

# CHEMISTRY & BIOLOGY INTERFACE

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## A novel study of heavy metals remedial activities through fungi immobilized polymeric film

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**Abstract:** Now a days, pollution occurs due to heavy metals is a most important problem because it results in toxicity, risk to the survival for humans and disrupting activities of ecological balance. Presence of toxic metals in the environment result in bioaccumulation, geo-accumulation and bio-magnification processes which have intense public health and ecological and implications. In the present research work, copolymer of lactic acid and maleic anhydride with butylene glycol has been prepared and distinguished using spectroscopic and other analytical techniques. The prepared copolymer has been used in preparation of films of different formulations & characterised, then fungal degradation was studied by using different fungal species such as *T. lignorum*, *A. Versicolor*, *A. niger*, *Alternaria alternata*, *Rhizopus sps.* etc. Degree of the degradation was analysed by percentage weight loss and SEM micrographs. *A. niger* have degrade the films mostly & maximal weight loss observed in film D. The degradation of copolymer having high lactic acid amount was faster than copolymer having low lactic acid amount. The effect of metals i.e. presence or absence on the degradation of films has done. Polymeric films that are treated with fungal strain (*A. niger*),  $\text{Cr}^{6+}$  &  $\text{Cd}^{2+}$  metal ions, degradation rate was 3–5 % higher than in absence of metal while in  $\text{Zn}^{2+}$ , rate is 1 – 2% higher. Negligible effect had shown in  $\text{As}^{3+}$  metal ion.

**Key-words:** Lactic acid, Maleic anhydride, Butylene glycol, fungal degradation and toxic metal.

**1. Introduction:** in the development of biodegradable plastics, a growing interest has been generated as it improves degradability of plastic products in composts and landfills [1].

Due to this interest, more and more scientists are spending time in amending the conventional material so

that the products obtained will be more convenient to use & can design polymer compound from the naturally present materials. Biodegradable plastics which have been used as a source of energy & carbon by the microorganisms are disposing favourably in environment [2].

Due to the new emergence of

biodegradable polymers, there is an urgent need in addressing their environmental performance for their complete degradation in nature [3]. It happens very less that polymer is safe before the biodegradation process but it can turn toxic during the degradation process [4].

The absolute requirement of our environment to promote a sustainable development is the elimination of wide range of wastes and pollutants with low environmental impact from our society [5]. Biological processes are playing important part in the degradation and removal of contaminants [6].

Microbial degradation is an intensive process which is reducing the amount of wastes as it doesn't aid in preservation of the non-renewable resources. It can occur either by activity of the enzyme or by products (such as peroxides and acids) discharged by micro-organisms (fungi, yeast, bacteria, etc) [7-8]. Microbial exudates are creating a micro-environment in which certain polymers are becoming chemically unstable. Bioplastics has been the need of hour and usage of lactic acid in bioplastic production has opened a new side to the field [9]. They have proven advantageous over the relative plastics as they degrade easily in environment without producing reverse effects.

## 2. Experimental

### 2.1 Materials required

The  $\pm$  DL Lactic Acid [AR] & Butylene Glycol have taken by Merc, Maleic anhydride by Aldrich chemical company. Stannous chloride has used as catalyst and procured by Qualigens. The

reagents received were used as such.

### 2.2 Synthesis of Copolymer

The copolymer of lactic acid with maleic anhydride and butylene glycol has synthesized through the condensation of Lactic acid (5 mol) with Maleic Anhydride (5 mol) and Butylene Glycol (5 mol) in the round bottom flask having three necks. Thermometer was put on first neck, stirrer to second and the Dean Stark to the third neck. In the first step of the process, temperature maintained constant for 8 hours at 120°C while in the second step, reaction continues for 10 hours at 280°C, 0.1% SnCl<sub>2</sub> was add on as an activator by constant agitation. The product was obtained and characterized.

### 2.3 Characterization of copolymer

Copolymer so obtained has been characterized for acid value, number average molecular weight, hydroxyl value [10]. The spectroscopic techniques are used in the characterization of compound such as UV-Visible spectroscopy, Infrared spectroscopy, SEM etc. These techniques are helpful to identify the structure of compound.

### 2.4 Casting of Films

In the present research work, films were casted by crosslinking of copolymer with the acrylic acid & methyl methacrylate [10] as presented in the table 1.

**Table 1: Different Formulations of Film**

Ingredients	Weight (gm)	Weight (gm)	Weight (gm)	Weight (gm)
	A	B	C	D

Copolymer	10	20	30	40
Methyl Metha Acrylate	5	6	7	8
Acrylic acid	5	6	7	8
Benzoyl peroxide	0.4	0.44	0.48	0.52
Dimethyl aniline	1.5	1.25	1.0	0.75

## 2.5 Characterization of Films

The casted films were observed for transparency, colour, shrinkage and smoothness before & after degradation. These properties of the films were observed for fungal degradation after a period of 15, 30, 45 and 60 days.

## 2.6 Biodegradation of Films

The biodegradability of films has been tested by various fungal strains like *A. niger*, *A. versicolour*, *A. fumigatus*, *T. lignorum*, *Rhizopus sp.*, *Alternaria alternata* and *Mucor sp.* etc. Identified fungal strains have taken from Microbiology Lab, Department of Microbiology, MIET, Meerut (U.P) India. The fungal cultures have maintained at 40°C, in a tub test with PDA medium.

### 2.6.1 Degradation in absence of metals

The work is done as follows:

#### (i) Preparation of potato dextrose broth (PDB)

For 30 minutes, 50 gm potato slices were boiled in distilled water and then filter the potato extract by using muslin cloth in a flask from which starch have been extracted and add 5 gm dextrose. Add some water to make up the volume

1 litre. The sterilization of medium has preceded by autoclaving the solution for 30 minutes at 121°C.

#### (ii) Fungi Culture

Fungi growth increases at their ambient temperature. Fungi culture harvested from the media plate and inoculated.

#### (iii) Inoculation of fungi to the sample

20–25 ml of the prepared medium filled in the petriplates cleanly. Each petridish was centrally impregnated through fragments of mycelium, spores from fungus culture and then put for incubation in the BOD incubator for one week at  $27 \pm 1$  °C in an inverted position. Then cylinder of agar from the periphery of growing culture has taken with the help of flamed cork borer dipped in alcohol and transfer the cylinder to the centre of PDA agar plates labelled as blank (having media & film of different formulations) and sample (the fungi spread on the film of different formulations), kept at the room temperature for 60 days. The films have removed from the mixture, water-washed and put for drying under vacuity. The degraded films have determined by the % weight loss & SEM analysis.

### 2.6.2 Degradation in Presence of Metals (Chromium, Cadmium, Zinc and Arsenic)

#### Preparation of metal solution:

The stock solution of Cr (VI) metal has been made by dissolving 1000 mg potassium dichromate ( $K_2Cr_2O_7$ ) in 10 ml double distilled water. By using metal free distilled water, working concentration of chromium solutions have made from the

stock solution.

- **For other metals, the process is same as performed with chromium metal.**

### (i) Potato dextrose broth preparation-

For 30 minutes, 50 gm of potatoes have boiled in 250 ml of metal solution (0.1ppm) and then filtered by using muslin cloth. Volume of the filtrate made to 250 ml and then, add dextrose (5 gm) and rose bengal. The sterilization of medium was done by autoclaving for 30 minutes at 121°C.

### (ii) Fungi Culture

Fungi growth increases at their ambient temperature. Fungi culture harvested from the media plate and inoculated.

### (iii) Inoculation

Films of different formulations were inoculated onto the test medium with concentration  $1\text{mg ml}^{-1}$  of  $\text{Cr}^{6+}$ . pH of solution maintained at  $5.2 \pm 0.2$ . The incubation of cultures was done at 15, 20 and  $30\text{ }^\circ\text{C} \pm 1^\circ\text{C}$  and period of growth have noticed at different periods. The degraded films have analysed by weight loss and SEM micrographs.

- **For other metals, the process is same as performed with chromium metal.**

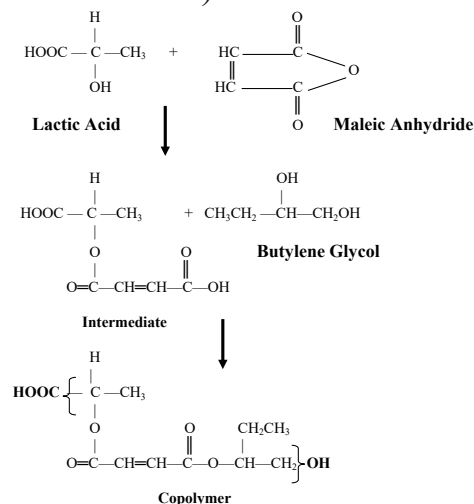
### 2.7 Testing of films after degradation

After degradation, the films will be tested for properties such as mechanical and thermal.

## 3 Result & Discussion

### 3.1 Synthesis of Copolymer

During copolymerization, throughout the process temperature was maintained at 170-190°C. After completion of reaction, chloroform and methanol were added in the mixture which results in generation of the 2 layers like non-copolymerized waste residues present in the upper part and yellowish precipitate present at the bottom part which termed as copolymer. The copolymer obtained by the copolymerization process was creamy coloured and fruity smell confirms presence of ester. The synthesized copolymer contains carboxylic group on the 1 hand & hydroxyl group on another hand that means the reaction is taking place at the same rate. Reaction mechanism has been shown in figure 1 (Reaction scheme).



**Figure 1: Synthesis of Copolymer**

### 3.2 Characterization of Copolymer

The solubility of copolymer has determined in different solvents and represented in the table 2.

**Table 2: Solubility of copolymer in different solvents**

Solvent Used	Copolymer
Water (Cold/Hot)	Insoluble
Ethanol	Soluble
Carbon Tetrachloride	Insoluble
DMSO	Partially soluble

**Acid number, Mn, Hydroxyl value of synthesized copolymer**

Copolymer was characterized for various properties like Mn, acid number and hydroxyl value as shown in the table 3.

**Table 3: Acid number, Hydroxyl value & Mn of copolymer**

Copolymer	Acid Value (mg/gm)	Hydroxyl No. (mg/gm)	Mn
Copolymer	0.057	0.051	7702

Acid number & hydroxyl value of copolymer are almost equal which means that the reaction is taking place at same rate from both the ends.

**Viscosity of Copolymer**

Viscosity of copolymer in different solvents has shown in table 4.

**Table 4: Viscosity of Copolymer in different solvents at temperature 34°C**

S.No	Solvent Used	Density (g/cm <sup>3</sup> )	Flow time (seconds)	Viscosity (poise)
1	Methanol	0.724	910	230

2	Ethanol	0.963	850	165
3	T.H.F	1.254	715	115
4	D.M.S.O	1.361	960	245

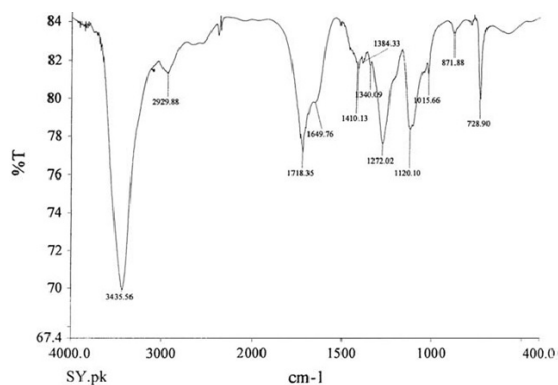
The viscosity of copolymer in DMSO is too high as resin is partially soluble with DMSO.

**U.V Visible Spectrum Analysis**

U.V visible spectrum of synthesized copolymer  $\lambda_{\max} = 297.2 \text{ nm}$ , The graph of copolymer shows strong absorption in the region 275 - 300 nm which might be due to presence of carbonyl group in the synthesized copolymer with unsaturation in conjugation.

**FTIR Analysis**

FTIR spectrum of copolymer has obtained to characterize various groups present in it and collect sufficient information about product. IR spectra of copolymer have presented in figure 2.

**Figure 2 – F.T.I.R Spectrum of Copolymer**

The different peaks were assigned to corresponding functional group summarized in the

Table 5.

**Table 5-Variou s Peaks Obtained in the F.T.I.R Spectra of Copolymer**

Wave Number (cm <sup>-1</sup> )	Functional Group	Types of Vibrations
1120, 1272	Ester	C—O stretching
1718	Unsaturated Ester	C=O stretching
1410	Alkane	C—H stretching
3435.56	Alcohol (Free)	O—H stretching

The distinct band present at 1718cm<sup>-1</sup> may leads to presence of the C=O stretching in  $\beta$ -unsaturated ester. Presence of bands at 1120 and 1272cm<sup>-1</sup> predicted C—O stretching of ester. One wide peak present at 3435.56cm<sup>-1</sup> may leads to the O—H stretching.

### 3.3 Casting & Characterization of Films

The films have characterized for different properties like hardness, specific gravity, tensile strength, elongation breakdown, thickness etc have mentioned in the table 6.

**Table 6: Testing of films before Degradation**

Film	Thickness ( $\mu$ m)	Hardness	Specific Gravity	Tensile strength (kg/mm <sup>2</sup> )	Elongation At break (%)
A	33	32	0.87	42	63
B	31	30	0.89	39	77
C	28	29	0.9	38	95
D	32	26	1.2	41	120

There is a gradual decrease in hardness values as monomer concentration is increasing from film A to D. Specific gravity of all films is approximately same but film A of all the copolymers has the minimum value of specific

gravity. This might be due to decrease in the crosslinking of copolymer with monomers. Oligomer of high molecular weight produces crosslinked elastic web while the oligomers of low molecular weight produces crosslinked smooth network.

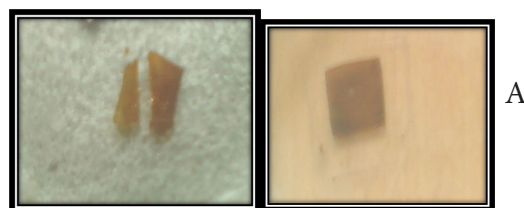
Hence, more LA concentration leads a more elastic film which has a higher effect of impedance and lower scrape impedance. As the concentration of LA decreases, crosslink density increases which can be associated with generation of the acrylic phase.

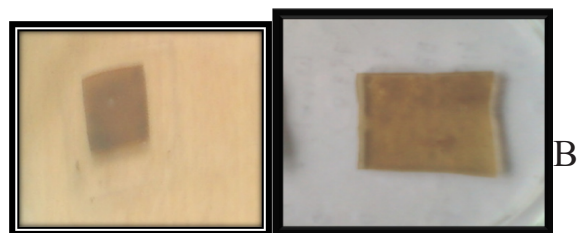
At higher lactic acid concentration, Film D synthesized from all the copolymers was soft and elastic this happens because long chain of copolymer provides more elasticity in the organisation which lowers the crosslink frequency.

Film A has highest crosslink density and tensile strength but lower elongation breakdown. Film D has lower crosslink density resulting in low value of tensile strength with greater elongation at break.

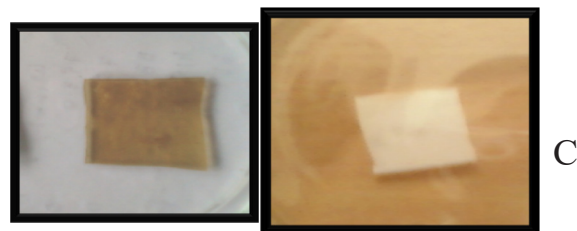
### 3.4 Biodegradation of Casted Films

The films have degraded by different fungal species. The photographs of films have taken after a gap of 90 days and presented in figure 3.

**Before Degradation After Degradation**



Before Degradation After Degradation



Before Degradation After Degradation

Figure 3: Degradation of films (before & after) by fungus *A. niger*

- Weight loss of films after degradation

The degradation percentage has been calculated by the loss in weight of polymeric films after degradation (90 days) is presented in figure 4.

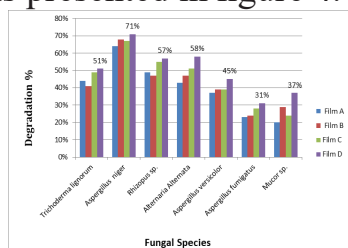


Figure 4: Degradation % of polymeric films (after 90 days)

Figure 4 shows high degradation rate of 71% occurs in Film D by *A. niger* in comparison of other fungal species obtained after 90 days. *A. alternata* shows degradation % of 51% in case of film D. The highest degradation rate was found in Film D degraded by different fungal strains as the samples having higher concentration of lactic acid had a higher ester bond cleavage amount and high rate of degradation. Deviation in degradation also related to water intake i.e. LA concentration increases; amount of the water uptake also increases.

### 3.4.1 Degradation of films in presence & absence of metals (Chromium (VI), Cadmium (II), Zinc (II) & Arsenic (III))

Microbial degradation of the polymeric films has proceeded by incubating the polymeric films with *A. niger* strain and metal ions (presence & absence) ( $\text{Cr}^{6+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{As}^{3+}$ ). Biodegradation was analysed by mean weight loss, visual observation and SEM micrographs.

- Mean weight loss of films after degradation in presence and absence of heavy metals (after 90 Days)

The degradation percentage of films has shown in table 7. Films that have treated with the fungal strain (*A. niger*),  $\text{Cr}^{6+}$  &  $\text{Cd}^{2+}$  metal ions, biodegradation rate are 3–5 % higher than without metal while in case of  $\text{Zn}^{2+}$ , rate is 1 – 2% higher. Negligible effect had shown in case of  $\text{As}^{3+}$  metal ion.

Films	Degradation in absence of metal	Degradation in the presence of metal			
	PD Broth	PD Broth + 0.1PPM Cr <sup>6+</sup>	PD Broth + 0.1PPM Cd <sup>2+</sup>	PD Broth + 0.1PPM Zn <sup>2+</sup>	PD Broth + 0.1PPM As <sup>3+</sup>
A	68%	70.58%	70.43%	68.90%	68.03%
B	71%	73.91%	73.30%	71.90%	71.10%
C	63%	65.46%	65.20%	63.91%	63.06%
D	72%	75.21%	74.89%	73.30%	72.09%

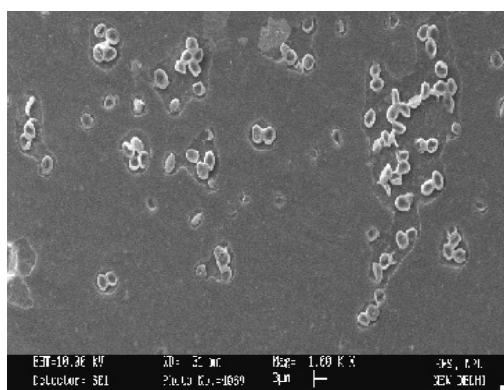
**Table 7: Degradation % of polymeric films (presence & absence of metal) after 90 days**

**Table 8: Degradation % of film D after degradation by *A. niger***

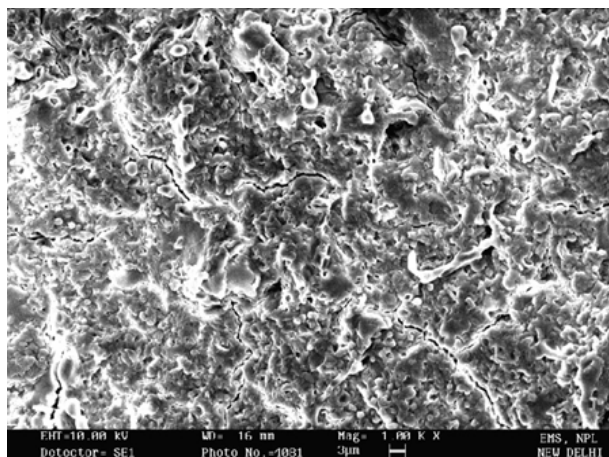
Film	15 days	30 days	45 days	60 days	90 days
D					
(presence of Cr <sup>6+</sup> )	10 %	33 %	42 %	68 %	75.21 %
(presence of Cd <sup>2+</sup> )	13 %	32 %	47%	65 %	74.89 %

- SEM analysis of film D before & after degradation

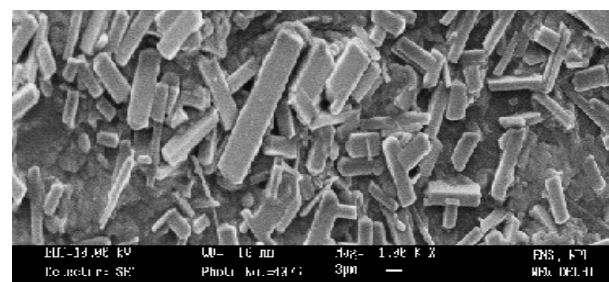
SEM micrographs were scanned before & after degradation at magnification 1000 X have been presented in figure 5.



**(a) Film D Before degradation**



**(b) Degradation of film D in presence of Cr<sup>6+</sup> metal ions**



**(c) Degradation of film D in presence of Cd<sup>2+</sup> metal ions**

**Figure 5: SEM micrographs of film D (presence & absence of metals) at magnification 1000 X**

The control i.e. films inoculated in broth had displayed normal view of the surface (figure 5 a) while the test (presence of metals) shows distinct type of surface morphologies. Films treated with Chromium (VI) & Cadmium (II) metals (figure 5 b & c) showed appreciable surface corrosion, cracks and folds as compared to the other metals i.e. Zinc (II) & Arsenic (III) which has showed small pores with irregular cases at the surface. This might be due to fungal extracellular



metabolites and fungal enzymes. In figure 5 b & c, micrographs have shown the presence of cracky initiation point which indicates that polymer has becoming brittle.

From these cracks, there has been initiation of microbial propagation. The adhesion and colonization by the microorganisms is initiation in the degradation of polymer. The presence of cavities and penetration were very high in the correlation of nutrients level present in culture medium and composition of the polymer.

### 3.5 Testing of degraded films (after 90 days)

Degraded films were tested for the various mechanical (tensile strength) and thermal properties are presented in tables 9 -13.

**Table 9: Testing of films before degradation**

Casted films	Thickness (µm)	Breaking Load (kg)	Elongation (mm)	Hardness	Specific gravity	Tensile Strength (kg/mm <sup>2</sup> )	Elongation Breakdown (%)
A	36	0.278	33	25	1.64	2.37	41
B	40	0.510	42	34	2.89	2.14	52
C	37	0.293	31	23	0.78	1.71	73
D	39	0.364	39	27	1.20	1.23	89

**Table 10: Testing of films after degradation in presence of Cr<sup>6+</sup> metal ions**

Casted films	Thickness (µm)	Breaking Load (kg)	Elongation (mm)	Hardness	Specific gravity	Tensile Strength (kg/mm <sup>2</sup> )	Elongation Breakdown (%)
A	23	0.311	11	15	0.99	21	49
B	36	0.432	23	29	1.71	37	63
C	21	0.281	14	18	0.43	21	78
D	30	0.396	15	21	1.60	22	98

**Table 11: Testing of films after degradation in presence of Cd<sup>2+</sup> metal ions**

Films	Thickness (µm)	Breaking Load (kg)	Elongation (mm)	Hardness	Specific gravity	Tensile Strength (kg/mm <sup>2</sup> )	Elongation Breakdown (%)
A	19	0.258	27	22	1.09	2.35	54
B	38	0.486	34	30	2.13	1.74	66
C	14	0.211	16	19	1.03	1.43	81
D	23	0.373	28	26	1.19	1.02	97

**Table 12: Testing of films after degradation in presence of Zn<sup>2+</sup> metal ions**

Films	Thickness (µm)	Breaking Load (kg)	Elongation (mm)	Hardness	Specific gravity	Tensile Strength (kg/mm <sup>2</sup> )	Elongation Breakdown (%)
A	30	0.312	29	18	1.13	2.67	53
B	37	0.503	38	30	2.50	2.38	74
C	24	0.273	24	22	0.19	1.22	82
D	31	0.396	37	27	1.20	1.14	91

**Table 13: Testing of films after degradation in presence of As<sup>3+</sup> metal ions**

Films	Thickness (µm)	Breaking Load (kg)	Elongation (mm)	Hardness	Specific gravity	Tensile Strength (kg/mm <sup>2</sup> )	Elongation Breakdown (%)
A	35	0.342	36	20	1.16	25	143
B	37	0.497	40	23	1.83	40	179
C	33	0.287	34	21	0.64	31	141
D	42	0.363	37	24	1.17	32	169

Specific gravity of the films is approximately same but incase of Chromium (VI) metal, film D has the highest value of specific gravity & hardness. This may be associated with decrease in the crosslinking of resin with the monomers.

Film D has lower crosslink density and tensile strength with higher value of elongation at break. Film A hadn't shows the sign of stratification because of low water intake as metal solution can't efficiently permeate inside the

films. Degradation process occurs on the exterior of film and if degrade interior of film than stratification occurs gently.

As the swelling in the film increases, mobility of polymer chains becomes greater. Chain flexibility is an important function because the chains must be able to reach and conform to conformation site present in enzyme afore enzyme activates segmentation of ester bond [11-12].

#### 4. Conclusion

In the present research work, copolymer of lactic acid, maleic anhydride & butylene glycol has synthesized and characterized by spectroscopic & analytical procedures. The copolymer have been used in preparation of the films with various formulations, characterised and their fungal degradation was studied by using different fungal species such as *T. lignorum*, *A. niger*, *A. versicolour*, *Alternaria alternata*, *Rhizopus* sps. etc. Extent of the degradation was analysed by weight loss and SEM micrographs.

*A. niger* has degraded the films mostly and maximum weight loss is observed in film D. The degradation of copolymer having high lactic acid amount was faster than copolymer having low lactic acid amount.

The effect of metals i.e. presence or absence on the degradation of films has done. Polymeric films that are treated with fungal strain (*A. niger*),  $\text{Cr}^{6+}$  &  $\text{Cd}^{2+}$  metal ions, degradation rate was 3–5 % higher than in absence of metal while in  $\text{Zn}^{2+}$ , rate is 1 – 2% higher. Negligible effect had shown in  $\text{As}^{3+}$  metalion.

The determination of effects of heavy metal ions on degradation of polymers yields conflicting results which can be associated to overgeneralization that comes from short-term studies that focuses on single parameter under the controlled conditions.

#### 5. Acknowledgement

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