Evaluation of Biodegradation in Aqueous Medium of Poly(Hydroxybutyrate-Co-Hydroxyvalerate)/Carbon Nanotubes Films in Respirometric System

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Abstract: Biodegradable polymers have been increasingly used for scientific and commercial applications because they are similar to some conventional thermoplastics and exhibit the ability of self-degradation. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanocomposites films with 1 and 2 wt% of carbon nanotubes (CNT) were prepared by solution mixing, followed by solvent evaporation. In this work, PHBV/CNT nanocomposites were submitted to biodegradation in an aqueous medium for 34 days through a respirometric system. Then, the PHBV films were analyzed by the CO₂ production and mineralization as a response of a microbial attack, which was monitored by back titration during all the experiment. The films were also characterized by measuring the weight loss; crystallinity was evaluated by differential scanning calorimetry (DSC) and the surface morphology by scanning electron microscopy (SEM). By analyzing the weight loss of the films, it was observed that adding CNT increases the resistance to biodegradation process. The obtained values of CO₂ production and mineralization of the samples, as well as the values of weight loss, showed that the biodegradation of PHBV/CNT nanocomposites was minor in comparison to neat PHBV. The addition of CNT in PHBV matrix influences the surface morphology, causing the presence of cavities and an increase of roughness.

Keywords: Poly(3-hydroxybutyrate-co-3-hydroxyvalerate); carbon nanotube; nanocomposites; biodegradation in aqueous medium

1 Introduction

Biodegradable polymers are increasingly inserted even more into the commercial world and have been explored and studied by the scientific community with a higher intensity in recent years, due to its extensive use, similarity to some conventional thermoplastic and ability to self-degradation. Accordingly, a biodegradable polymer that fits these conditions is poly (3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV [1,2].

The poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV, consists of a straight chain semicrystalline polyester belonging to the family of polyhydroxyalkanoates (PHAs). PHAs are synthesized by many bacteria in the form of intracellular granules for carbon and energy storage [3,4]. PHBV has attracted significant academic and commercial interests because of its biodegradability, biocompatibility and also because it has some properties similar to conventional thermoplastics [5,6]. In addition to satisfying the requirements that classify it as a biodegradable polymer, PHBV is
biocompatible and very similar to conventional thermoplastics, petroleum-based polymers [7,8,9]. It has an important use in the medical field, such as scaffolds [10] and tissue engineering [11].

However, its application is limited mainly by low mechanical resistance. Therefore, to improve the thermal and mechanical properties, some researches have studied the production of PHBV and carbon nanotubes (CNT) nanocomposites [12,13,14,15]. CNT have excellent thermal, electrical and structural properties that depend directly on their diameter and chirality, which justify their use in polymer nanocomposites including biodegradable polymers [16,17]. Carbon nanotubes were discovered by Radushke-vich and Lukyanovich [18] which reported a range of 50 nm of the CNT tubes and the electron transparency in-between the dark contrast representing the tube walls [19]. CNTs are formed by graphite sheets concentrically rounded; they are circular structures, with length in the range of microns, and diameter in nanometers. They are known due to the excellent conductivity [20] and high modulus of elasticity [21].

In this way, the mechanical properties of CNT are the main responsible for its use in polymer nanocomposites. Thus, many studies have been made to evaluate the effect of CNT addition into PHBV matrix, concerning several properties. Xu et al. [22] studied thermal properties like nonisothermal melt crystallization and subsequent melting behavior of neat PHBV and PHB/functionalyzed-Multi-walled carbon nanotubes (f-MWCNTs). Gui-Fang et al. [23] investigated the effect of CNT on PHBV matrices regarding the crystallization behavior. Jin-Le et al. [24] tested different concentrations of MWCNT in PHBV matrices for medical use. Ni et al. [25] evaluated crystallization and mechanical behavior in PHBV/CNT nanocomposites. Pellegrino et al. [26] verified dielectric behavior in polymeric matrix with the addition of CNT. Montanheiro et al. [27] studied thermal and electrical properties of PHBV/MWCNT nanocomposites. Nonetheless, the effects on the biodegradation process of PHBV/CNT nanocomposite have not been widely studied, although this information is essential for its implementation.

Degradation of polymers proceeds by one or several mechanisms including chemical and enzymatic hydrolysis, thermal and microbial degradation (microbial attack) [28]. The complete degradation of a polymeric material depends on the nature of the material (chemical structure) and on the environmental conditions to which it will be exposed, i.e., abiotic factors (light, humidity, and temperature) and biotic factors (bacteria and fungi). Initially, material fragmentation occurs, i.e., the material is reduced to small particles. In a second moment the mineralization occurs, in other words, the fragmented material will be digested by the microorganisms, resulting in carbon dioxide (CO₂), water (H₂O) and biomass [29,30].

The purpose of this study was to verify the influence of CNT in the PHBV matrix after biodegradation in an aqueous medium. The effects of biotreatment were discussed and evaluated by characterization methods such as the CO₂ production and mineralization, thermal analyses by differential scanning calorimetry (DSC), weight loss, and morphological changes by scanning electron microscopy (SEM), before and after biodegradation.

2 Experimental Part
2.1 Materials
Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with 4% of 3-hydroxyvalerate (HV) units and Mw 187,000 g mol⁻¹ was kindly supplied by PHB Industrial, São Paulo, Brazil. The CNT were provided by Co. Ltd. Korea. The solvent used for film production was chloroform from Synth.

2.2 Preparation of PHBV and PHBV/CNT Nanocomposite Films
The PHBV/CNT nanocomposites (1 wt% and 2 wt%) were prepared according to the methodology previously described by Montanheiro et al. [27]. Initially, PHBV was dissolved in chloroform (1:10 w/v), and the CNTs were previously dispersed in chloroform using a Sonics Vibra Cell (Model VC 750, 750 W, 20 kHz) ultrasonic processor with 25% of amplitude for 2 minutes. The suspension of CNTs and PHBV solution were mixed using the ultrasonic processor for 2 minutes. The final solution was cast onto Petri dishes covered
with aluminum foil to obtain films after solvent evaporation at room temperature for 12 hours.

2.3 Biodegradation in Aqueous Medium

The methodology of the biodegradation test in aqueous medium was already described by Corti and Chiellini [31] and Montagna et al. [32]. The tests were performed in a mineral salt aqueous medium at pH 7.4 ± 0.2. The mother solution to be used as incubation medium was prepared by adding 2 L of distilled water and the following amounts of salts: \( \text{KH}_2\text{PO}_4 \) (170 mg), \( \text{K}_2\text{HPO}_4 \) (436 mg), \( \text{Na}_2\text{HPO}_4 \) (668 mg), \( \text{NaH}_2\text{SO}_4 \) (20 mg), \( \text{NH}_4\text{NO}_3 \) (20 mg), \( \text{CaCl}_2 \) (72 mg), \( \text{MgSO}_4 \cdot 7\text{H}_2\text{O} \) (46 mg), and \( \text{FeCl}_3 \cdot 6\text{H}_2\text{O} \) (0.6 mg).

The microbial inoculum was prepared by addition of 30 g of forest soil and 300 mL of distilled water. The PHBV samples were individually sunk into Erlenmeyer flasks (250 mL) containing 150 mL of mineral salts solution and 1.0 mL of microbial inoculum. Cellulose was used as positive control. Each incubation flask was corked by a silicone rubber stoppers. The flasks were placed in an oven at 28°C ± 1°C for 34 days. During the period of the test the release of CO\(_2\) by the PHBV samples and cellulose were monitored by back titration with 0.11 molL\(^{-1}\) HCl solution, and prior to the titration, 1 mL of 35 mass/vol (%) BaCl\(_2\) solution was added to the NaOH (20 mL) used for the titration.

The amount of carbon dioxide produced and the mineralization during the incubation period were determined and the percentage of the theoretical quantity of CO\(_2\) produced in the blanks (liquid solution without any further carbon source) during the incubation time and sample mineralization were calculated.

2.4 Analytical Methods and Characterization

2.4.1 Determination of CO\(_2\) Generation during the Biodegradation in Aqueous Medium

The amount of CO\(_2\) produced during the metabolism of living organisms in the biodegradation in aqueous medium was calculated according to the method described in ISO 17556/2005 [33] and was based on some parameters reported by Montagna et al. [32].

The cumulative CO\(_2\) in contact with a basic solution (NaOH) produces Na\(_2\)CO\(_3\), which is precipitated with an aqueous solution of BaCl\(_2\) forming BaCO\(_3\), according to Reactions 1 and 2.

\[
\text{CO}_2 + 2\text{NaOH} \leftrightarrow \text{Na}_2\text{CO}_3 + \text{H}_2\text{O} \quad (1)
\]

\[
\text{Na}_2\text{CO}_3 + \text{BaCl}_2 \leftrightarrow \text{BaCO}_3 + 2\text{NaCl} \quad (2)
\]

Therefore, the amount of NaOH that did not react with the involved gas (CO\(_2\)) is reacted with the same amount of HCl in the titration of the acid solution, according to Eq. (1),

\[
m = \left( \frac{(2\text{CS} \times \text{VSO})}{\text{CA}} - (\text{VA} \times \frac{\text{VST}}{\text{VSZ}}) \right) \times \text{CS} \times 22
\]

where \( m \) is the mass of CO\(_2\) released in the biodegradation tests (mg), \( \text{CA} \) is the exact concentration of the HCl solution (0.11 molL\(^{-1}\)), \( \text{CS} \) is the exact concentration of the NaOH solution (0.25 molL\(^{-1}\)), \( \text{VSO} \) is the volume of NaOH solution at the beginning of the test (20 mL), \( \text{VST} \) is the volume of NaOH solution before the titration (20 mL), \( \text{VSZ} \) is the volume of the aliquot of NaOH solution used in the titration (20 mL), \( \text{VA} \) is the volume of HCl solution used for the titration (mL), and 22 is half the molarity of CO\(_2\).

The determination of the amount of CO\(_2\) generated in the biodegradation in aqueous medium was made in triplicates, and the average value was used. The CO\(_2\) values obtained from the test were used to calculate the mineralization of the samples, according to Eq. (2). This methodology used for the calculation of mineralization was based on the ASTM 5988-12 [34].

\[
\text{mineralization} \% = \frac{\text{CO}_2 \text{produced}}{\text{CO}_2 \text{theoretical}} \times 100
\]
The theoretical CO$_2$ mass (theoretical m$_{CO2}$) consists of the amount of CO$_2$ produced in the total biodegradation of a sample, i.e., assuming the conversion of any mass of carbon present in the sample (m$_C$) into CO$_2$. Firstly, m$_C$ is calculated from the mass of PHBV in each sample (m$_{PHBV}$), the molar mass of the mere PHBV with 4% HV (86.56 g mol$^{-1}$) and the mass of carbon present in one mole of PHBV 4% HV (48.48 g), being obtained from the structural chemical formula of PHBV (Eq. (3)). In the case of nanocomposite samples the mass of CNT was discounted to obtain m$_{PHBV}$.

$$m_C = \frac{m_{PHBV} \times 48.48}{M_{mere \ PHBV}}$$

In the case of cellulose control, m$_C$ is based on molar mass of the mere cellulose (162.00 g mol$^{-1}$) and mass of carbon present in one mole of cellulose (72.00 g mol$^{-1}$).

Thus, based on the stoichiometric relation of the reaction between C and O$_2$ producing CO$_2$, the theoretical mass of CO$_2$ was calculated as indicated in Eq. (4):

$$mCO_2\text{ theoretical} = mC \times \frac{44}{12}$$

2.4.2 Visual Analysis

Neat PHBV and PHBV/CNT films were photographed before and after biodegradation in aqueous medium tests, to observe the macroscopic surface modifications in the films.

2.4.3 Weight Loss

Weight loss analyses of PHBV films from biodegradation in aqueous medium test were carried out by weighing the films in an analytical laboratory balance (RADWAG AS 60/220.R2). The PHBV films were weighted before and after the end of the biodegradation in aqueous medium test. The percent weight loss determined as a function of the biotreatment with filamentous fungi was calculated using Eq. (5):

$$\text{Wt. loss (\%)} = \frac{W_0 - W_f}{W_0} \times 100$$

where W$_0$ is the initial weight of the PHBV films, and W$_f$ is the weight of the PHBV films at the time after the biodegradation in aqueous medium test. The experiment was performed in triplicate.

2.4.4 Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry analyses were performed on a Netzsch Phoenix DSC 2014 F1. Small amounts ($\approx$ 5 mg) of dried samples were placed into aluminum pans. It was carried out only one heating cycle, with a heating rate of 10 °C min$^{-1}$ from 25°C to 200°C, in nitrogen atmosphere with a gas flow of 20 mL min$^{-1}$. The melting temperature (T$_m$) and the melting enthalpy ($\Delta H$) were obtained. The crystalline content was obtained by Eq. (6), where $\Delta H$ is the total melting enthalpy and $\Delta H^0$ is the theoretical melting enthalpy value of 100% crystalline PHBV, which was taken as 109 J g$^{-1}$[35]:

$$Xc \ (\%) = \frac{\Delta H}{\Delta H^0} \times 100$$

2.4.5 Scanning Electron Microscopy (SEM)

The surface morphology of neat PHBV and PHBV/CNT nanocomposites films before and after the biodegradation in aqueous medium test were recorded by using FEI Inspect S50 scanning electron microscope, at the magnification of 1000x and with accelerating voltage of 7.5 keV. The films were sputtered with gold before SEM observation.
3 Results and Discussion

Fig. 1 shows the visual analysis of all films at the initial time and after biodegradation in aqueous medium during 34 days. At the initial time, all PHBV films presented a smooth and homogeneous surface. However, after biodegradation in aqueous medium test, it was visually observed that all the films became brittle, fragile, and suffered de-fragmentation on the surface due to biodegradation. These analyzed parameters of visual changes can indicate the beginning of a microbial attack. Neat PHBV films samples after biodegradation in aqueous medium showed on the surface the adhesion of a yellow colored biofilm.

Figure 1: Neat PHBV, PHBV/1% wt% CNT and PHBV/2 wt% CNT films at the initial time and after biodegradation in aqueous medium

Fig. 2 shows the weight loss percentage of PHBV films after being submitted to the biodegradation test in aqueous medium for 34 days. All samples showed significant weight loss values after the biodegradation test.

Compared with the PHBV/CNT nanocomposites, neat PHBV film had the highest values of mass loss, of 49% after biodegradation in aqueous medium. This fact may be related to the results of the degree of crystallinity (which is discussed in the following pages). The degradation in polymers occurs in the amorphous regions and subsequently in the crystalline regions, therefore, the crystalline regions control biodegradability, which means that the rate of degradation of a material will be strongly dependent on crystallinity and crystalline morphology [36,37].

After biodegradation in aqueous medium test, PHBV/CNT nanocomposites presented lower values of weight loss compared with neat PHBV, being 19.74% and 39.46% for PHBV/1 wt% CNT and PHBV/2 wt% CNT, respectively. However, these values showed that the increase of CNT concentration in PHBV matrix increases the weight loss when compare with the PHBV/1wt% CNT (less amount of CNT). In this case, it appears that the increase of CNT content has the effect of decreasing the resistance to biodegradation in aqueous medium process when added in higher amounts in the polymeric matrices of PHBV biopolymer. Therefore, the addition of higher content of CNT in PHBV matrix, the biodegradation properties begin to resemble the neat PHBV. In this way, this function of delaying the biodegradation of PHBV varied according to the concentration of CNT in the nanocomposite, which indicates that CNT may also work as an additive to control biodegradation timing, which would be of great importance in the application of this material.

Improvements in the final biodegradation of nanocomposites from the PHA family were observed by Maiti et al. [36], which evaluated the biodegradation of poly (hydroxybutyrate) (PHB)/layered nanoclay
nanocomposites. The authors reported that the biodegradation rate of PHB is significantly enhanced in the presence of nanoclay with near complete degradation (after 7 weeks) for PHB containing 2 wt% of nanoclay.

The results reported in this research are in agreement with those studied by Wang et al. [37], which prepared poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/organophilic montmorillonite (PHBV/OMMT) nanocomposites by solvent casting, the same methodology that we used in this article. The authors subjected the samples to biodegradation in soil suspension and observed that the biodegradability of PHBV/OMMT in soil suspension decreased with increasing amount of OMMT [38,39].

![Graph showing weight loss for neat PHBV, PHBV/1 wt% CNT and PHBV/2 wt% CNT nanocomposites](image)

**Figure 2**: Weight loss (%) for neat PHBV, PHBV/1 wt% CNT and PHBV/2 wt% CNT nanocomposites, after biodegradation in aqueous medium

Fig. 3 shows the cumulative CO₂ emissions vs. time of biodegradation in aqueous medium. Analyzing Fig. 3, it can be seen that the production of CO₂ as a product of the metabolic reaction by cellulose, a biodegradable material and used as a positive control, and values are similar when compared to CO₂ production of the other samples in aqueous medium, which confirms that the assay was performed in a viable manner [40]. The amount of CO₂ generated also depends on the initial mass of the samples, so the amount of CO₂ released does not reflect the total percentage of biodegradation of the sample.

During the biodegradation test, the values of CO₂ released by neat PHBV, PHBV/2 wt% CNT, and the positive cellulose control were similar during the test. The PHBV/1 wt% CNT sample presented lower values of CO₂ production than the others, and lower slope of the CO₂ production curve during the evaluated period, suggesting that the biodegradation for this sample was lower. In all cases it is verified that the biodegradation rate stabilizes between 31 days and 33 days; however it is expected total conversion of carbon to CO₂ since part of the PHBV polymer chain is entirely metabolized by the microorganisms. Based on the average values of initial samples mass and assuming the total conversion of the polymer chain of PHBV into CO₂ it was possible to calculate the mineralization of the samples after the biodegradation test (Tab. 1).

Therefore, Tab. 1 shows the average values of the samples mass (M₀), the PHBV mass (m_{PHBV}), carbon mass (m_c), the average mass values of CO₂ that would be produced assuming total conversion of the carbon present in the samples to CO₂ (theoretical m_{CO₂}) based on the mass of PHBV, medium values of CO₂ mass produced (m_{CO₂}) after biodegradation in aqueous medium test and the mineralization of the PHBV samples. Based on the results, it can be stated that the biodegradability of the PHBV matrix is maintained even with the addition of CNT at the concentrations studied (1 wt% and 2 wt% CNT). However, after the total test period, the biodegradation of PHBV nanocomposites was lower compared to neat PHBV. The decrease was more pronounced in the case of the PHBV/1 wt% CNT nanocomposite, which was 46%, whereas for the PHBV/2 wt% CNT nanocomposite this decrease was 30%, both compared to neat PHBV mineralization.

It was expected that the higher content of CNT presented the lower value of samples mineralization. However, higher concentrations of CNT may generate changes in morphology, such as increased roughness, which help to fix microorganisms and would have a positive effect on the biodegradation
process. This could be a possible explanation for the increased biodegradation of PHBV/2 wt% CNT samples compared to PHBV/1 wt% CNT samples. These claims can be observed in the superficial morphological analysis in Fig. 5.

![Graph showing CO₂ emissions](image)

**Figure 3:** Cumulative CO₂ emissions of cellulose (positive control), neat PHBV, PHBV/1 wt% CNT and PHBV/2 wt% CNT nanocomposites during biodegradation in aqueous medium vs. incubation time

| Biodegradation | Samples            | $m_0$ (g) | $m_{PHBV}$ (g) | $m_C$ (g) | $m_{CO_2}$ theoretical (g) | $m_{CO_2}$ produced (g) | Mineralization (%) |
|----------------|--------------------|-----------|----------------|-----------|---------------------------|-------------------------|-------------------|
| In aqueous medium | Cellulose          | 0.0383    | N              | 0.0170    | 0.0625                    | 0.0462                  | 74                |
|                 | Neat PHBV          | 0.0254    | 0.0254         | 0.0142    | 0.0523                    | 0.0450                  | 86                |
|                 | PHBV/1 % wt CNT    | 0.0367    | 0.0363         | 0.0204    | 0.0748                    | 0.0343                  | 46                |
|                 | PHBV/2 wt% CNT     | 0.0352    | 0.0348         | 0.0195    | 0.0716                    | 0.0429                  | 60                |

Table 1: Medium values of the samples mass ($m_0$), PHBV mass ($m_{PHBV}$), carbon mass ($m_C$), theoretical CO₂ mass (theoretical $m_{CO_2}$), CO₂ produced mass ($m_{CO_2}$ produced) and mineralization after biodegradation in aqueous medium

Fig. 4 shows the degree of crystallinity ($X_c$) of neat PHBV, PHBV/1 wt% CNT and PHBV/2 wt% CNT at the initial time and after biodegradation in aqueous medium. Neat PHBV showed a high degree of crystallinity at the initial time, which causes its fragility and low mechanical properties [41,42,43]. Furthermore, the $X_c$ values of the PHBV samples at the initial time decreased, as increased the CNT content in the PHBV matrix. This fact may be related to the inability of the polymer chains to be completely incorporated into the growing crystalline lamella, meaning that the amorphous part of the nanocomposites became larger than that of neat PHBV [44].

Similar behavior was observed by Carli et al. [45] that verified the decrease of $X_c$ for neat PHBV matrix with the addition of OMMT (organic modified montmorillonite) at the initial time, and made it difficult to crystallize the biopolymer due to the reduction of the polymer chains mobility, caused by the strong interaction between the nanofiller and the matrix.

Neat PHBV, after the biodegradation process presented reduction of $X_c$ values, these results were not expected, because theoretically, the rate of biodegradation is higher in the amorphous region than in the crystalline region. However, as the DSC analysis was performed only on the final samples of the biodegradation test, the difference between the rate of biodegradation of the amorphous and crystalline regions may have occurred earlier in a period of less than 34 days. The results of CO₂ production (Fig. 3)
showed that between 31 days and 33 days the rate of biodegradation stabilized, which would be a possible explanation for the values of $X_c$ obtained for neat PHBV.

PHBV nanocomposites containing 1 wt% of CNT obtained similar $X_c$ for the initial and final times, as well as the PHBV nanocomposites containing 2 wt% of CNT, have similar values of $X_c$ for the initial and final times of degradation, however, these values are lower than PHBV/1 wt% CNT. Thus, it is suggested that the higher the concentration of the nanofiller in the polymer matrix, the smaller is the $X_c$ value. The CNT present in the amorphous phase of the nanocomposites can turn them more resistant to the attack of the enzymes released by the microorganisms, so the amorphous and crystalline phase can be attacked at the same speed, justifying the fact that there are no significant differences between the $X_c$ values of the nanocomposites before and after 34 days of biodegradation. The literature reports cases in which during the biodegradation process the consumption of both phases, amorphous and crystalline, of the polymer occurs, verifying an even proportional decrease of both [46,47].

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**Figure 4:** $X_c$ for neat PHBV, PHBV/1 wt% CNT and PHBV/2 wt% CNT nanocomposites, before and after biodegradation in aqueous medium

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Fig. 5 shows the surface micrographs of neat PHBV, PHBV/1 wt% CNT and PHBV/2 wt% CNT nanocomposites at the initial time and after biodegradation in aqueous medium. Through SEM images it is possible to obtain information about the biodegradation mechanism, that is, more sophisticated observations. All the PHBV films at the initial time presented homogeneous and smooth morphological surface, being more evident in neat PHBV sample since the surface of the nanocomposites presented slight roughness and undulations influenced by the presence of the CNT.

The degraded surfaces are visible in all samples, and they appear to have suffered degradation, with typical pores with sizes of several microns. Therefore, after biodegradation in aqueous medium, the morphological surfaces were irregular with the presence of ripples and cavities.

Similar micrographs were observed by Shah et al. [48], that investigated the morphological surface of PHBV film buried in soil mixed with sewage sludge for 120 days by SEM, and the authors clearly observed evidence of degradation with pits, surface roughening, grooves, cavities, and disintegration.
4 Conclusions

It was concluded that the methodology used as a biodegradation test in aqueous medium employing the respirometric system was adequate for the evaluation of CNT effect into PHBV polymer matrices.

The results showed that the studied concentrations of CNT added in the PHBV matrix does not prevent its biodegradation, but increases its resistance to the attack of the microorganisms and can be used as an additive that controls the time of biodegradation of the matrix. However, it is not possible to state that the higher the concentration of CNT in the PHBV matrix, the more resistant to biodegradation the PHBV becomes. This is because other changes caused by the addition of CNT in the PHBV matrix such as the degree of crystallinity and roughness of the samples may have a positive effect on the biodegradation of the material.

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