

Biodegradation Behavior of Composite Films with Poly (Vinyl Alcohol) Matrix

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Abstract The main objective of this study was to develop biodegradable, composite materials, based on poly (vinyl alcohol), bacterial cellulose and chitosan for possible application in packaging industry. Two composite materials were prepared, one containing poly (vinyl alcohol) (PVA) and bacterial cellulose (BC), named PVA/BC, and the other containing PVA, BC but also chitosan (CTS), named PVA/BC/CTS. The biodegradation behavior was studied in a fed-batch bioreactor, in aerobic and anaerobic conditions, using activated sludge. Biodegradation tests were based on weight loss measurements. Structural changes were confirmed by Fourier transform infrared spectroscopy (FTIR) and the morphological ones by scanning electron microscopy (SEM). After 4 weeks, the biodegradation experiments have shown a relative high degradation of the PVA/BC/CTS film compared with the PVA/BC one. These results were confirmed by spectral analysis and also by SEM images. Besides, the SEM images revealed that biodegradation occurs also inside the composite materials, not only on the surface.

Keywords Biodegradation · Poly (vinyl alcohol) · Bacterial cellulose · Chitosan · Composite films

Introduction

Petroleum-based synthetic polymers have become an indispensable ingredient of human life due to versatility, flexibility, toughness, excellent barrier, physical properties and ease of fabrication. Over the past decades the production of these synthetic polymers has increased and, if the prediction is right, in the future it will increase even further. Due to remarkable properties, their degradation requires an extraordinary long time [1]. This fact leads to their accumulation in the environment, causing a great ecologic, economic and waste management concern.

In order to overcome this inconvenient, new polymers have been developed in the last few years, based on renewable and biodegradable materials, attracting widespread interest [2]. Blending natural polymers and synthetic ones is a simple and practical way to produce new materials [3]. Among synthetic polymers poly (vinyl alcohol) is known to be biodegradable and it blends with natural polymers (such as cellulose, starch, chitosan, soy protein isolate, fish myofibrillar protein) having promising industrial applications in many fields, including packaging industry, because of their biodegradability, biocompatibility, chemical resistance and excellent physical properties [2, 4–8]. Bacterial cellulose (BC), biosynthesized by several bacterial strains from which the *Acetobacter xylinum* is the most studied, represents another valuable candidate for composite materials. In comparison with plant cellulose, BC possesses higher water holding capacity, higher crystallinity, higher mechanical properties and a fine web-like network. It has a wide range of application in different areas, like biomedical, food industry, biotechnology and material science [9–12].

Biodegradation is a key property for novel materials. Nevertheless, biodegradability tests are necessary to

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estimate the environmental impact and to find solutions to avoid their accumulation in nature [13]. Several biodegradation studies, conducted in various conditions, are reported recently in literature. Zhang [14] studied PVA biodegradation in mixed bacterial culture, Scaffaro et al. [15] studied the biodegradation of a composite material obtained from a commercial biodegradable polymer, Mater-Bi® and wood flour in an active sewage sludge reactor, Su et al. [2] performed an aerobic biodegradation according to ASTM D 5338, Riaz et al. [16] studied the biodegradation behavior of bio-based epoxy and PVA in simulated soil burial condition, Jayasekara et al. [17] conducted biodegradation tests by composting for surface modified starch and PVA blended films, Julinova et al. [18] performed biodegradation tests in aerobic and anaerobic environment for some PVA/waxy starch blends and also biodegradation tests in aqueous environment with both unadapted and adapted activated sludge for PVA composite films [19] and, finally, Hrnčířík et al. [20] studied anaerobic biodegradation of PVA modified by extracellular polysaccharides.

The objective of the present study was to develop biodegradable composite materials, based on poly (vinyl alcohol), bacterial cellulose and chitosan and to measure the degree of biodegradation in both aerobic and anaerobic aqueous environment in the presence of activated sludge.

Experimental

Chemicals and Materials

Poly (vinyl alcohol) (PVA), average molecular weight (Mw) 85,000–124,000 g/mol, >99% hydrolyzed and chitosan (CTS), with the degree of deacetylation 85.0% and molecular weight of Mw = 330 kDa, were purchased from Sigma–Aldrich and used without further treatment or purification. Bacterial cellulose (BC) fibrils were produced in the Mass Transfer Laboratory of Chemical Engineering Department of “Politehnica” University of Bucharest, as it was described elsewhere [21]. Activated sludge used in aerobic and anaerobic biodegradation studies were kindly supplied by the Miercurea Ciuc and the Heineken Brewery wastewater treatment plants.

Film Preparation

A casting 4% PVA solution was made by dissolving PVA in distilled water at 90 °C. In the resulting solution, 10% wet BC fibrils (96% humidity), was dispersed under vigorous stirring.

Chitosan powder was added into 0.5% (v/v) aqueous acetic acid in order to reach a final concentration of 2% (w/v)

and the mixture was then stirred at 37 °C for approximately 3 h. The resulted solution was filtered through polyester cloth to remove residues of insoluble particles.

The PVA/BC dispersion and the chitosan solution, previously prepared, were blended in 1/1 (w/w) ratio in order to obtain a PVA/BC/CTS film forming composition.

In terms of dry mater the ratio between the components of blends are 1:0.1 (w/w) for PVA/BC and 1:0.1:0.5 (w/w) for PVA/BC/CTS. These compositions were chosen after preliminary experiments.

The resulting mixtures were cast onto Perspex plates with the aid of a casting knife and dried at room temperature for 24 h.

The films' thickness was measured with micrometer (Mitutoyo, Japan). The overall thickness was expressed as an average of 5 readings taken randomly on each film sample and was 0.12 ± 0.02 mm. Prior to the immersion in the bioreactor, the films were cut into 2×5 cm pieces and afterwards dried in vacuum oven at 60 °C until they reached a constant weight.

Film Characterization

Spectral Analysis

Fourier-transform infrared (FT-IR) spectral analysis was carried out with an IR Spectrometer (Jasco FT/IR6200) with Intron 1 Infrared Microscope with ATR-1000-VZ objective. The spectra represent the average of 50 scans recorded at a resolution of 4 cm^{-1} in the range of $4,000\text{--}500 \text{ cm}^{-1}$ with a DLATGS detector.

Morphological Analysis

The morphology of the plane surface of the films and also the fractured section of the biodegraded films were examined by a HITACHI S2600N scanning electron microscope (SEM), operated at 25 kV, at different magnifications. SEM micrographs were taken after coating the analysed surfaces with a thin layer (10–20 nm) of gold.

Swelling Degree

The swelling degree of the samples was characterized at 37 °C, for which samples of 2×2 cm were placed in deionized water. The experiments were carried out by measuring the weight gain as a function of immersion time in 20 mL water. The swelling degree was calculated by using the following equation:

$$\text{Swelling degree, [\%]} = (W_s - W_0)100/W_0 \quad (1)$$

where W_s is the wet weight after swelling for a predetermined time, W_0 is the original weight of the sample.

Water Vapor Permeability (WVP)

WVP of films was determined using a modified ASTM method (ASTM E 96-95, 1989) as described by Limpan et al. [4]. The film was sealed on an aluminium cup containing silica gel (0% RH) with silicone vacuum grease and rubber gasket. The cup was placed at 30 °C in a desiccator containing distilled water. The same cup was weighed at different time intervals over a 24 h period. WVP of the film was calculated as follows:

$$\text{WVP}, \left[\text{g m}^{-1}\text{s}^{-1}\text{Pa}^{-1} \right] = w l A^{-1}t^{-1}(P_2 - P_1)^{-1} \quad (2)$$

where w is the weight gain of the cup (g); l is the film thickness (m); A is the exposed area of film (m^2); t is the time of gain (s); $(P_2 - P_1)$ is the vapor pressure difference across the film (Pa). Three films were used for WVP testing.

Biodegradation Studies

Biodegradation tests were based on weight loss measurements, performed on the above described samples, after the immersion in an activated sludge fed-batch bioreactor, in aerobic and anaerobic aqueous environment, detecting the weight variations over an investigation time of 4 weeks. Inoculation took place in the form of activated sludge from a wastewater treatment plant. The bioreactor's working volume is 2.5 L and it was operated isothermal. Magnetic stirring assures a satisfactory homogenization of the bioreactor content. Table 1 presents the main process parameters of the fed-batch bioreactor. The bioreactor was fed with 2.2 L activated sludge. For each experiment 0.3 L synthetic medium was added, with the composition presented in Table 2.

After every week the bioreactor content was supplemented with 3 g of the synthetic medium. After 14 days pH correction was made with 15% sodium hydroxide, 20% acetic acid solution respectively.

Several samples were prepared for every composition. Every week, samples of each film were removed from the bioreactor, washed with tap water, then with distilled water and sterilized by UV irradiation (radiation wavelength 254 nm, exposure time about 20 min). After sterilization, the samples were dried in vacuum oven at 60 °C for 24 h,

and finally weighed, calculating the percent weight variation, WL, according to the following:

$$\text{WL}, [\%] = (W_0 - W_t) \cdot 100/W_0 \quad (3)$$

where W_t is the weight at time t and W_0 is the initial weight. After weighing, the samples were stored in a refrigerator until subjecting them to structural and morphological analysis. Meanwhile, a sample of each composite film type was immersed in distilled water and buffer solution of pH 8 respectively, for the same period of time as the prior investigated ones. Their weekly weight variations were measured and taken as calibration values to correctly evaluate the weight loss of the materials immersed in active sludge. It was found that the weight loss of these calibration samples was always below 1%, therefore the observed weight loss of the materials immersed in activated sludge can be attributed solely to bacterial activity.

Results and Discussion

Film Characterization

The FTIR spectra of composite films, presented in Fig. 1, allow identifying the main characteristic bands, which confirm the interaction between its components.

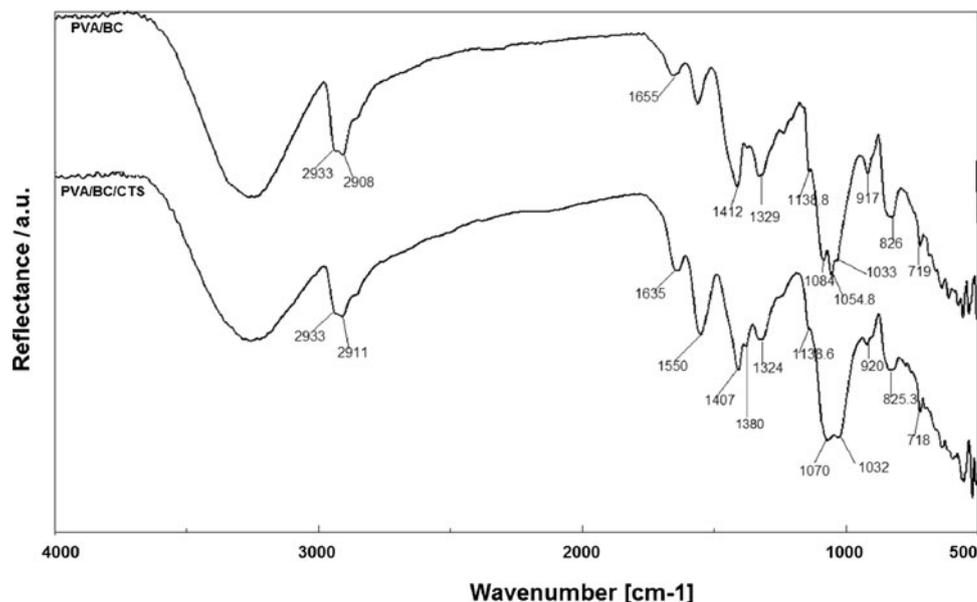
Table 2 Formulation of synthetic medium

Ingredient	Concentration, mg/L
Starch	122
Milk powder	116.19
Urea	91.74
Peptone	17.41
NH ₄ Cl	12.75
Soy oil	29.02
Sodium acetate	131.64
KH ₂ PO ₄	23.4
FeSO ₄ ·7H ₂ O	5.8
CuCl ₂ ·2 H ₂ O	0.536
MnSO ₄ ·7H ₂ O	0.108
NiSO ₄ ·6 H ₂ O	0.336

Table 1 Main process parameters of the bioreactor

Environment	Temperature [°C]	pH	Sludge concentration [g/L]	COD* [mg/L]	Air flow rate [L/min]
Aerobic	21 ± 1	6.5	3.08	826	4.5
Anaerobic	33 ± 1	8	8.77	826	–

* COD chemical oxygen demand

Fig. 1 FTIR spectra of composite films

A broad peak for PVA/BC composite film between 3,200 and 3,500 cm^{-1} can be attributed to O–H stretching in the molecule and to the hydrogen bridges built in film. This peak is even larger in the composite PVA/BC/CTS due to overlapping stretching of hydrogen bonds, O–H stretching and amine N–H symmetric vibration [2, 22, 23]. Both spectra show a peak at 2,933 cm^{-1} , attributed to aliphatic C–H stretching, at 2,908 and 2,911 cm^{-1} in PVA/BC, in PVA/BC/CTS composite attributed to asymmetric stretching of CH_2 respectively. A small peak at 1,655 cm^{-1} in PVA/BC composite is attributed to a deformation vibration of the absorbed water molecule in BC [24]. Muzzarelli et al. [25] assigned the absorption band at 1,381 cm^{-1} to C– CH_3 amide stretching [25]. According to Phisalopong and Jatupaiboon [26], the absorption bands around 1,650, 1,560 and 1,375 cm^{-1} are bands for amide group existing in chitosan molecule. Thus, the shoulder at 1,380 cm^{-1} from PVA/BC/CTS spectrum confirms the presence of chitosan. The absorption bands at 1,412 cm^{-1} in PVA/BC, 1,407 cm^{-1} in PVA/BC/CTS, respectively, can be attributed to O–H, C–H bendings from PVA structure and the absorption bands at 1,329 cm^{-1} , 1,324 cm^{-1} to δ (OH) with C–H wagging, respectively. A small shoulder at 1,138.8 cm^{-1} can be attributed to stretching of C–O (crystalline sequence of PVA). The peaks at 1,084 cm^{-1} in PVA/BC composite, shifted to 1,070 cm^{-1} in PVA/BC/CTS composite can be attributed to stretching of C=O and bending of OH (amorphous sequence of PVA), 917 cm^{-1} shifted to 920 cm^{-1} in PVA/BC/CTS to CH_2 rocking, and the peak around 826 cm^{-1} to C–C stretching [27, 28]. The sharp peak at 1,054.8 cm^{-1} and shoulder at 1,033 cm^{-1} present in the PVA/BC spectra can be assigned to the –C–O–C– in glycosidic bonds,

respectively to C–O stretching vibration of aliphatic primary and secondary alcohols in cellulose [29, 30].

Figure 2 presents the SEM images of the two composite films before starting the experiments. These photos are references for the changes of the polymers surface after biodegradation.

Water absorption (swelling) and water vapor permeability are important properties for practical applications of composite films. The swelling degree at 24 h and water vapor permeability of the composite films are presented in Table 3.

The swelling degree of the composite film PVA/BC has a lower value than the simple PVA, for which the swelling degree reported in literature is higher than 500% [31]. This means that the contents of BC enhanced the hydrophobicity of blend membranes as a result of the hydrophobic chains of bio-cellulose. By comparing swelling values of the two composite films it is easy to observe that the swelling degree of PVA/BC/CTS composite film is several times greater than the PVA/BC one; the presence of chitosan affected the water absorption by enhancing the composite hydrophilicity. These results confirmed the visual observations during experiments.

The water vapor permeability of the two composite films has quite the same magnitude, the values being within the limits reported in literature for pure PVA and pure CTS film [3, 4].

Aerobic Biodegradation

The weight loss of the composite films after 28 days degradation process in aerobic environment is presented in Table 4. One can see that the weight loss of PVA/BC/CTS

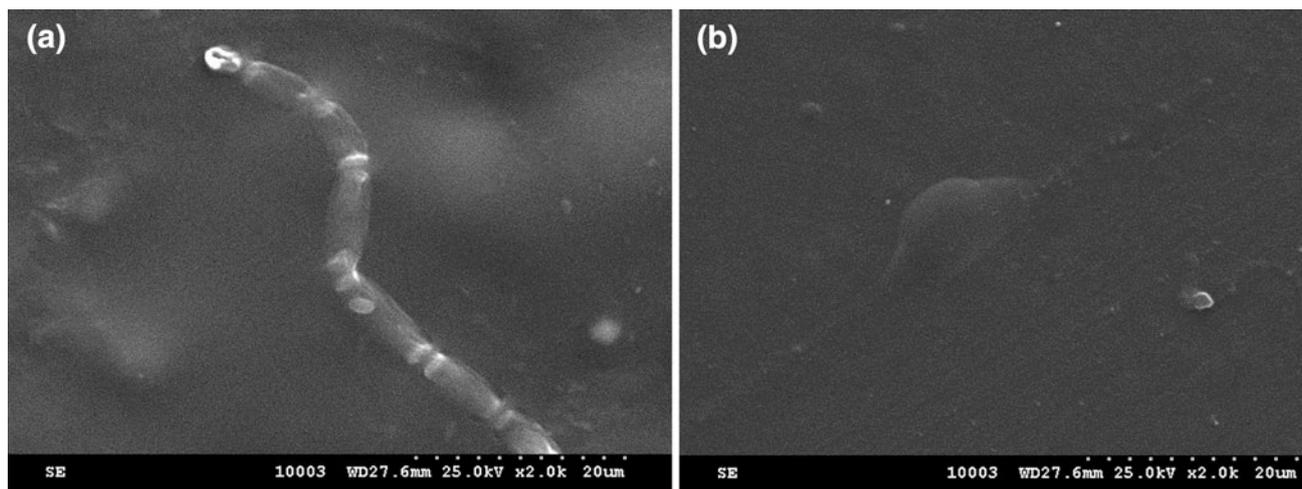


Fig. 2 The surface SEM images of the PVA/BC (a) and PVA/BC/CTS (b) composite films

Table 3 Swelling degree (SD) at 24 h and water vapor permeability (WVP) of composite films

Film type	SD [%]	WVP [$\text{g m}^{-1} \text{s}^{-1} \text{Pa}^{-1}$]
PVA/BC	190.6 ± 0.85	$5.14 \pm 0.45 \cdot 10^{-10}$
PVA/BC/CTS	449.0 ± 1.25	$3.72 \pm 0.39 \cdot 10^{-10}$

Values are means \pm standard deviation, $n=3$

Table 4 Weight loss during aerobic biodegradation

Film type	Weight loss [%]			
	7 days	14 days	21 days	28 days
PVA/BC	6.5	7.2	10.6	11.5
PVA/BC/CTS	20.3	29.3	29.6	33.2

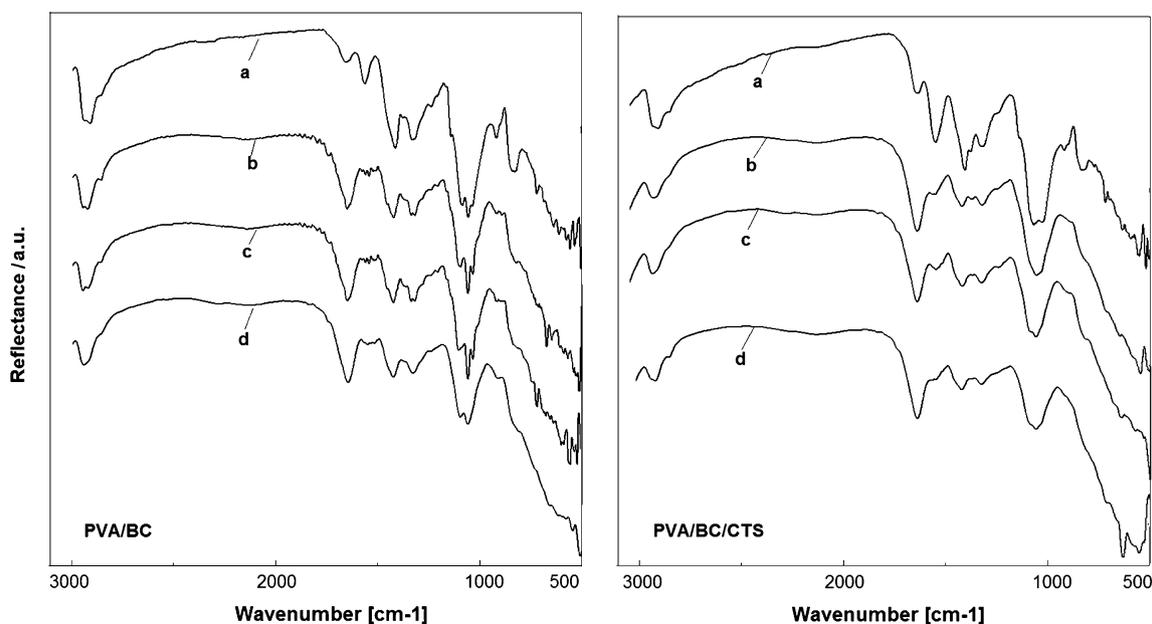


Fig. 3 FTIR spectra of composite films, a initial film, b after 7 days; c after 14 days and d after 28 days of degradation in aerobic environment

composite films is almost three times higher than the PVA/BC composites.

The structural changes of composite films were investigated using Fourier transform infrared spectroscopy in order to determine the effect of enzymatic attack during the aerobic biodegradation process. If we compare the spectra

of composite film samples before and after different periods of biodegradation, there are changes in peak heights as it can be seen in Fig. 3.

All spectra showed a reduction of asymmetric stretching of C–H absorbencies ($2,908\text{--}2,911 \text{ cm}^{-1}$) due to the degradation of the sample. In spectra of both film types a

sharp peak appears at $1,649\text{ cm}^{-1}$, accompanied by a small one at $1,558\text{--}1,560\text{ cm}^{-1}$ which can be attributed to amides I and II groups resulting from the protein production by microbial growth on films according to Klemenčič et al. [32]. A higher degree of film biodegradation is accompanied by a greater growth of microorganisms on the films, reflected in the larger increase of the band at $1,649\text{ cm}^{-1}$ [32]. Zhang [14] studied PVA biodegradation in mixed bacterial culture of *Bacillus* sp. and *Curtobacterium* sp., and observed absorbencies at $1,653$ and $1,385\text{ cm}^{-1}$, which were identified as those of β -diketone structure and the methyl formed by the scission of several carbon–carbon bonds, respectively. In our spectra the absorption band at $1,385\text{ cm}^{-1}$ was not observed. So, we consider that the observed absorption bands at $1,649\text{ cm}^{-1}$ and the small one at $1,558\text{--}1,560\text{ cm}^{-1}$ for all composite films could be mainly attributed to microbial metabolites. In case of the PVA/BC/CTS these bands increase due to the presence of the same type of functional groups from the CTS structure. The absorption peaks from 917 to 920 cm^{-1} and 826 to

825.3 cm^{-1} which are visible in the spectrum of the initial films practically disappeared in the spectra after 7 days, which indicates also the film degradation.

Several SEM images of composites, taken after 28 days, are given in Fig. 4. In comparison with the surface images of the initial films, one may observe that these samples are considerably destroyed. The same phenomena could be observed from the film edge's micrographs.

Anaerobic Biodegradation

Table 5 presents the weight loss of the composite film samples during degradation under anaerobic environment.

One can see that the degradation of PVA/BC/CTS composite is higher than the PVA/BC one, as it was observed also in aerobic environment. These data are in accordance with structural and morphological modifications, observed in FTIR spectra (Fig. 5), SEM pictures (Fig. 6), respectively.

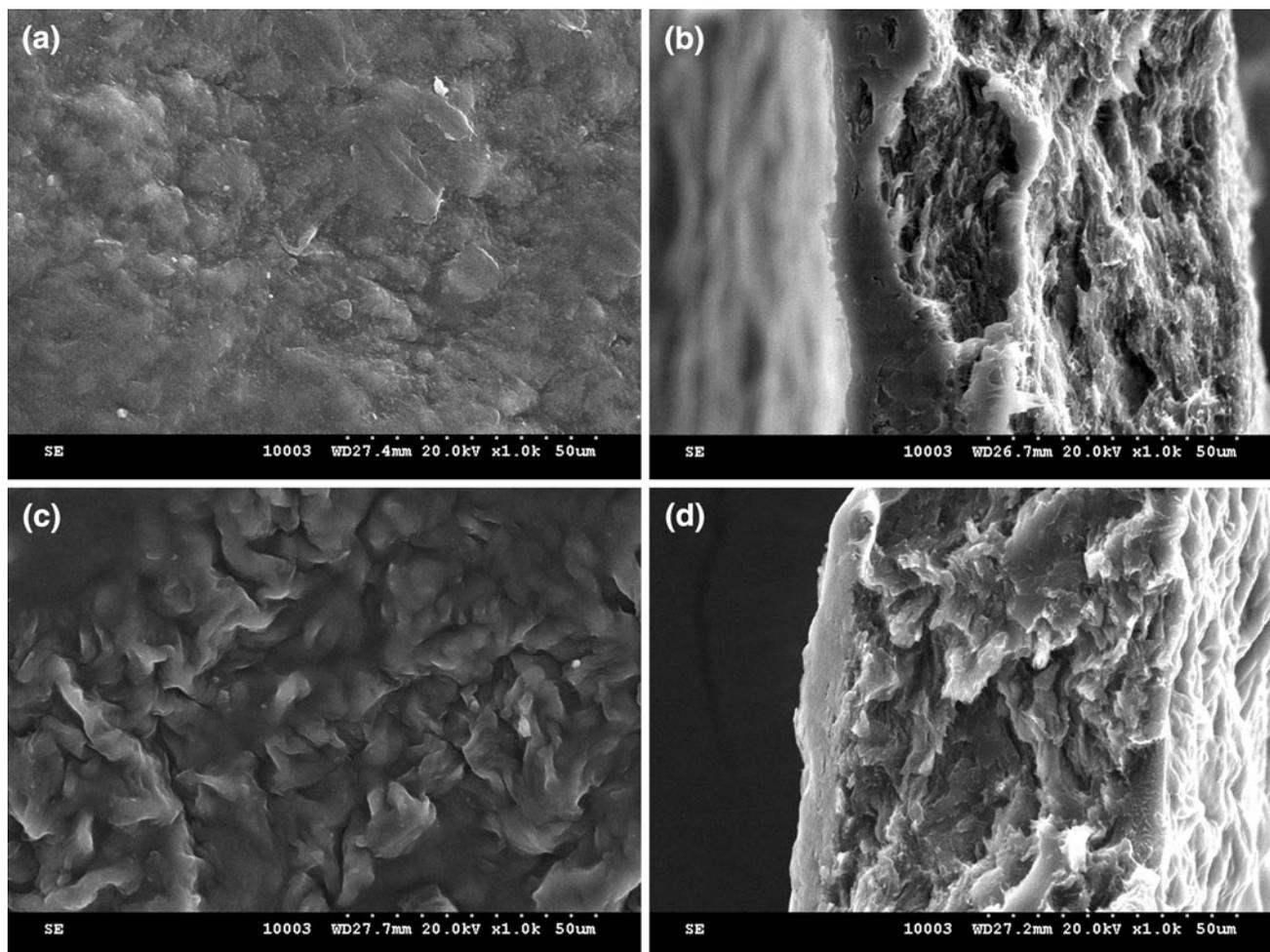


Fig. 4 The surface and edge SEM pictures of PVA/BC composite film (a, b), respectively PVA/BC/CTS (c, d) after 28 days of biodegradation in aerobic environment

The FTIR spectra recorded on samples after different periods of degradation in the fed-batch reactor, operated anaerobically, revealed the same modification as in aerobic conditions. One can observe an important reduction of absorbencies in the range of 2,933 and 2,908 cm^{-1} attributed to aliphatic C–H stretching and asymmetric CH_2 stretching, the appearance of a sharp peak at 1,648 cm^{-1} , a dramatically reduction of absorbencies in the range of 1,200–1,500 cm^{-1} and the disappearance of the absorption peaks from 917 to 920 cm^{-1} and 826 to 825.3 cm^{-1} .

The SEM surface images are relevant for the surface destruction of both composite films. The micrographs taken at films edge, also revealed degradation inside the polymeric material.

Comparing the biodegradation results in aerobic and anaerobic aqueous environment (Tables 4, 5) one can see a higher weight loss in anaerobic condition. This unexpected result could be a consequence of the different source of the activated sludge and its higher concentration in the bioreactor operated anaerobically (Table 1).

In both biodegradation studies, despite our expectations, there was observed that a higher degree of degradation affects the PVA/BC/CTS composite films than the PVA/

BC composite. The SEM pictures obtained after 28 days of degradation in both aerobic and anaerobic conditions reveal crystalline regions in the PVA/BC composite structure (Figs. 4a, b 6a, b) which can greatly reduce biodegradability [33]. The ability of cellulolytic enzymes to degrade cellulose can vary greatly with the physico-chemical characteristics of the substrate, of which the crystallinity degree is the most important structural parameter [9].

A study on the kinetics of thermal degradation of biopolymers showed that the thermal stability follows the order: cellulose > chitin > chitosan [34]. Unfortunately we found no reports in literature comparing the enzymatic degradation of cellulose and chitosan.

Furthermore, the chitosan can act as emulsifying agent [35], producing homogenous composite films (observed in SEM pictures, Fig. 2), which enhances the biodegradability. Also, a contributing factor to the advanced degradability of the PVA/BC/CTS composite films could be the presence of chitinase enzymes in the biodegradation environment. However, as this is only a presumption, for a better understanding, an advanced characterization of the activated sludge would be needed.

Table 5 Weight loss during anaerobic biodegradation

Film type	Weight loss [%]			
	7 days	14 days	21 days	28 days
PVA/BC	14.15	16.44	17.49	18.17
PVA/BC/CTS	33.08	35.37	35.64	37.6

Conclusions

Two composite films were prepared from poly (vinyl alcohol), a synthetic biodegradable polymer, bacterial cellulose and chitosan. The swelling degree and water vapor permeability of the two composites were determined.

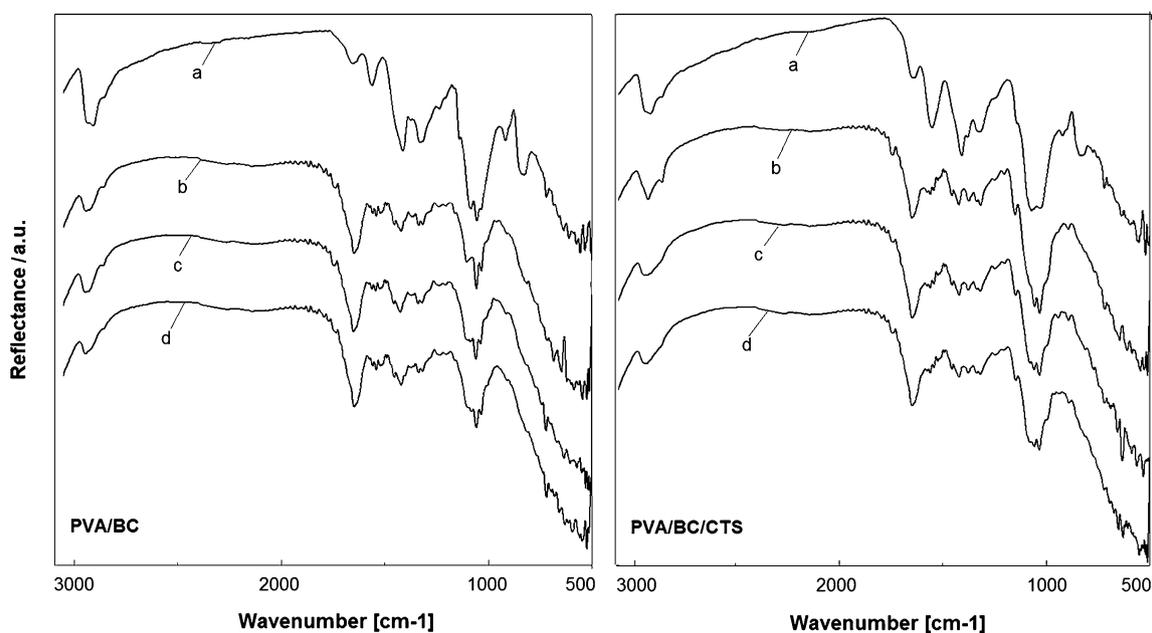


Fig. 5 FTIR spectra of composite films, *a* initial film, *b* after 7 days; *c* after 14 days and *d* after 28 days of degradation in anaerobic environment

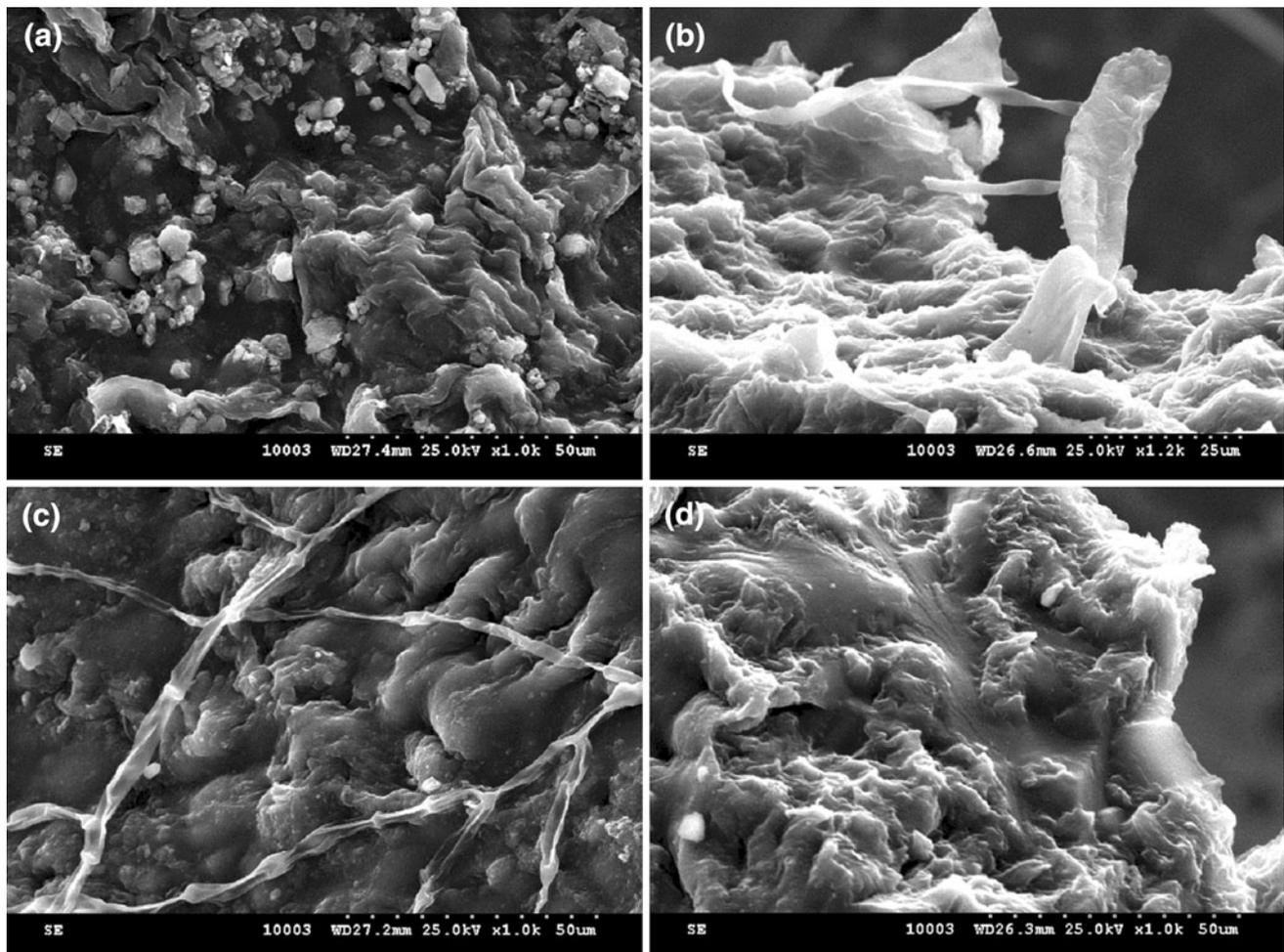


Fig. 6 The surface and edge SEM pictures of PVA/BC composite film (a, b), respectively PVA/BC/CTS (c, d) after 28 days of biodegradation in anaerobic environment

The structure and morphology of composite films were determined by FTIR spectral analysis and scanning electron microscopy. Biodegradation behavior was determined with activated sludge in aerobic and anaerobic conditions.

The results reported here indicate that the association of poly (vinyl alcohol), bacterial cellulose and chitosan can provide new biodegradable composite materials. After 28 days of degradation with activated sludge, the weight loss in anaerobic environment was higher than in aerobic one. The degradation of composite films not only at the surface, but also inside, was confirmed by FTIR spectral analysis and scanning electron microscopy.

The new developed composite materials, could find application in packaging industry as polymeric matrix for active packaging, due to their biodegradability.

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