Journal of Environmental Polymer Degradation, Vol. 1, No. 4, 1993

Degradation of Poly(β-Hydroxyalkanoates) and Polyolefin Blends in a Municipal Wastewater Treatment Facility

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Six types of plastics and plastic blends, the latter composed at least partially of biodegradable material, were exposed to aerobically treated wastewater (activated sludge) to ascertain their biodegradability. In one study, duplicate samples of 6% starch in polypropylene, 12% starch in linear low-density polyethylene, 30% polycaprolactone in linear low-density polyethylene, and poly(β-hydroxybutyrate-co-hydroxyvalerate) (PHB/V), a microbially produced polyester, were exposed to activated sludge for 5 months, and changes in mass, molecular weight average, and tensile properties were measured. None of the blended material showed any sign of degradation. PHB/V, however, showed a considerable loss of mass and a significant loss of tensile strength. In a second study, PHB/V degraded rapidly, but another type of microbial polymer which forms a thermoplastic elastomer, poly(β-hydroxyoctanoate), did not degrade. These results illustrate the potential for disposal and degradation of PHB/V in municipal wastewater.

KEY WORDS: Poly(β-hydroxyalkanoates); biodegradation; activated sludge; starch–polyolefin blends.

INTRODUCTION

Waste disposal is becoming a major concern because acceptable waste disposal sites are becoming scarce amid an increasing amount of waste. Plastics comprise about 18% of municipal solid waste [1] and are among the most visible and the least degradable. Among the strategies being considered to deal with this problem is the use of biodegradable plastics. Numerous attempts have been made to produce biodegradable plastics and films through blending of relatively small amounts of degradable materials with conventional plastics with limited success [2]. Some companies are now beginning to produce materials expected to be totally degradable [3].

In order for biodegradable polymers to degrade quickly and totally, the disposal environment must have sufficient and appropriate microbial activity. One approach to optimize degradation is to utilize environments in which microbial activity is already being managed. Currently, few landfills are so-managed, and the number of municipal solid waste (MSW) composting facilities is still limited. Therefore, municipal wastewater treatment facilities could be employed for biodegradation of some items which might logically be disposed in a municipal sewer system.

It is generally accepted that the microbially produced polyester, poly(β-hydroxybutyrate-co-hydroxyvalerate) (PHB/V), is biodegradable, but few quantitative studies of its degradation in specific environments have been reported. We have shown that samples composed of PHB/V deteriorate in leaf compost through a combination of biological and chemical degradation [4]. Doi *et al.* [5] have measured changes in mass, molecular weight distribution, and mechanical properties of polyesters exposed to seawater. Windeatt and Street [6] have demonstrated the rapid degradation and conversion to methane and carbon dioxide of poly(β-hydroxybutyrate) under anaerobic conditions typical of many small-

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1064-7564/93/1000-0269$07.00/0

scale wastewater treatment systems such as septic tanks. In this paper we report the results of two studies of the biodegradation of PHB/V in activated sludge. In the first study, we compare degradation of PHB/V to several blends of degradable polymers with polyolefins. In the second, the fate of PHB/V was compared to that of poly(β-hydroxyoctanoate) (PHO), another microbially produced polyester.

MATERIALS AND METHODS

Preparation and Exposure of Polymer Samples

Polyolefins were blended and supplied by Exxon Chemical Co. (Baytown, TX). Polypropylene (PP), blended with 6% corn starch, had a molecular weight average ($\overline{M}_w$) of 300,000 and was received as pellets. Linear low-density polyethylene (LLDPE; $\overline{M}_w$ 450,000) blended with 12% corn starch was received as plastic sheets. LLDPE contained 500 ppm each of Irganox 1076 and Irganox 168, the minimum amount needed for thermal stability during processing. About 9% (w/w) butene was present as copolymer, with 2.4 ethyl side chains per 100 carbon atoms. The LLDPE/starch was prepared by Exxon Chemical Co. from a master batch consisting of polyethylene and 43% (w/w) silane-treated corn starch, originally obtained from St. Lawrence Starch (Mississauga, Ontario, Canada). Polycaprolactone (PCL; $\overline{M}_w$ 36,000), obtained from Union Carbide (Danbury, CT), was blended with LLDPE by Exxon Chemical Co. to produce a material comprised of 30% PCL. PHB/V, lot PO5, was received as a technical grade power from ICI (Billingham, UK). Prior to use, PHB/V was purified by two cycles of dissolution in room-temperature chloroform, filtration, and reprecipitation into methanol. The resulting material had a $\overline{M}_w$ of 330,000 and a hydroxyvalerate content of 26.5 mol% as determined by gas chromatography [7].

PHO was prepared from *Pseudomonas oleovorans* grown with sodium octanoate as the carbon source [8]. The composition was determined to be 88% hydroxyoctanoate and 12% hydroxyhexanoate by gas chromatography.

In the first study, dogbone-shaped films, 6.5 cm long, 0.5 cm wide at the neck, and 0.5 mm thick, were prepared by compression molding in stainless steel templates. Powders (PHB/V), granules (starch/PP, PCL/LLDPE), and strips of sheet material (starch/LLDPE) were placed in the mold. Materials were heated to 10°C above the melting transition (T_m) of the highest-melting temperature component, subjected to 6000 psi, and cooled to room temperature with moderate air circulation. Small mounting holes were melted about 0.5 cm from both ends of each dogbone using a heated awl. The dogbones were then attached in duplicate to sample sticks, fashioned from 1.2-m-long wooden dowels, originally designed for use in leaf compost [4]. The sample sticks were then suspended by nylon strings from the side of the wastewater aeration basin so that all the samples were submerged in the uppermost 0.5 m of the activated sludge. Of the five pairs of samples originally exposed, two were lost during the course of the study.

For the second study, PHB/V films were prepared as above. A PHO film was solution cast from a 15% (w/v) chloroform solution, dried under vacuum overnight, and cut into strips approximately 45 × 9 × 0.9 mm. In an improved method, duplicate PHB/V and PHO films were stitched into four charcoal–fiberglass mesh bags (4 × 4-mm mesh) which were tied into a wire basket with fishing line. The basket was then submerged in the activated sludge as above. Water was able to flow freely through the mesh so that both sides of the samples were exposed to the activated sludge.

Incubations were carried out at the Springfield Regional Wastewater Treatment Plant serving the western Massachusetts cities of Springfield and Agawam. Incoming wastewater, after preliminary screening, flows into two (2.56×10^7-L capacity each) open aeration basins, where it combines with sludge returning from downstream in the process. Each basin has a holding time of approximately 24 h, after which the wastewater is clarified, treated, and discharged. Polymer samples were suspended in the initial aeration area, where the substrate concentration, and therefore the microbial activity, was expected to be the highest. At different times during the study, a sample stick or mesh bag holding duplicate samples of each material being tested was returned to the lab and the extent of degradation of the samples was determined as described below.

Control samples were also tested under sterile conditions during the study. Duplicate polymer samples were submerged in boiling 1% sodium dodecyl sulfate for 1 min, then rinsed with 10 ml sterile distilled water and placed in a closed vessel containing 500 ml wastewater sterilized at 121°C for 2 h. These samples were incubated at 15–25°C, which mimicked the temperatures in the wastewater aeration basin. Duplicate samples of PHB/V and PHO used in the second study were sterilized for 15 min in a solution of 1% formaldehyde, washed with sterile distilled water, and incubated similarly. Sterility was verified visually by the absence of turbidity; sludge as a culture medium would produce considerable microbial growth if sterile conditions were not maintained.

Measurements and Analyses

Upon being returned to the lab, polymer films were freed of excess debris, removed from the sample sticks, and soaked in a rapidly stirring solution of 1% Sparkleen detergent (Fisher Scientific) for 30 min. Films were then greatly scrubbed with a paint brush under distilled water to remove residual organic matter, blotted dry, and incubated in a vacuum oven at 50°C for 3 days. Sterile control samples were treated similarly. Samples in the second study were removed from the mesh bags, washed with distilled water, and treated as above.

Tensile properties were determined using an Instron Model 1321 tensile testing instrument. The gauge 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 and 5 min^{-1}, respectively. These crosshead speeds were chosen because they produced the optimum sensitivity for the samples being studied. The exact thickness of all samples was determined with a micrometer before performing tensile tests and the measured values were used in calculations of percentage elongation to break and tensile strength.

Gel permeation chromatography (GPC) was used to determine molecular weights of the PHB/V samples in the first group. Samples were analyzed 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 of chloroform, and sample concentrations of 15 mg/ml and injection volumes of 50 μl were used. Polymer samples were dissolved in warm chloroform and filtered. Polystyrene 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-Houwink constants for PHB of $a = 0.78$ and $k = 1.18 \times 10^{-3}$ ml/g [9].

RESULTS

Figure 1 shows the temperatures of the activated sludge lagoon during the two studies. Because of the high heat capacity of water and the relatively constant temperature at the source of the wastewater, the temperature of the activated sludge was relatively warm and increased very gradually with seasonal change. In the first study, the initial reading was taken in mid-February (ambient air temperature, $< 12°C$) and the last reading was obtained in mid-June (ambient air temperatures, $> 19°C$). The second study began in early June of the following year. The activated sludge temperature never rose above 22°C throughout the summer.

None of the polyolefin blend samples lost mass over the 138 days of the first study (Fig. 2). In contrast, the PHB/V samples lost over one-half their initial mass in

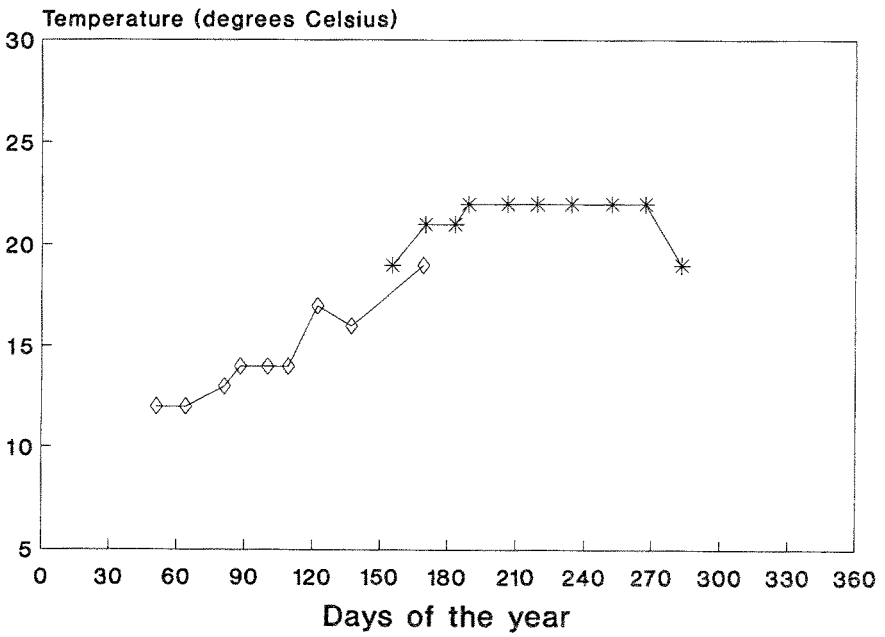

Fig. 1. Temperature of activated sludge from February through June 1990 (diamonds) and from June 1991 through September 1991 (stars).

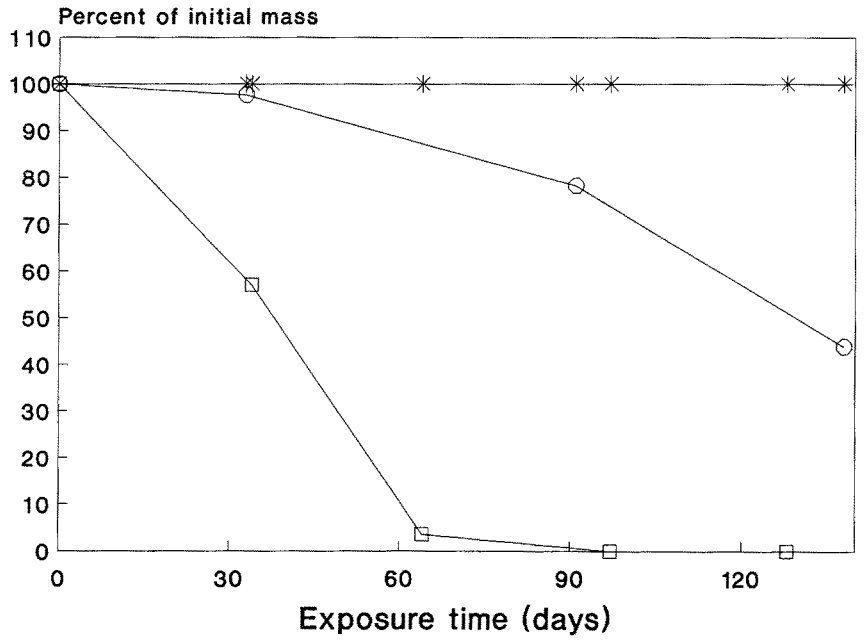

Fig. 2. Percentage of mass lost from samples with time of exposure to activated sludge. Circles, PHB/V, first study; squares, PHB/V, second study; stars, PHO and polyolefin blends. Values are the means of duplicate samples.

the same time period. Control PHB/V samples incubated in the same wastewater under sterile conditions at similar temperatures lost no more than 0.5% of their initial mass and appeared unchanged. Thus, the large loss of mass was clearly the result of biodegradation.

Tensile properties of the polymer samples were also measured. The starch/polyolefin blends showed no significant changes in either percentage elongation to break or tensile strength (Table I). Unexposed PCL/LLDPE samples did not have reproducibly measurable tensile properties, therefore exposed PCL/LLDPE samples were not examined. PHB/V samples exposed to activated sludge showed a large decrease in tensile strength and some variability in percentage elongation to break, whereas PHB/V samples exposed to sterilized sludge exhibited little change in tensile properties (Table I). Thus, the loss of strength of PHB/V samples in an activated sludge environment required biological activity.

No significant changes in molecular weight were observed for PHB/V samples exposed to either native or sterilized activated sludge (Table II). This observation indicates that chemical hydrolysis did not occur throughout the bulk of the samples. As shown in Fig. 3, the thickness of PHB/V samples decreased markedly with time of exposure as a result of surface erosion. Both of these results would be expected if the primary mechanism of degradation was through enzymatic attack, which is known to be a surface phenomenon [10].

Table I. Tensile Properties of Polymer Blends and PHB/V[a]

Exposure time (days)	Elongation to break (%)	Tensile strength (MPa)
	6% strarch/polypropylene blend	
0	5.5 ± 1.1[b]	26 ± 2[b]
33	7.0 ± 0.8	27 ± 1
91	6.5 ± 0.3	28 ± 0
138	6.7 ± 0.2	27 ± 0
111[c]	6.5[d]	27[d]
	12% starch/LLDPE blend	
0	730 ± 70[e]	15 ± 1[e]
33	670 ± 190	10 ± 5
91	790 ± 10	15 ± 0
138	760 ± 10	14 ± 1
111[c]	730 ± 90	14 ± 2
	PHB/V	
0	12 ± 2[b]	28 ± 1[b]
33	7 ± 1	24 ± 1
91	8 ± 2	20 ± 0
138	10[d]	10[d]
111[c]	10 ± 1	26 ± 1

[a] All value are the averages and standard deviations of measurements on duplicate samples except where noted otherwise.
[b] Average of four samples.
[c] Sterile control samples which were incubated for 111 days.
[d] Single sample; duplicate was broken.
[e] Average of three samples.

Table II. Molecular Weight Average of PHB/V Exposed to Activated Sludge[a]

Exposure time (days)	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
0	306,000	175,000	1.75
91	284,000	131,000	2.17
91	268,000	189,000	2.62
138	320,000	189,000	1.70
138	302,000	96,000	3.14
111[b]	361,000	140,000	2.58

[a] $\overline{M}_w$, molecular weight average; $\overline{M}_n$, number average.
[b] Sterile control sample, incubated in autoclaved sludge at about 18°C.

Although the exposure method in the second study was more convenient, there was no apparent difference in the degree of exposure of the samples to the surrounding wastewater between the two studies. The temperature, however, was both higher and more constant in the second study because of the time of year. Loss of mass of PHB/V was much more rapid than in the first study (Fig. 2). Less than 5% of the initial mass of samples remained after 2 months of exposure, and no material was recoverable after 3 months. In contrast, PHO samples showed no loss of mass over the 4 months of the study.

DISCUSSION

Municipal activated sludge (MAS) contains a highly diverse microbial population [11]. One would expect MAS from different geographical locations to have at least qualitatively similar microbial populations, reflecting the similarities in the organic materials that MAS is employed to degrade. We have shown that, in a temperate climate, temperature changes in activated sludge are mild and gradual. MAS is thus useful as an environment for biodegradability testing and may be useful for the disposal of some biodegradable materials.

Blends of polyolefins with the biopolymer starch and the synthetic, biodegradable polymer polycaprolactone did not degrade in the microbially active environment of activated sludge. Changes in neither mass nor tensile properties were observed. The failure of the polyolefin blends to lose mass can be attributed partly to the inaccessibility of the starch or polycaprolactone encased in the polyolefin matrix [12]. We have previously reported that very little of the degradable components of these blends were lost after 6 months of exposure to leaf compost [4]. The failure of the starch and PCL components to lose any mass in activated sludge may reflect both a difference in microbial population and a lower temperature in activated sludge compared to leaf compost.

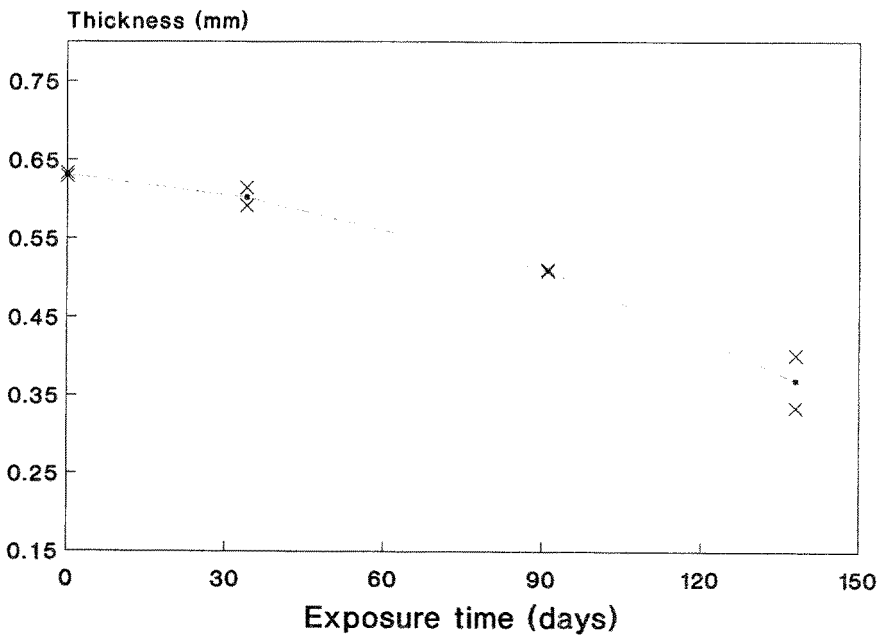

Fig. 3. Changes in thickness of PHB/V samples with time of exposure to activated sludge. X, individual duplicate samples; line is plotted through the average of duplicates. Values for sterile controls were within 2% of values for unexposed samples.

Polyolefin-blend samples used in this study did not contain any type of degradation-enhancing additives. Products comprised solely of starch and polyethylene were being actively marketed when this study was initiated. It is unlikely that the addition of a photosensitizing agent would have enhanced the degradation of such blends; submersion in the turbid activated sludge and accumulation of surface biofilm would have blocked absorption of the UV radiation required to initiate degradation [1]. Prooxidants such as fatty acids may have produced some brittleness in the starch–polyolefin blends with time, but heat dissipation by the wastewater would have limited prooxidant effectiveness [13].

PHB/V degraded during the exposure to activated sludge, losing both mass and tensile strength. Samples exposed to sterile sludge did not degrade, however, demonstrating that the degradation in activated sludge resulted entirely from biological activity. This result stands in contrast to the results obtained in leaf compost [4], in which PHB/V control samples incubated in sterile leaves at 55°C, while losing no mass, lost all tensile strength. This loss was accompanied by a twofold decrease in molecular weight, a change not seen in samples exposed to activated sludge. These results suggest that while abiological hydrolysis comprised a significant portion of the degradation observed during exposure to leaf compost, only biological degradation of PHB/V samples took place in activated sludge, a reflection primarily of the lower temperature of the latter environment.

Surface erosion of PHB/V samples during exposure, observed as a decrease in thickness of the samples exposed, likely was the result of enzymatic degradation by secreted depolymerases produced by microbes colonizing the surface of the samples. Enzymatic degradation is known to be a surface phenomenon [10]. *Alcaligenes faecalis*, a bacterium known to secrete an extracellular PHB depolymerase, was first isolated from activated sludge [14] and is likely one of many bacteria in activated sludge capable of degrading PHB/V.

There was clearly a temperature effect on the degradation of PHB/V. During the first study, the rate at which mass was lost increased with increasing temperature, with the rate highest above 17°C. At the temperature of the second study, 19–23°C, PHB/V degradation was very rapid. The higher temperature may have increased degradation directly by stimulating microbial metabolism and enzymatic activity or indirectly by increasing a population of microbes with greater activity against PHB/V.

The other bacterial polyester PHO did not lose any mass during the 4 months of the second study. It had been predicted that PHO would be less biodegradable than PHB/V because of the hydrophobicity resulting from the longer alkyl side chains of its monomer constituents [15]. Recently, the isolation of several microbes capable of growing with PHO as the sole carbon and energy source was achieved using static enrichment cultures [16]. One strain, identified as *Pseudomonas fluorescens*, was isolated from activated sludge. It seems likely that the failure of PHO to degrade in this study reflects the difficulty of microbial colonization, a critical first step in degradation, of a hydrophobic material in a dynamic aqueous system. Successful biodegradation of PHO in activated sludge may require measures to make the surface of the material more amenable to microbial attachment.

ACKNOWLEDGMENTS

We thank Dr. Richard Austin of Exxon Chemical Co., Dr. Nadia Lotti of the University of Bologna, Italy, and Anthony Curto and George Romano of the Springfield, MA, Department of Public Works for their assistance with this project. Dr. S. Antoun was funded by the University–Industry Cooperative Research Program, CUMIRP, of the University of Massachusetts and in part by the National Science Foundation. This work was done under contracts with Exxon Chemical Co. (MEW 8907.25) and Proctor and Gamble Co.

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