Bio-based poly(3-hydroxybutyrate)/thermoplastic starch composites as a host matrix for biochar fillers

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53 **Abstract**

54 Biochar is an excellent, but less-used candidate to serve as an alternative filler in poly(3-
55 hydroxybutyrate) (PHB)-based composites. Increasing amounts of biochar between 20 and 50 wt.%
56 were incorporated in PHB/char and PHB/thermoplastic starch (TPS)/char composites and its effects on
57 the microstructure, crystallization and thermal properties were investigated. PHB shows a significant
58 reduction in molecular weight after processing and the increasing amounts of biochar decreases this
59 even stronger. From thermogravimetric analysis, it was clear that the onset degradation temperature
60 of the PHB/char composites (255 °C) is only slightly influenced by the biochar particles up to 40 wt.%.
61 Contrastingly, this temperature reduces to 245 °C when 50 wt.% of biochar is added. Additional data
62 confirm that morphology and crystallization kinetics are enhanced up to 40 wt.% of biochar, while even
63 higher percentages of filler clearly have an opposite effect. Finally, this work reveals the ability of TPS
64 to work as an excellent intermediary between biochar and PHB at biochar concentrations up to 20
65 wt.%, where degradation and resulting reduction in molecular weight remains limited as compared to
66 that of the PHB/char sample. Furthermore, like biochar, TPS acts as a nucleation agent in the
67 composites and removes the influence of the biochar on the thermal stability of the composites.

68 **Key words:** Polymer composites, Thermal properties, Biochar, poly(3-hydroxybutyrate), Thermoplastic
69 starch

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1 Introduction

The use of biopolymers with fillers originating from residue streams is a promising alternative to replace fossil-based products that are widely used today [1]. Doing so would not only reduce the dependence on petroleum based materials, but would also improve the valorization of the used residual streams. Moreover, the biodegradability of biopolymers makes them favorable to be used as a host material for functional fillers in order to become available through gradual exposure and/or diffusion from the matrix material [2–4]. Therefore, the production of slow-release fertilizers based on biopolymers and a functional filler material can become a reality. However, a better insight in processing and in the interactions between specific fillers and polymer matrices remains critical for the formulation and the final properties and decomposition of these biocomposites materials.

Poly(3-hydroxybutyrate) (PHB) is an interesting candidate for its use in bio-based composites due to the fact that it can be easily processed and is better biodegradable than most other biopolymers [5]. Despite these advantages, the use of PHB is still limited due to its small operational processing window [6] and the elevated cost of the commercial product [7]. The use of an inexpensive filler in PHB based composites could therefore aid in the future applicability of this polymer as it would decrease costs and could improve thermal properties [8]. Several lignocellulosic fillers have already been considered as they are biodegradable and highly available, like potato waste fermentation residue [9], kenaf [10], flax fibers [11], and agave fibers [12]. Lignocellulose fillers are however often unsatisfactory due to their physico-chemical heterogeneity and most of all their incompatibility with most polymers due to their hydrophilic nature, often lacking good interface compatibility [13–15]. This lack of sufficient interfacial adhesion between the polymer and the fibers can be desired for increased biodegradation [16]. However, for the intended application as a slow-release fertilizer, biochar could be used as an improved filler material with likely a better compatibility with the polymer host matrix as compared to lignocellulosic fillers. After all, the fast decomposition of the composite and therefore reduced life span and premature erosion of the fillers is undesired in slow-release fertilizers [17, 18]. In semi-crystalline

polymers, heterogeneous nucleation can be initiated at the interface between the filler and the polymer and the quality of this interface determines the crystallization behavior of the composites and hence release rate of the fillers. Nevertheless, little is known about the characteristics of biocomposites based on biochar, especially with PHB.

The production of biochar is based on a process of dealkylation, dihydroxylation and aromatization of the initial lignocellulosic starting material [19]. Due to thermal degradation, the resulting biochar has a low hydrogen and oxygen content, leading to a high hydrophobicity [20, 21]. Furthermore, the surface properties and surface functional groups of the biochar can effectively be tuned by appropriate pyrolysis process parameters and the choice of precursor material [22]. If biochar could successfully be used as a filler to produce biocomposites, an additional advantage emerges in the biochars potential use as a slow release fertilizer. It was recently shown that PHB is indeed an interesting candidate as a matrix for controlled slow release of NPK fertilizers, positively affecting crop growth [4]. At the end of its life, the PHB matrix could even again be co-pyrolyzed with biomass to achieve a bio-oil with favorable characteristics as compared to conventional pyrolysis [23].

As previously discussed, PHB has a narrow processing window and is prone to thermal degradation during processing. This degradation is usually catalyzed in the presence of fillers [24] due to the presence of localized hotspots, which increase the process temperature due to internal friction. Recently, efforts have been made to increase the processing window of PHB by the preparation of copolymers with, for example, poly-(3-hydroxyvalerate) [25] or poly-(3-hydroxyhexanoate), which lower the melt temperature [26]. Thermoplastic starch (TPS) is another promising candidate for increasing the thermal processing window of PHB as it lowers the processing temperature and possibly decreases the formation of hotspots during processing [27]. However, unlike other biopolymers like polylactic acid (PLA) [28–30], little is known about the interaction between PHB and TPS, especially in the presence of fillers like biochar. Biochar particles and TPS might affect release and diffusion mechanisms in PHB composites, as well as the thermal stability and therefore ultimately

biodegradability of these composites. Therefore, the effect of biochar particles and TPS as a processing agent on the properties of PHB/char and PHB/TPS/char composites is investigated in this work. The composition, molecular weight, morphology, crystallization and thermal properties with increasing amounts of biochar and TPS fillers are discussed, as these properties impact either the production of the composites or the end-use case as a slow-release fertilizer.

2 Materials and Methods

2.1 Materials

The PHB (Biomer P226 in powder form, > 98 % purity, Biomer, Germany) has a density of around 1250 kg/m³, Young's modulus between 1140-1900 MPa (1mm/min), a tensile strength of around 25 MPa (50 mm/min), and an elongation of 6 to 9 % (50 mm/min). The TPS was obtained as the commercial product FlourPlast GP2 from Rodenburg Biopolymers (Oosterhout, The Netherlands). Biochar was made from a pine tree bark (*Pinus sylvestris*) residue stream at 450 °C and a residence time of around 12 min, using a lab-scale pyrolysis unit as described in [31]. Biochar was ground in a Retsch S1 planetary ball mill (Retsch, Haan, Germany) and sieved using a Retsch AS200 Basic sieving apparatus (Retsch, Haan, Germany) to achieve a mean particle size of 18.9 µm (P10 = 4.8 µm, P90 = 59.98 µm). Elemental analysis and ash content of the biochar indicated the presence of carbon (70 wt.%), oxygen (18 wt.%) and inorganics (8 wt.%). This matches well with the most commonly found surface functionalities in FT-IR: aromatic C-H, inorganic carbonates (e.g. CaCO₃), and C-O and C-OH groups. The biochar is further characterized by a low BET specific surface area of 2.40 m²/g, as defined by N₂ absorption and desorption isotherms at 77 K using a Tristar II 3020 surface area analyzer (Micromeritics, Georgia, U.S.A).

All materials were dried in a circulating hot air oven at 80 °C until constant weight before further processing. In **Table 1**, the different compositions for production of PHB/char and PHB/TPS/char composites are displayed. As the intended purpose of the produced composites is to serve as a slow-release fertilizer, the biochar concentration in the composites should be as high as possible without

greatly affecting the composites properties. To find this optimal biochar concentration, a screening study was performed, starting with 20 wt. % of biochar and increased this concentration up until 50 wt. %. The materials were all dry-mixed in the given weight ratios and later compounded by melt-extrusion in a 5 cm³ twin screw micro-compounder (DSM Xplore, Geleen, The Netherlands) for 5 minutes at a temperature of 180 °C and a screw speed of 200 rpm. Samples were all independently produced in threefold, cooled in air at room temperature, cut in small strands and mixed into one batch.

Table 1: Theoretical composition of PHB/char and PHB/TPS/char composites

Sample	PHB (%)	TPS (%)	Biochar (%)
PHB	100	0	0
PHB/TPS 25/75	25	75	0
PHB/TPS 50/50	50	50	0
PHB/char 80/20	80	0	20
PHB/char 70/30	70	0	30
PHB/char 60/40	60	0	40
PHB/char 50/50	50	0	50
PHB/TPS/char 40/40/20	40	40	20
PHB/TPS/char 35/35/30	35	35	30
PHB/TPS/char 30/30/40	30	30	40
PHB/TPS/char 25/25/50	25	25	50

2.2 Methods

Molecular weight analysis

The molecular weight of the virgin and processed samples was measured in duplicate with size exclusion chromatography (GPC, Waters Breeze, Milford, USA) at 35 °C. The composite samples are first dissolved in CHCl₃ and filtered over a 0.45 µm syringe filter. After precipitation, the biochar and TPS were removed as a non-dissolved fraction and the remaining PHB solution could be measured separately. A PSS SDV analytical precolumn, and 3 PSS SDV analytical columns (1000; 100,000 and 1,000,000 Å) were used, along with a 2414 refractive-index detector from Waters (Milford, USA) and a Waters 1515 isocratic high-performance liquid chromatography (HPLC) pump. Chloroform (HPLC-

197 grade, Biosolve) was used as the eluent at a flow rate of 1 mL/min for a total of 40 min. Polystyrene
198 standards with different molecular weights (580–1,930,000 g/mol) were used to calibrate. Statistical
199 differences between the samples is evaluated using a standard T-test ($\alpha = 0.05$).

200 **Thermogravimetric analysis**

201 Thermogravimetric analysis (TGA) (TA Q500, Zellik, Belgium) was performed during heating from room
202 temperature to 550 °C at a heating rate of 20 °C/min under 90 ml/min N₂-flow on a sample mass of 10
203 ± 0.1 mg.

204 **Scanning electron microscopy**

205 The composites morphology was evaluated using a TM3000 Tabletop Scanning Electron Microscope
206 (SEM) at 15 kV (Hitachi, Krefeld, Germany) on a cross-section that was fractured from the extruded
207 samples. Before imaging, samples were gold sputter coated using a jfc-1300 auto fine coater (Jeol,
208 Tokio, Japan) for 60 seconds at 30 mA, 0.05 mbar and a working distance of 4 cm.

209 **Differential scanning calorimetry**

210 Differential scanning calorimetry (DSC) was performed on 10 ± 0.1 mg of sample, using a DSC Q200 (TA
211 Instruments, New Castle, United Kingdom), running two heating cycles interrupted by a cooling cycle
212 between -50 °C and 200 °C at a constant heating and cooling rate of 20°C/min. For isothermal
213 crystallization, 10 ± 0.1 mg of sample was heated to 190 °C and kept at this temperature for 2 min.
214 Hereafter, the material was cooled at 100 °C/min to a crystallization temperature of 120 °C, followed
215 by an isothermal period of 30 min. The crystallinity level (X_c) of the composites was determined using
216 equation 1:

$$217 \quad X_c = \left[\left(\frac{\Delta H_m}{(1-\Phi)\Delta H_0} \right) \right] \times 100\% \quad (1)$$

Where ΔH_0 is the melting enthalpy of the entirely crystallized PHB (146 J/g) [32], and Φ is the fraction of filler and TPS. The isothermal crystallization behavior is further examined by the Avrami model to inspect the effects of nucleating agents, as was previously done for PHB [33].

Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FT-IR) was performed with a VERTEX 70 FT-IR spectrometer equipped with a deuterated triglycine sulphate detector (Bruker, Karlsruhe, Germany), in attenuated total reflection (ATR) mode using a diamond crystal (PIKE, Fitchburg, USA). A total of 32 scans per sample were obtained in the spectral region of 4000 – 600 cm^{-1} at a resolution of 4 cm^{-1} and 32 scans are acquired per sample. The crystallinity index according to FT-IR ($X_{\text{CFT-IR}}$) is calculated from the absorbance ratio of the crystalline band at 1230 cm^{-1} to the reference band at 1454 cm^{-1} (CH_3 asymmetric stretching, insensitive for crystallinity [34]). It should be noted that this is indeed a crystallinity index and not an absolute crystallinity.

3 Results and discussion

3.1 Molecular weight analysis

First, the molecular weight analysis of the extruded biocomposites with various compositions was evaluated and shown in **Fig. 1**, to determine the effect of processing parameters and fillers on the degradation of the polymer structure. The pristine PHB displayed a weight-average molecular weight (M_w) of 611 kg/mol, while in the processed PHB sample, M_w is only 463 kg/mol. In parallel, the polydispersity (M_w/M_n) decreases from 3.1 to 2.5 after processing. In parallel with literature, the reduction of PHB's molecular weight after processing at 180 °C is due to thermal and mechanical degradation, causing random chain scission of the six-member ring ester, and ultimately resulting in the formation of volatile acids which are liberated from the PHB, leading to a decrease in molecular weight [6, 35, 36].

Fig. 1: Mass-average molecular weight (M_w) of PHB in the prepared composites

When biochar is present in the PHB/char samples, the M_w drops to around 55-60 % of the molar mass of the processed PHB and remains relatively constant up until a biochar addition of 40 wt.%. In the PHB/char 50/50 sample, a more sudden drop in M_w is observed to only about 47 % of the molar mass of the processed PHB samples without biochar, indicating more severe degradation of the sample at the highest filler content. Contrastingly, polydispersity remains almost constant at 2.5 with the addition of increasing amounts of biochar. When TPS is added in the PHB/TPS/char samples, the M_w is significantly ($p < 0.05$) higher for the PHB/TPS/char 40/40/20 sample (297 kg/mol) than for the PHB/char 80/20 sample (260 kg/mol). In the PHB/TPS/char samples, the M_w decreases more gradually with the addition of more biochar to 204 kg/mol in the PHB/TPS/char 25/25/50 sample. These results indicate that at low biochar concentrations (20 wt.%), TPS prevents the decrease in molecular weight by acting as an intermediary between the biochar and PHB. According to literature, the lubricating action and/or higher thermal stability of TPS may enhance the processability [27]. When more biochar is added, however, this effect disappears and TPS is no longer capable of preventing the biochar from causing additional lowering of PHB's molecular weight.

The observed reduction in molecular weight due to the introduction of biochar in the samples can likely be explained by the fact that processing the composites in the presence of solid particles induces

higher shear stresses, causing local zones of overheating due to friction during processing [35, 37]. In these local hotspots, temperatures are likely higher than 180 °C, giving rise to the quantitative conversion of PHB to mainly crotonic acid (at 225 to 250 °C) [36]. The biochar particles likely also lead to higher mechanical forces on the PHB, contributing greatly to its decomposition [6]. Another reason for the two-fold decrease in molecular weight of PHB in the presence of biochar after processing, could be the presence of inorganic oxides (e.g. CaO, MgO, Al₂O₃,...) on the biochar surface. These oxides have been shown to destabilize PHB, leading to much easier formation of volatile products [38]. TPS can have a three-fold action in suppressing these effects of biochar. It can act as a plasticizer and lubricant, thereby reducing the viscosity of the biocomposite and reducing the stress transfer from the biochar to the PHT. Moreover, it could shield the PHB from the filler and its destabilizing oxides. However, the results in **Fig. 1** show that these effects only contribute at low biochar content.

3.2 Scanning Electron Microscopy

Macroscopic differences between the composites are shown in **Fig. S1**. It is clear that a similar homogeneous black color is observed, but differences in surface roughness arise when TPS is present. Morphological changes of the PHB/char and PHB/TPS/char composites are visualized by SEM microscopy. **Fig. 2a** shows that the biochar has different sizes, shapes and surface morphologies. Pure PHB shows a smooth and continuous polymer section in **Fig. 2b**, with several small cavities caused by air bubbles that are present in the sample. In **Fig. 2c**, it can be seen that there are also cavities present in the PHB/char 80/20 sample. Furthermore, it becomes clear that the biochar particles are well dispersed in the PHB/char 80/20 sample, causing minimal interference to the PHB surface and the PHB/char interface. For the PHB/char 50/50 sample shown in **Fig. 2d**, a completely different structure of agglomerated particles and polymer fragments can be observed. The char particles are more clustered and become less visible due to the chaotic structure that is caused by the possibly degraded PHB. This confirms previous assumptions for particle overloading and more severe thermal degradation of the polymer during processing, which is in good accordance with the results of the

molecular weight analysis. The processed TPS sample is shown in **Fig. 2e** and shows a heterogeneous morphology with alternating smooth surfaces and large air cavities. The PHB/TPS sample in **Fig. 2f** shows a different interface than pure PHB, as more thermoplastic zones with TPS appearing as particles are visible. These structures remain intact when a limited amount of biochar (20 wt.%) is added as can be seen in **Fig. 2g**. However, from **Fig. 2h** it is again clear that the char particles are more clustered and the composite's structure is more chaotic at higher biochar content, the latter probably being caused by the decrease in molecular weight of the PHB during processing of the composite.

Fig. 2: SEM images of char (a), PHB (b), PHB/char 80/20 (c), PHB/char 50/50 (d), TPS (e), PHB/TPS 50/50 (f), PHB/TPS/char 40/40/20 (g) and PHB/TPS/char 25/25/50 (h). Red arrows point to biochar particles in the matrix

3.3 Thermogravimetric analysis

Thermal stability of PHB/char and PHB/TPS/char composites is assessed with TGA and shown in **Fig. 3**. It is visible in **Fig. 3a** and **b** that PHB starts to decompose at around 255 °C. Thermal decomposition is finished at 315 °C, with a maximum weight loss at around 300 °C. The temperature at which the PHB/char composites start to degrade is only very slightly influenced by the char particles up to 40 wt.% of biochar, as shown in **Fig. 3a** and **b**. The initially stable and even slightly increased onset degradation temperature when biochar particles are incorporated in the PHB structure is likely caused by the increased thermal stability of the biochar particles as compared to PHB. Contrastingly, the onset degradation temperature reduces to 245 °C when 50 wt.% of biochar is added. The sudden decrease in onset degradation temperature at 50 wt.% of biochar addition is coinciding well with the sudden

decrease in molecular weight of the sample after processing. This decrease in onset and maximum degradation temperature of composites was previously found for increasing amount of biochar fillers in PLA [30].

Fig. 3: Thermogravimetric and Differential thermogravimetric analysis of the PHB/char composites (a, b respectively) and the PHB/TPS/char composites (c, d respectively) in the temperature range of 20 to 550 °C under nitrogen atmosphere.

All PHB/TPS/char compositions show an initial weight loss below 200 °C, corresponding to volatilization of water and plasticizer in TPS [39]. The influence of the biochar on the onset degradation temperature of the PLA/TPS/char composites is very small. All PHB/TPS/char composites start their decomposition at around 240 °C (just like the PHB/TPS 50/50 sample), indicating that TPS removes the influence of biochar on the thermal stability of the composites, as was also the case in PLA/TPS/char biocomposites [30]. The decrease in onset degradation temperature (240 °C) compared to that of both the PHB (255 °C) and PHB/char samples is caused by the decomposition of TPS [39]. It can be noted that, unlike in PHB/char 50/50, there is only a minor effect of the high amount of biochar in the PHB/TPS/char 25/25/50 sample on the onset degradation temperature. This indicates that the presence of TPS removes the influence of the high biochar loads. **Fig. 3d** shows that the temperature of maximum weight loss decreases from around 285 °C in PHB/TPS/char 40/40/20 to 275 °C in PHB/TPS/char 25/25/50, confirming the predominant influence of biochar fillers concentration on thermal stability

at high filler content. Biochar and TPS both decrease the thermal stability and therefore ultimately biodegradability of the composites.

Based on the clear differentiation between degradation steps in **Fig. 3**, a quantitative estimation of the composites composition can be made. When all PHB has degraded ($T > 400\text{ }^{\circ}\text{C}$ in a nitrogen atmosphere), only the biochar remains in the PHB/char composites. Therefore, the amount of biochar can be determined by TGA and is shown in **Table 2**. The PHB/char samples contain respectively 17 % (PHB/char 80/20), 27 % (PHB/char 70/30), 38 % (PHB/char 60/40) and 44 % (PHB/char 50/50) of biochar. The same observation can be made for the PHB/TPS/char samples ($T > 550\text{ }^{\circ}\text{C}$ in a nitrogen atmosphere). If the remaining TPS at $550\text{ }^{\circ}\text{C}$ (13 % in the pure TPS sample) is taken into account, this results in biochar contents of 18 % (PHB/TPS/char 40/40/20), 23 % (PHB/TPS/char 35/35/30), 35 % (PHB/TPS/char 30/30/40) and 44 % (PHB/TPS/char 25/25/40). All mixing ratios are slightly lower than their intended ratio, caused by some loss of the fine biochar powder during material loading in the micro-compounder.

3.4 Differential Scanning Calorimetry

The behavior of the PHB/char and PHB/TPS/char composites in the first and second heating cycles in differential scanning calorimetry (DSC) is visualized in **Fig. 4** and summarized in **Table 2**. When the PHB/char and PHB/TPS/char composites are heated from $-50\text{ }^{\circ}\text{C}$ to $250\text{ }^{\circ}\text{C}$ at $20\text{ }^{\circ}\text{C}/\text{min}$, only minor effects on T_g are observed due to the weak glass transition in the present PHB grade. The glass transition of PHB is usually located between 0 and $10\text{ }^{\circ}\text{C}$ and is often too weak to differentiate it from the baseline [40].

Table 2: Thermal characteristics of all composite materials obtained from DSC analysis. T_c is the non-isothermal crystallization temperature, ΔH_c is the non-isothermal crystallization enthalpy, T_m is melting temperature (measured in the second heating cycle in DSC), ΔH_m is the normalized melting enthalpy (measured in the second heating cycle in DSC), X_c is the crystallinity level determined from DSC, X_{cFT-IR} is the crystallinity level determined from FT-IR spectroscopy. TPS is thermoplastic starch and (-) indicates that this value could not be determined

Sample	Biochar (%)	T_m (°C)	ΔH_m (J/g)	T_c (°C)	ΔH_c (J/g)	X_c (%)	X_{cFT-IR}
PHB	0	170.3	70.6	70.3	50.3	48.4	5.5
TPS	0	-	-	-	-	-	-
PHB/TPS 50/50	0	169.9	57.0	82.0	40.5	39.1	-
PHB/TPS 25/75	0	168.3	108.4	80.5	62.0	74.2	-
PHB/char 80/20	17.0	170.7	71.2	96.3	66.4	48.7	3.3
PHB/char 70/30	26.9	170.8	72.8	91.3	64.8	49.9	4.4
PHB/char 60/40	37.8	169.8	75.4	89.2	69.0	51.6	2.8
PHB/char 50/50	44.1	166.6	71.6	92.6	63.9	49.0	3.0
PHB/TPS/char 40/40/20	22.9	170.0	80.9	100.4	79.0	55.4	-
PHB/TPS/char 35/35/30	27.8	169.0	81.9	102.9	76.9	56.1	-
PHB/TPS/char 30/30/40	38.7	168.0	69.7	97.1	62.3	47.7	-
PHB/TPS/char 25/25/50	46.9	166.1	60.5	93.7	61.2	41.4	-

When looking at the melting behavior of PHB/char samples in the second DSC heating curve, T_m first remained nearly identical to the one of pure PHB (170.3 °C) up to 40 wt.% of biochar addition. However, when even more biochar was added, T_m decreased sharply to 166.6 °C for PHB/char 50/50. It was previously found that melting temperatures decrease with decreasing molecular weight in PHB [41], therefore this result is again coinciding well with the decrease in molecular weight and the decreased onset degradation temperature that was found in TGA for this sample. The normalized melting enthalpy (to the amount of PHB in the composite) remains relatively unaffected by the amount of biochar filler.

The PHB/TPS/char composites show a similar thermal behavior in the melting temperature region in DSC. However, the decrease in T_m happens more gradually than in the PHB/char samples, in parallel with the results of the molecular weight analysis of these samples. Overall, **Fig. 4a** to **Fig. 4d** shows that there is a decrease in T_m and normalized ΔH_m , accompanied with peak broadening when more biochar is added to the composites. At 20 wt.% of biochar, the melting peak is relatively sharp in all

composites (with and without the addition of TPS), while the melting peak even shifts towards a double melting peak in PHB/char 50/50, PHB/TPS/char 25/25/50 and to a lesser extent in PHB/char 60/40 and in PHB/TPS/char 30/30/40 in both the first and second heating cycle. This phenomenon was also observed in PLA/char composites [30], and it is hypothesized that in the composites with high amounts of biochar, many small crystals are formed in the sample during the cooling cycle due to the nucleation ability of the biochar, as often seen when micron-scale fillers are added into polymers [42]. These crystals are not able to grow to large crystals as they are obstructed by the biochar. The multitude of small crystals melt at different lower temperatures, leading to multiple melting peaks in the second heating cycle [43]. **Fig. 4e** and **f** shows that the peak broadening and double melting peak behavior, together with a decrease in T_m is also present when no biochar is present and more TPS is added to the PHB/TPS composites. Even when no fillers (TPS or biochar) are added to PHB, there is evidence of a double melting peak, suggesting the subsequent melting and recrystallization of PHB crystals during the heating run [43, 44]. In PHB/TPS, the very broad melting peak in the first heating cycle is caused by the composition of the materials, as the first part of this peak is attributed to the escape of water and plasticizer, while the second part is the melting of the PHB [45].

The crystallinity level increased from pure PHB (48 %) until 40 wt.% of biochar addition in PHB/char 60/40 (52 %), indicating the nucleation ability of the biochar particles. It decreased again to 49 % when 50 wt.% of biochar was added. This decrease in crystallinity again strengthens the hypothesis that at 50 wt.%, the composite is overloaded with biochar particles and the crystalline structure is not able to develop. Interestingly, for the PHB/char samples, crystallinity level was always higher than that of the PHB without biochar, indicating a strong interaction between the biochar particles and PHB.

Indeed, there is a clear influence of TPS on the crystallization behavior of PHB, as evidenced by the very high crystallinity level (X_c) of PHB/TPS 25/75, likely caused by the formation of strong hydrogen bonds between starch and PHB, increasing its stability and system integrity [45]. However, **Table 2**

shows that at 50 % of TPS, this influence seems to be small, as X_c and T_m are comparable to that of pure PHB.

For the PHB/TPS/char samples, X_c increased until 30 wt.% of biochar addition in PHB/TPS/char 35/35/30 (56 %) and then decreased with more biochar addition. Crystallinity in PHB/TPS/char 35/35/30 is the highest of all samples, indicating a combined nucleation effect of biochar particles and TPS, while this effect decreases again at higher biochar contents. The lower X_c in PHB/TPS/char 30/30/40 and PHB/TPS/char 25/25/50 can possibly be explained by the hindered motion of the polymer segments due to the presence of high filler concentrations in the matrix, causing obstruction of the crystal growth and therefore overall crystallinity [46].

Fig. 4: Thermal behavior of the composites in the T_m region: DSC curves of the PHB/char samples in the first heating cycle (a), the PHB/char samples in the second heating cycle (b), the PHB/TPS/char samples in the first heating cycle (c), the PHB/TPS/char samples in the second heating cycle (d), the PHB/TPS samples in the first heating cycle (e), and the PHB/TPS samples in the second heating cycle (f)

Table 2 and **Fig. 5** illustrate that the crystallization temperature (T_c) in non-isothermal crystallization of the composite materials increased to 96.3 °C for PHB/char 80/20, compared to 70.3 °C for PHB without filler. In samples with more biochar, T_c decreased again, but remained a lot higher than this of PHB without biochar. The increase in T_c for the composite materials is again attributed to the nucleation effect of the biochar particles and strong interactions between PHB and microscale biochar particles. Based on these results, the biochar may act as a heterogeneous nucleation agent, reducing the energy barrier for PHB nucleation and increasing the nucleation rate. This nucleation effect was already found for biochar particles in PLA composites [30], and for up to 30 % of lignin rich residues (by-product of giant reed biomass microbially treated to release cellulose) with similar dimensions as the used biochar in PHB [15]. Although biochar presumably has less available surface functional groups, the surface functionalities of both the biochar and the lignin-rich residues used in [15] are similar (C-H, C-O and C-OH groups). The decrease in T_c when more biochar is added can be assigned to the agglomeration of biochar particles in the composite matrix at higher biochar concentrations, as was seen before in **Fig. 2d, h**. The agglomeration of biochar particles after all reduces the available particle surface area and thus its nucleation ability. Similar to ΔH_m , there is no significant trend observed in ΔH_c for the PHB/char samples as it remains around 65 J/g.

Table 2 and **Fig. 5** further show that the thermal behavior of PHB/TPS/char composites is related to this of the PHB/char composite materials, however a co-operative nucleation effect of the TPS is observed as T_c is always higher for the PHB/TPS/char composites than for the respective PHB/char composites. This co-operative effect is also evidenced by the fact that T_c is also higher than that of the PHB/TPS samples. Both T_c and ΔH_c decrease with the addition of more biochar particles, confirming agglomeration and likely the associated crystal growth restriction when too many char particles are present in the composite, as well as reduced nucleation ability of agglomerated biochar, as hypothesized before.

Fig. 5: DSC cooling curves of all composites in the crystallization temperature range: PHB/char samples (a), PHB/TPS samples (b), and PHB/TPS/char samples (c)

Isothermal crystallization experiments at 120 °C were performed to further investigate the effect of the biochar particles on the crystallization kinetics of the PHB/char composites. The crystallization over time at this constant temperature is visualized in **Fig. 6a** for both PHB and the composite materials. When X_t is plotted versus $(t - t_0)$ in **Fig. 6b**, a typical S-shape is observed, illustrating different stages of the crystallization process as a function of time. When this function is transformed into $\log[-\ln(1 - X_t)]$ vs $\log[t - t_0]$, a linear curve in the selected time range (-1.0 to 0.3) is obtained in **Fig. 6c**. The Avrami parameters k and n are shown in **Table 3**.

Fig. 6: Isothermal crystallization isotherms of PHB/char composites (a), relative crystallinity vs. time (b), and plots of $\log(-\ln(1-X_t))$ vs. $\log(t-t_0)$ for PHB/char composites at an isothermal crystallization temperature of 120 °C (c)

As can be seen in **Table 3**, the n values of the different composites are always between 2 and 3, suggesting a three-dimensional crystal growth of the polymer during isothermal crystallization with pre-formed nucleation sites in all cases [47]. There is a slight deviation between the results of $t_{1/2}$ as determined by the Avrami model and the experimentally determined $t_{1/2}$, however the observed trends are the same. The $t_{1/2}$ in **Table 3** shows a clear decrease in crystallization time from pure PHB to PHB/char 80/20, which clearly illustrates the nucleating ability of the biochar particles. This positive nucleation effect of the biochar particles remains present at concentrations of 20 and 30 wt.% of biochar and generally decreases due to agglomeration of biochar particles and decreased mobility of the polymer chains until a biochar percentage of 40 wt.%. When 50 wt.% of biochar particles are added to the PHB, $t_{1/2}$ is again lower than in the PHB/char 60/40 sample, coinciding with a higher T_c found in non-isothermal crystallization as shown in **Table 2**, and indicating an agglomerated distribution of char particles in the sample. The lower $t_{1/2}$ model of the PHB/char 60/40 sample is explained by its slower initial crystallization compared to this of PHB/char 50/50, although its slope in **Fig. 6c** is similar. The uneven distribution of char particles is further evidenced by the fact that both the S-curve in **Fig. 6b** and the logarithmic curve in **Fig. 6c** of the PHB/char 50/50 samples are very close to those of the

pristine PHB, indicating that a large fraction of PHB was not affected by char particles, in parallel with its agglomerated state.

Table 3: Avrami kinetic crystallization parameters for the different PHB/char composites

Sample name	n	K (min ⁻¹)	t _{1/2} model (min)	t _{1/2} exp (min)
PHB	2.7	0.1	1.9	2.1
PHB/char 80/20	2.5	0.6	1.1	0.7
PHB/char 70/30	2.8	0.3	1.4	1.6
PHB/char 60/40	3.0	0.1	2.0	2.5
PHB/char 50/50	2.9	0.2	1.6	1.4

3.5 Fourier Transform Infrared Spectroscopy

Next to DSC, crystallinity of PHB can be further accessed with FT-IR spectroscopy. All spectra of PHB samples were normalized against the reference peak at 1454 cm⁻¹, which represents the CH₃ asymmetric stretching and is insensitive to crystallinity, as suggested in [48] and can be seen in **Fig. 8**. A full assignment of all bands related to PHB is found in [49]. The major characteristic absorption bands for PHB concerning crystallinity are as follows: 1747 cm⁻¹ (amorphous C=O stretching), 1720 cm⁻¹ (crystalline C=O stretching), 1280 cm⁻¹ (crystalline C-O-C stretching), 1271 cm⁻¹ (amorphous C-O-C stretching), 1230 cm⁻¹ (crystalline C-CH₃ stretching), 1175 cm⁻¹ (amorphous C-O-C asymmetric stretching), and 980 cm⁻¹ (crystalline C-C stretching) [49–51]. The calculated ratio ($\frac{I_{1230}}{I_{1454}}$) represents X_{CFT-IR}, and is summarized in **Table 2** for all samples. As X_{CFT-IR} is a crystallinity index and not an absolute crystallinity, only trends can be compared between this parameter and X_c from DSC. Unlike in DSC measurements, the increase in crystallinity in PHB/char 80/20 and PHB/char 70/30 compared to pure PHB was not found in FT-IR. Like in DSC, however, the crystallinity index first increases up until 30 wt.% of biochar and then decreases again for 40 and 50 wt.% of biochar. This result indeed supports the previous hypothesis that biochar effectively acts as a heterogeneous nucleation agent for the PHB at concentrations up to 30 wt.%. At higher concentrations, the lower crystallinity suggests less efficient nucleation effects due to agglomeration of the biochar particles and the decrease in polymer molecular weight and related lower crystallinity [41], as illustrated before in **Fig. 2**.

When the other crystalline sensitive bands are considered, it becomes clear from **Fig. 7a** that in pure PHB, the crystalline band at 1720 cm^{-1} is highest and the amorphous band at 1747 cm^{-1} is lowest. The crystalline band first decreases in height for PHB/char 80/20, then increases for PHB/char 70/30 to finally decrease again for PHB/char 60/40 and PHB/char 50/50 in very good correlation with the crystallinity $X_{\text{CFT-IR}}$. The amorphous band at 1730 cm^{-1} follows the same, but opposite trajectory. **Fig. 7b** shows that these changes are much less obvious in the PHB/TPS/char composites, partly due to the interference of the C=O absorption band of TPS at 1730 cm^{-1} [52]. Even considering the overlapping band at 1730 cm^{-1} , these results are again in good correlation with the crystallinity $X_{\text{CFT-IR}}$ of all samples.

Fig. 7: FT-IR spectra in the region of $1650 - 1800\text{ cm}^{-1}$ of PHB/char (a), and PHB/TPS/char (b) composites normalized to the intensity of the CH_3 band at 1455 cm^{-1} . (A) indicates the amorphous band, whereas (C) indicates the crystalline band

From the FT-IR spectra at lower wavenumbers in **Fig. 8a**, it is clear that the crystalline band at 1280 cm^{-1} is again highest in PHB. This band drops significantly in height when biochar is incorporated into the composites and just like the bands at 1747 and 1720 cm^{-1} , first increases in intensity until 30 wt.% of biochar before decreasing in height and shifting more towards the amorphous band at 1271 cm^{-1} at higher biochar percentages. This same trend is also visible in the amorphous band at 1175 cm^{-1} and the crystalline band at 980 cm^{-1} . Before analyzing the PHB/TPS/char samples, first the characteristic bands of TPS in **Fig. 8b** have to be considered. Characteristic bands of TPS are found at 1365 cm^{-1} (C-H bending in CH_2 [53]), 1230 cm^{-1} (O-H bending [54]), 1075 cm^{-1} (C-H in methylene groups [55]), and

1015 cm^{-1} (C-O stretching of the anhydro glucose ring [56]). Due to the strong band at 1230 cm^{-1} of TPS, no conclusions can be made using the $X_{\text{CFT-IR}}$ parameter to access the influence of the biochar particles on the crystallinity of the PHB/TPS/char samples.

Fig. 8: FT-IR spectra in the region of 950 – 1500 cm^{-1} of PHB/char (a), and PHB/TPS/char (b) composites normalized to the intensity of the CH_3 band at 1455 cm^{-1} . (A) indicates an amorphous band, whereas (C) indicates a crystalline band

4 Conclusions

The effect of increasing amounts of biochar on the microstructure and thermal properties of bio-based PHB/char and PHB/TPS/char composites was investigated. Different composites with between 20 and 50 wt.% of biochar fillers were prepared by melt compounding. From molecular weight analysis it became clear that the processed PHB sample had a lower (463 kg/mol) M_w than the pristine PHB (611 kg/mol). Increasing the amounts of biochar particles from 20 to 50 wt.% lowered the M_w of the composites even more to between 284 and 218 kg/mol. Higher relative amounts of biochar negatively influenced the thermal stability of the samples and a strong interaction between the biochar fillers and

PHB was found as X_c and T_c were always higher for the composites than for the pure PHB. In the PHB/char 50/50 sample, it was found that 50 wt.% is an excess amount that leads to deterioration of the properties, as evidenced by molecular weight analysis, FT-IR, SEM, the sharp decrease in T_m (167 °C) and a decrease in crystallinity level (49 %) as compared to the other samples. When TPS was added to the samples, the decrease in T_m , like the decrease in M_w is more gradual as more biochar is added to the composites. Furthermore, molecular weight analysis showed that at low biochar concentrations (20 wt.%), TPS is able to assist in preventing the decrease of the molecular weight by acting as an intermediary between the biochar and PHB. Finally, it was found from TGA that TPS masks the influence of biochar on the thermal stability of the composites and a combined nucleation effect of the biochar and TPS was found up to 30 wt.% of biochar particles. In a future application step towards slow release fertilizers, these results provide valuable insights in how biochar particles and TPS might affect release and diffusion mechanisms in PHB composites, as well as the thermal stability and therefore ultimately biodegradability of these composites.

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