BIODEGRADABILITY TESTING OF MODIFIED PHA-BASED NANOMATERIALS BY STANDARD TEST

Pavla BENEŠOVÁ, Stanislav OBRUČA, Agáta WURSTOVÁ, Vojtěch KUNDRÁT, Ivana MÁROVÁ

Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 612 00 Brno, Czech Republic, E-mail: xcbenesova2@fch.vutbr.cz

Department of Food Chemistry and Biotechnology, Faculty of Chemistry, Brno University of Technology, Purkyňova 118, 612 00 Brno, Czech Republic, E-mail: xcbenesova2@fch.vutbr.cz

Abstract

Polyhydroxyalkanoates (PHA) are produced from renewable carbon resources by some microorganisms belonging to the *Bacteria* and *Archaea* domains of life. Mechanical properties of PHAs make them suitable replacements for petrochemically produced bulk plastics (polyethylene, polypropylene etc.), but in contrast to these commodity plastics PHA are completely biodegradable to carbon dioxide and water without formation of any toxic by-product. PHAs are environmentally compatible polymeric materials and can be processed into films and fibres. PHAs are naturally degraded by intracellular and extracellular PHA depolymerases.

The work was aimed to comparison of biodegradability of PHB nano-fibre material with different forms of PHA films. For biodegradability testing standard biodegradation test described in IS/ISO 20200 norm was chosen. Degradation test was performed in thermophillic conditions. Poly(3-hydroxybuytyrate), produced from waste frying oils by *Cupriavidus necator* H16, was tested in following forms: i. PHB nano-fibrous material formed by electro-spinning. ii. PHB processed into film by extrusion iii. PHB processed by extrusion and modified by commercially available plasticizer. In comparison with the PHB films nano-fibres exhibited the highest degree of degradation in the shortest time. Anyway, all of the samples were completely degraded during four weeks of standard biodegradation test.

Key words:

Polyhydroxyalkanoates (PHA), nano-textile, biodegradation

1 INTRODUCTION

Polymers are widely used in various industrial fields due to their merits, such as light weigh, resistance to chemicals, resistance to the environment, easy processing. It is difficult for polymers to be treated after use due to their resistance to the environment. When polymers are disposed of in a natural environment, they remain for a long time without degradation. One of the solutions to this problem of polymers after use is the development of biodegradable polymers [1]. Various types of biodegradable plastics have been developed. The biodegradable polymers can be classified according to their chemical composition, origin and synthesis method, processing method, economic importance, application. One of the biopolymers class are polyhydroxyalkanoates (PHA). In general PHA based materials are degradable in comparison with petrol plastic (e.g. polyethylen, polypropylen). The biodegradation test determines the degree of disintegration of test materials on a laboratory scale under conditions simulating an intensive aerobic composting process. Degradation of PHAs can take place in natural environments such as soil, water, and compost by microorganisms. Microorganisms can degrade PHAs under both aerobic and anaerobic conditions, using degradation fragments as source of energy and carbon. Under aerobic conditions PHAs are degraded to end

products – carbon dioxide and water; under anaerobic – to water and methane. Polyhydroxyalkanoates can also be degraded by thermal action and as a result of acid and alkaline hydrolysis [2,3].

1.2 Polyhydroxyalkanoates

Polyhyroxyalkaonates are microbial polyesters which are produced by biosynthetic function of a microorganism and readily biodegraded by microorganisms. As was mentioned before, PHA is family of intracellular biopolymers synthetized by many bacteria as intracellular carbon and energy storage granules. Depending on growth conditions, bacteria strain, and carbon source, the molecular weights of these polyesters can range tens into the hundreds of thousands [3].

PHAs are composed of 3-hydroxy fatty acid monomers, which from linear, head-to-tail polyester. PHA is typically produced as a polymer of 10^3 to 10^4 monomers, which accumulate as inclusions of 0.2-0.5 μm in diameter. These inclusions or granules are synthesized and stored by both gram-positive and gram-negative bacteria without hazardous effects to the hosts. PHA accumulation occurs when the cells experience a nutrient imbalance such as excess carbon with limited nitrogen, phosphorus or oxygen. PHAs containing up to C5 monomers are classified as short chain length (scl-PHA). PHAs whit C6-C14 and > C14 monomers are classified as medium chain length (mcl-PHA) and long chain length (lcl-PHA) PHAs, respectively. The best studied polymer within the PHA family is poly(3-hydroxybutyrate) (PHB), which has a model character for research on PHA. Copolymers from PHA can be formed containing 3-hydroxybutyrate, 3-hydroxyvalerate or 4-hydroxybutyrate monomers. The copolymers of PHB with hydroxyvaleric acid (PHB/HV) are less crystalline, more flexible, and more readily processible. On the other hand, they suffer from the same disadvantage of being too hydrolytically stable to be useful in short-term applications when resorption of degradable polymer within less than one year is desirable. Bacterially synthesized PHAs have attracted attention because they can be produced from a variety of renewable resources such as waste frying oils or lignocellulosic materials. PHAs are thermoplastic, biodegradable, biocompatible, and non-toxic [2, 4].

1.3 Biodegradation process

Microorganisms from families *Pseudonocardiaceae, Micromonosporaceae, Thermomonosporaceae, Streptosporangiaceae and Strepromycetaeae* predominantly degrade PHB in the environment. However, most PHA-producing bacteria are able to degrade the polymer inside the cells. Extracellular depolymerases degrading PHAs are present in the environment [2].

Microorganisms (bacteria, fungi, algae) recognize polymers as a source of organic compounds and energy that sustain them. Under the influence of intracellular and extracellular enzymes (endo- and exoenzymes) the polymer undergoes chemical reactions and degrades by process of scission of the polymer chain. The result of this process that can be affected by a great number of different enzymes are increasingly smaller molecules, which enter into cellular metabolic processes, generating energy and turning into water, carbon dioxide, biomass and other basic products of biotic decomposition. These products are non-toxic and occur normally in nature and living organisms. A process in which an organic substance (polymers) is converted to an inorganic substance (CO_2) is called mineralization. The decomposition of condensation polymers (e.g. polyesters and polyamides) takes place through hydrolysis. Microbes secrete extracellular enzymes that solubilise the polymer and these soluble products are then absorbed through their cell walls and utilised. The PHA depolymerase enzymes act on the polymer mainly by hydrophobic interactions. Degradation by these depolymerases initially produces oligomers. Some microbes produce an additional dimer hydrolase, which further breaks down the oligomers into the corresponding monomer [4, 5].

Several factors are affecting biodegradation of polymers; the most important of them are polymer structure, polymer morphology, molecular weight, Radiation and chemical treatments. Also environmental conditions like temperature, moisture, pH, nutrient supply affecting biodegradation process.

A combination of biodegradability and use of renewable resources to produce biodegradable plastic provides a unique possibility of aligning the entire life cycle of plastic with the natural cycles: plastics are produced from and return to the natural renewable resource [6, 7].

2. EXPRIMENTAL

2.1 Methods

Three types of test material based on polyhydroxyalkanoates were performed for biodegradability testing. First of them was PHB film made by evaporation of solvent and processed into the film. The second one was PHA film processed by extrusion. Finally, nano-fibrous textile was obtained by electrospinning. All of materials which were mentioned before were made of poly(3-hydroxybutyrate) produced by *Cupriovidus necator* H16. The biodegradation test was performed under thermophilic conditions described in the norm IS/ISO 20200. The composition of synthetic waste used in this method is described in Table 1. Composting reactor was a polypropylene box of the following dimensions: 30 x 20 x 10 cm. The box (Fig.1) was covered with a lid assuring a tight seal to avoid excessive evaporation. In the middle of the two 20 cm wide sides, a hole of 5 mm diameter shall be made approximately 6.5 cm from the bottom of the box. These two holes provide gas exchange between the inner atmosphere and the outside environment. Samples were checked and maintained according to the instruction defined in direction. Tested materials were cut up to give pieces with the dimensions defined in Table 2 [8].

Table 1: Composition of synthetic waste

Material	Dry mass [%]
Sawdust	40
Rabbit-feed	30
Ripe compost	10
Corn starch	10
Saccharose	5
Cornseed oil	4
Urea	1

Table 2: Dimensions of test materials

Thickness of test material	Dimensions of pieces [mm]
< 5 mm	25x25xoriginal thickness
> 5 mm	15x15xthickness (from 5 mm to 15 mm)

Fig. 1: Composting box with nano-fibrous textile

2.2 Results and discussion

Degradability of modified PHA-based materials was tested in this work. Samples were processed by various techniques, from the most easiest – evaporation of organic solvent, to processing of nano-fibrous by electro-spinning. Biodegradability testing was carried out according to standard test ISO 20200, duration of testing was 57 days. This time period was completely sufficient to finish of degradation of all testing materials. Composting process was maintained under thermophilic conditions at 58 °C. Disintegration of tested PHA materials was probably caused by influence of microorganisms originated from compost which were added to the synthetic waste (Fig. 1). Thanks to adjustment of the test we can assume that microbes which were responsible for successful degradation were of thermophilic character.

Table 3: Summary of test duration

<table>
<tr><th>Time of degradation</th><th>PHB film</th><th>PHB extruded film</th><th>PHB nanotextile</th></tr>
<tr><td>1. day</td><td colspan="3">Start of testing</td></tr>
<tr><td>5. day</td><td></td><td></td><td></td></tr>
<tr><td>10. day</td><td></td><td></td><td rowspan="10">DEGRADATION PRECESS OVER</td></tr>
<tr><td>15. day</td><td></td><td></td></tr>
<tr><td>19.day</td><td rowspan="8">DEGRADATION PROCESS OVER</td><td></td></tr>
<tr><td>23. day</td><td></td></tr>
<tr><td>24. day</td><td></td></tr>
<tr><td>30. day</td><td></td></tr>
<tr><td>38. day</td><td></td></tr>
<tr><td>46 day</td><td rowspan="2">DEGRADATION PROCESS OVER</td></tr>
<tr><td>57. day</td></tr>
</table>

The process of degradation was completely done in 57 days. Duration of experiment was determined by complete disintegration of tested materials. As shown in Table 3 film processed by extrusion was the most resistance material for degradation. Time of complete disintegration was 38 days. It was probably caused by impossibility of microbial colonization due to surface compatibility. The maximum thickness of all tested materials had extruded film. Hence, thickness could be one of the main factors influencing successful and rapid disintegration of biopolymers. Thanks to maximum surface and minimum thickness of nano-fibrous textile, in comparison with other tester materials, degradation was in this case the fastest one. After 15 days incubations PHB film was considered as completely decomposed.

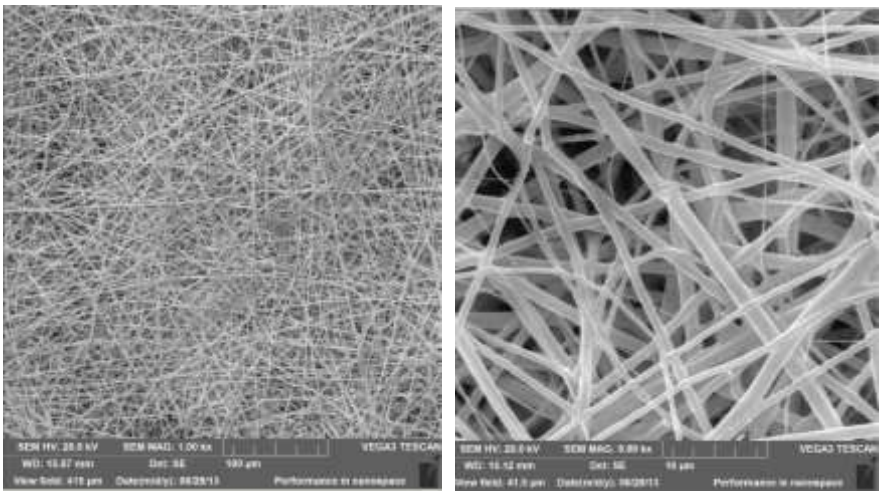

Figure 2: SEM scan of nano-fibrous textile before the test

Figure 3: Biodegradation process of PHA-based nano-fibrous textile a) 0th hour b) 5 days c) 8 days

Nano-fibrous textile was scanned by electron microscope to confirm the size of fibres. The nano-fibrous textile was made of PHB by electrospinning in cooperation with Masaryk University Brno. In Figure 3a) PHA-based nano-fibrous textile at 0th hour of testing is shown. Colonization of thermophilic microorganisms and secretion of hydrolytic enzymes on surface after 5 days of composting can be observed in Fig.3b). Considerable degree of disintegration is obvious after 8 days of incubation (Fig. 3c).

Success of degradation is apparently depending on surface and thickness of composting material. It's obvious that surface is very important factor for microbial colonization. Extruded film had the longest period of degradation and also smaller surface in contrast to nano-fibrous textile.

3. CONCLUSION

All of PHAs processed materials were fully biodegradable. We can conclude that degradation time was dependent on processing technique; also surface was of important influence on degradation process. From all testing materials nano-textile was the fastest degraded, approximately in 10 days. On the other hand, PHA which was modified by extrusion was degraded during 38 days in thermophillic conditions. The future experiments will be focused on biodegradability of PHA-based materials and their applications in simulating the natural environment.

ACKNOWLEDGEMENTS

This work was supported by project "Centre for Materials Research at FCH BUT" No. CZ.1.05/2.1.00/01.0012/ERDF.

REFERENCES

[1] FUNABASHI, Masahiro, Fumi NINOMIYA a Masao MASAO KUNIOKA. Biodegradability Evaluation of Polymers by ISO 14855-2. *International Journal of Molecular Sciences*. 2009, vol. 10, issue 8, s. 3635-3654. DOI: 10.3390/ijms10083635.

[2] VOLOVA, T. *Polyhydroxyalkanoates--plastic materials of the 21st century: production, properties, applications*. Hauppauge, N.Y.: Nova Science Publishers, c2004, xix, 282 p. ISBN 15-903-3992-4.

[3] PHILIP, S., T. KESHAVARZ, I. ROY, Guo-Qiang CHEN, G CHEN, G.G-Q. CHEN, M K COX, Kumar SUDESH, Kumar SUDESH, Ivan CHODAK, P.J. HALLEY a A.J. VARMA. Polyhydroxyalkanoates: biodegradable polymers with a range of applications. *Journal of Chemical Technology*. 2007, vol. 82, issue 3, s. 165-188. DOI: 10.1533/9781845690762.2.165.

[4] URIYAMONGKOL, Pornpa, Randall WESELAKE, Suresh NARINE, Maurice MOLONEY a Saleh SHAH. Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants — A review. *Biotechnology Advances*. 2007, vol. 25, issue 2, s. 148-175. DOI: 10.1016/j.biotechadv.2006.11.007.

[5] LYU, SuPing a Darrel UNTEREKER. Degradability of Polymers for Implantable Biomedical Devices. *International Journal of Molecular Sciences*. 2009, vol. 10, issue 9, s. 4033-4065. DOI: 10.3390/ijms10094033.

[6] GHANBARZADEH, Babak a Hadi ALMASI. Biodegradable Polymers. *Biodegradation - Life of Science*. InTech, 2013-06-14. DOI: 10.5772/56230.

[7] MÜLLER, Rolf-Joachim a Alexander STEINBÜCHEL. Biodegradability of Polymers: Regulations and Methods for Testing. *Biopolymers Online*. Weinheim, Germany: Wiley-VCH Verlag GmbH, 2005-01-15. DOI: 10.1002/3527600035.bpola012.

[8] IS/ISO 20200. Plastics - Determination of disintegration of plastic materials under simulated compositing conditions in a laboratory-scale test. India: Bureau of Indian Standards (BIS), 2004.