Environmental degradation of lignin/poly(hydroxybutyrate) blends

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Abstract

Blends of lignin and poly(hydroxybutyrate) (PHB) were obtained by melt extrusion. They were buried in a garden soil for up to 12 months, and the extent and mechanism of degradation were investigated by gravimetric analysis, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), X-ray photoelectron spectroscopy (XPS), scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FT-IR) over the entire range of compositions. The PHB films were disintegrated and lost 45 wt% of mass within 12 months. This value dropped to 12 wt% of mass when only 10 wt% of lignin was present, suggesting that lignin both inhibited and slowed down the rate of PHB degradation. TGA and DSC indicated structural changes, within the lignin/PHB matrix, with burial time, while FTIR results confirmed the fragmentation of the PHB polymer. XPS revealed an accumulation of biofilms on the surface of buried samples, providing evidence of a biodegradation mechanism. Significant surface roughness was observed with PHB films due to microbial attack caused by both loosely and strongly associated microorganisms. The presence of lignin in the blends may have inhibited the colonisation of the microorganisms and caused the blends to be resistant to microbial attack. Analysis suggested that lignin formed strong hydrogen bonds with PHB in the buried samples and it is likely that the rate of random chain scission of PHB is reduced, preventing rapid degradation of the blends.

KEYWORDS: Biopolymer; degradation; lignin; poly(hydroxybutyrate) (PHB); soil; thermal properties.
Producing biodegradable plastics and materials has been suggested in response to increased awareness of environmental hazards caused by disused plastics. One such material is poly(hydroxybutyrate) (PHB) which is totally biodegradable and is produced by the fermentation of sugars and other chemicals or produced from plants [1]. PHB has attracted commercial interest as a plastic material because its physical properties are remarkably similar to those of polypropylene (PP), even though the two polymers have quite different chemical structures. PHB exhibits a high degree of crystallinity, has a high melting point of approximately 173 °C, and most importantly, unlike PP, is biodegradable [2]. PHB has much potential in applications such as in packaging, pharmacology and medical applications [2].

Two major factors that currently inhibit the widespread use of PHB are its high cost and poor mechanical properties. The production costs of PHB are significantly higher than plastics produced from petrochemicals [2] and its stiff and brittle nature makes processing difficult which impedes its ability to handle high impact. Several attempts have been made to improve the physical properties by blending it with other biodegradable polymers found to be miscible with PHB, such as poly(ε-caprolactone) [3], poly(vinylidene fluoride) [4], poly(vinyl alcohol) [5], poly(lactic acid) [6], poly(vinyl acetate) [7], poly(vinyl phenol) [8], poly(DL-lactide)–co–poly(ethylene glycol) [9], and cellulose esters [10]. Recent studies have focussed on lignin, an amorphous natural polymer with hydrophobic behaviour [11]. This property makes it a suitable candidate for blending with PHB.

Biodegradation of polymer blends is determined by blend composition, composition of the blend surface, miscibility properties between components, phase structure (i.e., amorphous or crystalline) of the components and roughness of the blend surface. Woolnough et al., [12] studied the biodegradation of PHB and some other “green plastics” in garden soil by detecting mass loss, topographical changes and biofilm attachment, and found that PHB itself
has a better degradability than polyhydroxyoctanoate, poly-\( \text{DL} \)-lactide and ethyl cellulose. The study demonstrated a relationship between biofilm attachment, surface rugosity and polymer degradation.

The enzymatic degradation of blends of PHB with immiscible and miscible polymers by extracellular PHB depolymerase has been studied by Kumagai and Doi [13]. The degradation process was strongly dependent on the polymer type. The rate of degradation of PHB with the miscible poly(\( \varepsilon \)-caprolactone) was found to be dependent on a complex weight fraction relationship. With the immiscible polymer, poly(vinyl acetate), the weight loss decreased monotonously with increasing weight fraction of poly(vinyl acetate). Poly(vinyl acetate) which is non-degradable remained on the surface of the buried blends and protected the blends against any biodegradation.

Ikejima et al., [14] found that the degradation profile of the PHB/poly(vinyl alcohol) (PVA) blends buried in river water was dependent on blend composition because the micro-organisms utilising PVA are different from those utilising PHB. The blends with PHB-rich composition showed higher degradation rate and higher amount of lost material than pure PHB. Similarly, PVA-rich blends degraded faster than pure PVA. Ikejima et al., [14] suggested that the improved degradability is also partly due to reduced crystallinity of the minor components in the blend.

Avella et al., [15] showed that reinforcing PHB with a lignocellulose material (wheat straw) does not affect its biodegradation in long term soil burial tests. Scott [16] reported that lignocellulose materials, due to their hydrophobicity and chemical inertness, do not readily degrade either abiotically or biotically and when they do biodegrade, lignin tends to accumulate in the soil. However, filamentous basidiomycetous fungi are the most efficient micro-organisms that degrade lignin [17]. Chen et al., [18] have shown that lignin
The degradation reactions by these micro-organisms involve (a) oxidation, (b) demethylation or demethoxylation, (c) side-chain oxidation and (d) propyl side-chain cleavage between $C_\alpha$ and $C_\beta$. The absence of these microorganisms in soil significantly reduces lignin disintegration.

The aim of this work is to determine the environmental degradation of lignin/PHB blends and to determine the role lignin plays in either accelerating or retarding PHB degradation. The properties of the buried blends were examined by gravimetric, thermogravimetric analysis (TGA), differential scanning calorimetry (DSC), scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and Fourier transform infra-red spectroscopy (FTIR) over a wide range of blend compositions.
2. MATERIALS AND METHODS

2.1 PHB

Bacterial PHB was obtained from Sigma Aldrich (Castle Hill, NSW, Australia). The weight average molecular weight ($M_w$) reported by the supplier was 440,000 g/mol, while the number average molecular weight ($M_n$) was 260,000 g/mol.

2.2 Lignin

Lignin was extracted from sugar cane fibre (i.e., bagasse) obtained from Mackay Sugar Mill, (Mackay, QLD, Australia) by the soda process using 0.7 M sodium hydroxide solution. The procedure for lignin extraction and purification has been described elsewhere [19].

2.3 Lignin Characterisation

Lignin composition was determined by the methods described in the paper by Mousavioun et al. [19] and is presented in Table 1.

2.4 Blend Preparation

Lignin and PHB were dried at 100 °C and 40 °C respectively for 12 h and stored in desiccators under vacuum prior to use. The PHB/lignin blends with lignin contents from 10 wt% to 90 wt% were mixed in a Themo Scientific HAAKE Minilab II micro compounder (Karlsruhe, Germany) with a rotating twin screw mini-extruder using the procedure reported by El-Hadi Abdel Ghaffar [1]. To minimise PHB degradation, the temperature of the extruder was maintained at 175 °C for 2 min. Polymer blends were extruded as strands, then cooled and pelletised. The pellets were stored in a desiccator to avoid moisture absorption. Similar processing conditions were carried out for pure PHB. Previous work on PHB/lignin
blends showed that in blends containing up to 30 wt% lignin, lignin and PHB are miscible [19].

2.5 Polymer Film/Blend Fabrication

Polymer films were prepared by hot pressing the blends under 7.5 bar pressure at 175 °C. To produce film samples with the same thickness, a rectangular mould (45 mm × 30 mm) with the thickness of 100 μm was used. Film samples were prepared by compression moulding between two Teflon sheets. They were subsequently removed and dried under vacuum (48 h, 25 °C), before leaving for a further 24 h at 25 °C and at 30% relative humidity to constant weight. The films were then aged for three weeks to enable their crystallinity to reach an equilibrium value and then were carefully inserted in slide frames prior to burial.

2.6 In situ Biodegradation of Polymer Films in Soil

In situ environmental degradation was conducted on site in a garden soil (Pinjarra Hills Field Station, University of Queensland, Pinjarra Hills, QLD, Australia). The procedure followed was similar to that reported by Woolnough et al., [12]. The soil was sieved to particles of less than 2 mm in diameter and mixed completely before burial of samples. The samples were buried as per ASTM D 5988 in three 1.0 × 0.7 m² plots for 4, 8 and 12 months. The soil of each plot had a pH of 6.7 as measured according to ASTM D 4972, a temperature of 12–27 °C and water content that varied with rain patterns around 20%. Polymer films were buried at least 2 cm apart and 20 cm below the soil surface. The special arrangement of burial ensured that the total sample weight did not exceed 7.7 wt% of soil (ASTM D 6003). Every 2 months soil from one of the three plots was removed and the temperature, pH and water content were measured. The experiment was conducted over 52 weeks. At the end of each period, soil was removed from the polymer film by immersing in a solution containing 0.25% sodium hypochlorate, prior to drying under vacuum (84 h, 25 °C) and then weighed. The soil
removal protocol and preparation of films again followed the method suggested by Woolnough et al [12]. The estimate of standard deviation (absolute) for this technique (based on duplicate experiments, 12 degrees of freedom (df)) is 5% based on starting mass.

2.7 Thermogravimetric Analysis (TGA)

The thermal decomposition studies were carried out in a TA Instruments Q500 thermogravimetric analyser (New Castle, DE, USA). Approximately 10 mg of sample was weighed into an aluminium pan and analysed by TGA by the non-isothermal method. Heating was at a rate of 10 °C/min and was performed from ambient temperature to approximately 800 °C. The test was performed in an atmosphere of nitrogen, which was injected at a flow rate of 15 mL/min. A curve of weight loss against temperature was constructed from the data obtained by the instrument. The estimate of standard deviation (absolute) for the TGA technique (based on duplicate experiments, 10 df) is 0.2 wt%.

2.8 Differential Scanning Calorimetry (DSC)

Approximately 5 mg of sample was precisely weighed and then sealed in an aluminium pan. The pan was then placed in a TA Instruments DSC-Q2000 differential scanning calorimeter (New Castle, DE, USA) and heated from 0 °C to 175 °C at a heating rate of 10 °C/min (cycle 1). The test was performed in an atmosphere of nitrogen, which was injected at a flow rate of 15 mL/min. Samples were then cooled down at a rate of 30 °C/min to –20 °C/min (cycle 2). Samples were then reheated to 180 °C at a rate of 10 °C/min (cycle 3). The plot obtained from this second heating run shows the T_g as a step transition.

In addition to T_g, other thermal parameters of PHB/lignin blends were evaluated. Melting temperature (T_m), melting enthalpy (ΔH_m) and crystallinity (X_c) were extracted from DSC
thermographs. The crystallinity of a blend based on its PHB content was calculated using the following equation:

\[ X_c = \frac{\Delta H_m}{\Delta H_{\text{max}}} \frac{X_{\text{PHB}}}{X_{\text{PHB}}} \] (1)

where \( \Delta H_{\text{max}} \) is melting enthalpy of pure PHB; \( X_{\text{PHB}} \) and \( X_{\text{PHB}} \) are the crystallinity and mass ratio of PHB used in this study, respectively. The crystallinity ratio \( (X_{\text{cPHB}}) \) is a ratio of \( \Delta H_m \) of the sample PHB and that of 100% crystalline PHB \( (\Delta H_0) \). \( \Delta H_0 \) of PHB is assumed to be 146 J/g [20]. On this basis, the values of \( \Delta H_{\text{max}} \) and \( X_{\text{cPHB}} \) used in this study are 92 J/g and 63% respectively. The estimate of standard deviation (absolute) for this technique (based on duplicate DSC analyses, 15 df) for \( T_g \), is 5%, \( T_m \) is 5%, \( \Delta H_m \) and for \( X_c \) is 2%.

2.9 X-ray photoelectron spectroscopy (XPS) analysis

The XPS data were acquired using a Kratos Analytical Axis ULTRA X-ray photoelectron spectrometer (Manchester, UK), incorporating a 165 mm hemispherical electron energy analyser. The incident radiation was monochromatic Al K\( \alpha \) X-rays (1486.6 eV) at 150 W (15 kV, 10 mA) and at 45° to the sample surface. Photoelectron data were collected at a take off angle of theta equal to 90°.

Survey (wide) scans were taken at an analyser pass energy of 160 eV and multiplex (narrow) high resolution scans, which focus on a particular atom at 20 eV. Survey scans were carried out over 1200–0 eV BE range with 1.0 eV steps and a dwell time of 100 ms. Narrow high-resolution scans were run with 0.05 eV steps and 250 ms dwell time. Base pressure in the analysis chamber was 1.0 x 10^{-9} torr and during sample analysis 1.0 x 10^{-8} torr. Atomic concentrations were calculated using the Kratos Analytical Kratos Vision 2 software (Manchester, UK). The error in the measurement is ±10%.
2.10 Scanning electron microscopy (SEM) analysis

The morphology of the lignin/PHB blends (carbon coated) was examined using a FEI Quanta 200 Environmental scanning electron microscope (Hillsboro, OR, USA), at an accelerating voltage of 15 kV. To obtain better images of film topography, micrographs were taken at a tilt of 35°. Prior to analysis by SEM, photographs of the blends were taken with an Olympus BX41 System Light equipped with an Olympus Digital Camera (Melville, NY, USA).

2.11 Fourier transform-Infrared spectroscopy (FT-IR) analysis

Infrared spectra were collected using a Nicolet 870 Nexus Fourier transform infrared (FTIR) spectrometer equipped with a Smart Endurance single bounce diamond ATR accessory (Madison, WI, USA). Spectra were manipulated and plotted with the use of the Galactic Industries Corporation GRAMS/32 software package (Salem, NH, USA). Spectra were collected in the spectral range of 4000 to 525 cm\(^{-1}\), using 64 scans at 4 cm\(^{-1}\) resolutions with a mirror velocity of 0.6329 cm/s. The measurement time for each spectrum was around 60 s. The FTIR peaks were normalised with respect to the main peak at 1722 cm\(^{-1}\).
3. RESULTS AND DISCUSSION

3.1 Gravimetric Analysis

To investigate how the mass of buried samples changed with time, two approaches were used as shown in Figures 1 and 2. Figure 1 shows how blends of different compositions compare with PHB with respect to mass reduction in incremental periods of 4 months burial over 12 months. PHB films disintegrated and lost 45 wt% of mass within 12 months, while with the blend containing 10 wt% lignin, only 12 wt% of mass was lost during the same period.

Clearly there is a significant difference between the degradation rates of pure PHB compared to the blends. The obvious effect of even small concentrations of lignin to retard degradation of PHB is shown in a different plot of the gravimetric data in Figure 2. Figure 2 depicts the trends of actual mass loss of different lignin/PHB blends after 4 months, compared with the expected mass loss of those blends in which PHB is assumed to act as the only degradable component in blend (\(i.e.,\) a rule of mixtures plot). Figure 2 shows that once lignin is present in a blend, degradation is inhibited. This could arise either by a biochemical protection effect of lignin against attack by bio-organisms on PHB [21] or surface segregation of lignin from the blend inhibiting biofilm formation and access to PHB [22]. The possible surface segregation of lignin has been probed by XPS, as discussed later.

3.2 Thermogravimetric Analysis

The mass ratio of pure PHB, pure lignin and two blends of PHB with 30 and 60 wt% lignin respectively (after 4, 8 and 12 months burial time) during thermal degradation to 900 °C are given in Figure 3.

PHB is prone to thermal degradation and decomposes by a three-step mechanism. Firstly, in the temperature range of 170–200°C, volatile monomeric, dimeric, trimeric and tetrameric
species are formed (Figure 4). This mechanism of random chain scission (Figure 4) has been demonstrated by mass spectroscopy/FTIR [21; 22].

When the temperature is increased from 200–300°C, the second step of degradation occurs and PHB oligomers are broken down to free monomeric units of crotonic acid, pictured in Figure 5.

At the third step, when the temperature reaches 500 °C, the only species observed are carbon dioxide and propane. As shown in Figure 3, PHB appears to have two main overall degradation steps, while the degradation of the blends occur in several more stages [19]. The addition of lignin to PHB raises the degradation temperature of the blends relative to PHB.

Figure 3 also shows that all PHB samples (i.e., after 4, 8 and 12 months) have similar thermal degradation temperatures and profiles irrespective of burial time. A similar trend is followed by the blend containing 30 wt% lignin, implying that the blend maintains its structural features through the degradation period. However, with the blend containing 60 wt%, the longer the burial time, the lower the degradation temperature. The inference from this is that some structural changes, within the lignin-PHB matrix, are occurring within the burial time. It is probable that lignin itself is undergoing depolymerisation [16].

3.3 Differential Scanning Calorimetry (DSC)

Changes in transition temperatures by DSC analysis, give insight into physicochemical and structural changes that occur during degradation. Thus, in addition to the use of $T_g$, other thermal parameters such as melting temperature ($T_m$), apparent melting enthalpy ($\Delta H_m$) and relative crystallinity scale ($X_c$), permit useful information to be ascertained. The DSC data of some lignin/PHB blends after soil burials of 4, 8 and 12 months are summarized in Table 2. The blends containing up to 40 wt% lignin contain a single $T_g$ depicting miscibility
between lignin and PHB at these concentrations. Two $T_g$ values were obtained at higher lignin concentrations indicating immiscibility between lignin and PHB. As a consequence of these results, the observed differences in thermal degradation of the buried blend samples with time (Figure 3) are probably related to the compatibility between the two components.

Table 2 clearly shows that $X_c$ of PHB changes with lignin content. In lower lignin contents (i.e., miscible ratios of lignin/PHB blends) the addition of lignin increases the crystallinity of PHB. In contrast, higher lignin contents (i.e., immiscible ratios of lignin/PHB blends) have no effect on crystallinity of PHB. Weihua et al., [23] obtained similar results at lower lignin contents. The increase in PHB crystallinity at low lignin concentrations could in fact contribute to the resistance of these blends to environmental degradation relative to PHB.

In general, the buried samples have lower values of $X_c$, $\Delta H_m$ and $T_m$ than the virgin samples. The decrease in these values is due the loss of both amorphous and crystalline portions of PHB, in addition to the chain scissions of PHB polymer chains (associated with lower $\Delta H_m$ values).

To evaluate the interaction between PHB and lignin, a well-known equation of Kwei [24] was employed (Equation 2).

$$T_g (\text{blend}) = \frac{w_1 T_{g1} + K_w w_2 T_{g2}}{w_1 + K_w w_2} + q w_1 w_2$$ (2)

where $T_{g1}$ or $T_{g2}$ and $w_1$ or $w_2$ are glass transition temperatures of the pure components and their corresponding weight fractions, respectively. $K_w$ and $q$ are adjustable parameters.

A relatively higher $q$ indicates a stronger hydrogen bonding between those two components [25]. Using the data of Figure 1, together with the assumption that most of the observed degradation is due to PHB, the $q$ value that satisfies the evaluated $T_g$ values for the 4 month
buried blends is equal to 52. Mousavioun et al., [19] obtained a value of 22 for unburied blends. The significant increase in $q$ could represent a significant increase in hydrogen bonding between lignin and PHB after 4 months of burial time. The hypothesis is proposed that the less strongly bonded PHB is susceptible to degradation. Therefore, the hydrogen bonding of lignin with PHB plays a significant role to protect PHB against degradation.

3.4 XPS Analysis

Survey analysis of pure PHB and lignin are shown in Figure 6. PHB was found to contain a significant nitrogen peak with atomic concentration of 3.5%, and from the binding energies appeared to be present in the form of an amide or a polypeptide fragment of a protein. As noted in the experimental section, PHB is of bacterial origin and the nitrogen contamination is most likely a remnant of the fermentation and extraction process used to produce the PHB [22].

The multiplex carbon 1s scan of pure PHB portrayed in Figure 6(c) and Table 3 is comparable with the standard PHB which is reported by Beamson and Briggs [26] except for the effect of the peptide which results in a broadening of the C–O band in the spectrum due to an underlying C–N band at 286 eV in Figure 6(c). Table 3 also shows the main bands associated with lignin, with C–C linkage being the major band.

Table 4 clearly shows the differences between zero time and 4 months burial time for a blend containing 20 wt% lignin. No nitrogen or C–N signal was found with the blend at zero time, though these signals were observed with PHB alone (Table 3) and the blend after 4 months burial (Table 4). This is likely due to lignin masking these groups, but the former was removed with burial time revealing the groups. This result and lignin inhibition observed at low lignin content, tends to support the hypothesis of surface coverage mentioned in the earlier section.
The COO content dropped from 12.6% to 3.05% after 4 months burial time. This is suggestive of PHB hydrolytic degradation. Also, the atomic concentration of oxygen involved in O–C linkages have reduced from 11% to 6% further supporting this route for PHB degradation. The CO content increased from 14.7% to 21%, most likely indicating an increase in lignin content after 4 months burial time. Scott [16] reported accumulation of lignin in the soil, when a lignocellulosic material is buried, confirming the resistance of lignin to biodegradation in soils that do not contain filamentous basidiomycetous fungi. On the basis of these data, the hypothesis that lignin in the blend completely covered the surface of the film is not valid.

The nitrogen and C–N atomic concentrations increased from 3.5% and 2.8% for PHB to 5.6% (equivalent to 7% on PHB component) and 8.3% (10.4% on PHB component) in the blend (buried after 4 months) respectively. These results, therefore, show biofilm attachment resulting from fermentation, and indicate that microorganisms are involved in environmental degradation of PHB and lignin/PHB blends.

The results of Table 4 show evidence of some surface contamination with a silicate compound as seen by the presence of O-ex-silicate signal. This is indicative of silica/clay mineral. Total removal of clay and other surface contamination from buried samples is difficult, despite the cleaning protocol adopted in these trials.

3.5 FT-IR Analysis

Figure 7 shows the IR spectra of PHB, lignin and a 10 wt% lignin/PHB blend aged for 4 months. The IR spectrum of lignin shows a strong hydrogen bonded O–H stretching absorption around 3400 cm\(^{-1}\) and a prominent C–H stretching absorption around 2900 cm\(^{-1}\) [27]. The PHB spectrum exhibits two main peaks at 1733 cm\(^{-1}\) and 1722 cm\(^{-1}\), though the first peak at 1733 cm\(^{-1}\) is more of a shoulder to the main peak at 1722 cm\(^{-1}\). The peak at 1733 cm\(^{-1}\)
1 is associated with the amorphous component of PHB; the peak at 1722 cm\(^{-1}\) is associated with the crystalline component of PHB (in its preferred conformation) and the peak at 1655 cm\(^{-1}\) may be associated to the –C=C– stretching vibration [28]. However, because this band is broad it may well due to water associated with PHB [29]. The band at 1685 cm\(^{-1}\) has been reported to be a crystalline band, although its origin is not known [30]. All the buried blends contain some extra peaks compared with PHB and lignin. These peaks generally are seen from 3697 cm\(^{-1}\) to 3620 cm\(^{-1}\), which have been reported as indicative of kaolin [31], confirming that some soil remained on the films despite the cleaning protocol used.

To examine chemical changes in PHB and lignin/PHB blends buried samples, the IR spectra from 1850 cm\(^{-1}\) to 1400 cm\(^{-1}\) of the carbonyl stretching region of PHB were examined (Figure 8).

Figure 8 shows the intensity of the peak at 1685 cm\(^{-1}\) (a crystalline band of PHB) decreases with burial time. This is an indication of loss of PHB crystallinity with burial time and confirms the DSC data relating to \(X_c\). As the band at 1655 cm\(^{-1}\) is most likely associated with water in the film, the increase in intensity of this peak at 4 months and remained at that intensity up to 12 months is due to water absorption rather than an increase in the amount of –C=C– group.

The bands at 1510 cm\(^{-1}\) and 1450 cm\(^{-1}\) are characteristic to lignin [32]. The intensities of these peaks increased with burial time for the lignin/PHB blend. This means that the proportion of lignin in the blend increases with burial time as a result of PHB removal.

### 3.6 Macroscopic and Microscopic Changes

Photographs of Table 5 show significant degradation, as evident in the holes, and weight loss of PHB after 12 months. Photographs of blends of lignin/PHB show comparatively little or
no degradation (Table 5). These results confirm the gravimetric data that showed significant difference between the degradation of PHB compared to those of the blends. The rate of PHB degradation (Table 5 and Figure 1) follows similar trends as that reported by previous workers [32]. Woolnough et al., [12] reported that the rapid weight loss of PHB in garden soils is associated with high numbers of micro-organisms in the soil capable of degrading PHB. Thus, it is inferred that the very slow degradation of lignin/PHB blends, in this study, is due to the inhibitory effect of lignin on the microorganisms.

The SEM micrographs of Table 5 show that surface roughness increases with burial time for PHB and the blend containing 30 wt% lignin. However, the surface of the blend with higher lignin remained smooth after 12 months burial. The micrographs for PHB and the blend with 30 wt% lignin show that degradation is through surface erosion probably caused by enzymes produced by mobile micro-organisms. The SEM of PHB also reveals holes at various locations. This is likely to be areas where colonies of microorganisms were strongly attached during biofouling. So, while PHB may be associated with loosely and strongly associated microorganisms, some of the blends are probably associated in a similar way. The presence of lignin in the blends appears to reduce or prevent accumulation of the microorganisms (evident from the smooth surface for the 90 wt% lignin), and so plays a significant role against degradation of the blends.

### 3.7 Conclusion

PHB is the only component of lignin/PHB blends that is susceptible to biodegradation. Experimental investigations have revealed that lignin significantly reduced biodegradation of PHB. It forms miscible blends with PHB at low lignin contents; while at the same time increases the crystallinity of the blends. As such, the degradation of blends with low lignin content is more resistant to degradation than PHB. Lignin also forms strong association with
PHB, as revealed from the thermal studies, and so may inhibit PHB random chain scission rates of PHB. Lignin also protects PHB against biodegradation attack because it is recalcitrant to microorganisms present in the garden soil used in the present study.

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References


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*T$g$ without parentheses is pure PHB and the one with parentheses is that of lignin.
### Table 3  
XPS analysis of PHB and lignin

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<tr>
<th>Linkage</th>
<th>PHB Binding energy (eV)</th>
<th>Atomic %</th>
<th>Lignin Binding energy (eV)</th>
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</table>
Table 5  Photographs and SEM of PHB and lignin/PHB blends (scale bar = 15 mm for photos and 40 µm for SEM)

<table>
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<tr>
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<th>Photographs</th>
<th>SEM</th>
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<tr>
<td></td>
<td>Virgin</td>
<td>12 months</td>
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<tr>
<td>Pure PHB</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>30/70 Lignin/PHB</td>
<td>![Image]</td>
<td>![Image]</td>
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<tr>
<td>90/10 Lignin/PHB</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
**Figure 1**

- Pure PHB
- 10% Lignin
- 40% Lignin
- 60% Lignin
- 90% Lignin

**Figure 2**

- Actual mass ratio
- Expected mass ratio
Figure 3
Figure 4

Figure 5
Figure 6
Figure 7

Figure 8
FIGURE LEGENDS

Figure 1
Variation of mass ratio of PHB and blends over 12 months soil burial.

Figure 2
Actual and expected mass ratio of lignin/PHB blends after 4 months soil burial.

Figure 3
Thermograms of PHB, lignin and blends containing 30 and 60 wt% lignin after 4, 8 and 12 months soil burial.

Figure 4
PHB random chain scission at temperatures of 170 °C–200 °C.

Figure 5
PHB oligomer chain scission at temperature of 200 °C–300 °C.

Figure 6
Survey XPS of (a) PHB and (b) lignin and multiplex scans of carbon 1s region of (c) PHB and (d) lignin.

Figure 7
FTIR spectra of (a) PHB, (b) lignin and (c) 4 months buried, 10 wt% lignin/PHB blend.

Figure 8
IR spectra of the carbonyl stretching region of PHB at different burial times.

Figure 9
FTIR spectra of the carbonyl stretching region of 10 wt% lignin/PHB blend.