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Biodegradation of poly(ethylene terephthalate) modified with polyester "Bionolle®" by *Penicillium funiculosum*

Summary — The purpose of the study was to determine the degree of biodegradation of poly(ethylene terephthalate) films modified with "Bionolle®" polyester in comparison with films made of neat commercial poly(ethylene terephthalate). After 84-day incubation in the presence of filamentous fungi *Penicillium funiculosum* or their extracellular hydrolytic enzymes secreted by "Bionolle®" weight loss of the films was measured. The texture of the polymeric samples was observed with a scanning electron microscope. Important chemical changes of polymeric chains were detected by FT-IR and XPS analysis. Significant reduction in quantity of aromatic rings derived from terephthalic acid indicated that decomposition of films by fungi occurred not only due to hydrolytic enzymes but also oxidative ones. Moreover, we observed rather unilateral influence of poly(ethylene terephthalate) on biodegradation: addition of "Bionolle®" didn't accelerate significantly degradation of modified films and — what is more important — the presence of PET inhibited decomposition of easily biodegradable "Bionolle®".
Keywords: biodegradation, PET/"Bionolle®" composition, fungi *Penicillium funiculosum*, enzymes.

BIODEGRADACJA POLI(TEREFTALANU ETYLENU) MODYFIKOWANEGO POLIESTREM „BIONOLLE®" WYWOŁANA PRZEZ *PENICILLIUM FUNICULOSUM*

Streszczenie — Celem badań było porównanie stopnia biodegradacji folii wykonanych z poli(tereftalanu etylenu) (PET) i z PET modyfikowanego alifatycznym poliestrem „Bionolle®". Prowadzono trwającą 84 dni degradację folii spowodowaną grzybem mikroskopowym *Penicillium funiculosum* lub wydzielanymi przez niego zewnątrzkomórkowymi enzymami hydrolitycznymi indukowanymi w obecności „Bionolle®". Po tym czasie badano ubytek masy folii (tabela 1), obserwowano w skaningowym mikroskopie elektronowym (SEM) zmiany tekstury kompozycji (rys. 1), a także — metodami FT-IR (rys. 2 i 3) i spektroskopii fotoelektronów (XPS) (tabela 2 i 3 oraz rys. 4—7) zmiany chemiczne polimerów wchodzących w skład badanej kompozycji. Istotne zmniejszenie zawartości pierścieni aromatycznych pochodzących z kwasu tereftalowego w PET świadczyło o tym, że grzyb degradował tworzywo z wykorzystaniem nie tylko enzymów typu hydrolaz, ale także oksydoreduktaz. Dodatek „Bionolle®" nie przyspieszył biodegradacji folii politereftalanowych, a co więcej, obecność PET zahamowała rozkład tego łatwo biodegradowalnego poliestru alifatycznego.
Słowa kluczowe: biodegradacja, kompozycja PET/„Bionolle®", grzyb *Penicillium funiculosum*, enzymy.

Polymers, such as polyethylene, polystyrene, polypropylene or poly(ethylene terephthalate) (PET), are widely used in various fields and for this reason should be generally recycled. However this process is complex and has one major drawback: the materials are degraded during normal usage and by the recycling process [1]. In Poland, the PET packaging has a very short shelf life, therefore about 90 thousand tons of this plastic, representing 10 % of all waste plastic, are sent to landfill sites per year [2]. These materials, due to high molecular weight and hydrophobicity, are resistant to environmental factors and after usage they become a burdensome ballast to the environment [3]. It is known that fungi and bacteria, which are the major component of the biosphere, are responsible for breakdown of organic compounds and circulation of elements in the environment [4]. These microorganisms with the ability to adapt to diverse and changeable environmental conditions are able

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to degrade even totally new synthetic compounds. Therefore, attempts to raise the susceptibility of plastic to degradation are mainly aimed at the action of microorganisms. For this purpose, the modification of such synthetic polymers as polyethylene or polystyrene with *inter alia* natural compounds, *e.g.* starch, cellulose, lignin and poly(hydroxybutyrate) (PHB) is used [1, 5—10].

Boost to the vulnerability of PET to degradation is related mainly to increased sensitivity to enzymatic and/or non-enzymatic hydrolysis, and so far the main way to obtain biodegradable PET materials is copolymerization of PET with oxyethylene diols, adipic acid, L-lactic acid, ε-caprolactone, sebacid acid, oxybutylene diol, ethylene glycol or succinic acid [11—15]. Such copolymers undergo enzymatic decomposition by the action of esterases, lipases and cutinases [12, 16, 17].

In our prior works we examined polyethylene (PE) films modified with aliphatic synthetic polyester "Bionolle®". We found out that addition of the polyester has enabled a significant degradation of low-density polyethylene in PE/"Bionolle®" compositions. Films containing 40 % polyethylene and 60 % "Bionolle®" were completely degraded within 84 days of incubation with filamentous fungi *Penicillium funiculosum*, *Gliocladium solani* and *Talaromyces flavus*. One of the most active fungi turned out to be *Penicillium funiculosum* secreting both hydrolytic and oxidative enzymes [18—20]. Our findings are in agreement with the literature where *P. funiculosum* is described as an effective degrader of many natural and synthetic materials, namely wood, PE, poly(hydroxy alcanoate) or compounds like monocyclic and polycyclic aromatic hydrocarbons [21—25].

The aim of this work was to examine whether the modification of poly(ethylene terephthalate) with polyester "Bionolle®" accelerates the degradation of modified films using *Penicillium funiculosum* and to demonstrate if fungal extracellular enzymes secreted in the presence of polyester "Bionolle®" are able to catalyze the hydrolysis of ester bonds in PET as well as in "Bionolle®" [20].

EXPERIMENTAL

Materials

— PET with intrinsic viscosity 0.650 dl/g was obtained from Elana, Toruń, Poland; apart from ethylene glycol, for its preparation 1,4-dimethylcyclohexane was used.

— Pelletized "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.

Polymer blend preparation

Before use, polymers were dried: PET at 68 °C under vacuum for 4 h, while "Bionolle®" 3001 at 70 °C under vacuum for 2 h. Preparation of the blends was carried out in a two-step process. For example, in the case of PET mixture containing 50 wt. % of "Bionolle®" the composition was first homogenized, extruded and granulated by a Berstorff ZE-25x33D (GmbH, Germany) modular co-rotating twin-screw extruder (φ = 25 mm, L/D = 33). The temperature profile along the extruder barrel was as follows: 140/192/195/210/217/232/232/234/226/239 °C and the screw speed was 80 rpm. Next the film was prepared on Plasti-Corder PLV 151 (Brabender, Germany) single-screw extruder (φ = 18 mm, L/D = 24). The barrel temperatures were maintained at the level of 220/235/235/240 °C. In the same manner, from suitable quantities of starting polymers, the compositions 100/0, 90/10, 75/25 and 0/100 PET/"Bionolle®" were prepared at 210/220/230/230, 230/245/245/250, 220/235/235/240 and 210/230/230/235 °C, respectively; in all cases screw speed was 50 rpm.

Samples of films were then cut into strips size of 40 mm × 10 mm, weighed, disinfected with 70 % isopropyl alcohol and washed in sterile distilled water.

Microorganisms

Filamentous fungi *Penicillium funiculosum* was isolated from a dump in Sosnowiec and its identification was carried out by Institute for Ecology of Industrial Areas in Katowice, Poland. Fungi were maintained in test tubes containing Czapek-Doxa medium [$NaNO_3$ (2 g), KH_2PO_4 (0.7 g), K_2HPO_4 (0.3 g), KCl (0.5 g), $MgSO_4 \cdot 7 H_2O$ (0.5 g), $FeSO_4 \cdot 7 H_2O$ (0.01 g), sucrose (30 g), Bacto Agar (Difco) (20 g), distilled water (1000 ml), pH 6.0] [26]. Cultures were incubated at 30 °C and humidity up to 90 % for 2 weeks.

P. funiculosum spores used for the experiments were separated from hyphae by centrifugation at 4000 rpm and resuspended in surfactant (SDS) solution. The spore suspension concentration was adjusted to 10^6 spores/ml.

Agar plates biodegradation

Biodegradation of the films was performed in Petri dishes containing modified (sucrose-free) Czapek-Doxa medium. Each film was aseptically placed into the sterile medium and covered with 0.1 ml spore suspension. Five replicates were used for each film.

All film samples were incubated at 30 °C and humidity up to 90 % for 84 days, thereupon washed in distilled water to remove as much cell mass from the film as possible, submerged in 1 % Hg_2Cl_2 solution for 5 min to halt further action, thoroughly washed again and dried until a constant weight was obtained.

Enzyme induction and activity

Induction of polyester degrading extracellular hydrolases was conducted in flasks containing 1 g of sterilized — at temperature of 121 °C and under pressure of 1.2 atm

for 20 min — mineral liquid medium [$(NH_4)_2SO_4$ (1 g), $NaNO_3$ (1 g), KH_2PO_4 (1 g), K_2HPO_4 (1 g), $MgSO_4 \cdot 7\,H_2O$ (0.5 g), KCl (0.1 g), $ZnSO_4$ (0.07 g), $FeSO_4$ (0.01 g), $CaCl_2 \cdot 2\,H_2O$ (0.01 g), distilled water (1000 ml), pH 5.2] [27]. 0.25 g of "Bionolle®" and 10^5 spores of *P. funiculosum* were placed into each flask which were then incubated without shaking at room temperature for 7 days. At the end of the incubation period, to remove the mycelium, liquid cultures were filtered through Whatman paper and then through 0.45 μm millipore membrane for sterilization. The sterile culture filtrates were used for the estimation of the enzymes activity.

To determine the activity of esterase and lipase the *p*-nitrophenol butyrate (pNBP) and *p*-nitrophenol palmitate (pNPP) were used respectively. Activities were assayed in 3 ml reaction mixtures consisted of 1.9 ml 0.05 M phosphate buffer (pH 6.0), 0.1 ml pNBP or pNPP, and 1 ml of the sterile culture filtrates. The activity was monitored as an increase in absorption at 410 nm. Accordingly, control samples with or without enzymes, which were denatured by incubation at 100 °C for 10 min were prepared. One unit of activity (1 U) was defined as the amount of the enzymes releasing *p*-nitrophenol at a rate of 1 μmol·min^{-1} [17]. Protein concentration was determined using Bradford method with lysosime as standard.

Enzyme solution biodegradation

Enzymatic degradation of films was carried out in flasks containing 250 ml of the sterile culture filtrates with activity against pNBP of about 11 U/mg protein (esterase) and against pNPP of about 36 U/mg protein (lipase). In order to maintain constant enzymatic activity the filtrates were replaced in 7-day intervals. At the beginning of 84-day degradation period five samples of each film were aseptically placed into separate flasks. After the incubation all film samples were cleaned and sterilized as described for agar plates biodegradation.

Methods of testing

— The weight loss of stripes was measured to a precision of 0.1 mg using an analytical balance. The percentage weight change was calculated.

— Scanning electron microscopy (SEM, TESLA B340) analyzes were applied in order to observe microscopic images of the films' surface. Test samples were coated by exposing to a gold ion beam sputter using PELCO S.C. 6 at 25 mA current for 40 s and under 80 Pa pressure.

— Fourier transform infrared (FT-IR) measurements were carried out with a BIO-RAD spectrometer (model FTS 40A) in the range of 4000—650 cm^{-1}. The FT-IR spectra were recorded at a resolution of 2 cm^{-1} and an accumulation of 32 scans.

— The XPS spectra were obtained using the PHI 5700/660 Physical Electronics Photoelectron Spectrometer with monochromatized AlK_α X-ray radiation (1486.6 eV). The hemi-spherical mirror analyzer measured the energy of the electrons with the energy resolution of about 0.3 eV. The photoelectron emission from the surface area of 800 μm × 2000 μm was recorded. All measurements were performed under UHV (ultrahigh vacuum) conditions of order 10^{-8} Pa. In every case the neutralizer was used due to a charge effect which occurs among non-conducting samples. The binding energy was determined with reference to the C1s line at 284.8 eV of adventitious carbon. The Gaussian-Lorentzian functions were used to fit the XPS core level spectra.

RESULTS AND DISCUSSION

It is well known that the susceptibility of synthetic polyesters to the enzyme catalysis is determined by the conformation of polymer chains, their molecular structure, the length of individual segments and hydrophilicity-hydrophobicity balance within the main chain. In order to be biodegradable the polymer chain must by flexible enough to fit the enzyme active site. This is one of the main reasons why flexible aliphatic polyesters are prone to biotransformation and biodeterioration while more rigid aromatic polymers are not [29]. Since aromatic polyesters [like poly(ethylene terephthalate)] were found to be resistant to biodegradation, numerous efforts were taken to overcome their insensitivity by introducing aliphatic components into the aromatic polyester chain [30]. Below we present the main changes of some PET properties induced by such modification.

Weight and texture

Table 1 shows that after 84-day incubation with *Penicillium funiculosum* or a solution of its extracellular hydrolytic enzymes comparable and slight weight loss of PET was found. Incubation of "Bionolle®" with fungi caused approximately 30-fold higher weight loss compared to the treatment with enzymes (90. 28 % *vs* 2.95 %). Contrary to our expectations, regardless of the "Bionolle®" concentration, modification of PET did not significantly increase weight loss of materials. This small dif-

T a b l e 1. Weight loss of PET, PET/ "Bionolle®" compositions and "Bionolle®" after biodegradation using *Penicillium funiculosum* or its secreted hydrolytic enzymes

Composition PET/"Bionolle®", %	Weight loss, %	
	fungi	enzymes
100/0	0.08	0.06
90/10	0.07	0.05
75/25	0.21	0.11
50/50	0.19	0.14
0/100	90.28	2.95

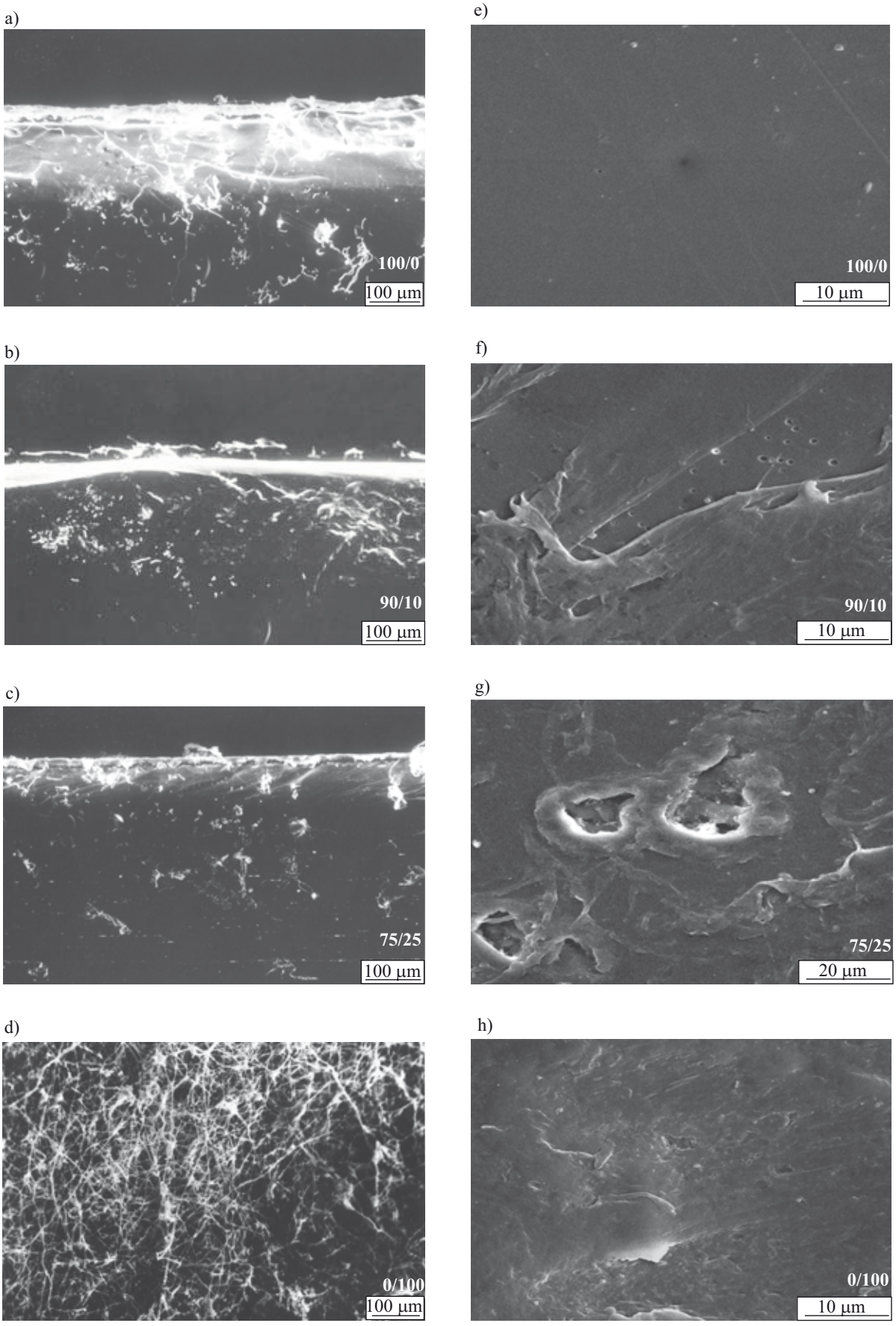

Fig. 1. SEM micrographs of films after biodegradation using: a), b), c), d) — Penicillium funiculosum and e), f), g), h) — hydrolytic enzymes; films notation according to Table 1

ference in the degree of degradation of the studied materials proved that the area available to the enzymatic attack was not increasing linearly with the content of "Bionolle®".

Similar effect was reported by a number of researchers involved in the examination of the biodegradation of PET copolyesters. However, there are no reports on the impact of PET kind on enzymatic degradation of its compositions.

So, enzymatic hydrolysis of copolyester of PET with ε-caprolactone was studied by Tokiwa and Suzuki [31]. As a result of polymer decomposition using lipase isolated from strain *Rhizopus delemar* they found that biodegradation rate decreased with increasing amount of PET in the sample. Similarly, Jun *et al*. [32] demonstrated that poly(ε-caprolactone) was degraded up to 93 % within 3 days in the presence of lipase originated from *Pseudomonas* sp. while the copolymer containing 50 % or more of terephthalate units was completely resistant to enzymatic attack; they explained the results as a reduction in chain flexibility of poly(ε-caprolactone) caused by introducing a rigid chain of PET. Nagata *et al*. [15] prepared aliphatic-aromatic PBST copolyesters [poly(butylene succinate-*co*-butylene terephthalate)] to investigate the effect of the aromatic units on the enzymatic degradation of the films and found that the highest degradation rate was observed for the film with the composition of 17 mol % of aromatic units. Furthermore, Witt *et al*. [33] by investigating the enzymatic degradation of aromatic oligomers containing different amount of terephthalate units showed that oligomers with more than two terephthalate units in the sequence remain almost unaffected.

Taking into consideration all these reports we assumed that small weight losses of examined films were caused by the amount of PET exceeding 50 % despite the fact that polyester "Bionolle®" was present in the films not as a copolymer fragment but as a filler.

The growth of *Penicillium funiculosum*, mainly in the form of spores scattered on the surface of PET film, resulted in the emergence of a few small holes (Fig. 1a), however, there were no changes in the PET texture after 84-day biodegradation in the enzyme solution (Fig. 1e). And again, just as shown for the weight loss, filamentous fungi colonizing all examined modified films decomposed their surfaces to a similar extent as for pure PET (Fig. 1b and c). On the other hand, enzymatic degradation of the film containing 10 % "Bionolle®" proceeded only locally (Fig. 1f), while in the composition with 25 % "Bionolle®" the entire surface was eroded and some large holes of about 10—20 μm were found (Fig. 1g). *Penicillium funiculosum* during growth on "Bionolle®" created a multi-layered dense network of hyphae (Fig. 1d), which hindered observation of the film's surface. The enzymes attacked the aliphatic polyester evenly over the entire surface and that is why the material became thinner (Fig. 1h).

Chemical structure

The FT-IR spectra of PET and modified PET before and after degradation are presented on Fig. 2 and Fig. 3, respectively.

Changes that occur in the PET and "Bionolle®" can be observed in the region 4000—650 cm^{-1} [34—36].

The FT-IR analysis of PET after 84-day incubation with *Penicillium funiculosum* revealed a decrease in intensity of the band at 3340 cm^{-1} assigned to the stretching of O-H of diethylene end-group and decrease in intensity of the band at 3060 cm^{-1} attributed to the stretching of aromatic C-H. A new band at 3030 cm^{-1} was found. The decrease in intensity of the band connected with aliphatic C-H stretching at 2960 cm^{-1} and 2880 cm^{-1} as well as decrease in intensity of all bands in the range 1900 cm^{-1} to 700 cm^{-1} were observed. The new band at 760 cm^{-1} appeared probably due to changes in the substitution patterns of the aromatic rings (Fig. 2, thin continuous line).

Enzymatic degradation of PET had an influence on shifts in wavenumbers of bands correlated with aliphatic C-H stretching from 2960 cm^{-1} to 2980 cm^{-1}, which were caused by oxygen building into the aliphatic chain. The carbonyl band at 1720 cm^{-1} was converted into several separated bands at 1754 cm^{-1}, 1736 cm^{-1}, 1720 cm^{-1}, 1700 cm^{-1}, 1685 cm^{-1}, 1675 cm^{-1}, 1654 cm^{-1} and 1635 cm^{-1}. Whilst aromatic skeletal stretching bands at 1615 cm^{-1}, 1450 cm^{-1}, 1430 cm^{-1} and 1410 cm^{-1} diminished, the new bands at 1650 cm^{-1}, 1560 cm^{-1}, 1540 cm^{-1} and 1520 cm^{-1} appeared. Considerable decrease of band intensity at 1270 cm^{-1} associated with CO-O stretching of ester group as well as decrease of intensity of bands at 1175 cm^{-1}, 1120 cm^{-1} and 1020 cm^{-1} characteristic of aromatic substitution pattern with 1,4-substitution and reduction of band at 980 cm^{-1} giving evidence of O-CH_2 stretching of ethylene glycol segment in PET were observed. Moreover, lower-

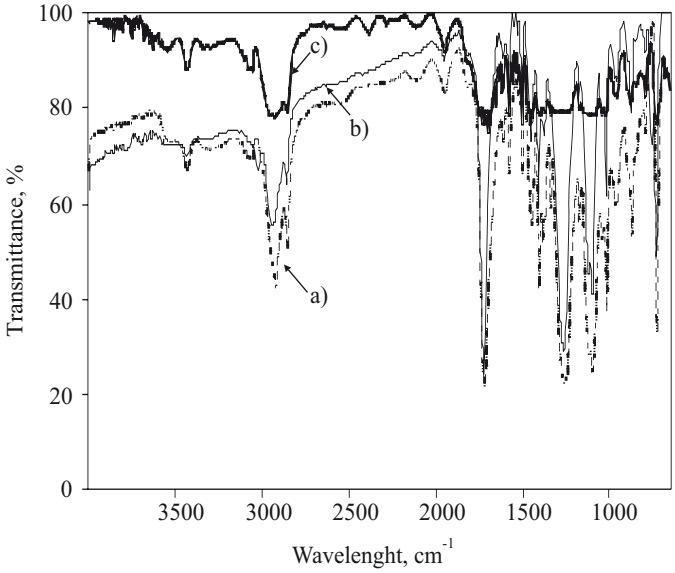

Fig. 2. FT-IR spectra of PET before a) and after biodegradation using b) Penicillium funiculosum, c) hydrolytic enzymes

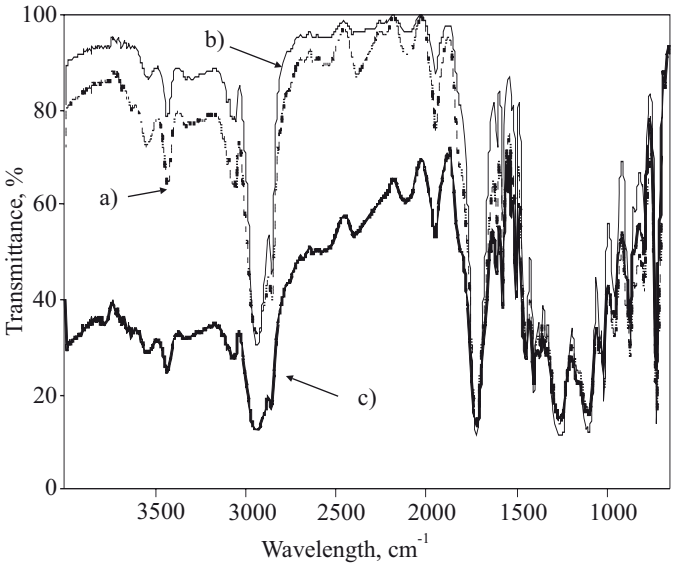

Fig. 3. FT-IR spectra of PET/"Bionolle®" composition (75/25) before a) and after biodegradation using b) Penicillium funiculosum, c) hydrolytic enzymes

ing band intensity at 850 cm^{-1} associated with C-H deformation of two adjacent coupled hydrogens in an aromatic ring and decrease of intensity of band at 730 cm^{-1} accompanied with change in its shape bound with out of plane deformation of the two carbonyl substituents in the aromatic ring were revealed (Fig. 2, thick continuous line). So, from the IR spectra it can be seen that *Penicillium funiculosum* was able to introduce chemical changes not only in aliphatic, but also in aromatic segments of unmodified PET.

In the spectrum of PET containing 25 % "Bionolle®" after 84-day incubation with fungi (Fig. 3b) the peaks at 2960 cm^{-1}, 1720 cm^{-1} and 1270 cm^{-1} slightly increased due to the degradation of PET and/or "Bionolle®". Changes in bands at 1410 cm^{-1}, 1380 cm^{-1}, 1120 cm^{-1} and 730 cm^{-1} indicated deterioration of PET.

After the enzymatic treatment of modified film (Fig. 3c) the change of shape of peaks at 1720 cm^{-1}, 1460 cm^{-1} and new bands at 1650 cm^{-1}, 1640 cm^{-1}, 1630 cm^{-1}, 1550 cm^{-1}, 1530 cm^{-1} assigned to unsaturated bonds or conjugated aromatic bonds were found.

Summarizing, when exposed to the direct action of *Penicillium funiculosum* or only its hydrolytic enzymes, both ester components of the modified films were decomposed. Surprisingly PET as a component of the modified film had been degraded to a limited degree in contrast with PET alone.

Biological transformations — XPS results

In contrast to the films incubated in the hydrolytic enzyme solution, more diverse chemical changes, indicating both the enzymatic hydrolysis and oxidation, were observed in the films incubated with *Penicillium funiculosum*. It is widely known that the secretion of proteins of various types of activity by living organisms could be influenced by many factors. One of them could be the presence of two different substrates — in our research aromatic PET and aliphatic "Bionolle®". Also interaction of the already secreted enzymes with macromolecules can inhibit or increase their activity [37], for example, by reducing the number of enzymes able to adsorb onto the surface of the material *via* binding domain. It could have occurred also during the enzymatic treatment only. Moreover, there is a possibility that the enzymes present in solution secreted by *Penicillium funiculosum* in the presence of "Bionolle®" have not been able to attack PET.

Kawai [38] studied the degradation of copolymers of PET with poly(ethylene glycol) using lipases originated from *Candida cylindracea*, *Fusarium heterosporum* and *Rhizopus arrhizus* and esterases isolated from *Pseudomonas* sp. and *Comamonas acidovorans*. As a result of hydrolytic biodegradation releasing of terephthalate units and poly(ethylene glycol) was observed. The highest degradation activity showed *Comamonas acidovorans* esterase. By contrast, hydrolytic activity of *Fusarium heterosporum* lipase toward polymer was not detected. Finally, the enzymatic degradation of PET and composition of PET with "Bionolle®" was led in the aquatic environment, therefore the observed changes indicating hydrolysis could also be caused by a purely abiotic process.

Abiotic but not enzymatic hydrolysis was reported by Witt *et al.* [33] who indicated that in compost there were no effective extracellular enzymes able to cleave the aromatic ester bonds of aliphatic-aromatic oligomers but in spite of these compounds disappeared, probably due to the chemical hydrolysis.

According to the literature, the enzymatic degradation of polymeric films usually proceeds on its surface since protein catalysts are not able to penetrate deeper parts of samples [39]. Thus it became essential to establish the nature of the changes on the surface of the test materials after the incubation with enzymes.

Table 2 shows atomic concentration of the examined films obtained by XPS analysis in the energy range of

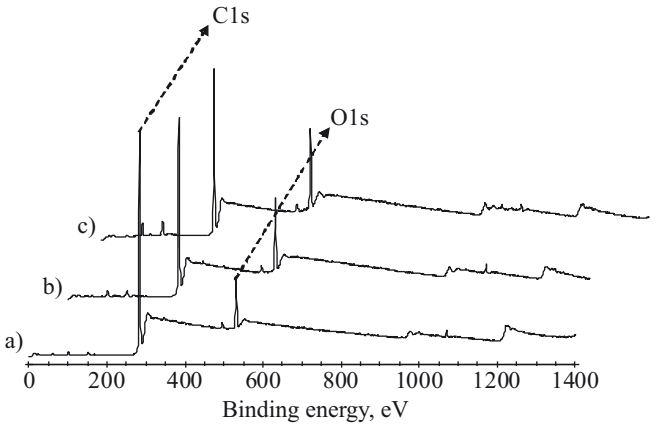

Fig. 4. XPS spectra of PET before a) and after biodegradation using b) Penicillium funiculosum, c) hydrolytic enzymes

T a b l e 2. Obtained by XPS changes of atomic concentrations of PET, PET/"Bionolle®" composition and "Bionolle®" and also of its O/C and N/C ratios caused by biodegradation using *Penicillium funiculosum* or its secreted hydrolytic enzymes

Atomic concentrations %	PET/"Bionolle®" composition, %								
	100/0			75/25			0/100		
	control	fungi	enzymes	control	fungi	enzymes	control	fungi	enzymes
C	87.40	75.61	80.09	86.12	76.76	85.53	80.32	67.03	72.73
N	0.56	0.57	0.56	0.68	0.30	0.43	0.23	4.6	1.34
O	9.40	16.32	14.93	10.16	16.56	10.12	18.51	27.37	24.17
Na	0.87	0.74	0.99	0.98	0.90	0.94	0.04	0.08	0.04
Si	0.99	6.41	2.91	1.24	5.22	2.22	0.76	0.51	1.12
S	0.18	—	0.12	0.18	—	0.12	0.02	0.21	0.09
Cl	0.10	0.04	0.05	0.09	0.03	0.04	0.01	0.01	—
Ca	0.12	—	0.27	0.44	0.18	0.26	0.06	—	0.33
F	0.04	0.09	—	0.06	—	0.16	—	—	0.07
P	0.06	—	0.04	—	—	0.14	0.04	0.17	0.11
Zn	0.03	0.22	0.03	0.05	0.06	0.04	0.02	0.02	—
O/C ratio	0.11	0.22	0.19	0.12	0.22	0.12	0.23	0.41	0.33
N/C ratio	0.006	0.008	0.007	0.008	0.004	0.005	0.003	0.069	0.018

0—1400 eV. The main elements detected with XPS were carbon and oxygen. Smaller amounts of nitrogen, sodium, silicon and trace amount of sulphur, chlorine, calcium, fluorine, phosphorus and zinc were also detected.

The enzyme adsorption is a key process during hydrolysis of water insoluble substrates [40]. The unspecific adsorption of proteins and other cellular components may interfere with the surface analysis by XPS. The adsorption of these macromolecules should be accompanied by a corresponding increase of O and N [41]. From our experiments on PET and modified PET it can be noted that such phenomenon has not occurred, so the analysis of the XPS spectra could be a good determinant of biological transformation of these polymers.

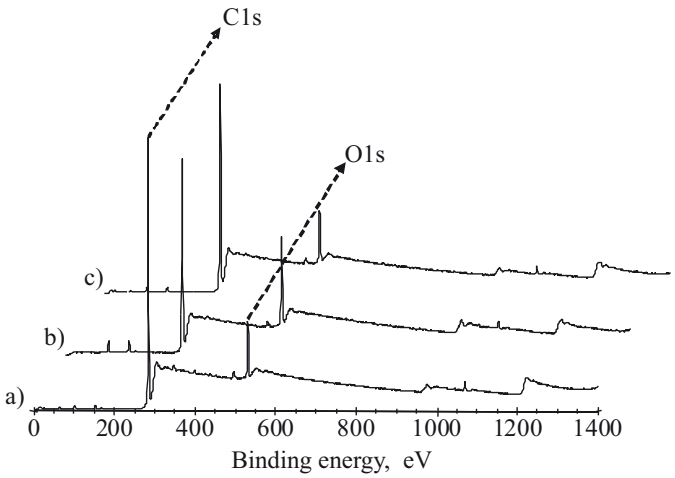

Fig. 5. XPS spectra of PET/"Bionolle®" composition (75/25) before a) and after biodegradation using b) Penicillium funiculosum, c) hydrolytic enzymes

Figs. 4 and 5 show the wide-scan XPS spectra of PET (Fig. 4) and modified PET (Fig. 5) films containing 25 % "Bionolle®" polyester before and after biodegradation. Both films had two main absorption peaks at about 285 and 532 eV corresponding to C1s and O1s, respectively. It was found that compared to the control samples, the oxygen content of films after biodegradation was increased as well as carbon content decreased.

The untreated PET samples and the film samples modified with 25 % "Bionolle®" (Figs. 6a and 6d) display a C1s spectrum with three distinctly discerrible peaks. The main peak located at 285.0 eV is assigned to the aromatic ring of terephthalic acid. The second peak centered at 286.6—287.1 eV is attributed to the carbon of glycol $O\text{-}CH_2\text{-}CH_2$ groups. The third peak centered at 288.8—289.0 eV is associated with the carbon involved in C=O ester and/or carboxyl groups.

The O1s (Figs. 7a and 7d) component at 531.9 eV corresponds to O=C in carboxylate, carbonyl or ester groups, the component at 533.4—533.5 eV to O-C=O.

Table 3 displays binding energies of the main XPS peaks.

Decrease in the percentage of C1s peak area of PET could be associated with the ability of *Penicillium funiculosum* to cleave and oxidize aromatic rings or C-C bonds present in ethylene glycol. Because the oxidation products are mainly carboxylic acids, ketones and aldehydes with various alkyl chain lengths, their increasing concentration was observed at 286.8 and 289.0 eV [42, 43]. Slight changes in the C1s peak after degradation of PET using enzyme solution occurred most likely due to the hydrolytic nature of enzymes acting as egzoenzymes cutting off the end group of terephthalic acid or due to the abiotic hydrolysis in the aquatic environment. The same phenomena were observed for the modified films.

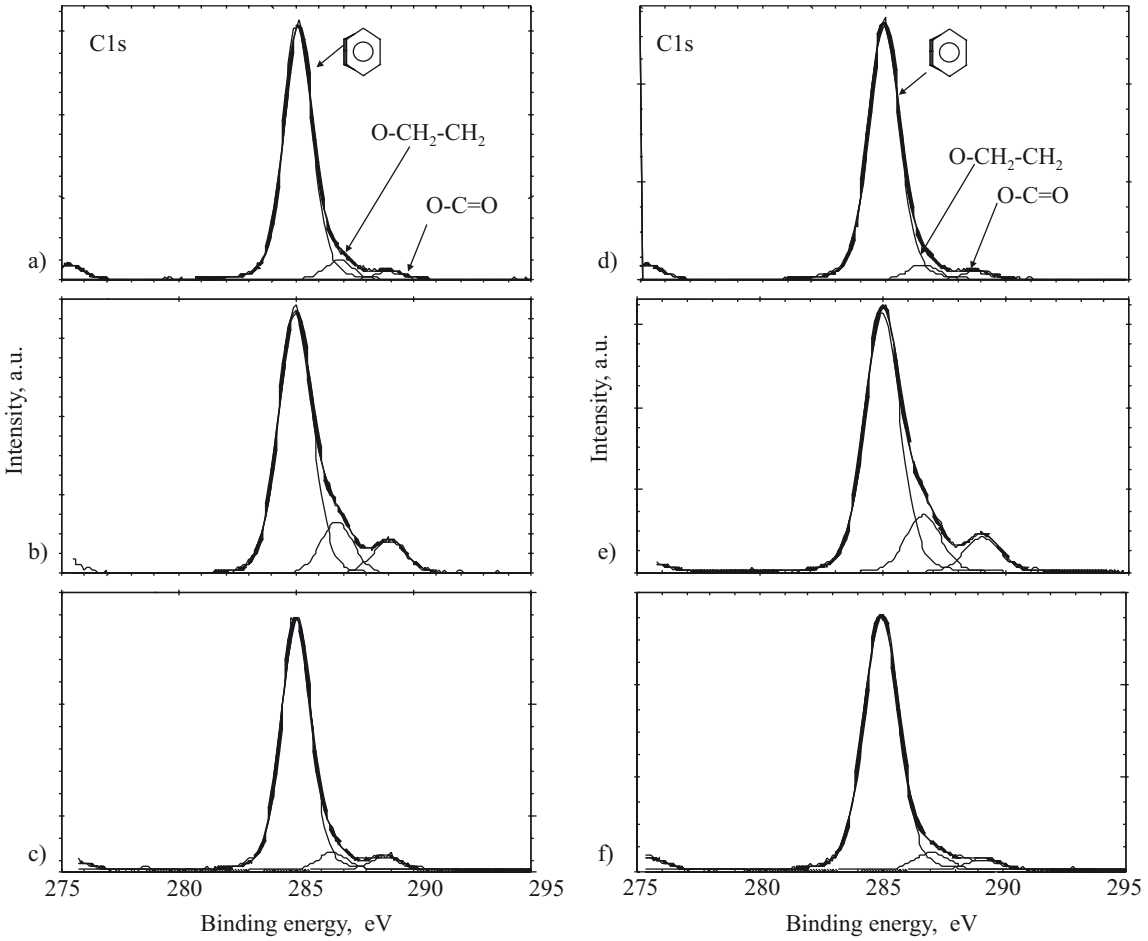

Fig. 6. XPS carbon spectra of PET before a) and after biodegradation using b) Penicillium funiculosum, c) hydrolytic enzymes and PET/"Bionolle®" composition (75/25) before d) and after biodegradation using e) Penicillium funiculosum, f) hydrolytic enzymes

T a b l e 3. Binding energies and area of main peaks of PET and PET/"Bionolle®" composition (75/25) obtained by XPS before and after biodegradation using *Penicillium funiculosum* or its excreted hydrolytic enzymes

Degradation	Binding energy, eV and peak area, %			
	PET/"Bionolle®" composition, %			
	100/0		75/25	
	C1s	O1s	C1s	O1s
Control	285.0 (90 %)	531.9 (82 %)	285.0 (91 %)	531.9 (60 %)
	286.6 (7 %)	533.4 (18 %)	286.6 (6 %)	533.4 (40 %)
	288.8 (3 %)		288.8 (3 %)	
Fungi	285.0 (76 %)	531.9 (75 %)	285.0 (74 %)	531.9 (64 %)
	286.8 (15 %)	533.4 (25 %)	286.6 (16 %)	533.5 (36 %)
	289.0 (9 %)		289.0 (10 %)	
Enzymes	285.0 (89 %)	531.9 (87 %)	285.0 (90 %)	531.9 (70 %)
	286.6 (6 %)	533.4 (10 %)	286.8 (6 %)	533.5 (30 %)
	288.8 (5 %)	534.2 (3 %)	289.1 (4 %)	

Analyzing the O1s line, it was found that fungal PET degradation proceeded mainly *via* oxidation so that the percentage of ester and carboxyl groups among others in the material increased. Biodegradation of pure PET with the enzyme solution or modified PET film, whether it was incubated with the enzymes or fungi, showed that the number of terminal carboxylic groups increased, probably due to the hydrolysis of intramolecular ester bonds [44, 45].

Based on the XPS results obtained after degradation of the films using fungi or only their hydrolytic enzymes we have come to interesting conclusions. Because of decreasing percentage of carbon in the sample, together with the increasing number of carbonyl bonds and increasing percentage of oxygen bound to the surface sample mainly in the form of C=O bonds, we assume that PET is for *P. funiculosum* a stronger inducer of oxidative enzymes than "Bionolle®" of the hydrolytic ones. These findings also explain why addition of even 50 % of "Bionolle®" to PET didn't increase weight loss of material when incubated with fungi, while in the presence of "Bionolle®" its hydrolytic activity allowed for complete degradation of the polyester.

During the incubation of PET with enzymes the amount of carbon in the plastic did not change and slight changes in the carbon peak, in contrast to the oxygen one, infallibly indicate hydrolysis of the film associated with the introduction of an additional oxygen atom from water. For the modified film only the percentage of C=O

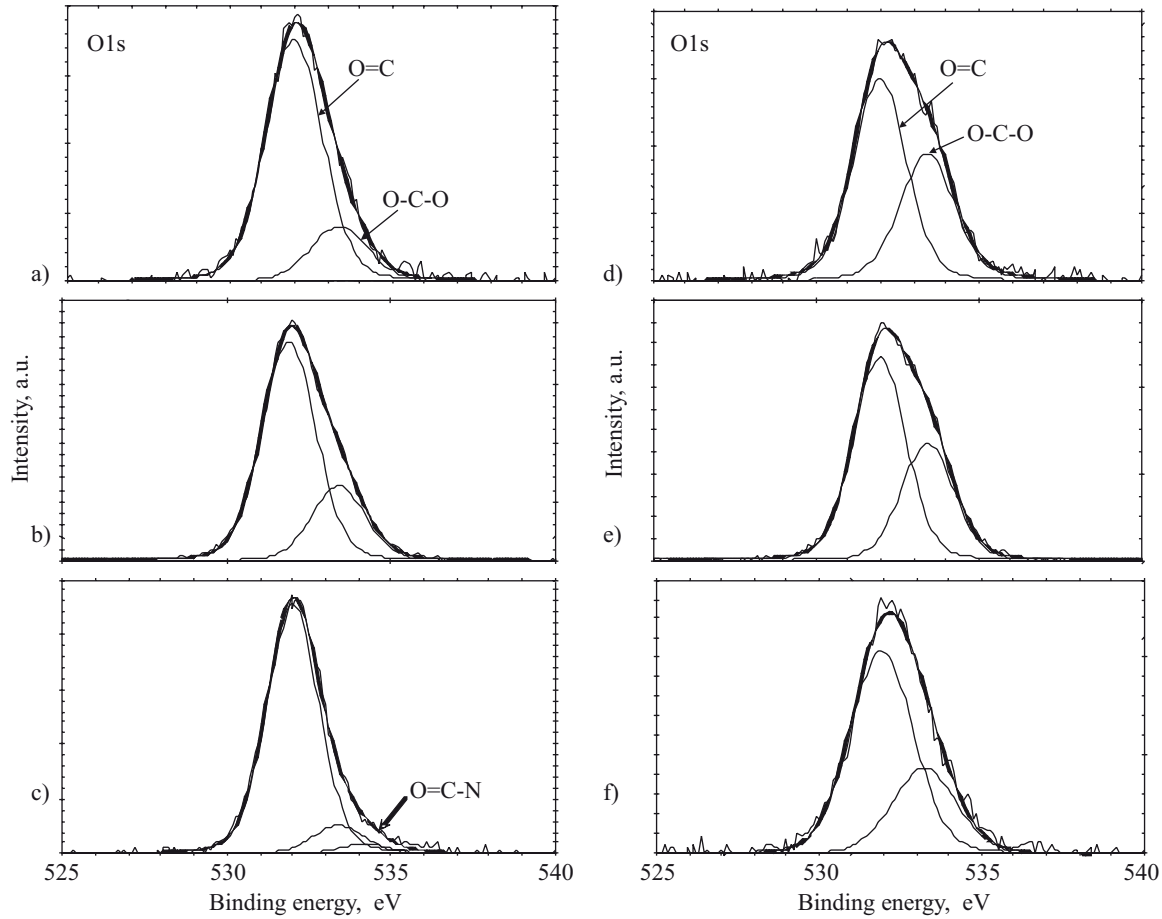

Fig. 7. XPS oxygen spectra of PET before a) and after biodegradation using b) Penicillium funiculosum, c) hydrolytic enzymes and PET/"Bionolle®" composition (75/25) before d) and after biodegradation using e) Penicillium funiculosum, f) hydrolytic enzymes

bonds in relation to O-C=O bonds increased. At the same time constant percentage content of the elements in the plastic could indicate that simultaneously with the slight hydrolysis of the surface connected with the introduction of oxygen, the release of short oligomers of succinic or adipic acid from composition resulting in reduction of carbon proceeded and hence — the ratio of these two elements remained constant.

In the previous studies on activity of *P. funiculosum* toward different polymers conducted also in our department, it appears that the fungi secrete not only poly(hydroxybutyrate) (PHB) depolymerase (esterase), but also several lipases [37]. Among them is an enzyme secreted in the presence of "Bionolle®" with capability of degrading poly(caprolactone) (PCL) and olive oil [46]. PET hydrolyzing enzymes have been reported among esterases (*Penicillium citrinum*), lipases (*Humicola* sp., *Candida* sp., *Pseudomonas* sp., *Thermomyces lanuginosus*) and cutinases (*Fusarium solani*, *Fusarium oxysporum*) [39]. However, Heumann *et al*. [47] showed that there is only marginal correlation between capabilities of enzymes to hydrolyze model substrates such as *p*-nitrophenyl butyrate, *p*-nitrophenyl decanoate or *p*-nitrophenyl palmitate and capability to hydrolyze PET fibers. Thus it seems that this feature should be treated more as individual feature of the single enzyme than the whole group.

Increasing oxygen content during the oxidation of materials is evident and clear and results from introduction of oxygen atoms into aliphatic chain or aromatic ring. The side effect of action of oxidative enzymes is in most cases reduction of molecular weight (*M*) of the polymer resulting from intramolecular random scission of linkages which is practically impossible to control and highly undesirable when enzymes are used as factors modifying plastic surface. As a result of this kind of treatment one can observe a very slight weight loss of the polymer but significant reduction of its molecular weight values.

When hydrolytic enzymes are used, two different effects can be observed depending on the mode of action. Endo-acting enzymes preferentially hydrolyze bonds located centrally in the polymer, leading to the so called surface functionalization not followed at once by the weight loss of product. On the contrary, "exo-wise" hydrolases act mainly on the chain ends, leading to the release of short chain oligomers and accompanying weight loss of the polymer [39]. Hence, a small weight loss of PET and modified PET films was probably associated not only with the secretion and action of oxidative enzymes, but also with insignificant or "endo-wise" mode of action of hydrolytic enzymes on PET.

CONCLUSIONS

Penicillium funiculosum and its extracellular hydrolytic enzymes secreted in the presence of "Bionolle®" showed activity towards both PET and PET modified with "Bionolle®". It cannot be stated that they were able to degrade films containing such high-molecular weight compound as PET, however surely we could observe at least modification of the polymeric samples. Noticeable reduction in the amount of aromatic rings originating from terephthalic acid determined on the basis of FT-IR and XPS spectra pointed out that while growing on films made of PET *Penicillium funiculosum* was destroying materials using oxidative rather than hydrolytic enzymes. Moreover it also became apparent that the addition of "Bionolle®" does not accelerate the degradation of PET film significantly, while the presence of PET inhibits deterioration of easily biodegradable "Bionolle®".

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