenolysis of Polyethylene and Polypropylene to Liquid Alkanes over Tunable Ruthenium-Based Heterogeneous Catalysts, 2021 AIChE National Conference, Boston, MA, November 2021.

Plastics recycling and upcycling, ACS Converge (via webinar), October 2021

Genetic tools and microbial engineering for biological production of sustainable fuels and chemicals, Presented to Weekly Seminar for DOE CCI/SULI Students. October 2021

Heterogeneous Catalytic Deconstruction and Upcycling of Waste Polyolefins, Biodesign Institute at Arizona State University, SM3 Seminar Series, October 2021.

Domestication of diverse non-model microbes for plastics upcycling and sustainable fuel and chemical production, Biological Sciences Departmental Seminar, Michigan Technical University. October 2021.

Catalysis for valorization of lignin and plastics, Great Plains Catalysis Society (via webinar), June 2021

The critical role of economic and environmental analysis to guide research in lignin valorization and plastics upcycling, Keynote Invited Lecture, ACS Green Chemistry and Engineering (via webinar), June 2021

Towards Intrinsically Circular Thermoplastics and Reprocessable Thermosets, Dow Chemical Company, virtual seminar, May 2021

Recent progress in performance-advantaged bioproducts and plastics upcycling, Arizona State University (via webinar), April 2021

Recent adventures in biomass conversion and plastics upcycling, Rutgers University (via webinar), April 2021

Recent adventures in biological plastics upcycling, MIX-UP Consortium (via webinar), April 2021

Framing challenges and opportunities for chemical recycling of waste plastics, ACS Presidential Symposium on Chemistry and the Future of Plastics (via webinar), April 2021

Recent updates in plastics upcycling from the BOTTLE Consortium, ExxonMobil Research and Engineering, April 2021

Design Principles and Synthetic Methodologies for Circular Polymers and Biodegradable Plastics, KAUST, Physical Science and Engineering Division, virtual seminar, April 2021

Heme and non heme iron enzymes and renewable carbon, University of San Antonio Texas, April 2021

A flexible kinetic assay efficiently sorts potential biocatalysts for BHET hydrolysis, Symposium on Biomaterials, Fuels, and Chemicals, April 2021

BETO 2021 Peer Review, virtual, March 2021

Design Principles for Circular Plastics with Tunable Properties, CellPress LabLinks: The Circular Plastics Economy: Linking Across Scales, virtual event with 440 registered attendees. March 2021.

Process analysis for enzymatic PET recycling, Global Research and Innovation on Plastics annual meeting (via webinar), March 2021

Polyolefin upcycling in the BOTTLE Consortium, Annual SPE meeting (via webinar), February 2021

Biological processes for lignin and plastics conversion, University of California Riverside (via webinar), January 2021