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BIODEGRADATION OF A STARCH CONTAINING THERMOPLASTIC IN STANDARDIZED TEST SYSTEMS

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Key Words: Biodegradation, Standard Test, Starch, Poly(ε-caprolactone)

ABSTRACT

Biodegradation of the commercial starch-based thermoplastic MaterBi (MB) ZI01U was investigated in six different test systems: (1) aqueous aerobic (AQ-AE), (2) aqueous anaerobic (AQ-ANA), (3) *in vitro* in the presence of the microorganism *Acidovorax avenae avenae* (MICRO), (4) controlled compost (COCO), (5) composting bins (COBI) and (6) high solids anaerobic digestion (HSAD). MB ZI01U was found to biodegrade in all test systems. Samples in the form of pellets, films and ASTM-D638 test bars were used. The composition of MB ZI01U in the course and at the end of the biodegradation experiments was monitored by thermogravimetric analysis. Rapid loss from MB ZI01U of the water-soluble plasticizer (glycerin) was invariably observed. In the anaerobic tests (AQ-ANA and HSAD), the ratio of the two polymeric components of MB ZI01U (starch and Poly-ε-caprolactone, PCL) strongly changed with increasing exposure time, indicating preferential degradation of starch. During the aerobic tests AQ-AE and MICRO the starch-to-PCL ratio remained practically constant and equal to the initial value (0.54). At the end of the COCO tests (45 days), only sample fragments were recovered, composed of plain PCL. After the COBI tests, where high temperatures (73°C) were reached during the thermophilic phase, no samples were retrieved. MB ZI01U showed the following weight loss per unit surface area: 1 mg/cm^2, both after one day of exposure to *Acidovorax avenae* in the MICRO test and after 5 days in the AQ-AE test system; 24 mg/cm^2, after 5 days of HSAD testing.

INTRODUCTION

The interest for biodegradable polymeric materials [1-3] has been growing in connection with waste management problems. Based on availability, low-cost and known environmental biodegradability, starch has been broadly employed in the last twenty years as an additive for synthetic polymers. Starting from the earlier attempts to use starch both as a granular filler [4] and in its gelatinized form [5], many starch-based biodegradable plastics have been described in the literature [6-9]. Thermoplastic starch blended with Poly(ε-caprolactone) is commercialized by Novamont under the trade name Mater-Bi ZI01U, and its biodegradation properties have been recently reported [10-11]. Mater-Bi ZI01U (MB) was investigated in the context of a research project focused on the development of standardized systems

for testing biodegradability of bioplastics [12. This work reports the biodegradation behavior of MB in six different (aerobic and anaerobic) test systems. Changes of composition during degradation in the different environments are also discussed.

EXPERIMENTAL

Materials

Mater-Bi (MB) grade ZI01U pellets and injection molded bars (ASTM-D638) were kindly supplied by Novamont. The cast film was produced on a standard laboratory chill roll extrusion unit (Plastik Maschinenbau) at 145°C.

The composition of MB ZI01U was determined by elementary analysis, obtaining the following values: C 52.67 %; H 8.19 %; O 39.00 %; N 0.14 %.

Poly-ε-Caprolactone (PCL) grade TONE-P-787 pellets were purchased from Union Carbide. The cast film was produced as described above, at a temperature of 160°C. The test bars (DIN 53 455) were produced according to the recommended processing parameters by using a standard injection molding machine (Boy 50 M).

Biodegradation Experiments

In Aqueous Environment Under Aerobic Conditions

The aerobic biodegradation tests were carried out according to the DIN 54900-2 draft [15]. In general 400 mg/L polymer in 250 mL phosphate buffered medium were inoculated with activated sludge from a wastewater treatment plant (30 mg/L MLSS) and incubated at 20°C. At the start and end of the test, pH, dissolved organic carbon (DOC) and biomass of the test solution were measured. Biomass was assessed by the protein content remaining after filtration through a 0,45 m cellu lose acetate filter [16, 17]. DOC was determined by a DOC-Analyzer (TOCOR 2, Maihak-Company, Hamburg), after filtration through a 0.45 m cellulose filter. Duplicate films of MB ZI01U were retrieved for each exposure time.

Calculation of the Theoretical Oxygen Demand (ThOD)

The ThOD was determined by calculating the amount of O_2 necessary for aerobic mineralization of the polymer, i.e. complete oxidation of C to CO_2. ThOD of the substance $C_cH_hO_o$ of a relative molecular mass M_r can be calculated according to:

$$\mathrm{ThOD} = 15{,}99 \times \frac{2c + 0{,}5h - o}{M_r} \tag{1}$$

The amount of O_2 consumed by the polymer degradation (after correction with the blank test) was expressed as a percentage of ThOD calculated with Equation 1:

$$\% \text{ ThOD} = \frac{(BOD_P - BOD_B) \times 100}{ThOD} \tag{2}$$

Degree of Biodegradation

The degree of biodegradation η_C regarding the carbon (including the biomass) was calculated as follows:

$$\eta_C = \frac{(C_{sampleox} + \Delta C_{biomass}) * 100}{C_{samplestart}} \tag{3}$$

with $Cs_{sample\ ox} = \%ThOD * C_{sample\ start} / 100$

$\Delta C_{biomass} = C_{biomass\ end} - C_{biomass\ start}$

In Aqueous Environment Under Anaerobic Conditions

The gas production of the anaerobic biodegradation was determined with an eudiometer. The polymer and mineral medium [18] with the anaerobic sludge (stemming from an anaerobic digester, Lehr- und Forschungsklärwerk, Universität Stuttgart) was prepared in gas-tight vessels normally with a volume of 250 mL and incubated at 35°C. First the medium was stripped with Ar. Small amounts of sodiumthionite were used in order to lower the redox potential. The inoculum concentration of anaerobic sludge was ≈5 g/L MLSS. In addition to this total solid, DOC, dissolved inorganic carbon (DIC) and proteins of each sample were determined.

Calculation of the Percentage Theoretical Biogas Production

The percentage theoretical biogas production (%Thgas) of a polymer was calculated by the amount of the theoretical gas production via the disproportionation equation [19] of the test substance:

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

The biogas produced was transformed to normal conditions and lead to %Thgas as follows:

$$\%Thgas = (\text{biogas produced} / \text{theoretical gas production}) * 100 \quad (5)$$

Carbon Balance

Anaerobic degradation of a polymer means biogasproduction and biomass formation. At the start and end, Equations 6 and 7 hold:

$$C_{input}: \quad DOC_{start} + DIC_{start} + C_{sample\ start} + C_{biomass\ start} \quad (6)$$

$$C_{output}: \quad DOC_{end} + DIC_{end} + C_{sample\ end} + C_{biogas} + C_{biomass\ end} \quad (7)$$

With $C_{input} = C_{output}$ in a closed system, Equation 13 is valid:

$$0 = DOC_{start} - DOC_{end} + DIC_{start} - DIC_{end} + C_{sample\ start} - C_{sample\ end} - C_{CO2} + C_{biomass\ start} - C_{biomass\ end} \quad (8)$$

The extent of biodegradation of the polymer can be calculated with Equation 9:

$$(C_{sample\ start} - C_{sample\ end}) / C_{sample\ start} = Biodeg$$

$$= (-DOC_{start} + DOC_{end} - DIC_{start} + DIC_{end} + C_{biogas} - C_{biomass\ start} + C_{biomass\ end}) / C_{sample\ start} \quad (9)$$

Abbreviations

Biodeg *100	Biodegradability of polymer examined in %
BOD_P	Biochemical oxygen demand of the polymer reaction mix at time t
BOD_B	Biochemical oxygen demand of the blank reaction mix at time t
C_{biogas}	C-content in biogas produced, referred to 1 L, at standard conditions in mg/L; at end
$C_{sample\ start}$, $C_{sample\ end}$	C-content in polymer in mg/L; at start, end
$C_{biomass\ start}$, $C_{biomass\ end}$	C-content in biomass in mg/L, evaluated via protein-analysis; at start, end
C_{CO2}	C-content of CO_2 produced in mg/L
DIC_{start}, DIC_{end}	Dissolved inorganic carbon in mg/L; at start, end

DOC_{start}, DOC_{end}	Dissolved organic carbon in mg/L; at start, end
MLSS	Mixed liquor suspended solids in mg/L
M_r	Relative molecular mass in g/mol
$Polymer_{start}$	Polymer concentration in mg/L; at start
$O_{2\ sample} - O_{2\ blank}$	mg/L O2 consumed in testing device: O_2-consumption polymer containing sample minus blank sample in mg/L O_2
ThOD	Theoretical oxygen demand in g O_2/g polymer
Thgas	Theoretical biogas production

In Vitro Using Microorganisms

Duplicate film samples of MaterBi ZI01U and PCL of typically 20x25mm^2 were mass calibrated, disinfected by flushing for 10 minutes in 70% ethanol, followed by rinsing twice in sterile distilled water for 10 minutes. The samples were either aseptically applied to minimal medium (0.1% NH_4CL, 0.05% $MgSO_4.7H_2O$, 0.005% ferriammonium citrate, in KH_2PO4-$Na2HPO_4$ buffer, 33 mM, pH 6.8) [13] or to sterile buffer without nutrients, in erlenmeyers of 250 ml, at a ratio of approximately 50 cm^2 total surface (both sides) per 100 ml. The medium was inoculated with a suspension of strain *Acidovorax avenae avenae* PHA 1183, prepared from cells grown on Nutrient Agar medium (Oxoid) during 3 days at 28°C. This bacterium was isolated from fresh water from the botanical garden of the University Gent, was identified by fatty acid analysis and some physiological features, is able to degrade PCL and starch [14], and has been deposited in the Culture Collection of the Laboratorium voor Microbiology (Universiteit Gent, Belgium) as LMG 17238. The erlenmeyers with medium and buffer were incubated at 28°C with rotary shaking at 125 rpm. Plastic samples were periodically retrieved aseptically, washed with distilled water, and dried *in vacuo* until constant weight. Growth of the cultures was determined by serial dilution plating on Nutrient Agar medium and counting of colony forming units per ml after 3 days of incubation at 28°C.

Controlled Composting

Method ISO CD 14855 (Evaluation of the ultimate aerobic biodegradability and disintegration of plastics under controlled composting conditions) was used.

Composting Bin

The composting bin test simulates as closely as possible a real and complete composting process in pilot-scale composting bins of 200 L. The test followed the CEN draft CEN TC261/SC4/WG2/97-04a (Evaluation of the disintegration of packaging materials in a bench-scale test under natural self-heating composting

conditions). Twelve test bars of MB-ZI01U and PCL-tone-P787 were mixed with about 100 kg of the organic fraction of fresh, pretreated municipal solid waste (biowaste), which was derived from the waste treatment plant in Brecht, Belgium. The mixtures were introduced in separate composting bins after which composting spontaneously started. The composting process was directed through air flow and moisture content. As in full-scale composting, inoculation, and temperature increase happened spontaneously. The test was scheduled to last 12 weeks, but was stopped after one week because the test bars had already disappeared .

High Solids Aanaerobic Digestion (HSAD)

Method ASTM D.5511-94 (Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under High Solids Anaerobic Digestion Conditions) was used.

Four test bars of MB ZI01U and PCL were retrieved for each exposure time.

The biodegradation results of standardized tests are reported as average values, the standard deviation being indicated in the Figures by the size of symbols or – where appropriate – by bars (see Figure Legend).

Characterization Methods

Thermal Properties

Thermogravimetric analysis (TGA) was carried out by means of a TA Instruments TGA 2950 (heating rate 10 deg/min; gas flow: nitrogen). Differential Scanning Calorimetry (DSC) was performed by means of a Du Pont 9900 thermal analyzer (heating rate 10°C/min).

Wide Angle X-ray Diffraction

WAXS measurements were carried out with a Philips PW1050/81 diffractometer controlled by a PW1710 unit, using a graphite monochromatized Cu k radiation (λ=0.1542nm; 40kV; 40mA). The percentage of crystallinity was calculated through graphical integration of the diffracted intensity data in the 2 θ range 10-60°C and subtraction of the amorphous scattering band intensity.

Scanning Electron Microscopy

MB ZI01U bars were freeze fractured as follows: samples, wrapped in paper tissue, were immersed in liquid nitrogen and, after ca. 1 minute, they were broken (at liquid nitrogen temperature) by hand under bending using two pairs of pliers. Before breaking, a small cut of ca. 0.3 mm was made on the tensile side of the samples.

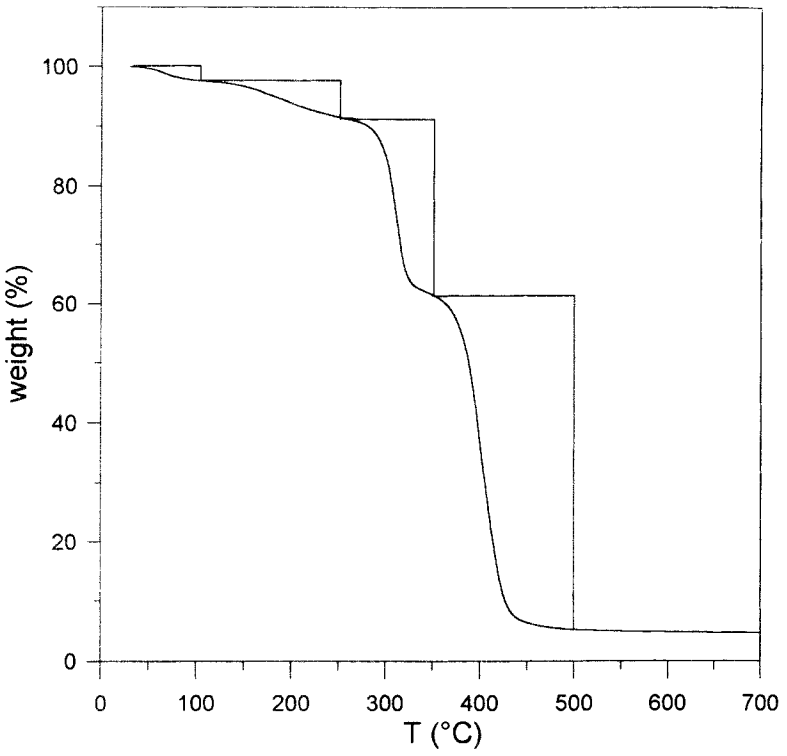

Figure 1. Thermogravimetric curve of MB ZI01U.

Samples were sputter coated for 600-800 seconds at 10-12 mA and 0.05 mbar in a Balzers SCD 004 Sputter Coater with platinum. Documentation of the samples was performed with a Hitachi S41C at an acceleration voltage of 12 kV. Working distances were typically 12-20 mm.

RESULTS AND DISCUSSION

Characterization of MB ZI01U

According to the manufacturers (Novamont), MB ZI01U is composed of starch, PCL and a 'natural' plasticizer (glycerin). Thermogravimetric analysis was used to quantify the components of MB ZI01U (Figure 1). Four weight loss steps were observed in the TGA curve. In order of increasing temperature, they were attributed to: 1) absorbed water (lost between room temperature and 120°C), 2) glycerin (boiling point 290°C [19]), 3) starch [20], 4) PCL (pure PCL, run in

identical experimental conditions, showed a single weight loss centered at 400°C). The magnitude of the weight loss steps was approximately 3% water, 7% glycerin, 30% starch, 56% PCL and 4% solid residue. By differential scanning calorimetry (DSC) and wide angle X-ray scattering (WAXS) only the presence of PCL was revealed in MB ZI01U. A melting endotherm at 60°C and a glass transition at -60°C in the DSC curve, and the typical PCL X-ray diffraction pattern [21] clearly identified the polyester. Quantification of the observed phenomena by evaluation of melting enthalpy, specific heat increment at T_g and degree of crystallinity yielded an estimate of the amount of PCL contained in MB ZI01U close to 55%, in good agreement with the weight loss by TGA. The T_g of PCL in MB ZI01U was the same as that of the pure polyester, indicating that glycerin (whose function is to plasticize starch [10]) did not interact with PCL.

The weight loss attributed to glycerin invariably disappeared from the TGA curve of MB ZI01U after biodegradation in all test system employed, suggesting that the plasticizer is easily extracted from MB ZI01U. Very fast extraction of glycerin was confirmed by experiments carried out in water, where no plasticizer was found in films of MB ZI01U after 5 minutes of immersion. In all MB ZI01U samples investigated (both before and after biodegradation) the two main weight losses in the TGA curve, due to thermal degradation of the two polymeric components (starch and PCL), were well resolved and easily quantified. The ratio of starch to PCL, determined after different exposure times, was used to monitor composition changes during the biodegradation process.

Biodegradation in Aqueous Environment

Aerobic Conditions

MB ZI01U films were tested in aqueous aerobic conditions over a period of two weeks. Samples were retrieved after different exposure times, washed, dried to constant weight and the starch-to-PCL ratio was determined by TGA. Weight loss and %ThOD were plotted as a function of time in Figure 2. MB ZI01U biodegraded in aerobic conditions, showing 33%ThOD and 51% weight loss after 12.5 days. The calculated degree of biodegradation η_C was 53%, in good agreement with the observed weight loss. Sample composition did not change appreciably during biodegradation, as shown by the starch/PCL data reported in Figure 2.

Anaerobic Conditions

Films and pellets of MB ZI01U were tested in aqueous anaerobic conditions over a period of 81 days. Samples were retrieved at the end of the test and the starch-to-PCL ratio was determined by TGA. The %Thgas of films and pellets was

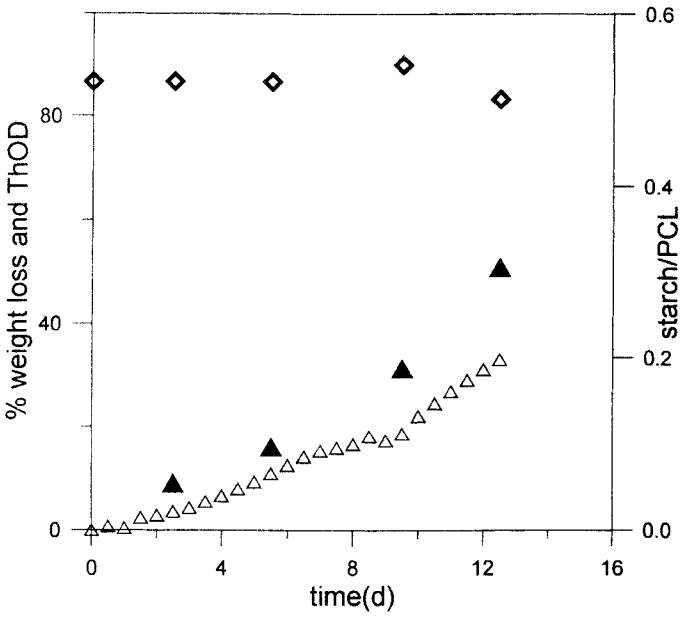

Figure 2. Biodegradation of MB ZI01U (film) as a function of time, in aqueous aerobic conditions. (Δ) ThOD, (▲) weight loss, (◇) starch-to-PCL ratio (by TGA). Symbol size reflects scatter of results.

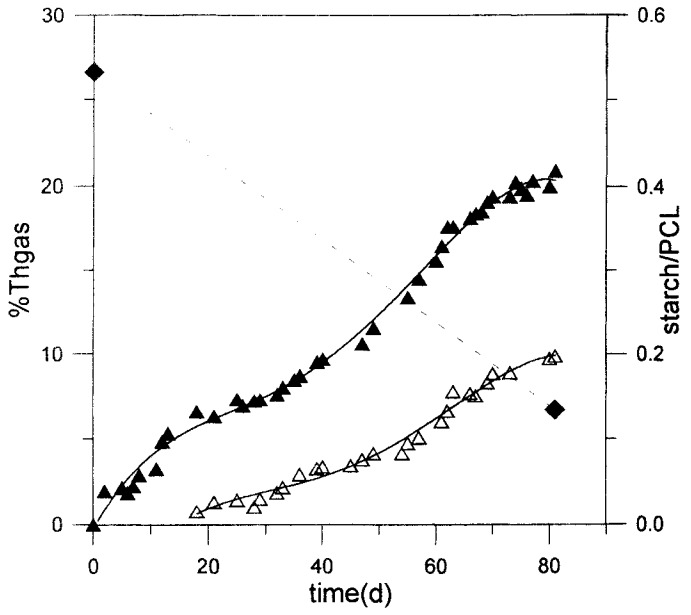

Figure 3. Biodegradation of MB ZI01U as a function of time, in aqueous anaerobic conditions. Theoretical gas of (▲) film and (Δ) pellets; (◆) starch-to-PCL ratio of film (by TGA).

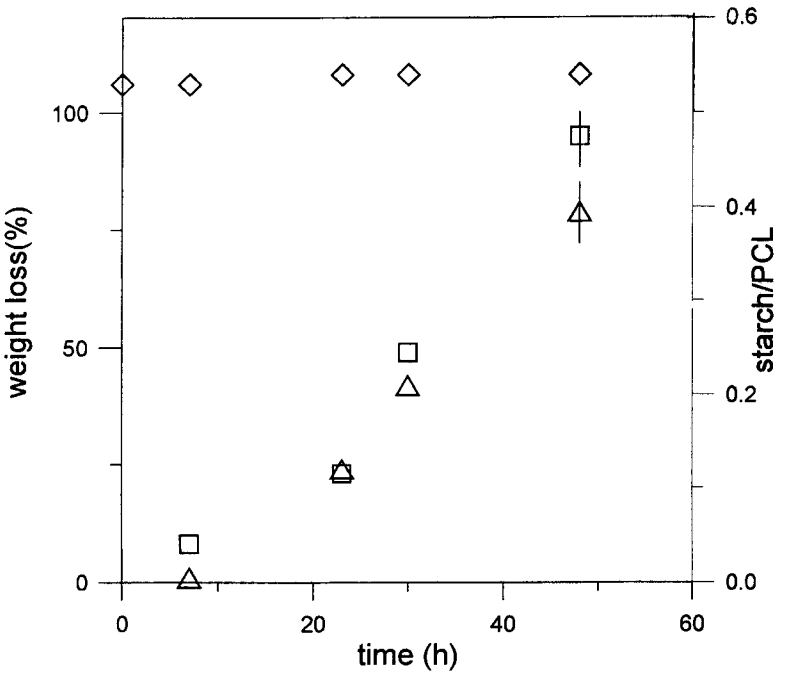

Figure 4. Biodegradation *in vitro* in the presence of *Acidovorax avenae* of films of MB ZI01U (□) and of PCL (Δ) as a function of time. (✧) starch-to-PCL ratio of MB ZI01U (by TGA). Symbol size reflects scatter of results, with the exception of the longest degradation time, where bars indicate maximum deviation from the average.

plotted as a function of time in Figure 3. Biodegradation of MB ZI01U took place in anaerobic conditions, with 21% and 10%Thgas developed by films and pellets after 81 days of exposure, respectively. The difference of biodegradation rate of the two samples was attributed to the larger exposed surface area (per unit weight) of films compared to pellets. At the end of the experiment, the films of MB ZI01U had a starch/PCL ratio much lower than before biodegradation (0.13 vs. 0.54, Figure 3). After 81 days, the composition of the outer layer of the pellets (starch/PCL 0.14) was similar to that of the film, whereas at the pellet core starch depletion was much less drastic (starch/PCL = 0.34).

Biodegradation *In Vitro* Using Microorganisms

Figure 4 shows the weight losses of MB ZI01U and PCL film in medium inoculated with *Acidovorax avenae avenae* PHA 1183. Growth of the culture is shown in Figure 5. The disinfection procedure resulted in 7% weight loss of the MB

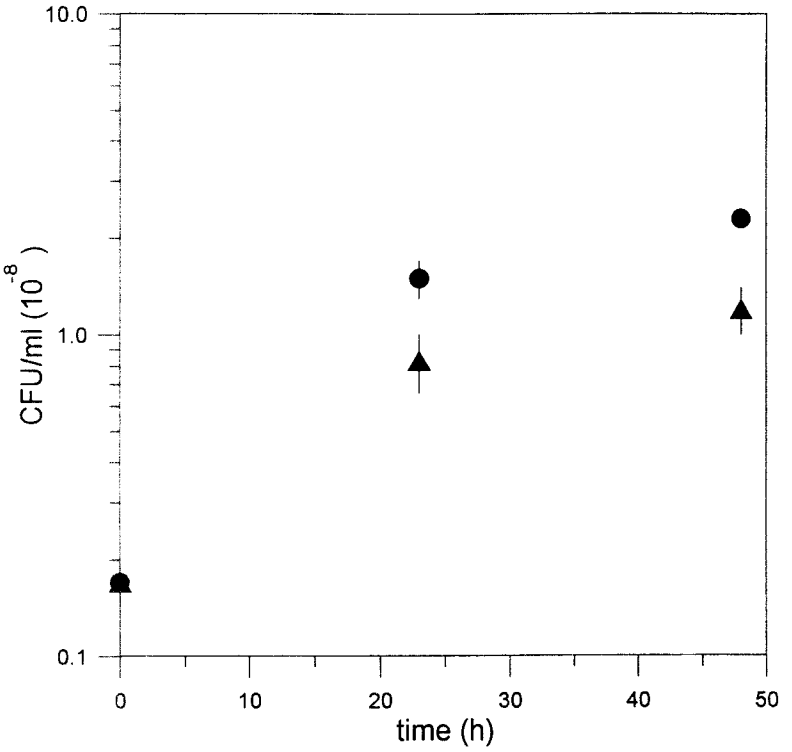

Figure 5. Growth of *Acidovorax avenae* (colony forming units per ml) in the presence of films of MB ZI01U (●) and PCL (σ).

ZI01U samples, while the weight loss of PCL samples was less than 1%. TGA measurements on disinfected MB ZI01U films showed that glycerin had been completely extracted upon disinfection. No additional weight losses were recorded during further incubation in buffer for 48 hours. The samples incubated in the presence of the bacterial culture showed very rapid weight loss, with only about 6% and 21% of the initial weight remaining after 48 hours, for MB ZI01U and PCL, respectively. In the presence of both plastics, the cell density of the culture increased by a ten-fold (Figure 5), indicating that the plastic materials were used as sole sources of carbon for growth. As the strain PHA 1183 is able to utilize starch as well as PCL [14], the ratio of these components in the MB ZI01U film remained constantly around 0.54 during degradation.

Biodegradation Tests in Solid Environments

Composting Bin

During biodegradation experiments in composting bins, in the thermophilic

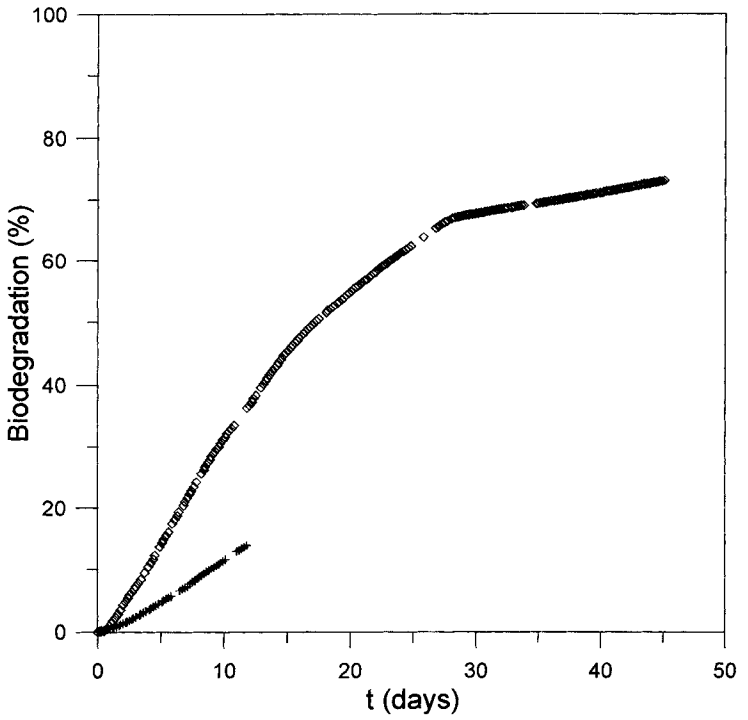

Figure 6. Biodegradation of bars of MB ZI01U (◆) and of PCL (+) in controlled composting as a function of time.

phase the temperature strongly increased (from T=53°C, 1st day, to T=73°C, ninth day). MB ZI01U bars became very soft owing to melting of PCL component (T_m of PCL = 60°C) and could not be retrieved from the test vessel. The same observations were made for PCL.

Controlled Composting

Both MB ZI01U and PCL bars were tested in controlled composting conditions and the biodegradation results were reported in Figure 6 (in both tests after 45 days cellulose biodegradation was over 80%). After 12 days, the experiment on PCL was interrupted, no samples being retrieved, because of complete disintegration. The test on MB ZI01U lasted 45 days; only fragments of the original MB ZI01U bars were recovered at the end of the experiment. TGA analysis of the retrieved fragments revealed that they were composed of pure PCL. The results of Figure 6 show that during the first 12 days the biodegradation rate of MB ZI01U was threefold that of PCL.

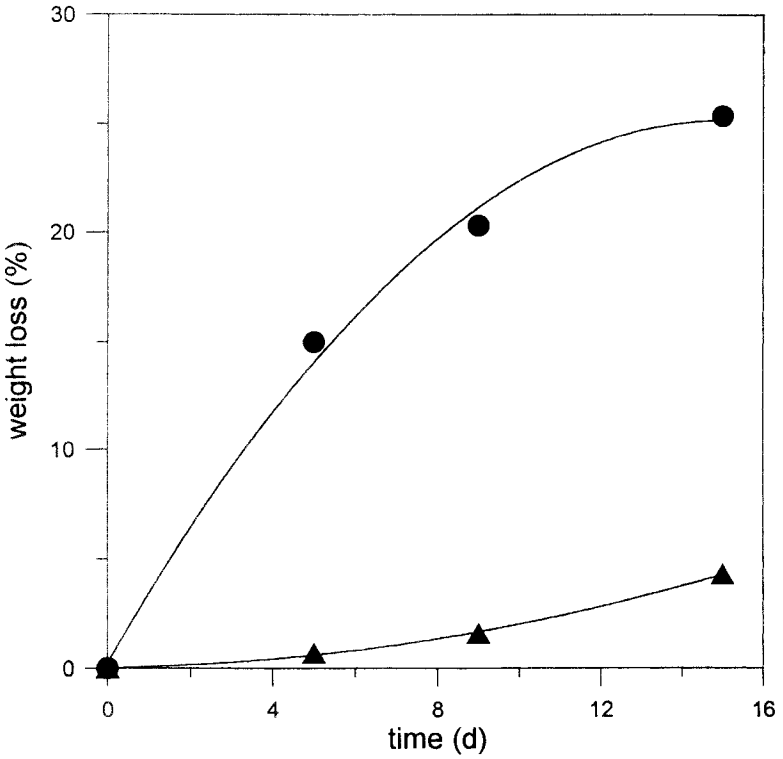

Figure 7. Biodegradation of bars of MB ZI01U (●) and of PCL (▲) in high solids anaerobic digestion, reported as weight loss vs. time. Symbol size reflects scatter of results.

High Solids Anaerobic Digestion

MB ZI01U bars were tested in HSAD conditions over a period of 15 days. PCL bars were also tested for the sake of comparison. Samples were retrieved after different exposure times, washed, and dried to constant weight. Weight loss of MB ZI01U and PCL was plotted as a function of time in Figure 7. In HSAD conditions both MB ZI01U and PCL biodegraded, but at remarkably different rates. The weight loss of MB ZI01U after 15 days was 6-fold that of PCL (25% vs. 4%). In biodegraded MB ZI01U bars the starch-to-PCL ratio was determined by TGA both at the surface layer and at the core of the bar. The TGA curves of MB ZI01U bars after 15 days of exposure to HSAD are shown in Figure 8. Only the thermal degradation step typical of pure PCL was observed in the TGA curve of the outer layer of the bar, whereas the core showed two well defined weight loss steps, due to

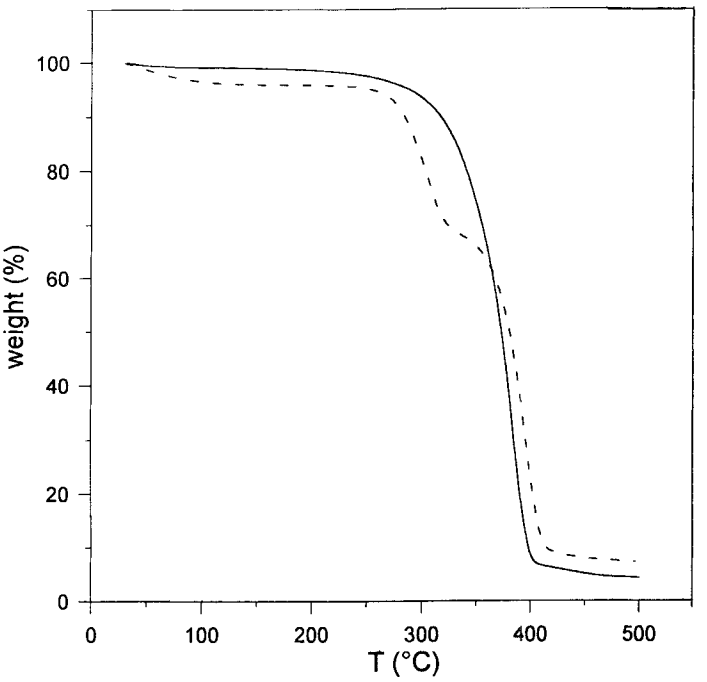

Figure 8. TGA curves of MB ZI01U bar after 15 days in high solids anaerobic digestion: (—) outer layer of test bar; (---) core of test bar.

thermal decomposition of starch and PCL. The starch-to-PCL ratio at the core was the same as that of MB ZI01U before biodegradation. Comparison of the TGA curves of Figure 8 with the curve of non-degraded MB ZI01U in Figure 1 demonstrates the mentioned absence of plasticizer in biodegraded MB ZI01U, and shows the presence at the test bar core of a considerable amount of water (weight loss between room temperature and 120°C) associated with starch.

MB ZI01U bars were freeze fractured after 15 days of exposure to HSAD, and the fracture surfaces were investigated by SEM (Figure 9). In micrographs (a) and (b), the cut used to initiate fracture is observed on the left-hand side of the fracture surface. At the right hand side of the cross-sections the area underneath the surface exposed to HSAD appears quite different before (a) and after biodegradation (b). A magnification of an area of micrograph (b), taken at a depth of about 800 μm from the exposed surface (marked c), is shown in Figure 9 (c). In the center of micrograph 9 (c) a vertical line ideally separates the rough but compact surface on the left from the rough but heavily porous structure on the right. Closer to the

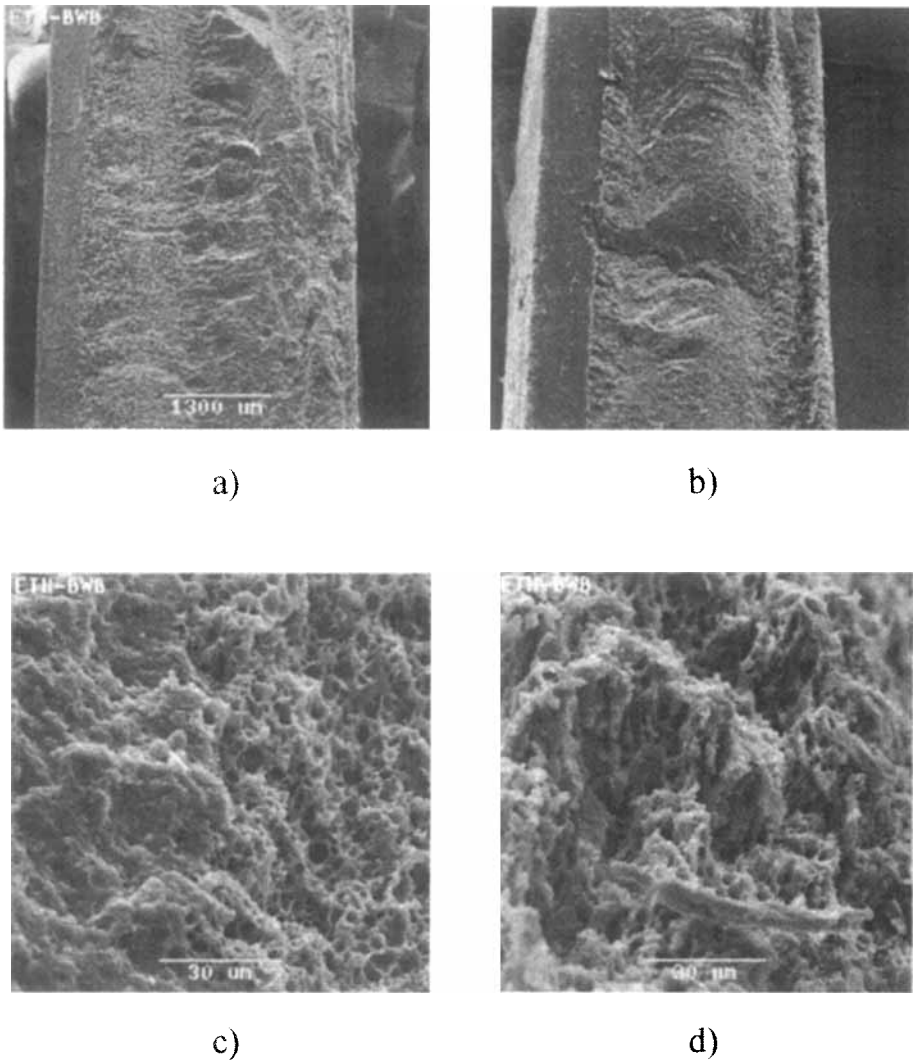

Figure 9. SEM pictures of freeze fractured MB ZI01U bar: *a)* before biodegradation (x20), *b)* after 15 days in HSAD (x20). *c)* and *d)* are magnifications (x1000) of micrograph *b)* taken 800µm *(c)* and 60µm *(d)* underneath the exposed surface.

exposed surface of the bar (i.e 60 μm deep, see Figure 9d) deeper cavities are observed on the fractured cross-section. This latter region represents the so-called outer layer of the bar which was shown by TGA to be composed of pure PCL. At depths higher than 800 μm (bar core) the aspect of the cross section was the same as that on the left-hand side of Figure 9(c) and composition (by TGA) was the same as non-degraded MB ZI01U (apart from glycerin loss).

MB ZI01U was found to biodegrade in all test systems investigated: aqueous aerobic, aqueous anaerobic, *in vitro* with microorganisms (*Acidovorax avenae*), controlled compost, composting bins and high solids anaerobic digestion. In all cases, the plasticizer (glycerin) was lost from MB ZI01U during the very early stages of the test.

In anaerobic conditions remarkable changes of composition occurred during biodegradation. In aqueous anaerobic tests (where pure PCL did not biodegrade) and in HSAD (where pure PCL biodegraded, but at a much slower rate than MB ZI01U), preferential degradation of the starch fraction of MB ZI01U occurred. In MB ZI01U pellets, at the end of aqueous anaerobic experiments very little starch was left in the outer layer, whereas starch depletion at the granule core was much less drastic. Similarly, MB ZI01U test bars after high solids anaerobic digestion had no starch left at the surface (constituted by a pure PCL porous matrix, as shown by SEM) but the same starch content as non-biodegraded MB ZI01U at the core. It is clear that biodegradation of starch domains located far from the surface is conditioned by their accessibility which depends on domain connectivity, rate of penetration of enzymes in the partially degraded (i.e. cavitated) polymer matrix, solubility and diffusion rate of biodegradation products, etc. As a consequence, the starch concentration profile in thick samples subjected to biodegradation tests was found to decrease from a maximum at the core to a minimum at the surface.

The most drastic composition change after biodegradation of MB ZI01U was observed, however, in an aerobic environment (controlled composting), where after 45 days only fragments constituted of pure PCL were recovered.

A different behavior was found when MB ZI01U biodegraded in aqueous aerobic environment and *in vitro* with microorganisms. The starch-to-PCL ratio of MB ZI01U did not change appreciably during biodegradation in these aerobic tests systems, showing that in the bioplastic the two polymeric components biodegraded at the same rate. This observation did not necessarily imply that in these test systems pure starch and pure PCL should biodegrade at the same rate. In the specific case of the *in vitro* tests, where results on pure PCL run in identical conditions were available, it was found that the biodegradation behavior of the

polyester did not differ remarkably from that of MB ZI01U. In general terms, it is expected that the biodegradation behavior of multi-component materials will result from a complex combination of parameters such as distribution, accessibility and relative biodegradation rate of the components.

It would be very interesting to compare the biodegradation rate results obtained in this work for MB ZI01U in the different test systems investigated. However, direct comparison is prevented by the fact that the type of sample used (film, pellet, test bar) often differed from test to test. It is known that enzymatic degradation of solid substrates is a surface phenomenon [1], and that the area exposed to the biodegrading environment plays a very important role in determining the biodegradation rate. Hence, a comparison of the biodegradation rate in different test systems of MB ZI01U (film and test bar) was attempted by using weight loss data, where available, after normalization with respect to the initial sample surface area. Only short exposure times were considered, where area changes were assumed to be negligible. The results of these calculations showed that MB ZI01U lost 1 mg/cm^2 after one day of exposure to *Acidovorax avenae* in the *in vitro* test and after 5 days in the aqueous aerobic test system, whereas after the shortest exposure time to the HSAD test (5 days) the weight loss of MB ZI01U was 24 mg/cm^2.

CONCLUSION

Biodegradation of the commercial starch-based thermoplastic MB ZI01U was studied in six standardized test systems. In addition to assessing MB ZI01U biodegradation in the different environments, changes of composition during the biodegradation process were also evaluated. Glycerin, which acts as a plasticizer for starch and is easily extracted by water, was rapidly lost by MB ZI01U during biodegradation in all environments. The starch-to-PCL ratio strongly changed during biodegradation in the anaerobic test systems (aqueous and HSAD), indicating preferential degradation of starch. Conversely, in the aqueous aerobic test and in the test with *Acidovorax avenae* both PCL and starch in MB ZI01U biodegraded at the same rate, their relative content remaining constant over the whole course of the biodegradation experiments.

This work has demonstrated the usefulness of associating analytical tools able to monitor composition changes to standardized test methods for the assessments of biodegradability. In plastics with complex formulations this allows to follow biodegradation of each component and quantify its contribution to the overall biodegradation behavior.

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