Conditions for a Microbial Consortium for the Biological Degradation of Plastic Polymers

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Abstract: Plastic pollution is one of the most serious environmental problems the world faces. Conventional approaches to plastic waste, such as landfilling, incineration and recycling are not sufficient to maintain an equilibrium between plastic production and decomposition. Since plastics can provide an energy source for microbes, biotic degradation could contribute a solution towards excessive plastic waste. Over the last 30 years, hundreds of species have been described to degrade one or more of the six most common polymer types. By combining multiple types of species to form consortias, the efficiency of degradation can be synergistically increased. In this study, we consider the factors that would contribute to a microbial consortium, and what combinations of species would most effectively degrade plastic polymers. Abiotic factors such as temperature, oxygen level, pH, UV exposure, moisture level, carbon availability and abundance of trace elements are considered. The origin of individual microbes is considered with regard to their compatibility. The usefulness and shortcomings of reported degradation efficiencies is discussed. A theoretical consortium is recommended, taking into account individual strain decomposition efficiency and the comprehensiveness of plastic degradation.

1 INTRODUCTION

Plastic pollution is one of the greatest environmental concerns of our time, adversely affecting most biomes and wildlife across the globe. The growth in volume of plastic production alongside the longevity of most plastic polymers have both contributed to this issue. Annual plastic production has increased rapidly since 1940, with global plastic production reaching 365 million tons in 2019. The most common plastics polymers, which account for about 80% of worldwide plastic production, are polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC), polystyrene (PS), polyethylene terephthalate (PET) and polyurethane (PU). It has been predicted that in the next decade, if there are no improvements to manage the pollution, 99 million tons of plastic waste will end up in the environment by 2030.

Due to its longevity in nature, and its propensity to be used as a disposable item, the output of plastic polymers vastly outpaces its natural degradation. Plastic can take from 20 to 500 years to decompose, depending on its structure and chemical composition. The longevity of plastic is estimated to be hundreds to thousands of years, and is likely to be even longer in deep sea and non-surface polar environments.

2 METHODS

Conventional approaches to plastic waste, including landfilling, incineration and recycling, all come with their own drawbacks. Land filling can lead to contamination of earth's surface and result in anaerobic production of methane gas which contributes to climate change. Furthermore, because of the large footprint required by landfills, the habitat of animals may be affected and displaced. In addition, landfilling produces leachate that pollutes the surrounding water and soil. The incineration of waste plastic material produces toxic gases, which can result in human health complications such as lung disease and carcinomas. During the process of

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burning plastic, dangerous chemicals like hydrochloric acid, sulfur dioxide, dioxins, furans and heavy metals can be discharged. These kinds of emissions are known to be carcinogenic and can cause respiratory diseases. The burning of polystyrene polymers from food packaging releases styrene which can be absorbed through the skin and the lungs. When it comes to plastic recycling, there are several problems that need to be addressed. First, it generates economically low yields, with complications such as the removal of dyes, fillers and other additives increasing the cost of recycling. Second, individual polymer types require their own specific pathways to be recycled, therefore plastic waste needs to be sorted before efficient processing can take place. These factors make recycling suboptimal for many countries (Suchismita Satapathy 2017).

The abiotic degradation of plastics takes place through four main pathways, oxidation, photolysis, hydrolysis and mechanical shearing (Figure 1). The mechanical shearing of polymers is influenced by a wide variety of environmental factors such as weathering and freeze thaw cycles. Plastic waste gradually experiences cracking, surface erosion, abrasion, and breakdown to mesoplastics (~5–20 mm), large microplastics (~1–5 mm), small microplastics (~20–999 µm), and nanoplastic (<1 µm) sized pieces. In the presence of water, hydrolysis of those plastics with heteroatoms in their backbone causes cleavage of ester bonds, allowing the rapid breakdown of PET or PU to their monomer constituents. Exposing polymers to UV radiation causes photodegradation, and is considered the primary method of degradation for discarded plastic pollution. UV radiation provides the energy to break C-H or C-C bonds, forming a highly reactive radical group. Alternatively, UV radiation can lead to the dechlorination of a C-Cl bond in PVC, releasing chlorine. The first step of polymer oxidation is free radical formation, often initiated by UV mediated breaking of C-C and C-H bonds. The carbon centered free radicals that are generated can then react with free oxygen in the environment, leading to the formation of peroxy radicals; or react with the polymer, causing the formation of more carbon centered free radicals. In an effort to quench the free radicals, this often results in chain linking and chain scission reactions in the polymer backbone.

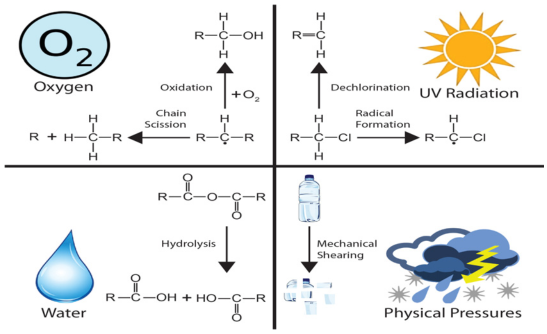

Figure 1: Four major pathways of abiotic polymer degradation. "R" represents a polymer chain.

Plastics present a poor food source for most organisms, but there are those that have evolved the cellular machinery required. Plastic polymers synthesised from fossil fuels are a relatively new addition to world ecosystems, and generally represent an inefficient reservoir of energy. As such, there has not been the opportunity for biology to evolve an effective means of degrading them. The millions of tons of plastic that ends up in the sea, landfills and other environments has created a reservoir of potential energy for microbial life. The microbial degradation of plastic plays a not insignificant role in polymer degradation, with over 400 species having been identified to date. The general biodegradation mechanism for polymer breakdown can be summarised in five steps; secretion, adsorption, release, uptake and degradation (Figure 2). Enzymes such as PETase, Cutinase, or perhaps alkane hydroxylases are excreted by the cell into the extracellular. These enzymes will then adsorb to the hydrophobic polymer surface, a process facilitated by the formation of biofilms. The enzymes secreted can then bind to their potential substrates, the polymers, and interact with them. Enzymes can successfully break down the large polymers into smaller segments or monomers which are then released. These smaller fragments can then be uptaken into the cell through specific importers. Once in the cytoplasm, additional specific enzymes may be required to enact modification before the polymer fragments can be incorporated into cellular metabolism.

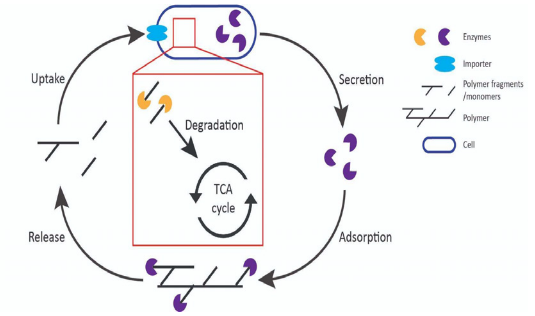

Figure 2: Proposed mechanism for microbial led biodegradation of polymers.

Microorganisms will often exist in nature in established consortiums, allowing for powerful benefits to all its members, including the capacity to degrade substances. Cultures with multiple species have better communal properties like robustness and division of labor. Various components are required for the biological breakdown of plastics (Figure 2), and dividing their production over multiple species could lead to synergistic effects. Robustness is the trait of microbes which enables them to survive in varieties of unstable environments. Such a property is presented in lichens, a composite organism which can include bacteria, fungi, and algae. When algae and fungi are alone, they both suffer from oxidative damage during desiccation. But when in a consortium, both enable up-regulation of protective systems. Without the contact with fungus, the algae can tolerate only low levels of light, and their photoprotective systems are not upregulated; in the absence of algae, the glutathione-based antioxidant system of fungus is slow and invalid. There are already some successful applications of consortium in the field of plastic degradation. Individual bacterial strains were isolated from various faecal samples, and tested both individually and in consortium for their capacity to degrade polyethylene or polyethylene and polypropylene. Individually, degradation rates ranged from 15.5% to 29% over the course of the assay, but in consortiums of four they achieved 75% efficiency of degradation for polyethylene and 56% for polypropylene.

3 DISCUSSIONS

In January 2021, Gambarini et. al. published an extensive and theoretically comprehensive database describing every microbial strain that has been reported to degrade any type of plastic polymer to date. This database is continually updated as new publications come out. For this work, we have analysed all species from this database that degrade one or more of the 6 predominant types of "non-biodegradable" plastics; PE, PP, PVC, PS, PET or PU.

Incorporating organisms across the different kingdoms into a consortium will likely allow for a wider degradation coverage of polymer types and additives. Since different types of organisms with varying enzymes and chemical substances have the ability of degrading different parts of the plastic, they are able to decompose larger proportions of plastic if they can work as a consortium. As a comparison, if a human wants to share a banana with a chimpanzee, they are only able to consume the banana pulp; if the human can share the banana with ants which can consume the skin, the whole banana may be decomposed in a rapid fashion. The degradation of plastic can be divided into several steps, such as degrading long chains and small chains, or the backbone of a polymer vs the monomers released, or a hydrophobic bacteria that facilitates interactions between the polymer and other organisms. Different kinds of organisms can be responsible for each part. For example, some insects have been identified that are able to chew plastic debris into smaller parts, assisting with mechanical shearing, before the bacteria in their gut break down the plastic enzymatically.

Microbial strains isolated from a similar environmental location have a higher chance of successfully cultivating in a single, compatible, minimal media. When considering a consortium, organisms isolated from the same kind of environment are likely to have a higher compatibility than organisms collected from disparate environments. For example, Samples collected from water (particularly ocean water) are unlikely, though not impossible, to be compatible with samples from soil. In addition, an organism's capacity to grow in a minimal medium with a limited carbon source will likely contribute to its efficiency at degrading plastic polymers. With plastic polymers generally being a poor source of energy, if an alternative energy source is present then it will be consumed preferentially first. Therefore, if an organism has been shown to grow successfully in a minimal medium with plastic polymers as the sole carbon source, this would be a positive sign. The growth environment should also contain the essential trace elements, such as alkali metals, transition metals or phosphates. Since there is little experimental evidence observing consortiums of polymer degrading microbes growing together, it is hard to say which strains will be compatible so it is worth trying out a variety of combinations.

For a consortium to be effective, all organisms must be able to successfully grow at equivalent temperatures, with higher temperatures being preferable for thermodynamic optimisation. The higher the temperature the organism can survive at, more likely that faster degradation will occur. The thermal compatibility of different organisms is heavily dependent on the environmental niche that organism was isolated from, and its thermophilic potential.

Additional environmental factors to be considered are pH and oxygen level. A significant part of polymer degradation is hydrolysis (Figure 3), which can be influenced by the pH value. In an acidic environment, the carbonyl group of an ester bond will be protonated, allowing for an easier nucleophilic attack. This process is particularly relevant for polymers with heteroatoms in their backbones, such as PET and PU (Fig 1). Oxygen is also an important consideration for the degradation of plastic, playing a role in both abiotic and biotic degradation. For abiotic degradation, oxidation and incorporation of oxygen into the polymer structure both require the presence of oxygen. Biotically, for most microbes, oxygen is preferential or even necessary for survival. It is also worth noting that the vast majority of polymer degradation studies have been carried out in an aerobic environment, as experimental convenience favours this condition. It is possible that anaerobic metabolism could offer alternative pathways.

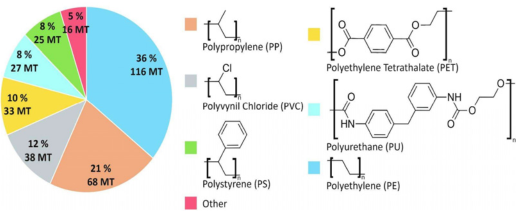

Figure 3: The proportion of waste, non-fibrous plastic mass attributed to the most common forms of plastic, from 2015. MT= million tons.

Reported degradation efficiency gives an indication of the potential of an organism, but comes with the considerable caveat that degradation studies are far from standardized, or even reliable. Theoretically, we would choose bacteria that have a higher degradation rate over a shorter period of time, for a more efficient breakdown of material. However, many reported strains have no data supporting their degradation rate, and those that do differ vastly in the substrates used or the methodology carried out. In addition, different studies vary in their pretreatment of the plastic substrate used with thermal, acidic UV or no treatment employed. It can be difficult to determine if an observed weight loss or CO_2 capture is due to the degradation of the polymer additives in the plastic. Moreover, degradation rates are often reported as a single value after a time course, and therefore it cannot be determined if degradation will continue at the same rate or if an asymptote is reached.

To create the optimal conditions for the biodegradation of polymers, a variety of potentially incompatible environmental factors will need to be considered and balanced. These factors include temperature, oxygen level, pH, UV exposure, moisture level, carbon availability and abundance of trace elements. In order to control the temperature, heating elements or ventilation can be used to maintain the temperature within an optimum range. To prevent an oxygen gradient forming, mechanical rotation may be necessary, although this may become prohibitively expensive on larger scales. pH will need to be monitored and kept within biologically acceptable limits using additives. UV levels are expensive to control artificially therefore natural light will be a more viable method. Moisture levels can be controlled using the addition of water. The consortium should be provided with the necessary trace elements generally required for microbial growth, and should not be provided with an alternative carbon source such as sugars or fats that would be consumed preferentially over the polymers. It is important to keep in mind that exposure to sunlight as a UV source will provide complications when trying to maintain an optimum temperature and moisture level, therefore a balance must be struck.

Genera that occur more frequently could be more reliable and compatible, with multiple related species all reported with degradation capacity. When strains capable of degrading polymers are analysed, some genera occur much more frequently than others. The three most common genera are Aspergillus, Bacillus, and Pseudomonas. There are 16, 18 and 19 species for each, reported across 18, 18 and 20 publications, respectively. Species that originate from the same genus will have closer genotypes, which means that their habitat and environmental needs for trace elements will be more compatible, so being able to select multiple species from the same genus could prove advantageous. It could be argued that because these genera are found more frequently to degrade plastics, they are the genera which are best adapted for it. However, it is more likely that laboratory based screening has a strong bias for strains that thrive in standard laboratory conditions.

Several species have been identified that have been observed to degrade multiple types of polymer, which could prove advantageous when considering a consortium. Only three types of bacteria or fungi have the ability to break down three or more types of plastic *Bacillus cereus*, *Bacillus gottheilii*, and *Phanerochaete chrysosporium*. *Bacillus cereus* and *Phanerochaete chrysosporium* have each been observed to degrade three types of polymer (PE, PET and PS and PE, PP and PVC respectively), while *Bacillus gottheilii* degrades 4 types of polymer (PE, PP, PS and PET) If all these species can be utilized, then with the help of just these three species, five of the six main plastics can be broken down. Since two of them are from the same genus, they are likely to be compatible and both of them are isolated from soil/sediment environments. With a smaller number of species, biotic degradation and the use of consortia could be more practical for large-scale use. However, since related research is still limited, some organisms that can break down multiple plastics may not have been observed to yet.Although reported degradation rates are not always reliable or comparable, there are some species with such a high reported rate that they are worth additional consideration. For example, four species that have a reported weight loss for PE above 50% are *Penicillium chrysogenum*, *Penicillium oxalicum*, *Microbacterium paraoxydans*, and *Pseudomonas aeruginosa*. The two fungal species, *P. chrysogenum* and *P. oxalicum*, were found to degrade 55 % and 59 % of a PE sheet over a 90 day period. P. aeruginosa and *M. paraoxydansare* bacteria found to degrade PE with a 50.5% and 61% weight loss recorded after 60 days at room temperature. These four species have all been isolated from soil samples, and have an optimal growth temperature of around 28 °C and have similar growth conditions, so it would be reasonable to have them in the same consortium. However, it is important to remember that methodologies for degradation rates vary greatly between publications, making exact comparisons and conclusions hard to draw. Nevertheless, it is probably worth considering these four species for a consortium that is required to degrade PE.

In order to select the right organisms for a consortium, compatibility, efficiency and degradation comprehensiveness need to be considered. As previously mentioned, *B. cereus* and *B. gottheilii* are both soil microbes capable of degrading multiple types of polymer. *Ideonella sakaiensis* is soil microbe whose PET degrading activity is well characterized, as is *Acinetobacter baumannii*. The soil fungus *Aspergillus flavus* could be considered for the decomposition of PU. High levels of PE degradation could be covered by one or more of the soil microbes *P. chrysogenum*, *P. oxalicum*, *M. paraoxydans*, or *P. aeruginosa*. By using these organisms the six predominant forms of plastic (PE, PP, PU, PS, PVC and PET) can be degraded by a range of organisms all capable of growing in a similar environment.

4 CONCLUSIONS

If implemented correctly, and at a significant scale, biodegradation of plastic waste through microbial consortiums could present an efficient, economical and environmentally sound response to the world's ever increasing plastic waste crisis. However, we do not recommend that this approach be taken on as an alternative to reducing the current polymer production levels, rather as a method for reducing the waste that already exists.

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If any, should be placed before the references section without numbering.

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