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Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and wheat straw fibre composites: thermal, mechanical properties and biodegradation behaviour

M. AVELLA, G. LA ROTA, E. MARTUSCELLI, M. RAIMO
Istituto di Ricerca e Tecnologia delle Materie Plastiche-Via Toiano, 6-80072 Arco Felice (Na)-Italy
E-mail: raimo@mail.irtemp.na.cnr.it

P. SADOCCO, G. ELEGIR, R. RIVA
Stazione Sperimentale per la Cellulosa, Carta, Fibre Tessili Vegetali e Artificiali-Piazza Leonardo da Vinci 26, 20133 Milano
E-mail: sscctlib@icil64.cilea.it

The thermal and mechanical behaviour of a biotechnological polyester (poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV) reinforced with wheat straw fibres has been investigated. In order to improve chemico-physical interactions between the components, the reinforcing agent has been previously submitted to a treatment with high temperature steam leading to fibres richer in cellulose and more reactive. The addition of straw fibres has been found to increase the rate of PHBV crystallisation, while it does not affect the crystallinity content. Furthermore, the comparison of the mechanical properties has shown that the composites exhibit higher Young moduli and lower values of both the stress (σ_B) and strain (ε_B) to break than the neat matrix of PHBV. The biodegradability in different environments by means of short and long term tests has been studied. It has been observed that the presence of straw does not affect biodegradation rate evaluated in liquid environment and in long term soil burial tests. In the composting simulation test the rate of biodegradation is reduced for composites with more than 10% of straw content. The morphology of the composites has also been investigated and correlated to the biodegradation process. © *2000 Kluwer Academic Publishers*

1. Introduction

Poly(3-D-hydroxybutyrate) (PHB) is the foremost polymer of poly(β-hydroxyalkanoates), a series of microbial polyesters behaving like synthetic thermoplastic materials. They are produced by bacterial fermentation under restricted feeding conditions, promoting the accumulation of high amount of storage products in microorganisms. Notwithstanding the high cost of poly(hydroxyalkanoates) respect to conventional thermoplastics, their production is commercially attractive because of their important characteristics of biodegradability and biocompatibility [1, 2].

Poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV), a statistical copolymer of 3-hydroxybutyrate and 3-hydroxyvalerate, presents over PHB homopolymer the advantages of improved thermostability and toughness [3]. The high price of the copolymer still prevents its large use, even in applications where biodegradability is needed. However, if the cost of the material were reduced by adding a cheaper product that simultaneously would extend polymer performances, it could become competitive and converted in everyday articles.

In a previous work [4], PHB/wheat straw fibre composites were prepared up to 50% by weight of straw and their thermal and impact behaviour investigated. This study has shown that 20% of straw is the optimum content for toughening PHB, filling the gap of its poor impact resistance. Being a poly(hydroxyaldehyde), cellulose is very effective in forming intermolecular hydrogen bonds with polyesters. However, before the mixing with polyester matrices, the interacting hydroxyl groups of cellulose have to be exposed, by a steam explosion process (SEP) [5], removing the non fibrous components (hemicellulose, lignin, pectin, wax) of the vegetal product. In this process cellulosic material is heated with saturated water vapour and after few minutes submitted to a sudden release of pressure. The consequent adiabatic expansion of the water within the vegetal tissues produces cellulose almost free of amorphous components. SEP also causes morphological and structural changes that enhance the ability of cellulose to form interfacial bonds with thermoplastics. Cellulose can be readily hydrolysed by cellulases, which are excreted by a wide variety of bacteria and fungi (6). Thus, biodegradable matrices like poly(hydroxyalkanoates) reinforced with wheat straw fibres are fully environmentally biodegradable composites.

The aim of the present work was to study morphological, thermal and tensile properties of PHBV/SEP wheat straw composites, as well as to determine the biodegradation rate of the material in different bench scale environments.

2. Experimental

2.1. Materials

Poly-β-hydroxybutyrate-β-hydroxyvalerate copolymer (PHBV) with molecular weight of 400,000 daltons was provided by Zeneca Bio Products (Cleveland, England). This copolymer contains 5% mol of β-hydroxyvalerate units.

The wheat straw (Italian and EC sources) was submitted to steam explosion treatment in a Deltalab EC 300 apparatus. The biomass was heated at 210 °C with saturated water vapour for 120 seconds; after this permanence time, the pressure was suddenly released to obtain an adiabatic expansion of the water present in the wood tissues. The resulting exploded straw fibres were dried at 80°C until they reached constant weight. This treatment removes a significant part of the non-fibrous components (lignin and hemicellulose) from the cellulose also causing morphological changes which can favour interfacial adhesion with plastic materials.

2.2. Samples preparation

The straw fibres (2–3 mm length) were mixed with powder PHBV and the heterogeneous materials kept under stirring for 3 hours. Then it was compression moulded under the following conditions:

$$T = 180^\circ\mathrm{C}, \qquad p = p_{\mathrm{atm}} \text{ for 3 minutes}$$

$$T = 180\,^\circ\mathrm{C}, \qquad p = 10^7 \text{ Pa for 1 minute}$$

The moulded composite was then die water cooled to room temperature . The percentage by weight (10, 20 and 30 wt%) of the straw fibres and the respective codes of the composites are reported in Table I. Sheets having thickness of 1 mm were cut to obtain dumb-bell specimens for tensile tests.

2.3. Techniques

Thermal analysis was performed by using a Mettler TA-3000 differential scanner calorimeter equipped with a control and programming unit and a calorimetric cell operating under nitrogen atmosphere. In order to determine the crystallinity content, the following procedure was performed: first heating from 30°C to 210°C, (RUN I) then cooling down to −50°C (RUN II) and finally re-heating to 210°C (RUN III). The scan rate was 10°C/min throughout. To obtain the glass transition temperature, the samples were submitted to the following thermal treatment: heating from 30°C to 210 °C at a scan rate of 20 °C/min, cooling down to −50°C with a scan rate of −50°C/min, and re-heating from −50 °C to 200 °C at a scan rate of 20 °C/min.

Scanning electron microscopy (SEM) was carried out by using a Philips 501 SEM, after vacuum metallization of the samples by means of a Polaron Sputtering apparatus with Au-Pd alloy.

An Instron machine was used to perform room temperature tensile tests according to ASTM-D256 standard method. The adopted cross head speed was 1 mm/min. All the mechanical parameters were derived averaging at least six experimental values for each composition.

2.4. Evaluation of the biodegradability

2.4.1. Modified Sturm test

A modification of the Sturm test was adopted [7]. The experiment was carried out in 5 litre flask containing 3 litre mineral medium with no carbon sources except the powdered polymeric samples (200 kg/m^3). The samples were analysed in duplicate. The CO_2 trapping system, for the absorption of the biologically produced gas, consisted of a series of three gas-washing bottles, each filled with 10^{-4} m^3 of a 0.05 M $Ba(OH)_2$ solution. CO_2 evolution during microbial degradation was monitored by titration of the residual $Ba(OH)_2$ with 0.2 M HCl. The amount of CO_2 released was calculated as a percentage of the total CO_2 that the test material can theoretically produce referred to its carbon content (%Th CO_2). The carbon percentage was determined taking into account the inorganic content of materials. Whatman cellulose, a well-known commercial available biodegradable product, was used as reference material.

The inoculum was prepared as follows: a sample of activated sludge, derived from an aerobic waste-water

TABLE I Thermal parameters of PHBV/SEP straw composites

Weight percentage of straw fibres	Codes of composite materials	RUN I			RUN II		RUN III					
		ΔH_m (J/g)	T_m (°C)	X_c (%)	ΔH_c (J/g)	T_c (°C)	$\Delta H_c'$ (J/g)	T_c' (°C)	ΔH_{tot} (J/g)	ΔH_m (J/g)	T_m (°C)	X_c (%)
0	Neat PHBV	64	166	44	20	56	24	56	44	68	160	47
10	PHBV/SEP Straw 90/10	66	165	45	59	74	4	92	63	75	164	51
20	PHB/SEP Straw 80/20	64	164	44	57	73	—	—	57	77	163	52
30	PHBV/SEP Straw 70/30	63	164	43	58	72	—	—	58	81	164	55

treatment plant not treating any industrial effluents, was aerated overnight and allowed to settle for 2 h. The supernatant contained 20 000 colony forming units per mm^3 as determined by conventional plate counting using plate count agar (Oxoid) as culture medium. 3×10^4 mm^3 of supernatant was used to inoculate 3×10^6 mm^3 of mineral medium (1% inoculum).

2.4.2. Compost test

This test was a modification of the ASTM D5338 [8] procedure of controlled composting. A 3 litre reactor was filled with 2×10^6 mm^3 of mature compost and thermostated at $50 \pm 2°C$. The system was continuously aerated with previously water-saturated and thermostated pressurised air. The test specimens, having the same initial shape (i.e. the same surface of exposure to the biodegradation treatment), were buried inside the reactors. The samples were withdrawn from the compost reactors at different times, washed with distilled water and dried at 60°C to constant weight.

The mature compost used in this test was kindly supplied by Centro Ricerche Produzioni Animali (CRPA) of Reggio Emilia (Italy). It was produced at the Platform of the Composting Plant of Limidi di Soliera, Modena (Italy), from a mixture of residual sludges (municipal sewage treatment plant), grass and wood chips (maintenance of civic parks). The compost was stored indoors for several months. Prior to use, the compost humidity was adjusted to 60%.

2.4.3. Soil burial test

Specimens of the investigated materials were buried in rectangular boxes (300 × 400 × 70 mm) filled with garden soil. The boxes were stored in the laboratory for six months at a temperature of 23°C. The soil humidity was kept at 21%. The samples were withdrawn from the soil after three and six months of incubation, then they were washed with distilled water and dried at 60°C to constant weight and the percentage of weight loss was determined.

3. Results and discussion

3.1. Thermal properties

Changes in energetic and transition temperature of processes like glass transition, melting and crystallisation revealed by DSC analysis, lead to consider chemico-physical and structural variations caused by the presence of the straw fibres.The thermal analysis of PHBV/SEP Straw composites was carried out on specimens according to the specifications reported in the experimental part. As an example, the DSC curves for PHBV and PHBV/SEP Straw 80/20 are shown in Fig. 1. From the DSC curves the temperatures T_m and T_c were obtained as the maxima of the first order transition peaks, while the apparent crystallisation ΔH_c or fusion ΔH_m enthalpies were calculated from the area of the peaks. The crystallinity is defined as:

$$X_c = \frac{\Delta H_m}{\Delta H_m^*} \times 100$$

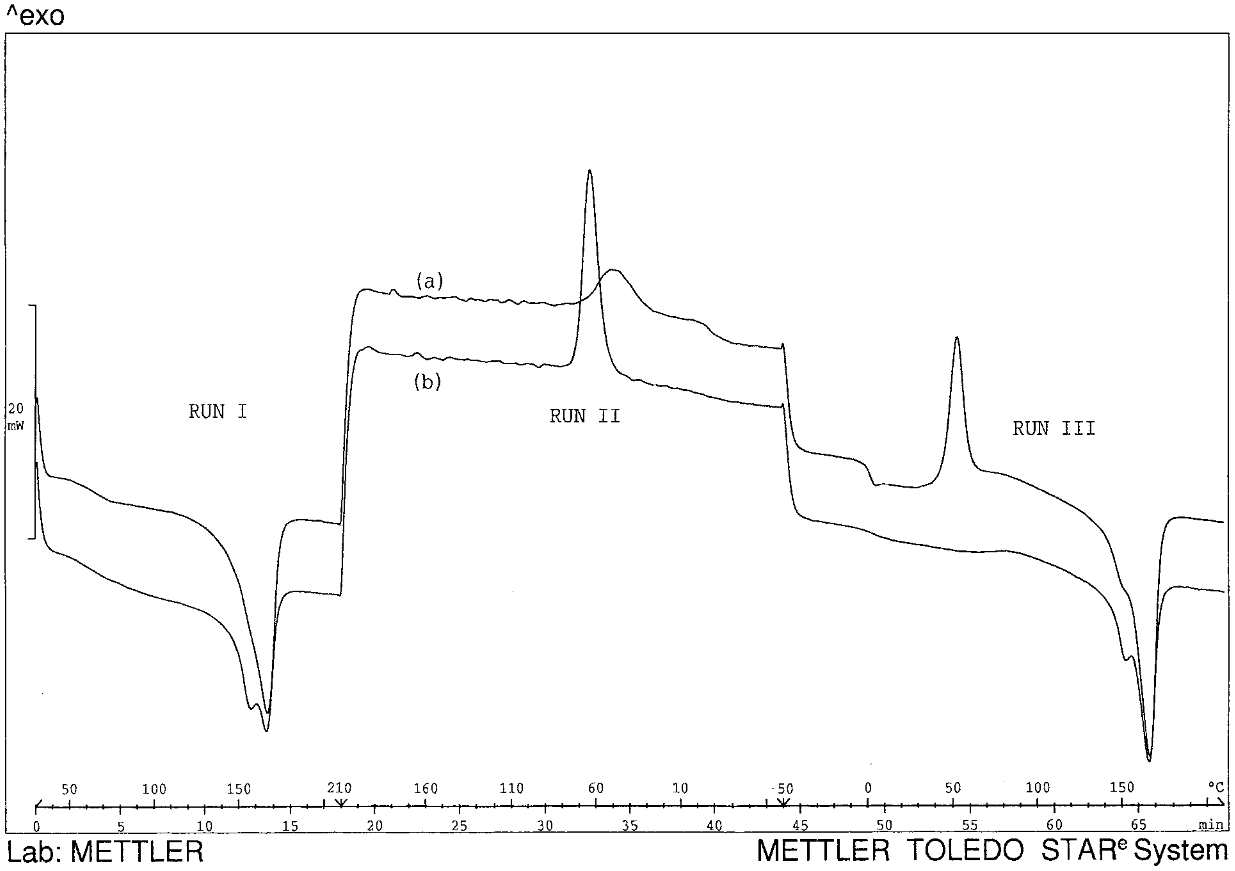

Figure 1 DSC curves for (a) PHBV and (b) PHBV/SEP Straw 80/20 submitted to the following thermal program: 30°C to 210°C at (RUN I); 210°C to −50°C (RUN II); −50°C to 200°C (RUN III); the scan rate was 10°C/min throughout.

where ΔH_m is the apparent fusion enthalpy and ΔH_m^* the theoretical fusion enthalpy of a 100% crystalline polymer. A value of 146 J/g [9], for ΔH_m^* of PHBV was used. This value, found for PHB homopolymer by Barham *et al.*, is considered as a good approximation of the ΔH_m^* for PHBV samples [10], provided that the copolymers have a low percentage of hydroxyvalerate units. Indeed, statistical copolymers of PHBV have been found to show the phenomenon of isodimorphism [11]: the crystal lattice of a component undergoes a deformation in order to allow the accommodation of the other monomer units in the same cell. The entity of such changes depends on the amount of comonomer and so the cell parameters should not significantly vary at low percentage of hydroxyvalerate. Thus the use of the value of ΔH_m^* found for PHB homopolymer, even if causes a little systematic error, allows to obtain a relative crystallinity scale useful to compare the samples. The values of the apparent crystallisation (ΔH_c) and fusion (ΔH_m) enthalpies together with the transition temperatures (T_c and T_m), taken as the maxima of the peaks, and the crystallinity content X_c are reported in Table I for non-biodegraded materials. The enthalpies are expressed as joule per grams of PHBV. The total crystallisation enthalpy $\Delta H_{tot} = \Delta H_c + \Delta H_c'$ is also reported.

By comparing the values of X_c and ΔH_m referring to RUN I, it comes out that the moulded samples reach the same crystallinity content, independently of the composition. However, the presence of steam exploded straw influences the crystallization behaviour of PHBV. As a matter of fact, comparing the data reported in RUN II (crystallization) of Table I, a clear difference between neat PHBV and PHBV/SEP Straw composites emerges. Indeed the crystallization from melt of neat PHBV occurs only partly (45% of the total) in RUN II (primary crystallization) and proceeds during RUN III (secondary crystallization), where a crystallization peak after a well evident glass transition step is found (Fig. 1). This fact indicates that the sample remains still largely amorphous after the non isothermal crystallization occurring in RUN II. Thus it is able to recrystallize from the amorphous phase during the re-heating in RUN III. On the contrary, the thermograms related to RUN III of PHBV/SEP Straw composites do not present exothermic peaks, since the crystallization process is almost complete at the end of RUN II. Furthermore, owing to the low amount of non crystalline material, the glass transition step is scarcely visible in RUN III. Moreover, the composites are found to crystallize at higher temperatures than the neat PHBV, demonstrating a nucleating effect of the straw exploded fibres. Finally, the composite materials may reach, at least in short times, a greater amount of crystallinity than the neat matrix, as shown by the comparison between the global crystallisation enthalpies of neat PHBV (44 J/g) and the crystallization enthalpies evolved from the composites in RUN II, being in this case neglectable the secondary crystallization in RUN III.

Concerning the influence of the straw on the glass transition temperature T_g of PHBV, it has been found that T_g slightly increases with increasing the straw content (Table II). This finding, previously observed [4] and supported by infrared spectroscopy investigations, may be explained by an intermolecular interaction between CO groups of the PHBV and OH groups of the straw that decreases the molecular flexibility of the polymer chains involved in hydrogen bonding.

TABLE II Glass transition and fusion temperatures of PHBV/SEP Straw composites

Composition	T_g (°C)	T_m left (°C)	T_m right (°C)
Neat PHBV	1	—	166
PHBV/SEP Straw 90/10	1	157	165
PHBV/SEP Straw 80/20	3	155	164
PHBV/SEP Straw 70/30	6	155	164

Besides the different crystallisation behaviour, neat PHBV and composite samples exhibit a further difference concerning the melting behaviour. The thermograms of fusion of PHBV (corresponding to RUN I) show a unique peak for neat PHBV, while a double peak of fusion appears for PHBV/SEP Straw composites. The two temperatures corresponding to the relative maxima of the fusion peaks are reported in Table II for each composition. Both higher (T_m right) and lower (T_m left) temperatures are independent of the straw content.

The presence of a double peak can be explained in the following two ways. 1) The presence of the straw causes the formation of crystals having different degree of perfection [12]. Less perfect crystals, being also less stable from a thermodynamic point of view, melt at lower temperature than more perfect crystals. The fusion of these crystallites is followed by recrystallization on more stable crystals. The simultaneous occurrence of two phenomena respectively endothermic and exothermic during the reorganisation of crystals, leads to a global endothermic double peak. 2) Presence of two populations of crystals which melt at different temperatures [13]. Deeper investigations are needed to discriminate between such hypotheses. Wide angle X-Ray diffraction analysis of the samples is still in course and will be the object of a future paper.

3.2. Investigation on biodegradability

The neat PHBV and PHBV/SEP Straw composites were submitted to laboratory biodegradation tests simulating different environments. The results of the test conducted in liquid environment (modified Sturm test), using milled samples are shown in Fig. 2. Although bacterial PHBV is an easy degradable polymer it can be noted that its degradation proceeds slower than cellulose reference material. However, CO_2 recovery from neat PHBV and PHBV/SEP Straw composites was very similar ranging from 55 to 65% within 48 days. In contrast, the pure SEP Straw sample was degraded to a lower rate (30% of CO_2 recovery) than neat PHBV and PHBV/SEP Straw composites. These results are not surprising since lignocellulosic materials (SEP Straw) are usually biodegraded quite slowly, due

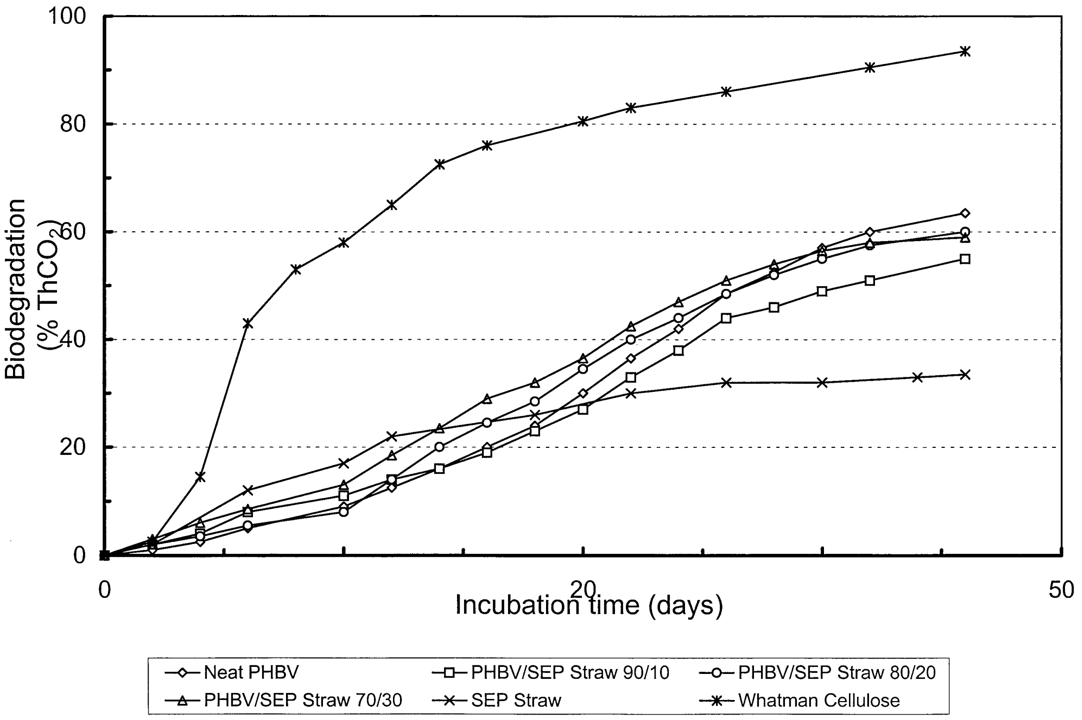

Figure 2 Respirometric mineralization of PHBV and PHBV/Straw composites in liquid environment (modified Sturm test). Biodegradation is reported as percentage of carbon dioxide evolution with respect to the theoretical amount of carbon dioxide in the samples (%$ThCO_2$). The theoretical carbon dioxide was calculated by elemental analysis.

TABLE III Percentage of weight loss of neat PHBV and PHBV/SEP Straw composites, in the simulating compost test. The % of weight loss values are the average of at least ten specimens and the calculated standard deviations are also reported

Incubation time (days)	PHBV	90/10	80/20	70/30
15	11 ± 2	11 ± 2	10 ± 1	11 ± 2
22	23 ± 5	21 ± 10	14 ± 1	16 ± 2
36	35 ± 5	36 ± 8	20 ± 2	21 ± 2
42	N.D.	N.D.	38 ± 2	37 ± 1
67	N.D.	N.D.	44 ± 2	47 ± 3

N.D.: Not detectable due to the high level of disintegration.

to the structural and chemical complexity of the material. Particularly one component, lignin, is very recalcitrant to microbial degradation and more easily degraded in solid state fermentation rather than liquid culture [14]. In the Sturm test, the neutral pH and the liquid environment is more favorable to bacterial growth causing the development of a mixed bacterial population able to degrade cellulose and hemicellulose components of the material but not lignin. Fungi, which are the main lignin destroyers, are not favored in these conditions. Data obtained in this trial suggest that in this environment PHBV in the composite is readily available to the enzymes produced by microorganisms. Probably, the milling treatment of samples causes an easy accessibility to PHBV in the material increasing also the available surface for degradation [15].

Specimens of neat PHBV and PHBV/SEP Straw composites were also submitted to a compost simulation test evaluating the % of weight loss (Table III). In this environment neat PHBV and 90/10 composites were degraded to a similar extent. The degraded samples appeared heterogeneously eroded and presented several holes of different sizes and deepness such that specimens degraded to about 40% of weight loss could be hardly handled. Because of the greater disintegration the weight loss determination after 42 and 67 days of incubation was not performed. Therefore, we considered these latter samples not only degraded but also disintegrated to a significant level according to CEN/TC 261/SC4/WG2/N97-04 definition "the physical falling apart into very small fragments of packaging or packaging material". The composites with higher SEP Straw content (80/20 and 70/30 PHBV/SEP Straw) were degraded to a lower rate and did not disintegrate to a significant level even at 67 days of incubation. In this case the specimens appeared degraded more homogeneously than 90/10 composite although they reached relatively high levels of weight loss, 44 and 47% respectively. The greater straw content in the composite probably causes a more regular erosion of the PHBV matrix leading to a more pronounced resistence at disintegration phenomena. It has to be underlined that also the composting environment, although it is a type of solid state fermentation, does not overcome the problem of lignin degradation; being characterized by neutral to basic pH the lignin degrader white rot fungi are not favoured as above mentioned for the Sturm test [16].

A long term disintegration test (soil burial test conducted in garden soil) was also performed comparing neat PHBV and 70/30 PHBV/SEP Straw composite. In contrast to the results obtained with the composting simulation test, no significative difference between the samples was revealed in this experiment with respect to degradation rate. After 3 months of incubation neat

PHBV and PHBV/SEP Straw 70/30 composite reached 11 ± 1 and 10 ± 2% of weight loss, respectively. Even following 6 months of incubation the degradation of the two materials was similar, showing losses of 20 ± 5% and 23 ± 5%. These data suggest that lignin degradation takes place in this environment allowing a similar degradation rate for both samples. However, the % of weight loss reached by the specimens after 6 months of incubation was significantly lower, compared to that observed in the composting environment probably due to the lower number of microorganisms and the lower oxygenation of the soil burial test.

The polymeric specimens degraded at different extent during the compost test, were examined by scanning electron microscopy (SEM). Figs 3 and 4 show the surface of neat PHBV and 70/30 composite, respectively, before (a) and following about 30% degradation (b). Initially the surface of the neat PHBV and of the composite specimens appear essentially smooth. As a consequence of microbial erosion, in the case of neat PHBV the spherulitic texture is clearly observed. As already reported in the literature [17, 18] the micrograph reported in Fig. 3b confirms the preferential erosion of the interlamellar phase, in fact bundles of lamellar crystals, denuded of the cementing amorphous phase, can be seen over the eroded surface. The biodegraded composite materials do not show a well evident spherulitic morphology due to the presence of SEP straw fibers. The presence of the SEP straw fibers on the specimen surface indicates the preferential erosion of the PHBV component by the microorganisms.

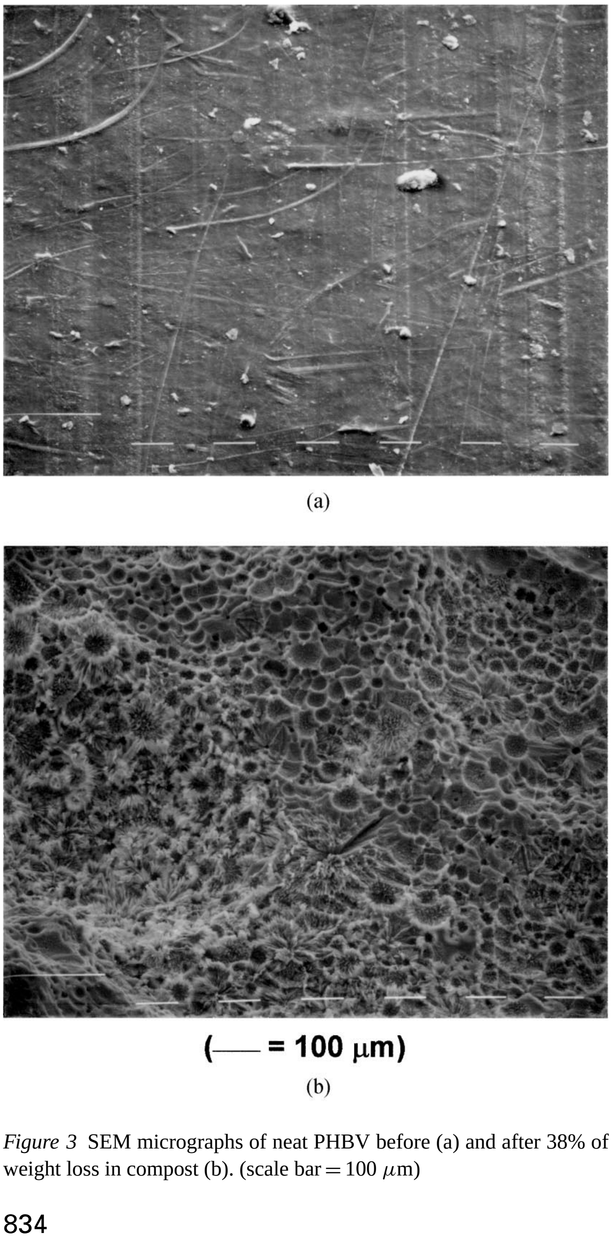

Figure 3 SEM micrographs of neat PHBV before (a) and after 38% of weight loss in compost (b). (scale bar = 100 μm)

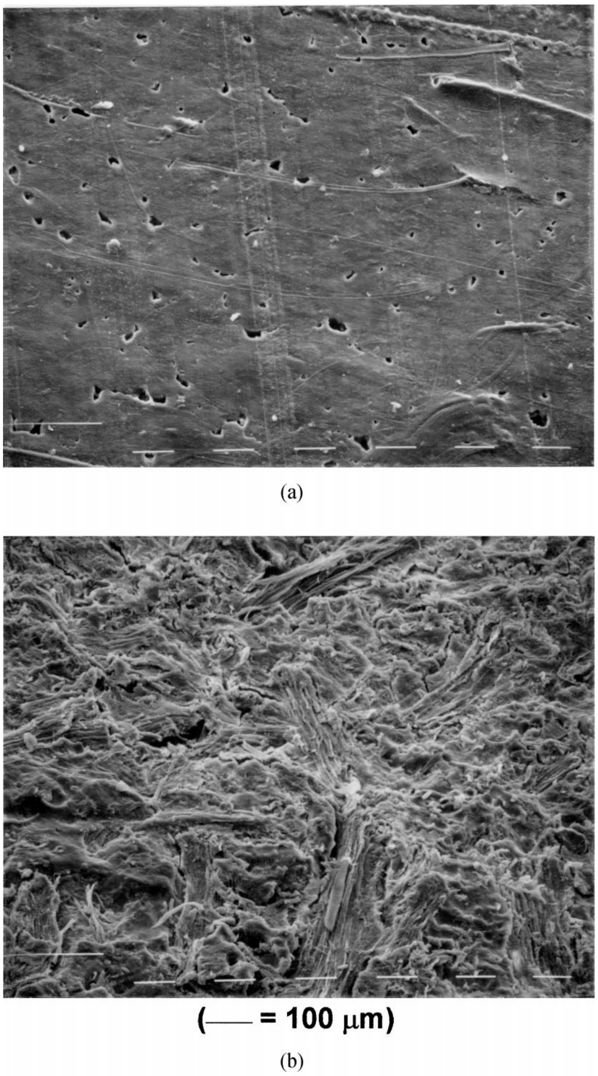

Figure 4 SEM micrographs of PHBV/SEP Straw 70/30 before (a) and after 24% of weight loss (b) (scale bar = 100 μm)

3.3. Mechanical behaviour

Biodegradation of polymers can be advantageously followed by measuring the change in tensile properties. A significant decrease in such properties could be accounted for the removal of polymer molecules or chain cleavage. In Table IV are reported the Young's Modulus (or elasticity modulus) E, the stress to break σ_B and the strain to break ε_B of samples degraded to different extents, up to 35% of weight loss, during the compost test. Highly degraded samples (45% of weight loss) were not subjected to mechanical test due to apparatus limits.

Concerning the non-degraded materials, the addition of straw fibres causes an enhancement of the Young

TABLE IV Mechanical parameters of PHBV/SEP Straw systems

	Biodegradation (%)	E (GPa)	σ_B (MPa)	ε (%)
Neat PHBV	0	1.4 ± 0.2	19 ± 2	2.4 ± 0.5
	15 ± 5	1.1 ± 0.2	13 ± 4	1.5 ± 0.5
	25 ± 5	1.0 ± 0.2	10 ± 4	1.3 ± 0.5
	35 ± 5	0.9 ± 0.1	10 ± 3	1.1 ± 0.4
PHBV/SEP 90/10	0	1.4 ± 0.1	14 ± 3	1.7 ± 0.3
	15 ± 5	1.1 ± 0.1	11 ± 2	1.3 ± 0.3
	25 ± 5	0.9 ± 0.2	10 ± 1	1.6 ± 0.5
	35 ± 5	0.8 ± 0.2	5 ± 3	0.9 ± 0.3
PHBV/SEP 80/20	0	1.5 ± 0.1	14 ± 1	1.3 ± 0.2
	15 ± 5	1.2 ± 0.3	9 ± 2	1.1 ± 0.3
	25 ± 5	1.1 ± 0.1	6 ± 2	0.9 ± 0.2
	35 ± 5	0.6 ± 0.3	1 ± 0.5	0.4 ± 0.1
PHB/SEP 70/30	0	1.7 ± 0.1	14 ± 1	1.4 ± 0.2
	15 ± 5	1.3 ± 0.2	9 ± 1	1.0 ± 0.2
	25 ± 5	1.2 ± 0.2	8 ± 1	0.9 ± 0.2
	35 ± 5	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.04

modulus and a decrease of σ_B and ε_B. Obviously, the biodegradation lowers the values of all these parameters. In Fig. 5 the relative parameters $E \times 100/E^0$, $\sigma_B \times 100/\sigma_B^0$ and $\varepsilon_B \times 100/\varepsilon_B^0$ (the values E^0, σ_B^0, ε_B^0 refer to the non-degraded samples) are reported as a function of the composition for PHBV/SEP Straw composites biodegradated to 35%. While the modulus E is not very much affected by the initial composition of the material for biodegradation levels less than 35%, samples with 35% of weight loss and high straw content show pronounced drops. A similar behaviour has been observed for σ_B and ε_B. This significant drop of the mechanical parameters of highly degraded composites is likely a consequence of the deterioration of the matrix rather than the degradation of the lignocellulosic component.

4. Conclusions

The thermal characterization has evidenced that the presence of straw fibres increases the rate of PHBV crystallization, due to a nucleating effect, while the thermal parameters such as crystallinity remain unchanged. This finding could advantageously affect the processing of the composites. Besides the cost reduction of expensive PHBV, the straw component permits preparation of more rigid materials as shown by the mechanical properties of composites.

The presence of straw does not reduce the biodegradation rate of PHBV/Straw composites in liquid environment and in a long term soil burial test as well. However, 80/20 and 70/30 PHBV/Straw composites showed, in a composting simulation test, a lower rate of biodegradation compared with neat PHBV and 90/10 composite.

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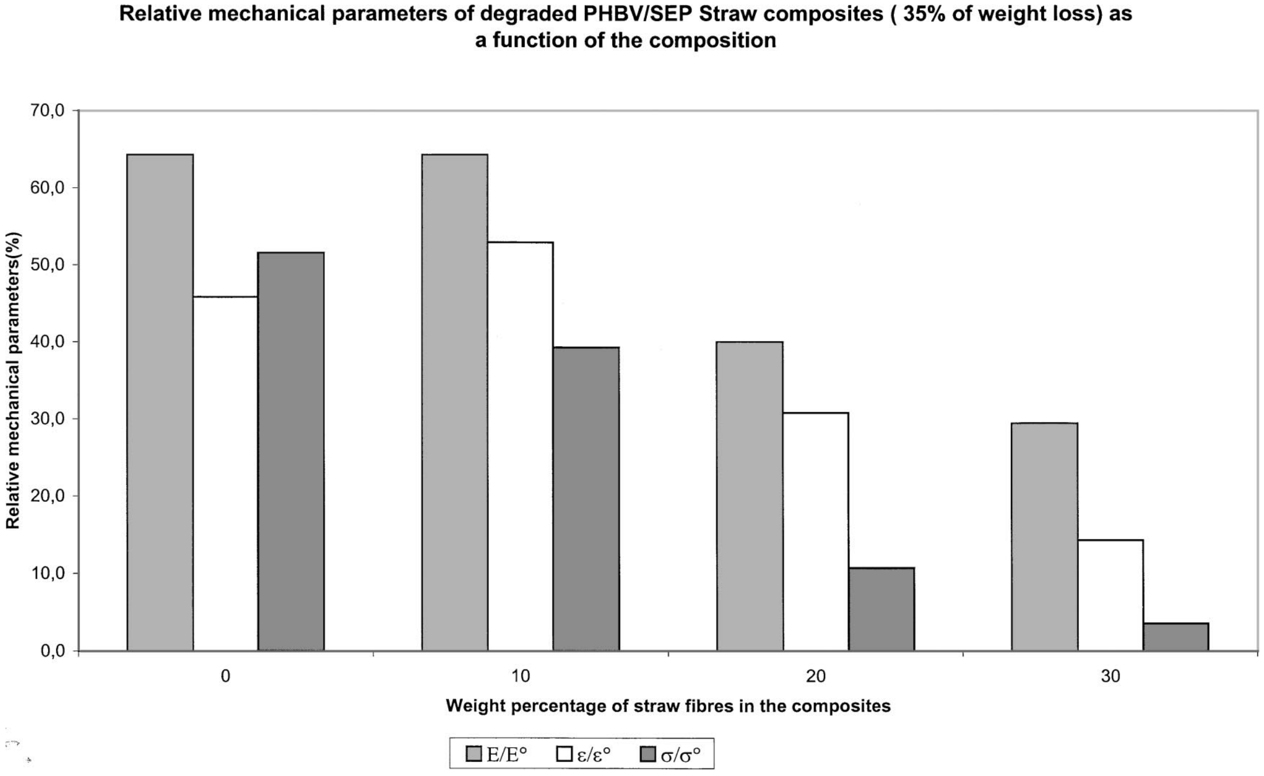

Figure 5 Relative percentage values Y × 100/Y^0 of modulus (a), stress (b) and strain (c) versus the initial composition for biodegraded samples (35% of weight loss).

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