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Der Pharmacia Lettre, 2017, 9 [11]: 63-69
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Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Interaction of Heavy Metals in Fungal Degradation of Polymeric Materials

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ABSTRACT

Plastics materials are one of the most popular in the present world but the accumulation of it in the environment leading to long-term environment, economic and waste management problems. In present research paper, microbial degradation of polymeric films was carried out by incubating films with Aspergillus niger strain in presence and absence of heavy metals (Cr, Cd, Zn, As). Biodegradation was measured in terms of mean weight loss, visual observation and Scanning Electron microscopy (SEM). Polymeric films that are incubated with A. niger in presence of chromium (Cr) & cadmium (Cd) metals, biodegradation rate are 3-4% higher than without metal after 60 days and in case of zinc (Zn) & arsenic (As) metals, negligible effect had shown. Further SEM analysis confirmed the degradation by revealing the presence of porosity and fragility of the fungal degraded polymer surface.

Keywords: Heavy metals, Copolymer, Polymeric films, Biodegradation.

INTRODUCTION

When biodegradable polymers disposed in the environment (e.g. compost, soil, or waste water) is utilized by the indigenous microorganisms as sources of carbon and energy [1]. There is a need to address environmental performance of the biodegradable polymers particularly the time required for their complete disintegration in nature [2]. It is a very rare case that the polymer is safe before biodegradation but may turn toxic during degradation process [3].

There is a growing interest in the manufacturing of biodegradable plastics that enhance the degradability of other plastic products in landfills and composts under natural conditions [4]. Enzymatic activities lead to microbial degradation of plastics which results in chain cleavage of the polymer into oligomers and monomers after which they are further metabolized by the microbial cells. Aerobic metabolism results in the generation of carbon dioxide and water [1], while anaerobic metabolism generates carbon dioxide, water, and methane as the end products, respectively. In the process of depolymerisation, two categories of enzymes are actively involved: extracellular and intracellular depolymerases [5]. Depolymerisation results due to various physical or biological forces [6]. The physical forces such as temperature, moisture, pressure etc. cause mechanical damage to the polymer while biological forces like enzymes and other microbial metabolites catalyze the process.

Increased industrialization has affected the ecosystem through waste disposal which contains toxic metal contaminants [7]. Degradation of such heavy metals is not possible as these cannot be converted into non-toxic end products like CO_2 and H_2O [8]. Since heavy metals are major pollutants of both terrestrial and aquatic (even in treated waste water) ecosystem, novel technologies for the removal of metals are still managing to register the success [9].

Heavy metal contamination is a major global concern because it leads to toxicity and threat to human life and environment [10,11]. Release of heavy metals in the environment without proper treatment pose a significant threat to public health because of its persistence, biomagnification and accumulation in food chain. Two major constraints of metal treatment are non-biodegradability and sludge production.

The goal of microbial remediation from contaminated soils and sediments are to immobilize the metal *in situ* to reduce metal bioavailability and mobility [12]. At low metal concentration, low cost and higher efficiency make biotechnological processes attractive in comparison to physico-chemical methods for the removal of heavy metals [13]. Biological approaches for metal detoxification afford the potential for selective removal of toxic metals in a range of bioreactor configurations [14]. Prokaryotic and eukaryotic microorganisms are capable of binding metals as cations to the cell surface in a passive process [15].

This study investigated the ability of *A. niger* to grow and degrade a broad range of substrates. The research work examines the effect of the *A. niger* attack on the polymer surface in the presence and absence of heavy metals by qualitative assessment of films.

MATERIALS AND METHODS

The synthesis and characterization of copolymer have already been published [16].

Microbial degradation of polymeric films

The biodegradation of polymeric films was tested using *Aspergillus niger*. Fungi are widely used in biodegradation process due to their source of diverse enzymes [17].

Microorganisms and cultivation conditions

The microorganism used for biodegradation was *Aspergillus niger*, from Microbial Type Culture Collection (MTCC), Chandigarh, India. The fungal cultures were maintained at 40℃, in a tub test with dextrose-agar-potato medium.

In the liquid cultivation, basic mineral medium contains (g/L): 1.0 NH_4NO_3; 1.0 K_2HPO_4; 0.5 $MgSO_4 \times 7\ H_2O$; 0.5 KCl; 2.0 yeast extract; pH 6. The culture was carried out during a month on a rotary shaker at 200 rpm and 28°C in 300 mL flasks containing 50 mL of the medium.

Degradation in absence of metals

The pre-weighed films of 1 cm diameter were aseptically transferred to the conical flask containing 50 mL of broth medium, and separately inoculated with the fungal strain. Four flasks were maintained for each treatment and left in a shaker for 4-5 days. After sufficient growth of the fungus, the films were treated with 0.5 N NaOH and kept in a boiling water bath for 15 min to kill the fungal spores. Films were washed twice with tap water and thereafter with double distilled water until the pH reaches 7. The films were then air dried, kept in a hot air oven at 80°C for overnight and was examined for weight loss. From the data collected, weight loss of the degraded films was calculated. Further the surface of degraded films was analyzed by SEM.

Degradation in presence of metals (Chromium, Cadmium, Zinc and Arsenic)

Metal solution preparation

The stock solution of Cr (VI) metal was prepared by dissolving 1000 mg of potassium dichromate ($K_2Cr_2O_7$) in 10 ml of double distilled water. Working concentrations of chromium solutions with different initial concentrations were made from the stock solution using metal free distilled water.

The fungal strain and film was amended in PDA medium with a concentration of 1mg mL^{-1} of Cr^{6+}. The pH of the medium was maintained at 5.2 ± 0.2. The plates were then incubated at 27°C for 4-7 days and observed for the visible fungal growth.
For other metals, the process is same as performed with chromium metal.

Weight loss

Weight loss of the films was calculated by the following expression:

$$\text{Weight loss} = \frac{\text{Weight of sample (Before degradation - After degradation)} \times 100}{\text{Weight of sample before degradation}}$$

Scanning Electron Microscopy (SEM)

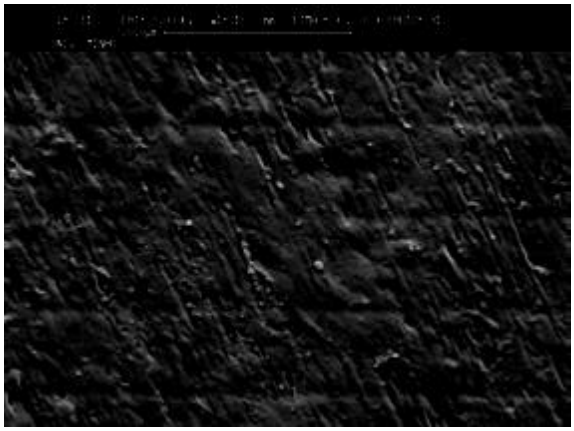
The Scanning Electron Microscopic (SEM) analysis was performed to analyze the surface texture of untreated and treated films against metals using JEOL JSM-840 scanning electron microscope. Micrographs of the samples were taken at different magnifications to identify the changes occur during degradation process.

RESULTS

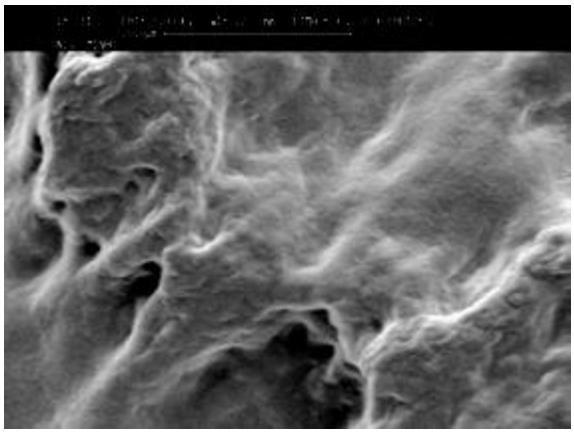
SEM micrographs of polymeric films in presence and absence of metals after 60 days

SEM analysis was done at different magnifications has been shown in Figure 1. The control of films inoculated in PD broth displayed a normal surface view in comparison to the test i.e., in presence of metals show distinct surface morphologies. The

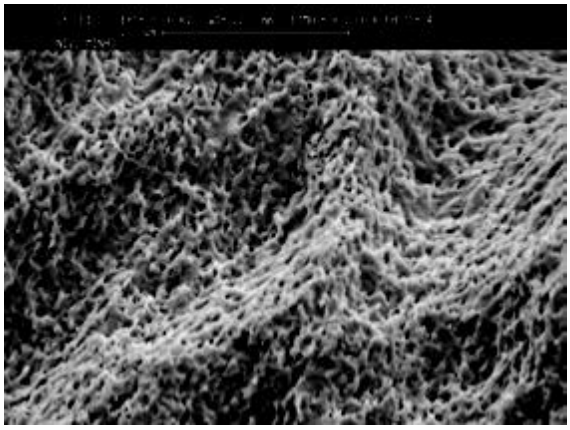
films treated with metals Chromium and Cadmium (Figures 1A and 1B) showed appreciable surface corrosion, folding and cracks in comparison to other metals Zinc and Arsenic which shows some small pores with irregular cases at the surface represented in Figures 1C and 1D. This may be due to the reduction of CrO_4^{2-} to Cr $(OH)_3$, or fungal extracellular metabolites and fungal enzymes. In Figure 1E, micrographs show clear crack initiation points, indicating that the polymer has become brittle. Also, the microbial propagation has been initiated from these cracks. Such colonization and adhesion by microorganisms are a fundamental prerequisite for biodegradation of the polymer. Penetration and cavities were higher in correlation with composition of polymer and nutrients level in culture medium. Microorganisms that colonize the polymer surface can probably adhere by means of extracellular polymeric substances.



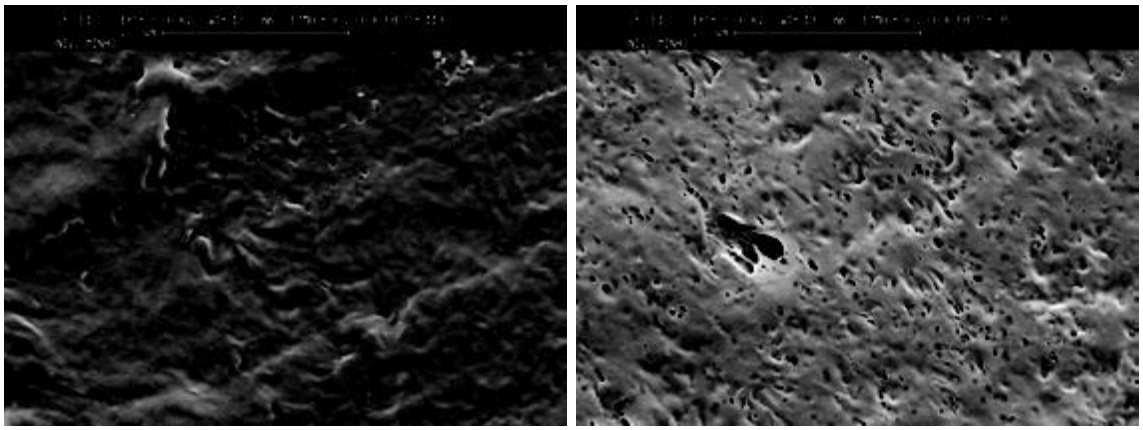
A. Absence of metals



B. Chromium



C. Cadmium



D. Zinc E. Arsenic

Figure 1: SEM micrographs of degraded films in presence and absence of metals at magnification 2000x.

Weight loss after biodegradation of films in presence and absence of metal (after 60 days)

The percentage weight loss of film is presented in Table 1. Polymers that are treated with chromium (Cr) and cadmium (Cd) metals and fungus, biodegradation rate are 3-4% higher than without metal and in case of zinc (Zn) and arsenic (As) metals negligible effect had shown.

Table 1: Percentage weight loss of copolymer after biodegradation in presence and absence of metal after 60 days.

	Biodegradation in absence of metal	**Biodegradation in presence of metal**			
Casted Film	PD Broth	PD Broth + 0.1PPM Cr	PD Broth + 0.1PPM Cd	PD Broth + 0.1PPM Zn	PD Broth + 0.1PPM As
A	69.35%	73.61%	74.00%	68.23%	63.42%

Testing of degraded films (60 days)

Degraded films have been tested for various mechanical (tensile strength) and thermal properties are presented in Table 2. The results of this test are a useful measure of relative resistance in various grades of polymers. The specific gravity of the films is almost same but in case of chromium metal, film has the lowest value of specific gravity and hardness. This may be attributed due to decrease in the crosslinking of resin with the monomers.

Table 2: Testing of films after degradation in presence and absence of metals (60 days).

Degradation of films in absence of metals	**Hardness**	**Specific Gravity**	**Tensile Strength (Kg/Sq. mm)**	**Elongation Breakdown (%)**
	28	0.89	2.31	80
Degradation of films in presence of metals	**Hardness**	**Specific Gravity**	**Tensile Strength (Kg/Sq. mm)**	**Elongation Breakdown (%)**
Chromium	25	0.81	2.28	77
Cadmium	26	0.83	2.17	73
Zinc	27	0.84	2.00	72
Arsenic	25	0.88	2.09	79

DISCUSSION

Microorganisms play a significant role in the biological decomposition of materials in natural environment. The mechanism behind degradation process is not exactly known and the changes like surface of plastic material has turned from smooth to rough, molecular weight reduction, erosion on the surface of polyethylene is due to microorganisms [18]. During the degradation process, exo-enzymes releases from microorganisms can break complex polymers into smaller molecules of short chains, e.g., dimmers, monomers etc. which are very small to cross semi-permeable outer membranes of the microbes, and then utilized as carbon and energy sources [19]. Hence, further studies on release of microbial enzymes in degradation of the plastic materials will pave way for finding technology for degrading these environmentally hazardous plastic materials.

CONCLUSION

Microbial degradation of polymeric films was carried out by incubating them with *A. niger* in presence and absence of heavy metals (Cr, Cd, Zn, As). Biodegradation was measured in terms of mean weight loss. Polymeric films that are treated with *A. niger* in presence of chromium (Cr) & cadmium (Cd) metal, biodegradation rate are 3-4% higher after 60 days than without metals and in case of zinc (Zn) & arsenic (As) metals negligible effect had shown. Further SEM (Scanning electron microscopy) analysis confirmed the degradation by revealing the presence of porosity and fragility of the fungal degraded polymer surface. The control of films inoculated in PD broth displayed a normal surface view. The films treated with metals (Cr and Cd) showed appreciable surface corrosion, folding and cracks in comparison to other metals (Zn and As). This may be due to the reduction of CrO_4^{2-} to $Cr(OH)_3$, or fungal extracellular metabolites and fungal enzymes.

ACKNOWLEDGEMENTS

The corresponding author is thankful to DST, New Delhi for providing financial assistance for the research work.

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