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The Susceptibility of Polyethylene Modified with Bionolle to Biodegradation by Filamentous Fungi

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Abstract

The aim of this investigation was the biodegradation of low-density polyethylene (LDPE) film modified with Bionolle by fungi. The samples in the form of composite films were prepared by homogenization and extrusion. The effect of polymer composition on mechanical properties was determined. We proved that the composite film had higher tensile strength and elongation at break than LDPE. Examined films were incubated in the presence of the fungi *Aspergillus niger* and *Penicillium funiculosum* isolated from a dump. Degree of colonization and surface morphology of the films with an optical microscope and a scanning electron microscope were observed. The effect of microorganisms' action on the samples by the loss of mass and FTIR spectroscopy was estimated. The results of the study have proven that both fungi were capable of degrading polyethylene. *P. funiculosum,* which is well known for secreting various polymer-degrading enzymes totally assimilated the composite film containing 40% of this highly hydrophobic polymer. It was concluded that polyethylene film modified with 60% (wt/wt) Bionolle can be degraded within 90 days according to European directive 94/92/EC.

Keywords: biodegradation, polyethylene, polyester Bionolle, filamentous fungi

Introduction

Many of the physical and chemical properties of plastics make them ideal materials for a variety of products and applications. Most widely used alkane-derived plastics are considered to be resistant to environmental factors like heat, oxygen, moisture, stress, macroorganisms, ultraviolet light or microorganisms [1]. However, when plastics are used in a disposable product, their resistance to degradation becomes a disadvantage [2]. To enhance the environmental degradation of polyethylene a number of different approaches are used, such as copolymerization or compounding with additives susceptible to any of these factors [3]. Polyethylene is believed to be catabolized by microbes at the chain ends. Because of its high molecular weight and hydrophobicity, this process is slow and may take hundreds of years [4,5]. The main problem is degree of hydrophobicity of the polyethylene chains, which impact directly on the rates of depolymerization caused by enzymes [6,7]. To enhance its hydrophilicity a variety of additives are used. The most popular additive is starch, which makes such plastic more susceptible to microbial attack; however, it may cause poor mechanical properties of composite material [7,8].

On the contrary, aliphatic polyesters presently constitute the most attractive class of artificial polymers which can be degraded in contact with living tissues or under environmental conditions [9]. These compounds are of interest for outdoor applications such as packagings or mulch films, although they are still too expensive [10]. Plastic formulations containing different quantities of polymers that are more expensive but susceptible to a microbial

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attack, and more resistant polymers such as polyolefins, seemed to be excellent materials for controllable lifetime plastics in the biological environment. There have been many reports of acceleration of LDPE biodegradation, but none of them shows completely biological assimilation of polymeric samples. Authors describe so-called biodisintegration process, i.e. partial decomposition of modified LDPE materials. Although it can lead to reducing a huge amount of packaging wastes, it does not solve the problem of their presence. Until now, there is no evidence of a significant biodegradation of polyolefin fragments produced by disintegration.

In this paper, we focus on the aerobic microbial degradation of low-density polyethylene (LDPE) modified with synthetic polyester. These polymer materials, trademarked 'Bionolle' such as polybutylene succinate (PBS), polybutylene succinate adipate copolymer (PBSU·AD) and polyethylene succinate (PES) have been produced through polycondensation reaction of glycols with aliphatic dicarboxylic acids used as principal raw materials since 1990 [11,12].

In the previous study on the modified polyethylene films it was demonstrated that only a 30% addition of Bionolle slightly accelerated biodegradation of low-density polyethylene. It was claimed that microorganisms primarily decomposed polyester, then a porous matrix of polyethylene [13].

This paper presents results of investigations on the biodegradation of polyethylene film containing 60% (wt/wt) of Bionolle by the fungi *A. niger* and *P. funiculosum*.

Materials and Methods

LDPE GGNX 18D-003 type with melt flow rate (*MFR*) of 0.3 g/10 min. was obtained from "Blachownia" Chemical Works, Kędzierzyn-Koźle. Peletized Bionolle (Co-PBSU·Ad) was received from Showa Denko Europe, GmbH. This aliphatic polyester with *MFR* about 1.5 g/ 10 min. is well known as Bionolle grade 3001. Polyester-polyethylene film was prepared containing 60% (wt/wt) Bionolle. The LDPE and Bionolle powder were homogenized in a Co-Knetter Buss high-speed mixer at 170°C. The homogenized material was further processed on a PLV 151 type Plasti-Corder extruder for the production of films. The films were prepared with ratio of 33 rpm and 220, 230, 230, and 235°C set temperatures. Polyethylene film without any additives was used as a control material. LDPE films compounded with Bionolle were obtained from the Institute of Plastics and Paint Industry in Gliwice.

The mechanical properties of the films were evaluated on the dumbbell shape samples from universal test machine INSTRON 4466 with 5 kN load cell at a crosshead speed of 50 mm $min.^{-1}$ at ambient temperature (22ºC). The tensile values were calculated from arithmetic average of five measurements.

Two strains of fungi *Aspergillus niger* and *Penicillium funiculosum* were used in this study. Fungi were isolated from a dump in Sosnowiec. Identification of these strains was carried out by the Institute for Ecology of Industrial Areas in Katowice. Both filamentous fungi are also recommended for determining resistance of synthetic polymeric materials to fungi [14].

Fungi were maintained in test tubes with Czapek-Doxa medium and to produce conidia cultures were incubated at 30°C and humidity up to 90% for 2 weeks.

A. niger and *P. funiculosum* spores were harvested in physiological solution containing 0.01 g of SDS $liter^{-1}$ and separated from hyphae by filtration through three layers of lens tissue paper. Spores were centrifuged at 4,000 rpm for 4 min., washed three times and resuspended in SDS solution to quantity 10^6 spores ml^{-1}.

Biodegradation of the films was performed in Petri dishes containing modified sucrose-free Czapek-Doxa medium.

Cut into strips (ca. 40 mm x 10 mm) preweighed samples of films were disinfected by 70% isopropyl alcohol and washed with sterile distilled water. Each film was then aseptically transferred and individually placed into sterile medium. Five replicates were used for each film. Two types of control specimens were applied. Zero controls comprised uncultured materials. Uninoculated controls were incubated in medium moistened with 1% aqueous solution of mercurous chloride to prevent them from acting on microorganisms. All film samples were incubated at 30°C and humidity up to 90% for a minimum of 10 days up to 90 days. Samples were removed every 10 days. Thereupon polymer slabs were washed with distilled water to remove as much cell mass from the residual film as possible, submerged in 1% Hg_2Cl_2 solution for 5 min. to halt further action and thoroughly washed again then blotted dry and dried until a constant weight was obtained.

After a period of incubation with fungi, polymeric films were observed with an Olympus SZH optical microscope. To evaluate the degree of colonization, the number of conidia and hyphae invading the samples was quantified. Five-grade scales of invasion ranging from 0 to 4 were established as a function of fungi observed on the surface of the films. Grade 0 indicated absence of invasion; grade 1 was considered to correspond to a low attack with a maximum 25% of the film surface covered with fungi, grade 2 indicated an expansion of moderate intensity with a maximum 50% of the film covered with fungi, grade 3 expressed a high degree of colonization over 50%, and grade 4 denoted the growth of fungi occupying the whole surface of the specimen.

The weight loss of each strip was measured to a precision of 0,1 mg to an analytical balance. The percentage weight change was calculated.

Scanning electron microscopy (SEM) analyzers were applied in order to observe microscopical images of the fungal growth or attachment on the surface of films and performed with a TESLA B340 SEM. Test samples were coated by exposing a gold ion beam sputter using PELCO S.C. 6 at 25 mA current for 40 s. Micrographs of the samples were taken at different magnifications to identify

Table 1. The composition and mechanical properties of film samples.

Ingredients of extrusion mixture (%[wt/wt])		Mechanical properties	
Bionolle	LDPE	Tensile strength (MPa)	Elongation at break (mm)
100	0	52.5 ± 3.9	878.0 ± 38.3
60	40	17.9 ± 0.6	616.1 ± 38.9
0	100	15.8 ± 1.2	474.3 ± 44.5

Value are means ± S.D.

cracks, holes and other changes on the surface during the degradation process.

Fourier transform infrared (FTIR) measurements were carried out with a BIO-RAD spectrometer (model FTS 40A). The FTIR spectra were recorded at a resolution of 2 cm^{-1} and an accumulation of 32 scans.

Results

Bionolle film yields tensile strength and elongation at break around 52.5 MPa and 878.0%. The LDPE film used in this study has significantly lower properties than that of Bionolle. It is also interesting to note that in the presence of copolyester, tensile strength and elongation at break of polyethylene film improved up to 17.9 MPa and 616.1%, respectively. The composition and mechanical properties of Bionolle, LDPE and LDPE modified with Bionolle films are shown in Table 1.

Initially, the growth of *A. niger* was restricted to the edges of LDPE samples. After 20 days further colonization occupying a maximum 25% polymer surface was recorded. The quantity of conidiophores with pigmented conidia increased with time (Phot. 1a). *P. funiculosum* invaded the surface of LDPE films from the outset and by the end of cultivation it covered about 50% of the samples (Phot. 1b). No hyphae were observed. The appearance of *A. niger* mycelium on the surface of modified film proceeded within 2 days. Fungi gradually colonized samples

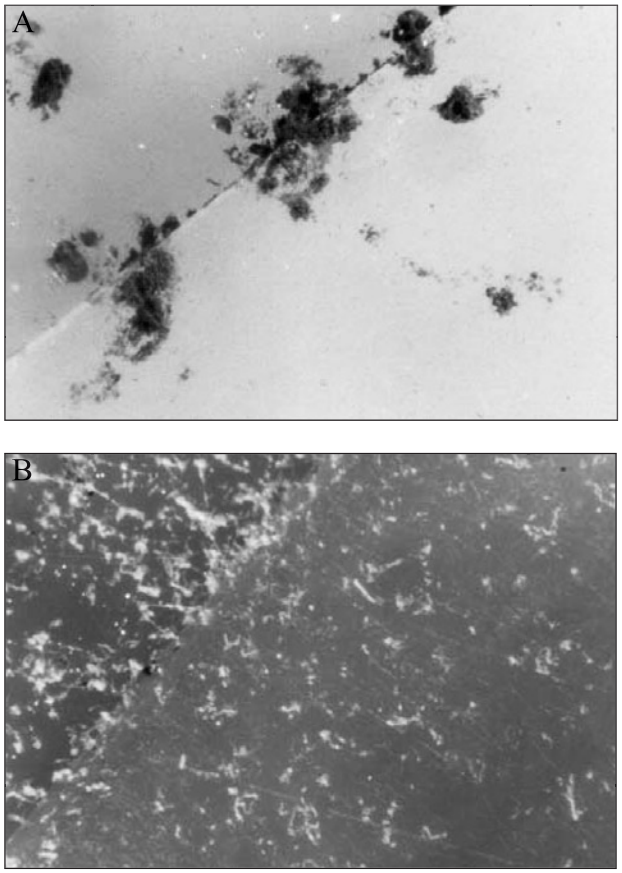

Photo. 1. a) *Aspergillus niger* b) *Penicillium funiculosum* conidiophores growing on LDPE film after 60 days of incubation.

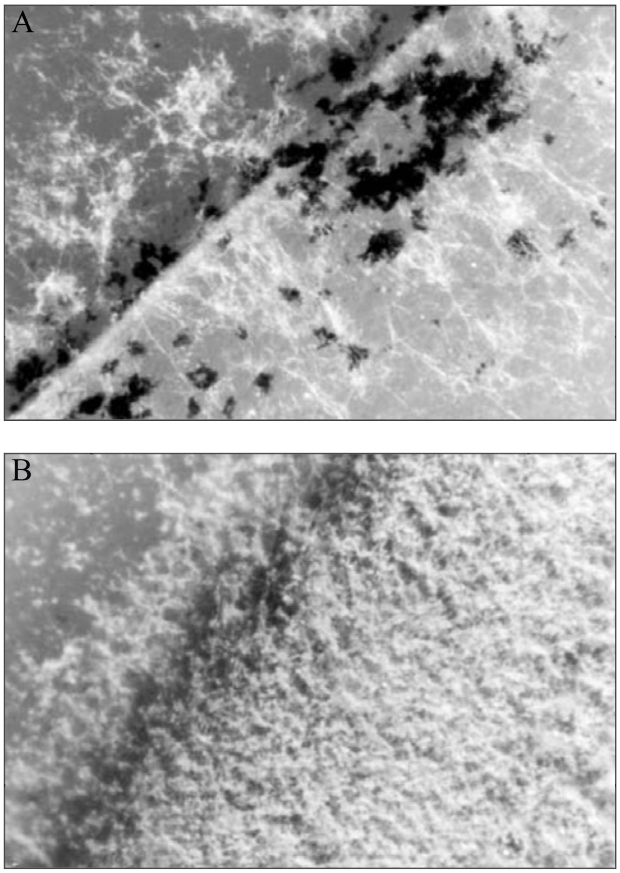

Photo. 2. a) *Aspergillus niger* b) *Penicillium funiculosum* mycelium growing on modified LDPE film after 60 days of incubation.

Table 2. The fungal growth rate on the film samples.

Cultivation time (days)	*Aspergillus niger*		*Penicillium funiculosum*	
	Growth scale			
	LDPE	modified LDPE	LDPE	modified LDPE
10	1	1 s m	1 s	4 s m
20	1 s	2 s m	1 s	4 s m
30	1 s	2 s m	1 s	4 s m
40	1 s	3 s m	1 s	4 s m
60	1 s	3 s m	1 s	4 s m
90	1 s	3 s m	2 s	4 s m

s – fungal expansion on the surface of the film samples, m - mycelium

over 50% in the middle of an incubation period. A high-density colony of *P. funiculosum* covering 100% of the samples during the first 10 days of experiments was observed. Photograph 2 shows (a) *A. niger* and (b) *P. funiculosum* mycelium expanding the surface of modified film specimens after 60 days of incubation. The fungal growth rate on the film samples according to the five-grade scale (see in Material and Methods) illustrates Table 2.

After removal of the fungi from polymeric strips a range of macroscopic changes was observed. LDPE film did not exhibit any color or shape changes after 90 days of incubation with fungi. An unaided visual observation of modified polyethylene film incubated with *A. niger* revealed irregular yellow and brown spots on the surface of the samples. The absence of small fragments of the films was observed after 20 days of cultivation. During a further 70 days the edges of the samples become thinner (Phot. 3a). After the first 10 days the samples incubated with *P. funiculosum* had whitened parts which indicated biodegradation over about 80% of the sample area. During the next weeks of cultivation examined films exhibited further color changes and shape loss due to fungal growth (Photo 3b).

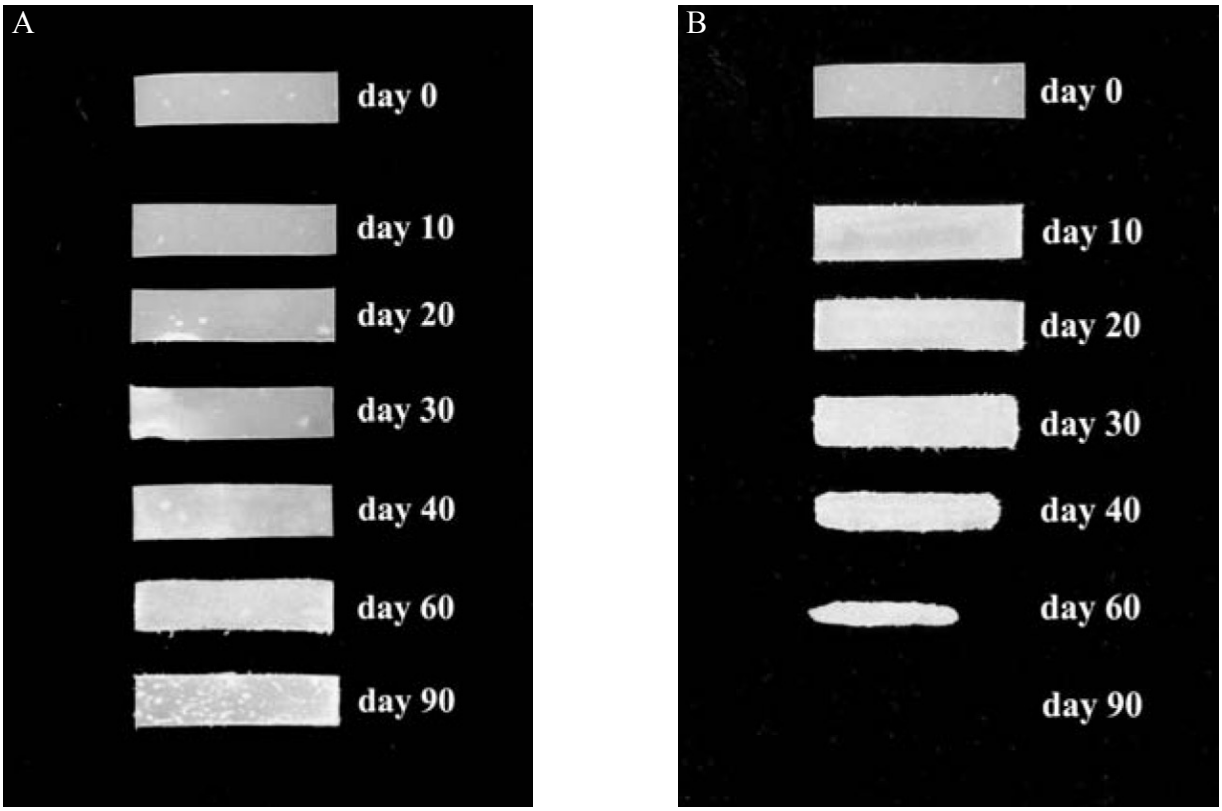

Photo. 3. Photographs of the modified LDPE film after biodegradation with a) *Aspergillus niger* b) *Penicillium funiculosum*.

Table 3. The percentage weight loss of the polymers due to fungal degradation.

Cultivation time (days)	*Aspergillus niger*		*Penicillium funiculosum*	
	Weight loss (%)			
	LDPE	modified LDPE	LDPE	modified LDPE
10	0	1.04	0	6.08
20	0.09	1.14	0.13	30.28
30	0.14	2.26	0.14	49.22
40	0.29	3.49	0.23	75.06
60	0.33	6.12	0.27	93.44
90	0.81	7.53	0.35	100

In the case of polyethylene strips incubated with *A. niger* no weight loss was detected at the beginning of the experimental period. The percentage of weight loss increased significantly after 60 days of cultivation. During the first 60 days polyethylene film lost only 0.3% while it lost nearly 0.5% within the next 30 days. LDPE film inoculated with *P. funiculosum* exhibited little steady weight loss over the cultivation period. Filamentous fungi caused 0.13 and 0.35% of weight loss after 20 and 90 days, respectively. *A. niger* assimilated about 1; 2.3 and 7.5% of modified film after 10, 30 and 90 days of cultivation, respectively. Around 6% weight loss was caused by *P. funiculosum* over a 10-day period. Thereafter, the rate of consumption increased to attain 93.44% after 60 days. The percentage weight loss of the polymer due to fungal degradation after correction for weight loss of the controls is shown in Table 3.

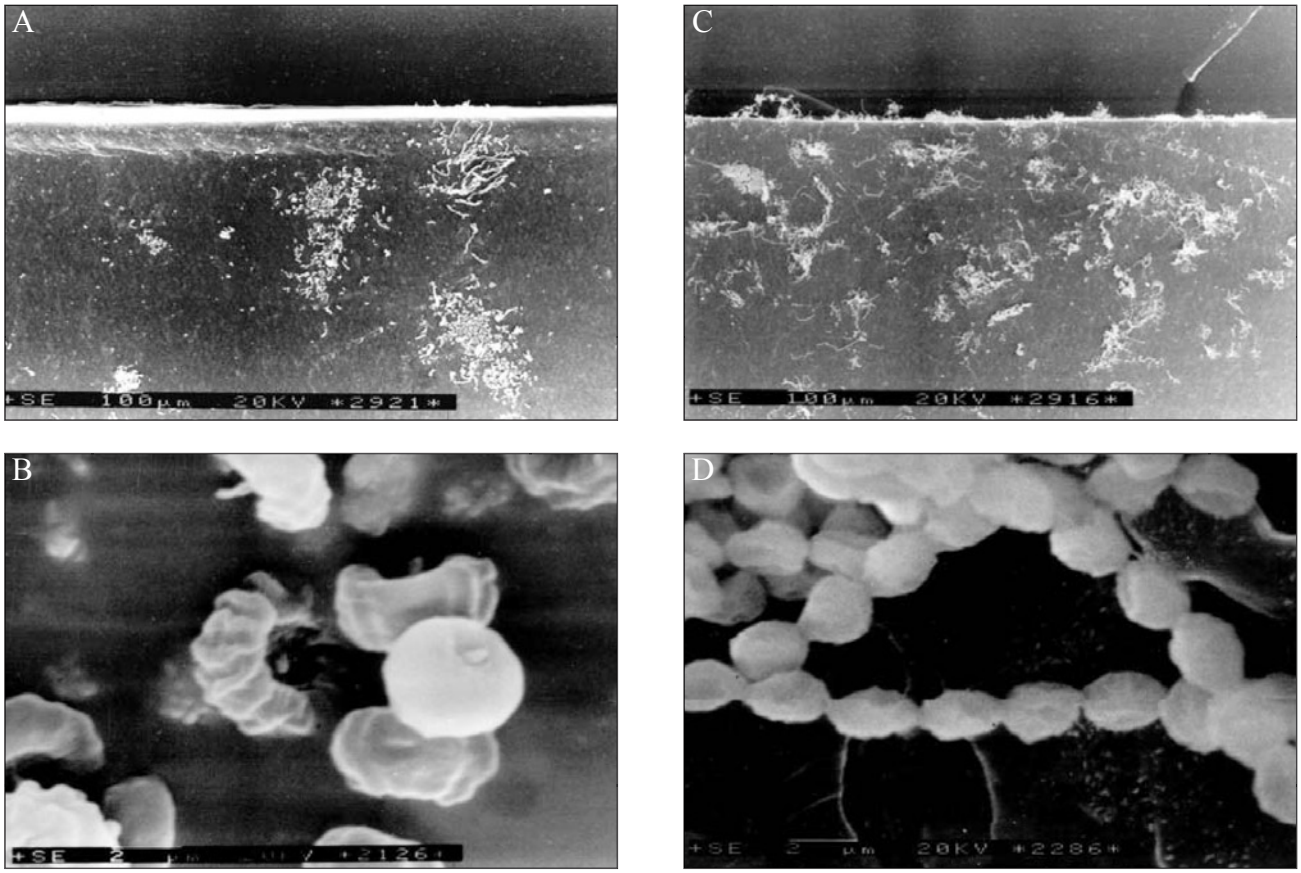

Photo. 4. SEM micrographs of a), b) *Aspergillus niger* and c), d) *Penicillium funiculosum* growing on LDPE film after 40 days of incubation with fungi.

In order to analyze morphology of fungal growth on films, microscopic surface images were obtained using scanning electron microscopy. Initially only the edges of the LDPE film were locally colonized by *A*. *niger*. Thereafter, massive generation of tyrelike conidia dispersed on the surface was observed (Phot. 4a and 4b). The sample area was almost as smooth as non-aged original film. *P*. *funiculosum* colonized the edges and surface of samples. Phot. 4c and 4d show both hyphae and conidia scattered on the strips. After 90 days of incubation sample appeared smooth. Phot. 5a reveals that surface of the modified film subjected to *A*. *niger* was significantly eroded after 10 days. Different degradation patterns and cracks formed over the whole surface. A number of filaments and holes kept growing rapidly with time were observed (Phot. 5b and 5c). *P*. *funiculosum* created complex-networks on the modified samples and the formation of biofilm was observed (Phot. 6a). The long thin filaments were observed after 10 days but generative forms evolved after 30 days of incubation (Phot. 6b). The dense network of filaments covering the film made it impossible to observe its surface morphology (Phot. 6c). In this case it was necessary

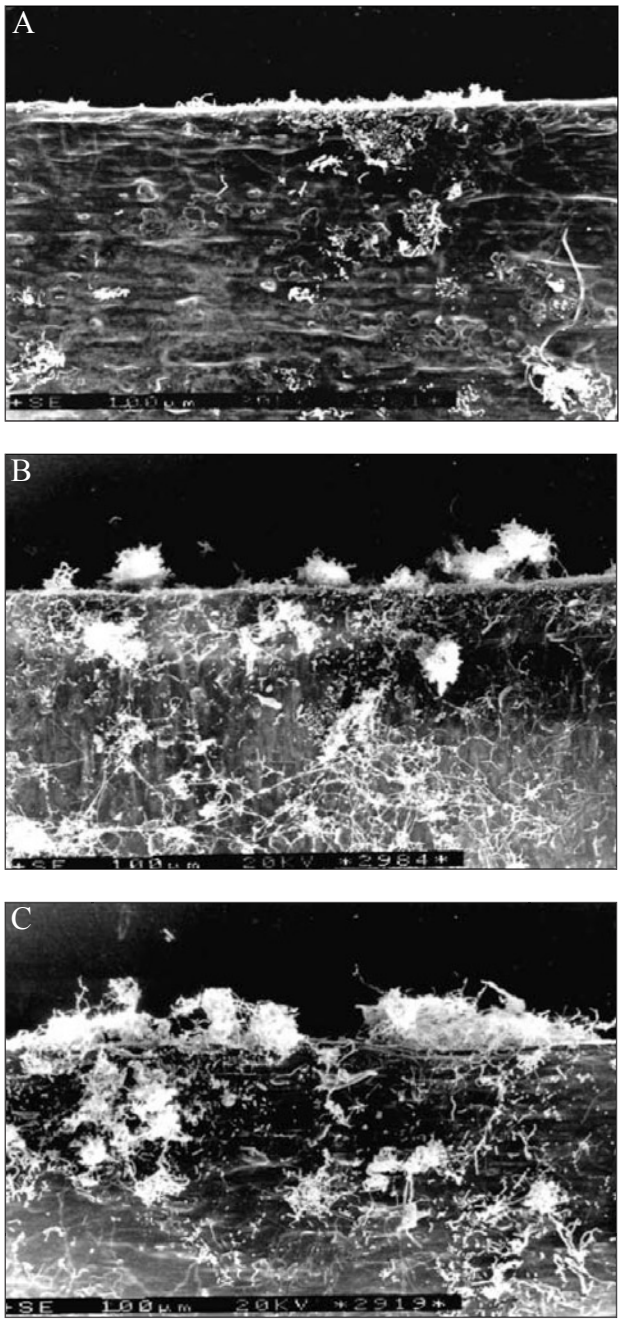

Photo. 5. SEM micrographs of *Aspergillus niger* incubated on the modified LDPE film within a) 10 days; b) 30 days; c) 60 days.

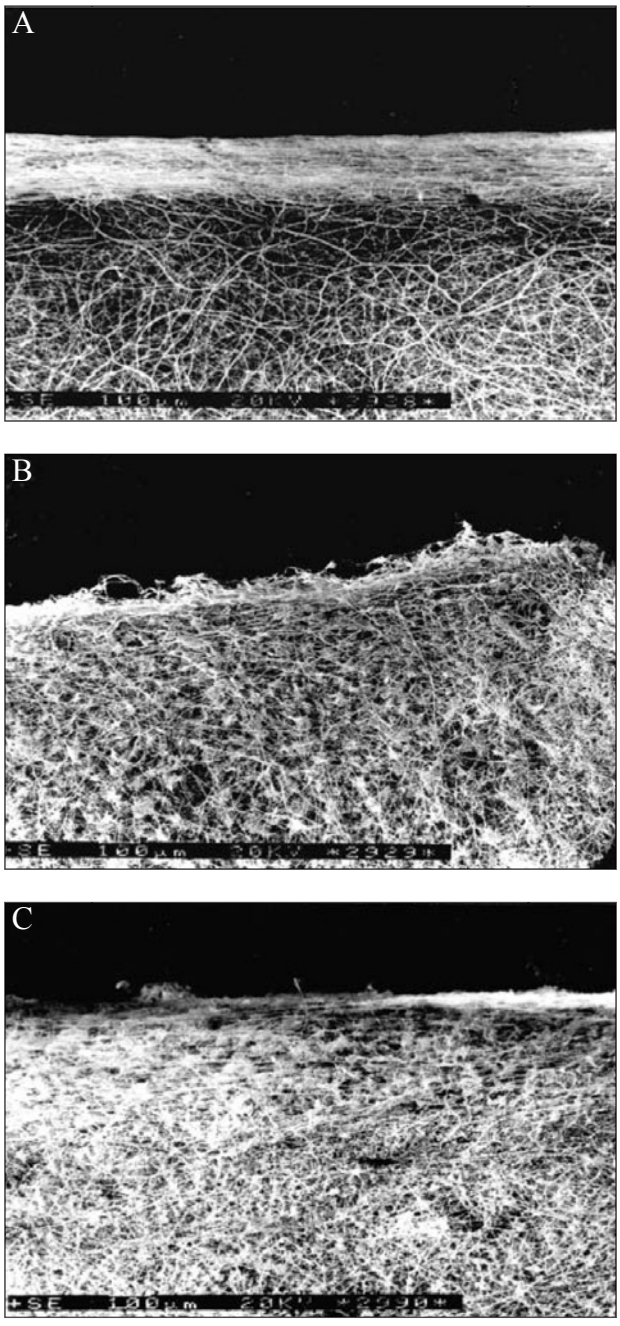

Photo. 6. SEM micrographs of *Penicillium funiculosum* incubated on the modified LDPE film within a) 10 days; b) 30 days; c) 60 days.

to remove residual mycelium from the samples. It was found that after 30 days of inoculation the samples of modified film were deeply degraded (Phot. 7a). After 60 days only a thin layer with long cracks of about 50-100 μm was observed (Photo 7b).

FTIR spectra showed little changes in the LDPE film after 30, 60 and 90 (Fig. 1) days of biodegradation with (a) *A. niger* or (b) *P. funiculosum*. The slight increase in the 1715 and 3500 cm^{-1} bands and a decrease in the 1465 cm^{-1} band were observed. As shown in Fig. 2a, FTIR spectra of modified film after 30 days of incubation with *A. niger* indicated slightly increased absorption C=O and C-O stretching bands in 1750-1700 and 1190-960 cm^{-1} region, respectively. At the same time the peak of C-H bending band at 1465 cm^{-1} decreased and a new weak band near 1410 cm^{-1} and wide O-H stretching band near 3600-3000 cm^{-1} were observed. After 60 days of incubation intensification of these changes was found (Fig. 2b). During the next 30 days FTIR spectra revealed further decrease of the peak at 1465 cm^{-1} and simultaneously two new wide peaks at 1640 and 1550 cm^{-1} due to C=O un-stretching and C=C unconjugated bond appeared. The strong set of C-O stretching band in 1190-960 cm^{-1} region changed its shape (Fig. 2c). FTIR spectra showed similar biodegradation mechanism after 30 and 60 days of cultivation with *P. funiculosum* (Fig. 3a and 3b). Peaks at 1750-1700, 1320 and 1190 cm^{-1} increased while the peak at 1465 cm^{-1} decreased.

Discussion

In the first step of this study some mechanical properties of films were examined. Good tensile properties of modified polyethylene film (Table 1) indicated that such film formulation containing 60% (wt/wt) Bionolle could be used for production of various products for many applications.

Next, the ability of *A. niger* and *P. funiculosum* to grow on the polymer surface was tested. Earlier publications interpreted the growth of microorganisms on polyolefins, e.g. polyethylene as being limited to the microbial action on the surface of an inert support without impact on the polymers [15]. However, it was found that polyethylene is not only colonized but also biodegraded by various fungi mostly belonging to the genera *Aspergillus*, *Fusarium* or *Penicillium* [15,16].

A. niger colonized LDPE film by forming small colonies of conidiophores scattered on the surface of polymeric samples (Phot. 4a). Part of *P. funiculosum* conidiophores was dispersed on the LDPE surface in a uniform

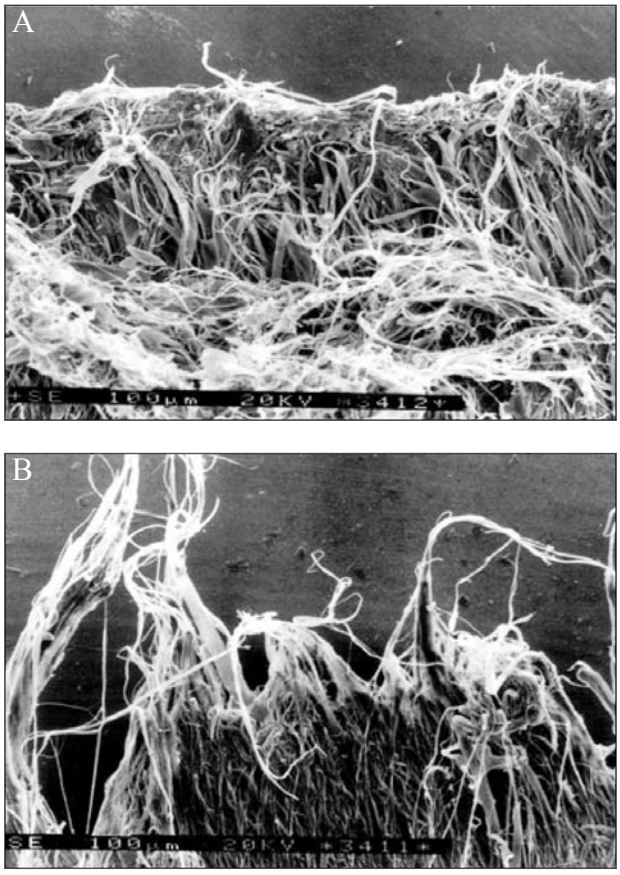

Photo. 7. SEM micrographs of surface morphology modified LDPE film after degradation by *Penicillium funiculosum* a) on day 30 and b) on day 60.

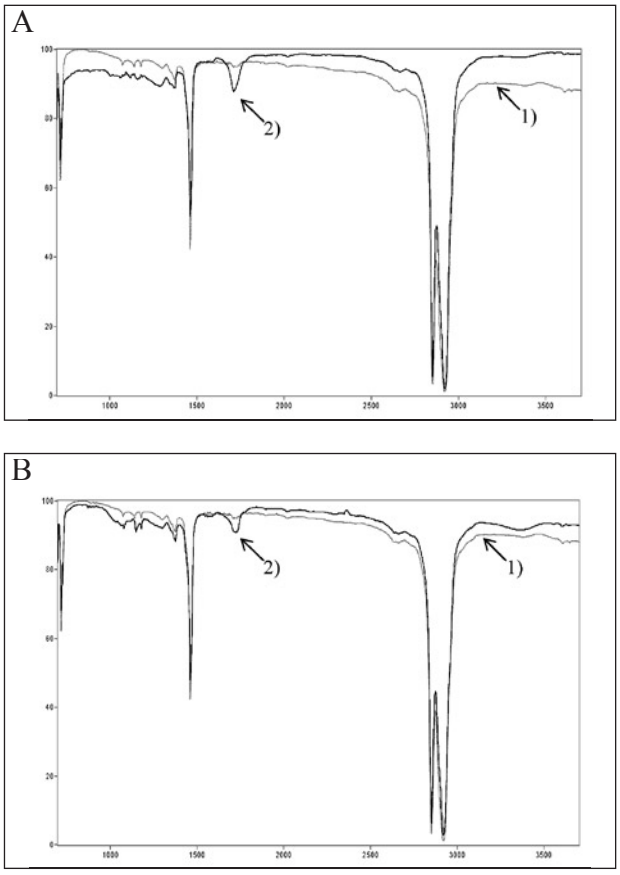

Fig. 1. FTIR spectra of LDPE film 1) before and 2) after 90 days of incubation with a) *Aspergillus niger* and b) *Penicillium funiculosum.*

way, whereas the other one grew on the edges of samples (Phot. 4c). Neither *A. niger* nor *P. funiculosum* developed mycelium (Table 2). *A. niger* and *P. funiculosum* formed dense mycelium on the surface of modified LDPE film. *P. funiculosum* was a more efficient invader than *A. niger* (Photo 5 and Photo 6). During growth on the films different morphological forms of fungi were observed. *A. niger* produced both white hyphae and brown conidiophores from the outset (Photo 2a), while *P. funiculosum* generated only long white filaments. Visible conidiophores were produced in further weeks (Photo 2b). Kim *et al.* [17] showed that chemical composition, structure of polymer and its hydrophilicity affected the morphology of the fungus growing on such an artificial medium. It is also well known that fungi produce mainly generative forms such as conidia under stressful conditions [18]. In accordance with visible and SEM observations we suppose that polyethylene was a poor sole carbon and energy source for filamentous fungi (Photo 1). Judging from these observations, we also concluded that polyethylene film containing Bionolle was more assimilable to *P. funiculosum* compared with *A. niger* (Photo 2).

After a proper cultivation period (10-90 days) the percentage weight loss of polymers was measured. It was noted that *A. niger* and *P. funiculosum* showed similar biodegradation rates of LDPE film within 60 days of incubation (Table 3). Thereafter, the acceleration of the biodegradation process due to *A. niger* action was observed. Such induction periods fit in the results obtained for various fungi [17]. Probably *P. funiculosum* required a longer adaptation period to decompose low-density polyethylene film in a more efficient way. In contrast to the outcome achieved for LDPE, the weight loss of modified polyethylene film subjected to *A. niger* was lower than expected. SEM micrographs revealed holes and cracks on the surface

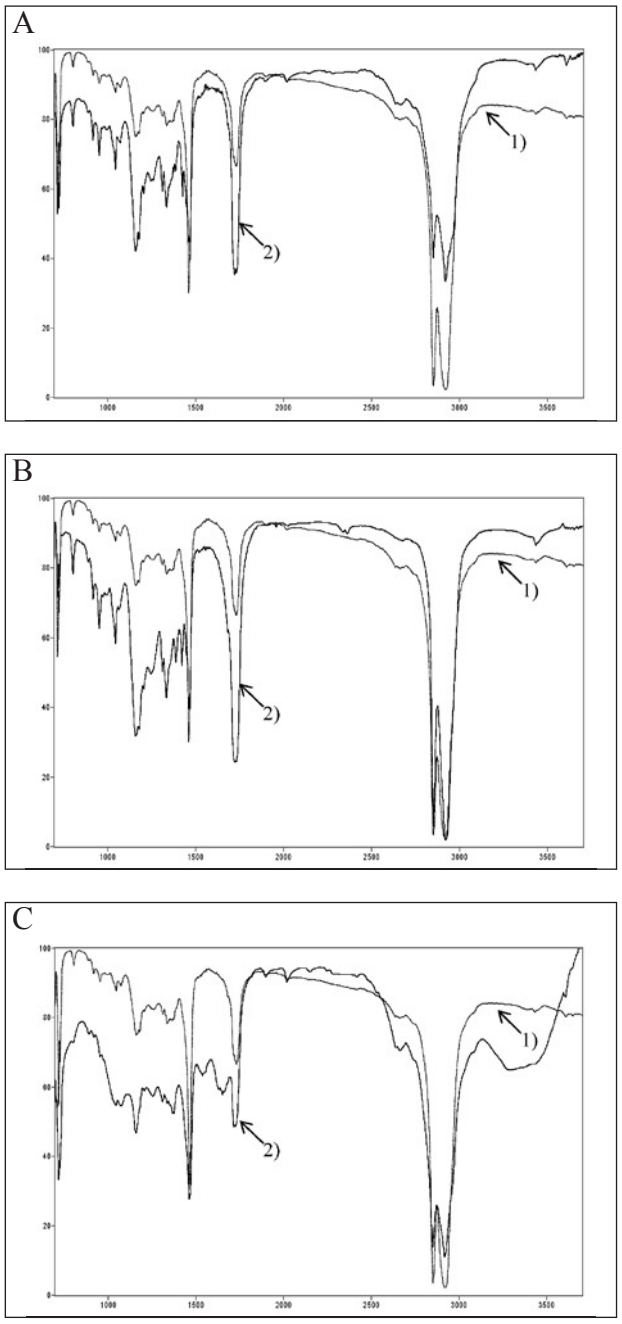

Fig. 2. FTIR spectra of modified LDPE film 1) before and 2) after incubation with *Aspergillus niger* within a) 30 days, b) 60 days and c) 90 days.

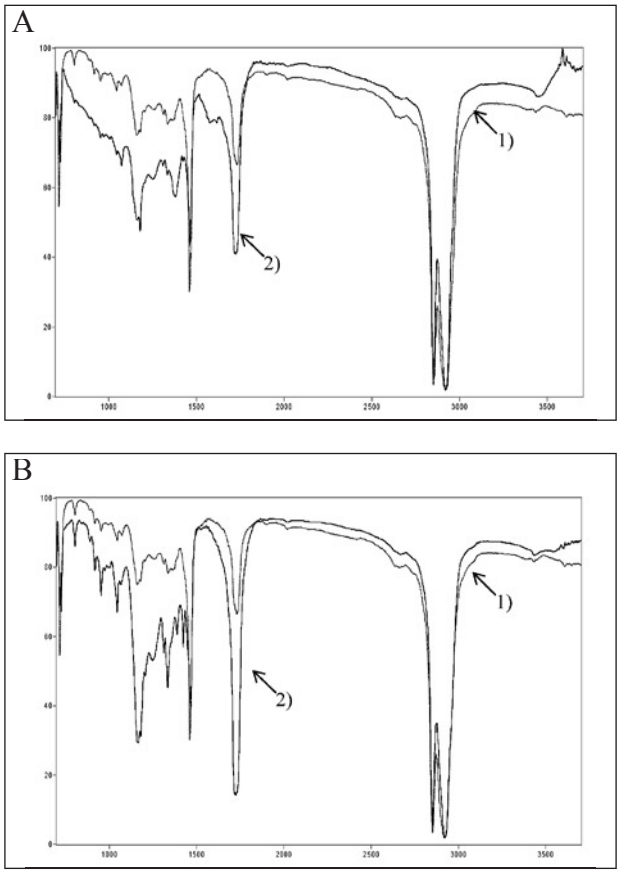

Fig. 3. FTIR spectra of modified LDPE film 1) before and 2) after incubation with *Penicillium funiculosum* within a) 30 days and b) 60 days.

of polymeric samples after 10 days of incubation (Photos 5 and 6). Surface erosion proceeded steadily and slowly. Surprisingly, in studies conducted with *P. funiculosum* biodegradation process leading to assimilation of the polyester accompanied consumption of polyethylene. The structure of the film resembled a sieve (Photo 7). As described in other reports [19, 20, 21], the biodegradability of the synthetic aliphatic polyesters (e.g. Bionolle) is strictly connected with the similarity of their individual structural units to those of poly(β-hydroxybutyrate) (PHB). Eucaryotic PHB depolymerases from *Alternaria* sp., *Penicillium funiculosum*, *P. notatum*, *P. simplicissimum*, *Eupenicillium* sp., *Aspergillus terreus*, *A. fumigatus*, *Fusarium equiseti*, and *Paecilomyces lilacinus* [22-26] play an important role in the biodegradation of these bacterial and other synthetic polyesters. Additionally, fungi belonging to the genus *Penicillium* are capable of biodegrading hazardous substances and many plastics including poly(ethylene terephtalate) (PET), poly(ε-propiolactone) (PCL) or poly(β-propiolactone) [23]. The different species of fungi belonging to the genus *Aspergillus* have been described as degraders of DDT, plasticized PVC, polyethylene and Bionolle [17,25]. In an earlier report [13] we showed that the Bionolle polymer itself showed 100 and 85% weight loss after 84 days of incubation with *P. funiculosum* and *A. niger*, respectively. As these data reveal *A. niger* and *P. funiculosum* are able to secrete enzymes almost equally decomposing this polyester. In this study we demonstrated that *P. funiculosum* was able to utilize polyester in the presence of polyethylene. On the contrary, *A. niger* degraded modified film at approximately one-eleventh the rate of Bionolle. Ikejima *et al*. [27] also reported that the non-biodegradable components sometimes inhibit decomposition of the degradable components.

In order to analyze if the biodegradation proceeded in both polyethylene and polyester components, FTIR spectra of the films were recorded. The special interest was focused on forming carbonyl groups –C=O (1715 cm^{-1}) as a result of enzymatic oxidation of LDPE. We also paid attention to absorption bands of hydroperoxide –OH (3500 cm^{-1}) and of –C-O- (1100 cm^{-1}) caused by alcohol, which indicate faster degradation rates of the carbon chains.

FTIR analyses affirmed that polyethylene itself was a sole carbon and energy source for fungi. The oxidative enzymes led to the formation of ketone, aldehyde and ester groups along the polymer chains (Fig. 1). It was revealed that *P. funiculosum*, which degraded whole samples of modified films, simultaneously removed polyester and polyethylene, but in the case of *A. niger* it was not so obvious. From FTIR spectra plotted in Fig. 2 it was apparent, therefore, that Bionolle as the major component in the samples stimulated degradation of polyethylene matrix by *A. niger*.

Unlike some investigations [1,2,13], we observed that modification of polyethylene with compound susceptible to fungal attack does not inhibit its biodegradation.

Acknowledgements

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