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The fate of 'biodegradable' plastics in municipal leaf compost

David F. Gilmore[a], S. Antoun[b,*], Robert W. Lenz[b], Steve Goodwin[c], Richard Austin[d] and R. Clinton Fuller[a]

[a] *Department of Biochemistry,* [b] *Department of Polymer Science and Engineering and* [c] *Department of Microbiology, University of Massachusetts, Amherst, MA, USA and* [d] *Exxon Chemical Co., Baytown Polymers Center, Baytown, TX, USA*

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SUMMARY

Blends of starch with polypropylene, starch with polyethylene, polycaprolactone with polyethylene, and a copolymer of β-hydroxybutyrate and β-hydroxyvalerate (PHB/V) were exposed to degrading leaves in a municipal leaf composting operation. Every month for 6 months, duplicate samples were analyzed for changes in weight and tensile properties, and many of these samples were further analyzed for changes in molecular weight and surface morphology. All results were compared to controls which were incubated for 6 months in moist, sterile leaves at a leaf compost temperature. Very little change was noted for any of the polyolefin blends over the 6-month period. In contrast, PHB/V samples showed massive deterioration with substantial weight loss. Although there was a decrease in molecular weight and a loss of tensile properties in leaf-exposed PHB/V films, the sterile control films also showed similar changes, but without weight loss. Of the microbial isolates from film surfaces, only fungi possessed PHB/V depolymerase activity.

INTRODUCTION

In response to an increasing shortage of landfill space and concerns about environmental quality, producers of plastic packaging have engaged in active marketing of products touted to be 'degradable' or 'biodegradable'. Most of these products are either starch blended with linear low density polyethylene, with or without pro-oxidant additives, or polyethylene containing ultraviolet light-absorbing substituents designed to initiate photo-oxidation of the polymer (photo-degradables). Many of the studies carried out to demonstrate ultimate biodegradability of these products point to a consumption of the starch portion of blends by microbes or a lowering of molecular weights of the polyethylene through photo- or pro-oxidant action, and conclude that the polymer chains will ultimately become small enough that microorganisms can consume them [4,5,15]. However, there is no evidence that biodegradation of intact polyolefins occurs, and early formulations of starch/polyolefin leaf compost collection bags did not deteriorate effectively [10]. The environmental organization Greenpeace has summarized data on this lack of biodegradability and produced a report for the educated consumer [19]. Lack of a clear definition of biodegradability has hindered communication in this field. We consider a polymer to be biodegradable if it can be broken down, by biological activity, into subunits which can be metabolized to CO_2 and H_2O. For polymers of biological origin, weight loss is presumptive evidence of biodegradation.

As part of our studies on the synthesis and biodegradation of the poly(β-hydroxyalkanoates) (PHA), a family of microbially produced polyesters [5], we initiated a comparative study of the biodegradability of what have been popularly called 'biodegradable plastics'. We tested blends of starch with polypropylene and with polyethylene, neither containing added pro-oxidants; a blend of polycaprolactone (PCL; a synthetic, biodegradable polyester; [17]) with polyethylene; and poly(β-hydroxybutyrate-co-hydroxyvalerate; PHB/V), a PHA. Samples of each were exposed to decomposing leaves at a municipal leaf composting operation.

MATERIALS AND METHODS

Polyolefins were blended and supplied by Exxon Chemical Co., Baytown, TX. Polypropylene (PP) blended with 6% corn starch, had a molecular weight (MW) of 300000 and was received as pellets. Linear low density polyethylene (LLDPE; MW 450000) blended with 12% corn starch was received as plastic sheets. LLDPE con-

Correspondence to: R.C. Fuller, Department of Biochemistry, University of Massachusetts, Amherst, MA 01003, USA.
* Permanent address: Department of Chemistry, Faculty of Science, University of Cairo, Giza, Egypt.

tained 500 ppm each of Irganox 1076 and Irganox 168 for thermal stability. About 9% (w/w) butene was present as copolymer with 2.4 ethyl side chains per 100 carbon atoms. A masterbatch consisting of polyethylene and 43% (w/w) silane-treated corn starch was obtained from St. Lawrence Starch, Mississauga, Ontario, and further blended with polyolefins. A blend of 30% polycaprolactone (PCL), MW 36000, was obtained from Union Carbide, Danbury, CT, and blended with LLDPE. Poly(β-hydroxybutyrate-co-hydroxyvalerate) (PHB/V), lot P05, was received as a technical grade powder from ICI, Billingham, UK. Prior to use, this material was purified by two cycles of dissolution in room temperature chloroform, filtration, and reprecipitation into methanol. The resulting material had a MW of 330000 and a β-hydroxyvalerate content of 26.5 mol% as determined by gas chromatography [4].

Standard dogbone-shaped films, 8.5 cm long, 0.5 cm wide at the neck, and 0.5 mm thick, were prepared by compression molding in stainless steel templates. Polymers were heated to 10 degrees above their T_m, subjected to 6000 psi, and cooled to room temperature with moderate air circulation. Holes were melted about 0.5 cm from both ends of each dogbone using a heated awl. Sample sticks (Fig. 1) were fashioned from 1.2 m long wooden dowels. Samples were then attached in duplicate, by means of 1/4″ No. 4 brass screws, at positions 15 and 23 cm from the pointed end of each of the seven sample sticks.

During the autumn months, leaves collected in and around the city of Springfield, MA were piled into windrows, long mounds measuring about 2 m high, 3 m wide, and 30 m long. Sample sticks with attached plastic samples (Fig. 1) were inserted about 0.6 m into the windrows about 1 m up from the base of the pile. Approximately every 2 weeks, the sticks were removed and the windrows were 'turned' by machine (Scarab leaf compost turner, Model 18, Scarab Manufacturing, White Deer, TX), a process which mixed and shredded the leaves. This turning operation is crucial as it prevents compaction and subsequent anaerobiosis, controls moisture content, and increases both the surface area and the homogeneity of the decomposing material (Department of Environmental Protection, Commonwealth of Massachusetts, unpublished data). Immediately after the sample windrow had been turned, the sample sticks were replaced as near to their original location as possible.

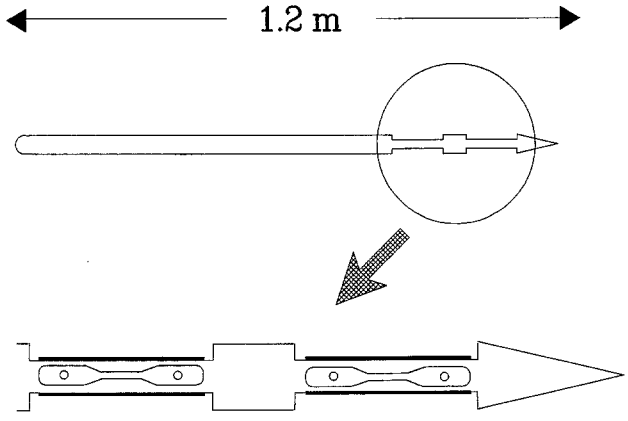

Fig. 1. Schematic of sample sticks showing attachment of dogbone-shaped polymer samples with screws to a 1.2 m long wooden dowel.

Once each month for 6 months, one sample stick was returned to the lab and the polymer samples were analyzed; thus, each time point was represented by a different, duplicate set of samples, not continually re-exposed samples. A re-exposure protocol was also followed in which one particular sample stick was periodically removed, the samples were cleaned, weighed and reattached, and the stick was returned to the compost.

Sterile control samples were prepared by submerging duplicate polymer samples in boiling 1% sodium dodecyl sulfate for 1 min, rinsing them with 10 ml sterile distilled water, and burying them in bags of wet leaves (approx. 0.6 kg/bag) which had been autoclaved for 17 h. These bagged samples were kept sealed at 55 °C for 6 months in polyethylene trash bags (double-bagged), then assayed for degradation by the techniques described below. Unexposed control samples kept dry and at room temperature for 6 months were similarly assayed.

Polymer films to be assayed were freed of excess debris and soaked in a rapidly stirred solution of 1% Sparkleen detergent (Fisher Scientific) for 30 min. Films were then gently scrubbed with a paint brush under distilled water to remove traces of soil, blotted dry, and incubated in a vacuum oven at 50 °C for 3 days.

An Instron tensile testing machine was used for the determination of tensile properties. The gage length for all dogbone samples was 2.0 cm. The crosshead speed for the starch/PP blend and for PHB/V was 0.5 cm/min, with a strain rate of 0.25 min^{-1}. The crosshead speed and strain rate for the LLDPE blends were 10 cm min^{-1} and 5 min^{-1}, respectively. These crosshead speeds were chosen because they produced the optimum sensitivity for the samples being studied. The thickness of all samples was determined with a micrometer before performing tensile tests.

Gel permeation chromatography (GPC) was used for the determination of molecular weights of samples exposed to leaf compost. Molecular weights of polyolefin-containing samples were determined at Exxon Chemical Co. Molecular weights of the PHB/V samples were determined using a Waters Model 6000A solvent delivery system with a Model 401 refractive index detector and 2 Ultrastyragel columns in series. The flow rate was 1.0 ml min^{-1} of chloroform, and sample concentrations of 15 mg ml^{-1} and injection volumes of 50 μl were used. Polysty-

rene standards obtained from Polysciences, Warrington, PA, were used to calibrate the instrument, and values more closely approximating true molecular weights were obtained by correcting results using the Mark-Houwinck constants for PHB of $a = 0.78$ and $k = 1.18 \times 10^{-3}$ ml/g [18].

Scanning electron microscopy (SEM) was used for analysis of sample morphology and colonization. For the examination of polymer surfaces, samples were washed free of debris as described above and sputter coated with a 200 Å coating of pure gold. For viewing cells attached to polymer surfaces, samples were gently washed with distilled water, dried, and gold coated.

Media used for manipulation of leaf compost microbes were as follows: Basal medium consisted of 0.9 g NaCl, 1.0 g NH_4Cl, 0.1 g Na_2SO_4, 0.2 g $MgCl_2 \cdot 6H_2O$, 0.05 g $CaCl_2 \cdot 2H_2O$, and 10 ml of trace minerals solution [13], plus 0.3 g KH_2PO_4, 0.56 g $Na_2HPO_4 \cdot 7H_2O$, and 20 ml of vitamin solution [21], sterilized and added separately, per 1 liter of medium. Three rich media employed were Trypticase Soy agar (TSA), Saboraud Dextrose agar (SA), and Malt Extract-Yeast Extract agar (MEYEA). PHB/V used in overlay plates was ground with a mortar and pestle, suspended in basal medium, and sterilized by heating it at 80 °C for 1 to 2 h.

Microorganisms attached to PHB/V films which had been exposed to leaf compost for 1–3 months were soaked in basal medium, then scraped with a sterile glass rod, and the suspension was streaked onto TSA, SA, and MEYEA plates. These initial cultures were incubated at 30, 45, and 60 °C, then refrigerated when growth was apparent.

Microbes capable of depolymerizing PHB/V were detected by a modified clear zone method [9]. Colonies from the initial plates were suspended in melted basal medium agar (50 °C) containing powdered, sterilized PHB/V (0.1%) as sole carbon source, and these suspensions were quickly poured over a basal medium agar bottom layer. These overlay plates were incubated at the initial growth temperatures of the colonies and periodically checked for clearing of the polymer. Colonies appearing responsible for polymer degradation were isolated on rich medium, and their PHB/V degrading abilities were confirmed by the production of clear zones upon transfer back to PHB/V overlay plates.

RESULTS

The temperature of the leaf compost windrow remained near 40 °C for 3 weeks because of low moisture content in the leaves and unseasonably cold ambient air temperatures (−10 °C initially). When the ambient air temperature rose above freezing, water was mixed into the windrow, and microbial metabolic activity raised the compost temperature to 63 °C. Thereafter, the compost temperature ranged from 42 to 52 °C.

Biodegradation of starch/polyolefin blends

Weight loss by starch/polyolefin samples was minimal, not greater than 1 mg for starch/PP or 6 mg for starch/LLDPE samples (Table 1). Assuming no loss of the polyolefin, a maximum loss of 16% of the total starch in a starch/LLDPE sample was observed. There was no correlation between weight loss and time of exposure. Samples of neither starch/polyolefin blend showed significant microbial colonization upon examination by SEM, although partial degradation of some starch granules was visible on the surfaces of starch/LLDPE samples (data not shown).

None of the values obtained from measuring the three tensile properties of the starch/polyolefin samples (elongation to break, modulus, and tensile strength) differed significantly from values obtained from unexposed control samples (Table 2). Molecular weight determinations of the polyolefin portions of the samples revealed no measureable changes. These data indicate that no significant degradation of the starch/polyolefin samples occurred during the 6 months exposure to leaf compost.

Biodegradation of PCL/LLDPE

Weight loss of PCL/LLDPE samples did not correlate well with time of exposure. The greatest weight loss was

TABLE 1

Weight loss of samples during exposure

Days exposed	St/PP		St/LLDPE		PCL/LLDPE		PHB/V	
	mg	%	mg	%	mg	%	mg	%
34	1	0.3	5	1.6	4	1.3	18	3.9
	1	0.3	5	1.6	4	1.3	16	3.6
68	1	0.4	5	1.6	7	2.2	25	5.3
	1	0.4	6	1.8	7	2.2	61	14.2
98	1	0.4	2	0.6	4	1.2	76	17.2
	nd	nd	nd	nd	nd	nd	nd	nd
126	1	0.3	1	0.3	11	3.4	46	10.3
	0	0.0	1	0.3	12	4.0	99	20.7
154	1	0.4	5	1.6	4	1.2	50	11.4
	1	0.3	5	1.5	6	1.9	93	19.8
186	1	0.3	2	0.6	7	2.3	nd	nd
	1	0.3	2	0.7	8	2.6	265	58.8
Sterile	0	0	0	0	0	0	1	0.2
Controls	0	0	0	0	0	0	2	0.4

%, weight loss as percentage of original weight of sample. nd, not determined; samples were used for SEM analysis and were not available for weight loss determination. Sterile controls were exposed to sterile leaves for 186 days.

TABLE 2

Changes in tensile properties of starch/polyolefin blends during exposure to leaf compost

Days of exposure	% Elongation to break		Modulus (MPa)		Strength (MPa)	
	St/PP	St/PE	St/PP	St/PE	St/PP	St/PE
0	5.48[a]	730[b]	739[b]	181[b]	25.5[a]	14.7[b]
34	5.00	630	989	214	29.0	17.4
68	7.29	694	546	152	27.4	12.8
98	5.46	633	614	137	25.0	19.8
126	6.62	665[c]	715	133[c]	28.5	nd[d]
154	6.54	666	666	156	29.4	13.0
186	5.92	683	722	240	26.9	13.2
SC[e]	5.83[c]	723	654[c]	152	27.9[c]	14.0

All values are the average of determinations of duplicate samples except as indicated: [a] = average of 4; [b] = average of 3; [c] = single sample; [d] nd = not determined; [e] SC = sterile controls, exposed to sterile leaves for 186 days.

observed in samples exposed 126 days (Table 1); these lost 3.4 and 4% of their initial weights. If we assume no loss of LLDPE, then not more than 13% of the total PCL was removed from PCL/LLDPE blend samples. PCL forms an immiscible blend with LLDPE, with spheres of PCL encased in a matrix of LLDPE (data not shown) and blebs of PCL extruded to the surface during compression molding (Fig. 2a). Upon exposure to leaf compost, these blebs were largely removed (data not shown), presumably by the microbes embedded in the biofilm which covered large portions of PCL/LLDPE samples (Fig. 2b).

The PCL/LLDPE samples had poor tensile properties, probably as a consequence of the immiscibility of the polymers. Because of tear failure, we were unable to obtain reliable tensile measurements.

Changes in the molecular weight of PCL during exposure to leaf compost were observed but could not be quantified because of the significant overlap in the traces of PCL and LLDPE (Fig. 3). After 2-months exposure, a lower molecular weight shoulder, corresponding to PCL, emerged from the LLDPE peak. After 6 months of exposure (Fig. 3), a significant amount of PCL had been degraded to lower molecular weight fragments but without further weight loss. Because a small shift to lower molecular weight fragments occurred in sterile control samples also (data not shown), it appears that at least a portion of the degradation of PCL was a result of abiotic hydrolysis.

Biodegradation of PHB/V

Samples of PHB/V exposed to decaying leaves for only 1 month showed visible colonization by fungi and significant loss of weight. Weight loss generally increased with time of exposure (Table 1), and samples had begun to disintegrate after 6 months. Sterile control samples, however, lost less than 0.5% of their initial weights.

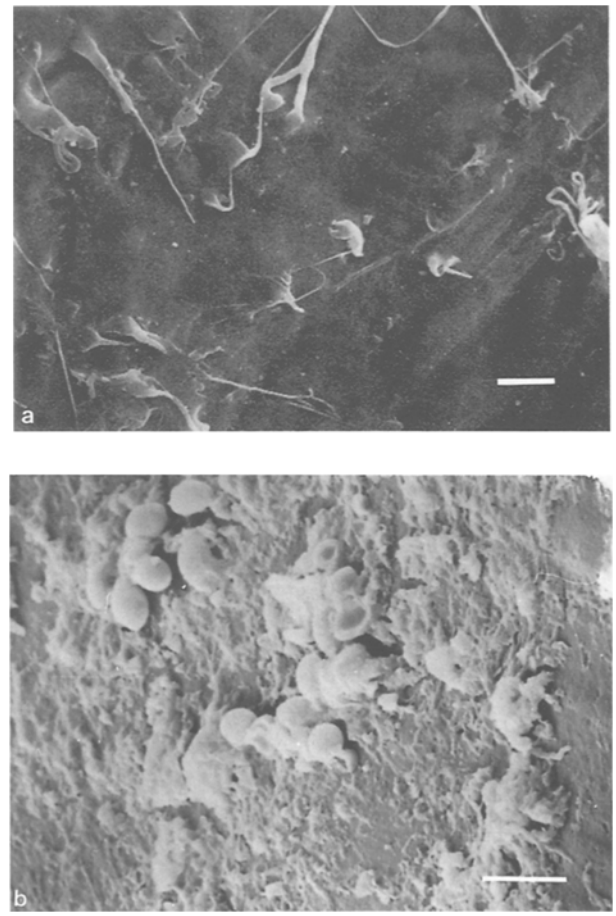

Fig. 2. SEM of PCL/LLDPE samples. (a) Samples after 68 days of exposure, thoroughly washed, and (b) after 98 days of exposure, gently rinsed. Bar = 10 μm.

Prior to exposure, the surfaces of PHB/V films were flat and featureless when viewed by SEM. After 2 months of exposure, thoroughly washed films showed extensive grooves and pits (Fig. 4a). Examination of films exposed for 3 months and lightly washed revealed colonization by fungi and bacteria (Fig. 4b), the probable cause of such pits.

Tensile properties of PHB/V films changed rapidly. Extensibility decreased by nearly one half after only 1 month of exposure, and both extensibility and strength decreased to zero after 4 months (Fig. 5). These changes cannot be solely attributed to biodegradation, however, as sterile control samples become very brittle over the 6-month period and had no measureable extensibility or strength.

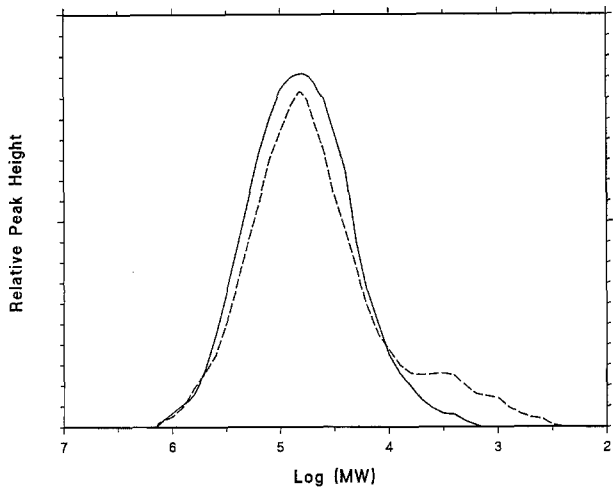

Fig. 3. GPC traces of PCL/LLDPE. Solid line, unexposed control sample; dotted line, sample exposed for 186 days.

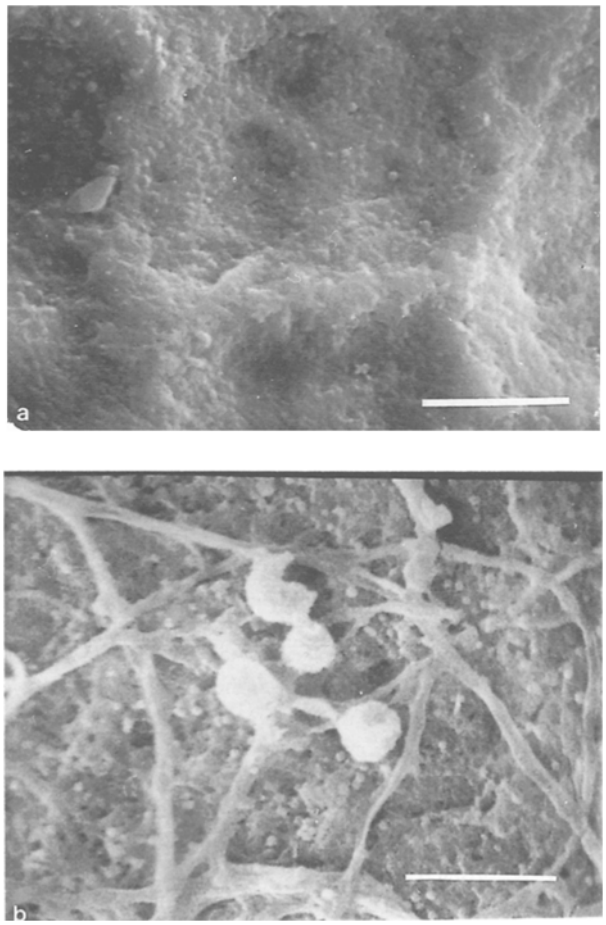

Fig. 4. SEM of PHB/V samples. (a) Surface of sample exposed for 68 days, viewed after thorough washing, and (b) colonized surface of sample exposed for 98 days; washing to remove some biofilm was necessary in order to visualize individual organisms. Bar = 10 μm.

Molecular weights of PHB/V decreased steadily during the first 4 months of exposure to leaf compost without significant change in polydispersity (Fig. 6). The molecular weight of sterile PHB/V incubated aseptically also showed a substantial decrease, suggesting that much of the molecular weight decrease observed in samples exposed to compost resulted from abiotic hydrolysis. This decrease in molecular weight was correlated to the loss of tensile strength ($r = 0.98$) (Fig. 7).

Effect of exposure protocol

Individual samples were exposed to compost for periods of time then removed from the study rather than re-exposing a few samples several times during the study. This was done to avoid the possibility of delays in biodegradation that might have occurred during reestablishment of biofilms on the surfaces of washed samples. Such a possibility was investigated by following a re-exposure protocol with one set of samples. The weight loss per

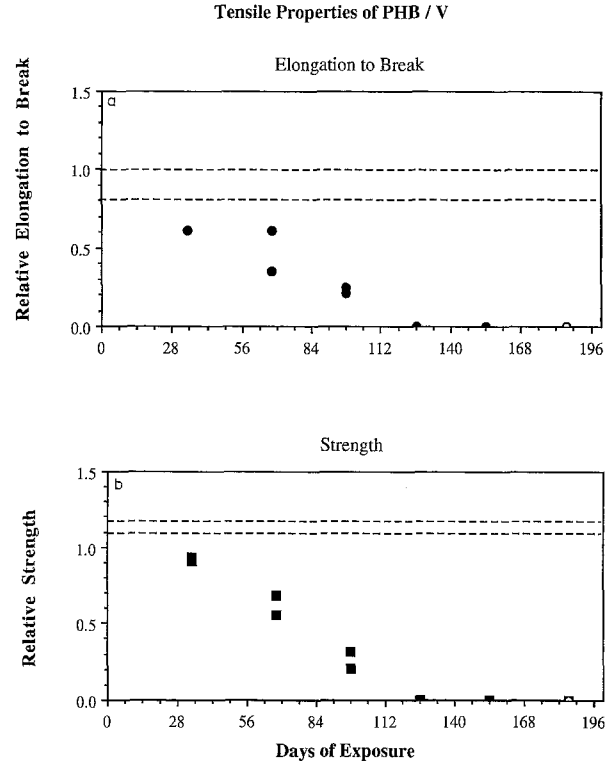

Fig. 5. Changes in tensile properties of PHB/V with time of exposure. All data are expressed relative to the average of the unexposed control values for that assay. Standard deviations of unexposed control values are plotted as dashed lines across each graph. Closed symbols, values for individual exposed films; open symbols, values for sterile control samples (incubated 186 days). (a) Percent elongation to break, control values = 12.2 ± 1.9% (b) Strength, control values = 28.4 ± 1.0 MPa.

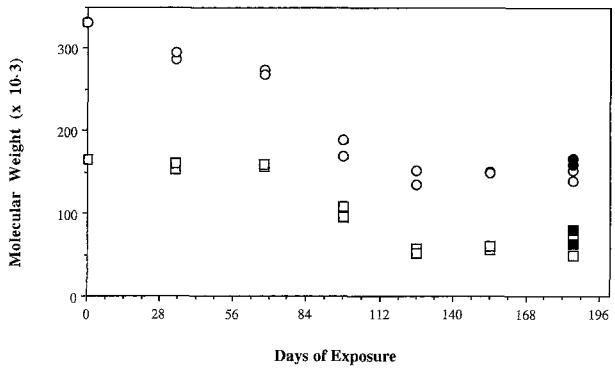

Fig. 6. Change in weight average (MW) and number average (MN) of PHB/V samples with time of exposure. Circles, MW; squares, MN. Open symbols, exposed samples; closed symbols, sterile control samples.

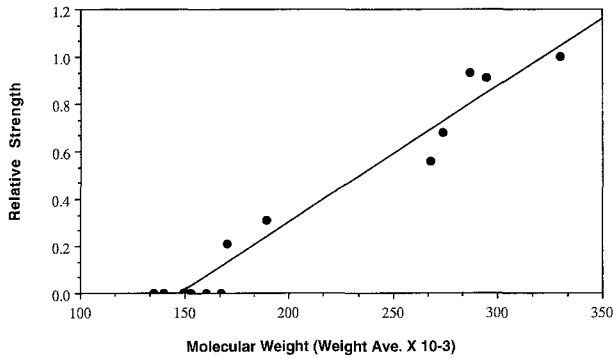

Fig. 7. Relationship between strength and molecular weight (MW) of PHB/V samples.

month of re-exposed PHB/V and PCL/LLDPE samples over the first 4 months was always less than the calculated weight loss per month of samples that had not been removed and re-exposed. Thus, re-exposure of washed samples would have caused an underestimation of the rate of biodegradation.

Microbial isolates

From heterotrophic enrichment plates, 221 independent isolates were obtained of which 193 were bacterial species and 28 were fungal species. These isolates were screened for the ability to depolymerize PHB/V using overlay plates. The screening provided 22 confirmed depolymerizing isolates, all fungi, isolated at 45 or 37 °C. During the first screening on PHB/V overlays, several depolymerizing cultures were found to contain both bacterial and fungal species. Each of these cultures was eventually separated into a pure bacterial culture and a pure fungal culture. In each case only the fungal culture produced PHB/V depolymerase. However, the mixed cultures appeared to clear the PHB/V overlays more rapidly than the fungal isolate alone. This apparent syntrophy is the subject of continuing research. During the 6 months of the composting process, the bacterial flora consisted of a wide range of bacterial genera including *Pseudomonas*, *Bacillus*, and *Klebsiella*. However, no bacterial isolates capable of depolymerizing PHB/V were found using the isolation and enrichment procedures of this study.

DISCUSSION

To our knowledge, this is the first study to compare directly and quantitatively the biodegradability of starch/polyolefin blends and microbially synthesized plastics. Because only duplicate, thick samples (0.5 mm) were tested, the possibility that slow degradation of the polyolefin samples occurred cannot be eliminated. However, this study was designed to measure significant, easily detectable degradation, and duplicate samples were sufficient to show such degradation of PHB/V samples and the lack of such degradation of polyolefin blends. The thickness of the samples prevented physical abrasion from being an important factor, focusing attention on *bio*degradation.

Because the starch/polyolefin samples were without pro-oxidant additives, any changes in tensile properties of the samples or in molecular weights of the polyolefins resulted solely from the microbial removal of starch and any subsequent enhancement of polyolefin biodegradation. Our results are in agreement with those of Austin [2], who used a mixed microbial culture to study degradation of 6% starch/LLDPE films, and Ianotti et al. [12], who tested 3, 6, and 9% starch/LLDPE blends in a controlled soil environment. Neither found significant changes in tensile properties of samples nor in the molecular weights of the LLDPE portion, nor removal of more than 30% of the starch. In blends containing less than 40% starch, most of the starch is encased in LLDPE and not likely accessible to microbial enzymes (Wool et al. [22]).

Regardless of the extent of starch removal, degradation of LLDPE would not occur, as polyethylene must be extensively oxidized and reduced in molecular weight before biodegradation can be detected [1]. Leaf collection bags currently marketed contain enough oxidant to promote rapid disintegration [11], but the potential toxicity and biodegradability of these breakdown products needs further study.

Polycaprolactone, a synthetic polyester, is biodegradable when placed in soil [17]. Fungi and some bacteria capable of PCL degradation have been isolated by enrichment culture [3]. When PCL is blended with a non-degradable material such as LLDPE, however, it becomes inaccessible to microbial enzymes [17]. In our study, no more than 13% of the PCL was removed from samples.

This amount of weight loss is consistent with the removal of the PCL blebs from the surfaces of samples by leaf compost microorganisms.

PHAs are inherently biodegradable, being bacterial storage polymers. PHB is hydrolyzable by microbes other than those producing the polymer [6]. The biodegradation in soil of solution-cast and compression-molded films of PHB, 0.2 to 0.3 mm thick, has been studied [11]. No changes in molecular weight were noted, but the films lost appreciable weight and had disintegrated by 52 weeks. Fungi were reported to be mainly responsible for the attack. Sufficient radiolabeled CO_2 was recovered to account for the sample weight loss, demonstrating that the ultimate fate of the polymer was total metabolism. Similar published data are not available for PHB/V, but ICI claims a 50% loss of carbon recovered as $^{14}CO_2$ after 16 weeks exposure to moist soil (ICI Americas Inc., 1988, product brochure). Kunioka et al. [14] exposed a 50:50 PHB/V copolymer to laboratory-maintained soil and sludge, and reported qualitative evidence of degradation.

Our conclusion that PHB/V samples exposed to leaf compost lost weight as a result of biodegradation is supported by several observations. Throughout most of the study, samples were intact and coated with biofilm, ruling out a physical abrasion mechanism. Fungi were observed on film surfaces, and several were isolated and had PHB/V depolymerase activity. Samples incubated in sterile leaves did not lose weight, demonstrating that biological activity was required for weight loss to occur.

The large weight loss observed between 5–6 months of exposure may have resulted from an increase in the activity of anaerobic microbes, as the windrows were infrequently aerated at this time. Degradation of PHB under anaerobic conditions has been reported [20].

We report a clear correlation between decreases in tensile properties and in molecular weight. Such decreases also occurred in PHB/V samples incubated for 6 months at 55 °C in an environment devoid of biological activity. Exposure of PHB/V to warm aqueous conditions leads to hydrolysis to lower molecular weight fragments as observed by Doi et al. [8], but Miller and Williams [16] did not see a decrease in tensile properties when they exposed PHB/V to similar conditions. Embrittlement, without molecular weight change, can result from biodegradation in soil at 25 °C [11]. Molecular weight changes as a result of biodegradation would not be expected, as enzymatic degradation is a surface phenomenon [8], and polymer chains in the bulk of a sample would not be affected.

We conclude that PHB/V degraded by a combination of biological and chemical mechanisms. Hydrolytic degradation physically weakened the material and reduced the molecular weight of the polymer, making more chain ends available for enzymatic attack, and microbes enzymatically degraded the polymer and metabolized the products. This combination of abiotic and biological degradation may be important in the total degradation of PHA and its assimilation into the environment.

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