

Effect of biobased fillers nature on biodeterioration of hybrid polyethylene composites by mold fungi

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Abstract. The paper is devoted to investigation of deterioration of natural fillers and polyethylene composites on their basis (polyethylene/filler=70/30) due to the action of mold fungi. The fillers chemical composition, dimensional parameters and biodegradability have been analyzed as factors exert a considerable impact on composite materials biodeterioration. It has been found that the principal factor determining the biodeterioration of polyethylene/filler composites by mold fungi is chemical composition of a filler and, in turn, its biodegradability. The excess of holocellulose content over lignin content and high protein content in a filler are able to induce biofouling of the polymeric composite materials. The presence of soluble and easy hydrolysed fraction in a filler increases its availability in a polymeric matrix. According to the study results, most effective natural fillers as additives stimulating polyethylene composites biodegradability are milled straw of seed flax and hydrolyzed keratin of bird's feather.

1. Introduction

Development of composite materials on the basis of traditional synthetic polymers and various additives initiating process of biodegradation is the most technologically and economically advantageous direction of biodegradable polymers creation. Oxo-additives, polymers synthesized from renewable vegetable raw materials by biotechnological methods and natural polymers are most often used as the additives initiating degradation of polyolefins in the environmental conditions.

Hybrid composite materials based on polyolefins with natural fillers such as starch [1], wood flour [2], natural rubber [3], chitosan [4], various natural fibers [5-8], etc. have prospects for wide use. Addition of various production wastes as natural fillers allows not only to provide biodegradability and to partially replace non-renewable petrochemical raw materials on renewable but also to reduce their cost [9]. In most cases, production of similar composites based on polyolefins and natural additives does not require cardinal change of production process that also causes profitability of their production.

The presence of a filler leads to existence of large surface contact area with polymeric matrix through which destroying agents can get into the polymer [10]. Thus, a natural additive acts as an initiator not only biological destruction but also polymer oxidizing. Besides, natural fillers being hydrophilic and easily assimilated by microorganisms promote increase in adhesion of microorganisms to polyolefins surface [11]. The result is an expedited attachment of microorganisms to the surface of composite material with subsequent biofouling. To a greater extent, properties and ability of hybrid composite materials based on polyolefins and natural fillers to be degraded and



assimilated by microorganisms is defined by content and chemical composition of a filler, and also dimensional parameters and shape of its particles [12].

The main objective of this study was to investigate the influence of chemical composition and dimensional parameters of biofillers on biodegradability of polyolefin composites. Additionally the authors place emphasis on the correlation between ability to biodeterioration of individual fillers and composites on their basis.

2. Materials and methods

Objects of the research were fillers and composite materials on the basis of low-density polyethylene (LDPE) (TM 15803-020, JSC Neftekhimsevilen) with the following molecular mass characteristics: $M_w=1.0 \times 10^5$ Da, $M_n=1.5 \times 10^4$ Da, $M_w/M_n=6.9$ (GPC at $(140 \pm 2)^\circ\text{C}$, solvent is 1,2,4-phenyl trichloride). Available natural raw materials, which mainly are waste of various productions, were used as fillers: powdered cellulose (hereafter cellulose) ("Politsell PTsS", TU 5410-029-32957739), native corn starch (starch) (GOST 51953), milled straw of seed flax (flax) (LM98 grade), milled chaff of winter wheat (wheat) (Krasnodarskaya-99 grade), wood powder of fir-tree breeds (wood), hydrolyzed keratin of bird's feather to oligopeptides (feather).

The filler content for all the composite materials based on LDPE was 30 wt.%. Obtaining of the composites was carried out by means of a Plasti-Corder mixer torque rheometer (Brabender type) in the atmosphere of gaseous argon at a temperature of $(140 \pm 2)^\circ\text{C}$ and with rotors speed of 30 rpm within 5 minutes. A weighted amount of LDPE was placed in the mixing chamber, 2 minutes later filler was added. After cooling, materials were crushed by means of a knife mill RM 120 (Vibrotechnik, LLC, Russia), and then subjected to pressing by a manual hydraulic press PRG-10 (VNIR, LLC, Russia) at a temperature of $(140 \pm 2)^\circ\text{C}$ and load of 70 kgf/m^2 on a cellophane substrate, followed by quenching in water at $(20 \pm 2)^\circ\text{C}$. Finally, film samples with the thickness of $(130 \pm 10) \mu\text{m}$ were obtained. The thickness of the films was measured by a micrometer screw gage.

The analysis of chemical composition was carried out by infrared spectroscopy on a FT-IR spectrometer Perkin Elmer Spectrum 100 (Germany) at a temperature of $(22 \pm 2)^\circ\text{C}$ in the range of wave numbers $4600 \leq \nu \leq 650 \text{ cm}^{-1}$ by a method of frustrated total internal reflection.

Thermal stability of fillers was determined by a method of thermogravimetric analysis (TGA) on a derivatograph Netzsch TG 209 F1 Iris (Germany) at a heating speed of $20^\circ\text{C}/\text{min}$ in a temperature interval of $25\text{-}800^\circ\text{C}$.

Microscopic investigation was carried out using an optical microscope Axio Imager Z2m, Carl Zeiss (Germany) with Axio Vision software at magnification of 50x, 200x, 500x in transmitted and reflected light.

Mycological examination was carried out at the premises of the mycology and algology department of Lomonosov Moscow State University. Biodegradability of the fillers was estimated on water and agar media with 30 wt.% of a filler according to GOST 9.049 and ISO EN 846 with the use of *Aspergillus niger*, *Aspergillus terreus*, *Penicillium chrysogenum*, *Aspergillus flaus*, *Penicillium cyclopium* and *Paecilomyces variotii* test fungi cultures, which are the most universal decomposers of carboniferous substances. As control environments, Czapek's medium and wort agar on the basis of malt extract were used. The pH index was determined by means of pH chemical agent test strips.

Microbiological testing of the composite materials was carried out with *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride* and *Paecilomyces variotii* test cultures according to GOST 9.049 and ISO EN 846. The incubation of the samples inoculated by spore suspension of micromycetes (1×10^5 spor/ml) was carried out in the conditions of relative humidity more than 90% at $(22 \pm 2)^\circ\text{C}$ within 84 days with intermediate studies in 28 and 56 days. The fungal development on a sample surface was estimated by a rating scale according to ISO EN 846. The gain of fungal biomass was determined by the difference in weight of a sample with fungal mycelium and a cleared sample (washing in ethyl alcohol (95 vol.%)).

3. Results and discussion

The most important characteristics of fillers for polyolefins are decomposition temperature, particles shape and size, chemical composition, and biodegradation rate under the influence of microorganisms. The basic physical and chemical properties of the fillers under investigation are given in Table 1.

The fillers have various degree of dispersion and dimensional parameters of particles (length (L) and diameter (D), length to diameter ratio (L/D -ratio)) that can exert a considerable impact on materials structure, properties, and biodegradability [7]. Oblong soft fibers with a wide range of length ($L/D \approx 10$) are typical for the cellulose. Largely, particles of the milled flax straw have rigid oblong cylindrical units ($L/D \approx 8$). The particles size and shape of the milled wheat chaff and wood powder are similar with elliptical shape of particles ($L/D \approx 3$). The starch filler and hydrolyzed feather have small spherical particles of 10-20 μm with a tendency to large agglomerates formation (up to 250 μm). The presence of a soluble and easy hydrolyzed fraction in some of the fillers (flax, wheat and feather) could effect on the weight loss under soil medium resulting from removing of the available fraction.

Table 1. Properties of the fillers applied for polymeric composites.

Filler	Decomposition temperature (TGA), °C ($\Delta \pm 3^\circ\text{C}$)	Dimensional parameters of particles, μm ($\Delta \pm 5\mu\text{m}$)	Easy hydrolysable fraction, wt.% ($\Delta \pm 5\text{wt.}\%$)	Crystallinity index, D_{1423}/D_{895} (IR)	Holocellulose/ lignin, D_{1370}/D_{1510} (IR)	Lignin/ hemicellulose D_{1595}/D_{1645} (IR)
Cellulose	290	$L=150-500$ $D=20-40$ $L/D \approx 10$	5	0.76	-	-
Flax	240	$L=100-400$ $D=25-50$ $L/D \approx 8$	25	1.33	1.14	1.05
Wheat	270	$L=80-100$ $D=20-40$ $L/D \approx 3$	30	1.12	1.96	0.64
Wood	275	$L=70-120$ $D=20-40$ $L/D \approx 3$	10	0.87	1.05	1.45
Starch	290	$L=10-20$ $D=10-15$ $L/D \approx 1$	Gel formation	-	-	-
Feather	190	$L=10-20$ $D=10-20$ $L/D \approx 1$	55	-	-	-

One of the major factors determining a rate of biodegradation of synthetic polymers filled by natural components is the ability of microorganisms to use substances forming part of the fillers as a nutrient source [13]. For studying biological utilization rate of the fillers by microorganisms, the analysis of their chemical composition was carried out by means of IR-spectroscopy and TGA, as well as an assessment of fillers biofouling by mold fungi cultures.

According to TG and DTG-curves received by the TGA method the starch and hydrolyzed keratin fillers contain about 10 wt.% of easily volatiles as shown by an endothermic peak in the area of $(70 \pm 15)^\circ\text{C}$ [14]. The summarized peak of lignocellulose complex decomposition (hemicellulose, cellulose and lignin) with a maxima at temperatures of 307°C , 320°C and 345°C are observed on the DTG-curves of flax, wheat and wood, respectively. It is important to note that a decrease of thermal stability of cellulosic components compared to pure cellulose occurs due to the presence of water and hemicellulose in flax, wheat and wood [15]. In consequence of hemicellulose destruction in these fillers, non-flammable gases are evolved that is capable to accelerate the process of cellulose depolymerization [16]. Increasing the oxidation time of a carbon residue and share of a non-

combustible ash content for the cellulosic fillers (10-13 wt.%) compared with pure cellulose (5 wt.%) indicates the presence of lignin. The existence of a narrow exothermic peak on the DTG-curve of the flax straw decomposition in the area of $(450\pm 5)^\circ\text{C}$ can be referred to fats oxidation being its part [17].

In accordance with the results of IR-spectroscopy (ATR) of the powdered fillers, the peculiarities of chemical composition were determined (Figure 1). The ratio of absorption bands intensities of 1015 and 1047 cm^{-1} , which are responsible for amorphous and organized structures of starch, respectively [18], is equal to 1.65 that gives evidence of its poorly organized structure. The comparison of IR-spectra of the cellulosic fillers was carried out. From the ratio of band intensities of D_{1370}/D_{1510} [19] it was stated that the ratio of holocellulose/lignin increases in the series of wood-flax-wheat. The ratio of lignin and hemicellulose in the fillers was determined by the ratio of band intensity of 1595 cm^{-1} in xylans (fluctuation of $\text{C}=\text{O}$ in ester groups) and band intensity of 1645 cm^{-1} in pectins, which are the basic components of hemicellulose [20]. It was shown that lignin share compared to hemicellulose content in the cellulosic fillers increases in the series of wheat-flax-wood. According to a ratio of the optical density values of D_{1423}/D_{895} , the crystallinity index of the fillers increases in the series of cellulose-wood-wheat-flax.

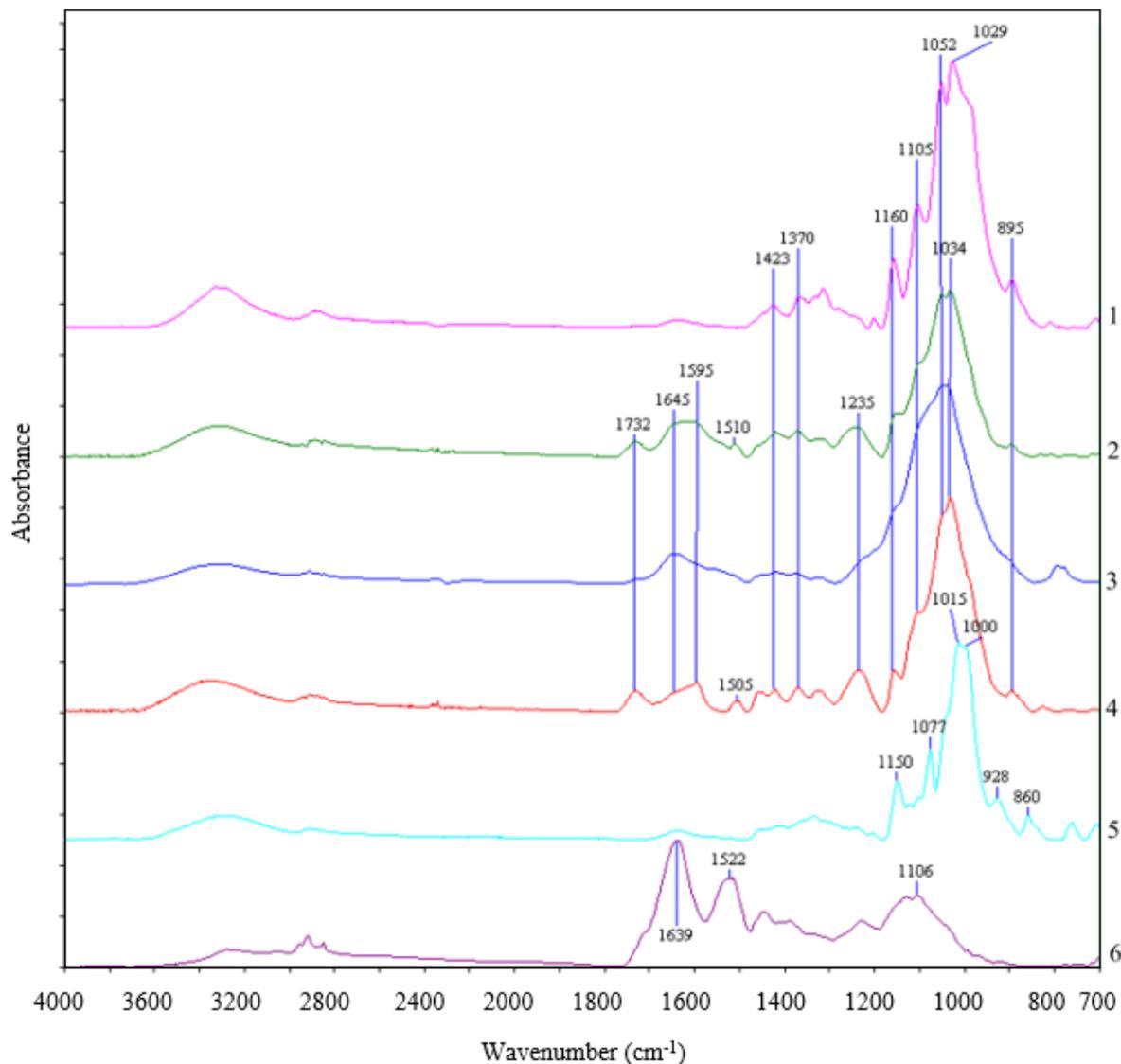


Figure 1. FTIR-spectra of the powdered fillers: cellulose (1), flax (2), wheat (3), wood (4), starch (5), feather (6).

According to the results of microbiological test of individual fillers, it was determined that mold fungi are capable to use all the tested fillers as a carbon source. However, the dynamics of micromycetes growth of on aqueous and agar media with different fillers under the identical conditions of incubation and the same filler content are not identical (Figure 2 (a)). The highest values of summarized biomass gain – $(36\pm 0.5)\text{wt.}\%$ and $(38\pm 0.5)\text{wt.}\%$, respectively, were observed on the 14th day after test cultures inoculation of aqueous media with flax straw and hydrolyzed feather keratin. The wheat chaff and wood powder fillers were characterized by a biomass cumulative value of $(27\pm 0.5)\text{wt.}\%$. The lowest value of biomass gain from all the tested fillers was observed on media with cellulose and starch – $(23.5\pm 0.5)\text{wt.}\%$ for the majority of test cultures. In case of cultivation of such cultures as *A. terreus*, *P. chrysogenum*, *A. flaus* and *P. cyclopium* on the hydrolyzed feather containing peptides, alkaline products of micromycetes metabolism were accumulated. The flax straw and hydrolyzed feather under the action of *A. niger* were characterized by the most intensive reduction of pH index.

Dynamics of mold fungi growth on agar media with different fillers has a complex character. As an example, growth curves of inoculated colony of *A. terreus* on agar media with 30 wt.% of fillers are given (Figure 2 (b)). A high intensity of a colony growth for the majority of mold fungi cultures was characteristic for media with hydrolyzed feather keratin (growth rate on this substrate was 3-4 mm/day) that is apparently connected with a high content of low molecular peptide components in it. The surface of agar media with cellulose and starch at the incubation period of 28 days was characterized only by separated areas of sporulation formation. The maximum colonization of agar media with introduction of cellulosic components was determined for cultures of *A. terreus* and *P. chrysogenum*. High rate of micromycetes growth was typical for medium with seed flax straw additive that could be caused by a high content of hydrolyzed holocellulose, and the presence of fat component. It should be noted that growth curves of the majority of cultures (*A. niger*, *A. terreus*, *P. cyclopium*, *P. variotii*) on media with wheat (Figure 2 (a) and (b)) had the existence of a long lag phase (up to 11 days) after that colony growth occurred. The wood of fir-tree species also had reduced biodegradability, apparently, due to a high lignin content.

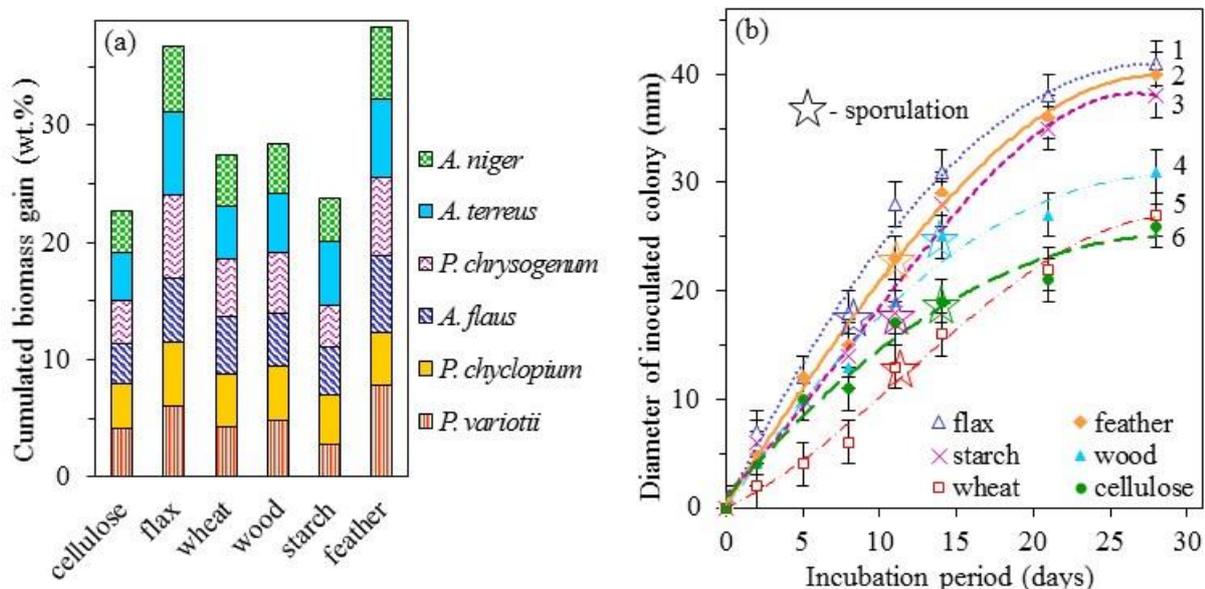


Figure 2. Cumulated biomass gain of mold fungi test cultures (wt.% of filler weight) on aqueous media with 30 wt.% of fillers on the 14th day after cultures inoculation (a); Growth curves of inoculated colony of *Aspergillus terreus* on agar media with 30 wt.% of fillers: flax (1), feather (2), starch (3), wood (4), wheat (5), cellulose (6) (b).

The fungal growth assessment of such test cultures as *A. niger*, *P. chrysogenum*, *T. viride* and *P. variotii* on film samples of LDPE/filler composites was carried out (Figure 3). It was experimentally shown that the film sample of neat LDPE is not nutrient medium for mold fungi (intensity of fouling is 0-1 point) (Figure 4 (a)). For all the LDPE/filler composites, it was observed the growth of fungal mycelium with sporulation development that argues for the content of enough nutrients, which provide fungal growth. The difference in the intensity of micromycetes growth on surface of the materials depending on the type of a filler was noted that is coherent with the results of biofouling assessment of individual fillers. Therefore, composite materials filled by cellulose contain nutrients, which provide low growth of mold fungi with fouling of less than 20% of the material surface (1-1.5 points depending on culture) (Figure 4 (b)). Filling of LDPE by starch also promoted insignificant increase in biodegradability of materials in comparison with neat LDPE (1.5-2.5 points depending on culture). At the same time the existence of additives like flax, wheat and wood flour in LDPE matrix promoted more intensive fungal growth (2.5-3.5 points) (Figure 4 (c)). The hydrolyzed bird feather containing proteinaceous component and having high ability to washing away from a polymeric matrix is easily available source of carbon for micromycetes. Therefore, the intensity of fungal growth on the LDPE/filler composites is connected with chemical nature and availability of a filler.

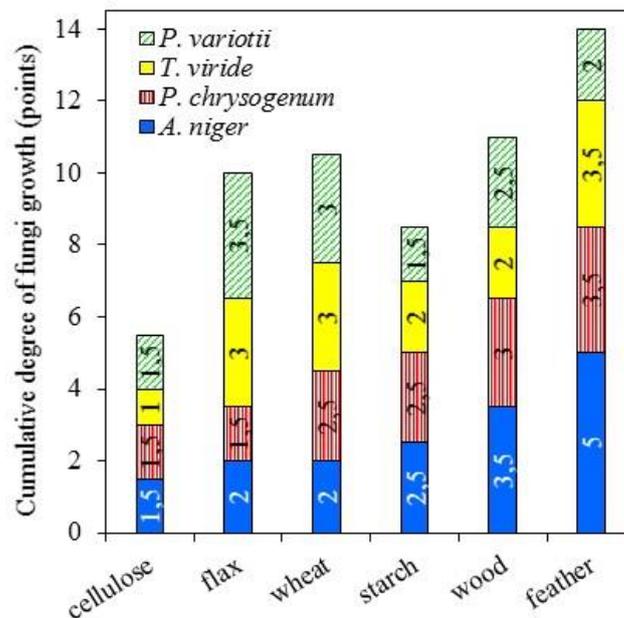


Figure 3. Dependence of cumulative degree of fungal growth on filler nature in the LDPE/filler composites.

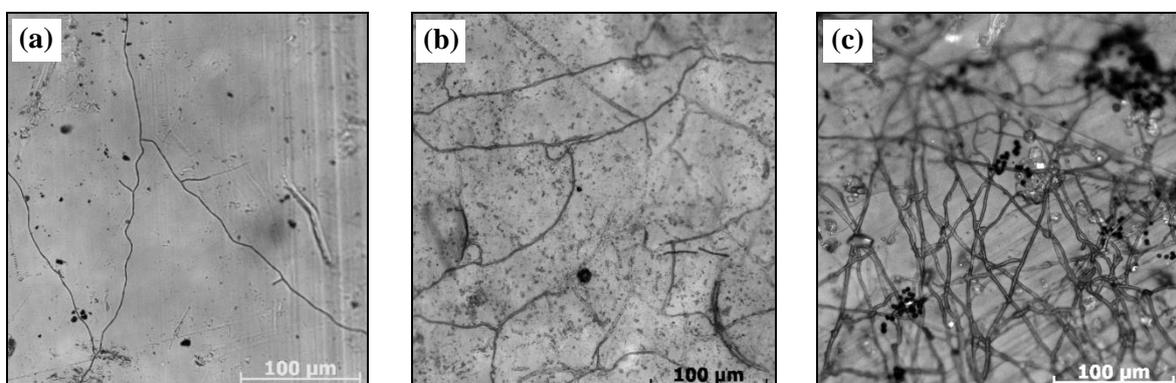


Figure 4. Microphotographs of LDPE (a), LDPE/cellulose (b), LDPE/flax (c) samples after 84 days from inoculation by spore suspension of *Aspergillus niger* culture (reflected light, 200x).

4. Conclusion

Novel hybrid polyethylene composites filled by natural powdered components (30 wt/%) have been investigated. Natural fillers and polymer composite materials on their basis have been destructed by mold fungi. The chemical compositions of the fillers, their dimensional parameters and biodegradability have been analyzed as factors exert a considerable impact on the biodegradability of composite materials. The data of biodegradability test for individual fillers is consistent with the data of biodegradability test for the LDPE/filler = 70/30 composites. It has been found that biodegradability of the composites under mold fungi action is preeminently affected by not materials structure, but chemical composition of a filler.

Protein content and presence of soluble and easy hydrolyzed fraction in the fillers are able to increase biodegradability of hybrid polyethylene composites. High rate of micromycetes growth on the PE/flax composites could be caused by the excess of hydrolyzed holocellulose content over lignin content, and presence of fat component. The intensity of biodegradability of the bird feather filler containing proteinaceous component and composites on its basis is twice as much as the other fillers. Easy hydrolyzed fraction (more than 10 wt.%) of fillers incorporated in the polyethylene matrix makes composites an easily available source of carbon for micromycetes. Higher L/D-ratio of filler particles could induce biofouling of the polymeric composite materials due to their accessibility.

According to the paper results, most effective natural fillers as additives stimulating polyethylene composites biodegradability are milled straw of seed flax and hydrolyzed keratin of bird's feather.

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