Journal of Environmental Polymer Degradation, Vol. 3, No. 3, 1995

Assessing the Biodegradation Potential of Polymers in Screening- and Long-Term Test Systems*

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This paper presents a test scheme for assessing the biodegradation potential of polymers, starting with aquatic screening systems (aerobic and anaerobic) and continuing to long-term systems. At the end of the scheme the material has to prove its behavior under the relevant disposal conditions. Aerobic screening was performed mainly under aquatic conditions, but also in soil, using BOD-respirometry. Carbon balances were performed to obtain a better evaluation of the biodegradation potential. Under anaerobic conditions, biodegradation in an aquatic medium was followed by measuring CH_4 and CO_2 production. Polymers not fully degraded in the screening systems were tested in aquarium systems for at least 1 year. Biodegradation was followed by monitoring the DOC released in the water, mass loss, and microbial growth on the samples and in the water as well as via FTIR spectroscopy and SEM pictures. Results are presented for the polymers PHB, PHBV, PCL, Mater-Bi AI05H and ZF03U, and Bioceta. By combining the data from the screening with the aquarium system, a good picture of the degradation behavior of the polymers is obtained.

KEY WORDS: BOD respirometer; anaerobic biodegradation; poly(β-hydroxybutyrate); poly(ε-caprolactone); Bioceta; Mater-Bi; aquarium test system.

INTRODUCTION

During the last 40 years, plastics have become an essential component of most of the articles used in everyday life. While the versatility and convenience of plastics are still important, increasing public awareness of the environmental stability of plastics has caused some concern.

So-called "biodegradable" plastics have been found on the market for a few years now. The basic equations raised refer to the extent of biodegradation and the test methods used for assessing biodegradability. Several committees throughout the world are discussing how biodegradable plastics should be defined and what test systems are most applicable. But there is a shortage of data that describe the behavior of biodegradable polymers in biodegradation test systems.

At the Institute of Water, Wastewater Engineering, and Refuse Disposal at Stuttgart University, we had the chance to work on a 3-year project, supported by the German Ministry of Research and Technology, to assess the biodegradation of different types of biodegradable polymers in screening, long-term, and actual disposal systems. A test scheme was proposed that starts with aquatic screening systems (aerobic and anaerobic) and continues to long-term systems. At the end of the scheme, the biodegradability of the material must be established under the relevant disposal conditions.

This paper presents some of the data we obtained with poly(β-hydroxybutyrate) (PHB) and its copolymer poly(β-hydroxybutyrate-co-β-hydroxyvalerate) (PHBV), poly(ε-caprolactone) (PCL), Mater-Bi AI05H (starch and natural additives plus vinyl-alcohol/ethyl-

*Paper presented at the Bio/Environmentally Degradable Polymer Society—Third National Meeting, June 6–8, 1994, Boston, Massachusetts.

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1064-7546/95/0700-0133$07.50/0

ene copolymer), Mater-Bi ZF03U (starch, natural additives, and PCL as main component, vinyl-alcohol copolymer as minor component), and Bioceta (cellulose diacetate with approximately 20% natural additives) under aerobic and anaerobic screening conditions and in a long-term aerobic system (aquarium).

EXPERIMENTAL

Materials

PHB G08 powder (M_w, 539,000; M_n, 131,000; mean particle size, 200 μm), PHBV films with a 10% HV content (17 μm thick), and PHBV testbars with 4.2% HV (surface, 18 and 39 cm^2 each) were provided by Zeneca Bioproducts, Billingham, UK. Bioceta-films (90 μm thick) were made available through Tubize Plastics in Tubize, Belgium, Mater-Bi films (AF06H and ZF03U; 35 μm thick) and Mater-Bi testbars (AI05H; surface, 15 cm^2 each) were provided by Novamont, Italy. Films made of PCL P-787 and of PCL P-787 blended with 30% LLDPE (55 μm thick) and PCL P-787 powder (particle size, 250 μm) were provided by Union Carbide.

Aerobic Screening

The aerobic screening was performed in an automatized BOD respirometer (BSB-Digi, J. Otto GmbH, Bodelshausen, Germany) with 500-ml flasks containing 250 ml medium. Medium was according to the modified MITI test [1], except for stock solution A, which was made up of a stronger phosphate buffer with a higher N content (37.5 g KH_2PO_4, 69.7 g $Na_2PO_4 * 2\ H_2O$, and 15 g NH_4Cl in 1 L deionized water). To inhibit nitrification 10 ml of an allylthiourea solution (0.025 g in 100 ml distilled water) was added. Reactors were inoculated with activated sludge or a 1:1 mixture of activated sludge with sewage effluent [1% (v/v)]. Samples were introduced as powder or films (5 × 5 mm) and incubated at 20°C in the dark. At least two parallel tests with samples and two blanks (medium plus inoculum) were set up. Before and after incubation the following parameters were determined: DOC (dissolved organic carbon) of the 0.45-μm-membrane filtrate, with DOC Analyzer TOCOR Maihak, Hamburg. via CO_2-infrared gas analyzer UNOR 6N; pH; biomass-carbon, numbered by determining the protein content (modified Lowry method [2]), assuming that bacteria consist of 50% protein as well as 50% carbon—thus the amount of protein determined was set equal to the amount of biomass carbon; and nitrate and nitrate concentration, with ion chromatography on a 690 IC Metrohm system [3] with conductivity detection at 35°C.

Theoretical oxygen demand (ThOD) of the substance $C_cH_hO_oN_nP_pS_sNa_{na}X_x$ (where X = halogen) of relative molecular mass M_r was calculated according to [4]

$$\mathrm{ThOD} = \frac{16[2c + 0.5(h - x - 3n) + 3s + 2.5p + 0.5na - o]}{M_r}$$

This calculation implies that carbon is mineralized to CO_2, hydrogen to H_2O, phosphorus to P_2O_5, and sulfur to an oxidation state of +6, halogens are eliminated as hydrogen halides, and nitrogen to ammonia.

Biodegradation was calculated by relating the ThOD to BOD after incubation and transforming the percentage ThOD into oxidized polymer carbon:

$$C_{oxP} = \frac{\%\ \mathrm{ThOD} \times C_P}{100} = \text{oxidized polymer carbon}$$

Biomass carbon and DOC increase were added to calculate the biodegraded polymer carbon:

$$\%\ \text{biodegraded } C_P = C_{oxP} + \Delta C_B + \Delta\mathrm{DOC}$$

C_P = polymer carbon

C_B = biomass carbon

δC_B = C_B after incubation − C_B before incubation

ΔDOC = DOC after incubation − DOC before incubation

Anaerobic Screening

The anaerobic test system consisted of 300-ml flasks filled with 250 ml anaerobic phosphate-buffered medium [5] containing trace elements and vitamins for anaerobic bacteria [6, 7]. The test flasks were connected via Viton tubes to a gas collection system filled with acidified 20% NaCl solution. The medium was inoculated with domestic sewage sludge (100 ml/L), and samples were added to the test flasks as powder or films (5 × 5 mm). Samples and blanks (medium plus inoculum) were tested in two parallel trials incubated at 35°C.

Degradation was followed by biogas production and measured volumetrically through daily readings of the amount of NaCl solution displaced. Biogas was analyzed regularly for CH_4 and CO_2 content through gas chromatography via thermal conductivity on activated carbon (2-m column) (temperature, 150°C detector, 150°C injector, 80°C oven; bridge current, 180 mA). Before and after incubation DOC, pH, and protein content were measured (see above methods).

Theoretical values for CH_4 and CO_2 (biogas) production were calculated using the equation [8]

$$C_nH_aO_b + \left[n - \frac{a}{4} - \frac{b}{2}\right] H_2O - \rightarrow \left[\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right] CO_2 + \left[\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right] CH_4$$

Biodegradation was expressed as a percentage of the theoretical values for Biogas (ThBiogas) and methane ($ThCH_4$). Because of the good solubility of CO_2 in aqueous media, CO_2 was also determined in the medium via acidification or by DIC analytics (dissolved inorganic carbon, measured at TOCOR 2 via CO_2-infrared gas analyzer; see DOC above). To exclude pressure and temperature effect, biogas volume readings were transformed to standard conditions:

$$V_0 = V \times \frac{(p_L - p_W) \times T_0}{p_0 \times T}$$

V_0 = ml standard volume biogas
V = ml volume biogas readings
p_L = mbar air pressure at time of gas reading
p_W = mbar water pressure, temperature dependent
T_0 = standard temperature; T_0 = 273 K
p_0 = standard pressure; p_0 = 1013 mbar
T = K temperature of biogas at time of gas reading

Percentage of theoretical biogas and methane production were calculated as follows

$$\% \text{ ThBiogas} = \frac{\text{ml Biogas (Test)} - \text{ml Biogas (Blank)}}{\text{ml ThBiogas}} \times 100$$

$$\% \text{ ThCH}_4 = \frac{\text{mmol CH}_4 \text{ (Test)} - \text{mmol CH}_4 \text{ (Blank)}}{\text{mmol ThCH}_4} \times 100$$

Aquarium System

The aquarium system consisted of 13 L of continuously aerated phosphate-buffered mineral medium (15.09 g KH_2PO_4, 134.6 g $Na_2PO_4 * 2H_2O$ in 13 L deionized water plus 13 ml each of solutions B to E of the medium for modified MITI test [1]). The following parameters were determined regularly: DOC, pH, total bacterial number via most-probable-number (MPN) method in nutrient broth (Merck 5443), number of PHB-degrading and starch-degrading organisms on PHB- and starch-agar plates (see agar plates preparation), and fungi on malt agar plates (Merck 5398). Bacterial numbers were determined in the water and on the samples. Biofilms were scratched from the polymer surface with a sterile fork into sterile NaCl solution (0.9%) for bacterial counts.

Agar Plate Preparation

PHB-Agar. Dissolve 3.5 g K_2HPO_4, 1.5 g KH_2PO_4, 0.1 g $MgSO_4 \times 7H_2O$, 1 g NaCl, 1 g $(NH_4)_2SO_4$, and 15 g agar-agar in 800 ml distilled water; dissolve 0.5 g PHB powder in 200 ml distilled water and emulsify for 15 min under vacuum in an ultrasonic bath; mix both solutions after being autoclaved separately and then pour into petri dishes.

Starch-Agar. Dissolve 3.5 g K_2HPO_4, 1.5 g KH_2PO_4, 0.1 g $MgSO_4$, 1.0 g NaCl, 1 g $(NH_4)_2SO_4$, 2 g starch, and 15 g agar-agar in 1 L distilled water, pH 7.2.

SEM

SEM pictures were taken by the Fraunhofer Institute of Food-Technology and Packaging in Munich, Germany [9].

RESULTS AND DISCUSSION

Aerobic Screening System

PHB and PCL were fully biodegraded during the aerobic screening process, while with Mater-Bi and Bio-

ceta biodegradation of only the readily degradable part of the polymers was detected. PHB powder (200 mg/L) and PCL powder (400 mg/L) were tested in mineral salt medium inoculated with 1% (v/v) of a mixture of activated sludge and sewage effluent (1 : 1). PHB degraded rapidly, after a lag phase of 3.5 days, to an extent of 79% ThOD. For PCL the lag phase lasted 6 days, and after 20 days the degree of biodegradation was 87% ThOD (Fig. 1).

Films of PHBV (200 mg/L) and Mater-Bi ZF03U (800 mg/L) were incubated with a 1% (v/v) activated sludge inoculation. After a short lag phase of 2 days PHBV degraded rapidly to 70% ThOD. Mater-Bi ZF03U showed a longer lag phase (5.5 days) but reached a similar degree of degradation as PHBV after 25 days. PCL films had the longest adaptation phase (15 days) but, once started, degraded rapidly to 82% ThOD. Only Bioceta films (600 mg/L) and Mater-Bi AF05H films incubated in sewage water started degrading without a visible lag phase; however, biodegradation stopped at 22% ThOD for Bioceta and at 30% ThOD for Mater-Bi AF05H (Fig. 2).

PHB, PCL, and Mater-Bi ZF03U exhibited similar extents of biodegradability as determined in the aerobic percentage ThOD screening test. Looking at the carbon balance, which includes biomass and DOC, PHB and

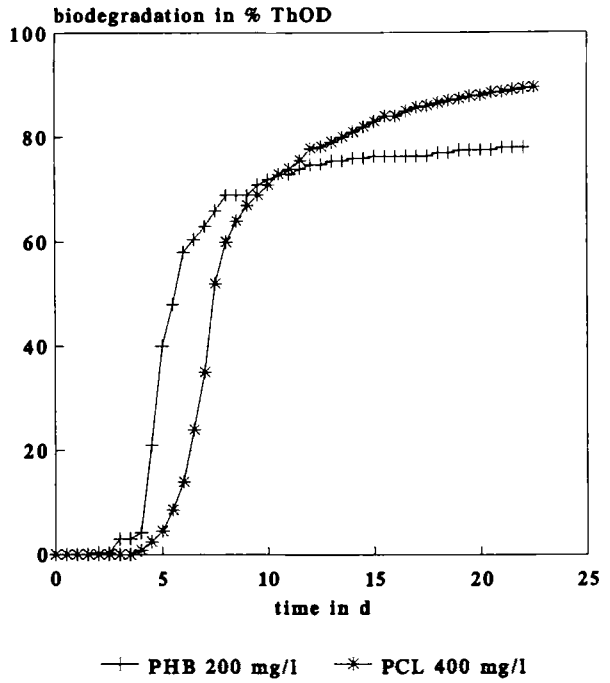

Fig. 1. Biodegradation as percentage ThOD of PHB and PCL powder under aerobic screening conditions. Mineral salt medium with 1% (v/v) mixture of activated sludge and sewage effluent.

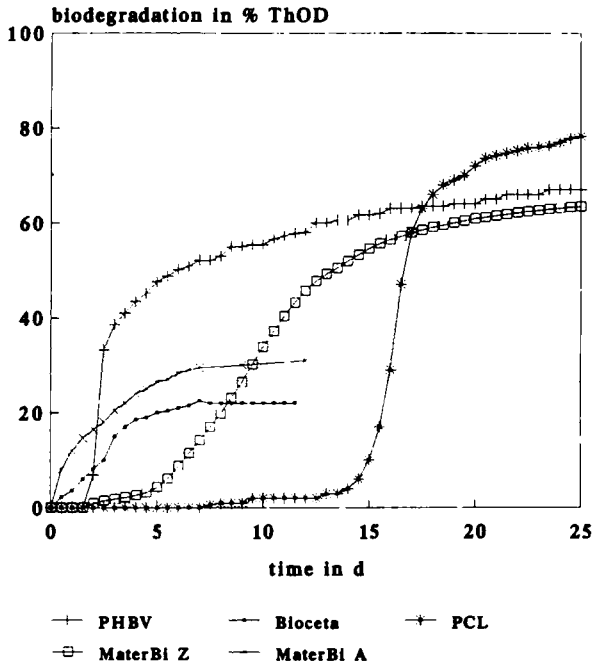

Fig. 2. Biodegradation under aerobic screening conditions of PHBV and Mater-Bi ZF03U films inoculated with 1% (v/v) acitvated sludge, PCL films inoculated with 1% (activated sludge + sewage effluent; 1 : 1), and Bioceta and Mater-Bi AF05H films incubated in sewage water.

PCL were fully biodegraded (Table I). Carbon not being oxidized was found primarily in biomass and low molecular weight soluble carbon (DOC). No visible sign of the polymers remained after screening was completed. The biodegradation of Mater-Bi ZF30U also resulted in an increase in biomass, but the C balance shows that 77% of the polymer carbon could be detected as being oxidized (BOD) or transfered in biomass or DOC. These data indicate that approximately 23% of the carbon remained as polymer, which was still visible as intact film in the reaction vessel after incubation.

These data show that the BOD parameter in itself is not sufficient to prove total biodegradation, especially if the polymer consists of more than one compound. Differences in biomass production can lead to apparently high values of percentage ThOD but might hide important differences in the real degree of biodegradation. The importance of including biomass production for calculating the degree of biodegradation was also demonstrated by Müller et al. [10].

Anaerobic Screening System

PHB homopolymer (400 mg/L) was tested for anaerobic biodegradation several times using the same me-

Table I. Balance: Comparing C Input (Polymer C + Biomass C + DOC) with C Output After Incubation (BOD Calculated as Oxidized Carbon + Biomass C + DOC)[a]

	Polymer	mg/L	Polymer C (mg/L)	Biomass C (mg/L)	DOC (mg/L)	Oxidized C (mg/L)	Total mg/L	Total %
Input	PHB powder G08	200	111.6	7.0	8.0		126.6	100
Output				21.0	10	88.2	119.2	94
Input	PCL-787 powder	404	255	7.2	2		264.3	100
Output				23.2	7.9	221	252.1	95
Input	PHBV films	201	112.5	1.4	2.3		116.5	100
Output				21.1	21.8	85	127.9	110
Input	PCL-787 film	400	252	6.6	7.5		266.1	100
Output				22.0	15.0	207	244.0	92
Input	Mater-Bi ZF03U	800	448	1.4	2.3		452	100
Output				26.2	12.7	307	345.9	77

[a]Incubation time: PHB powder, PHBV film, and PCL film, 30 days; PCL powder, 18 days; Mater-Bi, 64 days.

dium (Fig. 3). Biogas production started after a lag phase of 3 and 4 days. After 10 to 20 days of incubation, biogas production (determined volumetrically) reached a plateau of 60% ThBiogas. Because CO_2 is very soluble in water (145 ml CO_2 is dissolved in 250 ml water at 35°C and 1013 mbar, compared to only 6.5 ml CH_4), the gas volume measured in the head space above the medium will not reflect the total amount of CO_2 produced. Therefore, after incubation the dissolved CO_2 was measured as DIC or the medium was acidified with HCl and the dissolved CO_2 purged out of solution and measured volumetrically. Through this, the degree of degradation was calculated to be around 80% ThBiogas.

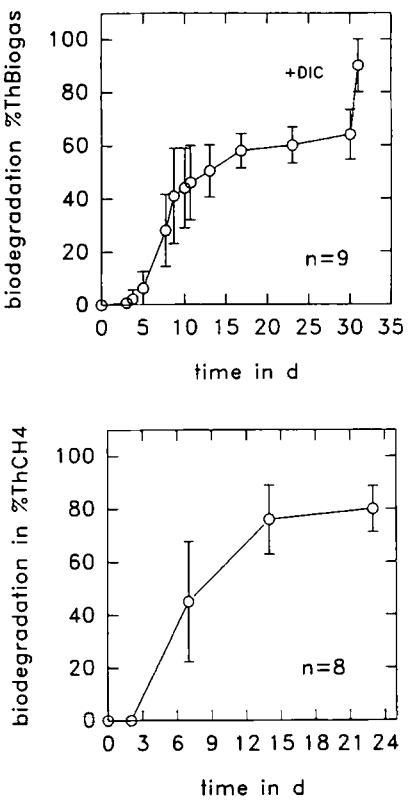

Fig. 3. Biodegradation of PHB powder (400 mg/L) in the anaerobic screening system: biodegradation as percentage ThBiogas (top) and as percentage $ThCH_4$ (below); average curve with confidence limits.

Methane is the important end product of anaerobic biodegradation and was therefore considered to be a separate parameter for determining the degree of biodegradation. For PHB, the average degradation grade was around 80% $ThCH_4$, thus matching the percentage ThBiogas when dissolved CO_2 was included (Fig. 3). Budwill *et al.* [11] also demonstrated the anaerobic biodegradation of PHB, detecting 90% ThBiogas after 21 days of incubation in an anaerobic mineral medium inoculated with domestic sewage sludge.

Films of Mater-Bi Z30U (600 mg/L) were less biodegradable under anaerobic conditions than under aerobic conditions. Including the DIC-values, Mater-Bi was degraded to 22% ThBiogas after 60 days of incubation, and biogas production apparently had stopped at day 45 (Fig. 4). The last CH_4 measurement, at 34 days, had yielded only 10% $ThCH_4$ (Fig. 5).

Bioceta had degraded to a degree of 28% ThBiogas, including DIC values, after 60 days (Fig. 4). At this time, biogas production, however, was still increasing slowly. After 34 days, the amount of CH_4 produced was only 13% $ThCH_4$ (Fig. 5).

Biogas production in reactors containing PCL films never exceeded the values reached in the blank, under anaerobic conditions during 60 days of incubation.

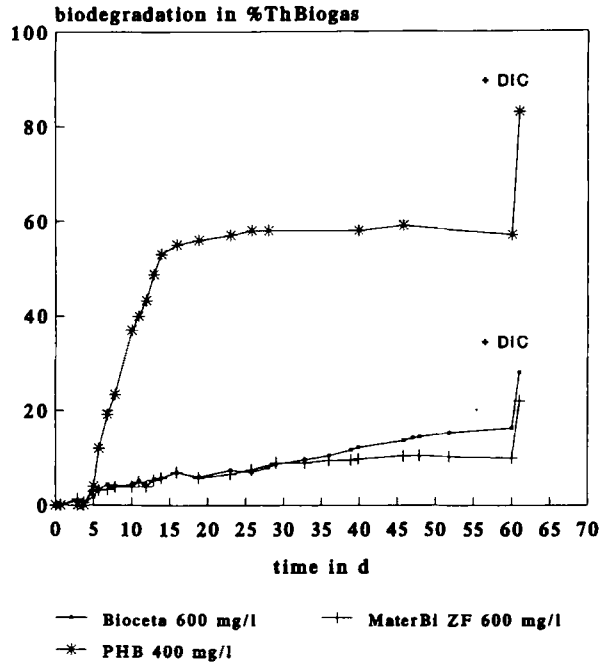

Fig. 4. Biodegradation of PHB powder and Bioceta and Mater-Bi ZF30U films under anaerobic conditions as percentage ThBiogas including the DIC content of the medium after incubation.

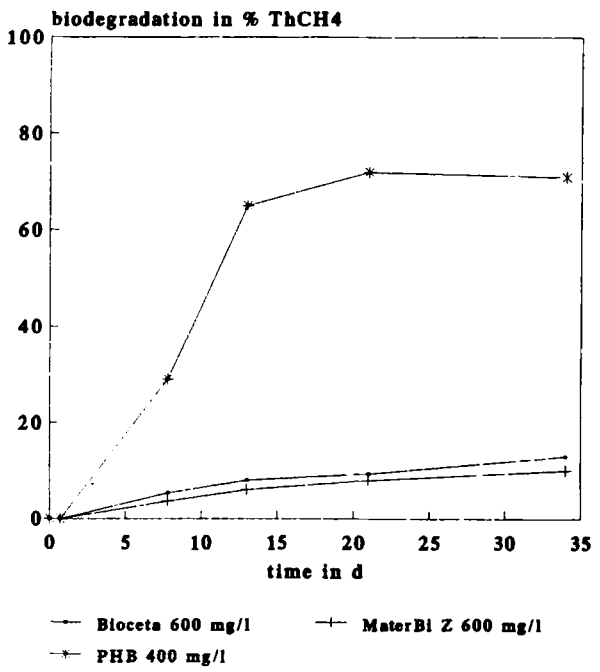

Fig. 5. Biodegradation of PHB powder and Bioceta and Mater-Bi ZF30U films under anaerobic conditions as percentage $ThCH_4$.

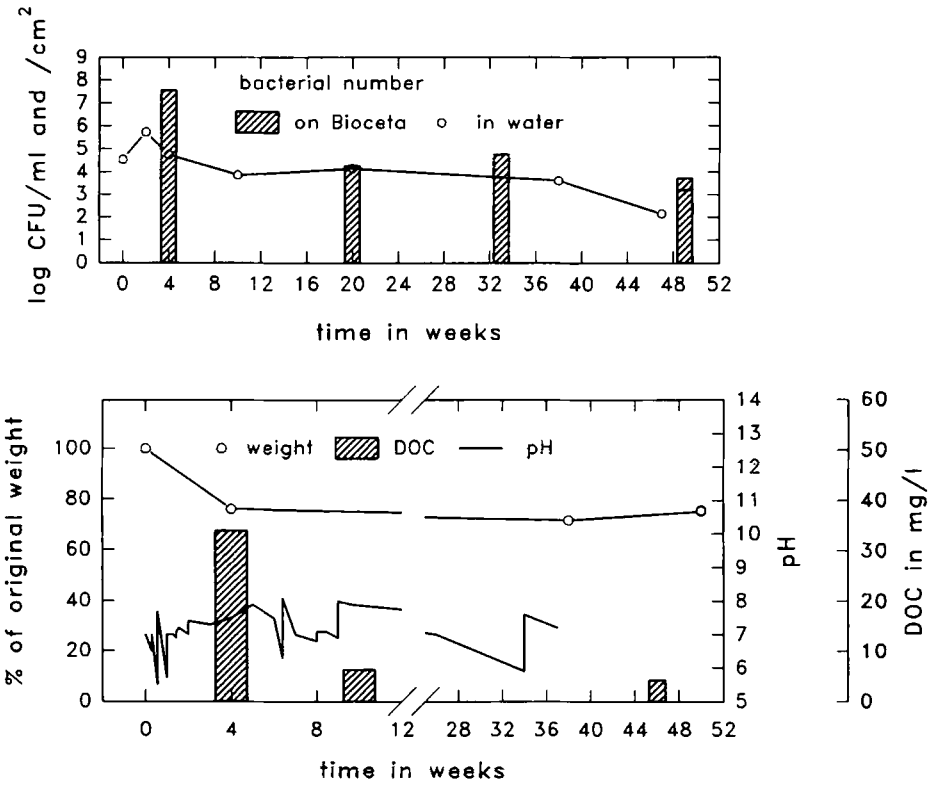

Fig. 6. Bioceta films (90 μm thick) incubated in an aerobic aquarium system: development of the microbial population (CFU, colony forming units) on the films and in the water. Weight loss as percentage, DOC, and pH development during incubation. The x axis was changed to point out the decreasing pH values at the beginning of incubation.

Aquarium Test Systems

Aquarium systems are used to simulate surface water environments. Often several polymers are tested in parallel in the same system [12, 13] and degradation is characterized by weight loss related to the polymer surface. We used small (13-L) aquariums and tested only one type of polymer at a time so that data concerning pH, DOC, and bacterial population development could provide additional information regarding the degradation behavior.

During the first 4 weeks of incubation with Bioceta films, a large increase in DOC was detected. The pH value of the medium had to be neutralized due to a severe decrease in the first week. The only significant weight loss that occurred during the incubation time of 52 weeks was also measured after the first 4 weeks of incubation (Fig. 6). During these first 4 weeks the highest bacterial numbers were counted both on the sample and in the water (Fig. 6). SEM pictures show that the original film had no visible surface corrosion but that cracks started to develop by the end of the 4-week incubation (Fig. 7).

With Mater-Bi AF-test bars weight loss was continuous, with peak rates during the first 20 weeks of incubation. Bacterial numbers were much higher on the testbars than in the water and consisted mostly of starch degrading organisms (Fig. 8). The regular hill structure of the original surface appeared to become smoother after 4 weeks of incubation and eventually showed ir-

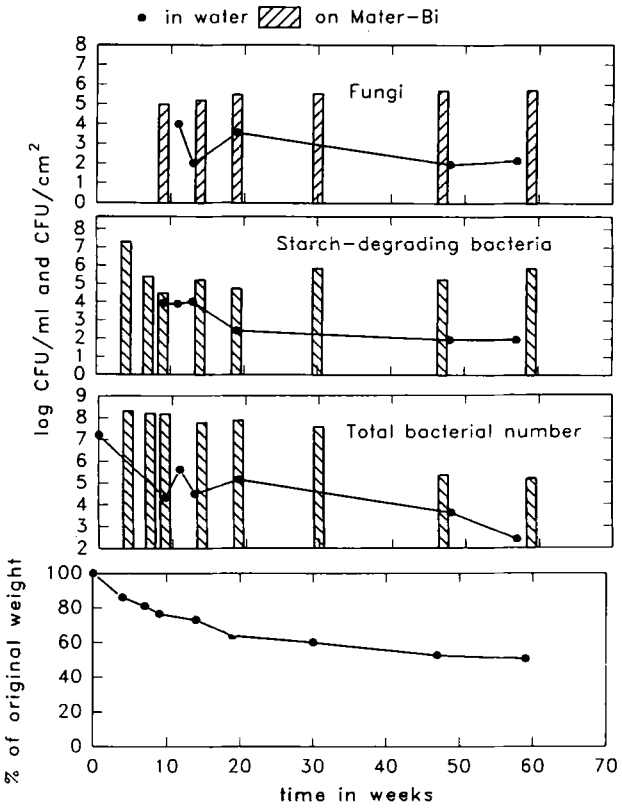

Fig. 8. Mater-Bi AI05 testbars incubated in an aerobic aquarium system: development of the microbial population (fungi, starch degrading bacteria, and total bacterial counts expressed as CFU = colony forming units) on the sample and in the water and weight loss as percentage during the incubation time of 59 weeks.

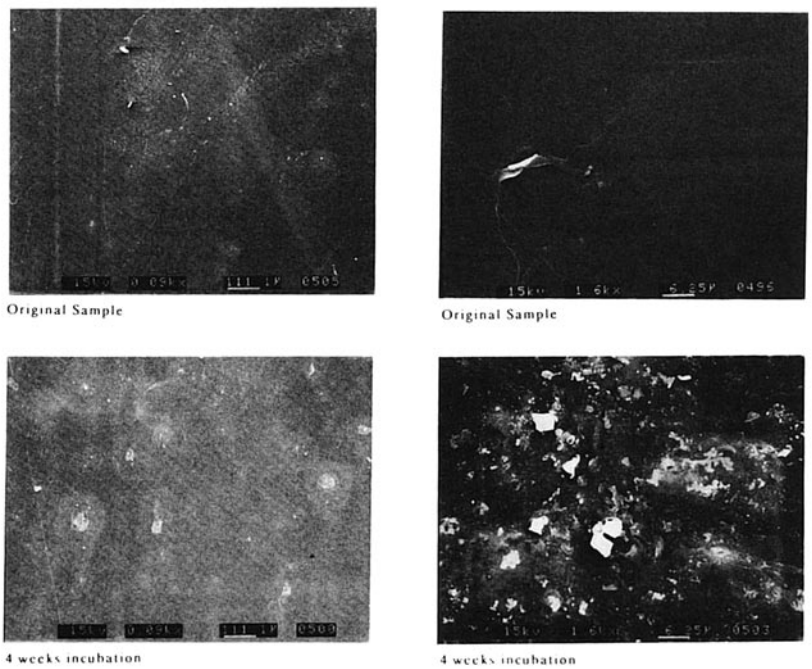

Fig. 7. SEM pictures of the original Bioceta film and after being incubated in the aerobic aquarium system for 4 weeks. Magnification, 0.09 (left) and 1.6 (right).

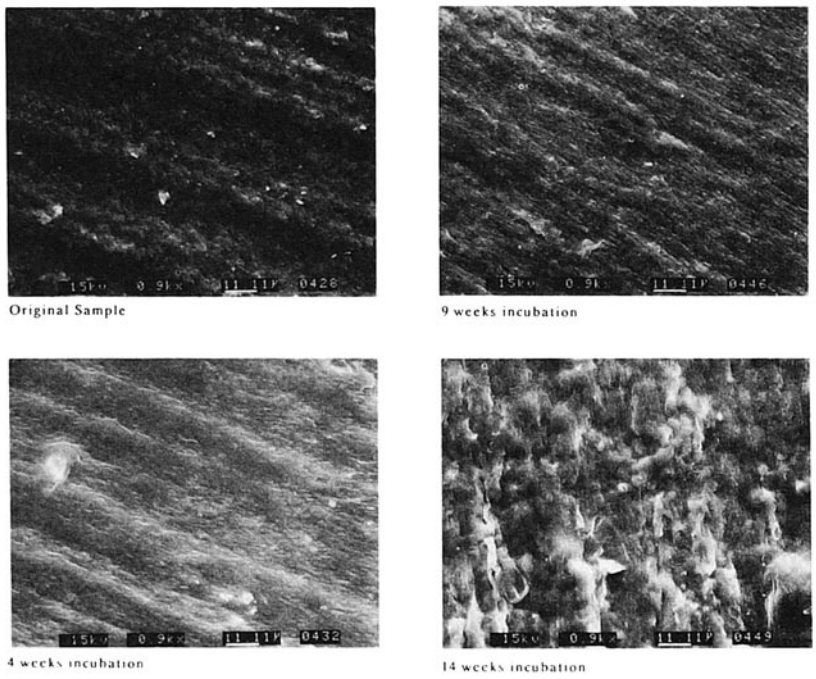

Fig. 9. SEM-pictures of the original Mater-Bi testbar and after being incubated in the aerobic aquarium system for 4, 9, and 14 weeks. Magnification, 0.9.

regular inlets (Fig. 9). IR spectroscopy made clear that after 50 weeks of incubation the starch component had disappeared in the polymer to a depth of 500 μm [9]. This gives the overall picture that starch was degraded from the surface; the open pores that formed probably filled with water and became channels for exoenzymes, which degraded the starch located deeper in the polymer [14]. As Siehr [15] showed for Mater-Bi in aquatic systems, starch also tends to diffuse out of the polymer compound into the surrounding water. As no increase in DOC was noticed, however, the starch was probably metabolized right at the polymer surface.

PHB testbars were fully degraded during 1 year. Total bacterial numbers were always higher than the PHB-degrading organisms, indicating that—through extracellular enzyme activity—enough substrate is available for the growth of other bacteria (Fig. 10). The polymer surface became highly corroded during 18 weeks of incubation (Fig. 11).

PCL films made of 100% PCL were fully biodegraded after 8 weeks (Fig. 12). After 4 weeks, the films had lost 80% of their original weight, and after 8 weeks they were no longer detectable in the water; the DOC had not increased (data not shown). Thus PCL was fully biodegraded. With only 30% LLDPE in the polymer, the biodegradation rate was slowed and only 25% weight

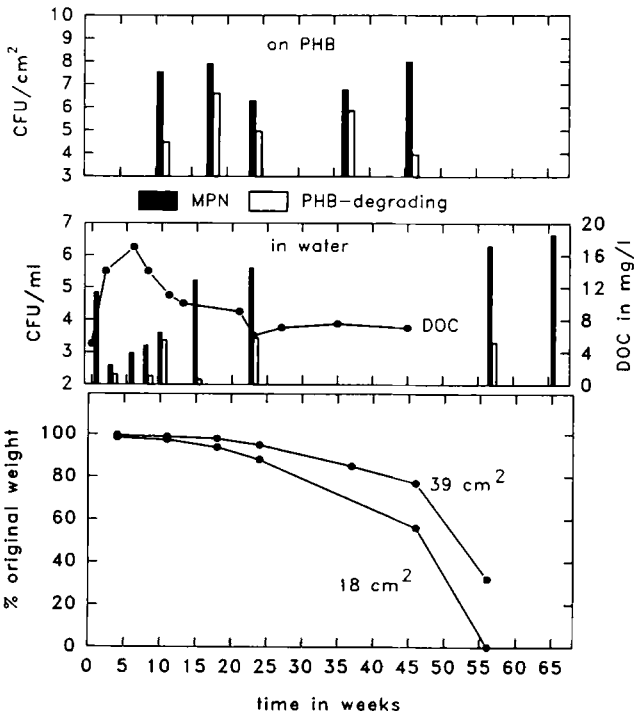

Fig. 10. PHBV testbars incubated in an aerobic aquarium system: development of the microbial population (PHB degrading bacteria and total bacterial counts expressed as CFU = colony forming units) on the sample and in the water and weight loss as percentage during the incubation time of 56 weeks.

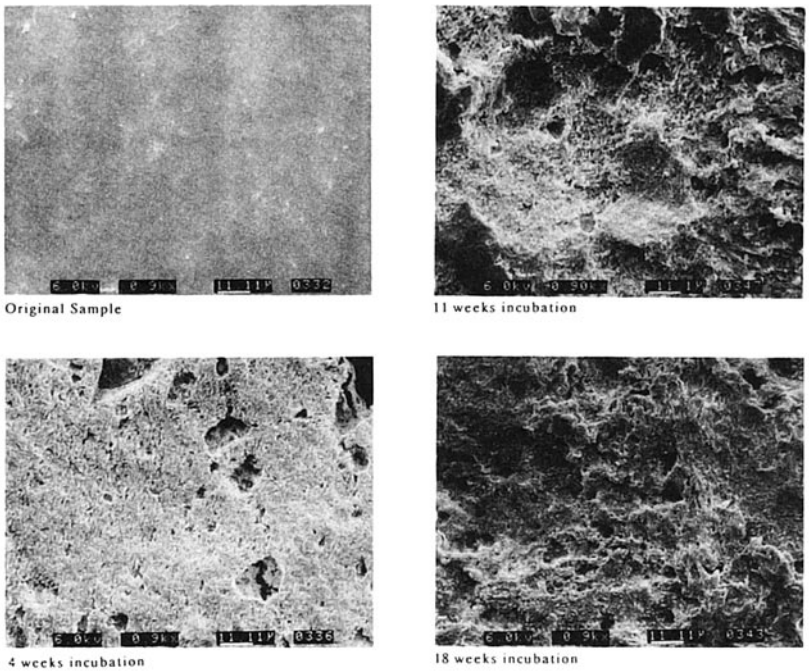

Fig. 11. SEM pictures of the original PHBV testbar and after being incubated in the aerobic aquarium system for 4, 11, and 18 weeks. Magnification, 0.9.

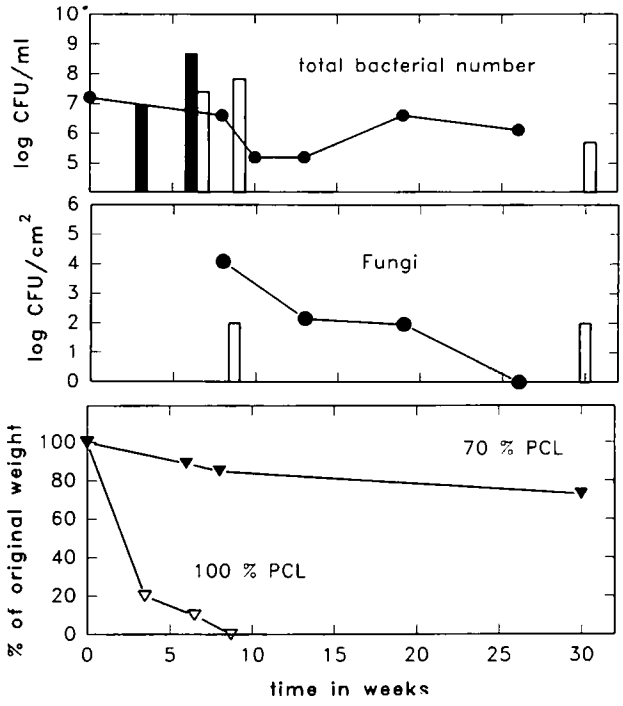

Fig. 12. Films of PCL and PCL with LLDPE (7:3), 55 μm thick, incubated in an aerobic aquarium system: development of the microbial population (CFU = colony forming units) on the films and in the water and weight loss (%). Filled circles, in water; filled columns, with 100% PCL; open columns, with 70% PCL.

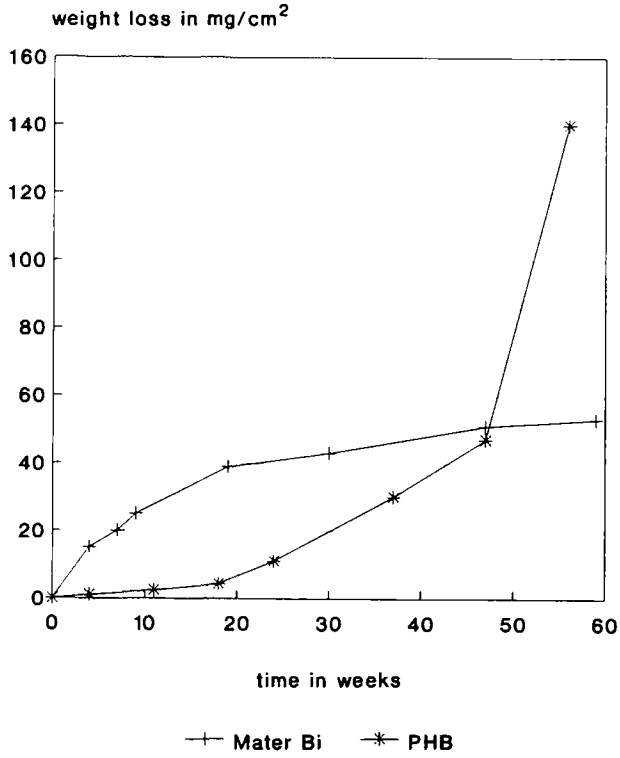

Fig. 13. Weight loss (mg/cm²) of PHBV and Mater-Bi AI05H testbars incubated in an aerobic aquarium system.

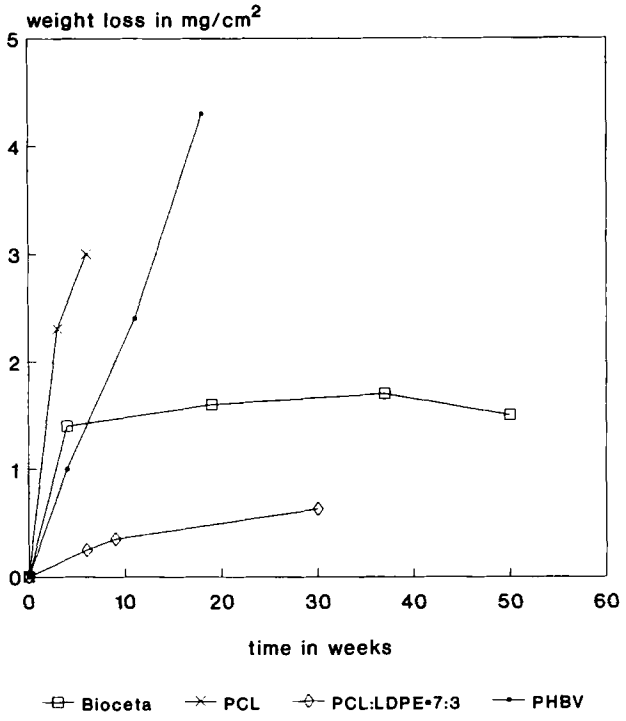

Fig. 14. Weight loss (mg/cm^2) of PHBV testbars and films of Bioceta, PCL, and PLC + LLDPE (7:3) incubated in an aerobic aquarium system.

loss was reached after 30 weeks (Fig. 12). Total bacterial numbers were higher on the films than in the water during the first 10 weeks of incubation (Fig. 12).

For comparing the biodegradation values of the different polymers, weight loss was related to the surface area of the polymer and expressed as milligrams per square centimeter. Figures 13 and 14 show the data for all the polymers being tested in the aquarium system. Results obtained with the Mater-Bi AI05H and PHBV testbars (Fig. 13) demonstrate that Mater-Bi AI05H degraded more rapidly at the beginning of the incubation but then degraded very slowly. Conversely, the degradation rate for PHBV was very low at the beginning but increased with continuing incubation (Fig. 13). The PHBV data also are presented in Fig. 14 at a higher resolution, showing, in more detail, weight loss at the beginning of the incubation in comparison with PCL and Bioceta. The 100% PCL films apparently were degraded more rapidly than the PHBV. Bioceta, which started out with a high rate weight loss, seemed to stop degrading after having lost one of its components, which had led to an increase in DOC until it was presumably metabolized by the microbial flora.

CONCLUSION

Initial testing of polymer biodegradation in screening systems helps to reduce costs and provides answers regarding the easily biodegradable part of a plastic material. Our results indicate that C balances are necessary to determine the degree of biodegradation, especially when the tested polymer consists of more than one component. As biodegradation can be very different under anaerobic conditions (as in the case of PCL), screening for biodegradability should always include an anaerobic method.

For polymers that show only partial biodegradation in the screening system, long-term testing should follow. The aquarium system described herein allows the biodegradation process to be followed through weight los and DOC to show the accumulation of dissolved polymer compounds. In this way biodegradation can be quantified.

Results from the screening system matched well those received in the long-term aquarium system. Especially for Mater-Bi and Bioceta, the biodegradation process could be elucidated through polymer analytics after incubation in the aquarium system. It became obvious that one component of Bioceta was dissolving in the surrounding medium (increase in DOC) and was then metabolized by the bacteria. This process led to a rapid weight loss of the films, the percentage of weight loss matching the percentage of ThOD in the screening system. The residual polymer seemed to take much longer to degrade. For Mater-Bi AI05H, the starch component was easily degraded, the residual polymer needed much longer, and degradation of the synthetic part could not be demonstrated, even through the aquarium system.

Soil burial systems are another possibility for long-term systems. But without using expensive labeling methods, no quantitative answer of biodegradation can be given, as biomass and/or DOC are difficult to quantify in soil systems.

In addition to the biodegradation tests mentioned, we propose to test the behavior of the polymer product under the relevant disposal conditions. Composting, for example, requires more than the biodegradability of a material. Biodegradation has to be completed in a defined time frame; the material must disintegrate rapidly so as not to disturb the composting process. The polymer components as well as the biodegradation products must meet the quality and ecotoxicity requirements defined by the appropriate authorities.

Therefore, no single test system will yield all the answers needed, before a material can be called biode-

gradable. Only a set of test systems will meet the specific demand. Many data need to be collected to identify the behavior of polymers in various systems, which, in turn, will help to define an appropriate testing scheme for evaluating biodegradation of plastics.

ACKNOWLEDGMENTS

This work was supported by the German Ministry of Research and Technology, Bonn.

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