

Water Resistance, Mechanical Properties, and Biodegradability of Poly(3-hydroxybutyrate)/Starch Composites

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ABSTRACT: Composites of poly(3-hydroxybutyrate), P(3HB), and starch were prepared by solution casting technique. To improve adhesion of starch to P(3HB), stearic acid was added as a compatibilizer and glycerol as a plasticizer. The water resistance, mechanical, and biodegradable properties of the P(3HB)/starch composites were studied. Diffusion and penetration coefficients of water increased with increasing starch content in the composites. The results showed that the elastic modulus and strain at rupture of the P(3HB)/starch composites were enhanced by increasing starch content upto 10 wt % and the tensile strength increased from 21.2 to 93.9 MPa. The presence of

starch content higher than 10 wt % had an adverse effect on the mechanical properties of the investigated composites. The biodegradation rate using Actinomycetes increased proportionally to the starch content in the composite and accelerated in a culture medium of pH \approx 7.0 at 30°C. Enzymatic degradation experiments showed that lipase produced by *Streptomyces albidoflavus* did not degrade P(3HB)/starch composites. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 115: 2813–2819, 2010

Key words: poly(3-hydroxybutyrate); starch; composites; mechanical properties; water resistance; biodegradability

INTRODUCTION

Synthetic polymers, such as polyethylene, polypropylene, and polystyrene, are widely used in daily life, in food industry, biomedical field, and agriculture. A heavy environmental pollution accompanies their uses because they need hundreds of years to degrade, and the disposal of waste plastics has become a serious problem.¹ To increase the biodegradability of these synthetic polymers, efforts have been made to blend starch with some thermoplastics, such as low-density polyethylene,² polyvinyl alcohol,³ and polystyrene,⁴ polycaprolactone^{5,6} and polyethylene vinyl alcohol.^{7–9} A thermoplastic polymer from a renewable source would be better for the development of a biodegradable plastic. Polyhydroxyalcanoates (PHAs) biopolymers from renewable resources attracted much attention in the last few years as biocompatible and biodegradable thermoplastics with potential application in agriculture, marine, and medicine fields.¹⁰

Poly(3-hydroxybutyrate) P(3HB), the most common member of (PHAs) family, is accumulated as intracellular granules by bacteria from more than 75

different genera. The main advantage of P(3HB) over other types of biodegradable plastics is that they do not require special environmental conditions to degrade. They can undergo rapid biodegradation under aerobic and anaerobic conditions, thus solving the problem of vanishing landfill space. Landfills are one of the safest and least expensive ways to deal with the disposal of polymers.¹¹ However, some characteristics of P(3HB) limit its applications, such as high crystallinity, poor processability, and high fragility. To improve P(3HB) characteristics, physical mixtures or blends can be prepared with other biodegradable polymers which enhance the range of its applications and control its profile of biodegradation.³⁶ It was also recognized that the high cost of biopolymers is not due to the cost of raw materials for polymer synthesis but is mainly attributable to low production volumes.³⁷

One possible way to obtain low-cost biodegradable polymer composite is the use of biodegradable fillers such as starch. The use of starch as a filler in high-cost biodegradable plastics is preferred because it is totally biodegradable, inexpensive, and available in large quantities.¹⁴ Synthetic polymer/starch composites initially attracted some research interest but their development was not as expected, mainly because of their deficiencies with regard to mechanical properties and water resistance.¹⁵ Therefore, many investigations have been conducted during

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the last decade on thermal, mechanical, and gas and water permeability properties of synthetic polymer/starch composites as affected by composition, moisture content, and plasticizers.^{16–18} Studies on starch-based polyethylene blends showed that higher the starch content worse the performance of the composite materials but higher their biodegradability. However, the addition of water, plasticizers, and/or compatibilizers to these blends exhibited improved properties.^{19,20}

The main purpose of this study was to prepare P(3HB)/starch composites varied in starch content, to investigate their mechanical and swelling in water properties, and to evaluate the capability of Actinomycetes to degrade this polymer under different cultural conditions.

EXPERIMENTAL

Materials and films preparation

The poly(3-hydroxybutyrate) used in this study was purchased from Copersucar (Brazil) as a white powder produced naturally from the bacterial fermentation of sugarcane. P(3HB) has an average molecular weight $M_w = 4 \times 10^5$ g mol⁻¹; degree of crystallinity 60%; a glass transition temperature, T_g , at -2°C; and a melting temperature, T_m , at 180°C.

Solutions of P(3HB) in chloroform were used as a continuous matrix and different amounts of commercial pure grade of corn starch, obtained from The Egyptian Company for Starch and Glucose, were added to form P(3HB)/starch filled composites. To improve adhesion of filler (starch) to polymer matrix (P(3HB)), stearic acid was added as a compatibilizer and glycerol as a plasticizer. The mixture is then stirred for 6 h. at 40°C using a magnetic stirrer. P(3HB) and P(3HB)/starch films of 0.3 mm in thickness were prepared by solution casting in Petri dishes and allowed to dry slowly at 30°C in a vacuum desiccator. Sheets were then cut into small pieces and used for degradation and characterization studies.

Swelling in water test

Circular samples of radius (0.45 cm), punched out from the specimen sheets by means of a sharp edged steel die, were vacuum dried at room temperature for 24 h. They were then immersed in diffusion bottles containing water and kept in a thermostatically controlled oven at 30°C. Water swollen samples were taken out at constant time intervals, the solvent adhered to the samples was removed by using a tissue paper and quickly weighed on an electronic balance with an accuracy of $0.0002 \times$ g. The water

uptake, $Q_t(\%)$, was calculated using the gravimetric method according to the following equation:

$$Q_t(\%) = \frac{m_s - m_p}{m_p} \times 100 \quad (1)$$

where, m_p and m_s are the mass of the sample before and after swelling.

Morphology

The surface morphology of the P(3HB) composite containing 10 wt % starch, before and after biodegradation, and its tensile rupture surface was investigated using scanning electron microscope (SEM). The SEM photomicrographs were taken in a JEOL 35 CF model. Before taking the photographs, the samples were sputter coated with gold for 5 min under high vacuum in an automatic sputter coater.

Mechanical properties test

The stress-strain measurements were performed at 30°C using a locally made tensile testing machine in which a precise digital force gage was attached. A motor with micro switch system was attached to the test machine to control the strain rate and the readings were recorded as a function of time. The strain rate throughout the experiment was maintained at 10 mm/min. The samples used in mechanical measurements were strips of dimensions $20 \times 4 \times 0.3$ mm.

Microbiological degradation test

Actinomycetes were isolated from clayed fertile agriculture soil samples (humus content 5%, water content 42%, pH 6.7) on starch nitrate medium. A thin starch nitrate agar medium without carbon source was poured and solidified. P(3HB) and P(3HB)/starch composites were then over layered on the basal layer and allowed to solidify. The over layered plates were inoculated in the form of a streak with a loopful of fresh inoculum of isolates and incubated at 30°C. The biodegradation of P(3HB) composites was monitored by the weight loss of the composites as a function of time.

The effects of different carbon and nitrogen sources on P(3HB) and P(3HB)/starch composites degradation by Actinomycetes were studied. In these experiments, different carbon and nitrogen sources were added to P(3HB) composites in the culture media. These culture media were inoculated with the test organism and incubated at 30°C and 120 rpm for 15 days.

The effect of pH on P(3HB) composites degradation by Actinomycetes was also studied using

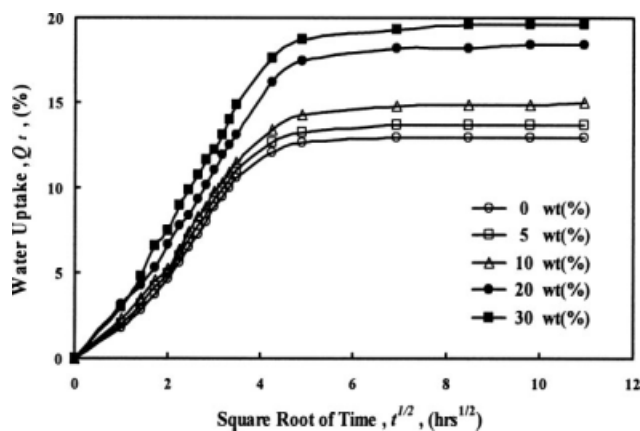


Figure 1 Dependence of the degree of swelling on time for different concentrations of starch.

buffered starch nitrate without carbon source supplemented with P(3HB) composite films. The following buffer systems were utilized: citrate–phosphate buffer pH 4.0 and 5.0, phosphate buffer pH 6.0 and 7.0, and tris-HCl buffer pH 8.0 and 9.0. Incubation was carried out at 30°C and 120 rpm for 15 days. To determine the optimal incubation temperature for P(3HB) by Actinomycetes, the organism was grown in starch nitrate medium without carbon source supplemented with P(3HP) composite films. The incubation was carried out at different temperatures (30, 35, 40, and 45°C) and 120 rpm for 15 days.

Enzymatic degradation test

Polyester film (2 × 0.75 cm; initial weight 20–30 mg) was incubated, in duplicate, at 30°C, in vials containing 1.5 mL of 0.1M potassium phosphate buffer (pH = 7.4), in presence of lipase. The lipase used was extracted from the *Streptomyces albidoflavus* with an activity 400 mU/mL. The film was removed from the enzymatic solution after 24 h, washed with distilled water, and then dried to a constant weight. The biodegradation was evaluated by monitoring the normalized weight.

RESULTS AND DISCUSSION

Swelling in water

Swelling in water and degradability are the most important properties for biodegradable materials.²¹ Polymer films were degraded by surface absorption of moisture and microorganisms, such as fungi and bacteria. The water absorbed on the materials allowed the microorganisms to grow and to utilize the material as an energy source. Figure 1 shows the effect of starch content in film matrix on its degree of swelling $Q_t(\%)$ in water. It can be observed that $Q_t(\%)$ increased gradually during the first 2 days

until it reached a plateau, and this behavior is similar for both P(3HB) and its composites with starch. The water uptake $Q_t(\%)$ of the P(3HB) film was lower than those of the P(3HB)/starch composites, indicated that the hydrophobicity of P(3HB) decreased by the addition of starch. Generally, the presence of hydroxyl groups on the surface of the starch granules increases the affinity for water. Therefore, higher the starch content, more is the water uptake in the high-starch content composites.

The transport behavior of water through the P(3HB)/starch composites is of great technological importance and it plays a vital role in its biodegradability and applicability. The permeation of water molecules through polymers generally occurs through a diffusion mechanism, i.e., the penetrant water molecules are first absorbed by the polymer followed by diffusion through the polymer. The net transport through the polymer depends on the difference in the amount of penetrant molecules between the two surfaces. Hence, the permeability is given by the following²²:

$$P = D.S \quad (2)$$

where D is the diffusivity and $S = m_s/m_p$ is the solubility.

The diffusion coefficient D can be calculated using the equation as follows²³:

$$\frac{Q_t}{Q_\infty} = 1 - \frac{8}{\pi^2} \sum_{n=0}^{\infty} \frac{1}{(2n+1)^2} \exp\left(\frac{-D(2n+1)^2\pi^2 t}{h^2}\right) \quad (3)$$

where t is the time and h is the initial thickness of the polymer membrane. A simplified form of eq. (3) for relatively short-time swelling is given by the following²⁴:

$$D = \pi \left(\frac{h\theta}{4Q_\infty} \right)^2 \quad (4)$$

where θ is the slope of the initial linear portion of the sorption curve $Q_t(\%)$ versus $t^{1/2}$.

When the samples immersed in water, starch granules at or near the surface absorb water rapidly, whereas water absorption is slower for granules buried within the interior of the P(3HB) matrix. Figure 2 represents the variation of the diffusion and penetration coefficients with starch content in the investigated composites. It is clear that both diffusion D and penetration P coefficients increased gradually with increasing starch content upto 10 wt % of starch but at higher concentrations of starch (20 and 30 wt %), a drastic increase in both D and P was observed. The increase in diffusion and penetration coefficients in starch-loaded P(3HB) is logical

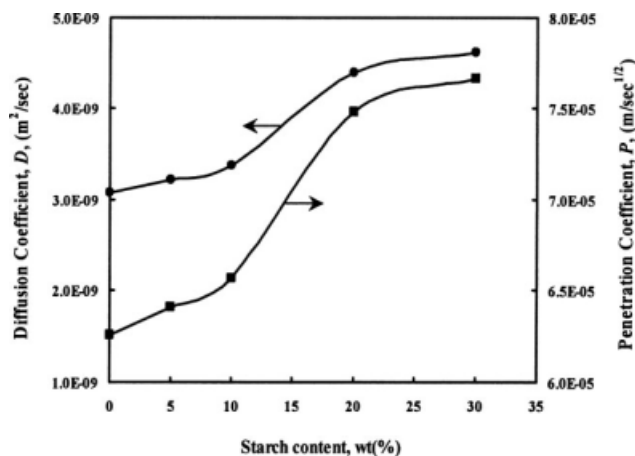


Figure 2 Dependence of the diffusion and penetration coefficient on the concentration of starch.

because the water absorption increased in the presence of hydrophilic starch filler. The drastic increase in the diffusion and penetration coefficients that observed when the starch content exceeded 10 wt % can be attributed to the capillary moisture formed by water on a starch basis.²⁰ These results indicate that the increase in starch reduces the water resistance of the P(3HB)/starch composites specially at concentrations higher than 10 wt %.

Mechanical properties

Stress–strain curves of P(3HB)/starch composites are shown in Figure 3. Each curve consists of two distinct regions, the linear and the plastic regions. With increasing starch content, the tensile properties of the composites were altered. The stiffness of the samples increased with increasing the concentration of starch upto 10 wt % and decreased by further addition of starch. The use of compatibilizers and

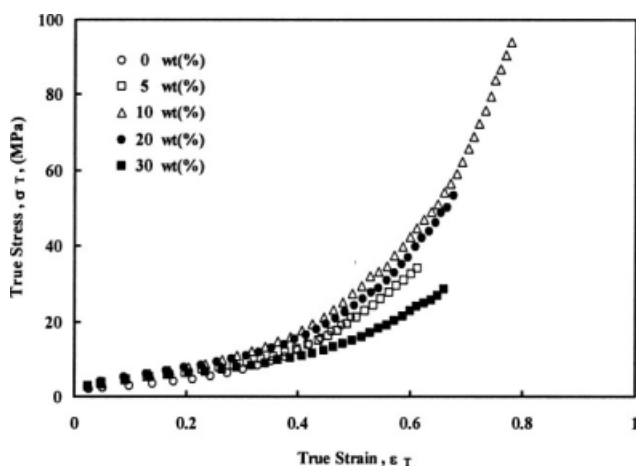


Figure 3 Stress–strain curves of P(3HB)/starch composites.

plasticizer leads to finer morphology and may produce higher mechanical properties.^{25–27} The mechanical properties of the composites can be improved by the strong interfacial tension between the filler and matrix being overcomes.^{28–30} Reducing the interfacial tension and strengthening the interaction between polymer phases can transfer the internal stresses from the filler to the matrix and, consequently, enhance the strength of the composites.³¹ Glycerol and stearic acid were used as a plasticizer and compatibilizer, respectively, in the investigated composites. P(3HB)/starch composites have been found to exhibit good mechanical properties at low concentrations of starch upto 10 wt %. The mechanical properties of the composites, at 10 wt % starch content, seemed to possess a good combination of stiffness, strength, and toughness.

The dependence of the elastic modulus E , tensile strength σ_R , and strain at rupture ϵ_R , on starch content is shown in Figure 4. From this figure, it is clear that 10 wt % is the optimum concentration of starch at which a maximum of all the plotted mechanical parameters occurs. The first increase in both E and σ_R is due to the increase in the viscosity of the matrix by suspension of starch granules, which is responsible for the decrease in deformability at a certain stress. The decrease in both E and σ_R at 20 and 30 wt % of starch implied the weak adhesion between P(3HB) matrix and starch particles at relatively high concentrations of starch. The increase in ϵ_R could be due to the penetration of the plasticizer (glycerol) in the composite network leading to “filling” of the voids and thus reducing considerably the number of existing defects and the entanglement of chain segments by the physical crosslinking with starch granules. A compact structure is formed by further increasing of the starch content which resulted in the disruption of P(3HB) network

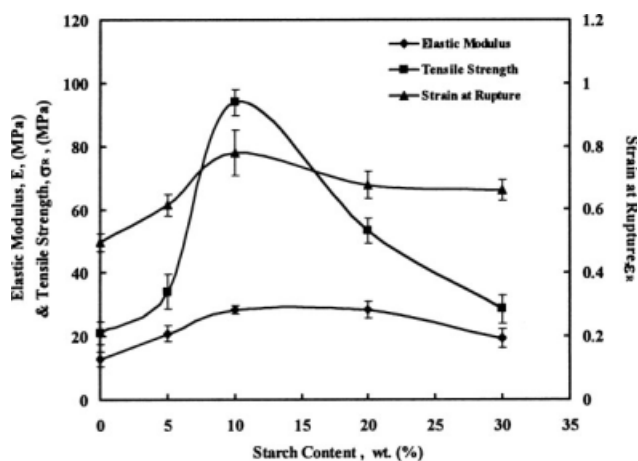


Figure 4 Dependence of the elastic modulus E , tensile strength σ_R , and strain at rupture ϵ_R , on the concentration of starch.

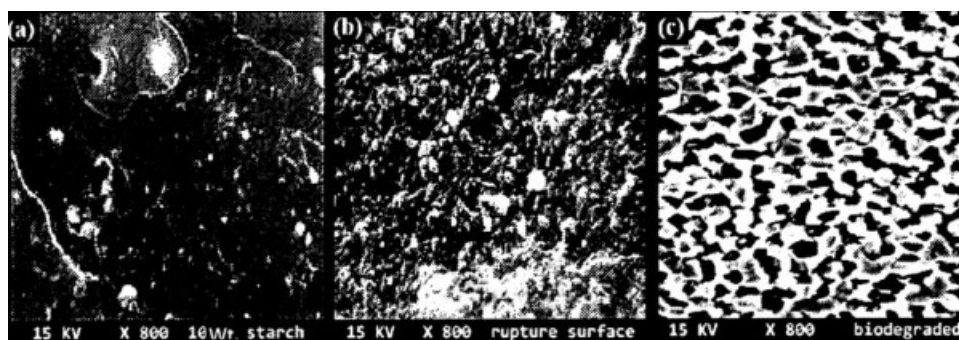


Figure 5 SEM micrographs of the (a) sample surface, (b) tensile rupture surface, and (c) biodegraded surface of the P(3HB)/starch composite containing 10 wt % starch.

continuity by the occluded filler particles (starch), leading to the decrease in ϵ_R .¹⁹

Figure 5(a,b) shows the SEM micrograph of the surface and tensile rupture surface of the sample containing 10 wt % starch. It can be seen clearly that there is a relatively good dispersion and interfacial adhesion between starch granules and P(3HB) matrix. The starch granules are shown to be embedded in the P(3HB) matrix even after the rupture process.

Microbiological degradation behavior

The percentage of weight loss of polymer versus time plots is shown in Figure 6. Weight loss, due to the utilization of the P(3HB) and its composites by Actinomycetes, was used to estimate the biodegradation of these composites, since there is no significant weight loss in the controls and the well-known ability of P(3HB) to biodegrade. All the plots of Figure 6 clearly indicate rapid initial weight loss of the material in the first 15 days of the incubation period followed by a slow degradation until the end of the experiment. The results indicate that the higher the starch content, the faster will be the rate of biodegradation. This is because the presence of starch increases the moisture content into the samples due

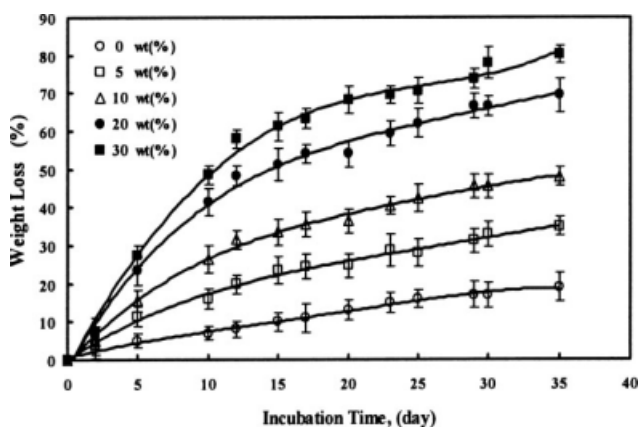


Figure 6 The percentage of weight loss of P(3HB)/starch composite versus the incubation time with Actinomycetes.

to its hydrophilic nature and thus allowed the growth of microorganism on the composite film. Also, the crystallinity of P(3HB) is highly decreased by the addition of starch, allowing a faster microbiological degradation. All the composites presented morphological modifications throughout the biodegradation test. The SEM micrograph of the surface of the biodegraded composite containing 10 wt % of starch represented in Figure 5(c).

Degradation of P(3HB)/starch composites was affected significantly when the composites containing medium was supplemented with different carbon sources. All carbon sources, inhibited P(3HB) utilization as shown in Figure 7(a) and this is in

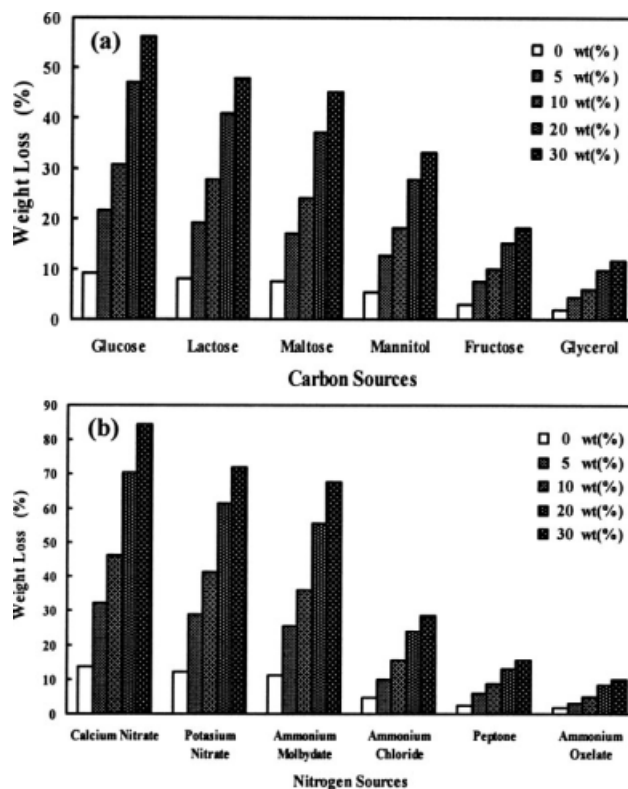


Figure 7 Effect of different (a) carbon sources and (b) nitrogen sources on the biodegradation of P(3HB)/starch composites.

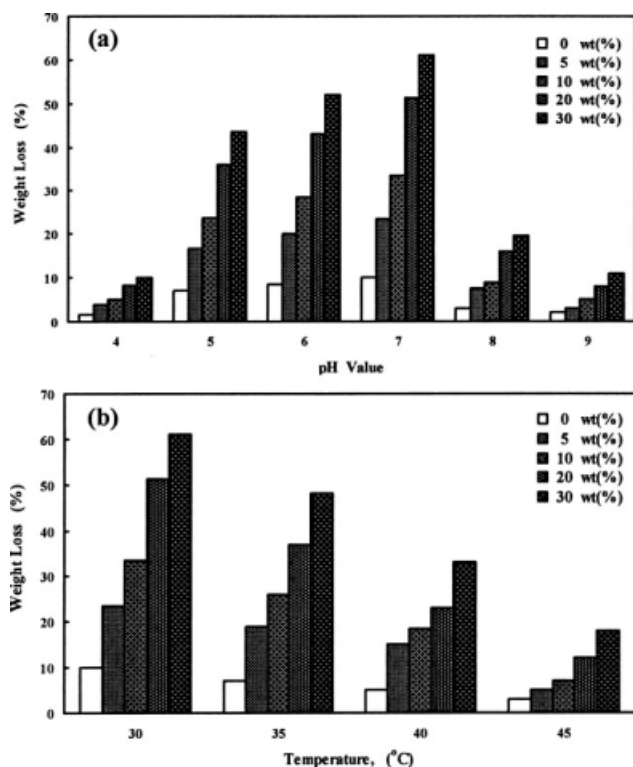


Figure 8 Effect of different (a) pH values and (b) temperature on the biodegradation of P(3HB)/starch composites.

agreement with the results obtained by Manna and Paul.³² The reduction of weight loss indicates repression of P(3HB) depolymerase, the extent of such repression appeared to be influenced by the type of carbon source supplemented in the medium.

Degradation of P(3HB)/starch composites was also affected significantly with different nitrogen sources in the medium. Peptone, ammonium chloride, and ammonium oxalate when supplemented in the medium lowered the degradation of P(3HB) composites, while an increase in the degradation was observed when calcium nitrate, potassium nitrate, or ammonium molybdate were supplemented in the medium as shown in Figure 7(b). Bonartseva et al. reported that P(3HB) degradation observed by the dominant species (*Pseudomonas fluorescens* and *Pseudomonas stutzeri*) was 7.3 times lower and there was a decrease in temperature from 20°C to 5°C in the presence of nitrate.³³

The optimum pH value for degradation of P(3HB) was 7.0 as shown in Figure 8(a). In contrast to our findings, Klingbeil et al. found that *Streptomyces exofolius* produce P(3HB) depolymerase at 40°C and pH value between 8.5 and 9.0.³⁴ The optimum pH of P(3HB) depolymerase produced by *Pseudomonas picketti* and *Penicillium funiculosum* have pH optima between 5.5 and 7.0.³⁵

Degradation of P(3HB) under laboratory conditions was recorded after 15 days of incubation at 30,

35, 40, and 45°C. Most of P(3HB)/starch composites test pieces lost weight during incubation, but the degree of weight loss varied widely with the incubation temperatures as seen in Figure 8(b). This result indicated the mesophilic nature of the degraded organism.³²

Enzymatic degradation behavior

The weight of P(3HB) and its composite films were not affected in presence of lipase produced by *S. albidoflavus*. In contrast to our finding, Rizzarielle et al. reported that the copolymers included P(3HB) showed enzymatic degradation which was evaluated by the weight loss of the P(3HB) samples.³⁶ It was also reported that extracellular hydrolytic enzyme such as lipases are able to digest films of aliphatic polyesters.³⁷ The chemical, physical, and molecular weight properties of polyester have a strong influence on its biodegradability. Lipases can hydrolyze aliphatic polyester other than optically active polyesters such as P(3HB) and poly(L-lactide).

CONCLUSIONS

P(3HB)/starch composites, plasticized with glycerol and compatibilized with stearic acid, were prepared, and their mechanical, water resistance, and biodegradation properties were studied and correlated to their composition. The diffusion and penetration coefficients of water showed a drastic increase when the starch content exceeded 10 wt %. The mechanical properties of P(3HB)/starch composites were improved with addition of starch upto 10 wt %, while at higher starch content, a reasonable decrease in the mechanical parameters was observed. The microbiological degradation of P(3HB)/starch composites using Actinomycetes was confirmed by weight loss measurements and showed a remarkable increase with increasing starch content. The presence of glucose, as a carbon source, or calcium nitrate, as a nitrogen source, at a cultural medium of pH \approx 7.0 at 30°C was the optimum conditions for the biodegradation of the investigated composites.

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