Elucidation of biological processes for polyethylene deconstruction

Ross R. Klauer
Advisors: Dr. Mark A. Blenner; Dr. Kevin V. Solomon
Committee Members: Dr. Aditya M. Kunjapur, Dr. LaShanda T.J. Korley, Dr. Jason P. Gleghorn

Tuesday, September 23, 2025 at 10:00 am (ET)
FinTech 414 | https://udel.zoom.us/j/3295278709?omn=91066294371| Password: Plastics

---

Plastics waste is accumulating at exponential rates in the environment, causing negative environmental effects such as the release of toxic xenobiotics through additive leaching and increases in greenhouse gas levels through environmental plastics degradation and microplastics toxicity to CO2-fixing phytoplankton. Moreover, plastics waste directly impacts human health by (1) causing food chain instability from the worldwide transport of microplastics and (2) by increasing the likelihood of cardiovascular events and strokes upon microplastics entry into human arteries. Therefore, waste plastics represent an ideal feedstock for (bio)manufacturing due to their abundance, intrinsic low value, and the ability to mitigate adverse environmental and human health consequences from their environmental removal. However, commonly used mechanical recycling approaches lead to lower quality plastics with each iteration of the recycling process, limiting their use. Moreover, chemical recycling and upcycling strategies rely on high temperatures and pressures that demand significant energy inputs, often produce unwanted byproducts, and require use of expensive rare earth metal catalysts, limiting their feasibility in practice. Biological plastics waste deconstruction and upcycling offer more sustainable and economically competitive approaches to plastics waste management by operating at ambient temperatures and pressures, using inexpensive-to-produce enzymes, and limiting unwanted byproducts. The economic feasibility of biological plastics deconstruction is demonstrated by recent successes in the enzymatic deconstruction of poly(ethylene terephthalate) (PET). Engineered PET hydrolases are being employed at industrial scale to convert PET into monomers for recycling, with the first commercial enzymatic PET recycling plant opening in France in 2026.

The promise of enzymatic plastics deconstruction has led to a rapid increase in the number of reported plastics deconstructing microbes and enzymes. However, the biological deconstruction of non-PET plastics remains highly controversial due to conflicting and irreproducible results in the literature. This irreproducibility stems largely from (1) a lack of standardized substrates that enable direct, cross-study comparison and (2) false positive reports of plastic active enzymes resulting from a lack of key controls in experimental designs that leads to data misinterpretation. In this thesis, we highlight this irreproducibility by using carefully selected controls to show that key results from the literature were false positive claims of deconstruction. We use these learnings to propose a set of standards and best practices for biological plastics deconstruction. These standards include the selection of additive-free substrates with material properties that promote bioavailability to minimize false-negative reporting. Additionally, the proposed standards provide

guidelines for appropriate experimental design and materials characterization analysis by suggesting the use of inactivated enzyme controls, promoting appropriate substrate washing, and highlighting common pitfalls in analyzing spectroscopy and microscopy data. These proposed best practices can greatly increase the rate at which biological plastics deconstruction advances by permitting direct cross-study comparisons and limiting false positive reporting.

Using the developed 'best practice' methodologies, we searched the gut of plastic-fed yellow mealworms for enzymes that participate in the deconstruction of low-density polyethylene (LDPE), one of the most widely used and discarded plastics globally. The yellow mealworm gut microbiome was selected due to its reported ability to deconstruct plastics at rates much higher than those in marine or soil environments. We first confirmed the need for a healthy gut microbiome in mealworm LDPE deconstruction by abolishing the LDPE deconstruction phenotype upon gut sterilization. We thus mined the guts of LDPE-fed mealworms and discovered dye decolorizing peroxidases (DyP) that oxidize LDPE, initiating biodeconstruction. DyPs are necessary for LDPE deconstruction in mealworm guts, as LDPE deconstruction is greatly inhibited in mealworms co-fed competitive DyP inhibitor reactive black 5. LDPE-active DyPs contain a divergent hydrophobic loop region that mediates binding and can be used to modulate activity and LDPE oxidation by DyPs is driven by surface exposed, radical-harboring residues proximal to the active site that permit long range electron transport and polymeric substrate oxidation. This work presents evidence for biological LDPE deconstruction, enzymatic LDPE oxidation, and identifies targets for further development towards scalable biological LDPE upcycling.

The hydrophobic loop region and electron transport system in DyPs present promising enzyme engineering targets. However, the throughput of commonly used spectroscopic techniques is too low to test libraries of engineered enzyme mutants. Therefore, we developed a microtiter plate based assay to screen for LDPE oxidizing enzymes in an effort to improve discovery and engineering rates of LDPE-oxidases. We use 4-hydrazino-7-nitro-2,1,3-benxoxadiozole hydrazine (NBD-H) which reacts with LDPE-aldehydes, a predominant DyP product, to form fluorescent hydrazones. We confirm that the fluorescence from hydrazone formation is a reliable measure of LDPE oxidation by showing that fluorescent signal from plasma-oxidized positive control LDPE films correlates strongly with widely used carbonyl index measurements for oxidation. More importantly, we demonstrate that the NBD-H probe reliably identifies LDPE-active DyPs, serving as an effective screening tool for enzymatic LDPE oxidation. The use of the NBD-H assay enables the screening of thousands of enzymes in 24 hours relative to the tens of enzymes that can be screened by commonly used spectroscopic approaches, thus enabling enzyme engineering efforts to propel enzymatic plastics deconstruction closer to industrial use.

In summary, the work in this thesis confirms that yellow mealworms deconstruct LDPE, identifies a subclass of DyPs as responsible for initiating that deconstruction via oxidation, and delineates novel methods and engineering targets to further improve LDPE bio-oxidation and -deconstruction towards industrial relevance.