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Biodegradation of new series of aliphatic copolyesters by fungi

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ABSTRACT

Biodegradable aliphatic random copolyesters poly(butylene succinate-co-butylene adipate), PBSA and poly(butylene succinate-co-butylene azelate), PBSAz were synthesized from 1,4 butane diol, succinic acid and adipic acid/azelaic acid through direct melt polycondensation with Titanium Tetra isopropoxide (TTiPO) as a catalyst. These polyesters were characterized by Viscosity measurements, ^{1}H NMR spectroscopy, Gel Permeation Chromatography (GPC) and X-ray diffraction (XRD) analysis. Thermal properties have been analysed using Differential Scanning Calorimetry (DSC). We have studied the biodegradation behaviour of these polyesters by *Aspergillus versicolor* fungi. The fungal degradation rates for the polyesters were observed and compared. The changes in the structure morphology during the degradation process were studied by scanning electron microscopy (SEM). It appears that the key factor affecting the degradation was its crystallinity. This kind of novel biodegradable polymers are expected to have potential applications as environment friendly materials.

Key words: Biodegradable polyesters, fungal degradation, *Aspergillus versicolor.*

INTRODUCTION

The problems of environmental pollution and waste management from the bio-resistant synthetic plastics have become increasingly serious in near several decades. Many countries put much attention and effort on the reusing and recycling of plastic waste. In the mean time, the development of biodegradable polymers offers an alternative way to solve the problems. Biodegradable polymers had been developed and applied in many fields likewise packaging, agriculture, sanitary and other biomedical applications nowadays. Among these biodegradable materials, the family of aliphatic polyesters appears to be the most attractive and promising because of their susceptibility to biological attacks[1-3].

In recent years, there has been a great need for biodegradable polymeric materials, since waste polymers cause serious destruction of the environment. A number of biodegradable aliphatic polyesters, such as poly(hydroxy butyrate) (PHB),poly(L-lactic acid) (PLLA), poly(3-caprolactone) (PCL) andpoly(1,4-butanediol succinate) (PBS), have been developed and some of them are now commercially available because of their good biodegradability. However, their lower thermal and mechanical properties provide obstacles to their commercialization[4].

Much work has been done on the degradation of aliphatic polyesters by bacteria from compost, soil or freshwater[5-7]. However, reports on

the fungal degradation of these polyesters and the related hydrolytic enzymes are relatively rare and not well documented. Considering that fungi play a significant role in degrading polyesters. Little attention has been devoted to the fungal degradation[8]. Actually, fungi have particular interest in the biological process of solid-state substrate. In soil systems, the fungal biomass generally exceeds the bacterial biomass, and the organic matter in acidic soils is predominantly decomposed by fungal strains, which exhibit a greater tolerance to acid conditions compared with bacteria[9,10]. Rhee *et al*., showed that fungi are the dominant contributors to the degradation of cellulosic materials in soil ecosystems[11]. Jian-Hao Zhao *et al*., also found that the in situ biodegradation of PBSA film by fungi was much faster than by actinomycetes[12]. It is obvious that fungi play a considerable role in decomposing polymer materials.

Considering the high commercial potential of aliphatic copolyesters and their interesting properties, the present investigation deals with the synthesis and characterization of new type of biodegradable polymer with considerable fungal degradability was prepared from suberic acid, succinic /adipic acid and 1,4 butanediol .The structure of their repeating units and the effect of the copolymer composition on the physical and thermal properties. We investigated the degradation behaviours of these polyesters by Aspergillus versicolor strain and discussed the degradation mechanism.

MATERIAL AND METHODS

Materials

Succinic acid (MERCK AR grade), Adipic acid (LANCASTER AR grade) and Azelaic acid (LANCASTER AR grade) were recrystallized from deionised water and used. 1,4-Butanediol (LANCASTER, AR grade) was dried with CaO overnight and then distilled under reduced pressure. Titanium Tetra isopropoxide (TTiPO) , used as catalyst, purchased from LANCASTER was used as such, Aspergillus versicolor strain purchased from IMTECH Chandigar,INDIA was used for degradation studies. All the other materials and solvents used were of analytical grade.

Synthesis of copolyesters

The copolyesters were synthesized by two step melt polycondensation method. As an example, the synthesis of poly(butylene succinate-co-butylene azelate), PBSAz have been described. The polycondensation flask was a three necked one, equipped with a nitrogen inlet, a condenser and a thermometer. A magnetic stirrer is used to stir the mixture. The reaction mixture consists of 0.2 mol of 1.4-butanediol, 0.1 mol of succinic acid and 0.1 mol of azelaic acid.

The reaction mixture is purged with nitrogen and heated in an oil bath. The temperature of the reaction mixture was raised to 150°C in 20 minutes. Then, the temperature was gradually raised in 10°C steps every minute to the final reaction temperature of 210°C to remove water being the esterification byproduct. When water ceased to be generated, a predetermined amount of Titanium Tetra isopropoxide (TTiPO) catalyst was added to the reaction mixture. Subsequently, the pressure of the reaction system was gradually decreased, and condensation polymerization was continued at 210°C under a final reduced pressure lower than 0.5mm Hg. Finally, the reaction was terminated when the rotation of the mechanical stirrer was stopped. The viscous slurry was cooled in the flask under nitrogen atmosphere. The crude polyester was dissolved in chloroform and then poured into excess of dry cold methanol to purify the polyesters. The precipitated polyester was dried in a dessiccator to constant weight.

Gel Permeation Chromatography (GPC)

The molecular weights of copolyesters were analysed by gel permeation chromatography using a Shimadzu instrument equipped with a pump and a refractive index detector. Tetrahydrofuran was used as a mobile phase with a flow rate of 1.0 ml/min. Polystyrene standards from shapodex was used for calibration.

Intrinsic Viscosity

The intrinsic viscosity, [h] of polymer solutions in chloroform was measured at 30°C in a constant temperature bath using Ubbelohde Viscometer.

Nuclear Magnetic Resonance (NMR)

^{1}H NMR spectra were obtained with a JEOL Model GSX300 MHz NMR spectrometer using $CDCl_3$ as solvent and TMS as internal standard respectively. The measurement were carried out at room temperature.

Differential Scanning Calorimetry (DSC)

The DSC scans were recorded at a heating rate of 10°C/min using a Perkin-Elmer Pyris I analyser. Indium was used as the calibration standard.

X-ray Diffraction Analysis

A siemens D 500 diffractometer with Cu K_a filtered radiations was used for assessing the crystallinity of the polymers. The samples were scanned over the range of 2q angle, from 5° to 80°.

Fungal Degradation studies

Copolyester thin films were obtained by hot pressing method. The thin films of area 10 x 10 mm^2 and about 200mm thickness. *Aspergillus versicolor* fungai strain was diluted with sterilized water and inoculated into 100 ml basal medium containing 200 mg polyesters film as the sole carbon source. The composition of the basal medium was: $(NH_4)_2SO_4$ 1.0 g, KH_2PO_4 0.2 g, K_2HPO_4 1.6 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, NaCl 0.1 g, $CaCl_2 \cdot 2H_2O$ 0.02 g, $FeSO_4 \cdot 7H_2O$ 0.01 g, $Na_2MoO_4 \cdot 2H_2O$ 0.5 mg, $Na_2WO_4 \cdot 2H_2O$ 0.5 mg and $MnSO_4$ 0.5 mg in one litre of distilled water. The pH was adjusted to 7.0. Meanwhile, a control experiment without inoculation of the strain was carried out.

Weight Loss

Weight loss of a polymer is a direct measure of its degradation. Polymer films were removed under sterile conditions from the culture medium at regular intervals, washed with distilled water for several times to remove the mycelia on the surface. Then the films were dried to constant weight under vacuum at 50 °C. The weight loss percentage (*D*) was calculated as

$$D = \frac{m_0 - m_t}{m_0} \times 100\%$$

where m_0 is the weight of original films, m_t is the weight of residual films after degradation for different times.

Scanning Electron Microscope (SEM)

The effect of biodegradation upon the polymer surfaces was examined using JEOL, Model JSM-840A SEM microscope. Prior to the analysis, the samples were coated with gold to avoid charging under the electron beam.

RESULTS AND DISCUSSION

Intrinsic viscosity, molecular weights and elemental analysis

The polyesters PBSA and PBSAz were synthesized by a two step melt polycondensation method. The intrinsic viscosity, the molecular weights and the polydispersity index of the copolyesters are presented in Table 1.

Table 1: Instrinsic viscosity and molecular weights of the copolyesters

Polymer	Intrinsic viscosity, [h](dl/g)	Mn	Mw	Poly dispersity index
PBSA	0.72	8426	10448	1.24
PBSAz	0.86	9826	13525	1.37

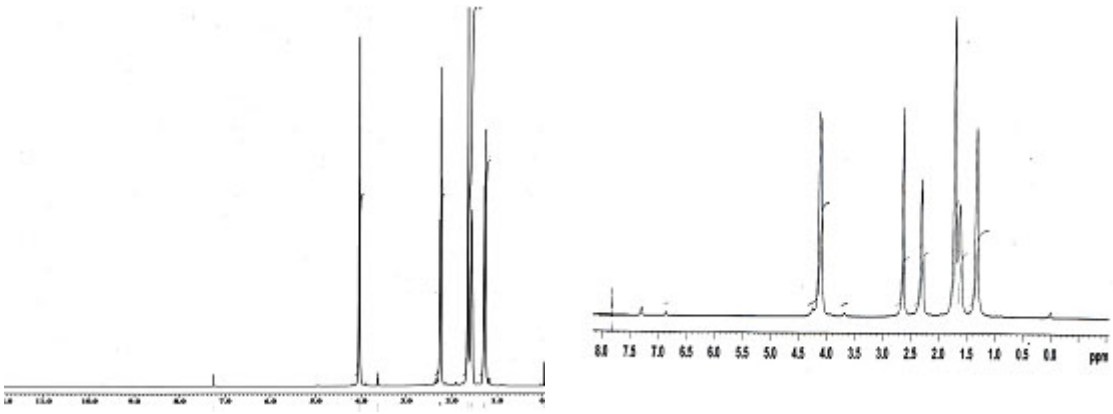
Table 2: Elemental Analysis

Polymer	Formula	% of carbon		% of hydrogen
PBSA	$(C_{18}H_{28}O_8)n$	Calcd	58.06	7.50
		Found	58.00	7.21
PBSAz	$(C_{21}H_{34}O_8)n$	Calcd	60.86	8.93
		Found	60.21	8.61

The extent of polymerisation was determined by the molecular weight and the intrinsic viscosity of the polymers. The intrinsic viscosity[h] of these polyesters PBSA and PBSAz were 0.64dL/g and 0.73dL/g respectively. In all GPC measurements, a single peak which differs in magnitude and elution time was received.

^{1}H NMR

The structure of the repeating units of the polymers are confirmed by elemental analysis and NMR spectroscopy. The elemental analysis values of the resulting polymers were in good agreement with the calculated values for the proposed structures Table 2.



PBSA PBSAz

Fig. 1: ^{1}H NMR spectrum of PBSA, PBSAz

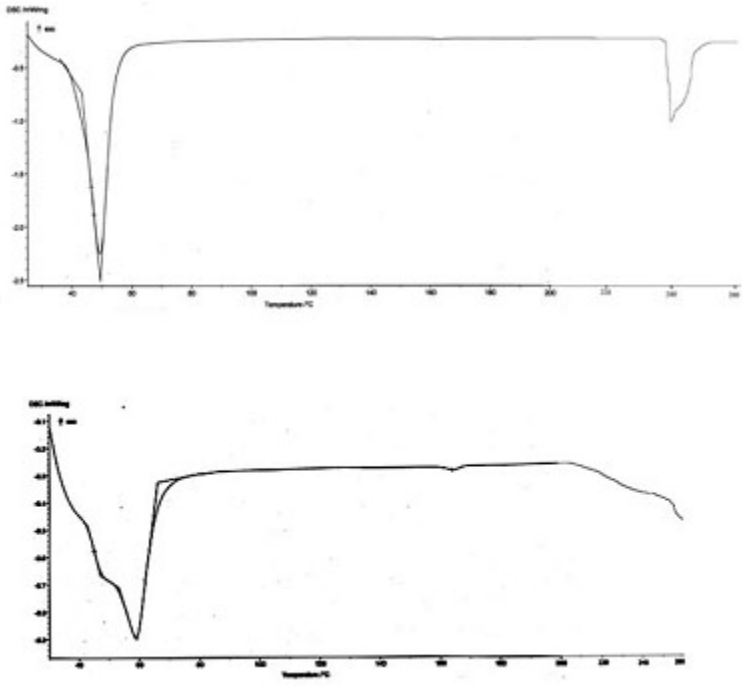


Fig. 2: DSC thermogram of PBSA and PBSAz

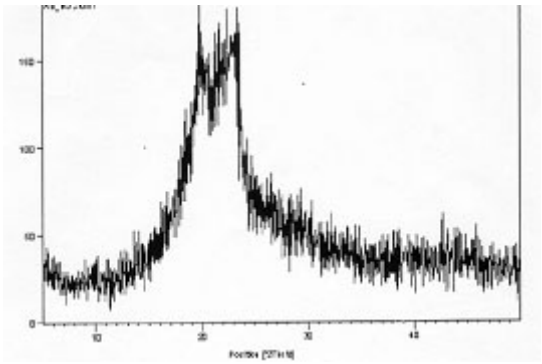
A ^{1}H NMR spectrum of the polyesters are shown in Fig. 1. The peak at d = 2.63 ppm was attributed to methylene protons of dicarboxylic acid at d = 1.63-1.71 ppm due to ester moieties, respectively. In addition the peak at d = 1.7 ppm was attributed to central methylene protons of 1,4–butane diol while d=4.1 ppm is due to terminal methylene groups of 1, 4–butanediol moiety.

Thermal properties

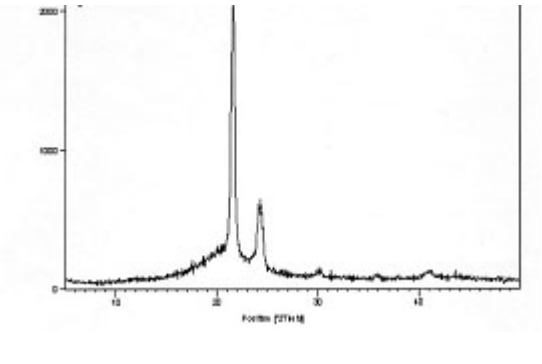
The thermal property data of the synthesized polyesters are summarized in Table 3 and the corresponding thermograms are shown in Fig. 2.

PBSAz is a highly crystalline polymer exhibiting a melting point (Tm) of 44.9°C and a heat of fusion of 18.69 J/g. It is worth noting than an increase of methylene units in the chain increases the crystalline nature of the resulting copolyester.

The glass transition temperature (Tg) values of the PBSA and PBSAz are 43.2, and 42.7°C respectively. The glass transition temperature (Tg) of polymers is closely related to the flexibility of the chains because a high Tg is generally assumed to be connected with relatively high barriers of bond rotations. The results show that the Tg values decreases with increase in methylene units. This indicates that the increase in the length of flexible spacers reduced the proportion of mesogens and decreased the rigidity of polymers. Hence, the Tg values decrease from PBSA to PBSAz.



PBSA



PBSAz

Fig. 3: X-Ray diffractogram of PBSA and PBSAz

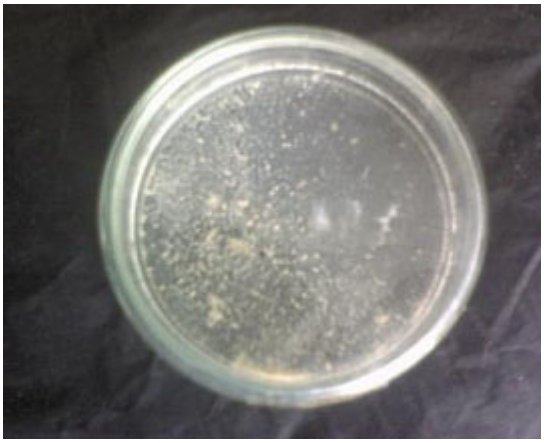


Fig. 4: Subculture formation during the course of degradation using *Aspergillus versicolor* fungi

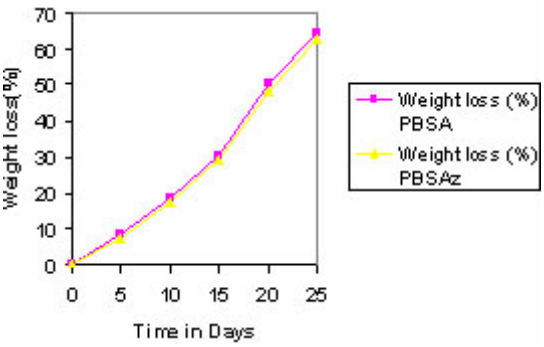


Fig. 5: Fungal biodegradation of PBSA and PBSAz

X-ray diffraction analysis

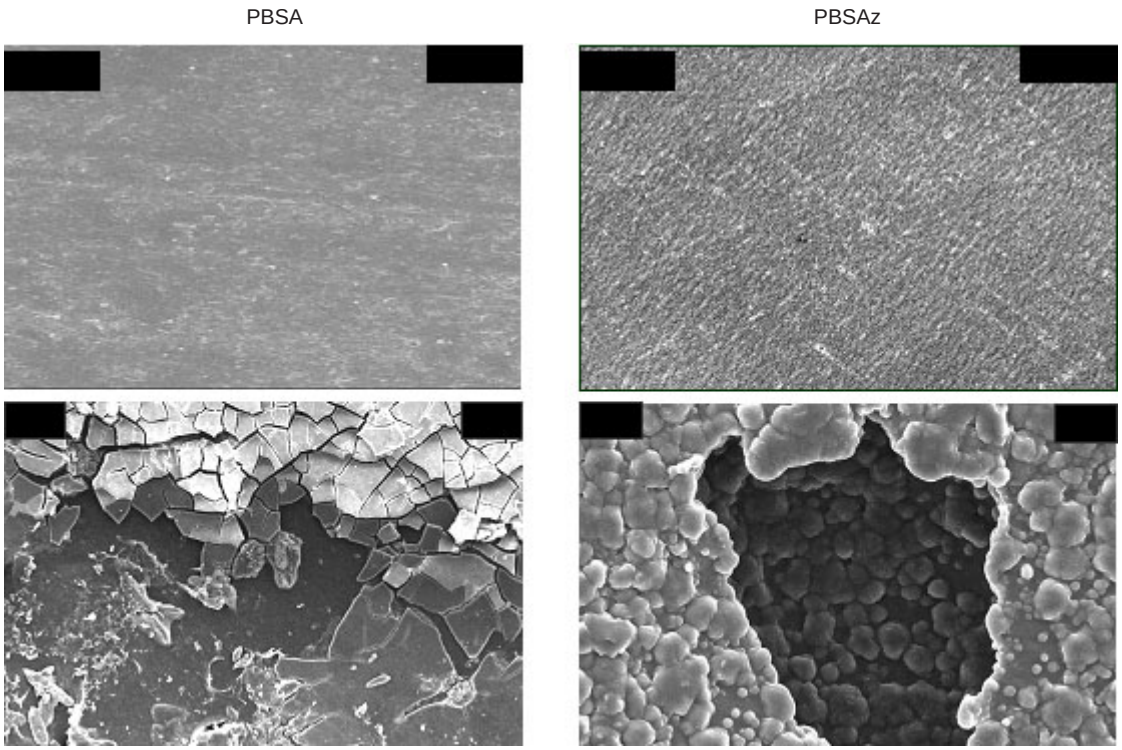
X-ray diffractogram of the synthesized polymers are shown in Fig.3. The crystalline nature of polyesters was determined from X-ray diffractogram. Gaussian curves are used to describe the amorphous phase and all crystal reflections of a diffractogram[32]. In the X-ray diffractogram, the intensity of diffraction peaks increases with the increase in the length of the flexible spacer group. This is in accordance with the study of B.K. Chen *et al*. [33]. This indicates that the crystallinity of the polymer increases with the length of flexible segments. From the X-ray diffratogram, it is observed that PBSAz is highly amorphous in nature than PBSAz.

Biodegradation by fungal species

The hydrolytic degradation of the polymers was investigated by *Aspergillus versicolor* fungi. The weight loss of thin films inoculated with *Aspergillus versicolor* fungi is plotted against the degradation time. Fig. 4 shows the sub culture of *Aspergillus versicolor* at 30°C. A number of colonies and clear zones were observed on the plate.

The biodegradation of the copolyesters is determined by monitoring the weight loss percentage and erosion of thin films with definite time intervals. The degradation of the polyesters without the addition of fungi was also carried out and the weight loss percentage of polyesters was found to be almost negligible. The weight loss percentage of the thin films of polyester samples were analysed using *Aspergillus versicolor* fungi and presented in the following fig.5

During fungal degradation the weight loss is due to the disintegration of the polymer thin films caused by the cleavage of polymer chains. The physical properties like crystallinity and degree of polymerisation of synthesised polyesters seem to be predominant factors which control the hydrolytic degradation.



(a) Before degradation

(b) After degradation using *Aspergillus versicolor* fungi

Fig. 6: SEM micrographs of PBSA, PBSAz Polyester during fungal degradation

Table 3: Thermal properties of the synthesized co-polyesters

Polymer	Tg (°C)	Tm (°C)	Td (°C)	DHm (J/g)
PBSA	43.2	44.1	190.2	16.42
PBSAz	42.7	44.9	212.5	18.67

From the reports it is concluded that the polymer degradation usually proceeds in a selective manner, with the amorphous region being preferentially degraded as compared to crystalline ones. From the investigation of biodegradation using *Aspergillus versicolor* fungi, it is clear that the PBSA undergoes more degradation which indicates the less crystalline nature of PBSA.

SEM Observations

SEM micrographs are used to study the morphology of the aliphatic polyester films during fungal degradation. Further, the biodegradation of the polyesters was confirmed by SEM. As seen in the micrographs of Fig.6, the hydrolytic action of the *Aspergillus versicolor* fungi led to the formation of a rough surface with large cavities on the film surface of the polyesters.

SEM studies were done after biowashing the samples by water and ethanol (50:50). Biodegradation was appeared clearly on the surface. SEM micrographs were scanned before and after biodegradation. Fig. 6 indicates the SEM micrographs of the copolymer taken before and after 25 days of biodegradation. After 25 days of inoculating the. *Aspergillus versicolor* fungi to the copolymer has biodegraded. SEM Migrographs reveal that sphericalholes probably resulting from localized enzymatic action with colonization of fungi.

CONCLUSION

The high molecular weight aliphatic random copolyesters derived from Succinic acid, Adipic acid, Azelaic acid and 1,4-Butanediol were synthesized by the two step melt polycondensation method in the presence of a highly effective catalyst TTiPO at 215°C. The synthesized polyesters were characterized by thermal and spectral methods of analysis. The biodegradation of these polyesters was investigated using the *Aspergillus versicolor* fungi.

The results indicate that the highest degradation was observed for the PBSA polyester as it was expected due to lower crystallinity and flexibility of chain backbone. The major factor which controls the biodegradation rate is how tightly the polymer chains are fixed in the crystalline regions of the polymer material. This is characterized by the melting point of the material. The order of fungal biodegradation of the copolyesters was PBSA > PBSAz. This investigation clearly demonstrates that the fungal degradation of polyesters by *Aspergillus versicolor* fungi is predominantly controlled by the degree of crystallinity of the material and structural differences in the polymer chain.

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