DOE Bioenergy Technologies Office (BETO) 2021 Project Peer Review

Bioconversion of Heterogeneous Polyester Wastes to High Value Chemical Products

9 March 2021

Performance-Advantaged Bioproducts, Bioprocessing Separations, and Plastics

Margaret Sobkowicz
University of Massachusetts Lowell

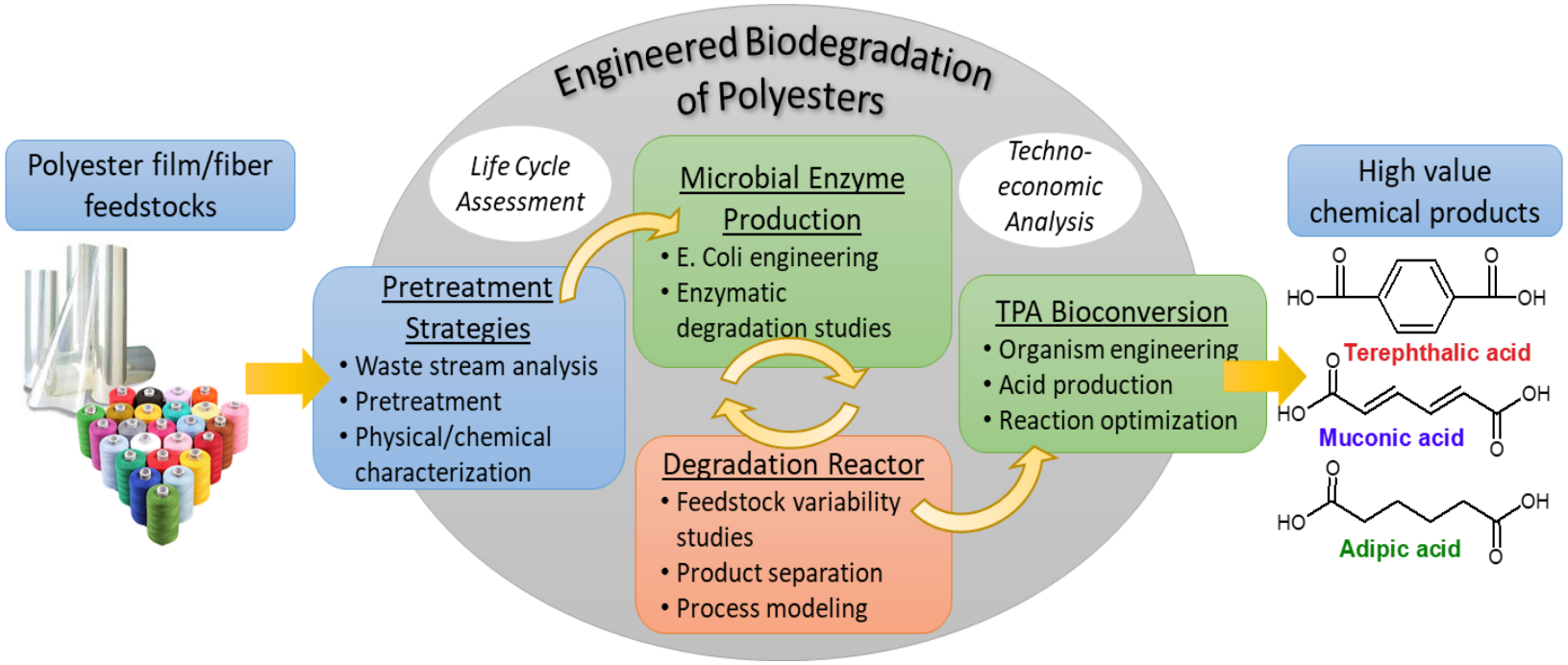
UMASS LOWELL

NREL
NATIONAL RENEWABLE ENERGY LABORATORY

Learning with Purpose

Project Overview

Integrated thermomechanical pretreatment, enzymatic hydrolysis and bioconversion to recycle polyester waste to high value chemicals



Project Objectives

- → Determine most prevalent forms of poly(ethylene terephthalate) (PET) film and fibers in post-consumer and industrial waste streams; classify their physical characteristics before and during biodegradation
- → Explore pretreatment and reactor design to increase efficiency of terephthalic acid (TPA) production
- → Design bacterial strains that produce key enzymes for biodegradation and bioconversion of PET into TPA
- → Devise biochemical conversion strategy for producing high-volume products from TPA
- → Determine conversion kinetics, efficiency and separations strategies for target products
- → Evaluate the economics and environmental impact of the process

Overview: Problem Statement

- Conventional recycling is underperforming globally in dealing with plastic waste
- Poly(ethylene terephthalate) production: >30 Mtons annually, on the rise
- ~50% PET produced is bottles; rest is fiber, sheet and mixed materials, not suitable for mechanical recycling

- Biological advanced recycling technologies maturing, but not optimized for heterogeneous and diverse PET waste streams
  - Enzyme production activity & efficiency improvements needed
  - New reactor design concepts and scale-up needed
  - Analysis relative to status quo needed

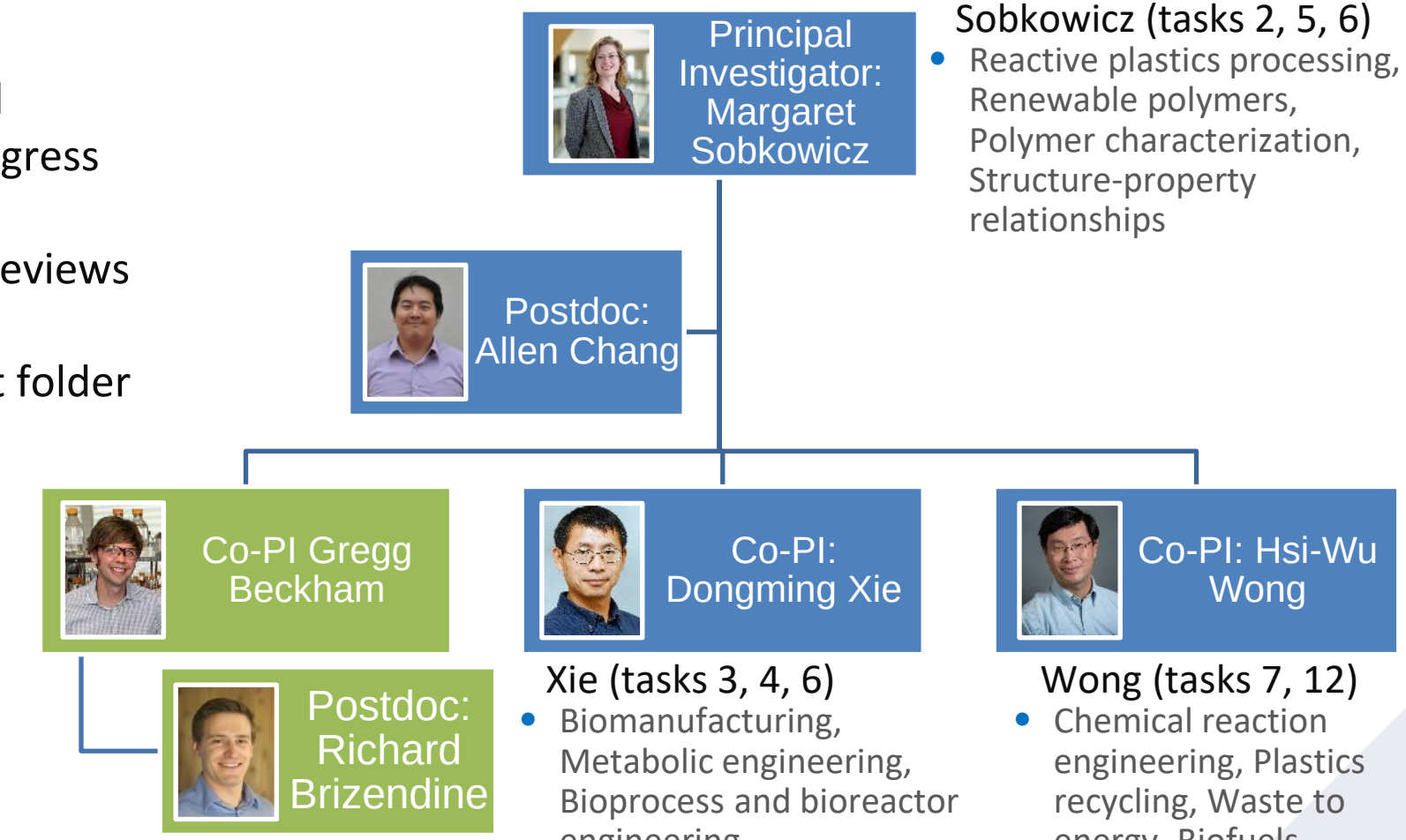
1 – Management

Project Management Activities

- Biweekly meetings: all personnel provide updates on research progress and milestones
- Quarterly project management reviews with BETO
- File and data sharing: SharePoint folder

Risk mitigation

- PI addresses upcoming milestones at biweekly meeting
- Task leads suggest changes to approach to address risks
- Changes discussed with BETO at quarterly update meetings



2 – Approach

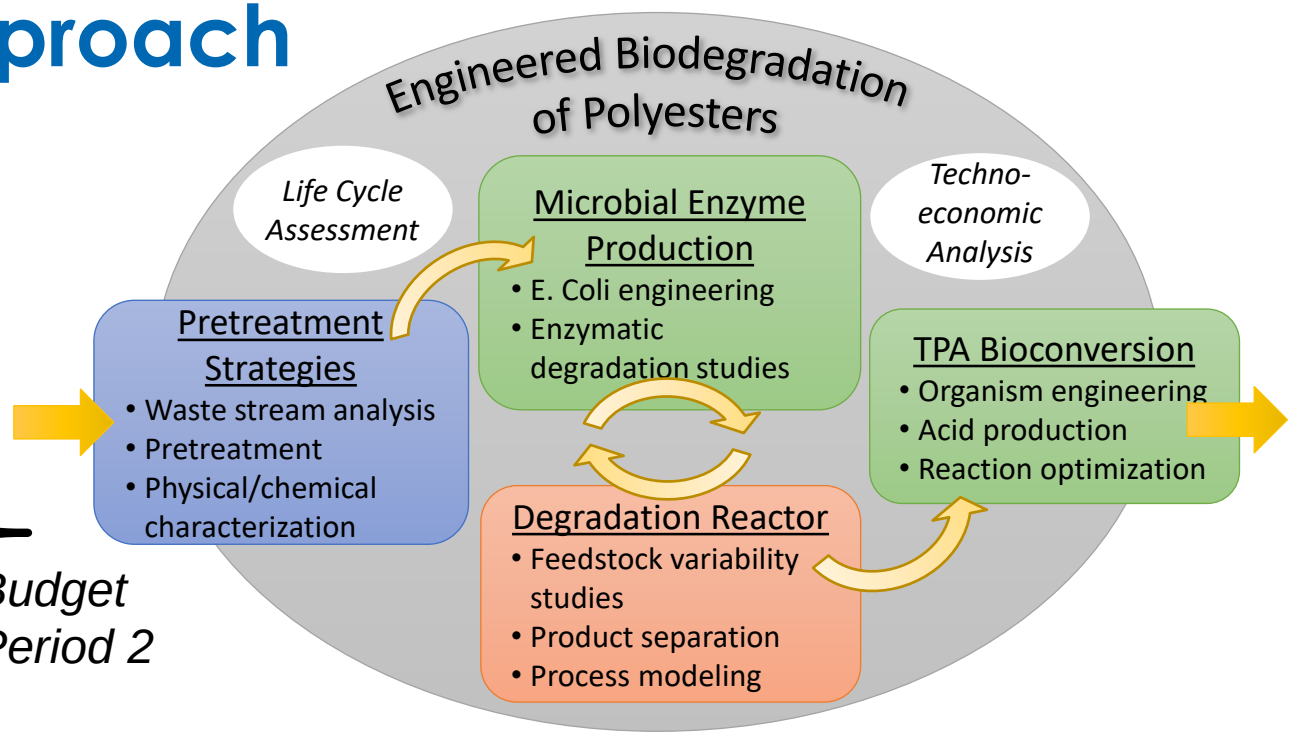
Technical Tasks Overview

- 2. Select and acquire forms of PET waste not recycled; analyze properties of the PET samples
- 3. Express several high efficiency enzymes in *E. coli* and *B. subtilis*
- 4. Identify best PET hydrolase or enzyme recipe
- 5. Explore pretreatment strategies for biodegradation
- 6. Design enzymatic degradation experimental system
- 7. Design reactor system to maximize degradation efficiency

Budget Period 2

- 8./10. Create/modify techno-economic model
- 11. Scale up experimental reactor system
- 12. Optimize bacterial expression system

Budget Period 3



Risks/Challenges Identified

- Mechanical grinding process energy intensive
- Biological conversion technologies not optimized (low efficiency and rate)
- ~50% PET produced is bottles; rest is fiber, sheet and mixed materials; impurities have unknown impacts

2 – Approach

Go/No-Go Decision Points

- G/NG 1: Initial Verification: Verified baseline data on PET composition, biodegradation enzyme activity and project targets – completed 5/20/2020
- G/NG 2: Intermediate Verification – planned Q4 2021
  - At least 50% biodegradation of amorphous PET in one week in shake flask setup
  - Functional initial reactor with volume > 1L; produces 20% conversion of PET to monomers in one week
  - At least 15% higher PET conversion using intermittent grinding
  - A 20% increase of TPA yield using the combination of size reduction and in situ product removal

End of Project Goals:

1. 20% increased rate of degradation over current enzymatic and microbial approaches for three forms of waste PET not suitable for mechanical recycling
2. Conversion yield of 70% in one day for three PET waste streams that are both crystalline and amorphous, and contain trace impurities
3. Technoeconomic analysis that identifies the process path to achieve $2/kg terephthalic acid starting from at least one form of PET waste

3 – Impact

Project success will result in:

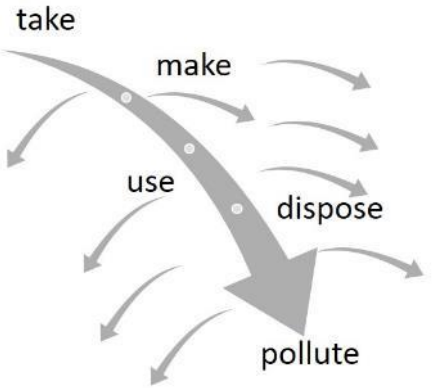
1. Improved system integration and scale-up of enzymatic bioconversion for efficient plastic recycling
2. Increased range of suitable plastic substrates for microbial conversion
3. Optimized metabolic engineering to produce high-value chemical products (TPA and derivatives)

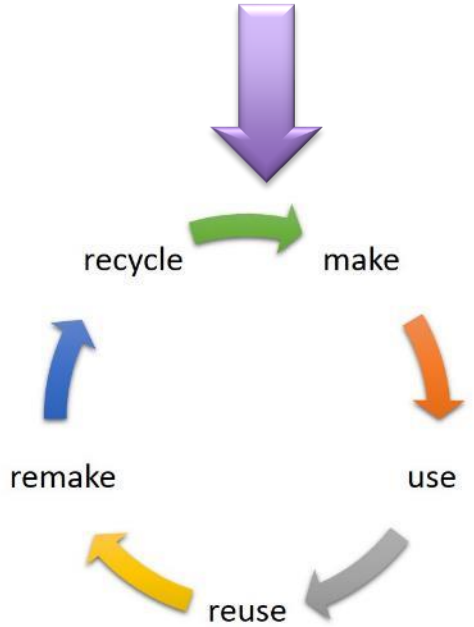
Energy impacts include:

- Reduce energy requirements by over 60% relative to fossil feedstocks to produce new plastic materials
- 1,000–2,000 gallons gasoline saved by recycling one ton of plastics
- Estimated 80% savings of energy consumption during mechanical recycling
- Reduced CO_2 and methane generation from landfilled plastics

Results dissemination:

- Journal article in progress for submission to Biomacromolecules (IF: 6.1)
- Planned presentation at American Chemical Society Spring meeting





Progress Task 2: Evaluate PET waste streams and characteristics for degradation

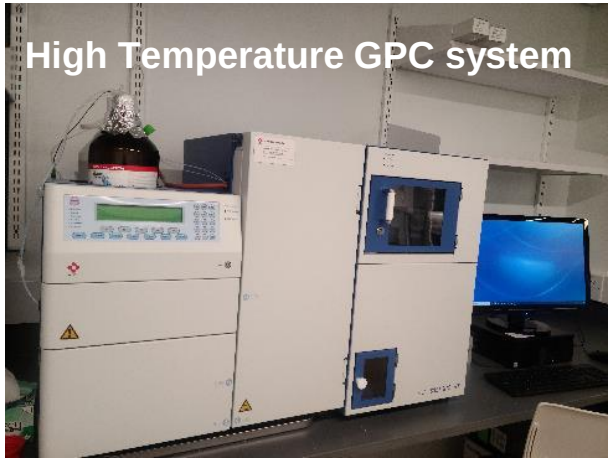
PET sources identified: film grade PETG (Indorama), reprocessed fiber (Unifi, over 30% crystalline), several virgin grades, and post-consumer bottle

Name	Description	Manufacturer/ supplier	Tg [C]	Tm [C]	Crystallinity [%]	Intrinsic Viscosity [dL/g]
VPET	Pre-crystallized granules used for blow molding	DAK Americas	80.5	235	39.2	0.85
RPET	Recycled PET Bottle Flakes after washing/shredding	Post-consumer, Plastics Forming Enterprise	81.8	244.48	30.46	0.75
GPET	PET Granules glass reinforced	Sigma-Aldrich	52.3	214.8/ 237.6	23.61	
PPET	PET powder, 300 um	Goodfellow	81.2	244.09	28.08	
PETG-F	Virgin PETG pellets used for film extrusion	Indorama XPURE 4004	82.5	254	23. 7	0.725
FPET	Recycled fiber PET yarn	Unifi				

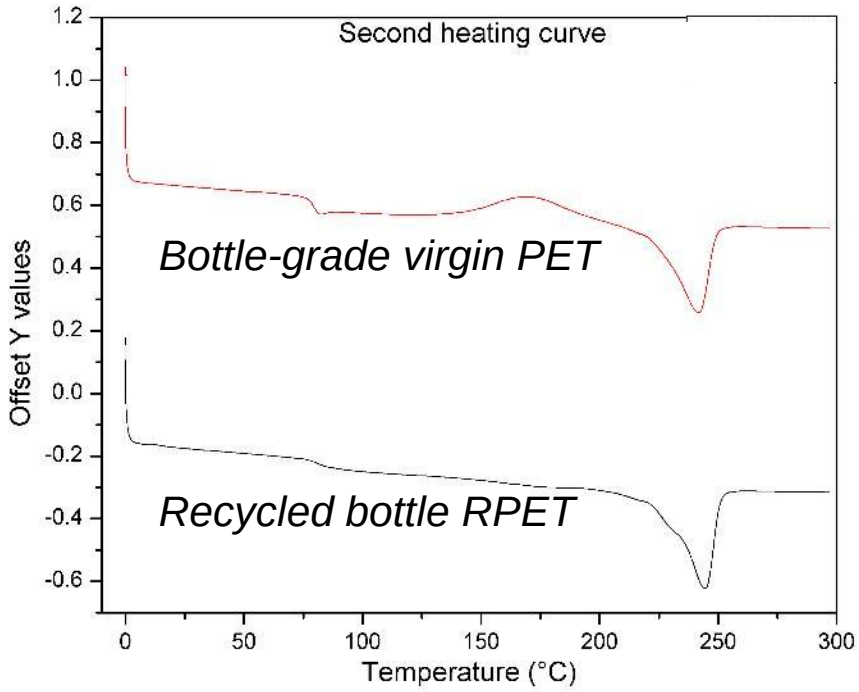
Milestone 2.1: (M8) Provide list of at least ten PET formulations common in post-consumer waste; obtain at least three industry-relevant materials
Milestone 2.2: (M10) Provide thermophysical characteristics of at least five common PET formulations for evaluation in the enzymatic degradation process

Progress Task 2: Evaluate PET waste streams and characteristics for degradation

- Physical characterizations to differentiate waste streams and predict degradation performance
  - Differential scanning calorimetry for crystallinity, melt and glass transitions
  - Spectroscopy for copolymer composition
  - Intrinsic viscosity and gel permeation chromatography (GPC) for molecular weight



	Virgin PET	Dried RPET	Vacuum dried RPET
Relative Viscosity	1.63	1.42	1.35
Intrinsic Viscosity (dl/g)	0.85	0.75	0.54
Wt Avg Mol Wt. (Mw)	62500	51300	32000
Crystallinity (%)	39.2	30.5	--



Link physical properties to degradation kinetics → ***Molecular weight & crystallinity vary across PET sources***

Progress Task 3. Create PET Hydrolase expression system in *E. coli*

Leaf compost cutinase (LCC) previously demonstrated as most efficient thermophilic hydrolytic enzyme for PET

Four enzymes successfully expressed in *E. coli:*

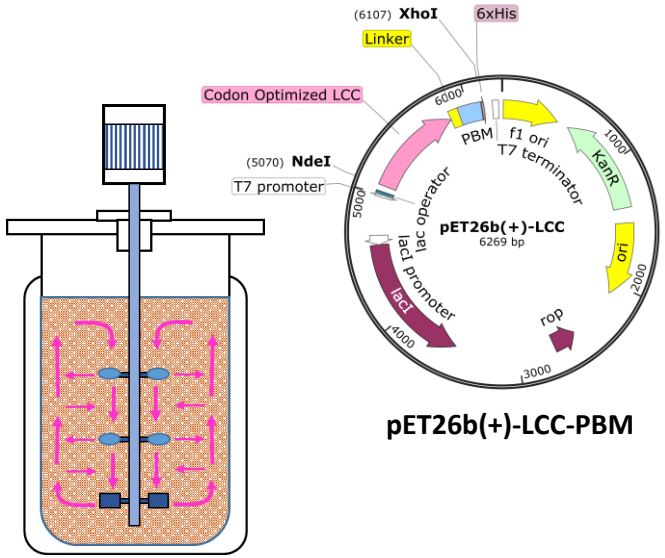
① **pET26b-ECLCC** (Expression of LCC Enzyme)

② **pET26b-pelB-ECLCC** (Expression of Secreted LCC Enzyme)

③ **pET26b-ECLCC-Linker-PBM** (Expression of LCC-PBM Fused Enzyme)

④ **pET26b-pelB-ECLCC-Linker-PBM** (Expression of Secreted LCC-PBM)

pelB=secretion sequence; PBM=binding module



pET26b(+)-LCC-PBM

Ongoing:

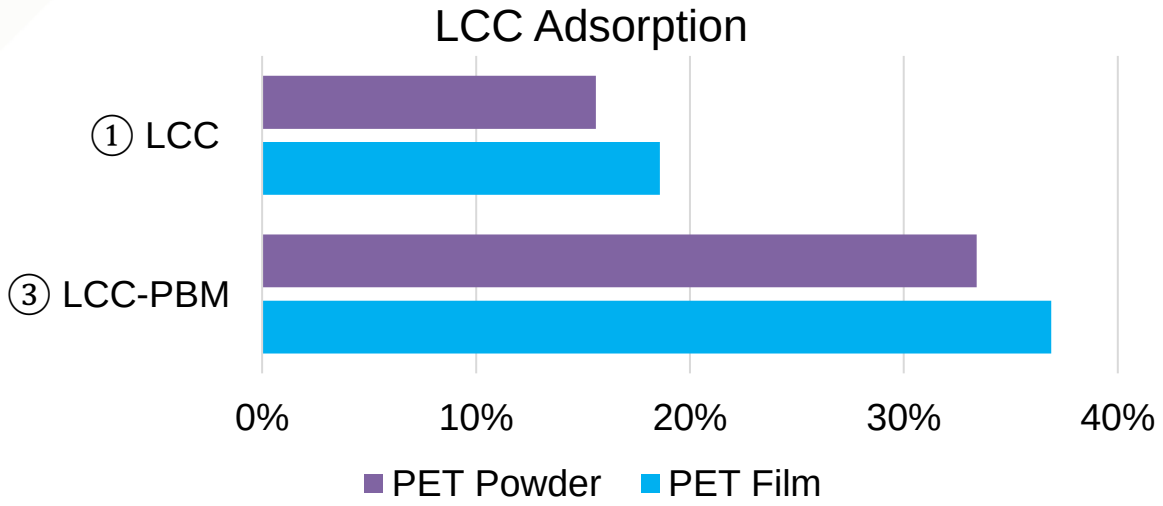
- Investigate the use *B. subtilis* expression system for higher yield of enzyme production
- Production of PETase/MHETase and comparison with LLC variants

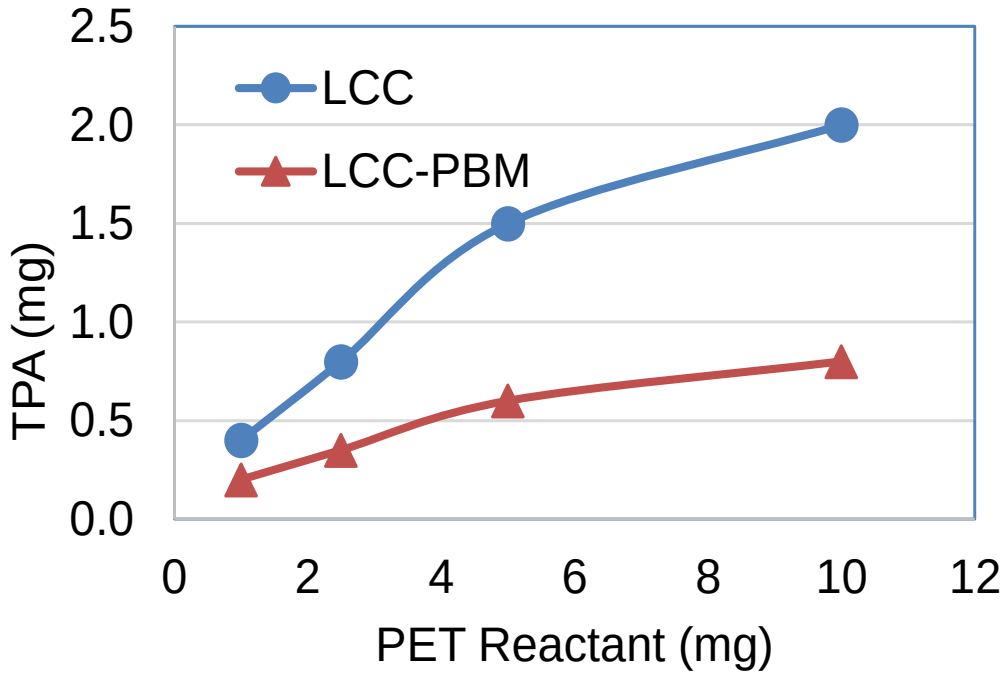
	Intra-cellular	Extra-cellular
BL21(DE3) Transformation	**LCC Titer (mg/mL)**	**LCC Titer (mg/mL)**
① pET26b-ECLCC	**0.066**	**-**
② pET26b-pelB-ECLCC	**0.064**	**0.012**
③ pET26b-ECLCC-Linker-PBM	**0.114**	**-**
④ pET26b(+)-pelB-ECLCC-Linker-PBM	**0.077**	**0.014**

Binding module & secretion sequence incorporated in LCC → ***Enzymes 2 & 4 secrete ~20% of enzyme***

Progress Task 4. Identify the best PET hydrolase or an enzyme recipe to degrade PET polymer into monomers TPA and EG

- Investigate and compare PET degradation efficiency for enzymes produced by *E. coli* strains
  - TLC quick analysis, HPLC for quantification
- TPA yield begins to level off over 1500:1 PET:enzyme ratio
- Binding module increases binding but not TPA yield





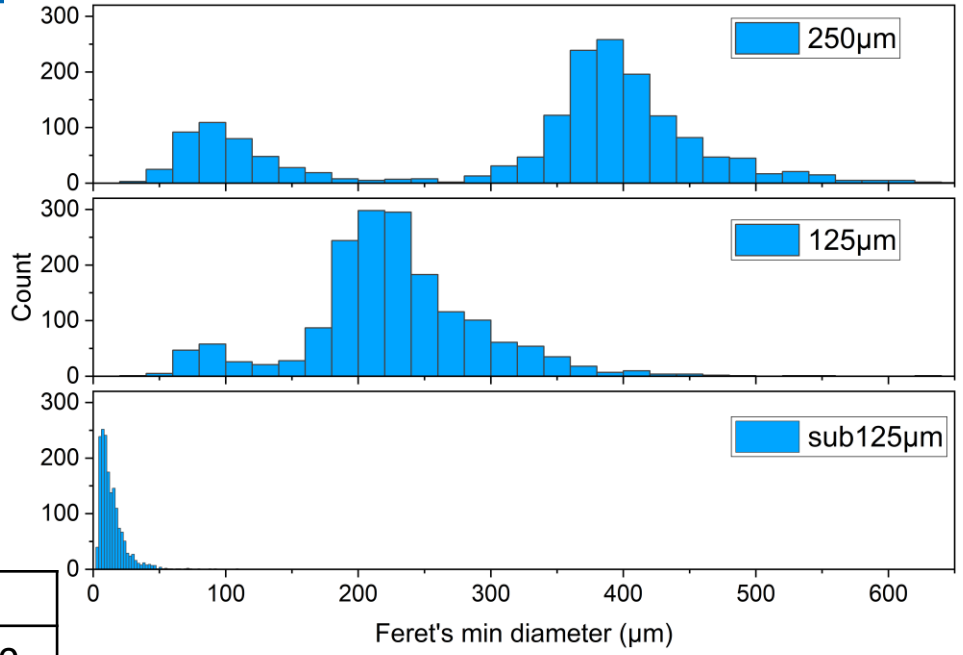
Enzyme efficacy studied via two chromatographic assays → ***Lower TPA yield with binding module could indicate other products***

Progress Task 5. Explore pretreatment strategies to prepare PET for biodegradation

Study relationships among surface area, molecular weight, crystallinity and enzymatic degradation

→ Feedstock grinding for high surface area

→ High speed extrusion for molecular weight reduction



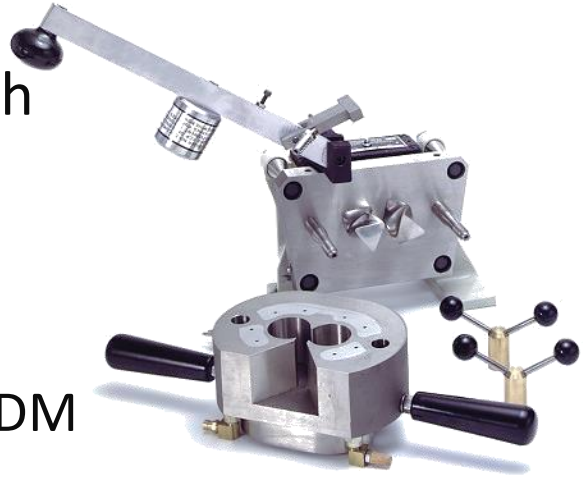
Conditions	IV	Mn	Mw
9g/m, 200rpm	0.41	14590	21240
15.48g/m, 200rpm	0.50	19430	28880
9g/m, 4000 rpm	**0.37**	**12800**	**18460**
15.48g/m, 4000 rpm	0.37	13030	18810
15.48g/m, 4000rpm, (No vacuum)	0.38	13390	19370

Increasing surface area and reduced crystallinity for faster depolymerization → ***Cryogrinding and sieving yields distinct size fractions; extrusion decreases MW by ~50%***

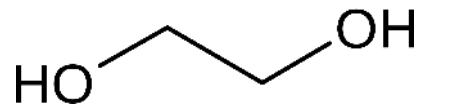
Progress Task 5. Explore pretreatment strategies to prepare PET for biodegradation

Molecular weight and structure modification by melt processing with added ethylene glycol (EG) and cyclohexanedimethanol (CHDM)

- Internal batch mixer to process RPET with 1 wt% and 4 wt% EG and CHDM at 50 rpm and 280 °C up to 30 minutes
- Molecular weight decreased by 80% and T_g lowered 10 °C with 4% CHDM



Sample	T_g (°C)	T_m (°C)	H_f (J/g)	Crystallinity (%)	IV (dL/g)	M_n (g/mol)
RPET	81.8	244.5	42.4	30.46	0.83	28800
EG 0.01	79.4	248.4	46.4	33.1	0.38	8900
EG 0.04	77.2	248.6	47.6	33.9	0.33	7000
CHDM 0.01	76.4	244.4	40.8	29.1	0.37	8300
CHDM 0.04	72.5	237.7	42.0	29.9	0.29	5900



EG

CHDM

Reactive melt processing to alter PET chain structure for enzyme accessibility → ***Linear diol reduces MW, cyclic diol decreases glass transition temperature***

Progress Task 6. Establish enzymatic degradation experiment system

Design of Experiments to develop baseline batch kinetics for enzyme-PET waste combinations

Shake Flask Experiments (LCC systems)

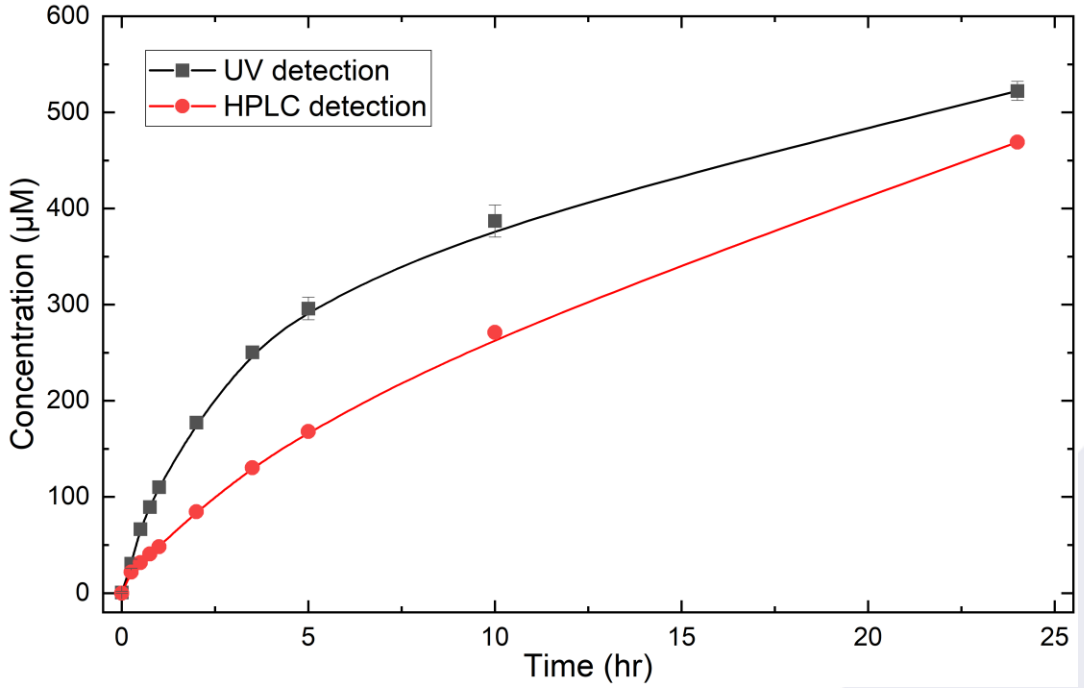
- 250-mL Flask containing 50 mL buffer: 100 mM potassium phosphate (pH 8)
- Reaction Condition: 1.4 µM Enzyme, 100 mg PET, at 65 °C for 48hr

Tube Experiments (PETase-MHETase systems)

- 5-mL Tubes containing 1 mL buffer:
  50 mM Glycine-NaOH (pH 9)
- Reaction Condition: 4.0 µg Enzyme, 5 mg PET,
  at 40 °C for 48hr

Characterizations

- Weight loss and crystallinity measurements
- HPLC measurements for product identification
- UV spectroscopy for quick comparative kinetics tests

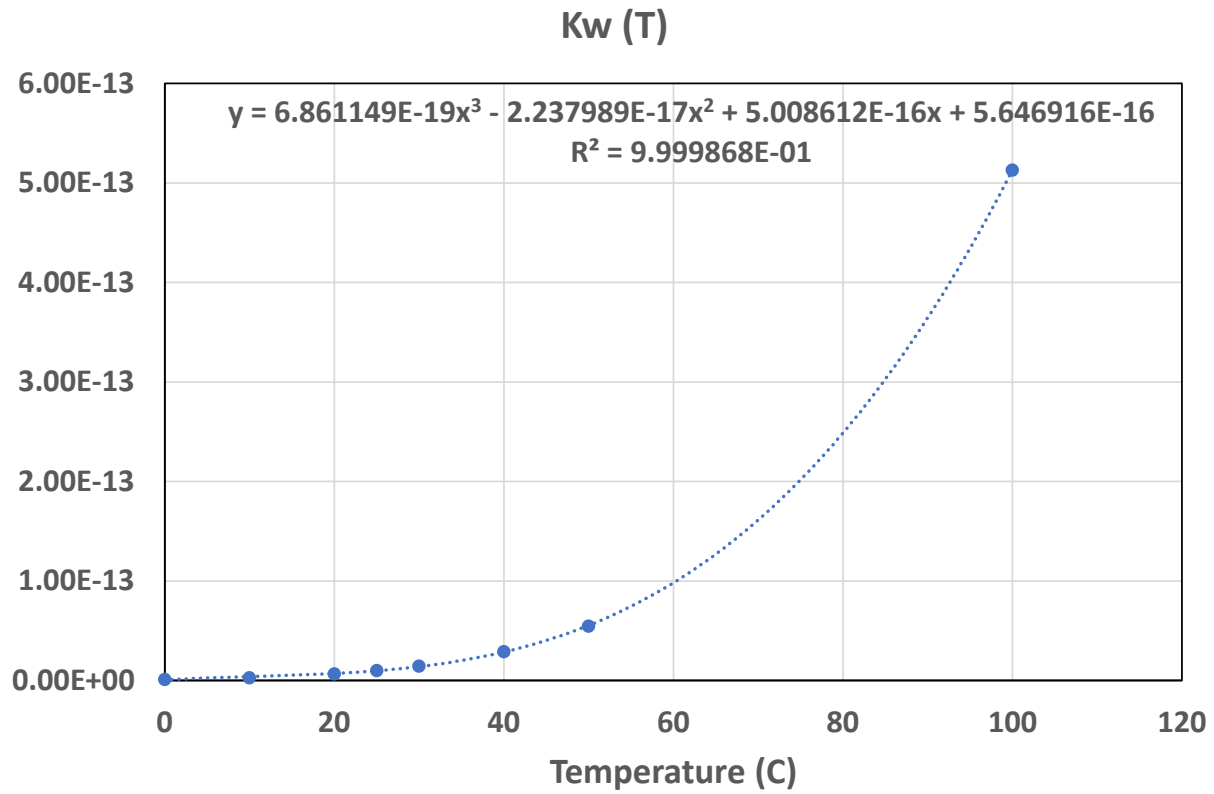


Determine conditions for reaction screening → ***Two reaction sizes and conditions established; two assay tests verified***

Progress Task 7. Design reactor system to maximize degradation efficiency

Design in situ product removal system

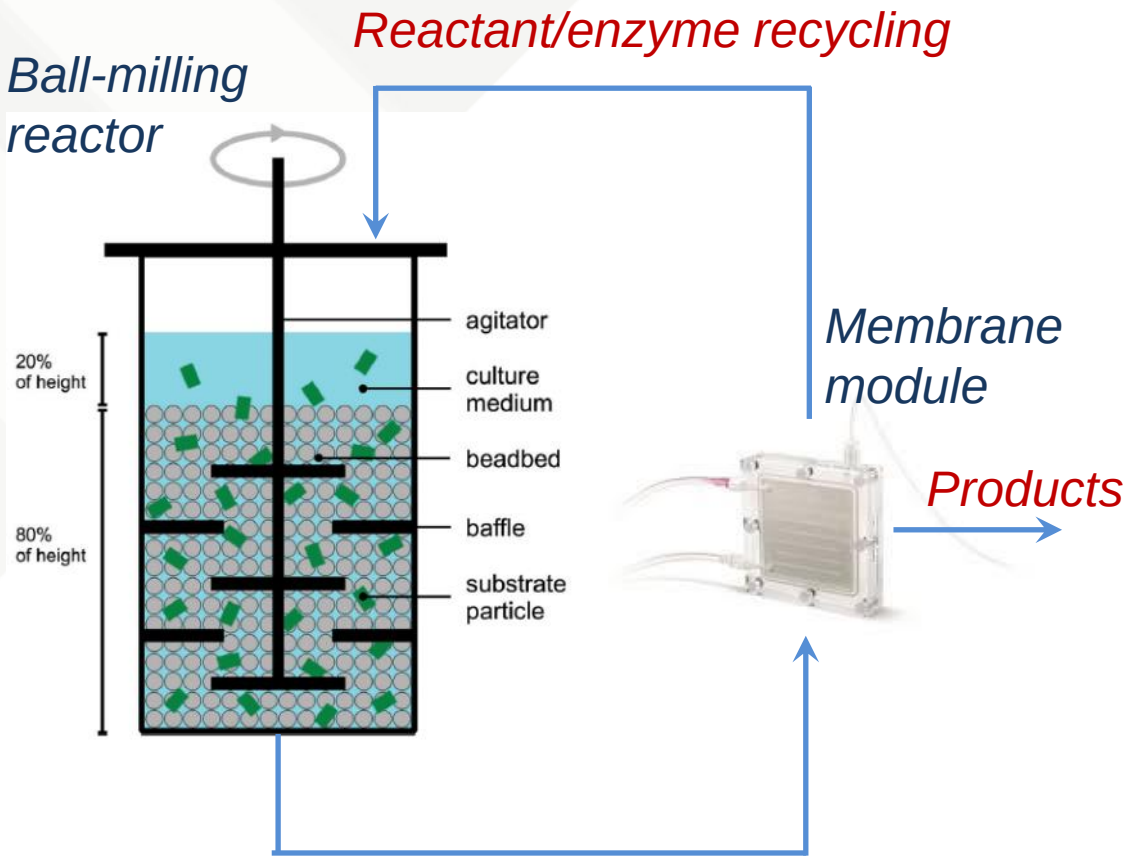
- Calculated partition coefficient (Kw) of TPA versus temperature



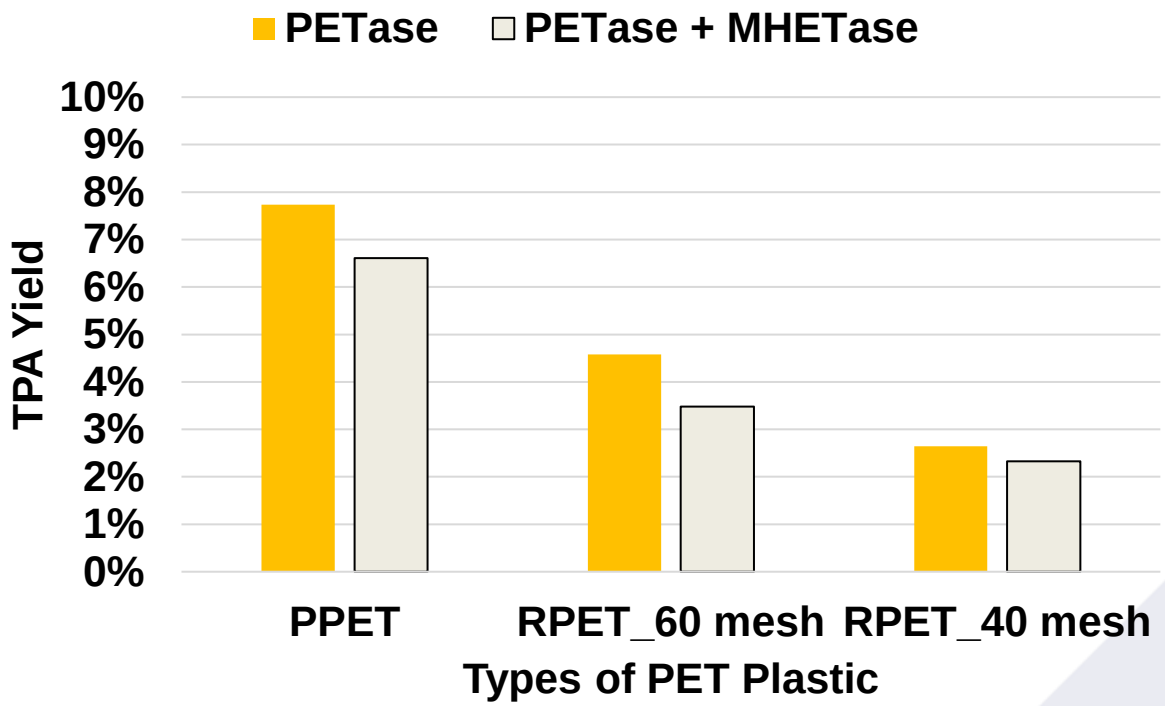
PET	192	g/mol
TPA	166	g/mol
EG	62	g/mol
Solution	**1**	**L**
Conversion	**90**	**%**
Temperature	**65**	**C**
Kw	1.27E-13	
pH	**8**	
pKa, 1	3.51	
pKa, 2	4.82	
[OH-]	1.270E-05	M
[H3O+]	1.000E-08	M
[HA-]/[H2A]	3.090E+04	
[A2-]/[HA-]	1.514E+03	
[A2-]/[H2A]	4.677E+07	
[H2A] Fraction	**2.137E-08**	
Start		
PET	**21.33**	**g**
End		
TPA	**16.6**	**g/L**
EG	**6.2**	**g**

Preliminary calculations for product equilibria @ reactor conditions → ***Given hypothetical input conditions, TPA solubility is 16.6 g/L***

Progress Task 7. Design reactor system to maximize degradation efficiency



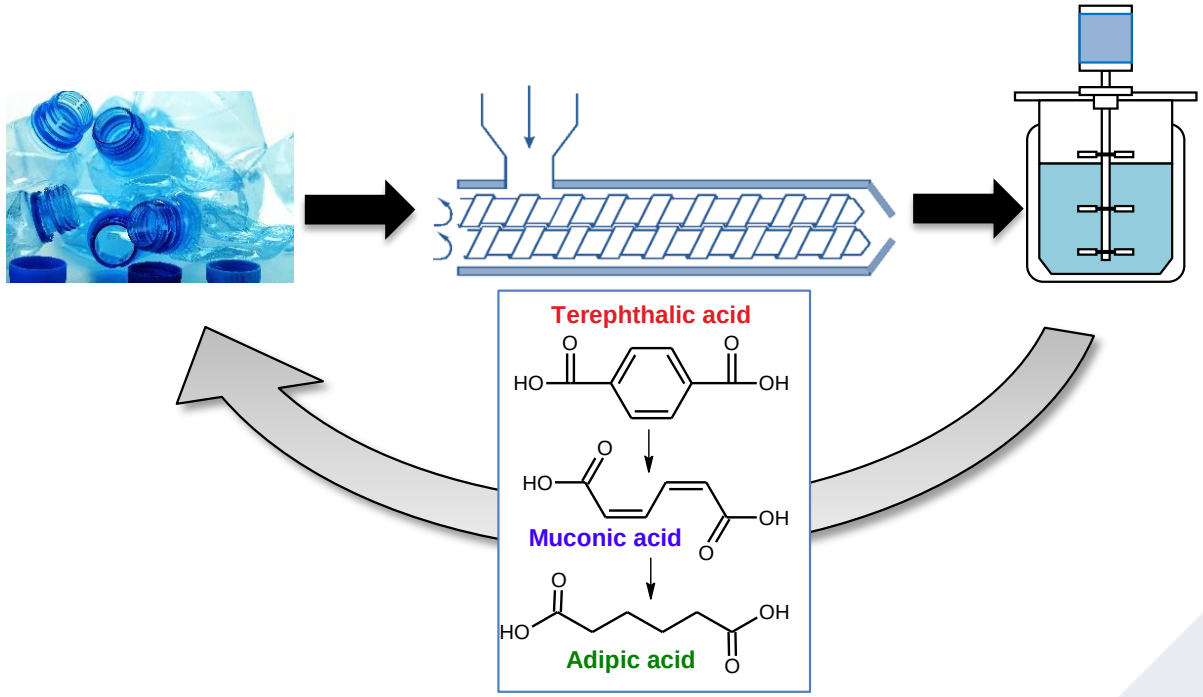
Design a ball-milling reactor for simultaneous feedstock grinding and enhancement of mixing between the enzyme and the substrate



Investigate performance depending on particle size, enzyme recipe → ***Smaller particle size yields more product; still investigating mechanisms in two-enzyme system***

Summary

- Heterogeneous and diverse PET sources identified and characterized
- Mechanical and chemical pretreatment has potential to improve efficiency of enzymatic hydrolysis
- Enzyme modifications for optimal deconstruction performance
- Reactor design, scaleup, and integration to lower cost of value-added chemicals
- Project is on schedule with progress toward upcoming milestones



Quad Chart Overview

Timeline

- Start data: 1 April 2020
- End date: 31 March 2023

	FY20 Costed	Total Award
DOE Funding	$87,537	*$1,140,814*
Project Cost Share	$34,576	$420,423

Project Goal

The overall goal of this project is to study and develop a biochemical conversion process for microbial production of specialized degradation enzymes for recalcitrant polyesters, and bioconversion of the degradation products to high value chemicals.

End of Project Milestones

- A 20% increased rate of degradation over current enzymatic and microbial approaches for three forms of waste PET not suitable for mechanical recycling
- A technoeconomic analysis that identifies the process path to achieve $2/kg TPA starting from at least one form of PET waste
- A conversion yield of 70% in one day for three PET waste streams that are both crystalline and amorphous, and contain trace impurities

Project Partners

National Renewable Energy Laboratory

Funding Mechanism

DE-FOA-0002029
Topic Area AOI 8b: Designing novel methods for deconstructing and upcycling existing plastics
FY2019

Additional Slides

Responses to Previous Reviewers' Comments

- Initial Verification Go/No-Go Review:
  - Emphasize work with cost-advantaged PET waste sources
  - Focus more strongly on LCC as best performing enzyme in prior literature. Work with PETase/MHETase for comparison.
  - Screen degradation on structurally contrasting PETs to provide rationale for pretreatments
  - Refocus to intermittent grinding rather than in situ ball milling due to energy intensity concerns
  - Move efforts in Technoeconomic analysis to begin earlier in the project (M3)
  - Modify to reduce effort in bioconversion of TPA to *c,c*-muconic acid and adipic acid
  - Pilot scale reactor size modified from 5 L to 2 L
  - Added work on enzyme expression system to BP3

Publications, Patents, Presentations, Awards, and Commercialization

- None published to date