

**The Plastic Problem – A Review of Current Methods of Plastic Waste Management
and the Merits of Microbial Plastic Degradation
By: Mariam Mahboob (iGEM Toronto)**

Introduction:

Over the past century, there has been a shift in the industrial market in terms of the materials most abundantly being used for products across the world. Due to its low cost, high efficiency in production, and relative thermal stability, plastics have replaced traditional glass and paper products in various industries, including packaging, household utilities, and construction. [1] A specific type of plastic, Poly(ethylene terephthalate) (PET), constituted 7% of the plastic material demand in 2015, which is approximately 18.8 million tons of the total 269 million tons of all plastic produced that year. [1] Due to its highly aromatic and nonreactive properties, PET is difficult to degrade and accumulates in the environment which has become a global concern, classified as “plastic pollution”.

Most of this plastic waste arises from terrestrial and marine sources, through either inappropriate dumping of domestic and industrial products or through poor containment of waste by established plastic waste management systems. [2] As conventionally understood, the abundance of plastic debris in the environment results in hazardous effects on the ecosystems that thrive off of the planet’s resources. Some of the more imminent physical dangers of plastic debris in the environment include potential ingestion or entanglement by vulnerable species, including but not limited to sea turtles, fur seals, sharks, and marine birds. [3] Plastic particles in the ocean have also been shown to contain high levels of organic pollutants, such as polychlorinated biphenyls (PCBs), nonylphenol (NP), organic pesticides such as dichlorodiphenyltrichloroethane (DDT), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), and bisphenol A (BPA) – many of which can then undergo significant rates of biomagnification, posing significant developmental and neurological

impairments to humans amongst other species. [4] Furthermore, through the existence of plastic debris, direct challenges to maintaining ecological diversity have also increased as colonisation and dispersal of invasive species by adherence to circulating plastic waste has become more abundant. [3]

Designing and implementing universal plastic waste management strategies proves challenging due to issues in quantifying levels of plastic pollution across a wide variety of environments and the impediments in recovering end products for economic incentives, as per the theory of circular economies. [5]

Currently, there are three established methods of plastic waste management across the world: landfills, incineration, and recycling. Each are explored below.

Landfills:

Plastic landfills are isolated areas of land that are purposefully established for the containment of waste

away from communities. [2] Landfills often occupy essential space that could be used for greater purposes for a growing human population, such as

agriculture. [5] Although they are generally meant to exist for a relatively short time frame due to supposed degradation of waste materials, plastic landfills persist for up to 20 years or longer, as many plastics contain components that do not degrade well naturally. [2] Thermo-oxidative degradation processes that can break down some plastic components through UV-activated incorporation of oxygen molecules for polymer chain deterioration are also significantly hindered in landfills due to the highly anaerobic environment. [5] Conclusively, plastic debris also releases harmful toxins, such as BPA, that increases the production of hydrogen sulphide, which in high quantities can be lethal. [6]



Incineration:

Incineration is another widely established method that counteracts issues abundant with landfills in the sense that it uses less space and there is a possibility of energy recovery in the form of heat. [7] It is also potentially the most suitable way for dealing with highly mixed plastics, such as electronic or electrical waste. [5] However, incineration also produces numerous harmful compounds such as heavy metals, toxic carbon and oxygen based free radicals, and greenhouse gases that are released into the atmosphere, which contribute to the depletion of the ozone layer. [8]

Recycling:

Recycling is the more efficient method of the three established plastic waste management techniques. There are two main approaches used for recycling PET: chemical and mechanical. [2] Chemical procedures include performing chemolysis of one compound, resulting in the depolymerization of the plastic. Depending on the substance used for chemolysis (e.g.



water for hydrolysis, methanol for methanolysis, etc.), different monomer units are formed, yet almost all can be recovered as polymerisation materials to produce new plastics. [2] This procedure, however, is very expensive.

[2]

Mechanical procedures include removing contaminated material from the plastic by sorting plastic wastes, separating PET-plastics, grinding PET into flakes, washing flakes with a strong detergent, and then drying at high temperatures using friction. [5] Unfortunately, in this process, the presence of additives and impurities heavily complicates the recycling procedure, often decreasing the yield and quality of the recovered product. [2]

Furthermore, the recycling program has been largely ineffective in the grand scale of environmental concern initiatives due to the rate of plastic production being significantly higher – and continuously increasing – in comparison to the relatively slow increasing rates of recycling. [1]

Discrepancies in Recycling Waste Management Techniques:

Alongside the above-mentioned concerns with the current recycling waste management procedures, discrepancies amongst the classifications of various types of recyclables across different municipalities, provinces or states, and countries provide a further barrier for effective solutions to the plastic pollution issue.

Within a nation, as individuals exercise their free movement, they bring accustomed practices in waste management techniques from one location to the next – many of which may not be applicable to their new place of residence. For instance, while the City of Toronto considers black lids of cardboard coffee cups and the cups themselves to be unrecyclable waste material, in the same province, the City of Waterloo considers these items to be recyclables. [9, 10] A person moving from one location to the next may not be aware of this shift in rules due to the logical fallacy of both cities being within the same region.

Various nations also have different standards of acceptable end products generated for revenue purposes. Recently, China, the world's largest importer of recyclable material, has begun to ban imports of paper with more than 0.5 percent contamination. [11] As nations struggle to meet these varying standards due to inner discrepancies in their waste management systems, more potential reusable materials such as plastics are placed in landfills and incineration centers, adding further challenges to existing environmental concerns. [11]

Merits of Microbial Degradation and Utilising Synthetic Biology:

As previous mechanisms of plastic-waste management prove largely inefficient in solving the plastic problem, there has been a shift towards considering biodegradation through microbial organisms. [12] Biodegradation is a considerably cheaper process, more efficient, opens the possibilities to more pathways for obtaining useful end products, and often does not produce secondary pollutants such as the ones found in landfills or after incineration has occurred. [13] The significant metabolic diversity found within bacteria also professes a wider variety of potential tools for solving the plastic concern. [12]

PETase, A Glimpse at the Possible Future:

Studying the degradability of a wide range of polymers, Zheng *et al* (2005) uncovered that polymers with pure carbon backbones are resistant to most methods of degradation, but polymers with heteroatoms in their backbones, such as polyesters and polyamines, have higher susceptibility to potential degradation, and subsequently, use of metabolism for microbials. [14] PET, however, remains an issue as its aromaticity prevents traditionally readily degradable polymer chains, such as ester bonds, from being deteriorated. [15]

In 2016, Yoshida *et al* screened microbial communities exposed to PET in the environment, and isolated consortium 46 that contained plastic degrading abilities to a significant rate. [16] Upon subculturing multiple subconsortiums from the original consortium 46, the researchers discovered that one of the subconsortium lost its plastic degrading abilities. The plastic degrading abilities were lost due to the absence of the bacteria *Ideonella Sakaiensis*, which uses the resulting monomers formed after PET degradation as its primary source of metabolism. *I. Sakaiensis* secretes PETase, which hydrolyses PET to produce MHET. MHET is

then hydrolysed by MHETase, a lipoprotein, to produce two monomers TPA and EG, which pose no known threat to the environment. [16]

PETase is highly thermally unstable, though certain mutations performed in our project may ensure greater thermostability and increased catalytic activity, for the potential integration of PETase-mechanisms in new biodegradable industrial procedures. Hence, our proposed industrial utilisation of microbes that secrete PETase, a novel plastic-degrading enzyme, offers a potential solution for the aforementioned global issue, paving the path to substantial environmental protection.

References

- [1] Taniguchi, I., Yoshida, S., Hiraga, K., Miyamoto, K., Kimura, Y., & Oda, K. (2019). Biodegradation of PET: Current Status and Application Aspects. *ACS Catalysis*, 9(5), 4089-4105. doi:10.1021/acscatal.8b05171
- [2] Webb, H.; Arnott, J., Crawford, R., Ivanova, E. (2012). Plastic Degradation and Its Environmental Implications with Special Reference to Poly(ethylene terephthalate). (2012) *Polymers*, 5 (1), 1–18 DOI: 10.3390/polym5010001.
- [3] Gregory, M.R. (2009). Environmental implications of plastic debris in marine settings—Entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philos. Trans. R. Soc. B* **2009**, 364, 2013–2025.
- [4] Rios, L. M., Moore, C., & Jones, P. R. (2007). Persistent organic pollutants carried by synthetic polymers in the ocean environment. *Marine Pollution Bulletin*, 54(8), 1230-1237. doi:10.1016/j.marpolbul.2007.03.022
- [5] Hopewell, J., Dvorak, R., & Kosior, E. (2009). Plastics recycling: challenges and opportunities. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 364(1526), 2115–2126. doi:10.1098/rstb.2008.0311
- [6] Xu, S., Zhang, H., He, P., & Shao, L. (2011). Leaching behaviour of bisphenol A from municipal solid waste under landfill environment. *Environmental Technology*, 32(11), 1269-1277. doi:10.1080/09593330.2010.535175.
- [7] Sinha, V., Patel, M. R., & Patel, J. V. (2008). Pet Waste Management by Chemical Recycling: A Review. *Journal of Polymers and the Environment*, 18(1), 8-25. doi:10.1007/s10924-008-0106-7
- [8] Astrup, T., Møller, J., & Fruergaard, T. (2009). Incineration and co-combustion of waste: Accounting of greenhouse gases and global warming contributions. *Waste Management & Research*, 27(8), 789-799. doi:10.1177/0734242x09343774

- [9] City of Toronto. (2019, August 06). What Goes in the Blue Bin? Retrieved from <https://www.toronto.ca/services-payments/recycling-organics-garbage/houses/what-goes-in-my-blue-bin/>
- [10] Blue Box. (n.d.). Retrieved from <https://www.regionofwaterloo.ca/en/living-here/blue-box-recycling.aspx>
- [11] Many Canadians are recycling wrong, and it's costing us millions | CBC News. (2018, April 09). Retrieved from <https://www.cbc.ca/news/technology/recycling-contamination-1.4606893>
- [12] Iranzo M., Sainz-Pardo I., Boluda R., Sánchez J., Mormeneo, S. (2001). The use of microorganisms in environmental remediation. *Annals of Microbiology*. 51.
- [13] Pieper, D. H., & Reineke, W. (2000). Engineering bacteria for bioremediation. *Current Opinion in Biotechnology*, 11(3), 262-270. doi:10.1016/s0958-1669(00)00094-x
- [14] Zheng, Y., Yanful, E. K., & Bassi, A. S. (2005). A Review of Plastic Waste Biodegradation. *Critical Reviews in Biotechnology*, 25(4), 243-250. doi:10.1080/07388550500346359
- [15] Müller, R., Kleeberg, I., & Deckwer, W. (2001). Biodegradation of polyesters containing aromatic constituents. *Journal of Biotechnology*, 86(2), 87-95. doi:10.1016/s0168-1656(00)00407-7
- [16] Yoshida, S., Hiraga, K., Takehana, T., Taniguchi, I., Yamaji, H., Maeda, Y., Oda, K. (2016). A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*, 351(6278), 1196-1199. doi:10.1126/science.aad6359

MICROBIAL PLASTIC BREAKDOWN: INDUSTRIAL CONCERNS AND ENVIRONMENTAL IMPACT

By: Kate MacQuarrie

Consequences of environmental release of PET

Poly(ethylene terephthalate) (PET) has been one of the most widely used synthetic plastic polymers in the world since it became widespread during the 1970s [1]. This ubiquity is the result of PET's properties, including plasticity, transparency, and durability, which make it a favourable compound for diverse plastic products like containers and clothing¹. Since PET is so

widely used, it is frequently released into the environment both when it is manufactured and disposed [2].

The same properties that make PET a desirable plastic also mean that it is prone to accumulation in the environment, as its stable aromatic structure makes it resistant to enzymatic biodegradation². Additionally, PET has a high molecular weight, and a very crystalline structure: two properties which makes it less degradable [1,3]. As a result, when PET is introduced into the environment, it is not broken down naturally and remains a hazard to plant and animal life for hundreds of years [1].

On land and in the water, plastics like PET are harmful to flora and fauna. They injure and are consumed by many animals, and then are in turn consumed by humans when we eat those animals [4]. Plastics are also physically broken down by the elements until they are in the form of very small microplastics and pieces that can enter drinking water systems and the air, causing toxic effects and respiratory issues [5]. The vast accumulation of PET is especially problematic in marine environments, as marine life often ingests so much plastic that it causes starvation due to a reduction in appetite [5].

Industrial importance of increasing PETase's thermostability

As a result of the many negative effects of plastic pollution in conjunction with our current reliance on plastics, efforts have been made to break down the waste rather than allowing it to damage the environment. Plastics can be broken down through several methods, including thermolysis, photolysis, chemical degradation, and biodegradation [6]. Of these options, biodegradation is optimal, as it is efficient both environmentally and economically in terms of breaking down a polymer into monomers that can then be reused [3]. However, until recently,

PET has not been able to be degraded enzymatically. As PET is an aromatic polyester, in order to break up the polymer, the ester bonds between monomers must be cleaved by a hydrolase [1].

PETase, an enzyme secreted from the bacteria *Ideonella sakaiensis*, which is part of a microbial consortium found in plastic bottle waste, is currently the most effective source of PET hydrolase. The secreted enzyme degrades PET into mono-2-hydroxyethyl terephthalate (MHET), bis(2-hydroxyethyl) terephthalate, and terephthalic acid (TPA). A second enzyme which is also secreted by *I. sakaiensis*, MHETase, then breaks MHET down into TPA and ethylene glycol: the two monomers of PET [2]. As PETase's substrate, PET serves as a carbon source for the enzyme [1]. PETase has thus far been most effective on the polymer at lower temperatures around 30 degrees Celsius. PETase is structurally unstable and denatures at higher temperatures, thereby losing its enzymatic ability [7]. One unique feature of PETase is its two disulfide bridges. While these structures are thought to be important to its enzymatic activity, they are also susceptible to breaking down at elevated temperatures, which likely contributes to this negative characteristic [8].

The fact that PETase is not thermostable means that it is limited in terms of its potential to degrade PET industrially, as many industrial processes are done at high temperatures. Furthermore, plastics are more easily degraded at the glass transition temperature; a temperature at which PETase would denature [9]. Melting plastics at these elevated temperatures makes them easier to break down as it provides more surface area with which the hydrolase enzyme can interact and reduces its crystallinity [3]. PETase's low structural stability also limits its capacity to be purified for use in industry [7]. Furthermore, polyethylenes like PET can benefit from being blended with additives prior to biodegradation, another process that could increase the temperature of the process and denature the enzyme [3]. As there is so

much PET in the environment, and as it continues to be manufactured in large quantities, it is crucial that the plastic can also be recycled in large quantities. This need for industrial polyester recycling necessitates improvements with respect to PETase's thermostability.

References

- [1] Austin, H. P. et al. Characterization and engineering of a plastic degrading aromatic polyesterase. *Proceedings of the National Academy of Sciences of the United States of America*, 115(19), E4350-E4357 (2018).
- [2] Yoshida, S. et al. A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science*, 351, 1196-1199 (2016).
- [3] Tokiwa, Y., Calabia, B. P., Ugwu, C. U., Aiba, S. Biodegradability of plastics. *Int. J. Mol. Sci.*, 10(9), 3722-3742 (2009).
- [4] Johnston, I. (2017). How plastic is damaging planet earth. Available at <https://www.independent.co.uk/environment/plastic-how-planet-earth-environment-oceans-wildlife-recycling-landfill-artificial-a7972226.html>
- [5] Parker, L. (2019). The world's plastic pollution crisis explained. Available at <https://www.nationalgeographic.com/environment/habitats/plastic-pollution/>
- [6] Nithin, B., Goel, S. Degradation of plastics. *Advances in Solid and Hazardous Waste Management*. 235-247 (2017).
- [7] Seo, H. et al. Production of extracellular PETase from *Ideonella sakaiensis* using sec-dependent signal peptides in *E. coli*. *Biochemical and Biomedical Research Communications*, 508(1) 250-255 (2019).
- [8] Vieille, C., Zeikus, G. J. Hyperthermophilic enzymes: sources, uses, and molecular mechanisms for thermostability. *Microbiol. Mol. Biol. Rev.*, 65(1) 1-43 (2001).
- [9] Haider, T. P. et al. Plastics of the future? The impact of biodegradable polymers on the environment and on society. *Angewandte Chemie*. (2018).

Microbial Plastic Breakdown: Societal Concerns and Scientific Communication

By: Iris Liu (iGEM Toronto)

Synthetic plastic pollution has been an ongoing serious environmental issue due to the overuse of plastics for numerous purposes such as industries, packaging,

agriculture, and daily practices. In addition to overuse, plastic bags need around 1000 years to fully break down into organic matter [1]. Not only does plastic pollution negatively impact terrestrial and marine ecosystems, but also promote climate change due to the release of harmful byproducts while burning plastic. As synthetic plastic pollution continues to pose an environmental threat, there is a shift in focus towards utilizing microbes to degrade plastics. This article will be focusing on a few current microbial degradation strategies, such as using biodegradable plastics, and specific organisms that can degrade synthetic plastic, and how these strategies affect the possibility of adopting PETase as a bioremediation method.

One current solution to solve the issue of plastic pollution is manufacturing biodegradable plastics, which are natural polymers that can be degraded by organisms once disposed of into the environment [2]. These biodegradable plastics are made from plant materials or extracted from microorganisms such as starch, oils, chitin, cellulose, and polyhydroxy butyrate (PHB) [3]. As these plastics are made from natural materials, they have many advantages in terms of reducing CO₂ and other greenhouse gas emission levels, waste pollution and production, decomposition time, and energy consumption during production [4]. In addition to improving environmental conditions, manufacturing biodegradable plastics may also benefit the economy by creating new job opportunities and increasing new industries and exports. While biodegradable plastics may appear to be a viable solution to the harmful effects of using synthetic plastics, there are multiple shortcomings of adopting this method. As biodegradable plastics require natural methods to decompose, the correct temperature and humidity

are necessary [4]. The production of biodegradable plastics can be about 50% more expensive than the production of synthetic plastic [5]. In addition, more cropland will be needed to produce ingredients, which can cause a few ethical considerations to arise. In countries where hunger is an issue and resources are scarce, is the use of cash crops to produce plastics rather than feeding the people justifiable? While the method of adopting biodegradable plastics can be considered a positive impact towards the environment, there are still multiple drawbacks that could be further investigated and improved.

From the current methods of microbial degradation of plastics, we can utilize their strengths and weaknesses to analyze the suitability of adopting PETase as a bioremediation strategy. PETase is an enzyme that is originally found in *Ideonella sakaiensis*, which is a type of bacteria that can digest PET plastic found in PET contaminated sediments in Japan. This method can allow us to directly degrade PET plastics, without needing to create new types of plastic that can be more costly in terms of production. However, similar to the use of biodegradable plastics, direct degradation of PET reduces recycling. As a result, more plastics need to be produced. In addition, the use of PETase also relies heavily on direct action of people. For instance, if people discard PET products as litter, this method of bioremediation will not serve its purpose. In conclusion, utilizing PETase to tackle the problem of plastic pollution can be a suitable bioremediation strategy, if we strictly implement the procedures of enzyme degradation.

References

[1] Kale S, Deshmukh AG, Dudhare MS, Patil VB. MICROBIAL DEGRADATION OF PLASTIC: A REVIEW. In 2015.

[2] Gross RA, Kalra B. Biodegradable Polymers for the Environment. Science. 2002 Aug 2;297(5582):803–7.

[3] Pathak VM, Navneet. Review on the current status of polymer degradation: a microbial approach. Bioresour Bioprocess. 2017 Mar 23;4(1):15.

[4] Ayres C. 22 Advantages and Disadvantages of Biodegradable Plastics [Internet]. ConnectUS. 2019 [cited 2019 Jul 10]. Available from:
<https://connectusfund.org/7-advantages-and-disadvantages-of-biodegradable-plastics>

[5] The truth about bioplastics [Internet]. [cited 2019 Jul 11]. Available from:
<https://phys.org/news/2017-12-truth-bioplastics.html>