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Comparative Biodegradation Study of Starch- and Polylactic Acid–Based Materials

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The degradation of starch- and polylactic acid–based plastic films by microorganisms extracted from compost was studied in a liquid medium. The various degradation products produced were measured throughout the duration of the experiment, and total carbon balances were estimated. For an easily biodegradable material, the evolution of the way carbon repartitioned between different degradation products was quite similar whatever the experimental condition or the type of substrate. On the other hand, for a resistant material exposed to these microorganisms, the nature of the biodegradation depended strongly on the experimental conditions. In the latter case, a differential scanning calorimetry analysis confirmed the importance of the applied norm on the state of the residual material. The consequences for improved methods of estimation of biodegradability of these materials are discussed.

KEY WORDS: Starch; polylactic acid; biodegradation; ASTM norms; CEN norms.

INTRODUCTION

A biopolymer-based material may be expected to exhibit a partial intrinsic biodegradability because its constituents are not completely chemically modified throughout the manufacturing process. Even according to this hypothesis, the biodegradable character of the material must be demonstrated. All common methods used to estimate the biodegradability of an insoluble polymer are based on estimation of the percentage of mineralization of the material's carbon content by the end of the experiment (e.g., [1] and [2]). In a previous study [3], we demonstrated, by using thermoplastic starch as a substrate under aerobic conditions, that the two common standard biodegradability methods, ASTM and ISO/CEN, applied in liquid or solid media, gave comparable final mineralization percentages with different kinetics applying for carbon dioxide release. These percentages, up to 60%, are in accord with that expected for a biodegradable substrate; but we may ask two questions. First, is the remaining carbon in an acceptable environmental form? Second, do degradation studies of more resistant biopolymers conducted in the presence of the same microorganisms give the same results?

The answer to the first question requires one to identify and measure the entire repartitioning of the material's carbon fraction among various degradation products, namely: carbon dioxide, biomass formed by abstraction of some of the material's carbon, soluble organic compounds, and possibly nondegraded material. Sometimes, this analysis work was performed in liquid medium [4, 5], and those authors emphasized that it is necessary to develop better evaluation methods for a polymer's biodegradation potential.

The aim of the present work was to compare the biodegradation of two biopolymer-based materials in liquid media. The first material was chosen as likely to be

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easily biodegradable in order to test the methodology. The second corresponded to a not easily biodegradable film. In these two cases, the influence of both the condition of the substrate and the applied standard methods were examined.

EXPERIMENTAL

Equipment

Biodegradation was conducted in two-liter bioreactors (type CMF100, Chemap, Switzerland). Temperature was controlled by a double jacket water flow. The process for the data acquisition of the CO_2 percentage has been previously described [6].

Materials

The films were made by an extrusion process using a single screw extruder (type S 2032, SCAMIA, France). The extruder characteristics and the film manufacturing conditions have been previously described [7]. The composition (by weight) of the starch-based film (thickness: 870 $\pm$ 50 μm) was, to the nearest 1%: cornstarch (type Chamtor, Bazancourt, France) 74%, glycerol (99.5% pure) 10%, and water 16%. The polylactic acid (PLA) film (thickness: 660 $\pm$ 50 μm) was made by single extrusion without inclusion of plasticizers. The initial product (type Eco PLA, Dow Cargill, USA) was formed from L-lactic acid (95%) and D,L-lactic acid (5%). The films were stored at 23°C under a relative humidity of 50%. Their humidity percentage (starch: 10.5 $\pm$ 0.3%; PLA: 0%) was measured by the Karl Fischer method [8]. The total carbon contents of the films on a dry weight basis (starch: 39.0 $\pm$ 0.1%; PLA: 50.1 $\pm$ 0.1%) were determined by elemental analysis of crushed and dehydrated samples.

For both materials, the studies were carried out with about 2- $\times$ 2-cm pieces of film. The weight of each piece ranged between 0.4 and 0.5 g. For the starch-based material, the substrate was also studied in a crushed form (A10 grade crushing, Janke and Kunkel mill, IKA, Labortechnik).

Inocula

The inocula comprised an extract of microorganisms obtained from a mature compost of household refuse. Only the fraction sieved through a 1-cm sieve was used, in accord with usual American and European procedures. Inorganic materials such as glass or metal were removed before sieving. The characteristics of the sieved fraction (by weight) were: dry matter content, 52.7%; ashes, 34.7% pH, 8.2; and C:N ratio, 28.9 Carbon dioxide production over 10 days was 104 mg $CO_2.g^{-1}$ of organic matter. These values are in accordance with the norm recommendations.

To prepare the extract of microorganisms, 15 g of compost was added to 150 mL of Ringer liquid (MERCK Eurolab, Fontenay sur Bois, France). The mixture was shaken for 1 h, and then filtered to exclude the particles larger than 0.125 mm. The filtrate was centrifuged at 10,000 rpm for 20 min (type MR 1822 centrifuge, JOUAN, Saint Herblain, France). The supernatant was discarded, the solid residue was dispersed in Ringer liquid and then centrifuged again under the same conditions. The final solid residue was dispersed in Ringer liquid to form an inoculum that contained less than 100 mg carbon.L^{-1}.

Degradation Conditions

According to the adopted experimental protocol, either a 58°C constant temperature was maintained throughout the 45 days of experiment time (ISO/CEN European standards) or a temperature profile was applied according to the American standard, namely: day 1, 35°C; days 2–5, 58°C; days 6–28, 50°C, and days 28–45, 35°C [1].

Oxygenation was provided by bubbling air (0.5 L/min^{-1}) through the liquid medium. This flow provided a 6% oxygenation minimum and permitted assessment of the percentage of CO_2 produced (scale 0–2% by volume).

The percentage of the material's carbon converted to CO_2 (percentage mineralization–Cg-) was defined by the relation:

$$Cg\ (\%) = 100*(nCO_2e - nCO_2t)/nCO_2th$$

where nCO_2th is the theoretical amount of CO_2 in moles potentially available from the initial substrate, and nCO_2e and nCO_2t are the amounts of CO_2 product in the assay and control test reactor, respectively. The control test used liquid medium with added microorganism inoculum alone and provided a measure of the microorganisms' endogenous respiration. All the experiments were stopped when the difference ($nCO_2e - nCO_2t$) was less than 0.01% of the nCO_2th value.

The composition of the liquid medium corresponded to the DERRADJI procedure [6] with no carbon source. Starch- (15 g) or PLA-based (10.5 g) films were dispersed in 1.5 L of liquid medium. The reactor was finally seeded with 15 mL of the compost extract (1% of the total liquid volume). The assay experiment was conducted in triplicate for estimation of its repeatability.

The volume of each sample taken for measuring dissolved organic carbon, biomass weight, and dissolved species was 15 mL. A maximum of 10 samples limited the decrease in reaction volume to a maximum of 10%. The samples were centrifuged for 20 min at 1,000 rpm at 4°C. Then the upper liquid phase was filtered through a cellulose acetate membrane with 0.2-μm pore size (type Osmonics, USA). This filtrate was used for measurement of dissolved organic carbon (DOC) and high-performance liquid chromatography (HPLC) studies of the soluble degradation products.

For the DOC measurement, the inorganic carbon fraction was less than 20 mg C/L^{-1}, and hence was negligible by comparison with the organic carbon quantity (4 g/L^{-1}). However, before the DOC assays, the dissolved inorganic carbon was discharged by acidification. The dissolved fraction of organic carbon (Cs) was estimated with a total organic carbon analyser (1010 model, OI Analytical brand). For the HPLC studies, the chromatograph (SP 8880, Thermo Separation Products, Les Ullis, France) was equipped with an Aminex HPX 42A, 300- $\times$ 7.8-mm ID column (Biorad, Ivry sur Seine, France) and the temperature regulated with an oven (Crocosil). Ultrasonically degassed ultrapure water (Millipore Corporation, USA) was used as the mobile phase. The injection volume was 20 μL, and detection was achieved by refractometry. Standardization with various starch hydrolysates was carried out according to a previously described procedure [9].

Lactic acid was measured with an HPX 87H 300- $\times$ 7.8-ID column (Biorad) regulated to 40°C, and ultrasonically degassed 5×10^{-3} *M* sulfuric acid was used as the mobile phase. A flow rate of 0.6 mL/min^{-1} was maintained at a pressure of 900 psi. The lactic acid was measured with a UV detector (210-nm wavelength). Standardization with known concentrations of lactic acid showed a retention time of 13.6 min.

The residue was dispersed in 15 mL of Ringer liquid for the biomass measure. Biomass produced by the endogenous activity of the microorganisms in the control reactor was systematically subtracted from the corresponding experimental reactor values. Two different methods were used to make this measure of biomass (Cb). The first consisted of an evaluation of global protein content by the modified Lowry method. This was done with the micro SPITZER method [10] using the kit I for DC protein tests (Biorad Laboratories). After preparation of standards and samples according to the supplier's instructions, optical density measurements were performed at a wavelength of 750 nm (Uvikon 932 spectrophotometer, Kontron). The second method estimated the biomass by the dry matter technique using a sample volume of 10 mL in a cupel at a temperature of 105°C until constant weight (Mettler Toledo, AB104, Switzerland). From this, biomass was estimated on the basis of a 50% carbon content.

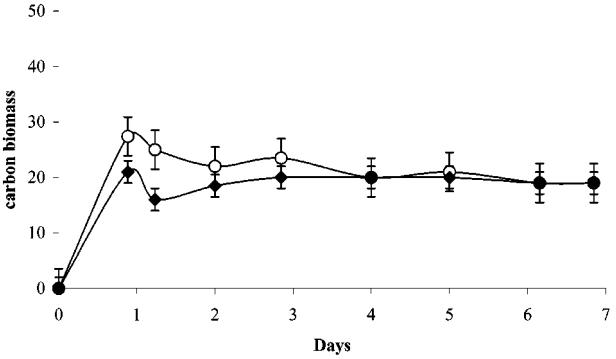

Fig. 1 Compared evaluation of the bioassimilated carbon (Cb) from the proteins dosage (♦) and dry matter measure (○) during the crushed starch substrate degradation according to the D-5209-92 ASTM norm.

The results from both these methods have been compared, for instance, with measurements for crushed starch according to the standard ASTM method [1] (Fig. 1). At the end of the experiments, the values obtained by each method were roughly the same. At the start of the experiments, the differences among the initial values could be explained by the fraction of residual starch that was still present at the beginning of the biodegradation and therefore led to an overestimate of the biomass by the dry matter technique. As the highest accuracy was obtained using the protein measurement method (Table I), it was selected for the studies conducted in liquid media.

Carbon Balances

The measured values for Cg, Cb, and Cs percentages allowed estimation of the carbonaceous fraction of the different degradation products:

$$Cd = Cg + Cb + Cs$$

The difference between Cd and 100% arose from either experimental inaccuracies or a nonbiodegraded fraction Cnd of the material. Finally, the biodegradation percent-

Table I. Carbon of the Biomass Measured at the End of the Experiment in Liquid Medium from Protein Dosage or Dry Matter Measure.*

Norm	ASTM D-5209-92		ISO/CEN 14852	
Starch	Crushed	Film	Crushed	Film
Proteins	18,0 ± 0,7	14,3 ± 1,0	16,1 ± 0,1	18,2 ± 1,9
Dry matter	19,4 ± 2,6	12,4 ± 2,2	14,6 ± 1,2	14,8 ± 4,1

* The results are expressed as carbon percentage of the substrate carbon.

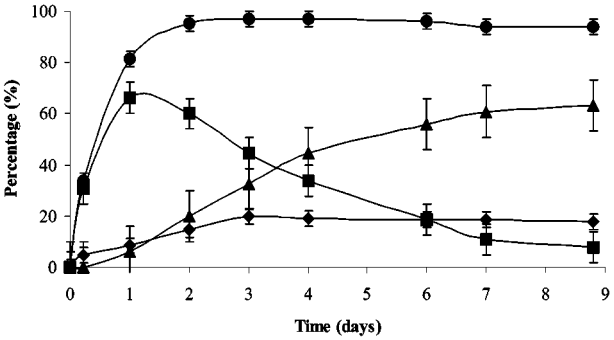

Fig. 2 Carbon balance in liquid medium: starch-based film degradation according to the 14852 ISO/CEN norm, (▲) Cb biomass carbon. (♦) Cg carbon dioxide carbon, (■) Cs DOC, (●) degraded carbon.

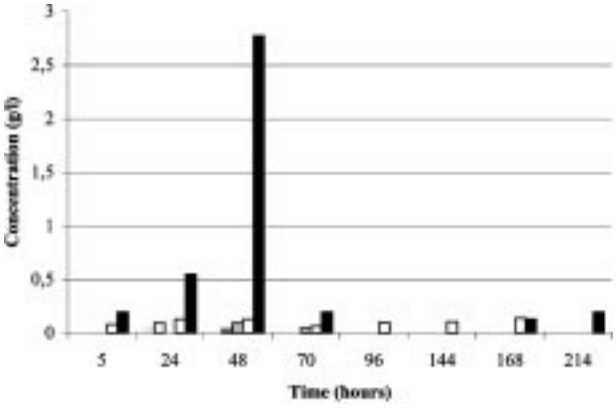

Fig. 3 Evolution of the sugars quantities released during the starch-based film degradation in liquid medium according the 14852 ISO/CEN norm. ■ Glucose, □ maltose, ■ maltotriose, maltotetraose.

age was defined as the sum of the mineralization (Cg) and bioassimilation (Cb) percentages.

Degraded Film Studies

From the weights and the numbers of film pieces at the beginning of the tests, it was possible to measure the mass loss undergone by the material when the experiment was completed. After drying at 23°C and 50% relative humidity, all the recovering material at the end of the test was weighed on precision scales (Mettler Toledo AB104, Switzerland).

The thermal characteristics of the polymers were determined with a modified differential thermal analyser (Universal V1.9D TA Instrument, USA) refrigerated by liquid nitrogen circulation. The analyses were carried out on pieces of film taken from the degradation medium during the experiment that were stored at 23°C and 50% relative humidity. Two scans were performed (at a temperature ramp rate of 10 °C/min^{-1}). The first (from ambient temperature up to 200°C) allowed elimination of the material's thermal history. The samples were cooled quickly at 0°C and scanned again to 200°C.

RESULTS AND DISCUSSION

Easy Biodegradable Material: Thermoplastic Starch

Evolution of Cg, Cb, and Cs During a Test

Figure 2 shows the percentages of carbon repartitioned among the various degradation products of the starch-based pieces of film. As previously noted, this experiment was carried out in accord with the ISO/CEN 14852 standard. By the second day, Cd had already attained about 100% of the initial carbon of the sample. Hence, all of the starch was degraded in accord with the visual disappearance of the pieces of film. The soluble carbon species (Cs) reached a maximum percentage in the first day of the experiment. The microorganisms obviously quickly assimilated the film metabolically. The biomass grown by them from the material's carbon, as estimated by the protein technique, tended quite quickly to a maximum.

The evolution of soluble sugars produced from the biodegradation of the starch-based film, as shown in Fig. 3, was contributed to by a significant release of glucose over the first two days. This agreed roughly with the Cs curve maximum (see Fig. 1). At that time, the sum of the various oligomers as measured by HPLC represented only 35% of the initial material carbon, compared with 60% for the Cs value (see Fig. 1). The difference was probably due to a higher degree of polymerization of sugars and also to enzymes released by the microorganisms during their attack on the material.

Duration of the Experiments

The duration of the experiments by comparison with previous studies was invariably shorter (less than 17 days, Table II). Taking into account the readily biodegradable character of the material, this was to be expected.

Comparative Results According to the State of the Substrate and the Applied Conditions

Table 2 shows the Cg, Cb, and Cs percentages obtained at the end of the starch-based material biodegradation experiments. The precisions of the different percentages (Cg, Cb, and Cs) were estimated to be less than a few percent. These experiments were conducted according to ASTM and ISO/CEN standards and used

Table II. Influence of the Substrate State and the Applied Norm on the Repartition of the Substrate Carbon Among the Different Degradation Products of the Starch-Based Material at the End of the Experiment

Substrate	Medium	Norm	Duration (hours)	Cg	Cb	Cs	Cd
Film	Liquid	CEN 14852	9	69	18,5	7,5	95
		ASTM D5209-92	16	67,5	14,5	10	92
Powder	Liquid	CEN 14852	10	70	16	6	92
		ASTM D5209-92	7	71	18,5	5,5	95

Cb, Bioassimilation percentage; Cd, degraded forms; Cg, final mineralization percentage; Cs, soluble carbon species.

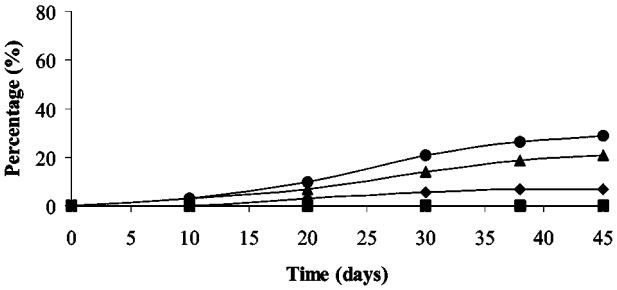

Fig. 4 Carbon balance in liquid medium: PLA film degradation according to the 14852 ISO/CEN norm. (■) Cb biomass carbon. (π) Cg carbon dioxide carbon, (■) Cs DOC, (●) degraded carbon. Cb was evaluated from the proteins content determination.

two different physical states of the material, i.e., film and powder forms.

The final mineralization percentage (Cg) was always greater than 60%, the minimum assigned value for a biodegradable material [1, 2]. Moreover, the percentage of biodegradation (Cg + Cb) including the microbial bioassimilation of the material carbon was between 82% and 90%.

Few soluble species were still present at the end of the test: less than 10% at the most for the film experiment and the ASTM standard (powder) experiment. Finally, the sum of the degraded forms (Cd) was close to 100%. This result confirmed the reliability of the measures of biodegraded forms, the difference from the 100% theoretical value being within the estimated precision of the different measurements.

The repartitioning of biodegradation products was approximately the same whether the material was film or powder. Only the soluble species (Cs) were present in higher concentration when the substrate had the form of a film. Apparently, those species were extracted more slowly from the film and therefore assimilated later.

For materials in the same state, the comparative differences between each standard method fell within the limits of the measurement uncertainties. Therefore, it seems that the American and European standard methods produced the same degree of mineralization and values for percentage biodegradation, whatever the degradation medium. Moreover, the degree of global biodegradation (Cd) was also independent of the applied standard method.

Polylactic Acid: A Biomaterial That Is Not Easily Biodegradable

Carbon Balance

The application of the ASTM standard method did not produce biodegradation of pieces of PLA film, because all the measured values for the biodegradation products were effectively zero. Moreover, the film pieces were wholly recovered at the end of the experiment. With the ISO/CEN standard method, the percentage biodegradation after 45 days was found to be significant: 30% including 23% mineralization (Fig. 4). The residual carbon in the remaining material was evaluated by residue weighing to be 67% of the initial carbon. The missing 3% probably corresponded to unrecovered fine fragments of the residual material. In fact, the initial film was turned into a powder, as shown Fig. 5. HPLC analysis of the very small soluble fraction produced (Cs) indicated release of small quantities of lactic acid during the experiment: 0.02 g/L^{-1} at most. This indicates that the microorganisms assimilated the lactic acid as soon as it was released.

These differing results from applying the two standard methods are understandable because the temperature profile was different between them. Globally, the temperature is higher in the ISO/CEN method, especially at the beginning

Fig. 5 Evolution of the physic state of the PLA film pieces during their degradation in liquid medium according to the 14852 ISO/CEN norm.

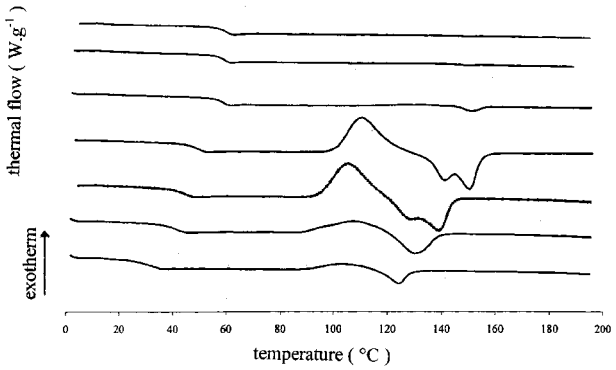

Fig. 6 Thermograms evolution with the time of PLA film pieces degraded according to the ISO/CEN 14852 norm.

of the test. This allowed an important abiotic degradation of the substrate to occur before biodegradation.

DSC Study of the Residual Material

The evolution of the DSC thermograms of the PLA biodegradation conducted in liquid medium according to the ISO/CEN standard method (Fig. 6) showed the amorphous character of the starting material. Its calculated percentage crystallinity was very low, as seen in Table III (0.2%). This meant that the PLA was in an essentially amorphous state before the biodegradation experiment. Then, for the ISO/CEN-based test, Tg values decreased strongly throughout the experiment in accord with the molar mass of the PLA present. This indicates the PLA had undergone an important physical degradation throughout the experiment conducted according to the ISO/CEN standard. From the fourteenth day, two peaks appeared. These exothermic and endothermic peaks corresponded first to a crystallization of the material during its degradation and second to the melting of the PLA. With increasing time, the extent of the degradation was characterized by simultaneous decreases of Tg and Xm.

Table III. DSC Thermograms Evolution of PLA with the Time for Degradation Experiments According to the ASTM and ISO/CEN Norms

Norm	ASTM D-5209-92		ISO/CEN 14852	
Time (days)	Tg (°C)	Xm (%)	Tg (°C)	Xm (%)
0	59,4	0,2	59,4	0,2
1	59,0	0,3	58,9	0,4
5	57,5	0,6	58,3	1,9
14	56,8	5,8	49,3	33,6
24	54,3	22,8	44,3	32,9
31	45,0	31,4	40,8	15,4
45	32,2	33,5	30,3	6,6

For the ASTM standard method, the results in Table III show a weaker shift in Glass Transition Temperature (Tg) toward low temperatures and a slower increase in Xc during the experimental period. These results were in agreement with an absence of mineralization throughout the test.

CONCLUSION

Estimation of material biodegradability is based on measures of the substrate's degree of carbon mineralization. The present work showed a relative independence of the results obtained, regardless of whether the applied standard method was American or European when the material was an easily biodegradable material such as a starch-based one. However, conversely, resistant materials, such as polylactic acid–based materials gave various values of percentage mineralization according to whatever standard method was used. The temperature of the medium seemed to be the major factor in explaining the observed differences.

The best way to estimate the biodegradable character of a material would be to evaluate, in addition, the biomass formed from its destruction. In fact, if at the end of a standard test, the test microorganisms assimilated a non-negligible fraction of the material's carbon, then it would be necessary to take this into account in the estimation of biodegradability. The present study has shown that the bioassimilation percentage (Cb) represented between 21% and 27% of the corresponding percentage mineralization (Cg). For materials that are not easily biodegradable, it seems to be very important to measure the Cg + Cb quantity to give a better appreciation of the biodegradation caused by microorganisms.

Finally, knowledge of the material's behaviour under exposure to microorganisms ideally requires identification of the various degradation products in order to perform a carbon balance. This study constituted one attempt that indicated methodological improvements are still required because the actual balances obtained were still too inaccurate. Possible improvements could be based on a better determination of Cb. The same method is not necessarily the most suitable for substrates in different physical states. Moreover, the accuracy of the biomass determination was still poor. A possible way to validate biomass estimation methods would be to use substrates containing a radioactive element. This approach would validate the carbon balance in its entirety. We plan to use this approach in our next study.

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