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Influence of photodegradation with UV radiation in biotreatment with *Paecilomyces variotti* on PHBV/GNS nanocomposites

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Abstract: Graphite nanosheets (GNSs) and poly(hydroxybutyrate-*co*-hydroxyvalerate) (**PHBV**) nanocomposites were prepared by solution casting method. This study aimed to evaluate the effects of previously phototreatment with ultraviolet (UV) radiation on the biotreatment with *Paecilomyces variotti* of neat **PHBV** and **PHBV**/GNS nanocomposites. Some samples of **PHBV** film were submitted only to biotreatment with *P. variotti* during 120 days; other samples were subjected to phototreatment (UV radiation) for 30 h followed by biodegradation assessment with *P. variotti* for a period of 120 days. The effects of biotreatments on thermal properties were studied through differential scanning calorimetry. The **PHBV** films were monitored by weight changes as a function of time. Also, their surfaces were examined after the tests using scanning electron microscopy, contact angle and roughness measurements. The level of oxidation was recorded by means of carbonyl index evaluation by Fourier transformed infrared spectroscopy spectroscopy. The phototreatment of **PHBV** films influenced the process of adhesion and colonisation by *P. variotti* on the surface of the films, and enhanced morphological and structural changes.

1 Introduction

In recent years, there is growing interest in replacing petroleum-based synthetic polymers by biodegradable materials from renewable resources for applications as eco-friendly biomedical materials. Good properties performed by these materials combined with biodegradation property make them promising alternatives to the problem of environmental pollution [1–3]. Poly(hydroxybutyrate-*co*-hydroxyvalerate) (**PHBV**) and copolymers from polyhydroxyalkanoates family are among the most widely used biodegradable polymers. They are produced by bacterial fermentation mediated by synthase enzyme and using renewable feedstock such as sugar or enzyme-thinned starch. This biodegradable polymer has attracted large scientific interest due to their biodegradable, biocompatible and absorbable properties [4, 5].

Biodegradable polymers have limited use, due to their low performance of mechanical properties, electrical conductivity, thermal stability, hardness and low melt viscosity [6]. In this way, to solve part of this problem, research is being conducted with the aim to improve these properties, as the development of nanocomposites using a biodegradable polymer matrix such as **PHBV** [7, 8].

Therefore, polymer nanocomposites have assumed great importance in the past years, as the addition of nanoparticles in polymer matrix improves the final properties of the materials and opens new possibilities for the applications in engineering, physics, chemistry and biology [9]. Currently, there are many nanoparticles with extraordinary properties that have been used in several studies such as nanoclays, carbon nanotubes, graphite nanosheets (GNSs), nanocellulose and others [1, 4, 10–14].

In this research project, the GNSs were chosen to be incorporated into **PHBV** matrix, to obtain a biodegradable nanocomposite. Graphite is an allotropic form of carbon that is found naturally. It is abundant and has exceptional strength in the graphitic plane, low weight and high electrical conductivity. Thus, it has attracted great interest as reinforcement for polymers because of the ability to improve their mechanical, physical, chemical, magnetic and electrical properties [14–16].

Recently, several micro-organisms have been reported for degradation of plastics [17–19]. The fungal species identified, *Aspergillus niger*, *Amauroclopius ornatus*, *Aspergillus nidulans*, *Penicillium funiculosum, Janibacter cremeus*, *Chaetomium globosum, Aspergillus flavus*, *Aspergillus candidus*, *Paecilomyces variotti* and *Aspergillus glaucus* are the most frequently cited species [20, 21]. These fungal species are found in terrestrial environments such as soil or dead vegetable matter and perform the function of mineralising organic carbon in nature. The fungi can also be found in various habitats, mainly living in freshwater and also in marine environments [22, 23].

The biodegradation of polymeric materials is due to some morphological and biochemical characteristics. Specific enzymes that degrade waste plastics are produced and secreted into the medium, in order to provide monomers, which in turn can act as a carbon source for the micro-organisms growth. The final product of biological degradation is mostly microbial biomass, water and carbon dioxide. In addition, filamentous fungi are less sensitive to changes in nutrients, aeration, pH and temperature, growing in inhospitable habitats and extreme environments. Thus, they are able to colonise and breaking down the macromolecules of the polymers. Also, fungal hyphae increase the contact of fungi and the waste, enhancing the biodegradation [18]. Some species of filamentous fungi such as *Aspergillus*, *Penicillium* and *Paecilomyces* are capable of degrading polymeric waste.

The filamentous fungus chosen to be used in this research was the *P. variotti*, as it is commonly found in soil, indoors, plants, animals and unpasteurised food. Besides rapid growth, and ability to grow at low oxygen levels, its hyphae, conidia and ascospores are thick walled, promoting thermotolerance [24].

Photodegradation or phototreatment is an important degradation process, in which the polymeric material is exposed to ultraviolet (UV) rays [25, 26]. The UV radiation oxidises the polymer chain due to dissolved oxygen in the environment, causing breaking of bonds and formation of functional groups, i.e. it increases the level

of degradation of the polymeric material. Thus, the photodegradation process can be considered one of the initial steps in the biodegradation. It increases the amount of low molecular weight materials by the breaking of bonds (oxidation of the polymer chain) promoting the contact surface by the molecular weakening and consequently enhances the hydrophilicity of the polymer by the introduction of carbonyl groups [27, 28]. This oxidative treatment can be performed in laboratory scale, also called artificial exposure, by controlled degradation conditions using UV radiation lamps.

In this way, the purpose of this paper was to verify the influence of photodegradation in the biotreatments with *P. variotti* on neat **PHBV** and **PHBV**/GNS nanocomposites. The effects of both treatments (photodegradation followed by biotreatment with *P. variotti*) were discussed and evaluated by characterisation methods such as thermal analyses by differential scanning calorimetry (DSC), weight loss, degradation level by means of carbonyl index (CO_i) determined by Fourier transformed infrared spectroscopy (FT-IR), morphological changes by scanning electron microscopy (SEM), surface roughness, contact angle, before and after photodegradation and biotreatments.

2 Experimental results

2.1 Materials

PHBV with 4% of 3-HV units and MW 187.000 g mol^{-1} was kindly supplied by PHB Industrial, São Paulo, Brazil. The graphite used for the preparation of GNSs was natural graphite flake from Sigma Aldrich (332461). Concentrated sulphuric acid and concentrated nitric acid from Chemical Company of Brazil Vetec were used as chemical intercalant and oxidiser to prepare expanded graphite. The solvent used for film production was chloroform ($CHCl_3$) from Synth. *P. variotti* was cultivated with potato dextrose agar (Kasvi, Company from Brazil). Saline solution [sodium chloride (NaCl) 0.9%] was prepared with reagents (Synth, Company from Brazil) and Tween 80 was purchased (Alamar Tecno-Científica, Diadema-Brazil).

2.2 Obtainment of the GNS

The methodology of obtaining the GNSs was already described by Montagna *et al.* [15]. The process of preparing the GNSs was carried out in three steps: intercalation (chemical exfoliation), expansion (thermal treatment) and GNSs obtainment (physical treatment by exfoliation ultrasound).

2.3 Preparation of **PHBV** and **PHBV**/GNS nanocomposite films

The **PHBV**/GNS nanocomposites (0.25, 0.50, 0.75 and 1.00 wt%) were prepared according to the methodology described by Montagna *et al.* [10]. Initially, the GNSs and $CHCl_3$ (Synth) (1:10 w/v) were sonicated for 4 h in an ultrasonic bath (Unique, USC 1450). **PHBV** was solubilised in $CHCl_3$ (1.1:10 w/v) at 40°C. The system remained under stirring until the entire polymer mass was solubilised and resulted in a viscous solution. Subsequently, the solution containing the GNSs was stirred with the **PHBV** solution for 3 h at 60°C. The final solution was cast onto Petri dishes covered with aluminium foil to obtain films after solvent evaporation at room temperature for 12 h.

2.4 Photodegradation/phototreatment

Laboratory scale photodegradation tests were based on ASTM G154 [29]. Neat **PHBV** and **PHBV**/GNS nanocomposite films were exposed to photodegradation with UV lamp (Germicidal Lamp (GL), 20 W) for 30 h before being subjected to biodegradation with the filamentous fungus *P. variotti* during 120 days. The phototreatment was performed on **PHBV** samples and nanocomposites, in order to evaluate and compare the influence of photodegradation in the adhesion process and colonisation of the surface of the films.

2.5 Fungal-growth test on solid medium

The methodology used for the fungal-growth test was based on ASTM-ISO 846-1997 [30] and Montagna *et al.* [19]. Initially, *P. variotti* was cultivated in glass tubes containing potato dextrose agar (Kasvi, Curitiba – Brazil). After 15 days of incubation (28 ± 1°C), 5 ml of saline solution (NaCl 0.9%) containing 50 µl Tween 80 (Alamar Tecno-Científica, Diadema – Brazil) were added to the glass tube. The spore suspension was prepared by scraping the fungal growth from agar surface. After 15 min, the spores in the supernatant were counted using a Newbauer's chamber and adjusted to 10^6 spores/ml. Then, 0.1 ml of spore suspension were plated on potato dextrose agar plates and incubated for 5 days at 28 ± 1°C. On the sixth day of growth, the films of neat **PHBV** and **PHBV**/GNS nanocomposites, with and without phototreatment by UV radiation during 30 h, were added to each agar plate. The Petri dishes containing the samples were incubated at 28 ± 2°C during 120 days, and were removed in the periods of 30, 60, 90 and 120 days. At the end of the experiment, the samples were cleaned with ethanol–water mixture (70/30 v/v) for 1 min, to prevent the continuation of microbial attack on the **PHBV** films, and then dried at room temperature in a desiccator for 3 days, in order to remove excess of humidity from the **PHBV** films. This paper was performed in triplicate.

2.6 Analytical characterisation

2.6.1 Differential SC: DSC analysis were performed on a Netzsch Phoenix DSC 2014 F1. Small amounts (≃5 mg) of dried samples were placed into aluminium pans. Heating scan was performed at a heating rate of 10°C min^{-1} from 25 to 200°C, in nitrogen atmosphere with a gas flow of 20 ml min^{-1}. The melting temperature (T_m) and the melting enthalpy (ΔH) were obtained. The crystalline content was obtained by (1). **PHBV** films were removed at each period of 30, 60, 90 and 120 days to carry the characterisations. The melting enthalpy of the 100% crystalline **PHBV** ($\Delta H°$) was taken as 109 Jg^{-1} [31]

$$X_c(\%) = \frac{\Delta H}{\Delta H^{\circ}} \times 100 \tag{1}$$

2.6.2 Weight loss: Weight loss analyses of **PHBV** films from biotreatment and photodegradation/biotreatments studies were carried out by recording the films weight in an analytical laboratory balance (RADWAG AS 60/220.R2). The **PHBV** films were weighed before and after the end of the treatments (bio and photodegradation/biotreatment). The per cent weight loss determined as a function of the biotreatment with filamentous fungi was calculated using (2), where W_0 is the initial weight of the films and W_f is the weight of the films at the time after the biotreatment with filamentous fungi and after phototreatment followed by biotreatment with the fungi. The experiment was performed in duplicate

$$W_t.\ \text{loss}\,(\%) = \frac{W_0 - W_f}{W_0} \times 100 \tag{2}$$

2.6.3 Fourier T-IR spectroscopy: FT-IR measurements were carried out with a Shimadzu IR Affinity-1 spectrometer. The spectra were taken as an average of 32 scans with 2 cm^{-1} resolution, and were scanned from 4000 to 400 scans. The CO_i was calculated according to (3), where $A1$ is the ratio of the optical density of the absorption band of carbonyl at 1720 cm^{-1} crystalline phase and 1751 cm^{-1} to the amorphous phase and $A2$ is the optical density of the absorption band at 1380 cm^{-1} (attributed to the symmetric vibration CH_3 crystalline phase of the **PHBV**). **PHBV** films were removed at each period of 30, 60, 90 and 120 days to carry the characterisations

$$\text{IC} = \frac{A1}{A2} \tag{3}$$

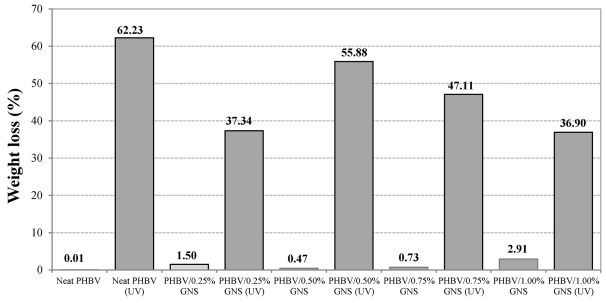

Fig. 1 *Weight loss (%) for neat PHBV, PHBV/0.25 wt%,* ***PHBV****/0.50 wt%,* ***PHBV****/0.75 wt% and* ***PHBV****/1.00 wt% films, after biotreatment with P. variotti and after photodegradation (UV) followed by biotreatment with P. variotti*

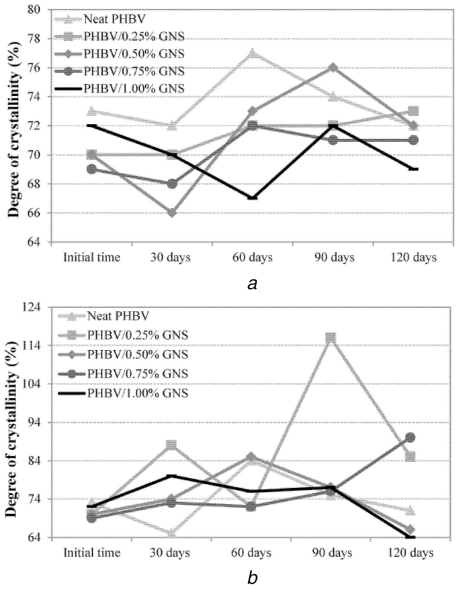

Fig. 2 *Degree of crystallinity (X_c) of neat* ***PHBV*** *and* ***PHBV****/GNS nanocomposites*

(a) At the initial time and after biotreatment with *P. variotti*, ***(b)*** At the initial time and after photodegradation followed by biotreatment with *P. variotti*

2.6.4 Contact angle measurement: The **PHBV** and nanocomposite film surfaces before and after biotreatment with filamentous fungi and after phototreatment by UV during 30 h, followed by biotreatment with the fungi, were submitted to measurements of contact angle, carried out using a Ramé-Hart, mod. 500, advanced goniometer. The wetting liquid was distiled water with the sessile drop method (1.0 µl, 25°C). Calculations were averaged on three measurements out at appropriate samples.

2.6.5 Surface roughness: The surface roughness was quantified through perfilometry using a WYKO NT1100 optical profiling system (Veeco, Tucson, AZ). Three measurements of roughnesses for each sample were performed on a displacement of the ferrule at the centre and both ends of the samples.

2.6.6 Scanning EM: The surface morphologies of neat **PHBV** and **PHBV**/GNS nanocomposite films before and after the biotreatment and phototreatment followed by biotreatment were recorded by using a Quanta FEG 250 SE microscope, at the magnification of 1000× and with accelerating voltage of 7.5 keV. The films were sputtered with gold before SEM observation.

3 Results and discussion

Weight loss is a relatively sensitive, frequently and directly used method for determining the weight changes and measure the degradability caused by environmental degradation conditions and microbial attack of polymers after treatments [32]. Fig. 1 shows the weight loss percentage of neat **PHBV** and **PHBV**/GNS nanocomposite films after biotreatment with *P. variotti* during 120 days and after phototreatment (UV) for 30 h followed by biotreatment with *P. variotti* during 120 days.

It is noted that all **PHBV** films after biotreatment showed a slight weight loss, almost imperceptible and neat **PHBV** film showed the lowest percentage of weight loss. **PHBV**/GNS nanocomposite films present greater values of weight loss when compared with neat **PHBV** film. In this case, the result indicates that the biodegradation of **PHBV**/GNS nanocomposites was faster than neat **PHBV**, because the presence of GNS in **PHBV** matrix. In this case, the matrix was more easily attacked by micro-organisms, i.e. the nanofillers in the polymer behaved as a defect by interfering on the morphology, consequently increasing the roughness and the hydrophilicity of the film (results discussed below). Thus, the nanocomposites were more susceptible to fungal colonisation [33].

Nonetheless, **PHBV** films exhibited significant weight loss after exposure to phototreatment followed by biotreatment with filamentous fungi, markedly in neat **PHBV** film. This significant weight loss after phototreatment/biotreatment may be related to the influence of UV radiation, which influenced the breaking of molecular bonds of polymer, consequently leading to loss of physical integrity of the film. Thus, the fragments were metabolised by the fungi, resulting on fungal cell growth and adhesion of the fungi on the surface of the films, which contributed to fungal colonisation.

According to Sudhakar *et al.* [34] when the loss of physical integrity of the polymeric material occurs, weight loss can be detected.

Fig. 2 shows the degree of crystallinity (X_c) of all **PHBV** samples, before and after biotreatments with *P. variotti* during 120 days and after photodegradation (UV) for 30 h followed by biotreatment with *P. variotti* during 120 days.

It can be observed that the addition of GNSs in the **PHBV** matrix at the initial time resulted in decrease of X_c values of the **PHBV**/GNS nanocomposites. This fact may be associated with a large number of nucleation centres, due to the presence of GNSs in **PHBV** matrix, which caused a greater number of crystal defects, reducing the degree of crystallinity of the nanocomposite [35].

The X_c increases after the treatments (photodegradation and biotreatment) may correspond to a degradation of the amorphous phase in the **PHBV** films. Fig. 2*a* shows the X_c values of the **PHBV** films during the biotreatment with *P. variotti.* Both **PHBV** films exhibited decrease in X_c values after 30 days of biotreatment. After this first period (30 days), there was an increase in the X_c values until 90 days. However, after this period, there was a decrease until the end of biotreatment (120 days). This may be due to the fact that induction of crystallinity increases until it reaches a constant value. This crystallinity limit value can be attributed to the increased presence of chemical defects (hydroperoxides and carbonyl groups) in the molecules [36].

Fig. 2*b* shows the X_c values of photodegraded **PHBV** films with UV radiation for 30 h and exposed to biotreatment with *P. variotti* for 120 days. All **PHBV**/GNS nanocomposites showed higher X_c values after 30 days of biotreatment.

The decrease in X_c values during the biotreatment may be related to the influence of UV radiation which may have affected regions of amorphous **PHBV**, breaking their polymer chains, followed by recrystallisation of smaller chains. Thus, the remaining polymer has led to an organisation with increased crystallinity. However, the amorphous regions have certain limit, and after a crystallisation time the degree of crystallinity tends to decrease due to the high level of degradation [37, 38].

The results indicate that there are greater X_c values in **PHBV** films previously photodegraded with UV radiation for 30 h. This fact may be related to UV radiation and heat condition to which the films were exposed, which allowed the breakdown of connections and rearrangements in the polymer chain by favouring the formation of functional groups such as hydroxyl, carbonyl and carboxyl [39, 40]. Thus, the **PHBV** films were subjected to the first stage of degradation, which is related to the breaking of covalent bonds, in which the energy of C–H bond is smaller tertiary carbon

Table 1 CO_i of amorphous and crystalline phases, calculated for neat **PHBV** and **PHBV**/GNS nanocomposites at the initial time, after biotreatment with *P. variotti* and after photodegradation followed by biotreatment with *P. variotti*

Samples		Initial time	After 30 days		After 60 days		After 90 days		After 120 days	
			Bio.*	Photo./Bio.**	Bio.*	Photo./Bio.**	Bio.*	Photo./Bio.**	Bio.*	Photo./Bio.**
neat **PHBV**	amorphous phase (1751/1380)	1.00	0.95	1.05	1.00	1.08	1.00	1.07	1.02	1.09
	crystalline phase (1720/1380)	1.00	0.95	1.05	1.00	0.99	1.02	1.00	1.04	1.08
PHBV/0.25% GNS	amorphous phase (1751/1380)	0.89	1.06	1.01	0.98	1.07	0.99	1.03	1.01	1.04
	crystalline phase (1720/1380)	0.88	1.05	1.01	1.02	1.00	1.05	1.03	1.05	1.03
PHBV/0.50% GNS	amorphous phase (1751/1380)	0.99	0.97	1.05	0.81	1.06	1.03	1.07	1.07	1.05
	crystalline phase (1720/1380)	0.96	1.02	1.02	0.88	0.99	0.99	0.98	1.06	1.03
PHBV/0.75% GNS	amorphous phase (1751/1380)	0.91	0.90	1.05	1.02	1.08	1.02	1.06	1.01	1.05
	crystalline phase (1720/1380)	0.90	0.90	0.97	1.02	0.89	1.01	0.97	1.00	1.00
PHBV/1.00% GNS	amorphous phase (1751/1380)	0.87	1.00	1.06	1.02	1.08	0.98	1.07	1.01	1.05
	crystalline phase (1720/1380)	0.81	1.01	0.99	1.02	0.96	1.00	0.94	1.01	1.02

*Bio.: Biotreatment with *P. variotti* during 120 days;

**Photo./Bio.: Photodegradation for 30 h followed by biotreatment with *P. variotti* during 120 days.

atoms. This implies that it can be broken more easily than the C–H bonds of primary and secondary carbons. This breaking generated reactive species that were responsible for the propagation process by facilitating and promoting the biodegradation process with *P. variotti* [41].

Rosa *et al.* [42] studied the biodegradability of poly-*β*-hydroxybutyrate and poly-*ε*-caprolactone in soil composting before and after irradiations, using a Weather-Ometer. These authors noted that the photodegradation influenced the increase in crystallinity, as crosslink reactions were very common during the experiment.

The level of oxidation of **PHBV** films was determined by means of CO_i determined by FT-IR spectroscopy, in order to better understand the structural changes in the **PHBV** films after photodegradation and biotreatments. Table 1 shows the changes of CO_i of neat **PHBV** and **PHBV**/GNS nanocomposite films at initial time and during the biotreatment with *P. variotti* during 120 days and during photodegradation for 30 h followed by biotreatment with *P. variotti* during 120 days.

Prior to the exposure to the treatments (photodegradation and biotreatment), all **PHBV** nanocomposites exhibited a reduction in the level of oxidation, because the presence of GNS influenced the effects of thermal oxidation, with decreasing values of CO_i.

Table 1 shows the carbonyl group evolution after biotreatment and phototreatment followed by biotreatment with *P. variotti* of neat **PHBV** and **PHBV**/GNS nanocomposite films. The intensification and the appearance of the carbonyl group indicate and confirm that degradation occurred, leading to a change in the polymer chemical structure [43].

It was noted that the CO_i values in amorphous phase are higher than in the crystalline phase. This behaviour is attributed to the first to be consumed and is the amorphous phase of the polymers resulting in an increase in the portion of the crystalline phase in the remaining films after exposed to the treatments (bio and photodegradation/biotreatment) [44].

It was verified that after both treatments (bio and photodegradation/biotreatment), the nanocomposites showed similar lower values of CO_i in both phases, crystalline and amorphous, comparing with neat **PHBV** films. This may be related to the presence of GNS dispersed in the **PHBV** matrix, which would be interacting with **PHBV**, not only changing their morphology but also promoting the biodegradation. Therefore, it is noted that the presence of GNS in the **PHBV** matrix did not delay the process of carbonyl groups formation throughout exposure time. These findings suggest that GNS does not act as an efficient physical barrier and thus neither can interfere in the penetration of UV radiation during the phototreatment nor with the adhesion of micro-organisms during the biotreatment with *P. variotti* [45].

The contact angle measurements were performed with the aim of evaluating the hydrophobicity of **PHBV** films after treatments (phototreatment and biotreatment).

According to the literatures [33, 46, 47], the hydrophobicity is an important property of the surface in biodegradation studies, because the relation between surface hydrophobicity and micro-organisms will determine the extent of colonisation on the polymer substrate. More hydrophilic surfaces are more easily colonised by micro-organisms.

Table 2 depicts the results of the characterisation of **PHBV** films by means of contact angle measurements. All **PHBV** films presented contact angle values similar at the initial time and before exposure to the treatments, which are underlining hydrophilic character. The presence of GNS in the **PHBV** matrix resulted in the absence of reduction in contact angle values (∼1%), with the exception of **PHBV**/1.00 wt% GNS film, which showed a slight increase of 2%.

As result of exposure to degradation processes, phototreatment and biotreatment in this case, the tendency significantly reduces the values of the contact angle [48].

All the **PHBV** films after exposure to biotreatment with *P. variotti* during 120 days, showed a significant reduction in the contact angle values (Table 2 and Fig. 3). The reductions were 17, 21, 13, 12 and 26% for neat **PHBV**, **PHBV**/0.25 wt% GNS, **PHBV**/0.50 wt% GNS, **PHBV**/0.75 wt% GNS and **PHBV**/1.00 wt % GNS, respectively. Therefore, the decrease in contact angle indicates that the surfaces of **PHBV** films have become more hydrophilic due to microbial action (adhesion, colonisation and erosion).

Moreover, after the biotreatment, the presence of GNS in the **PHBV** matrix did not affect the values of contact angle, because all samples showed a significant decrease in contact angle measurements.

Table 2 shows the contact angle measurements of **PHBV** films after photodegradation during 30 h followed by biotreatment with *P. variotti* for 120 days. A significant reduction can be observed in contact angle values in comparison with the **PHBV** films at the initial time. This reduction was 22, 23, 25, 34 and 36% for neat **PHBV**, **PHBV**/0.25 wt% GNS, **PHBV**/0.50 wt% GNS, **PHBV**/0.75 wt% GNS and **PHBV**/1.00 wt% GNS, respectively. A tendency of decrease was observed in the values of the contact angle as a function of increase in the GNS content in **PHBV** matrix. This fact can be related to the phototreatment with UV radiation, in which the **PHBV** films were subjected prior to biotreatment with *P. variotti*. The phototreatment oxidised the surface of **PHBV** films and facilitated fungi colonisation, reflecting an increase in wettability of the **PHBV** surface [49].

The reduction of contact angle values or increased wettability can be attributed to increase of surface roughness. In fact, according to the literature, higher contact angle of a hydrophobic surface can be related to increase in surface roughness, whereas the contact angle of a hydrophilic surface decrease with increasing roughness of the polymer sample surface after the process of degradation [50].

Table 2 Contact angle of neat **PHBV** and **PHBV** nanocomposites with different contents 0.25, 0.50, 0.75 and 1.00 wt% of GNS at initial time, after biotreatment with *P. variotti* and after photodegradation followed by biotreatment with *P. variotti*

Samples		Contact angle, deg
neat **PHBV**	initial time	81.9
	biotreatment	68.0
	phototreatment/biotreatment	64.3
PHBV/0.25 wt% GNS	initial time	81.1
	biotreatment	63.9
	phototreatment/biotreatment	62.7
PHBV/0.50 wt% GNS	initial time	81.0
	biotreatment	70.3
	phototreatment/biotreatment	60.6
PHBV/0.75 wt% GNS	initial time	80.8
	biotreatment	70.8
	phototreatment/biotreatment	53.3
PHBV/1.00 wt% GNS	initial time	83.6
	biotreatment	62.1
	phototreatment/biotreatment	53.8

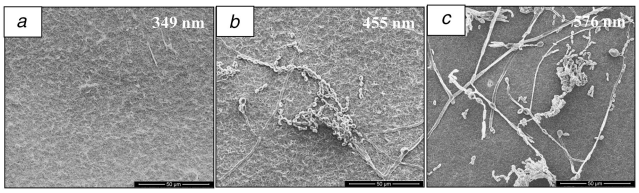

Fig. 3 *SEM micrographs and average roughness values of neat **PHBV***
(a) At the initial time, ***(b)*** After biotreatment with *P. variotti*, ***(c)*** After photodegradation followed by biotreatment with *P. variotti*

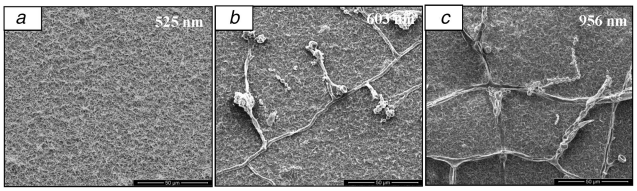

Fig. 4 *SEM micrographs and average roughness values of **PHBV**/0.25 wt % GNS*
(a) At the initial time, ***(b)*** After biotreatment with *P. variotti*, ***(c)*** After photodegradation followed by biotreatment with *P. variotti*

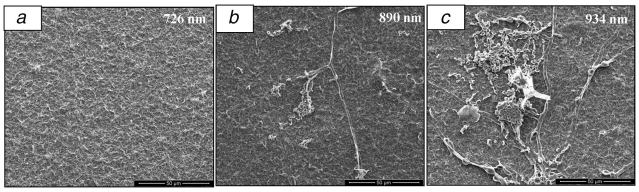

Fig. 5 *SEM micrographs and average roughness values of **PHBV**/0.50 wt % GNS*
(a) At the initial time, ***(b)*** After biotreatment with *P. variotti*, ***(c)*** After photodegradation followed by biotreatment with *P. variotti*

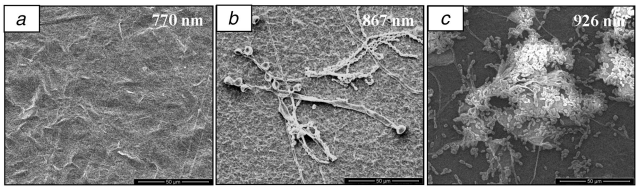

Fig. 6 *SEM micrographs and average roughness values of **PHBV**/0.75 wt % GNS*
(a) At the initial time, ***(b)*** After biotreatment with *P. variotti*, ***(c)*** After photodegradation followed by biotreatment with *P. variotti*

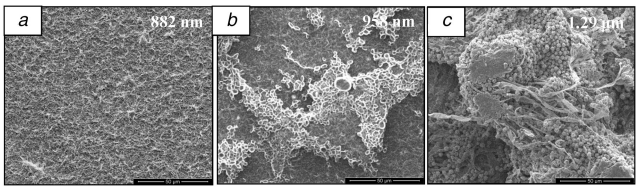

Fig. 7 *SEM micrographs and average roughness values of **PHBV**/1.00 wt % GNS*
(a) At the initial time, ***(b)*** After biotreatment with *P. variotti*, ***(c)*** After photodegradation followed by biotreatment with *P. variotti*

The previous study on the effect of different degradation conditions on the oxidative degradation of polyethylene (PE) film samples containing pre-oxidant additives [32], reported that the temperature and relative humidity may influence the film wettability. This modification in wettability may increase the vulnerability to microbial colonisation.

Matsunaga and Whitney [49] studied the influence of modified surface condition by UV irradiation and the ability of micro-organisms to colonise low-density PE (LDPE). These authors verified decrease in the contact angle on the pre-treated LDPE film. Possibly, these treatments oxidised the polymer's surface and enhanced microbial colonisation, reflecting an increase in wettability of the polymer's surface.

Figs. 3–7 show the surface micrographs and the roughness average of **PHBV** films, at the initial time, after biotreatment with *P. variotti* and after photodegradation followed by biotreatment with *P. variotti*. It was possible to observe the changes in the surface morphology of all **PHBV** films after biotreatment and phototreatment/biotreatment.

It could be seen that the **PHBV** films at the initial time (micrographs 'A' of Figs. 3–7), before treatment, have a uniform surface with small undulations, which may be due to the preparation method used. The roughness values presented by **PHBV**/GNS nanocomposite films at the initial time, presenting a gradual and significant increase as the concentration of GNS in this **PHBV** matrix; with the increase being of 50, 108, 120 and 152% for **PHBV** films containing 0.25, 0.50, 0.75 and 1.00 wt% of GNS, respectively, when compared with neat **PHBV** at the initial time. The increase of imperfections and surface roughness facilitates microbial action on the surface of the film, i.e. enhances the colonisation of micro-organisms by exposing the polymer to fungal hydrolases that act on the carbons of the carbonyl groups [51].

Micrographs 'B' of Figs. 3–7 show the surface morphology of **PHBV** films biotreated for 120 days with *P. variotti*. Undulations, roughness and surface adhesion of micro-organisms can be observed on the surface of all the **PHBV** films. Hyphae of the colonising organism may be observed, particularly in the **PHBV**/1.00 wt% GNS film. This film showed the highest roughness and

the lowest contact angle, consequently the higher degree of microbial adhesion. This result, in association with the other characterisations, was able to evidence that the fungi were capable of a plentiful colonisation of the **PHBV** film surfaces and also promoted further oxidation of the polymer chains.

The micrographs of surface morphology of **PHBV** films after phototreatment by UV radiation for 30 h followed by biotreatment for 120 days are shown in micrographs 'C' of Figs. 3–7. It is observed that there were no perceived changes on the surface of these films, when compared with the micrographs of **PHBV** films just after the biotreatment. Superficial fungal growth occurred, and penetration of hyphae was observed in the most oxidised samples. It was also noted an increase in the roughness in all **PHBV** films, and higher number of adhered microbial cells on the surface of **PHBV**/0.75 wt% GNS and **PHBV**/1.00 wt% GNS films. This observation may be related to the higher roughness values and contact angle of these samples. Therefore, due to phototreatment with exposure to UV radiation, **PHBV** films were fragile and damaged. The reduction of hydrophobicity, evidenced by contact angle measurements and increased roughness, promoted higher colonisation of micro-organisms.

Therefore, the most likely is that the changes occurred in the molecular structures of **PHBV** films. The evidences can be, as noted in Fig. 2, with increasing the values of X_c; moreover, in Table 1, with increasing CO*i* values, These values indicate that they are chemically bound to the main chain formed during the UV exposure. Carbonyl groups being the main group formed and they are of fundamental importance at the start of biodegradation. This fact contributed to support the idea that the pretreatments enhanced polymer biodegradation process [2].

4 Conclusions

In this investigation, the influence of phototreatment with UV radiation and phototreatment with UV radiation followed by biotreatment with *P. variotti* of neat **PHBV** and **PHBV**/GNS (0.25, 0.50, 0.75 and 1.00 wt%) nanocomposites was studied. The nanocomposites were prepared in film form by a solution casting.

PHBV films after exposure to phototreatment with UV radiation followed by biotreatment with filamentous fungi exhibited significant weight loss, in particular in neat **PHBV** film. Fragmenting and loss of physical integrity of the film were observed as product of UV radiation and consequent breaking of molecular bonds of the polymer films. Fragments were subsequently metabolised by the filamentous fungi.

SEM micrographs indicated an extended decomposition and adhesion of fungi on the surface of all **PHBV** films. However, this fungal adhesion and surface degradation were more evident in **PHBV**/1.00 wt% GNS. This fact may be related to the reduction of contact angle values that increased the wettability, and increase of surface roughness observed in these films. **PHBV**/GNS nanocomposites after the treatments (bio and photodegradation/ biotreatment) showed similar or lower values of CO*i* in both phases (crystalline and amorphous) comparing with neat **PHBV** films. The presence of GNS in the **PHBV** matrix did not interfere with the penetration of UV radiation during the phototreatment and with the adhesion of micro-organisms during the biotreatment with *P. variotti*.

X_c values determined by DSC suggested that the photodegradation influenced the increase in crystallinity in all **PHBV** films. During the phototreatment, the UV radiation affected regions of amorphous **PHBV**, breaking their polymer chains, followed by recrystallisation to smaller chains. Thus, the remaining polymer has led to an organisation with increased crystallinity. However, the amorphous regions are limited, and after crystallisation time, degree of crystallinity tended to decrease due to the high level of degradation.

The phototreatment of the **PHBV** samples influenced the fungal adhesion and colonisation of the films' surface. Furthermore, *P. variotti* has the ability to degrade nanocomposite based on polyesters. This observation indicates that this filamentous fungus might be useful for the treatment of biodegradable polymeric waste or bioremediation in the environments contaminated with polymeric waste.

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6 References

[1] Yu, H.-Y., Qin, Z.-Y., Yan, C.-F., *et al.*: 'Green nanocomposites based on functionalized cellulose nanocrystals: a study on the relationship between interfacial interaction and property enhancement', *ACS Sustain. Chem. Eng.*, 2014, **2**, (4), pp. 875–886

[2] Muniyasamy, S., Ofosu, O., John, M.J., *et al.*: 'Mineralization of poly(lactic acid) (PLA), poly(3-hydroxybutyrate-*co*-valerate) (PHBV) and PLA/PHBV blend in compost and soil environments', *J. Renew. Mater.*, 2016, **4**, (2), pp. 133–145

[3] Shah, A.A., Hasan, F., Hameed, A., *et al.*: 'Biological degradation of plastics: a comprehensive review', *Biotechnol. Adv.*, 2008, **26**, (3), pp. 246–265

[4] Choi, W.M., Kim, T.W., Park, O.O., *et al.*: 'Preparation and characterization of poly(hydroxybutyrate-*co*-hydroxyvalerate) – organoclay nanocomposites', *J. Appl. Polym. Sci.*, 2003, **90**, pp. 525–529

[5] Ambrosio-Martín, J., Gorrasi, G., Lopez-Rubio, A., *et al.*: *J. Appl. Polym. Sci.*, 2015, **42101**, pp. 1–11

[6] Sanchez-Garcia, M.D., Lagaron, J.M., Hoa, S.V.: 'Effect of addition of carbono nanofibers and carbono nanotubes on properties of thermoplastics biopolymers', *Compos. Sci. Technol.*, 2010, **70**, pp. 1095–1105

[7] Wang, B.-J., Zhang, Y.-J., Zhang, J.-Q., *et al.*: 'Crystallization behavior, thermal and mechanical properties of PHBV/graphene nanosheet composites', *Chin. J. Polym. Sci.*, 2013, **31**, (4), pp. 670–678

[8] Sridhar, V., Lee, I., Chun, H.H., *et al.*: 'Graphene reinforced biodegradable poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) nanocomposites', *Express Polym. Lett.*, 2013, **7**, (4), pp. 320–328

[9] Peponi, L., Puglia, D., Torre, L., *et al.*: 'Processing of nanostructured polymers and advanced polymeric based nanocomposites', *Mater. Sci. Eng.*, 2014, **85**, pp. 1–46

[10] Montagna, L.S., Montanheiro, T.L.A., Machado, J.P.B., *et al.*: 'Effect of MWCNT functionalization on thermal and electrical properties of PHBV/ MWCNT nanocomposites', *J. Mater. Res. Technol.*, 2014, **30**, (1), pp. 55–65

[11] Montanheiro, T.L.A., Cristóvan, F.H., Machado, J.P.B., *et al.*: 'Effect of MWCNT functionalization on thermal and electrical properties of PHBV/ MWCNT nanocomposites', *J. Mater. Res. Technol.*, 2014, **30**, pp. 1–11

[12] Wang, H., Qiu, Z.: 'Crystallization behaviors of biodegradable poly(L-lactic-acid)/graphenes oxide nanocomposites from the amorphous state', *Thermochim. Acta*, 2011, **526**, pp. 229–236

[13] Barret, J.S.F., Abdala, A.A., Srienc, F.: 'Poly(hydroxyalkanoate) elastomers and their graphenes nanocomposites', *Macromolecules*, 2014, **39**, pp. 3926–3941

[14] Kashi, S., Gupta, R.K., Baum, T., *et al.*: 'Morphology, electromagnetic properties and electromagnetic interference shielding performance of poly lactic/graphenes nanoplatelet nanocomposites', *Mater. Des.*, 2016, **95**, pp. 119–126

[15] Montagna, L.S., Fim, F.C., Galland, G.B., *et al.*: 'Synthesis of poly(propylene)/graphite nanocomposites by in situ polymerization', *Macromol. Symp.*, 2011, **299/200**, pp. 48–56

[16] Gheno, G., Basso, N.R.S., Hübler, R.: 'Polyaniline/graphite nanocomposites: synthesis and characterization', *Macromol. Symp.*, 2011, **299–300**, pp. 74–80

[17] Lipsa, R., Tudorachi, N., Darie-Nita, R.N., *et al.*: 'Biodegradation of poly(lactic acid) and some of its based systems with *Trichoderma viride*', *Int. J. Biol. Macromol.*, 2016, **88**, pp. 515–526

[18] Passos, T.M.: 'Biodegradação de filmes de polihidroxibutirato-co-hidroxivalerato (PHBV), polietileno de baixa densidade (PEBD) e blenda de PEBD/PHBV (70/30), com fungos específicos'. MSc thesis, Universidade Estadual Paulista Júlio de Mesquita Filho, Brazil, 2013

[19] Montagna, L.S., Montanheiro, T.L.A., Borges, A.C., *et al.*: 'Biodegradation of PHBV/GNS nanocomposites by *Penicillium funiculosum*', *J. Appl. Polym. Sci.*, 2016, **134**, p. 44234

[20] Upreti, M.C., Srivastava, R.C.: 'A potential Aspergillus species for biodegradation of polymeric materials', *Curr. Sci.*, 2003, **84**, pp. 1399–1402

[21] Narayan, R.: 'Fundamental principles and claims of biodegradability – sorting through the facts, hypes and claims of biodegradable plastics in the marketplace', In TAPPI 2nd Annual PaperCon'09 Conference - Solutions for a Changing World, 2009, pp. 28–31

[22] Muenee, S., Chiemchaisri, W., Chiemchaisri, C.: 'Microbial consortium involving biological methane oxidation in relation to the biodegradation of waste plastics in a solid waste disposal open dump site', *Int. Biodeterioration Biodegradation*, 2015, **102**, pp. 172–181

[23] Zafar, U., Nzeram, P., Langarica-Fuentes, A., *et al.*: 'Biodegradation of polyester polyurethane during commercial composting and analysis of associated fungal communities', *Bioresour. Technol.*, 2014, **158**, pp. 374–377

[24] Houbraken, J., Varga, J., Rico-Munoz, E., *et al.*: 'Sexual reproduction as the cause of heat resistance in the food spoilage fungus *Byssochlamuys*

spectabilis (*Anamorph paecilomycesvariotii*)', *Appl. Environ. Microbiol.*, 2008, **74**, pp. 1613–1619
[25] Adema, K.N.S., Makki, H., Peters, E.A.J.F., *et al.*: 'The influence of the exposure conditions on the chemical and physical changes of polyester-urethane coatings during photodegradation', *Polym. Degradation Stab.*, 2016, **123**, pp. 13–25
[26] Montagna, L.S., Catto, A.L., Rossini, K., *et al.*: 'Evaluation of the effect of organic pro-degradant concentration in polypropylene exposed to the natural ageing'. AIP Conf. Proc., 2014, vol. **1593**, pp. 329–332
[27] Rupp, B., Ebner, C., Rossegger, E., *et al.*: 'UV-induced crosslinking of the biopolyester poly(3-hydroxybutyrate)- co-(3-hydroxyvalerate)', *Green Chem.*, 2010, **12**, pp. 1796–1802
[28] Montagna, L.S., Pezzin, A.P.T., Forte, M.M.C., *et al.*: 'Comparative study of degradation of PP modified with an organic pro-degradant subjected to natural and artificial ageing', *Polyols J.*, 2016, **3**, pp. 93–101
[29] American Society for Testing Materials, ASTM G154 – Standard Practice for Operating Fluorescent Ultraviolet (UV) Lamp Apparatus for Exposure of Nonmetallic Materials
[30] Int. Standard ISO 846/1997 – Plastics – Evaluation of the action of microorganisms
[31] Vidhate, V., Innocentini-mei, L., Souza, N.A.D.: 'Mechanical and electrical multifunctional poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) multiwall carbon nanotube nanocomposites', *Polym. Eng. Sci.*, 2012, **52**, pp. 1367–1374
[32] Chiellini, E., Corti, A., D'Antone, S., *et al.*: 'Oxo-biodegradable carbon backbone polymers – oxidative degradation of polyethylene under accelerated test conditions', *Polym. Degradation Stab.*, 2006, **91**, pp. 2739–2747
[33] Sen, S.K., Raut, S.: 'Microbial degradation of low density polyethylene (LDPE): a review', *J. Environ. Chem. Eng.*, 2015, **3**, pp. 462–473
[34] Sudhakar, M., Doble, M., Murthy, P.S., *et al.*: 'Marine microtemediated biodegradation of low- and high-density polyethylenes', *Int. Biodeterioration Biodegradation*, 2007, **61**, pp. 203–213
[35] Hou-Yong, Y., Zong-Yi, Q., Bin, S., *et al.*: 'Reinforcement of transparent poly(3-hydroxybutyrate-co-3-hydroxyvalerate) by incorporation of functionalized carbon nanotubes as a novel bionanocomposite for food packaging', *Compos. Sci. Technol.*, 2014, **94**, pp. 96–104
[36] Gleadall, A., Pan, J., Atkinson, H.: 'A simplified theory of crystallisation induced by polymer chain scissions for biodegradable polyesters', *Polym. Degradation Stab.*, 2012, **97**, (9), pp. 1616–1620
[37] Rabello, M., White, J.R.: 'Crystallization and melting behaviour of photodegraded polypropylene – I chemi-crystallization', *Polymer*, 1997, **38**, pp. 6379–6397
[38] Montagna, L.M., Catto, A.L., Forte, M.M.C., *et al.*: 'Comparative assessment of degradation in aqueous medium of polypropylene films doped with transition metal free (experimental) and transition metal containing (commercial) pro-oxidant/prodegradant additives after exposure to controlled UV radiation', *Polym. Degradation Stab.*, 2015, **120**, pp. 186–192
[39] Espí, E., Salmerón, A., Fontecha, A., *et al.*: 'The effect of different variables on the accelerated and natural weathering of agricultural films', *Polym. Degradation Stab.*, 2007, **92**, pp. 2150–2154
[40] Corti, A., Muniyasamy, S., Vitali, M., *et al.*: 'Oxidation and biodegradation of polyethylene films containing pro-oxidant additives: synergistic effects of sunlight exposure, thermal aging and fungal biodegradation', *Polym. Degradation Stab.*, 2010, **95**, pp. 1106–1114
[41] Rivaton, A., Gardette, J.L., Mailhot, B., *et al.*: 'Basic aspects of polymer degradation', *Macromol. Symp.*, 2005, **225**, pp. 129–146
[42] Rosa, D.S., Calil, M.R., Guedes, C.G.F., *et al.*: 'The effect of UV-B irradiation on the biodegradability of poly-*β*-hydroxybutyrate (PHB) and poly-*ε*-caprolactone (PCL)', *J. Polym. Environ.*, 2001, **9**, (3), pp. 109–113
[43] Sam, S.T., Ismail, H., Ahma, Z.: 'Soil burial of polyethylene-g-(Maleic anhydride) compatibilised LLDPE/soya powder blends', *Polym. Plast. Technol. Eng.*, 2011, **50**, (8), pp. 851–861
[44] Masood, F., Yasin, T., Hameed, A.: 'Comparative oxo-biodegradation study of poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate/polypropylene blend in controlled environments', *Int. Biodeterioration Biodegradation*, 2014, **87**, pp. 1–8
[45] Araújo, A.S.S.: 'Thermal and UV stability of PLA nanocomposites'. MSc thesis, Universidade do Minho, Portugal, 2002
[46] Al-Makhlafi, H., McGuire, J., Daeschel, M.: 'Influence of preadsorbed milk proteins on adhesion of Listeria monocytogenes to hydrophobic and hydrophilic silica surfaces', *Appl. Environ. Microbiol.*, 1994, **60**, (10), pp. 3560–3565
[47] Wang, R., Neoh, K.G., Shi, Z., *et al.*: 'Inhibition of Escherichia coli and Proteus mirabilis adhesion and biofilm formation on medical grade silicone surface', *Biotechnol. Bioeng.*, 2012, **109**, (2), pp. 336–345
[48] Belibel, R., Avramoglou, T., Garcia, A., *et al.*: 'Effect of chemical biodegradable polymers on surface energy: a static contact angle analysis of polyester model films', *Mater. Sci. Eng., C.*, 2016, **59**, pp. 998–1006
[49] Matsunaga, M., Whitney, P.J.: 'Surface changes brought about by corona discharge treatment of polyethylene film and the effect on subsequent microbial colonization', *Polym. Degradation Stab.*, 2000, **70**, pp. 325–332
[50] Schusser, S., Menzel, S., Bäcker, M., *et al.*: 'Degradation of thin poly(lactic acid) films: characterization by capacitance-voltage, atomic force microscopy, scanning electron microscopy and contact-angle measurements', *Eletrochim. Acta*, 2013, **113**, pp. 779–784
[51] Lucas, N., Bienaime, C., Belloy, C., *et al.*: 'Polymer biodegradation: mechanisms and estimation techniques', *Chemosphere*, 2008, **73**, pp. 429–442