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Degradation of Starch–Poly(β-Hydroxybutyrate-Co-β-Hydroxyvalerate) Bioplastic in Tropical Coastal Waters

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Extruded bioplastic was prepared from cornstarch or poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV) or blends of cornstarch and PHBV. The blended formulations contained 30 or 50% starch in the presence or absence of polyethylene oxide (PEO), which enhances adherence of starch granules to PHBV. Degradation of these formulations was monitored for 1 year at four stations in coastal water southwest of Puerto Rico. Two stations were within a mangrove stand. The other two were offshore; one of these stations was on a shallow shoulder of a reef, and the other was at a location in deeper water. Microbial enumeration at the four stations revealed considerable flux in the populations over the course of the year. However, in general, the overall population densities were 1 order of magnitude less at the deeper-water station than at the other stations. Starch degraders were 10- to 50-fold more prevalent than PHBV degraders at all of the stations. Accordingly, degradation of the bioplastic, as determined by weight loss and deterioration of tensile properties, correlated with the amount of starch present (100% starch >50% starch > 30% starch > 100% PHBV). Incorporation of PEO into blends slightly retarded the rate of degradation. The rate of loss of starch from the 100% starch samples was about 2%/day, while the rate of loss of PHBV from the 100% PHBV samples was about 0.1%/day. Biphasic weight loss was observed for the starch-PHBV blends at all of the stations. A predictive mathematical model for loss of individual polymers from a 30% starch–70% PHBV formulation was developed and experimentally validated. The model showed that PHBV degradation was delayed 50 days until more than 80% of the starch was consumed and predicted that starch and PHBV in the blend had half-lives of 19 and 158 days, respectively. Consistent with the relatively low microbial populations, bioplastic degradation at the deeper-water station exhibited an initial lag period, after which degradation rates comparable to the degradation rates at the other stations were observed. Presumably, significant biodegradation occurred only after colonization of the plastic, a parameter that was dependent on the resident microbial populations. Therefore, it can be reasonably inferred that extended degradation lags would occur in open ocean water where microbes are sparse.

In recent years there has been growing public concern over environmental deterioration associated with the disposal of conventional plastics. For marine waters, an international accord (the MARPOL [marine pollution] Treaty) has been ratified to deal with sources of pollution, including plastics. The MARPOL Treaty is short for the MARPOL Protocol of the International Convention for the Prevention of Pollution from Ships. Annex V of the MARPOL Treaty includes a restrictive policy for disposal of plastics and garbage from ships. In order to bring Annex V into effect, the U.S. Congress passed the Marine Plastic Pollution and Control Act (Public Law 100-220), which after presidential signature became effective on 31 December 1988. This act specifically prohibits overboard disposal of plastics anywhere in the world by United States vessels or by any vessel within United States waters out to 200 miles. While the MARPOL Treaty exempts public vessels, Public Law 100-220 directs all federal agencies (including the Coast Guard and Navy) to be in compliance with Annex V by 31 December 1998, a date which includes an extension granted to the Navy.

Polyhydroxyalkonoates (PHAs) are microbial polyesters which have received considerable attention as biodegradable alternatives to conventional plastics. Copolymers of hydroxybutyrate and hydroxyvalerate, including poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV), form plastics having good mechanical qualities and have been commercially marketed. Several articles pertaining to the applications, manufacture, and properties, including biodegradability, of PHBVs have been published (7, 16–18). The primary deterrent to widespread use of PHBVs has been and remains the high cost of these plastics compared to conventional plastics, regardless of the potential benefits to the environment.

Various efforts have been made to incorporate low-value materials into PHBVs to reduce their overall cost (13, 14, 22, 24, 25, 30). Cornstarch is a particularly attractive filler for PHBV-based composites (1, 2, 5, 10, 26, 28). Starch is a bulk commodity, which makes it inexpensive and available in sufficient volume for the large-scale production common to the plastics industry. It is a renewable material that is derived from a crop grown in surplus by American farmers. Starch is highly biodegradable, although little information regarding this property in marine environments is available. Moreover, methods for blending starch with a number of polymers to produce blown film and extruded composites have been developed (9, 11, 12, 21, 25, 28). Utilizing starch as a PHBV filler, unfortunately, results in a loss of mechanical properties due to poor adhesion between starch granules and the PHBV matrix (15, 29). This limits the proportion of starch which can be incor-

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porated to 25 to 30% for practical applications. However, Shogren (25) showed that the tensile strength (TS) and percent elongation (% E) at break, common measurements of mechanical properties, are improved by coating starch with polyethylene oxide (PEO) before the starch is incorporated into PHBV-based composites; it was postulated that PEO enhances adhesive interactions.

A good general rule, often paraphrased by researchers in the field of biodegradable plastics, is as follows: if nature makes it, nature will degrade it. This rule leads to the prediction that starch-PHBV blends should be highly biodegradable since both starch and PHBV are biopolymers. However, the rule must be applied with a cautionary qualifier: while nature's materials are biodegradable, this quality may be lost upon processing. There have been few reports regarding marine degradation of either starch or PHBV, and we know of no studies that have addressed the marine performance of blends of these compounds. Furthermore, to comply with the Marine Plastic Pollution and Control Act, as well as Annex V of the MARPOL Treaty, the degradable nature of starch-PHBV plastics must be validated. Due to their high level of biological activity, tropical coastal waters were selected as the first marine environment used to assess the performance of starch-PHBV plastics, including formulations which include PEO.

MATERIALS AND METHODS

Materials. Pearl cornstarch (Buffalo 3401) was purchased from CPC International, Englewood Cliffs, N.J., and was vacuum dried at 110°C (40°C for PEO-coated starch) for 1 day before use. The high-amylose (70% amylose) cornstarch used was Amylomaize VII obtained from American Maize-Products (now Cerestar), Hammond, Ind. The PHBV used was nucleated BIOPOL resin containing 12% valerate (molecular weight, 690,000) and was obtained from Zeneca Bioproducts (now Monsanto Chemical Company, St. Louis, Mo.). PEO (molecular weight, 4×10^6) was obtained from Aldrich Chemical Company, Milwaukee, Wis.

Sample preparation. Six different formulations were prepared. These formulations included 100% PHBV and 100% starch, as well as blends containing 50 and 30% granular pearl starch and 50 and 30% PEO-coated pearl starch (9% PEO). Triacetin (10%) was added to PHBV-containing formulations to plasticize the PHBV. The 100% starch formulation was made with high-amylose starch (27) and was adjusted to a moisture content of 25% and pH 7 by adding 0.06 M NaOH.

Details concerning the extrusion procedures used for starch-PHBV (25) and high-amylose starch (27) have been described elsewhere. Briefly, the ingredients were mixed in a cake mixer and were extruded with a model PL2000 single-screw extruder (C. W. Brabender Instruments, Inc., South Hackensack, N.J.) equipped with a slit die (2.54 by 0.051 cm). Ribbons were taken off with an air-cooled belt. The high-amylose starch ribbon was annealed at 60°C and a relative humidity of 95% for 1 day to increase the crystallinity (27) and thus prevent dissolution in water.

Tensile test specimens that were approximately 10 to 12 cm long and 2.5 cm wide representing each formulation were numbered, weighed, and placed in color-coded, nylon mesh jackets. The jackets each contained six pouches (one pouch for each formulation); each pouch was about 17 by 15 cm, and the mesh size was 0.4 cm^2. The pouches were sewn shut. The jackets were then individually placed in polypropylene baskets with openings small enough to retain the jackets but large enough to allow free exchange of water.

Sampling sites. Four locations in tropical coastal waters were established as study stations. Station 1 (the mangrove interior station) was located in a series of small canals running between three mangrove islands. Plastic baskets containing samples were placed at an intermediate depth (0.5 m) between the bottom and the surface at a juncture of the interior canal system. Wave action was essentially nonexistent this far into the mangrove. The pH varied 0.6 U, and the lowest value (pH 7.5) was observed after a significant rainfall. The highest seawater temperature (32°C) was recorded at the beginning of the study (2 November 1995), and the seawater temperature gradually declined to the minimum value recorded (26°C) during the late winter.

Station 2 (the mangrove edge station) was located along a fringe of mangrove where samples were more readily exposed to the fluxes of larger mangrove lagoons. The sample baskets were placed at an intermediate depth (0.7 m). Despite its more open location compared to station 1, wave action was minimal at this station, and the seawater pH remained essentially constant at 7.9. The seawater temperatures recorded at station 2 mirrored those observed at station 1.

Station 3 (the reef shoulder station) was offshore among the coastal reefs. The sample baskets were placed 1.0 m from the bottom at a depth of 1.9 m in order to minimize physical damage to the samples due to wave action. The seawater pH was nearly constant at station 3 and higher (pH 8.1 to 8.3) than the pHs at the other stations. The seawater temperature varied from 29°C in November to 25°C in the late winter.

Station 4 (the deeper-water station) was offshore in relatively open water. The sample baskets were placed 1.0 m from the sandy bottom, which reduced the immediate physical effects of wave action and resulted in relatively constant values for the seawater pH (ca. pH 8.1) and temperature (ca. 26°C) throughout the study period.

Determinations of biodegradation. Triplicate samples (three baskets, each containing one jacket) were retrieved from each site for every time point, as indicated below. After retrieval, the baskets were taken within 2 h to the University of Puerto Rico Marine Science Station in Parguera, Puerto Rico, where the nylon jackets were removed and gently rinsed with fresh water. The rinsed jackets were dried for 1 day at room temperature and then shipped overnight to the U.S. Department of Agriculture laboratory in Peoria, Ill., where test specimens were removed from the pouches and cleaned with distilled water. After drying and weighing, the percent weight loss was calculated for each specimen.

The TS and %E of biodegraded specimens and unexposed controls were also determined. Samples were equilibrated at 23°C and 50% relative humidity for 28 days prior to testing. The tests were conducted with a model 4201 universal testing machine (Instron Corp., Canton, Mass.). A gauge length of 10 mm and a crosshead speed of 20 mm/min were employed.

To determine the losses of starch and PHBV from partially degraded matrices individually, the residual PHBV was extracted with dichloromethane. Soluble PHBV was separated from insoluble carbohydrate by this procedure. The separated contents were recovered, dried, and weighed, and the final polymer concentrations were calculated as the percentage of starch and the percentage of PHBV in each degraded specimen. Specimen weight losses due to biodegradation of starch or PHBV individually were calculated as $L_p = A_p - B_p\,(1 - L_s/100)$, where L_p is the weight percentage of the specimen lost due to degradation of polymer p (either starch or PHBV), A_p is the percent concentration of polymer p initially present in the undegraded specimen, B_p is the percent concentration of polymer p remaining in the degraded specimen, and L_s is the percent weight loss of the specimen.

Microbial counts, temperature, and pH. Water samples were obtained from each site. A sterilized 1-liter screw-cap flask was placed close to the baskets containing samples. The cap was removed, water was allowed to fill the flask, and the cap was replaced. The temperature of the water next to the baskets was then measured with a mercury thermometer after 5 min (for equilibration). Within 2 h, the flasks containing water samples were returned the laboratory, where aliquots were withdrawn and plated onto media as described below. The pH values of water samples were then determined immediately.

The microbial populations in water samples were characterized by standard spread plate methodology by using three different media. The media were incubated at 25°C. The first medium used was marine agar (Difco Laboratories, Detroit, Mich.) and was reconstituted by following the manufacturer's instructions, except that the pH was adjusted to 8.0. This medium was used to evaluate the population in general. The second medium was used to evaluate the population of starch degraders; it consisted of artificial seawater (ASW) (see below) (6) supplemented with 0.5% pearl starch as a sole carbon source and 0.1% ammonium chloride as a nitrogen source. The third medium was used to evaluate the population of PHBV degraders; it consisted of ASW supplemented with 0.5% PHBV and 0.1% ammonium chloride. Agar (Difco Laboratories) was added to the latter two media at a final concentration of 1.5%. When necessary, inocula were diluted with ASW. ASW contained (per liter of distilled H_2O) 18.8 g of $NaCl_2$, 0.4 g of KCl, 1.9 g of $MgSO_4 \cdot 7H_2O$, 1.5 g of $MgCl_2 \cdot 6H_2O$, 0.4 g of $CaCl_2 \cdot 2H_2O$, 4.9 g of HEPES, 10 ml of solution A, and 1 ml of a trace metal solution. Solution A contained (per liter of H_2O) 2 g of $K_2HPO_4 \cdot 3H_2O$, 1.0 g of Na_2CO_3, 0.4 g of sodium citrate, 0.3 g of $Fe_2(SO_4)_3$, and 0.05 g of EDTA. The trace metal solution contained (per liter of H_2O) 2.9 g of H_3BO_3, 1.8 g of $MnCl_2 \cdot 4H_2O$, 0.2 g of $ZnSO_4 \cdot 7H_2O$, 0.04 g of $Na_2MoO_4 \cdot 2H_2O$, 0.05 g of $CoSO_4 \cdot 7H_2O$, and 0.08 g of $CuSO_4 \cdot 5H_2O$. The pH of ASW was adjusted to 8.0 with NaOH.

RESULTS

Study area and stations. The location selected for this study was a tropical coastal mangrove stand on the southwest coast of Puerto Rico near the town of Parguera (Fig. 1). This mangrove community is located in an area with low human population density. The principal variables that affect its growth and stability appear to be rainfall and temperature. The prevailing ocean currents along the coast generally flow from east to west in this area. Of the four study stations established, two were within the mangrove stand. The remaining two stations were off the coast, south of the mangrove stand among the fringing coastal reefs. The data in Table 1 characterize these stations.

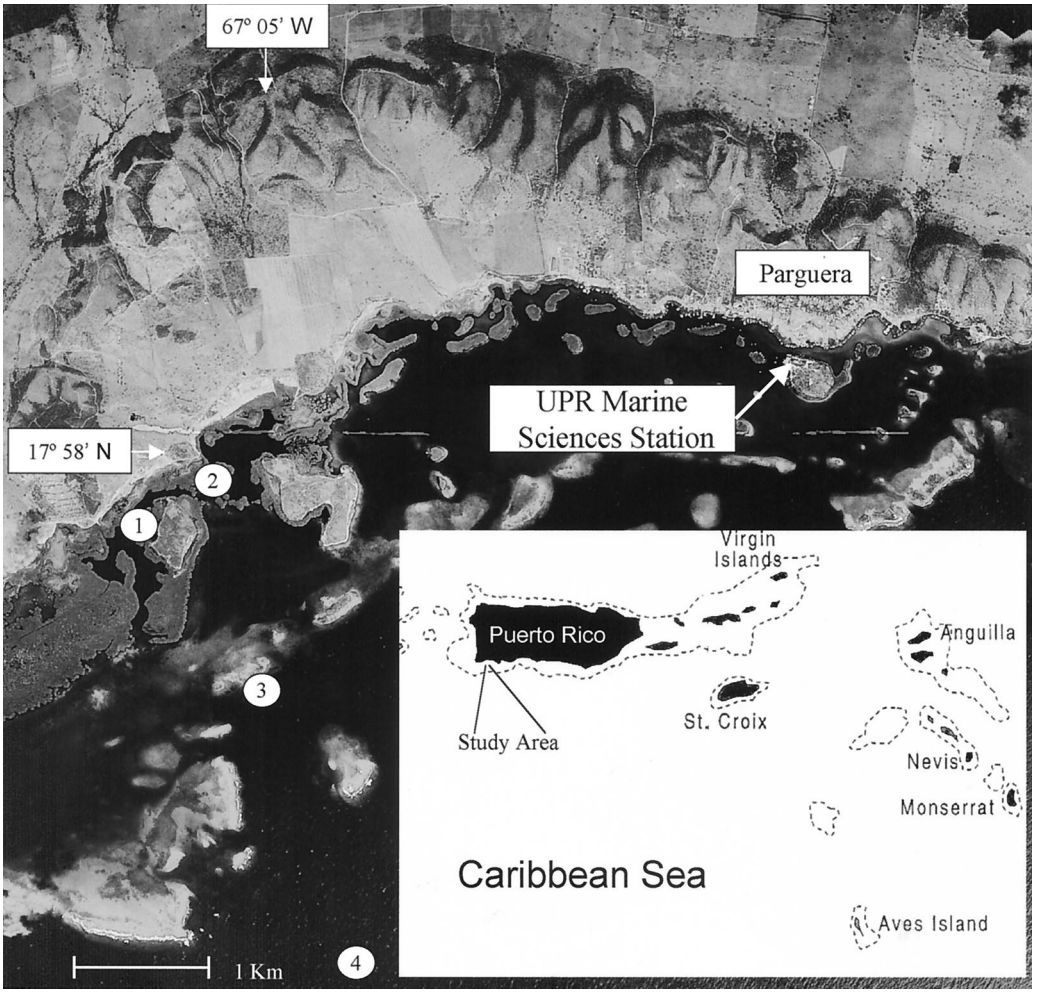

FIG. 1. Satellite photograph showing the southwest coast of Puerto Rico near the University of Puerto Rico (UPR) Marine Science Station in Parguera. The locations of the four study stations are indicated.

Microbial enumeration. Table 2 shows the mean number of CFU determined for seawater samples taken at each station, along with the standard deviations obtained over the course of the study. The standard errors of the assay were insignificant compared to the deviations in population densities observed over time. The data revealed several noteworthy points. First, as indicated by the large standard deviations, at all stations there were considerable changes in the microbial populations over time. The highest counts were obtained at zero time (data not shown), which coincided with the end of the rainy season. Second, the densities of all of the populations exhibited the following relationship: station 3 (reef shoulder) $\geq$ station 2 (mangrove edge) $\geq$ station 1 (mangrove interior) $>$ station 4 (deeper water). Third, there were numerous microbes capable of growth on starch. Microbes capable of growth on PHBV were about 1 order of magnitude less prevalent than starch growers. This difference was accentuated when clearing zones associated with individual colonies were enumerated (data not shown). Clearing zones, which were considered evidence of extensive polymer degradation, were associated with more than 50% of the colonies grown on starch plates, while only about 10% of the colonies grown on PHBV plates exhibited clearing. Well-developed starch clearing zones appeared within a few days, while the PHBV clearing zones generally appeared after 1 week of incubation. However, clearing by individual PHBV-grown colonies often resulted in large zones, which were easily enumerated by visual observation of the bottom side of a plate, as shown in Fig. 2.

Environmental performance of bioplastics. Table 3 provides data pertaining to the loss of TS and %E in samples after marine exposure. The data in Table 3 are data from station 2 but are representative of the data obtained for all stations, which exhibited only minor differences. Samples consisting of 100% starch, which had very poor physical properties to begin with, were too deteriorated by day 25 to obtain physical data. The mechanical deterioration of blends was proportional to the amount of starch present, and significant deterioration

TABLE 1. Station characterization

Station	Description	Depth/sample depth (m)	pH	Temp (°C)
1	Mangrove interior	1.0/0.5	7.5–8.1	26–32
2	Mangrove edge	1.3/0.7	7.8–8.0	26–32
3	Reef shoulder	2.9/1.9	8.1–8.3	25–29
4	Deeper water	12.1/11.1	8.0–8.2	25–27

TABLE 2. Microbial enumeration

Station	10^2 CFU/ml (mean ± SD)		
	Marine broth	Starch minimal medium	PHBV minimal medium
1	71.7 ± 44.3	52.0 ± 41.8	6.9 ± 2.4
2	140.7 ± 132.6	97.7 ± 79.8	27.1 ± 21.6
3	396.7 ± 409.1	370.7 ± 359.4	29.0 ± 7.0
4	8.7 ± 4.2	9.3 ± 5.7	0.8 ± 0.7

occurred by day 25 in all starch-containing formulations. Interestingly, at first the TS of 100% PHBV was enhanced by placement of the preparation in seawater, and significant losses of mechanical properties were observed only after 150 days of exposure to the environment. The TS and %E values for PEO-containing samples (coated starch) decreased more rapidly than the values for the corresponding uncoated formulations. These findings, however, reflected the higher initial values obtained when PEO was utilized. After marine exposure, the absolute TS and %E values for corresponding formulations were very similar, suggesting that PEO deteriorated rapidly.

The weight loss data were in agreement with the data for the loss of mechanical properties. Figure 3 shows weight loss data obtained for all six formulations exposed to the environment at the four stations. Samples composed of 100% starch degraded rapidly at all of the stations; about 2% of the weight was lost per day, and the samples were completely decomposed within 100 days. This was somewhat expected given the high numbers of starch-degrading microorganisms present (Table 2). In contrast, 100% PHBV ribbons lost weight slowly. Rates of weight loss between 0.05 and 0.12%/day were obtained for the different stations. Values between the values obtained for the 100% formulations were obtained for starch-PHBV blends, and these values positively correlated with the amount of starch present. Incorporating PEO into blends slightly retarded degradation or had little effect on degradation rates. The weight loss curves obtained for station 4 exhibited an initial lag period, which probably reflected this station's low microbial population compared to the other stations (Table 2). Interestingly, biphasic weight loss curves were obtained with starch-PHBV blends, a phenomenon observed to some degree at all stations.

We presumed that the biphasic weight loss resulted from two separate biodegradation processes in which starch and PHBV were degraded at different rates. In order to verify this presumption and then estimate biodegradation profiles and lifetimes of individual polymers, the 30:70 starch-PHBV blend from station 2 (which exhibited pronounced biphasic weight loss [Fig. 3B]) was selected for further investigation. The weight loss data was fitted to a mathematical model that assumed that the specimen weight loss process was the sum of two independent biodegradation processes, both of which could be expressed as sigmoidal exponential functions of time. The fitted curve was then deconvoluted to extract the two underlying degradation profiles (the starch and PHBV degradation profiles), as shown in Fig. 4. The mathematically extracted degradation profiles agreed closely with the experimentally determined weight loss profiles of the individual component polymers (Fig. 4). The individual polymer profiles revealed that the starch in the blend biodegraded much faster than the PHBV during the first few days of marine exposure. Significant PHBV degradation was delayed for more than 50 days until more than 80% of the starch was consumed; then measurable PHBV degradation occurred, and the rate of PHBV degradation rapidly accelerated. Accordingly, the maximum rate of starch degradation occurred during the first few days of exposure, while the maximum rate of PHBV degradation occurred 143 days later. The profiles computed predicted that the starch in the 30:70 blend would be completely degraded in 174 days, while residual PHBV would persist for 1,009 days. The model also predicted that the starch and PHBV in the blend had half-lives of 19 and 158 days, respectively.

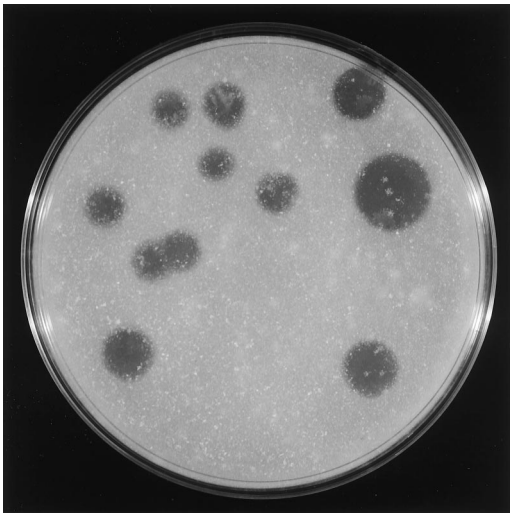

FIG. 2. Bottom side of a typical minimal medium plate used for enumeration of PHBV-degrading microbes.

DISCUSSION

Workers in various laboratories have documented that PHAs are biodegradable (7, 16–18), and PHBV is used commercially in Europe and is marketed principally on the basis of its biodegradability. The data obtained in this study substantiate the fact that PHBV can be degraded in the ocean, a finding supported by little previous data. However, the rate at which PHBV was degraded in the tested marine environments was rather slow (ca. 0.1% of the weight was lost per day). Similar slow rates have been observed in terrestrial environments (11), as well as freshwater and marine environments (19, 20). Many factors, including surface area, temperature, microbial density and composition, enzyme percolation, microbe infiltration, glucose repression of PHA esterase activity, etc., influence the rate at which PHBV is degraded. Thus, other workers (3, 19) have reported faster rates of PHBV degradation, and extremely rapid degradation (loss of 1% of the weight per day)

TABLE 3. Deterioration of physical properties after environmental exposure at station 2

Formulation	Day 25		Day 75		Day 150		Day 380	
	TS	%E	TS	%E	TS	%E	TS	%E
100% PHBV	103[a]	87	105	89	37	35	—[b]	—
30% Starch	70	56	31	39	—	—	—	—
30% PEO-coated starch	60	47	24	35	—	—	—	—
50% Starch	32	40	—	—	—	—	—	—
50% PEO-coated starch	18	26	—	—	—	—	—	—
100% Starch	—	—	—	—	—	—	—	—

[a] The data are percentages of the initial (zero-time) values.
[b] —, severely deteriorated sample for which data could not be obtained.

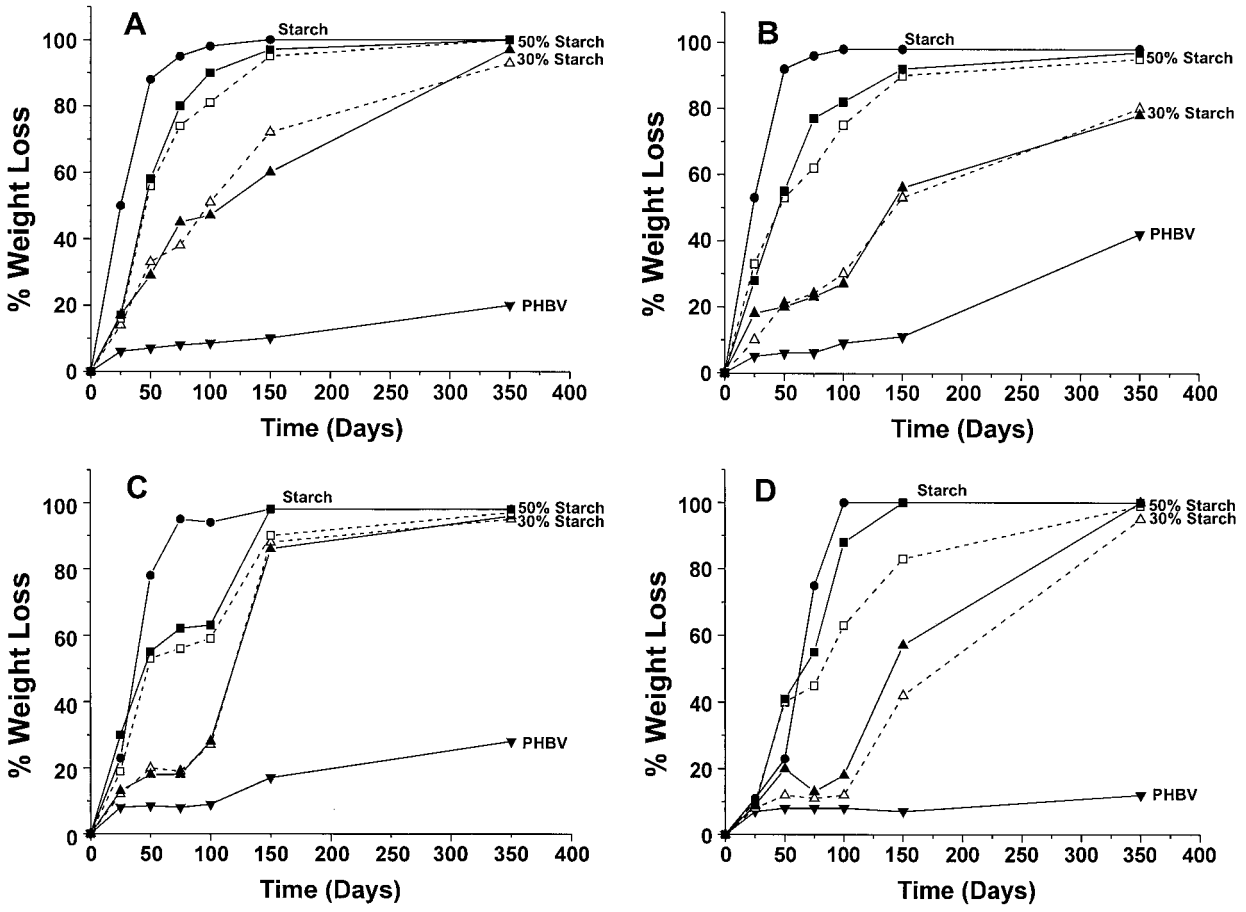

FIG. 3. Weight loss of bioplastic formulations. (A) Station 1. (B) Station 2. (C) Station 3. (D) Station 4. Symbols: ●, 100% starch; ■, 50% starch–50% PHBV; □, 50% PEO-coated starch–50% PHBV; ▲, 30% starch–70% PHBV; △, 30% PEO-coated starch–70% PHBV; ▼, 100% PHBV.

was observed in activated municipal sewage sludge by workers in our laboratory (11). Therefore, the degradation rate is affected significantly by the environment. An important marine environment, which has not been tested to date, is the sediment. It is possible that relatively rapid PHA degradation occurs in coastal marine mud, in which the microbial populations and metabolic activities are high.

Compared to PHBV, starch was degraded rapidly (ca. 2% of the weight was lost per day) at all of the marine stations studied. The starch degradation data correlated well with the microbial enumeration data (Table 2), which revealed the presence of numerous starch-degrading microorganisms. Indeed, the number of CFU observed on starch minimal medium was nearly the same as the number of CFU observed on marine agar, which is a commonly utilized standard medium for marine microbes.

As expected, blended formulations containing starch and PHBV were degraded at rates which were between the rates observed for formulations containing 100% starch and 100% PHBV. Several studies (8, 14, 24, 29) have documented that starch is degraded more rapidly than PHBV. However, biphasic degradation (Fig. 3), in which the initial degradation of starch was followed by degradation of PHBV (Fig. 4), has not been observed previously. This phenomenon can be attributed to fact that there were far more starch-degrading microbes than PHBV degraders (Table 2), as well as to the fact that more than 90% of the colonies that grew on PHBV also grew on starch, as revealed by replicate plate experiments (data not shown). It will be interesting to test the isolates for glucose repression of PHA esterase activity, which could explain the significant lag in PHBV degradation observed with the starch-PHBV blends. Wool and coworkers (4, 23) have shown that for

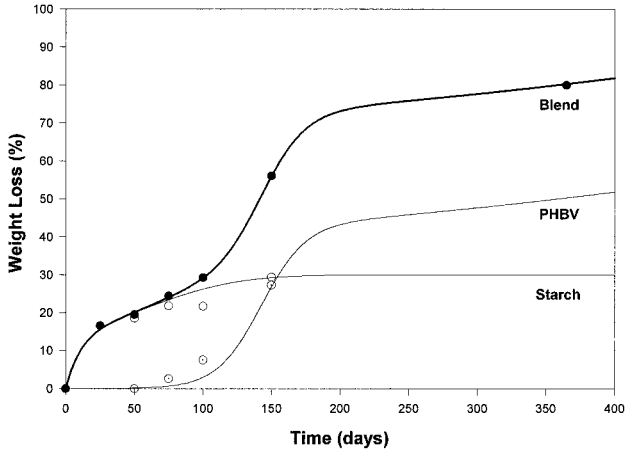

FIG. 4. Computed polymer weight loss profiles (curves) for a 30% starch–70% PHBV blend. Data are from station 2. Symbols: ●, total weight loss for test specimens; ○ and ⊙, experimental weight loss for individual starch (○) and PHBV (⊙) polymers obtained by dichloromethane extraction of test specimens, plotted after the starch and PHBV curves were determined with the mathematical model.

starch-polyethylene blends, little starch is digested by acid, by enzymes, or in soil when the starch volume fraction is less than 31%, while degradation is extensive above that level. These authors pointed out that this corresponds to the percolation threshold for a cubic lattice or, in other words, the volume fraction of cubes at which all cubes are connected by at least one face-to-face contact. Our results for the 50% starch formulations (45 volume%) are consistent with this theory. Starch-PHBV formulations containing 30% starch (27 volume%) are, however, below the threshold, yet the starch is still rapidly and completely degraded. This implies that there are mechanisms by which microorganisms and/or enzymes penetrate "PHBV walls" separating starch granules.

Shogren (25) showed that PEO improves the mechanical properties of starch-PHBV blends, probably by enhancing the adherence of granular starch to PHBV. In compost, incorporation of PEO into such blends had no effect on the biodegradation rates (8). However, in municipal sludge, incorporation of 9% PEO into starch-PHBV blends significantly slowed biodegradation (11). It was postulated that the difference in the results resulted from the extreme difference in oxygenation between the two environments. Since PEO is readily oxidized into low-molecular-weight fragments, abiotic oxidation would probably occur in oxygen-rich compost and would be unlikely in oxygen-poor sludge. The slight to nonexistent retardation of degradation observed for PEO-containing samples in the ocean (Fig. 3) lends further credence to the abiotic oxidation postulated, since marine waters are oxygenated but the availability of oxygen is not as great as it is in compost. The environmental fate of PEO and the environmental fate of triacetin (used to plasticize PHBV) have been discussed elsewhere (25). However, a series of laboratory experiments with marine cultures is under way to confirm that these compounds are completely mineralized and do not form toxic moieties during the process.

The four stations used in this study represent a continuum of at least three environments common in tropical coastal waters, mangrove, reef, and deeper water. It is interesting that the ratio of PHBV-degrading microbes to starch-degrading microbes was fairly constant, 1:10 (1:50 when clearing zone data were used), for all stations. Coincidentally, the rate at which bioplastic formulations were degraded was also fairly constant for all stations. However, the onset of degradation at station 4 (offshore, deeper water), where the microbial population was lower than the populations at the other stations, lagged. Significant degradation coincided with the formation of biofilms on the plastic (data not shown). It can be reasonably inferred that degradation occurs only after the bioplastic is colonized and that the degree of colonization is dependent upon the resident microbial populations. Therefore, significant lag periods would be expected in open ocean waters, where microbes are sparse. A reasonable strategy to reduce the lag in degradation at sea would be to colonize (inoculate) bioplastics before disposal overboard. Identification, isolation, and characterization of microorganisms suitable for this purpose is a focus in our laboratories, particularly considering that, as shown in this study, starch-PHBV plastics meet the marine degradability criterion set forth by the MARPOL Treaty and that these formulations can produce bioplastics of reasonable quality.

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