

Impact of Heavy Metals (Cr & Zn) on Fungal Biodegradation of Polymers

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ABSTRACT: Heavy metals are natural constituents of the earth's crust, but indiscriminate human activities have drastically altered their geochemical cycles and biochemical balance. This results in accumulation of metals in plant parts having secondary metabolites, which is responsible for a particular pharmacological activity. Prolonged exposure to heavy metals such as cadmium, copper, lead, nickel, and zinc can cause deleterious health effects in humans. In the present work, polymeric resin has been synthesized and characterized using analytical techniques. The synthesized resin has been used for the development of films. The impact of metals (presence or absence) on biodegradation of films has been studied. Polymer that is treated with Cr metal and fungus, biodegradation rate are higher 2-5% than without metal. In case of Zn metal negligible effect is shown.

KEYWORDS: Biodegradation, Heavy metals, Copolymer, Films

I. INTRODUCTION

With the growth of industry, there has been a considerable increase in the discharge of industrial waste to the environment, chiefly soil and water, which has led to the accumulation of heavy metals, especially in urban areas. Slow depletion of heavy metals also takes place through leaching, plant uptake, erosion and deflation. The indiscriminate release of heavy metals into the soil and waters is a major health concern worldwide, as they cannot be broken down to non-toxic forms and therefore have long-lasting effects on the ecosystem. Many of them are toxic even at very low concentrations; arsenic, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, zinc etc are not only cytotoxic but also carcinogenic and mutagenic in nature [1]. However, the increasing concentration of several metals in soil and waters due to industrial revolution has created an alarming situation for human life and aquatic biota.

Metal ions are not only valuable intermediates in metal extraction but also important raw materials for technical applications.

The levels of heavy metals in the environment have been seriously increased during the last few decades. Beside the natural activities, almost all human activities also have potential contribution to produce heavy metals as side effects. Migration of these contaminants into non-contaminated areas as dust or leachates through the soil and spreading of heavy metals containing sewage sludge are a few examples of events contributing towards contamination of the ecosystems [2]. Several methods already used to clean up the environment from these kinds of contaminants, but most of them are costly and difficult to get optimum results. Currently, biodegradation is an effective and affordable technological solution used to extract or remove inactive metals and metal pollutants from contaminated soil. The chemical technologies generate large volumetric sludge and increase the costs [3]; chemical and thermal methods are both technically difficult and expensive [4].

Polyethylene terephthalate (PET) is a thermoplastic resin of the polyester family and is used in synthetic fibers. PET is produced by the polymerization of ethylene glycol and terephthalic acid [5]. Ethylene glycol and terephthalic acid, when heated together under the influence of chemical catalysts, produces PET in the form of a molten, viscous mass that can be spun directly to fibers or solidified for later processing as a plastic. It is synthesized by the

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transesterification reaction between ethylene glycol and dimethyl terephthalate [6]. The presence of a large aromatic ring in the PET repeating units gives the polymer notable stiffness and strength [7].

This research work employs the usage of copolymerization technique to produce bioplastic consisting of lactic acid and PET to finally yield the polymer. The synthesized resin has been used for the development of films of different formulations. The impact of metals (presence or absence) on biodegradation of films has been studied using fungus *Trichoderma lignorum*. Polymers that are treated with Cr metal and fungus, biodegradation rate are higher 2-5% than without metal. In case of Zn metal negligible effect is shown. Metals affect biodegradation by impacting both the physiology and ecology of degrading microorganisms. The bioavailable fraction of heavy metals is the key factor in risk assessment analysis, varies with speciation, nature of applied metal salt, medium components/soil type, pH, redox potential and other environmental variables. Thus, no single parameter can be adequate to generalize heavy metal toxicity, necessitating the need for a battery of assays with sensitive, reliable and ecologically relevant biological tools to provide an understanding of environmental situations in which biodegradation is unexpectedly low, such as in co-contaminated environments [8].

II. MATERIALS & METHODS

1. SYNTHESIS OF COPOLYMER

40 g of PET was taken and cut into very thin films. The cut pieces were taken in a 500 mL two necked round bottom flask with a reflux condenser attached to it. Add 70 mL of ethylene glycol to it which was dried before followed by the maintenance of a constant temperature of 180°C in an oil bath for about 5 h. In this, zinc acetate was used as the catalyst and the amount taken is 0.2% by weight of PET used which will finally result in the production of BHET (Bis-2-hydroxyethyl terephthalate).

35 g of BHET react with lactic acid in the presence of stannous chloride which was taken to be around 0.5 to 0.8 weight % along with p-toluene sulphonic acid[9]. This reaction mixture was again taken in a 500 mL round bottom flask which was to be immersed in an oil bath at a temperature of 180 to 210°C kept for 6 to 8 h. As the process continues, water and glycol was removed from the reaction and the remaining mixture was dissolved in chloroform which was later precipitated in methanol filtered and dried at 90°C [10].

2. CHARACTERIZATION

Copolymer obtained was characterized using FTIR helps in identifying the structure of compound.

F.T.I.R SPECTROSCOPY

The FTIR spectra of synthesized copolymers were recorded using Perkin Elmer model spectrum BX Series FTIR. The spectra were recorded by using KBr Pellets. A total scan 16 scan per sample at resolution of 4 cm⁻¹ were obtained over mid-IR region of 4000–400 cm⁻¹.

3. BIODEGRADATION

Fungi were procured from Deptt. Of Microbiology, C.C.S. University Campus, Meerut. In this work, synthesized sheets were biodegraded by fungi.

a. BIODEGRADATION WITHOUT USING METAL

This work is done in the following steps:

i. Preparation of PDB (Potato Dextrose Broth)

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The potatoes (50 gm) were boiled in 250 ml of distilled water for 30 min and then filtered through muslin cloth. The volume of filtrate was made upto 250 ml and then dextrose (5 gm) and rose bengal was added. The medium was sterilized by autoclaving at 121°C for 30 minutes.

ii. Inoculation

In incubation chamber, took 4 conical flasks and add 30 ml of broth in each conical flask. Inoculate broth with fungus *Trichoderma lignorum* and add films of different formulations in each conical flask.

iii. Incubation

Incubate these conical flasks in incubator at 20°C for 60 days and then samples were biowashed with water and dried under vacuum. The biodegraded copolymers were examined for weight loss.

b. BIODEGRADATION USING METAL (CHROMIUM AND ZINC)

The potatoes (50 gm) were boiled in 250 ml of 0.1ppm Cr solution for 30min and then filtered through muslin cloth. The volume of filtrate was made upto 250 ml and then dextrose (5 gm) and rose bengal was added. The medium was sterilized by autoclaving at 121°C for 30 minutes. Inoculation and incubation is same as above.

For Zinc metal, the process is same as of chromium metal.

4. WEIGHT LOSS

Weight loss was calculated by the following expression:

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

III. EXPERIMENTAL RESULTS

1. SYNTHESIS OF COPOLYMER

During the copolymerization process, the temperature was maintained at 180-200°C throughout the process. After completion of the copolymerization, both chloroform and methanol were added resulting in the formation of two layers with a white precipitate at the bottom part and in the upper part, all the non-copolymerized waste residues were obtained. After filtering and drying of the precipitate, a white powder was obtained which had a melting point of 170°C confirming the formation of copolymer and on melting it at the characteristic melting point, thin films were obtained. The thin films were rigid and tensile in nature even after two weeks. The plastic film produced after melting the powder product showed increased stability. Films were casted by crosslinking of resin with acrylic acid and methyl metha acrylate (MMA) monomers. The casted films were 2 mm in thick and designated as A, B, C, and D. The amount of lactic acid (10 gm in each) was kept constant while maleic anhydride and propylene glycol (2, 2.5, 3, 3.5 gm in each) was varied.

2. CHARACTERIZATION OF COPOLYMER

a. F.T.I.R SPECTRUM ANALYSIS

The FTIR spectrum of the product obtains in order to characterize various groups present and gather sufficient information about the product. Fig. 1 shows IR spectrum of the product obtained by polymerization of lactic acid.

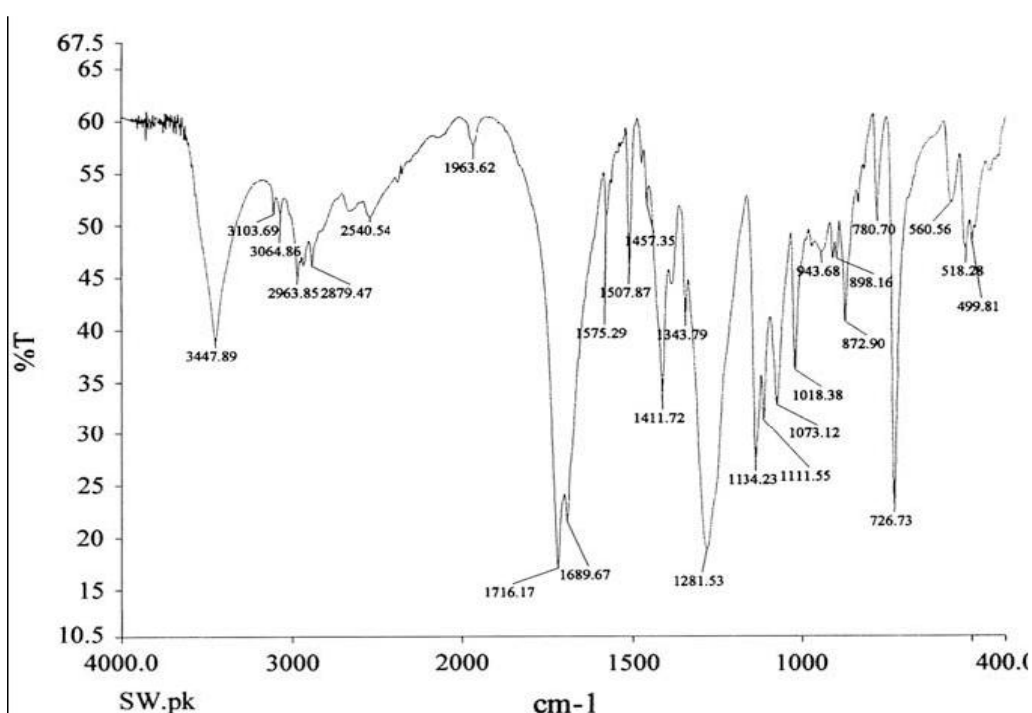


Fig 1: FTIR spectra of copolymer

The FTIR spectra of the product obtained by polymerization of lactic acid shows the presence of a sharp band at 1726 cm^{-1} may be attributed to presence of C=O stretching in a β -unsaturated ester (Figure 1). The C–O structure of ester appears at 1096 cm^{-1} , due to free alcoholic group O–H structure band appears at 3435.96 cm^{-1} .

3. CHARACTERIZATION OF FILMS

The hardness, specific gravity, tensile strength and elongation breakdown of films is shown in Table 1. The results obtained from this test are a useful measure of relative resistance to indentation of various grades of polymers.

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Table 1: Hardness, Specific gravity, Tensile strength and Elongation breakdown of films

Casted Films	Hardness	Specific Gravity	Tensile Strength	Elongation Breakdown (%)
A	26	0.87	2.33	80
B	28	0.89	4.36	120
C	32	0.9	3.2	96
D	32	1.2	4.87	112

As the amount of the acrylic acid increases in the film, hardness also increases. The specific gravity of the films is almost same but the film A has the lowest value of specific gravity & hardness. This may be attributed due to decrease in the crosslinking of resin with the monomers.

4. WEIGHT LOSS AFTER BIODEGRADATION IN PRESENCE AND ABSENCE OF METAL (AFTER 60 DAYS)

The percentage weight loss of copolymer in presented in Table 2. Copolymer that are treated with Cr metal and fungus, biodegradation rate are higher 2-5% than without metal. In case of Zn metal, negligible effect is shown. Metals affect biodegradation by impacting both the physiology and ecology of degrading microorganisms. Metals inhibit biodegradation through interaction with enzymes directly involved in process or through interaction with enzymes involved in general metabolism. In either case, inhibition is mediated by the ionic form of metal [11]. Medium pH also dramatically impacts solution-phase metal concentrations. As pH increases, metals tend to form insoluble metal oxides and phosphates, result in decreased solution-phase metal concentrations [12].

Table 2: Biodegradation of copolymer in presence & absence of metal after 60 days

Casted Films	Biodegradation in absence of metal	Biodegradation in presence of Metal	
	PD Broth	PD Broth + 0.1PPM Cr	PD Broth + 0.1PPM Zn
A	66.66%	71.42%	65%
B	75%	75.50%	75%
C	61.53%	64.70%	64%
D	70%	72.72%	68%

Polymer that is treated with Cr metal and fungus, biodegradation rate are higher 2-5% than without metal. In case of Zn metal, negligible effect is shown. Determination of the effects of heavy metals on the biodegradation of polymers

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often yields conflicting results. Such inconsistencies can be attributed to overgeneralization of the outcomes from short-term laboratory studies that focus on a single parameter under controlled conditions.

IV. CONCLUSION

Biodegradation of plastics is a heterogeneous process because of water insolubility and size of polymer molecule, microorganisms are not able to pick up the polymer directly into the cells but first have to secrete extracellular enzyme which depolymerise the polymer outside the cells. Copolymer has been biodegraded by fungus *T. lignorum* in the presence of metal (chromium or zinc). Polymer that is treated with Cr metal and fungus, biodegradation rate are higher 2-5% than without metal. In case of Zn metal, negligible effect is shown. Determination of the effects of heavy metals on the biodegradation of polymers often yields conflicting results. Such inconsistencies can be attributed to overgeneralization of the outcomes from short-term laboratory studies that focus on a single parameter under controlled conditions. The bioavailable fraction of heavy metals is the key factor in risk assessment analysis, varies with speciation, nature of applied metal salt, medium components/soil type, pH, redox potential and other environmental variables. Thus, no single parameter can be adequate to generalize heavy metal toxicity, necessitating the need for a battery of assays with sensitive, reliable and ecologically relevant biological tools to provide an understanding of environmental situations in which biodegradation is unexpectedly low, such as in co-contaminated environments.

Thus, the bioplastic produced will be an opening to the near future in providing a successful sustainable environment for coming future generations.

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REFERENCES

- [1] Salem, H.M.; Eweida, E.A.; Farag, A, "Heavy metals in drinking water and their environmental impact on human health", ICEHM; Cairo University: Giza, Egypt; pp. 542-556, 2000.
- [2] Gaur, A., Adholeya, A, "Prospects of arbuscularmycorrhizal fungi in phytoremediation of heavy metal contaminated soils", Current Science, vol. 86, no. 4, pp. 528-534, 2004.
- [3] Rakhshae, R., Giahi, M., Pourahmad, A, "Studying effect of cell wall's carboxyl-carboxylate ratio change of Lemna minor to remove heavy metals from aqueous solution", Journal of Hazardous Materials, vol. 163, no. 1, pp. 165-173, 2009.
- [4] Hinchman, R.R., Negri, M.C., Gatlif, E.G, "Phytoremediation: using green plants to clean up contaminated soil, groundwater, and wastewater, Argonne National Laboratory Hinchman, Applied Natural Sciences, Inc, 1995.
- [5] Chetna S, Madhuri S, "Studies on biodegradation of PET : A synthetic polymer", J. Microbiol. Biotechnol. Res. 2(2):248-257, 2012.
- [6] Zulfiqar A, Faqir MA, Tahir Z, "Production of lactic acid from corn cobs hydrolysate through fermentation by *Lactobacillus delbrukii*.", Afr. J. Biotechnol. 8(17):4175-4178, 2006.
- [7] Fariyal A, Dennis DE, "Self-porating polymersomes of PEG-PLA and PEG-PCL hydrolysis triggered controlled release vesicles", J. Contrld. Release. 96:37-53, 2004.
- [8] Olaniran AO, Balgobind A, Pillay B, "Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies", International Journal of Molecular Sciences, 10197-10228, 2014.
- [9] Achanai B, Nattawut C, TanitJ, Sasirada W, Sureerat J, "Preparation and characterization of PET-PLA copolyester from waste PET and Lactic acid", Chiang Mai J. Sci. 38(4):619-624, 2011.
- [10] Jin SC, Jae HP, Suk JM, "Transformation of carboxylic acid salts to aldehydes by stepwise reduction with borane and oxidation with pyridiniumchlorochromate", Bull. Korean Chem. Soc. 22(10):1089-1092, 2001.
- [11] Angle, J.S., Chaney, R.L, "Cadmium resistance screening in buffered minimal media", Applied Environmental Microbiology, 55, 2101-2104, 1989.
- [12] Hahne HCH, Kroontje W, "Significance of pH and chloride concentration on behaviour of heavy metal pollutants: mercury, cadmium, zinc, and lead", J Environ Qual, 2:444-450, 1973.