

## Supporting information

# Sustainable Food Packaging Using Modified Kombucha-Derived Bacterial Cellulose Nanofillers in Biodegradable Polymers

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## METHODS

### Scanning electron microscopy (SEM)

SEM was performed to observe microstructural features of nanocomposite films, which were prepared for the gas permeability test. Nanocomposite films were immersed in liquid nitrogen for 30 minutes, then broken manually by bending. High-vacuum secondary electron imaging of the fractured surface was performed using an Aprea VS SEM (Thermo Scientific, the Netherlands) at an acceleration voltage of 1 kV.

### Differential Scanning Calorimetry

The thermal behavior of the nanocomposites was measured using the differential scanning calorimeter (DSC) under nitrogen atmosphere. The samples of  $8.5 \pm 0.5$  mg were heated from 25 to 190 °C and kept under isothermal conditions at 190 °C for 3 min to eliminate the thermal history. Then the samples were cooled to 25 °C and reheated to 190°C. The cooling and heating rate was 10 °C·min<sup>-1</sup>. The first and second heating curves were recorded. For each sample, the reported value is the average of at least three measurements. The crystallinity ( $\chi_c$ ) of the PHBV and PLLA phases was calculated by:

$$\chi_c (\%) = \left( \frac{\Delta H_m - \Delta H_{cc}}{\Delta H_m^0 \times w_f} \right) \times 100\%$$

where  $\Delta H_m$  is the melting enthalpy,  $\Delta H_{cc}$  is the cold crystallization enthalpy,  $w_f$  belongs to the weight fraction of polymer and  $\Delta H_m^0$  is the melting enthalpy of completely crystallized polymer (100% crystalline). For PLLA  $\Delta H_m^0 = 93$  J/g<sup>1</sup> and for PHBV due to low content of HV the theoretical fusion enthalpy  $\Delta H_m^0$  is chosen as 146 J/g, which corresponds to 100% crystalline PHB.<sup>2</sup>

### **Water uptake**

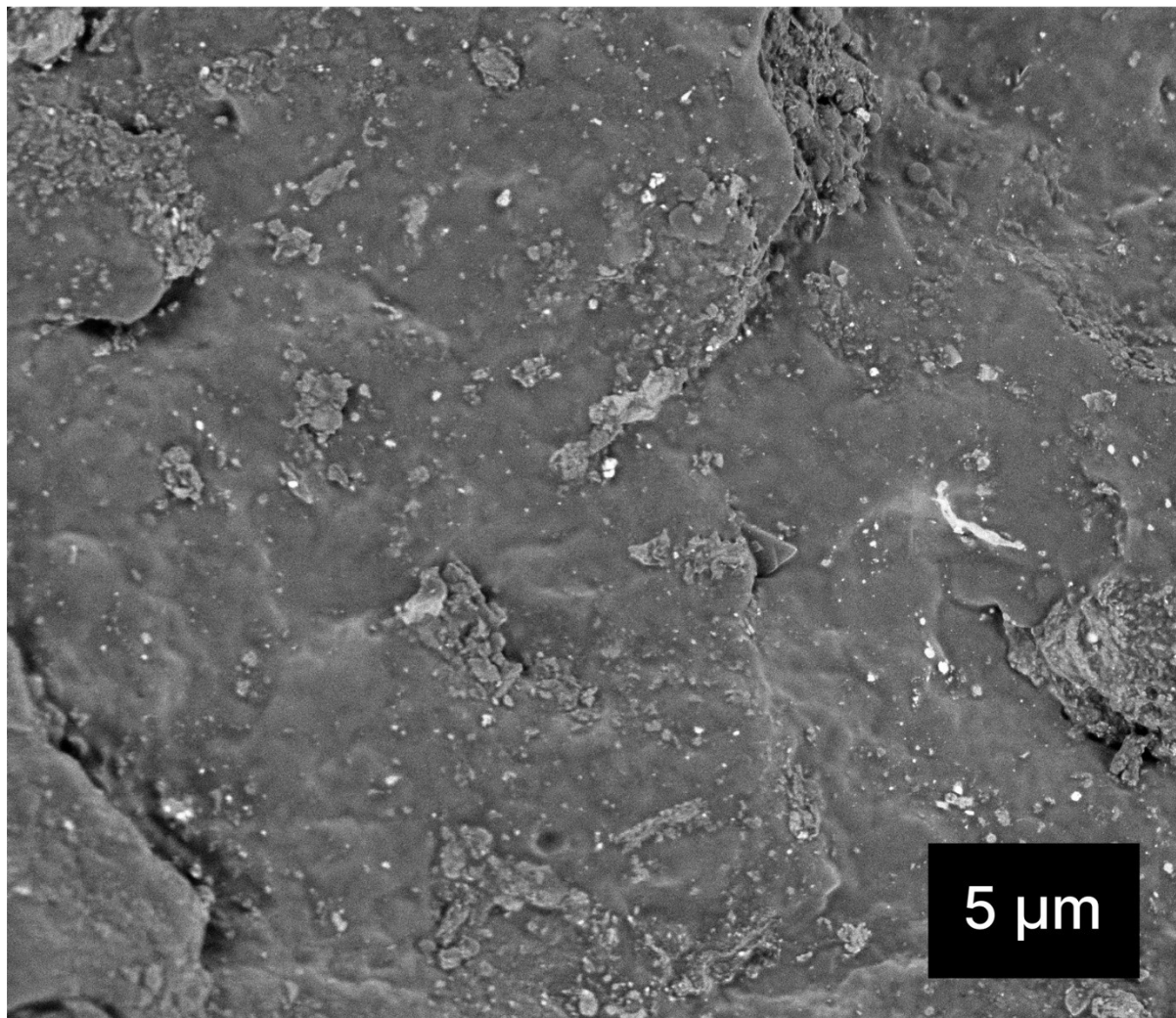
To examine the water uptake, samples were dried for 72h, then immediately weighed ( $W_0$ ) and immersed in 80 ml of MilliQ water at  $23.1 \pm 0.1$  °C for 14 days. After every 24h, the samples were taken out from water, surface dried with tissue, and weighed ( $W_1$ ) using an electronic semi-microbalance Sartorius R160P (Sartorius GmbH, Goettingen, Germany). For each sample, the reported value is the average of five tests. The water absorption ( $W_a$ ) of the samples was calculated with the following equation:

$$W_a = \frac{W_1 - W_0}{W_0} \times 100\%$$

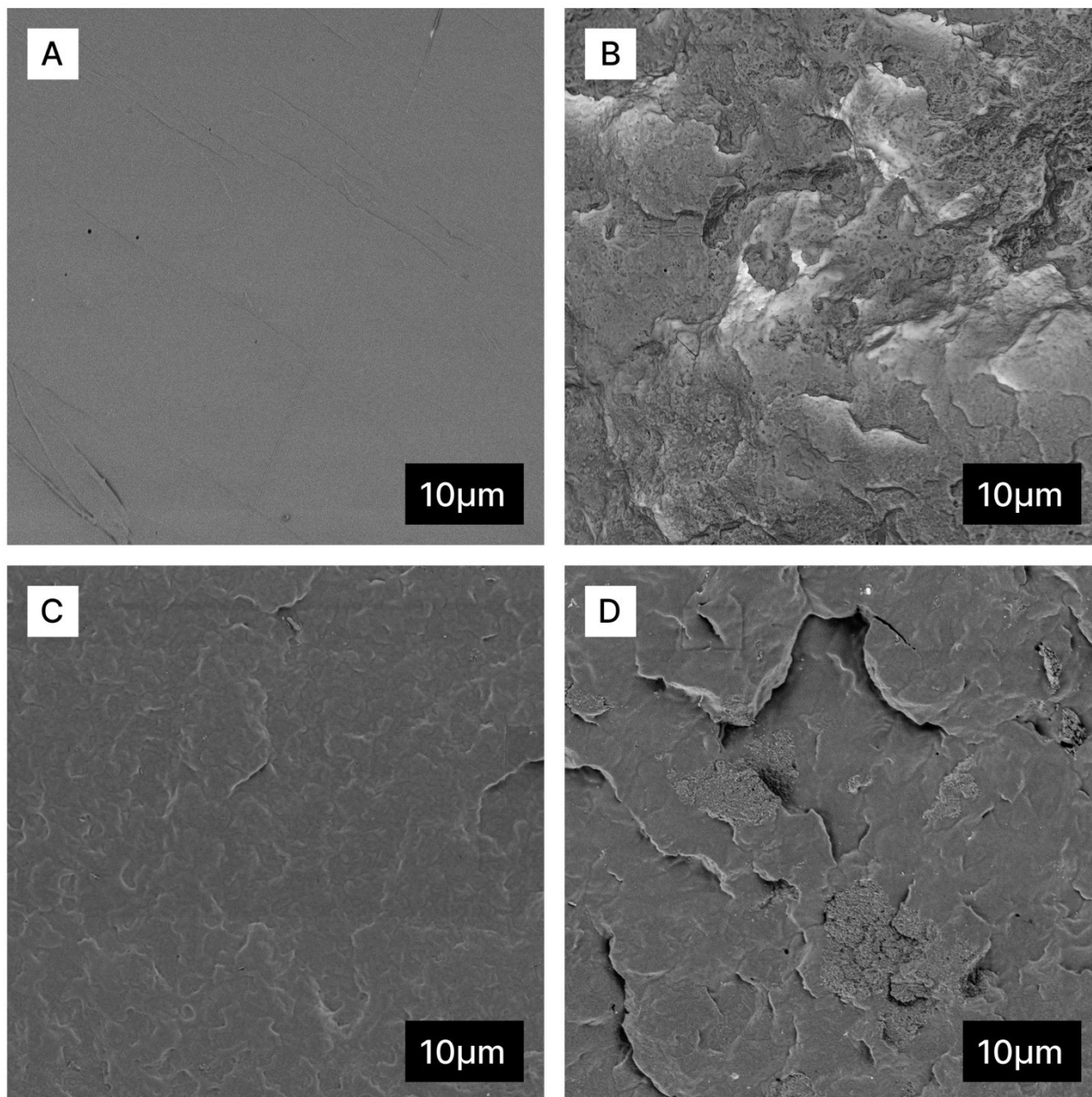
### **Biodegradation tests**

In order to evaluate the biodegradation properties of the filler and nanocomposites, a four-stage research scheme was implemented in accordance with the general recommendations of EN 13432 standard, which provides guidelines for test selection and test parameter design. Therefore, for the purpose of this study only the (idealized) biodegradation test according to OECD 301B (modified Sturm test) was required. All biodegradation test were set up as aquatic aerobic biodegradation test. We used the mineral medium as recommended in 500 mL glass bottles (Shott) equipped with the fitting gas washing unit. Test volume was 400 mL plus 10 mL of an mixed bacteria inoculum plus the amount of reference or test substance which was equivalent to ca. 0.3 to 0.6 g carbon. The carbon content of the substances was determined by element analysis with the photo-oxidation principle. Inocula were either enriched sewage sludge (settled for four hours, supernatant decanted and residue with roughly 2-3 % dry solid matter used) for mesophilic test conditions or an elutriate from organic waste compost (by suspending active compost in warm tap water, removing stones and swimming particles and using the rest as it is) for thermophilic test conditions. Each test setup

consisted of a blanc, a reference substance and any number of test substance, each of them in threefold replicates. In case of limited sample amounts, only twofold determinations were done.



**Figure S1.** SEM micrograph of cryogenically fractured film of unpurified PHBV/OLLA-g-BC contains boron nitride and antioxidant additives from the manufacturer.



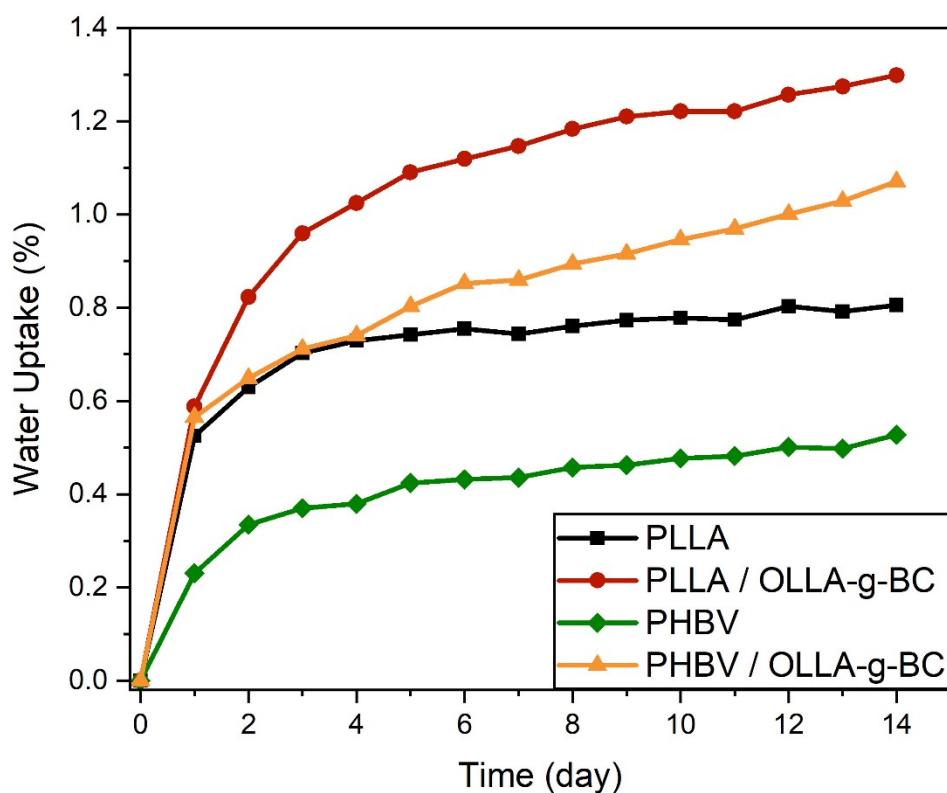
**Figure S2.** SEM micrographs of cryogenically fractured films of (A) PLLA, (B) PLLA/OLLA-g-BC, (C) PHBV\* and (D) PHBV\*/OLLA-g-BC.

\* – The commercially available PHBV Enmat Y1000P contains boron nitride and antioxidant additives from the manufacturer (Figure S1). Therefore prior to production of film for SEM characterization PHBV was additionally purified by dissolution in chloroform and centrifugation.

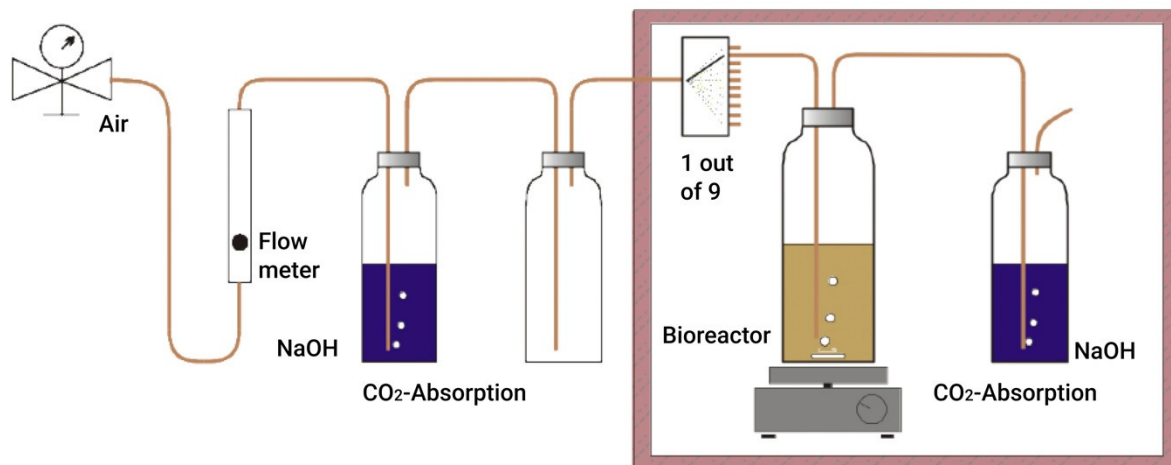
However, the remaining nano-sized particles of additive are shown in Figure S2D, which were not removed after centrifugation.

**Table S1.** Density of the PLLA, PLLA/OLLA-g-BC (5wt.%), PHBV and PHBV/OLLA-g-BC (5wt.%).

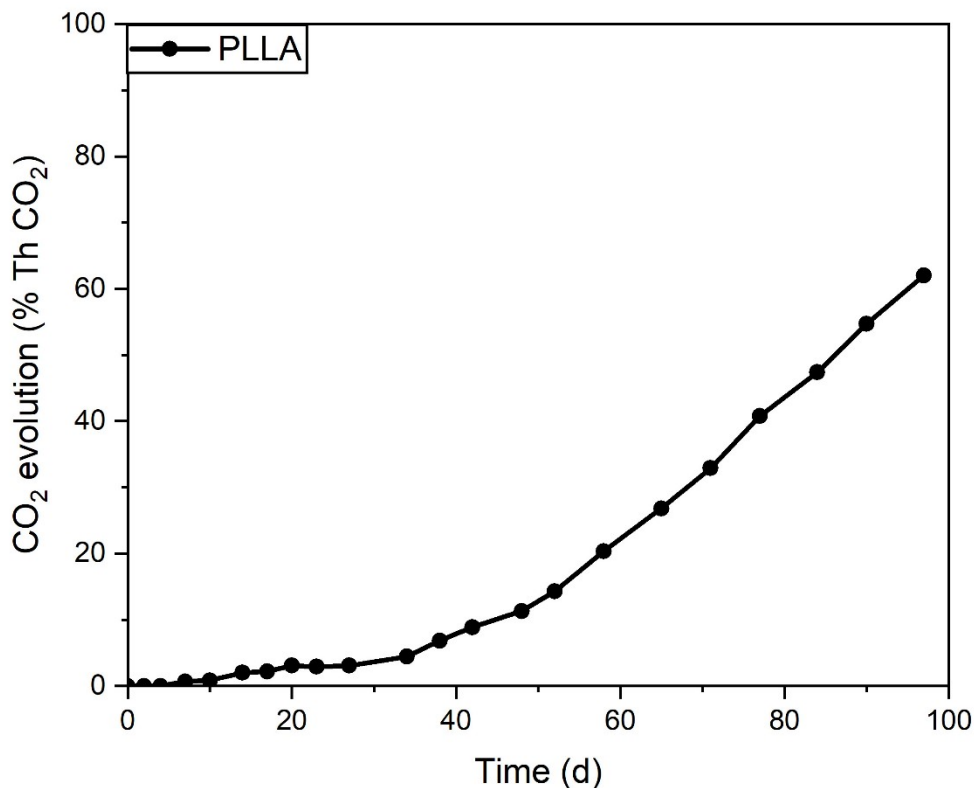
Sample	Density (g/cm <sup>3</sup> )
PLLA	1.211 ± 0.006
PLLA/OLLA-g-BC	1.218 ± 0.010
PHBV	1.190 ± 0.013
PHBV/OLLA-g-BC	1.206 ± 0.009



**Figure S3.** Water absorption profiles for PLLA, PLLA/OLLA-g-BC, PHBV and PHBV/OLLA-g-BC at 23 °C.



**Figure S4.** General setup of the biodegradation tests according to OECD 301B, the temperature of bioreactor was set to either 22°C or to 58°C.



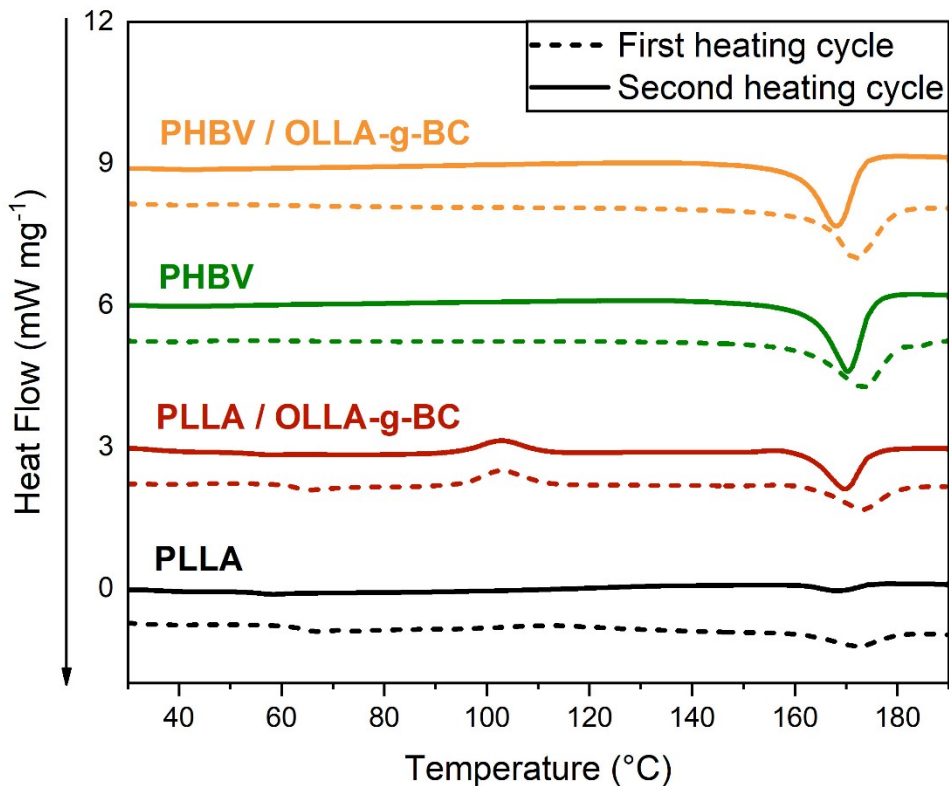
**Fig**

**ure S5.** CO<sub>2</sub> evolution of pure PLLA polymer in an aquatic test under thermophilic (58 °C) aerobic conditions as a result of biodegradation by a mixed bacterial inoculum deriving from biowaste compost.

### **Crystallization and melting of nanocomposites**

DSC was used to evaluate the effect of OLLA-g-BC on the melting and crystallization behavior of PLLA and PHBV. The thermograms obtained from the first and second heating cycles are presented in Figure S6, and the corresponding properties were compared with those of neat PLLA and PHBV (Table S2).





**Figure S6.** DSC first and second heating cycles of PHBV, PHBV/OLLA-g-BC, PLLA and PLLA/OLLA-g-BC.

The amorphous regions of the polymers are more susceptible to enzymatic, moisture, and oxygen penetration during biodegradation and gas transport. From the DSC curves of the first heating cycle we could identify a crystalline fraction of polymers in the nanocomposites after extrusion and compression molding. The cold crystallization exotherm at 103 °C for PLLA / OLLA-g-BC was narrower than that for PLLA around 113 °C, indicating the formation of crystals with a more homogeneous size distribution. Moreover, the lower cold crystallization temperature indicated accelerated crystallization induced by OLLA-g-BC, acting as a nucleating agent for PLLA.<sup>3</sup>

After the first heating cycle, the thermal history of the extruded polymers was erased and the thermograms obtained from the second heating cycle of the nanocomposites were recorded. The

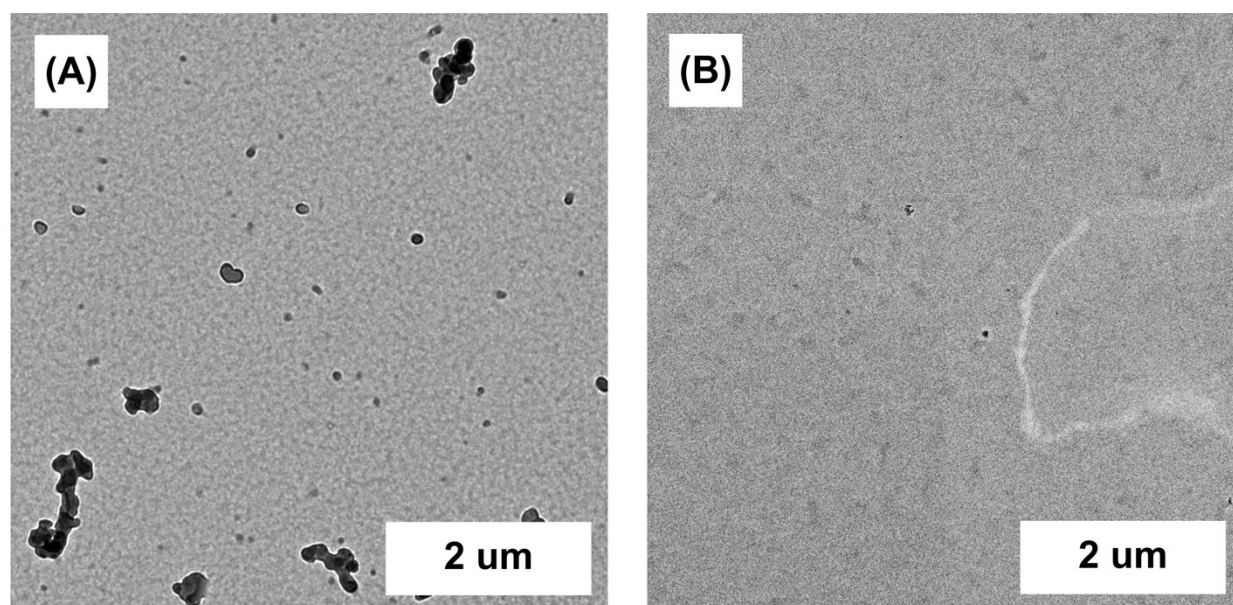
cold crystallization peak was absent for PLLA in the second thermal cycle. However, a pronounced peak was still observed for PLLA / OLLA-g-BC composites at roughly the same temperature as in the first thermal cycle. Similar results were previously observed for PLLA composites containing a microcrystalline cellulose-based filler.<sup>4</sup> The PLLA melting endotherm at 168.5 °C was narrower but had higher enthalpy for PLLA / OLLA-g-BC than for pure PLLA. In the second thermal cycle, this difference was even more pronounced suggesting that the OLLA-g-BC significantly increased the crystallinity of PLLA in the thermal cycle, in agreement with the literature data on modified microcrystalline cellulose fillers.<sup>4,5</sup>

In agreement with the literature, the PHBV-based samples only showed melting peaks in both heating cycles. Our measured melt enthalpy of pure PHBV correlated with literature.<sup>6</sup> The melting enthalpy and temperature of PHBV decreased when OLLA-g-BC was added, which could be attributed to possibility that the nanofiller suppresses PHBV molecular chain rearrangement during crystallization, resulting in a lower degree of crystallization. The crystallinity of the PHBV nanocomposite might be affected by the presence of boron nitride nanoparticles already present in the pure PHBV films, which could cause nucleation and masking any effect caused by the cellulose-based filler materials.<sup>7</sup>

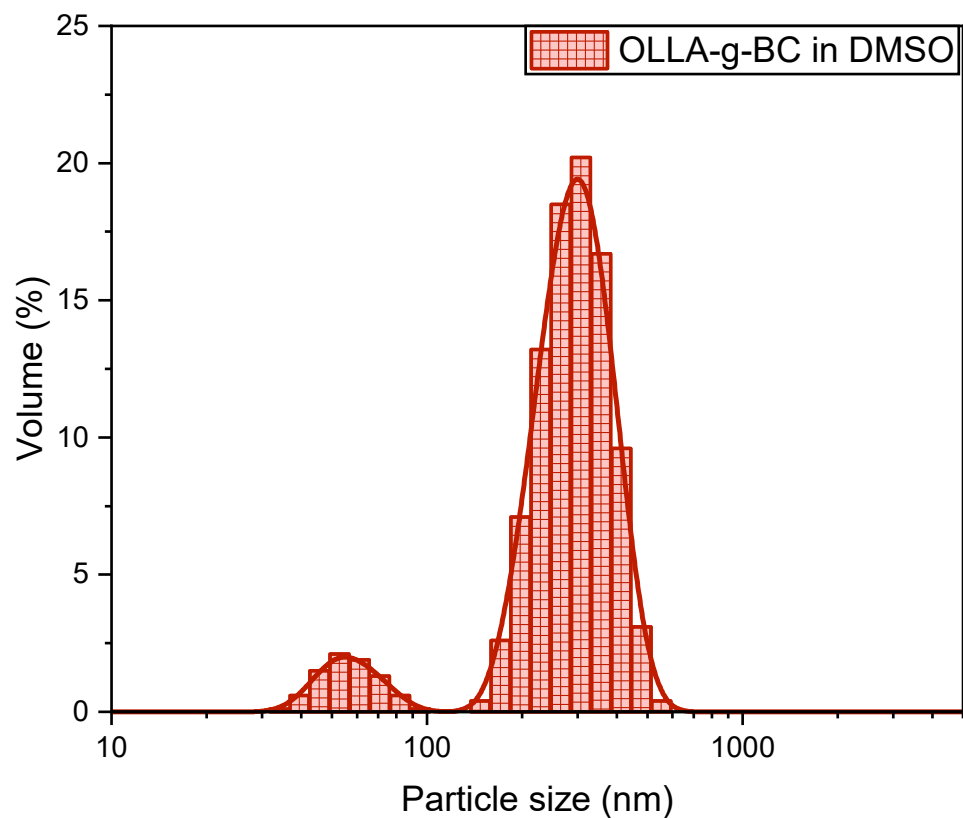
**Table S2.** Thermal characteristics of PLLA and PHBV and their nanocomposites.

Sample	$w_f$ (%)	First heating cycle			Second heating cycle		
		$T_{m1}$ (°C)	$\Delta H_{m1}$ (J/g)	$\chi_{c1}$ (%)	$T_{m2}$ (°C)	$\Delta H_{m2}$ (J/g)	$\chi_{c2}$ (%)
PLLA	100	171.7 ± 0.6	5.8 ± 0.4	6.2	169.4 ± 0.7	7.3 ± 0.2	7.8
PLLA/ OLLA-g-BC	95	172.5 ± 0.6	10.6 ± 0.7	12.0	170.9 ± 0.8	23 ± 2.4	25.7

<b>PHBV</b>	98.5	172.6 ± 0.7	75 ± 2.4	52.0	170.4 ± 0.5	88 ± 1.1	61.3
<b>PHBV/ OLLA-g-BC</b>	93.6	171.3 ± 0.4	69 ± 2.8	50.1	169 ± 1.4	79 ± 2.7	58.2



**Figure S7.** TEM micrographs of (A) generated BC nanoparticles, (B) OLLA-g- BC nanoparticles.



**Figure S8.** Volume-weighted size distribution measured by DLS for OLLA-g-BC in DMSO. Aggregates or particles larger than 100 nm comprised ~92% of the particle volume.

## REFERENCES

- 1 E. W. Fischer, H. J. Sterzel and G. Wegner, *Kolloid-Zeitschrift & Zeitschrift für Polymere*, 1973, **251**, 980–990.
- 2 P. J. Barham, A. Keller, E. L. Otun and P. A. Holmes, *J Mater Sci*, 1984, **19**, 2781–2794.
- 3 A. N. Frone, S. Berlioz, J.-F. O. Chailan and D. M. Panaitescu, *Carbohydr Polym*, 2013, **91**, 377–384.
- 4 S. Hua, F. Chen, Z. Y. Liu, W. Yang and M. B. Yang, *RSC Adv*, 2016, **6**, 1973–1983.
- 5 A. Gupta and V. Katiyar, *ACS Sustain Chem Eng*, 2017, **5**, 6835–6844.

- 6 W. V. Srubar, S. Pilla, Z. C. Wright, C. A. Ryan, J. P. Greene, C. W. Frank and S. L. Billington, *Compos Sci Technol*, 2012, **72**, 708–715.
- 7 E. L. Sanchez-Safont, J. Gonzalez-Ausejo, J. Gamez-Perez, J. M. Lagaron and L. Cabedo, *J Renew Mater*, 1970, **4**, 123–132.