Mater-Bi: Properties and Biodegradability

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This paper examines the biodegradability of a new class of materials based on starch and vinyl-alcohol copolymers, which have been commercialized under the Mater-Bi trademark. Particular attention is given to the biodegradation process for natural and synthetic components of different Mater-Bi grades for film blowing in an aerobic respirometric test, in a SCAS (semicontinuous activated sludge) test, and by submersion in lake water. The correlation between morphology and biodegradation behavior is also considered. Taking into account the prior art on biodegradation of insoluble substrates, a two-step mechanism is proposed for Mater-Bi products.

KEY WORDS: Biodegradability; methods; starch; vinyl-alcohol copolymers; morphology.

INTRODUCTION

The aerobic and anaerobic digestion of soluble substrates has been widely investigated and the degradation processes involved have been mostly understood; on the other hand, only a little is known about biodegradation mechanisms of even simple insoluble substrates.

Cellulose and similar materials are transformed into soluble and assimilable saccharides by means of an initial hydrolytic step, carried out by extracellular enzymes (cellulase, etc.) [1].

The biodegradation of a wide range of insoluble substrates, such as long-chain hydrocarbons and fatty acids, is more complex. Available data imply an initial oxidative process, catalyzed by intracellular enzymes and coenzymes. In this case, three main mechanisms have been observed [2].

1. *Homogeneous:* The substrates are used by bacteria in a dissolved form and the degradation rate depends on the very reduced solubility of such substrates in water [3–6].
2. *Pseudosolubilization:* Microorganisms can produce metabolites which help the dissolution of the solid substrates in water environment [7–9].
3. *Interfacial adsorption:* The microorganisms,

which normally live in water, can be adsorbed at the substrate surface. The substrate digestion can presumably occur thanks to adsorption/desorption processes of substrate molecules on the cellular wall, at the adsorption site [10, 11].

The three processes for insoluble substrates may also apply to polymeric materials.

In this case, aspects such as molecular weight, crystallinity, orientation, porosity, wettability, and chemical structure can strongly affect the biodegradation behavior [12–15].

Albertsson and Ljungquist [12, 13] studied poly(tetramethylen-adipate) biodegradation as a function of crystallinity and molecular weight. They observed an initial phase of abiotic hydrolysis of amorphous regions of the polymer and a subsequent oligomer attack by microorganisms.

Benedict *et al.* [14, 15] observed faster biodegradation of high molecular weight poly ε-caprolactone in comparison with one at a low molecular weight, due to the higher crystallinity shown by the latter.

The influence of the physical state on the biodegradation behavior of a crystalline polymer such as PVA (polyvinyl-alcohol) at a high hydrolysis degree, insoluble in cold water, and of the same polymer solubilized in hot water has been monitored by means of an aerobic respirometric method [16]. After 28 days the solubilized material gave a biodegradation index of about 80%,

compared with about 5% for the same product not solubilized and strongly crystalline.

The above examples point out that, for insoluble materials, biodegradation depends on both chemical structure and physical state. It means that biodegradation tests in a specific environment may indicate only an apparent biodegradability of the material.

This paper presents some preliminary experimental evidence of the complex interactions occurring between starch and vinyl-alcohol copolymers, able to influence the microstructure of different Mater-Bi grades. Attention is also given to the role of microstructure in determining the biodegradation rate of this class of products. The hypothesis of a different biodegradation mechanism for starch and vinyl-alcohol copolymers is supported by aerobic tests such as modified Sturm, SCAS, and submersion in lake water.

EXPERIMENTAL

Materials

The following materials were used in this study.

- Mater-Bi AF05H, an industrial grade for film blowing. It contains about 60% starch and natural additives and 40% (w/w) vinyl-alcohol/ethylene 60/40 mol/mol copolymer. It was used in the form of a microdispersion (particle aggregates <30 μm) obtained by boiling 2 g of a film of 30 μm in 150 ml of distilled water, under stirring, for 5 h. Intrinsic viscosity, GPC, and IR spectroscopy of the microdispersion did not reveal changes of the ratio between synthetic and natural components after boiling or alteration of the molecular weight and distribution of the synthetic fraction.
- Films of Mater-Bi AF10U, an experimental grade for film blowing. This grade differs from AF05H only by the amount of natural component (70%).
- Mater-Bi AF08H powder (<90 μm, obtained by grinding in liquid nitrogen). It contains the same natural/synthetic ratio as for AF05H. The synthetic copolymer average composition is, in this case, 80/20 mol/mol vinyl-alcohol/ethylene.
- Vinyl-alcohol/ethylene, 60/40 mol/mol copolymers in powder form at different molecular weights and crystallinities, as reported in Fig. 6.

Methods

Aerobic Respirometric Test

The test applied in the present work is a modification of the Sturm test proposed by OECD [17]. It implies the automatic control of air flow and of pH and the use of a composite inoculum prepared mixing activated sludge from a municipal waste water depurator with activated sludge produced by a laboratory acclimation unit. Net CO_2 evolved from the specimens, corrected for the CO_2 produced by the reference, was determined as the percentage of total CO_2 that the test material can theoretically produce, based on its carbon content ($ThCO_2$). The procedure was checked by analyzing, in parallel, well-known reference substances (sodium acetate, paper).

In the case of microdispersed AF05H slurry, the aerobic test was followed for 10 months and stopped when the CO_2 production was still relevant so as to have a significant residual quantity for analysis (Fig. 6). Two concentrated specimens were considered: specimen 1, 360 ml soil with an initial material content of 60 mg; and specimen 2, 540 ml soil with an initial material content of 90 mg.

Specimen 1. The recovery of the residual organic material after biodegradation was carried out by the following procedure: sludge filtration; drying; solubilization of the dry residue (natural and synthetic component) with 2 ml of deuterated DMSO at 150°C; filtration to eliminate the microbial matter; addition of 2 μl of benzene as internal standard for quantitative evaluation of the residue; proton NMR of the solution and, as a crossverification, solution recovery by insolubilization of the synthetic material (acetic acid/sodium acetate buffer, pH 5); and filtration and weighing.

Specimen 2. The recovery procedure was simplified to obtain only gravimetric data. The residual organic material, in this case, was obtained as follows: sludge extraction with DMSO, insolubilization of the organic material with buffered water, and recovery and weighing.

SCAS

An aerated semicontinuous activated sludge unit of 25 L was used. The unit was fed daily with peptone solution, meat extracts, and polymeric substances similar to the test materials. It was well aerated and maintained at temperatures between 20 and 25°C. The films

to be biodegraded were fixed between two nets on stainless -steel frames and introduced into the SCAS unit. The biodegradation trend was monitored by weight loss, optical microscopy, FTIR, NMR, and elemental analysis of the samples, when possible, washed, and ultrasonicated.

Biodegradation in Lake Water

Films of AF10H, together with a negative standard (PE), were introduced in a plexiglass cylinder, which was made buoyant by a life-belt; it had a solid cap at the top and a net at the bottom to permit water circulation and to avoid sample loss. The apparatus was introduced in a test tank of 20 × 20 × 5 m in Maggiore Lake (Italy) for 22 months. The biodegradation trend was carried out as for SCAS samples.

GPC Test

The organic residues of AF05H (specimens 1 and 2 mixed together) and of vinyl-alcohol copolymers, before and after aerobic respirometric test, were acetylated for the GPC analysis. Acetylation was carried out by adding 10 ml pyridine and 10 ml acetic anhydride to the organic residues and heating at 100°C for 30 min. The acetylated specimen was dried under vacuum. The completeness of acetylation was verified by NMR. The residue was analyzed by GPC in tetrahydrofurane (THF).

The molecular weight of the acetylated vinyl-alcohol copolymers was determined by the Mark–Houwink equation [18]:

$$n = KM^\alpha$$

where $K = 1.49 \times 10^{-4}$ and $\alpha = 0.698$.

Scanning Electron Microscopy (SEM)

After drying and gold plating, a drop of AF05H microdispersed slurry was analyzed with a Model Stereoscan 260 scanning electron microscope from Cambridge Instruments.

Transmission Electron Microscopy (TEM)

Microtomed slices of Mater-Bi films, fixed in an epoxy resin, were analyzed with a Philips EM-300 transmission electron microscope.

RESULTS

Mater-Bi Microstructure

As reported under Experimental, the starch component of AF05H films does not dissolve even boiled in water under stirring. Under these conditions a microdispersion is produced constituted by microsphere aggregates whose individual particle diameter is under 1 μm (Fig. 1). A droplet-like structure is also confirmed by TEM analysis of AF05H film slices (Fig. 2). The droplet size is comparable with that of the microdispersion obtained on boiling.

A general study of AF05H shear flow characteristics [19] shows a strong pseudoplastic behavior at high shear stresses as well as yield stress at lower ones (Fig. 3). The Merschel–Bulkley model (nonlinear Bingham fluid) can well describe its viscous behavior over a wide range of shear rates [20]. High levels of melt elasticity were indicated by exit pressure data (Fig. 3), whereas its recoverable fraction was almost negligible (low die swell) (Fig. 4).

The droplet-like morphology observed for AF05H also explains [19–21] its peculiar viscous and elastic behavior. This microstructure is probably due to the ability of starch, and mainly of amylose, to generate not only hydrophilic but also hydrophobic interactions, as already observed in the literature for butanol/starch complexes [22].

In AF05H the vinyl-alcohol copolymer, working as butanol, makes starch insoluble in both hot and cold water. Suitable manipulations of natural and synthetic components can provoke alterations of the balance between hydrophobic and hydrophilic interactions, reducing the ability to generate complexes.

The increased hydrophilicity of the synthetic copolymer, for example, tends away from the droplet-like morphology, generating layered structures as shown by TEM analysis of AF08H Mater-Bi films (Fig. 5). In this case no microspheres are produced on boiling and the starch component becomes partially soluble.

A model can be proposed for the droplet-like morphology, considering large individual amylopectine molecules interconnected at several points per molecule as a result of hydrogen bonds (hydrophylic interactions) and entanglements [19] by chains of amylose/vinyl-alcohol copolymer complexes (hydrophobic interactions). Further studies are in progress in order to confirm such a model.
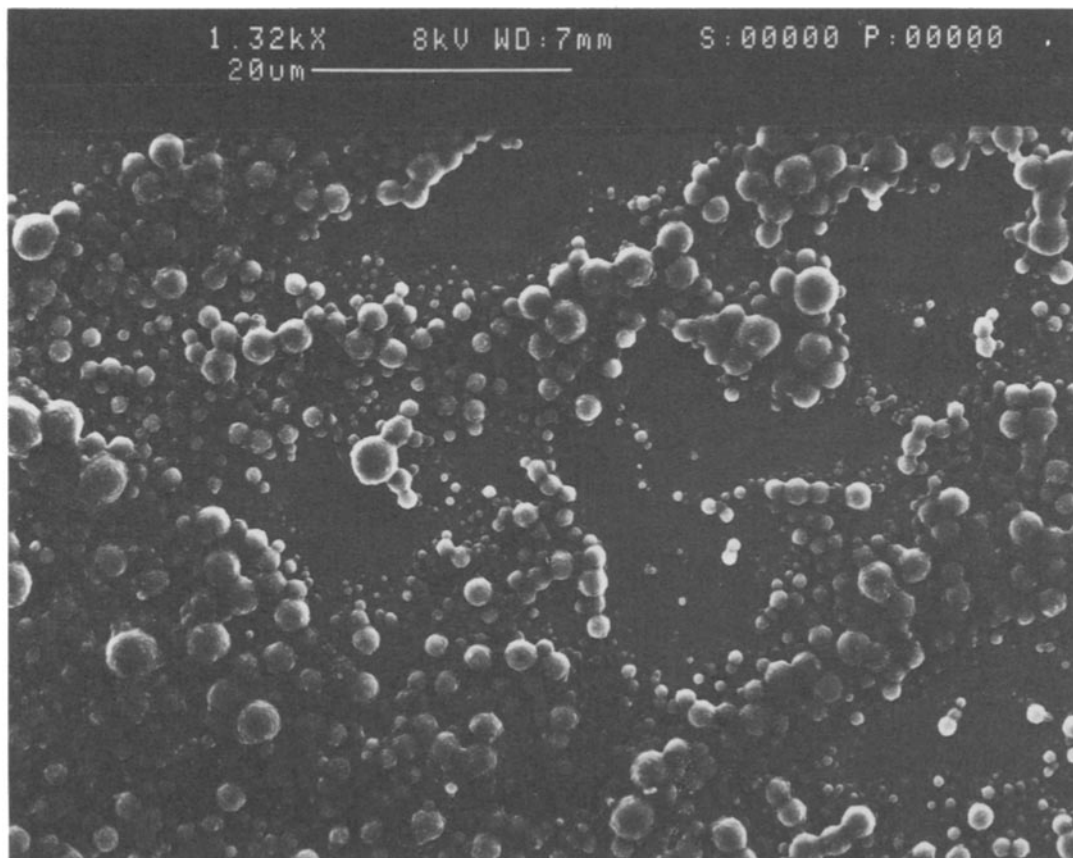
Fig. 1. SEM analysis of a microdispersion of AF05H obtained by boiling a film of 30 μm in water. 1320×.

Aerobic Respirometric Test

AF05H

The microdispersed AF05H fraction of specimen 1 (Experimental) was analyzed both qualitatively and quantitatively after 10 months in the aerobic respirometer. The TOC (total organic carbon) value of the aqueous filtrate was 11.4 ppm, while that of the reference blank test was 11 ppm. This suggests that practically all the organic material is present in the sludge as a solid.

The NMR spectrum, obtained for the residue of specimen 1, coincides with that of the synthetic component of AF05H (vinyl-alcohol copolymer) in the presence of starch traces (peaks between 5 and 5.6 ppm). The presence of starch after 10 months of biological treatment may be explained on the basis of hydrophobic interactions between the polysaccharide and the synthetic component in agreement with the observed droplet-like structure, making the polysaccharide itself not

completely accessible to the enzymatic attack. FTIR studies are in progress to confirm this hypothesis.

Using benzene as internal standard in the deuterated DMSO solution, it was determined, through NMR, that 5.0 mg of synthetic polymer remained. This result was then confirmed gravimetrically by insolubilizing the resin by adding aqueous buffer (acetic acid/sodium acetate, pH 5) and finding 5.56 mg of residual material. This was equal to about 9.27%, by weight, of the initial specimen. From these data, about 10% residual starch was estimated.

From specimen 2, treated according to the method described under Experimental, 7.93 mg of organic residue (8.81% of the initial specimen) was obtained. This agreed with the data for specimen 1.

Therefore, 10 months of exposure in the aerobic respirometric test degraded about 91% (w/w) of the initial material, leaving a residue composed of at least 90% synthetic component. On the basis of the initial AF05H composition, 80%, by weight, of the synthetic fraction was degraded during the test period.
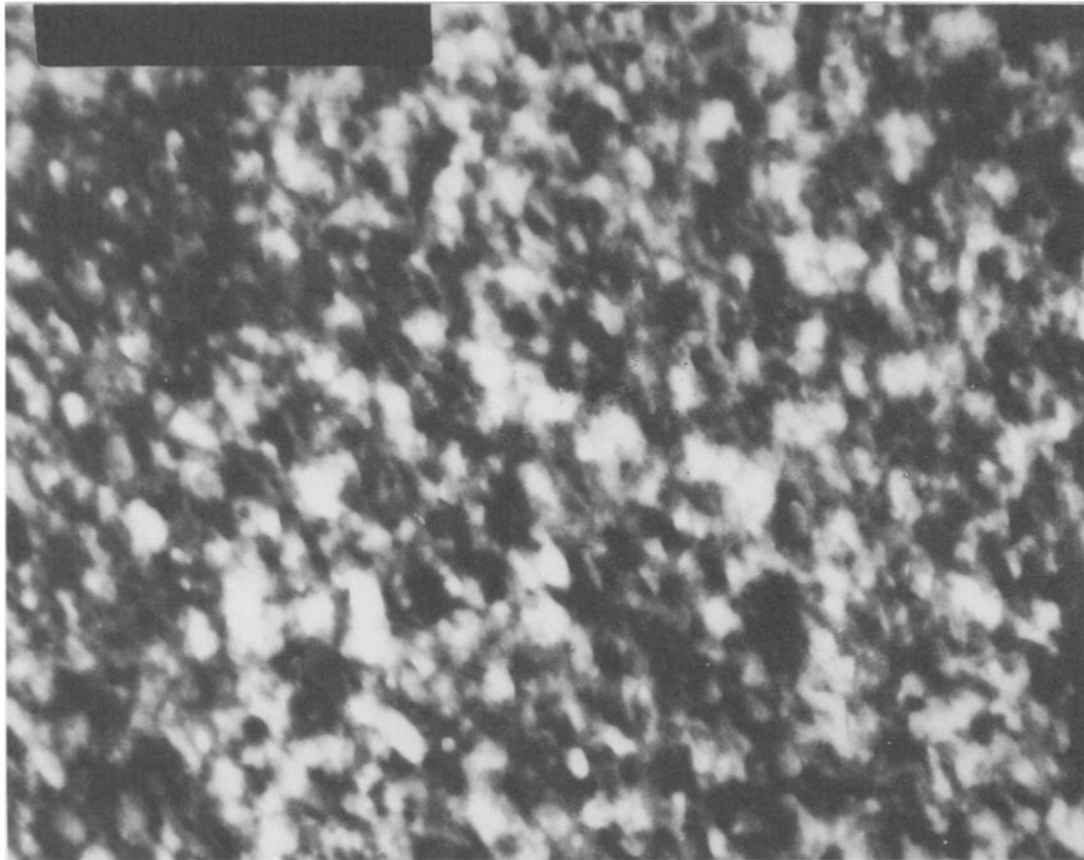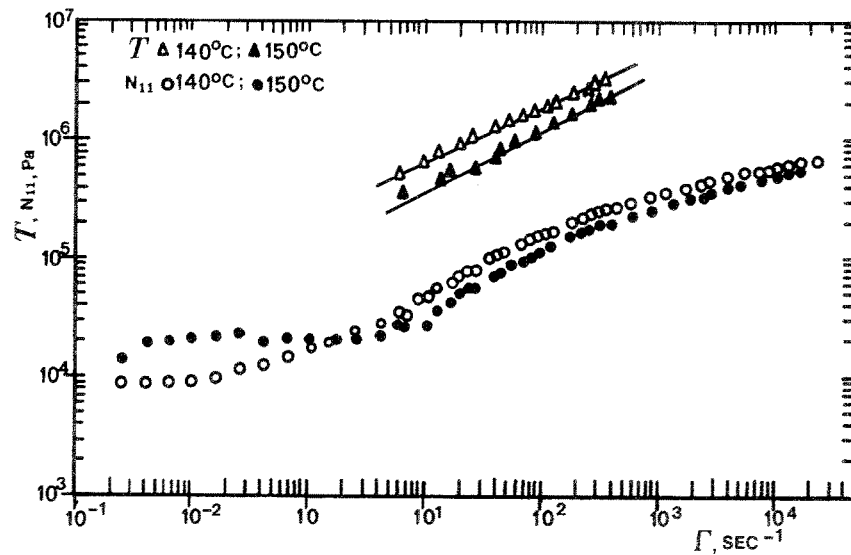
Fig. 2. TEM analysis of an AF05H film slice. 7500×.

Fig. 3. Shear stress (τ) and first normal stress difference (N_{11}) vs wall shear rate for AF05H. First normal stress difference was estimated from exit pressure values [19].
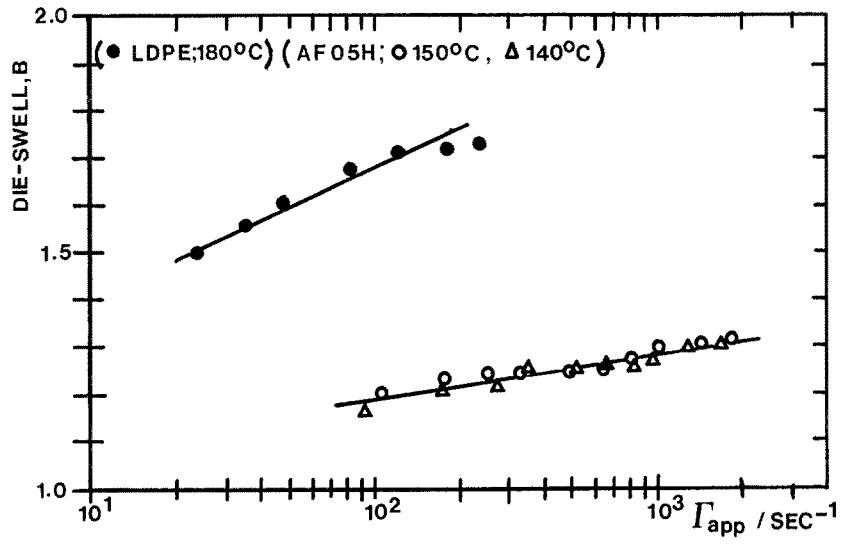
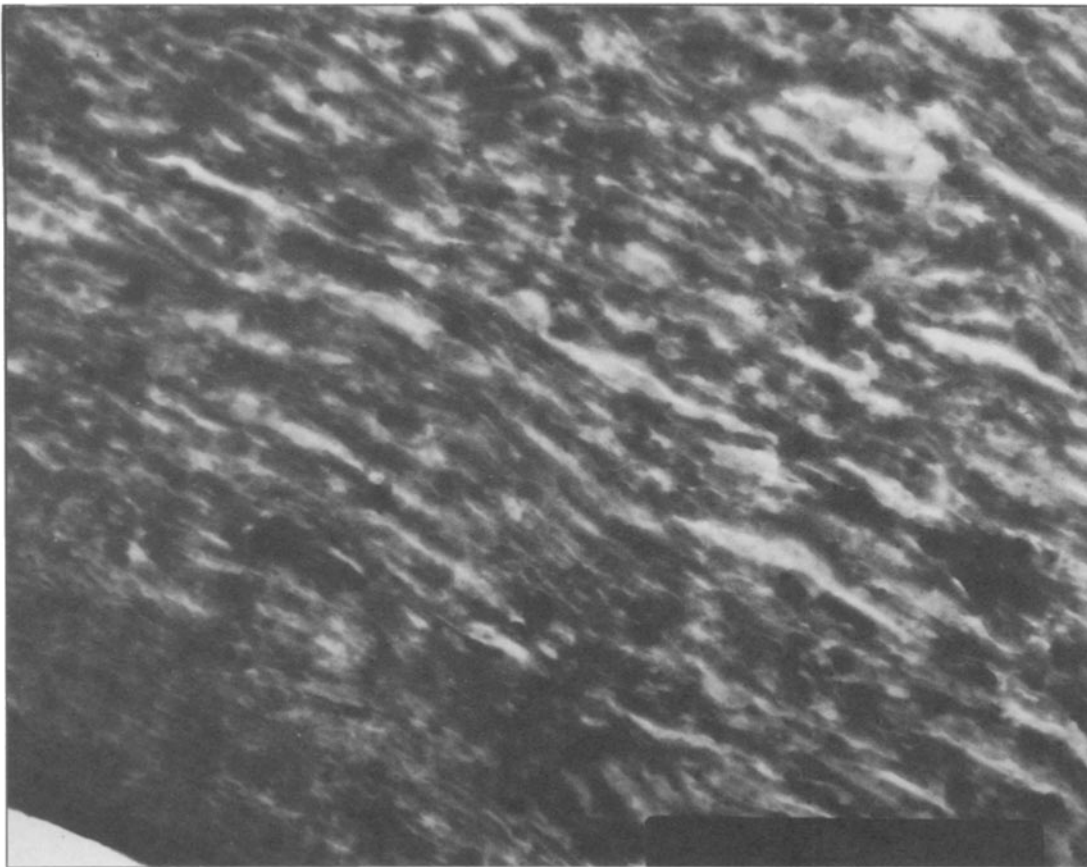**Fig. 4.** AF05H swelling ratio (die–swell) vs apparent shear rate.

Fig. 5. TEM analysis of an AF08H film slice. 7500×.

Comparison Between Analytical and Biological Data.

The biodegradation index $(CO_2/ThCO_2)$ on stopping the biological treatment was 78.5%. This value cannot be directly compared with the analytical weight loss data. The synthetic component, due to its lower oxygen percentage, may produce a higher CO_2 quantity than the average one produced by AF05H. A homogeneous comparison requires the conversion of the biological results of CO_2 evolution into degradated mass. Therefore, a second curve of weight loss was obtained from the experimental $CO_2/ThCO_2$ curve (Fig. 6), taking into consideration the different contribution of the two components to theoretical CO_2 and assuming that, up to a complete degradation of the 60% (w/w) natural component, no degradation of the synthetic part occurs.

The evaluation of the total degraded mass equal to about 84%, obtained through such an extrapolation, is reasonably aligned with the 91% obtained through gravimetric analysis and NMR, taking into account the test length.

These data support the hypothesis of a different degradation kinetic of the two components and prove the validity of modified Sturm test in monitoring the biodegradation rate of insoluble substrates.

GPC analysis of the organic residue after aerobic respirometric test and of the initial AF05H synthetic component, after acetylation and solubilization in THF, gave a decrease in average molecular weight of about 13%, together with a shift in the elution peak maximum from 81,500 to 51,000. The molecular weight distribution widened from 4.46 to 5.80.

AF08H

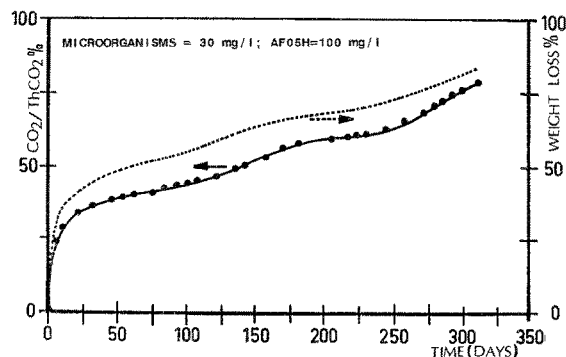This experimental grade has the same composition as AF05H, but as shown under Experimental, it con-

tains a more hydrophilic vinyl-alcohol copolymer. As a consequence, a layered morphology has been observed instead of the AF05H droplet like structure.

CO_2 evolution in this case shows an initially higher biodegradation kinetic (Fig. 7) in comparison with AF05H due to the increased availability of starch. This result is only an example of the role played by the microstructure in determining the biodegradation behavior of Mater-Bi products.

Vinyl-Alcohol Copolymers

Three products were analyzed: P17 and P8 in the same test and N17 in a second test. All three products have the same chemical composition.

In Fig. 8 the $CO_2/ThCO_2$ index is shown. The delay in CO_2 evolution of P17 and P8 is probably due to the acclimatization of unacclimatized bacteria. In the case of N17, acclimatized bacteria were used and CO_2 evolution was observed immediately after the test startup. The test was repeated several times with good reproducibility. The results in Fig. 8 clearly point out that the crystallinity of the synthetic component plays a fundamental role in determining its biodegradation kinetic. Due to its mainly amorphous state, P17 biodegrades at a higher speed than N17, which is characterized by a lower molecular weight and higher superficial area.
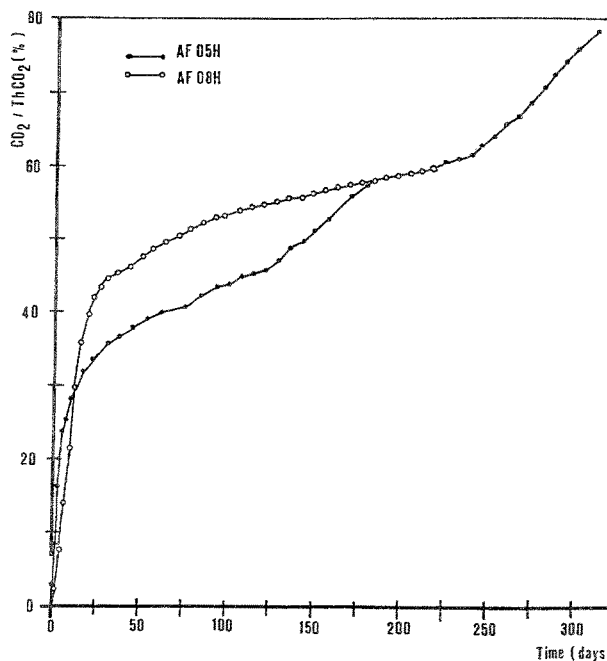

Fig. 6. CO_2 evolution for AF05H in an aerobic respirometric test (solid line). Weight loss curve extrapolated on the basis of a two-step degradation model (dashed line).

Fig. 7. CO_2 evolution of AF08H compared with that of an AF05H microdispersion in an aerobic respirometric test.
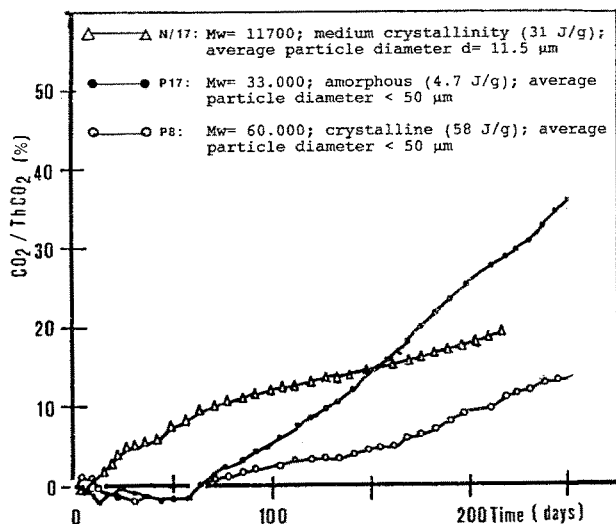
Fig. 8. CO_2 evolution of vinyl-alcohol copolymers in the aerobic respirometric test.

Figure 9 gives a comparison of the molecular weight distribution peak of acetylated N17 before and after aerobic respirometric test. A significant increase in molecular weight can be observed, from 11,720 to 34,710. In spite of the increased molecular weight, N17

samples still under testing continue to exhibit a constant CO_2 evolution.

SCAS Test and Biodegradation in Lake Water

Figure 10 shows micrographs of Mater-Bi AF10U aged for 75 and 175 days. The observed weight losses were 70 and 74%, respectively. A weight loss difference of only 4 points, however, does not correlate with the drastic morphological changes pointed out in Fig. 10.

IR spectroscopy (band at 1652 cm^{-1}) and elemental analysis (N content grows from 0.17 to 0.88% in the 75- to 175-day period) demonstrated the presence of about 10% cellular material in the residual specimen. Proton NMR spectroscopy also indicated the presence of about 11% starch. This first set of SCAS data includes the following observations.

- Initially biodegradation of the natural component occurs without significative bacterial growth on the specimen surface. The film in this case shows a reduced thickness but no holes.
- Later, at 75 days, the degradation of the synthetic part, estimated at about 20% (w/w), is ev-
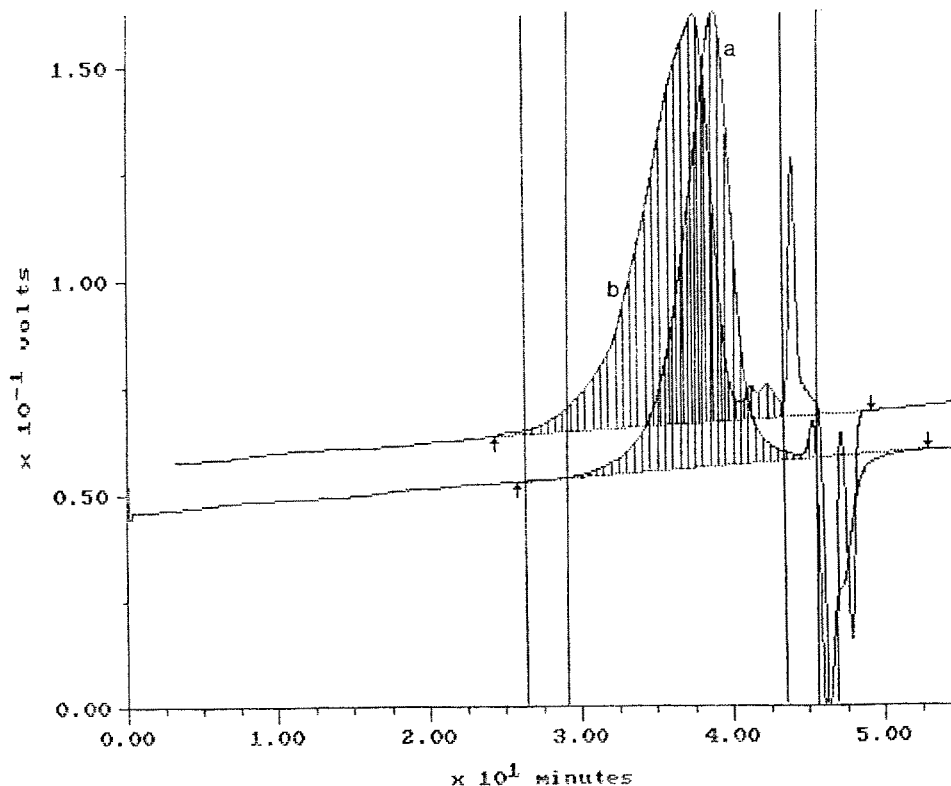

Fig. 9. GPC analysis of N17 before (A) and after (B) 160 days of aerobic biodegradation.
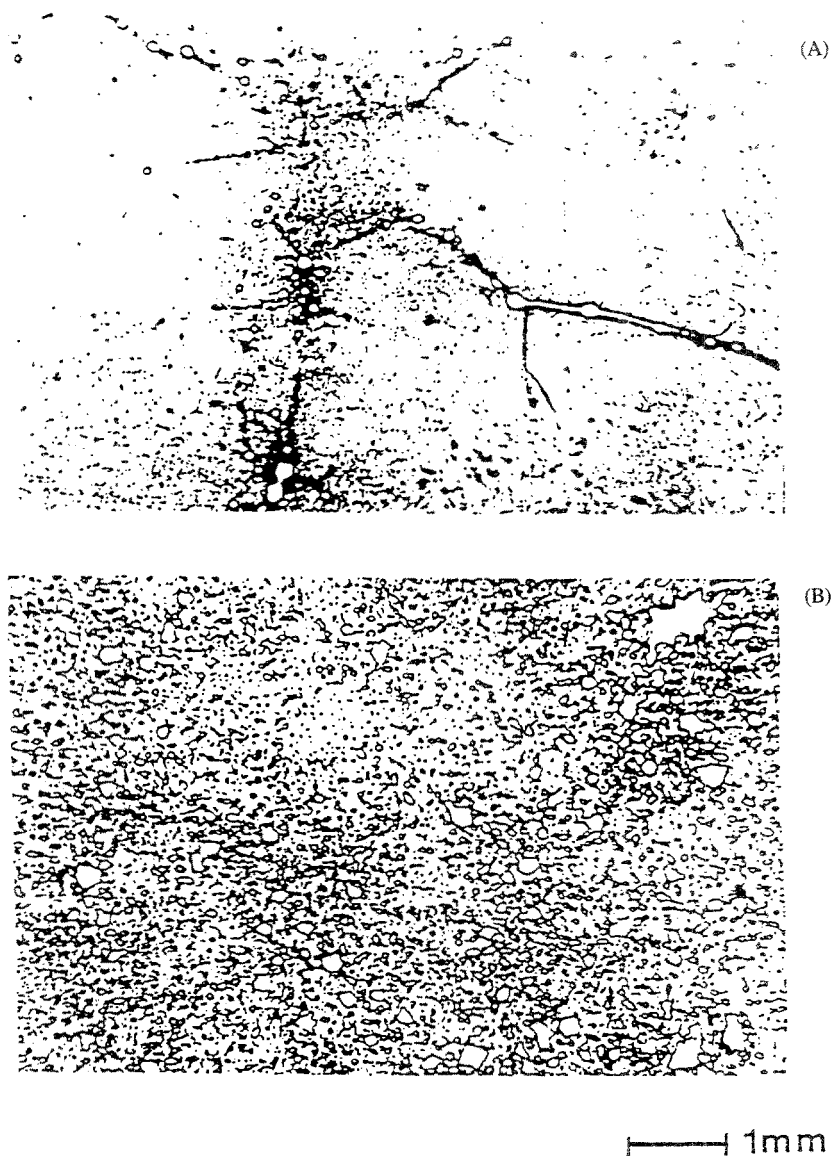
Fig. 10. SCAS test of AF10U film after (A) 75 days (weight loss of 70%) and (B) 175 days
(weight loss of 74%).

ident with the growth of microorganisms on the film surface and with the generation of regular holes. In this second step analysis of weight loss alone is not sufficient to evaluate the correct degradation degree as microbial matter penetrates the sample.

The test was continued until complete disintegration of the sample occurred at about 500 days.

In Table I simple weight loss data, without considering the contribution of microbial growth, are reported for AF10U in the SCAS test and in lake water. From Figs. 10 and 11 it is possible to note a parallel behavior

Table I. AF10U SCAS Test and Biodegradation in Lake Water

Biodegradation time (days)	Weight loss (%)	
	SCAS	Lake water
75	70	—
210	77	63
460	83	
660	Not found	79

of the sample in the two environments: the films do not show holes and strong growth of microbial material before a significant portion of the natural component is

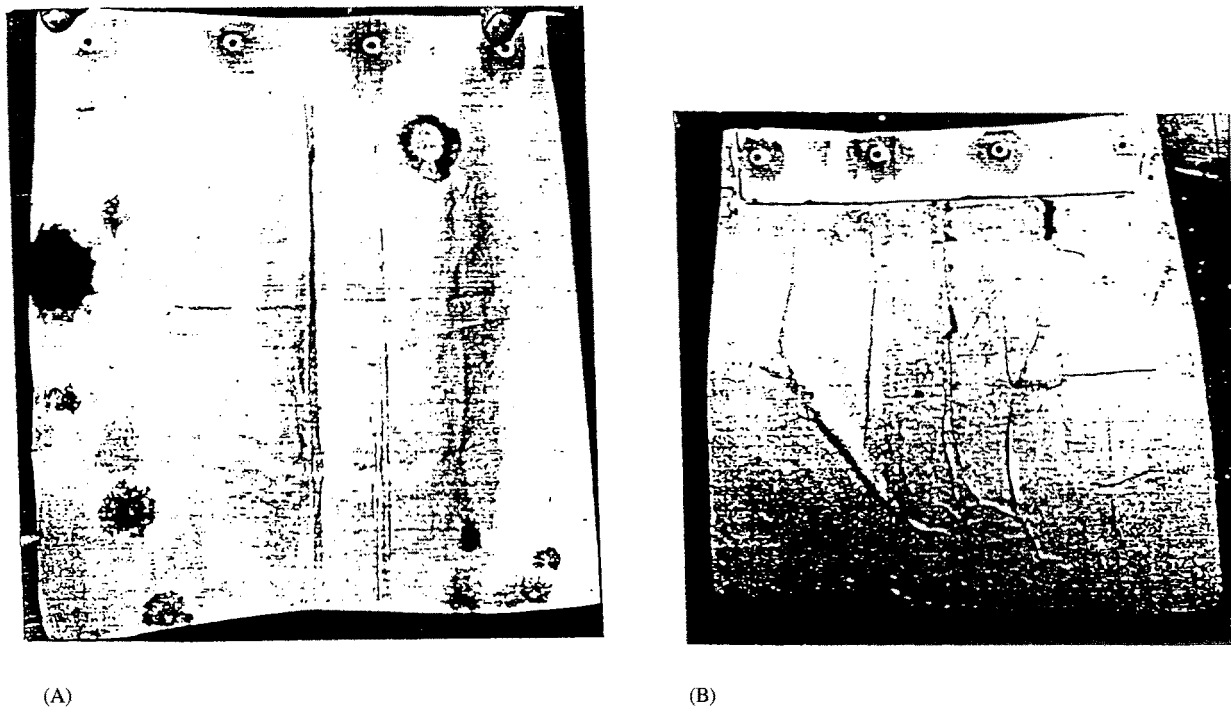(A)   (B)

Fig. 11. AF10U film after (A) 7 months and (B) 22 months of lakewater aging.

degraded. The film becomes full of holes and is covered by microbial material when the biodegradation level of the synthetic component becomes relevant. It seems from the data reported here that the SCAS test can be considered a good simulation for evaluating degradability in a watery environment.

These last experimental results support the hypothesis of a substantially different biodegradation mechanism for the two Mater-Bi components: the natural component, even if significantly shielded by the interpenetrated structure, is first hydrolyzed by extracellular enzymes; the synthetic component is biodegraded at a slower rate by microorganisms absorbed on the plastic surface—biodegradation is made easier by the increase in available surface during the hydrolysis of the natural component.

CONCLUSIONS

Mater-Bi products constitute a wide family of biodegradable materials mainly from a natural origin. They exhibit different microstructures—from a droplet-like (AF05H) to a layered one (AF08H)—probably as a result of hydrophobic and hydrophilic interactions between the natural and the synthetic components. A

model can be proposed to explain the droplet-like morphology considering large individual amylopectine molecules interconnected at several points per molecule as a result of hydrogen bonds (hydrophilic interactions) and entanglements by chains of amylose/vinyl-alcohol copolymer complexes (hydrophobic interaction). Varying the natural and synthetic components, it is possible to alternate the balance between hydrophobic and hydrophilic interactions so that the droplet-like morphology can be substituted by a layered structure. Further research is needed to confirm such a model.

The different aerobic biodegradation behaviors shown by AF05H and AF08H draw attention to the role of microstructure in determining the biodegradation rate of this class of products. Ten months of aerobic biological digestion in a high-sensitivity respirometric test degraded more than 90% (w/w) of AF05H Mater-Bi grade. Furthermore, its synthetic component was degraded about 80% (w/w), even though the test was terminated while CO_2 evolution was still relevant.

The aerobic respirometric test performed on vinyl-alcohol copolymers (Mater-Bi synthetic components) demonstrated a significant biodegradability, whose rate is influenced more strongly by the crystallinity than by the molecular weight. SCAS digestion and biodegradation in lake water of the experimental-grade AF10U

support the hypothesis of a substantially different bio-degradation mechanism for the two Mater-Bi components.

- The natural component, even if significantly shielded by the interpenetrated structure, is hydrolyzed most rapidly, probably by extracellular enzymes.
- The synthetic component seems to be biodegraded through a superficial adsorption of microorganisms, made easier by the increase in available surface occurring during hydrolysis of the natural component.

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