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Insights into the Biodegradation of PHA / Wood Composites: Micro- and Macroscopic Changes

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Abstract

This study presents a comprehensive analysis of the microscopic and macroscopic changes during the biodegradation of composites of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) and wood flour (WF) in soil, to provide insights into the biodegradability of this emerging material. Composite samples with different wood contents (0, 20 and 50 wt%) were buried in soil at a field trial site in a subtropical region of Australia, alongside polylactic acid (PLA) and polyethylene (PE) based wood composites as reference. The degradation rate of the PHBV/WF composites increased with wood content, with weight loss after 12 months being five times greater for PHBV/50 wt% WF than for neat PHBV plaques under identical conditions. The mechanical properties of neat PHBV were retained after 12 months of soil burial. However, PHBV/50 wt% WF lost its mechanical integrity after 12 months, despite only a 13% loss in weight. PLA and PE composites by contrast showed only slight decreases in mechanical

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properties, which could be associated with moisture induced degradation at the surface only. It is proposed, with evidence from optical microscopy, that for the PHBV-based samples, localised stress loosened the interface, allowing channels for the bacteria and fungi to access PHBV in the bulk of the matrix for local enzymatic biodegradation. With this network of interconnecting pores and cracks, crack propagation and mechanical failure would readily result from an applied stress. Overall, although the erosion rate for the exposed PHBV surfaces in the different samples could be similar, the accessible surface area in PHBV/50 wt% WF composites is significantly larger following local biodegradation and subsequent void formation than for neat PHBV and thus delivers a higher biodegradation rate.

Keywords:

Polyhydroxyalkanoate (PHA); Biocomposites; Biodegradation; Degradation mechanism

1. Introduction

Wood plastics composites (WPCs) are found in many applications in daily life, from household furniture to structural components of buildings. In recent years, concerns over the large amount of solid waste associated with conventional polymers have raised public awareness of the extensive use of petroleum-derived polymers, which have been the primary polymer matrices for WPCs. Research has turned to the use of biodegradable polymers, such as polylactic acid (PLA) and polyhydroxyalkanoate (PHA), in WPCs. These biopolymers are attractive because they have been shown to have useful mechanical properties and are easy to process.

PHA is of particular interest as it can be synthesized intracellularly by a range of bacteria and archaea from carbon-rich resources and is biodegradable in the presence of bacteria, fungi or

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enzymes [1]. Furthermore, short chain length-PHAs, including poly(3-hydroxybutyrate) (PHB) and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) have physical and mechanical properties similar to polypropylene [2]. With a melt viscosity lower than traditional polyolefins [3], PHAs also have a potential advantage in the processing of composites. Another important advantage of PHA over PLA is their much faster biodegradation rate in soil under ambient conditions and their biodegradability in the marine environment [4]. It has been shown that PLA is relatively resistant to microorganisms that are present in soil, as soil bacteria can only produce a very few natural PLA-degrading enzymes [5, 6]; PLA degrades rapidly only in soil or compost at elevated temperatures (i.e. >45^{o}C) [7]. Unlike PLA, PHA can be degraded readily under ambient conditions through enzyme-catalysed hydrolytic chain scissioning, producing monomers which can be further metabolised to methane under anaerobic conditions and to carbon dioxide and water under aerobic conditions [8]. The biodegradation of PHA can occur in many environments [8], such as in marine waters [9], activated sludge [10], and soil [11, 12].

The current literature has demonstrated the potential of and the rising interest in biodegradable PHA-based WPCs. Common PHA matrices used in WPCs include PHB and its copolymer PHBV. However, the majority of the studies have focused on improving the interfacial adhesion between wood and polymer and thus the overall mechanical properties through various compatibilisation techniques [13-15]. Only a few studies have characterised the biodegradation of PHA-based WPCs in soil, with the focus being on the effect of the filler. These studies have produced mixed results. Avella et al., for example, investigated the biodegradation of PHBV/wheat straw composites containing 30 wt% of wheat straw, and found that the weight loss of the composites was similar to that of the neat PHBV after 6 months of incubation in soil under controlled temperature and humidity conditions in an indoor environment [16]. By contrast,

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Peterson et al. reported that the biodegradation rate of PHBV/wood fibre composites increased with increasing wood content up to 15 wt% loading and then decreased gradually when the loading was further increased to 25 wt% [17]. Contradictory results were reported in studies on the biodegradation of PHBV/peach palm particle composites [18] and PHB/wood flour composites [19, 20] in soil under an indoor environment. However, all these studies have shown that a higher biodegradation rate, based on weight loss and visual inspection, was observed from the composites with higher fibre contents. Overall, however, while biodegradability has been highlighted as a unique advantage of PHA-based WPCs, there have been limited insights into the biodegradation kinetics and mechanisms of these hybrid materials [15]. Also, the underlying effects of wood fillers on biodegradation have been poorly explained. An investigation into the role of wood flour in promoting biodegradation is needed in order to provide a better understanding of the end-of-life behaviour.

Overall, advances in material development are far ahead of an understanding of lifetime analyses of this emerging biodegradable composite material. It is therefore necessary to fully characterise the biodegradability of PHA/wood flour composites in practical real-life contexts such as in soil under ambient outdoor conditions. In this work, PHBV/wood flour (WF) composite samples were placed under the soil at a fully monitored field in sub-tropical Queensland (27.5^{o} S, 152.9^{o} E). Three wood content levels, 0, 20 and 50 wt%, were chosen to assess the effect of wood content, and to represent a commercially relevant range. Polylactic acid (PLA) and polyethylene (PE) composites containing 50 wt% WF were used as experimental reference materials. The objective of this study was to comprehensively characterise the biodegradability of PHBV/WF composites in soil through monitoring the microscopic (micro-structure), macroscopic (weight loss, mechanical properties) and chemical (oxygen consumption) changes over time upon soil

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burial, with results combined to provide insights into the role of wood flour on the biodegradability of WPCs.

2. Experimental procedure

The materials, composite processing and characterisation methods used in this study are also described in full in another parallel study on the stability of PHBV/WF composites under natural weathering [21].

2.1. Materials

Unstabilised PHBV with 1 mol% HV content in powder form was supplied by TianAn Biopolymer, China under the trade name of ENMAT Y1000. PLA (Ingeo^{TM} 2003D) and HDPE (EL-Lene H5818J) pellets were obtained from Natureworks, USA and SCG Chemicals, Thailand respectively. *Pinus radiata* wood flour (WF) sieved to below 300 μm was obtained from Micromilling, Australia.

2.2. Composite extrusion

The composite materials were prepared by a two-step process, consisting of dry mixing and melt compounding. The formulations with associated sample identifications are summarised in Table 1. PHBV and WF were first dried separately at 105^{o}C in a vacuum oven at a vacuum of 80 kPa for 24 h. The dried PHBV and WF were then premixed according to the formulations as shown in Table 1 by manual stirring with a Homemaker kitchen blender at 200 rpm for 2 mins. The mix was then fed to a EuroLab 16 XL 40:1 co-rotating twin screw extruder. Flood feeding and a decreasing temperature profile with a maximum barrel temperature of 180^{o}C and a die temperature of 160^{o}C were implemented. The screw profile consisted of forward conveying elements only, with no mixing zones. The extruder was running at a screw speed of 100 rpm, which resulted in a residence time of around 1 min. A slit die with cross sectional dimension of

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25 by 2 mm was fitted to yield rectangular strips. The rectangular strips were then melt-pressed at 180°C with 5 tonnes of force for 2 mins to produce consistent flat sheets with a thickness of 1.6 mm. Due to differences in polymer form and properties, the extruder set-up was modified for the production of PE and dried PLA composites with dried wood flour. Two Gravimetric spring feeders were calibrated to deliver a 50/50 polymer to wood ratio by weight. A screw speed of 100 rpm, a maximum barrel temperature of 210°C and a die temperature of 160°C were employed for both PLA and PE composites. A kneading section was incorporated to facilitate mixing between the polymer pellets and wood flour. The rectangular strips were then melt-pressed at 200°C with 5 tonnes of force for 2 mins to produce consistent flat sheets with a thickness of 1.6 mm.

Table 1

2.3. Field trial

The melt-pressed sheets were laser cut to produce 70 x 15 x 1.6 mm rectangular specimens for soil burial. ASTM G160-12 standard was used as a basis for the biodegradation tests but natural outdoor burial conditions were used instead of a fully controlled environment. The samples were buried under the soil (at more than 7 cm in depth) at a fully monitored field trial site in sub-tropical Queensland (27.5° S, 152.9° E). On-site weather data including air temperature and total rainfall were recorded. An average temperature of 19.8°C and a total rain fall of 890 mm were observed throughout the burial period. Specimens were collected after 1, 2, 3.5, 6 and 12 months for physical and mechanical characterisation.

2.4. Characterisation methods

2.4.1. Moisture content and weight loss

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Before soil burial, the specimens were first dried in a vacuum oven at 80°C at a vacuum of 80 kPa for 24 hours. The samples were then pre-weighed and marked with unique identification codes. At each time-point, 3 buried specimens were collected, wiped with a cloth, and weighed, before drying in the vacuum oven at the same settings as above. The moisture content of the recovered samples was determined by dividing the weight loss following drying by the weight before drying. The dry weight loss values of the samples were determined using the following equation:

$$\%\ Dry\ weight\ loss = \frac{Dry\ weight\ after\ soil\ burial - Dry\ weight\ before\ burial}{Initial\ dry\ weight\ before\ burial} \times 100\%$$

2.4.2. PHA content determination

A chloroform extraction method was used to determine the PHA content in the composites after soil burial. 50 mg of composite samples were cut into small pieces and placed into 25 mL capped glass tubes. 10 mL of chloroform (Sigma-Aldrich, USA) was then added. The tubes were placed in a heating block at 80°C for 2 hours to allow for complete dissolution. The samples were then filtered through 0.22 µm Teflon disc filters and washed with excess chloroform, before pouring into a tared petri dish. The solution was allowed to cool and evaporate for at least 24 hours at room temperature, and then dried in the vacuum oven at 60°C at vacuum of 80 kPa for 12 hours. The weight of the dried extracted polymers was then determined by subtracting the weight of the dried empty petri dish from the total weight.

2.4.3. Gel permeation chromatography (GPC)

The molecular weight of PHA was determined using gel permeation chromatography (GPC). Samples were added to HPLC grade chloroform to achieve a concentration of 2.5 mg/mL in capped glass tubes. The solution was heated on a dry heating block at 80°C for 30 min or until

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fully dissolved. A HPLC solvent delivery system (Waters 1515) was used with conjunction with auto-injector (Wisp 717). A column set consisting of a guard column (Waters Styragel (20 μm, 4.6 mm × 30 mm)) followed by a series of columns in series: Waters Styragel HR5 (5 μm, 7.8 mm × 300 mm), Waters Styragel HR4 (5 μm, 7.8 mm × 300 mm) and Waters Styragel HR1 (5 μm, 7.8 mm × 300 mm) was kept at 30°C. A refractometer, at 30°C, was used for detection. A chloroform flow rate of 1 mL/min was used for the analysis. Narrowly distributed molecular weight polystyrene standards were used for calibration.

2.4.4. Scanning electron microscopy (SEM)

Samples were imaged by JOEL 6460 and JOEL 6610 scanning electron microscopes under secondary electron mode. The top and bottom surfaces were exposed for imaging. They were coated with ~30 nm of Iridium and vacuum dried at room temperature for at least 24 hours before imaging. Image acquisition was done on the Iridium-coated samples at 5 kV accelerating voltage and approximately 10 mm working distance.

2.4.5. Optical microscopy

Composite samples were first fractured to expose the cross-section. The samples were then embedded in polyester resin and were polished to remove observable scratches from the surface. Images of the cross-section of the composites were obtained using a polarised optical microscope (Olympus BX50).

2.4.6. Mechanical testing

Prior to any characterisation, the samples were dried in a vacuum oven at 60°C at a vacuum of 80 kPa for 24 hours to remove any absorbed moisture. The final drying before mechanical testing provided a common basis and conditions from which to compare the influence of environmental

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exposure on material properties. Tensile mechanical tests were performed according to ASTM D638 standard on an Intron 5584 with a 1-kN electronic load cell. Sections of extruded rectangular strips were laser cut into Type V dogbone-shaped specimen. Tests were performed at a rate of 1 mm/min until fracture. A video extensometer was used to obtain an accurate strain value across the narrow region. Five replicas were performed for each sample set.

2.5. Lab-scale anaerobic biodegradation characterisation using OxiTop WTW system

The rates of aerobic biodegradation of the composite samples in soil were analysed using the OxiTop WTW system. The ASTM D5988-12 standard was used for this study with the following changes to allow for direct comparison to the field trial, in that the soil used was collected from the field trial site and the samples with the same dimensions were used. Thus, for each test the 70 x 15 x 1.6 mm rectangular specimens were placed into 200 g of the soil with a moisture content of 80% of soil water holding capacity. The water holding capacity of soil was determined based on the weight of the soil saturated by water and the dry weight of the soil. The soil sample was saturated with water from another container at the same level where the water level was kept in the middle of the packed soil until equilibrium.

This mixture was placed in a 1.0 L OxiTop bottle with 50 mL of 0.5 M KOH solution in a 100 mL beaker to act as a CO_2 trap. PHA20W, PHA50W, PLA50W and PE50W were assessed along with a negative control (soil only) and a positive control (starch powder - Gelose 80). For starch powder, 0.8 g of Gelose-80 powder was placed into 200 g of soil with 80% of the soil water holding capacity.

Chemical changes upon biodegradation were captured by the measurement of the evolution of CO_2 and thus indirectly the consumption of oxygen by soil microorganisms. With a CO_2 trap in place, the consumption of the oxygen during the biodegradation of PHA and wood generated a

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negative pressure inside the sealed jar, which was automatically measured by the jar tops. The OxiTop-C heads recorded a data point every 6.6 hr (396 min). The pressure data was corrected against the negative control. All tests were run in duplicate over a period of 12 months.

The following calculations were performed to estimate the percent degradation data from the pressure data with several key assumptions:

- First, it is assumed that the CO_2 production rate by the microorganisms is directly proportional to the degradation of the carbon content inside the composites.
- Second, it is assumed that the starch was fully degraded when the pressure reading in that vessel stabilised. The corrected pressure curves of the starch samples showed that a plateau was achieved after 400 days and the pressure reading at that point was used for calculation.

Using the pressure at this end point, the pressure change per carbon content was estimated by dividing the end pressure value from the model by the theoretical amount of carbon content in starch. The % carbon degraded was then calculated using the following equation:

$$\%\, C\ degraded = \frac{Amount\ of\ carbon\ released\ (calculated\ from\ pressure\ change\ data)}{theoretical\ amount\ of\ carbon\ content\ in\ whole\ sample}$$

3. Results

3.1. Physical appearance

Figure 1 shows the visual appearances of both surfaces of the initial and buried samples at five different exposure times (1, 2, 3.5, 6 and 12 months). All samples darkened throughout the burial period, which was brought about by the attachment of soil on the sample surfaces when in contact. Neat PHBV (PHA0W) samples were still intact after 12 months of burial and lacked any obvious signs of biodegradation on inspection. PHA20W had visibly rougher surfaces when compared to PHA0W, and PHA50W had the most severe damage after 12 months. As can be

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seen in the 3rd column of Figure 1, the materials at the edges of PHA50W samples were eroded. Such erosion is likely linked to the expected bacterial attack and/or fungal (enzymatic) degradation which is common in the soil environment [22]. PLA50W and PE50W samples also had rougher surfaces after 12 months but showed minimal signs of biodegradation on inspection. No notable differences were observed between the PLA50W and PE50W samples after soil burial.

Figure 1 (1.5-column fitting)

3.2. Micro-structure of surface by scanning electron microscope (SEM)

A closer inspection of the surfaces of the buried samples was conducted using SEM under secondary electron mode, with a view to identifying sites of bacterial or fungal activity. The first two columns of Figure 2 show the SEM micrographs of the surfaces of all samples before and after 12 months of soil burial at low magnification (100x), whereas the images in the third column are samples after 12 months at higher magnification (1000x), zooming on the obvious biodegradation sites. Despite the observed low weight loss, neat PHBV (PHA0W) clearly had rougher surfaces with dents after 12 months of soil burial. The surfaces of PHA20W were also significantly rougher after soil burial. A network of fungal hyphae filaments was embedded within the matrix along with the presence of spores.

PHA50W, PLA50W and PE50W started off with notable surface roughness and dents, which were attributed to the imperfect mixing and consequently inhomogeneous nature of the composites post processing. After 12 months, wood particles in the PHA50W sample were clearly exposed by the surface erosion of PHBV. A site with simultaneous wood and PHBV

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biodegradation was captured at higher magnification, and biodegradation of the wood particles was evidenced by a depression in the middle of the particles, which were wrapped by a network of fungal hyphae filaments and spherical spores. Such a network of long, filamentous fungal hyphae and spherical spores is also seen in decayed woody materials [23, 24].

When comparing the surface roughness between PHA50W, PLA50W and PE50W after 12 months, PLA50W and PE50W were relatively smoother than that of PHA50W. The observed flat, smooth areas of the PLA50W and PE50W in the beginning remained smooth after 12 months. One can conclude that the biodegradation of PLA and PE was limited in soil under ambient temperatures. At high magnification, a network of fungal hyphae filaments and spores was observed within the depression of these materials leading to the assumption that these microorganisms are responsible for the degradation of wood. Erosion of the PLA50W and PE50W surfaces was found to be located predominantly at the surface depressions created during processing.

Figure 2 (1.5-column fitting)

3.3. Quantification of degradation by weight loss

Dry weight loss has been widely established as a direct indication of biodegradation and/or fragmentation of polymers and composites upon soil burial or composting. The percent dry weight loss of all samples is plotted against the soil burial time in Figure 3.

Figure 3 (single-column fitting)

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Roughly linear weight loss trends were observed for all samples, suggesting that a surface erosion mechanism was dominating throughout the testing period [25]. PHA0W was found to have only a 2.6 ± 0.1% weight loss after 12 months. However, higher weight loss values were obtained in past studies. For example, it has been reported that melt-processed PHB specimens in dumbbell shape (injection moulded, unknown thickness) lost 7% of their initial weight after 200 days (~6.7 months) in loamy soil at 28^{o}C [26]. Another study showed that PHB (solvent-cast film, 0.1 mm thickness) had a 16% weight loss in soil at a field during the summer season in Russia [27]. Likewise, PHB (compression moulded plaque, 1 mm thickness) showed 11% weight loss after 6 months in boxes filled with garden soil in an indoor environment at 23^{o}C [16]. Another study investigated the biodegradation of PHBV in anaerobic conditions and showed that 100% mass loss was observed after 4 weeks of burial in anaerobic digester sludge with mineral media at 37^{o}C [28]. This deviation from the literature could be explained by the different thicknesses between sample sets [29], different medium and the use of different processing methods [12], which can lead to differences in surface area and bulk polymer density, as well as crystallinity and porosity (surface and bulk), as examples. The varying moisture content levels across studies could also contribute to the observed differences.

The dry weight loss values increased with increasing wood content. Among all PHBV WPCs, PHA50W recorded the highest weight loss values among all samples at every time point, which aligned with the results from visual inspection. A 12.7 ± 0.9% weight loss was achieved after 12 months. PHA20W achieved a percent weight loss of only approximately half of that of PHA50W after 12 months (6.7 ± 0.4%). Same trends were also observed from the anaerobic biodegradation study on PHBV composites with oak wood flour that higher fragmentation rate and mass loss

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rate was observed from the composites with 20 wt% oak wood flour when compared to neat PHBV [28].

PLA50W (3.7 ± 0.6%) and PE50W (3.9 ± 0.7%) had similar percent weight losses after 12 months. It is well-established that conventional PE is non-biodegradable within this timeframe. More interestingly, there was no difference when PLA was used as the polymer matrix instead of PE. It has been reported, in a field study, that the biodegradation rate of PLA film in soil is much slower than PHA [30]. PLA is known to be readily degradable in soil or compost only at elevated temperatures (> 45^{o}C) [7]. Under these ambient outdoor conditions, the biodegradation rate of PLA is comparable to PE, which is very slow, at least initially before autocatalytic depolymerisation starts to accelerate the bulk erosion of such materials. In these cases, it is likely that it was the wood component that underwent biodegradation during soil burial [31].

3.3.1. Degradation rate

The degradation rates of PHA0W, PHA20W and PHA50W were estimated based on the as-obtained weight loss data from the 12-month field trial assuming that the samples (both PHA and wood) only undergo surface erosion throughout the period (linear weight loss). The degradation rates, in mg/day, were normalised by dividing these weight loss values by the exposed surface area of the specimen, in cm^2, to account for the edge effects. The as-calculated values are presented in Table 2. The addition of wood flour resulted in a linear relationship between the degradation rate and wood content (R^2 values: 0.998). This linear relationship implies that the inclusion of wood provides a larger surface area for both surface erosion of PHA and wood along the interface rather than a shift to bulk erosion of the PHA phase [25]. This is aligned with the suggested degradation mechanism.

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In order to provide insights into the real-life environmental impact of the melt-processed neat PHA and its composites, their maximum lifetime was estimated (Table 2). The time needed for melt-processed PHA0W, PHA20W and PHA50W slabs (dimensions: 70 x 15 x 1.6 mm; initial weight: ~ 2 g) to degrade was calculated based on the weight loss data assuming only a surface erosion mechanism (linear weight loss rate) throughout. According to this calculation, it would take 34 years to degrade a melt-processed neat PHBV slab. The lifetime was halved when 20 wt% of wood flour was added and was further decreased to 7.5 years when 50 wt% of wood flour was included. The lifetime of PHA-based composites could thus be adjusted for specific applications by altering the wood content. It is worth noting that these values over-estimate the actual lifetime of the neat PHA and composites, as simultaneous surface and bulk erosions occur when the thickness of the samples or of the exposed interior regions are lower than the critical thickness (Figure 9), resulting in an exponential decrease in weight and thus acceleration of the degradation.

Table 2

3.3.2. PHA content after 12 months

In order to understand whether the weight losses observed from the composites were associated with the biodegradation of PHA or wood, the polymer content of the PHA composites after 12 months were quantified using a chloroform extraction method (Table 3). The theoretical PHA contents, assuming that the weight loss observed was associated with only PHA or only wood, were also determined as a comparison. The PHA content in PHA20W after 12 months was 74 ± 1%, which is lower than the theoretical value if the weight loss observed was associated with

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PHA only (79%). The lower experimental PHA content compared to expectation could be attributed to residual PHA which was not extracted, and to solution losses during the pouring and filtering processes. Nevertheless, comparing the PHA content before and after soil burial could still provide insights into the location of degradation. PHA20W, for example, had a lower PHA content after 12 months, which suggests that more PHA was degraded than wood. With a high PHA to wood ratio, the wood flour could be surface wetted/coated by the PHA such that the microorganisms were not able to access it. As can be seen in Table 3, the experimentally determined PHA content of PHA50W after 12 months fits within the window between the theoretical values. This implies that both wood and PHA have degraded throughout the 12 months in soil.

Table 3

3.3.3. Quantification of degradation by molecular weight analysis

Since PHA degrades via a surface erosion mechanism rather than through bulk erosion, due to enzymatic hydrolysis at the surface, the molecular weight loss of the PHA-containing samples was not significant. The number-average molecular weight ($\overline{M_n}$), weight-average molecular weight ($\overline{M_w}$) and polydispersity (PDI) of PHA in all PHA-containing samples are shown in Figure 4a, 4b and 4c, respectively. PLA50W was used as a reference. The molecular weight data showed similar trends to the weight loss results. Only slight decreases in both $\overline{M_n}$ and $\overline{M_w}$ were observed from PHA0W after 12 months of soil burial. It is however noted that the $\overline{M_n}$ of PHA0W had increased slightly after 3.5 months. It is known that the lower molecular weight chains are more readily degraded [32], as well as being more concentrated in the amorphous

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regions which are in turn more rapidly degraded than the crystalline regions. In addition, lower molecular weight chains can more readily migrate, and may relocate to the surface where they again can be readily degraded by extracellular enzymes. As $\overline{M_n}$ is more sensitive to the changes to the proportion of low molecular weight chains, an increase in $\overline{M_n}$could be associated with the digestion of the lower molecular weight chains.

The decreases in both the $\overline{M_n}$ and $\overline{M_w}$ of PHBV throughout 12 months of soil burial became more severe with increasing wood content, although were still not significant. It is worth noting that with surface erosion of PHA polymers, the material properties of the bulk polymer (such as mechanical properties and molecular weights) will remain relatively unchanged, at least until bulk depolymerisation starts to take place.

The PLA in PLA50W showed similar $\overline{M_n}$ and $\overline{M_w}$ values throughout the soil burial period, showing that PLA underwent minimal biodegradation in soil after 12 months under ambient conditions and/or was only surface eroded. Finally, the PDI values for all samples were similar throughout the 12-month soil burial period (as can be seen as can be seen from Figure 4(c), suggesting that the distributions of molecular weight were similar at all time-points.

Figure 4 (2-column fitting)

3.4. Quantification of biodegradation through oxygen consumption

Due to the limited availability of the Oxitop apparatus and the need to be selective in the samples analysed, the biodegradation in the laboratory of only the following materials were studied: PHA20W and PHA50W for understanding the role of wood content, PLA50W and PE50W for comparing between polymer matrices at a given 50 wt% wood content, plain soil as a negative

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control and starch as a positive control, as described in section 2.5. The percentage of carbon degraded for PHA20W, PHA50W, PLA50W and PE50W was plotted against time (Figure 5), showing the curves of both duplicates. The biodegradation of PHA20W and PHA50W was initiated after around 50 days with a linear increase in % degraded throughout the incubation period of 12 months. This lag time has been reported to be associated with the time needed for adherence of microorganisms on the surface [33]. Large variations between the duplicates were observed, which is common for biological activities. In contrast to the results from dry weight loss in the field trial, the biodegradation rates were similar between the two wood contents, wherein PHA20W and PHA50W were on average 36% and 35% degraded after 12 months, which is higher than the weight loss values from the field. Without both air-cooling and sunshine when compared to the field trial, the samples were exposed to soil at higher moisture content, wherein the presence of moisture has been shown to accelerate the biodegradation of both wood [34] and PHA [35].

PLA50W and PE50W took a much longer time to degrade than PHA, with biodegradation not being initiated until after 320 days. This result aligns with the trends observed from the field trial in that the wood particles were partially protected from bacterial and fungal attack by the non-degradable polymer matrix.

Figure 5 (1.5-column fitting)

3.5. Micro-structure of cross-sections by optical microscopy

To understand the surface effect of the biodegradation process, the micro-structures of the cross-sectional surfaces were analysed. The optical microscopy images of the cross-sections before

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burial and after 12 months are presented in Figure 6. As can be seen from the first row in Figure 6, neat PHBV (PHA0W) was intact and free of obvious porosity in the bulk both before and after soil burial. The only sign of biodegradation was the formation of rough surfaces along the edges, which is an evidence of the surface erosion mechanism for PHA. There was a visual difference between surface and bulk in the PHA20W sample (second row, Figure 6). The surface erosion of PHA and possibly degradation of accessible wood flour near the surface introduced cracks around the edges for a depth of approximately 200 μm whereas the bulk was still intact with minimal obvious porosity. The bacterial or fungal attack was limited to the surface by the loose network of wood flour such that it lacked a clear pathway for water to penetrate through the samples. However, for PLA50W and PE50W, porosity was observed within the bulk in all samples containing 50 wt% wood flour, independent of polymer type, after 12 months of soil burial. This porosity could be attributed to the flaws produced during processing and also the degradation of wood particles by microbial attack.

When comparing between the images of PHA50W before and after soil burial (third row, Figure 6), more cracks and porosity were observed after soil burial. Figure 7 shows the optical microscopy image of the cross-section of PHA50W at higher magnification showing the network of cracks at the edges. The cracks penetrated through to the bulk of the composite sample. It can be seen from Figure 7 that the cracks run particularly along the wood-PHA interface, as annotated by the black arrows. The black regions in Figure 7 represent the edges of the cracks and the white regions are the loosened/opened channels developed from the initial cracks. For PLA50W (forth row, Figure 6), the observed porosity is most likely due to the flaws formed during processing, as black regions were also seen in the sample before burial. This agrees with the weight loss results in that the biodegradation of wood in PLA50W is low. PE50W (fifth row,

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Figure 6) also had similar intact microstructures before and after soil burial. Signs of wood degradation in PLA50W and PE50W were only evidenced by the higher porosity near the surface, as can be seen from the optical microscopy images.

Figure 6 (1.5-column fitting)

Figure 7 (1.5-column fitting)

3.6. Moisture content

The samples were placed underground at a site with an accumulated rainfall of 890 mm throughout the burial period, allowing for water absorption by the hydroscopic wood flour. The moisture contents were therefore determined to connect the role of moisture to the biodegradation of the composites. Figure 8 shows the water absorption profile of all samples against soil burial time. PHA0W showed a slight and gradual increase in moisture content throughout the 12 months reaching a maximum moisture content of 1.0 ± 0.2% after 12 months, showing that PHA has very limited water uptake, which may be associated with surface roughness. PHA20W had the same profile as PHA0W for the first month, then the moisture content increased rapidly over the next 2 months and then stabilised at 5.7% ± 0.5%. The moisture uptake of all composites containing 50 wt% wood flour increased rapidly after 1 month which implies that the wood flour was not completely sealed by the polymer matrices. PHA50W achieved a moisture content of 18.4% ± 0.6% after 12 months of soil burial. Similar equilibrium moisture contents were observed from water immersion studies in the literature, with 18.8% moisture contents for PHBV composites with 50 wt% pine WF [36] and 4.8% for PHBV with 20

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wt% of oak wood flour [37]. Such similarity was not surprising given that the water from rainfall can be trapped under the soil and provide a moist environment. However, higher initial absorption rates were observed in water immersion studies due to the higher water content in the environment. In the current study, the moisture content profiles after 1 month were different for each polymer matrix. PHA50W showed a gradual increase in moisture content over the 12 months. No further increase was observed from PE50W and PLA50W after 1 month. The saturated moisture content for PLA50W and PE50W were 13% ± 1% and 11% ± 1%, respectively, which were lower than the value of PHA50W after 12 months.

Figure 8 (single-column fitting)

3.7. Mechanical properties

The mechanical properties of the samples were characterised before and after 12 months of soil burial, with the tensile strength values (mean ± 95% confidence interval) of the composites being presented in Table 4. The tensile strength of neat PHBV (PHA0W) was maintained throughout the burial period. When wood was added, the mechanical stability of the samples under soil deteriorated: the tensile strength of PHA20W decreased, although the samples were still intact after 12 months. The moisture-induced mechanical deterioration [38] and the erosion of both wood and PHA as evidenced by the weight loss could be used to explain the lower mechanical stability, as well as surface cracks that induce crack propagation. Unlike PHA20W, PHA50W samples lost all their mechanical strength after 12 months (with the samples fracturing on being placed into the grips for testing). On the other hand, PLA50W and PE50W showed less decrease in tensile strength over time when compared to PHA50W.

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Table 4

4. Discussion

4.1. Biodegradation mechanism of neat PHA

Two mechanisms, surface and bulk erosion, are involved in the hydrolytic degradation of biopolymers, which is governed by the relationship between the thickness of the polymer (L) and the critical thickness (L_{crit}). For enzymatically catalysed hydrolysis, such as for PHA, the rate of hydrolysis at the surface is much greater than the uncatalysed rate of hydrolysis internally, and polymers are therefore eroded layer by layer from the surface to the core. The bulk remains intact as the microorganisms and their associated enzymes cannot access the bulk due to their size. The changes in mass, molecular weight and mechanical property against burial time are illustrated in Figure 9. When the surface eroded polymer reduces to a thickness that is lower than L_{crit}, the degradation mechanism shifts to a combined bulk and surface erosion where the material degrades internally at a significant rate and becomes subject over time to autocatalytic depolymerisation. The polymer is eroded in bulk throughout the matrix which results in an exponential decrease in mass, molecular weight and mechanical property as illustrated in Figure 9. PHA undergoes biodegradation via a surface erosion mechanism (with a linear degradation rate) until it reaches a critical thickness at which point bulk erosion (with an exponential degradation rate) is initiated [25]. Therefore, as for other surface eroding biodegradable polymers, the biodegradation rate of PHA depends on thickness.

Figure 9 (ref: [25]) (1.5-column fitting)

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The mechanism of surface erosion of PHA was demonstrated in the field trial results. After 12 months of soil burial, the mechanical strength was retained and only a slight loss in molecular weight was evidenced, whereas a linear mass loss trend was observed throughout the burial period. Without a void network across the sample, living microorganisms such as bacteria and fungi, and the relevant degradation enzymes that initiate the biodegradation, cannot access the bulk of the hydrophobic PHBV sample, thus resulting in surface erosion [39]. Therefore, biodegradation can only happen slowly on the surface of the PHBV. With a relatively low exposed surface area, this led to a low dry weight loss after 12 months in soil. The molecular weight and mechanical properties results are in line with the weight loss result in that the neat PHBV degraded by surface erosion, which did not affect the polymer chain lengths in the bulk.

4.2. Biodegradation mechanism of PHA-wood composites

The degradation mechanism becomes more complicated when wood flour is added into the system. The mass and molecular weight losses upon burial are expected to behave similarly to the surface erosion mechanism of biodegradable polymers. However, the mechanical properties would likely behave more like the bulk erosion mechanism, given that the loss of PHA and wood from surface erosion creates porosity and cracks within the composite matrix, leading to loss of mechanical integrity. As mechanical properties are more sensitive to the bulk properties among the three tested properties (mass, molecular weight and mechanical properties), the mechanical properties thus change in a manner reminiscent of bulk erosion, even if the underlying molecular changes are not driven by such processes.

The following mechanism is proposed based on the observations, with a schematic diagram that illustrates the putative mechanism being presented in Figure 10. In this model, initial surface erosion of PHA takes place when in contact with the enzymes associated with bacteria or fungi,

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which need a sufficiently wide porous pathway to access internal sites [40]. The moisture absorbed by the wood creates a local moist environment at the wood-polymer interface, likely promoting phenomena similar to crazing and localised environmental stress cracking, possible hydrolytic degradation and eventually loosens the interface. Through providing an open channel adjacent to the wood following polymer degradation and stress cracking, a pathway for bacteria and fungal hyphae to navigate into the bulk of the matrix. Thus, the surface erosion of both wood and PHA could happen along the wood-PHA interfaces, forming a network of cracks throughout the bulk.

The observed network of cracks along the interface with a penetrated depth of approximately 1 mm from the microscopy images of PHA50W after 12 months (Figure 7) was clear evidence for the proposed mechanism. The moisture content and mechanical properties results also provide support for the above mechanism. Unlike PLA50W and PE50W, PHA50W showed gradual increase in moisture content from 3.5 months to 12 months (Figure 8).

The porosity and cracks created by the PHBV and wood biodegradation allowed more water to be absorbed within the composite samples. With the presence of defects in the bulk of the composites, the samples could only bear a very low load. It should be noted that although the mechanical properties were completely lost within 1 year, PHA50W only achieved a 12.7% weight loss. This can be interpreted as the mechanical deterioration being not directly induced by the overall weight loss but through the introduction of cracks and porosity by the degradation of wood and PHBV in bulk. The swelling and moisture absorption of wood flour likely promotes localised stress cracking although such phenomena could possibly be mitigated by surface modifications of wood or the addition of compatibilisers which have shown to improve the mechanical properties [41] and reduce the rate of water absorption [42] of PHA-based

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composites. The biodegradation rate of PHA WPCs depend on the surface area of wood-PHA interface and thus the wood content. Although the surface erosion rate could be similar, the surface area for surface erosion in PHA50W is significantly larger than that of PHA20W and thus the overall degradation rate was higher, which was evidenced from the weight loss results. With fewer networks of cracks and less penetration through the composites matrix, as observed from the microscopy images, the mechanical property deterioration of PHA20W was not as severe as that of PHA50W.

For PLA50W and PE50W, the biodegradation of wood flour in composite systems was limited by the partial encapsulation of the wood by the non-degradable polymer matrix, such that most of the wood particles were not accessible by the surrounding living microorganisms. As a result, the composite's matrix remained intact in bulk with minimal networks of cracks such that low weight loss values were observed and their deterioration in mechanical properties was minimal. However, the localised stress induced by the moisture absorption of wood, as suggested in the proposed mechanism, also applies to PLA50W and PE50W. Given that moisture absorption was still seen in both PLA50W and PE50W, the encapsulation was not perfect and the wood particles were still partially accessible. The swelling and moisture absorption of wood flour likely promotes localised stress cracking. The observed slightly reduced mechanical strength could be due to the as-created cracks and the biodegradation of accessible wood particles. Therefore, it is concluded that the localised stress alone did not lead to the complete loss in mechanical properties. The combination of the cracks induced by localised stress and the surface erosion of wood and PHA along the opened channel contributed to the complete mechanical loss of PHA50W and thus they are the key activities involved in the biodegradation of PHA WPCs.

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Figure 10 (2-column fitting)

5. Conclusion

In this work, the biodegradability of neat PHBV and PHBV/WF composites upon outdoor soil burial were studied, using PLA/WF and PE/WF composites as reference materials. Rough surface morphologies were observed on the PHA0W plaques and on all the PHA WPCs after 3.5 months of soil burial. However, with a relatively low exposed surface area, only 2.6% weight loss was achieved for PHA0W after 12 months of soil burial. By contrast, the degradation rate of PHA/WF composites increased with increasing wood content. The weight loss after 12 months was approximately 2.5 times greater for PHA20W composites and 5 times greater for PHA50W than for neat PHA (PHA0W) under identical conditions. The molecular weight results are also in line with the weight loss results such that the loss in molecular weight was more pronounced with increasing WF content.

The mechanical properties of neat PHA were retained throughout the 12-month soil burial period. When 20 wt% of WF was added, the mechanical stability of the samples under soil deteriorated, but the samples were still intact after 12 months. More interestingly, PHA50W lost its mechanical integrity after 12 months although only a 13% weight loss was observed. By contrast, PLA and PE composites showed only slight decreases in mechanical properties over time, which could be associated with moisture induced localised stress, a phenomenon similar to environmental stress cracking. This alone did not lead to a complete loss of mechanical properties.

A network of cracks and voids along the WF-PHA interface with a penetration depth of approximately 1 mm was evident from the cross-section of PHA50W after 12 months. It is

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proposed that the localised stress loosened the interface, allowing channels for the bacteria and fungi to access PHA in the bulk of the matrix for local enzymatic biodegradation. With this network of interconnecting pores and cracks, crack propagation and mechanical failure would readily result from an applied stress. Although the erosion rate for the exposed PHA surfaces in the different samples could be similar, the surface area for surface erosion in PHA50W is significantly larger following void formation than for neat PHA and thus there is a higher biodegradation rate overall.

Acknowledgement

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Figure 1 Visual appearances of all unaged and buried samples at five different exposure times (1, 2, 3.5, 6 and 12 months) showing both surfaces (top surface in upper row, bottom surface in lower row).

Figure 2 SEM micrographs of the surface of all samples (in rows) before soil burial (left column) and after 12 months (middle column) at 100x magnifications and after 12 months at higher

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1000x magnification (right column) showing the details on the rough surfaces. Arrows indicate the fungal hyphae cells and circles indicate the fungal spores observed.

Figure 3 Percent dry weight loss of all samples (dimension: 70 x 15 x 1.6 mm, initial dry weight: approximately 2 g) plotted against soil burial time (data presented as mean values with 95% confidence interval).

Figure 4 The (a) number-average molecular weight ($\overline{M_n}$), (b) weight-average molecular weight ($\overline{M_w}$) and (c) polydispersity (PDI) of all unaged and buried samples against burial time.

Figure 5 Biodegradation rate of PHA20W (green), PHA50W (red), PLA50W (brown) and PE50W (blue) from CO_2 evolution data. Full and dashed lines represent the results from replicate (parallel) studies.

Figure 6 Optical microscopy images of the cross-sections of all samples (in rows) before soil burial (left column) and after 12 months (right column).

Figure 7 Optical microscopy images of the cross-sections of PHA50W after 12 months of soil burial at higher magnification. The arrows show the loosened/opened channels developed from the propagation of initial cracks.

Figure 8 Moisture uptake profile of all unaged and buried samples against burial time.

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Figure 9 The stages and mechanisms of biodegradation by hydrolysis of biodegradable polymers. Under a surface erosion mechanism driven by enzymatic hydrolysis, the polymer is eroded layer by layer and the core remains intact. When the sample is thin enough, both surface and bulk erosion take place throughout the matrix. [25] © Elsevier. Reproduced by permission from Elsevier.

Figure 10 Schematic diagram showing the biodegradation mechanism of PHA wood plastic composites.

Table 1 Composite formulations for soil burial study

Samples	PHBV (wt%)	PLA (wt%)	HDPE (wt%)	Wood flour (wt%)
PHA0W	100	0	0	0
PHA20W	80	0	0	20
PHA50W	50	0	0	50
PLA50W	0	50	0	50
PE50W	0	0	50	50

Table 2 Degradation rates and estimated maximum lifetimes of PHA0W, PHA20W and PHA50W under soil burial, based on the weight loss data from the field trial

Samples	Degradation rate $(mg/cm^2 - day)$	Estimated maximum lifetime (years)
PHA0W	0.0069	34
PHA20W	0.015	14.5

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PHA50W	0.030	7.5

Table 3 Experimentally determined PHA contents of PHA20W and PHA50W after 12 months of soil burial in comparison with the theoretical PHA content if the weight loss observed was associated with PHA only and with wood only

Samples	**Experimentally determined PHA content after 12 months (%)**	**Theoretical PHA content if weight loss observed was only from PHA (%)**	**Theoretical PHA content if weight loss observed was only from wood (%)**
PHA20W	74 ± 1	79	86
PHA50W	48 ± 3	43	57

Table 4 Tensile strength of the samples before and after 12 months of soil burial, and their corresponding percent change

Sample	**Tensile strength (MPa)**		**% change**
	Before soil burial	**After 12 months of soil burial**	
PHA0W	32 ± 1	33 ± 1	+3%
PHA20W	29 ± 1	24 ± 2	-17%
PHA50W	22 ± 2	*Fractured when placed onto the grips*	-100%
PLA50W	28 ± 2	21 ± 2	-25%
PE50W	12 ± 1	8 ± 1	-33%

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Graphical abstract

Highlights

- Comprehensive biodegradation analysis of PHA/wood composites was conducted
- Increase in wood content led to an increased rate of degradation
- PHA composites degraded much faster than PLA and PE composites in soil
- Degradation mechanism of composites of biopolymer and wood was proposed
- Localised stress loosened the interface allowing larger surface area for biodegradation

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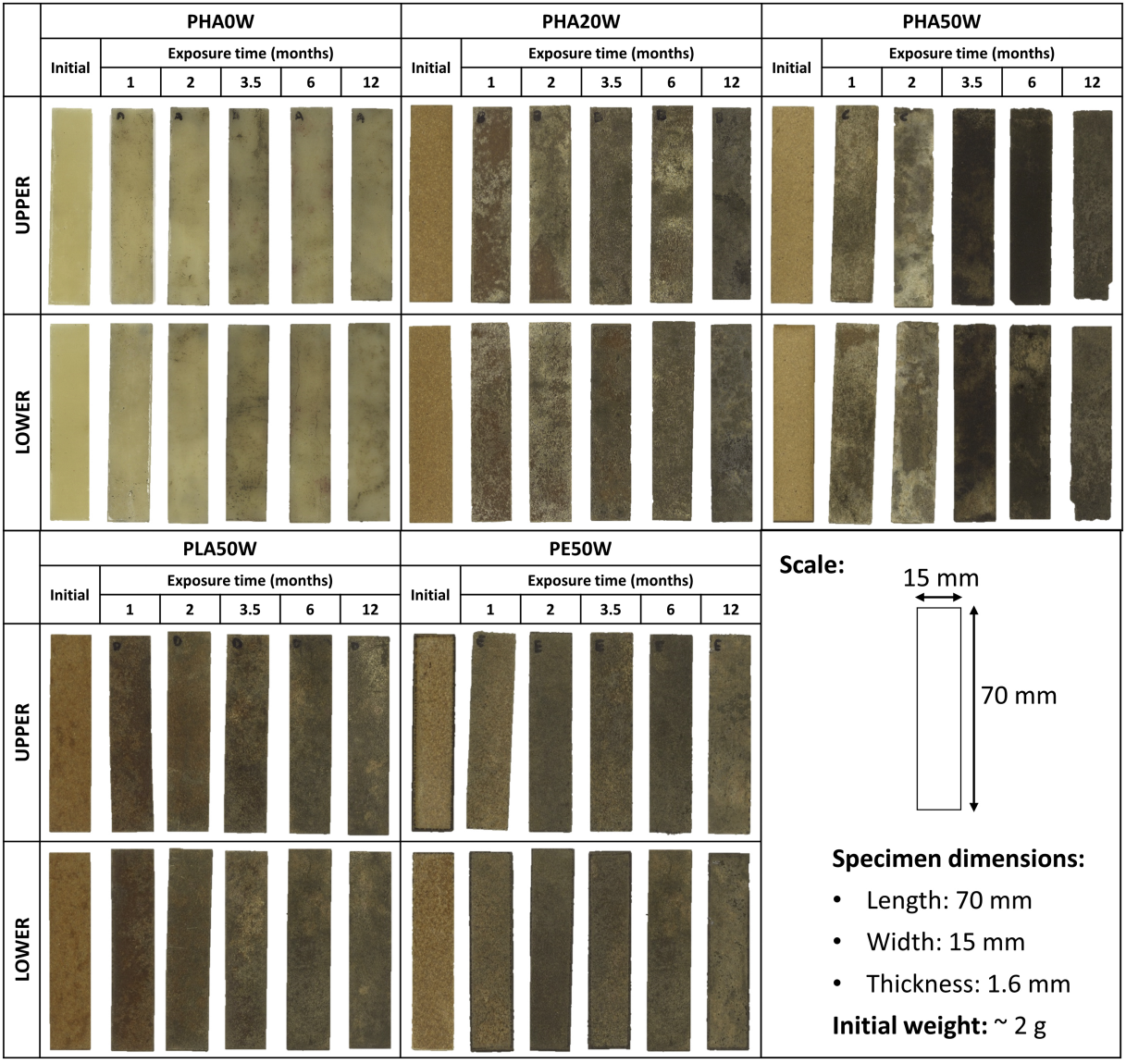
PHA0W
PHA20W
PHA50W
PLA50W
PE50W
Initial
Exposure time (months)
1
2
3.5
6
12
UPPER
LOWER
Scale:
15 mm
70 mm
Specimen dimensions:
• Length: 70 mm
• Width: 15 mm
• Thickness: 1.6 mm
Initial weight: ~ 2 g

Figure 1

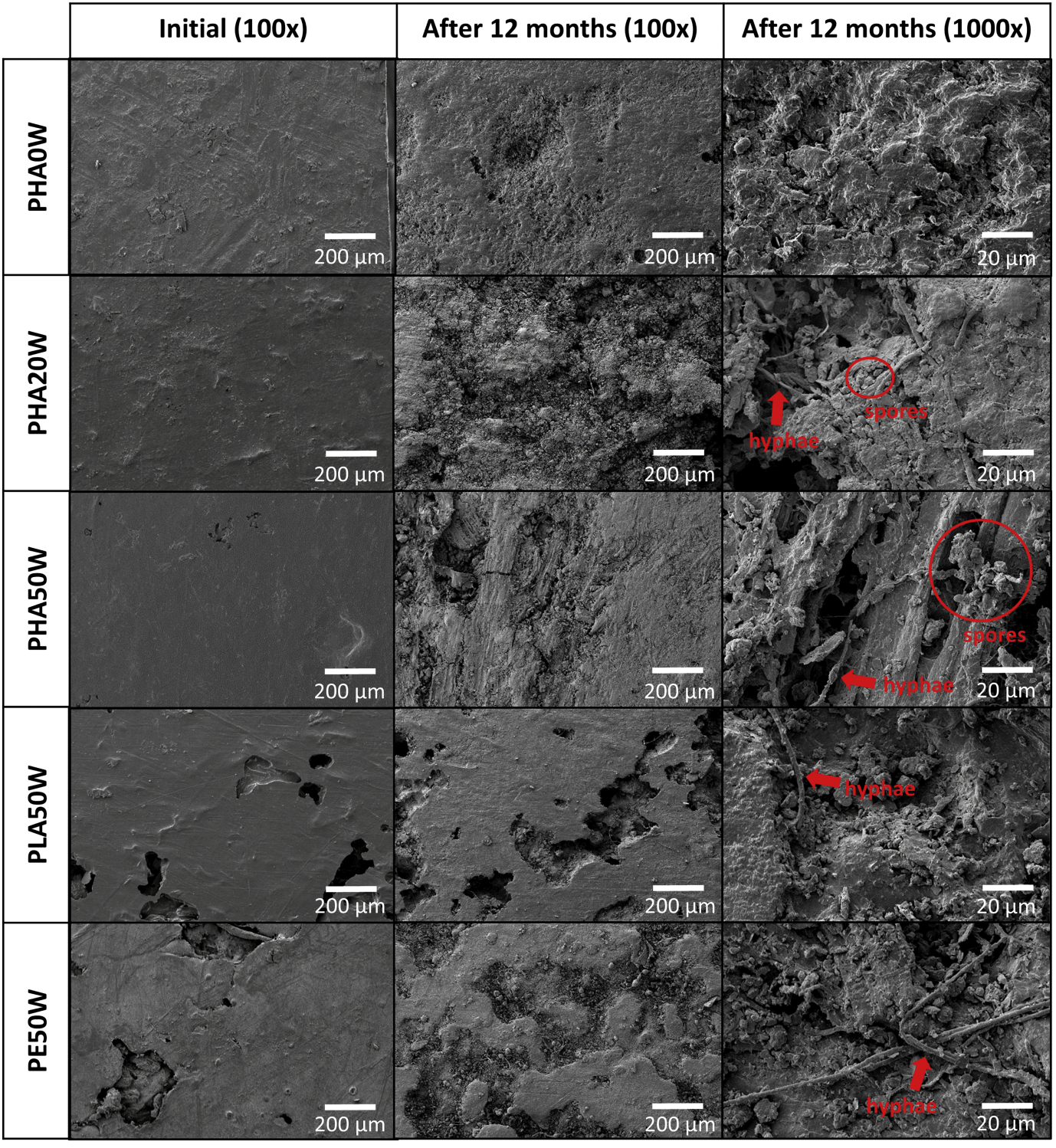
Initial (100x)
After 12 months (100x)
After 12 months (1000x)
PHA0W
PHA20W
PHA50W
PLA50W
PE50W
200 µm
20 µm
spores
hyphae

Figure 2

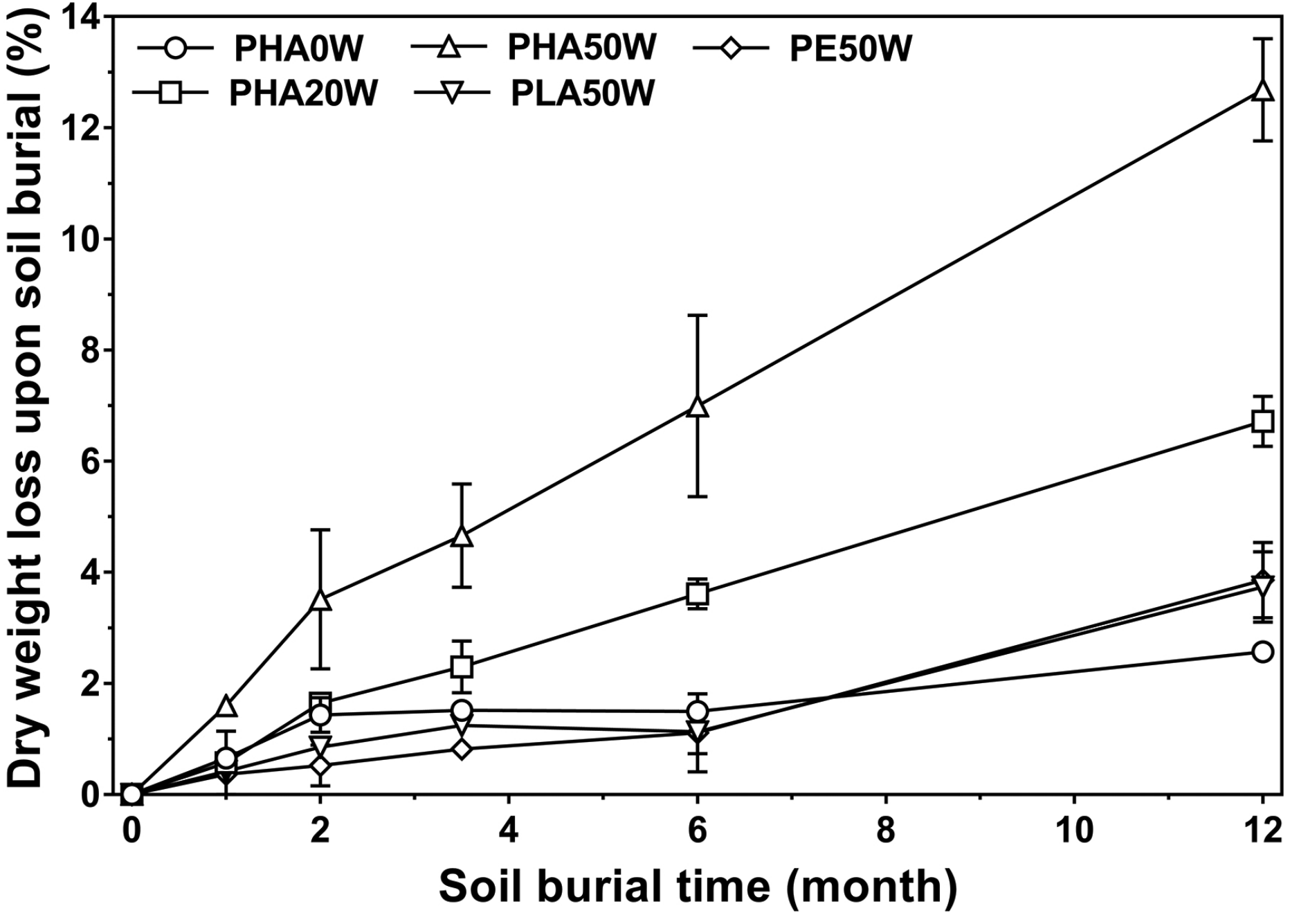
PHA0W
PHA50W
PE50W
PHA20W
PLA50W
Dry weight loss upon soil burial (%)
Soil burial time (month)
0
2
4
6
8
10
12
14

Figure 3

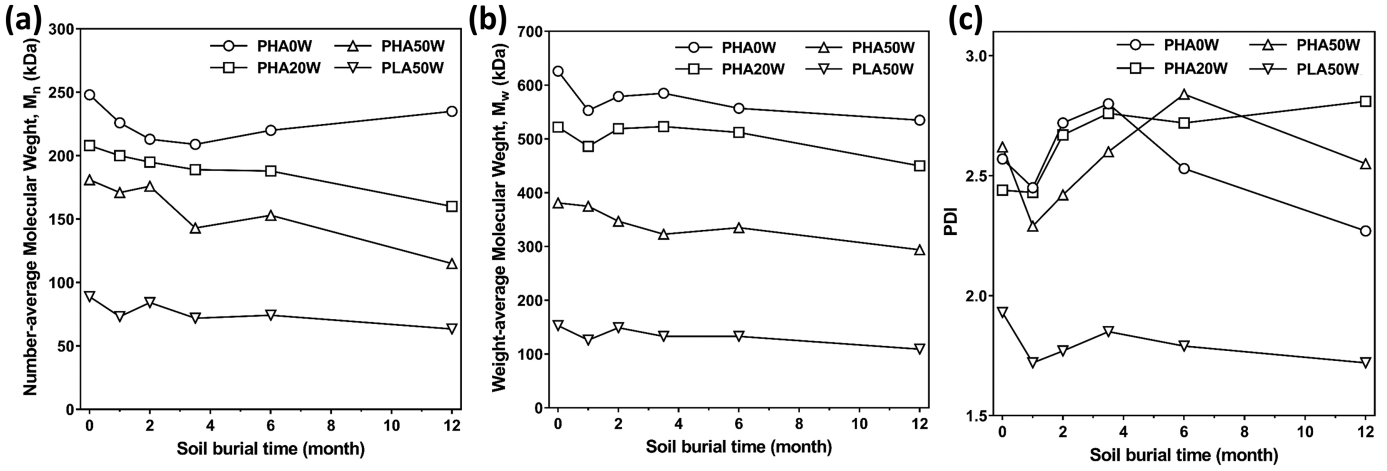
(a)
Number-average Molecular Weght, M_n (kDa)
PHA0W
PHA50W
PHA20W
PLA50W
Soil burial time (month)
(b)
Weight-average Molecular Weght, M_w (kDa)
PHA0W
PHA50W
PHA20W
PLA50W
Soil burial time (month)
(c)
PDI
PHA0W
PHA50W
PHA20W
PLA50W
Soil burial time (month)

Figure 4

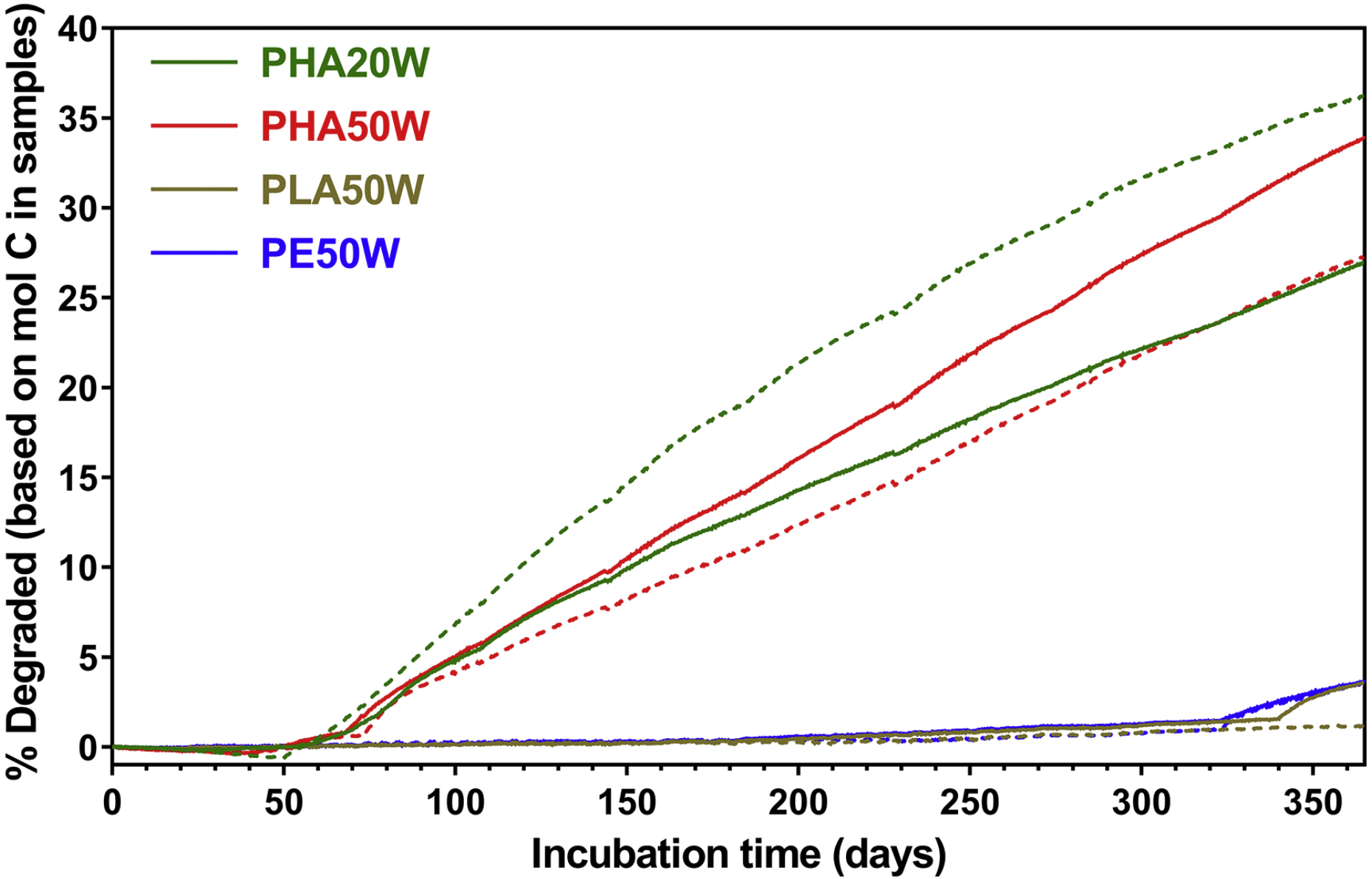
PHA20W
PHA50W
PLA50W
PE50W
% Degraded (based on mol C in samples)
0
5
10
15
20
25
30
35
40
0
50
100
150
200
250
300
350
Incubation time (days)

Figure 5

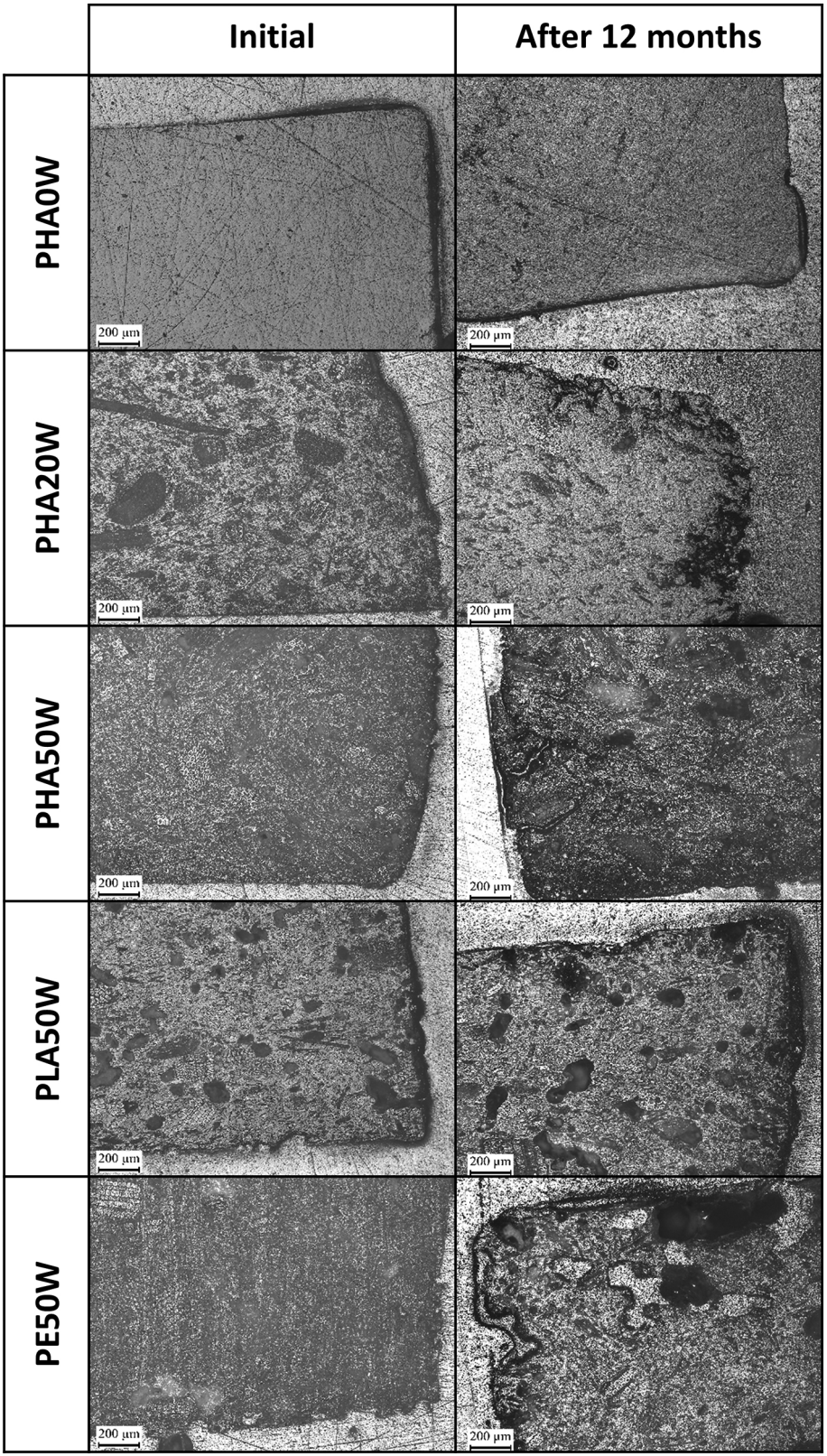
Initial
After 12 months
PHA0W
PHA20W
PHA50W
PLA50W
PE50W
200 µm

Figure 6

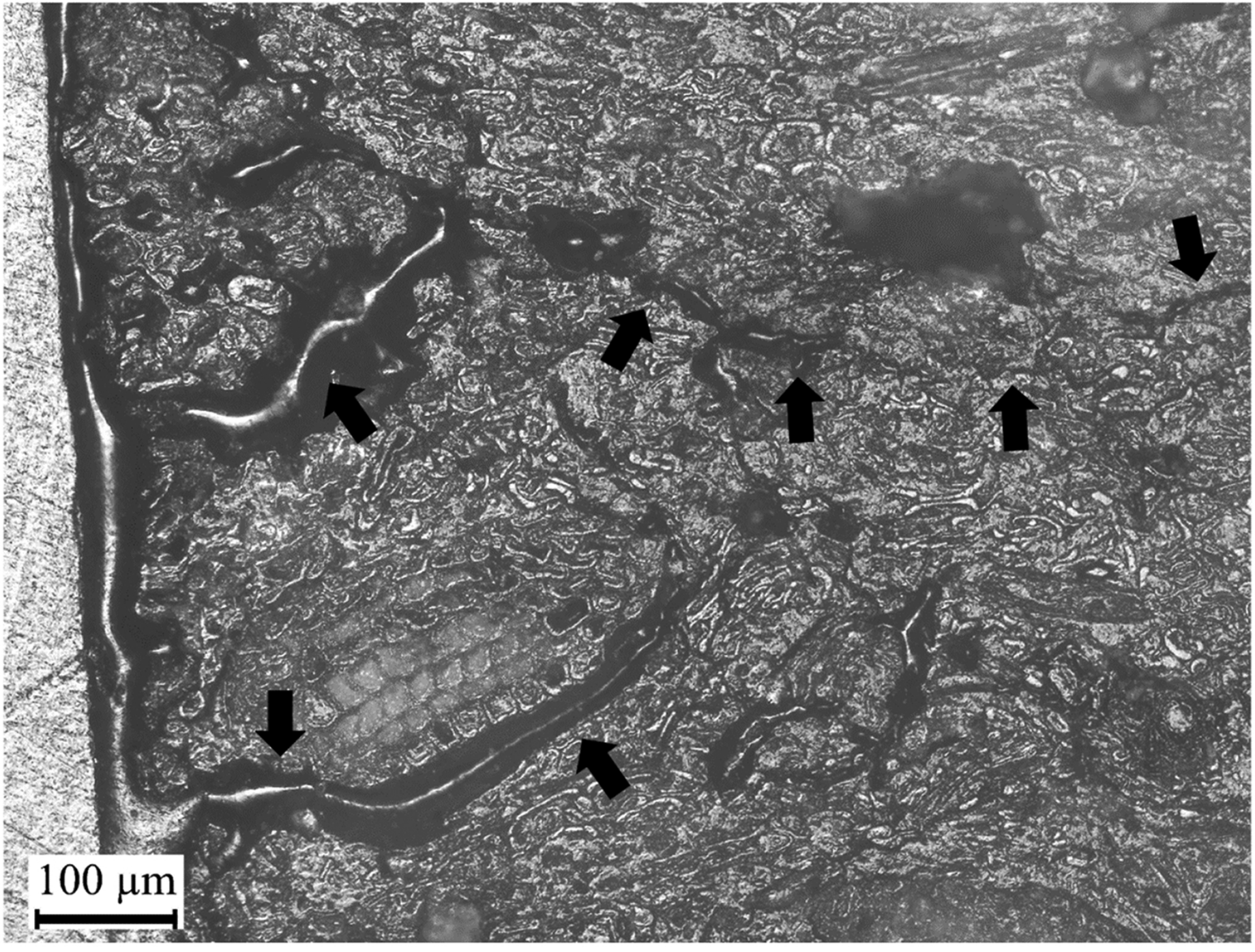

Figure 7

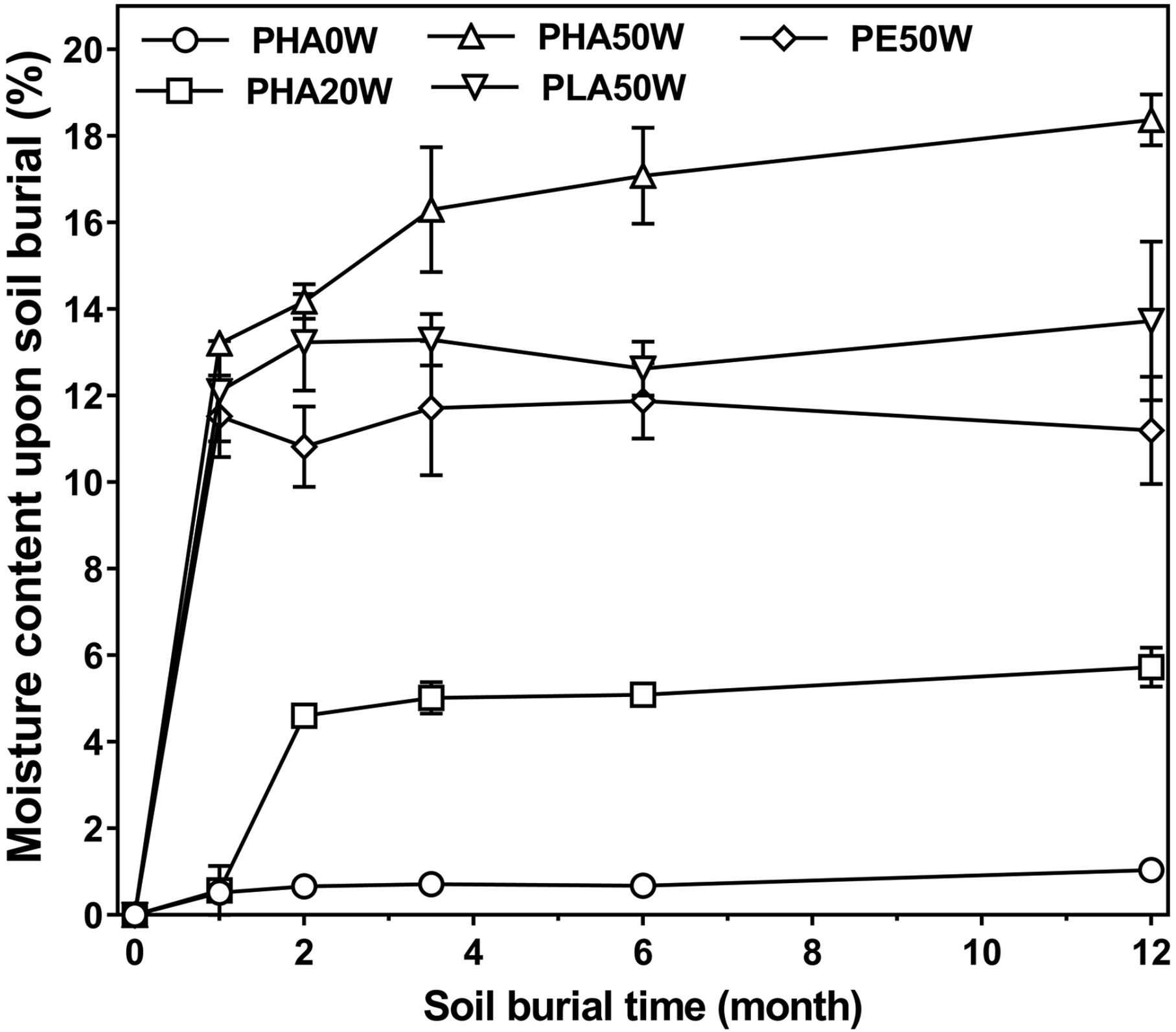
PHA0W
PHA50W
PE50W
PHA20W
PLA50W
Moisture content upon soil burial (%)
0
2
4
6
8
10
12
14
16
18
20
0
2
4
6
8
10
12
Soil burial time (month)

Figure 8

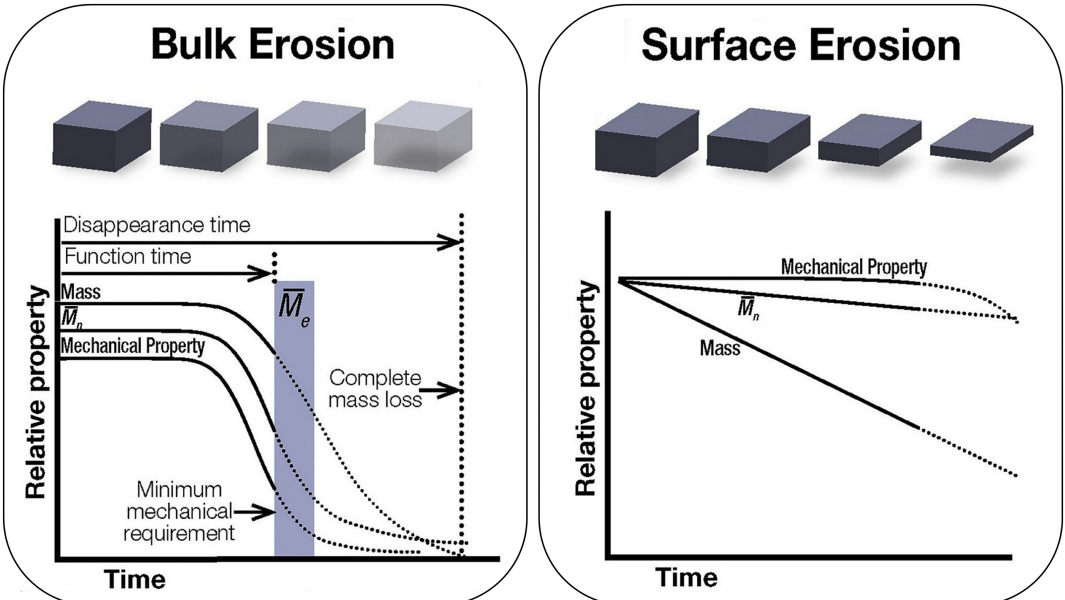
Bulk Erosion
Disappearance time
Function time
Mass
$\bar{M}_n$
Mechanical Property
$\bar{M}_e$
Complete mass loss
Minimum mechanical requirement
Relative property
Time
Surface Erosion
Mechanical Property
$\bar{M}_n$
Mass
Relative property
Time

Figure 9

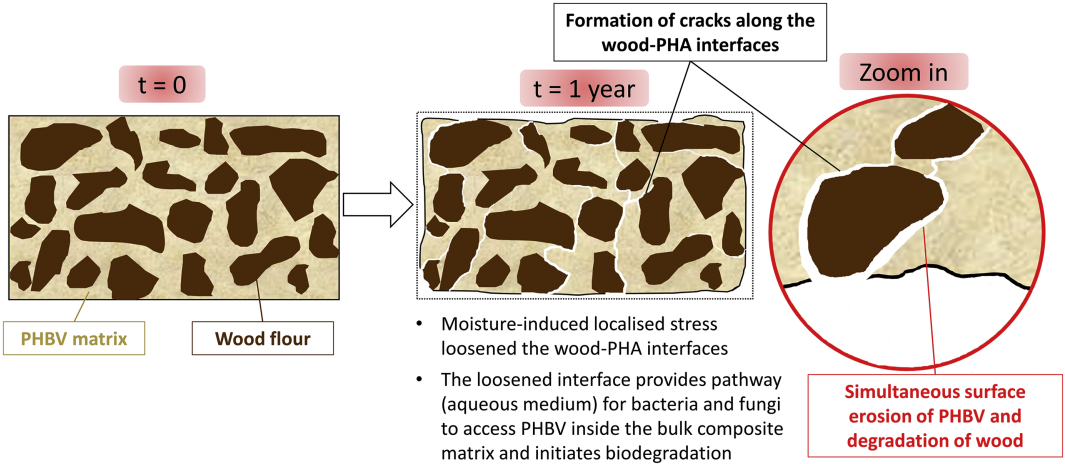
t = 0
t = 1 year
Formation of cracks along the wood-PHA interfaces
Zoom in
PHBV matrix
Wood flour
• Moisture-induced localised stress loosened the wood-PHA interfaces
• The loosened interface provides pathway (aqueous medium) for bacteria and fungi to access PHBV inside the bulk composite matrix and initiates biodegradation
Simultaneous surface erosion of PHBV and degradation of wood

Figure 10