Fungal Genomics
MCLS | Writing Assignment

Plastic Degrading Fungi

A solution to overcome the issues of degrading mixed plastics

By Janieke Klusener (6972721)
Supervised by Prof. dr. Han Wösten

January 19th, 2022

Abstract

With the high demand for plastics, comes the rapid accumulation of plastic waste. Unfortunately, the build-up of plastic is causing serious environmental harm to animal and human life, and to the entire global ecosystem. Current solutions to deal with plastic waste include landfill and incineration. However, these methods only exacerbate the issue by releasing toxins into the air and soil. Another option is recycling, though nowadays, many plastic products are composed of multiple layers of different polymers, which requires intensive labour to separate each plastic before it can be reused. This literature review explores an alternative solution that is eco-friendly and can deal with the issues of mixed plastics, namely bioremediation. Specifically, fungi have been shown to quickly colonise polymer surfaces and can extend and penetrate places that other microbes cannot. Additionally, researchers have demonstrated the ability of fungi to degrade different types of synthetic polymers. A possible way to resolve the issue of degrading mixed plastics is to mix enzymatic systems and metabolic pathways of different fungal isolates. In this review, three fungi are chosen based on their ability to breakdown more than one type of synthetic polymer. Together, these three fungal isolates should be able to degrade all the main types of plastics. The three fungi include: (1) *Fusarium solani*, which can degrade low crystalline polyethylene terephthalate (PET), high- and low-density polyethylene (HDPE and LDPE), polyvinyl chloride (PVC), and polyester polyurethane (PS-PUR), (2) *Phanerochaete chrysosporium*, is able to breakdown HDPE, PVC, polypropylene (PP), and polystyrene (PS), and (3) *Cephalosporium* sp., which has been shown to degrade PP and PS. Furthermore, the biodegradation pathways of each type of plastic will be discussed, and together, this information provides a theoretical reference for further exploration on fungal plastic biodegradation.

Laymen summary

Today, we cannot imagine living in a world without plastic. This material is durable, versatile, and low cost, which make it a valuable resource for a wide range of applications. It is used in packaging, infrastructure, agriculture, biomedicine, and many more areas. Unfortunately, with the high demand for plastic comes the rapid accumulation of plastic waste. By 2050, it is to be expected that 1,800 million tonnes (Mt) of plastic is produced, of which 66% will end up in the environment. As plastic polymers take a long time to be broken down, if broken down at all, it will persist and accumulate in the environment for many years. Unfortunately, plastic waste has harmful effects on terrestrial and marine life, as well as vegetation and human health. Various disposal methods have been implemented, such as landfill and incineration. Unfortunately, these techniques only add to the problem by releasing toxins into the air and soil. Another option is recycling; however, many products are layered with multiple types of plastics, making it almost impossible to separate the materials. In fact, only 15% of the recycled products are reused.

This literature review focuses on an alternative method, which is the most eco-friendly and can overcome the problems of mixed plastics. Bioremediation involves using microbes that breakdown plastic polymers into smaller fragments which it can use as nutrients for its own growth. Fungi have been shown to be very efficient in degrading plastics, as they are able to extend and penetrate places no other microbe can reach. Regarding mixed plastics, a possible solution would be to select fungi that are able to breakdown multiple types of plastics and then grow them together. As a result, all the main types of plastics are degraded by just a few fungi. The three fungi considered in this literature review include: (1) *Fusarium solani*, which can degrade low crystalline polyethylene terephthalate (PET), high- and low-density polyethylene (HDPE and LDPE), polyvinyl chloride (PVC), and polyester polyurethane (PS-PUR), (2) *Phanerochaete chrysosporium*, that is able to breakdown HDPE, PVC, polypropylene (PP), and polystyrene (PS), and (3) *Cephalosporium* sp., which has been shown to degrade PP and PS. In addition, the biodegradation of each type of plastic will be discussed. This review offers further insight on the current knowledge on fungal plastic degradation.

Table of contents

List of abbreviations

FESEM	Field emission scanning electron microscopy
FTIR	Fourier transform infrared
FsC	*Fusarium solani* cutinase
HDPE	High-density polyethylene
HiC	*Humicola insolens* cutinase
*hc*PET	High crystalline polyethylene terephthalate
*lc*PET	Low crystalline polyethylene terephthalate
LDPE	Low-density polyethylene
LiP	Lignin peroxidase
MI-PP	Pro-oxidant blended polypropylene
MnP	Manganese peroxidase
MSM	Minimal salt medium
Mt	Million tonnes
OMMT	Organically modified montmorillonite
PE	Polyethylene
PET	Polyethylene terephthalate
PLA	Poly(lactic acid)
PmC	*Pseudomonas mendocina* cutinase
PP	Polypropylene
PS	Polystyrene
PS-PUR	Polyester polyurethane
PUR	Polyurethane
PVC	Polyvinyl chloride
SEM	Scanning electron microscopy
ST-PP	Starch blended polypropylene
TDS	Total dissolved solid
T_g	Glass transition temperature
TGA	Thermo gravimetric analysis
T_m	Melting point
TPA	Terephthalic acid

1. Introduction

Synthetic plastics have become an unmissable product in our lives, and our dependence on the material only continues to increase [1]. Plastic is durable, versatile, low weight, and low cost, making it a valuable resource for a wide range of applications [2]. For instance, it is found in kitchens, vehicles, agriculture, and biomedicine (Fig. 1) [3]. In 2019, 368 million tonnes (Mt) of plastics were produced globally. Asia contributed 51% of the production, followed by North America with 19% and Europe with 16% [4]. Unfortunately, 8 Mt of plastics ends up in the ocean every year, adding to the enormous garbage patch that has a surface area of 1.6 million square kilometres [5]. By 2050, the expectation is the production of 1,800 Mt of synthetic plastic, of which 1,200 Mt will end up in the environment as plastic waste [6].

Plastic is non-biodegradable or has a very slow degradation rate, resulting in the accumulation of plastic waste that persists in nature [3]. Consequently, this causes serious environmental concerns for terrestrial and marine life, but also for vegetation and human health [7]. For example, plastic waste blocks the water and air from passing through the earth, thus depleting the water reserve underground. Moreover, when plastic is broken down by sunlight, small toxic parts are released, thereby contaminating the water and soil [8]. Not only do these particles affect soil fertility, but they also end up in the digestive systems of marine animals. This leads to the deaths of millions of animals, and slowly works its way up the food chain, at a point where human health is also affected [3,8].

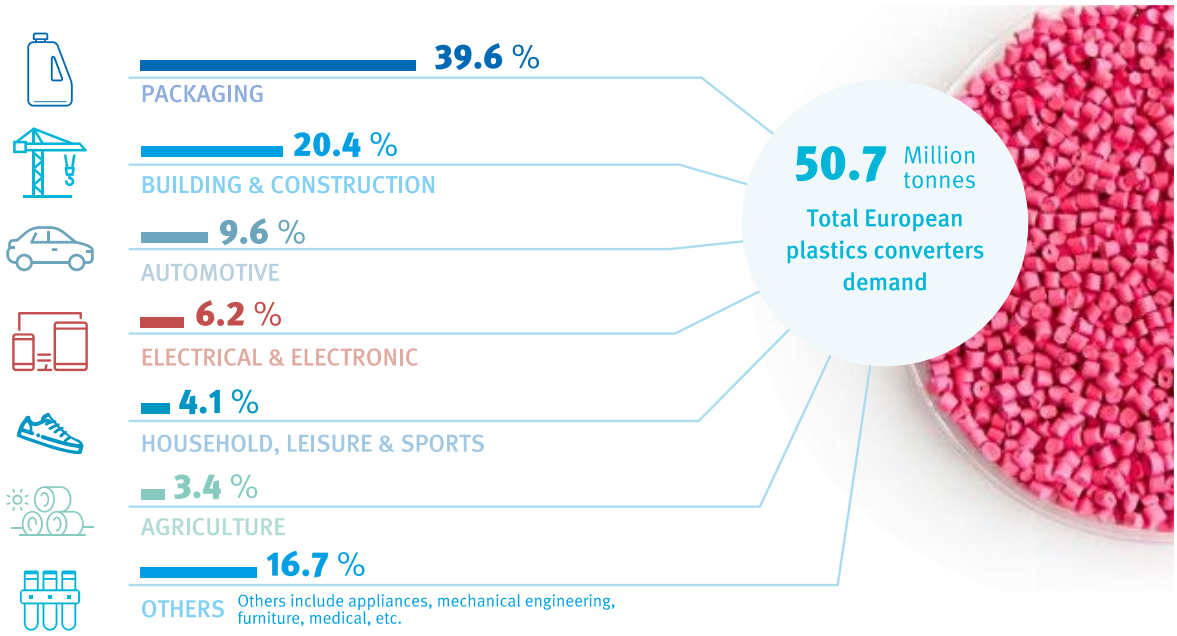


Figure 1. Europe contributed 16% of the global plastic production in 2019, of which most of the plastic demand came from the packaging industry [4].

Several waste management methods have been implemented on how to combat the accumulation of plastic waste. This includes landfill (65%), incineration (25%), and recycling (10%) [3]. These techniques have been used globally for years, however their negative impact on the environment is starting to show [1]. Landfills have become a landscape of derogation, where the soils are contaminated with plastic particles, and releases greenhouse gases and hazardous chemicals [2]. Similarly, the burning of plastic produces huge amounts of CO_2, dioxins and other toxic gases that can cause lung disease and cancer [8]. In addition, recycling would seem like the best alternative, however,

there is a lack of recycling technologies. Consequently, 10% of the plastic waste that is recycled, only 15% is reused more than once [9].

An alternative solution that is the most eco-friendly and widely accepted method is bioremediation. This technique involves the use of microorganisms that can colonise the surfaces of plastics, and through naturally occurring decaying processes, secrete enzymes. These enzymes can breakdown complex polymers into smaller molecules, which the microorganism can use for its own growth, and releases CO_2 and H_2O, under aerobic conditions (and CH_4 under anaerobic conditions) [2]. In this context, biodegradation is the safest, most natural way of disposing plastics, and it is cost-effective due to the abundance of microbes and dependence on natural processes [2,10].

Unfortunately, biodegradation of plastics has not been globally implemented yet. Bioremediation is not economical, instead, it is cheaper to burn plastics [11]. In order to globally implement this method, advances have to be made on how to effectively collect all disposed plastics and develop an efficient and large-scale bioreactor, which contains fungi that can degrade various types of plastics, under controlled conditions [12]. To achieve this, more research must be done on the use of fungi in plastic degradation. Currently, there is a gap of knowledge about the enzymes and mechanisms involved in biodegradation. Thus, more specific information must be collected on the different fungal species, their oxidative enzymes, chelators, and organic acids, in relation to plastic degradation [12].

To help gain information on this matter, this literature review will take a closer look at the degradation abilities of three fungi, namely *Fusarium solani*, *Phanerochaete chrysosporium*, and *Cephalosporium* sp. Together, these fungi can degrade the seven main types of plastics, which presents an effective alternative to overcome the issues of plastic waste. Furthermore, the mechanical pathways involved in the degradation of each plastic is discussed.

2. The main types of plastics

Depending on the chemical structure and properties of polymers, different synthetic plastics can be made (Table 1) [2]. Often, plastics are divided into two categories based on their thermal properties: thermoplastics and thermosets. Thermoplastics are a family of plastics that do not change chemical structure upon heating and can be remoulded several times [2,13]. Examples of thermoplastics are high- and low-density polyethylene (HDPE and LDPE), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), and polystyrene (PS) [14]. On the other hand, thermosets can only be moulded into one shape, as they do undergo chemical changes when heated. One example is polyurethane (PUR) [14]. Together, these seven types of plastics make up 92% of all plastics being produced, with PE being produced the most, followed by PP and PET (Fig. 2) [6].

The process of making petroleum-based plastics starts off with the refinement of two raw materials: crude oil and natural gas. These materials are refined through distillation and cracking, which results in intermediates that are required to form different polymers through reactions like polymerisation/polycondensation. Furthermore, plastic additives are incorporated to achieve the final functional properties, such as plasticisers, heat stabilisers, flame retardants, and pigments [2].

The most commonly used plastics are made up of polyolefins. Polyolefins are created by polymerising olefins, such as ethylene, propylene isoprenes, and butenes [15]. Their mechanical flexibility, energy efficiency and recyclability make them a highly wanted material that is utilised in all parts of our daily life [16]. Two examples of polyolefins that together are the most produced plastics are HDPE and LDPE (Fig. 2). These two polyethylene's (PE) are composed of long chains of ethylene monomers, and have a wide range of applications [17]. Specifically, HDPE has a low degree of branching, resulting in a high melting point (T_m), glass transition temperature (T_g), and crystallinity (Table 1). These properties make it very strong and resistant to moisture [7]. Therefore, HDPE is used

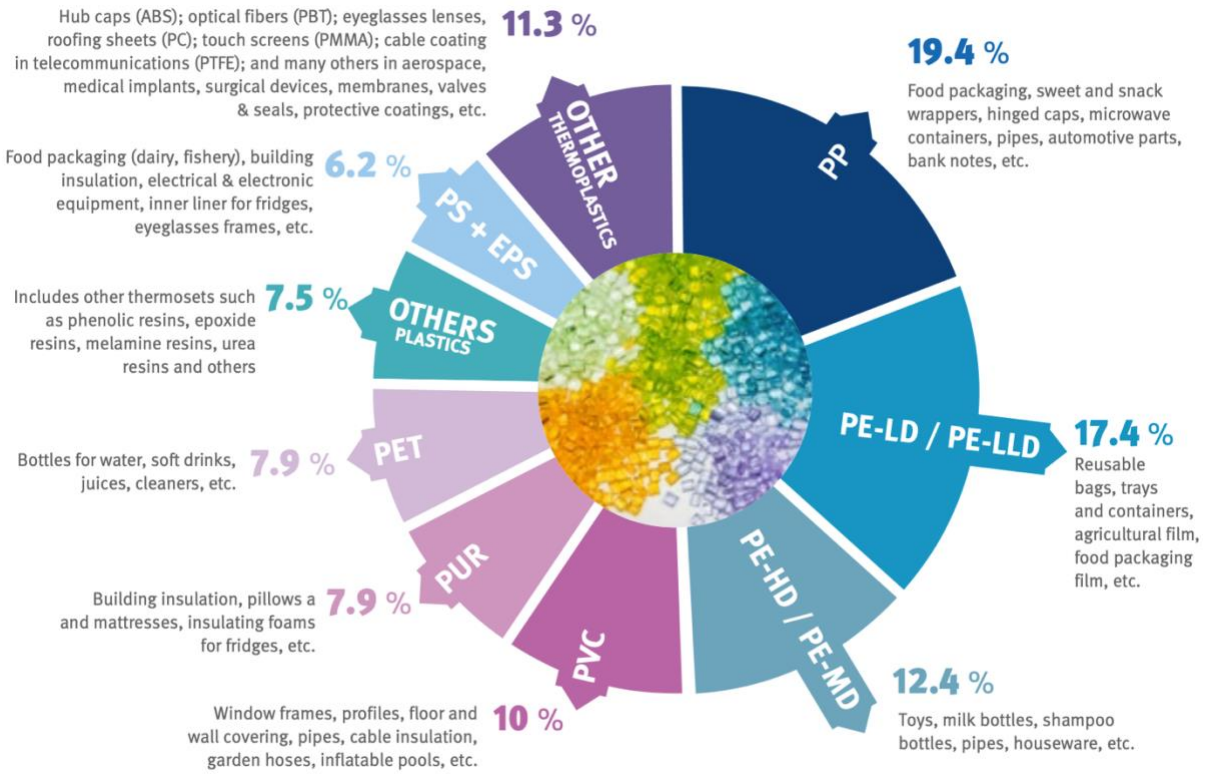
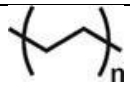
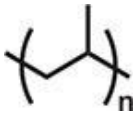
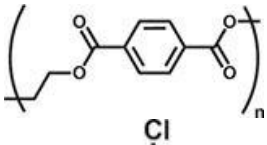
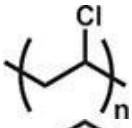
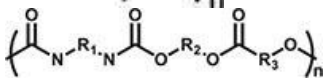


Figure 2. The usage of the main petroleum-based plastics in Europe in 2019 [4].

in the textile industry, toys, detergent bottles, and food packaging [18]. In contrary, LDPE has a higher degree of branching, resulting in a softer more flexible material (Table 1) [19]. It is commonly used in plastic bags and food packaging film [4]. Another type of polyolefin, and second most produced plastic is PP (Fig. 2). This type of plastic is one of the more durable kind, and because of its high heat resistance, it is often used in products that require it to withstand heat (Table 1) [19]. Examples include food packaging, plastic tubs, nonabsorbable sutures, and plastic pressure pipe systems [17].

Another common polymer is PET, which is composed of terephthalic acid (TPA) and ethylene glycol (Table 1) [20]. Due to its semi crystallinity, this plastic is strong and lightweight, making it an attractive material to use in drink bottles, microwavable packaging, and synthetic fibre [11]. Furthermore, PVC is made up of repeating chloroethyl monomers and is the plastic that is often used in building and construction is PVC (Table 1) [21]. Due to its resistance to chemicals and weathering, as well as its hardness and rigidness, it is the perfect material to use in plumbing pipes, window frames, flooring, and data cables (Ali et al., 2014; Suresh et al., 2017). PVC is also used for medical applications, because it can easily be cleaned, and it is resistant to germs [17]. Moving on, PS, also known as Styrofoam, is made up of aromatic styrene monomers (Table 1) [21]. Its rigid, tough, and lightweight, as well as its ability to insulate well allow its usage in cutlery, cups, CDs, and packaging foam [17]. Lastly, the only thermoset in this review is PUR (Table 1). PUR is obtained by condensing polyols and polyisocyanate, which can either be polyesters (PS-PUR) or polyethers and are linked by carbamate (urethane) [23–25]. This plastic is generally used in surface coatings and foams, such as cushioning foams and thermal insulation foams [17].

Table 1. Types and properties of the main types of synthetic plastics [26]. *T_m melting temperature, T_g glass transition temperature, X_C crystallinity*

Plastics	Abbreviation	Structure	T_m (°C)	Tg (°C)	X_C (%)	Recycling codes
High-density polyethylene	HDPE		200-300	-120	80-90	
Low-density polyethylene	LDPE		160-260	-120	45-65	
Polypropylene	PP		130	-10-18	60-70	
Polyethylene terephthalate	PET		260	80	40-60	
Polyvinyl chloride	PVC		100-260	60-70	-	
Polystyrene	PS		240	63-112	-	
Polyester polyurethane	PS-PUR		8-20 (soft)	-75 to -50 (soft) 185-205 (hard)	40-50	

2.1. Problems with synthetic plastics

Most of the plastic waste that ends up in nature are non-biodegradable. For example, the lifetime of PET is 20-25 years, while PVC shows no degradation even after 35 years [11,22]. Polyolefins, for that matter, can take up to 1000 years before they are broken down [3,27]. The recalcitrant nature of plastic is due to its high molecular weight, complex three-dimensional structure, and hydrophobic nature, all of which make it less susceptible to microbial attack [12]. More specifically, due to the hydrophobicity, biofilm formation or attachment by microorganisms is not possible, which is a prerequisite to biodegradation [27]. Furthermore, the main challenge of degrading plastics with common enzymes is that the structure of plastic does not lend itself to be hydrolysed. This is due to the extensive C-C backbone structure and lack of functional groups [9,10]. Another factor that affects the rate of biodegradation is that the T_m, crystallinity, and elasticity vary greatly between the different polymers (Table 1) [8]. Additionally, almost all plastics are formulated with additives, which help improve their final functional properties, but also slows down microbial degradation or even kill the microorganism [8,28]. Morover, plastic products are often layered with multiple diverse materials, including several different plastics, via co-extrusion or lamination [29,30]. Some examples of multi-layered products include bottles, bags, and food packaging [31]. These multi-material multi-layer structures are produced to help with rigidity or flexibility of the packaging, to prolong shelf-life, and provide heat resistance [30]. Unfortunately, layering different sorts of polymers makes it difficult to recycle the product, as thorough sorting is required before each polymer can be reused [9].

3. Bioremediation as the solution

The most eco-friendly and low-cost method to breakdown synthetic plastics is bioremediation. In comparison to bacteria, fungi have an advantage when it comes to plastic degradation. Most fungi that are capable of degrading plastics belong to the genera of ascomycetes, followed by basidiomycetes [12]. These organisms are found in many different places across the world, growing sometimes under the most extreme conditions. This has given them the ability to adapt to harsh environments, allowing them to survive under low nutrient conditions, as well as making them tolerant to certain pollutants [27]. In fact, fungi can convert these pollutants into non-toxic components and use them as nutrients for their own growth [2]. For example, in a carbon-deprived condition, fungi switch their metabolism to produce hydrolytic enzymes, which they secrete from the hyphae. These enzymes can solubilise complex organic polymers into smaller organic compounds that are absorbed back in through the hyphae as nutrients and energy, and release CO_2 and H_2O, under aerobic conditions [32,33]. Furthermore, fungi's extensive mycelial network allows hyphae to colonise surfaces quickly and penetrate places most other microorganisms cannot reach [10,34]. More so, adhesion is a prerequisite of biodegradation, and bacteria are dependent on its own properties and on the physiochemical surface of the polymer. Fungi, on the other hand, synthesise biosurfactants (i.e. hydrophobins), which help the fungus adhere to polymer surfaces and promotes fungal growth [2,35]. Also, no research has shown that bacteria degrade the polymers upon early attachment, while fungi do. All in all, the combination of a rich source of degrading enzymes, adsorption to polymer surfaces and the production of hydrophobins, provide fungi with a powerful system that can remove plastic waste from the environment [2,36].

However, one challenge of using microorganisms to degrade plastics is that fungi often only breakdown certain types of plastic. This would mean that before biodegradation can take place, each type of plastic would have to be separated, costing a lot of time. For mixed plastics, this is barely feasible because these products are a combination of different sorts of polymers [14,30]. A solution would be to identify a fungus that can breakdown more than one type of plastic and complement this fungus with one or two other fungi that breakdown a different variety of polymers.

4. Fungi that breakdown multiple plastic types

Only a few fungi can degrade multiple types of plastics, most of which belong to the ascomycetes. This literature review considers three fungi, each of which can degrade different variations of polymers. Collectively, they can breakdown all seven main types of plastics, which should tackle the problem of mixed plastics. The three fungi that will be discussed are *Fusarium solani*, *Phanerochaete chrysosporium*, and *Cephalosporium* sp.

4.1. *Fusarium solani*

The ascomycete genus *Fusarium* comprises over 200 species and belongs to the Nectriaceae family [37]. It is also known for being a species complex, meaning that this lineage is hard to distinguish or closely related taxonomically [38]. Therefore, instead of dividing species into taxonomic categories, *Fusarium* species are classified based on morphology. These macro- and micro-morphological phenotypes include colony colour, shape, size and spore development and structure [39]. This genus includes many pathogens of various crops but can also infect humans. Due to the vast number of species, *Fusarium* can be found in a vast range of habitats; from tropical regions to deserts [39].

The species that this paper will focus on is *F. solani*, which is most commonly found in soil and can also cause infections in plants and humans [37,40]. It secretes non-lignolytic enzymes and has been shown to degrade the highly toxic organopollutant polychlorinated biphenyls, which are commonly used in the industry and persist in the environment [12]. Apart from this, it is known to degrade low crystalline PET (*lc*PET), HDPE, LDPE, PVC and PUR.

Low crystalline PET. Ronkvist et al. (2009) compared the catalytic activity of cutinases that were obtained from three different fungal isolates on their ability to degrade *lc*PET (7% crystallinity). These fungi included *Humicola insolens* (HiC), *Pseudomonas mendocina* (PmC) and *F. solani* (FsC). Using a pH-stat to measure the NaOH consumption versus time, the catalytic activity of the three enzymes were analysed when incubated with pieces of PET. The researchers observed that after 96 h at pH 8, HiC was able to degrade 97% PET at 70°C. Meanwhile, PmC and FsC only degraded 5% of the polymer, at 50°C and 40°C, respectively. The researchers explain that HiC has a higher thermal stability, thus able to reach temperatures that are near *lc*PET's T_g of 75°C. At this transition temperature, the chains in *lc*PET become more mobile, therefore, allowing HiC to access PET ester groups. Furthermore, longer incubation times of PmC and FsC with the polymer did not increase weight loss. It was hypothesised that these two cutinases strongly adsorb to PET and saturate the available surfaces. These enzymes become deactivated after 48 h, blocking the soluble active enzymes still in the medium from access the polymer surface, resulting in no further degradation. Consequently, as the temperature-activity profiles of PmC (50°C) and FsC (40°C) did not reach PET's T_g and saturate all available polymer surfaces, *lc*PET was less susceptible to microbial degradation, explaining why they only degrade 5% of the film. Even though little to no polymer weight loss may occur, FsC can still be of use in the textile and biomedical world to modify PET surfaces by increasing hydrophilicity [11].

HDPE. A study conducted by Rani, Singh and Kumar (2020), demonstrated the ability of *F. solani* to degrade HDPE. The fungal strains were collected from dump sites and were selected by growing them on minimal salt medium (MSM) containing PE powder, which acted as the sole carbon source. After four weeks, the colonies that were able to grow were *Aspergillus fumigatus*, *Aspergillus flavus*, and *F. solani*. These strains were inoculated with HDPE strips in the shake flask method, and the dry weight of the remaining strip was measured up to 90 days. Compared to the other strains, *F. solani* showed the most degradation. To further confirm the activity of *F. solani*, field emission scanning electron microscopy (FESEM) was used to examine surface deformities in the deteriorating HDPE strips. Clear cracks and groves were visible in the plastic, which could be explained by enzymatic activity. The researchers concluded that *F. solani* was capable of degrading HDPE and was able to do so the fastest in comparison to any other identified isolate [7].

LDPE. The study by Rani and Singh (2017) looked at the degradation of LDPE. Fungal isolates were obtained from pollution sites, and they were grown on MSM, supplemented with PE powder. The colonies that were able to grow, were identified based on macro- and microscopic phenotypes. Next, known amounts of LDPE was placed in soil and inoculated with the selected fungi. To monitor the rate of degradation, the weight of LDPE was measured after the incubation with the fungal isolates. Out of the 13 fungi, *F. solani* had the highest rate of LDPE degradation (77.668%), followed by *Aspergillus fumigatus* (25.42%), *Aspergillus flavus* (12.269%), *Aspergillus terreus* (11.98%), and *Aspergillus niger* (7.176%) [35].

Das, Kumar and Das (2018) identified fungi present in samples from a dump yard. LDPE strips were inoculated with each strain in a flask for 60 days. The researchers revealed that *F. solani* was able to reduce the weight of the LDPE strips by 13%. To confirm this, the production of CO_2 was measured with Sturm tests and showed a bio-mineralisation effect of 19.27%. This demonstrates the rate of

polymer degradation which is converted into simple molecules, such as CO_2. Similarly, the pH changed from 7 to 7.9, revealing that there are chemical reactions occurring due to metabolic activity. FESEM showed a presence of pits and corrosion close to the fungal growth on the polymer surface. Lastly, Fourier transform infrared (FTIR) spectroscopy shows intensified changes in the peaks between the control and treated LDPE. The peaks at 1068 and 726 cm^{-1} corresponding to C-C stretching bonds and C-H rocking bonds became more prominent when the LDPE strips were inoculated with selected fungal strains. Furthermore, the researchers observed other peaks, namely those consistent with "hydroxyl O-H, aldehyde C-H, amide, C-N of amine, and C-O/C=O stretching" [10]. These changes in the peaks indicate oxidation and depolymerisation of the polymer. This study further demonstrates that *F. solani* can use LDPE as its carbon source and converts the complex polymers into simple molecules [10].

PVC. *F. solani* has also been shown to be able to degrade PVC. In this study, Sakhalkar and Mishra (2013) cut pieces from different types of plastic bags, which they buried in the soil for two months. After this period, they sorted the different plastic pieces along with the growing fungi and identified all the corresponding fungal species. The researchers characterised the fungi by placing them on PVC powder for 12 weeks and observing which ones were able to grow. This resulted in a total of 13 fungi that used PVC as their main carbon source. In sixth place was *F. solani*, which was able to degrade 0.240 g of PVC powder (no data provided on the start weight or timeframe). Furthermore, FESEM confirmed that the fungus was actively decomposing the polymer, as the researchers observed the hyphae breaking down the particles into finer sizes. Also, FTIR spectra showed a shift in the peaks of treated samples that corresponded to changes from a polymeric molecule to smaller units. Even though *F. solani* did not have the highest plastic degrading potential compared to some of the other fungi that were identified, this data still provides evidence that *F. solani* was able to breakdown PVC.

PS-PUR. In an experiment conducted by Ibrahim et al. (2011), several fungal isolates were collected from the soil, wall paints, plastic debris, and lamp posts. Several experiments were used to test the ability of these isolates in degrading PUR. In the shaking flask method, six fungi were able to degrade the PUR blocks, where *F. solani* showed the highest biodegradation of 100%. In the petri dish test, fungi were directly placed onto the PUR pieces, revealing a 72.5% weight loss by *F. solani*. To further confirm the results of the previous methods, the fungal isolates were added to the centre of a plate containing two agar media layers. The lower layer contained basal medium while the upper layer consisted of a polymer suspension. Only four fungi were able to degrade PUR, as indicated by clear zones, where *F. solani* cleared the most agar (70 mm). Ibrahim et al. (2011) reveal that *F. solani* was one of the only isolates that provided positive results of PUR biodegradation.

4.2. *Phanerochaete chrysosporium*

The white rot basidiomycete, *Phanerochaete chrysosporium* belongs to the Phanerochaetaceae and is found in forests throughout North America, Europe, and Iran, and it is known to breakdown both hard- and softwood [42]. It produces three extracellular lignolytic enzyme groups: lignin peroxidases (LiP), manganese peroxidases (MnP), and laccases, making it a perfect model organism for lignin-degrading enzymes [43]. These enzymes are also capable of mineralising recalcitrant organic pollutants, polycyclic aromatic hydrocarbons, and synthetic dyes [27,32]. In addition, *P. chrysosporium* has also been shown to degrade plastics, such as HDPE, PVC, PP, and PS.

HDPE. A study conducted by Iiyoshi et al. (1998), analysed the rate of degradation of HDPE by lignin-degrading fungi, under nitrogen- and carbon-limited conditions. The researchers compared the degradation potential of an unknown strain named IZU-154, *P. chrysosporium* and *Trichoderma*

versicolor. In addition, the enzymes related to HDPE's degradation were analysed. HDPE strips were placed on growing mycelium for 12 days. Depending on whether it was the nitrogen- or carbon-limited condition, concentrations of $(NH_4)_2SO_4$ and glucose differed, respectively. Under nitrogen-limited conditions, *P. chrysosporium* and IZU-154 both showed a similar reduction in the relative elongation and relative tensile strength, over time, indicating degradation of HDPE. On the other hand, *T. versicolor* degraded less HDPE, and showed fewer reduction in the relative elongation and relative tensile strength. Under carbon-limited conditions, IZU-154 had the most HDPE degradation, followed by *P. chrysosporium* and *T. versicolor*. However, a carbon deficiency led to lower values of biodegradation, indicating that a nitrogen-deficiency results in more efficient degradation. Furthermore, enzyme activity assays were used to identify whether any of the ligninolytic enzymes were involved in the degradation of HDPE. *T. versicolor* produced only large quantities of laccase, though no significant HDPE degradation was observed. In contrary, IZU-154 and *P. chrysosporium* produced high amounts of MnP, which was accompanied by significant levels of HDPE degradation. Upon the addition of manganese sulphate to *T. versicolor*, the rate of HDPE degradation was drastically accelerated. These findings demonstrate that laccase is not involved in HDPE degradation, whereas MnP seems to be an important factor in the degradation of HDPE [43].

PVC. In this study, Ali et al. (2014) collected fungal strains that were able to grow on PVC films, after 10 months of incubation. The researchers identified the strains as *P. chrysosporium*, *Lentinus tigrinus*, *A. niger*, and *Aspergillus sydowii*. After incubating thin films of PVC with each fungus, they turned from white to yellow, and there were clear fungal infestations on the surface of the polymer, which were accompanied by surface aberrations. Scanning electron microscopy (SEM) showed hexagonal rings in the PVC films, representing adherence and growth of the fungus. These rings were most apparent in the samples treated with *P. chrysosporium*. Similarly, the shake flask method revealed a significant colour change from white to brown, and deterioration was visible on the surface of the polymer. Furthermore, *P. chrysosporium* reduced the most molecular weight of PVC from 200,000 Da^{-1} to 178,292 Da^{-1}. Likewise, *P. chrysosporium* produced the most CO_2 (7.31 g L^{-1}), as indicated through Sturm tests. Moreover, FTIR spectroscopy and nuclear magnetic resonance showed a shift in the peaks, reflecting structural changes in the PVC. In conclusion, these findings provide strong evidence that *P. chrysosporium* was able to degrade PVC.

LiP is most likely the enzyme that is responsible for the degradation of the PVC films. In a study by Khatoon et al. (2019), they collected *P. chrysosporium* from contaminated soil, and placed it with PVC films in a shaker for two months. Due to the limited availability of carbon, *P. chrysosporium* excreted an enzyme, which was extracted and purified. Molecular weight determination revealed the presence of LiP. Sturm tests showed that PVC films treated with LiP produced 13.74 mg L^{-1} of CO_2 which was four times more than in the control. In addition, there was a 31% significant reduction in the weight of the PVC film. The degradation of film was confirmed with SEM, which clearly demonstrated deterioration on the polymer surface where it was exposed to the fungal filtrate. Furthermore, FTIR spectra reveals structural changes in the polymeric material, specifically in a peak corresponding to an alkenyl C-H stretch at 2943 cm^{-1}. Together, these findings indicate that LiP is involved in the biodegradation of PVC.

PP. In the experiment of Jeyakumar et al. (2013) the effects of pre-treatment (100°C or 10 days of UV) and blending of PP on biodegradation of fungi was studied. Two types of PP blends were tested: pro-oxidant blended PP (MI-PP) and starch blended PP (ST-PP). Blending PP with these components ensure the addition of functional groups, through oxidation, and decrease the hydrophobicity, thus making the polymer more susceptible to microbial attacks. Two fungi were used to study the effects, *P. chrysosporium* and *Engyodontium album*. Their mycelium was added to a flask and incubated for 12

months. In the pre-treated PP, FITR spectra showed clear peaks that corresponded to ketones and esters, at region 1700-1800 and 1300-1400 cm^{-1}, respectively. The formation of these peaks suggest that oxidation occurred in the polymer, leading to the presence of hydroxyl groups in the backbone. The positive effects of pre-treatment can be observed through SEM, where untreated PP appeared to have smooth surfaces, while treated PP had numerous cracks and grooves, accompanied by fungal propagation. In addition, laccase activity appeared to be the highest (1.8 nanokatals ml^{-1}) in MI-PP under UV conditions in *P. chrysosporium*. However, *E. album* had more degradation potential under the same conditions, as its gravimetric weight loss reached 18.8%, and only 9.42% in the case of *P. chrysosporium*. Similarly, the thermo gravimetric analysis (TGA) at 400°C showed 86.3% and 84.2%, for *E. album* and *P, chrysosporium*, demonstrating that *E. album* had a better thermal stability. Moreover, the low correlation between laccase activity of *P. chrysosporium* and the gravimetric weight loss (r = 0.49) most likely indicates that this fungus produces other enzymes at the same time [27]. Regardless, this research shows the ability of *P. chrysosporium* in degrading (treated) PP films.

PS. A study conducted by Milstein et al. (1992) analysed the effects of a copolymer with different proportions of lignin and PS on fungal biodegradation. By grafting lignin onto the backbone of a polymer, it is suggested to enhance the efficiency of plastic degrading by fungi. Three white rot fungi and one brown rot fungus were grown together with the copolymer containing either 10.3%, 32.2% or 50.4% lignin, on agar plates (for 68 days) or in liquid medium (for three weeks). The researchers observed that with increased lignin content, more copolymer was being degraded, where *P. chrysosporium* decomposed the most. The researchers ensured that both PS and lignin were being degraded, as verified through UV spectroscopy. On the other hand, PS as a homopolymer was not decomposed significantly by any fungi. Furthermore, SEM showed that the white rot fungi were releasing extracellular capsular material, of which *P. chrysosporium* had the most mycelial growth. It is suggested that this material helps the fungus adhere to the polymer surface and increases its oxidation potential. Not only did the researchers observe colonisation and propagation of the fungi, the polymer surface also had striations, pits and some decay. Furthermore, enzyme analysis showed that *P. chrysosporium* produced the most LiP and MnP. The researchers propose that these enzymes modify the lignin component in the copolymer, subsequently making the entire structure more susceptible to microbial degradation.

Another study by Shimpi et al. (2015) showed similar findings of *P. chrysosporium* breaking down PS more efficiently when composited with other components. Such components included poly(lactic acid) (PLA) (PS:PLA) and PS:PLA filled with organically modified montmorillonite (OMMT) (PS:PLA:OMMT). To investigate this, the researchers used spores from *P. chrysosporium* and added them to nanocomposite sheets which varied in the amount of PLA and OMMT. After 28 days in a shaking incubator, *P. chrysosporium* was able to grow on all compositions. The most degradation in PS:PLA was observed in the 30% composites (17%), and in the 5 phr composite of PS:PLA:OMMT (20.9%). SEM revealed rough surfaces due to fungal growth. Further analysis showed a reduction in tensile strength and elongation at the break, after treatment with *P. chrysosporium*. A decrease in these mechanical properties is caused by the fungus growing between the layers of polymers. In conclusion, Shimpi et al. (2015) demonstrate that the addition of bio-accessible materials to PS, helps *P. chrysosporium* in degrading the polymer. They propose that these nanocomposites change the structure of PS by making the polymer complex more hydrophilic, allowing fungal growth inside the polymer matrix, and activating hydrolytic degradation.

4.3. *Cephalosporium* sp.

The ascomycete genus *Cephalosporium* belongs to the Hypocreaceae family. This genus is comprised of about 100 species and are often saprophytic on dead plants and soil dwellers [45]. One species, for example *Cephalosporium gramineum*, is found in Japan and North America and is known as a pest of wheat and other grasses [46]. Another well-studied species is *Cephalosporium acremonium* and is known to produce cephalosporins, which are used for drug development due to their antibacterial properties [47]. For this literature review, the studies focus on *Cephalosporium* species (sp.) (NCIM 1251), and has been shown to degrade HDPE and PS.

HDPE. In one study by Chaudhary and Vijayakumar (2020), they incubated nitric-acid pre-treated HDPE samples with *Cephalosporium* sp. in a shaker for 20 days [18]. Based on weight reduction measurements, the fungus was able to degrade the HDPE films by 7.18%. Furthermore, the researchers observed changes in the pH, total dissolved solid (TDS) and conductivity. There was a reduction in the pH value (5.64 to 4.81), but an increase in TDS (0.650 to 0.731 ppm) and conductivity (0.499 to 1.424 μs) over time, which was an indication that *Cephalosporium* sp. was secreting enzymes and confirm the ability of the fungus to use the polymer as a carbon source. Moreover, SEM showed that the fungus was able to adhere to the HDPE films, a prerequisite to fungal degradation. Furthermore, X-ray diffraction studies revealed that there was a 14.67% decrease in crystallinity after incubation with the fungal strain. All in all, the researchers provide strong evidence of *Cephalosporium* sp.'s abilities in degrading HDPE film.

PS. Another study conducted by Chaudhary and Vijayakumar (2020) demonstrated *Cephalosporium* sp. breaking down PS [36]. The researchers incubated *Cephalosporium* sp. as well as another fungus, *Mucor* sp., with PS strips for eight weeks. Weight reduction measures showed that *Cephalosporium* sp. had more potential in degrading PS (2.17%) than *Mucor* sp. (1.81%). Similar to their previous experiment with HDPE, the pH decreased (7 to 5.91), while TDS (0.546 to 1.615 ppm) and conductivity (0.452 to 3.141 μs) increased. Again, these changes in values are indications that *Cephalosporium* sp. secreted enzymes and uses PS as a carbon source. Furthermore, SEM revealed a rough polymer surface after fungal treatment, while FTIR showed a shift in the position of the peaks, signifying the weakening of the bonds in the PS sample. In conclusion, the researchers demonstrate *Cephalosporium* sp.'s ability to degrade PS and us it as a carbon source for their own growth.

5. The biodegradation of synthetic plastics

The extracellular enzymatic system, such as the hydrolytic and oxidative system, are responsible for breaking down complex polymers [2]. In general, the process of plastic biodegradation starts with the colonisation of the fungus (Fig. 3). As the hyphae extend along the surface, it secretes specific enzymes that depolymerise and hydrolyse the polymers into oligomers or monomers. These low molecular weight fragments can be taken up by the fungus as energy for growth, and release CO_2 and H_2O under aerobic conditions (CH_4 under anaerobic conditions) [10,12].

The difference in the physio-chemical properties of the various polymers are what affects which enzymes and mechanisms are required, and influence the rate of biodegradation. These variables include reaction temperatures, crystallinity, polymer chain orientation, and hydrophobicity. For example, PET and PUR are easier to breakdown compared to PE, PS, PP, and PVC. This is because the backbone in the former two have hydrolysable chemical bonds (Table 1) [48]. The other types of polymers have strong C-C bonds that first should be oxidised, to facilitate the depolymerisation [25].

Therefore, pre-treatments, such as UV or thermal treatment, are essential to introduce oxygen molecules in these polymer samples [49]. In the next section, the biodegradation pathway for each type of plastic is discussed.

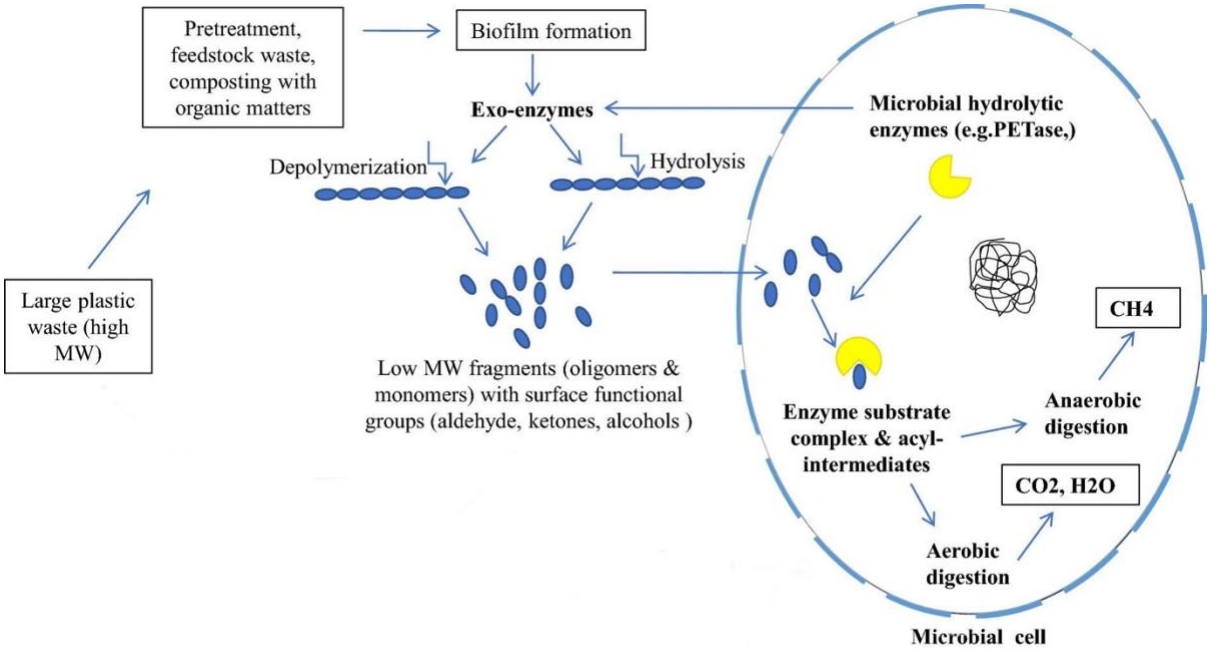


Figure 3. A representation of the general biodegradation pathway of plastic by microorganisms, mediated by environmental and biotic factors (modified from [1]). Plastic waste can be pre-treated to facilitate the colonisation by microorganisms. These microbes secrete enzymes that depolymerise and hydrolyse the long-chain carbon compounds into oligomers and monomers. These fragments can be taken up by the microbes as nutrients and converted into CO_2 and H_2O (and CH_4 under anaerobic conditions).

PET. Enzymes that have been identified that degrade PET are often serine hydrolases. These include, cutinases, carboxylesterases and lipases [13]. Cutinases have been shown to have the greatest potential for PET hydrolysis, by breaking the bonds of ester linkages [2]. Sanchez (2020) proposes how cutinases can be used to generate a PET catalytic cycle. Two cutinase molecules are activated which react with the carbonyl group of PET and form a serine-terephthalate complex and two ether compounds. These components go through a series of oxidation, hydrolysation and decarboxylation processes, producing a pyruvate and glyoxylate, which can then enter the Krebs cycle.

PE. One of the possible biodegradation pathways of PE, involves MnP. As suggested by Sanchez (2020), MnP gets activated in an acidic condition and start as a free radical. Once PE binds, the polymer is broken down into smaller pieces, such as ethanol and tetradecanoyl CoA. These molecules are oxidised or β-oxidised into acetic acid and acetyl CoA, respectively, and enter the Krebs cycle. Regarding the differences in degradation of LDPE and HDPE, LDPE has a branching system that is more accessible for oxidising enzymes due to the majority being amorphous with short branches [26]. HDPE on the other hand, has a higher molar mass, thus making it harder for microbes to access the polymer chains [26].

PS. No enzymes have been identified that initiate depolymerisation of PS, however, it is known that microorganisms bring about styrene metabolism [50]. A predominant pathway that has been shown to operate in bacteria involves the following enzymes: styrene monooxygenase, styrene oxide isomerase, phenylacetaldehyde dehydrogenase, and phenylacetyl coenzyme A ligase. Through a series of

reactions, PS is depolymerised into styrene, which is then oxidised to phenylacetate and can enter the Krebs cycle [13,50,51].

PS-PUR. Components important in the biodegradation of PS-PUR are the aromatic esters and the crystalline fraction [25]. No specific enzymes have been identified that degrade this polymer, but most likely, the ester bonds in the polyester polyol segment are hydrolysed by esterases and lipases [26]. The proteases also hydrolyse ester bonds, as well as amide and urethane bonds, while urea linkages are attacked by the ureases [25]. Nonetheless, there is still a lack of data on depolymerases that can cleave the robust urethane bonds [24].

PVC and PP. Lignin peroxidases have been shown to play a significant role in the degradation of PVC, however no schematic pathway has been proposed [32]. Similarly, no enzymes or pathways have been identified for the biodegradation of PP [26]. There are studies demonstrating the ability of microbes breaking down PVC and PP, though there needs to be more research done to identify the specific enzymes involved.

The biodegradation of each plastic generally follows the same catabolic pathway which yields energy that can be used for growth. However, having considered all the possible mechanisms for each type of plastic, it is clear that different enzymes and metabolic pathways are required for the degradation of different polymers. The aim of this literature review is to bring these systems together to overcome the issues of separating mixed plastics and creating an environmentally friendly waste disposal method. To achieve such an overview, a general perspective of all the papers used regarding fungal degradation can be seen below (Table 2).

Table 2. Biodegradation of the main types of plastics using *F. solani*, *P. chrysosporium*, and *Cephalosporium* sp.

Polymer used	Fungus Used	Substrate Preparation	Pre-treatment	Evaluation of Biodegradation	Degradation Achieved	Compared to other fungi & ranking	Medium	Temperature (°C)	pH	Timeframe (days)	References
HDPE	*F. solani*	HDPE strips	(i) 80°C for 120 h (ii) UV light for 10 days (iii) Nitric acid for 10 days	Shake flask method, weight loss, FESEM	Maximum degradation of treated & untreated HDPE: 2.65% & 1.69% after 60 days; 2.58% & 1.84% after 90 days	Yes (3) Best degrader	MSM	28	NA	60-90	[7]
	P. chrysosporium	Two strips: 1 x 6 cm 100 μm	No	Strips placed onto growing mycelium, under nitrogen- or carbon-limited conditions	Reduction in elongation & tensile strength, more so in nitrogen-limited than in carbon-limited condition. Same levels of degradation as IZU-154. Produced both LiP & MnP	Yes (3) Shares 1st place with IZU-154	MSM	30	4.5	12	[43]
	Cephalosporium sp.	4 x 4 cm 8 μm	Nitric acid	Shake flask method, gravimetric analysis, FTIR, SEM, X-ray diffraction	Weight loss of 7.18%	No	MSM	28	5.64	20	[18]
PVC	*F. solani*	100 mg polymer source	No	Agar plates, weight loss, SEM, FTIR	Weight loss of 0.24 g. No data on start weight or percentage of weight loss	Yes (10) 6th best degrader	MSM	37	NA	28-84	[41]
	P. chrysosporium	6 x 2.5 cm	No	Shake flask method, Sturm test, SEM, FTIR	Significant reduction of 178,292 Da^{-1} (control 200,000 Da^{-1})	Yes (4) Best degrader	MSM	30	NA	49	[22]
		0.1 g PVC film	No	Shake flask method, Sturm test SEM, FTIR	Weight reduction of 31%	No	MSM	25	5	28	[32]
PS	*P. chrysosporium*	Copolymer: LPS (10.3%, 32.2%, 50.4% lignin) Powder or 0.15 mm thick & 5-7 cm circular film Homopolymer: 0.25 mm thick & 7 cm in diameter	No	(i) Solid 2.5% agar (ii) Shake flask method Weight loss, UV spectroscopy, SEM, assay of enzyme activity	About 45% weight loss of PS in LPS 32 & LPS 50	Yes (/4) Shares 1st place with other fungus	MSM	25	NA	(i) 68 (ii) 21	[44]
		PS, PS:PLA, PS:PLA:OMMT Nanocomposite sheets	Blended	Shake flask method, weight loss, SEM, FTIR	17% degradation in the 30% composite PS:PLA 20.9% degradation in 5 phr PS:PLA:OMMT	No	MSM	Room temperature	7	28	[33]
	Cephalosporium sp.	4 x 4 cm	No	Shake flask method, FTIR, SEM, TGA	2.17% weight loss	Yes (/2) Best degrader	MSM	28	7	56	[36]

Polymer used	Fungus Used	Substrate Preparation	Pre-treatment	Evaluation of Biodegradation	Degradation Achieved	Compared to other fungi & ranking	Medium	Temperature (°C)	pH	Timeframe (days)	References
*lc*PET	*F. solani*	15 x 15 mm^2 250 μm	No	pH-stat measuring NaOH consumption versus time	Up to 5% weight loss	Yes (3) 2nd best degrader	Tris-HCl	40	8	4	[11]
LDPE	*F. solani*	Medium supplemented with LDPE powder (0.1%)	No	Agar plates, weight loss	Maximum degradation of 77.67%	Yes (5) Best degrader	MSM	35-30	NA	28	[35]
		1.5 x 1.5 cm	No	Shake flask method, weight loss, CO_2 evolvement test, FESEM, FTIR	Total weight loss of 13%	Yes (3) 3rd best degrader	NA	25	7	60	[10]
PS-PUR	*F. solani*	(i) 25 mg cube-shaped PS-PUR (ii) PS-PUR pieces (iii) 4 g/L powder in upper agar layer	No	(i) Shake flask method (ii) Direct plating (iii) 2-layered agar media	(i) 100% degradation (ii) 72.5% weight loss (iii) 70 mm diameter clear zone	Yes (4) (i & iii) Best degrader (ii) 2nd best degrader	Basal medium modified from [52,53]	30	NA	(i & ii) 21 (iii) 14	[23]
PP	*P. chrysosporium*	Pure PP, MI-PP, ST-PP 20 mg film	Blended Treated & untreated. 100°C or 10 days UV	Fungal biomass, gravimetric weight loss, SEM	In UV pre-treated MI-PP, 9.42% gravimetric weight loss & 84.2% TGA weight loss (at 400°C) (r = 0.49 correlation between laccase activity & weight loss)	Yes (2) 2nd best degrader	MSM	30	NA	365	[27]

MSM: minimal salt medium; FESEM: field emission scanning electron microscopy; SEM: scanning electron microscopy

6. Techniques to combine fungal cultures

It is more efficient to use a fungus that degrades multiple kinds of polymers, rather than mixing seven fungi for each type of plastic. Less organisms means fewer confounding variables. As such, three fungi that breakdown several types of plastics were considered in this literature review. Together, these fungal isolates degrade the seven main types of polymers. Furthermore, mixing the enzymatic systems and metabolic pathways requires little labour regarding separating and cleaning the plastic waste. Three strategies of mixing fungal strains are considered: (1) co-culturing, (2) sequential, and (3) knock-in.

Co-culturing. The first option would be to grow all three fungi together in a reactor that contains the plastic waste. The advantages of this technique are its efficiency and low cost. Co-culturing the isolates at the same time only requires single inoculation. After this, the mycelium can spread and start degrading the plastic. Moreover, co-culturing fungi can induce cell-cell interactions which may promote the activation of certain pathways that are often silent when grown under laboratory conditions [54]. Similarly, degradation may be promoted due to the synergy of the various enzymatic and metabolic pathways, which may increase the efficiency of utilising the substrate [9].

On the other hand, the disadvantage of co-culturing fungal isolates is that they are all grown under the same conditions. Variables such as the temperature, moisture and pH must be considered. For example, *P. chrysosporium* and a species of *Chrysosporium*, *C. gramineum*, produce the most enzymes around pH 5, whereas *F. solani* is most active at pH 7-8 (Table 2) [32,46,55]. In this way, the biodegradation of one fungus will be less efficient. Furthermore, the microbial communities have to be checked, whether these fungi are compatible. If they are not, the fungi will compete and initiate their defence pathways, meaning that the plastic degradation pathway will be less active. Ultimately, one of the fungal isolates will be eradicated, therefore also at least one type of plastic will not be degraded.

Sequential culturing. The issue with compatibility can be overcome by inoculating each fungus one after the other. Then, depending on the fungus that is grown at that time, conditions can be changed according to what is optimal for each isolate. However, one major issue of this method is that many plastic products are layered with different kinds of polymers. For example, food packaging is often composed out of multi-layered plastics, as it provides protection to the product inside, therefore enhancing shelf-life (See section 2.1) [30]. One likely arrangement is PET/PP/PE/polyamide [56]. If either *P. chrysosporium* or *Cephalosporium* sp. would be grown first, they both would be unable to degrade the food packaging from the outside and the inside, as they cannot breakdown PET or polyamide. Then, if *F. solani* was added last, it would breakdown PET, however, at the end of the cycle, both PP and PE would remain. This technique of sequentially growing each fungus on multi-layered plastics would be inefficient and require going back-and-forth between cultures. A possible solution to overcome this problem would be to pre-treat the plastic waste by shredding the products, thereby exposing more polymer surface including the different layers [57].

Knock-in enzymes. Instead of culturing all three fungi, another option would be to create a transgenic fungus that can produce all the enzymes that breakdown the different kinds of synthetic polymers. Ideally, a fungus is used that can withstand extreme conditions. This is because the biodegradation of plastics can be improved by incubating the fungus and polymer at temperatures close to the T_m and T_g of the polymer [11]. The next step would be to insert genes that code for the enzymes that are able to breakdown plastics. The advantage of this technique is that conditions can be made optimal, and there are no issues of compatibility or problems associated with multi-layered plastics. That said, not all

enzymes and genes involved in plastic degradation have been identified. One option would be to take the fungus with the unknown genome and place the already identified genes into this fungal isolate.

7. Discussion

There are some questions that need resolving before implementing mixing fungal cultures. More research needs to be conducted on whether one fungus can activate several enzymatic systems and metabolic pathways at the same time. Also, the duration of complete biodegradation of each type of plastic should be measured. Together, this information will provide insight into which technique of mixing fungal cultures is most efficient, and it will determine the ideal number of fungi that should be combined. For example, if multiple enzymatic systems cannot be activated simultaneously, it is not efficient to use the sequential and knock-in method, as the fungus can only breakdown each plastic one by one. On the other hand, if three fungi are grown together at the same time, then three types of plastics are broken down. Furthermore, research on the rate of degradation specifies the optimal number of fungi that can be mixed. For example, if the fungi are quick degraders, then more fungi can be combined.

The fungi selected in this literature review are not always the most efficient plastic degraders in comparison to other fungal isolates in terms of weight reduction. For example, *E. album* had twice as much PP degradation potential than *P. chrysosporium*, as shown by thermal gravimetric weight loss [27]. Similarly, *Aspergillus flavus* was able to degrade almost three times more PVC than *F. solani* [41]. Moreover, *F. solani* could not effectively degrade *lc*PET, as it lacked thermal stability and could not be incubated beyond 40°C [11]. Furthermore, no depolymerases have been identified that can degrade high crystalline PET (*hc*PET). There should not be a problem in breaking down PVC, as *P. chrysosporium* also breaks down this polymer, therefore complementing *F. solani*'s PVC degradation pathway. For *lc*PET and PP, an option could be to add additional fungi that are more efficient in degrading these polymers, such as *H. insolens* and *E. album*, respectively [11,27]. In the case of *hc*PET, more research must be conducted on other fungal species, or even bacteria, that are *hc*PET degraders. However, adding more fungi increases the chances of incompatibility or prolonging the degradation process.

The aim of this review is to find a solution to degrade the main types of plastic simultaneously to circumvent the issue of separating materials and removing the waste in an eco-friendly manner. Nonetheless, it is difficult to establish an optimal incubation condition that is ideal for the plastics and fungi. The biodegradation of plastic can be improved by incubating the fungus and polymer at temperatures close to the polymers' T_m and Tg. However, each polymer has its own thermal properties (Table 1). For example, the T_g of PET is 80°C, while the highest T_g is 112°C, for PS. This means that the fungi that are incubated, must have a high thermal stability to ensure accessibility to the polymer chains, thereby increasing plastic degradation. But researchers have demonstrated that the fungus' thermal stability limits the incubation temperature to reach optimal conditions. For example, *F. solani* was unable to reach PETs T_g, let alone 112°C [11]. Subsequently, out of the three techniques discussed on mixing fungal cultures, the knock-in method would be the best option if a fungus is selected that can endure extreme conditions. If a fungus can withstand 112°C, it will ensure that all the plastics can be optimally degraded.

Additionally, pre-treatments should be considered along with fungal biodegradation. Processes such as shredding, UV and thermal treatments have been shown to effectively help reduce polymer weight when treated with a fungus [7,27,49,57]. Especially for PE, PS, PP, and PVC, which have strong C-C bonds, these pre-treatments introduce oxygen molecules into the polymer backbone, thus facilitating the adhesion of the fungus and biodegradation. However, processes such as UV and thermal

treatments have a high cost, and their processes are becoming very complex and elaborate, thereby complicating scalability [57]. Hence, more research should be conducted on deciding which is the most efficient pre-treatment that compliments the degradation of polymers by fungi.

Still another issue remains. Nowadays there are almost no plastics produced that do not contain additives [28]. Additives are used to attain final functional properties such as flexibility or making products less flammable [58]. Unfortunately, these materials leach from plastic waste, even during recycling processes. Like plastics, they represent a hazard to the environment and organisms [28,59]. To prevent the harm of both plastics and additives, more research must be conducted on whether fungi that can degrade synthetic polymers, also breakdown additives.

8. Conclusion

A major issue concerning plastic waste is that these products are composed of multiple types of plastics. This makes it almost impossible to separate the materials for them to be processed properly for recycling. Unfortunately, other disposal waste options like incineration and landfill only add to the environmental problems by releasing toxins into the air and soil. An effective alternative to tackle plastic waste is bioremediation. Using microbe's natural polymer degrading processes, plastics can be broken down in an eco-friendly and low-cost manner. Most of all, fungi have been shown to be ideal degraders as they quickly colonise polymer surfaces and can reach places most microbes cannot. Moreover, some fungi degrade more than one type of plastic. This provides an ideal opportunity to identify these fungi and combine their abilities that together degrade the seven main types of plastics, without having to separate the materials.

In this literature review, three fungi were selected based on various research that demonstrate that they can degrade multiple kinds of plastics. *F. solani* breaks down *lc*PET, HDPE, LDPE, PVC, and PS-PUR. *P. chrysosporium* degrades HDPE, PVC, PP, and PS, while *Cephalosporium* sp. breaks down PP and PS. Combining the three fungi's enzymatic systems and metabolic pathways provides a synergy of processes that degrade the seven main types of plastics. Mixing the fungal isolates can be done by growing the cultures together at the same time, which is a very efficient method requiring little labour. The second option is to grow them one after the other to circumvent issues with compatibility and ensuring optimal conditions for all fungal isolates. The third option is to knock-in genes that are known to be involved in biodegradation into one fungus. Any of these three techniques are a promising solution to reduce plastic waste in a widely accepted manner that requires little labour, as no separation is required, and it has no harmful effects on the environment, and on animal and human health.

References

[1] S. Maity, S. Banerjee, C. Biswas, R. Guchhait, A. Chatterjee, K. Pramanick, Functional interplay between plastic polymers and microbes: a comprehensive review, Biodegradation. 32 (2021) 487–510. https://doi.org/10.1007/s10532-021-09954-x.

[2] C. Sánchez, Fungal potential for the degradation of petroleum-based polymers: An overview of macro- and microplastics biodegradation, Biotechnology Advances. 40 (2020) 107501. https://doi.org/https://doi.org/10.1016/j.biotechadv.2019.107501.

[3] M.K. Sangale, M. Shahnawaz, A.B. Ade, Potential of fungi isolated from the dumping sites mangrove rhizosphere soil to degrade polythene, Scientific Reports. 9 (2019). https://doi.org/10.1038/s41598-019-41448-y.

[4] PlasticsEurope, Plastics - The Facts 2020. An analysis of European plastics production, demand and waste data, 2020.

[5] L. Lebreton, B. Slat, F. Ferrari, B. Sainte-Rose, J. Aitken, R. Marthouse, S. Hajbane, S. Cunsolo, A. Schwarz, A. Levivier, Evidence that the Great Pacific Garbage Patch is rapidly accumulating plastic, Scientific Reports. 8 (2018) 1–15.

[6] R. Geyer, J.R. Jambeck, K.L. Law, Production, use, and fate of all plastics ever made, Science Advances. 3 (2017) e1700782.

[7] A. Rani, P. Singh, R. Kumar, Microbial deterioration of high-density polyethylene by selected microorganisms, Journal of Applied Biology & Biotechnology Vol. 8 (2020) 64–66.

[8] S.K. Kale, A.G. Deshmukh, M.S. Dudhare, V.B. Patil, Microbial degradation of plastic: a review, Journal of Biochemical Technology. 6 (2015) 952–961.

[9] H. Ballerstedt, T. Tiso, N. Wierckx, R. Wei, L. Averous, U. Bornscheuer, K. O'Connor, T. Floehr, A. Jupke, J. Klankermayer, L. Liu, V. de Lorenzo, T. Narancic, J. Nogales, R. Perrin, E. Pollet, A. Prieto, W. Casey, T. Haarmann, A. Sarbu, U. Schwaneberg, F. Xin, W. Dong, J. Xing, G.-Q. Chen, T. Tan, M. Jiang, L.M. Blank, MIXed plastics biodegradation and UPcycling using microbial communities: EU Horizon 2020 project MIX-UP started January 2020, Environmental Sciences Europe. 33 (2021) 99. https://doi.org/10.1186/s12302-021-00536-5.

[10] M.P. Das, S. Kumar, J. Das, Fungal-mediated deterioration and biodegradation study of low-density polyethylene (LDPE) isolated from municipal dump yard in Chennai, India, Energy, Ecology and Environment. 3 (2018) 229–236. https://doi.org/10.1007/s40974-018-0085-z.

[11] Å.M. Ronkvist, W. Xie, W. Lu, R.A. Gross, Cutinase-Catalyzed Hydrolysis of Poly(ethylene terephthalate), Macromolecules. 42 (2009) 5128–5138. https://doi.org/10.1021/ma9005318.

[12] K.D. Hyde, J. Xu, S. Rapior, R. Jeewon, S. Lumyong, A.G.T. Niego, P.D. Abeywickrama, J.V.S. Aluthmuhandiram, R.S. Brahamanage, S. Brooks, A. Chaiyasen, K.W.T. Chethana, P. Chomnunti, C. Chepkirui, B. Chuankid, N.I. de Silva, M. Doilom, C. Faulds, E. Gentekaki, V. Gopalan, P. Kakumyan, D. Harishchandra, H. Hemachandran, S. Hongsanan, A. Karunarathna, S.C. Karunarathna, S. Khan, J. Kumla, R.S. Jayawardena, J.K. Liu, N. Liu, T. Luangharn, A.P.G. Macabeo, D.S. Marasinghe, D. Meeks, P.E. Mortimer, P. Mueller, S. Nadir, K.N. Nataraja, S. Nontachaiyapoom, M. O'Brien, W. Penkhrue, C. Phukhamsakda, U.S. Ramanan, A.R. Rathnayaka, R.B. Sadaba, B. Sandargo, B.C. Samarakoon, D.S. Tennakoon, R. Siva, W. Sriprom, T.S. Suryanarayanan, K. Sujarit, N. Suwannarach, T. Suwunwong, B. Thongbai, N. Thongklang, D. Wei, S.N. Wijesinghe, J. Winiski, J. Yan, E. Yasanthika, M. Stadler, The amazing potential of fungi: 50 ways we can exploit fungi industrially, Fungal Diversity. 97 (2019). https://doi.org/10.1007/s13225-019-00430-9.

[13] A. Amobonye, P. Bhagwat, S. Singh, S. Pillai, Plastic biodegradation: Frontline microbes and their enzymes, Science of The Total Environment. 759 (2021) 143536. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.143536.

[14] I. Turku, T. Kärki, K. Rinne, A. Puurtinen, Characterization of plastic blends made from mixed plastics waste of different sources, Waste Management & Research. 35 (2017) 200–206.

[15] M. Al-Ali AlMa'adeed, I. Krupa, Introduction, in: M. Al-Ali AlMa'adeed, I. Krupa (Eds.), Polyolefin Compounds and Materials: Fundamentals and Industrial Applications, Springer International Publishing, Cham, 2016: pp. 1–11. https://doi.org/10.1007/978-3-319-25982-6_1.
[16] G. Zanchin, G. Leone, Polyolefin thermoplastic elastomers from polymerization catalysis: Advantages, pitfalls and future challenges, Progress in Polymer Science. 113 (2021) 101342. https://doi.org/https://doi.org/10.1016/j.progpolymsci.2020.101342.
[17] S.K. Ghosh, S. Pal, S. Ray, Study of microbes having potentiality for biodegradation of plastics, Environmental Science and Pollution Research. 20 (2013) 4339–4355.
[18] A.K. Chaudhary, R.P. Vijayakumar, Effect of chemical treatment on biological degradation of high-density polyethylene (HDPE), Environment, Development and Sustainability. 22 (2020) 1093–1104. https://doi.org/10.1007/s10668-018-0236-6.
[19] T. Hardin, Plastic: It's not all the same, Plastic Oceans International. (2021).
[20] A.M. Al-Sabagh, F.Z. Yehia, Gh. Eshaq, A.M. Rabie, A.E. ElMetwally, Greener routes for recycling of polyethylene terephthalate, Egyptian Journal of Petroleum. 25 (2016) 53–64. https://doi.org/https://doi.org/10.1016/j.ejpe.2015.03.001.
[21] D. Danso, J. Chow, W.R. Streita, Plastics: Environmental and biotechnological perspectives on microbial degradation, Applied and Environmental Microbiology. 85 (2019). https://doi.org/10.1128/AEM.01095-19.
[22] M.I. Ali, S. Ahmed, G. Robson, I. Javed, N. Ali, N. Atiq, A. Hameed, Isolation and molecular characterization of polyvinyl chloride (PVC) plastic degrading fungal isolates, Journal of Basic Microbiology. 54 (2014) 18–27.
[23] I.N. Ibrahim, A. Maraqa, K.M. Hameed, I.M. Saadoun, H.M. Maswadeh, Assessment of potential plastic-degrading fungi in Jordanian habitats, Turkish Journal of Biology. 35 (2011) 551–557.
[24] A. Magnin, L. Entzmann, E. Pollet, L. Avérous, Breakthrough in polyurethane bio-recycling: An efficient laccase-mediated system for the degradation of different types of polyurethanes, Waste Management. 132 (2021) 23–30. https://doi.org/https://doi.org/10.1016/j.wasman.2021.07.011.
[25] R. Wei, W. Zimmermann, Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: how far are we?, Microbial Biotechnology. 10 (2017) 1308–1322. https://doi.org/10.1111/1751-7915.12710.
[26] J. Ru, Y. Huo, Y. Yang, Microbial Degradation and Valorization of Plastic Wastes, Frontiers in Microbiology. 11 (2020) 442. https://doi.org/10.3389/fmicb.2020.00442.
[27] D. Jeyakumar, J. Chirsteen, M. Doble, Synergistic effects of pretreatment and blending on fungi mediated biodegradation of polypropylenes, Bioresource Technology. 148 (2013) 78–85.
[28] J.N. Hahladakis, C.A. Velis, R. Weber, E. Iacovidou, P. Purnell, An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling, Journal of Hazardous Materials. 344 (2018) 179–199. https://doi.org/https://doi.org/10.1016/j.jhazmat.2017.10.014.
[29] E. Mount, Chapter 6 - Coextrusion equipment for multilayer flat films and sheets, in: J.R. Wagner (Ed.), Multilayer Flexible Packaging, William Andrew Publishing, Boston, 2010: pp. 75–95. https://doi.org/https://doi.org/10.1016/B978-0-8155-2021-4.10006-1.
[30] C.T. de M. Soares, M. Ek, E. Östmark, M. Gällstedt, S. Karlsson, Recycling of multi-material multilayer plastic packaging: Current trends and future scenarios, Resources, Conservation and Recycling. 176 (2022) 105905. https://doi.org/https://doi.org/10.1016/j.resconrec.2021.105905.
[31] B.A. Morris, The science and technology of flexible packaging: multilayer films from resin and process to end use, William Andrew, 2016.
[32] N. Khatoon, A. Jamal, M.I. Ali, Lignin peroxidase isoenzyme: a novel approach to biodegrade the toxic synthetic polymer waste, Environmental Technology. 40 (2019) 1366–1375. https://doi.org/10.1080/09593330.2017.1422550.
[33] N. Shimpi, M. Borane, M. Kadam, S. Mishra, Effect of Organically Modified Montmorillonite (OMMT) on Biodegradation of PS: PLA and PS: PLA: OMMT using, Polymer Composites. (2015). https://doi.org/10.1002/pc.23694.

[34] S. Zahra, S.S. Abbas, M.-T. Mahsa, N. Mohsen, Biodegradation of low-density polyethylene (LDPE) by isolated fungi in solid waste medium, Waste Management. 30 (2010) 396–401. https://doi.org/https://doi.org/10.1016/j.wasman.2009.09.027.
[35] A. Rani, P. Singh, Screening of Polyethylene Degrading Fungi from Polyethylene Dump Site, International Journal of ChemTech Research. 10 (2017) 699–704.
[36] A.K. Chaudhary, R.P. Vijayakumar, Studies on biological degradation of polystyrene by pure fungal cultures, Environment, Development and Sustainability. 22 (2020) 4495–4508. https://doi.org/10.1007/s10668-019-00394-5.
[37] A. Šišić, J. Baćanović-Šišić, A.M.S. Al-Hatmi, P. Karlovsky, S.A. Ahmed, W. Maier, G.S. de Hoog, M.R. Finckh, The 'forma specialis' issue in Fusarium: A case study in Fusarium solani f. sp. pisi, Scientific Reports. 8 (2018) 1252. https://doi.org/10.1038/s41598-018-19779-z.
[38] M. Chen, J. Zeng, G.S. de Hoog, B. Stielow, A.H.G. Gerrits Van Den Ende, W. Liao, M. Lackner, The 'species complex' issue in clinically relevant fungi: A case study in Scedosporium apiospermum, Fungal Biology. 120 (2016) 137–146. https://doi.org/https://doi.org/10.1016/j.funbio.2015.09.003.
[39] A.M.S. Al-Hatmi, A.H.G.G. van den Ende, J.B. Stielow, A.D. van Diepeningen, K.A. Seifert, W. McCormick, R. Assabgui, T. Gräfenhan, G.S. de Hoog, C.A. Levesque, Evaluation of two novel barcodes for species recognition of opportunistic pathogens in Fusarium, Fungal Biology. 120 (2016) 231–245. https://doi.org/https://doi.org/10.1016/j.funbio.2015.08.006.
[40] M. Homa, L. Galgóczy, P. Manikandan, V. Narendran, R. Sinka, Á. Csernetics, C. Vágvölgyi, L. Kredics, T. Papp, South Indian Isolates of the Fusarium solani Species Complex From Clinical and Environmental Samples: Identification, Antifungal Susceptibilities, and Virulence, Frontiers in Microbiology. 9 (2018) 1052. https://doi.org/10.3389/fmicb.2018.01052.
[41] S. Sakhalkar, R.L. Mishra, Screening and Identification of Soil Fungi with Potential of Plastic Degrading Ability, Indian Journal of Applied Research. 3 (2013) 62–64.
[42] D. Singh, S. Chen, The white-rot fungus Phanerochaete chrysosporium: conditions for the production of lignin-degrading enzymes, Applied Microbiology and Biotechnology. 81 (2008) 399–417.
[43] Y. Iiyoshi, Y. Tsutsumi, T. Nishida, Polyethylene degradation by lignin-degrading fungi and manganese peroxidase*, Journal of Wood Science. 44 (1998) 222–229.
[44] O. Milstein, R. Gersonde, A. Huttermann, M.-J. Chen, J.J. Meister, Fungal biodegradation of lignopolystyrene graft copolymers, Applied and Environmental Microbiology. 58 (1992) 3225–3232.
[45] A.J.S. Alves, J.A. Pereira, T. Dethoup, S. Cravo, S. Mistry, A. Silva, M.M.M. Pinto, A. Kijjoa, A new meroterpene, a new benzofuran derivative and other constituents from cultures of the marine sponge-associated fungus Acremonium persicinum KUFA 1007 and their anticholinesterase activities, Marine Drugs. 17 (2019) 379.
[46] M.C. Quincke, T.D. Murray, C.J. Peterson, K.E. Sackett, C.C. Mundt, Biology and control of cephalosporium stripe of wheat, Plant Pathology. 63 (2014) 1207–1217.
[47] Britannica, The Editors of Encyclopaedia, Antibiotic, Encyclopedia Britannica. (2021). https://www.britannica.com/science/antibiotic (accessed December 21, 2021).
[48] N. Mohanan, Z. Montazer, P.K. Sharma, D.B. Levin, Microbial and Enzymatic Degradation of Synthetic Plastics, Frontiers in Microbiology. 11 (2020) 2837. https://www.frontiersin.org/article/10.3389/fmicb.2020.580709.
[49] A.K. Chaudhary, K. Chaitanya, R.P. Vijayakumar, Synergistic effect of UV and chemical treatment on biological degradation of Polystyrene by Cephalosporium strain NCIM 1251, Archives of Microbiology. 203 (2021) 2183–2191. https://doi.org/10.1007/s00203-021-02228-3.
[50] B.T. Ho, T.K. Roberts, S. Lucas, An overview on biodegradation of polystyrene and modified polystyrene: the microbial approach, Critical Reviews in Biotechnology. 38 (2018) 308–320.
[51] N.D. O'Leary, K.E. O'Connor, A.D.W. Dobson, Biochemistry, genetics and physiology of microbial styrene degradation, FEMS Microbiology Reviews. 26 (2002) 403–417. https://doi.org/10.1111/j.1574-6976.2002.tb00622.x.

[52] T. Nakajima-Kambe, Y. Shigeno-Akutsu, N. Nomura, F. Onuma, T. Nakahara, Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes, Applied Microbiology and Biotechnology. 51 (1999) 134–140.
[53] N. Şahin, A.Ü. TAMER, Isolation, characterization and identification of thiram-degrading microorganisms from soil enrichment cultures, Turkish Journal of Biology. 24 (2000) 353–364.
[54] R. Serrano, V. González-Menéndez, L. Rodríguez, J. Martín, J.R. Tormo, O. Genilloud, Co-culturing of Fungal Strains Against Botrytis cinerea as a Model for the Induction of Chemical Diversity and Therapeutic Agents, Frontiers in Microbiology. 8 (2017) 649. https://doi.org/10.3389/fmicb.2017.00649.
[55] P. Patel, Influence of Carbon-Nitrogen Supplements and pH on Growth of Sugarcane Stem rot Pathogen Fusarium Solani NVS671, Journal of Fungal Diversity. 1 (2020) 27–32.
[56] Cordis, A new method for separation full recovery of multilayer packaging waste to create high value materials - LAMPACK, (2019). https://cordis.europa.eu/project/id/736010 (accessed January 4, 2022).
[57] A. Lee, M.S. Liew, Ecologically derived waste management of conventional plastics, Journal of Material Cycles and Waste Management. 22 (2020) 1–10. https://doi.org/10.1007/s10163-019-00931-4.
[58] C. Sánchez, Microbial capability for the degradation of chemical additives present in petroleum-based plastic products: A review on current status and perspectives, Journal of Hazardous Materials. 402 (2021) 123534. https://doi.org/https://doi.org/10.1016/j.jhazmat.2020.123534.
[59] L. Hermabessiere, A. Dehaut, I. Paul-Pont, C. Lacroix, R. Jezequel, P. Soudant, G. Duflos, Occurrence and effects of plastic additives on marine environments and organisms: A review, Chemosphere. 182 (2017) 781–793. https://doi.org/https://doi.org/10.1016/j.chemosphere.2017.05.096.