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# The Invisible Plastic Pollution

## How washing your clothes is destroying the environment

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**Plastic is a low-cost versatile material that is used in every part of life, from vital medical supplies to textiles and packaging. Over 400,000,000 tonnes of plastic are produced each year and this is predicted to double by 2040 (Carniel *et al.,* 2021). Plastic is a polymer meaning it is made up of the same repeating molecules, which are known as monomers.**

**To form plastic, monomers are joined through a chemical reaction at a high temperature to form a large polymer (e.g., polyethylene terephthalate (PET)). In theory, the bond holding the monomers together can be broken; however, due to its rigid structure PET is highly resistant to degradation.**

**Current recycling methods usually involve storing the plastic in landfill or shredding the plastic into smaller fragments, before being melted down to form reusable pellets. However, this recycled plastic is a downgrade of the original item, with limited uses. The majority of PET (also known as polyester) is made new with only 1% of collected textiles recycled back into textiles.**

### The impact of clothes

Synthetic plastic textiles such as PET are widely used in the textiles industry. In 2020, 57,000,000 tonnes of polyester were produced, making it the most used fibre globally (Textile exchange.org, 2021). Polyester is mass produced in fast fashion due to its versatile properties and cheap price. Despite the usefulness, it leaves a trail of environmental destruction throughout its life cycle, before eventually littering ecosystems.

Synthetic textiles pile up in landfill; however, a more subtle plastic pollutant is microplastics. Microplastics are small plastic particles less than 5 mm long - every plastic eventually forms a microplastic (Zhu *et al.,* 2021). Due to their small size most of them are invisible to the eye, causing widespread pollution that is difficult to remove.

Microplastics are produced when plastic undergoes mechanical strain. This occurs throughout the production, use and disposal of plastics. Synthetic fibres (e.g., PET) release microplastics during every wash. These particles are released into the environment, surpassing current waste-water filtration systems. The amount of people who wash synthetic fibres every day mean microplastics have accumulated exponentially in the environment. Microplastics are toxic and can be ingested by wildlife, infiltrating the food chain. This build-up means microplastics are in the products we consume. These can be harmful for human health (e.g., increased cancer risk) (Campanale *et al.,* 2020).

### The solution: biodegradation using PETases

Biodegradation offers a method to remove and prevent build-up of microplastics (and potentially a new recycling method). Biodegradation is a process by which enzymes break up a polymer (e.g., PET) back into its original monomer form.

Enzymes are biological catalysts that speed up reactions by reducing the amount of activation energy needed for a reaction to occur. They bind to the substrate (in this case, plastic) to reduce the energy required for a reaction to occur, thus making it more favourable. This offers a mechanism to turn the polymer PET back to the original monomers.

Enzymes are formed by many amino acids joining together to form a chain. This folds up into a globular structure due to interactions between the amino acids which hold this shape. The three-dimensional shape of every enzyme is different and will create an active site that is specific to the substrate the enzyme will bind – this is the lock and key mechanism. Specific enzymes (lock) interact at the active site (keyhole) with the specific substrates (key), meaning only the desired reactions are sped up.

PETase is an enzyme specific to degrading PET: it breaks down PET to form its original (non-plastic) monomers (Carniel *et al.,* 2021). To reduce the amount of

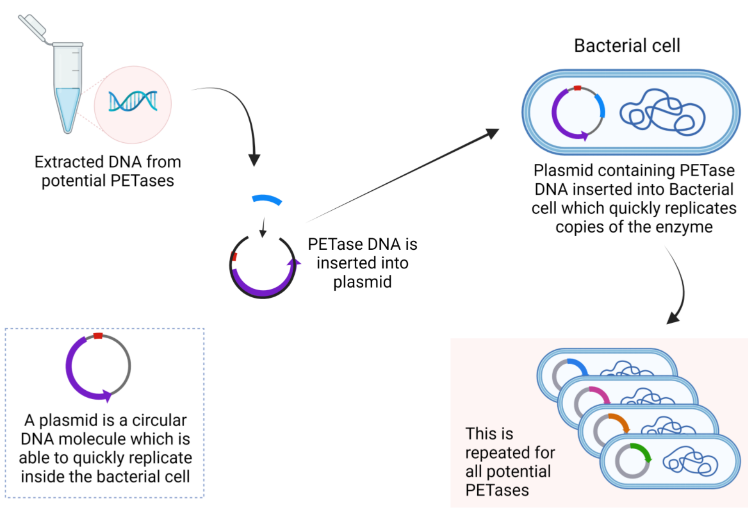


**Figure 1.** How DNA is added to bacterial cells for quick replication of PETase DNA sequences. The DNA can then be 'expressed', which means the bacterial cells then make the enzyme coded by the DNA. *Figure created using BioRender.com.*

microplastics released from washing polyester, PETase could be added to filters or washing powder to reduce the amount of microfibres that are released into the environment. This would mean microplastics would be broken down as they are released preventing harm to ecosystems.

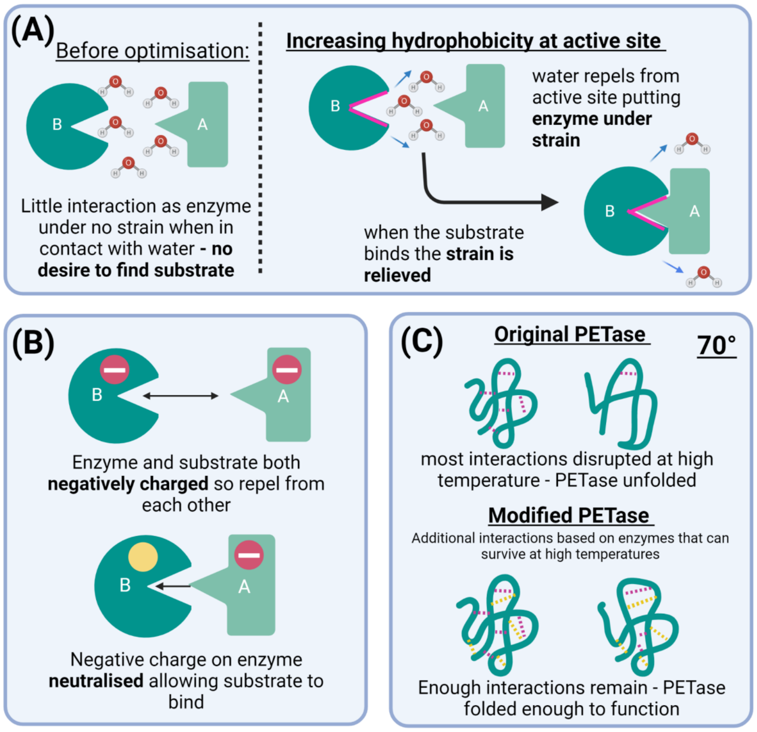


**Figure 2.** Types of PETase optimisation. (A) Increasing hydrophobicity of the active site (B) Altering the charge of the PETase to prevent repulsion. (C) Increasing the stability of PETase at higher temperatures. *Figure created with BioRender.com.*

### Discovering PETases

To discover enzymes capable of degrading PET, researchers have collected samples of micro-organisms living in a high concentration of plastic (Danso *et al.,* 2018). These organisms are more likely to have started developing enzymes with the ability to break down PET. The DNA of these organisms is extracted and analysed to find the DNA sequence. As DNA codes for proteins, the DNA extracted from these organisms can be compared to a database of different enzyme sequences to highlight any enzymes that are likely to have PET degrading properties. These sequences are placed into bacterial cells which allows production of the enzyme from the DNA sequence, creating an abundance of the enzyme (Figure 1). These enzymes are then screened using high-throughput methods to assess which ones have PET degradation activity. These methods mean a wide range of enzymes can be quickly screened simultaneously for activity. The candidates found to have the most activity undergo further investigation and optimisation for use in industry.

### Optimising PETases

PETases have been found to show degradation properties in some microorganisms; however, as plastic is a relatively new material, these microorganisms are yet to evolve efficient enzymes that could be used in industry. Therefore, the best candidates (found through screening) need to be optimised. These optimisations are done through computer modelling which predicts the outcome of modifications (altering the PETase DNA sequence). If alterations are successful, the enzyme can be tested in industrial conditions.

Enzyme efficiency is improved when there is more substrate binding. As this binding takes place at the active site, efficiency can be improved by optimising the active site. This is done by increasing the hydrophobicity; a more hydrophobic active site means the active site will try and repel from water, in a similar way to oil in water. This hydrophobicity makes the active site strained when unbound (in water) and unstrained when bound to PET, therefore increasing the likelihood of binding substrate (Figure 2a) (Zhu *et al.,* 2021).

Enzyme-substrate binding also depends on the electrostatic interactions between PET and PETase. These interactions depend on the charge of the interacting parts; like magnets, when two negative forces interact, they repel. To prevent the substrate and enzyme repelling, the enzyme can be altered to have a neutral charge (Figure 2b) (Son *et al.,* 2020). These optimisations "speed up" the evolution process allowing PETases to degrade PET more effectively.

The degradation of PET is most efficient at higher temperatures, where the PET structure is more likely to bind to the enzyme. However, the PETase structure unfolds at higher temperatures, reducing functionality. Using knowledge of enzymes that function at hotter temperatures, the PETase can be optimised to increase the number of interactions holding the structure together (Figure 2c) (Ma *et al., 2018)*. This increases the enzyme efficiency and allows use in industrial conditions (e.g., a warm washing machine) – however, finding enzymes able to withstand these conditions is challenging.

### Conclusion

Revolutionary new techniques to discover and optimise PETases unlocks new opportunities to prevent and mediate PET pollution. Adding these enzymes to washing detergents removes microplastics at the major source without damaging clothes (PETases only target microplastics). PETases in washing machine filters also ensures reduced exposure to wildlife preventing plastic entering the ecosystems and causing health hazards. These enzymes offer a sustainable way to prevent the daily release of microplastics and remove the accumulation in the environment (Figure 3).

On a larger scale PETases show promise to degrade larger plastics. However, there are outstanding challenges preventing them being implemented on this level.

Optimisation methods would need to improve to produce an enzyme efficient enough, and these enzymes would need to be contained to prevent damage to non-discarded plastic products.

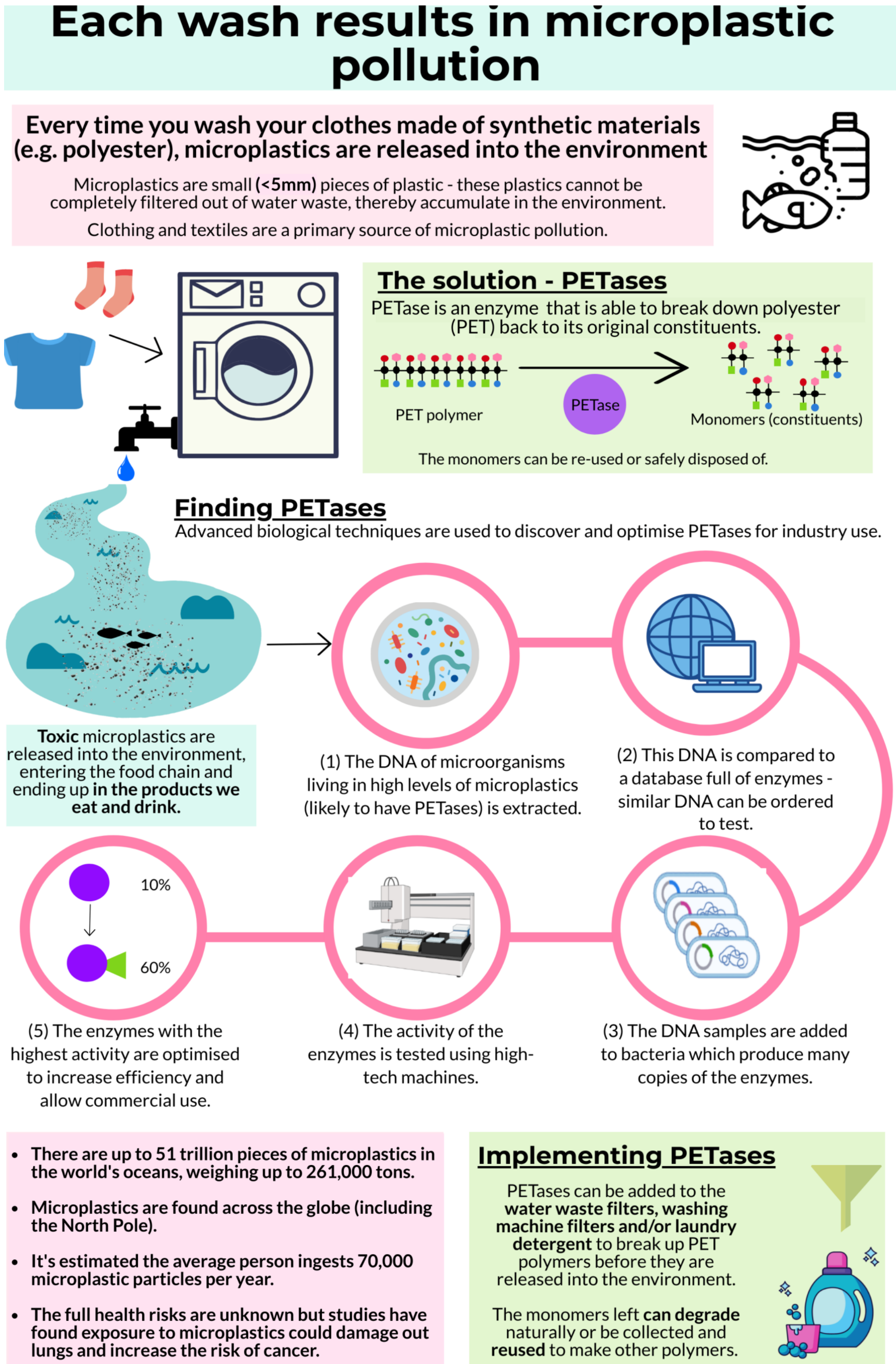


**Figure 3.** Each wash results in microplastic pollution. Infographic displays the impacts of microplastics and how PETases offer a solution, summarising how PETases are discovered and optimised for commercial use. *Infographic created using Piktochart.com.*

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