

# Plastic bioremediation: An exploration of current developments

A master essay



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## Summary

The development of synthetic plastics revolutionized the economy of the world. From greater ease of packaging consumer goods to car parts and medical components, this broad range of fossil-based materials has become a staple of our civilization. However, such widely used products also create equally vast amounts of waste. Which, due to its non-biodegradable nature, has not yet fully understood, but already severely damaging effects on the natural world. With microplastics being found from the deepest oceanic gorges to the stratosphere, and even within human bodies, time is short to find alternatives to our current plastic economy.

Nature provides, as numerous organisms have already been identified which are capable of bioremediating various plastic products to varying degrees and efficiencies. Therefore, in this essay the current state of development and discovery of effective plastic bioremediating organisms for the most common plastic wastes is discussed.

Polyethylene (PE) will be discussed in great depth, showing the different bioremediative strategies with- and without plastic pre-treatment, both for bacteria and fungi; as well as the metabolic pathways of a bioengineered organism capable of converting polyethylene into the biodegradable polymer PHB. The more challenging plastic polypropylene (PP) will also be described, with a known and promising metabolic pathway opening the door to industrial breakdown of this plastic. And lastly, the optimization of rapid -and almost total polyethylene terephthalate (PET) biodegradation by a bacterium will be described; the products of which were shown to be usable for PET reconstitution.

All the developments described in this essay show both the great leaps that are being made in the field of plastic bioremediation, and the challenges currently faced. But also provides a glimpse into a future of complete plastic economy circularization.

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## Introduction

Before the development and industrial-scale application of modern plastics in the 20<sup>th</sup> century, there were very few moldable materials besides ceramics and glass that could be used for consumer goods. Metals had their own draw-backs, notably expense and weight, which collectively limited the consumer economy to higher strata of society. Trade was still widespread, but options for truly large-scale industry producing luxury items for the masses only truly began with the development of lightweight moldable (semi-)synthetic plastics (Science History Institute, n.d.).

First, the discovery of the vulcanization process in 1839 made rubber more resilient, already leading to innovations in the car industry (Tang et al., 2019). Next, the accidental discovery of the polymer nitrocellulose in 1846 (Mattar et al., 2020) eventually resulted in the development of celluloid (developed approximately in 1862) which was used for various goods like ping-pong balls to photographic film (Reilly, 1991). And eventually materials like bakelite (discovered in 1907) and other phenolic resins were used in things like early phones and car parts (Klun et al., 2022). Or nylon (discovered in 1930), which is still in use today in clothing, tents, ropes, etc. After this, plastic types were developed faster and faster, applied with countless purposes in almost every facet of society (Matthies & Seydl, 1986).

But despite these wonderful characteristics, plastic's potentially catastrophic drawback is caused by its inability to effectively biodegrade combined with its potential harmfulness to nature. For example, when plastic ages it may disintegrate into microplastics which may enter the air and water, and reach the gills of fish or accumulate in grazing animals, which in turn results in the consumption of those plastic-laden animals by their predators and the consequent transfer of plastic up the entire food-chain (Crawford & Quinn, 2017; Hollman et al., 2013). In this way, and through other natural transfer methods like wind and water, microplastics have spread far and wide. In fact, microplastics have been found in arctic snow (Bergmann et al., 2019), deep-sea beds (Chiba et al., 2018), and on mountain peaks (Napper et al., 2020), as well as in the gut and tissues of many species, including humans (American Chemical Society, 2020; Ragusa et al., 2021). While it is not fully known what the effects of microplastics are in nature, it has been described that a poison is a matter of dose (SAPEA, 2019). And combined with the prediction that by 2050 there will be a greater mass of plastic in the ocean than fish, the development of means to both recycle and more renewably dispose of plastics in various environments is becoming extremely pressing (Ellen MacArthur Foundation, 2016).

This essay will explore the composition of the most ubiquitous types of plastic, the dangers thereof, and the ways in which microorganisms, engineered or otherwise, may aid mankind in cleaning the stain of plastic waste from the Earth's waters and soil.

## Chapter 1: Plastic

While in the public eye plastic may be thought of as a collective name for lightweight malleable materials like that used in toothbrushes and children's toys, the reality is somewhat more complicated. Many types of plastic have been developed, each with their own physical characteristics, sometimes unexpected applications, and most importantly their own chemical structures. This latter point also means both the production and subsequent degradation of each plastic type may be vastly different, and one method to remove plastic from nature may leave the other types unaffected. It is therefore important to start the story of plastic bioremediation at the very beginning, at the synthesis of different plastics, how raw materials are processed to produce these materials.

### 1.1. Plastic synthesis

The raw materials needed for plastic synthesis are surprisingly relatively singular in origin, as all modern plastics are originally generated from carbon sources like plant-matter, natural gas or oil. For brevity only the production from petroleum oil will be described, though natural gas specifically is rich in light hydrocarbons which are also ideal for plastic polymerization.

Petroleum oil consists of hydrocarbons of various length and composition, and is first distilled in a process known as atmospheric distillation. Here the oil is heated and injected into a distillation tower where the vapor will rise and eventually condense as it cools, the time required depending on the boiling point of the liquid. As such various fractions can be extracted from the oil, like naphtha, one of the least dense hydrocarbon mixtures and the one used for plastic production. When describing this material the chemical composition may differ due to the non-standardized use of the term, but is generally seen as hydrocarbons of 5-9 carbon atoms in length (Carruthers et al., 2018).

The naphtha chains are further fragmented using a steam-cracker, with a process called pyrolysis; which produces products like ethane, propene, butylene and various aromatics (Sadrameli, 2016).

These small hydrocarbons can then be used as the basis for addition polymerization, polycondensation or polyaddition to synthesize various kinds of plastics. The main principles of all three are simple, where simple monomeric hydrocarbons are chained together into longer polymers. The three methods produce plastics with wildly differing characteristics however (CK-12 Foundation, 2022).

Addition polymerization involves the reaction of small monomers at high pressure, heat and with the aid of a catalyst (usually a peroxide). Depending on the starting monomers this may either result in the formation of a homodimer (like polystyrene) containing one type of monomer, or a copolymer (like nitrile rubber) containing different monomers in repeating sequences. Examples of plastics synthesized with addition polymerization are polystyrene (PS), polypropylene (PP) and polyethylene (PE) (Cheng et al., 2016).

Polycondensation works similarly, though a secondary molecule (like water) is also generated, which may generate thermoplastics in the case of a linear chain forming, or thermosets when the reaction yields a branched structure. Examples hereof are the thermoplastics acrylic and nylon. These materials are highly heat-resistant (Bhat & Kandagor, 2014).

Polyaddition is nigh identical to addition polymerization, though it also involves the migration of hydrogen atoms without the formation of secondary products. An example of this is polyurethane (PU), shown below in Figure 1 (Krol, 2006).

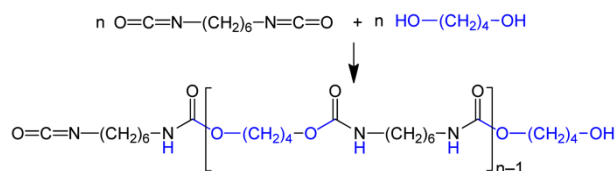


Figure 1: Simplified polyaddition reaction for the formation of polyurethane. Image retrieved from (Wikipedia, n.d.), with supporting information from (Krol, 2006).

Synthesis of plastic in this way comes naturally with great flexibility, as blends of different monomers can be used to insert various nitrogen, oxygen and sulfur atoms into the hydrocarbon chain. Various side chains may also be included, greatly modifying the properties of the materials (Wang et al., 2018). Various classifications of plastics therefore arise, allowing for greater ease of identification of materials.

For example, acrylics like polymethyl methacrylate, also known as plexiglass, contain a  $-\text{COOCH}_3$  and a  $-\text{CH}_3$  side chain resulting in the plastic becoming transparent and brittle (Ali et al., 2015; Polymer Database, 2021b). Alternatively, a material like polyethylene does not possess any side chains and is opaque and far more flexible (see Figure 2 for a comparison in polymer structure) (Polymer Database, 2021a; Roy et al., 2011).

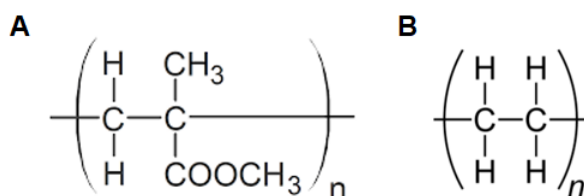


Figure 2: The chemical structures of polymethyl methacrylate (A) and polyethylene (B) (Polymer Database, 2021a, 2021b)

Not only through the modification of input monomers can these plastics be formed, but synthesis products may also be melt-blended in a process known as compounding where, by adding different chemicals and polymers, the properties of the final product may be modified, for example to increase thermostability when adding zinc urate to PVC (Ye et al., 2018). This demonstrates the sheer capacity for variety of plastics, as nearly any material demand can be met with plastic, up to and including conductivity for in the use of electronics (Otero, 2016).

## 1.2. Conventional recycling strategies

Naturally, the blending and addition of chemicals also complicates recycling prospects of the materials, as greater amounts of processing may be needed to fully separate the constituent plastic types and filter out undesired chemical residues. For the less complex thermoplastic blends, or even pure variants, traditional (also known as mechanical) recycling can be employed. This method at its most basic level involves the separation and cleaning, followed by the mechanical shredding and reshaping (usually into pellets) of plastic waste. These pellets are then melted, after which the molten plastic can be reformed into new products through injection molding. Though in principle all thermoplastic plastics can be recycled in this way, like water bottles made out of polyethylene terephthalate (PET), the material must be decontaminated as mentioned before, decreasing the quality of the reformed product. As such, many plastics which are theoretically recyclable may possess prohibitively high cleansing costs, lowering its financial feasibility (Ragaert et al., 2017).

For thermoset plastics and other more complex mixes of plastic types this method is not possible, hence advanced (chemical based) recycling methods may be employed. Included here are three

common types: Gasification, chemical recycling and pyrolysis (Dayana et al., 2016; Ragaert et al., 2017; Saebea et al., 2020).

Gasification involves the conversion of plastic into gas which may be used to collect energy (Saebea et al., 2020). Chemical recycling reverts plastic polymers to monomers through chemical means. And pyrolysis fully reverts plastic back into crude oil (Dayana et al., 2016; Ragaert et al., 2017). Though the specifics of these chemical processes are beyond this essay, of note is that these methods involve extensive use of chemicals, high temperatures and pressure, which all require complex machinery. With only a fraction of plastic waste being viable to be recycled in this manner this means that the costs (both in resources and energy) of effectively recycling most plastics are prohibitive (Ragaert et al., 2017). As a result, more efficient methods of plastic degradation and potentially recycling are being sought, for which nature often provides great potential and inspiration.

### 1.3. Plastic variants

Bacteria and other microorganisms already function as the fundament of Earth's ecosystem, recycling organic matter which may then rise up higher into the food chain. With their high adaptability to nutrient- and energy availability there have already been reports of plastic-degrading microorganisms, which may be adapted for industrial use (Saritha et al., 2021). Such organisms will likely adapt to specific types of plastic, as it has been described in this chapter that there is great chemical variation in polymer structure and reactivity. Hence, to determine the viability of bioremediation of plastics, potentially with bioengineered microorganisms and/or enzymes, these different types of plastic must first be described. Though to limit the scope of this work, there will be a focus on the primary plastic waste types. The most used plastic types, their market sector share and simplified production share can be seen below in Table 1 (Geyer et al., 2017; Schwarz et al., 2019), and the simplified structures of these materials can be seen in Figure 4 (Mohanani et al., 2020).

Table 1: Shares of plastic production per type per market sector in the US, Europe, India and China between 2002 and 2014, and a visual representation of the relative shares arranged by size. In descending order of production share these materials are: polypropylene (PP) (21%), low-density polyethylene (LDPE) (20%), high-density polyethylene (HDPE) (16.3%), polyvinyl chloride (PVC) (11.8%), polyethylene terephthalate (PET) (10.2%), polyurethane (PUR) (8.2%), and polystyrene (PS) (7.6%) (Geyer et al., 2017)

Market Sector	LDPE, LLDPE	HDPE	PP	PS	PVC	PET	PUR	Other	Total
Transportation	0.1%	0.8%	2.6%	0.0%	0.3%	0.0%	1.6%	1.4%	6.7%
Packaging	13.5%	9.3%	8.2%	2.3%	0.9%	10.1%	0.2%	0.1%	44.8%
Building and Construction	1.1%	3.3%	1.2%	2.2%	8.1%	0.0%	2.4%	0.5%	18.8%
Electrical /Electronic	0.5%	0.2%	0.9%	0.6%	0.4%	0.0%	0.4%	1.0%	3.8%
Consumer & Institutional Products	2.9%	1.7%	3.8%	1.8%	0.6%	0.0%	1.0%	0.2%	11.9%
Industrial Machinery	0.2%	0.1%	0.2%	0.0%	0.0%	0.0%	0.3%	0.0%	0.8%
Other	1.7%	0.9%	4.2%	0.7%	1.4%	0.0%	2.5%	1.7%	13.2%
<b>Total</b>	<b>20.0%</b>	<b>16.3%</b>	<b>21.0%</b>	<b>7.6%</b>	<b>11.8%</b>	<b>10.2%</b>	<b>8.2%</b>	<b>4.9%</b>	<b>100.0%</b>

PP 21.0%	LDPE/LLDPE 20.0%	HDPE 16.3%	PVC 11.8%	PET 10.2%	PUR 8.2%	PS 7.6%	Other 4.9%
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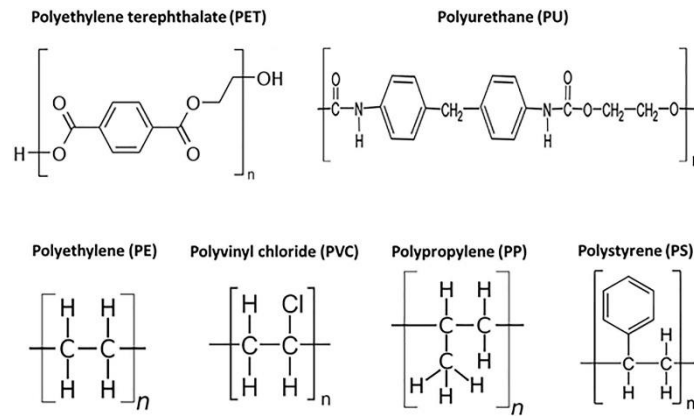


Figure 4: Chemical structures of the 6 most common synthetic plastics: PET, PU, PE, PVC, PP and PS (Mohanani et al., 2020).

As can be seen in Table 1, the vast majority of plastic produced is in the packaging sector, for which LDPE/HDPE, PP and PET hold the greatest shares. These are also the greatest sources of plastic waste in nature, where only fractions of the waste generated are either incinerated or recycled, while the vast majority is simply discarded into landfills or into rivers which spread the plastic across the world. In numbers, from 1995 to 2015 8300 Mt plastic was produced, of which 2600 Mt were in-use stocks, 600 Mt was recycled (which accounts for approximately 7.2%), 800 Mt was incinerated, and the remaining 4900 Mt was discarded (Geyer et al., 2017) (See Figure 3). As such, the focus of this work will be on LDPE, HDPE, PP and PET, though as will be seen in the next chapter, for its uniquely challenging characteristics PE will be discussed in most detail.

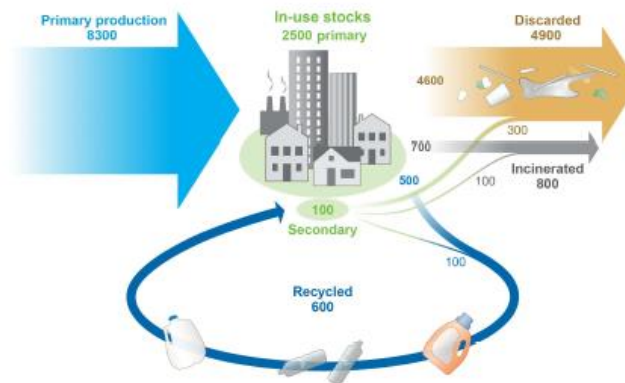


Figure 3: Global production of plastics, the shares recycled, incinerated and discarded between 1995 and 2015 (Geyer et al., 2017).

## Chapter 2: Plastic bioremediation overview

As described above, LDPE/HDPE, PP and PET are the most prevalent types of plastic waste. Hence in chapter 3 these plastic types and the bioremediation thereof will be discussed in depth. However, the bioremediation of plastic tends to follow a common trend, due to shared characteristics of the material.

### 2.1. General trends of plastic-bioremediative organisms and enzymes

The general plastic bioremediation pathway can be separated into two main processes: The physical, chemical and/or biological fragmentation of a plastic mass into smaller molecules, followed by the assimilation and enzymatic degradation of these fragments (see Figure 5).

The fragmentation may be mediated by extra- and/or intracellular enzymes, for example using the hydrolase enzyme class, capable of utilizing water to cleave hydrocarbon chains. The extracellular variants of the enzymes involved usually function by utilizing hydrophobic regions close to the active site to adhere to the hydrophobic polymers, followed by hydrolysis of the carbohydrate chain to form dimers or monomers which may then be absorbed by the organism. Though other methods of fragmentation are of course possible.

The absorbed molecules are enzymatically mineralized<sup>1</sup> stepwise into various end-products like CO<sub>2</sub>, H<sub>2</sub>O, CH<sub>4</sub> and N<sub>2</sub>. The specific enzymes involved in these processes depend on the organism, and perhaps most importantly, the polymers digested (Kaushal et al., 2021).

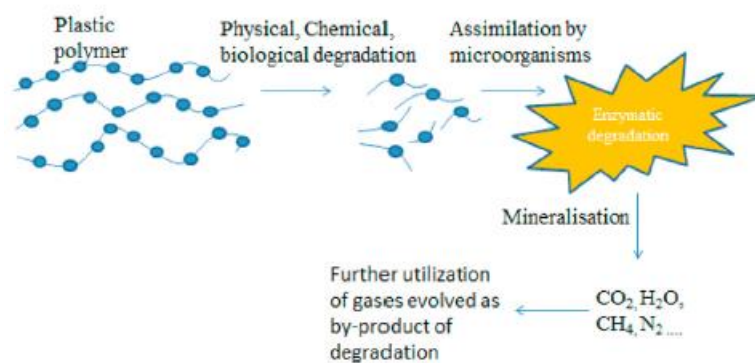


Figure 5: Generalized process of plastic bioremediation. (Kaushal et al., 2021)

Some of the most well studied of these plastic-degrading enzymes come from 2 distinct microorganism types: Fungi and bacteria. Fungi can produce depolymerases, peroxidases, laccases and cutinases as well. And bacteria can produce PETases (also known as PET hydrolases), alkane hydroxylases, laccases, cutinases, and carboxylesterases. Naturally this is not an exhaustive list, but it provides guidelines to determining the mechanisms of plastic bioremediation by these kinds of microorganisms by looking for clues of enzyme activity. This will be described in more detail in the following chapter (Kaushal et al., 2021).

<sup>1</sup> Mineralization: The process by which biological molecules are converted into gasses, water, salts and minerals.

## 2.2. Difficulty with characterization of plastic bioremediation mechanisms

Before various plastic bioremediation-capable species and strategies will be discussed, it must be noted that it has been found there is a great lack of characterization of the exact mechanisms of plastic bioremediation. Many of the papers in this field lack an in-depth analysis of enzymatic activity and metabolic processes, specifically of the initial fragmentation step. Indeed, such mechanistic studies appear to be far less numerous than reports on bioremediative species discovery, but are nonetheless equally important to enable targeted metabolic- and enzymatic engineering of plastic degrading microbe species and proteins.

The reasons for this are myriad, though primarily due to the nature of plastic itself. Namely, plastics are often solid, chemically inert and hydrophobic masses with incredibly limited bioavailability (only the surface layer). This causes plastic degradation to be a slow process with limited detection methods, which usually depend on mass decrease (e.g. gravimetry), surface modification (E.g. AFM<sup>2</sup>, SEM<sup>3</sup>), biomass increase, physical characteristic modification, or metabolite detection (Krueger et al., 2015; Yang et al., 2014).

Though the former methods possess limited explanatory power, as plastic surface modification and mass decrease may have unrelated and unidentifiable causes, metabolite detection is of more interest. Unfortunately, metabolite detection often depend on the monitoring of metabolites like CO<sub>2</sub>, dissolved O<sub>2</sub>, and sometimes CH<sub>4</sub>. But these metabolites are not exclusively tied to plastic degradation, which makes tracking plastic biodegradation using metabolite detection prone to various errors and false-positives (Eubeler et al., 2009; Krueger et al., 2015).

More detailed analysis of the alterations to the plastic's chemical structure is possible, however, and this may be the strongest elucidator of the mechanics of bioremediation. Methods like FTIR<sup>4</sup>, micro-ATR<sup>5</sup>, NMR<sup>6</sup> and MS<sup>7</sup>-based methods may reveal how plastic is chemically modified, for example through the formation of carbonyl groups, dehydrogenation, or other modifications. These signs of bioremediation mechanisms are often scarce, again due to the slow speed of bioremediation. And require significant diversity of evidence to form coherent and credible theories, which limits the feasibility and specificity of bioremediation characterization (Eubeler et al., 2009; Krueger et al., 2015). Therefore, in the following chapter, various papers will be discussed to show progress made so far, and suggest various species of interest to be researched in greater depth in the future.

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<sup>2</sup> AFM (Atomic Force Microscopy): A form of microscopy where a probe sensitive to the repulsive force of a sample scans the surface of said sample to determine the surface contours.

<sup>3</sup> SEM (Scanning Electron Microscopy): A microscopy method where a surface is scanned using a concentrated beam of electrons.

<sup>4</sup> FTIR (Fourier Transform InfraRed): A spectroscopy method where infrared radiation is sent through a sample, and the IR radiation that passes through is measured to generate spectral data of a sample.

<sup>5</sup> Micro-ATR (Attenuated Total Reflection): A form of FTIR using the properties of internal reflection of radiation within a sample to directly examine solids or liquids.

<sup>6</sup> NMR (Nuclear Magnetic Resonance): A spectroscopy method used to observe the magnetic resonances of materials.

<sup>7</sup> MS (Mass Spectrometry): Used to identify molecules within a sample using mass-to-charge ratios.

## Chapter 3: Bioremediation of plastic variants

### 3.1. LDPE and HDPE

First are the most common plastic waste variants, LDPE and HDPE<sup>8</sup> (Low-density-, and high-density polyethylene, collectively called PE). These are widely used as benchmark plastics, with incredibly wide applications like the previously mentioned children's toys, foldable chairs, etc. Both polymer types consist of long chains of hydrocarbons, with the prime difference being that HDPE is linear, and LDPE has long interconnected branches. HDPE's unbranched nature allows for greater molecular stacking and as the name implies, higher polymer density (see Figure 6). This generates more rigid crystalline plastic structures and as such greater tensile strength in the material, as well as UV resistance unlike LDPE (Sam et al., 2014). It is also harder and more opaque than LDPE, allowing it to be used in products children's toys, disposable suits, bottle crates, and milk jugs (A&C Plastics Inc., n.d.). LDPE on the other hand possesses a more branched chemical structure, providing the material with greater flexibility, resilience, and can be made more translucent. This makes LDPE ideal for use in general purpose trays, snap-on lids, plastic wrap, and six-pack rings for example. Though it is also widely used as packaging film in agriculture. (Sam et al., 2014; Sen & Raut, 2015).

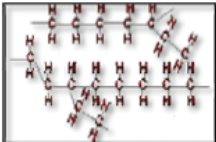
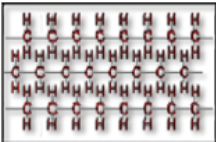
Property	Low-density polyethylene (LDPE)	High-density polyethylene (HDPE)
Schematic Diagram		
Density	0.91–0.94 g/cm <sup>3</sup> lower density than HDPE	0.95–0.97 g/cm <sup>3</sup> Higher density than LDPE
Melting point	~115 °C	~135 °C
Crystallinity	Low crystallinity (50–60% crystalline)	Highly crystalline (>90% crystalline)
Flexibility	More flexible than HDPE due to lower crystallinity	More rigid than LDPE due to higher crystallinity
Transparency	Good transparency since it is more amorphous (has noncrystalline regions) than HDPE	Less transparent than LDPE because it is more crystalline
Heat resistance	Retains toughness and pliability over a wide temperature range, but density drops off dramatically above room temperature	Useful above 100°C
Chemical properties	Chemically inert Exposure to light and oxygen results in loss of strength and loss of tear resistance.	Chemically inert

Figure 6: Comparison of the chemical structure, stacking structure and other characteristics of LDPE and HDPE (Sam et al., 2014).

This latter point makes LDPE difficult to recycle, as when used in agriculture the LDPE film becomes contaminated with organic matter which may make it unfit to be recycled into packaging for household use as has been mentioned in a previous chapter. Proper cleaning will be required, resulting in increased cost and lessened financial viability of recycling. Consequently, in the US only 6.2% of LDPE waste was recycled in 2015 (EPA, 2015; Korol et al., 2021).

HDPE is marginally easier to process in recycling plants due to its increased rigidity and limited use in food-packaging, with a recycling rate of 10.3% in the US in 2015 (EPA, 2015). As such, it is easier to be mechanically chewed into pellets and then molten down with virgin plastic to increase reliability and structural integrity before being shaped into new plastic products (Ragaert et al., 2017).

<sup>8</sup> Low Molecular Weight Polyethylene (LMWPE) and Linear Low-Density Polyethylene (LLDPE) are also variants of PE, but are less common, so will not be discussed in this essay.

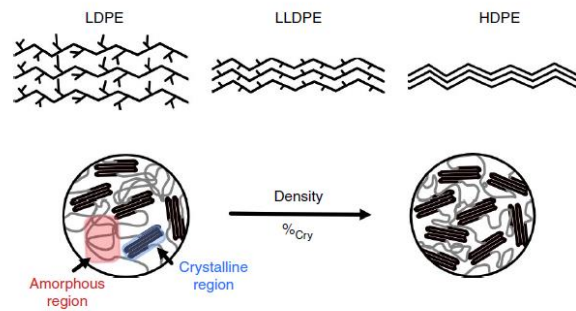


Figure 7: Structural comparison between LDPE, LLDPE and HDPE and their relation to the ratio between structurally crystalline and amorphous regions in the chemical make-up. (Cowan et al., 2021)

### 3.1.1. Known bioremediative strategies

An alternative is the processing of LDPE and HDPE with micro-organisms, which may turn the previously complicative nature of plastic films into a benefit, as films have a large surface-mass ratio allowing for the easy access of the plastic by micro-organisms.

#### *Bacterial bioremediation of PE with pre-treatment*

The first method is using bacteria, which, due to the notoriously chemically inert and hydrophobic nature of PE plastics, often requires pre-treatment to be effective.

One example of this is a study Rajandas et al., performed with *M. paraoxydans* and *P. aeruginosa*, where LDPE was first dissolved in O-Xylene at 70°C followed by recrystallization and mechanical powdering. The powder was pretreated with 65% nitric acid solution for 10 days to oxidize the LDPE, with the replacement of pretreatment solution every 2 days. This oxidation step is important, since enzymes require reactive groups in the plastic to effectively mediate reactions, and oxidation forms double-bonds within the carbon-chains of the LDPE which allows for various digestion strategies (the strategies used by *M. paraoxydans* and *P. aeruginosa* have not yet been elucidated). The pretreated LDPE had to be washed before addition to a bacterial broth, where it functioned as the sole carbon source. The result was the formation of a biofilm on the LDPE, considered to be an important indicator that a microorganism is capable of using a high hydrophobicity and high molecular weight material such as plastic as a source of nutrition (Sivan, 2011). Consequently a decrease in LDPE mass of 61% by *M. paraoxydans* was detected in 2 months, while *P. aeruginosa* decreased the mass by 50.5% in the same timeframe (see Figure 8) (Rajandas et al., 2012).

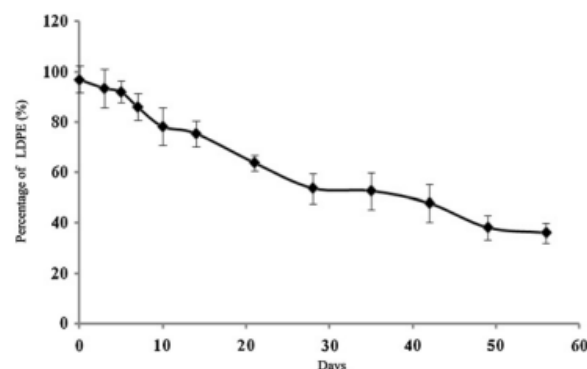


Figure 8: Biodegradation of pre-treated LDPE by *M. paraoxydans* over 2 months (Rajandas et al., 2012).

### Bacterial bioremediation of PE without pre-treatment

The plastic bioremediation process described above is far more passive compared to conventional plastic processing methods. However, it still requires the use of chemicals, limiting the competitiveness compared to other methods of recycling and degradation. Ideally, bacteria would not need pretreatment to remediate plastics, and would instead somehow pretreat the material themselves. Once again nature provides, as two affiliate strains of *Stenotrophomonas* sp. and *Achromobacter* sp. were found by Dey et al. on waste dump sites which could do just that. Though like before, the exact chemical and enzymatic processes have yet to be elucidated, these aerobic bacterial species were found to be able to grow on LDPE film in basal medium at 30°C, and appear to be capable of degrading the material quite effectively. Through FTIR analysis it was inferred this was likely made possible by generating and releasing radical oxygen species into the environment around the bacteria (perhaps by first producing extracellular enzymes which produce radicals), which upon contact with the plastic oxidize the carbon chains. The oxidized groups may have then been transformed into various functional groups which increase the chemical reactivity of the material and decrease hydrophobicity, allowing for other bacterial enzymes to fragment the chains. This may have been mediated by ester hydrolysis and alcohol formation based on the functional groups detected in the reaction mixtures after plastic degradation. The result of degradation appeared to be smaller fatty acids, esters, ketones and other such small chains, which could be used by the bacteria as carbon sources (see Figure 9) (Dey et al., 2020).

The rate of degradation was found to be quite low, however, with only approximately 8% being degraded in ~45 days, rather than 50-65% in ~70 days when chemical pretreatment is performed. And additionally, sterilization of the LDPE waste may be necessary to prevent disruption of the relatively fragile biofilm by other microorganisms. Though the use of simple basal medium, and minimal temperature control required still makes these strains highly attractive, especially if bioengineering can be applied to increase the degradation rates (Dey et al., 2020; Rajandas et al., 2012).

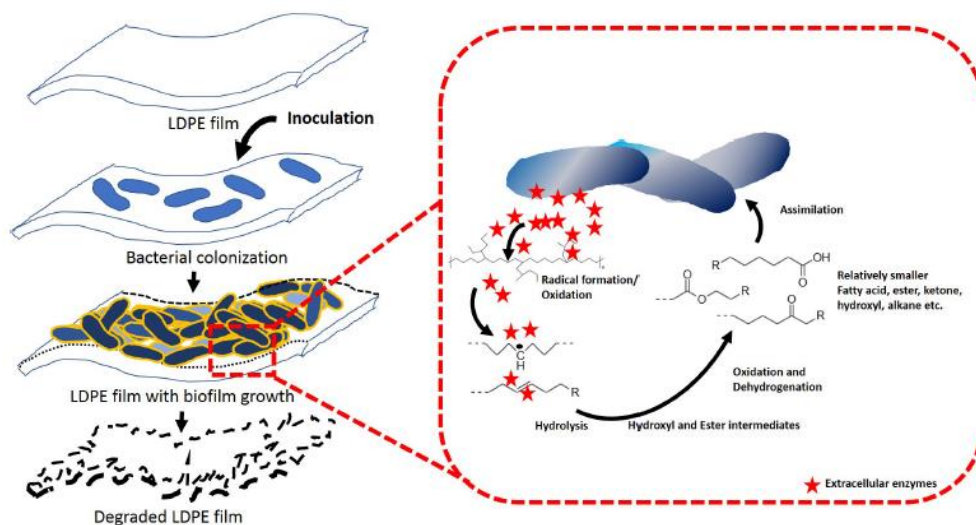


Figure 9: The proposed mechanism of LDPE degradation by *Stenotrophomonas* sp. and *Achromobacter* sp. LDPE is inoculated by the bacteria, after which radical oxygen species are formed which oxidize the LDPE allowing for hydrolysis, oxidation and dehydrogenation. This forms smaller fatty acids, esters, ketones, etc. (Dey et al., 2020)

### Bioremediation of LDPE and HDPE with fungi

Now, while the chemical structure of LDPE and HDPE are almost identical, and therefore may be able to be bioremediated by the same micro-organisms, this has not been tested for the previously described papers. In a study by Ojha et al., fungal samples were isolated from soil of a plastic dumping ground and used for a primary screening to select for HDPE and LDPE degradation capacity. This was performed by first pre-treating the plastics with tween 80, bleach and water to physically soften the material and then sterilized. The fungi isolates were incubated with and without shaking at room temperature for 30 days, with added LDPE and/or HDPE as the only carbon sources. The 5 most effective strains were incubated in larger flasks with HDPE, LDPE or a combination of both for 90 days without shaking, and plastic mass loss was determined every 30 days. Alongside dry mycelium weight analysis, FTIR-, AMF-, and FE-SEM<sup>9</sup> analysis to determine the degree of plastic degeneration. From these analyses two strains of exceptional HDPE and LDPE degradation capacity were identified: *Penicillium oxalicum* NS4 (KU559906) and *Penicillium chrysogenum* NS10 (KU559907). For strain NS4 36,60% of LDPE and 55,34% of HDPE was consumed by the fungus, while for strain NS10 34.35% of LDPE and 58,60% of HDPE was consumed, both over 90 days (Ojha et al., 2017).

Notably, like hypothesized before, the same strains were capable of bioremediating both types of polyethylene with similar efficiency. This suggests that perhaps with further experimentation the

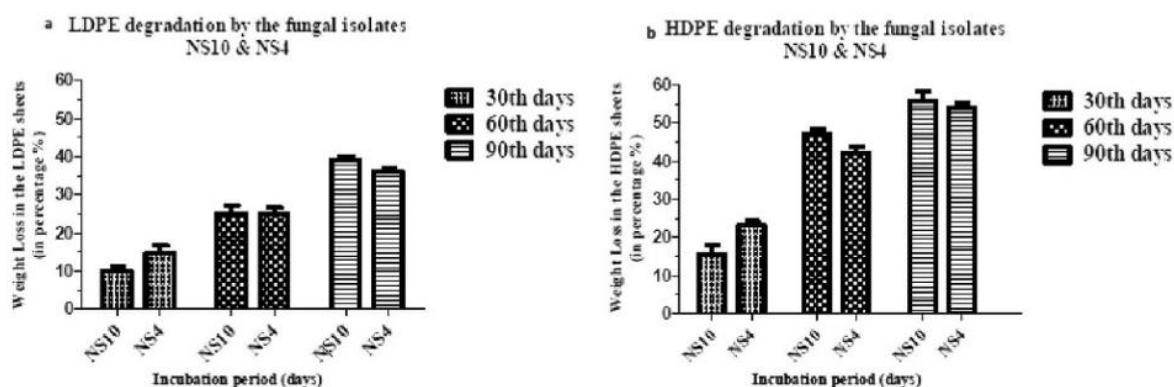


Figure 10: LDPE and HDPE degradation by 2 fungal isolates (*Penicillium oxalicum* NS4, and *penicillium chrysogenum* NS10) (Ojha et al., 2017).

bacteria described before may prove to be capable of degrading not only LDPE but also HDPE. Moreover, the fungal strains were simply isolated from soil samples, and had not undergone any form of engineering to increase LDPE or HDPE degradation capacity, and yet is capable of digesting plastic at a very high rate already. Some benefits of fungi over bacteria for plastic degradation are their ability to secrete extracellular polymers which may stimulate biofilm formation, and the penetrative- and distribution abilities of fungal hyphae, which is particularly beneficial for more resilient plastics (Volke-Sepulveda et al., 2002).

Directed evolution strategies may therefore allow these fungi to be optimized for industrial use in plastic recycling, after which the fungal biomass may be re-used for any number of purposes. For example, fungi are high in protein, and as such may serve as animal-feed supplements or fertilizer (Asadollahzadeh et al., 2018). Or may also be used as the basis for other bioplastics if fungal metabolism is engineered to increase polysaccharide production (Cottet et al., 2020).

<sup>9</sup> FE-SEM (Field Emission Scanning Electron Microscopy): Similar to SEM, but a different method of electron generation.

### 3.1.2. Metabolism of PE fragmentation products

The above papers show that plastic biofragmentation is possible, despite difficulties elucidating the exact mechanisms thereof. With this in mind, the next step in plastic bioremediation is the processing of these plastic fragments. The most promising approach here is through comparison, since the metabolic pathways involved in PE fragment bioremediation likely have great overlap with the far better characterized metabolism of another group of hydrocarbons: linear *n*-alkanes. After all, fragmented PE is chemically identical to this group of metabolites<sup>10</sup>. Additionally, it has been observed that the same species capable of degrading PE are also able to consume *n*-alkanes like paraffin, providing greater support for the assumption that the metabolic pathways are related, if not identical (Haines & Alexander, 1975; Yoon et al., 2012).

The metabolism of *n*-alkanes involves the hydroxylation of carbon bonds through the formation of primary/secondary alcohols, and the subsequent oxidation into hydrophilic carboxylic acids. These fatty acid analogues can either be used directly by the organism, or processed further through the  $\beta$ -oxidation pathway into *de novo* fatty acid synthesis or the TCA cycle. See Figure 11 for an illustration of the degradation and assimilation of various hydrocarbons by different microorganisms (Alvarez, 2003; Montazer et al., 2018).

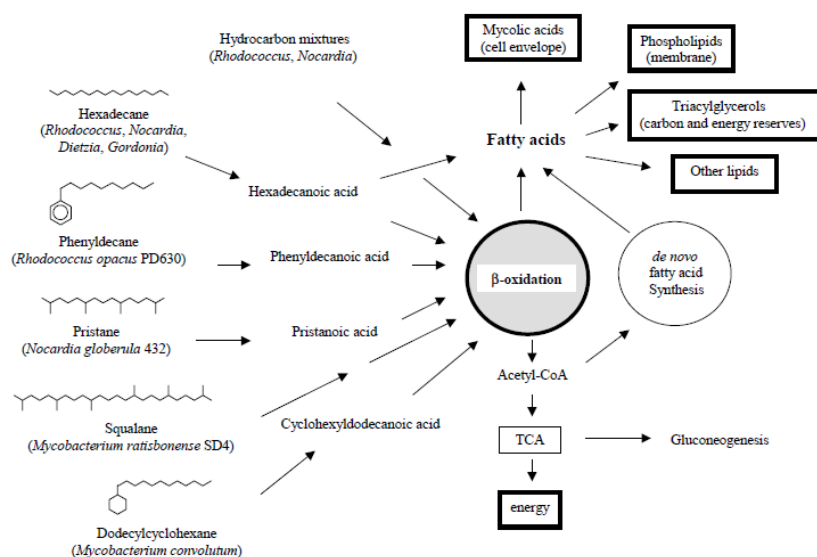


Figure 11: Summarized degradation and assimilation pathways of different hydrocarbons by bacteria (Alvarez, 2003).

This is, as previously stated, a strong parallel to the general biodegradation process of plastics of various types. Specifically hexadecane has the greatest chemical similarities to paraffin and fragmented PE, consisting of a linear saturated hydrocarbon. The catabolism of this molecule (as illustrated in Figure 12) is thought to involve hydroxylation of *n*-hexadecane, forming hexadecanol, which in turn can be dehydrogenated to hexadecanal, and lastly dehydrogenated again into hexadecenoic acid. These steps involve alkane hydroxylase, hexadecanol dehydrogenase, and aldehyde dehydrogenase respectively; though the exact enzymes used differ per organism (Feng et al., 2007; Meng et al., 2017; Yoon et al., 2012).

<sup>10</sup> *n*-alkanes are simply saturated hydrocarbons, making polymers like polypropylene (PP) and polyethylene (PE) *n*-alkanes as well (Zakaria and Barbora, 2018).



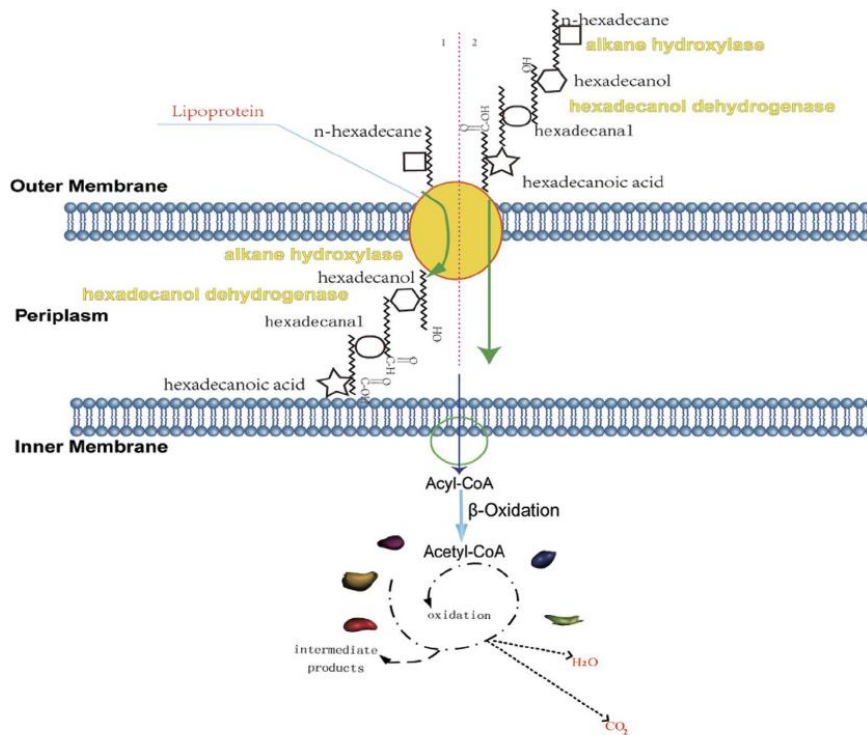


Figure 12: Metabolic processing of *n*-hexadecane into the  $\beta$ -oxidation cycle. Section 1 and 2 show two possible mechanisms, though differ only in the location of the process (Meng et al., 2017).

Already this provides a target for genetic engineering, as shown by both Feng et al. and Yoon et al. The former discovered an organism (*Geobacillus thermodenitrificans*) capable of efficiently degrading *n*-hexadecane and a number of other oil-based hydrocarbons, and performed genome analysis to determine the enzymes and metabolic pathways involved in these processes. For example the alkane hydroxylase *LadA*, which in its purified form could hydroxylate *n*-hexadecane into 1-hexadecanol in little more than 12 minutes (see Figure 14) (Feng et al., 2007).

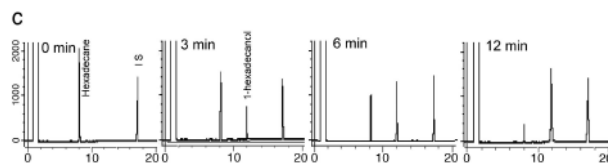
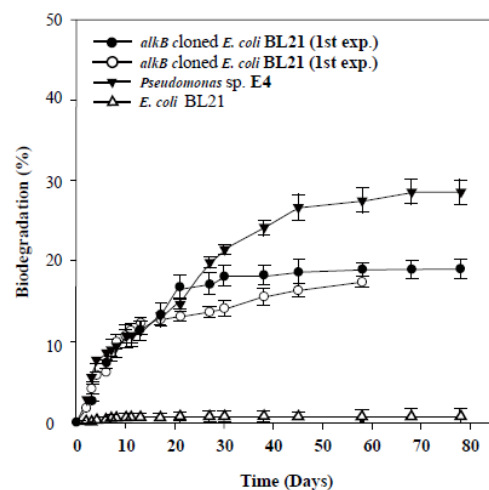


Figure 13: Figure 14: GC chromatograph of the hydroxylation of *n*-hexadecane by purified *LadA* (Feng et al., 2007).

Yoon et al. built upon this, by expressing the alkane hydroxylase *alkB* from *Pseudomonas* sp. E4, first identified and characterized by (Belhaj et al., 2002), in *Escherichia coli* strain BL21. This recombinant *E. coli*, which was previously unable to process PE, was now able to mineralize 19.3% of LMWPE into  $\text{CO}_2$  during 80 days of incubation at  $37^\circ\text{C}$  (See Figure 15) (Yoon et al., 2012). This shows that even if it is somewhat slow, simply engineering an organism with this one enzyme will already allow for biodegradation of PE.

Figure 15: Biodegradation of LMWPE by *alkB* cloned *E. coli* BL21, compared with *Pseudomonas* sp. E4 and non-recombinant *E. coli* BL21 (Yoon et al., 2012).



### 3.1.3. Bioconversion of synthetic plastics into biodegradable polymers

Besides simply degrading PE for use as an energy source, a study by Montazer et al. has also shown it is possible for bacteria to utilize the carbohydrates won from plastic degradation in the formation of biodegradable biopolymers like PHA (polyhydroxyalkanoates). This material possesses physical characteristics similar to PET and PP, and can function as a thermoplastic material in bottles and food-packaging, but is also a great material for medical equipment due to its biocompatibility. As such, more than simply breaking down harmful plastics, or even reverting the long hydrocarbon chains into monomers, this species could be engineered to convert harmful synthetic plastics into virgin biodegradable plastic clumps. In essence, a true form of bio-recycling (Montazer et al., 2018).

The group analysed the LDPE bioremediative capacity of a number of organisms by growing these organisms for 21 days in basal medium using 1% of LDPE as the sole carbon source; then performing a growth curve analysis (Figure 16A), a gravimetric analysis on LDPE biomass decrease (Figure 16B), a gas chromatography flame ionization detection (GC-FID) on the supernatant of the culture to determine the presence LDPE hydrolysis metabolites (Figure 16C), and a GC on the biomass to determine PHA accumulation and chain length (Figure 16D) (Montazer et al., 2018).

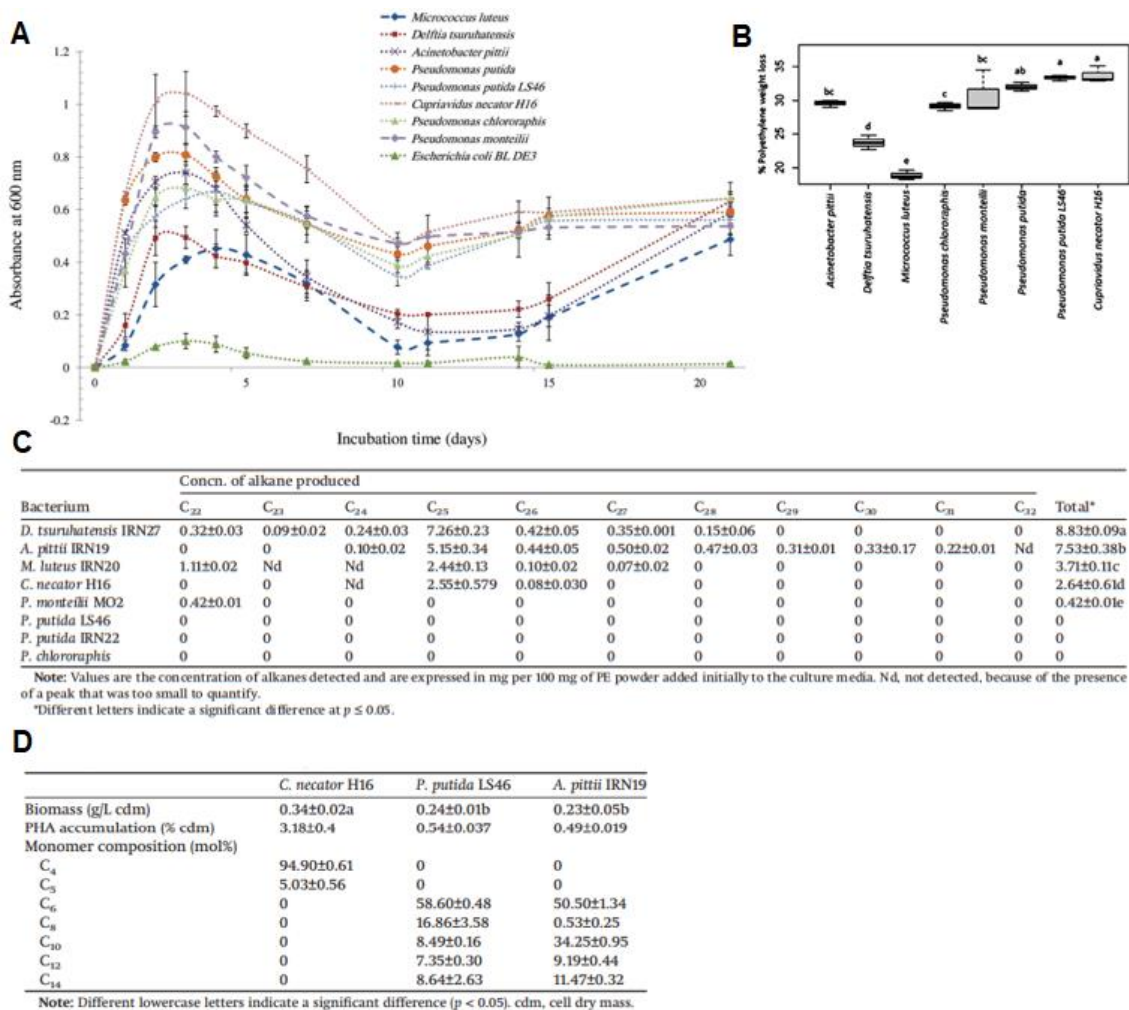


Figure 16: A) Absorbance (OD600) change of 9 potential LDPE bioremediative species growing in basal medium supplemented with 1% LDPE as the sole carbon source, incubated for 21 days. B) Gravimetric analysis of LDPE mass decrease by the same species. C) GC-FID analysis results of the supernatant of the cultures, showing alkane production associated with LDPE hydrolysis. D) Biomass change, PHA accumulation and PHA chain lengths of the 3 most promising species (Montazer et al., 2018).

These results show that most of the tested species can indeed grow in medium where LDPE is the only carbon source. And *Cupriavidus necator* H16, *Pseudomonas putida* LS46, and *Acinetobacter pittii* IRN19 were all able to degrade LDPE quite quickly; showing decreases in LDPE dry-weight of approximately 34%, 34% and 30% respectively, all over 21 days. These are already massive increases over the other papers described before (Montazer et al., 2018).

However, not all of the tested species produced LDPE hydrolysis products in the supernatant, which was explained by Montazer et al. as a likely product of the LDPE synthesis process. Though LDPE is defined as a branched polymer of saturated hydrocarbons, often-times its synthesis modifies the branches, resulting in more reactive groups like unsaturated bonds or oxygen groups being present for easier hydrolysis by enzymes. This allows even less LDPE bioremediative species to feed off of the plastic, even if it will not result in measurable LDPE hydrolysis products in the supernatant (Montazer et al., 2018).

The three highly LDPE biodegrading species appear to accumulate PHA as well, as *C. necator*, *P. putida* and *A. pittii* were able to produce approximately 0.34 g/L, 0.24 g/L and 0.23 g/L of polyhydroxybutyrate (PHB) in 21 days (Montazer et al., 2018).

Though this is not fast enough for industrial application, it does act as a proof-of-concept for the direct conversion of untreated plastics into biodegradable polymers. Which, with sufficient bioengineering (like directed evolution or targeted mutation), may revolutionize plastic recycling. Not just for LDPE, but other synthetic polymers as well; since if a plastic as chemically inert as LDPE can be bioremediated, others are not beyond the scope of biology either.

### 3.2. Polypropylene (PP)

The bioremediation of polypropylene is another example of this, which will not be discussed in as great a depth as PE, but still requires mention for its greatest share in plastic waste globally. Mainly, this synthetic polymer fibre is used in plastic tote bags, clothes, rope and twine; while its waterproof qualities also help it find application in the marine sector. This characteristic can be attributed to a chemical structure not unlike PE, consisting of a polymer of saturated hydrocarbon subunits. These vary with PE only by its repeating  $\text{CH}_3$  side-chains (as seen in ), creating a less sturdy, but more stiff and rigid material; though this difference is minimal, which is a great challenge for the recycling of PE/PP plastic blends (Aumnate et al., 2019).

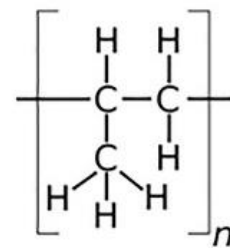


Figure 17: Chemical structure of polypropylene (PE) (Mohanani et al., 2020).

#### 3.2.1. Known bioremediative strategies

The bioremediation of PP appears to be similarly tied to the processes involved in PE degradation, as was shown by Tamnou et al., who compared the biodegradation of LDPE and PE by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These organisms were selected for their adaptability and known production of various enzymes involved in hydrolysis and oxidation, which as mentioned before are vital steps in hydrocarbon catabolism (Brunelle et al., 2022).

The organisms were cultured in mesophilic conditions in basal medium, using small LDPE- and PP sheets as the sole carbon sources, and utilizing different initial pH of 5, -7, and -9 to compare environmental effects. Gravimetric analysis was performed on the plastic mass change (to determine the PWADR<sup>11</sup>), electrical conductivity assays on the solution to approximate reaction activity over time (ECAIR<sup>12</sup>), and biomass growth to show cell culture viability (CAAIR<sup>13</sup>). The data (see Figure 18) shows that both species are quite capable of degrading both LDPE and PP, with both causing similar decreases in LDPE and PE mass. Though there are apparent differences in both changes of solution electrical conductivity and biomass growth, as *P. aeruginosa* seems to grow more quickly on the plastic- and is

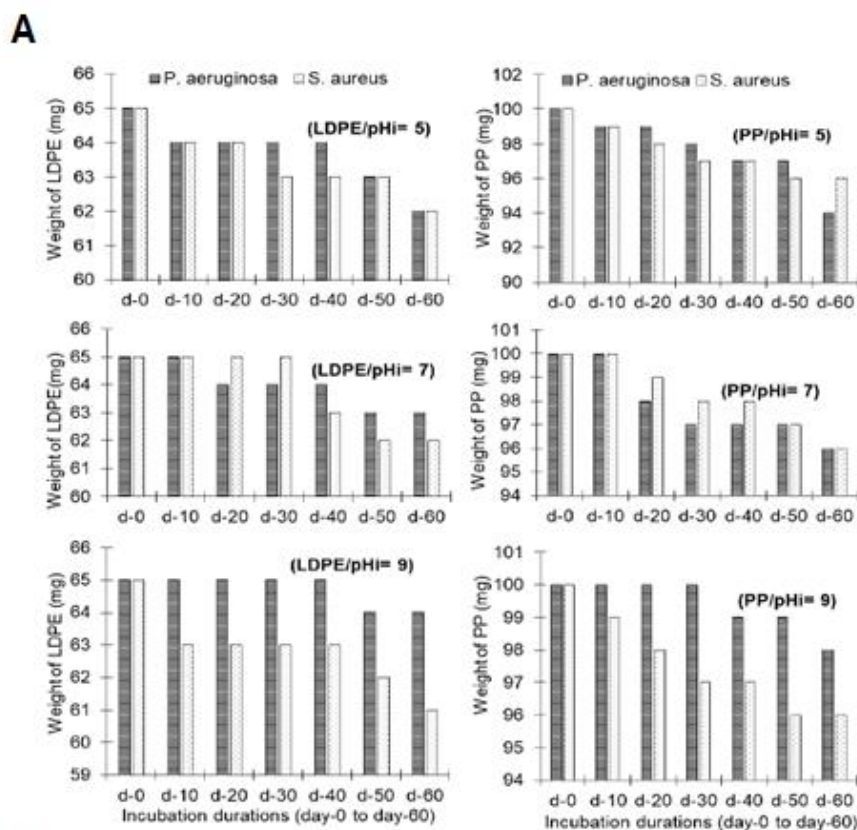
<sup>11</sup> PWADR: Polymers Weight Apparent Decreasing Rates.

<sup>12</sup> ECAIR: Electrical Conductivity Apparent Increasing Rates.

<sup>13</sup> CAAIR: Abundance Apparent Increasing Rates.

more resilient against pH differences than *S. aureus*. Though *S. aureus* increased the conductivity of the solution more, potentially caused by the greater biomass increase and its related secretions (Brunelle et al., 2022).

All in all, this quite clearly shows that the breakdown and catabolism of PP is likely very similar to that of PE described before. This is supported by a study by Boyd et al., who investigated the inhibition of the CoM-dependent propylene metabolism. Though for brevity the exact pathway will not be discussed, not unlike the catabolism of PE, PP is first processed by an alkane monooxygenase, followed by various hydrocarbon modification enzymes. PP is not cycled into the Acyl-CoA pathway however, instead being used to generate NADH in a self-feeding system, with acetoacetate as a by-product (Analog et al., 2010). This makes PP bioremediation less likely to be used for biopolymer production, unlike PE, but still shows that the material can be effectively degraded by various microorganisms; and perhaps with bioengineering can be plugged into the formation of other useful materials.



**B**

Type of polymer and pH value		Parameters considered and the bacterial species present in solution during					
Type of Polymer	pHi value	PWADR (mg/10days)		ECAIR ( $\mu\text{S}/\text{cm}/10$ days)		CAAIR (CFU/10 days)	
		<i>P. aerug.</i>	<i>S. aureus</i>	<i>P. aerug.</i>	<i>S. aureus</i>	<i>P. aerug.</i>	<i>S. aureus</i>
LDPE	pHi= 5	-0.4 ( $r^2=0.796$ )	-0.4 ( $r^2=0.9$ )	122.36 ( $r^2=0.936$ )	195.21 ( $r^2=0.889$ )	185.18 ( $r^2=0.923$ )	24.10 ( $r^2=0.163$ )
	pHi= 7	-0.4 ( $r^2=0.892$ )	-0.6 ( $r^2=0.802$ )	102.39 ( $r^2=0.957$ )	175.96 ( $r^2=0.956$ )	65.82 ( $r^2=0.194$ )	116.79 ( $r^2=0.883$ )
	pHi= 9	-0.2 ( $r^2=0.625$ )	-0.5 ( $r^2=0.790$ )	32.89 ( $r^2=0.053$ )	75.92 ( $r^2=0.684$ )	102.61 ( $r^2=0.630$ )	34.39 ( $r^2=0.332$ )
PP	pHi= 5	-0.9 ( $r^2=0.878$ )	-0.7 ( $r^2=0.940$ )	91.42 ( $r^2=0.655$ )	203.39 ( $r^2=0.844$ )	297.61 ( $r^2=0.173$ )	4.53 ( $r^2=0.658$ )
	pHi= 7	-0.7 ( $r^2=0.867$ )	-0.7 ( $r^2=0.960$ )	102.11 ( $r^2=0.860$ )	171.57 ( $r^2=0.628$ )	48 ( $r^2=0.342$ )	67.64 ( $r^2=0.861$ )
	pHi= 9	-0.3 ( $r^2=0.778$ )	-0.7 ( $r^2=0.940$ )	99.28 ( $r^2=0.581$ )	95.53 ( $r^2=0.648$ )	79.07 ( $r^2=0.282$ )	16.25 ( $r^2=0.448$ )

*P. aerug.*= *Pseudomonas aeruginosa*

Figure 18: Biodegradation of LDPE and PP by *P. aeruginosa* and *S. aureus*. A) The decrease in dry-weight of the plastics was measured over 60 days at different pH, growing at mesophilic conditions using the plastics as the sole carbon sources. B) The exact measured plastic mass decrease (PWADR), solution electrical conductivity (ECAIR), and biomass growth (CAAIR) per 10 days (Brunelle et al., 2022).

### 3.3. Polyethylene terephthalate (PET)

The last plastic type that will be discussed here is the thermoplastic PET, a material widely used in plastic bottles when in solid form (to which 30% of PET production is dedicated), and in clothing as fibers (more than 60% of PET production) (Li-na, 2013). With such a widespread application of the material, its contribution to plastic waste is therefore also significant, making it one of the primary plastic targets for biological degradation. A goal made quite difficult by the chemical structure of the polymers, as unlike the relatively straightforward structure of PE and PP, PET subunits contain a terephthalate group as seen in Figure 19; which prevents easy processing by most enzymes (Alyamaç-Seydibeyoğlu, 2004).

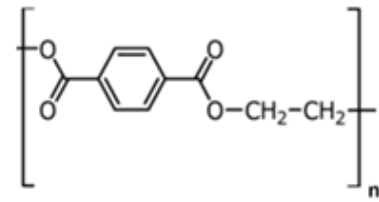


Figure 19: Chemical structure of polyethylene terephthalate (PET) (Alyamaç-Seydibeyoğlu, 2004).

Despite its significant chemical and structural differences, the physical properties of PET are somewhat similar to those of PE. The main differences are that PE is opaque unless treated with significant additives while PET is transparent, and HDPE is more durable and temperature resistant, but less rigid (Taniguchi et al., 2019).

#### 3.3.1. Known bioremediative strategies

But unlike the previously discussed plastic types, PET has been far more deeply researched in regards to enzymatic breakdown by various organisms. It was found that numerous species of bacteria and fungi were able to produce enzymes like cutinases, lipases, esterases and PETases; which are able to depolymerize PET quite effectively (See Figure 20). These enzymes have evolved to accommodate high-molecular-weight compounds within their enzyme-surface-oriented active sites, granting them some sequence-, structural- and functional identity (Carr et al., 2020; Maurya et al., 2020).

The mechanism of depolymerization is most interesting here, as cutinase, lipase, and PETase are able to hydrolyze PET into BHET (bis(hydroxyethyl)terephthalate), MHET (mono(hydroxyethyl)terephthalate), TPA (terephthalic acid), and EG (ethylene glycol), as seen in Figure 20.

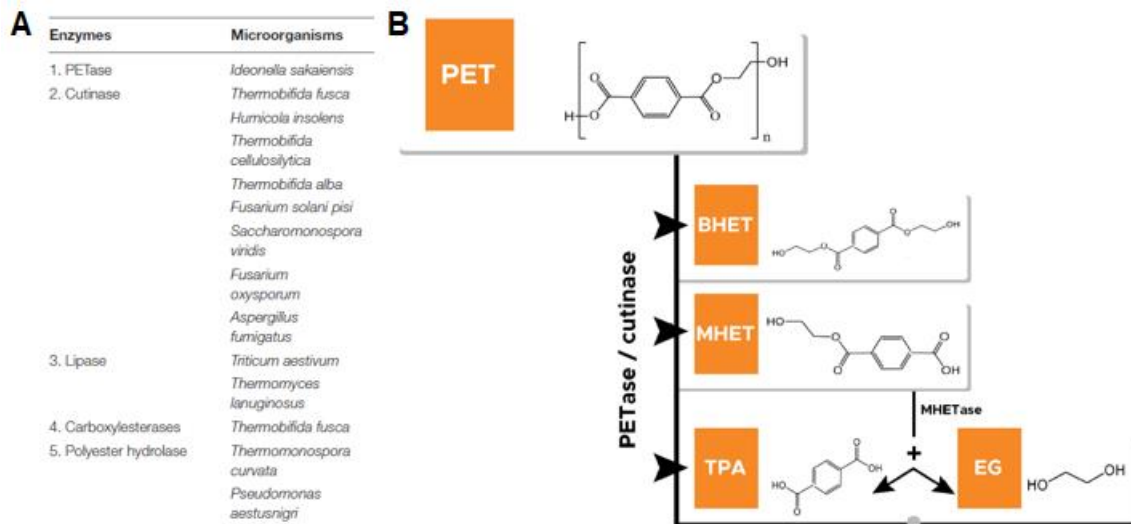


Figure 20: A) A list of some of the more promising PET-degrading enzymes, and the associated organisms. See the paper by Maurya et al. for more detail. B) Enzymatic degradation of PET by PETase, cutinase, lipase and PETase into BHET, MHET, TPA and EG. MHETase is also capable of breaking down MHET into the latter two molecules (Maurya et al., 2020).

Though for brevity the exact mechanism will not be discussed here (and was discussed in depth in the reviews by Maurya et al. and Carr et al.), it is of note that many of these enzymes require quite specific environmental parameters to function effectively. The most important of which is high temperature (up to 80°C), to make the highly hydrophobic PET mass more accessible for the enzymes (Maurya et al., 2020; Tournier et al., 2020). Naturally, if such enzymes are to be employed on an industrial scale, some enzyme engineering is required. This has been quite successful already, as seen in a work by Tournier et al., where leaf-branch compost cutinase (LCC) from an unknown prokaryote<sup>14</sup>, with an already high PET depolymerization activity was optimized further (Tournier et al., 2020).

The original enzyme was able to break down even bottle-grade PET at a rate of 81.8 mg/h/mg<sub>enzyme</sub> at 65°C, though setting the reaction conditions to those optimal to PET enzymatic accessibility stopped the reaction after 3 days; when only 31% of the PET had been broken down. Reaction products were found to have no inhibitory effect, nor was plastic crystallinity affected. In the end it was found that the reaction could resume by adding more LCC, suggesting the thermostability of the enzyme was the limiting factor (Tournier et al., 2020).

As a result, both the activity and thermostability were optimized through targeted mutagenesis of identified positions of importance, generating a library of variants which were then tested for depolymerization activity. The most promising variants showed significant increases in effectiveness and thermostability, as can be seen in Figure 21. Here it was shown that adding a disulfide bridge to key residues within the enzyme results in an increase in melting temperature of 9.8°C. In addition, the mutagenesis raised the PET depolymerization rate from 25.7 g<sub>hydrolyzed PET</sub>/L/h (consuming 53.9% of the PET in 18 hours) for the WT enzyme, to a rate of 36.2 g<sub>PET</sub>/L/h (consuming 92.3% of PET in 15 hours) for the WCCG variant. Of note is that this was optimized for 72°C and an enzyme concentration of 3 mg<sub>enzyme</sub>/g<sub>PET</sub>, though the WT enzyme was only tested for 1 mg<sub>enzyme</sub>/g<sub>PET</sub> (Tournier et al., 2020).

This shows that relatively simple mutations were able to quite drastically improve PET bioremediation. However, the study went beyond mere PET degradation, and also showed they were able to recycle the terephthalic acid which is generated from the degradation into new PET; with all characteristics of import being within US government regulatory guidelines.

As such, using this enzyme it was shown that a circular PET economy is not only theoretically possible, but within arm's reach.

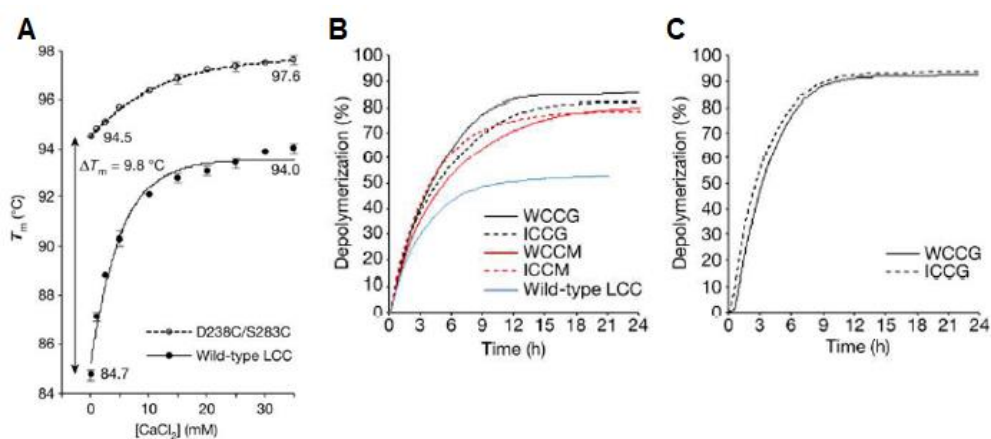


Figure 21: The optimization of LCC for improved PET degradation and thermostability. A) Comparison between WT LCC and the variant where a disulfide bridge was added, increasing the thermostability by nearly 10°C. B) Comparison between WT LCC and some of the most promising variants from the targeted mutagenesis library, with 1 mg<sub>enzyme</sub>/g<sub>PET</sub>. C) Comparison between the two most effective optimized LCC variants with 3 mg<sub>enzyme</sub>/g<sub>PET</sub> (Tournier et al., 2020).

<sup>14</sup> The uniprot accession number of the LCC used in this study was G9BY57, but links to an unidentified organism.

## Conclusion

In this essay a number of possible biosynthetic strategies to mitigate the severe effects of synthetic fossil-based plastics upon our biosphere were discussed. It was seen that though plastics like polyethylene (PE), polypropylene (PP) and polyethylene terephthalate (PET) are incredibly resistant against natural decomposition, numerous organisms are constantly being found that are able to not only aid in biodegrading this material, but actively consume it for various purposes. Though fungus was most capable of catabolizing wide varieties of fossil-based products, it was bacteria that provide the most easily engineered enzymatic targets for application to industrial-scale bio-recycling. With the metabolic pathway for PE biodegradation even being transplanted into *E. coli* BL21, and another strain being able to be biologically convert PE into PHB as a biodegradable alternative to synthetic plastics. PP, despite its similar chemical- and physical characteristics to PE, was found to be distinct in both its bioremediative challenge, and opportunity. As organisms found to be able to process PE could often do so with PP as well. However, the metabolic pathways were distinct, mostly using the catabolites as sources of NADPH, and resulting in the later products of the acyl-CoA pathway (predominantly acetoacetate). This metabolic pathway may yet be integrated in larger inter-connected processing networks, not unlike how PE could be converted into PHB.

The developments in PET bioremediation were no less promising, with a single enzyme having been engineered to depolymerize the plastic at incredible rates; degrading more than 90% of PET in a suspension within 15 hours. And the products of this reaction were able to be used to re-synthesize PET on an industrial scale into a state indistinguishable with virgin PET synthesized from fossil-based hydrocarbons, providing great opportunity to develop a truly circular PET economy.

In short, through the many papers described in this essay, it becomes clear that synthetic biology is beginning to quietly revolutionize the way in which our entire global plastic economy functions.

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