Microbial Degradation, Recycling and Upcycling of PET Wastes: A Mini-Review

Debananda S. Ningthoujam

Abstract

More than 350 million tons of plastics are produced globally per annum. The major kinds of plastics include polyethylene (PE), polypropylene (PP), polystyrene (PS), polyurethane (PU), polyvinyl chloride (PVC), and polyethylene terephthalate (PET). PET is one of the predominant plastics used in textiles and packaging. PET wastes have been recycled using mechanical and chemical methods. However, these techniques are costly, non-eco-friendly, and may generate toxic by-products. Hence, intensive research is underway to develop biological methods of PET depolymerization. Several PET-digesting enzymes have been isolated from bacteria, actinobacteria and fungi. However, bacterial enzymes suffer from low efficiency, slow reaction rates, recalcitrance to high crystallinity PET, and lack of robustness to range of pH and temperatures. In contrast, actinobacterial enzymes show higher thermostability but they also have their own limitations. More recently, improved enzymes have been produced using rational, AI directed and even directed evolution strategies. Some of these enzymes have demonstrated rapid depolymerization of low-to-medium crystallinity PET wastes. A French group has shown efficient depolymerization of PET bottles and recycling into virgin PET bottles. A British team has produced food, vanillin, from PET waste using an engineered microbe for the first time and other groups have shown the feasibility of upcycling (valorization) of PET waste into various value-added products. This minireview will highlight the recent developments in microbial degradation, recycling, and upcycling of PET wastes.

Keywords: Polyethylene terephthalate, PET, enzyme engineering, recycling, upcycling, *Ideonella sakaiensis*, LCC.

Abbreviations: AI, Artificial intelligence; BHET, Bis-(2-hydroxyethyl) terephthalate; BKA, Beta-keto adipate; EG, Ethylene glycol; IsPETase, *Ideonella sakaiensis* PETase; LCC, Leaf compost cutinase; MHET, Mono-(2-hydroxyethyl) terephthalate; ML, Machine learning; PET, Polyethylene terephthalate; PHL, Polyester hydrolase Leipzig7; TPA, terephthalic acid.

Significance | Ecofriendly degradation of PET by genetically engineered microbes

*Correspondence: **Professor Debananda S. Ningthoujam**, Department of Biochemistry, Manipur University Canchipur, Imphal 795003, India. **E-mail:** debananda.ningthoujam@gmail.com; **Contact no.:** +919862027271

Edited by **Dr. Md. Fakruddin, North South University, Bangladesh**, and accepted by the Editorial Board December 28, 2022 (Received for review December 6, 2022)

Introduction

The annual production of plastics exceeds 350 million tons (Carr et al., 2020). As very few plastics are recycled, they get incinerated or landfilled, and s significant portion falls into the world's oceans and other water bodies generating microplastics that threaten aquatic life forms.

The major kinds include PE, PP, PS, PU, PVC, and PET. PET is predominantly used in making clothes, textiles and bottles, and other packaging materials. PET is currently one of the most extensively used plastics (Liu et al., 2022). About 70 million tons of PET are produced globally per year for use in textiles and packaging (Tournier et al., 2020). The techniques of degradation are in the most advanced stage for one kind of plastic i.e. poly (ethylene terephthalate) compared to other plastic types.

Strategies for PET degradation

These include mechanical, chemical, and biological/enzymatic

Author Affiliation:
Department of Biochemistry, Manipur University, Imphal 795003, India.

Please cite this article:
Ningthoujam DS (2022). Microbial degradation, recycling and upcycling of PET wastes: A Mini-Review. *Microbial Bioactives, 5*(2), 219-224.

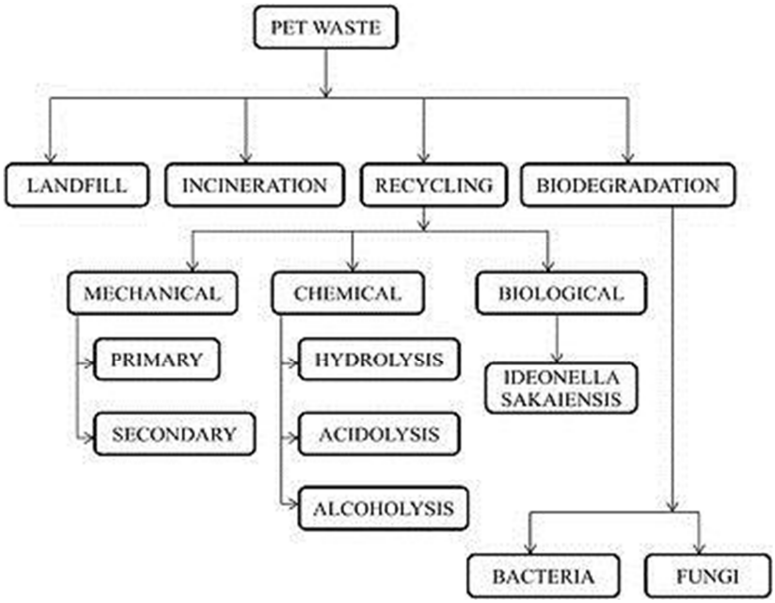

Figure 1| **Various Methods of PET Degradation (Courtesy: Google Creative Commons)**

Table 1| **Methods for PET disposal (Samak et al., 2020)**

Method	Brief Description	Extent of Recycling	Advantages	Disadvantages
Mechanical	Sorting, shredding, melting, and recycling	Limited to monolayer plastics	Fast and cost-effective	Eco-unfriendly may generate infectious agents and VOCs, loss of quality, and recalcitrance to multilayer plastics
Chemical	Depolymerization to monomers via hydrolysis, glycolysis, and ethanolysis	Limited to condensed polymers	Simple and moderately fast	Eco-unfriendly may generate infectious agents and VOCs, cost, and energy-intensive
Biological	Polymer bond cleavage into monomers via microbial enzymes	Limited to certain types of plastics only	Ecofriendly, products could be used for various applications	Time-consuming when compared with other methods

Table 2| **Bacterial species degrading PET**

Microorganism	Enzyme	Substrate	Temperature (°C)	Reference
Bacillus subtilis	BsEstB	3PET	40-45	(Ribitsch et al., 2011)
Clostridium bolulinum	Cbotu_EstA	PET film	50	(Biundo et al., 2018)
Ideonella sakaensis	IsPETase	Low crystallinity PET/bottle-grade, high crystallinity PET	20-45	(Yoshida et al., 2016)
O. antarctica BB-8	PET5	PET nano agar	50	(Danso et al., 2018)
Pseudomonas aeustunigri	PE-H	Amorphous PET film	30	(Bollinger et al., 2020)
Pseudomonas mendocina	PmC	PET nano agar	50	(Danso et al., 2018)
Vibrio gazogenes	PET6	PET nano agar	50	(Danso et al., 2018)
Uncultured bacterium from marine metagenome	PET2	PET nano agar	50	(Danso et al., 2018)
Uncultured bacterium in leaf branch compost metagenome	LCC	Amorphous PET film	50-70	(Sulaiman et al., 2012)
Uncultured bacterium in compost metagenome	PHL7	PET film	50	(Tiso et al., 2021)

methods. Mechanical and chemical methods are energy-intensive and may generate environmental pollutants (Figure 1 & Table 1). Landfilling and marine disposal threaten marine and terrestrial life forms. There is also the problem of microplastics. Recycled plastic produced via traditional strategies is of low quality and the process can run only for a few cycles. Intensive research is being conducted on microbial degradation, recycling, and upcycling of plastic wastes. The best option is using whole cell systems or enzymatic methods which can degrade, recycle and upcycle PET wastes for a truly circular economy.

Several bacterial species have been shown to degrade PET (Table 2). PET-digesting enzymes have been obtained from these microbial degraders. PET hydrolases may be PETases, cutinases, lipases, esterases, or carboxylesterases. Most enzymes from mesophilic species suffer from low efficiency, slow reaction rates, recalcitrance to high-crystallinity PET, and lack of robustness to a range of pH and temperatures. Some enzymes have been identified through a metagenomic approach e.g., LCC and PHL7. LCC has been the most efficient and robust enzyme but a recently discovered enzyme, PHL7, seems to be more robust than even LCC. It would be interesting to see how effective this new enzyme or its variants would be for large-scale depolymerization of PET wastes.

Thermostable PET-digesting enzymes have been obtained mostly from actinobacterial genera such as *Saccharomonospora, Thermomonospora,* and *Thermobifida* (Table 3).

Although bacterial/enzymatic recycling of PET wastes is an attractive option, it faces limitations such as low efficiency, low reaction rates, low tolerance to a range of temperatures and pH levels, and recalcitrance to high crystallinity PET. Therefore, PET-digesting enzymes have been engineered to address these problems. Some of these use rational engineering approaches such as site-directed mutagenesis or AI/ML-aided engineering. Some recent strategies included the directed evolution of PET-degrading enzymes (Table 4).

Lu et al. (2022) used a machine learning platform to engineer a robust and active hydrolase called FAST-PETase that showed better hydrolytic activity to PET between 30 °C and 50 °C and a wide range of pH levels and thus holds potential for enzymatic PET recycling at the industrial scale (Lu et al., 2022). An improved PET hydrolase able to achieve 90% PET depolymerization in 10 hours has been reported (Tournier et al., 2020). This group improved both the activity and thermostability of LCC through enzyme engineering. They reported that biologically recycled PET generated from PET waste using this enzyme has the same properties as petrochemical PET. The French firm Carbios has recently set up a demonstration plant for recycling PET bottles via this engineered enzyme.

A highly thermostable IsPETase enzyme called HotPETase has also been developed through directed evolution (Bell et al., 2022). It can operate at the glass transition temperature of PET. HotPETase can depolymerize semicrystalline PET better than other enzymes. Other groups have also developed improved versions of PET depolymerizing enzymes through engineering (Table 4).

Recycling PET wastes by thermomechanical or chemical means are not attractive. It's costly and can generate pollutants. Worse, the quality of recycled plastic decreases in each cycle compared to virgin ones. O late, the enzymatic route has been investigated for recycling plastic wastes. Some groups have attempted to use the monomers from PET not to produce fresh plastic (recycling) but convert them into high-value products (valorization, upcycling) (Table 5).

For the first time, PET waste has been upcycled into food (vanillin, a component of ice cream) by Sadler and his colleagues (Sadler & Wallace, 2021). They have valorized terephthalic acid (TPA), a breakdown product of PET, into vanillin using a genetically enzymes *E. coli* carrying genes for the enzymes required for bioconversion of TPA into E. Coli. Other groups have valorized PET waste into catechol and related aromatic compounds. Similarly, there are many other reports on upcycling of PET into bioplastics (polyhydroxy alkanoic acid. PHA), muconic acid and beta-keto adipic acid (BKA, a nylon monomer), and other high-value products. (Table 5).

Future Perspectives

Urgent upscaling of PET repolymerization by effective engineered enzymes is warranted. At the same time, we need to explore novel microbes and enzymes for PET degradation which have higher efficiency, faster reaction rates, and robustness to a wide range of temperatures and pH values. It is also critically important to look for enzymes that can depolymerize high-crystallinity PET. More research is also needed for not just recycling PET wastes but also upcycling them into value-added products. Mixtures of microbes and/or cocktails of enzymes must also be developed to address mixtures of plastic wastes in a real-world situation. The future looks bright for microbial degradation, recycling, and upcycling of PET wastes.

Conclusion

Though traditional PET wastes have been degraded via mechanical and chemical methods, biological methods are preferred. Quite a few bacteria and actinobacteria capable of digesting PET have been discovered. However, they suffer from slow reaction rates, low efficiency, recalcitrance to high crystallinity PET, and lack of robustness to a wide range of temperatures, and pH levels. Hence, enzyme engineering is being pursued to improve PET-digesting enzymes, especially IsPETase, LCC, and PHL7. Soon, a significant fraction of PET wastes could be recycled into virgin PET products using engineered enzymes and/or microorganisms. In addition, PET waste is being upcycled into high-value products such as vanillin, nylon monomers, muconic acid, glycolic acid, and other such products. We may anticipate the feasibility of large-scale recycling and upcycling of PET wastes soon.

Table 3| **Actinobacterial species able to degrade PET**

Microorganism	Enzyme	Substrate	Temperature (°C)	Reference
Saccharomonospora viridis	Cut190	Amorphous PET film and package-grade PET	60-65	(Kawai et al., 2014)
Thermomonospora curvata	Tcur0390	PET nano suspension	50	(Wei et al., 2014)
T. curvata	Tcur1278	PET nanospheres	60	(Wei et al., 2014)
Thermobifida fusca	Tfca	Cyclic PET trimers	50-60	(Billig et al., 2010)
T. fusca	Tfcut1	PET film	55-65	(Then et al., 2015)
T. fusca	Tfcut2	PET film PET nanospheres	55-65	(Then et al., 2015)
Thermobifida fusca	TfH (BTA-1)	Bottle-grade PET (10% crystallinity)	55	(Müller et al., 2005)
T fusca	Thf42_Cut1	3PET and PET film	50	(Herrero Acero et al., 2011)
T fusca	BTA-1	Bottle grade PET	65-70	(Müller et al., 2005)
Thermomonospora alba	Tha_Cut1	3PET	50	(Ribitsch, Herrero Acero, et al., 2012)
Thermobifida cellulolytica	Thc_Cut1	3PET and PET film (37% crystallinity)	50	(Herrero Acero et al., 2011)
T. cellulolytica	Thc_Cut2	3PET and PET film	50	(Herrero Acero et al., 2011)
Thermobifida halotolerans	Thh_Est	3PET	50	(Ribitsch, Acero, et al., 2012)
Thermomonospora alba	Est119	PET film	50	(Thumarat et al., 2012)

Table 4| **Engineered enzymes for improved PET degradation**

Enzyme	Chassis	Substrate	Effects	Reference
IsPETase	*E coli*	PET	Increased PETase activity	(Joo et al., 2018)
IsPETase	*E coli*	PET	4.13% higher crystallinity loss	(Austin et al., 2018)
IsPETase	*E coli*	PET	Enhanced activity at 30 degrees and pH9	(Liu et al., 2022)
IsPETase	*E coli*	PET	Increased PETase activity	(Ma et al., 2018)
FAST-PETase	*P. putida*	PET	Functional, active, stable, and tolerant PETase (FAST)	(Lu et al., 2022)
HotPETase	---	PET	Thermostable PETase variant	(Bell et al., 2022)
Cut190	*E coli*	PET	Enhanced cutinase activity at 70, Ph 6.5-8	(Kawai et al., 2014)
Cut190	*E coli*	PET	Increased enzyme affinity and activity	(Kawabata et al., 2017)
MHETase	*E coli*	MHET, BHET	Enhanced enzyme activity	(Palm et al., 2019)
PE-H	*E coli*	Amorphous PET	Enhanced activity	(Bollinger et al., 2020)
LCC	*E coli*	PET	90% PET degradation in 10 h	(Tournier et al., 2020)
IsPETase	*E coli*	Semi-crystalline PET	Increased thermostability at 60 for 3 d	(Cui et al., 2018)

Table 5| **Some examples of PET upcycling**

Microbe/enzyme used	Substrate	Key monomer	High-value products	Reference
IsPETase and *P. putida*	PET	TPA	BKA-monomer for nylon and other value-added products	(Lilli Manolis Sherman, 2022)
Genetically engineered *E coli*	PET	TPA	vanillin	(Sadler & Wallace, 2021)
Y lipolytica and *P stutzeri*	PET, BHET	TPA and EG	PHA	(Tiso et al., 2021)
P umsongensis	PET	TPA	PHA	(Tiso et al., 2021)
Engineered *P. putida*	PET	TPA and EG	Muconic acid	(Liu et al., 2022)
Engineered *E coli*	PET	TPA	5 products: GA, pyrogallol, Catechol, muconic acid, and vanillic acid	(Kim et al., 2019)
Gluconobacter oxydans	PET	EG	Glycolic acid (exfoliant)	(Kim et al., 2019)

Author Contribution

Conceptualization, experimentation, analysis and interpretation of data, drafting and critical revision of the manuscript were done by DSN.

Acknowledgment

This study was funded in part by a CSSP grant from Bibliotheca Alexandrina and supported by grant No. 12050137 from the National Research Centre. Deep appreciation to Prof. Suey-Sheng Kao for providing the original key of the Chinese species.

Competing financial interests

The authors declare that they have no potential conflict of interest in publishing this research output.

References

Austin, H. P., Allen, M. D., Donohoe, B. S., Rorrer, N. A., Kearns, F. L., Silveira, R. L., Pollard, B. C., Dominick, G., Duman, R., El Omari, K., Mykhaylyk, V., Wagner, A., Michener, W. E., Amore, A., Skaf, M. S., Crowley, M. F., Thorne, A. W., Johnson, C. W., Woodcock, H. L., … Beckham, G. T. (2018). Characterization and engineering of a plastic-degrading aromatic polyesterase. *Proceedings of the National Academy of Sciences*, *115*(19). https://doi.org/10.1073/pnas.1718804115

Bell, E. L., Smithson, R., Kilbride, S., Foster, J., Hardy, F. J., Ramachandran, S., Tedstone, A. A., Haigh, S. J., Garforth, A. A., Day, P. J. R., Levy, C., Shaver, M. P., & Green, A. P. (2022). Directed evolution of an efficient and thermostable PET depolymerase. *Nature Catalysis*, *5*(8), 673–681. https://doi.org/10.1038/s41929-022-00821-3

Billig, S., Oeser, T., Birkemeyer, C., & Zimmermann, W. (2010). Hydrolysis of cyclic poly(ethylene terephthalate) trimers by a carboxylesterase from Thermobifida fusca KW3. *Applied Microbiology and Biotechnology*, *87*(5), 1753–1764. https://doi.org/10.1007/s00253-010-2635-y

Biundo, A., Reich, J., Ribitsch, D., & Guebitz, G. M. (2018). Synergistic effect of mutagenesis and truncation to improve a polyesterase from Clostridium botulinum for polyester hydrolysis. *Scientific Reports*, *8*(1), 3745. https://doi.org/10.1038/s41598-018-21825-9

Bollinger, A., Thies, S., Knieps-Grünhagen, E., Gertzen, C., Kobus, S., Höppner, A., Ferrer, M., Gohlke, H., Smits, S. H. J., & Jaeger, K.-E. (2020). A Novel Polyester Hydrolase From the Marine Bacterium Pseudomonas aestusnigri – Structural and Functional Insights. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.00114

Carr, C. M., Clarke, D. J., & Dobson, A. D. W. (2020). Microbial Polyethylene Terephthalate Hydrolases: Current and Future Perspectives. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/fmicb.2020.571265

Cui, Q., Cheng, H., Xiong, R., Zhang, G., Du, R., Anantpadma, M., Davey, R. A., & Rong, L. (2018). Identification of diaryl-quinoline compounds as entry inhibitors of ebola virus. *Viruses*, *10*(12). https://doi.org/10.3390/v10120678

Danso, D., Schmeisser, C., Chow, J., Zimmermann, W., Wei, R., Leggewie, C., Li, X., Hazen, T., & Streit, W. R. (2018). New Insights into the Function and Global Distribution of Polyethylene Terephthalate (PET)-Degrading Bacteria and Enzymes in Marine and Terrestrial Metagenomes. *Applied and Environmental Microbiology*, *84*(8). https://doi.org/10.1128/AEM.02773-17

Herrero Acero, E., Ribitsch, D., Steinkellner, G., Gruber, K., Greimel, K., Eiteljoerg, I., Trotscha, E., Wei, R., Zimmermann, W., Zinn, M., Cavaco-Paulo, A., Freddi, G., Schwab, H., & Guebitz, G. (2011). Enzymatic Surface Hydrolysis of PET: Effect of Structural Diversity on Kinetic Properties of Cutinases from Thermobifida. *Macromolecules*, *44*(12), 4632–4640. https://doi.org/10.1021/ma200949p

Joo, S., Cho, I. J., Seo, H., Son, H. F., Sagong, H.-Y., Shin, T. J., Choi, S. Y., Lee, S. Y., & Kim, K.-J. (2018). Structural insight into molecular mechanism of poly(ethylene terephthalate) degradation. *Nature Communications*, *9*(1), 382. https://doi.org/10.1038/s41467-018-02881-1

Kawabata, T., Oda, M., & Kawai, F. (2017). Mutational analysis of cutinase-like enzyme, Cut190, based on the 3D docking structure with model compounds of polyethylene terephthalate. *Journal of Bioscience and Bioengineering*, *124*(1), 28–35. https://doi.org/10.1016/j.jbiosc.2017.02.007

Kawai, F., Oda, M., Tamashiro, T., Waku, T., Tanaka, N., Yamamoto, M., Mizushima, H., Miyakawa, T., & Tanokura, M. (2014). A novel Ca2+-activated, thermostabilized polyesterase capable of hydrolyzing polyethylene terephthalate from Saccharomonospora viridis AHK190. *Applied Microbiology and Biotechnology*, *98*(24), 10053–10064. https://doi.org/10.1007/s00253-014-5860-y

Kim, H. T., Kim, J. K., Cha, H. G., Kang, M. J., Lee, H. S., Khang, T. U., Yun, E. J., Lee, D.-H., Song, B. K., Park, S. J., Joo, J. C., & Kim, K. H. (2019). Biological Valorization of Poly(ethylene terephthalate) Monomers for Upcycling Waste PET. *ACS Sustainable Chemistry & Engineering*, *7*(24), 19396–19406. https://doi.org/10.1021/acssuschemeng.9b03908

Lilli Manolis Sherman. (2022). *"First" Bacteria to Upcycle Single-Use PET Heading to Space*. Plastic Technology. https://www.ptonline.com/blog/post/first-bacteria-to-upcycle-single-use-pet-heading-to-space

Liu, P., Zheng, Y., Yuan, Y., Zhang, T., Li, Q., Liang, Q., Su, T., & Qi, Q. (2022). Valorization of Polyethylene Terephthalate to Muconic Acid by Engineering Pseudomonas Putida. *International Journal of Molecular Sciences*, *23*(19), 10997. https://doi.org/10.3390/ijms231910997

Lu, H., Diaz, D. J., Czarnecki, N. J., Zhu, C., Kim, W., Shroff, R., Acosta, D. J., Alexander, B. R., Cole, H. O., Zhang, Y., Lynd, N. A., Ellington, A. D., & Alper, H. S. (2022). Machine learning-aided engineering of hydrolases for PET depolymerization. *Nature*, *604*(7907), 662–667. https://doi.org/10.1038/s41586-022-04599-z

Ma, Y., Yao, M., Li, B., Ding, M., He, B., Chen, S., Zhou, X., & Yuan, Y. (2018). Enhanced Poly(ethylene terephthalate) Hydrolase Activity by Protein Engineering. *Engineering*, *4*(6), 888–893. https://doi.org/10.1016/j.eng.2018.09.007

Müller, R.-J., Schrader, H., Profe, J., Dresler, K., & Deckwer, W.-D. (2005). Enzymatic Degradation of Poly(ethylene terephthalate): Rapid Hydrolyse using a Hydrolase fromT. fusca. *Macromolecular Rapid Communications*, *26*(17), 1400–1405. https://doi.org/10.1002/marc.200500410

Palm, G. J., Reisky, L., Böttcher, D., Müller, H., Michels, E. A. P., Walczak, M. C., Berndt, L., Weiss, M. S., Bornscheuer, U. T., & Weber, G. (2019). Structure of the plastic-degrading Ideonella sakaiensis MHETase bound to a substrate. *Nature Communications*, *10*(1), 1717. https://doi.org/10.1038/s41467-019-09326-3

Ribitsch, D., Acero, E. H., Greimel, K., Eiteljoerg, I., Trotscha, E., Freddi, G., Schwab, H., & Guebitz, G. M. (2012). Characterization of a new cutinase from Thermobifida alba for PET-surface hydrolysis. *Biocatalysis and Biotransformation*, *30*(1), 2–9. https://doi.org/10.3109/10242422.2012.644435

Ribitsch, D., Herrero Acero, E., Greimel, K., Dellacher, A., Zitzenbacher, S., Marold, A., Rodriguez, R. D., Steinkellner, G., Gruber, K., Schwab, H., & Guebitz, G. M. (2012). A New Esterase from Thermobifida halotolerans Hydrolyses Polyethylene Terephthalate (PET) and Polylactic Acid (PLA). *Polymers*, *4*(1), 617–629. https://doi.org/10.3390/polym4010617

Ribitsch, D., Heumann, S., Trotscha, E., Herrero Acero, E., Greimel, K., Leber, R., Birner-Gruenberger, R., Deller, S., Eiteljoerg, I., Remler, P., Weber, T., Siegert, P., Maurer, K.-H., Donelli, I., Freddi, G., Schwab, H., & Guebitz, G. M. (2011). Hydrolysis of

polyethyleneterephthalate by p-nitrobenzylesterase from Bacillus subtilis. *Biotechnology Progress*, *27*(4), 951–960. https://doi.org/10.1002/btpr.610

Sadler, J. C., & Wallace, S. (2021). Microbial synthesis of vanillin from waste poly(ethylene terephthalate). *Green Chemistry*, *23*(13), 4665–4672. https://doi.org/10.1039/D1GC00931A

Samak, N. A., Jia, Y., Sharshar, M. M., Mu, T., Yang, M., Peh, S., & Xing, J. (2020). Recent advances in biocatalysts engineering for polyethylene terephthalate plastic waste green recycling. *Environment International*, *145*, 106144. https://doi.org/10.1016/j.envint.2020.106144

Sulaiman, S., Yamato, S., Kanaya, E., Kim, J.-J., Koga, Y., Takano, K., & Kanaya, S. (2012). Isolation of a Novel Cutinase Homolog with Polyethylene Terephthalate-Degrading Activity from Leaf-Branch Compost by Using a Metagenomic Approach. *Applied and Environmental Microbiology*, *78*(5), 1556–1562. https://doi.org/10.1128/AEM.06725-11

Then, J., Wei, R., Oeser, T., Barth, M., Belisário-Ferrari, M. R., Schmidt, J., & Zimmermann, W. (2015). Ca 2+ and Mg 2+ binding site engineering increases the degradation of polyethylene terephthalate films by polyester hydrolases from Thermobifida fusca. *Biotechnology Journal*, *10*(4), 592–598. https://doi.org/10.1002/biot.201400620

Thumarat, U., Nakamura, R., Kawabata, T., Suzuki, H., & Kawai, F. (2012). Biochemical and genetic analysis of a cutinase-type polyesterase from a thermophilic Thermobifida alba AHK119. *Applied Microbiology and Biotechnology*, *95*(2), 419–430. https://doi.org/10.1007/s00253-011-3781-6

Tiso, T., Narancic, T., Wei, R., Pollet, E., Beagan, N., Schröder, K., Honak, A., Jiang, M., Kenny, S. T., Wierckx, N., Perrin, R., Avérous, L., Zimmermann, W., O'Connor, K., & Blank, L. M. (2021). Towards bio-upcycling of polyethylene terephthalate. *Metabolic Engineering*, *66*, 167–178. https://doi.org/10.1016/j.ymben.2021.03.011

Tournier, V., Topham, C. M., Gilles, A., David, B., Folgoas, C., Moya-Leclair, E., Kamionka, E., Desrousseaux, M.-L., Texier, H., Gavalda, S., Cot, M., Guémard, E., Dalibey, M., Nomme, J., Cioci, G., Barbe, S., Chateau, M., André, I., Duquesne, S., & Marty, A. (2020). An engineered PET depolymerase to break down and recycle plastic bottles. *Nature*, *580*(7802), 216–219. https://doi.org/10.1038/s41586-020-2149-4

Wei, R., Oeser, T., Then, J., Kühn, N., Barth, M., Schmidt, J., & Zimmermann, W. (2014). Functional characterization and structural modeling of synthetic polyester-degrading hydrolases from Thermomonospora curvata. *AMB Express*, *4*(1), 44. https://doi.org/10.1186/s13568-014-0044-9

Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Toyohara, K., Miyamoto, K., Kimura, Y., & Oda, K. (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*, *351*(6278), 1196–1199. https://doi.org/10.1126/science.aad6359