Anaerobic Biodegradation of Polyhydroxybutyrate in Municipal Sewage Sludge

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Abstract: Anaerobic biodegradation in sewage sludge of polyhydroxybutyrate (PHB) was investigated. Evolved gaseous carbon was measured to assess biodegradability according to ASTM D5210. Mass-loss experiments were performed to determine degradation kinetics. Changes in the polymer properties were investigated. The impact of a natural plasticizer [tributyl citrate (TBC)] on biodegradation was determined. Polylactic acid was also biodegraded for comparison. Melt-pressed plates of PHB (with thicknesses of 0.24, 0.5, 1.2, 3.5, and 5.0 mm) were biodegraded to investigate the relationship between initial mass:initial surface area ratios and decay rates. Scanning electron microscopy micrographs of degraded specimens were recorded for visual illustration of the degradation process. A relationship between initial mass:initial surface area and degradation rates indicates that the thickness and surface area of the material affect its degradation. The degradation rates were impacted by the sewage sludge activity. TBC additive hindered PHB's rate of degradation. Thermal properties, molecular bonding, and molecular weight measured by differential scanning calorimetry, Fourier transform infrared, and size exclusion chromatography, respectively, were only slightly affected by biodegradation, indicating that recycling PHB will not affect its performance.

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Introduction

The interest in the biodegradation of bioplastics has increased due to the environmental issues associated with the production and disposal of petroleum-based plastics and the finite supply of and reliance on foreign oil to produce these commodity plastics (i.e., Gomez-Martinez et al. 2009; Sobkowicz et al. 2008; Waltz 2008; Yu and Chen 2008; Fritz et al. 2001; Brandl and Püchner 1992). Although a generally accepted definition of bioplastics does not exist, the denomination is commonly applied to plastic materials made from renewable resources. Most bioplastics will biodegrade in natural environments. The European Bioplastics Organization includes in this category plastics from renewable resources that meet the Standard EN 13432:2000 criteria of compostability and degradability. Biodegradability as defined by this standard requires 90% mineralization of the material after six months under the procedures described by EN 14046 (also published as ISO 14855-1:2005). The ASTM D883-00 (ASTM International 2000a) defines a biodegradable plastic as one in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi, and algae. Under this standard, a degradable plastic should be designed to undergo a significant change in its chemical structure under specific environmental conditions. Additionally, ASTM D6400-04 (ASTM International 2004) mandates for products consisting of more than one polymer, an overall 90% conversion of organic carbon and a minimum conversion of 60% of each polymer that comprises more than 1% of the sample. The end products of bioplastic biodegradation are nontoxic and already occur naturally in the environment: carbon dioxide (CO_2) and water (H_2O) in aerobic environments and CO_2 and methane (CH_4) gas in anaerobic environments. The potential toxicity of degradation by-products from additives (i.e., plasticizers) can be avoided by using naturally derived compounds.

Polyhydroxyalkanoates (PHAs) are a family of biodegradable thermoplastic polymers (polyesters) originally produced naturally by some bacteria as part of their energy storage mechanisms (i.e., Williams and Peoples 1996). The most studied of the PHAs (i.e., Jendrossek and Handrick 2002; Imam et al. 1998; Reischwitz et al. 1998; Budwill et al. 1992; and Luzier 1992) are polyhydroxybutyrate (PHB) and its copolymer PHB-*co*-hydroxyvalerate (PHBV). PHA biodegradation is often studied in vitro using isolated microorganisms identified as PHA degraders (i.e., Freier et al. 2002; Jendrossek and Handrick 2002). Under this protocol,

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Table 1. Materials Tested in the Three Gas Production (EXP1, EXP2, and EXP3) and Two Mass Loss (ML1 and ML2) Experiments; Numbers Represent the Size (Width/Length) of the Plates in Centimeters and Numbers in Parentheses Represent the Number of Plates per Flask

	Experiment				
Material	EXP1	EXP2	EXP3	ML1	ML2
PHB powder	Yes	Yes	Yes	—	—
PHB plates					
0.24 mm	—	—	—	—	2.55/2.55 (2)
0.5 mm	—	—	—	2.5/2.5 (5)	—
1.2 mm	1.1/4.5 (4)	1.1/4.5 (2)	1.1/4.5 (2)	1.5/7 (2)	1.6/1.6 (1)
3.5 mm	—	—	—	12.7/6.4 (5)	—
5.0 mm	—	—	—	—	1.5/1.5 (1)
1.2-mm 85%PHB/15%TBC plates	—	—	1.1/4.5 (2)	—	1.6/1.6 (1)
Cellulose powder	Yes	Yes	Yes	—	—
LDPE beads	Yes	—	—	—	—
1.2-mm PLA plates	—	—	1.1/4.5 (2)	—	1.6/1.6 (1)

the degradation rates can be determined with very reproducible results for specific experimental conditions. In vitro studies provide information about changes in degradation patterns due to environmental conditions and the effect of degradation products on the environment. However, these studies provide limited information on biodegradation by the microorganisms' consortium that can be found in natural environments.

Some PHA biodegradation studies have been performed under natural environmental condition, including soil, freshwater, saltwater, and/or municipal waste environments consisting of mixed cultures of bacterial species (i.e., Imam et al. 1998). One municipal waste environment that has attracted great interest for the PHA biodegradation is anaerobic sewage sludge. Several authors have shown that PHA thin films and powder will completely degrade and often show higher biodegradability than other biodegradable polymers (i.e., Abou-Zeid et al. 2001; Reischwitz et al. 1998; Imam et al. 1995; Briese et al. 1994; Budwill et al. 1992) making them good candidates for the substitution of petrochemical plastics in disposable products. Luzier (1992) found that the time to complete biodegradation of 1-mm-thick PHBV molding in anaerobic sewage was 6 weeks, compared to 40 weeks in estuarine sediment, 60 weeks in aerobic sewage, 75 weeks in soil, and 350 weeks in sea water. While there are many studies on PHB degradation, particularly in the form of powder or thin films (i.e., Rutkowska et al. 2008; Woolnough et al. 2008; Abou-Zeid et al. 2001; Reischwitz et al. 1998; Imam et al. 1995; Briese et al. 1994; Budwill et al. 1992), few if any look at the impact of the initial mass:initial surface area on the degradation rate of the bioplastic.

The objectives of this work were to (1) develop a kinetic relationship between initial mass:initial surface area and biodegradation rate of PHB and (2) assess the impact of the additive tributyl citrate (TBC) on the biodegradation of PHB.

Materials and Methodology

Three anaerobic biodegradation experiments based on biogas production (EXP1, EXP2, and EXP3) and two based on mass loss (ML1 and ML2; in parallel with EXP1 and EXP3) were performed on PHB plates of various thicknesses to determine the impact of the initial mass:initial surface area ratio on the degradation kinetics (Table 1). The biogas production experiments were performed according to the standard test method for determining the anaerobic biodegradation of plastic materials in the presence of municipal sewage sludge [ASTM D5210-92 (ASTM International 2000b)] in a buffered diluted sewage sludge media (1/10 dilution) with added macronutrient and micronutrient. The test is designed to classify plastic materials that are more or less biodegradable relative to a positive standard (cellulose) in an anaerobic environment. The mass loss experiments were performed using the same media as in the biogas production experiments.

Melt-pressed plates from a finely powdered (13-μm particle size) bioplastic supplied by Metabolix, Inc., and identified as PHB were used as test specimens for the degradation studies. The PHB identification was confirmed by wide angle x-ray diffraction (WAXD) analysis (Siemens/Bruker D5000 x-ray diffraction) using Cu $K\alpha$ radiation (wavelength$=$1.5406 Å) at angles of diffraction from 2° to 50°. The diffractogram was compared with the Jade database (Materials Data, Inc.) using the Siemens Kristalloflex V3.1 software. Sigmacell cellulose powder (20-μm particle size) was used as a positive control to verify viability of the sludge inoculum. Melt-pressed plates of polylactic acid (PLA) (Natureworks) and an 85% PHB/15% TBC (Acros Organics) blend were tested for biodegradation in addition to the PHB plates in EXP3 to study the effect of the additive on the degradation of the PHB and to compare the PHB degradation with that of the PLA, a bioplastic that has been proposed as a reference material for international standards of biodegradation (i.e., Kunioka et al. 2006). TBC is a naturally derived nontoxic citrate ester that has been approved as an additive/plasticizer for polymers (i.e., Labrecque et al. 1997). Low density polyethylene (LDPE) (Dow Chemical, Inc.) beads served as a negative control in EXP1. Chemical, thermal, and physical properties of the test materials were determined before and after the degradation experiments.

Material Preparation

The PHB powder was melted at 179°C (355°F) for 5–10 min (longer for thicker plates) and pressed into plates of different thicknesses (0.24, 0.5, 1.2, 3.5, and 5 mm) in aluminum molds using a carver laboratory press. The prepared plates were cured for at least seven days to allow for complete chemical equilibrium and crystallization prior to use in the biodegradation experiments and characterization analyses. The PLA plates (1.2 mm thick) were prepared using the same procedure as for the PHB plates except that the melting temperature was 149°C (300°F). The

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85% PHB/15%TBC (w/w) was mixed (Hakke Rehocord 90) at 60 rpm and 175°C and then pressed into 1.2-mm-thick plates under the same conditions as the PHB plates.

Material Characterization

Chemical Properties

The effects of the biodegradation processes on the molecular bonding characteristics were investigated by Fourier transform infrared (FT-IR) spectroscopy (Bruker Tensor 27 microspectrometer) for the raw PHB powder on the undegraded and degraded PHB plates (0.24, 1.2, 3.5, and 5 mm thick) and 85% PHB/15% TBC blend on 1.2-mm-thick plates. Spectrograms were processed using OPUS Version 4.2 software.

Molecular weight was measured by size exclusion chromatography (SEC) (Agilent) for the PHB powder and all undegraded and degraded plates to determine the effects of biodegradation on the molecular weight of PHB of different thicknesses. For all samples, a 5-mg mL^{-1} solution of the plastic was prepared using chloroform ($CHCl_3$) as the solvent. For the separation, a guard column (5 μm, 50×7.8 mm) and three Phenogel 300×7.8 mm columns (Phenomenex, Torrance, Calif.) were used: (1) 5 μm, 500 Å (1–15 K); (2) 5 μm, 10–4 Å (5–500 K); and (3) 10 μm, MXM (100–10,000 K). Three detectors in series were used: (1) Wyatt Heleos multiangle light-scattering detector equipped with a QUELS DLS detector; (2) Wyatt ViscoStar viscosity detector; and (3) Wyatt rex differential refractive index detector. All separations used an injection volume of 100-μl chloroform (1 mL min^{-1}) as the eluent.

Thermal Properties

Thermal analyses [ASTM D3418-03 (ASTM International 2003)] were performed by differential scanning calorimetry (DSC) (TA Instruments DSC 2920) on the PHB powder and all undegraded and degraded plates to study the effect of the biodegradation process in the thermal properties of the PHB plates, particularly the crystallinity. A heating rate of $5^\circ\mathrm{C\ min}^{-1}$ in a nitrogen atmosphere was used. Sample sizes were approximately 5–10 mg. The samples were heated to 200°C and held for 0.5 min, cooled to −20°C and stabilized for 1 min, and reheated to 200°C. Melting temperature (T_m), melting enthalpy (ΔH_m), crystallization temperature (T_c), and crystallization enthalpy (ΔH_c) were obtained from the thermograms using the TA Universal Analysis V3.9A software. T_m and T_c were taken as the onset points of melting and crystallization, respectively.

Physical Properties

Scanning electron microscopy (SEM) images of undegraded and degraded melt-pressed plates of the PHB and the 85% PHB/15% TBC blend sputter-coated with gold were recorded using a Stereoscan 260 scanning electron microscope. The images provide a visual assessment of the effect of the initial mass:initial surface area ration on the degradation patterns of the plates.

Biodegradation Studies

Biogas Production Experiments

The plate sizes (length, width, and thickness) for all experiments are summarized in Table 1. For EXP1 and EXP2, sewage sludge was obtained from an anaerobic digester located at the Central Wastewater Treatment Facility, Baton Rouge, La. For EXP3, sewage sludge was obtained from an anaerobic digester located at the North Wastewater Treatment Facility, Baton Rouge, La. Both sludge sources had solid retention times of approximately 15–30 days and were filtered through a 2-mm sieve prior to use in the experiments. For the PHB and cellulose powders, the concentrations of polymer carbon (C) in the sewage media were 500, 1,000, and $1{,}000\ \mathrm{mg\ L}^{-1}$ for EXP1, EXP2, and EXP3, respectively. For all melt-pressed plates, the polymer carbon loading in the anaerobic sewage sludge media was based on plate sizes.

The biogas experiments were performed in triplicate. Serum bottles of approximately 240-mL volume containing 150 mL of the prepared diluted sludge media were used for each replicate. The media were prepared according to the ASTM D5210-92 (ASTM International 2000b) and transferred to the serum bottles under anaerobic conditions. The headspace within each bottle was sparged with nitrogen (N_2) gas in EXP1 and EXP2 and with a 70% N_2/30% CO_2 gas mixture [as recommended in the ASTM Standard 5210-92 (ASTM International 2000b)] in EXP3 to prevent reentry of oxygen (O_2) that had been purged from the media during preparation. The initial pH of the resulting media ranged between 7.0 and 7.5 for all experiments. The polymer carbon concentration of the PHB plates was calculated for each bottle to obtain an initial plastic surface area:sewage sludge volume of $0.3\ \mathrm{cm}^2\ \mathrm{mL}^{-1}$. The target polymer carbon concentrations were $8{,}000\ \mathrm{mg\ L}^{-1}$ (four plates) for the PHB plates in EXP1 and $4{,}000\text{–}4{,}500\ \mathrm{mg\ L}^{-1}$ (two plates) for the remaining materials (PLA, 85% PHB/15% TBC) and experiments.

The serum bottles containing the media and test specimens were incubated in the dark at 35°C until gas production due to degradation ceased. The evolved biogas (CH_4 and CO_2) was collected by an apparatus consisting of inverted graduated cylinders connected to the serum bottles by Tygon tubing. Generated gas volumes were measured daily. Maximum theoretical gas production (ThGP) at STP was calculated based on the original mass of the test materials

$$\mathrm{ThGP(mL)} = \mathrm{mass(mg)} \times (\%\ \text{by weight carbon}) \times \frac{1\ \text{mmol gaseous carbon}}{12\ \text{mg carbon}} \times \frac{22.4\ \mathrm{mL}}{\text{mmol gaseous carbon}} \tag{1}$$

When the experiments were concluded, the mean gas volume of each treatment was corrected for vapor pressure at 35°C and the average temperature of the graduated cylinders throughout the experiments and compared to the theoretical volume to assess biodegradability of the test materials.

The composition of the biogas was analyzed by gas chromatography (GC) using an SRI 8610C gas chromatograph equipped with a thermal conductivity detector. The analysis was performed at least once per week but only when sufficient amounts of biogas were produced to warrant a sampling event. The GC data served as an indication of the microbial consortia in the sewage sludge (i.e., the relative presence of methanogens versus CO_2-producing microbes).

The sludge media were analyzed for dissolved organic carbon (DOC) and biomass carbon predegradation and postdegradation to facilitate mass balance calculations for EXP2 and EXP3. No mass balance analysis was performed in EXP1 as the gas production was below the 70% required by the ASTM standard. The results of the EXP1 were used only to compare them with the concurrent ML1 experiment. The total solid content, predegradation and postdegradation, was also determined. The mean carbon content for each treatment was calculated and mass balances were performed (C=carbon)

$$C_{\text{input}} = C_{\text{output}} \tag{2}$$

where

$$C_{\text{input}} = C_{\text{polymer}} + C_{\text{biomass}} + C_{\text{DOC}} \tag{3}$$

$$C_{\text{output}} = C_{\text{polymer}} + C_{\text{biomass}} + C_{\text{DOC}} + C_{\text{biogas}} \tag{4}$$

C_{biomass} represents the determined carbon content of sludge solids, including bacteria.

Mass Loss Experiments

To develop a kinetic relationship of biodegradation coefficients and initial mass:initial surface area ratio, two mass loss experiments (ML1 and ML2) were performed in parallel to EXP1 and EXP3 using the same sewage sludge media and preparation as the biogas production experiments. ML1 was performed in quintuplicate and ML2 in triplicate.

For ML1, five 1-L erlenmeyer flasks each containing PHB melt-pressed plates of 0.5, 1.2, and 3.5 mm thickness [5, 2, and 5 plates, respectively (Table 1)] were incubated in the media at 35°C and in the dark. Prior to incubation, the specimens were rinsed with Type I analytical water, oven dried at 60°C, cooled in a dessicator, and weighed. The flasks contained the same ratio of initial plastic surface area:sewage sludge volume as the reactors in the parallel biogas production experiment (EXP1) to allow easy comparison of relation of biodegradation coefficients and initial mass:initial surface area ratio between the two studies. One flask was removed from the incubator during each sampling event (weeks 2, 4, 8, 12, and 19).

For ML2, PHB plates with thicknesses of 0.24, 1.2, and 5 mm were subjected to degradation in the anaerobic sewage sludge medium. The 1.2-mm-thick plates served as a reference between the two mass loss experiments. Melt-pressed plates of NatureWorks PLA (1.2 mm thick) and the 85% PHB/15% TBC blend (1.2 mm thick) were also tested for biodegradation. All plate sizes were tested in triplicate. The samples were not grouped as in the ML1 experiment as plates of different formulations were tested. Ninety-nine 250-mL flasks with sewage sludge media and either the PHB plates with thicknesses of 0.24, 1.2, or 5 mm, 1.2-mm NatureWorks PLA plates, or 1.2-mm 85% PHB/15% TBC blend plates were prepared (Table 1). In this experiment, the comparison with the parallel biogas production experiment (EXP3) was done through the ratio of plastic mass to sewage sludge volume of $(4.0\text{–}4.5) \times 10^3$ mg L^{-1} instead of the surface area as in the ML1 experiment to determine if the relationship between initial mass:initial surface area was due to differences in polymer mass to sludge volume ratio. The sampling schedules for the 0.24-mm PHB plates were 5, 10, and 15 days and 3, 4, 6, 8, and 10 weeks of exposure. Sampling times for all other sizes and types of plastic occurred at 2, 4, 8, 12, 16, and 20 weeks of exposure except that an additional sampling event occurred at 32 weeks for the 5-mm PHB plates.

During the sampling events the plastic was strained from the media with an 850-μm sieve, rinsed well with Type I analytical water to remove the microbial film, oven dried at 60°C, cooled in a desiccators, and weighed prior to mass loss determination. Mean mass loss for each thickness and material was calculated by subtracting the remaining mass from the initial mass of the plate. The mean mass loss was plotted versus time to allow calculation of maximum degradation rate coefficients. The degradation rate coefficients were regressed against initial mass:initial surface area ratios to establish a relationship that could be used to predict degradation of plastics with different thicknesses. All analyses were performed using SigmaPlot (Version 9.01, 2004 Systat Software, Inc.). The remaining plastic was then subjected to DSC, FT-IR, SEC, and SEM analyses. These analyses required destructive sampling of the sample.

Table 2. Percentages of Net Theoretical Gas Production (%ThGP) Observed for the Specimens Exposed to the Anaerobic Sludge Media of the Three Biogas Production Experiments

	Mean values ± standard deviation; $n=3$		
Experiment/ material	Maximum gas production (ml)	Theoretical gas production (ml)	%ThGP
	EXP1[a]		
PHB powder	88.8 ± 0.5	140 ± 0.1	63.4 ± 0.4
Cellulose powder	78.1 ± 2.6	140 ± 0.1	55.7 ± 1.8
PHB plates	1,632 ± 157	2,235 ± 78	73. ± 6.2
	EXP2[a]		
PHB powder	191 ± 12.7	280 ± 0	68 ± 4.5
Cellulose powder	202 ± 29.2	280 ± 0	72.1 ± 10.4
PHB plates	785 ± 20.7	1,170 ± 8.5	67.1 ± 1.3
	EXP3		
PHB plates	1,052 ± 99.9	1,271 ± 109	82.8 ± 2.3
Cellulose powder	206 ± 5.6	280 ± 0.1	73.6 ± 2
85% PHB/15% TBC plates	1,049 ± 147	1,121 ± 47	93.8 ± 15.7
PLA plates	14 ± 9.8	1,162 ± 32	1.2 ± 0.9

[a] CO_2 corrected.

Results and Discussion

Biodegradation Studies

Biogas Production Experiments

In EXP1, peak gas production from biodegradation of the PHB powder and cellulose was observed after 13 and 16 days, respectively, including a 4 day lag time. Degradation of the PHB plates required much more time (approximately 85 days) due to their higher initial mass:initial surface area ratio and the larger amount of mass to be degraded. Budwill et al. (1992) found a %ThGP > 80% for PHB powder degraded for 16 days by a methanogenic sludge inoculum mineral media. Puechner et al. (1995) found also a %ThGP > 80% for PHB powder after 20 days in water with PHB degrading bacteria. The lower %ThGP (Table 2) obtained in this work may be due to differences in the bacterial consortium present in the media. One notable observation from EXP1 is that both the PHB powder and plates degraded more thoroughly than the cellulose powder, which is known to be biodegradable under anaerobic conditions [ASTM Standard 5210-92 (ASTM International 2000b) requires a production of 70% of the ThGP with the cellulose to consider the test valid]. These results coincide with Mezzanotte et al. (2005) who found that in industrial sludge, polycaprolactone had a higher biodegradation than cellulose. In municipal sludge, the biodegradability of cellulose reported by some authors varies from 60 to 100% (i.e., Mezzanotte et al. 2005; Degli-Innocenti et al. 1998; Shin et al. 1997; Wang et al. 1994). The polyethylene beads used as negative controls expectantly exhibited no biodegradation.

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Table 3. Carbon Mass Balances of EXP2 and EXP3 Comparing C Input ($C_{polymer}$+$C_{biomass}$+DOC) with C Output after Incubation ($C_{polymer}$+$C_{biomass}$+C_{DOC}+C_{biogas}); Values Shown Are Averages for Triplicate Bioreactors for Each Polymer Tested for Biodegradation

	Polymer	Polymer (mg l^{-1})	$C_{polymer}$ (mg l^{-1})	$C_{biomass}$ (mg l^{-1})	C_{DOC} (mg l^{-1})	C_{biogas} (mg l^{-1})	C_{tot} (mg l^{-1})	C_{tot} (%)
				EXP2				
Input	Blanks	—	—	799	66.9		866	100
Output				734	22.0	65	821	94.8
Input	PHB powder	1,835	1,000	799	66.9		1,866	100
Output			—	878	30.1	704	1,612	86.4
Input	Cellulose powder	2,381	998	799	66.9		1,864	100
Output			—	897	44.0	721	1,662	89.2
Input	PHB plates	7,668	4,225	799	66.9		5,091	100
Output			—	864	37.8	2,803	3,705	72.8
				EXP3				
Input	Blanks	—	—	886	13.7		900	100
Output				1,034	7.4	23	1,064	118
Input	PHB plates	8,467	4,665	886	13.7		5,565	100
Output			—	1,094	21.0	3,783	4,898	88.0
Input	Cellulose powder	2,381	998	886	13.7		1,898	100
Output			—	1,125	12.2	751	1,888	99.5
Input	85% PHB/15% TBC plates	7,126	4,004	886	13.7		4,904	100
Output			—	965	157.4	3,761	4,883	99.6
Input	PLA plates	8,353	4,151	886	13.7		5,051	100
Output			4,151	1,017	5.7	51	5,225	103

In EXP2, peak gas production from the biodegradation of the PHB and cellulose powders was observed after 12 and 19 days, respectively, with a lag time of 2 days. Gas production from the degradation of the PHB plates proceeded much longer (approximately 65 days) than the powders. Similar to EXP1, the %ThGP of the positive control (cellulose powder) was slightly less than 70%. The use of high purity N_2 gas instead of a 70% $N_2/30\%$ CO_2 for sparging in EXP1 and EXP2 may have caused some produced CO_2 gas to redissolved into the sewage sludge media as has been observed by other authors (i.e., Gartiser 1998). Subsequently, the results of EXP1 and EXP2 were adjusted using Henry's law for solubility of CO_2 in water (Table 2) as described by Puechner et al. (1995) and Reischwitz et al. (1998). The gas produced from all specimens reached approximately 70% of ThGP, similar to the cellulose positive control.

In EXP3, peak gas production from biodegradation of the cellulose powder was observed after 30 days, including a lag time of 4 days. The viability of the sludge inoculum for assessing biodegradability was verified since 73.6% ThGP was observed for the positive control. Gas production from the degradation of the PHB plates and the 85% PHB/15% TBC blend plates reached plateaus by 80 and 190 days, respectively, with a lag time of 4 days. These data show that the PHB requires more than twice as long to degrade when blended with 15% TBC. However, the 85% PHB/15% TBC blend appears to have exhibited superior biodegradability (93.8%) compared with the pure PHB (82.8%) on the basis of the observed gas production (Table 2), although the difference was not statistically significant. The PLA showed no significant gas production in excess of the blank controls, indicating that it had undergone no degradation in the sewage sludge media throughout the exposure period. This is consistent with results reported by Shin et al. (1997) who did not observe any significant biodegradation of poly-L-lactic during 100 days of exposure to anaerobic sewage sludge using the same ASTM procedure. The longer times required to degrade the specimens in EXP3, compared with EXP1 and EXP2, indicate that the sewage sludge used was a slower degrading sludge, although it seemed to degrade the substances more thoroughly based on gas evolution.

The carbon mass balance of EXP2 (Table 3) shows that 73–95% of the carbon could be accounted for, depending on the sample. The carbon mass balance EXP3 showed that 88% of the carbon could be accounted for the PHB plates and approximately 100% for the cellulose powder, the 85% PHB/15% TBC blend plates, and the PLA plates. The reactors containing the 85% PHB/15% TBC blend plates had much larger amounts of dissolved carbon (DOC) remaining at the end of the experiment than the PHB plates, which may indicate that some degradation products were not completely biodegraded and remained in the sludge medium. These results concur with the observations of Bonartseva et al. (2003) who indicated that the degradation of PHB under anaerobic conditions can lead to accumulation of acetate, butyrate, and propionate in the medium.

Mass Loss Experiments

A gradual decomposition was observed in all of the PHB and 85% PHB/15% TBC blend plates in both mass loss experiments (Fig. 1). The results for ML1 indicated a 100% mass loss by week 12 for the 0.5- and 1.2-mm-thick plates, while the 3.5-mm plates were fully biodegraded by week 19 (Table 4). Mass loss with respect to initial surface area increased more rapidly throughout the degradation period for the thicker plates than the thinner plates. This may be attributed to a decrease in the mass:initial surface area ratio of the thicker plates as the degrading microorganisms burrowed into these plates and increased the surface area. Although the same phenomenon would have occurred for the thinner plates, the effect would be more pronounced for the thicker plates due to the higher availability of mass for burrowing.

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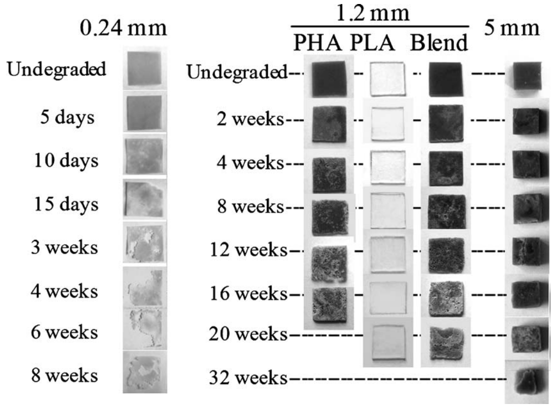

Fig. 1. Visual comparison of the undegraded and degraded PHB plates with thicknesses of 0.24, 1.2, and 5 mm and the PLA plates and 85% PHB/15% TBC blend plates from ML2. Times of exposure are indicated.

The 0.24-mm PHB plates investigated in ML2 reached 100% degradation after 10 weeks of exposure (Table 4). This result is lower than the 100% mass loss within six weeks obtained by Abou-Zeid et al. (2004) for compression-molded PHB films of similar thickness degraded in an anaerobic sewage sludge medium. The 1.2-mm PHB plates showed 100% degradation by 20 weeks, while the 5-mm PHB plates and 1.2-mm 85% PHB/15% TBC blend plates both showed significant but not complete degradation within the time frame of the experiment. Imam et al. (1995) obtained a mass loss ranging from 48 to 78% with <0.16-mm-thick injection-molded composites of PHBV and native cornstarch (30 and 50%) and PHBV with cornstarch precoated with 12% polyethylene oxide degraded in municipal activated sludge for 35 days.

The 1.2-mm PHB plates for ML1 show a faster degradation than the PHB plates of the same thickness in ML2. This may indicate a difference in the activity of the sludge. The comparison of the 1.2-mm PHB and the 1.2-mm 85% PHB/15% TBC blend plates provides some interesting observations. Within the first month of degradation, no difference was observed in mass loss. During the second month, the PHB degraded twice as fast as the 85% PHB/15% TBC blend, with the gap closing again thereafter. While the mechanisms are not known at this time, it can be stated that the additives had an effect on the degradation process. In practical terms, however, the difference is only a month or two. No mass loss occurred for the PLA, instead a slight gain of mass was observed throughout most of the experiment except for a slight loss and zero gain at 12 and 16 weeks, respectively. The gain of mass may indicate that the material had absorbed water that could not be removed by oven drying of the PLA, a phenomenon also observed previously by other authors with several polymers (i.e., Brandl et al. 1995).

For all plate thicknesses, the rate of degradation exhibited a sigmoidal behavior, with an initial lag, followed by exponential mass loss, and then a decrease in the mass loss rate (Fig. 2). The degradation rates of the exponential phase of the degradation curve for all the plate sizes and materials used in ML1 and ML2 are presented in Table 5. The magnitude of the degradation rate coefficient of the 1.2-mm PHB plates from ML2 was approximately 56% of that obtained for ML1, thus, indicating that while the same ASTM standard was used, the activity of the sludge had a tremendous impact on the degradation kinetics. The slower degradation observed in ML2 for the thicker plates, even when the

Table 4. Percentages of Degradation Observed for the PHB Plates Exposed to the Anaerobic Sewage Sludge Media of Both the First and Second Mass Loss Experiments and the PLA and 85% PHB/15% TBC Blend Plates of the Second Experiment Exposed for the Times Indicated

ML1	Mass loss percentage		
Exposure time	0.5 mm	1.2 mm	3.5 mm
2 weeks	20.3	12.3	4.6
4 weeks	48.4	46.4	16.5
8 weeks	97.3	97.2	64.3
12 weeks	99.5	100	92.5
19 weeks	100	100	99.7

ML2	PHB			PLA	1.2-mm 85% PHB/ 15% TBC
Exposure time	0.24 mm	1.2 mm	5 mm	1.2 mm	
5 days	6.6	—	—	—	—
10 days	22.0	—	—	—	—
2 weeks	—	8.1	2.2	−0.2	6.4
15 days	54.2	—	—	—	—
3 weeks	53.7	—	—	—	—
4 weeks	73.4	14.5	7.3	−0.3	13.3
6 weeks	82.9	—	—	—	—
8 weeks	94.1	68.2	22.5	−9.2	32.4
10 weeks	100	—	—	—	—
12 weeks	100	79.1	26.8	0.1	54.4
16 weeks	100	73.2	48.8	0.0	60.4
20 weeks	100	100	59.4	−0.2	85.5
32 weeks	100	100	68.9	—	—

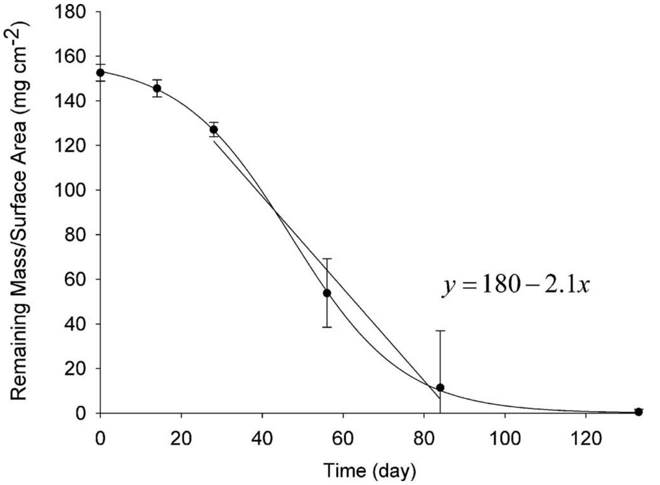

Fig. 2. Illustrative example of the mass loss curves obtained in ML1. For all treatments, the mass loss exhibited a sigmoidal degradation behavior.

total initial polymer carbon was the same for all thicknesses; we indicate that the degradation is not dependent only on the polymer carbon mass.

The analysis of covariance showed that the degradation coefficients in ML1 for all three thicknesses of PHB tested were significantly different, indicating a relationship between initial mass:initial surface area and the degradation coefficient. For ML2, due to the lower degradation coefficients, the ANCOVA was only able to conclude that the degradation coefficients for the 5-mm PHB and 1.2-mm PHA/15% TBC blend were significantly different.

A relationship between the degradation rate coefficients and the initial mass:initial surface area ratios was established. This relationship followed saturation kinetics, with degradation initially increasing with increasing initial mass:initial surface area ratio, and a subsequent stabilization of the degradation rate

$$v = \frac{v_{\max} x}{k_{\mathrm{m}} + x} \tag{5}$$

In this model, v represents the degradation rate coefficient (mg cm^{-2} d^{-1}); x represents the initial mass:initial surface area ratio (mg cm^{-2}); $v_{\max}$ (mg cm^{-2} d^{-1})=maximum degradation rate coefficient; and k_{m} (mg cm^{-2})=half saturation constant. For ML1 $v_{\max}$ is 4.6 mg cm^{-2} d^{-1} and k_{m} is 186 mg cm^{-2} (Fig. 3, top curve). The $v_{\max}$ calculated for ML2 (Fig. 3, bottom curve) was 1.1 mg cm^{-2} d^{-1}, with a k_{m} of 31.9 mg cm^{-2}. ML2 resulted in a much smaller $v_{\max}$ and k_{m} (25 and 17% of k_1 and k_{m} from ML1, respectively). The sludge in the first mass loss experiment degraded the PHB more quickly than the sludge in the second study (Fig. 3).

Table 5. Degradation Rate Coefficients of All Materials Tested in the First and Second Mass Loss Experiments

Experiment/ test material	Initial mass:initial surface area ratio (mg cm^{-2})	Degradation rate coefficient (mg cm^{-2} d^{-1})
	ML1 Experiment	
PHB 0.5 mm	31.7	0.58
PHB 1.2 mm	66.0	1.29
PHB 3.5 mm	152.6	2.07
	ML2 Experiment	
PHB 0.24 mm	12.3	0.32
PHB 1.2 mm	66.7	0.73
PHB 5 mm	183	0.95
1.2-mm 85% PHB/15% TBC	66.7	0.38
PLA 1.2 mm	66.7	0.00

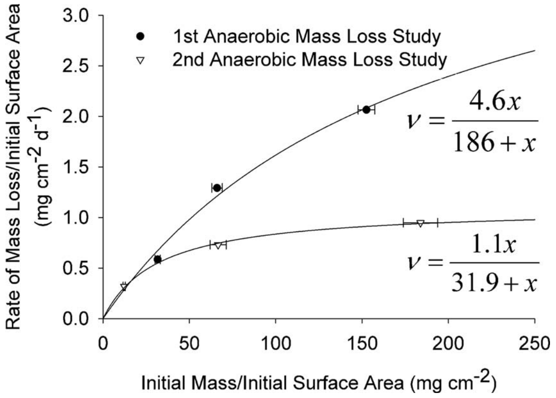

Fig. 3. Relationships between the degradation rate coefficient (mg cm^{-2} d^{-1}) and the initial mass:initial surface area ratio (mg cm^{-2}) for the three different plate sizes of PHB degraded in ML1 and the three different plate sizes degraded in ML2

The difference in degradation rates obtained in ML1 and ML2 may be attributed to a difference in the sludge activity. These results indicate that caution should be used when interpreting and comparing data obtained under different conditions. The relationship between degradation rate and initial mass:initial surface area ratio indicates that this ratio should be taken into account when calculating degradation times for PHB based products.

Characterization Analyses

Chemical Properties

FT-IR analyses indicate that biodegradation appeared to have very little effect on the spectrograms of the PHB bioplastic, suggesting that the cleavage of the different types of bonds is similar throughout the polymer during the degradation process. These findings are consistent with those of Day et al. (1994), who found no difference in the FT-IR of PHBV due to degradation. One of the more notable differences observed in the degraded plastic spectrograms was the appearance of peaks specific to peptide groups at wave numbers of 1,535 and 1,642 cm^{-1}, most likely from proteins of the degrading bacteria in the sludge medium.

Molecular weight determinations of the undegraded PHB plates and those with 0.5, 1.2, and 3.5 mm thickness (M_w of the undegraded plates ranged from 1.2×10^5 to 1.9×10^5 Da) degraded during ML1 indicated that biodegradation had little to no effect on the molecular weight (M_w) of the PHB bioplastic. Other investigators working with PHB and PHBV copolymers under anaerobic conditions have shown similar results (i.e., Doi et al. 1990; Day et al. 1994; Mergaert et al. 1994). The lack of change in M_w observed has led researchers to believe that the polymer is degraded enzymatically at the surface to lower-molecular-weight fragments that are rapidly metabolized by the degrading microorganisms causing mass loss but no effect on the molecular weight of the remaining polymer. This will indicate that the remaining plastic could be recycled and reused.

Table 6. DSC Thermal Data of the Undegraded Bioplastics

	First heating		Cooling	
Experimental material	T_m (°C)	ΔH_m (J g^{-1})	T_c (°C)	ΔH_c (J g^{-1})
PHB powder	165.0	106.1	104.1	82.9
0.24-mm PHB	167.6	89.9	102.2	74.3
1.2-mm PHB	165.6	91.9	103.3	82.7
3.5-mm PHB	168.4	99.2	103.6	82.4
5-mm PHB	163.5	104.1	103.3	73.3
85% PHB/15% TBC	153.4	81.9	101.7	59.2

Thermal Properties

An interesting trend was noticed in the ΔH_m values for the PHB plates. The ΔH_m during the first heating increased with the thickness of the PHB plates, ranging from approximately 90 J g^{-1} for the 0.24-mm plates to 104 J g^{-1} for the 5-mm plates (Table 6). This means the crystallinity of the PHB was dependent on the plate thickness and ranged from approximately 62% for the 0.24-mm plates to 71% for the 5-mm plates as determined from the ratio $\Delta H_f / \Delta H_f^0$, where ΔH_f and ΔH_f^0 are the observed enthalpy of fusion and the enthalpy of fusion of perfect PHB crystals (146 J g^{-1}) as reported by Barham et al. (1984), respectively. The difference in crystallinity can be attributed to the differences in cooling rates of the melt-pressed plates as the thinner plates cooled faster than the thicker plates. It is known that the crystallization rate of PHB increases with increasing heating/cooling rates, while the half-time of crystallization decreases (An et al. 1998) and, as a result, the crystallinity decreases (Chen et al. 2005).

The TBC additive in the 85% PHB/15% TBC plates lowered the melting and crystallization temperatures and enthalpies (T_m, T_c, ΔH_m, and ΔH_c) compared to those of the pure PHB. This indicates a lower degree of crystallinity for the blend due to the addition of TBC that nonetheless showed a lower biodegradation rate. The lower crystallinity reduces the brittleness of the PHB and makes the material easier to process. Lower values of T_m with the TBC additive were also observed by Labrecque et al. (1997) with TBC/PLA blends.

Biodegradation appeared to have only minor effects on the thermal properties of the PHB. For some samples from both mass loss experiments, a slight decrease in the crystallization enthalpy (ΔH_c) and the melting enthalpy (ΔH_m) was observed. This was more noticeable for the 1.2-mm PHB plates in the first mass loss experiment and the 0.24-mm PHB plates degraded in the second mass loss experiment at eight weeks just before biodegradation was complete. This decrease is most likely due to proteins from the degrading microorganisms and possibly other debris becoming incorporated into the polymer. The observed decreases in ΔH_m after degradation are consistent with data reported in literature by Tercjak et al. (2003), who degraded blends of a commercial plastic (Biopol) and polyamide 11 in compost.

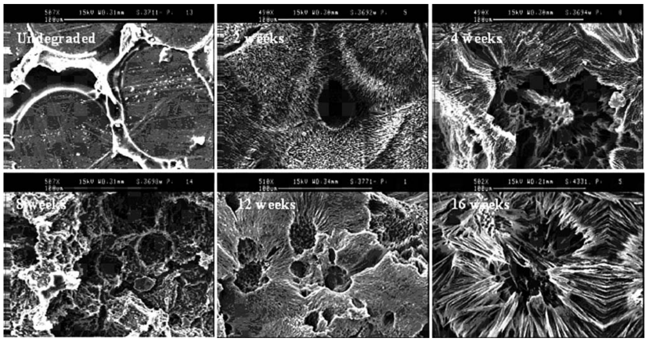

Fig. 4. SEM photographs of 3.5-mm-thick melt-pressed PHB plates undegraded and after 2, 4, 8, 12, and 12 weeks of anaerobic degradation in ML2. Captions indicate time of degradation.

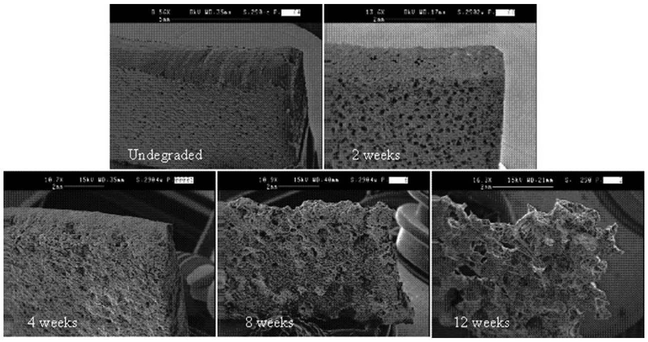

Fig. 5. SEM images (500× magnification) of 1.2-mm melt-pressed PHB plates undegraded and after 2, 4, 8, 12, and 16 weeks of exposure in ML2. Captions indicate time of degradation.

Physical Properties

A gradual decomposition and increasing porosity of the PHB plates over time was observed in ML1 and ML2. Illustrative examples of these changes are shown in the SEM micrographs of the 3.5-mm PHB plates degraded in ML1 (Fig. 4) and SEM micrographs of the 1.2-mm PHB plates degraded during ML2 (Fig. 5). For the 3.5-mm PHB plates (Fig. 4) a progressive increase in the porosity can be observed, with larger pore away from the surface after four weeks. The surface of the plates is almost completely eroded by week 8. By 12 weeks, the plate loses its shape. The ML2 1.2-mm PHB plates (Fig. 5) showed some holes in the surface after two weeks, which were most likely remnants of the large crevasses seen on the undegraded material which surrounded spherulitic structures within the material. Most degradation at this point appeared to have occurred primarily at the surface, which became very matte. By week 4, microbial attack within the material was apparent with lesions becoming larger, more abundant, and more scattered. By week 16, the remaining surface structures had changed their appearance to a network of narrow and scattered sickles, with regions of the material most likely lamellar crystals, left behind by microbes that favored the more amorphous regions. This is consistent with the findings of Li et al. (2007) who reported preferential degradation of the amorphous phase by bacterial PHB depolymerases. For ML1, the surface change of the plates followed the same pattern as in ML2 but proceeded at a faster rate. In contrast, for the 85% PHB/15% TBC blend, the degradation appeared to be mainly at the surface of the plate. This may explain why the degradation of the blend was slower than that of the pure PHB. The difference in degradation patterns emphasizes the importance of testing biodegradable materials after the incorporation of additives to ensure that the resulting product biodegradability is maintained.

Conclusions

This study clearly shows a relationship of the anaerobic degradation coefficients of PHB with the initial mass:initial surface area ratio. These results show that the thickness of the material with respect to mass changes the rate of degradation and should be considered in the calculations for disposable products. In addition, the mass reduction of the PHB and the degradation rates change with the specific sludge being used. This indicates that a series of tests should be performed to determine the average degradation rates and the percent of biodegradation that can be expected in a given degradation environment. The TBC additive reduces the degradation rate, but not the maximum degradation of the PHB, indicating that natural additives can be used to modify the characteristics and degradation rate of the PHB, without impairing its final biodegradation. The degradation studies have shown that the PHB bioplastic degrades at least as well as cellulose in anaerobic sewage sludge. The similarity of the molecular weight before and after degradation indicates that the PHB degradation proceeds from the surface and the remaining plastic can be recycled.

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