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Assessment of the impact of bacteria Pseudomonas denitrificans, Pseudomonas fluorescens, Bacillus subtilis and yeast Yarrowia lipolytica on commercial poly(ether urethanes)

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a b s t r a c t

The assessment of the impact of the bacteria Pseudomonas denitrificans, Pseudomonas fluorescens, Bacillus subtilis and yeast Yarrowia lipolytica on commercial poly(ether urethanes) Tecoflex® and Tecothane® is presented. The polyurethane samples were incubated with pure cultures of the microorganisms at 30 °C for five months. The changes in the chemical structure of the polymers were evaluated using loss of weight and contact angle measurements, infrared spectroscopy (ATR-FTIR), mass spectrometry (Py-MS), differential scanning calorimetry (DSC) and the thermogravimetric analysis (TG). In addition, scanning electron microscopy (SEM) and atomic force microscopy (AFM) were applied for imaging changes in surface morphology of the poly(ether urethanes). Comparative analysis of these polyurethane features before and after incubation with the microorganism cultures showed that Tecoflex® was less stable than Tecothane®. This can be explained by the presence of aromatic rings within the diphenylmethane dii-socyanate group in the chemical structure of the latter. Bacterial strains of Bacillus subtilis and Pseudo-monas fluorescens showed a much more prominent destructive effect compared to the strain of yeast Y. lipolytica.

Keywords: Poly(ether urethanes), Biodegradation, *Pseudomonas denitrificans, Pseudomonas fluorescens, Bacillus subtilis, Yarrowia lipolytica*

1. Introduction

In recent years, more and more attention is paid to the biodegradability of polymeric materials, mainly due to an increase in their use, causing a growth of waste littering landfills. The awareness of this growing problem which poses a serious threat to the environment, leads scientists to look for new solutions to polymer waste treatment including methods involving biological degradation by microorganisms. On the other hand, for polymers used as biomaterials in medicine, their resistance to biodegradation is often more important. Progress toward biodegradable polymers is a great challenge that requires engaging several fields of science: biology, chemistry and biotechnology.

Biological degradation (biodegradation) of polymeric materials takes place under the destructive influence of microorganisms involving a number of biochemical reactions. The decisive role in the biological degradation of polymeric materials in the natural environment is played by microorganisms such as bacteria, fungi, protozoa and algae [1–4].

Polyurethanes (PU) comprise an important group among synthetic polymers in respect of their widespread application and unique properties. They are synthesized from diisocyanates, polyols

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(polyesterol or polyetherols), diols or diamines. Thanks to specific chain structures they are characterized by high resistance towards stretching, and at the same time, are adjustable in a wide range of hardness with good resistance to abrasive wear. Although less thermally stable, with different chemical and biological resistance, these polymers are widely used in many industries as foams, elastomers, adhesives, paints, leather-like materials, fibers, chemicals in construction and coatings, and more recently as biomaterials in medicine [5].

Extensive use of polyurethanes requires knowledge of their susceptibility to biological degradation in order to establish the chemical structures that determine the mechanical properties that do not change in exploitation conditions (in soils for example). PU degradation in the natural environment is dependent on the chemical structure, abiotic and biotic factors.

Howard et al. [6] and Rowe et al. [7] showed that the presence of poly(ester urethane) Impranil DLN ™ in medium has a positive effect on the growth of soil bacteria colonies of *Bacillus* sp.. Cosgrove et al. [8] have identified that the poly(ester urethane) Impranil DLN ™ placed in soil shows a decrease in mechanical properties mainly due to fungi of *Geomyces pannrorum* and *Phoma* sp.. However, degrading properties of the strains *Corynebactrium* sp. [9]. and *Comamonas acidovorans TB-35* [10] isolated from soil have been reported towards the poly(ester urethane) synthesized from poly(diethylene glycol adipate) with 2,4-tolylene diisocyanate. Gautam et al. [11] described the possibility of degrading the waste of poly(ester urethane) foams used in the automotive industry by cultures of *Pseudomonas chlororaphis ATCC 55729*. In turn, Ibrahim et al. [12] marked a degradability of poly(ester urethane) by the fungus *Alternaria solani* isolated from soil. Upreti et al. [13] investigated the susceptibility of a poly(ether urethane) synthesized from polyetherol with cycloaliphatic diisocyanate to degradation affected by the fungus *Aspergillus foetidus* isolated from soil. Umar S. Suresh et al. [14] confirmed in their study the degradation in soil of poly(ester urethane) synthesized from poly(propylene sebacate) with 4,4′-methylene diphenyl diisocyanate and 1,4-butanediol. Kim et al. [15] determined the biodegradability of poly(ester urethanes) of different chemical compositions under aerobic conditions applied according to standards [16]. Urgun-Demirtas et al. [17] showed resilience of commercial poly(-esterether urethane) foams against biological activity of anaerobic microorganisms both in the laboratory and under a layer of soil originating from landfills. Shah et al. [18] rated the destructive act of the bacteria *Bacillus* sp. *AF8, Pseudomonas* sp. *AF9, Micrococcus* sp. *10, Arthrobacter* sp. *AF11, Corynebacterium* sp. *AF12* present in the soil on poly(ester urethane) (4,4′-methylene diphenyl diisocyanate/poly(butylene adipate)/1,4-butanediol) film. Further studies [19–21] highlight a susceptibility to degradation among poly(ester urethanes) by microorganisms from soil. Matsumiya et al. [22] demonstrated that an *Alternaria* sp. was capable of defacing polyether PU. There is a need to undertake further research to assess the impact of microorganisms on poly(ether urethanes), in particular, the ones that are commercially available.

Microorganisms used in this study were selected based on a literature review. They belong to popular strains existing in soil (bacteria *Pseudomonas* and *Bacillus subtilis*) that are likely to have contact with polyurethane materials. Demonstrating that these microorganisms are capable of degradation of polyurethanes would open new ways of polyurethane biological recycling, and the affirmation of resistance to degradation of the above mentioned polyurethanes could help to extend areas of their application [23,24].

An important feature of the ascomycetous yeast *Yarrowia lipolityca* (synonym: *Candida lipolytica*) is the ability to use substrates of different composition as carbon and energy sources, such as paraffin oils, while collecting a large amount of biomass [25]. This allows for the use of *Y. lipolityca* deposited on polyurethane foams to neutralize oil-contaminated areas [26]. *Yarrowia lipolytica* are microorganisms having the capacity to secrete a large number of enzymes: lipases, proteases, esterases. They are frequently used in degradation studies of different groups of polymers.

The aim of this study is to determine the degradative activity of these microorganisms on segmented commercial poly(ether urethanes).

2. Materials and methods

2.1. Materials

Poly(ether urethane) (PEU) of commercial type Tecoflex® and Tecothane®, products of Lubrizol company (U.S.A.), were used in this study. Tecoflex® (EG -80A, EG) is synthesized from 4,4′-methylenebis(cyclohexyl) isocyanate (HMDI) and Tecothane® (TT-1085A, TT) from 4,4′-methylene diphenyl diisocyanate (MDI). In the synthesis of both polyurethanes polytetrahydrofuran (PTHF) was used as polyetherol and 1,4 butanodiol as a chain extender (Fig. 1). The samples of poly(ether urethanes) (1 mm thickness, 10 × 10 mm) were obtained by the process of extrusion and were incubated with a culture of microorganisms after sterilization in ethanol for 1 h.

2.2. Microbial growth media

The bacteria *Pseudomonas fluorescens* ATCC 13525, *Pseudomonas* sp. (*Pseudomonas denitrificans*) ATCC 19244, *Bacillus subtilis* and yeast *Yarrowia lipolytica* (*Candida lipolytica*) CBS 224 were obtained from the Collection of Microorganisms of the Institute of Cell Biology, Lviv, Ukraine.

Medium SB composition contained per 1 L of distilled water: 20 g glucose, 3 g $(NH_4)_2SO_4$, 0.5 g KH_2PO_4, 0.2 g $MgSO_4 \times 7\ H_2O$, 0.2 g $CaCl_2 \times 6\ H_2O$, 2 μg biotin, 0.06 mg H_3BO_3, 0.04 mg $CuSO_4 \times 5\ H_2O$, 0.05 mg $MnSO_4 \times 7\ H_2O$, 0.12 mg $(NH_4)_6Mo_7O_{24} \times 4\ H_2O$, 0.3 mg $ZnSO_4 \times 7\ H_2O$, 3.9 μg iron as $FeSO_4 \times 6H_2O$, and 20 g agar.

Medium NB contained 20 g glucose, 8 g bacto nutrient broth and 10 g NaCl per 1 L of distilled water.

2.3. Biodegradation test

Flasks with TT and EG samples were embedded in cultures of bacteria *Pseudomonas fluorescens* (50 μg/ml; TTPf, EGPf), *Pseudomonas denitrificans* (50 μg/ml; TTPd, EGPd), *Bacillus subtilis* (50 μg/ml; TTBs, EGBs) on NB medium (TTNB, EGNB) and yeast *Yarrowia lipolytica* (50 μg/ml; TTYl, EGYl) on SB medium (TTSB, EGSB) were incubated on a shaker at 30 °C for a period of five months. Every 3 days, the culture medium was removed and replenished with new cultures of suitable microorganisms (50 μg/ml). After the experiment, the samples were washed 3 times with distilled water, dried at 20 °C, and kept for further analysis. The experiments were carried out in three repetitions. The controls (TT control, EG control) were TT and EG samples not incubated with either microorganism cultures nor in NB and SB medium.

2.4. Experimental techniques

Each of the PU (TT and EG) were analyzed before and after incubation in cultures of microorganisms using the following experimental techniques. Changes in the chemical structure of the examined poly(ether urethanes) were examined using indirect methods, i.e. DSC, TG, ATR-FTIR, Py-MS, and the mass loss analysis. Surface imaging was performed by means of SEM and AFM.

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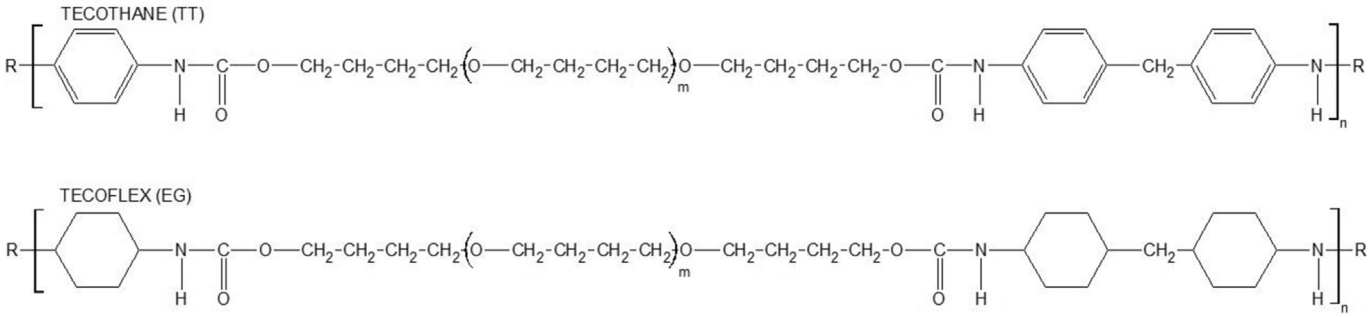

Fig. 1. Schematic structure of macromolecules Tecothane® (TT) and Tecoflex® (EG).

Additionally, each of the PU after incubation in cultures of microorganisms was washed with 70% ethanol solution and then repeatedly with distilled water to remove contaminants from microorganisms from the surface of the polymer before contact angle, SEM or AFM analysis.

2.4.1. Differential scanning calorimetry (DSC)

A thermal analysis was carried out using differential scanning calorimetry (DSC) and a flow calorimeter (DSC 204 F1 Phoenix®, Netzsch, Germany). The temperature range was from −85 °C to 150 °C at the heating rate 10 °C/min.

2.4.2. Thermogravimetric analysis (TG)

The thermal stability of the polymers was determined using a TG 209 F3 apparatus (Netzsch, Germany). Tests were carried out under nitrogen between temperatures of 40−600 °C with a heating rate of 20 °C/min.

2.4.3. Infrared spectroscopy (ATR-FTIR)

FTIR spectra of TT and EG samples were collected using a spectromicroscope (Nicolet iN10 MX N10, Thermo Scientific, Madison, WI, USA) equipped with a one-bounce diamond crystal of the ATR accessory (Smart Orbit). For each spectrum 68 scans were co-added in the range from 600 to 4000 cm^{-1} at a resolution of 4 cm^{-1}.

2.4.4. Py-MS analysis

Structural changes of the PUs were determined using pyrolysis mass spectrometry (Py-MS). A mass spectrometer (model MX-1321 Bagle, Russia) was used, according to the method described in Ref. [27] which allowed us to identify components of the gases from mass to charge (m/z) ratios ranging from 1 to 400. The changes in intensity of the secretion products, the total ion current (J), and the composition of ion fragments produced at different temperatures were analyzed.

2.4.5. Mass loss

The change in weight (Δm) of the samples was calculated from the following equation [1]:

$$\Delta m[\%] = (m_0 - m_1)*100\%/m_0$$

where: m_0 - mass of PEU before incubation and m_1-mass of PEU after incubation in microorganism cultures.

2.4.6. Contact angle

The contact angles of poly(ether urethanes) film surfaces were determined for water (distilled) using an electronic strain gauge extensometer (CAM 200, KSV NIMA, Instruments Ltd. Helsinki, Finland).

2.4.7. Scanning electron microscopy (SEM)

The morphology of poly(ether urethane) surface morphology of samples was examined using a scanning electron microscope (Phenom G2 pro, Phenom-World BV, The Netherlands). Excessive charging of the sample surface by electrons was prevented so that the test material did not have to be prepared in advance by coating with a conductive layer.

2.4.8. Atomic force microscopy (AFM)

Sample surfaces of the poly(ether urethanes) were imaged by means of an atomic force microscope (Innova AFM, Bruker, Tucson,USA) in intermittent contact mode (Tapping Mode).

3. Results and discussion

3.1. Thermal analysis

The results of DSC thermograms analysis of the poly(ether urethane) TT and EG materials are shown in Table 1. Basing on the DSC thermograms the glass transition temperature of the polyurethane soft phase was defined. The curves of changes in mass as a function of temperature (TG) and the weight change derivatives (DTG) are summarized in Table 1. On the basis of the TG curve, temperatures at 5%, 10% and 50% weight loss were determined. From the DTG curves, temperatures of maximum rate of degradation of the hard phase (T_{maxI}) and the soft phase (T_{maxII}) were determined.

3.1.1. TT samples

The glass transition temperature of polyurethane TT soft-phase after incubation in *Bacillus subtilis, Pseudomonas denitrificans, Pseudomonas fluorescens, Yarrowia lipolytica* increased by 1.9−3.5 °C compared to the control sample (TT control) (Table 1). The soft-phase T_g growth indicated that the flexibility of soft polyurethane segments decreased. The segment flexibility reduction could be caused by a variety of factors including (i) a change in the degree of soft-ordering associated with the creation of physical interactions, (ii) a change in the level of interactions between segments, (iii) a change in the chemical structure of macromolecules e.g. resulting from the formation of chemical crosslinking bonds, or due to dissolution of low molecular weight products or by decreasing molecular weight of segments. It can be assumed that the 50% weight loss temperature determined by TG corresponds to the soft phase degradation. For materials after incubation, this temperature decreased by 5.1−12.4 °C compared to the control samples. The T_{maxII} for these materials was also reduced by about 12 °C. These changes indicate that the length of flexible polyurethane segments after incubation has changed.

Changes were also observed in the PU hard phase after incubation. The T_{maxI} decreased by 0.9−7.6 °C compared to the control

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Table 1
Thermal analysis data of the poly(ether urethanes) TT and EG before and after incubation in microorganism cultures: *Bacillus subtilis* (TTBs; EGBs), *Ps.fluorescens* (TTPf; EGPf), *Ps. denitrificans* (TTPd; EGPd), *Yar. lipolytica* (TTYl; EGYl) for five months.

Sample	T_g (°C)	Temperature (°C) weight loss			T_{max} (°C)	
		5%	10%	50%	I peak	II peak
TT control	-52.1	324.7	338.2	401.1	371.8	429.4
TTBs	-48.6	329.0	341.6	392.4	370.9	417.1
TTPf	-50.2	327.0	339.9	396.0	369.0	417.5
TTPd	-48.2	332.5	345.3	395.3	372.9	419.8
TTYl	-49.0	326.4	336.6	388.7	364.2	417.4
EG control	-49.1	310.0	327.1	411.8	344.1	423.0
EGBs	-42.0	322.5	331.6	372.7	-	380.2
EG Pf	-44.0	321.0	332.7	382.4	356.5	391.3
EGPd	-47	340.6	355.3	404.9	-	417.3
EGYl	-48.8	333	346.5	402.6	359.8	410.6

sample (Table 1). These changes indicate that during the incubation some processes facilitating hard phase degradation occurred. They could be caused by reduction of the number of hydrogen bonds between rigid segments.

3.1.2. EG samples

Incubation of EG samples in the environment of microorganisms caused changes in their soft phase similar to those in the TT samples, however, the degree of these changes are more apparent. The phenomena that occurred in the soft phase caused an increase in T_g by 5–7.1 °C and T_{maxII} by 31.7–42.8 °C. The temperature of 50% weight loss also increased by 29–39 °C (Table 1). These changes indicated that in the samples exposed to after incubation the flexible segments became shorter or some of them degraded and were transferred to solution.

Somewhat different were the changes of the hard phase in EG samples after incubation as compared to those observed for TT. The temperatures at 5% and 10% weight losses were higher by 12 and 5 °C, respectively, as determined in TG measurements. Also T_{maxI} increased by about 12 °C. These changes suggested that degradation of the EG hard phase during incubation was not very effective. This may be explained by an increase in the amount of hydrogen bonds between the rigid segments in EG, resulting in some additional phase separation.

Biodegradability of poly(ether urethanes) evaluated by measuring their thermal stability determined by TG, DSC, DTG analysis are very scarcely found in the literature. However, a shift towards lower temperatures of the weight loss maximum rate for both urethane bonds (rigid segment) and ester bonds (flexible segment), and the changes in the glass transition temperature of poly (ester urethanes) after incubation in compost was demonstrated by Wojturska et al. [28]. The work pointed out that observed changes may be due to a decrease in the molecular weight of the samples tested after degradation in composting conditions. Changes in thermal properties of poly (ester urethanes) after incubation in culture of the fungus *Aspergillus flavus* were also reported by Mathur and Prasad [29].

In summary, determination of T_g by the DSC technique for the examined samples before and after incubation in the degrading medium allowed us to characterize indirectly the progress of the poly(ether urethanes) biodegradation. The observed elevation of T_g may indicate an increase in the rigidity of the polymer due to limited mobility of macromolecules.

3.2. ATR-FTIR spectroscopy

FTIR spectroscopy has been widely used for assessing biodegradability of polymers, including polyurethanes [30]. Relevant information may be derived following changes in the absorbance pattern of bands assigned to specified functional groups present in poly(ether urethanes).

3.2.1. TT samples

The ATR-FTIR spectra (Fig. 2) of TT samples showed the following major bands: υ (NH) at 3324 cm^{-1}, υ (CH_2) at 2952 cm^{-1}, the carbonyl bond υ (C = O) non hydrogen-bonded at 1732 cm^{-1} and υ (C = O) hydrogen-bonded at about 1704 cm^{-1} which are associated with urethane hard segments. At lower frequencies we could identify were the aromatic ring (C=C) band at 1598 cm^{-1}, bands at 1533 cm^{-1} and 1311 cm^{-1} assigned to δ (NH) + υ (CN) vibrations of urethane group, a band at 1225 cm^{-1} due to υ (CN), a band at 1112 cm^{-1} corresponding to the non-connected by hydrogen bond υ (COC), at 1083 cm^{-1} from hydrogen bonded υ (C-O-C) stretching, and at 983 cm^{-1} owing to the C-O-C group vibrations [9,31]. The band at 1598 cm^{-1} attributed to aromatic ring carbons in the poly(ether urethane) TT should not change during incubation in a biological environment, according to the literature [32], and thus it was adopted as the internal standard. The other absorbance peaks were thus normalized against it.

After incubation of TT in cultures of *Bacillus subtilis*, *Pseudomonas fluorescens* and *Pseudomonas denitrificans,* the only decrease in absorbance was observed in the band assigned to υ (C-O-C) group bound by hydrogen bonds (Fig. 2). This change could be caused by the oxidative activity of enzymes released by micro-organisms towards PU sample. In another study [33] it was suggested that poly(ether urethanes) may be degraded only to a small extent, however, isolation and identification of their hydrolysis products was possible. The polymer interaction with *Yarrowia lipolytica* culture also resulted in a decrease in absorbance of the C-O-C band, also suggesting possible mechanism of oxidation. The intensity of absorbance of the N-H vibration bands and C = O carbonyl group was not observed in relation to the control, suggesting that hydrolysis of urethane binding of TT samples after exposure to

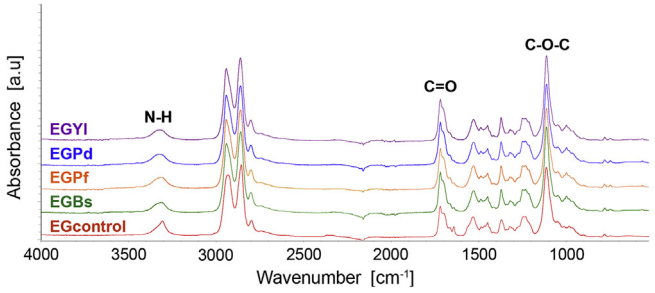

Fig. 2. ATR-FTIR spectra of poly(ether urethanes) TT: before (TTcontrol) and after incubation in microorganism cultures: *P. fluorescens* (TTPf), *Bacillus subtilis* (TTBs), *Ps. denitrificans* (TTPd), *Yar. lipolytica* (TTYl) for five months.

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bacteria and yeast did not proceed.

3.2.2. EG samples

The infrared spectrum of the EG sample (Fig. 3) showed prominent bands υ (NH) at 3324 cm^{-1}, υ (CH_2) at 2850 cm^{-1}, υ (C = O) hydrogen-bond unbound at 1717 cm^{-1}, υ (C = O) hydrogen bond bound at 1704 cm^{-1}, υ (C-C) in the cyclohexane ring at 1321 cm^{-1}, and at 1532 cm^{-1} assigned to δ (NH), υ (CN) of the urethane group. At lower frequencies there were also present bands at 1231 cm^{-1} due to υ (CN), and at 1112 cm^{-1} υ (COC) due to hydrogen-bond unbound. The stretching (υ) vibrations of (COC) hydrogen-bond bound group corresponded to the 1048 cm^{-1} wavenumbers [32]. In the case of this polymer, the absorption of band υ (C-C) at 1320 cm^{-1} was taken as the internal standard, in comparison to which other signals were normalized. The band does not alter its intensity during incubation in a biological environment [31,34].

The intensity of the absorbance at 3316 cm^{-1} υ (NH) decreased to the largest extent for EG incubated in the culture of *Bacillus subtilis* as compared to the control sample EG, while for the band at the 1717 cm^{-1} (due to υ (C = O) of urethane group) the largest increase in the intensity of absorbance was observed (Fig. 3). Also, an increase in the absorbance was found for the hydrogen-bond bounded C = O in the urethane group at 1700 cm^{-1}. The observed changes in the infrared absorbance pattern may indicate that the microbial activity was the cause of the polymer degradation, particularly through urethane-bond hydrolysis. It should be taken into account that released NH group could be assimilated by microorganisms as a source of N after the exhaustion of the resources in the culture medium. Similar spectral changes were observed for the samples of EG after incubation in the culture of *Pseudomonas fluorescens* while they were relatively smaller in the case of *Pseudomonas denitrificans*. Similar consequences of microbial activity towards the urethane bonds, i.e. modifications in the absorbances at 3316, 1717 and 1700 cm^{-1} were observed when polyurethane EG was incubated in the culture of *Y. lipolytica.*

3.3. Py-MS analysis

The use of mass spectrometry with sample pyrolysis enabled evaluation of changes in the chemical structure of the polymers based on the analysis of their degradation at high temperatures. The results obtained with this technique (at the same ionization energy) may indicate degradation of the polymer that was confirmed by an increase in value of the total ion current. Such a change might be due to the release of larger amounts of shorter fragment ions more susceptible to ionization.

3.3.1. TT samples

The thermograms of TT polyurethane samples after incubation for a period of 5 months in culture medium and the culture of microorganisms were characterized by a two-stage course of the curve (Fig. 4). Curves of changes in the total ion current of released volatile products during thermal degradation of the polymer (Fig. 4) confirmed the model of a 2-phase structure of polyurethanes. The thermograms showed two peaks (Fig. 4). The first one, referring to the distribution of hard segments, has a value greater than the maximum thermal degradation of the polyether soft segments of TT. Furthermore, the curves have a smooth transition from the first to the second peak, which can be explained by the biphasic morphology of polyurethane structure, where parts of hard domains are randomly distributed in a soft matrix.

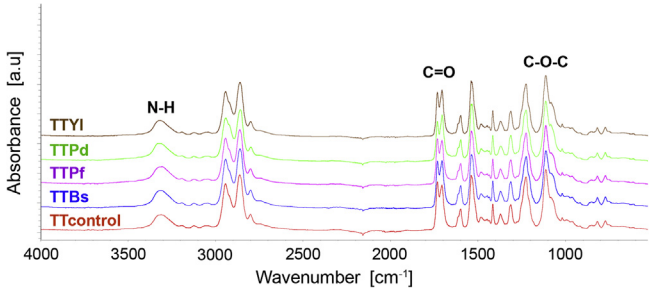

Fig. 3. ATR-FTIR spectra of poly(ether urethanes) EG: before (EG control) and after incubation in microorganism cultures: *P. fluorescens* (EGPf), *Bacillus subtilis* (EGBs), *Ps. denitrificans* (EGPd), *Yar. lipolytica* (EGYl) for five months.

We observed an increase in value of the total ion current for both phases I and II for TT incubated in the culture of *Bacillus subtilis* (an increase for both steps, and the first was higher compared to the second stage) and *Pseudomonas fluorescens* (the same for both stages), *Yarrowia lipolytica* (small growth) compared with the control sample. In the case of *Pseudomonas denitrificans* (hard segments only) this indicates that the microorganisms caused changes in the supermolecular segment structure of PU. Increased total ion current value of the second stage is due to the release of more fragment ions originating from the thermal decomposition of PTHF polyol. Changes in the flexible segment confirmed the results obtained for these samples with the use of TG, DTG and ATR-FTIR techniques.

3.3.2. EG samples

The increase in the total ion current for aliphatic poly(ether urethane) EG was also observed for both phases (I and II) for EG incubated with *Bacillus subtilis* (the largest) and with *Pseudomonas fluorescens*, compared with the control sample (Fig. 5). In contrast, for the incubation in the culture of *Pseudomonas denitrificans*, the increase in total ion current occurred only for the stage I (a rigid segment). Samples EG incubated in culture *Yarrowia lipolityca* also showed an increase in the total ion current for the two phases but the magnitude was lower in comparison with incubation in the bacterial cultures.

Generally, incubation of EG with the microorganisms resulted in changes in the polyurethane matrix in both segments. The *Pseudomonas denitrificans* culture exclusively affected only the hard segments that were shown by the increase in the intensity of separating the fragment ions. The increase in the value of the total ion current at the same ionization energy may indicate a partial degradation of the polyurethane. Changes in the supermolecular structure of polyurethane EG described above were also confirmed by the analysis of the results obtained by ATR-FTIR and the thermal analysis.

3.4. Mass loss

The observed changes in the sample mass resulting from exposure to the microbial cultures are presented in Table 2. Aromatic polyurethane TT samples did not show any reduction in mass after incubation in cultures of *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pseudomonas denitrificans* and with *Yarrowia lipolytica.* In contrast, some increase in the mass was observed that could be explained by a deposition of the bacteria microorganism at the surface of the samples. In turn, in the case of the aliphatic polyurethane EG, the mass loss occurred for each of the bacterial treatments, higher for *Bacillus subtilis* than for *Pseudomonas fluorescens* and *Pseudomonas denitrificans*. Exposure to yeast *Yarrowia lipolytica* caused a mass loss that was slightly lower compared to the bacterial impact. These results may indicate an initial stage of degradation of the aliphatic polyurethane and elution of low molecular weight fragments from within the sample into solution.

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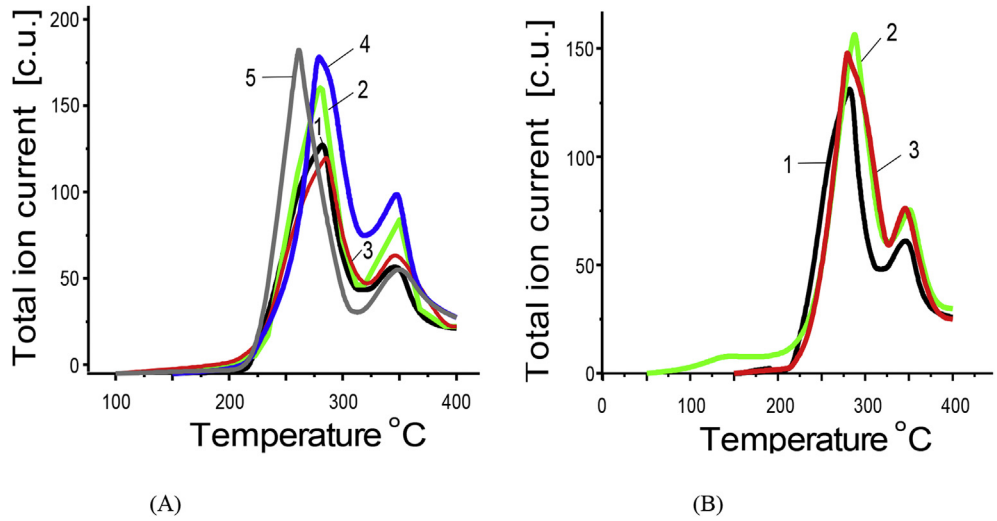

Fig. 4. Curves of changes in the total ion current as a function of temperature during thermodestruction of Tecothane before (1-TT control; A) and after incubation in NB medium (3-TTNB; A), in microorganism cultures: *Bacillus subtilis* (4 –TTBs; A), *Ps.denitrificans* (5-TTPd; A), *Ps.fluorescens* (2-TTPf; A) and before (1-TTfab; B), and after incubation in medium SB (3-TTSB; B) in culture *Yar. lipolytica* (2-TTYl; B) for five months.

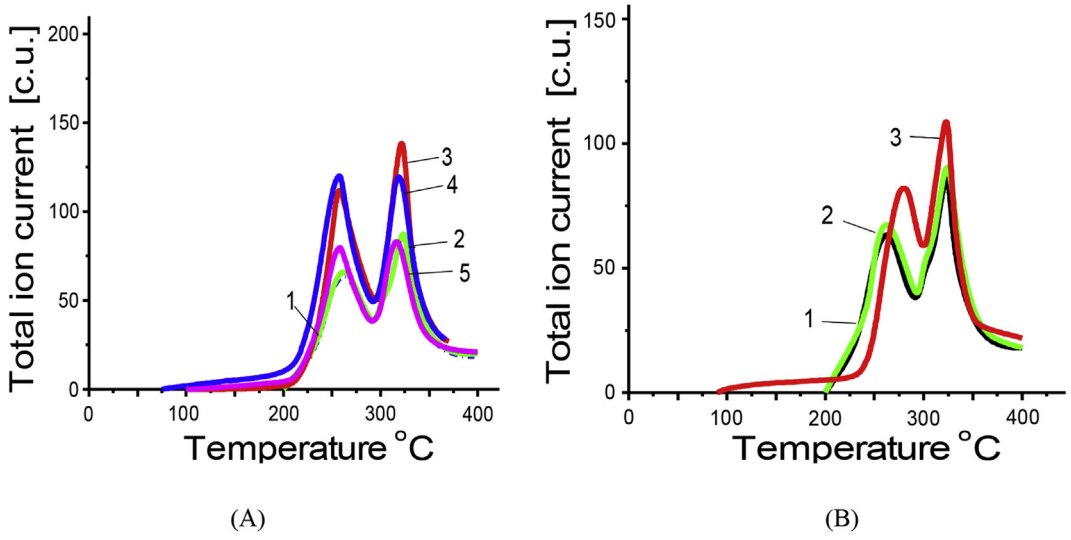

Fig. 5. Curves of changes in the total ion current as a function of temperature during thermodestruction of Tecoflex® before (1-EG control; A) and after incubation in NB medium (2-EGNB; A) in microorganism cultures: *Bacillus subtilis* (3-EGBs; A) *Ps.denitrificans* (5-EGPd; A) and *Ps.fluorescens* (4-EGPf; A) and before (1-EGcontrol; B), and after incubation in medium SB (2-EGSB; B), in culture *Yar. lipolytica* (3-EGYl; B) five months.

3.5. The water contact angle

Poly(ether urethanes) samples TT and EG showed changes in the water contact angle on their surfaces owing to exposure to the microorganisms, depending on the type of the incubation culture and polymer. Only for samples incubated in *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Yarrowia lipolytica* cultures were contact angle measurements made as the above described results indicated that these microorganisms were considerably active in the polyurethane sample degradation. For TT, which was originally characterized by a moderate water contact angle, both incubation in *Pseudomonas fluorescens* and *Yarrowia lipolytica* increased the value of this parameter. The TT showed thus moderately hydrophilic nature due to its aromatic chemical structure, which might hinder the adhesion of microorganisms on the surface of the polymer (higher than TT control). The measured values of the water contact angle were influenced, however, by the microorganism environment, possibly by the activity of secreted enzymes, hydrophobic interactions and van der Waals forces at the border of the outer layer of PU and microorganisms.

EG polymers which are characterized by a hydrophobic surface, hinder the adsorption of microorganisms and secreted enzymes on the surface of the polymer as evidenced by the relatively large water contact angle (98 θ) (Table 3). After incubation in cultures of *Bacillus subtilis* and *Yarrowia lipolytica* for a period of five months, the contact angle changed only slightly but in the case of *Pseudomonas fluorescens* this value was significantly reduced (Table 3). The change in the contact angle value reflects the changes in physicochemical nature of the surface, such as an increase in hydrophilicity and roughness that could result from the impact of the microorganisms and the activity of secreted enzymes.

3.6. Scanning electron microscopy (SEM)

Electron microscopy is a useful tool for examining changes in the morphology of poly (ether urethanes) and helps to assess the

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Table 2
Change of mass TT and EG before and after incubation in microorganism cultures: *Bacillus subtilis* (TTBs; EGBs), *Ps.fluorescens* (TTPf; EGPf), *Ps. denitrificans* (TTPd; EGPd), *Yar. lipolytica* (TTYl; EGYl) for five months.

Sample	Δ mass differences [g] ± SD Δ mass differences % ± SD
TTBs	0.0033 ± 0.0003 +6.8% ± 1%
TTPf	0.0026 ± 0.0002 +5.3% ± 1%
TTPd	0.0012 ± 0.0002 +2.5% ± 0.5%
TTYl	0,0033 ± 0,0004 +6.7% ± 1%
EGBs	-0,0072 ± 0,0003 -10.5% ± 1.5%
EGPf	-0.0049 ± 0.0003 -7.2% ± 1%
EGPd	0.0019 ± 0.0003 -2.8% ± 0.5%
EGYl	0.0033 ± 0.0003 -5.2% ± 1%

Table 3
Water angle contact on the surface of TT and EG before and after incubation in microorganism cultures: Bacillus subtilis (TTBs; EGBs), Ps.fluorescens (TTPf; EGPf), Ps. denitrificans (TTPd; EGPd), Yar. lipolytica (TTYl; EGYl) for five months.

Sample	Water angle contact θ [deg] ±SD
TT control	83.0 ± 4.2
TTPf	82.0 ± 4.1
TTBs	90.0 ± 4.5
TTYl	89.0 ± 4.5
EG control	98.0 ± 4.9
EGPf	83.0 ± 4.2
EGBs	97.0 ± 4.9
EGYl	94.0 ± 4.7

heterogeneity of surface pores or the presence of defects formed in the structure of the polymer. This technique has been successfully applied to evaluate the biodegradation course of these types of polymer [35–38]. Only a few microcavities and craters appeared on the surface of polyurethane TT incubated in the culture of *Bacillus subtilis* and *Pseudomonas fluorescens* (Fig. 6). In contrast, EG sample (Fig. 6) incubated in the same cultures had a larger number of small holes, cracks, cavities and surface irregularities resulting from the interaction of microorganisms.

3.7. Atomic force microscopy (AFM)

Employing SEM, we could observe small changes in the surface morphology of polyurethanes and thus we decided to perform further analysis using AFM at much higher magnification. AFM images collected for the TT samples before (a) and after incubation with cultures of *Bacillus subtilis* (b) and *Pseudomonas fluorescens* (c) are presented in Fig. 7. Several distinct changes in the morphology were observed on the surface of polyurethane TT after incubation in the microorganism environment. Particularly, AFM images showed disappearance of structures resembling boundaries of domain aggregates. In addition, the width of the borders of domain aggregates changed (Fig. 8b). All these changes may indicate initiation of surface erosion of the analyzed samples. AFM imaging of EG samples before (Fig. 8a.) and after incubation in the culture of *Bacillus subtilis* (Fig. 8b) showed the presence of visible cracks, numerous micro-folds and micro-pits which demonstrated surface corrosion. The structural changes of the sample surface of EG samples were much more prominent compared to the samples of poly (ether urethanes) of aromatic TT, which remained almost unchanged.

4. Conclusions

Prediction of potential microbial degradability of polyurethanes requires understanding physico-chemical mechanisms at the interface between the polyurethane substrate and the microorganisms. Examination of aromatic and aliphatic polyurethanes incubated with microbial cultures showed that both TT and EG undergo small but noticeable changes on the film surface as detected by multiple techniques. Exposure of TT samples to cultures of bacteria: *Bacillus subtilis*, *Pseudomonas denitrificans*, *Pseudomonas fluorescens*, and the yeast *Yarrowia lipolytica* resulted in an increase in the glass transition temperature of the polymer and lowering the temperature of weight loss that could be due to rinsing of the resulting low molecular weight polymer fractions. This indicates a partial degradation of polyurethane chains that was confirmed by a marked reduction in the temperature of maximum degradation rate of the soft phase and a decrease in the absorbance bands of the C-*O*-C in the infrared spectra. The degradation within the soft segments could involve oxidative processes caused by the digesting enzymes exuded by the microorganisms.

Changes in the soft segments were also confirmed by Py-MS measurements, which showed an increase in the intensity of the total ion current at the second stage of the thermodestruction after incubation in cultures of *Bacillus subtilis* and *Pseudomonas fluorescens*. In the case of TT samples exposed to bacteria and yeast, no changes of band intensities for N-H and carbonyl C = O groups were observed compared to the control excluding, seemingly, the possibility of hydrolysis urethane bonds. For polyurethane TT, weight loss was not observed, but rather a slight increase. This could result from some water absorption by the polymer at the initial stage of degradation or formation of a biofilm made by microorganisms. Changes in the morphology of the samples surface observed by SEM and AFM also indicated surface erosion. All these evidences lead to the conclusion that bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* showed the highest degradative activity for poly(ether urethane) TT among examined microorganisms.

Similar relationships could be observed for the aliphatic poly(-ether urethane) EG. The changes in the glass transition temperature and reduced temperatures of weight loss after incubation with the bacteria *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pseudomonas denitrificans*, and the yeast *Yarrowia lipolytica* in comparison to the control sample indicated a progressive degradation of the polymer. This degradation was mainly due to bond breakdown of the soft phase, as evidenced by reduced temperature of maximum degradation rate of the soft segments and the decrease in IR band intensities of ether groups. ATR-FTIR analysis also revealed changes in the vibration of urethane bonds: a decrease in the intensity of bands for N-H and an increase in the intensity of bands for C = O.

Changes in the degradation of polyurethane EG within both soft and hard segments were confirmed by the increase in the total ion current of the first and the second stage of polymer degradation after incubation with the culture of *Bacillus subtilis*. *Pseudomonas fluorescens* and *Yarrowia lipolytica* as compared with control sample. However, in the case of *Pseudomonas denitrificans* culture, there was an increase in total ion current only for the first stage, associated with the degradation of the hard segments. This type of degradation was also confirmed by changes in the intensity of infrared absorption bands assigned to functional groups within the rigid and flexible segments.

The weight losses, changes in the water contact angle and in the surface morphology following incubation in cultures of

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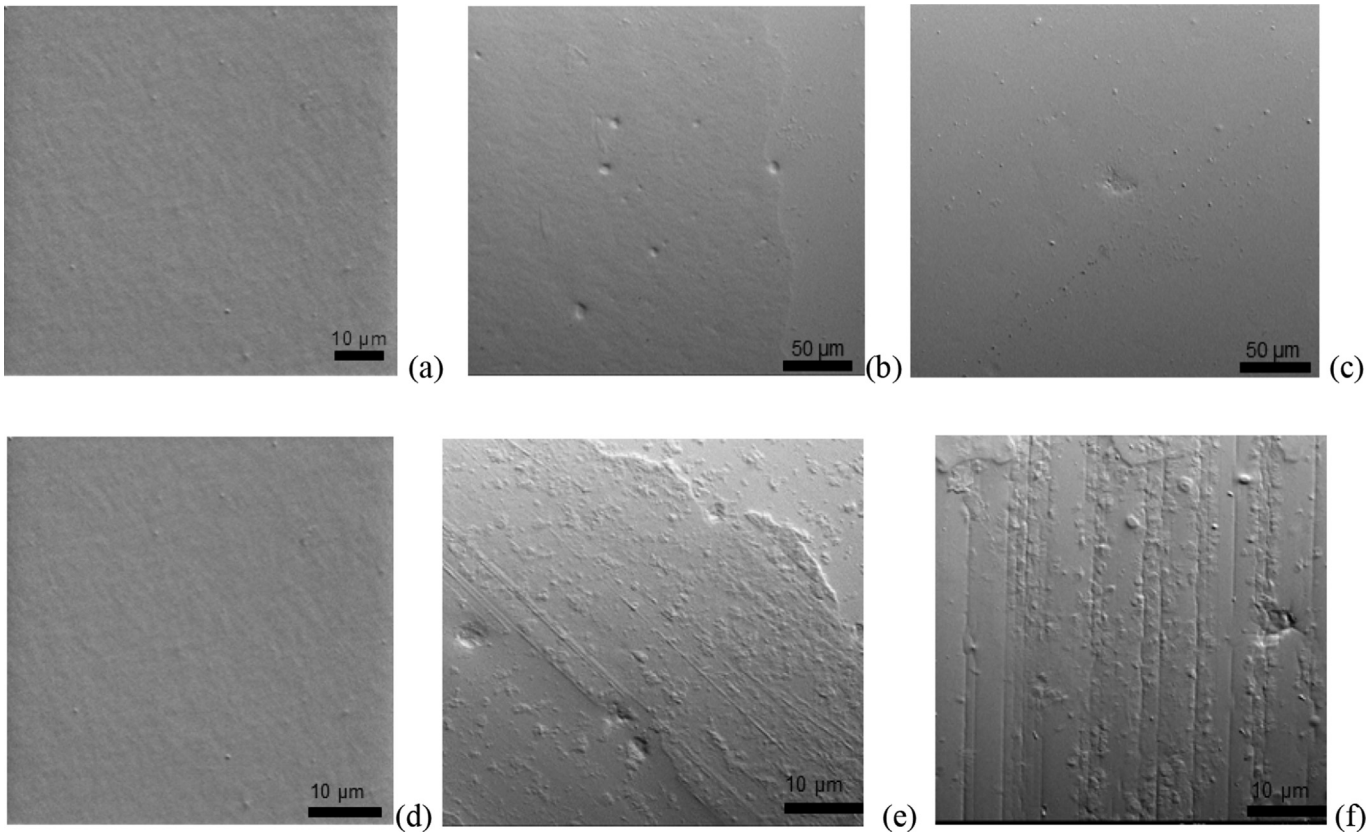

Fig. 6. The SEM images of TT before (a) and after incubation in microorganism cultures: *Bacillus subtilis* (b), *Ps.fluorescens* (c) and the SEM images of EG before (d) and after incubation in microorganism cultures: *Bacillus subtilis* (e), *Ps.fluorescens* (f) for five months.

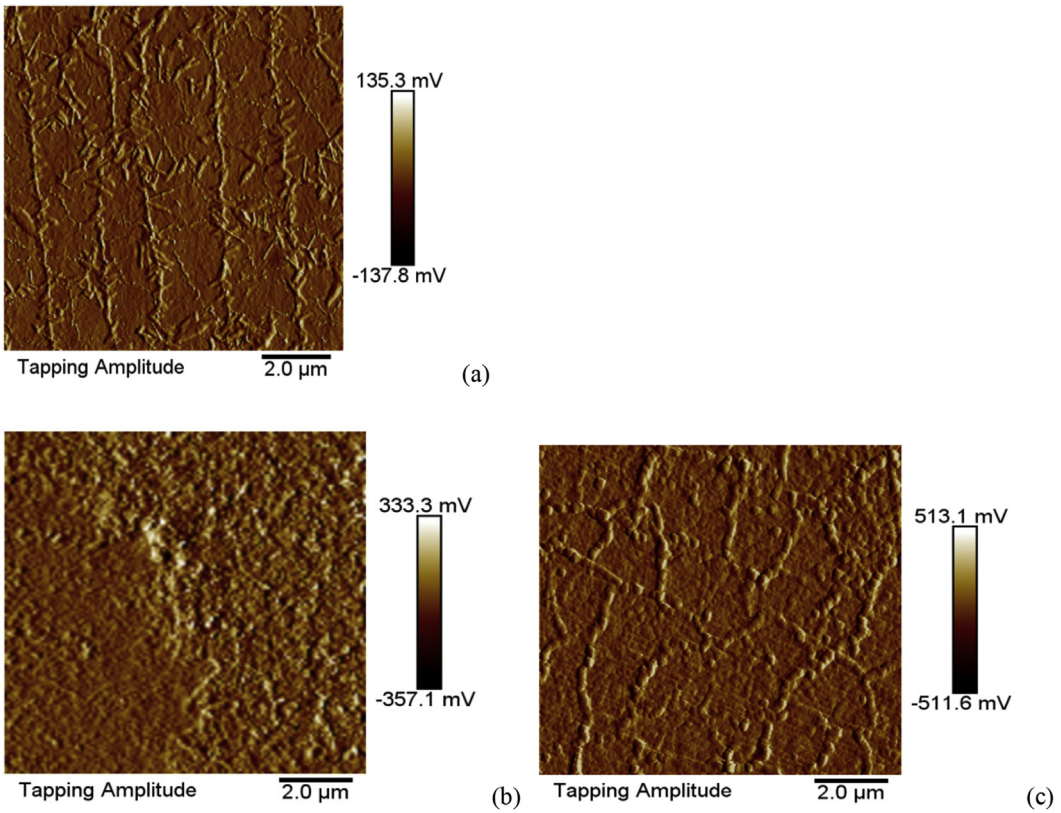

Fig. 7. The AFM images for the TT before (a) and after incubation in microorganism cultures: *Bacillus subtilis* (b), *Ps. fluorescens* (c) for five months.

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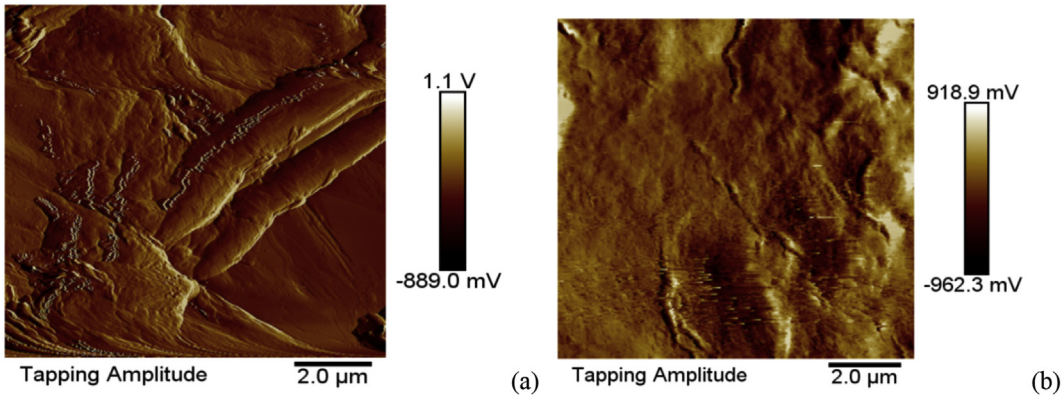

Fig. 8. The AFM images for the EG before (a) and after incubation in microorganism culture: *Bacillus subtilis* (b) for five months.

microorganisms also confirm that the aliphatic polyurethane EG is more susceptible to the influence of biological environment than TT synthesized from the aromatic diisocyanate. Among the microorganisms studied, cultures of *Bacillus subtilis* and *Pseudomonas fluorescens* showed the highest degradative activity against the polymer.

In summary, poly(ether urethanes) derived from aromatic diisocyanate are more resistant to microorganisms than polyurethanes obtained from the aliphatic diisocyanates. This may result from the presence of benzene rings linked directly to urethane groups that spherically impedes access of enzymes secreted by microorganisms to the intramolecular bonds of the polymer chemical structure. Among tested microorganisms, bacteria *Bacillus subtilis* and *Pseudomonas fluorescens* showed the highest degradative activity against analyzed poly(ether urethanes). The results allow us to conclude that polyurethanes exhibit generally significant resistance to biological agents, possibly owing to their hydrophobicity, presence of ether bonds and specific chemical structure of substrates used in the process of their synthesis [5].

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