Plastic Pollution: Novel Enzymes and Bioremediation

What are Plastics and Microplastics?

Plastics are synthetic polymers composed of carbon chains with oxygen, hydrogen, nitrogen, chlorine, fluorine, phosphorous, silicon and sulphur built around them. Today the raw materials to create plastics are derived from hydrocarbons readily available in natural gas, oil and coal to produce polymers like PE, PETE, PP, PVC, PS, PLA, PBS and PU [1]. The durability of these polymers is invaluable in the short term but can have devastating effects on the environment in the long term: of all the plastic produced in the world, only 21% is successfully tackled with and the rest still exists in the environment or is disposed in land area [2]. Microplastics are the main concern to human health which are produced from the degradation of plastics in the environment. They enter the human body via ingestion or inhalation where their decomposition exposes new surface areas and continued leaching of additives from the core. This can cause many negative health impacts including, but not limited to, inflammation, oxidative stress and necrosis; these are linked to cancer, cardiovascular diseases and auto-immune conditions [3].

Plastic Pollution and Management

Plastic pollution is a problem that haunts the future of our planet; around 8.3 billion tonnes have been produced in the last 70 years by packaging, electronic industries, building, construction, healthcare, and transportation [4]. There has been extensive research into techniques to dispose of excess plastic including recycling, incineration, landfills, pyrolysis and bioremediation; recycling has the downside of producing sulphur, carbon and other greenhouse gasses during its essential melting process and consecutive rounds of recycling also create lower quality plastics (‘*downcycling’)*; incineration facilities can be expensive to build and maintain, relative to other techniques and they release ash waste that can harm people and the environment; landfills are contaminating to soil, water, wildlife and are partially responsible for climate change via methane production; and pyrolysis is the process of converting gasses and fatty oils to recover crude petrochemicals and obtain hydrocarbons which is an efficient method of waste management but requires a high capital cost [5].

This leads us to biodegradation (via bioremediation), the focus of this article, which is a plausible method of sustainable plastic management, currently repressed by our knowledge on the global diversity of plastic-degrading enzymes [9].

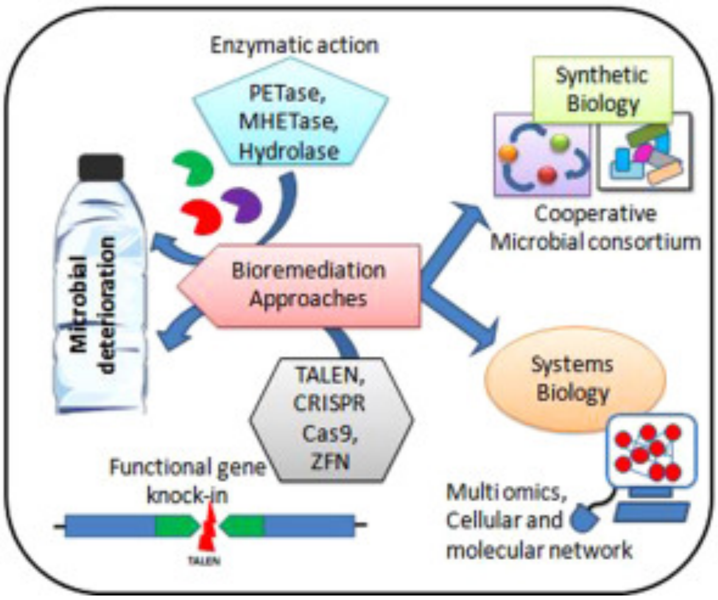

Figure 1 Bioremediation Approaches: enzymatic action, systems biology, synthetic biology, functional gene knock-in, microbial deterioration [7].

What is Bioremediation?

Bioremediation refers to the process where microorganisms decompose waste. It is a branch of biotechnology that follows the principles of detoxification and decontamination via the utilisation of microorganisms to biodegrade all-natural compounds. These microorganisms use plastics as their sole carbon source for their survival and can flourish on plastic waste under certain conditions that facilitate their growth [6]. The mechanisms of plastic polymer separation are facilitated by their exposure to heteroatomic molecules like nitrogen and oxygen and the presence of a carbon-carbon double bond [7]. The general approaches of Bioremediation are outlined in **Figure 1**.

The Micro-Bug that Eats Plastic – Case Study

The first natural microorganism that eats plastic (via bioremediation) was discovered at recycling facilities based in Osaka (Japan) in 2016. It was a bacterium named *Ideonella sakaiensis* which produced a pair of enzymes that could digest PET (polyester) plastic into a food source [10]. PET is one of the most prevalent plastics used across the world due to its high energy efficiency; a clear, strong and lightweight material used for packaging food, beverages, mouthwash, cooking oils, hand soap and more. Although it has a high recycling rate in Europe (around 52%) PET usually takes 450 years to degrade; *Ideonella sakaiensis* could break down a small film of low-grade PET in around 6 weeks [11]. In 2018 scientists attempted to tweak the microorganism's enzymes to understand its evolutionary connection to another bacterial enzyme that breaks down cutin (a natural polymer used by plants as a protective coating). This led to an unintentional discovery of a new, more efficient, enzyme (*PETase*) that could degrade PET 20% faster. They discovered further potential for this enzyme to degrade 10-100 times faster in its viscous state (above 70^{o}C) and in 2020 a biotechnology company, *Carabios*, introduced mutations for a novel PET-degrading enzyme, stable at 72^{o}C, that could degrade 0.9 tonnes of waste plastic bottles (PET) in 10 hours [13].

These PET-degrading enzymes are revolutionary in plastic degradation to easily biodegradable bacterial 'food sources' and even have the potential to turn plastic back into its original components. This proposes a novel biological recycling method that could circumvent the problem of '*downcyling'* where many recycled PET plastics have regeneration potential limited to opaque fibres for clothing or carpets rather than new food-grade PET products. Furthermore, the new enzyme can be produced at scale using fungi at a cost just 4% of virgin plastic made from oil, although overall cost is slightly higher as heating and grinding up of PET bottles is required before addition of the enzyme [12][13].

Recent Bioremediation Research

Following the remarkable discoveries from 2016-2020, a study published in the *mBio Journal* in 2021 used more than 200 million genes taken from global environmental DNA sampling projects to compile a catalogue of over 30,000 nonredundant enzyme homologues with the potential to degrade 10 different plastic types (false positives were controlled for using gut microbiome data) [8][9]. This study found that one in four organisms analysed carried a suitable enzyme for bioremediation; the number and type of enzymes was also proportional to the amount/type of plastic pollution in different locations [9]. Professor Aleksej Zelezniak at *Chalmers University of Technology in Sweden* referred to this phenomenon as '*a significant demonstration of how the environment is responding to the pressures we are placing on it*'. Almost 60% of the new enzymes did not fit into any known enzyme classes which suggests revolutionary new mechanisms of plastic degradation introduced by evolution. The mechanisms of the most promising plastic degrading enzymes could potentially be harnessed by deep learning and genetic engineering to design efficient microbial communities that can tackle plastic pollution of specific polymer types [14].

Challenges of Synthetic Bioremediation

Designing enzymes for bioremediation has several challenges. First of all, due to the limited metabolic capacities of individual organisms, many complex natural and synthetic compounds are biodegraded by multiple consortia rather than individual strains; microbial assemblies are hard to recreate synthetically. The importance of assemblies is particularly relevant for biodegradation of polyester polyurethane and polyacrylic polymers [15]. Furthermore, there is a great disparity of functional differences between soil and ocean microbiomes as large fluctuations in temperature, salinity and mechanical forces in the ocean lead it to intrinsically processing many polymer-degrading properties; this depicts the importance of replicating specific conditions to harness full potential of microorganisms for bioremediation [14]. For many enzymes, a viscous state can be highly beneficial for enhanced function and some studies have found methods to transplant mutant enzymes into 'extremophile bacteria' that can withstand such conditions of high temperature, but this is complex and hard to do on a large scale [16]. Enhancing the speed of an enzyme also comes with many challenges. For example, scientists have tried creating super-linked enzymes composed of a plastic-degrading enzyme and an enhancing enzyme that speeds up the breakdown of chemical groups liberated by the first enzyme; however, these 'super-enzymes' can be too large for a bacterium to create [17].

Conclusion

The utilisation of biological plastics has overwhelming potential and as it becomes more plausible as there is more financial backing. The biotechnology company, *Carbios,* that introduced the first enzymatic technology to create PET bottles from 100% recycled plastics is supported in a consortium by multiple Food & Beverage Giants since March 2022: *Nestlé Waters*, *PepsiCo*, *Suntory Beverage* and *L'Oréal* [18]. German researchers have also discovered a bug that feeds on toxic polyurethane (freezer and fridge insulation) and a wax moth larva has been found that can eat polyethene polymers (plastic bags); as biology evolves, there is ever more potential to discover new enzymes suitable for degradation of a wider range of plastic polymers [13]. With a global ocean sampling revealing over 40 million mostly novel non-redundant genes from 35,000 species in 2015, there is clearly much more to explore in the ocean alone [19]. In a nutshell, bioremediation is a beautiful blend of natural mechanisms and artificial engineering/enhancement that has the potential to sustainably save our world from a crisis of plastic pollution. The potential of this ground-breaking process lies in our gene editing ability to enhance enzyme candidates to their full potential for polymer degradation.

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