Biodegradation of polyethylene modified with Bionolle® polyester

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Synthetic polymers with a carbon chain and high molecular weight are regarded in the literature as compounds that are resistant to the action of microorganisms [1]. In order to speed up their biodegradation they are modified with polymers whose chain contains groups that are sensitive to enzymatic attack by microorganisms [2]. The polymers employed for modifying polyethylene (PE) have included natural polymers, such as starch [3-5], cellulose [6] or gelatin, and synthetic polymers, including aliphatic and aromatic polyesters [7]. The biodegradability of polyesters is probably connected with their chemical similarity to the naturally occurring poly(b-hydroxybutyrate) (PHB) of formula (I).

\[
\text{CH}_2\text{O-CH-CH}_2\text{-COOH} \quad (I) \\
\text{PHB}
\]

\[
\text{H-}	ext{CH}_2\text{CH}	ext{-CH}_2\text{-CH}	ext{-CH}_2\text{CH}	ext{-COOH} \quad (II) \\
\text{Bionolle}^®
\]

One such polymer is the synthetic aliphatic polyester Bionolle®, obtained via the polycondensation of glycols with dicarboxylic acids [8-10]. The properties of Bionolle® [formula (II)] – a thermoplastic with melting point in the range 90-120°C, glass transition temperature from -45 to -10°C and density of 1.25 g/m³ – are similar to the properties of LDPE.

Therefore we chose this polyester as a modifier of PE. The aim of the work described in the present article was to determine the susceptibility of polyethylene films modified with Bionolle® polyester to biodegradation caused by various microscopic fungi.

EXPERIMENTAL

Materials

As starting materials we used polyethylene (LDPE) of type GGNX 18-D003 with a melt flow index (MFI) of 0.3 g/10 min (manufacturer: ZCh Blachownia, Kedzierzyn-Kozle) and Bionolle® polyester, type #3001 with MFI of 1.5 g/10 min (manufacturer: Showa Denko Europe, GmbH).

Modification of PE

In the first instance we prepared a concentrate in the form of granules consisting of 60 wt.% Bionolle® and 40 wt.% PE. For this purpose the individual components were mixed together in a high-speed mixer and were then submitted to plasticising and homogenisation in a type PR 46 “Ko-Kneter Buss” extruder (from the Buss company) at a temperature of 170°C. The 60% concentrate obtained was diluted with polyethylene to concentrations representing 10%, 15%, 20% or 30% of Bionolle®. The individual compositions were submitted to an extrusion process, using a laboratory extruder (“Plasti-Corder PLV 151” from the company Brabender), equipped with a slot die. The extrusion conditions were: temperature of the individual zones of the
barrel 220, 230, 230 and 235°C, rotary speed of the screw 33 rev/min, torque 32-46 Nm (Table 1).

The flat composite films obtained in this way in the form of strips were submitted to biodegradation studies. The following were used as control samples in the degradation process: PE film that had not been modified with polyester, and film made from the Bionolle\textsuperscript{®} polyester itself.

**Strains of fungi**

<table>
<thead>
<tr>
<th>Component</th>
<th>Content of the component, wt.%</th>
</tr>
</thead>
<tbody>
<tr>
<td>film 0</td>
<td>100</td>
</tr>
<tr>
<td>film I</td>
<td>90</td>
</tr>
<tr>
<td>film II</td>
<td>85</td>
</tr>
<tr>
<td>film III</td>
<td>80</td>
</tr>
<tr>
<td>film IV</td>
<td>70</td>
</tr>
<tr>
<td>film V</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1 Composition of the films used for the degradation studies

The fungi Aspergillus terreus, Aureobasidium pullulans, Paecilomyces varioti, Penicillium ochrochloron, Scopulariopsis brevicaulis and Trichoderma viride were obtained from the Institute of Fermentation Technology and Microbiology of Lodz Polytechnic; the fungi Aspergillus niger and Penicillium funiculosum were isolated from a refuse dump in Sosnowiec and were identified at the Institute of Ecology of Industrialised Sites in Katowice. These are fungi that require few nutrients, are of wide natural occurrence, and are capable of degrading many natural and synthetic compounds [11].

**Microbiological media**

The fungi Aspergillus niger, Aspergillus terreus, Paecilomyces varioti, Penicillium funiculosum, Penicillium ochrochloron and Scopulariopsis brevicaulis were grown on slants containing Czapek-Dox substrate (PN-85/C-90080) with the composition: 2 g NaNO\textsubscript{3}; 0.7 g KH\textsubscript{2}PO\textsubscript{4}; 0.3 g K\textsubscript{2}HPO\textsubscript{4}; 0.5 g KCl; 0.5 g MgSO\textsubscript{4}·7H\textsubscript{2}O; 0.01 g FeSO\textsubscript{4}·7H\textsubscript{2}O; 20 g sucrose; 20 g "Bacto Agar" (Difco); 1000 ml distilled water. The reaction of the substrate was adjusted to pH = 6.0-6.5.

Modified Czapek-Dox medium was used for growing the strain Trichoderma viride. Modification was based on replacing the sucrose with cellulose in the form of strips of Whatman No. 2 filter paper.

The strain Aureobasidium pullulans was cultivated on modified Martin glucose-peptone substrate [12] with the composition: 1 g KH\textsubscript{2}PO\textsubscript{4}; 0.5 g MgSO\textsubscript{4}·7H\textsubscript{2}O; 5 g peptone (Difco); 10 g glucose; 20 g "Bacto Agar" (Difco); 1000 ml distilled water. Modification was based on removing Rose Bengal and aureomycin from the substrate.

**Experimental method**

For visual assessment of fungal growth we prepared film samples with the dimensions 40 x 40 mm (PN-85/C-89080), which were sterilised by immersion in 70% isopropanol, then rinsed in sterile distilled water and placed on modified Czapek-Dox substrate in Petri dishes. Modification of the substrate was based on removing sucrose from it. The films were inoculated with a suspension of spores at a concentration of 10\textsuperscript{6} per millilitre. The dishes were incubated in a "Binder" type BD 240 incubator at a temperature of 30°C and in conditions of 90% relative humidity. The growth of the fungi on the test samples was observed directly in a type SZH 10 stereoscopic microscope (magnification 50x).

For assessment of weight changes, film samples were cut out with sides 40 x 10 mm (PN-85/C-89080). The samples were submitted to the same procedure as in the method of visual assessment. The weight changes were determined after 28, 56 and 84 days. Compact and uniform mycelium, observed in the stereoscopic microscope, was removed from the surface of the film with tweezers. To destroy the residual biomass of the microorganisms the samples were immersed in a solution of mercuric chloride. The film was dried to constant weight in a desiccator, then the percentage weight loss of the polymers was calculated.

Micrographs of the polymer films were made using a TESLA BS 340 scanning electron microscope. The samples were coated with technical-grade gold by vapour deposition of the metal in type "PELCO S.C. 6" apparatus for 40 s in conditions with current intensity of 25 mA, at a pressure of 0.8 hPa.

The polymer films were analysed in the infrared, using a type FTS 40A spectrophotometer from Biorad; spectra were recorded in the range 3700-700 cm\textsuperscript{-1} with resolution of 2 cm\textsuperscript{-1} and with number of scans equal to 32. The samples were dissolved in decalin with addition of dimethylformamide at 70°C and after evaporation of the decalin they were analysed in the form of membranes on sodium chloride plates.

**RESULTS AND DISCUSSION**

**Growth of microorganisms on the films**

On PE film (film 0 according to Table 1), after 28 days of incubation we observed slight growth of Aspergillus niger, Penicillium funiculosum and Trichoderma viride (Figure 1a) and of a mixed population of fungi.

On film 1 after the same incubation time we found growth of Aspergillus niger, Penicillium funiculosum, Trichoderma viride and a mixed population of fungi. The strains of Aureobasidium pullulans, Penicillium ochrochloron and Paecilomyces varioti observed in the
stereoscopic microscope after 56 days of incubation populated locally small fragments on the edges of the films. Growth of Aspergillus terreus was not found.

After 28 days of incubation, all the fungal strains exhibited the ability to grow on film II. For a period of 56 days, growth of Aspergillus terreus (Figure 1b), Aureobasidium pullulans, Paecilomyces variotii and Penicillium ochrochloron was only observed on the periphery of the film, whereas that of the other fungi was also observed on their surface. All the fungal strains populated less than 25% of the surface of the films.

On film III, after 28 days of incubation best growth was exhibited by Aspergillus niger, Penicillium funiculosum and the mixed population of fungi. The strain Penicillium funiculosum (Figure 1c) in the form of mycelium populated more than 25% of the film, whereas the mixed population of fungi, without a visible hyphal structure, occupied less than 25% of sample surface.

On film IV, after 28 days of incubation, the culture of Trichoderma viride grew in aggregations on the periphery of the material. The other fungi populated the edges and surface of the film, but only Penicillium funiculosum, Aspergillus niger (Figure 1d) and the mixed population of fungi populated more than 25% of its surface.

After 28 days of incubation, the strains Penicillium funiculosum and Scopulariopsis brevicaulis and the mixed population of fungi covered over 50% of the surface of samples of film V.

Weight loss of the polymers

The percentage weight loss of the polymer films as a result of the action of microorganisms is shown in Table 2.

Thus, during 84 days of incubation, only the fungi Aspergillus terreus and Aureobasidium pullulans did not cause weight loss of PE. The largest weight losses – 0.21% and 0.16% – were caused by Aspergillus niger and the mixed population of fungi, respectively. Paecilomyces variotii and Penicillium ochrochloron did not cause weight loss of film I after 28 days, and Aspergillus terreus and Aureobasidium pullulans – during 56 days of incubation. After 84 days, Penicillium funiculosum caused a 0.23% weight loss of the polymers, whereas Aspergillus niger and the mixed population of fungi degraded 0.21 and 0.20% of film mass, respectively. Aspergillus terreus did not degrade film II during the first 28 days of incubation; after 84 days Penicillium funiculosum had degraded 0.39%, and the mixed population of fungi had degraded 0.31% of film mass. The other fungi caused a slow, steady loss of film mass throughout the experiments.

All the strains of fungi and their mixed population were characterised, after 28 days of incubation, by the ability to degrade film III. After 84 days, Aspergillus niger

Figure 1 Fungi growth on modified polyethylene films after 28 days of incubation: a) Trichoderma viride on film I, b) Aspergillus terreus on film II, c) Penicillium funiculosum on film III, d) Aspergillus niger on film IV
and *Penicillium funiculosum* had caused a 0.5% weight loss, whereas the strain *Aureobasidium pullulans* was characterised by the weakest capacity to degrade, and only broke down 0.07% of the mass of the polymers.

Among the modified films, the largest weight losses of the polymers as a result of the action of microorganisms were found for film IV. *Penicillium funiculosum* and the mixed population of fungi caused weight loss of the polymers of 10.07% and 8.01%, respectively. 

It was found that *Penicillium funiculosum* after 56 days, and the mixed population of fungi after 84 days, caused complete degradation of film V (pure polyester). Over a period of 84 days, *Aspergillus niger* degraded 84.4% of the mass of the polyester. This may indicate that these strains secrete enzymes that break down the ester bonds in Bionolle®.

### Condition of the sample surface

On the surface of film 0 that had been submitted to the action of microscopic fungi, only the strain *Penicillium funiculosum* caused degradative changes that were visible in the form of fissures.  

All the fungi, except *Aureobasidium pullulans*, caused changes in film I after 84 days of incubation, in the form of holes, fissures and flaking.

On film II, *Aspergillus niger* produced a few hyphae and conidiophores, on the other hand the entire fissured surface of the plastic was covered with spores. *Aureobasidium pullulans, Penicillium ochrochloron, Scopulariopsis brevicaulis (Figure 2a)* and *Trichoderma viride* caused changes in the structure of the film only at its periphery, whereas *Penicillium funiculosum* and the mixed population of fungi degraded the entire surface of the plastic.

Under the action of *Aspergillus niger, Penicillium ochrochloron, Trichoderma viride* and the mixed population of fungi, film III broke down into two layers. The strains *Paecilomyces variotii* and *Penicillium funiculosum (Figure 2b)* populated the edges and surface of the film. Cracking of the sample occurred as a result of their activity.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Film 0 time, days</th>
<th>Film I time, days</th>
<th>Film II time, days</th>
<th>Film III time, days</th>
<th>Film IV time, days</th>
<th>Film V time, days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>28</td>
<td>84</td>
<td>28</td>
<td>84</td>
<td>28</td>
<td>84</td>
</tr>
<tr>
<td><strong>weight loss, %</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>0.08</td>
<td>0.21</td>
<td>0.20</td>
<td>0.21</td>
<td>0.08</td>
<td>0.22</td>
</tr>
<tr>
<td><em>Aspergillus terreus</em></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.06</td>
<td>0.00</td>
<td>0.11</td>
</tr>
<tr>
<td><em>Aureobasidium pullulans</em></td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.02</td>
<td>0.10</td>
</tr>
<tr>
<td><em>Paecilomyces variotii</em></td>
<td>0.00</td>
<td>0.04</td>
<td>0.00</td>
<td>0.12</td>
<td>0.03</td>
<td>0.16</td>
</tr>
<tr>
<td><em>Penicillium funiculosum</em></td>
<td>0.00</td>
<td>0.05</td>
<td>0.02</td>
<td>0.23</td>
<td>0.25</td>
<td>0.39</td>
</tr>
<tr>
<td><em>Penicillium ochrochloron</em></td>
<td>0.00</td>
<td>0.03</td>
<td>0.00</td>
<td>0.16</td>
<td>0.09</td>
<td>0.22</td>
</tr>
<tr>
<td><em>Scopulariopsis brevicaulis</em></td>
<td>0.00</td>
<td>0.03</td>
<td>0.04</td>
<td>0.12</td>
<td>0.06</td>
<td>0.17</td>
</tr>
<tr>
<td><em>Trichoderma viride</em></td>
<td>0.00</td>
<td>0.03</td>
<td>0.02</td>
<td>0.12</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Mixed population of fungi</td>
<td>0.08</td>
<td>0.16</td>
<td>0.12</td>
<td>0.20</td>
<td>0.17</td>
<td>0.31</td>
</tr>
</tbody>
</table>
All the fungal strains caused delamination of film IV. Fissures and holes of varying dimensions were observed on the surface of the samples. After incubation with *Aspergillus niger* (Figure 2c), elongated polymer threads populated by fungal spores were observed on the sample surface. *Penicillium funiculosum* and the mixed population of fungi produced well-developed mycelium, located on the edges. *Penicillium ochrochloron* (Figure 2d) caused delamination of the film into flakes, and populated – in the form of chains of spores – mainly the fissures in the plastic. As a result of the action of *Aspergillus terreus*, fissures of various dimensions formed on the film surface, *Aureobasidium pullulans* caused delamination of the edge part of the film and flaking of its surface, and *Scopulariopsis brevicaulis* populated the entire surface in the form of entangled aerial hyphae.

**IR analysis**

In order to verify whether, in PE films modified with Bionolle®, degradation affected the polyester exclusively, or the polyethylene as well, IR analysis was carried out on films that had been submitted to the action of fungi. All the types of films in Table 1 were analysed after 84 hours of incubation with *Penicillium funiculosum*, which was characterised by the highest biodegradation activity. Analysis of the FT-IR spectrum of films containing 10-20% polyester showed that C=O (ketone or aldehyde) groups appeared in them in the range 1700-1800 cm⁻¹ (Figure 3a).

![Figure 2](image1.png) Scanning electron micrographs: a) Scopulariopsis brevicaulis on film II, b) Penicillium funiculosum on film III, c) Aspergillus niger on film IV, d) Penicillium ochrochloron on film IV

![Figure 3](image2.png) FT-IR spectra a) film I, b) film IV: 1 - before degradation, 2 - after 84 days of incubation with Penicillium funiculosum
These new groups probably derived from breakdown of the polyester, as no changes were observed in the absorption bands that are characteristic of PE.

Analysis of the FT-IR spectrum of film IV showed that a number of new bands appeared in the range 800-1800 cm⁻¹ and 3100-3600 cm⁻¹ (Figure 3b). The ketone, aldehyde, ester, acid, ether and hydroxyl groups found here point to degradation of both the polyethylene and the polyester.

CONCLUSIONS

Growth of microscopic fungi on polyethylene/polyester films increases with the content of polyester in the film. The strains Aspergillus niger, Penicillium funiculosum and the mixed population of fungi populate the film samples to the greatest extent; growth of the remaining fungi is slow, but definite.

The largest weight loss occurs in films containing 30% polyester. The smallest weight losses of the film – 0.64% and 0.66% after 84 hours of incubation – are caused by the strains Aureobasidium pullulans and Trichoderma viride respectively, whereas the strain Penicillium funiculosum and the mixed population of fungi are characterised by the highest degradation activity (10.07% in the case of Penicillium funiculosum).

The change in sample surface and the morphology of the fungi depend on the percentage content of polyester in the film. The changes are visible in the form of fissures, holes and flaking of the film. The surface changes are greatest for samples with 20% and 30% content of Bionolle®, the action of the microorganisms causes delamination of these. Conidiophores with conidia and individual hyphae are observed on the surfaces of films containing 10, 15 and 20% polyester. Mycelia do not appear until the films have a polyester content of 30%.

Analysis of FT-IR spectra showed that biodegradation of film with a low content of Bionolle® (10-20%) takes place as a result of elimination of the polyester from the polymer matrix, whereas significant chemical changes do not occur in PE. In films containing 30% Bionolle®, both the polyester and PE undergo chemical changes.

The results of this research show that PE films modified with Bionolle® polyester are biodegraded more quickly than polyethylene alone.

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(No date given)