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Growth of selected fungi on biodegradable films

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Abstract. This study presents the data summary on growth speed of selected species of fungi on some of biodegradable polymer materials. Growth rate was assessed on films composed of poly(lactide), poly(ε-caprolactone) and poly(hydroxybutyrate) after a month of incubation in 24°C. To assess growth of fungi optical microscopy on densitometric measurements were used. Through these analyses the best growth was confirmed for fungus: *Chaetomium globosum* (ATTC 6205) on a film made of poly(ε -caprolactone).

Keywords: fungi, poly(lactide), poli(ε-caprolactone), poly(hydroxybutyrate), biodegradable films.

1. Introduction

Polymeric materials play an important role in industry as they have been used for decades in packaging, architecture and medical industry (Kumar et al., 2007; Shah et al., 2008; Żakowska, 2009; Richert, 2017). For the last few years biodegradable polymeric materials are specifically gaining interest (Shogren, 1997; Hakkarainem et al., 2000; Tsuji et al., 2006; Rhim, 2009; Strömberg & Karlsson, 2009; Richert, 2017). Biodegradable polymers can be obtained from crude oil like poly(ε-caprolactone) (PCL) or from renewable resources, which is characteristic for aliphatic polyesters such as poly(lactide) (PLA) and poly(hydroxybutyrate) (PHB) (Manna, 2000; Tokiwa & Calabia, 2006; Wada et al., 2007; Morawska & Krasowska, 2017; Qi et al., 2017). The most common among biodegradable polyesters is PLA. It undergoes complete biodegradation in the time span of 6 to 24 months (Tokiwa & Calabia, 2006; Richert, 2017). PCL is used very commonly as a biodegradable component of many different films, and it accelerates their degradation (Tokiwa & Calabia, 2006). PHB is known in microbiology as a reserve material of numerous bacteria and its concentration in bacterial cells varies from 1 to 30% of dry matter (Jayasekara et al., 2005).

The aim of the analyses was to select the species which are the most beneficial for processes of biodegradation of polymeric materials such as: PLA, PCL and PHB. The attempt was made to select fungi whose growth would be possible in the presence of biodegradable polymeric materials.

2. Material and methods

The subject of the study was biodegradable films made of:

a) Poly(lactide) (PLA), type 2003 D (NatureWorks®, USA). Material marked with symbol (PLA);
b) Poly(ε-caprolactone), type CAPA™ FB100 (Rerstorp, UK). Material marked with symbol (PCL);

c) SoGreen®-2001a (Tianjin Green BioMaterial Company, China) a polymeric mixture composed of poly(3.4-hydroxybutyrate) and poly(lactide). Material marked with symbol (PHB).

The subject of the study was also microorganisms. In this study six strains of fungi from worldwide (ATCC) and Polish (IOR) collections were used. As these fungi are used in microbiological tests that aim at assessing their effect on plastics (PN EN ISO 846 (2014); PN EN 15457 (2014)), there emerged a research need to test and verify these microorganisms against biodegradable polymeric materials. The following types of strains were used for this study: *Chaetomium globosum* (ATTC 6205), *Fusarium culmorum* (IOR 1913), *Penicillium pinophilum* (ATCC 36839), *Paecilomyces variotti* (ATCC 18502), *Trichoderma viridae* (ATCC 9645), *Aspergillus niger* (ATCC 6275).

Cultures of every fungal strain were established in Petri dishes containing optimal agar medium with the composition according to the norm PN EN ISO 846 (2014). The cultures were maintained in 24°C for 1 week. After this time, suspensions with $1.5 \cdot 10^8$ spores/ml were prepared (PN EN ISO 846 (2014); PN EN 15457 (2014)). Later, 100 µl of suspension from every strain of fungi were used to inoculate test tubes with Czapek-Dox no glucose medium with the composition [g/l]: $MgSO_4 \cdot 7H_2O$ – 5.0, Na_2HPO_4 – 1.0, KCL – 0.5, $NaNO_3$ – 3.0, $Fe(SO_4)_3 \cdot 7H_2O$ – 0.01, agar – 15.0. Two test series were prepared:

1. Control samples, which consisted of medium with fungus inoculum (symbol in Figure 1: "Fungi").
2. Test samples, which, apart from medium and fungus inoculum, also included tested film fragments sized 1.5 cm x 1.5 cm (symbols in Figure 1: "PLA + fungi", "PCL + fungi", "PHB + fungi").

The experiment was conducted in three repeats for every configuration. Such prepared cultures were incubated in 24°C on laboratory shaker Unimax1010 with heating module (Heidolph) [100 rpm] for a month. After this time, optical density of suspensions was measured and microscopy analyses were performed.

Turbimetric measurements were conducted on Densi-La-Meter® II (Erba Lachema) densitometer. In microbiology, McFarland Standards are used as a reference to adjust the turbidity of microbial suspensions (Libudisz et al., 2009; PN EN ISO 846 (2014)). Table 1 shows the McFarland Standards.

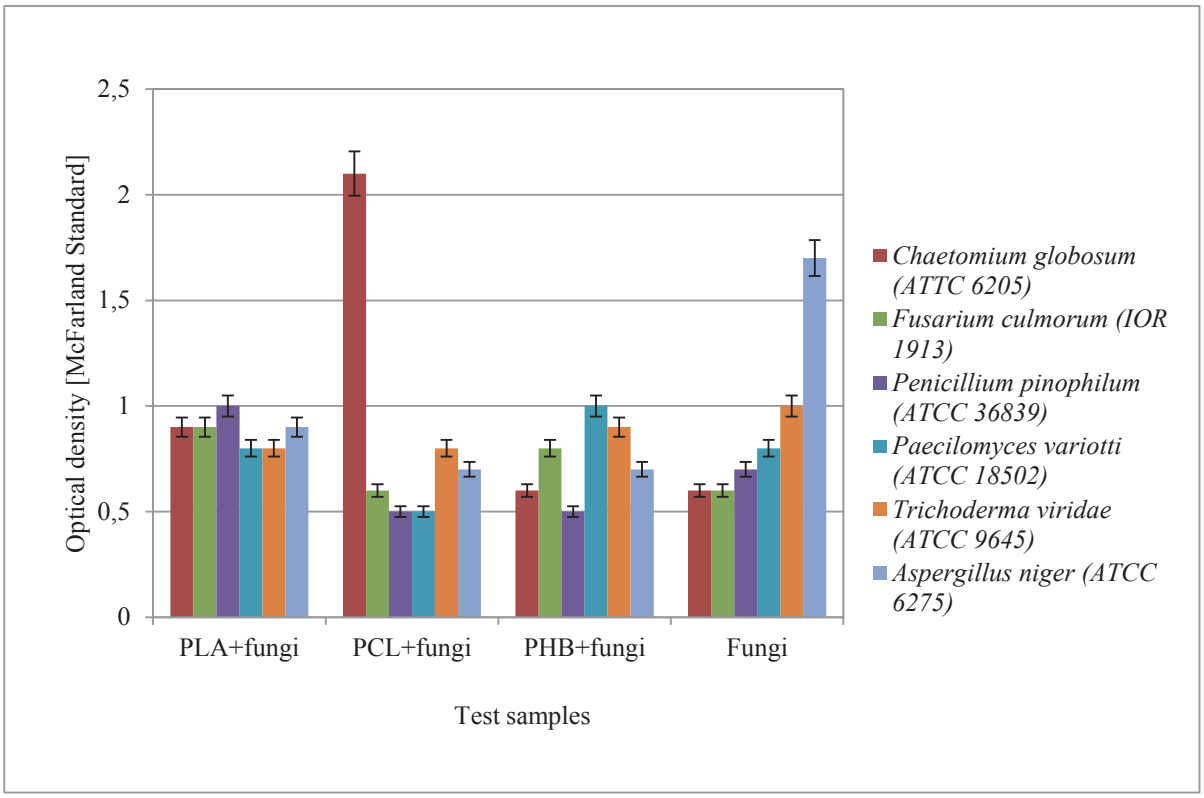


Figure 1. Optical density of the culture fluid after a monthly incubation in the presence of samples PLA, PCL, PHB and fungi as well as the fungi themselves

Table 1. McFarland Standards

McFarland Standards	Approximate microbial suspension/ml
0.5	$1.5 \cdot 10^8$
1.0	$3.0 \cdot 10^8$
2.0	$6.0 \cdot 10^8$
3.0	$9.0 \cdot 10^8$

Microscopic observations of mycelium growth on the films were conducted with the use of Olympus SZX12 stereoscopic microscope with image magnification 120x, with ARTRAY Model ARTCAM 300MI camera.

3. Results and discussion

The results of measurements of optical density of films in the presence of fungi are shown on Figure 1. It describes differences between the optical density of medium containing test materials (PLA, PCL, PHB) and fungi, and the medium containing only fungi: *Chaetomium globosum* (ATTC 6205), *Fusarium culmorum* (IOR 1913), *Penicillium pinophilum* (ATCC 36839), *Paecilomyces variotti* (ATCC 18502), *Trichoderma viridae* (ATCC 9645), *Aspergillus niger* (ATCC 6275). Table 2 shows compared results of changes on the surfaces of films under the influence of six strains of fungi.

In Figure 1, changes in the growth of individual fungal strains were observed depending on the type of material tested. Analysis of changes in the surface structure (Table 2) confirmed the highest activity of the *Chaetomium globosum* strain, which was characterized by the largest increase on the surface of the PCL film.

In our research it was proved that the fungi that best developed on the film made of poly(ε-caprolatone) (PCL) was: *Chaetomium globosum* (ATTC 6205) (Fig. 1, Table 2), it has the highest optical density above 2 McFarland (Fig. 1). I turn, *Fusarium culmorum* (IOR 1913) was the most grown on the surface of films marked as PLA and PHB. In several cases (Fig. 1), a higher optical density was noted in case of a variant containing only medium and fungal inoculum (without a film). These results indicate that fungi *Trichoderma viridae* (ATCC 9645) and *Aspergillus niger* (ATCC 6275) do not degrade PLA, fungi *Paecilomyces variotti* (ATCC 18502), *Penicillium pinophilum* (ATCC 36839) and *Aspergillus niger* (ATCC 6275) do not decompose PCL, nor do they degrade the material marked with the PHB symbol.

Presented result of this study (Fig. 1, Table 2) concerning growth of fungi on the surface of biodegradable polymeric materials prove that both environment of culture and material are not without a meaning for the process of fungi growth. This is particularly evident for PLA, PCL and PLA, PHB samples where the growth of *Chetomium globosum* and *Fusarium culmorum*, respectively, is the highest compared to other microorganisms (Table 2).

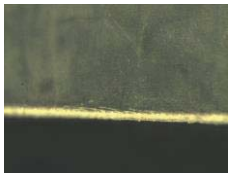
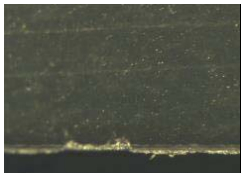
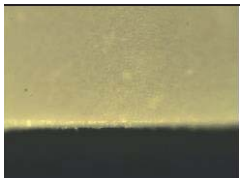
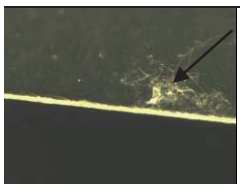
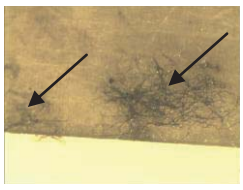
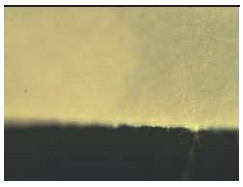
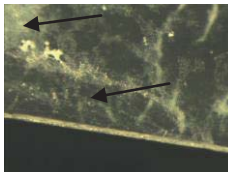
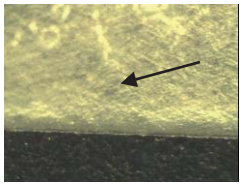
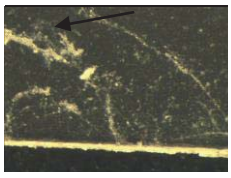
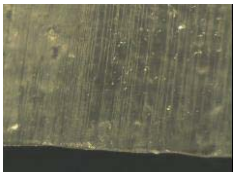
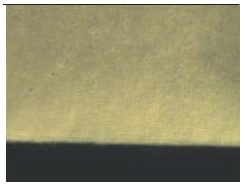
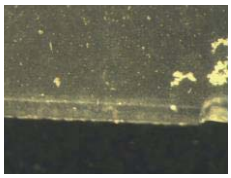
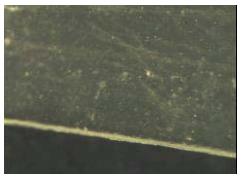
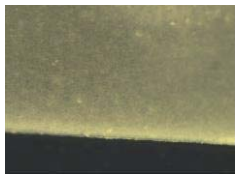
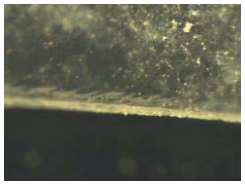
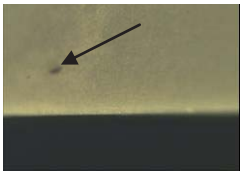
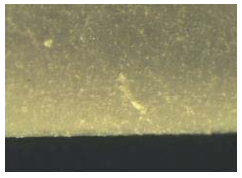
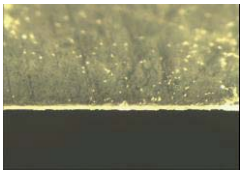
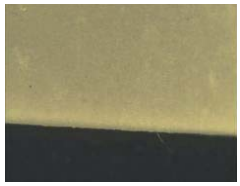
Material susceptibility to biodegradation is verified with use of normalised research methods. Each of these techniques consists in submitting the polymeric material to the microorganisms. Microorganism growth on the film sample is tested on liquid and solid surface depending on the experimental technique. Very often, such analyses use medium that does not contain a carbon source (Jayasekara et al., 2005).

Levinskaitė (2018) research showed that the fungi *Penicillinum. chrysogenum*, *P. spinulosum*, *P. verruculosum*, and *P. variabile* developed well on the majority of the substrata such as paraffin, chitin, leather. The use of the tested substrata shows that most of these fungi, isolated from materials hardly suitable to fungal nutritive requirements, can utilize different carbon sources. According the author These fungi can be considered good decomposers of materials occurring in nature as wastes.

The development of fungi on polymeric materials can be determined using different techniques, the growth of microorganisms itself depends mostly on experimental conditions (Redlak et al., 2001; Morawska & Krasowska, 2017). It should be noted, however, that unification of experimental conditions for different strains can be problematic. According to Nishida & Tokiwa (1993), determination of microorganism quantity is an effective and proper method of assessing the suitability of a strain in polymeric material biodegradation.

Araceli et al. (2011) proved that out of several fungi examined, *Aspergillus flavus* (ATCC 6051) caused a polyurethane mass decrease by over 60% after a month of incubation. Torres et al. (1996) while conducting research on PLA proved that only two out of fourteen strains (*Fusarium moniliforme* i *Penicillium roqueforti)* showed the ability to use PLA lactic acid. By 2006, the *Tritirachium album* (synonym *Engyodontium album*) was the only described fungus degrading L-PLA (Tokiwa & Calabia, 2006).

Table 2. Analysis of changes in surfaces of the films after month of incubation with fungi (the largest growth of fungi was marked with the arrows)

	PLA	**PCL**	**PHB**
Control (without fungi)			
Chaetomium globosum (ATTC 6205)			
Fusarium culmorum (IOR 1913)			
Penicillium pinophilum (ATCC 36839)			
Paecilomyces variotti (ATCC 18502)			
Trichoderma viridae (ATCC 9645)			
Aspergillus niger (ATCC 6275)			

Dey et al. (2012) research showed that in incubated films in the presence of microorganisms, changes appear in form of cracks, pores, crevices, bulges, holes, dark spots and the presence of microorganisms themselves, which can colonise a given surface of the film and create a biofilm. In the study Janczak et al. (2014) growth of the fungi such as *Clitocyte*, *Laccaria laccata* and *Trichoderma viridae* on type of films: polyethylene (PE), poly(ethylene terephthalate) (PET), polycaprolactone (PCL), polylactide (PLA), polyhydroxybutyrate (PHB) was analysed. *Laccaria laccata* showed the highest growth activity on polymers. The analysis with the microscope confirmed the highest activity of this fungi. On the surface of PCL, especially were visible deep cracks. In our research the highest activity of fungi (*Chaetomium globosum* and *Trichoderma viridae*) also was observed on PCL surface.

Numerous scientific papers on the search for fungi capable of growing on polymeric materials indicate the need for this kind of research (Torres et al., 1996; Dey et al., 2012; Morawska & Krasowska, 2017; Apetrei et al., 2018; Barnharst et al., 2018; Gibas et al., 2018; Levinskaite, 2018). Due to the protection of natural environment, especially heathlands, including plants and lichens (Adamska & Deptuła, 2015a, 2015b; Adamska et al., 2015), it seems appropriate to look for new recycling methods. It is also necessary to search for microorganisms capable of settling in the environment burdened with polymeric waste and to use them for waste utilisation through biodegradation processes (Tokiwa & Calabia, 2006).

4. Conclusion

The applied method allowed to evaluate the tested fungal strains in terms of their use in biodegradation processes. The fungi that was the most developed on the surface of PCL was *Chaetomium globosum*, while on the surface of PLA and PHB it was *Fusarium culmorum*. The analysis of the surface of the film carried out with the use of an optical microscope confirmed in a fairly good way the results of the research obtained from densitometric measurements.

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