Scientific Bulletin, Series F, Biotechnologies, Vol. XVI, 2012
ISSN Online 2285-5521, ISSN-L 2285-1364

COLONIZATION AND DEGRADATION OF POLYETHYLENE COMPOSITES BY FUNGAL STRAINS ISOLATED

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Key words*: biodegradation, biofilm, composites, low density polyethylene, SEM*

Abstract

Polyethylene is one of the most inert plastic materials characterized by a high molecular weight, complex three dimensional structures and hydrophobic nature. These features interfere with its availability to microorganisms and cause serious environmental problems. One of the viable alternatives to accelerate the attack of microorganisms to LDPE is the addition of natural polymers; like starch, wood flour or cellulose fibers to guarantee at least a partial biodegradation. Wood flour is considered an excellent fillers for LDPE because of their low density, low cost, high strength, desirable fiber aspect ratio, flexibility during processing and biodegradability. The objective of this study is to investigate polyethylene based composites biodegradability. Several fungal strains were isolated after exposure period to soil burial tests. Strains from existing microbial collection were also tested. Polyethylene degradation ability of microorganisms was evaluated by weight loss and scanning electron microscopic (SEM) study of plastic strips after 3 months incubation in pure shake culture conditions. SEM analysis showed a thick network of fungal hyphae forming a biofilm on the surface of the plastic pieces. The colonization extent varied from strain to strain. There was observed a small difference but no significant in the weight of polyethylene composites before and after incubation with microorganisms. Strains tested are capable to form an adherent biofilm on the surface of LDPE composites. It is a slow process but these experiments give the evidences of biodegradation of LDPE composites.

INTRODUCTION

Polyethylene (PE) occupies an important position representing the majority of thermoplastics currently used as food packaging materials. It can be classified as high density and low density polyethylenes (HDPE and LDPE). LDPE is characterized by good toughness, resistance to chemicals, flexibility and clarity. The high consumption of these polymers leads to negative environmental impact through the accumulation and disposal of plastic wastes. Degradation of waste plastics through ability of microorganisms to use it as carbon and energy source becomes a viable alternatives to deal with such problems [1, 2]. Biodegradation of polyethylene materials could be enhanced by the following approaches, (a) to exploit the microorganisms in degrading polyethylene and (b) to develop artificial polymers susceptible to biodegradation. The suitable microbial strains for biological degradation of polymeric materials could be selected from existing microbial collection or obtained after isolation from waste contaminated soil. The mixture of the conventional plastic with the biodegradable polymer is believed to produce a type of plastic material with an improved biodegradability. Natural polymers, such as starch and lignocellulosic fibers from agroindustrial or agricultural residues are good fillers for thermoplastics [3]. The objective of this study is to investigate biodegradability of polyethylene based composites using several microbial strains either isolated from plastic samples buried in soil, either from existing microbial collection. The biodegradation experiments were performed using composites containing LPDE, wood flour and glycerol. The

extent of biodegradation was evaluated by comparing the initial and final dry weights of polyethylene before and after incubation with microorganism. Morphological aspects were revealed by scanning electron microscopic (SEM) analysis.

MATERIAL AND METHOD

Polymeric substrates

Blend films with 75% LPDE, 15% wood flour and 10% glycerol were obtained by baking a mixture of components, and mixing on a Brabender Plastograph, followed by calendering and extrusion as films or sheets. The films were cut into pieces 2 cm x 2 cm and sterilized at UV light for 10 minutes. Each film was then aseptically transferred and individually placed into sterile medium.

Microorganism and growth conditions

Aspergillus niger 105 and *Phanerochaete chrysosporium* belong to Microbial Collection of INCDCP-ICECHIM. The isolated strains were: *Fusarium sp.* (strain 2, 6 and 9), *Aspergillus sp (*strain 5*), Penicillium sp. (*strain 7*).* All strains were maintained at 4^{o}C in tub test with dextrose-agar-potato medium. The composition of ¼ diluted Sabouraud medium was as follows (g/l): 2.5 peptone; 40,0 D+glucose; . pH medium 6.0. The medium was autoclaved at 121^{o} C for 20 minute. After inoculation, the cultures were carried out in 300 ml Erlenmayer flasks containing 50 ml of the liquid medium, on a rotary shaker at 160 rpm and 28◦C for 3 months. Experiments were performed in duplicate. The microbial culture without polymeric samples was used as control. After incubation, the pieces of polymer were taken out from the culture and repeatedly rinsed with distilled water, dried at 35^{o} C and use for evaluation of biodegradation efficiency.

Morphological analysis (Scanning electron microscopy)

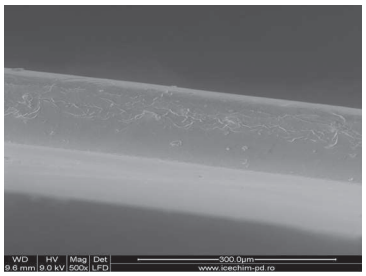
The observations of the film surfaces were performed with scanning electron microscope FEI-QUANTA 200. For SEM analysis, the film samples were dried and placed on metallic support, aluminum standard stub. The samples were performed at 10-15kV and 50-120 Pa using a Large-Field detector. Micrographs of the samples were taken at different magnifications to identify changes on the surface during the degradation process.

Determination of weight loss

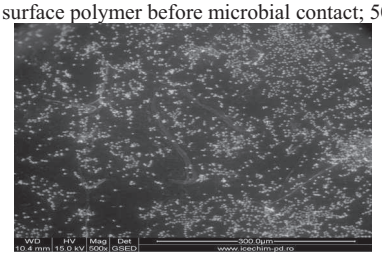
Recovered plastic samples were analyzed for degradation by weight loss before and after microbial treatment. The percentage weight loss of the inoculated plastic samples is given by the formula % Weight loss = (final weight – initial weight)/ initial weight x 100.

RESULTS AND DISCUSSIONS

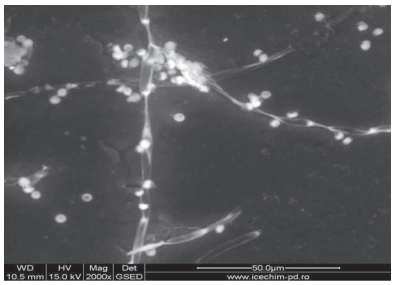
In previous paper, LDPE sample buried in soil for 3 months was used as source for isolation of fungal microorganisms [4]. The present tests were carried out with isolated strains: *Fusarium sp.* (strain 2, 6 and 9), *Aspergillus sp (*strain 5*), Penicillium sp. (*strain 7). Also, strains from microbial collection such as *Aspergillus niger* 105 [5] and *Phanerochaete chrysosporium* were tested. Morphological studies of the fungi and blend surface structure were carried out by scanning electron microscopy (SEM). The SEM micrographs of polymer composites are shown in Fig. 1-5.



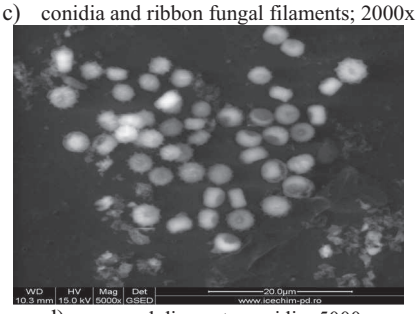
a) surface polymer before microbial contact; 500x



b) agglomeration of conidia; 500x



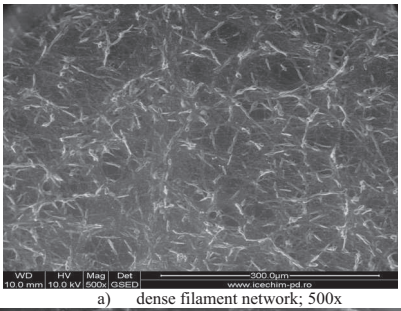
c) conidia and ribbon fungal filaments; 2000x



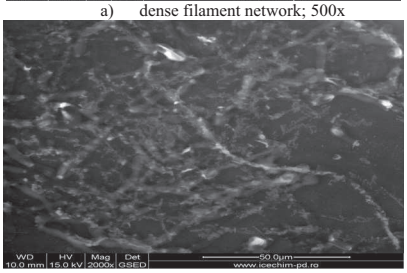
d) rare and disparate conidia; 5000x

Fig. 1. SEM micrographs of plastic film. *Aspergillus niger* 105 (b, c) and isolated *Aspergillus* sp. 5 (d) growing on polymer surface

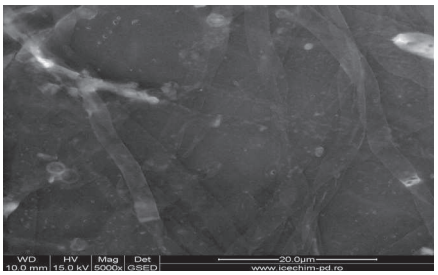
Strai*n Aspergillus niger* 105 was capable to grow and develop a dense filaments network on surface of film composite. The soil isolated strain *Aspergillus* sp. 5 presented a lower ability to produce a microbial biofilm.



a) dense filament network; 500x



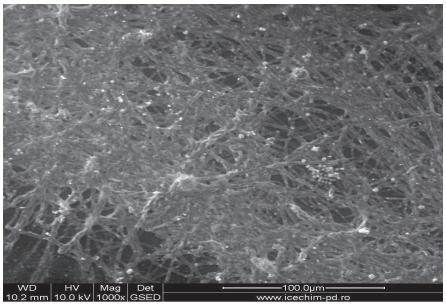
b) fungal filament network; 2000x



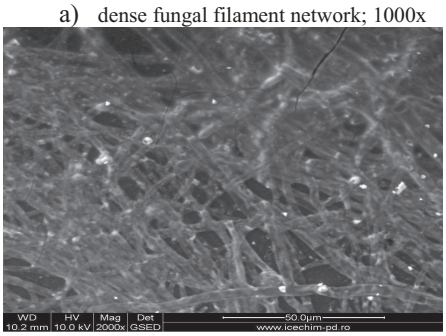
c) ribbon fungal filaments; 5000x

Fig. 2. SEM micrographs of plastic film. *Fusarium s*p. strain 2 (a) and strain 6 (b; c) growing on polymer surface

Fusarium strain 2 and 6 were isolated from the samples soil buried. The fungal networks developed on polymeric surface were less dense as comparative with *Aspergillu*s 105.



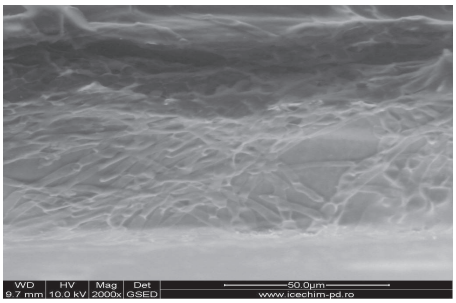
a) dense fungal filament network; 1000x



b) fungal filaments network; 2000x

Fig. 3. SEM micrographs of plastic film. *Penicillium s*p. strain 6 growing on polymer surface

More surface agglomerations of filaments could be seen in the case of *Penicilium* (Fig. 3). Examination of *Phanerochaete chrysosporium* grown on polymer surface is presented in Figure 4.



a) dense network of ribbon filaments

Fig. 4. SEM micrographs of plastic film. *Phanerochaete chrysosporium* growing on polymer surface

The incubation period was not enough to highlight the effect of microorganism towards a material polymeric containing polyethylene. However, the SEM micrographs indicate a microbial adhesion and biofilm formation on the polymer surface films. Biofilm formation depends on microbial origin, and there are microorganisms able to form a biofilm on polymer surface. This phenomenon is considered to be a preliminary step in polymer biodegradation, the biological attack of polymer begins with the colonization of microorganism on polymer film surface. After three months of incubation with microorganism, expected phenomena such as exfoliation, peeling and holes in the film structure were observed on a small scale. The weight loss is used as a quantitative measure for polymer degradation. Results of experiments are presented in Table 1.

Table 1. Weight loss of polymeric samples after incubation with microbial strains

Microorganism	Weight loss (%)*
Aspergillus niger 105	5.95
Phanerochaete chrysosporium	4.25
Fusarium sp strain 2	4.05
Fusarium sp strain 6	4.00
Fusarium sp strain 9	3.90
Aspergillus sp strain 5	3.85
Penicillium sp. strain 7	2.70

* - average of three determinations

As it can be seen, the values of weight loss are relative low and not so relevant for biodegradation process. The highest decrease of weight was obtained after incubation with *A. niger 105*. The activity of *Aspergillus sp.* in biodegradation of polyethylene has been confirmed by many reports [6, 7].

CONCLUSIONS

In this study, we have evaluated the degradation of plastic by measuring its weight loss and observing the film surface by SEM. The weight loss of polymeric samples after microbial incubation was not significant because of the high percentage of resistant polyethylene in composites. According to SEM micrographs it might be assumed that *Aspergillus niger* 105 is capable to produce a thin and dense filaments network on polymer surface. The weight loss results corroborated with those from SEM analysis suggest the beginning of a slow process of polymer biodegradation.

ACKNOWLEDGMENTS

The authors would like to thank Marius Ghiurea for SEM micrographs.

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