

Biodegradation of Starch–Poly(β -hydroxybutyrate-co-valerate) Composites in Municipal Activated Sludge¹

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Injection-molded composites were prepared by blending PHBV⁵ with native cornstarch (30% and 50%) and with cornstarch precoated with PEO as a binding agent. These composites were evaluated for their biodegradability in municipal activated sludge by measuring changes in their physical and chemical properties over a period of 35 days. All composites lost weight, ranging from 45 to 78% within 35 days. Interestingly, the extent and rate of weight loss were quite similar in PHBV composites with no starch, with 30% starch, and with 50% starch. Weight loss was slowest in PHBV blends prepared with PEO-coated starch. For all samples, the weight loss was accompanied by a rapid deterioration in tensile strength and percentage elongation. The deterioration of these mechanical properties exhibited a relative rate of PHBV > starch-PHBV > PEO-coated starch-PHBV. Changes in starch/PHBV composition after biodegradation were quantified by FTIR spectroscopy. Increasing the starch content resulted in more extensive starch degradation, while the PHBV content in the blends became less susceptible to hydrolytic enzymes.

KEY WORDS: Starch; poly(β '-hydroxybutyrate-co- β -hydroxyvalerate); poly(hydroxyalkanoates); plastic; blends; composite; biodegradation; activated sludge.

INTRODUCTION

Utilization of agricultural commodities such as corn is a primary focus of research at the USDA Laboratory

in Peoria, Illinois. In the early 1980s, using extrusion methods, a technology was developed [1, 2] to incorporate up to 40% cornstarch into blown plastic films. This technology provided the opportunity to utilize renewable agricultural material available in surplus quantities and replace, at least in part, some of the conventionally used, petroleum-based resins. Furthermore, plastics containing plant-derived materials are generally regarded as potentially biodegradable, a characteristic which is considered environmentally friendly. Studies have been undertaken to develop methods for measuring starch degradation [3–5] and to evaluate the biodegradability of starch-containing plastics under laboratory conditions, as well as in the natural environment [6–10]. Results obtained from several studies revealed that, while starch in all plastics degraded to a certain extent, the susceptibility of starch to microbial attack in different plastic blends was rather variable [11–13]. In this regard, attempts have also been made to access the relationship between starch degradation and the ability of starch-degrading microorganisms to colonize surfaces of starch–plastic [14], as well as certain derivitized starches

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⁵*Abbreviations used:* % E, percentage elongation; PHBV, poly(β -hydroxybutyrate-co- β -hydroxyvalerate); PEO, poly(ethylene oxide); FTIR, Fourier transform infrared; TS, tensile strength; SEM, scanning electron microscopy; PHA, poly(hydroxyalkanoates); DO, dissolved oxygen; TSS, total suspended solid; TVSS, total volatile suspended solid.

[15]. The ultimate purpose of such work was to produce surfaces with enhanced ability to attract or repel starch-hydrolyzing microbes.

Considerable efforts are now under way, particularly in industrialized countries, to engineer plastic blends incorporating a wide variety of renewable materials having useful thermoplastic properties [16]. These renewable materials derived from plant, animal, and microbial sources can be used to develop substantially biodegradable plastic blends suited for specific single use applications. Since the addition of biological material generally alters the mechanical properties of the plastic blend, thus affecting the performance of such plastics, it is important that each material be evaluated with respect to its physical, chemical, and biological properties. These include thermoplasticity, compatibility, mechanical properties, choice of plasticizers, binders, biodegradability, etc.

Zeneca Bioproducts Inc., Wilmington, DE (formerly ICI America, Inc.), has developed novel microbial fermentation processes for obtaining high yields of the copolymer, PHBV⁵ [17], currently being marketed under the trade name BIOPOL. This family of bacterially derived polyesters not only is biodegradable, but has excellent mechanical properties well suited for manufacturing single use consumer articles. Unfortunately, PHBV is also higher in cost than conventional plastic resins. Under a cooperative agreement between the USDA and Zeneca, blends of PHBV with granular cornstarch are being prepared to yield quality composites at a low cost. Using a combination of polymers, plasticizers, and binding agents, starch-PHBV formulations of improved mechanical properties were prepared [18]. Little is known regarding the performance and environmental biodegradability of such blends. In this paper, we describe studies in which selected starch-PHBV formulations were exposed to municipal activated sludge and evaluated for biodegradation.

EXPERIMENTAL

Materials. Pearl cornstarch (Buffalo 3401, 10% moisture) was purchased from CPC International, Englewood Cliffs, NJ, and dried before use. PHBV was nucleated BIOPOL resin with 12% valerate (MW 690,000) from Zeneca Bioproducts. PEO was obtained from Aldrich Chemical Co., Milwaukee, WI.

Extrusion Processing and Injection Molding. Detailed methods for extrusion and injection molding have been described elsewhere [18]. Briefly, ingredients

were mixed in a cake mixer and extruded using a Brabender PL2000 single-screw extruder (C. W. Brabender Instruments, Inc., South Hackensack, NJ) equipped with a strand die. Strands were cooled and pelletized prior to injection molding. ASTM D-638 Type V tensile specimens were molded using a Rabbit HY-4 (MCP Equipment LTD, Staffordshire, England).

Mechanical Testing. Tensile specimens were equilibrated at 23°C and 50% relative humidity for 28 days prior to testing. Tests were conducted on an Instron Universal testing Machine (Model 4201, Instron Corp., Canton, MA) using a gage length of 10 mm and a cross-head speed of 20 mm/min, for tensile measurements.

FTIR Analyses. The analyses of starch and PHBV components in composites were performed with a KVB/Analect RFX-75 FTIR spectrometer, as described earlier [7].

Scanning Electron Microscopy. Subsequent to tensile strength measurements, samples were mounted on aluminum stubs with graphite-filled tape and vacuum coated with gold-palladium. Samples were then examined using a JEOL JSM 6400V scanning electron microscope (JEOL Inc., Peabody, MA).

Biodegradation Studies. Tensile specimens were numbered, dried, and weighed. Each specimen in duplicate was placed together into a color-coded jacket (7.5 cm²) prepared from commercially available nylon tulle with pores large enough (approx. 0.1 mm²) to contain the specimen but allowing unrestricted flow of fluids across the pores. One jacket of each specimen was put into a larger nylon bag and sewn shut. A total of six bags, each containing five specimens in duplicate, was fastened to a nylon rope and lowered into the Peoria Sanitary District effluent ponds, a first step in the sanitation process. These ponds are kept at a rolling churning motion for aeration and have the highest dissolved oxygen levels and bacterial counts at this stage of the sanitation process. Ropes were tied in such a way as to allow nylon bags to have a small amount of movement with the currents, without being washed away. At a given time point, a bag was retrieved and placed in a large zip-lock plastic bag and transported to the laboratory for further processing.

Once in the laboratory, specimens were removed from the nylon jackets, placed into a beaker containing 500 ml of 3% hydrogen peroxide, covered, and allowed to stand for 60 min with occasional gentle swirling. Tensile specimens were then removed from the jackets

and placed in sterile petri dishes. Specimens were dried in an oven (60°C, 24 h) and allowed to cool to room temperature before weights were recorded.

Subsequent to weight measurements, specimens were first tested for mechanical properties and the broken pieces recovered after mechanical testing were used for FTIR and SEM analyses. To determine the percent weight loss in specimens due to shaking alone, controls specimens were suspended in a flask containing 250 ml of sterile distilled water and placed on a shaking orbit incubator (30°C, 180 rpm). Samples were retrieved at time points and processed as test samples to determine the weight loss.

RESULTS

A total of five blends, each containing about 10% triacetin as a plasticizer, was prepared (Table I). The composite specimen designated sample A was a PHBV control with no added starch. Specimens B and C were starch-PHBV blends with 30 and 50% starch contents, respectively. Specimens D and E were identical to samples B and C, except that the starch used in these samples was coated with PEO (Table I). The PHBV composites had the highest TS (24 MPa) and % E (38). Both TS and % E were drastically reduced when PHBV was blended with cornstarch. The deterioration in mechanical properties was greater in the blends with higher amounts of starch content. However, only a little loss in mechanical properties, particularly TS, was observed in starch-PHBV blends prepared with PEO-coated starch (Table I).

Biodegradation studies were conducted during the summer months of 1993. Table II summarizes the dates over which these experiments were conducted. Exposure times are given in parentheses. Some routinely measured biochemical parameters (data provided to us

Table I. Formulations of Composite Blends and Mechanical Properties

Sample	Starch ^a	PHBV ^a	Triacetin ^a	Tensile strength (MPa)	% elongation at break
A	0	100	10	24	38
B	30	70	10	15	21
C	50	50	10	10	11
D	30 ^b	70	10	19	21
E	50 ^b	50	10	18	21

^aParts by weight.

^bContains 9% PEO, MW 4×10^6 , based on starch weight.

Table II. Test Schedule and Some Environmental Parameters of the Sanitary Pond^a

Test date 1993	(day)	DO (mg/L)	Temp (°C)	TSS (mg/L)	TVSS (mg/L)
July 7	(00)	4.6	22	2000	1600
July 14	(07)	4.7	23	1840	1400
July 21	(14)	5.1	22	1220	950
July 28	(21)	5.6	22	1620	690
Aug. 4	(28)	4.6	23	2820	1820
Aug. 11	(35)	4.9	23	2120	1470

^apH values of the effluent pond averaged between 7.6 and 7.8 during the course of the experiments.

as a courtesy of the Greater Peoria Sanitary District) in the effluent ponds are also given in the table. These effluent ponds reflect the highest amount of biological activity during the water treatment process. Although dissolved oxygen (DO), total suspended solid (TSS), and total volatile suspended solid (TVSS) values fluctuated, they remained within the range considered typical. The temperature of the effluent pond varied between 22 and 23°C which is somewhat lower than normal and can be attributed to the heavy rainfall in the Midwest area during the summer of 1993.

Initial weight losses in all samples ranged from 10 to 20% within 7 days of exposure (Fig. 1). Thereafter, the weight losses in PHBV composites containing 0%, 30%, or 50% starch occurred rapidly and at relatively

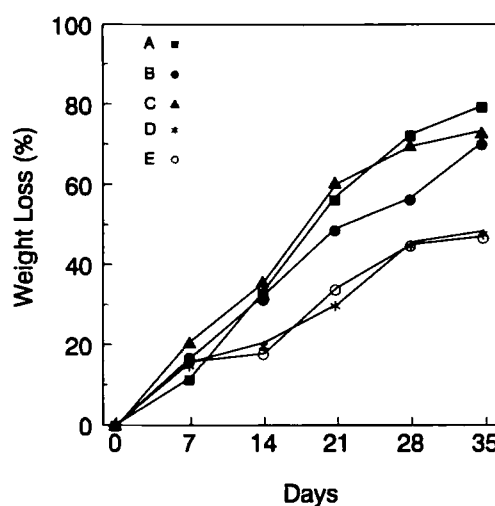


Fig. 1. Weight loss (%) in blends exposed in municipal activated sludge over a period of 35 days. PHBV (A); PHBV with 30% starch (B); PHBV with 50% starch (C); PHBV with PEO-coated 30% starch (D); PHBV with PEO-coated 50% starch (E). Each data point in the figure is an average of multiple samples having <5% margin of error.

similar rates, achieving respective total weight losses of 78, 68, and 70% in these specimens by day 35 (Fig. 1). Starch-PHBV blends in which the starch was precoated with PEO exhibited steady, but relatively slower weight losses. Total weight losses by day 35 were about 48% in composites with 30 and 50% PEO-coated starch.

During the 35-day exposure all of the specimens suffered progressive losses in mechanical properties, namely, TS and % E (Figs. 2A and B). Results indicate a trend similar to the weight loss data, exhibiting a rapid loss of 95 to 100% in TS and % E in PHBV alone or 50–75% with 30 and 50% starch by 7 days of exposure. The loss of mechanical properties was more gradual in PHBV blends containing PEO-coated starches, still retaining measurable amounts of TS and % E by the exposure time of 21 days and beyond (Figs. 2A and B).

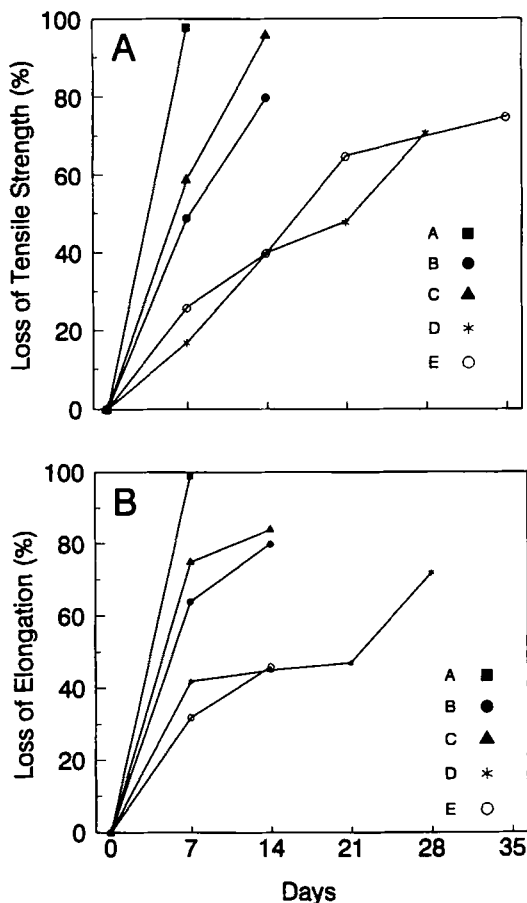


Fig. 2. Loss of tensile strength (%) at break and elongation (%) in blends exposed in municipal activated sludge over a period of 35 days. PHBV (A); PHBV with 30% starch (B); PHBV with 50% starch (C); PHBV with PEO-coated 30% starch (D); PHBV with PEO-coated 50% starch (E).

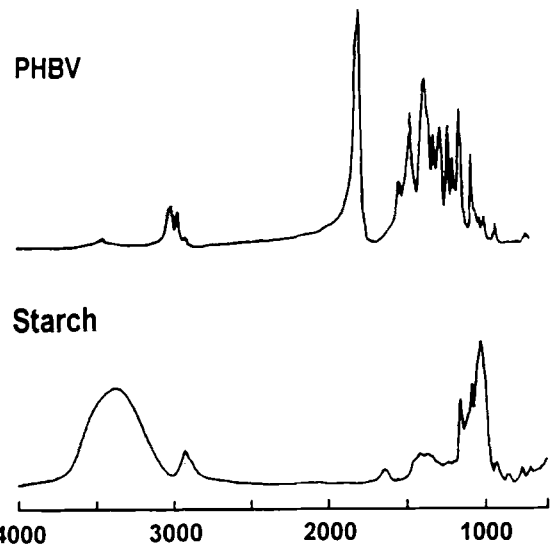


Fig. 3. FTIR spectra of pure starch and PHBV (formulation A) showing the wavenumber on the *x* axis and absorbance on the *y* axis.

The relative amounts of materials depleted from the starch-PHBV blends exposed to municipal sludge was also evaluated using Fourier transform infrared (FTIR) spectroscopy. Figure 3 shows the respective spectra of starch and PHBV alone, showing distinct absorption peaks characteristic of each material. Examples are a broad OH absorption peak centered around 3400 cm^{-1} , a characteristic set of strong C-O bands between 960 and 1190 cm^{-1} in the fingerprint region of the starch, a characteristic carbonyl peak (1725 cm^{-1}) in PHBV, and methylene absorption peaks (2980 cm^{-1}) common to both starch and PHBV. In both 30 and 50% starch-containing composites, diminution of peak absorption was greater in the hydroxyl and fingerprint region of the starch, relative to the carbonyl peak of PHBV after 35 days of exposure. The loss of starch was considerably greater in blends with a higher starch content (Fig. 4). Diminution of starch peak absorption was considerably less in blends prepared with PEO-coated starches exposed of 35 days (Fig. 5).

Starch and PHBV changes were quantified from the FTIR spectra to determine the individual weight losses of starch and PHBV in the composites after 35 days exposure (Table III). The relative losses were measured by a modification of the general FTIR absorbance ratio method [19, 20], using the ratio of the starch C-O absorbance at 1021 cm^{-1} to the PHBV methylene absorbance at 2980 cm^{-1} . This estimation of the starch/PHBV ratio was sufficiently accurate for this work since the PHBV absorbance at 1021 cm^{-1} was low compared to the starch absorbance at this frequency. Based on these

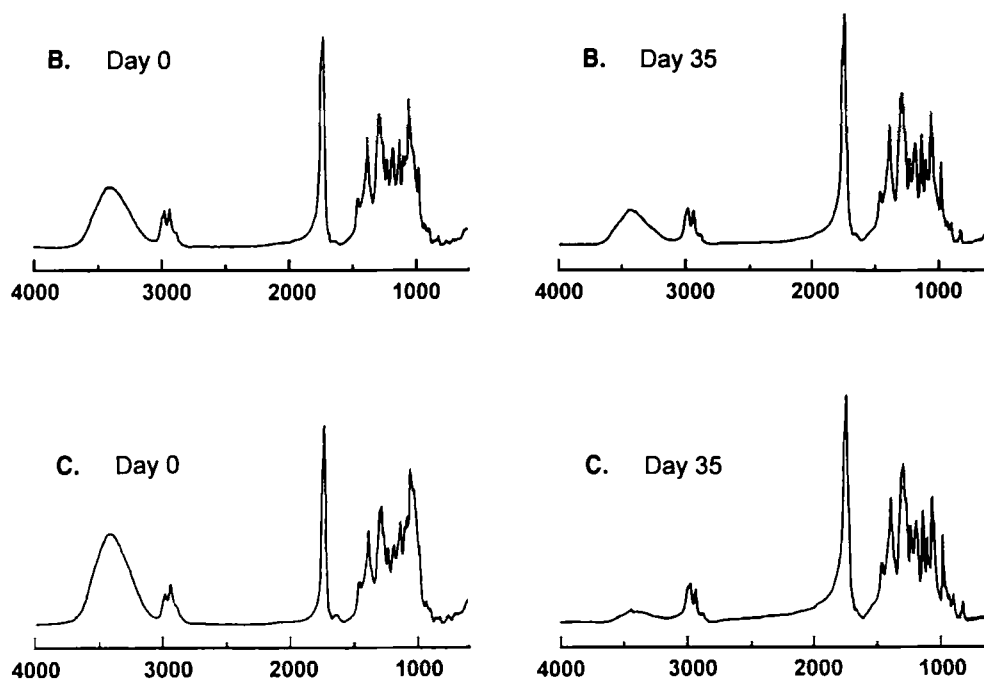


Fig. 4. FTIR spectra of formulations B and C exposed for 0 days (control) and 35 days in municipal activated sludge. x axis, wavenumber; y axis, absorbance.

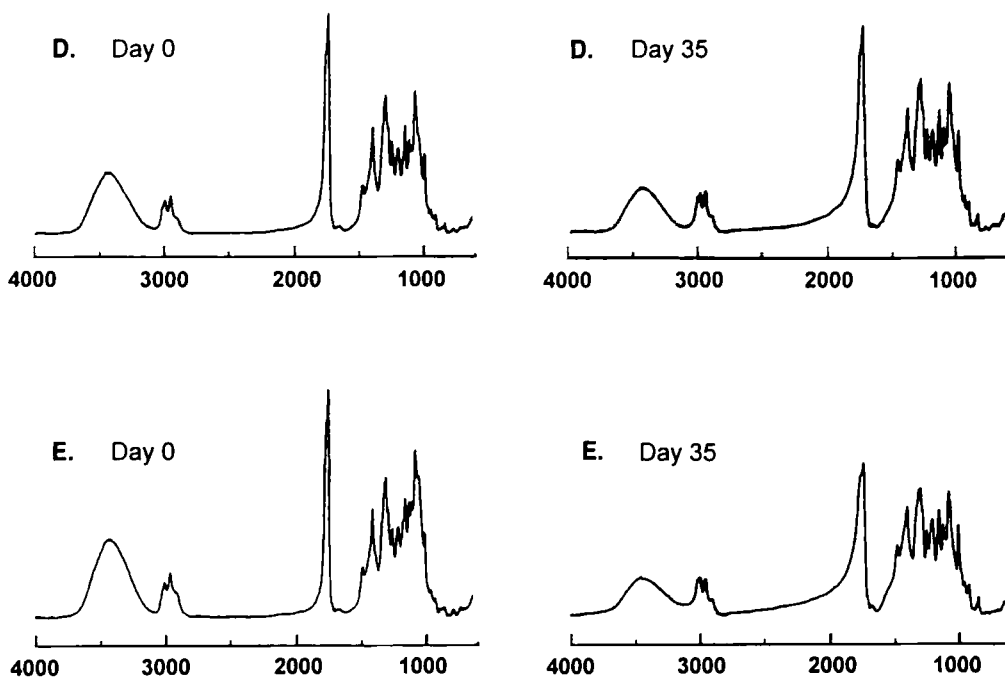


Fig. 5. FTIR spectra of formulations D and E, each exposed for 0 days (control) and 35 days in municipal activated sludge. x axis, wavenumber; y axis, absorbance.

Table III. Estimated Weight Losses of Starch and PHBV from FTIR Spectra

Sample ^a	Total wt. loss (%) ^a	Starch wt. loss (%)	PHBV wt. loss (%)
B (30%) ^b	68	79.4	63.1
C (50%)	70	89.8	50.2
D (30%) ^c	48	52.2	46.2
E (50%) ^c	59	73.0	45.0

^a 100 (1 - end weight ÷ initial weight).

^b Percentage in parentheses indicates the amount of starch present in PHBV composite.

^c PEO-coated starch.

estimates, weight losses of 79 and 63% were observed for starch and PHBV, respectively, in blends with 30% starch. Interestingly, increasing the starch content to 50% resulted in a relatively increased weight loss for starch, while the relative weight loss for PHBV decreased considerably. On the other hand, composites prepared with PEO-treated starch exhibited a comparatively reduced weight loss for starch as well as for PHBV. Again, increased starch content contributed to relatively higher weight loss of starch only with little or no change in the loss of PHBV.

Scanning electron micrographs revealed a homogeneous distribution of starch granules as a filler in PHBV blends (Figs. 6A and 6B). Upon starch degradation, surfaces of these plastic matrices become apparent with smaller and deeper holes resulting from the depletion of starch granules (Figs. 6C and D). Even after starch degradation, the surface of the starch-filled specimens appeared smooth. On the other hand, in PHBV specimens with no starch (Fig. 7A), depletion of material was visibly more uniform, producing large cavities on the surface, indicating that the enzyme was able to penetrate much deeper in these specimens (Figs. 7B and C). SEM also confirmed that the extent of starch removal was far less pronounced where PEO-coated starch was used in blends (Figs. 8A and B).

DISCUSSION

In the activated sludge environment, PHBV composites with 0%, 30%, or 50% starch consistently all showed rapid deterioration, as indicated by both the weight loss (Fig. 1) and the loss of mechanical properties (Figs. 2A and B). Despite some variability, the differences in the rates and the extent of weight losses in these blends by day 35 did not appear to be statistically

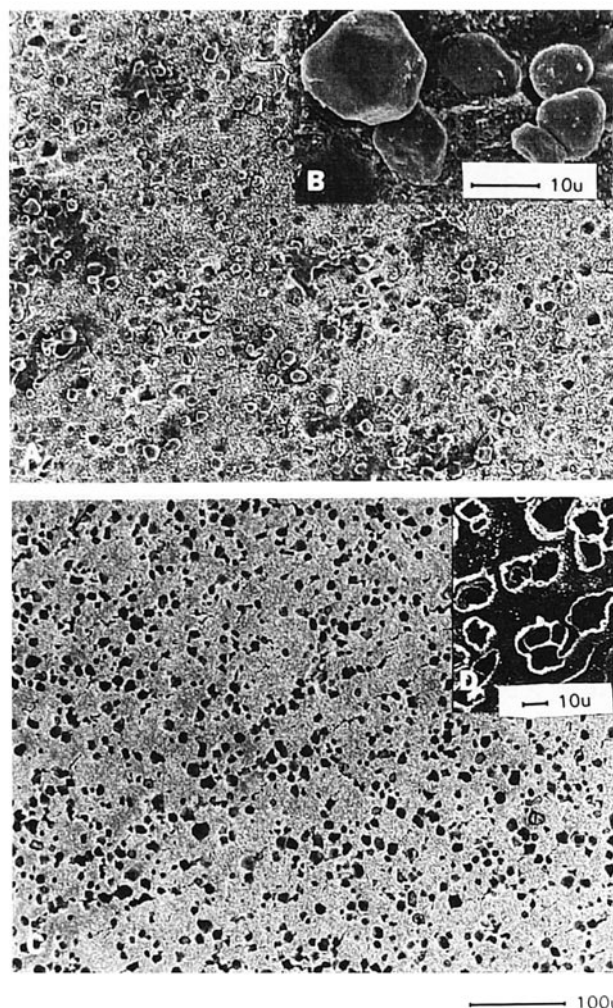


Fig. 6. Scanning electron micrograph of an unexposed PHBV blend with 30% granular starch at low (A) and high (B) magnification, and the same blend exposed for 35 days, when most of the starch has been depleted, shown at low (C) and high (D) magnification.

significantly ($P \geq 10$). These results were rather surprising since Ramsay *et al.* [12] have reported earlier that in solvent-cast and heat-compressed PHBV films, degradation was slower in pure PHBV and increased rapidly with increased starch content in the blends.

The unexpectedly rapid degradation of pure PHBV compared to PHBV with starch may be attributed to the peculiarity of this environment, since the activated sludge in the effluent pond is a highly enriched environment in terms of microbes, hydrolytic enzymes, oxygen content, and organic matter and, together with stable temperature and pH (Table II), provided a conditioned biological medium with an enhanced capability of degrading certain natural substrates very rapidly. Rapid degradation of PHBV composites with no added starch

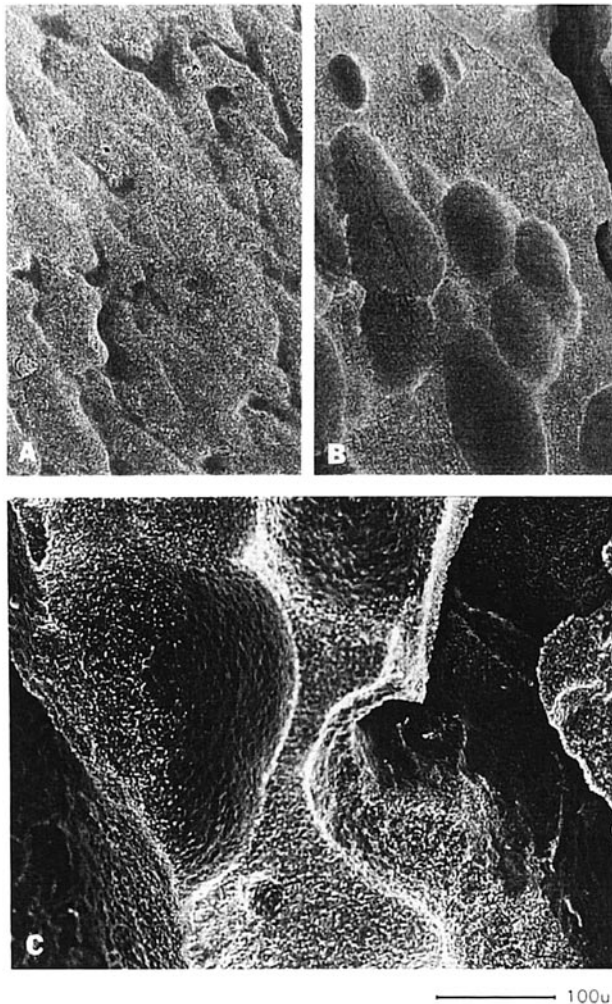


Fig. 7. Scanning electron micrograph showing a progressive degradation of PHBV blend with no added starch at day 0 (A), day 14 (B), and day 35 (C).

in activated sludge could be due to the large amount of PHA depolymerases activity present in this environment. This, however, needs to be verified. Ramsay *et al.* [12] have studied both PHA degradation and the lack of PHA degradation under anaerobic conditions and correlated it with the amount of PHA depolymerase present in these two environments. The PHA producing microorganisms are abundantly present in activated sludge, particularly PHA depolymerase producing *Alcaligenes* species [21]. Doi *et al.* [22] studied the hydrolytic and enzymatic degradation of PHA-containing films and fibers and found that enzymatic degradation of PHA depolymerase was two to three orders of magnitude faster than simple hydrolytic degradation, and most of the enzymatic degradation occurred on the surface of the film and fiber.

Depending on the methods and conditions used for processing and manufacturing the PHBV composites, the resulting materials may differ considerably with regard to PHBV morphology and degradability. Parikh *et al.* [23] found that the processing history and surface properties have a large influence on PHBV crystallinity; in particular, the surfaces of the injection-molded samples showed faster rates of degradation than the cores of the samples. Also, samples annealed at higher temperatures exhibited a significantly altered crystalline morphology, causing large changes in the interaction between enzymes and polymer. Based on SEM analyses, Nishida and Tokiwa [24] also proposed two possible mechanisms of microbial degradation of PHB. One was where degradation occurred homogeneously all over the PHB surface by diffused extracellular depolymerase which preferentially hydrolyzed the amorphous region, leaving the crystalline portion intact. The other was a nonpreferential spherical degradation of the PHB surface causing deep spherical holes on the surface resulting from colonization of hydrolyzing bacteria. Furthermore, these authors suggested that the crystalline structure of PHB influenced the physiological behavior of the degrading bacteria on the PHB surface.

After a polymer blend has been subjected to degradation, weight loss can serve as a good indicator of degradation, but not all of the weight loss in a biopolymer matrix can be attributed to the biodegradation process. The weight loss data often reflect an 8 to 15% margin of error, since it is difficult to account for the materials directly deposited on the polymer surface by microbes and macroorganisms in the vicinity, as well as the material lost due to the separation of smaller fragments or materials leaching out of the intact specimen. We have found that test specimens placed in a flask containing 250 ml of sterile distilled water alone and agitated at 180 rpm in a incubator at 30°C for 35 days lost weight ranging from 9% for PHBV blends with no starch to 16% for starch-PHBV blends (data not shown).

PEO appeared to enhance the binding of granular starch to PHBV, improving the mechanical properties of the blends [18] and causing a much slower rate of biodegradation compared to blends prepared with non-coated starches (Figs. 1, 2, and 5). These results suggest that PEO probably protected starch by providing a barrier to hydrolytic enzymes. However, PEO is also soluble in water to some extent, and whether this affects the release of granular starch from the matrix is not clear. Also, little is known about the triacetin and triacetin:PHBV ratios and what, if any, role this plasticizer plays in biodegradation of starch and PHBV in these

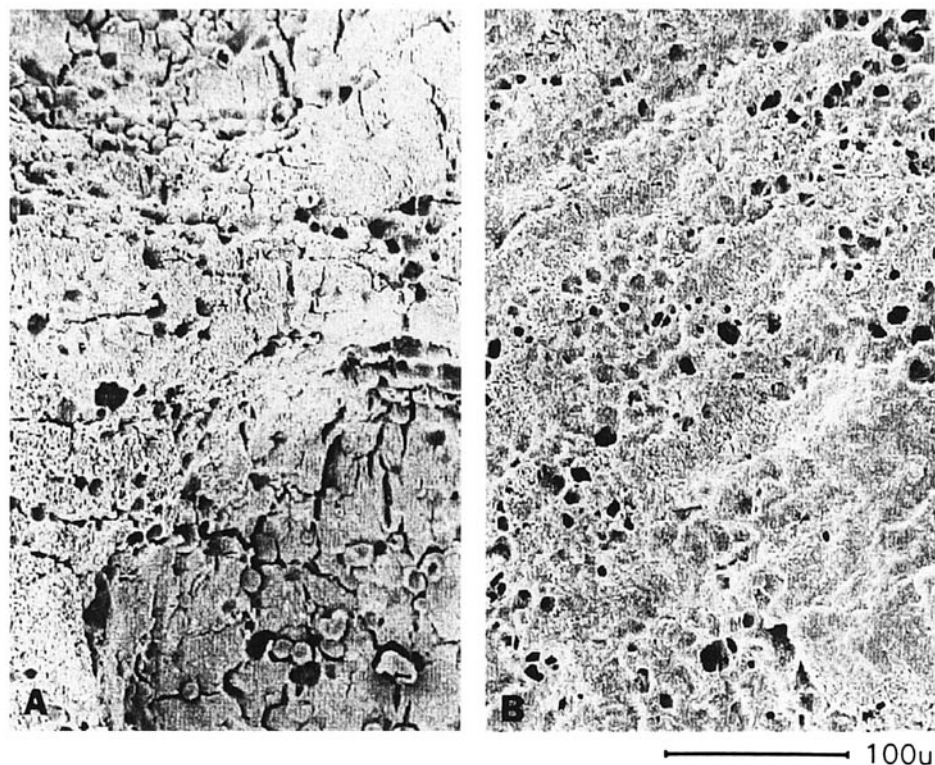


Fig. 8. Scanning electron micrograph of PHBV blends with PEO-coated 30% (D) and 50% (E) starches after 35 days of exposure.

composites. It has been reported that some of the starch forms V-type exclusion complexes with EAA, thus escaping the hydrolytic enzymes [4, 11, 25–27]. It is not known whether starch can also form exclusion complexes with triacetin, the plasticizer used in these formulations.

The FTIR spectroscopic technique does not directly measure the loss of materials as a result of biodegradation but, rather, semiquantitatively estimates the relative amounts of certain chemical species that remain in the test specimen after degradation. The FTIR spectra in Figs. 5 and 6 showed a significant reduction in absorption in the hydroxyl and fingerprint regions of starch relative to the carbonyl peak of the PHBV, suggesting rapid degradation of starch in these composites. The weight loss data indicated that PHBV from composite blends degraded rapidly, however, relative losses measured from the FTIR spectra in Table III suggested that, compared to PHBV, more starch was depleted from these blends. In addition, blends with 50% coated starch provided some protection to PHBV from hydrolytic microbes and enzymes. Furthermore, both weight loss and FTIR (Figs. 1 and 4) data indicate that some resid-

ual starch remained undegraded in the samples even after 35 days of exposure.

SEM analyses revealed that upon removal of starch granules, the matrix appeared to have large and deep holes throughout, creating a matrix with inconsistent properties, thus effectively contributing to the deterioration of the mechanical properties of the plastic blend. This would explain why blends with 50% starch showed more rapid deterioration than blends containing only 30% starch. On the other hand, as proposed by Nishida and Tokiwa [24], micrographs of PHBV blends with no starch showed significantly disrupted surface layers as well as deep spherical holes due to biodegradation of PHBV, causing thinning of the matrix and a concomitant loss of mechanical properties.

This study suggests that it is possible to blend starch and PHBV to manufacture composites of reasonable physical properties and that the municipal activated sludge is a suitable environment for rapid degradation of composite materials prepared by PHBV either alone or in combination with granular starch. Furthermore, the use of PEO-coated starches in these blends slows the deterioration process to some extent.

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