JOURNAL OF
EMERGING INVESTIGATORS

Article

Enzymatic pathway for polystyrene degradation using saliva of greater wax moth *Galleria mellonella*

Ishaan Bharadwaj[1], Joaquim I. Goes[2]

[1] Millburn High School, Millburn, New Jersey

[2] Marine Biology and Paleoenvironment, Lamont Doherty Earth Observatory at Columbia University, Palisades, New York

SUMMARY

Plastic pollution poses a major environmental challenge due to the persistence of polymers that take thousands of years to decompose. Disposal methods of plastics face several bottlenecks, including resource-intensive processes and limited recycling capabilities, making biodegradation an appealing, eco-friendly, and cost-effective alternative. This study investigates the potential of waxworm saliva, the secretion of Galleria mellonella (G. mellonella, commonly known as the greater wax moth) in its larval stage, to degrade polystyrene (PS), a polymer known for its resistance to environmental breakdown. We hypothesized that G. mellonella saliva has the direct ability to depolymerize PS. To test this, we collected saliva from G. mellonella larvae and applied it to commercially available PS beads. Spectrofluorometric analysis revealed emission peaks corresponding to styrene, the monomeric form of polystyrene, suggesting that G. mellonella saliva can depolymerize PS. This study demonstrates that waxworm saliva is capable of degrading PS, underscoring its potential for industrial applications in plastic waste management. We will conduct further analysis to identify the specific proteins/enzymes responsible and confirm the full range of degradation by-products.

INTRODUCTION

The global production of plastic has grown significantly, rising from about 1.5 million tons in 1950 to 368 million tons by 2019 (1). Most used plastics are discarded into the environment, with approximately 103.4 million tons accumulating in landfills and 19-23 million tons entering aquatic systems annually, leading to a significant increase in environmental pollution due to the non-degradable nature of most plastics (2,3). Biodegradation of polymers like polyethylene (PE) and polystyrene (PS) by organisms has been an appealing approach to addressing plastic pollution due to its environmentally friendly nature (4).

Because more research has been conducted on PE, it provides a useful context for understanding the degradation of other polymers, such as PS. In landfills, PE oxidation, the first step in the biodegradation of PE, relies on abiotic factors, like sunlight and heat (5). However, these natural processes can take years to break down the long-chain polymers, necessitating a more efficient solution to overcome this initial step (5). Several insect species have been found to possess the ability to biodegrade various polymers, including the Indian mealworm (*Plodia interpunctella*), the yellow mealworm (*Tenebrio molitor*), superworms (*Zophobas atratus*), and the lesser wax moth *(Achroia grisella*) (1). The greater wax moth (*Galleria mellonella*) is a cheap, readily available pest of apiaries, and its larvae have been found to degrade polymers very quickly due to the structural similarity between beeswax and polymers, as both contain long hydrocarbon chains (1,6). Studies have tested whether biodegradation of PE occurs through waxworms, specifically *G. mellonella* (6). The process of biodegradation of polymers has been studied in detail, and gut microbiota has been found to play an important role in the process of degradation. Bacterial genera of *Escherichia-Shigella*, *Asaia*, and *Acinetobacter* are predominant in *G. mellonella* and were found to break PE down to ethylene glycol (7). However, it is not clear whether the microbiome of *G. mellonella* is the sole factor that plays a role in the degradation process of PE. *G. mellonella* saliva, which contains high levels of enzymes, and no other structures such as vesicles or bacteria, has been found to oxidize and depolymerize PE, converting the polymers into ketones and other compounds (5).

PS, in particular, is a very harmful polymer to the environment. Although it makes up only 10% of the plastics produced, PS is not suitable for reuse and it is rejected by most recycling facilities (8). This highlights the urgent need to find a mechanism for the biodegradation of PS. With PS, regardless of whether the *G. mellonella* microbiota was present, PS broke down into its monomer styrene as well as its oligomers (1). The caterpillar's physiological adaptations may work in synergy with the microbiome to accelerate degradation. When PS beads were force-fed into the larvae with removed microbiota, analytical methods still indicated depolymerization of PS into styrene in the intestines, suggesting depolymerization was driven by the host's enzymes and not its microbiome (1). However, in the presence of its microbiota, *G. mellonella* further broke down styrene into 2-hydroxyphenylacetate, 4-hydroxybenzoate, and acetyl-CoA (1). We hypothesized that *G. mellonella* saliva alone can depolymerize PS into its monomer, styrene. While previous studies have examined PS degradation by whole waxworms, none have focused specifically on the role of saliva. To test this hypothesis, we applied saliva collected from *G. mellonella* larvae to PS beads and analyzed the samples using fluorescence spectroscopy. We found that the saliva successfully depolymerized PS, with emission peaks corresponding to styrene. These findings suggest that waxworm saliva contains enzymes capable of breaking down PS, highlighting its potential for future use in plastic waste management.

https://doi.org/10.59720/25-034

Figure 1: Browning, suggestive of oxidative activity, occurred between treated samples compared to the untreated sample. Representative image of three PS beads with various degrees of browning. The untreated PS bead is labelled as "1". Two PS beads in the image (labeled 2 and 3, indicated below the beads in red) were treated with 30 µL of G. mellonella saliva each and left at room temperature for one hour. The image contains two of 20 total beads with saliva from 20 individual larvae.

RESULTS

No previous research has examined whether *G. mellonella* saliva alone can depolymerize PS. We hypothesized that waxworm saliva can depolymerize PS into its monomer, styrene. To test this, we collected saliva from the buccal opening of the larvae and applied it to commercially available PS beads. We allowed the saliva to sit on the PS beads for one hour under ambient conditions before analysis. We observed a visible browning of the saliva when applied to PS beads. The color change suggests a possible oxidative reaction occurring at the surface of the polymer. Additionally, we observed varying degrees of browning when we used saliva from different worms to treat the PS beads, suggesting differences in enzymatic compositions between individual worms. While we tested saliva from 20 larvae throughout our experiments, the figure shows two representative samples, each treated with saliva from a different worm and applied to a separate bead **(Figure 1)**. When we applied saliva from the same larva to different beads, the color of browning remained consistent. We analyzed the samples using excitation-emission (EEM) fluorescence spectroscopy to observe changes in emission when degradation occurred. We used untreated PS beads as the control group. We suspended both control and treated PS beads in Tris-HCl buffer, vortex-mixed them, and used a spectrofluorometer to observe degradation products in solution. We compared the fluorescence spectra against a solution of styrene in methanol, prepared in the lab using a standard high-purity styrene solution. The untreated sample showed no peak while the treated sample displayed a clear peak at around excitation 275 nm and emission at around 330 nm, definitively indicating that degradation had occurred and produced fluorescent products absent in the untreated control **(Figures 2,3)**. It also shows that degradation occurs at room temperature within an hour of treatment with saliva. Further analysis showed that the degradation was consistent across different solvent environments used in the tests. The treated sample showed similar emission values in both the Tris-HCl buffer and methanol, which further confirms that the saliva has the inherent capability to degrade plastic independently of the solvent medium (**Figures 3,4**). The degradation process was further confirmed by comparison with the styrene monomer standard. The emission peaks of treated PS in methanol solvent matched the emission peaks of the styrene standard in methanol at around 305 nm, consistent with the established emission peak of styrene at 307 nm, suggesting that the depolymerization process broke the polymer down into its monomeric form **(Figures 4,5)**. This is an important finding to indicate that waxworm saliva does not simply break down the polymer into random, various compounds but consistently fragments it into its monomeric styrene form.

DISCUSSION

This study aimed to determine whether *G. mellonella* saliva alone has the capability to depolymerize PS into its monomer, styrene, under natural conditions. To test this, we collected saliva from the buccal opening of the larvae and applied it to commercially available PS beads. Our spectrofluorometer results support the hypothesis that waxworm saliva contains biological components capable of depolymerizing PS without the need for pretreatment. Using the waxworm saliva to test the degradation of PS offered key findings about the capability of the waxworm saliva to degrade the polymer within the first hour. This finding indicates that depolymerization can occur without any pretreatment in natural conditions, as well as at room temperature. The consistent results across different

https://doi.org/10.59720/25-034

solvent media also confirmed that the degradation process is not influenced by solvent conditions. Spectrofluorometer analysis shows the same peaks as styrene monomer, highly indicative of systematic depolymerization of the polymer. The biological components in the saliva overcame the time-consuming initial oxidation step and subsequently depolymerized polystyrene into its monomer, styrene, achieving both processes in one treatment. Compared to mechanical, thermal, and chemical degradation methods such as pyrolysis or UV degradation, this biodegradation method operates under ambient conditions and eliminates the production of more microplastics. This method also utilizes the affordable and readily available *G. mellonella* larvae. This research addresses a critical gap in current literature by directly providing evidence that it is the *G. mellonella* saliva that depolymerizes polystyrene into its monomer styrene.

Previous literature has established that *G. mellonella* gut microbiota has the ability to metabolize styrene into various compounds, but the direct cause of the initial depolymerization step (PS into styrene) has not been confirmed (1). This research supports a two-step degradation mechanism of PS in *G. mellonella*: (1) enzymatic depolymerization of PS into styrene by *G. mellonella* saliva, and subsequently (2) degradation of styrene by gut microbiota harbored by *G. mellonella.* One limitation in the use of waxworm larvae is that they can have different enzyme compositions, as indicated by varying levels of browning on PS beads. This can be avoided by identifying the proteins in *G. mellonella* saliva and testing PS with lab-grown cultures. The next step would be to conduct proteomic analysis to identify the enzymes present in the saliva, and subsequently culture them and conduct experiments testing their roles in polystyrene depolymerization. Additional experiments can also be conducted on the potential enzymes identified to determine the optimal time, pH, and temperature conditions for the degradation of PS by measuring the amounts of products formed. Conventional cultivation-based methods to identify biocatalysts are time-consuming and the potential to identify one that can depolymerize plastic is very low. Using deep learning, specific enzyme sequences, for example, can be compared to databases of known plastic-degrading enzymes (such as a potential *G. mellonella* saliva enzyme) to predict whether they have the capability to degrade other types of plastics, without conducting resource-consuming physical experiments. This study highlights the possible use of bioremediation for plastic degradation to reduce the polymer into its monomeric form. Further studies can also identify other biocatalysts with the capability to degrade PS, as well as other plastics, creating sustainable solutions to manage the global issue of plastic pollution.

MATERIALS AND METHODS

Waxworm Maintenance and Saliva Collection

G. mellonella larvae were sourced from Carolina Biological Supply Company and kept at room temperature in sterile conditions. To induce saliva secretion, larvae were mechanically stimulated for a few seconds using a specially customized glass Pasteur pipette until buccal secretion was observed. Once a droplet of saliva formed at the buccal opening, the larva was brought to the PS bead, allowing the saliva to transfer by contact without physically touching the larva to the bead. Each treated bead received saliva from a single larva. In total, saliva from 20 larvae was used to treat 20 individual PS beads. We ensured approximately equal-volume droplets of saliva were transferred from each larva to

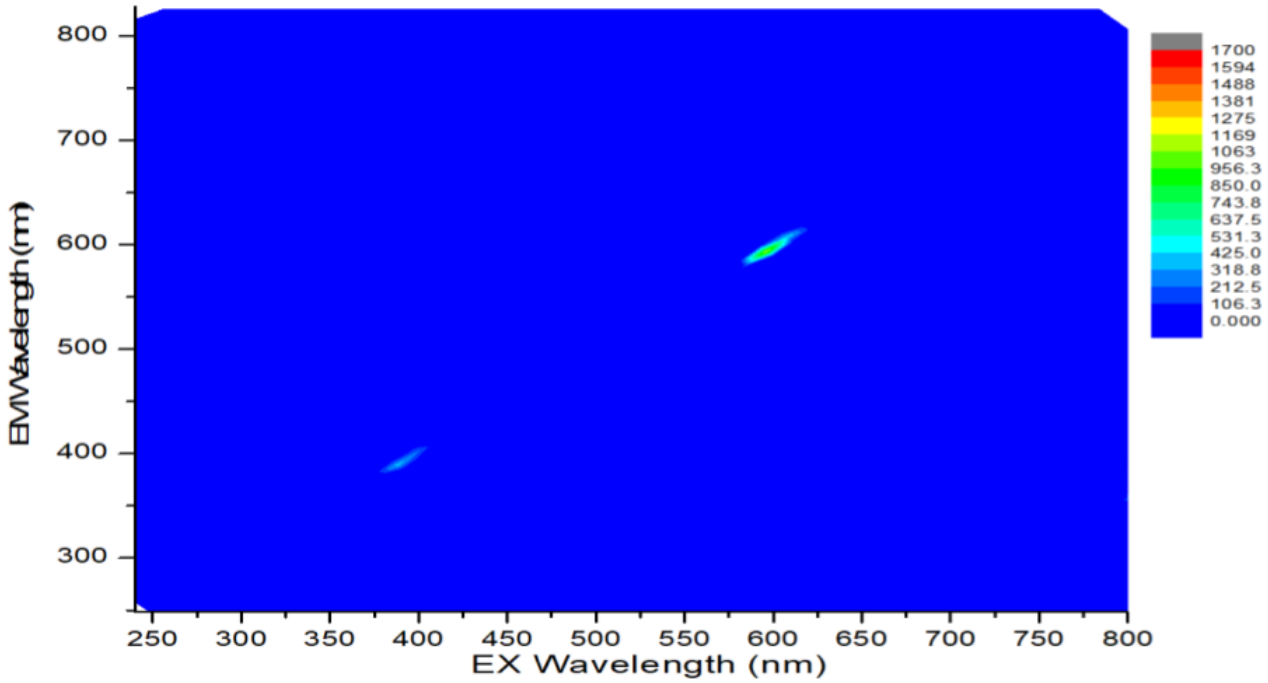

Figure 2: Spectrofluorometric analysis of an untreated PS bead. Shown is an excitation-emission matrix fluorescence plot of an untreated PS bead suspended in Tris-HCl buffer. No peak was detected. The data represent a single measurement.

https://doi.org/10.59720/25-034

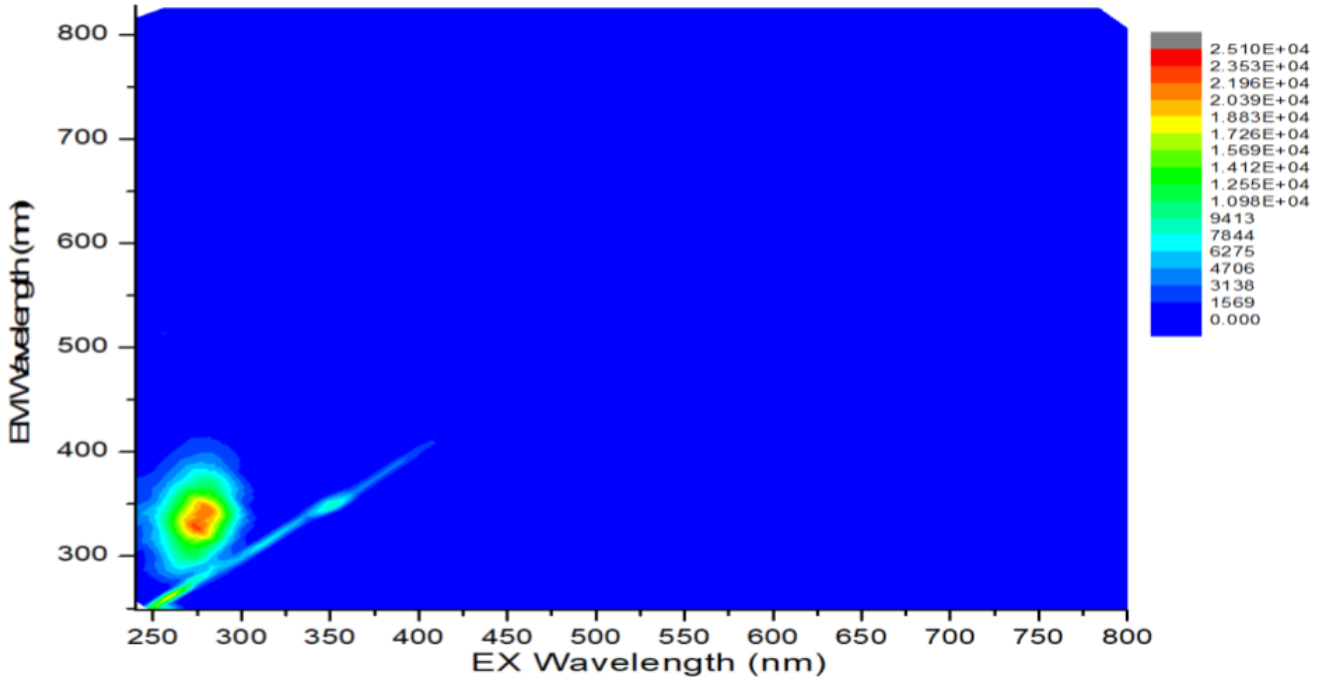

Figure 3: Spectrofluorometric analysis of a treated PS bead. Shown is an excitation-emission matrix fluorescence plot of a treated PS bead suspended in Tris-HCl buffer. A peak was detected at around excitation 275 nm and emission at around 330 nm. Data represent a single measurement.

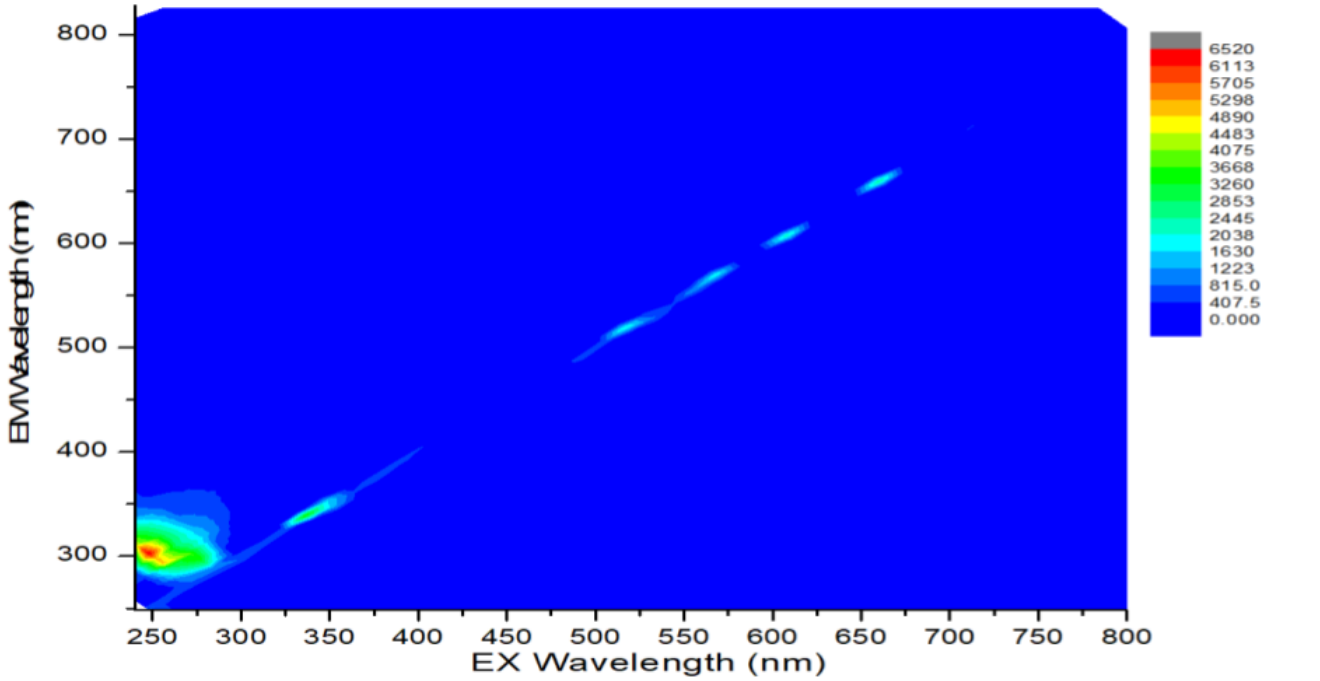

Figure 4: Spectrofluorometric analysis of a treated PS bead. Shown is an excitation-emission matrix fluorescence plot of a treated PS bead suspended in methanol solvent. A peak was detected at around excitation 250 nm and emission at around 310 nm. Data represent a single measurement..

https://doi.org/10.59720/25-034

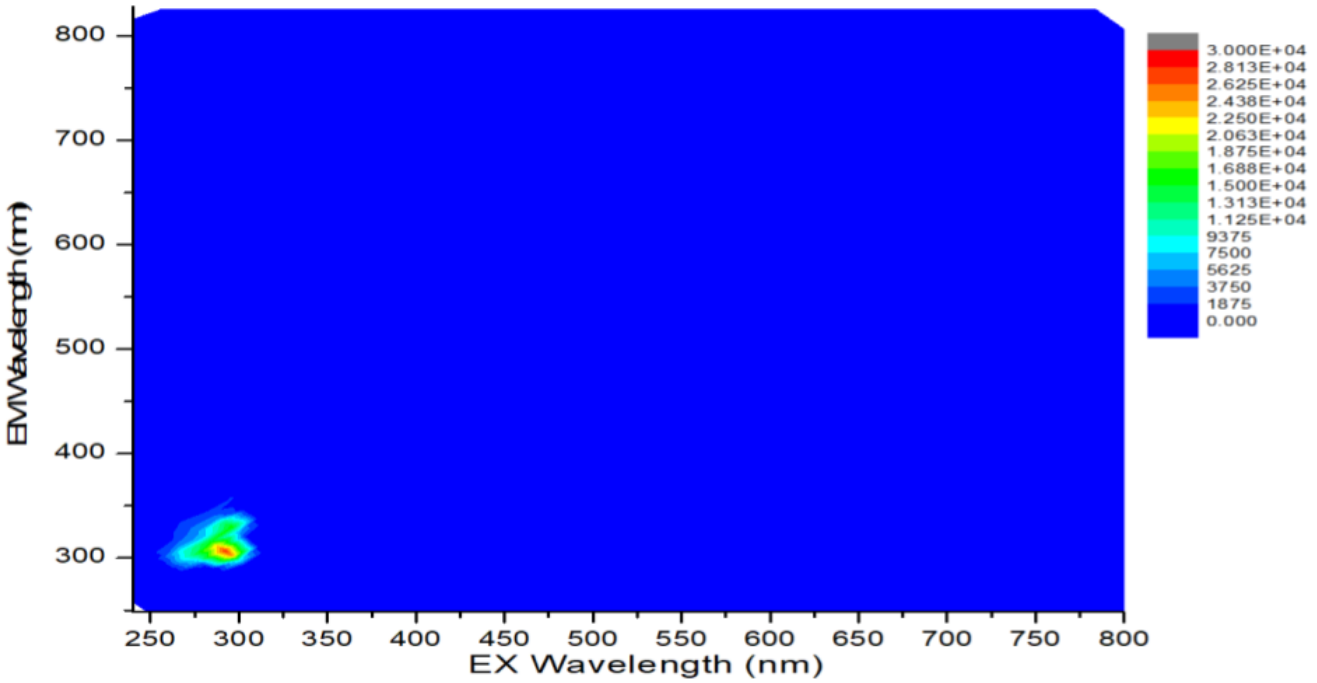

Figure 5: Spectrofluorometric analysis of the styrene standard solution. Shown is an excitation-emission matrix fluorescence plot of the styrene standard in methanol solvent. A peak was detected at around excitation 290 nm and emission at around 310 nm. Data represent a single measurement.

the substrate by visually estimating droplet size to maintain consistency across samples. All tools, including the pipette, working surface, and gloves, were sterile. Only freshly secreted saliva was used.

Substrate Selection and Treatment

Expanded PS beads (approximately 0.1 inch in diameter) were purchased from Michaels Stores Inc. (Product ID: 10524374, Item name: Foam Mini Balls by Ashland®), and equal-length beads were selected as the degradation substrate. These were chosen for their small size, allowing for easy fit into standard small-volume vials for vortex mixing without additional processing and cutting. The small size also offered a high surface-area-to-volume ratio, promoting contact between saliva and the bead surface. Each bead was treated with approximately 30 µL of freshly collected *G. mellonella* saliva. No specific method was used to control the distribution of saliva across each bead, as the purpose of the experiment was to determine the presence of degradation and not its rate or extent. Control and treated PS beads were left for 1 hour at room temperature to allow for hypothesized enzymatic interaction and polymer breakdown. Untreated control beads were exposed to similar conditions as treated beads throughout the experiment.

Buffering and Sample Preparation

Post-treatment, the PS beads (both control and treated) were submerged in 1M Tris-HCl buffer (pH=8) to preserve any hypothesized degradation by-products. To ensure uniform dispersion of the degradation products throughout the buffer, the samples were vortexed for 1 minute at medium speed.

Analytical Techniques

Each sample type (control and treated) was tested in triplicate (n=3) using spectrofluorometric analysis to detect any depolymerization of PS into its monomeric form, styrene. Fluorescence excitation-emission matrices were acquired using a HORIBA Fluoromax® spectrofluorometer paired with the HORIBA Aqualog® software (version 4.2).

The styrene standard solution was prepared by mixing 1 mL of pure styrene (≥99%, stabilized with 4-tert-butylpyrocatechol, Sigma-Aldrich, CAS 100-42-5) with 4 mL of methanol (Certified ACS grade, Fisher Chemical, CAS 67-56-1, ≥99.8% purity) to yield a final volume of 5 mL. All handling of styrene and methanol was performed in a certified chemical fume hood using appropriate protective equipment, including nitrile gloves, a lab coat, and safety goggles, due to the flammability and inhalation hazards of both compounds. The standard solution was prepared and immediately analyzed using the spectrofluorometer on the same day.

ACKNOWLEDGMENTS

The authors would like to thank Mr. Christopher J. Cook for his continuous support and mentorship throughout the research process. The authors also gratefully acknowledge the Lamont-Doherty Earth Observatory (LDEO), Columbia University Climate School, and the National Oceanic and Atmospheric Administration (NOAA) for their support of this research on plastic waste mitigation.

Received: December 25, 2024
Accepted: June 1, 2025
Published: September 10, 2025

https://doi.org/10.59720/25-034

REFERENCES

1. Wang, Shuai, *et al*. "Complete Digestion/Biodegradation of Polystyrene Microplastics by Greater Wax Moth (*Galleria mellonella*) Larvae: Direct In Vivo Evidence, Gut Microbiota Independence, and Potential Metabolic Pathways." Journal of Hazardous Materials, vol. 423, Sep. 2021, https://doi.org/10.1016/j.jhazmat.2021.127213.
2. Borrelle, Stephanie B., *et al*. "Predicted Growth in Plastic Waste Exceeds Efforts to Mitigate Plastic Pollution." Science, vol. 369, 2020, pp. 1515–1518, https://doi.org/10.1126/science.aba3656.
3. Houssini, K., Li, J., and Tan, Q. "Complexities of the Global Plastics Supply Chain Revealed in a Trade-Linked Material Flow Analysis." Communications Earth & Environment, vol. 6, 2025, article 257, https://doi.org/10.1038/s43247-025-02169-5.
4. Lear, Gavin, *et al*. "Plastics and the Microbiome: Impacts and Solutions." *Environmental Microbiome*, vol. 16, no. 2, Jan. 2021, https://doi.org/10.1186/s40793-020-00371-w.
5. Sanluis-Verdes, Anahí, *et al*. "Wax Worm Saliva and the Enzymes Therein Are the Key to Polyethylene Degradation by *Galleria mellonella*." *Nature Communications*, vol. 13, Oct. 2022, https://doi.org/10.1038/s41467-022-33127-w.
6. Bombelli, Paolo, *et al*. "Polyethylene Bio-Degradation by Caterpillars of the Wax Moth *Galleria mellonella*." *Current Biology*, vol. 27, no. 8, Apr. 2017, pp. R283–R293, https://doi.org/10.1016/j.cub.2017.02.060.
7. Cassone, Bryan J., *et al*. "Role of the Intestinal Microbiome in Low-Density Polyethylene Degradation by Caterpillar Larvae of the Greater Wax Moth, *Galleria mellonella*." Proceedings of the Royal Society B: Biological Sciences, vol. 287, Mar. 2020, https://doi.org/10.1098/rspb.2020.0112.
8. Hidalgo-Crespo, José, *et al*. "Circular Economy of Expanded Polystyrene Container Production: Environmental Benefits of Household Waste Recycling Considering Renewable Energies." *Energy Reports*, vol. 8, Feb. 2022, pp. 306–311, https://doi.org/10.1016/j.egyr.2022.01.071.
9. Spínola-Amilibia, Mercedes, *et al*. "Plastic Degradation by Insect Hexamerins: Near-Atomic Resolution Structures of the Polyethylene-Degrading Proteins from the Wax Worm Saliva." Science Advances, vol. 9, Sep. 2023, https://doi.org/10.1126/sciadv.adi6813.
10. Yang, Yu, *et al*. "Biodegradation and Mineralization of Polystyrene by Plastic-Eating Superworms Zophobas atratus." Science of the Total Environment, vol. 708, Jan. 2020, https://doi.org/10.1016/j.scitotenv.2019.135233.

Copyright: © 2025 Bharadwaj and Goes. All JEI articles are distributed under the attribution non-commercial, no derivative license (http://creativecommons.org/licenses/by-nc-nd/4.0/). This means that anyone is free to share, copy and distribute an unaltered article for non-commercial purposes provided the original author and source is credited.