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Fungal bioremediation of polyethylene: challenges and perspectives

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Abstract

Plastics have become ubiquitous in both their adoption as materials and as environmental contaminants. Widespread pollution of these versatile, man-made, and largely petroleum-derived polymers has resulted from their long-term mass production, inappropriate disposal, and inadequate end of life management. Polyethylene (PE) is at the forefront of this problem, accounting for one third of plastic demand in Europe in part due to its extensive use in packaging (European Parliament, 2020). Current recycling and incineration processes do not represent sustainable solutions to tackle plastic waste, especially once it becomes littered, and the development of new waste-management and remediation technologies are needed. Mycoremediation (fungal-based biodegradation) of PE has been the topic of several studies over the last two decades. The utility of these studies is limited by an inconclusive definition of biodegradation and a lack of knowledge regarding the biological systems responsible. This review highlights relevant features of fungi as potential bioremediation agents, before discussing the evidence for fungal biodegradation of both high- and low-density PE. An up-to-date perspective on mycoremediation as a future solution to PE waste is provided.

Keywords: bioremediation, plastic, polyethylene, biodegradation, fungi.

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Introduction

In a landmark study, Geyer *et al.* (2017) reported that of the 6.8 billion metric tonnes of plastic waste produced prior to 2015, approximately 20% of this was removed by recycling or incineration. The study concluded that if current production and waste management trends are maintained, 12 billion metric tonnes of plastic will be deposited in landfills or left unchecked to enter the environment by 2050. In efforts to establish new technologies for plastic waste management, considerable research investment has assessed whether microorganisms can utilise the commonly polluting polymers, and in doing so offer an environmentally friendly and sustainable solution to the current plastic crisis.

Although the term 'plastic' encompasses a wide range of high molecular weight polymers, the commercial plastics employed throughout society, for example as food and drink packaging, fibres for clothing, and materials in the construction industry, are limited to a handful of types. Of these, the simple alkyl-chain polymer polyethylene (PE) has the highest overall global production rate driven in part by its use in packaging (*Fig.1*) (Geyer *et al.,* 2017). PE is synthesised from ethylene into various forms (notably high-density PE (HDPE) and low-density PE (LDPE), but also other forms such as linear low density PE (LLDPE)) which differ in their degree of branching, molecular packing, and hence overall crystallinity and material density (*Fig.2*). Like other plastics, HDPE and LDPE are semi-crystalline in structure and the percentage crystallinity ($\%_{Cry}$) has a direct impact on polymer properties. For example, plastics with higher crystallinity tend to be more rigid, due to the stronger intermolecular forces associated with close chain packing.

Plastic waste has become omnipresent in natural and urban environments, and the impacts of its accumulation are yet to be fully understood. The world's oceans and waterways act as sinks for anthropogenic litter, with an estimated 12 million metric tonnes of plastic entering the marine environment in 2010 alone and this figure anticipated to increase by an order of magnitude by 2025 (Jambeck *et al.,* 2015). Abiotic processes (e.g. UV exposure and physical abrasion) drive the fragmentation of plastic debris into minute particles known as microplastics (diameter < 5mm) (Song *et al.,* 2017), and it is thought that most environmental plastic is present in this form, populating benthic regions and sedimentary habitats (Thompson *et al.,* 2004). Production figures are reflected in the composition of microplastic debris, which comprises predominantly PE, with other commodity polymers such as polypropylene (PP), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) at lower levels (Cheang *et al.,* 2018; de Haan *et al.,* 2019). The immediate harm caused from ingesting larger plastic items has been well documented in marine species (Avio *et al.,* 2017), though the consequences of long-term chronic exposure in human and wildlife populations remain unclear (Prata *et al.,* 2020). Aside from direct exposure, the mismanagement of plastic waste is linked to major health implications and increased

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mortalities in populations of low- and middle-income countries as a result of its open burning and blocking of waterways leading to increased rates of respiratory and waterborne diseases, respectively (Williams *et al*., 2019).

At present, solutions to tackle the global plastic waste crisis are neither sufficient nor sustainable. Recycling is effective in delaying disposal, though it can only reduce future plastic waste generation if it is able to replace primary plastic production, and less than a fifth of all plastic waste produced has been recycled to date (Geyer *et al.,* 2017). Although the recycling of polyolefins like HDPE and LDPE is widely available, it typically requires the addition of virgin material, and cannot be applied indefinitely due to an eventual reduction in the quality of recycled plastic (Garcia and Robertson, 2017). Incineration can be used to produce energy alongside waste disposal; however, only a fraction of plastic is treated in this manner and controlling the release of toxic combustion products (e.g. dioxins) is dependent on plant emission technologies, which may not be accessible for developing nations (Nkwachukwu *et al.,* 2013).

This review considers adaptations of fungi that may be inherently suited to the degradation of high molecular weight polymers, along with providing an up-to-date overview of PE mycoremediation studies together with the techniques and conditions used to assess biodegradation. Bacterial bioremediation is not included as this has been the subject of numerous other reviews (e.g. Restrepo-Flórez *et al.,* 2014; Krueger *et al.,* 2015a; Ghatge *et al*., 2020) and is outside the scope of this review. The limited knowledge surrounding the identification of pathways and processes involved is highlighted as an area of this field which requires greater attention before mycoremediation can be considered as a conceivable technology for plastic waste management.

Biodegradation of plastics: the challenge of PE

PE represents a considerable challenge to microorganisms due to its hydrophobicity, large molecular dimensions, and lack of reactive functional groups in the polymer backbone. Microbial consumption of long chain aliphatic compounds is well documented (Haines and Alexander, 1974), and bacteria and fungi have been shown to consume n-alkanes such as tetratetracontane ($C_{44}H_{90}$ = 619 g mol^{-1}; Sakai *et al.,* 1994) and pentacontane ($C_{50}H_{102}$ = 703 g mol^{-1}; Yamada-Onodera *et al.,* 2002), respectively. Although n-alkanes resemble LDPE and HDPE, the molecular weights of the polymers are considerably higher (LDPE ≤ 50,000 g mol^{-1} and HDPE ≤ 200,000 g mol^{-1}) due to the lengths of carbon chains involved (Kurtz, 2004). A stepwise model is proposed for the microbial biodegradation of high molecular weight polymers (*Fig.3*; adapted from Lucas *et al.,* 2008, Restrepo-Flórez *et al.,* 2014, and Wei and Zimmerman, 2017).

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Introduction of functional groups (e.g. carbonyl (C=O) and hydroxyl (O-H) groups) into PE via oxidative processes marks the first stage of its degradation (*Fig.3*). For effective utilisation, the long hydrocarbon chains are likely to be cleaved to produce monomeric, dimeric, and oligomeric breakdown products of an amenable size for assimilation through the cellular membrane (*Fig.3*). Biological depolymerisation (referred to as biofragmentation) has been shown for PUR and PET via the activity of secreted extracellular enzymes (Nimchua *et* al., 2007; Russel *et al.,* 2011; Austin *et al.,* 2018), although there is currently no evidence that confirms specific catalytic mechanisms in the fragmentation of the polyolefin backbone (Wei and Zimmerman, 2017). The biodegradation of plastics has the potential for desirable outcomes such as mineralisation of the polymer into simple products (H_2O, CO_2); or the depolymerisation and/or valorisation of breakdown products for re-use (*Fig.3*) (Ru *et al.,* 2020; Tournier *et al.,* 2020). Early experiments involving long-term soil incubations of radiolabelled ^{14}C-PE (Albertsson, 1978; Albertsson and Bánhidi, 1980) are suggestive of microbial degradation being restricted to the low molecular weight fraction, and substantial assimilation and mineralisation of this highly recalcitrant polymer remains to be documented.

Studies on plastic biodegradation, including that of PE, have investigated both bacterial and fungal species as potential degraders (example reviews include Restrepo-Flórez *et al.,* 2014; Krueger *et al.,* 2015a; Danso *et al.,* 2019; Montazer *et al.,* 2020; Ru *et al.,* 2020). The saprotrophic lifestyles, extracellular oxidative machinery, and morphological characteristics of fungi suggest them as a promising and relatively unexplored avenue for PE breakdown, and several filamentous species have been reported to deteriorate PE to some extent (see supporting information: *Table S1* and *Table S2* for a detailed summary).

Nature's recyclers: fungi as degraders

Fungi are a group of highly diverse eukaryotic organisms that are ubiquitous to almost all aerobic and several anaerobic ecosystems, wherein they function as saprobes, symbionts and parasites (Peay *et al.,* 2016). The fungal saprobes are typically filamentous, exhibiting active translocation of resources around the mycelial network to achieve growth into poorly bioavailable substrates (Lindahl and Olsson, 2004).

Wood-rotting fungi have evolved different mechanisms to utilise lignocellulose as a growth substrate. White-rot fungi produce a range of extracellular oxidoreductases that target lignin and counter its heterogeneity with their low substrate specificity (Pollegioni *et al.,* 2015). Lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase (*Fig.4*) are the best studied of these oxidoreductases and have different substrate ranges dependent on their respective redox potentials (reviewed in Harms *et al.,* 2011 and Pollegioni *et al.,* 2015). Studies linking the biodegradation of PE to any one enzyme are scarce,

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however, the activities of LiP, MnP, and laccase have all been associated with the biodegradation of PE by fungal species (Iiyoshi *et al.,* 1998; Mukherjee and Kundu, 2014; Kang *et al.,* 2019; Zhang *et al.,* 2020) and some efforts at enhancing their action have been made. All three enzymes most typically attack the phenolic subunits of lignin but may also attack non-phenolic subunits – which PE most closely resembles – with the addition of mediators (Datta et al., 2017; Kumar and Chandra, 2020). In particular, the addition of small organic compounds (1-hydroxybenzotriazole, Fujisawa *et al*., 2001; ethanol, Volke-Sepúlveda *et al*., 2002; lignin and hemicellulose residues, Mukherjee and Kundu, 2014; sucrose, Sáenz *et al*., 2019) to enhance PE biodegradation has led to some success. Additionally, several extracellular fungal hydrolases and cutinases have shown activity against polymers such as PET and PUR (e.g. Nimchua *et al.,* 2007; Russel *et al.,* 2011; Ferrario *et al.,* 2016).

The non-enzymatic mechanism by which brown-rot fungi attack lignocellulose is comparatively less well understood. Initial lignin disruption is thought to be driven via the action of hydroxyl radical (OH•) formation from extracellular Fenton chemistry which centres around the production of reactive oxygen species from H_2O_2 and Fe^{2+} (*Fig.4*). Fenton-driven oxidation of high-molecular weight polymers, and the potential of brown-rot fungi as biodegradation agents, remains relatively overlooked. Krueger *et al.* (2015b) reported the depolymerisation of a water-soluble polystyrene analogue (polystyrene sulfonate) through the application of hydroquinone-driven Fenton reactions originating from *Gloeophyllum trabeum*. Transferring this approach to polystyrene achieved poor results, highlighting the obstacle that bioavailability plays in the biodegradation of these polymers (Krueger *et al.,* 2017).

Filamentous species of fungi possess a family of small globular proteins (< 20 kDa) known as hydrophobins that form amphipathic monolayers at hydrophobic-hydrophilic interfaces. Hydrophobins are suggested to play a role in improving nutrient utilisation via the formation of compact substrate-enzyme-fungus relationships (Brown *et al.,* 2016). Reducing the hydrophobicity of high molecular weight plastics may be beneficial for promoting enzymatic degradation by bridging the solid-liquid interface. The importance of surface interactions is further evidenced by the improved degradation of PE following the addition of surfactants (Albertsson *et al.,* 1993; Yamada-Onodera *et al.,* 2001; Orr *et al.,* 2004).

The assimilation of higher n-alkanes is common amongst species of ascomycetous and basidiomycetous yeasts, and this is mainly believed to be due to the action of cytochromes P450, especially CYP52 family members, in the primary hydroxylation step (Prenafeta-Boldú *et al*., 2019). A role has also been shown for CYP52 in n-alkane hydroxylation in various filamentous species (Huarte-Bonnet *et al*., 2018). Subsequent oxidation by alcohol dehydrogenase and aldehyde dehydrogenase forms fatty acids, which enter the β-oxidation pathway as their coenzyme A derivatives (Prenafeta-Boldú et al., 2019) for assimilation.

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Regardless of whether the primary oxidation step is catalysed by a cytochrome P450 or by a lignin-degrading enzyme, it is likely that similar processes could be used for the later steps in the degradation of PE, especially with the involvement of Baeyer-Villiger monooxygenases (Butinar et al., 2015) to convert ketone intermediates into hydrolytically cleavable esters.

Evaluation of current experimental approaches to determine biodegradation

A synopsis of relevant studies on the biodegradation of HDPE and LDPE by fungi is provided in the supporting information (*Table S1* and *Table S2*, respectively). Studies were limited to those published in peer reviewed journals within the last two decades and that used two or more techniques to evaluate biodegradation.

Isolating fungal strains from plastic-abundant landfill (Esmaeili *et al.,* 2013; Muhonja *et al.,* 2018) or heavily polluted natural environments (e.g. mangrove stands; Ameen *et al.,* 2015) is a common starting point. This is followed by an enrichment step where PE is present as the sole source of carbon and fungal strains are selected based upon growth rate, or the clearing of PE granules from agar (Brunner *et al.,* 2018). Using either of these tests as conclusive evidence for biodegradation is not sufficient, though they remain useful as a screening step. Biodegradation assays are predominantly carried out in a carbon-limited medium where PE is the primary source of carbon, though additional substrates/co-metabolites (Volke-Sepúlveda *et al.,* 2002; Mukherjee and Kundu, 2014; Kang *et al.,* 2019) or surfactants (Yamada-Onodera *et al.,* 2001), and the overall optimisation of nutritional content (Iiyoshi *et al.,* 1998; Ojha *et al.,* 2017; Sáenz *et al.,* 2019) have all been used to facilitate, and in some cases improve, the biodegradation process. A second point of difference lies with the choice of PE itself. Additives (e.g. UV stabilisers, antioxidants) in HDPE and LDPE are a possible source of interference when using commercially available polymers as test pieces. Additive-free PE can be obtained via recrystallisation of virgin PE powder using xylene (Hasan *et al.,* 2007), though this method of preparation may cause fragmentation or other effects on the polymer structure (Montazer *et al.,* 2020), and may not be reflective of plastic used in industry. Finally, the plastic may be subject to physical (e.g. UV-irradiation, γ-irradiation, thermal oxidation) or chemical (application of strong acids, e.g. HNO_3) pre-treatment (PT) prior to fungal incubation. Early studies highlighted PT as a way of increasing the propensity for biodegradation through promoting oxidation of the polymer and hence increasing the susceptibility to biological attack (Albertsson *et al.,* 1987).

(Bio)deterioration

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Evidence for the deterioration of PE is obtained through measuring changes in both the chemical and physical properties of the polymer. The non-destructive method of Fourier-Transform Infrared Spectroscopy (FTIR) can be used to detect chemical changes in the composition of PE after abiotic/biotic treatment. Studies which perform a PT step report the introduction of C=O groups (Raut *et al.,* 2015; Sheik *et al.,* 2015; Awasthi *et al.,* 2017), or likewise an increase in the carbonyl index (C.I. – the ratio of C=O peaks to CH_2; Balasubramanian *et al.,* 2014). Changes in the level of unsaturation (C=C) are also detected, with an increase following PT attributed to photochemical cleavage (Manzur *et al.,* 2004; Esmaeili *et al.,* 2013). Upon biotic treatment, a decrease in C=O and/or further increase in level of unsaturation is frequently observed, with the proposed explanation being that the carbonyl groups undergo enzymatic attack (Yamada-Onodera *et al.,* 2001; Volke-Sepúlveda *et al.,* 2002; Balasubramanian *et al.,* 2014; Mukherjee and Kundu, 2014). Chemical changes have been detected following the fungal treatment of PE without it having first undergone PT (Paço *et al.,* 2017; Das *et al.,* 2018; Muhonja *et al.,* 2018), though it remains unclear whether this is a product of fungal oxidative damage or an artefact of interactions with dissolved/atmospheric oxygen present in the system (Devi *et al.,* 2015). Rouillon *et al.* (2016) reported that using C.I. to quantitatively determine polyolefin oxidation may not be as sensitive as once thought due to oxidation products (e.g. acetic acid) entering the gas phase in the early stages of the photodegradation process. The authors instead suggested measuring the decrease in CH_3 absorbance as this parameter was better correlated to changes in crystallinity and molecular weight. Regardless of the proxy chosen to measure oxidation, it is important to utilise this analytical technique in combination with others. For example, contact angle measurements report on the hydrophilicity of PE (where smaller contact angles indicate greater spreading of water droplets, and hence greater hydrophilicity), and hence the level of surface oxidation. Awasthi *et al.* (2017) reported a reduction in the contact angle of HDPE from 98.6 ± 3.5° to 67 ± 2.6° after incubation with *Rhizopus oryzae*. It has however been noted that the usual binary interpretation of static contact angles (<90º being hydrophilic and >90º being hydrophobic) is simplistic, and that additional information about surface wettability and surface adhesion can be obtained from the advancing and receding contact angles respectively during the measurement (Law, 2014).

High resolution imaging can give supporting evidence of both the physical association of mycelia with the surface of PE (Gajendiran *et al.,* 2016), and upon removal of biomass, the localised development of pits, cracks, and material corrosion (Paco *et al.,* 2017; Das *et al.,* 2018; Sáenz *et al.,* 2019). Increased surface penetration (Manzur *et al.,* 2004) and colonisation (Raut *et al.,* 2015) by fungi have been reported following PT, and when nutritional conditions were enhanced (i.e. the addition of co-metabolites) a higher degree of surface deterioration was witnessed (Volke-Sepúlveda *et al.,* 2002; Kang *et al.,* 2019). Coupling

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imaging with atomic force microscopy (AFM) can further validate the surface deterioration of PE via fungal colonisation (Gajendiran et al., 2016). In general, though, imaging tends to provide qualitative or semi-quantitative information about the deterioration of the plastic.

Many reports of plastic becoming brittle, soft, or impressionable following its extended incubation with fungi allude to a deterioration of the mechanical properties of PE (Iiyoshi *et al*., 1998; Mathur *et al*., 2011; Esmaelie *et al*., 2013; Raut *et al*., 2015; Awasthi *et al*., 2017). Measuring changes in tensile strength, percentage elongation at break, and Youngs Modulus (or Secant modulus for non-Hookean polymers) can be achieved using a tensometer and can provide valuable insights into the level of physical deterioration present throughout the polymer. Care should be taken to account for variation in the results that may be caused by irregularities in the untreated polymer structure itself, or by using non-standard testing parameters. It is important to perform any test under standardised conditions and at an appropriate loading rate pull speed since the tensile strength and deformation mechanisms of thermoplastics are highly time and temperature dependent. Standard test pieces and pull conditions are designed to minimise the effects of stress concentrations enabling comparative testing regimes (Higgins, 1997). The parameters used vary widely between papers reporting biodegradation (e.g. Iiyoshi *et al*., 1998 and Esmaelie *et al*., 2013), rarely fulfilling engineering standardised protocols for testing, which presents a significant challenge for interpretation of results.

(Bio)fragmentation

Due to its simplicity, weight loss is routinely measured in PE biodegradation studies. The microbial degradation of plastic is a relatively slow process, and therefore, although chemical and physical (i.e. mechanical/surface) deterioration may be prevalent, detecting material loss after long periods of biotic treatment can return limited results (Krueger *et al*., 2015a). Comparing weight loss figures in the studies reviewed (Table S1 & Table S2) does, however, indicate increased LDPE biodegradation over HDPE, enhancement of biodegradation following a PT step (Balasubramanian *et al.,* 2014; Sheik *et al.,* 2015), and variation in biodegradative capacity within and between genera (Alsherehrei, 2017; Muhonja *et al.,* 2018; Sáenz *et al.,* 2019). In a small subset of studies, considerable (>40%) weight losses have been reported over 15–90 days (Mukherjee and Kundu, 2014; Raut *et al.,* 2015; Alsherehrei, 2017; Paço *et al.,* 2017). Although an inexpensive and accessible measurement, weight loss must not be relied upon as the sole confirmation of biodegradation, as measurements may be bolstered by the leaching or fungal consumption of chemical additives (Danso *et al.,* 2019; Montazer *et al.,* 2020).

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Perhaps a more substantial measurement to determine material loss/depolymerisation of PE is that of molecular weight distribution. Plastics exhibit polydispersity; that is, they are composed of polymer chains of varying length and degree of branching, with the molecular weight distribution of these chains reflecting the overall properties of the polymer (e.g. ultra-high molecular weight PE is made up of on average longer chains than HDPE or LDPE). This property can be described in two ways; M_n (number-averaged molar mass) and M_w (weight-averaged molar mass), with the former being calculated from the mole fraction distribution (i.e. M_n = total mass/total number of molecules), and the latter calculated from the weight fraction distribution (M_w takes into account that larger molecules contain more mass than smaller molecules and therefore is weighted according to weight fractions). These values may be measured experimentally by size exclusion chromatography (Yamada-Onodera *et al.,* 2001; Zahra *et al.,* 2010), or indirectly via rheological assessments such as viscosity (high viscosity indicates high molecular weight; Sheik *et al.,* 2015). Early experiments involving the solvent extraction of HDPE to deprive it of its low molecular weight fraction (i.e. shorter polymer chains) resulted in a significant reduction in the level of biodegradation, suggesting that microorganisms were restricted to acting upon these shorter chains in a similar manner to the metabolism of n-alkanes (Albertsson and Banhidi, 1980). Measuring the molecular weight distribution is therefore an important method of determining whether biodegradation is limited as described above, or whether it takes place on a more global manner for species capable of fragmenting the polymer. Recently, *Aspergillus flavus* isolated from the gut of the PE-devouring wax moth *Galleria mellonella* was shown to significantly reduce both the M_w and M_n of HDPE particles over 28 days, with the up-regulation of two laccase-like multicopper oxidases (Zhang *et al.,* 2020).

Compositional changes to the semi-crystalline structure of both HDPE and LDPE (i.e. $\%_{Cry}$, mean crystallite size) have been detected using X-ray diffraction (XRD; Esmaeili *et al.,* 2013; Raut *et al.,* 2015), wide-angle X-ray scattering (WAXS; Manzur *et al.,* 2004), differential scanning calorimetry (DSC; Volke-Sepúlveda *et al.,* 2002; Sheik *et al.,* 2015), FTIR (Devi *et al.,* 2015), and Raman spectroscopy (Kang *et al.,* 2019). An increase in crystallinity following fungal incubation is reported in several studies (Sheik *et al.,* 2015; Devi *et al.,* 2015; Kang *et al.,* 2019), with the consumption of the more amenable amorphous region of the polymer presented as a possible explanation (*Fig.5*). Such regions are generally made up of shorter branches, with reduced molecular packing which allows increased enzymatic access, and contain a greater number of terminal carbon groups that are more susceptible to photo-oxidation (Gewert *et al.,* 2015). Moreover, the insolubility of oxygen within crystalline phases (Iida *et al.,* 1987), coupled with a higher degree of structural impurities in amorphous regions (Huzayyin *et al.,* 2010), will further increase oxidation in the latter, and hence provide a more substantial location for fungal attack. In some cases, this

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initial increase in $\%_{Cry}$ is followed by a decrease such as that observed by Manzur *et al.* (2004) after biotic treatment of thermally pre-treated LDPE film with *A. niger* and *Penicillium pinophilum*. Similar overall decreases in $\%_{Cry}$, together with a decreased thickness of the crystalline lamellar and an increase in the mean crystallite size, have also been reported by Volke-Sepúlveda *et al.* (2002). Overall decreases in $\%_{Cry}$ tend to be reported following longer incubation periods (Volke-Sepúlveda *et al.,* 2002; Manzur *et al.,* 2004; Esmaeili *et al.,* 2013). Biodegradation likely takes places preferentially in the amorphous regions due to increased susceptibility to enzymatic degradation (*Fig.5*), thereby explaining the increase in $\%_{Cry}$ which is regularly observed within a 90-day period. During extended incubation periods, smaller crystals located at the amorphous-crystalline interface may undergo breakdown, explaining both the $\%_{Cry}$ decrease and overall increase in the average crystallite size observed by Volke-Sepúlveda *et al.* (2002) and Manzur *et al.* (2004) (*Fig.5*). An outlier to this proposed model is Raut *et al.* (2015), wherein a decrease in both $\%_{Cry}$ and average crystallite size was observed following a 90-day incubation of pre-treated LDPE with *Curvularia lunata*. Despite some evidence pointing towards tentative reductions in crystalline regions, these highly organised, tightly packed molecular networks are unfavourable to substantial attack from larger macromolecular enzymes due to restricted access. Lucas *et al.* (2008) highlighted the ability of wood rotting fungi to encourage the generation of free radicals (e.g. OH•) and oxidants (e.g. Mn^{3+}, H_2O_2) which can access the semi-crystalline structure of lignocellulose, a process which may circumvent the problems associated with bulky enzyme access to crystalline regions of PE.

Assimilation and mineralisation

The identity of assimilated chemical species in the case of PE biodegradations remains largely undetermined, however, GC-MS has been used to detect possible breakdown products following its fungal treatment (Balasubramanian *et al.,* 2014; Muhonja *et al.,* 2018; Sangale *et al.,* 2019). Incubation of pre-treated HDPE with *A. terreus* resulted in the detection of several straight chain and branched carboxylic acids (ranging from acetic acid ($C_2H_4O_2$), up to docosanoic acid ($C_{22}H_{44}O_2$)), together with their corresponding alkanes (Balasubramanian *et al.,* 2014). Known plastic additives such as phthalate esters and their derivatives (e.g. phthalic acid) have also been detected (Sangale *et al.,* 2019). The passage of fragmentation products through the fungal cell membrane may occur in a manner similar to that of the active transport mechanism which operates in the n-alkane assimilating *Yarrowia lipolytica* (Bassel and Mortimer, 1985). Additionally, the production of biosurfactants for the purpose of hydrocarbon emulsification and subsequent membrane sorption has been implicated in yeasts (Hisatsuka *et al.,* 1977; Cirigliano and Carman, 1984), and in a number of filamentous species (Kirk and Gordon, 1988; Al-Hawash *et al.,* 2018). Based on the model of n-alkane metabolism, once across the cellular membrane breakdown

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products become oxidised (where appropriate) and converted to acyl-coenzyme A derivatives, which undergo β-oxidation to acetyl-CoA, eventually entering the tricarboxylic acid (TCA) cycle leading to ATP synthesis. It is worth noting that despite these promising characteristics, at the time of writing the literature search found no examples of yeasts successfully biodegrading PE.

Direct evidence for assimilation and mineralisation of PE in the form of isotopic radiolabelling is confined to a series of early, long-term experiments (Albertsson, 1978; Albertsson and Bánhidi, 1980; Albertsson *et al.,* 1987). When investigating the biodegradation of ^{14}C-HDPE buried in soil, Albertsson (1978) recorded minimal release of $^{14}CO_2$ over a period of two years (0.5% of total polymer radioactivity). A basal evolution of $^{14}CO_2$ was recorded in the absence of microbial growth indicating an abiotic background breakdown. Hypothesising that the low level of biotic mineralisation was a result of biodegradation being limited to the more accessible short-chain fraction of HDPE, Albertsson and Bánhidi (1980) deprived ^{14}C-HDPE of this molecular weight fraction using cyclohexane and observed a 50% reduction in $^{14}CO_2$ evolution. Quantifying the consumption or production of O_2 and CO_2, respectively, may also be used to estimate the mineralisation of PE. This is commonly carried out by capturing and subsequently measuring CO_2 produced within an enclosed biodegradation system (where PE is the sole carbon source) either via titrimetry (Esmaeili *et al.,* 2013; Ameen *et al.,* 2015; Gajendiran *et al.,* 2016; Das *et al.,* 2018) or through GC-MS (Volke-Sepúlveda *et al.,* 2002; Manzur *et al.,* 2013). The variation in which authors report CO_2 evolution (e.g. % biodegradation of the sample, g L^{-1} CO_2 evolved) makes it difficult to compare results, however, in most cases the level of mineralisation remains small even over incubation periods of months to years.

Measuring $^{14}CO_2/CO_2$ evolution primarily accounts for carbon which undergoes full aerobic metabolism, whereas it is possible that the atoms may be incorporated into phospholipids or stored as carbohydrates. In the case of co-metabolic breakdown, PE breakdown products may not undergo substantial assimilation by the degrading organism at all, therefore providing a possible explanation for some of the disparity in reported mineralisation levels. Utilising a stable isotopic labelling technique (e.g. ^{13}C) coupled with compound specific isotope analysis would prove a useful tool to effectively track the fate of PE breakdown products in the context of fungal biodegradation. This has already been successful in tracking the fate of PE microplastic in microbial food webs (Taipale *et al.,* 2019).

Future perspectives and conclusions

Plastics are superior to many of the materials they have come to replace and have provided ways of keeping food fresher for longer, reducing potential for contamination in clinical equipment, and the

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formulation of strong yet low-weight materials for construction. Human reliance on plastics is unlikely to diminish, therefore new, more sustainable technologies are required for their end-of-life management. The ability of diverse fungal species to induce changes in the chemical and physical properties of both HDPE and LDPE, fragmentation of the polymer chains, and (to a lesser extent) the assimilation and mineralisation of this polymer is evident from the biodegradation studies presented. Before mycoremediation can become a tangible solution for reducing plastic waste, several advances must be made in this research field. As Montazer *et al.* (2020) have recommended, studies would benefit from the development of a standardised methodology, the adoption of a clear definition of biodegradation itself, and maximising the number of techniques employed to detect biodegradative processes. Emphasis is placed regarding the latter point as this will allow differentiation between the detection of superficial surface deterioration and the more desirable biofragmentation and utilisation of the plastic itself that is necessary for substantial remediation to be achievable.

A principal point of future investigation lies in elucidating and understanding the biological systems responsible for the chemical and physical damage observed. To date, very few enzymes (bacterial or fungal origin) have been implicated in polymer degradation, and those that have been characterised are confined to the more susceptible, hydrolysable backbones of PET and PUR (Wei and Zimmerman, 2017). A number of studies have measured deterioration of PE with a concomitant increase in the activity of LiP, MnP, and laccase (Nwogu *et al.,* 2012; Mukherjee and Kundu, 2014; Sowmya *et al.,* 2015, Kang *et al.,* 2019; Zhang *et al.,* 2020), and in addition the cell-free application of such enzymes has led to measurable molecular weight changes (Iiyoshi *et al.,* 1998; Ehara *et al.,* 2000; Fujisawa *et al.,* 2001). As discussed by Krueger *et al.* (2015a), the direct oxidation of the carbon backbone by ligninolytic machinery is questionable due to the much higher redox potentials required. It is more likely that these enzymes can act upon functional groups introduced into the polymer chain via oxidation-promoting processes. Further investigations aimed at understanding their catalytic contributions are merited. Utilising 'omic' methods to achieve a global picture of pathway up-regulation, nutrient fluxes, and enzyme induction represents a powerful approach to achieve a better understanding of the biology of mycoremediation. Although the major goal of these studies reviewed here is the remediation of PE waste, understanding the process that biodegradation of plastics may have on the environment is of great importance from an ecological perspective. Relatively few studies have assessed the potential toxicity of PE breakdown products (Shahnawaz *et al.,* 2016; Sangale *et al.,* 2019), and this remains an important area of investigation.

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Conflict of Interest

SCM is a Trustee of SfAM. No other conflict of interest declared.

Author contributions

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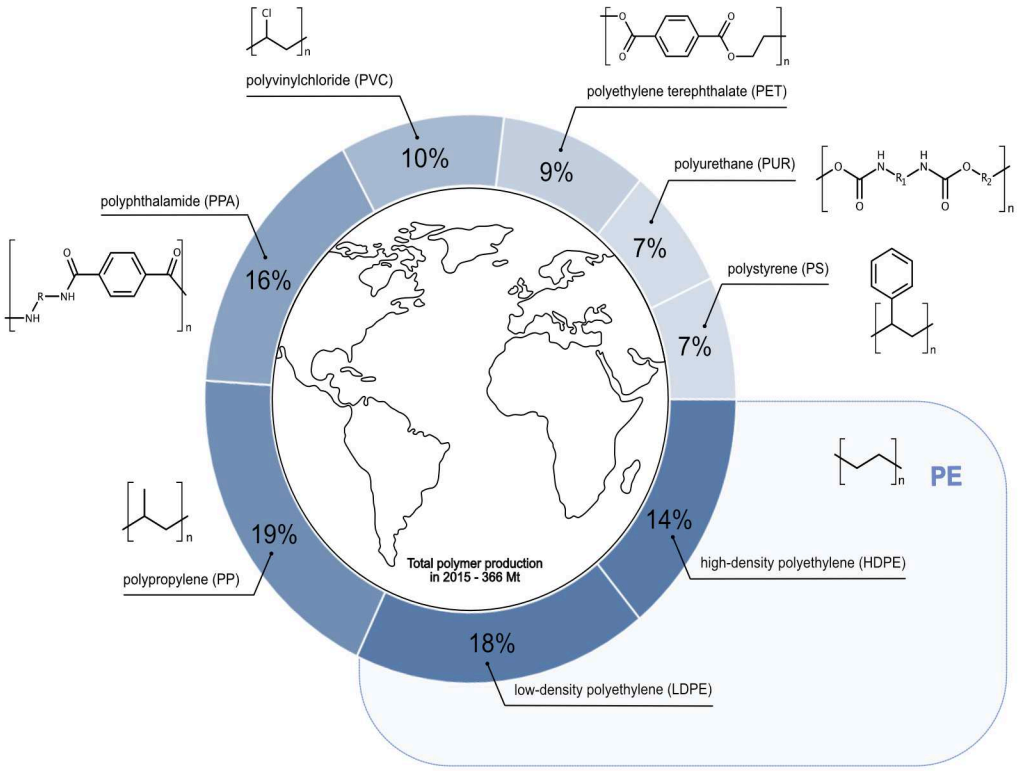

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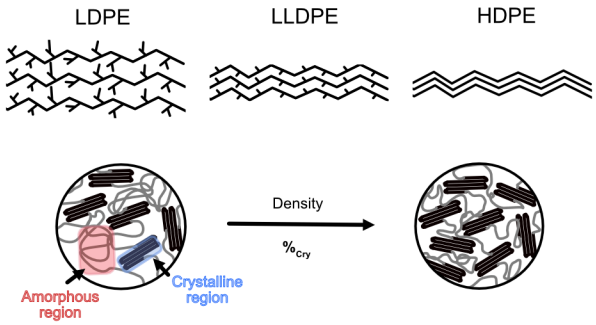

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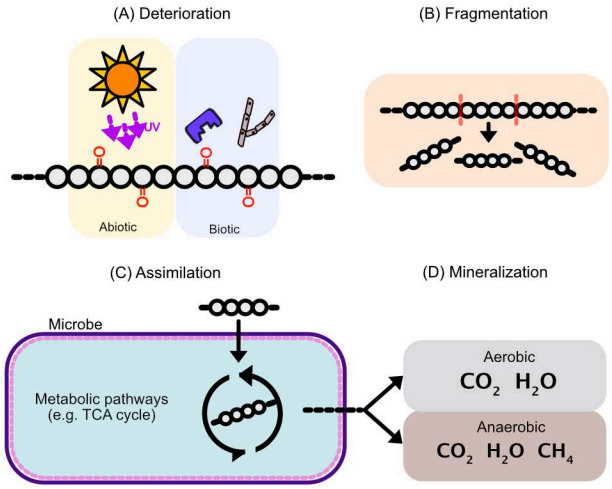

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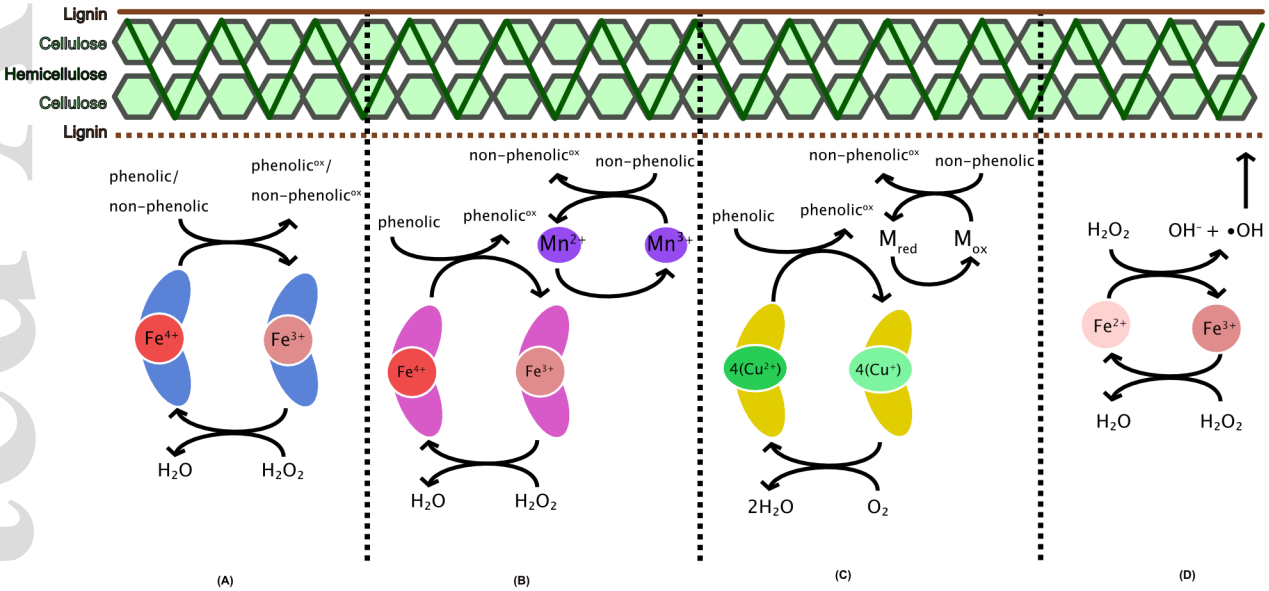

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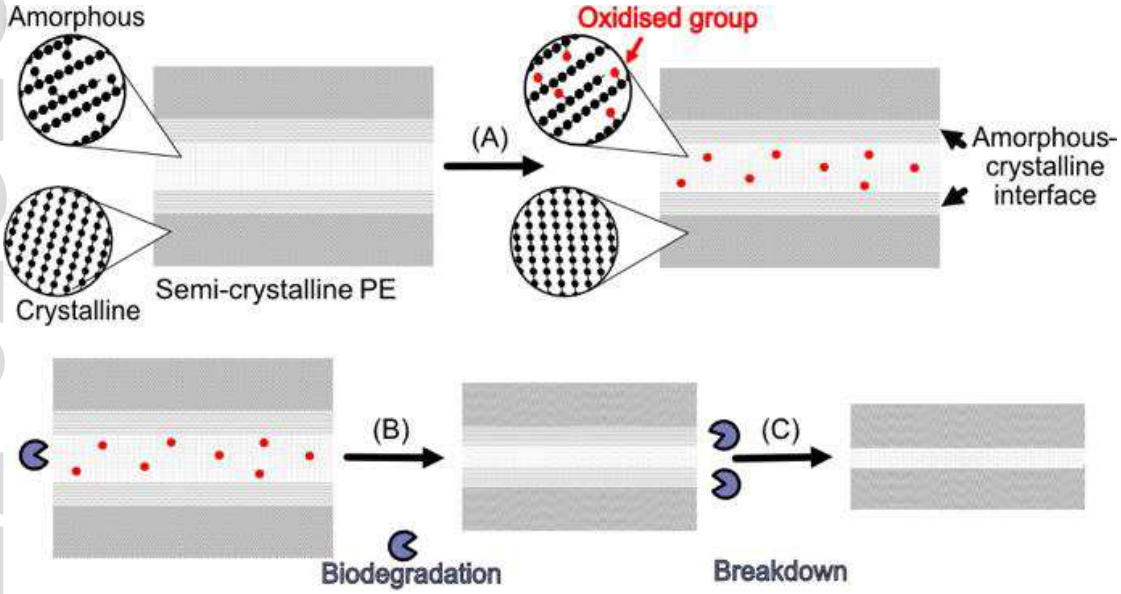
Figure 5



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