Biodegradation Kinetics of Antimicrobial Composite Films Based on Polyvinyl Alcohol-bacterial Cellulose

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New antimicrobial material based on bacterial cellulose-polyvinyl alcohol and benzoic acid was developed. Their biodegradability kinetics was studied by compost method for small sheets of processed material. Weight loss and spectrophotometric analyses were used to characterize the presence and dynamics of biodegradation of these materials. The macrostructure of the biodegraded materials was observed by optical microscopy. The results proved that the materials are promising candidates for biodegradation. All the films lost different percentages from their initial weights and FTIR spectra of the materials before and after degradation indicated scission of chemical bonds. Also, the antimicrobial activity against Escherichia Coli K12-MG1655 was studied. The composite films containing benzoic acid revealed strong inhibitory effect against studied bacteria.

Keywords: biodegradation kinetics, antimicrobial films, benzoic acid, bacterial cellulose, polyvinyl alcohol

Food packaging industry demands new eco-friendly materials in order to replace the actual plastics derived from petroleum sources which put real environmental problems. Bio-based polymers are good candidates for obtaining new biodegradable packaging materials [1]. Bacterial cellulose (BC) is a bio-based polymer produced by *Acetobacter xylinum* (reclassified as *Gluconacetobacter xylinus*) in static or agitated cultures. Under static conditions, a hydrated membrane of bacterial cellulose is formed at the medium-air interface. In agitated culture, BC is produced as granule, stellate, or fibrous strand [2]. Bacterial cellulose has unique properties such as high mechanical strength and an extremely fine and pure nanofiber network structure. Due to these properties, BC represents an ideal candidate as raw material to obtain high-strength composites, including those which can be used as packaging materials [3-5]. Additionally to these unique properties, bacterial cellulose has low cost of production on large scale because it can be obtained from waste products such as molasses or corn steep liquor [6, 7]. Bacterial cellulose was used in composition with polyvinyl alcohol in order to obtain potential biodegradable materials. Polyvinyl alcohol (PVA) is a hydrophilic biodegradable synthetic polymer and its biodegradability was intensively studied by different methods, mainly enzymatic [8] and in different types of blends with starch [9-11], collagen [12] and soy protein isolate [13].

Also, biodegradability of composite films based on bacterial cellulose and polyvinyl alcohol was studied by action of *Aspergillus niger* [14]. Concurrently with environmental requests, consumers demand minimally processed and safety food products, which implies development of new packaging materials with special properties. New antimicrobial packaging materials are developed in order to maintain the quality and safety of the food products, without altering the natural properties [15]. Antimicrobial particles for food preservation were also developed [16]. In order to replace classic preservatives which may have negative effect onto human health, new antimicrobial compunds are developed [17,18].

Composite materials containing PVA/BC and benzoic acid as antimicrobial agents were obtained. In present work the biodegradability kinetics of these composite materials was studied by compost method. Composting of materials allows disposal of biodegradable packages and is a process which consume less energy than sorting and reprocessing for recycling. By composting, the organic material is decomposed principally by microorganisms, but also earthworms, small insects, and other soil inhabiting organisms [19].

The antimicrobial effect of the composite films was tested against *Escherichia Coli* K12-MG1655. The biodegraded films were characterized by Fourier transformed infrared spectroscopy (FTIR) and weight loss dynamics. The structure of the films was also studied with optical microscopy.

Experimental part

Materials

Polyvinyl alcohol (PVA), average molecular weight (M_w) 85,000–124,000 g/mol and benzoic acid 99% purity were purchased from Sigma–Aldrich and used without further treatment or purification. Bacterial cellulose (BC) was obtained in a rotating reactor on a modified Hestrin-Schramn (MHS) medium containing 2.0% (w/v) fructose. The *Acetobacter* sp. strain used for BC production was isolated from the traditionally fermented vinegar in Microbiology Laboratory of Chemical Engineering Department of Politehnica University of Bucharest. The gel-like BC pellicles, obtained after 7 days, were purified by boiling in a 0.5 N aqueous solution of NaOH for 1 h. The BC thin sheets were then washed with deionized water several times until *p*H of water became neutral. BC pure pellicles were grinded and used as fibrils.

Preparation of composite films

Bacterial cellulose wet fibrils (95.7% humidity) were dispersed under vigorous stirring into a PVA 5% (w/w) aqueous solution. BC wet fibrils were added in a proportion of 10% (w/w). As antimicrobial agent was added benzoic

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acid in different proportions: 0.1% (w/w) and 0.5% (w/w). As control sample was studied the composite material without benzoic acid. The resulting mixture for each recipe was cast onto a Teflon sheet with the aid of a casting knife and dried at room temperature for 48 h. The dried films' thickness was measured with a micrometer (Japan). The thickness was expressed as an average of five readings taken on each film sample.

Antimicrobial activity of biobased composites

The antimicrobial activity of composite materials containing benzoic acid was tested against *Escherichia Coli* K12-MG1655. The culture medium (Luria – Bertani) was inoculated with 1% suspension of microorganisms. The sterilized film was introduced, in sterile conditions, in each inoculated tube with 5 mL fresh suspension of *E. coli*. All tubes were incubated in a temperature-controlled shaker. The incubation temperature was 30 ^{0}C. Samples were taken over time and the optical density was measured at 600 nm.

Biodegradability tests

The biodegradability potential of bacterial cellulose based composite films was tested by modified compost method [20]. Samples of each film were buried in compost maintained at different conditions. One compost pot was maintained in atmospheric conditions of temperature and humidity, noted with C, other two pots were maintained in laboratory controlled conditions, at room temperature, and different relative humidity of the compost, 66% RH – pot B and 20% RH – pot A. The biodegradation of the films was quantified by weight loss of the samples after different time intervals (7, 15, 29, 61, 90, 176 days). The surface modifications suffered by the materials buried in the compost for different periods of time were studied with an optical microscope (Olympus BX51).

The chemical structure of the biodegraded materials was studied by Fourier transform infrared spectroscopy. FTIR spectra of the composites were obtained with a FTIR spectrometer (Jasco FT/IR6200) with Irtron μ Infrared Microscope with ATR-1000-VZ objective. The spectra were the average of 50 scans recorded at a resolution of 4 cm^{-1} in the range from 4000 to 500 cm^{-1} with a TGS detector.

Results and discussions

Antimicrobial activity of biobased composites

The results of the microbiological tests are presented in figure 1 for all the composite films, with and without antimicrobial agent.

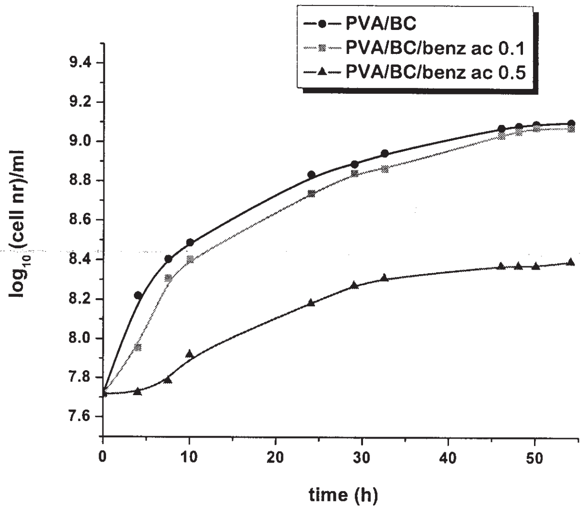

Fig. 1. Growth of *E. coli* in the presence of composite films

The microbiological tests revealed the antimicrobial activity of the composite films containing benzoic acid against *Escherichia Coli* K12-MG1655. The film with 0.1% content of benzoic acid only decelerated the growth of bacterial cells. Film with 0.5% content of active substance inhibited the growth of *E. coli*. These results show that the composites obtained can be used as antimicrobial materials. With respect to biodegradability the presented growth dynamics of E.coli shows a living system. So it is possible the microorganism attack on our tested films and consequently their degradation can go in bio active as is the compost case.

Biodegradability tests

Figure 2(a) presents a comparison between the weight loss of the three composite films during time inside compost pot A. Figures 2(b) and 2(c) present the biodegradation results obtained inside compost pot B, respectively compost pot C.

In pot A (RH = 20%) and C (atmospheric conditions), the film with PVA/BC/benzoic acid had the highest weight loss, followed by PVA film. In pot B (RH = 66%), was observed the highest biodegradability character for simple PVA film, phenomenon explained by the hydrophilic behaviour of the material. Also, pot B created the most favourable environment for biodegradation, because it revealed the highest weight loss values of the simple PVA and PVA/BC/benzoic acid films, more than 10 %.

In all three pots the composite material PVA/BC had the lowest weight loss, even lower than PVA/BC/benzoic acid film. Because of water solubility of benzoic acid, the compound diffused from the material inside compost and created available spaces for biodegradation by microorganisms.

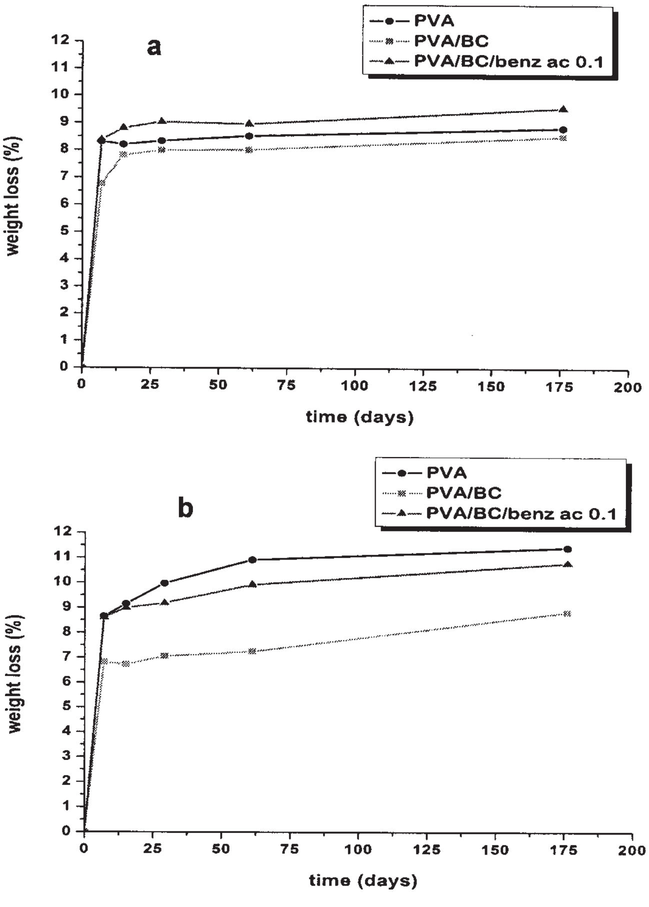

Fig. 2. Weight loss dynamics reported to dry composite materials (degradation: in pot A (a), pot B (b)

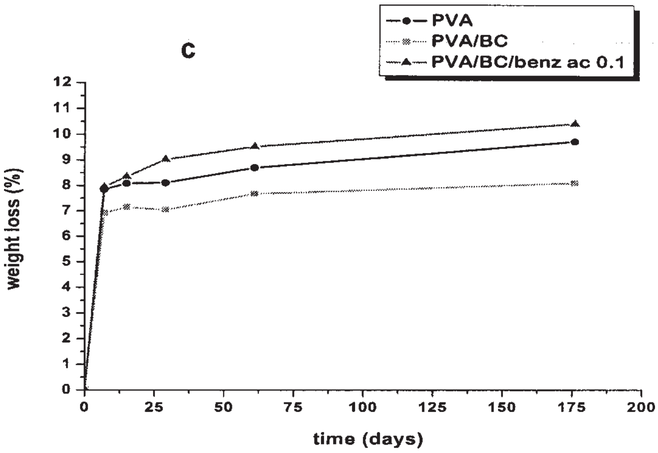

Fig. 2. Weight loss dynamics reported to dry composite materials (degradation: in pot C (c))

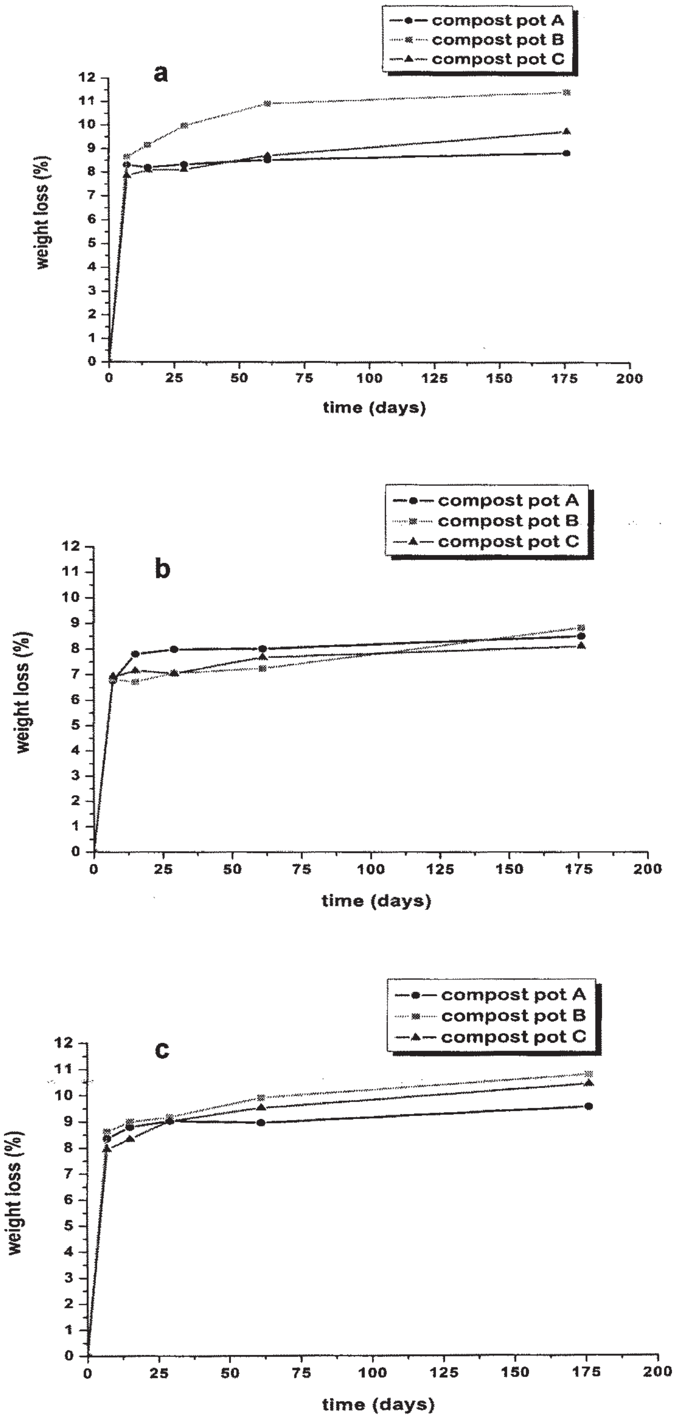

Fig. 3. Weight loss dynamics reported to dry PVA film (a), dry PVA/BC composite (b) and dry PVA/BC/benzoic acid composite (c)

Figure 3(a) compares the degradation during time for the simple PVA film inside the three different compost pots. The same type of comparison is recaptured also in figures 3(b) and 3(c) for PVA/BC film, respectively PVA/BC/benzoic acid film.

Figures 3(a), (b) and (c) confirms that for each type of film, pot B had the highest biodegradability effect on the materials. This is due to the higher humidity of the compost inside the pot. But, the results obtained for pot C, which was maintained under atmospheric conditions, are comparable with the ones from pot B. If we analyze the tendency and to values of weight loss dynamics from figures 2 and 3 then we immediately obtain the conclusion that here we have a very slowly degradation. Really, when the dried films are placed in compost they support a rapid process of water sorption when the samples mass increases nearly to maximum swelling (fig. 4). Simultaneously take place a rapid release of antimicrobial agent. So in about 10-12 h we have the composite films in the state to support the biodegradation process. As shown figures 2 and 3 it take place with an important rate in the first seven day. After that it obeys a linear time evolution with very low intensity. In a future paper it will be done a detailed mathematical model to the above phenomenological model. When it changes the representation from figures 2 and 3, by swelling consideration, the area of weight loss from 70 to 80% will characterize the degradation with low intensity. This advanced polymer degradation is sustained by the following reported data. The analysis of films biodegradation by optical microscopy reveals the modifications suffered by the macrostructure of the composites. Also it sustains an advanced biodegradation.

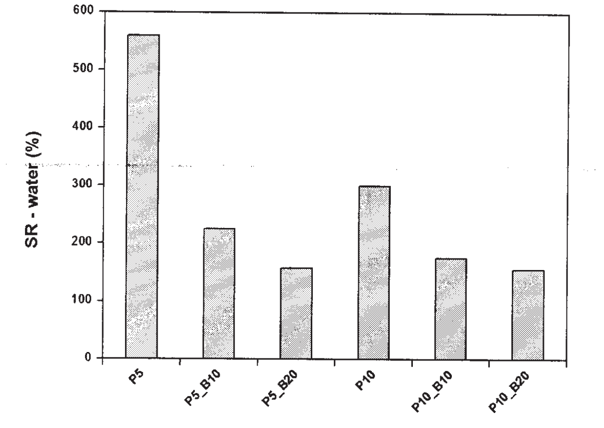

Fig. 4. Swelling ratio in water of PVA-BC based films (P5-PVA films from 5% solution, P5-B10 films from 5% PVA and 10% wet BC solution, etc.

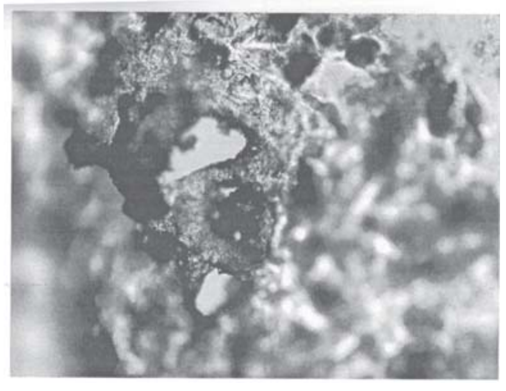

Fig. 5. Biodegradation of simple PVA film after 176 days inside compost pot A recorded with camera-linked light microscope (10× magnification)

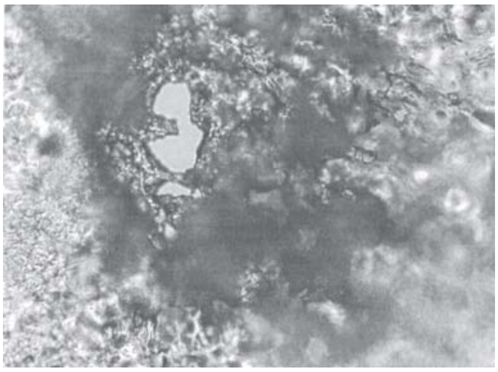

Fig. 6. Biodegradation of PVA/BC composite film after 176 days inside compost pot C recorded with camera-linked light microscope (20× magnification)

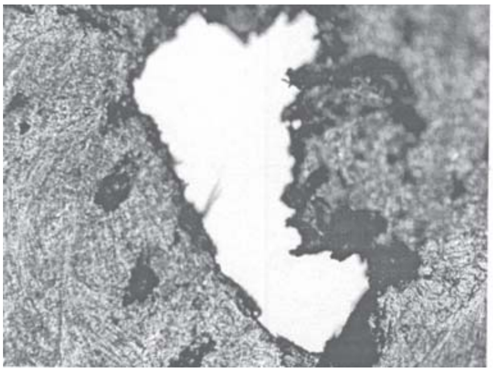

Fig. 7. Biodegradation of PVA/BC/benzoic acid composite film after 176 days inside compost pot B recorded with camera-linked light microscope (5× magnification)

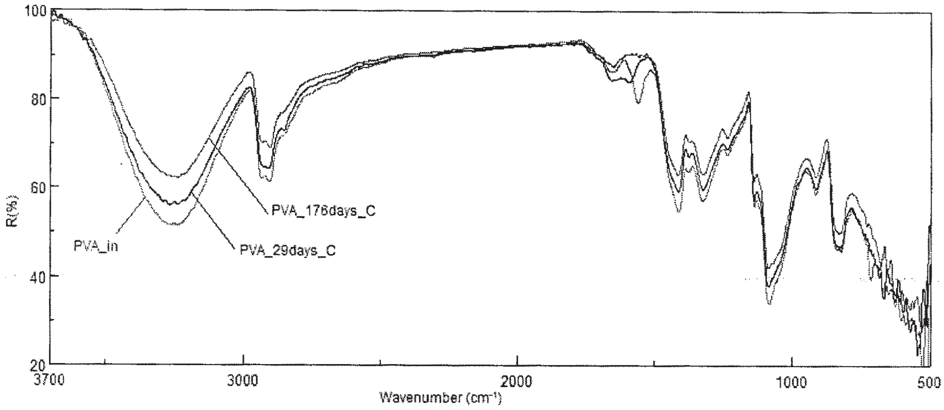

Fig. 8. FTIR spectra of simple PVA film - before biodegradation and after 29 and 176 days of maintaining into compost pot C

Figure 5 presents a picture from optical microscope of simple PVA film after 176 days of biodegradation in compost pot A. The microorganisms destroyed the crystalline structure of the polymer and perforated the material.

The appearance of biodegraded PVA/BC composite film for 176 days inside compost pot C is revealed by figure 6. Also, in this case is observed the perforation of the biobased material.

Figure 7 illustrates the appearance of PVA/BC/benzoic acid composite film after 176 days of biodegradation inside compost pot B. The material was destroyed by the degradation process.

Fourier transform infrared (FTIR) spectroscopy has shown to be a very powerful technique for studying biodegradability of some blends because certain chemical group in each polymer has a characteristic infrared absorption, and the decrease of these absorption peaks or changes in their peak ratios with time can provide useful information on the extent of polymer biodegradation [21]. Attenuated total reflectance – Fourier transform infrared (ATR–FTIR) spectroscopy was used for characterization of films based on polyvinyl alcohol and bacterial cellulose. FTIR spectra of simple PVA film before biodegradation and after 29 and 176 days of degradation into compost pot C (atmospheric conditions) are illustrated in figure 8. PVA's characteristic absorption peaks are found at 3239.8 cm^{-1} (OH stretching), 2908.1 cm^{-1} (CH$_2$ stretching), 1412.6 cm^{-1} (CH$_2$ bending), 1235.1 cm^{-1} (OH plane bending), 1138.7 cm^{-1} (C-C and C-O-C stretching), 1082.8 cm^{-1} (C-O stretching), 917.9 cm^{-1} (CH$_2$ rocking) and 824.4 cm^{-1} (C-C stretching) [22,23].

The intensity of the peaks is lower according to the time interval of PVA film biodegradation, including the peak at 1138.7 cm^{-1} which is sensitive to PVA crystallinity. The decreasing of peaks is observed due to broken of the structural bonds, which confirms that the chemical structure of PVA has been affected by the microorganisms from the compost.

Figure 9 shows FTIR spectral data of initial PVA/BC composite film and after different time intervals of biodegradation into compost pot B (66% RH). FTIR analysis of the initial composite film revealed the characteristic peaks for bacterial cellulose found at 3229.2 cm^{-1} (O-H stretching), 2912.9 cm^{-1} (C-H stretching), 1414.5 cm^{-1} (C-H bending), 1317.1 cm^{-1} (CH$_2$ bending), 1031.7.8 cm^{-1} (C-O stretching vibration of aliphatic primary and secondary alcohols) 911.2 cm^{-1} (C-H bending vibration from β-anomeric link) and 816.7 cm^{-1} (C-C stretching) [22]. Characteristic peaks of BC are at the same wavelengths with PVA's main peaks conducting to wider bands for the composite material due to higher number of the same functional groups vibrating at specific wavelengths.

The peak from 1647.8 cm^{-1} represents water molecules in the amorphous region [22]. Intensity of this peak increases with the biodegradation period. A possible explanation can be the absorption of water molecules by the composite film due to the high level of humidity inside compost.

Spectrum of the composite film PVA/BC after 61 days of biodegradation revealed new peak at 1234.2 cm^{-1} which is characteristic to simple PVA film for OH plane bending vibrations. The composite film subject to 176 days of biodegradation presents a FTIR spectrum with higher number of new peaks comparing with initial film, respectively 1235.1 cm^{-1}, 1138.7 cm^{-1} and 1087.6 cm^{-1}. All these new peaks are characteristic to simple PVA film. The peaks at 3229.2 cm^{-1} and 2912.95 cm^{-1} from the initial composite material spectrum shifted to 3232.1 cm^{-1}, respectively 2907.1 cm^{-1} in the spectrum of film after 176 days of biodegradation. The new shifted peaks are also, specific for simple PVA film.

This phenomenon suggests that microorganisms from the compost attacked firstly the functional groups specific

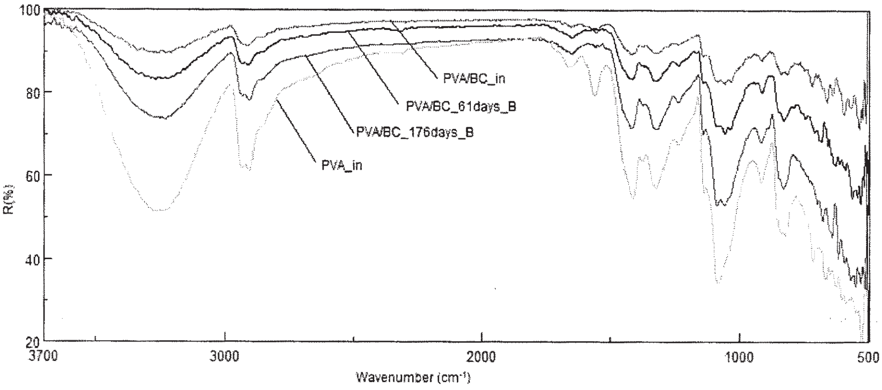

Fig. 9. FTIR spectra of PVA/BC composite film - before biodegradation and after 61 and 176 days of maintaining into compost pot B

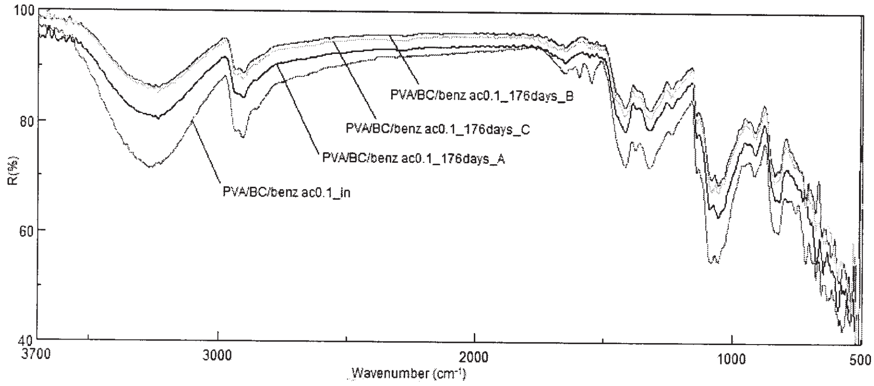

Fig. 10. FTIR spectra of simple composite film containing 0.1 % benzoic acid – before biodegradation and after 176 days of degradation into the three compost pots

to bacterial cellulose revealing the characteristic groups of simple PVA film.

FTIR spectra of PVA/BC/benzoic acid composite film before biodegradation and after 176 days of degradation into the three compost pots are presented in figure 10. The intensity of the peaks is lower according to the humidity of the compost, respectively the spectrum with the least intensive peaks is for the film biodegraded 176 days in compost pot B (with the highest humidity). These spectra confirm the results obtained by weight loss, namely that the highest biodegradability of the films was obtained in compost pot B (66 % RH).

Conclusions

Three data sorts have been done with the aim to characterize the biodegradation kinetics of composite films based polyvinyl alcohol-bacterial cellulose (PVA/BC) system. The experimental investigation concerning the Escherichia coli growth on composite PVA/BC films, with benzoic acid as antimicrobial species, shows that these films can be attacked by microorganisms. The weight loss dynamics of water saturated films at composting conditions shows that in the first 7 days the biodegradation is very active. The penetration of tested films after long composting periods has been observed by means of microscopic analysis. FTIR spectra confirmed the results obtained by weight loss and microscopic analysis. The films present the highest biodegradation rate inside of compost with the biggest content of humidity (66% RH).

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