DOE Bioenergy Technologies Office (BETO) 2023 Project Peer Review

Bioconversion of Heterogeneous Polyester Wastes to High Value Chemical Products

7 April 2023

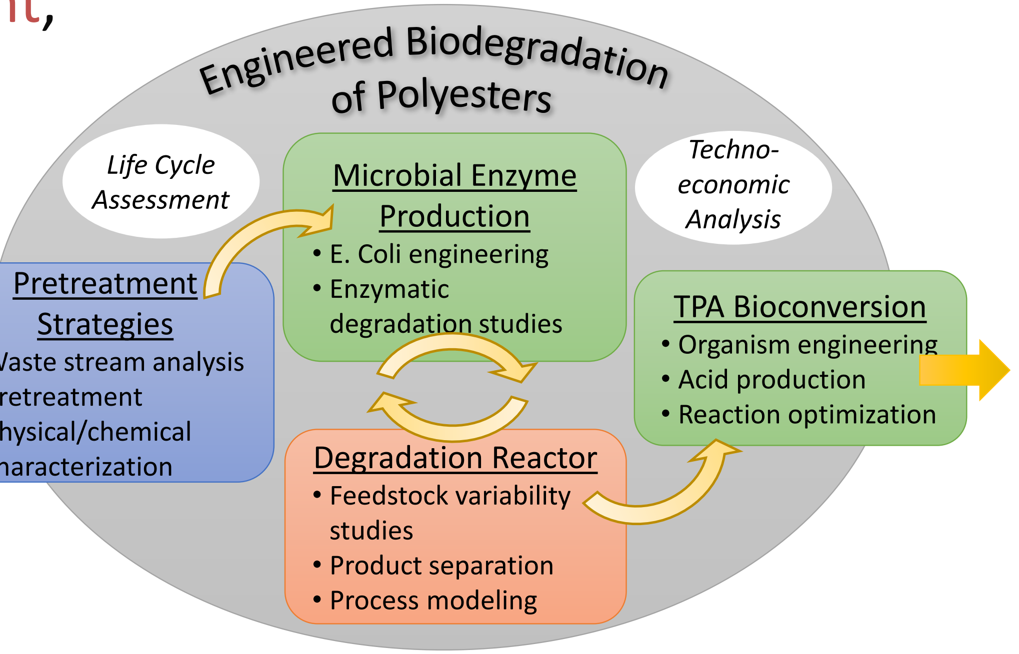
Performance-Advantaged Bioproducts, Bioprocessing Separations, and Plastics

Margaret Sobkowicz
University of Massachusetts Lowell

Project Overview

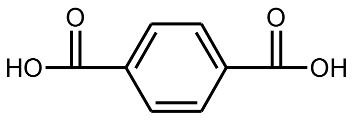
Integrated thermomechanical pretreatment, enzymatic hydrolysis and bioconversion to recycle polyester waste to high value chemicals

Diverse Polyester feedstocks



High value chemical products

Terephthalic acid



Project Objectives

- Determine most prevalent forms of poly(ethylene terephthalate) (PET) film and fibers in post-consumer and industrial waste streams; physical characterization before and during deconstruction
- Explore pretreatment and reactor design to increase efficiency of terephthalic acid (TPA) production
- Design bacterial strains that produce key enzymes for bioconversion of PET into 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 – Approach

- Couple plastics processing pretreatment, innovative reactor design, and bioprocess engineering for systems integration of process steps, guided by technoeconomic analysis
- Challenges:
  - Limited ability of enzymes to depolymerize crystalline domains
  - High cost of pretreatment steps and coupled size reduction and crystallization in PET substrates
  - Product bottleneck – high TPA concentration slows conversion, requires base addition
- G/NG 2: Intermediate Verification – completed 01/14/2022
  - Demonstrated 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 grinding
  - A 20% increase of TPA yield using the combination of size reduction and in situ product removal
- Diversity, Equity and Inclusion:
  - Diverse project team (44% underrepresented minorities)
  - UML faculty participate in Active Bystander training → enhances faculty awareness of microaggressions toward underrepresented groups and teaches strategies for mitigating their impact
  - Graduate student training under development

1 – Approach: Project 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


Principal Investigator: Margaret Sobkowicz

Sobkowicz (tasks 2, 5, 6, 11)
- Reactive plastics processing, Renewable polymers, Polymer characterization, Structure-property relationships


Postdoc: Allen Chang


Co-PI Gregg Beckham


Co-PI: Dongming Xie


Co-PI: Hsi-Wu Wong


Postdoc: Natasha Murphy

Xie (tasks 3, 4, 6, 12)
- Biomanufacturing, Metabolic engineering, Bioprocess and bioreactor engineering

Wong (tasks 6, 7, 11)
- Chemical reaction engineering, Plastics recycling, Waste to energy, Biofuels

Beckham (tasks 8/10, 11, 12)
- Waste valorization, Plastics upcycling, Metabolic engineering, Bioprocess development, Molecular simulation

Progress - Summary of Technical Achievements

Pretreatments

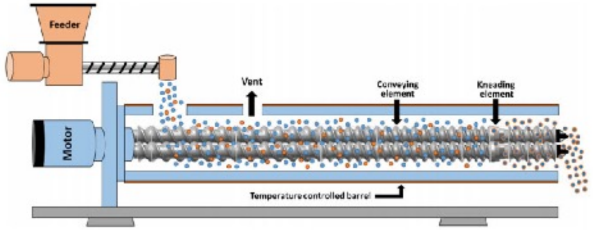
Effects of melting pretreatments on enzymatic depolymerization of PET[1]

Increasing screw speed → increases shear, specific mechanical energy, crystallinity

→ decreases molecular weight

Increasing throughput → little impact on crystallinity

→ decreases specific mechanical energy



Conclusion: low screw speed, high throughput provides optimal deconstruction

Effects of substrate particle size on enzymatic depolymerization of PET[2,3]

Increasing specific surface area → increases initial depolymerization rate, crystallinity

→ decreases total extent of conversion

Particle size reduction method is significant

Grinding: fast, high throughput. Increases crystallinity

Pelletizing: slower, energy intensive. Less impact on crystallinity

[1]Akanksha Patel, et al. *ACS Sustainable Chemistry & Engineering* **2022** *10* (41), 13619-13628. 10.1021/acssuschemeng.2c03142

[2]Chang, A.C., et al. *Macro Molecular Rapid Comm* **2022**. 43: 2100929. 10.1002/marc.202100929

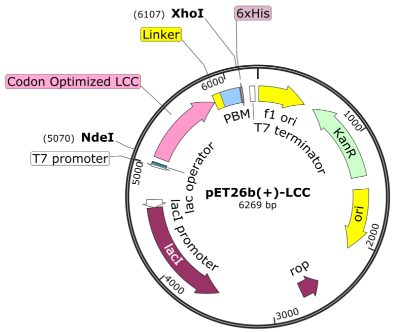
[3]Richard K. Brizendine et al. *ACS Sustainable Chemistry & Engineering* **2022** *10* (28), 9131-9140 10.1021/acssuschemeng.2c01961

Progress - Summary of Technical Achievements

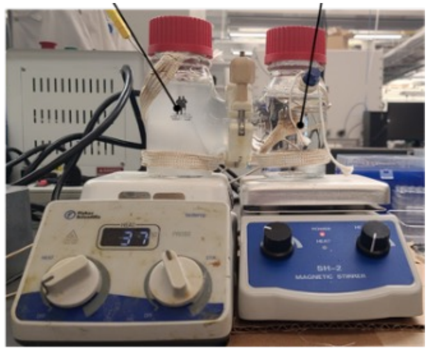
Bacterial expression

Enzyme Selection, Optimization, and Production toward Biodegradation of Waste Poly(ethylene terephthalate) at Scale[1]

- LCC (ICCG) and variants (LCC, PelB-LCC, LCC-PBM, PelB-LCC-PBM, PBM-LCC, PelB-PBM-LCC) were expressed in *E. coli* BL21(DE3), which produces > 1 g/L target enzyme in 1-L fed-batch bioreactor in 24~36hr
- PelB-LCC shows > 20% PET degradation rate over the original LCC while also increases the enzyme secretion



pET26b(+)-LCC-PBM

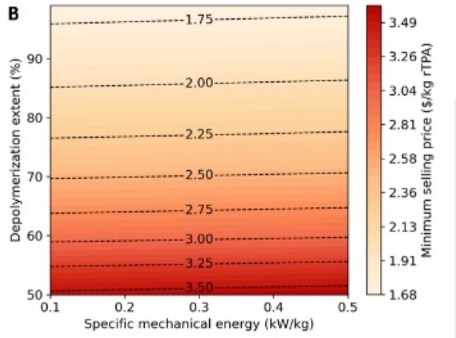


Reactor advancements

In situ product removal (ISPR) via membrane separation tested at 55 °C. Removal of products from enzyme/substrate reactions increases overall extent of the reactions and TPA yields compared to those without product removal. Scalability studies showed effect of surface-to-volume ratio on efficiency of ISPR system.

Technoeconomic Analysis

TEA case studies utilizing pretreatment improvements have shown reductions in MSP of rTPA as compared to previously published models[2]



1. Soong YHV, Abid U, Chang A, Ayafor C, Patel A, Xu J, Lawton C, Wong HW, Sobkowicz M, Xie D. Enzyme Selection, Optimization, and Production toward Biodegradation of Waste Poly(ethylene terephthalate) at Scale. Authorea. December 05, 2022. (10.22541/au.167023666.65844453/v1)
2. Singh et al. Joule (2020) 5, 9, 2479-2503 (10.1016/j.joule.2021.06.015)

UMASS LOWELL

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
Am-PET	Amorphous PET film	Goodfellow	52.3		~2-3	
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			35+	

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:*

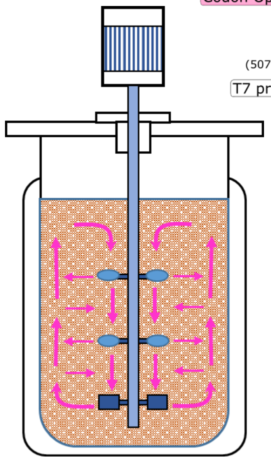
①**pET26b-ECLCC** (Expression of LCC Enzyme)

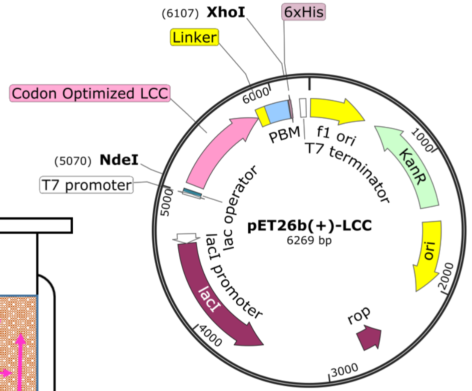
②**pET26b-pelB-ECLCC** (Expression of Secreted LCC Enzyme)

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=hydrophobic binding module





pET26b(+)-LCC-PBM

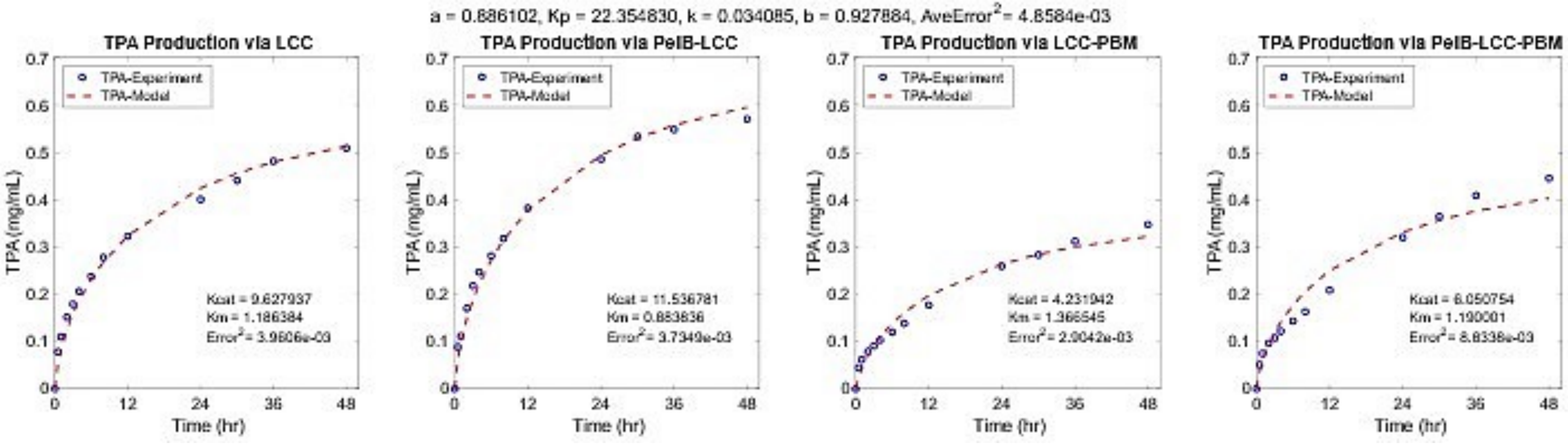
Ongoing:

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

BL21(DE3) Transformation	Intra-cellular LCC Titer (mg/mL)	Extra-cellular 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***

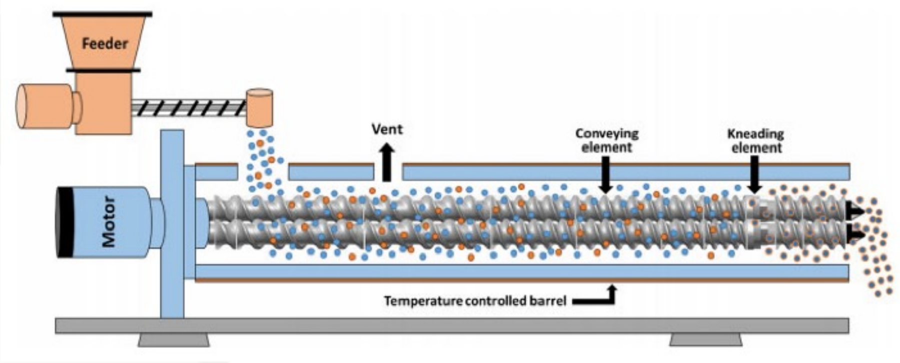
Progress Task 4. Identify the best PET hydrolase or an enzyme recipe to degrade PET polymer into monomers TPA and EG

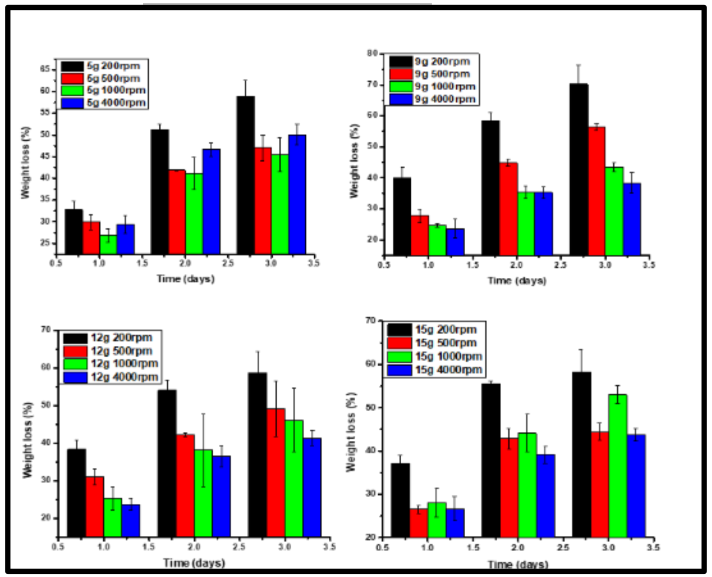


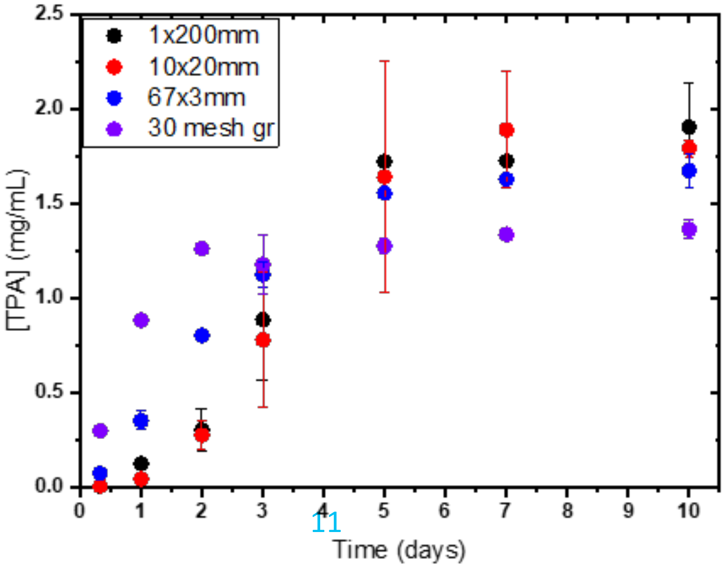
$$-\frac{dS}{dt} = \frac{K_{cat}\, E_t\, S}{K_m + bE_t + S} \cdot \frac{1}{1 + K_P P} \cdot e^{-kt} \qquad \frac{dP}{dt} = -a\frac{dS}{dt}$$

Turnover rate (K_{cat}): PelB-LCC > LCC > PelB-LCC-PBM > LCC-PBM
Michaelis constant (K_M) : PelB-LCC < LCC < PelB-LCC-PBM < LCC-PBM

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







Optimal extrusion conditions

- Low screw speed for less mechanical shear, yields higher MW which in turn limits crystallization in quenching step
- High throughput minimizes SME

Impact of surface area (form factor)

- Increasing surface area increases rate; may also increase crystallinity from shear heating during grinding

Progress Task 6. Establish enzymatic degradation experiment system

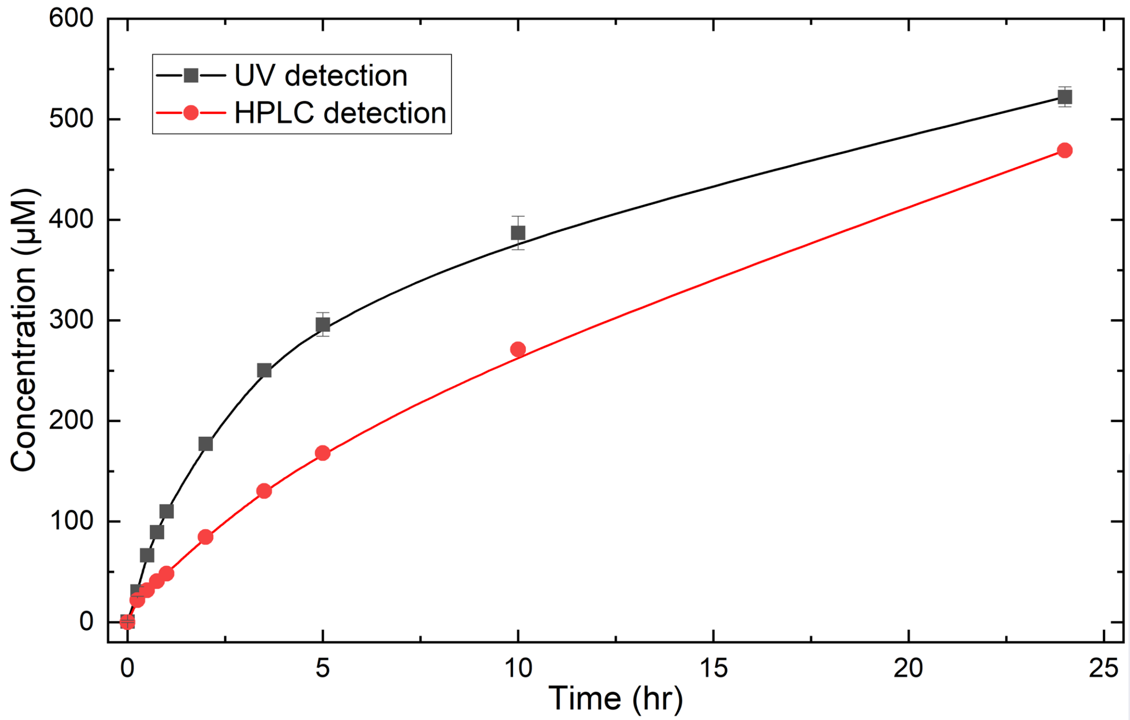
Design of Experiments to develop baseline batch kinetics for enzyme-PET waste combinations

Shake Flask Experiments:

- 250-mL Flask containing 50 mL buffer
- Reaction Condition: 1.4 µM Enzyme, 100 mg PET, at 65 °C for 48h
- Buffer: **LCC System: 100 mM potassium phosphate (pH 8)**
  PETase-MHETase systems: 50 mM Glycine-NaOH (pH 9)

Tube Experiments:

- 5-mL Glass Tubes containing 1 mL buffer
- Reaction Condition: 4.0 µg Enzyme, 5 mg PET, at 40 °C or 65 °C for 48hr
- Buffer: **LCC System: 100 mM potassium phosphate (pH 8)**
  PETase-MHETase systems: 50 mM Glycine-NaOH (pH 9)



Characterizations to track depolymerization

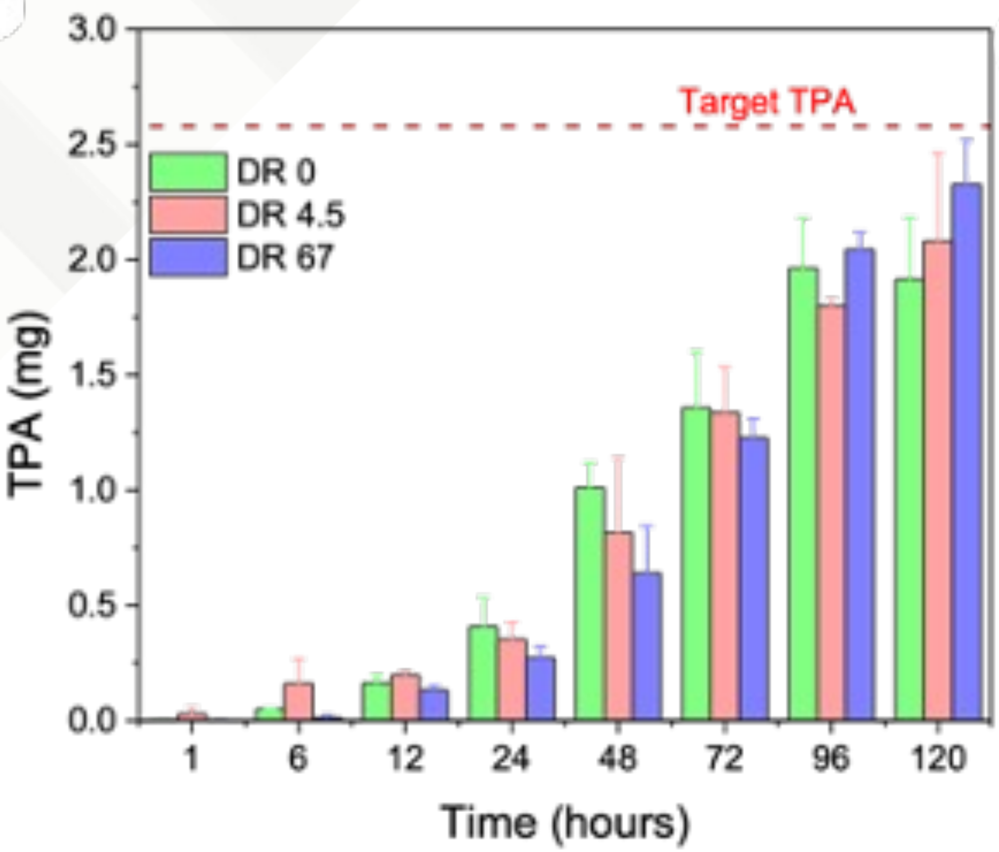
- Weight loss and crystallinity measurements
- HPLC measurements for product identification
- UV spectroscopy for quick comparative kinetics tests

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

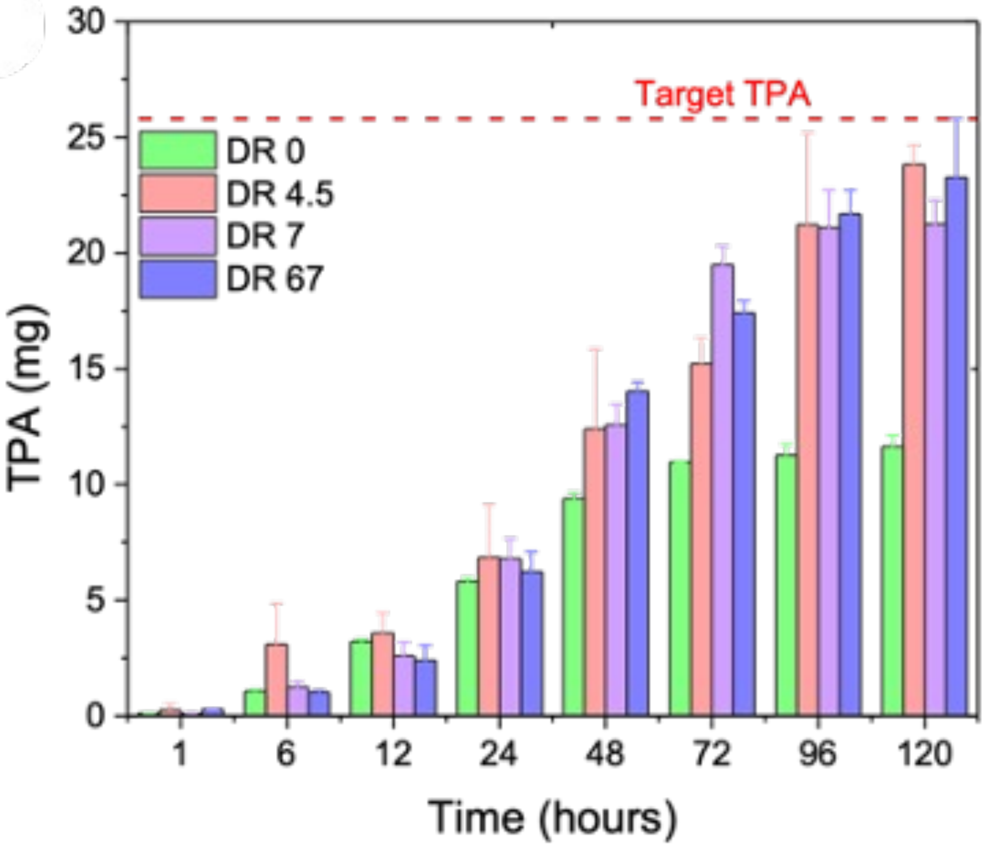
Progress Task 7. Design reactor system to maximize degradation efficiency

Membrane system explored for in situ product removal, found efficiency improved

[S]=1 mg/ml, [E]=0.1 μM purified LCC-ICCG, T = 55 °C



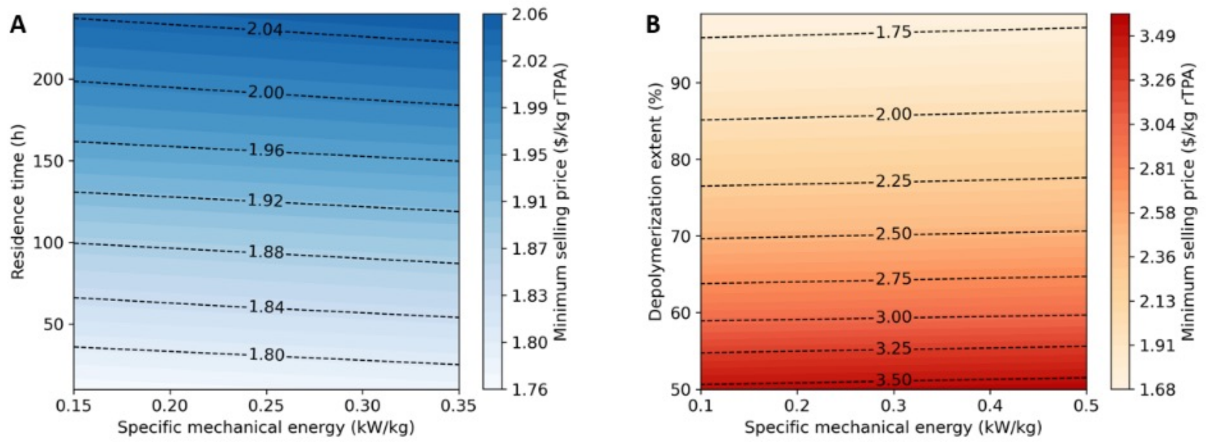
[S]=10 mg/ml, [E]=1 μM purified LCC-ICCG, T = 55 °C

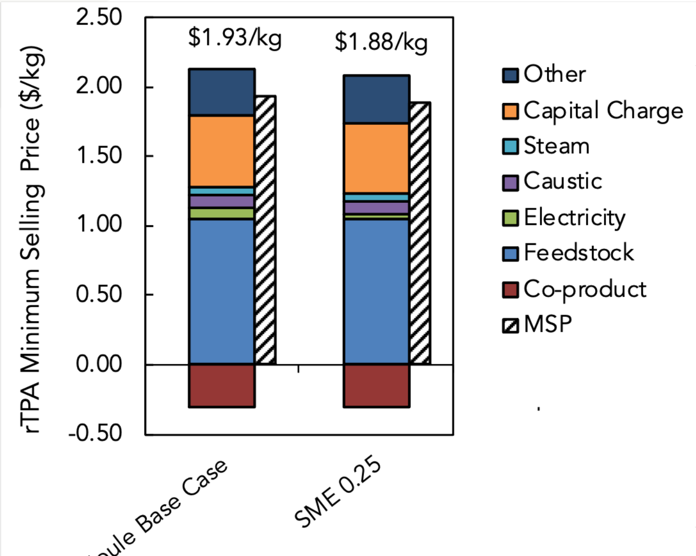


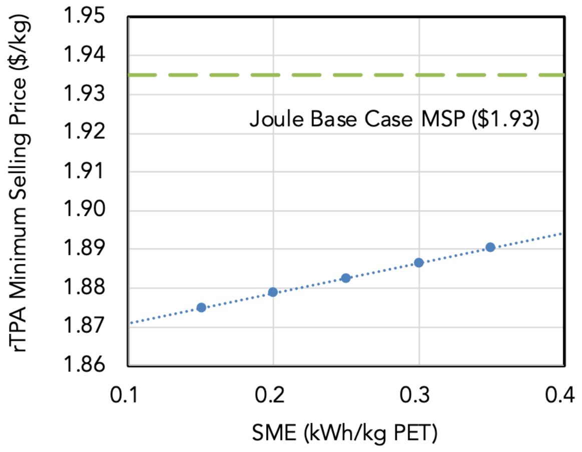
Progress Task 8/10. Modify techno-economic (TEA) and life cycle assessment models to incorporate process intensification

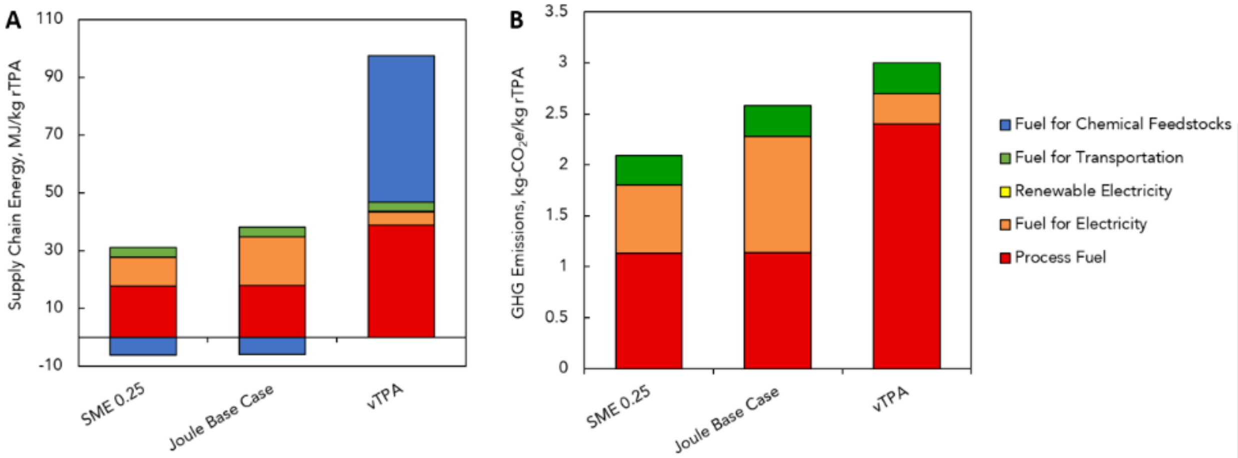
Comparison of Joule base case (2021) and UML extruded pelletized substrates with average SME 0.25 kWh/kg PET

Improvements in MSP &environmental impact resulting from lowered electricity costs for pelletizing vs. cryogrinding





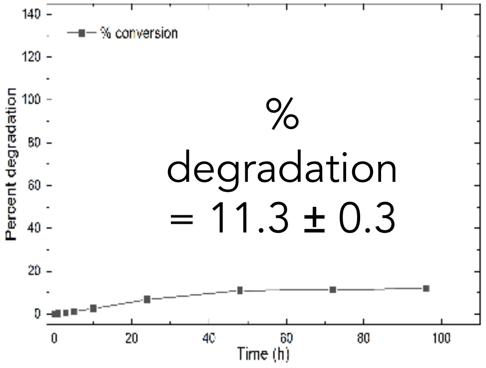




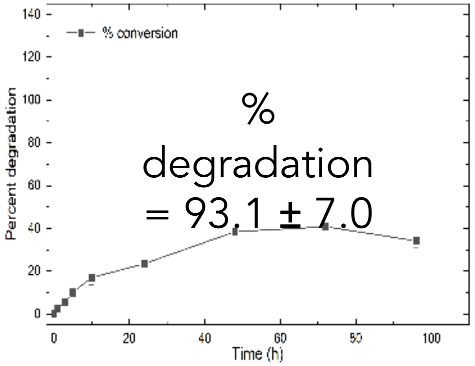
Progress Task 11. Establish a pilot-scale experimental reactor system for enzymatic biodegradation of PET plastic samples

Extrusion pretreatment comparisons

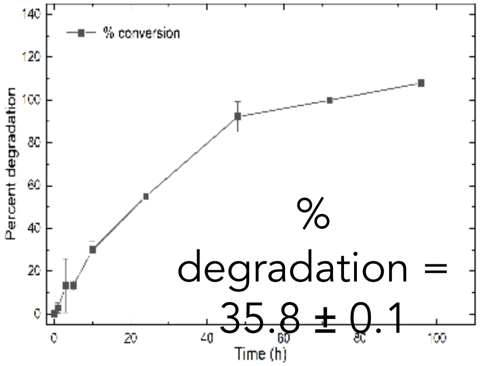
Chunks | Chopped strands | Ground chunks



% degradation = 11.3 ± 0.3

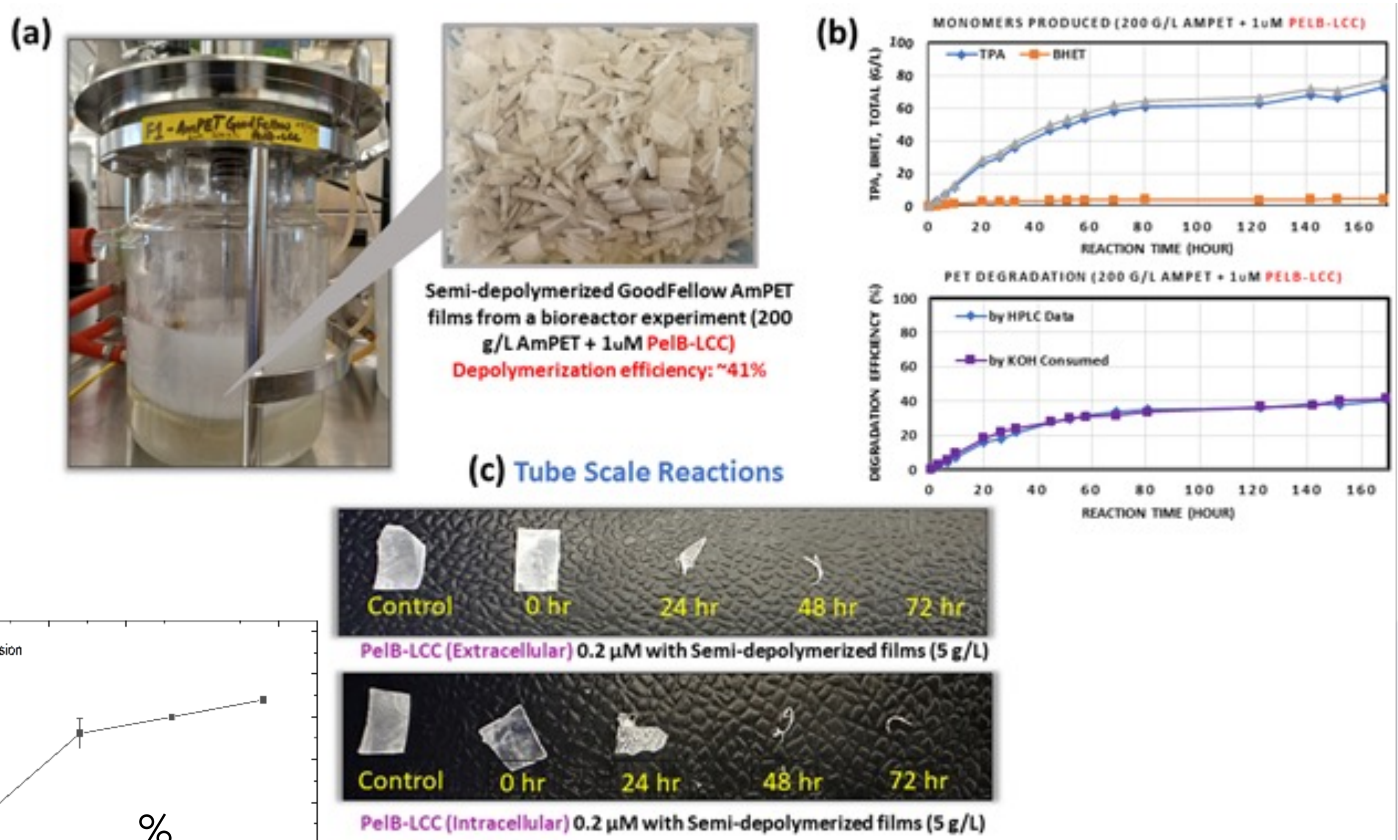


% degradation = 93.1 ± 7.0

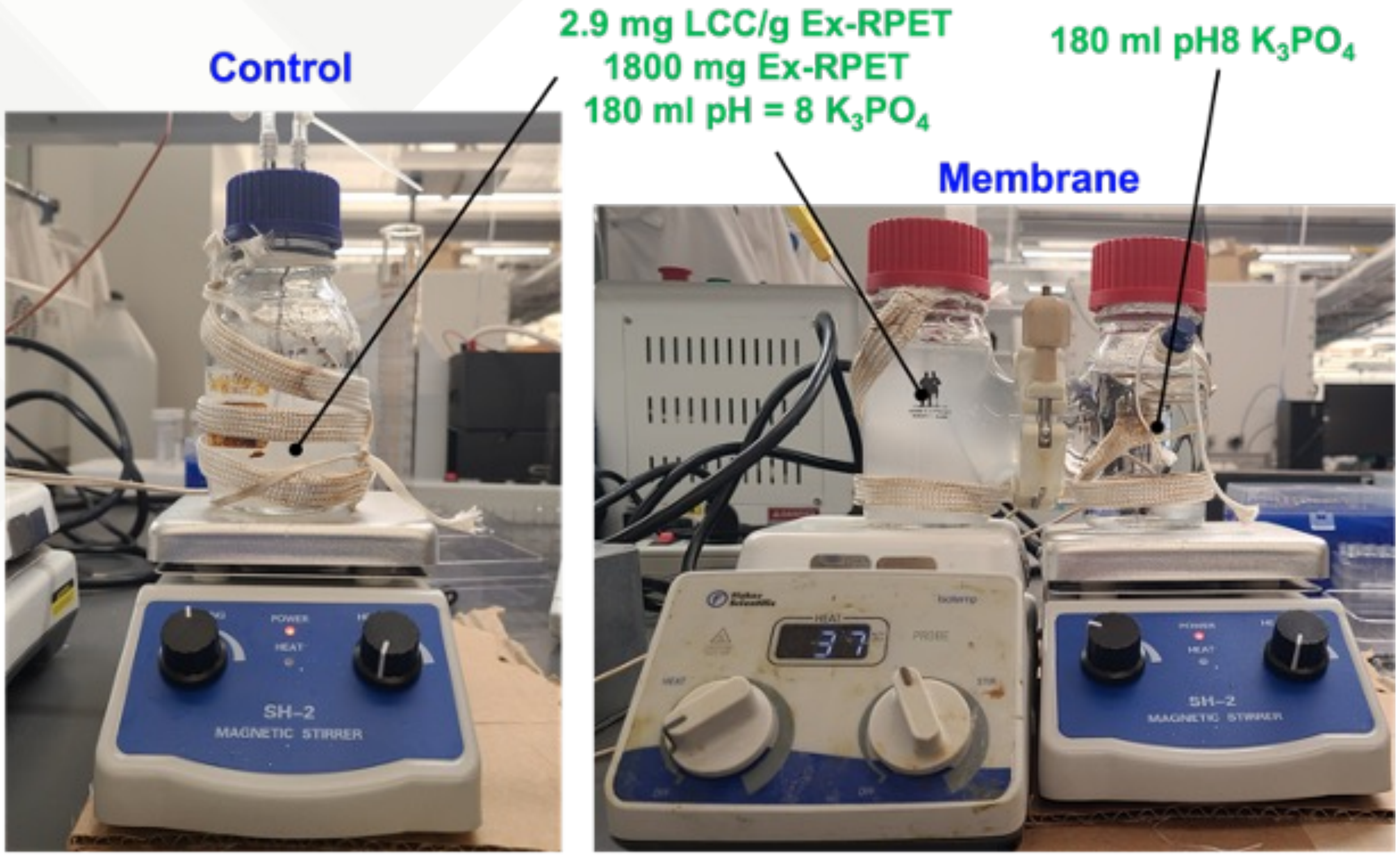


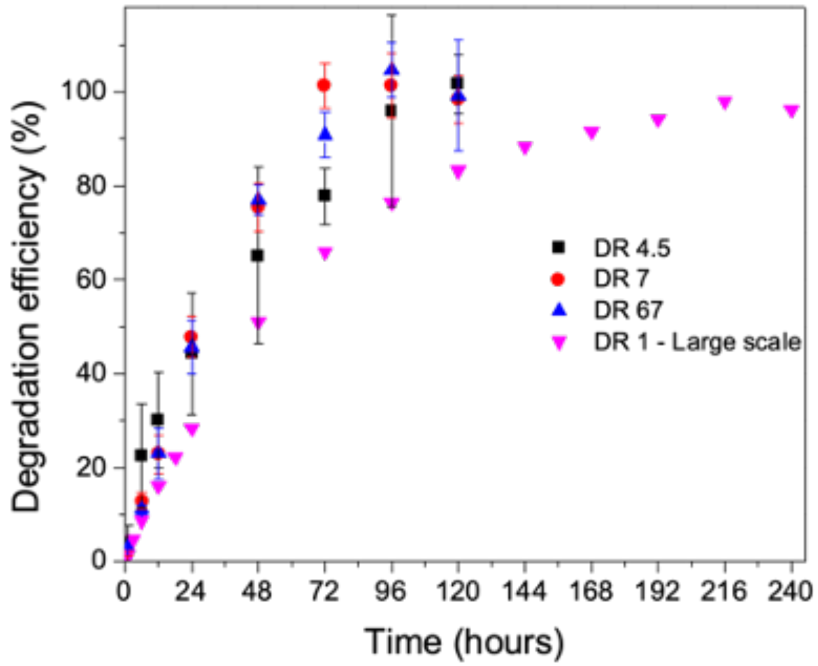
% degradation = 35.8 ± 0.1

Film substrate trials



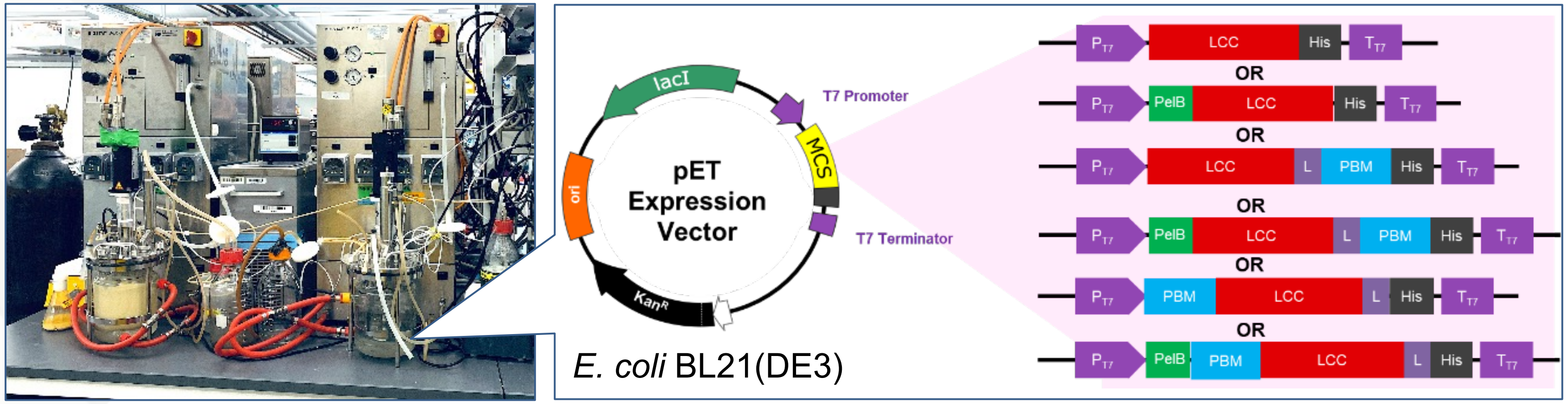
Progress Task 11. Establish a pilot-scale experimental reactor system for enzymatic biodegradation of PET plastic samples

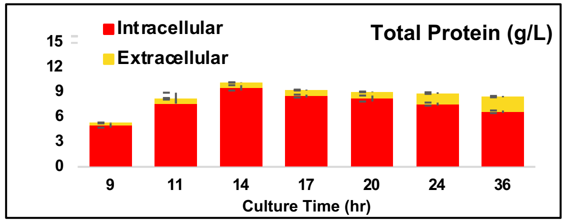


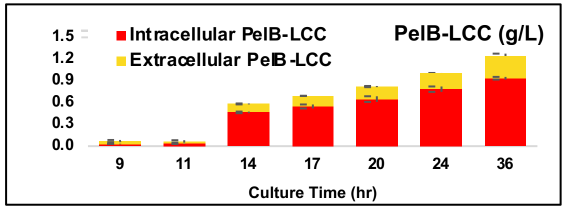


Larger scale studies suggest that membrane surface-to-volume ratio affect the effectiveness of the ISPR system

Progress Task 12. Optimize the bacterial expression system







Tasks in progress

- *E. coli* BL21(DE3) and *Bacillus subtilis* system compared for expression of LCC; *E. coli* BL21(DE3) demonstrated more efficient expression of LCC enzymes
- 1-L fed-batch bioreactor conditions developed to produce target enzyme > 1 g/L within 24 hours
- Novel PelB-LCC demonstrated > 20% PET degradation efficiency than LCC-ICCG
- Ongoing testing of PelB-PBM-LCC to compare activity with LCC-ICCG and PelB-LCC

3 – Impact

Project success will result in:

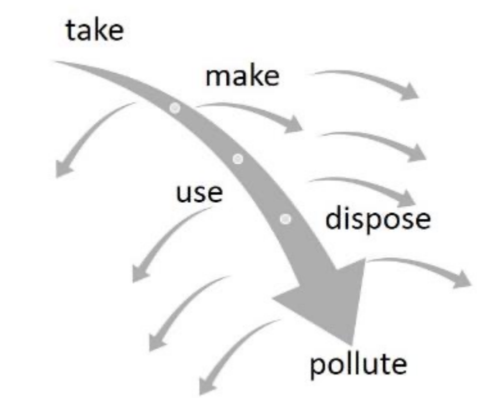
1. Improved system integration and scale-up of enzymatic bioconversion for efficient plastic recycling
2. Increased range of suitable plastic substrates for microbial conversion
3. Optimized metabolic engineering to produce high-value chemical products (TPA and derivatives)

Energy impacts include:

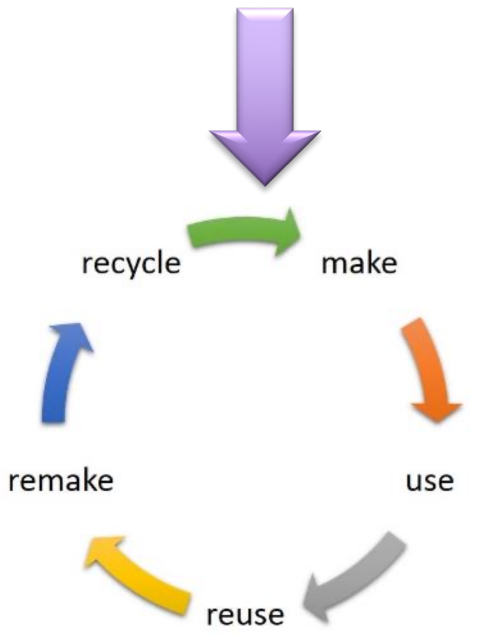
- Reduce energy requirements by over 60% relative to fossil feedstocks to produce new plastic materials
- 1,000–2,000 gallons gasoline saved by recycling one ton of plastics
- Savings of energy consumption during alternative chemical recycling processes
- Reduced CO_2 and methane generation from landfilled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

Results dissemination:

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



CC 3.0 Catherine Weetman 2016



Publications, Patents, Presentations, Awards, and Commercialization

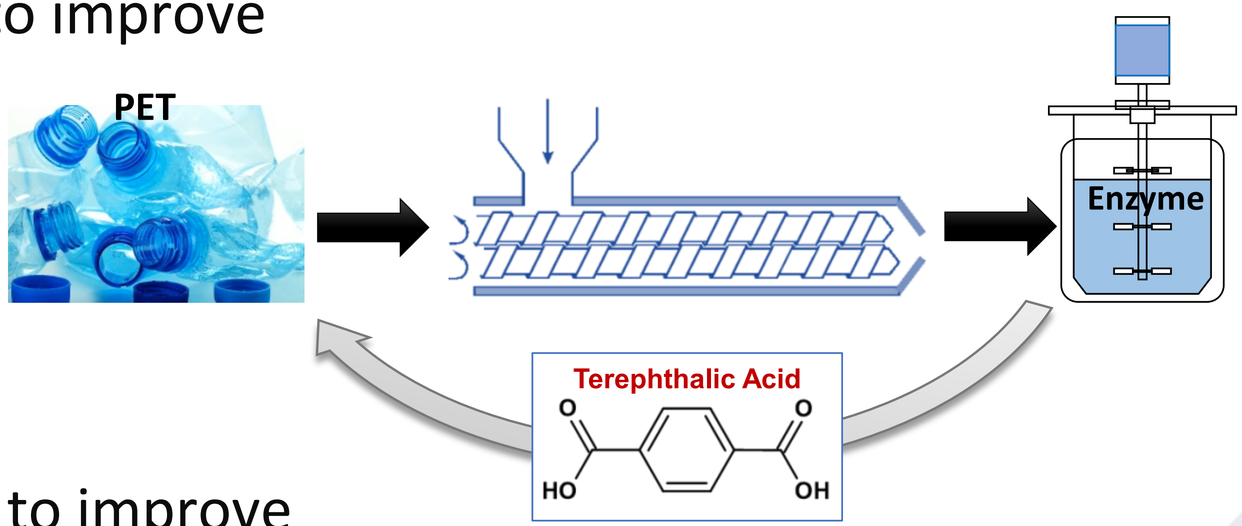
1. Ya-Hue Valerie Soong, Muhammad Umer Abid, Allen Chang, Hsi-Wu Wong, Margaret SobkowiczKline, Dongming Xie. "Efficient Biodegradation of Poly(ethylene Terephthalate) At Scale" SIMB Annual Meeting, San Francisco, Aug 7-11, 2022.
2. Christian Ayafor, Allen C. Chang, Akanksha Patel, Richard Brizendine, Gregg Beckham, Dongming Xie, Margaret J. Sobkowicz, Hsi-Wu Wong. "In situ product-removal for the enzymatic biodegradation of poly(ethylene terephthalate) (PET) via a membrane reactor". AIChE 2022
3. Allen C. Chang, Akanksha Patel, Sarah Perry, Yahue V. Soong, Christian Ayafor, Richard Brizendine, Gregg Beckham, Hsi-Wu Wong, Dongming Xie, Margaret J. Sobkowicz. "Pretreating polyester wastes for enzymatic depolymerization to recover high value products". Green Chemistry and Engineering 2022
4. Allen C. Chang, Akanksha Patel, Sarah Perry, Yahue V. Soong, Christian Ayafor, Hsi-Wu Wong, Dongming Xie, Margaret J. Sobkowicz. Understanding processing tradeoffs in the enzymatic degradation of Polyethylene terephthalate. *Macromolecular Rapid Communications* **2022,** 43(13), 2100929 https://doi.org/10.1002/marc.202100929
5. Allen C. Chang, Akanksha Patel, Sarah Perry, Yahue V. Soong, Christian Ayafor, Hsi-Wu Wong, Dongming Xie, Margaret J. Sobkowicz. "Exploring the effects of substrate size on depolymerization behaviors and costs of recycled PET". ACS San Diego 2022
6. Akanksha Patel, Allen C. Chang, Sarah Perry, Yahue V. Soong, Christian Ayafor, Hsi-Wu Wong, Dongming Xie, Margaret J. Sobkowicz. "Physio-chemical modification of Polyethylene terephthalate (PET) for improved enzymatic recycling". ACS San Diego 2022
7. Richard K. Brizendine, Erika Erickson, Stefan J. Haugen, Kelsey J. Ramirez, Joel Miscall, Davinia Salvachúa, Andrew R. Pickford, Margaret J. Sobkowicz, John E. McGeehan*****, and Gregg T. Beckham**.** Particle Size Reduction of Poly(ethylene terephthalate) Increases the Rate of Enzymatic Depolymerization But Does Not Increase the Overall Conversion Extent. *ACS Sustainable Chem. Eng.* **2022**, 10, 28, 9131–9140 https://doi.org/10.1021/acssuschemeng.2c01961

Publications, Patents, Presentations, Awards, and Commercialization, cont.

8. Akanksha Patel, Allen C. Chang, Sarah Perry, Yahue V. Soong, Christian Ayafor, Hsi-Wu Wong, Dongming Xie, Margaret J. Sobkowicz. “Improving enzymatic depolymerization of post-consumer PET via pretreatment methods”. Polymer Processing Society (PPS-37) 2022
9. Akanksha Patel, Allen C. Chang, Sarah Perry, Ya-hue V. Soong, Christian Ayafor, Hsi-Wu Wong, Dongming Xie, Margaret J Sobkowicz, “Melt Processing Pretreatment Effects on Enzymatic Depolymerization of Poly(ethylene terephthalate)”. ACS Sustainable Chem. Eng. **2022** 10(41) 13619-13628. https://doi.org/10.1021/acssuschemeng.2c03142
10. Akanksha Patel, Allen C. Chang, Margaret J. Sobkowicz, “Modifying Polyethylene terephthalate through reactive twin screw extrusion to improve enzymatic degradation” Bioenvironmental Polymer Society Conference Virtual at Rowan University, June 2021
11. Margaret J. Sobkowicz, Akanksha Patel, Ya-Hue Soong, Dongming Xie, Hsi-Wu Wong, Richard Brizendine, Allen C. Chang, Gregg Beckham “Polyester Recycling to Recover High Value Chemicals: Coupled Mechanical, Chemical and Biological Conversion” ACS Green Chemistry and Engineering Conference Virtual June 2021
12. Akanksha Patel, Allen C. Chang, Margaret J. Sobkowicz “Depolymerization Kinetics of Recycled Polyethylene Terephthalate During Melt Mixing” SPE ANTEC 2021 Virtual April 2021
13. Ya-Hue Valerie Soong, Margaret J Sobkowicz, and Dongming Xie, “Recent Advances in Biological Recycling of Polyethylene Terephthalate (PET) Plastic Wastes”. Bioengineering. **2022** 9(3): 98. https://doi.org/10.3390/bioengineering9030098.
14. Ya-Hue Valerie Soong, Akanksha Patel, Na Liu, Hsi-Wu Wong, Dongming Xie, Margaret J. Sobkowicz, Efficient Biodegradation of Poly(ethylene terephthalate) with Leaf-Branch Compost Cutinase. AIChE Annual Meeting, Boston, MA, Nov 7-11, 2021.

Summary

- Heterogeneous and diverse PET sources identified and characterized; crystallinity, molecular weight, and particle size manipulated and tracked
- Mechanical pretreatment has potential to improve efficiency of enzymatic hydrolysis with minimal energy input
- Enzyme modifications and expression in *E. Coli* show improved production efficiency and deconstruction performance
- In situ product removal a viable strategy to improve deconstruction efficiency and reduce need for pH adjustment
- Reactor design, scaleup, and system integration to lower cost of value-added chemicals



Quad Chart Overview

Timeline
- *Project start date: 1 April 2020*
- *Project end date: 30 June 2023*

	FY22 Costed	Total Award
DOE Funding	*(10/01/2021 – 9/30/2022)* *$317,100*	*(negotiated total federal share)* *$1,500,811*
Project Cost Share *	$61,032	$420,421

TRL at Project Start: 2-3
TRL at Project End: 4-5

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
- Unifi (material donation only)

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

Progress since peer review 1 (03/09/2021)

Project milestone	Due date	Percent complete	Date Completed	On track?
Milestone 1.0: Initial Verification, Go/No-Go 1	06/30/2020 (FY2020 Q1)	100%	05/20/2020 (FY2020 Q1)	Y
Milestone 2.1: Provide list of at least ten PET formulations common in post-consumer waste and secure commitments to obtain at least three industry-relevant waste materials from waste providers.	11/30/2020 (FY2020 Q3)	100%	10/31/2020 (FY2020 Q2)	Y
Milestone 2.2: Provide thermophysical characteristics of at least five common PET formulations used in industry for evaluation in the enzymatic degradation process.	01/31/2021 (FY2021 Q4)	100%	01/31/2021 (FY2021 Q4)	Y
Milestone 3.0: Functional *E. coli* chassis for production of LCC at a rate of 1 g/L from glucose within 36 h.	03/31/2021 (FY2021 Q4)	100%	03/31/2021 (FY2021 Q4)	Y

Milestone 4.0: Produce comparative kinetic data (including kcat and Km) of the degradation process for at least three individual enzymes and top three selected enzyme mixtures.	09/30/2021 (FY2021 Q6)	100%	09/30/2021 (FY2021 Q6)	Y
Milestone 5.0: Increase enzymatic degradation rate by at least 10% for three PET waste types by employing pretreatment strategies.	09/30/2021 (FY2021 Q6)	100%	09/30/2021 (FY2021 Q6)	Y
Milestone 6.0: Initial kinetic data on enzyme performance for at least two PET resins with differing crystallinity and with metallic and dye impurities.	09/30/2021 (FY2021 Q6)	100%	09/30/2021 (FY2021 Q6)	Y
Milestone 7.1: Obtain comparative data showing at least 15% faster attaining of maximum conversion by reducing the feedstock particle size and provide energy calculations for the grinding pretreatment.	12/31/2021 (FY2021 Q7)	100%	12/31/2021 (FY2021 Q7)	Y

Milestone 7.2: Obtain optimal operation conditions of the reactor system for a TPA yield that is 20% higher than in batch reaction.	12/31/2021 (FY2021 Q7)	100%	12/31/2021 (FY2021 Q7)	Y
Milestone 9.0: Go/No-go 2 M21	12/31/2021 (FY2021 Q7)	100%	01/14/2022 (FY2022 Q8)	Y
Milestone 10.0: Test validity of $2/kg initial target for TPA cost based on developed TEA, and deliver a framework and initial predictions of environmental impact of the process under investigation.	09/31/2022 (FY2022 Q10)	100%	09/31/2022 (FY2022 Q10)	Y
Milestone 11.0: (M30) Attain 70% enzyme conversion efficiency in one day in pilot scale reactor.	09/31/2022 (FY2022 Q10)	100%	09/31/2022 (FY2022 Q10)	Y
Milestone 12.0: (M36) An *optimized bacterial expression system* that expresses LCC and other enzymes at a rate of 1 g/L from glucose within 24 h.	03/31/2023 (FY2023 Q12)	80%	--	N
Milestone 13: Final Verification	03/31/2023 (FY2023 Q12)	0%	--	N

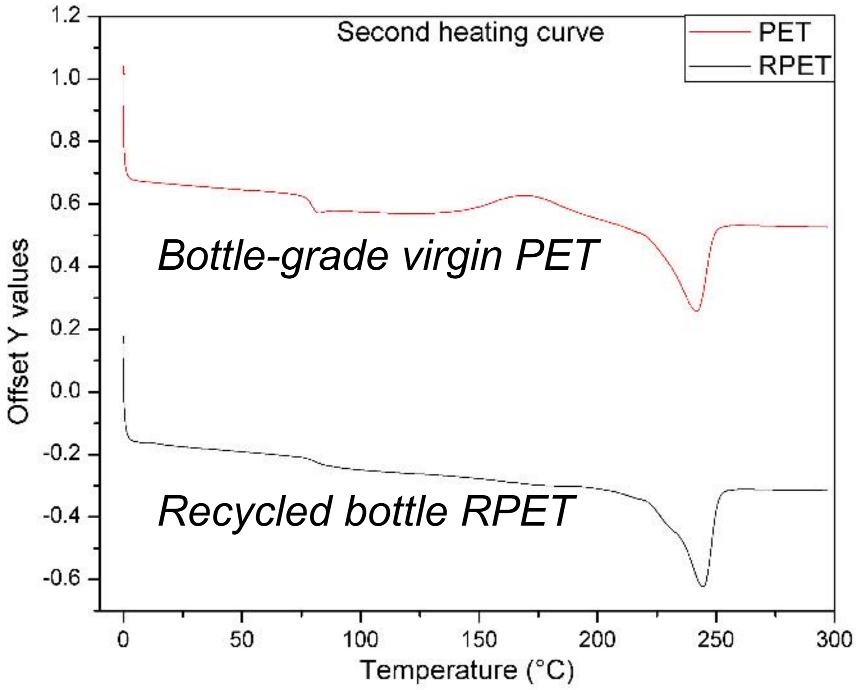
Task 2: PET waste streams and characteristics

Code	Description	Manufacturer/ supplier	Tg	Tm	Crystallinity	IV	Mn (kDa)	Mw (kDa)	PDI
VPET	pre-crystallized PET granules used for blow molding	DAK Americas	80.5	235	39.2	0.85	35.2	69.2	.97
RPET	Recycled PET Bottle Flakes acquired after washing/shredding	Post-consumer from Plastics Forming Enterprise	81.8	244.5	30.5	0.75	35.9	68.7	1.91
GPET	PET Granules with glass reinforcement	Sigma-aldrich	52.3	214.8/ 237.6	23.6	0.51	32.3	58.3	1.81
PPET	Amorphous PET powder, 300 um	Goodfellow	81.2	244.1	28.1	0.75	27.3	52.0	1.91
FPET	recycled fiber PET yarn	Prepared at UML	73.6	240	26.8	0.80	15.5	28.5	1.84
PETG-F	Virgin PETG pellets used for film extrusion	Indorama XPURE 4004	82.5	254	23.7	0.73	25.4	50.4	1.99

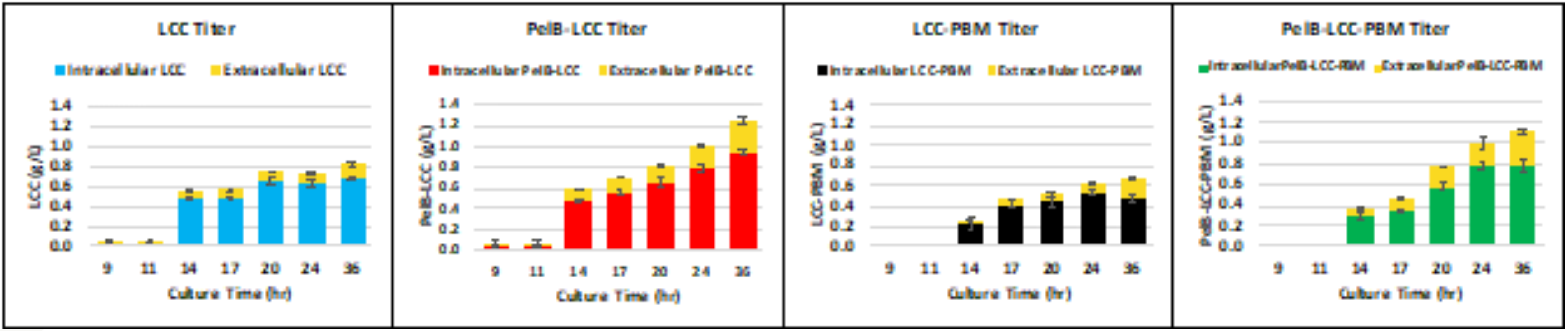
Progress Task 2: Evaluate PET waste streams and characteristics for degradation

- 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	--



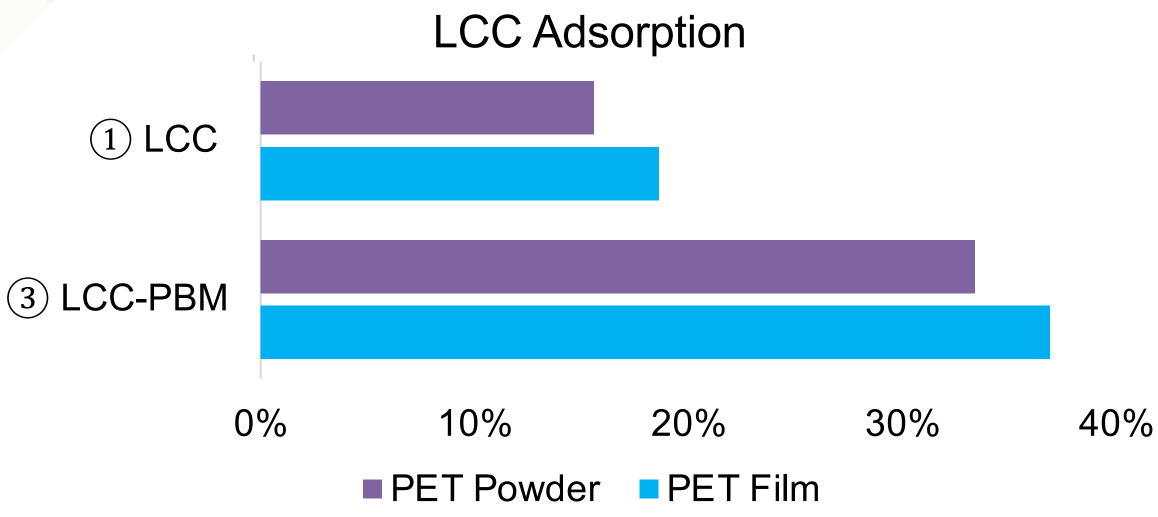
Task 3. Create PET Hydrolase expression system in *E. coli*

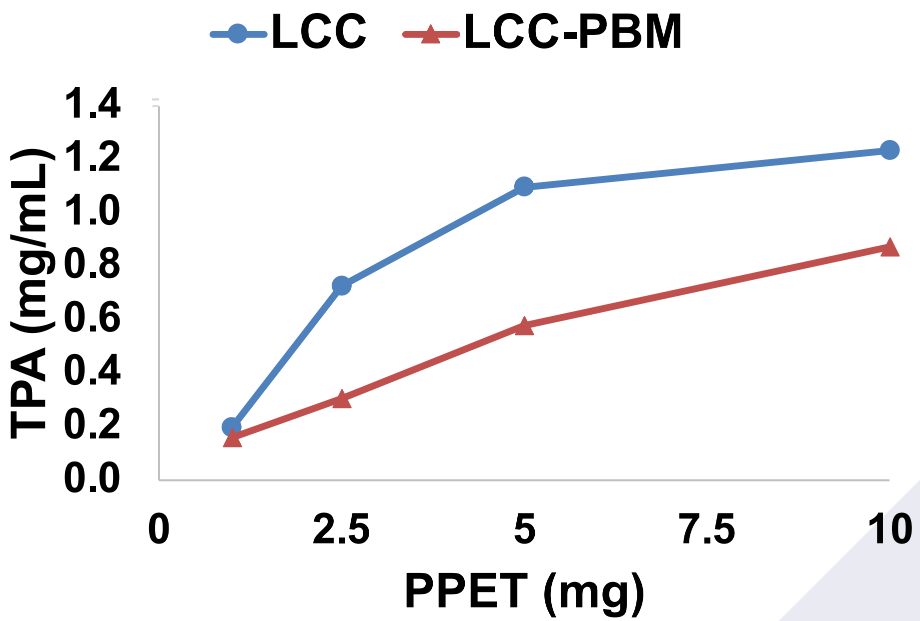


We have successfully demonstrated total protein production up to 10~12 g/L by the *E. coli* strain under 1-L fed-batch bioreactor conditions. The target PelB-LCC titer reached ***1.24 g/L within 36 hours***. Approximately 0.31 g/L produced PelB-LCC was secreted to the fermentation medium. The secreted, non-purified LCC enzymes in the fermentation medium can be directly used for further PET biodegradation experiments.

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



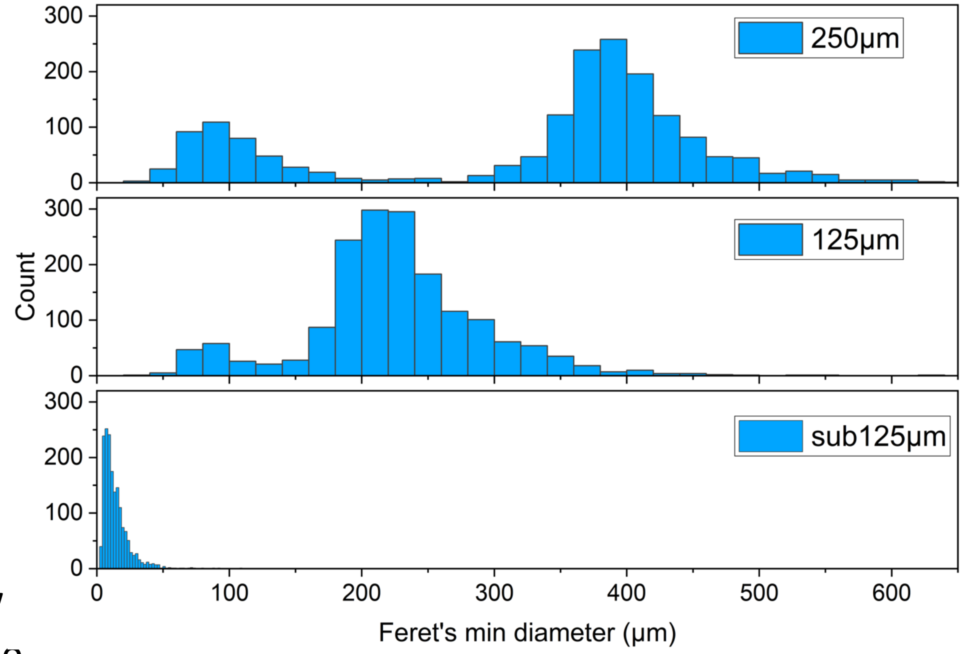


Task 5. Explore pretreatment strategies to prepare PET for biodegradation

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

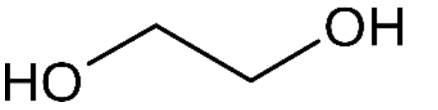
Increasing surface area and reduced crystallinity for faster depolymerization → ***Cryogrinding and sieving yields distinct size fractions; extrusion decreases MW by ~50%***

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