Małgorzata Lichocik, Monika Owczarek, Patrycja Miros, Krystyna Guzińska, Agnieszka Gutowska, Danuta Ciechańska, *Izabella Krucińska, **Piotr Siwek

Instytut Biopolimerów i Włókien Chemicznych,
ul. M.Skłodowskiej-Curie 19/27, 90-570 Łódź,
E-mail: lab@ibwch.lodz.pl

***Department of Material and Commodity Sciences and Textile Metrology,**
Lodz University of Technology,
ul Żeromskiego 116, 90-924 Łódź, Poland.

****University of Agriculture in Krakow,**
Faculty of Horticulture,
Department of Vegetable and Herbal Plants,
Al. 29 Listopada 54, 31-425 Kraków, Poland

Impact of PBSA (Bionolle) Biodegradation Products on the Soil Microbiological Structure

Abstract
An impact of the decomposition products of Poly(butylene succinate adipate) (PBSA) (Bionolle#3001) in the form of polymer chips and nonwovens made of the polymer upon the qualitative and quantitative microbiological profile of the soil in which samples of the materials were incubated was investigated. The mass loss of the polymer and nonwovens as a result of their biodegradation proceeding in the soil was compared. Two kinds of the soil were applied in the biodegradation tests: garden soil and an agriculture soil taken from the experimental field plots of the Agriculture University (AU) of Cracow. The AU soil despite low moisture content is fertile and holds a great amount of microorganisms. The garden soil does not offer favourable conditions for the biodegradation process, confirmed by a low mass loss in the incubated Bionolle#3001 samples.

Key words: *biodecomposition, aliphatic polyesters, soil biodegradation, microorganisms, soil environment.*

Introduction

One of the big problems of the environment nowadays is the accumulation of huge amounts of non-biodegradable and hazardous plastic waste. Materials that after their final use would in short time undergo a complete decomposition in the soil yielding harmless decomposition products capable of stimulating the growth of plants are searched [1].

The soil environment is a dynamic system dependable on a number of biotic and abiotic factors like physical-chemical conditions, vegetation, microorganisms activity, soil fauna and hydrothermal conditions [2]. The soil is composed of solid part in 50%, soil air in 35% and soil solution in 15%.

In the solid part, mineral and organic colloids in the form of humus compounds which are the nutritive and energy substances for the soil microorganisms are to be distinguished. The soil solution contains dissolved sugars, aminoacids and mineral salts: ammonium, potassium, phosphorous nitrates and others serving as source of nutrients for the microorganisms The soil air fills the voids of the soil; it contains O_2, CO_2, N_2 and NH_3 [3]. The soil is an environment of many microorganisms much diversified in regard to biochemical properties.

Some of them synthesize antibiotics like medical penicillin G and V produced by *Penicillium chrysogenum* and vacomycin by *Streptomyces orientalis* [4]. Others are capable of producing vitamins, polysaccharides, organic acids and enzymes which play an important role in the microbiological degradation of the polymeric materials like esterases and basic proteases produced by *Bacillus sp,* fungi of the *Tritrachium sp* kind and actinomycetes of the *Amycolatopsis sp.*[5, 6] *Paenibacillus sp* families [7, 8].

Poly(butylene succinate adipate) (PBSA) is manufactured by Showa Highpolymer Co. Ltd. under the trade name Bionolle#3001. It is a biodegradable, thermoplastic aliphatic polyester prepared by the reaction of 1,4-butandiol with aliphatic di-carboxylic acids such as succinic- and adypic acid [9, 10].

The producer recommends Bionolle #3001 to be used in standard polyolefin processing equipment with only minor modification. It finds utility in the manufacture of such materials as blown film, monofilaments, flat yarn, sheets and blow-molding [11]. Bionolle may also be used in the production of nonwovens by spun-bonding and melt-blowing [12, 13]. The producer claims the polymer to be entirely biodegradable in compost medium, moist soil, activated sludge and water [14]. Soil microorganisms metabolize PBSA to water and carbon dioxide [9].

An important role in the biodecomposition of Bionolle play the extracellular depolymerases enzymes delivered by bacteria of the *Pseudomonas sp*., *Alcaligenes sp* strains, actinomycetes of the *Streptomyces sp.* family and the eukariotic depolymerases delivered by the fungi *Penicillium funiculosum* and *Aspergillus fumigatus* [15].

The soil biodegradation rate depends upon many factors (both biotic and abiotic) i.a. the granulometric and mineral composition, content of organic substance, moisture, pH, redox potential, sorption properties and complexes of the soil microorganisms [16].

It was an aim of the work to define the qualitative and quantitative impact the degradation products of PBSA (Bionolle#3001) raw material and nonwovens thereof on the soil microbiological profile resulting from the incubation of the materials in the soil. One more goal was to assess the degradation efficiency of PBSA and nonwoven thereof caused by the soil incubation. The activity of the commercial garden soil and the agriculture soil of the experimental fields of the University was also assessed.

The investigation concerned soil microorganisms that are capable of *in vitro* growing on synthetic substrates.

The biodecompoition of the PBSA samples induced by selected microorganisms strains was tested. No special attention was given to the number of the soil microorganisms in the course of the biodegradation of the polymer and nonwovens materials.

New data concerning applied or proposed to application materials and products (nonwovens) in respect to soil biodegradation susceptibility is presented in

this article. Interesting results indicating the usefulness of prepared procedures that serve to define the microbiological profile in the course of biodecomposition are shown.

Materials and methods

Materials

- Granulated polymer poly(butylene succinate adipate) PBSA under the trade name Bionolle#3001 made by Showa Highpolymer Co Ltd, Japan was used in the investigations. It was characterized by: a melt flow index (MFI) of 1.4 g/10 min measured at 190 °C and 2.16 kg load [17], and Mn = 52 000 (numerical average molecular mass), Mw = 112 300 (weight average molecular mass) [18].

The chemical formula of PBSA is shown below:
-O-CH_2- CH_2- CH_2- CH_2-O-CO-CH_2-CH_2-CO- CO-CH_2- CH_2- CH_2- CH_2-CO [19].

- A nonwoven material made of Bionolle#3001 prepared by spunbonding was also examined. It was characterized by: surface mass of 53.1 ± 2.5 g/m^2, diameter of the fibre of 6.40 ± 0.22 μm, thickness of the nonwovens of 0.23 ± 0.01 mm and crystallinity of the nonwovens of 42.2%.

The nonwovens were produced on the technology spun-bond line constructed by Central R&D Hub of Textile Machinery "POLMATEX-CENARO", Poland, equipped with a 467 hole spinneret. The nonwovens were formed at the following conditions:

- processing temperature (polymer melt) 234 ± 0.5 °C;
- cooling air temperature 20 °C;
- throughput per hole 0.09 g/min;
- fiber take-up speed 1792 m/min;
- calander temperature 65 °C;
- nonwovens take up speed 2.4 m/min.

Two kinds of soil were used: an universal commercial garden soil with humidity of 60 - 80%, and pH of 6.0 - 6.5, appropriate for flowers and vegetables (garden soil), and a soil drawn from the experimental fields of the Agriculture University, Cracow, dark brown in colour with a large content of clay and humidity of 16 - 18%, (agriculture soil). In contact with water the soil turned into nubble form.

Table 1. *Microbiological substrate and dilution of the soil solution used in the deep inoculum in a given method.*

Substrate	Microorganisms expected to grow in the given substrate	Dilution
Nutrient PCA	Total numer of microorganisms	10^3-10^7
	Number of spore microorganisms	10^3-10^6
Nutrient PM3A – Agar Sabouraud substrate C with chloramfenikole	Moulds and yeast	10^1-10^3
Nutrient with addition of nystatin – 1250 units of nystatin-100μl/100ml nutrient PCA	Bacteria and actinomycetes	10^3-10^6

In both soils, prior to the biodegradation tests, the microbiological activity was estimated. The agriculture soil contained a higher amount of microorganisms than the garden soil: 1.8 × 10^7 cfu/g and 2.2 × 10^6 cfu/g, respectively.

Methods

The degree of degradation of the PBSA polymer and nonwovens at simulated soil conditions was estimated by measuring the mass loss

A testing procedure was prepared for the purpose which enables to estimate the degree of degradation of plastics and nonwovens in soil medium in lab scale. The procedure is based on Polish standards: PN ISO 11266:1997 [20], PN-EN ISO 11721-1:2002 [21], PN-EN ISO 11721-2:2002 [22].

The procedure describes a method to estimate the biodegradation degree of polymeric and textile materials by estimating the destruction of the tested material in laboratory conditions simulating an intensive aerobic soil action. The two soils, garden and agriculture one, were used as inoculum. The amount of decayed material is taken as the mass loss used to calculate the decomposition degree. Reactors with the tested material are placed in a thermal chamber at constant temperature of 30 ± 2 °C and humidity measured prior to the process start. Before the test, the samples were weighed and inserted in a marked polyester net resistant to biodegradation. The samples were prepared in 3 repetitions. The reactor in which the incubation was made, was filled with soil up to 1/3 of its volume. The tested samples were immersed in the soil separated from each other by layers of the soil to provide a direct soil-to-sample contact. The reactors were closed with a lid to prevent an excessive evaporation of the soil. The reactors were weighed and placed in the thermal chamber. Humidity of the soil was intermittently measured by weighing the reactors, and made up with water to the original weight of the reactors. During the incubation the samples were weighed at time intervals of 1, 4, 8, 12, 16, 20, 24 weeks from the beginning of the test. After a given time the samples were dried to constant weight in a vacuum dryer.

The test is deemed finished if a 100% mass loss occurs in a time shorter than 24 weeks.

Estimation of the morphology structure of the polymer and nonwovens

Scanning electron microscopy (SEM) was used to inspect the morphology changes proceeding in the course of the biodegradation.

Estimation of the total number of microorganisms in the soil used in the examination of the biodegradation of the polymer and nonwovens samples

The soil used in the testing of biodegradation degree ought to show an adequate microbiological activity of no lower than 10^6 cfu/g (cfu – colony forming unit).The amount of the microorganisms in the soil was estimated according to the procedure "Estimation of total number of microorganisms in soil and compost" based on standards PN-EN ISO 4833:2004 [23], PN-ISO 7218:2008 [24] and PKN-ISO/TS 19036:2011 [25].

Analysis of the soil microbiological profile

The analysis for the agriculture soil in the course of the biodegradation process was performed. The soil was shaken in normal saline for 2 minutes. From the prepared solution, a number of 10-fold diluted solutions were made. From each of the diluted solution a deep inoculum was made (the inoculum was done in two repetitions for a given dilution) using defined substrates (***Table 1***). The soil solution was heated to 85 ± 2 °C for 30 minutes in order to detect spore forms and, then, a number of the 10-fold dilutions were prepared and the inoculum was done.

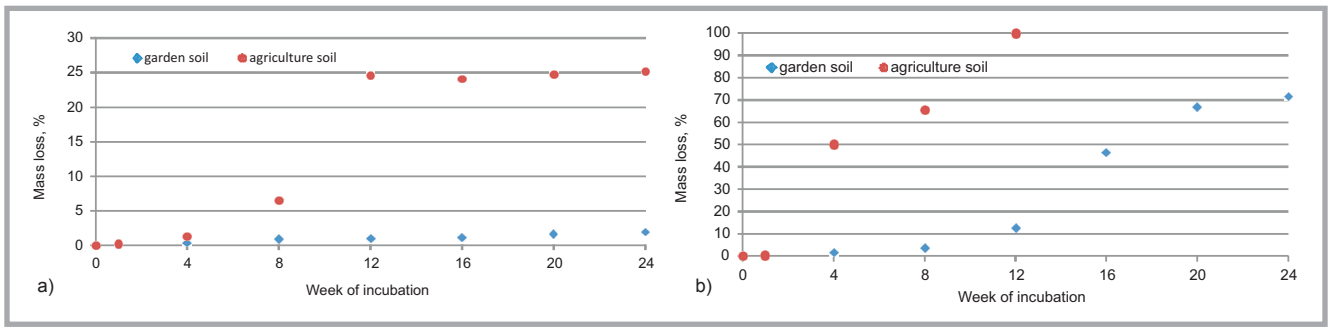

Figure 1. *Dependence of the mass loss of Bionolle#3001: polymer (a) and nonwovens (b) on the biodegradation time in garden and agriculture soil at 30 ± 1 °C.*

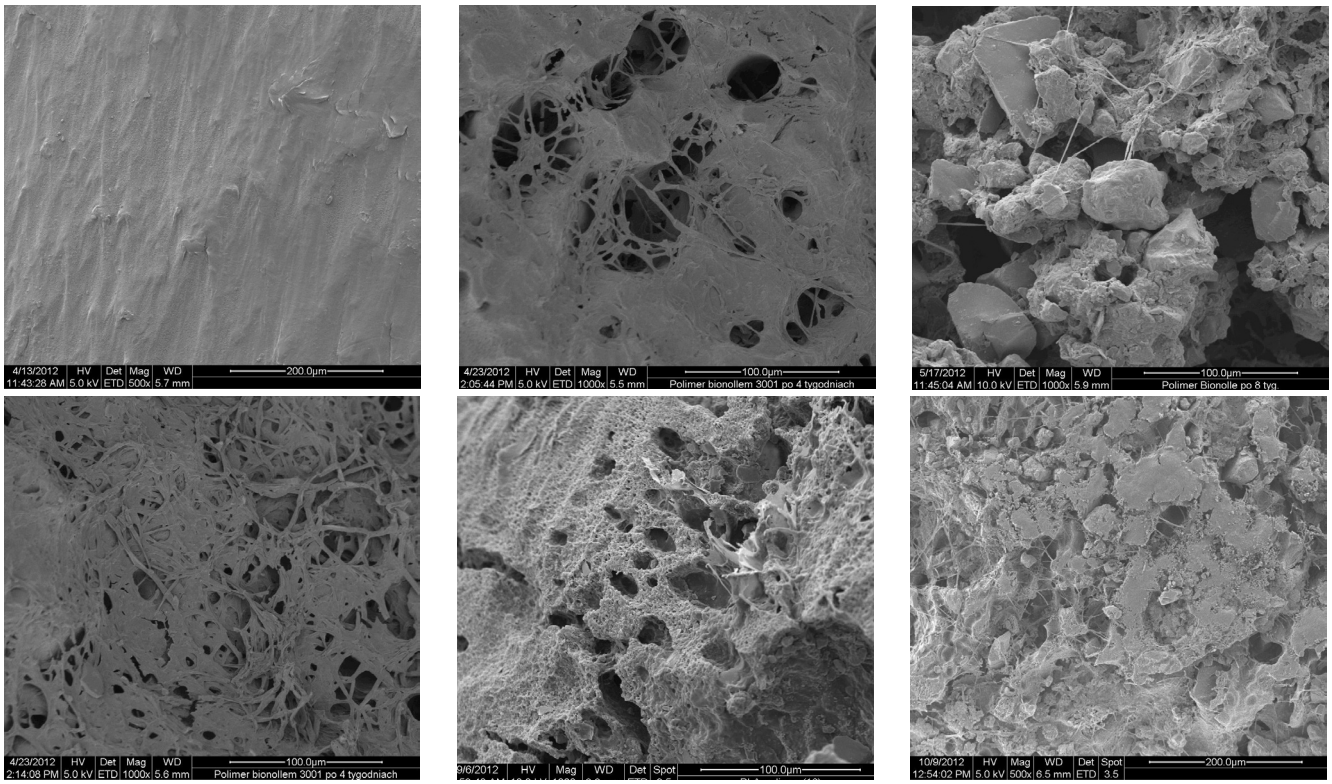

Figure 2. *SEM images of changes on Bionolle#3001 polymer surface in dependence on biodegradation time in agriculture soil.*

All Petri dishes with the deep inoculum were incubated for 72 hours at 30 ± 1 °C. After the incubation has finished, the colonies grown on the dishes with the given substrate were counted and the number of the singular microorganism groups was estimated. The fixed preparations coloured by Gram & Writz method (to detect spores) prepared from the singular microorganisms grown on the dishes were inspected by means of an optical microscope.

The number of microorganisms was calculated from the ***Equation 1***:

$$N = \frac{\Sigma(n1+n2) \times 10 \times 10^{f}}{2.2} \quad (1)$$

where:

N – number of microorganisms per 1 gram of the soil, cfu/g;

n1, n2 – cfu number on the dishes for two countable neighbouring dilutions;

10^{f} –lowest countable dilutions;

2.2 – in the case when both two dishes of the given dilution are countable (if only one dish is countable the number 1 is inserted instead of 2).

Results and discussion

Examination of the biodegradation degree of the polymer PBSA and nonwovens made thereof

Biodegradation of the PBSA polymer and nonwovens was investigated during the incubation in the garden and agriculture soil. The processes were compared in both of the soils and the more effective one was indicated. In the agriculture soil, the polymer degraded to a considerable degree, while in the garden soil the process proceeded with difficulty (***Figure 1.a***).

The mass loss of the polymer in the agriculture soil is much higher than in the garden soil. After a 24-weeks (that time is fixed in the method) incubation time, the loss amounted to 25.2% in the agriculture soil and a mere 2.0% in the garden soil. The results point the agriculture soil a favourable medium for the biodegradation of the tested polymer.

The biodegradation of Bionolle#3001 polymer proceeding in the agriculture soil is also witnessed by SEM images revealing numerous perforations on the surface of the material. (***Figure 2***).

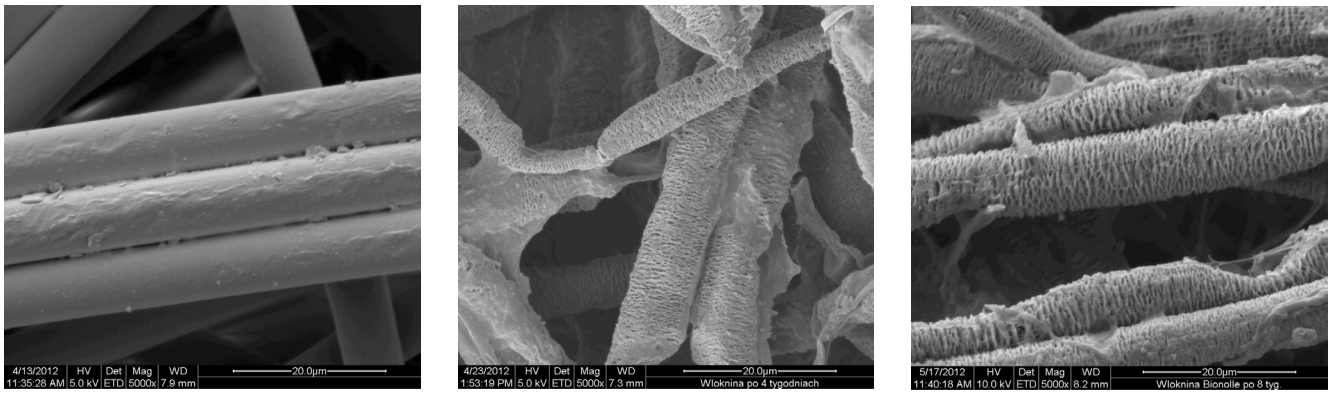

Figure 3. *SEM pictures of the surface of nonwovens made of Bionolle#3001 depending on time of incubation in agriculture soil; a) 1, b) 4 and c) 8 week.*

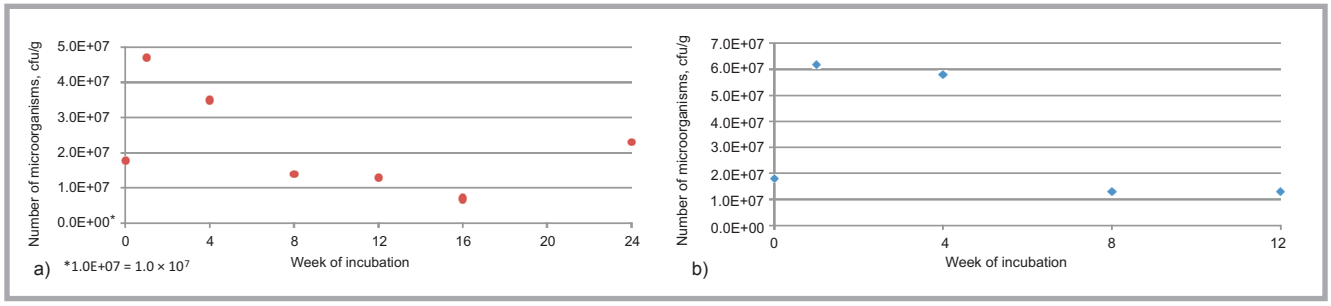

Figure 4. *Assessment of the total number of microorganisms counted per 1 gram of soil, in which the biodegradation of the Bionolle#3001: polymer (a) and nonwovens (b) - culture on medium PCA - proceeded depending on incubation time.*

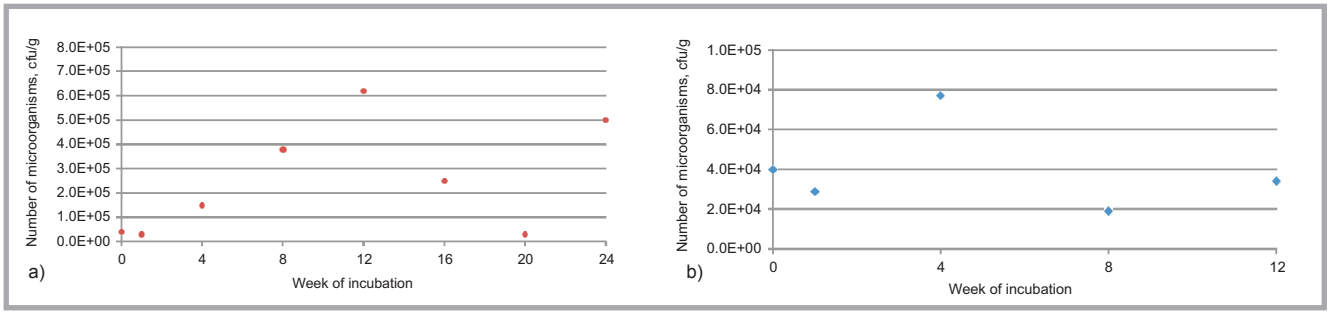

Figure 5. *Assessment of fungi number per 1 gram of soil, in which the biodegradation of Bionolle#3001: polymer (a) and nonwovens (b) proceeded (culture on medium PM3A), depending on incubation time.*

Biodegradation examination of the nonwovens of Bionolle#3001 in the two soils was carried out in the same time and conditions as for the PBSA polymer. Again, the biodegradation was more effective in the agriculture soil than in the garden one (***Figure 1.b***).

Mass loss of the nonwovens incubated for 12 weeks in the agriculture soil was 100% (complete degradation), while in the garden soil in the same time it was only 12,5%. After finished incubation (24 weeks), the mass loss of the nonwovens in the garden soil reached 71.7%.

The biodegradation of Bionolle#3001 nonwovens proceeding in the agriculture soil is documented with SEM images (***Figure 3***).

Investigation into the influence of the biodegradation products upon the changes of the soil microbiological profile

The analysis was made for the agriculture soil. Soil is a dynamic system where qualitative and quantitative changes proceed depending on the laboratory conditions: temperature, humidity, presence of decomposition products of the nonwovens/polymer and side products of the metabolic pathways. ***Figures 4 - 8*** present the changes in the microbiological profile in the agriculture soil in presence of the incubated biodegrading Bionolle#3001 polymer and nonwovens samples.

After the first week of incubation in the agriculture soil with polymer and nonwovens samples, a growth phase in the total number of microorganisms could be observed for the nonwovens to the amount of 6.2×10^7 cfu/g and for the polymer to 4.7×10^7 cfu/g (***Figure 4***). The microorganisms were that time supposedly in the logarithmic growth phase consuming as a source of carbon the soil organic substance and succinic- and adipic acid radicals remaining after the hydrolysis of the nonwovens/polymer. A decrease of the microbiological activity was observed for both materials during further incubation. The supposed reason

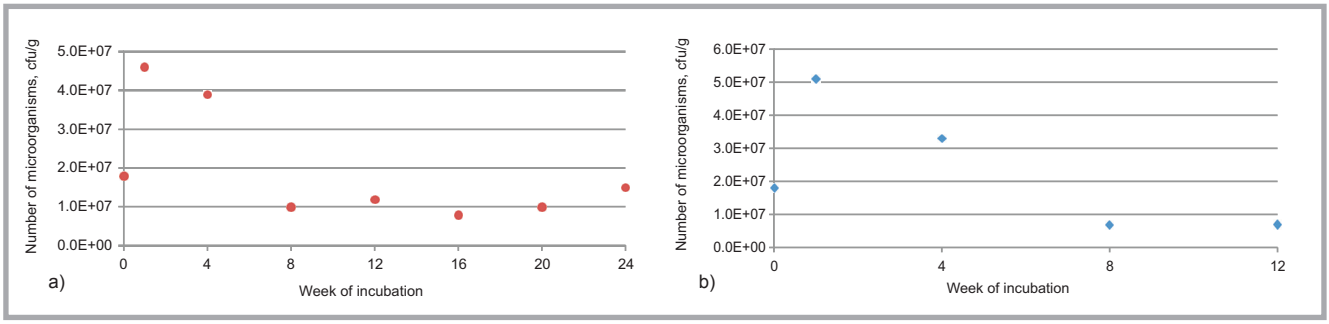

Figure 6. *Assessment of bacteria and actinomycetes number per 1 gram of soil in which the biodegradation of Bionolle#3001 polymer (a) and nonwovens (b) proceeded (culture on PCA medium with addition of nystatin) depending on incubation time.*

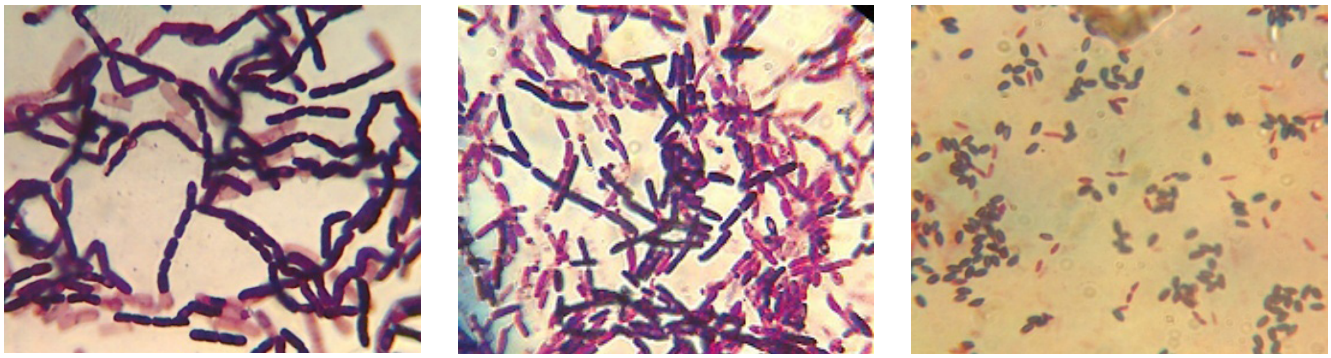

Figure 7. *Optical microscope images, magnification-1000×, inspection made with the use of immersion oil; a) Gram-positive aerobic bacilli (drawn from a round, mat wrinkled colony after boiling from PCA dish) fixed and stained by Gram method, b) Gram-positive bacilli drawn from colonies grown on PCA medium with addition of nystin), preparations fixed and stained by Gram method. c) Spores of bacilli drawn from a colony grown on PCA medium, preparations fixed and stained by Writz method.*

is the drying up of the soil environment as well as the accumulation of side products of the microorganisms metabolism negatively affecting themselves.

Soil is abode to a multitude of microorganisms i.e. mould spores which at adequately low pH of the soil turn to a vegetative form. Fungi spores are capable of surviving at adverse environment conditions (heat, acids). ***Figure 5*** present the assessment of fungi number in the soil environment with incubated polymer and nonwovens

The number of fungi in the soil environment (***Figure 5***) with the incubated polymer and nonwovens samples initially increased. In the soil with Bionolle polymer it increased to the 12^{th} incubation week reaching the level of 6,2 x 10^5 cfu/g and then it decreased to 2.9 × 10^4 cfu/g after 20 weeks of incubation. The growth of fungi in the soil was most probably related to the emergence of the monomeric succinic and adipic acids which acidify the soil environment. In the soil with incubated nonwovens, the fungi number increased up to the 4^{th} week to the level of 7.7 × 10^4 cfu/g, then, it dropped to resume increase after the 8^{th} week of incubation. The testing lasted till the 12^{th} week when the nonwovens underwent complete degradation.

Bacteria and actinomycetes are a family of microorganisms for which a neutral pH is optimal. Actinomycetes have the faculty of synthesizing antibiotics and geosmin which has the earthy flavor and aroma thanks to which the culture of the actinomycetes on synthetic cultures was detectable. The bacteria and actinomycetes profile in the agriculture soil with incubated Bionolle polymer and nonwovens is presented in ***Figure 6.***

The number of bacteria and actinomycetes in the soil with the incubated polymer and nonwovens grows after the first incubation week and amounts to 4.6 × 10^7 cfu/g, and 5.1 × 10^7 cfu/g, for the soil with polymer and nonwovens, respectively. Next, the level of bacteria and actinomycetes goes down. Later on in the 16^{th}, week, the microbiological activity increases slightly in the soil with the polymer.

The increase of the microbiological activity is most probably caused by the fact that the liberated succinic and adipic acid radicals are exploited as source of carbon, while an excessive amount of the monomers acidifies the environment limiting the bacteria and actinomycetes vitality.

Microscopic identification of bacteria isolated on the substrate

Fixed preparations were made and stained in turns in crystal violet, Lugol solution, decolourated with ethanol and stained with basic fuchsin (staining by Gram method).

The Gram –positive bacteria (mainly bacilli of the Bacillus family) transform in adverse conditions to spores resistant to heat (85 °C) for 30 minutes and take the malachite green in the "hot staining".

Staining of spores by Writz method is presented in ***Figure 7***.

The aerobic bacilli produce the biodegradation – promoting proteases and esterases enzymes. The number of the microorganisms spores in the agriculture soil with Bionolle#3001 polymer and nonwovens is presented in ***Figure 8*** (see page 184).

The number of microorganisms spores (***Figure 8.a***) in the soil with polymer de-

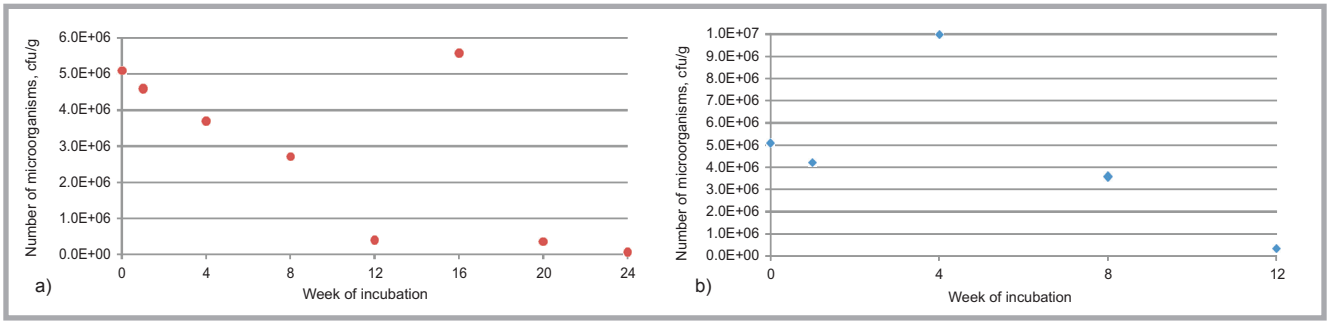

Figure 8. *Assessment of total number of microorganisms spores per 1 gram of soil in which the biodegradation of Bionolle#3001: polymer (a) and nonwovens (b) - culture on PCA medium - proceeded depending on incubation time.*

creased to the 12th week to the level of 4.0×10^5 cfu/g and then, raised in the 16th week of incubation to 5.6×10^6 cfu/g and decreased again to 8.0×10^4 cfu/g. In the soil with the nonwovens (***Figure 8.b***), the number of microorganisms spores increased to the 4th week to – 1.0×10^7 cfu/g, and then, decreased to the level of 3.5×10^5 cfu/g.

The biodegradation of both Bionolle#3001 polymer and nonwovens in the agriculture soil is composed of two processes: hydrolytic degradation and biodegradation induced by microorganisms producing an adequate set of enzymes. The mechanism of the microorganisms –induced degradation is not known, yet it appears that the PBSA decomposition products stimulate the growth of *Bacillus pumilus* bacteria on synthetic substrates [13]. Radicals of the succinic and adipic acids are delivered in the course of the hydrolysis which are probably devoured by the microorganisms (first by bacteria and actinomycetes) as nutriment and source of carbon.

It may be assumed that the appearance of the potential source of nutriment creates optimal conditions for the existence of microorganisms which enter into the phase of logarithmic growth and start to metabolize enzymes – the basic proteases and esterases which, in turn, enhance the hydrolytic degradation. After a certain time, assumedly, as a result of the increase in the concentration of the bacteria and actinomycetes metabolism products and radicals of the succinic and adipic acids, the number of bacteria and actinomycetes slightly decreases, and the amount of fungi increases which also play a certain role in the biodegradation of the polymer and nonwovens.

Conclusions

Biodegradation of the Bionolle#3001 polymer and nonwovens made thereof in the agriculture soil proceeds much faster than in the garden soil, The agriculture soil, though with a low moisture content, is fertile containing a much higher amount of microorganisms that promote the biodegradation of the polymeric materials, Samples of nonwovens made of the Bionolle#3001 were entirely decomposed after 12 weeks of incubation in the agriculture soil. The garden soil does not offer conditions for an effective run of the biodegradation. Samples of Bionolle#3001polymer in chips form degraded merely by 2% after 24 weeks of incubation in the garden soil.

The appearance of the polymer decomposition products is not neutral to the microbiological flora confirmed by the dynamic growth or drop of the singular groups of the microorganisms. Also other factors exert an influence on the microorganisms, notably an adequate moisture content or the concentration of side products delivered in the course of the microorganisms metabolism.

Bacteria are the dominant group amongst the soil microorganisms mainly the aerobic Gram – positive bacilli which take the violet hue in the Gram staining method and are capable of surviving in adverse environment conditions (temperature) which finds confirmation by the green spore hue in the staining by Writz method.

An effective method was prepared for the analysis of the microbiological profile in the soil in the course of the proceeding biodegradation. The method enables to define precisely the dynamic character of the soil environment and to analyze the soil in regard to the impact of the polymeric materials upon the environment micro-flora.

Acknowledgment

The work was completed in the Institute of Biopolymers and Chemical Fibers for the project "Biodegradable fibre products" BIOGRATEX-POIG.01.03.01-00-007/08, co-financed from EU funds within European Program of Regional Development – Innovative Economy.

References

1. Żuchowska D, Steller R, Meissner W. Polymeric composites prone to biodegradation (in Polish). *Polimery* 2007; 52; 7-8.
2. http://karnet.up.wroc.pl/~weber/powstaw1.html
3. Różalski A. *Exercise in general microbiology*. Ed. University of Lodz, Ed. IV, 2004.
4. Wakieć R. Separation of microorganisms from soil- potential producers of biologically active compounds (in Polish). In: *Biotechnologia leków*, 2006.
5. Nowak B, Pająk J. *Biodegradation of polylactide (PLA)* (in Polish), ISSN1733-4381, 2010; 12; 2: 1-10.
6. Pranamuda H, Tokiwa Y, Tanaka H. Polylactide Degradation by an Amycolatopsis sp. *Applied And Environmental Microbiology* 1997; 63; 4: 1637–1640.
7. Nowak B, Pająk J, Łabużek S, Rymarz G, Talik E. Biodegradation of poly(ethylene terephthalate) modified with polyester Bionolle® by Penicilliumfuniculosum. *Polimery* 2011; 56; 1.
8. Mayumi D, Akutsu-Shigeno Y, Uchiyama H, Nomura N, Nakajima-Kambe T. Identification and characterization of novel poly(DL-lactic acid) depolymerases from metagenome. *Appl Microbiol Biotechnol*. 2008; 79: 743-750.
9. http://www.showadenko.us/en/products/bionolle.html
10. Fujimaki T. Processability and properties of aliphatic polyesters, 'BIONOLLE', synthesized by polycondensation reaction. *Polymer Degradation and Stability* 1998; 59: 209 – 214.
11. http://www.showadenko.us/product/bionolle.php

12. Technical data sheet, Showa Highpolymer Co.LTD.
13. 5.WO 2008/008067 A1 to Wang at al., Biodegradable aliphatic polyester for use in nonwoven webs.
14. Łabużek S, Pająk J, Nowak B, Solga M. Investigation into the toxcicity of biodegradation products of the polyester Bionolle (in Polish). *Polimery* 2008; 53; 5.
15. Scherer TM, Clinton Fuller R, Lenza RW, Goodwinc S. Hydrolase activity of an extracellular depolymerase from Aspergillusfumigatus with bacterial and synthetic polyesters. *Polymer Degradation and Stability* 1999; 64: 267-275.
16. http://www.zgf.uni.wroc.pl/dydaktyka/przedmioty/Ochrona%20gleb/Ochrona%20gleb-PSOS-5.pdf
17. Ichikawa Y, Mizukoshi T. Synthetic Biodegradable Polymers, Advances in Polymer Science, Bionolle (Polybutylenesuccinate). Chemistry and Materials Science 2012; 24: 285-313.
18. Rizarelli P, Puglisi C, Montaudo G. Soil burial and enzymatic degradation in solution of aliphatic co-polyesters. *Polymer Degradation and Stability* 2004; 85: 855-863.
19. Hayase N, Yano H, Kudoh E, Tsutsumi C, Ushito K, Miyahara Y, Tanaka S, Nakagawa K. Isolation and characterization of Poly(Butylene Succinate-co-Butylene Adipinate) - degrading Microorganism. *Journal of Bioscience and Bioengineering* 2004; 97; 2: 131-133.
20. PN ISO 11266: 1997. *Soil quality Principles of laboratory testing of organic matter biodegradation in soil in aerobic conditions* (in Polish).
21. PN-EN ISO 11721-1:2002. *Textiles. Estimation of resistance to microorganisms action in textile materials with cellulose content. Method of soil burying Part I Assessment of decay- inhibiting finishing* (in Polish).
22. PN-EN ISO 11721-2:2002. *Textiles. Estimation of resistance to microorganisms action in textile materials with cellulose content. Method of soil burying Part 2 Identification of antifungal finishing considering its efficiency in time* (in Polish).
23. PN-EN ISO 4833:2004. *Microbiology of food and fodder-Horizontal method of estimating the number of microorganisms-Dish method at temperature of 30 °C* (in Polish).
24. PN-EN ISO 7218:2008. *Microbiology of food and fodder- General demands and principles in microbiological examination* (in Polish).
25. PKN-ISO/TS 19036:2011. *Microbiology of food and fodder-Guidelines for the assessment of measurement uncertainty* (in Polish).

Received 18.10.2012 Reviewed 18.12.2012