Scientific Bulletin. Series F. Biotechnologies, Vol. XXVI, No. 2, 2022
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

BIODEGRADATION OF NOVEL POLYLACTIC ACID BASED BIOMATERIALS BY DIFFERENT METHODS

Elisabeta Elena POPA[1], Maria RÂPĂ[2], Mihaela GEICU-CRISTEA[1], Amalia Carmen MITELUŢ[1], Paul-Alexandru POPESCU[1], Mihaela Cristina DRĂGHICI[1], Silvana Mihaela DĂNĂILĂ-GUIDEA[1], Mona Elena POPA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University Politehnica of Bucharest, Faculty of Material Science and Engineering, 313 Spl. Independentei, Bucharest, Romania

Corresponding author email: mihaela_geicu@yahoo.com

Abstract

The extensive production and usage of petroleum based plastic materials represent a great threat to the environment (both terrestrial and marine) due to their properties of slow degradation and landfill accumulation. Therefore, many researches were conducted in order to develop friendly packaging materials, which fulfil the current requirements in terms of biodegradability, bioavailability and compatibility. Among different types of biodegradable materials, polylactic acid (PLA) received more and more attention as green material due to the fact that it is derived from natural and renewable resources and present great physical-mechanical properties. This study aims to present the research conducted for the biodegradability degree determination of some novel biomaterials based on PLA that could be used as packaging materials. The newly developed materials were buried in a natural characterized soil and their biodegradability was determined after 30, 60 and 90 days of maintaining in soil. Furthermore, they were tested from a microbial colonisation and degradation point of view. The results showed low rates of biodegradability for PLA based samples.

Key words*: biodegradation, colonisation, fungi, polylactic acid, soil burial test.*

INTRODUCTION

Petro-chemical based packaging materials present many advantages such as low price, high manufacturing speed, great physical - mechanical and barrier properties (Iordache et al., 2018); however, they accumulate in the environment representing a great concern for both waste management industry and natural environment (Peng et al., 2021).

In recent years, consumer attention has been focused on environmentally friendly products, including packaging materials for their daily use commodities (Turco et al., 2021). Therefore, many studies were conducted for the development of packaging materials derived from natural and renewable resources with high-performing properties similar to conventional materials (Kalita et al., 2020) and environmentally friendly. Aliphatic polyesters in particular, represents the most promising polymers for obtaining biopolymers that fulfil the properties mentioned above (Turco et al., 2021). One of these polyesters is represented by polylactic acid (PLA), which is a renewable material (Jeon & Kim, 2013), considered one of the most promising alternatives to conventional petroleum based plastic materials (Castro-Aguirre et al., 2018). It has good processing properties, high transparency and is easy to process (Janczak et al., 2018). It is also commercially available at a large scale (Mistry et al., 2022), accounting for 25% of the total biopolymer production at a global level (Kalita et al., 2021; Sun et al., 2022). It can be used in various domains, such as food industry, agriculture, textile and medical or pharmaceutical fields (Lv et al., 2017; Freitas et al., 2017), being suitable for conventional plastic materials replacement (Boonluksiri et al., 2021).

Biodegradation of polymers represents a complex process based on the breaking of the molecular structure of the materials (Lammi et al., 2019). Under aerobic conditions two steps occur, namely fragmentation (or hydrolysis of

the polymer in short chains) and enzymes action (produced by microorganisms) which uses as source of energy hydrolysis products such as monomers, oligomers and dimers; following this process results H_2O, CO_2, CH_4 and biomass (Freitas et al., 2017; Janczak et al., 2018; Eslami & Mekonnen, 2022). Soil biodegradation is a method that has been widely applied in the last years on various biopolymers, due to researchers' interest over the biodegradation of different materials in environmental conditions. Another method that could be used for polymers biodegradation is the microorganism development observation on the polymer substrate (Janczak et al., 2018), namely colonisation analysis.
The aim of this study was to asses soil and microbial biodegradation on several polymeric materials based on PLA.

MATERIALS AND METHODS

Soil burial biodegradation test

The samples tested in this experiment are presented in Table 1.

Table 1. Tested samples based on PLA

Sample Code	Description
PLA	PLA film obtained by casting method (control sample)
PLA/Ns	PLA film covered with a nanoemulsion based on polyvinyl alcohol (APV) and nisin (Ns) by electrospinning process
PLA/Ne	PLA film covered with a nanoemulsion (Ne) based on dill essential oil and nisin encapsulated into APV by electrospinning process
PLA/AgNps	PLA film covered by electrospinning process with silver nanoparticles (AgNps) dispersed into APV

An active soil with a water content of (60 ± 5)% of the soil's water retention capacity was used within the experiments. The samples were cut in a rectangular form (3.0 x 1.5 cm). To determine the mass variations, the tested materials were kept at room temperature in a desiccator, until the mass of each sample reached a constant value (approximately after 48 hours), samples being weighed both at the beginning and at the end of the test period. The soil used in these experiments presented the characteristics described in Table 2.

Table 2. Characteristics of the soil used in the experiments

Ph	5.0-7.0 pH units
Total N in dry matter	max. 1.9%
P_2O_2 in dry matter	max. 0.5%
K_2O in dry matter	max. 0.9%
Content of combustion substances	min. 50%
Electrical conductivity	max. 1.2 mS cm^{-1}
Particles over 20 mm	max. 5%
Humidity	max. 65%

The microbiological activity of the soil was determined and analysis showed that the soil was active from a microbiological point of view (Figure 1).

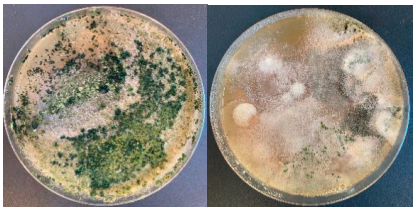

Figure 1. The visual aspect of the development of existing microorganisms in the soil

The polymer samples were buried in soil; the thickness of the layer covering the samples must not be greater than 12.5 cm. To ensure the circulation of oxygen, the containers were not hermetically closed. The containers were then stored at room temperature, which was monitored throughout the experiment (~ 25±1°C), for 30 days, 60 days and 90 days, respectively.
To examine the morphology of the studied samples, a FEI Inspect S50 scanning electron microscope was used, at accelerating voltages of 5 kV, at 200x magnification, in Low Vacuum.

Colonisation and in vitro biodegradation test

The method used was according to SR EN ISO 846:2000. The principle of this method consists of samples exposure to microorganisms' action, for a certain period of time at constant

temperature. Two minimal culture media were used - one without (M I) and one with carbon source (M II) -, both being prepared according to their recipes, sterilized at 121°C for 15 minutes, then cooled at approximate 45°C and poured into Petri dishes. Both *Aspergillus brasiliensis* ATCC 16404 and *Aspergillus terreus* fungi were grown on Potato Dextrose Agar (PDA) medium for 7-9 days and stored at 25°C, before use. The test samples (3.0 x 1.5 cm dimension) were added to the culture media just before the solidifying phase, and then each sample was inoculated in four points with the spore suspension. Two replicates were used for each sample.

The colonization of the samples was monitored by visual observation after 30 and 60 days of incubation in order to evaluate the degree of colonization. Five-grade scale of invasion ranging from 0 to 4 was established as a function of fungi observed on the surface of the films (Table 3).

Table 3. Evaluation of colonization degree over the studied polymeric materials

Growth intensity	Evaluation
0	absence of invasion
1	low attack with a maximum 25 % of the film surface covered with fungi
2	expansion of moderate intensity with a maximum 50 % of the film covered with fungi
3	high degree of colonization over 50 %
4	growth of fungi occupying the whole surface of the specimen

After 60 days of exposure to microorganism's action, the specimens were washed, submerged in ethylic alcohol and dried until a constant weight was obtained.

To determine the ***biodegradation rate*** for both soil burial test and microorganisms' action, the samples were weighed, and the mass variation (degree of biodegradation) was determined using the equation:

$$\Delta M_{biol.} = \frac{\Delta M_i - \Delta M_f}{\Delta M_i} \times 100$$

where: $\Delta M_{biol.}$ represents the variation in the mass of the samples (degree of biodegradation), ΔM_i represents the average of the initial masses of the samples and ΔM_f represents the average of the final masses of the samples (at the end of the maintenance period under controlled conditions).

RESULTS AND DISCUSSIONS

Soil burial biodegradation test - results

At the end of each test period (30 days, 60 days, 90 days), the studied polymeric materials were extracted from the soil, washed with distilled water to remove the soil and dried on filter paper. The samples were dried at room temperature and stored in a desiccator for 48 hours, until a constant mass was obtained.

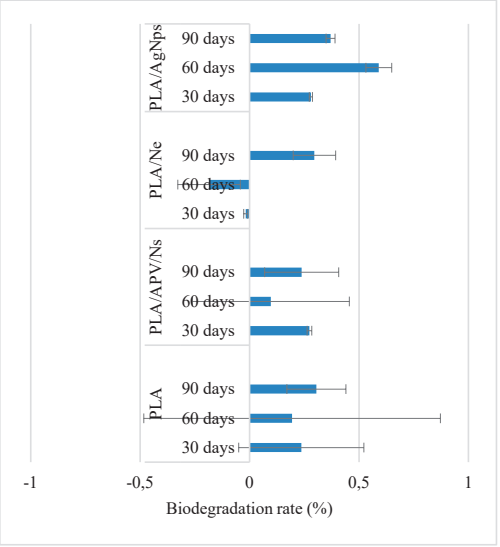

Figure 2. The weight loss values of the PLA based samples at the end of each testing period

Figure 2 presents the values obtained for the biodegradation rate of the PLA based samples. For PLA/Ne, an increase in weight was registered after 30 and 60 days of maintaining in soil, probably due to water absorption of the sample.

The PLA based samples presented low biodegradation rate, between -1.085% and 0.590%. These results are in accordance with other studies, accordingly the biodegradability of PLA taking a long time compared to other biodegradable biopolymers (Pattanasuttichonlakul et al., 2018; Boonluksiri et al., 2021; Mistry et al., 2022). It is mention that the composting is one of the most used methods of biodegradation for this type of material which

can be degraded within 180 days in these types of conditions (Kalita et al., 2021).

The morphology of the studied samples was determined using a scanning electron microscope (SEM). No modifications were observed on the surface of the tested samples at the end of the soil burial test (after 90 days). The analysis showed dense, continuous and smooth structure of the PLA based materials (Figure 3).

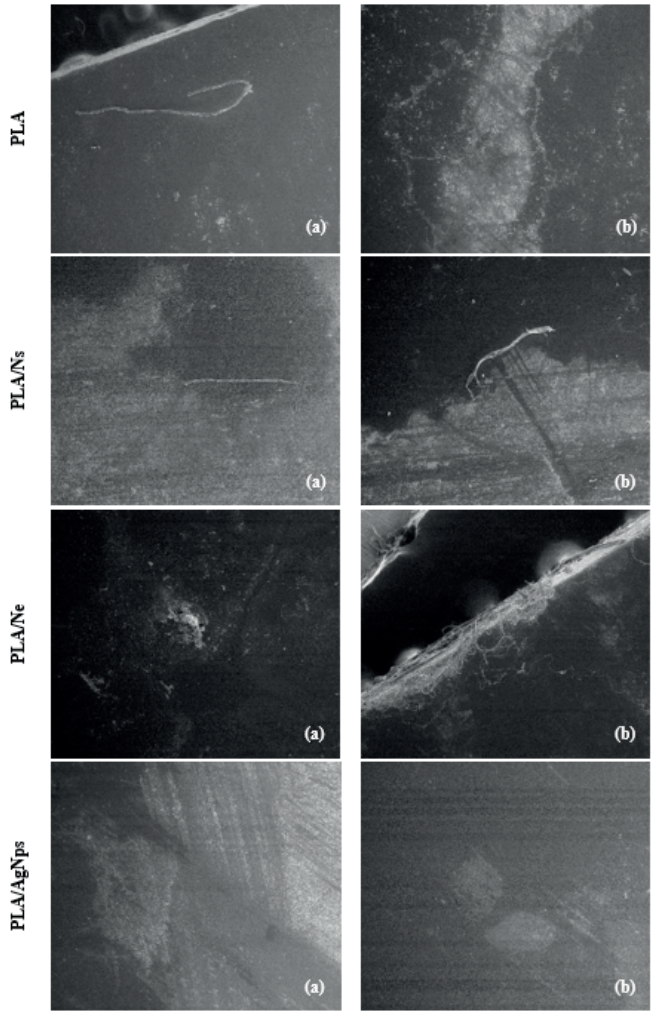

Figure 3. SEM morphology of PLA based samples at the beginning (a) and the end (b) of testing period

Colonisation and in vitro biodegradation test - results

The growth rate of both *Aspergillus brasiliensis* ATCC 16404 and *Aspergillus terreus* fungi on the surface of the studied polymeric samples is presented in Tables 4 and 5.

Table 4. The development degree of *Aspergillus brasiliensis* ATCC 16404 fungus on the surface of the tested polymeric materials

Sample	Incubation time (days)			
	M I		M II	
	30 days	60 days	30 days	60 days
PLA	0	0	0	1
PLA/Ns	1	1	1	2
PLA/Ne	1	1	1	2
PLA/AgNps	0	0	1	1

Table 5. The development degree of *Aspergillus terreus* fungus on the surface of the tested polymeric materials

Sample	Incubation time (days)			
	M I		M II	
	30 days	60 days	30 days	60 days
PLA	1	1	1	1
PLA/Ns	1	1	2	2
PLA/Ne	1	2	1	2
PLA/AgNps	0	0	0	1

The colonisation degree for the studied samples varied between 0 (absence of fungal growth) and 2 (expansion of moderate intensity with a maximum 50 % of the film covered with fungi), depending on the used culture media. The most susceptible samples to be colonised by both fungi were PLA/Ns and PLA/Ne samples. At the end of the testing period (after 60 days) on MII, both samples presented the highest amount of fungal growth on their surface compared to the other tested samples.

The colonisation degree was very low (0 on M I and 1 on M II) for PLA sample when *Aspergillus brasiliensis* ATCC 16404 was used and same values were obtained in the case of PLA/AgNps sample treated with *Aspergillus terreus*. Overall, the colonisation degree was higher for the samples inoculated and incubated on culture media M II, which contained carbon source for microorganism development. This result shows that in order to be colonised, the materials needed support in form of carbon source, found in the culture media. The aspect of the samples after 60 days of incubation is shown in Figure 4.

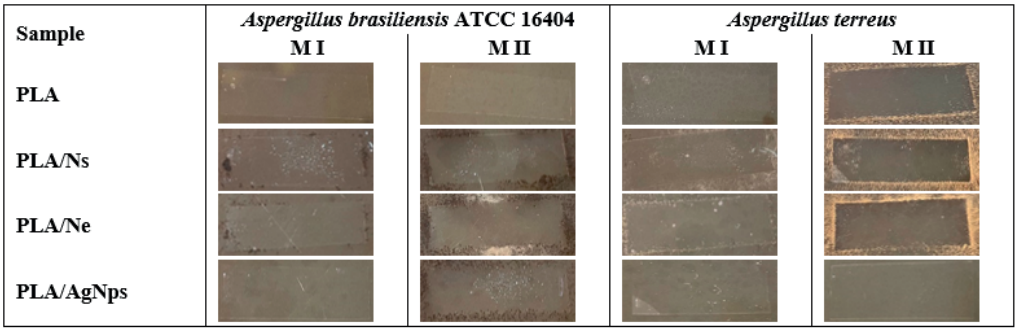

Figure 4. The aspect of PLA based materials after 60 days of incubation for colonisation assessment

At the end of the tested period, samples were taken out of the Petri dishes and cleaned, being stored in a desiccator until constant weight.

The *in vitro* biodegradation rate values for the studies samples are presented in Figures 5 and 6.

The results obtained for *in vitro* biodegradation using two fungal strains are in accordance with the colonisation rate of the studied samples (Table 4 and Table 5). The samples maintained on M II presented higher biodegradation rate compared to the samples exposed to M I, for both *Aspergillus brasiliensis* ATCC 16404 and *Aspergillus terreus*. The highest value for this parameter was determined for PLA/APV/Ns sample, of ~1.42% and ~4.61% on M II when inoculated with *Aspergillus brasiliensis* ATCC 16404 and *Aspergillus terreus,* respectively.

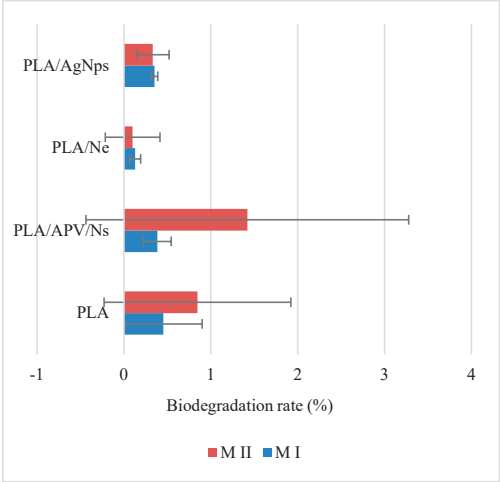

Figure 5. The *in vitro* biodegradation rate of PLA based samples by *Aspergillus brasiliensis* ATCC 16404 fungus

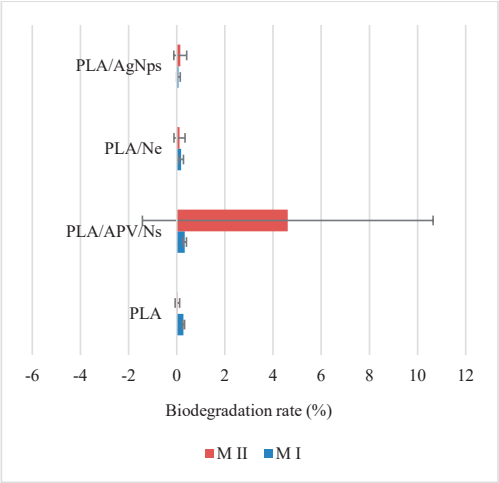

Figure 6. The *in vitro* biodegradation rate of PLA based samples by *Aspergillus terreus* fungus

Within natural environment there are few microorganisms that degrade PLA materials. Therefore, breaking down this polymeric matrix by microorganisms is realized to a lesser extent compared with other biopolymers (Janczak et al., 2018).

CONCLUSIONS

This study aimed at presenting the behaviour and biodegradation aptitude of some PLA based biomaterials approaching two biodegradation methods (soil burial biodegradation and degradation using microorganisms, namely two fungal strains of *Aspergillus brasiliensis* ATCC 16404 and *Aspergillus terreus*). The results are in accordance with other literature studies, PLA being a great biopolymer that could be used to replace conventional packaging plastics. However, its degradation in environmental conditions take a long time, more suitable for PLA biodegradation being composting conditions. The highest biodegradation rate at the end of testing period for soil burial test was obtained for PLA/AgNps sample (0.37%). In the case of *in vitro* biodegradation using fungi, the highest rate of biodegradation was recorded for PLA/Ns (4.61%). The results presented in this study are promising, even though the biodegradation rate is slow, the PLA based materials showed biodegradation properties.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PED-2019-2878, within PNCDI III, contract no. 379PED/2020, acronym BIOMATFOOD.

REFERENCES

Boonluksiri, Y., Prapagdee, B., Sombatsompop, N. (2021). Promotion of polylactic acid biodegradation by a combined addition of PLA-degrading bacterium and nitrogen source under submerged and soil burial conditions. *Polymer Degradation and Stability*, *188*, 109562.

Castro-Aguirre, E., Auras, R., Selke, S., Rubino, M., Marsh, T. (2018). Enhancing the biodegradation rate of poly(lactic acid) films and PLA bio-nanocomposites in simulated composting through bioaugmentation. *Polymer Degradation and Stability*, *154*, 46-54.

Eslami, H., Grady, M., Mekonnen, T.H. (2022). Biobased and compostable trilayer thermoplastic films based on poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and thermoplastic starch (TPS). *International Journal of Biological Macromolecules*, *220*, 385–394.

Freitas, A.L.P., Filho, L.R.T., Calvao, P.S., de Souza, A.M.C. (2017). Effect of montmorillonite and chain extender on rheological, morphological and biodegradation behavior of PLA/PBAT blends. *Polymer Testing*, *62*, 189-195.

Iordache, O., Dumitrescu, I., Perdum, E., Mitran, E.C., Chivu, A.M.A. (2018). An overview on microorganisms derived bio-materials. *Scientific Bulletin. Series F. Biotechnologies*, *XXII*, 194-199.

Janczak, K., Hrynkiewicz, K., Znajewska, Z., Dąbrowska, G. (2018). Use of rhizosphere microorganisms in the biodegradation of PLA and PET polymers in compost soil. *International Biodeterioration & Biodegradation*, *130*, 65–75.

Jeon, H.J., Kim, M.N. (2013). Biodegradation of poly(L-lactide) (PLA) exposed to UV irradiation by a mesophilic bacterium. *International Biodeterioration & Biodegradation*, *85*, 289-293.

Kalita, N.K., Bhasney, S.M., Mudenur, C., Kalamdhad, A., Katiyar, V. (2020). End-of-life evaluation and biodegradation of Poly(lactic acid) (PLA)/Polycaprolactone (PCL)/Microcrystalline cellulose (MCC) polyblends under composting conditions. *Chemosphere*, *247*, 125875.

Kalita, N.K., Damare, N.A., Hazarika, D., Bhagabati, P., Kalamdhad, A., Katiyar, V. (2021). Biodegradation and characterization study of compostable PLA bioplastic containing algae biomass as potential degradation accelerator. *Environmental Challenges*, *3*, 100067.

Lammi, S., Gastaldi, E., Gaubiac, F., Angellier-Coussy, H. (2019). How olive pomace can be valorized as fillers to tune the biodegradation of PHBV based composites. *Polymer Degradation and Stability*, *166*, 325-333.

Lv, S., Zhang, Y., Gu, J., Tan, H. (2017). Biodegradation behavior and modelling of soil burial effect on degradation rate of PLA blended with starch and wood flour. *Colloids and Surfaces B: Biointerfaces*, *159*, 800–808.

Mistry, A.N., Kachenchart, B., Wongthanaroj, A., Somwangthanaroj, A., Luepromchai, E. (2022). Rapid biodegradation of high molecular weight semi-crystalline polylactic acid at ambient temperature via enzymatic and alkaline hydrolysis by a defined bacterial consortium. *Polymer Degradation and Stability*, *202*, 110051.

Pattanasuttichonlakul, W., Sombatsompop, N., Prapagdee, B. (2018). Accelerating biodegradation of PLA using microbial consortium from dairy wastewater sludge combined with PLA-degrading bacterium. *International Biodeterioration & Biodegradation*, *132*, 74–83.

Peng, B.Y., Chen, Z., Chen, J., Zhou, X., Wu, W.M., Zhang Y. (2021). Biodegradation of polylactic acid by yellow mealworms (larvae of *Tenebrio molitor*) via resource recovery: A sustainable approach for waste management. *Journal of Hazardous Materials*, *416*, 125803.

SR EN ISO 846/2000 MATERIALE PLASTICE - Evaluarea acțiunii microorganimelor (Plastics. Evaluation of the action of microorganisms)

Sun, Y., Wang, X., Xia, S., Zhao, J. (2022). Cu(II) adsorption on Poly(Lactic Acid) Microplastics: Significance of microbial colonization and degradation. *Chemical Engineering Journal*, *429*, 132306.

Turco, R., Zannini, D., Mallardo, S., Dal Poggetto, G., Tesser, R., Santagata, G., Malinconico, M., Di Serio, M. (2021). Biocomposites based on Poly(lactic acid), Cynara Cardunculus seed oil and fibrous presscake: a novel eco-friendly approach to hasten PLA biodegradation in common soil. *Polymer Degradation and Stability*, *188*, 109576.