Testing the Effectiveness of PET Plastic-Eating Microbes at NAU

Justin D. Case[1] and Stephanie K. Hurst[1*]

[1]Department of Chemistry and Biochemistry, Northern Arizona University, Flagstaff, AZ 86011-5698

Executive Summary

This project has been examining the effects of scaling successful experimental methods to a size that would aid NAU in reducing its carbon footprint as well as how the efficiency of the microorganisms involved would react.

Introduction

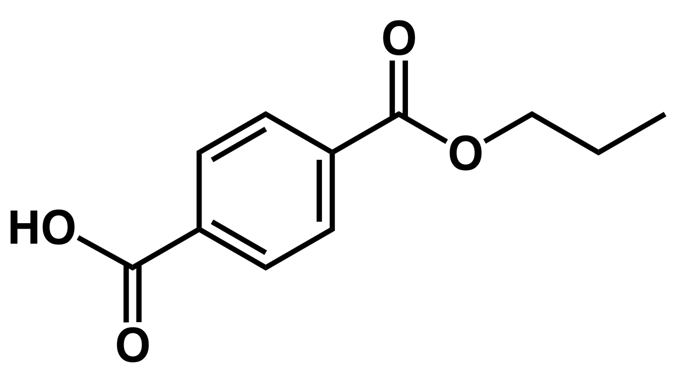


Figure 1: Chemical structure of PET monomer

Polyethylene terephthalate (PET) is a commonly used thermoplastic polymer resin found in everyday items such as clothing and containers for food and liquid.[1] Plastics, such as PET, have poor biodegradability and persist in landfills for extended periods of time. PET is chemically inactive and highly resistant to microbial attack.[1] The crystallinity of PET influences the density and hardness of the material increasing resistance to degradation.

Literature Survey

Figure 2: Single use PET bottles from the NAU, Flagstaff campus

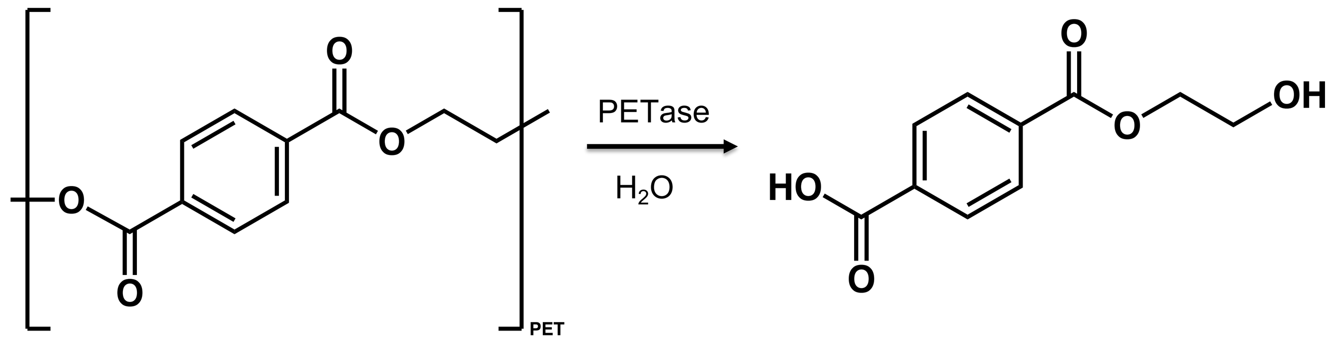
At Northern Arizona University (NAU), high-crystallinity PET exists in the form of single-use plastic drinking bottles, such as Pepsi, and "to-go" containers, seen right. Studies have shown the plausibility of using microorganisms to break down low-crystallinity PET. *Ideonella sakaiensis* is a microorganism capable of hydrolyzing PET using the enzymes, PETase and MHETase.[1-3] This hydrolysis performed by *I. sakaiensis*, could prove useful in pursuit of an effective process to degrade PET. The main goal is to provide a practical way of treating large quantities of plastic waste stored in landfills and produced on college campuses like NAU.

PETase has been seen to degrade low-crystallinity PET at a rate of 0.13 mg/cm^2 per day.[1] If the hydrolysis of high-crystallinity PET proves successful and practical, its application would assist in the degradation of the most common form of PET present at NAU.

Given the rate of degradation found at low-crystallinity PET, Higher crystallinity PET is expected to take longer to degrade. However, implementation of processing low-crystallinity PET could decrease the quantity of plastic waste extruded by NAU.

Literature Survey Cont.

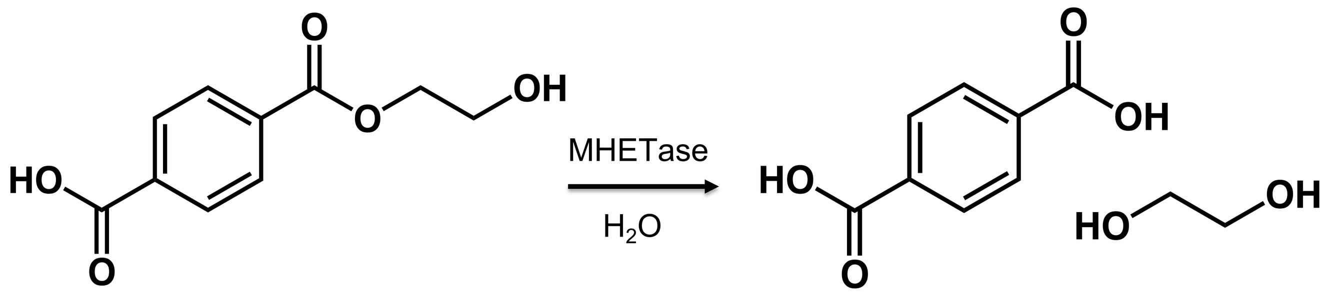
The hydrolysis for degrading PET is shown below in two steps. First with PET **(1)** which is broken down by PETase into MHET (Mono-(2-hydroxyethyl)terephthalic acid) **(2)** another large, breakdown resistant plastic. Second, MHET is then hydrolyzed further into ethylene glycol and terephthalic acid **(3)**.[4]



1 **2**

Scheme 1: Mechanism for degrading polymer **1** to monomer **2**

I. sakaiensis first uses the enzyme know as PETase and water to hydrolyze PET monomers into MHET.



2 **3**

Scheme 2: Mechanism for degrading monomers **2** to molecules **3**

The enzyme know as MHETase and water then hydrolyze MHET monomers into ethylene glycol and terephthalic acid. These molecules are much less resistant to microbial biodegradation compared to the PET and MHET polymers.

Discussion

The ability to degrade plastics that are resistant to other methods of degradation is an important advancement in recycling and the protection of the environment. However for this chemical scheme to become a staple in the recycling process it must be advanced even further.

Currently this process has been discovered in the last decade and scientists are only just beginning to understand the mechanism by which this process occurs. Meaning this process is still in its infancy where it could develop into a tool to be used in recycling however it is too early to tell.

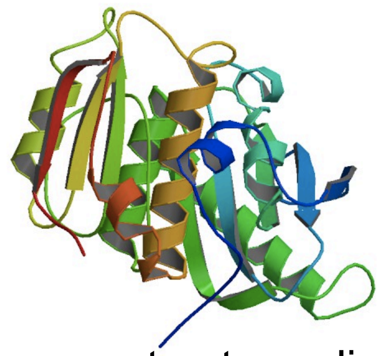
The plastic produced by NAU itself is estimated from the EPA reported amount generated by the country.[5] In 2017 the EPA reports 35.4 million tons of PET plastic was generated, dividing that evenly across the nation is 0.11 tons per person or 98.8 kilograms per person.

The population of NAU is 27,078 students meaning that NAU is estimated to produce 2948.5 tons of PET waste. The degradation rate for PET is 0.13 mg/cm^2 per day.[1] This is significantly larger than what the reported degradation rate could handle.

Discussion Cont.

This leads to the conclusion that the implementation of this bacteria is not a cost-effective solution. The cost of the bacteria is $2500 per gram and to scale it to be an effective solution it would be necessary thousands of kilograms to be purchased.

Figure 3: Crystal structure of PETase enzyme[8]



More money would need to be spent to construct a climate controlled facility to house the bacteria and the tons of PET waiting to be degraded. Then factoring in the maintenance costs of the building and the bacteria. All those factors make this waste reduction solution not effective. There are other cheaper and easier to implement solutions.

The campus could stop ordering these PET plastic bottles and containers to reduce the amount of PET waste generated. PET is also highly recyclable via meltdown and recreation or disinfection and does not require degradation and a simple solution could be to advertise more recycling on campus. Although there will always be recyclable materials in the landfill trash so perhaps a solution could be a separation center for campus trash.

Future Work

At this moment in time the ability of this bacteria is promising but not practical. Currently there are attempts to optimize this ability through gene modification and mutation of the enzymes themselves.[6-7] These advancements in the future may lead to a viable way to utilize the ability of these bacteria. Presently there are better solutions to the problem of PET waste that can be implemented with less time, money and effort for a similar effect. However, with the advancements being made there may be a use for these bacteria.

References

(1) Bornscheuer, U. T. *et al.*, Feeding on Plastic. *Science* **2016**, 351 (6278), 1154-1155
(2) Yoshida, S.; *et al.*, A Bacterium That Degrades and Assimilates Poly(Ethylene Terephthalate). *Science* **2016**, 351 (6278), 1196.
(3) Sagong, H.-Y. *et al.*, Decomposition of PET Film by MHETase Using Exo-PETase Function. *ACS Catal.* **2020**.
(4) Achilias, D. S.; Karayannidis, G. P. The Chemical Recycling of PET in the Framework of Sustainable Development. *Water Air Soil Pollut.* Focus **2004**, 385-396
(5) US EPA, O. Plastics: Material-Specific Data (accessed Apr 13, **2020**).
(6) Son, H. F.; *et al.*, Rational Protein Engineering of Thermo-Stable PETase from Ideonella Sakaiensis for Highly Efficient PET Degradation. *ACS Catal.* **2019**, 9, 3519–3526.
(7) Tournier, V.; *et al.*, An Engineered PET Depolymerase to Break down and Recycle Plastic Bottles. *Nature* **2020**, 580 (7802), 216–219.
(8) Han, X.; *et al.*, Structural Insight into Catalytic Mechanism of PET Hydrolase. *Nat. Commun.* **2017**, *8* (1), 1–6.

Acknowledgments

I would like to thank the NAU Green Fund for the opportunity to research this topic. I also want to thank Dr. Stephanie Hurst her support for my acceptance into her research group. I would also like to acknowledge the knowledge imparted to me by not only Dr. Hurst but by my excellent laboratory partners: Michael P. Moghadasnia, Ryan H. Evans, Patrick K. Cross, Tiphanie R. Pfefferle, Alexandra L. Novacek.

NAU NORTHERN ARIZONA UNIVERSITY

Green FunD