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**Microbial degradation of four biodegradable polymers in soil and compost
demonstrating polycaprolactone as an ideal compostable plastic**

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Abstract

Plastics are an indispensable material but also a major environmental pollutant. In contrast, biodegradable polymers have the potential to be compostable. The biodegradation of four polymers as discs, polycaprolactone (PCL), polyhydroxybutyrate (PHB), polylactic acid (PLA) and poly(1,4 butylene succinate (PBS) was compared in soil and compost over a period of more than 10 months at 25°C, 37°C and 50°C. Degradation rates varied between the polymers and incubation temperatures but PCL showed the fastest degradation rate under all conditions and was completely degraded when buried in compost and incubated at 50°C after 91 days. Furthermore, PCL strips showed a significant reduction in tensile strength in just 2 weeks when incubated in compost > 45°C. Various fungal strains growing on the polymer surfaces were identified by sequence analysis. *Aspergillus fumigatus* was most commonly found at 25°C and 37°C, while *Thermomyces lanuginosus*, which was abundant at 50°C, was associated with PCL degradation.

Keywords: *Aspergillus fumigatus*; Biodegradation; Compostable plastics; Polycaprolactone; *Thermomyces lanuginosus*

1. Introduction

Over the last six decades, the use of plastic materials has had a major impact on society and has become essential due to their extensive and diverse range of applications. There have been wide spread applications and usage of plastics because of their favourable mechanical and thermal properties and because plastics are cheap to manufacture, are stable and durable. The global production of plastics has increased every year (Tokiwa et al., 2009). For example, the annual production of petroleum-based plastics exceeded 300 million tonnes in 2015 (Emadian et al., 2017). Despite the applications and benefits of plastics, the recalcitrant nature of many plastics means that they stay in the environment for decades or even centuries. As a consequence, significant amounts of plastics accumulate in the environment and in landfills resulting in environmental pollution and waste management issues (Hopewell et al., 2009). Due to the growing environmental problems, biodegradable plastics are considered as one potential solution (Haider et al., 2019; Tokiwa et al., 2009; Zheng et al., 2005).

Biodegradable polymers are polymeric materials that can be decomposed into CO₂, methane, water, inorganic compounds or biomass by the action of microbial enzymes (Haider et al., 2019; Laycock et al., 2017; Song et al., 2009). The chains of these polymers can also be broken down by non-enzymatic processes, such as chemical hydrolysis (Tokiwa et al., 2009). There are many factors that can affect the degradation of biodegradable polymers such as molecular weight, surface area, and chemical structure of the polymer (Luyt and Malik, 2019; Tokiwa et al., 2009). A number of different types of

biodegradable polymers have been developed to date and several studies have investigated the degradability of these plastics. For example, polyhydroxybutyrate (PHB) is a naturally occurring polyhydroxyalkanoate-type polyester that is composed of small ($C_4H_6O_2$) monomer units (Supplementary Data Table S1), and can be produced from renewable resources such as starch and cellulose. PHB can be degraded by a range of bacterial species through the action of a PHB depolymerase enzyme (Reddy et al., 2003; Shimao, 2001; Tokiwa et al., 2009). In particular, PHB can be degraded in 5 – 6 weeks in microbiologically active environments such as compost (Siracusa et al., 2008). Polylactic acid (PLA) is an aliphatic polyester composed of small lactide ($C_3H_4O_2$) monomers (Supplementary Data Table S1) that can be produced by the fermentation of materials including starch, molasses and cellulose, and accounts for 24% of the global production of biodegradable polymers (Haider et al., 2019; Karamanlioglu et al., 2017). PLA can be degraded by abiotic and biotic processes including by a range of microorganisms (Nampoothiri et al., 2010; Shah et al., 2008), and can be fully degraded when compost temperatures reach 60°C and above (Pranamuda and Tokiwa, 1999; Shah et al., 2008).

Poly(1,4 butylene) succinate (PBS) is also an aliphatic polyester composed of butylene succinate ($C_8H_{12}O_4$) monomers (Supplementary Data Table S1). PBS can also be produced from various recycled materials and can be naturally degraded by bacteria and fungi (Kim et al., 2005). It has been observed that the degradation of PBS was higher in compost compared to natural soil due to the higher temperature and humidity conditions in compost

(Kim et al., 2005). Polycaprolactone (PCL) is a thermoplastic synthetic polymer composed of caprolactone ($C_6H_{10}O_2$) monomers (Supplementary Data Table S1). PCL is compatible with many other polymers and can be used for many applications (Borghesi et al., 2016; Fukushima et al., 2010; Vivi et al., 2019). Studies have reported that PCL is degradable in many natural environments such as soil, seawater and active sludge (Borghesi et al., 2016; Li et al., 2012; Nawaz et al., 2015). The hydrolysable ester linkage of this linear aliphatic polyester makes it susceptible to microbial degradation via lipase and esterase action (Albertsson et al., 1998).

Relatively little is known about the time required for these materials to be fully degraded in soil or compost, or the biotic and abiotic conditions that allow efficient degradation. Moreover, most previous studies have concentrated on monitoring degradation of plastic powders or plastic blends (biodegradable plastics mixed with starch or fibres or any other degradable materials). Therefore, the aim of this study was to determine the rate of degradation of four polymers, PCL, PHB, PLA and PBS as unblended discs or strips under both soil and compost conditions. These four polymers were chosen as they are among the most widely used by industry (Haider et al., 2019). Polymer degradation was examined over a range of temperatures within soil and compost. These temperatures are representative of mesophilic temperatures (25°C, 37°C, 45°C), which are typical within temperate soils, and thermophilic temperatures (50°C, 55°C), which can be achieved within composts. Furthermore, polymer degradation was examined under uncontrolled field soil conditions (Supplementary Data Table S2).

Various methods can be used to evaluate the degradation of biodegradable polymers including CO₂ evolution, clear zone formation, changes in mechanical properties (such as loss of tensile strength) and weight loss measurement (Shah et al., 2008). In this study changes in polymer mechanical properties and weight loss were used to evaluate the biodegradation of PCL, PHB, PLA and PBS (Supplementary Data Table S2). Moreover, this study intended to evaluate the biodegradation of PCL polymer as different geometric forms; thick discs, thin strips and powder. Finally, morphological and sequencing approaches were used to identify the principal colonising fungi strains and to examine whether some of these strains are important for mediating polymer degradation.

2. Materials and Methods

A summary of the different methodological approaches used to examine biopolymer degradation in soil or compost is presented in Supplementary Data Table S2.

2.1. Plastic materials, soil and compost

Granules (3 – 5 mm size) of PCL (M_w 80,000; Sigma-Aldrich, UK), PHB (M_w ~55,000; Sigma-Aldrich, UK), PLA (M_w ~30,000; Goodfellow Cambridge, UK), and PBS (M_w ~40,000; Goodfellow Cambridge, UK) were obtained for the preparation of polymer discs or strips (see Supplementary Data Table S1 for polymer granule characteristics). PCL powder with a particle size of < 600 µm

(M_w 50,000; Polysciences Europe, Germany) was obtained for preparing compost-PCL powder mixes.

Plastic sheets were prepared by melting the polymer granules in a halogen oven for 20 min at 80°C for PCL, 195°C for PHB, 180°C for PLA, and 210°C for PBS. Sheet thickness was determined by the depth of the glass casting tray. For soil or compost burial studies, the sheets were cut into either 1 cm diameter, 3 mm thick discs for the controlled condition experiments or into 5 cm diameter, 2.5 mm thick discs for the environmental burial (uncontrolled conditions) experiments using cork borers. PCL strips for tensile strength measurements were made by melting 12.5 g of PCL granules in 100 mL dichloromethane and pouring this into a levelled glass cast to then set to form a sheet with an even thickness of 0.4 – 0.5 mm. The sheet was cut into 6 cm x 0.5 cm strips. All strips had near identical maximum tensile strength characteristics (13.9 ± 0.4 MPa) indicating high quality control in strip preparation.

Commercial soil and compost (The Compost Shop, UK) was screened to remove large particles and material using a 7 mm sieve prior to use. The percentage moisture content and the water holding capacity (WHC) for the soil and compost was determined as described previously (Karamanlioglu et al., 2014). The moisture content of the compost and soil was 35% and 26%, respectively, and the water holding capacity was 75% and 60%, respectively, and the pH was 7.1 and 7.0, respectively.

2.2. Biodegradation of plastic discs under controlled conditions

In order to investigate the biodegradation of the plastics under controlled conditions, rectangular 1 L plastic boxes (Stewart, UK) with dimensions 16.5 cm x 11.5 cm x 5 cm were filled with 500 mL of soil or compost and seven pre-weighed plastic discs were buried vertically at 2 cm below the surface. The boxes were sealed with lids containing three 1 cm diameter air holes to allow for gaseous exchange and covered with a single layer of parafilm. Boxes were incubated at 25°C or 37°C (for soil) or 25°C, 37°C or 50°C (for compost) for 10 months. A temperature of 50°C was not used for soil because soil normally does not reach this temperature. Boxes were weighed every week to determine water loss by evaporation and replaced with sterile water using a fine spray. To determine changes in the weight of the plastic discs, discs were weighed then reburied periodically, every week (for compost) and every 3 weeks (for soil), after the removal of loosely bound soil or compost with a soft brush and excess moisture, weight loss was determined by:

$$\text{Weight loss (\%)} = (W_{dry} / W_{ini}) \times 100$$

Where W_{ini} is the initial weight of the disc before burial and W_{dry} is the dry weight of disc after burial.

2.3. Biodegradation of plastic discs in soil under environmental conditions

In order to investigate the biodegradation of the plastics under uncontrolled environmental conditions, plastic discs were buried in the soil in an elevated bed in a field environment (on The University of Manchester campus).

The polymer samples were randomly located within the bed and three replicates for each polymer were sampled then reburied every 2 months for PCL, PBS and PHB, and every 4 months for PLA, over a period of up to 21 months. Unburied control discs were kept in a dry indoor environment and were weighed periodically. Another set of control discs were incubated in sterile water at room temperature over 21 months and their weight was measured at the end of the period to determine if there was any effect of water on the degradation of the polymers.

2.4. Biodegradation of PCL strips in compost under controlled conditions

Rectangular 1 L plastic boxes (Stewart, UK) were filled with 400 mL compost and 40 pre-sterilized (70% ethanol washed) PCL strips were added to each box and buried in the compost. The boxes were sealed with lids containing three 1 cm diameter air holes to allow for gaseous exchange and covered with a single layer of parafilm. The boxes were incubated at 25°C, 37°C, 45°C, or 50°C for 10 weeks. At each week, four PCL strips were recovered, any compost sticking to the strips was removed and the maximum tensile strength of the strips was measured in order to determine loss of tensile strength during compost incubation. Measurements were performed using a T-series Tensile Test Machine (Tinius Olsen, UK) supported with QMAT Professional software. The load cell was 1 kN with a cross head speed of 10 mm min⁻¹. PCL strips were also kept dry in a petri dish at each temperature condition for control comparison. As an additional control, PCL strips were incubated in a compost

extract. The compost extract was prepared as described previously
(Karamanlioglu and Robson, 2013).

2.5. Biodegradation of PCL powder in compost under controlled conditions

Rectangular 1 L plastic boxes (Stewart, UK) were filled with 400 mL
compost that was mixed with 40 mL PCL granule powder (to give 10% PCL in
the compost mixture). The boxes were sealed with lids containing three 1 cm
diameter air holes to allow for gaseous exchange and covered with a single
layer of parafilm. The boxes were incubated at 25°C, 37°C, 45°C, 50°C and
55°C for 8 weeks and control boxes were incubated without PCL powder.
Samples of compost (1 g) were recovered every week from three different
random locations within each box. The samples (1 g) were mixed with 5 mL
dichloromethane solvent in a glass tube and left overnight. The solvent was
removed from the glass tube (avoiding any soil particles) and the solvent was
transferred to a glass petri dish and left until the PCL solidified to produce a
plastic sheet. The weight of the produced sheet was measured. This was
repeated at each weekly time points for 8 weeks for each of the temperature
treatments.

2.6. Scanning Electron Microscopy

Polymer discs recovered from compost and soil at all temperatures at
random time points were observed for fungal growth by scanning electron
microscopy (SEM) (FEI, QUANTA FEG 250, Netherlands). Air dried samples

were mounted for sputter coating with gold/palladium on a Quorum SC7620 sputter coater. The samples were then imaged at high vacuum at 10 kV.

2.7. Isolation and identification of fungal growth on the surface of polymers

Fungi were recovered from the surface of the polymer discs and this recovery was adapted using a previously described method (Cosgrove et al., 2007; Karamanlioglu et al., 2014). Polymer discs were recovered from all of the compost and soil treatments and placed in 1 mL phosphate-buffered saline. The surfaces of the discs were then scraped 3 times on both sides using a sterile razor blade. Biomass suspension obtained from the surface of the polymer discs were plated onto Potato Dextrose Agar (PDA) plates supplemented with chloramphenicol (50 µg mL⁻¹) after serial dilution and incubated at the same temperatures at which the discs were originally incubated.

2.8. Genomic DNA extraction from fungal mycelia

Distinct colonies from PDA plates were purified by sub culturing onto fresh PDA plates and incubated at their designated temperatures and then used for DNA was extracted from a loopfull of mycelium or spores exactly as described by Feng et al. (2010) except that the fungal material was homogenised twice for 30 s at 5000 rpm using a Bead Bug Microtube Homogenizer (Merck, UK) and 0.5 mm glass beads. DNA was quantified using a Nanodrop™ 1000 (Thermo Fisher Scientific, UK) and stored at -20°C in sterile water until use.

2.9. DNA amplification and sequencing of rRNA genes to identify fungal isolates

In order to identify the fungal isolates, the ITS1-5.8S-ITS2 rRNA gene fragment was amplified using the universal fungal primers (White et al., 1990) ITS1-F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS4-R (5'-TCCTCCGCTTATTGATATGC-3'). The PCR mixture contained 25 μ L MyTaq Red Mix (Bioline, UK), 1 μ L 100x Bovine Serum Albumin, 5 μ L of extracted DNA (75 – 100 ng μ L⁻¹), 2 μ L of each primer (10 μ M) and the total volume made up to 50 μ L with sterile water. The PCR cycle conditions were 35 cycles of 95°C for 1 min, 56°C for 15 s and 72°C for 10 s. The PCR products were purified using the QIAquick PCR purification kit (Qiagen,UK) according to the manufacturer's instructions. The purified PCR products were sequenced at The University of Manchester DNA sequencing facility. DNA sequences were then viewed using FinchTV and analysed by NCBI BLAST. Fungal strain isolates were identified from identical or closest match sequence present in the GenBank database.

2.10. Degradation ability of fungal isolates on PCL strips

The capability of fungal isolates recovered from the surface of PCL discs incubated in compost at 50°C to degrade PCL strips were determined using modified methods described by Cosgrove et al. (2010) and Crabbe et al. (1994). Wheat grains (200 g), used as a matrix for fungal growth, were autoclaved in a glass container then 150 mL of a yeast-extract-salt (YES) medium was added. Fungal isolates, PCL(A) and PCL(B), were grown on PDA media for one week, chopped and added to the sterile wheat-YES medium containers. One container was left uninoculated as a control. The containers were incubated at 50°C for

one week. PCL strips were surface sterilized with 70% (v/v) ethanol then added to the container to allow the fungal culture to grow on the strips for 3 weeks. The strips were recovered every 3 days and the tensile strength was measured as described in Section 2.4.

2.11. Statistical analysis

Data was analysed one-way ANOVA with a significance P value < 0.05 using SPSS.

3. Results and Discussion

3.1. Degradation of polymer discs buried in compost or soil under controlled conditions

The first set of experiments aimed to determine the weight loss of PCL, PHB, PLA and PBS materials in the form of thick discs following burial in compost or soil. Discs were recovered every week from compost and every three weeks from soil that were incubated at 25°C, 37°C and 50°C (compost only) and the percentage of the original disc weight calculated. All polymers showed degradation in compost at 50°C but the fastest rate of degradation occurred with PCL where complete degradation occurred under this condition after 91 days (Fig. 1). While a significant reduction in PCL disc weight was also observed at 25°C and 37°C in compost (Fig. 1a) and soil (Fig. 2a), the reduction was slower compared to 50°C and therefore temperature was a major factor in PCL degradation. PHB discs also showed a significant reduction in weight

under all conditions, and the rate of degradation at 37°C in compost (Fig. 1b) and soil (Fig. 2b) was equivalent to the PCL discs, although there was less reduction of PHB discs in comparison to PCL polymer at other temperatures, particularly at 50°C in compost. While no significant change was observed for PLA at 25°C and 37°C in both compost (Fig. 1c) and soil (Fig. 2c), a significant reduction was observed at 50°C in compost, with a degradation profile that was equivalent to the PHB discs. Finally, PBS discs displayed moderate degradation at 50°C in compost (Fig. 1d) and 37°C in soil (Fig. 2d). Surprisingly, there was no reduction in PBS disc weight at 37°C in compost, and there was no significant PBS reduction detected at 25°C in either substrate. A summary of the end point data is shown in Supplementary Data Table S3.

The rate of biodegradation of biodegradable plastics is influenced by a number of parameters including molecular structure, molecular weight, degree of crystallinity and melting temperature of the polymer (Chandra and Rustgi, 1998). For example, the presence of specific functional groups within the polymer chain will determine the degree of hydrolysis and therefore rate of degradation (Göpferich, 1996). Likewise, polyesters with side chains with show faster degradation than those without. If polymers such as PCL reduced crystallinity more than other polymers during the incubation period, this would also increase degradation rate (Tsuji and Miyauchi, 2001). Other factors that might also affect the rate of degradation include variation of the incubated environment (soil and compost) in terms of organic and inorganic compositions, the types of degrading microbiota and the incubation temperature (Manna and Paul, 2000). While PCL has the lowest melting temperature (~60°C) of all the

four polymers (Supplementary Data Table S1) it does not appear that the faster degradation of PCL is simply due to temperature sensitivity since PCL incubation at 50°C under dry (no compost/soil) conditions had no significant effect on the polymer. Here, PCL was shown to be degraded far more rapidly than PBS, PLA or PHB and thus is highly susceptible to biodegradation. Likewise, significant degradation of PCL was previously observed in compost at 40°C after 35 days (Fukushima et al., 2010) and in compost at 58°C after 47 days (Funabashi et al., 2007). Nishide et al. (1999) also found that PCL showed the fastest degradation rate at 52°C in aerobic soil. Therefore, temperature was clearly highly correlated to the rate of PCL degradation.

345

346

347 **Fig. 1.** Weight change over time of polymer discs buried in compost. PCL (a), PHB (b),
348 PLA (c) and PBS (d) discs were buried in compost in a laboratory microcosm and
349 incubated at 25°C, 37°C or 50°C and the mean weight remaining calculated at
350 approximately 7 d intervals. All data are mean percentage weight values $\pm$ standard
351 error of the mean of 7 replicate measurements.
352

Fig. 2. Weight change over time of polymer discs buried in soil. PCL (a), PHB (b), PLA (c) and PBS (d) discs were buried in soil in a laboratory microcosm and incubated at 25°C or 37°C and the mean weight remaining calculated at approximately 20 d intervals. All data are mean percentage weight values $\pm$ standard error of the mean of 7 replicate measurements.

In contrast, little degradation of PLA was observed either in soil or compost at 25°C or 37°C and degradation at 50°C only began rapidly after approximately 18 weeks. Fukushima et al. (2009) reported a significant reduction in the molecular weight of PLA when incubated in compost at 40°C for 17 weeks indicating significant depolymerisation. Moreover, it was shown that a significant weight loss in PLA was not observed until depolymerisation had occurred (Karamanlioglu and Robson, 2013). Likewise, it has been shown that breakdown of PHB was more enhanced by incubation of the polymer at higher

temperatures (Mergaert et al., 1994). Slow degradation of PBS (in comparison to PHB and PCL) has also previously been reported (Nishide et al., 1999). A key factor in this present study with regard to comparison with previous studies was the size and thickness of the disc used, here with an average thickness of 3 mm, in comparison to a much thinner polymer film (typically 0.2 mm thick) used in many other studies. Increased polymer thickness will greatly reduce the total contact between hydrolytic enzymes and the total surface area of the sample, thereby reducing degradation rate (Yang et al., 2005).

3.2. Degradation of polymer discs buried in soil under uncontrolled environmental conditions

Following on from the information gained from the first experiment showing differential degradation of the PCL, PHB, PLA and PBS polymers in compost and soil under controlled conditions, a second experiment aimed to compare the rate of degradation of the four polymers under natural environmental conditions. Therefore thick PCL, PHB, PLA and PBS discs were buried outside in natural soil then visualised and weighed over a period of 21 months. Control (unburied) discs were incubated in dry conditions at room temperature while additional control discs were placed in sterile water at room temperature. There was no significant difference in the weight of unburied discs for all polymers over the duration of 21 months (Fig. 3). Likewise, there was no weight change for discs incubated in water. There was no significant weight loss for the buried PHB, PLA and PBS discs but a significant reduction for the PCL discs (Fig. 3a). There was clear evidence of substantial fungal growth on the

surface of the PCL discs and some growth on the PBS discs, but PHB and PLA discs showed the least visual change (Supplementary Data Fig. S1).

Fig. 3. Weight change over time of polymer discs buried in soil under uncontrolled environmental conditions. PCL (a), PHB (b), PLA (c) and PBS (d) discs were buried in soil in a field environment and the mean weight remaining calculated at approximately 2 - 4 month intervals. Unburied (control) samples were kept dry at room temperature. All data are mean percentage weight values $\pm$ standard error of the mean of 3 replicate measurements.

Few studies have examined polymer degradation under natural conditions, but such understanding is very important in order to determine the nature and conditions of the environment where polymers can be disposed or where landfill sites should be made. The distinction in response of different polymers to natural field conditions in comparison to controlled soil and compost

incubations is likely explained in part by the differential temperature sensitivity of the polymers. The degradation of PCL only is likely explained by its higher degradation rate at the lower temperature of 25°C in soil (Fig. 2). However, other environmental factors that may explain the enhanced breakdown of this polymer cannot be discounted. PBS was also previously found to have significantly higher degradation in compost rather than natural soil because of the increased temperature and humidity conditions in the controlled chamber containing the compost (Kim et al., 2006). Likewise, degradation of PLA in a real soil environment over 11 months was very slow compared to a biopolymer such as cellulose, and this correlated to the low temperature of the natural environment (Rudnik and Briassoulis, 2011). Soil pH is also an important factor since this will also determine microbial activity (Emadian et al., 2017). It has also been argued that microbes that mediate the degradation of some polymers such as PBS are less common than microbes that can degrade polymers such as PCL (Ishii et al., 2008).

3.3. Loss of residual PCL powder following compost incubation

Since there was substantial weight loss of PCL polymer in compost in comparison to the other polymers, the breakdown of PCL in different geometric forms was further examined. First this was tested by quantifying degradation of very small (< 600 µm) PCL granules. This is relevant since many plastics are often fragmented or degraded, such as by weathering, into much smaller microplastics and it is of interest to determine whether such PCL microplastics are also efficiently degraded in compost. PCL powder was mixed with compost

to give a concentration of 10% PCL. The compost was again incubated at a range of temperatures, this time including up to 55°C. As seen by loss in PCL disc weight, incubation at the higher temperatures of 45°C and 50°C also gave rise to greater degradation of PCL powder, as indicated by substantial loss of residual PCL, in comparison to the more moderate reduction at 25°C and 37°C (Fig. 4). Degradation of the powder was rapid compared to the rate of degradation of the thick PCL discs in compost (Fig. 1a). The larger, thicker discs will initially be degraded by slower surface erosion. Furthermore, the small PCL powder granules will have increased surface area for colonization and degradation by microorganisms compared to the thick discs. Likewise, polymer geometries with a higher surface area to volume ratio will degrade faster (Bölgen et al., 2005). Surprisingly, the highest temperature of 55°C gave a slow degradation profile that was equivalent to the 25°C treatment. This might relate to the potential reduced abundance of microbial communities within the compost that can tolerate the higher temperature of 55°C. For example, fewer species of fungi have the ability to survive at temperatures between 45°C and 55°C (Maheshwari et al., 2000). Again this would indicate the importance of microbial activity rather than increased temperature itself for the degradation of the PCL polymer.

Fig. 4. Mean percentage of residual PCL powder remaining over time following incubation in compost. 10% PCL in compost was incubated at five different temperatures and recovered at 1 week intervals over 8 weeks for quantification. All data are mean percentage weight values $\pm$ standard error of the mean of 3 replicate measurements.

3.4. Loss of tensile strength of PCL strips following compost incubation

For the final method of characterization of PCL polymer degradation, a change in the mechanical properties of thin strips of PCL following compost incubation was quantified by use of maximum tensile strength measurement. There was a significant decrease in the tensile strength of all PCL strips incubated in compost (Fig. 5). This significant loss of strength was apparent by Week 5 for the strips incubated at 25°C and 37°C, and a near complete loss of tensile strength by the end of the burial period (Week 10) when the tensile strength reached 0.51 MPa after incubation at 25°C and 1.31 MPa after incubation at 37°C. This was in contrast to the tensile strength value before compost incubation, which was 14.18 MPa. Incubation at the two higher temperatures led to rapid loss in tensile strength of the strips, and a significant reduction by Week 2 (Fig. 5). At 45°C no intact strips could be recovered after 8

weeks, while at 50°C no intact strips could be recovered after 5 weeks. The loss
 of tensile strength at the lower temperatures was linear over time, indicating
 predominant surface erosion, while the curve profiles at 45°C and 50°C showed
 exponential decay (Fig. 5) indicative of bulk erosion (Göpferich, 1996). Control
 strips incubated dry, or in a compost extract for 10 weeks at the different
 temperatures showed no significant change in the tensile strength compared to
 the Week 0 value. This indicates that the polymer was mainly being degraded
 due to microbial action but not substantially due to abiotic hydrolytic
 disintegration, and not simply due to temperature. This further indicates a link
 between microbial activities at the higher temperatures facilitating PCL
 hydrolysis. Enzymatic activities will also be temperature dependent (Haider et al
 2019).

Fig. 5. Tensile strength measurements of PCL strips incubated in compost over time. PCL strips that were buried in compost at four different temperatures were recovered at 1 week intervals over 10 weeks for tensile strength measurement. At 45°C and 50°C the strips could be recovered only for 8 and 5 weeks, respectively before all strips were fully degraded. All data are mean values $\pm$ standard error of the mean of 4 replicate measurements.

Fig. 6. Fungal growth on the surface of polymer discs visualised by SEM. PCL (a), PHB (b), PLA (c) and PBS (d) discs were buried in soil at 25°C or compost at 50°C and discs were recovered for analysis. Unburied control discs from a dry indoor environment were compared to confirm for no background fungal growth.

3.5. Identification of fungal strains recovered from the surface of polymer discs buried in soil and compost

Visual inspection of recovered polymer discs indicated the presence of fungal growth (Supplementary Data Fig. S1). A more detailed analysis by SEM clearly showed fungal growth on the surface of all polymer discs following

incubation in soil and compost (Fig. 6). The different polymers and the different substrates (soil versus compost) yielded fungal growth with different morphological characteristics. In contrast, the control discs that were left unburied at room temperature were clear and had no fungal growth. A variety of microorganisms use the polymers as nutrients, particularly when under starvation and lacking essential nutrition (Roohi et al., 2017), thus they play a crucial role in the degradation of the polymers. However, there is a scarcity of information with regard to the organisms that are responsible for biodegradation (Suyama and Tokiwa, 1998).

Random discs were chosen from each substrate at each temperature for the isolation of the fungal strains. The discs were chosen according their appearance and how much fungi covered them. After fungal isolation and growth on PDA plates, distinct morphotypes were identified based on morphological variation of colonies (Supplementary Data Table S4). Only PCL discs provided fungal growth from all treatments with 1 – 3 morphotypes, followed PHB discs with 1 – 2 morphotypes from all treatments apart from 25°C soil. Fungi (3 morphotypes) could only be recovered from PLA discs from 50°C compost, and PBS discs provided just one morphotype each from 50°C compost and from 37°C soil.

In order to identify the fungal isolates, rRNA gene sequencing was performed. Most of the thermophilic isolates recovered from the surface of the PCL, PLA and PHB discs buried in compost at 50°C were identified as *Thermomyces lanuginosus*, with the exception of one isolate recovered from PBS, which could not be defined to a single species (Table 1). For the polymer

discs incubated at the lower temperatures, the most frequently recovered isolates were strains of *Aspergillus fumigatus* (Table 1). Isolates that were strains of *Fusarium* sp. were recovered from PCL and PHB at 25°C in both compost and soil. Furthermore, a strain that was identified as *Neocosmospora* sp. was found on the surface of PCL at 25°C in compost.

T. lanuginosus is a thermophilic fungus found in many habitats, with an optimum growth temperature of 50°C. *T. lanuginosus* produces heat-stable enzymes that can tolerate high temperatures in comparison to those produced by mesophiles (Singh et al, 2003). Previously *T. lanuginosus* was identified as the most frequently recovered isolate from the surface of PLA buried in compost at 50°C (Karamanlioglu et al., 2014). There is an important need to isolate thermophilic microorganisms that are capable of degrading polymers because of their importance in the composting process, which is considered as one of the most promising technologies in biodegradable polymer recycling (Chua et al., 2013; Tseng et al., 2007). In contrast, mesophilic fungi *Aspergillus* and *Fusarium* were previously found to be important degrading microorganisms of PCL (Chua et al., 2013). Both *A. fumigatus* and *F. solani* were also previously isolated from the surface of PLA when incubated at 25°C in soil and compost (Karamanlioglu et al., 2014).

Table 1. Fungal taxa assignments identified using ITS sequencing of rRNA gene amplified from samples isolated from the surface of polymer discs buried in soil and compost under controlled conditions. The closest sequence matches from the NCBI GenBank database determined by BLAST are shown.

Polymer and isolate condition	Strain isolate name	Strain identification	Sequence identity % to closest match (Accession number of closest match)
PCL Compost 50°C	PCL(A)	<i>Thermomyces lanuginosus</i>	99% (KT365229.1)
	PCL(B)	<i>Thermomyces lanuginosus</i>	98% (KY848520.1)
	PCL(C)	<i>Thermomyces lanuginosus</i>	99% (KT365229.1)
PHB Compost 50°C	PHB(H)	<i>Thermomyces lanuginosus</i>	99% (KT365229.1)
	PHB(I)	Undefined strain:	
		<i>Sordariales</i> sp.	99% (JN659492.1)
		<i>Scytalidium thermophilum</i>	99% (AB085928.1)
		<i>Chaetomium thermophilum</i>	99% (AB746179.1)
PLA Compost 50°C	PLA(E)	<i>Thermomyces lanuginosus</i>	97% (KT365229.1)
	PLA(F)	<i>Thermomyces lanuginosus</i>	99% (KT365229.1)
	PLA(G)	<i>Sordariales</i> sp.	100% (JN659504.1)
PBS Compost 50°C	PBS(K)	Undefined strain:	
		<i>Talaromyces pinophilus</i>	99% (MF686817.1)
		<i>Acremonium cellulolyticus</i>	99% (AB474749.2)
		<i>Penicillium pinophilum</i>	99% (AB474749.2)
PCL Compost 37°C	PCL(N)	<i>Aspergillus fumigatus</i>	99% (KX090325.1)
	PCL(O)	<i>Aspergillus fumigatus</i>	98% (KX090348.1)
PHB Compost 37°C	PHB(J)	<i>Aspergillus fumigatus</i>	99% (KR527135.1)
PCL Soil 37°C	PCL(P)	<i>Aspergillus fumigatus</i>	100% (KY450779.1)
	PCL(X)	<i>Aspergillus fumigatus</i>	99% (KR527135.1)
PHB Soil 37°C	PHB(M)	<i>Aspergillus fumigatus</i>	99% (KP724998.1)
PBS Soil 37°C	PBS(L)	<i>Aspergillus fumigatus</i>	100% (KF494830.1)
PCL Compost 25°C	PCL(Q)	<i>Neocosmospora ramosa</i>	99% (KY031973.1)
	PCL(R)	<i>Fusarium solani</i>	99% (KX929306.1)
	PCL(S)	<i>Aspergillus fumigatus</i>	99% (KP724998.1)
PHB Compost 25°C	PHB(V)	<i>Fusarium solani</i>	99% (KX929306.1)
	PHB(W)	<i>Aspergillus fumigatus</i>	100% (KX090348.1)
PCL Soil 25°C	PCL(T)	<i>Aspergillus fumigatus</i>	100% (KR527135.1)
	PCL(U)	<i>Fusarium solani</i>	99% (KM268689.1)

Microorganisms will adhere to the polymer surface making a biofilm and become responsible for the degradation of polymers by utilizing the hydrocarbons in the polymer backbone as a carbon source. The PCL discs attracted a significant amount of fungal biomass as determined from visual observations (Fig. 6; Supplementary Data Fig. S1) and had the greatest diversity of fungal taxa (Supplementary Data Table S4), but it is unclear what structural properties explain this. Hydrophobicity of the polymer surface is one key factor determining microbial attachment (Sarjit et al., 2015). Degradation of a number of polyester biodegradable plastics including those used in this study have been shown to be mediated by microbial extracellular esterases including lipases and cutinases causing hydrolysis of the ester linkages and progressive depolymerisation (Ishii et al., 2008; Nakamura et al., 2001; Numata et al., 2007; Shah et al., 2008; Tokiwa et al., 2009). Enzymatic degradation will be preferential in amorphous regions of the polymer but can also occur within crystalline regions (Castilla-Cortázar et al., 2012). Moreover, the molecular weight can influence the enzymatic biodegradation process such that the degradability will decrease with increased molecular weight. For example, high molecular weight PCL displays slower degradation by *Rhizopus delemar* lipase than low molecular weight PCL (Tokiwa et al., 2009).

3.6. PCL degradation by *Thermomyces lanuginosus* strains

PCL is known to be degraded at high temperature by bacteria (Emadian et al., 2017) but there have been no previous identification of fungal strains able to degrade PCL at high temperatures. The ability of the isolated *T. lanuginosus*

strains to degrade PCL at 50°C were investigated. Once again tensile strength measurement of thin PCL strips was quantified since this parameter allows accurate analysis of degradation rate over short time periods. Two strains named PCL(A) and PCL(B) were tested for their ability to degrade PCL strips using tensile strength measurements following 3-week incubation. Control strips incubated in growth medium without any fungal inoculation showed insignificant change in tensile strength. However, tensile strength of PCL strips incubated in the growth medium inoculated with the PCL(B) strain reduced significantly after just 3 d and dropped to approximately 3 MPa in 6 d in comparison to an initial tensile strength of approximately 14 MPa (Fig. 7). The reduction in tensile strength of the PCL strips incubated with the PCL(A) strain was not as fast but the degradation of PCL was nevertheless significantly enhanced compared to the control strips. The profile of degradation was equivalent to the incubation of the PCL strips in compost at 50°C, which shows exponential loss of tensile strength, however, the degradation rate was faster when the strips were incubated with the purified fungal strain (Fig. 7). This result clearly indicates that *T. lanuginosus* strains are capable of degradation of PCL at 50°C, and also suggests that the degradation induced by *T. lanuginosus* is largely mediating bulk erosion of the polymer. Future studies will aim towards identifying the exact mechanisms of *T. lanuginosus* induced degradation.

Fig. 7. The ability of fungal strains isolated from the surface of PCL in compost at 50°C to degrade PCL strips. Tensile strength data after 18 d incubation with *Thermomyces lanuginosus* strains PCL(A) and PCL(B) at 50°C. All data are mean values $\pm$ standard error of the mean of 3 replicate measurements.

4. Conclusions

With the increasing production and consumption of plastics in daily life, and the consequences of their disposal, there is a need to introduce new plastics that are environmentally friendly. The rate of biodegradation of four biodegradable polymers was investigated under controlled conditions and within the natural environment. Under both conditions PCL demonstrated more rapid degradation compared to PLA, PHB and PBS. Temperature was highly correlated to the rate of PCL degradation under controlled conditions due to microbial activity. Strains of *T. lanuginosus* grow on all tested polymers at high temperature, and are directly responsible for the degradation of PCL.

Competing interests

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

The supplementary data contains Supplementary Fig. S1 and Supplementary Table S1 – S4.

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783

Figure legends

Fig. 1. Weight change over time of polymer discs buried in compost. PCL (a), PHB (b), PLA (c) and PBS (d) discs were buried in compost in a laboratory microcosm and incubated at 25°C, 37°C or 50°C and the mean weight remaining calculated at approximately 7 d intervals. All data are mean percentage weight values $\pm$ standard error of the mean of 7 replicate measurements.

Fig. 2. Weight change over time of polymer discs buried in soil. PCL (a), PHB (b), PLA (c) and PBS (d) discs were buried in soil in a laboratory microcosm and incubated at 25°C or 37°C and the mean weight remaining calculated at approximately 20 d intervals. All data are mean percentage weight values $\pm$ standard error of the mean of 7 replicate measurements.

Fig. 3. Weight change over time of polymer discs buried in soil under uncontrolled environmental conditions. PCL (a), PHB (b), PLA (c) and PBS (d) discs were buried in soil in a field environment and the mean weight remaining calculated at approximately 2 - 4 month intervals. Unburied (control) samples were kept dry at room temperature. All data are mean percentage weight values $\pm$ standard error of the mean of 3 replicate measurements.

Fig. 4. Mean percentage of residual PCL powder remaining over time following incubation in compost. 10% PCL in compost was incubated at five different

temperatures and recovered at 1 week intervals over 8 weeks for quantification. All data are mean percentage weight $\pm$ standard error of the mean values of 3 replicate measurements.

Fig. 5. Tensile strength measurements of PCL strips incubated in compost over time. PCL strips that were buried in compost at four different temperatures were recovered at 1 week intervals over 10 weeks for tensile strength measurement. At 45°C and 50°C the strips could be recovered only for 8 and 5 weeks, respectively before all strips were fully degraded. All data are mean values $\pm$ standard error of the mean of 4 replicate measurements.

Fig. 6. Fungal growth on the surface of polymer discs visualised by SEM. PCL (a), PHB (b), PLA (c) and PBS (d) discs were buried in soil at 25°C or compost at 50°C and discs were recovered for analysis. Unburied control discs from a dry indoor environment were compared to confirm for no background fungal growth.

Fig. 7. The ability of fungal strains isolated from the surface of PCL in compost at 50°C to degrade PCL strips. Tensile strength data after 18 d incubation with *Thermomyces lanuginosus* strains PCL(A) and PCL(B) at 50°C. All data are mean values $\pm$ standard error of the mean of 3 replicate measurements.