



Morganton Scientific

North Carolina
School of Science
and Mathematics

Journal of Student STEM Research

An Examination of the Enzymatic Degradation of PET Plastic via PETase and MHETase

Ruby Allred¹  

¹North Carolina School of Science and Mathematics 

Abstract

Post-consumer plastic waste is a concerning issue in the world at large, and polyethylene terephthalate (PET) plastic is a key contributor of that waste. In 2016, Yoshida et al. discovered a system of enzymes, PETase and MHETase, in the bacteria *Ideonella sakaiensis*, which they claimed could degrade PET plastic. While true, this degradation only truly affects lower crystallinity PET (in the range of 2-3%), which is well below the crystallinity in which most PET plastic that is sold (a typical PET water bottle may be 31%). As such, while the surface of the plastic waste may be degraded, the core of the plastic tends to hold strong unless it is first melted into lower crystalline plastic. This study used a chimera of the two enzymes, developed by Knott et al., with the initial goal of testing various conditions for degradation. This work finds that these enzymes have a limited effect on the degradation of un-modified post-consumer PET plastic. While a minor increase in efficiency can be found in a solution which contains ethylene glycol, the rate of degradation is miniscule regardless.

Keywords Polyethylene terephthalate, Plastic degradation, Enzymatic degradation, MHETase, PETase

1. INTRODUCTION

Plastic has become a vital part of our global economy and society, and polyethylene terephthalate (PET) is one of the most common plastics in use, making up much of disposable packaging [1]. Of course, this causes massive amounts of waste, largely due to the fact that PET and other similar plastics are not easily broken down, and as such millions of tons of plastic waste are put into the ecosystem every year [2], [3], [4]. While the PET may be destroyed through intense heat and pressure or recycled through chemical processes, these are wasteful and often take up copious amounts of energy or use costly or hazardous chemicals [5], [6], [7]. Because of this the prospect of using enzymes to degrade the PET is appealing, both environmentally and economically, and a viable route toward this enzymatic degradation may involve the enzymatic system of PETase and MHETase. This system breaks down PET into terephthalic acid (TPA) and ethylene glycol (EG), which can be polymerized into PET, thus potentially creating a better closed loop. In addition, the EG could be sold for use in antifreeze or ballpoint pen ink, and the TPA can be utilized by bacteria such as *Rhodococcus sp* [8].

PETase and MHETase, discovered in *Ideonella sakaiensis* by Yoshida et al. in 2016, are notable because they are effective at 30 degrees celsius, as opposed to most other potential enzymes, which work best at PET's glass transition state (70 °C) [9]. Having a much lower temperature would mean less energy put into the process overall, which is environmentally preferable. Like with many other similar enzymes, the current main issue with PETase is that they require additional pretreatment for effective PET

Published Jun 27, 2024

Correspondence to
Ruby Allred
rubydiumallred@gmail.com

Open Access 

Copyright © 2024 Allred. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International](https://creativecommons.org/licenses/by/4.0/) license, which enables reusers to distribute, remix, adapt, and build upon the material in any medium or format, so long as attribution is given to the creator.

 Published by Curvenote

deconstruction, specifically they must be lowered in crystallinity, as PETase and MHETase are only truly effective at degrading PET at very low crystallinity (~3%) [10]. Post-consumer plastics are often much higher in crystallinity, for instance a standard plastic water bottle may hold a crystallinity of 31% [11]. As such, ways to decrease the crystallinity of PET in a quick, efficient, and environmentally conscious manner is an important step in the development of infrastructure for the enzymatic recycling of PET plastic.

Many facets of PET degradation have been studied, for instance Falkenstein et al. researched the effects of UV light on PET degradation, and found that the UV light highly crystallizes the PET, making it much more difficult to degrade [12]. PETase has also been modified to become more effective, typically being modified to be more like a cutinase, which has been proven to make it much more efficient, so developments are being made in that regard [10], [13]. Other methods of degradation have also been investigated, such as the use of ethylene glycol as an agent of degradation, which has limited but present effects at room temperature [14].

Overall, more research is presently needed in the determination of stronger methods for PET degradation without the use of high temperatures or harmful chemicals. This paper sets out to examine the use of various reagents and conditions on the enzymatic degradation of PET plastic without first pushing the PET past the glass transition state, and optimally with as low a heat as possible.

2. METHODS

2.1. Enzyme Broth Creation

The enzymes were created and used as described in Knott et al's paper. The specific enzyme used was the chimeric enzyme PCJ190, a combination of PETase and MHETase which was shown to hold increased degradation over wild-type PETase and MHETase enzymes on low crystallinity plastic [10]. Four broths which contained the enzyme in equal measure were created, one at 7.5 pH, one at 7.0 pH, one at 8.0 pH, and one at 7.5 pH containing an added volume of ethylene glycol equivalent to 10% of the total volume.

2.2. Materials Preparation for Degradation

The plastic which was degraded are small strips taken from various Sprite bottles bought at the same time from a local retailer. Specifically, areas of similar consistency, that being the area under the label but not affixed with the glue binding the label to the bottle itself, as that may cause inconsistencies in the samples. Samples of the plastic were cut out using a knife and scissors, with the intent that within a group of samples the sizes would be roughly the same. Some plastic was also ground using a combination of a coffee grinder and mortar and pestle, as a plastic grinder was unavailable.

2.3. Degradation Methods

Twelve 50 mL beakers which were split into six categories were each filled with 20 mLs of the enzymatic broth. The categories are as follows: 7.5 pH with plastic strips, 7.5 pH with ground plastic, 7.0 pH with plastic strips, 8.0 pH with plastic strips, 7.5 pH containing ethylene glycol with plastic strips, and 7.5 pH containing ethylene glycol with ground plastic. The plastic samples were dried in an oven at sixty degrees celsius for one day and then weighed before being placed into the beakers. The beakers were placed into a water bath and then left for a week, with occasional observation. After seven days the beakers were extracted from the water bath, at which point vacuum filtration was used to isolate the plastic samples from the broth. The plastic was then dried under the same conditions as above, and weighed again. Two trials were recorded, one at room temperature and one at thirty degrees celsius.

2.4. The Detection of Ethylene Glycol

Due to the low level of observed degradation on the plastic, CheMetrics Ethylene Glycol testing kits were obtained and used. The tests showed the presence of a glycol; however, it is possible that Sprite contains propylene glycol (a moderate sweetener used in certain soft drinks). Nevertheless, the presence of propylene glycol in Sprite is not disclosed [15]. In addition, the testing kits appear to be inconclusive in terms of exact concentrations, and as such the ethylene glycol testing kits are not a particularly viable method for determination of the extent of degradation.

3. RESULTS AND DISCUSSION

Due to the ineffectiveness of the ethylene glycol testing kits, the only reliable value which could be collected from the tests performed was the weight loss of the plastic samples. [Figure 1](#) and [Figure 2](#) display the percent change in plastic mass before and after the samples of the two recorded runs. As can be seen, while the data for the room temperature experiment is largely consistent with some degree of degradation, the one which was run at 30 °C largely gained in mass to some extent. This was likely due to the buffer salinity being too high, however multiple assays of this were performed and all returned some level of salt precipitate. As such, the data from 30 °C is unreliable, although it is interesting to note that in both experiments the standard conditions do to some extent degrade the plastic. [Figure 2](#)'s data is not presented to demonstrate the effectiveness of the degradation, but to warn of a potential flaw which can occur.

The unusually high peak of 7% mass loss seen in sample EGd-1 in [Figure 1](#) (ground plastic degraded in the ethylene glycol broth) could either be due to a large amount of degradation, or could be due to the loss of some particulates of the ground plastic, which may have occurred in several different steps of the process. It should be noted that the ethylene glycol solution containing ground plastic does appear to be a better performer in comparison to the 7.5 pH samples, however more thorough testing would need to be performed to truly prove a correlation.

4. CONCLUSION

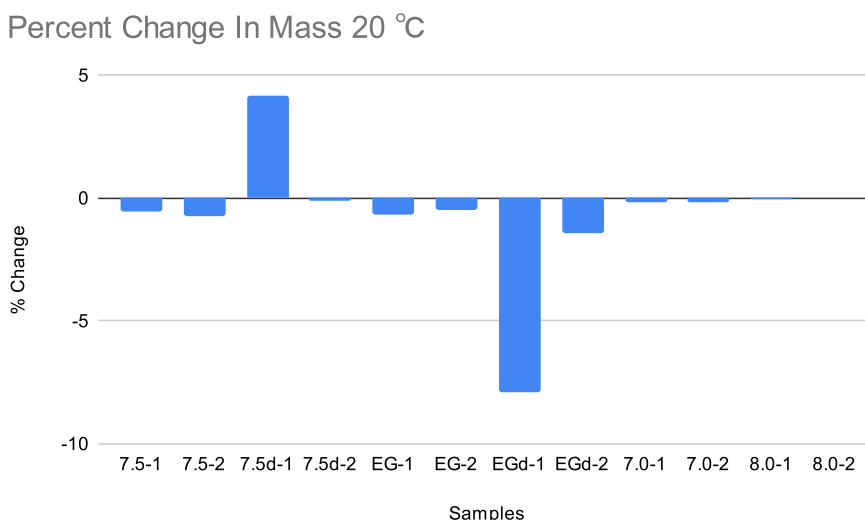


Figure 1. Percent mass loss of PET at room temperature. “d” signifies ground plastic.

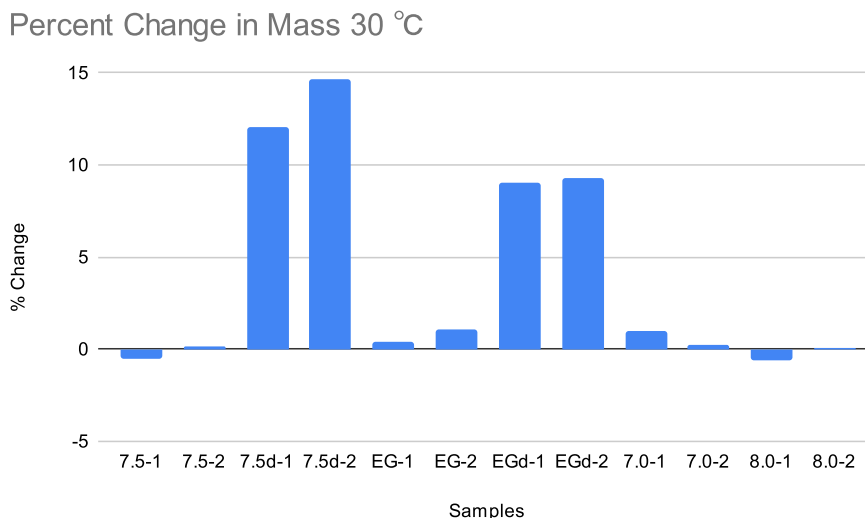


Figure 2. Percent mass loss of PET at 30 °C. “d” signifies ground plastic.

This lack of greater degradation of the plastic samples seems to point to PETase's inability to degrade post-consumer goods such as a Sprite bottle, even with aided help. The average of the data collected at room temperature is 0.66% degradation of the bottle, which is an incredibly low value. This does assume that the enzyme functioned properly, however there was substantial enough degradation to imply the presence of enzyme to some degree. To corroborate this, more tests could be done on the presence of the enzyme in the broths before degradation is underway. One way which will likely increase degradation is decreasing the crystallinity of the plastic, as stated before, however doing so would typically require that said waste be brought to its glass transition state, which, in the case of PET, is 70 °C. This is also the temperature at which various other potentially valuable PET degrading enzymes function best, such as leaf-cutter cutinase or polyester hydrolases. As such, the use of other enzymes may still be viable under many circumstances, and further examination should be done in regards to the efficacy of these alternatives. PETase and MHETase should not necessarily be a guiding mark for other enzymes to be modeled around, the enzymatic system itself requires much more modification if it is to be significantly useful.

In addition, enzymatic recycling is not a perfect solution to plastic waste. Simply because a method of recycling which may eventually become a dominant force exists, does not mean that corporations and other groups may continue to produce mass amounts of plastic waste without being held accountable for said plastic. Methods such as pyrolysis are often supported by corporations as being reliable, even when they are not entirely faultless [16]. PETase and MHETase could become part of a larger trend where the most known method of potential recycling is picked up by plastic producers as a way to appear more environmentally conscious and thus more marketable to the masses. The scientific community must remain aware and conscious of the viability of the methods larger groups may claim to be infallible.

Thanks go to Dr. Tandy Grubbs of the Research in Chemistry program at NCSSM-Morganton for permitting this research and allowing for the use of his lab. Thanks also go to the other members of the Research in Chemistry program for being supportive in this endeavour. Finally, thanks go specifically to Ian Richards, for proof reading this document and allowing for further discussion of the topic.

REFERENCES

- [1] R. Geyer, J. R. Jambeck, and K. L. Law, "Production, use, and fate of all plastics ever made," *Science Advances*, vol. 3, no. 7, 2017, doi: [10.1126/sciadv.1700782](https://doi.org/10.1126/sciadv.1700782).
- [2] J. R. Jambeck *et al.*, "Plastic waste inputs from land into the ocean," *Science*, vol. 347, no. 6223, pp. 768–771, 2015, doi: [10.1126/science.1260352](https://doi.org/10.1126/science.1260352).
- [3] A. O. C. Iroegbu, S. S. Ray, V. Mbarane, J. C. Bordado, and J. P. Sardinha, "Plastic Pollution: A Perspective on Matters Arising: Challenges and Opportunities," *ACS Omega*, vol. 6, no. 30, pp. 19343–19355, 2021, doi: [10.1021/acsomega.1c02760](https://doi.org/10.1021/acsomega.1c02760).
- [4] M. Bergmann *et al.*, "Plastic pollution in the Arctic," *Nature Reviews Earth & Environment*, vol. 3, no. 5, pp. 323–337, 2022, doi: [10.1038/s43017-022-00279-8](https://doi.org/10.1038/s43017-022-00279-8).
- [5] G. Pathak *et al.*, "Plastic pollution and the open burning of plastic wastes," *Global Environmental Change*, vol. 80, p. 102648, 2023, doi: [10.1016/j.gloenvcha.2023.102648](https://doi.org/10.1016/j.gloenvcha.2023.102648).
- [6] H. Jeswani *et al.*, "Life cycle environmental impacts of chemical recycling via pyrolysis of mixed plastic waste in comparison with mechanical recycling and energy recovery," *Science of The Total Environment*, vol. 769, p. 144483, 2021, doi: [10.1016/j.scitotenv.2020.144483](https://doi.org/10.1016/j.scitotenv.2020.144483).
- [7] J.-P. Lange, "Managing Plastic Waste Sorting, Recycling, Disposal, and Product Redesign," *ACS Sustainable Chemistry & Engineering*, vol. 9, no. 47, pp. 15722–15738, 2021, doi: [10.1021/acssuschemeng.1c05013](https://doi.org/10.1021/acssuschemeng.1c05013).
- [8] "Ethylene Glycol," 2022, [Online]. Available: <https://www.chemicalsafetyfacts.org/chemicals/ethylene-glycol/>
- [9] S. Yoshida *et al.*, "A bacterium that degrades and assimilates poly(ethylene terephthalate)," *Science*, vol. 351, no. 6278, pp. 1196–1199, 2016, doi: [10.1126/science.aad6359](https://doi.org/10.1126/science.aad6359).
- [10] B. C. Knott *et al.*, "Characterization and engineering of a two-enzyme system for plastics depolymerization," *Proceedings of the National Academy of Sciences*, vol. 117, no. 41, pp. 25476–25485, 2020, doi: [10.1073/pnas.2006753117](https://doi.org/10.1073/pnas.2006753117).
- [11] N. E. Wallace, M. C. Adams, A. C. Chafin, D. D. Jones, C. L. Tsui, and T. D. Gruber, "The highly crystalline <sc>PET</sc> found in plastic water bottles does not support the growth of the <sc>PETase</sc>-producing bacterium *Ideonella sakaiensis*," *Environmental Microbiology Reports*, vol. 12, no. 5, pp. 578–582, 2020, doi: [10.1111/1758-2229.12878](https://doi.org/10.1111/1758-2229.12878).
- [12] P. Falkenstein *et al.*, "UV Pretreatment Impairs the Enzymatic Degradation of Polyethylene Terephthalate," *Frontiers in Microbiology*, vol. 11, 2020, doi: [10.3389/fmicb.2020.00689](https://doi.org/10.3389/fmicb.2020.00689).
- [13] A. Barclay and K. R. Acharya, "Engineering Plastic Eating Enzymes Using Structural Biology," *Biomolecules*, vol. 13, no. 9, p. 1407, 2023, doi: [10.3390/biom13091407](https://doi.org/10.3390/biom13091407).
- [14] J. Huang *et al.*, "Depolymerization of polyethylene terephthalate with glycol under comparatively mild conditions," *Polymer Degradation and Stability*, vol. 208, p. 110245, 2023, doi: [10.1016/j.polymdegradstab.2022.110245](https://doi.org/10.1016/j.polymdegradstab.2022.110245).
- [15] Mauricio, "Carrier solvents in soft and energy drinks," 2022, [Online]. Available: <https://magritek.com/2022/02/08/undeclared-carrier-solvents-in-soft-and-energy-drinks/>
- [16] A. H. Tullo, "Amid controversy, chemical companies bet on plastics pyrolysis. Retrieved from," 2022, [Online]. Available: <https://cen.acs.org/environment/recycling/Amid-controversy-industry-goes-plastics-pyrolysis/100/i36>