Biodegradation of Starch and Acrylic-Grafted Starch by *Aspergillus niger*

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ABSTRACT: The biodegradation of starch and grafted starch by *Aspergillus niger* was examined. The grafted polymers were poly(methyl methacrylate) (PMMA) and poly(butyl acrylate) (PBA). Thermogravimetric analysis, Fourier transform infrared, and scanning electron microscopy were used to determine the morphology and degradation degree of each material. The temperature of maximum decomposition for starch decreased as enzymatic degradation proceeded, and it was completed on the 8th day of culturing in a liquid medium. Grafted samples with PMMA and PBA achieved degradation of their starch moiety. PBA in starch-*g*-PBA samples hindered the accessibility of the enzymes to the degradable material, and this resulted in a longer degradation time. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 2764–2770, 2003

Key words: biodegradable; graft copolymers; thermogravimetric analysis (TGA)

INTRODUCTION

Tougher legislation concerning environmental contamination has been passed because of the use of indestructible plastic materials. This has led to studies devoted to reducing this contamination through the use of completely biodegradable materials or by the incorporation of biodegradable components such as natural starch and cellulose into plastics to facilitate their degradation.[1–7] Starch is a polysaccharide composed of amylose and amylopectin chains that can be biodegraded by microorganisms such as fungi and bacteria, which are able to adapt to new substrates and produce a great variety of enzymes.[8] Starch has been used in composite materials as a filler to increase the degradation rate of synthetic polymers. Starch can be consumed by microorganisms; this increases the surface area of the polymer and thereby facilitate its degradation during exposure to environmental conditions. The material can be reduced into small particles causing minimal damage to the environment.[9,10] Nevertheless, because of the hydrophilic nature of starch and the poor interface adhesion of starch and polymers, most mixtures present deficient mechanical properties.[4,9] A way of increasing adhesion is to chemically modify the starch surface through the grafting of some polymers; this also modifies several of its physical properties, such as elasticity, absorbency, ion-exchange capacity, and thermal resistance.[11–14] There have been several studies on the biodegradation of composed materials in which starch or grafted starch is incorporated into a polymeric matrix [e.g., polyethylene, polycaprolactone, poly(vinyl alcohol), and acrylic acid copolymerized with polyethylene].[3–4,9,10,15–19] Nevertheless, few studies exist on the biodegradation of grafted starch. The biodegradation processes can significantly be influenced by the chemical constitution of the macromolecules. For this reason, studies on the biodegradation of grafted and ungrafted starch should be performed to clarify this process. Flaqué et al.[20] reported that grafted vinylic chains blocked the OH groups of cellulose and reduced the accessibility of the enzymes produced by the microorganism; this resulted in less biodegradation of grafted cellulose. We can expect this behavior for the biodegradation of grafted starch because of the very similar molecular structures (the same repetitive units) of cellulose and starch. To obtain a material with good mechanical properties that can be biodegraded when discarded and exposed to environmental conditions, we require a good balance between biodegradability and particle–polymer adhesion (both resulting from the polymer being grafted onto starch).

The biodegradation by *Aspergillus niger* of starch and starch particles grafted with acrylics is presented in this work. This fungus is commercially used to obtain the glucoamylase enzyme, which degrades starch. It can also produce other enzymes, such as α-amylase and α-glucosidase, involved in starch degradation.[21]

EXPERIMENTAL

Corn starch was acquired from Productos de Maíz, S. A. de C.V. (México City, México). The methyl

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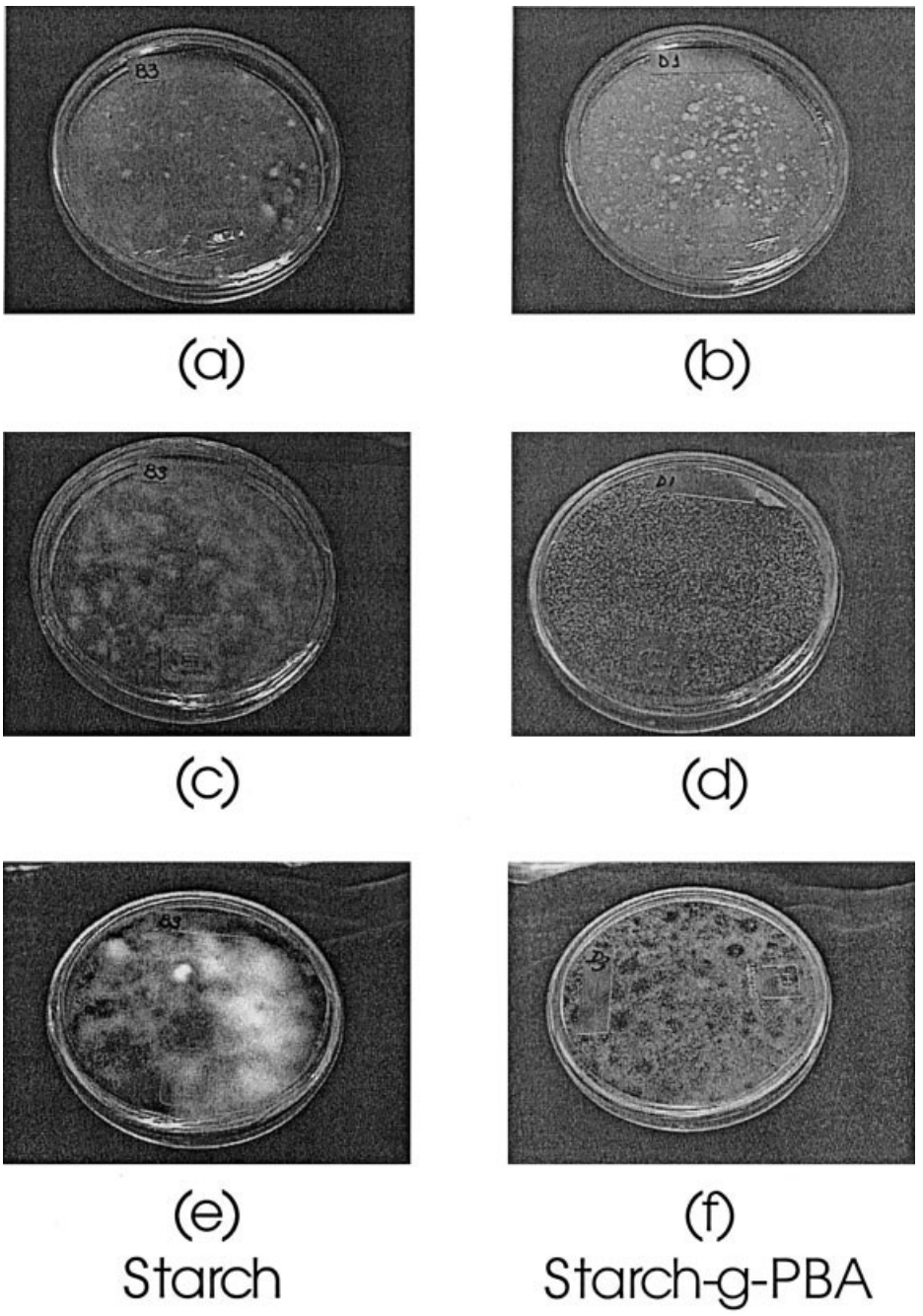

Figure 1 Photographs of petri dishes with starch (left) and PBA-grafted starch (right): (a,b) before biodegradation with *A. niger*, (c,d) after 5 days of culturing, and (e,f) after 20 days of culturing.

methacrylate (MMA) and butyl acrylate (BA) monomers were obtained from Aldrich (Milwaukee, WI); they were passed through an Aldrich column for the removal of inhibitors. Poly(methyl methacrylate) (PMMA)- and poly(butyl acrylate) (PBA)-grafted starches were obtained as granules according to a procedure reported in the literature.[22]

Sample sterilization

Starch and grafted starches were sterilized by immersion in a 70% (v/v) ethanol solution for 30 min. They were filtered and dried for 1 h under a laminar flow hood.

Biodegradation in an agar solid medium

The culture medium was prepared according to the G-21 ASTM standard, which is used to measure the resistance of synthetic polymeric materials to fungi.[22] The culture was prepared without a carbon source and sterilized by autoclaving at 121°C for 25 min. One layer of the medium was left to solidify in petri dishes. The starch or grafted starches were distributed over this layer. A second layer of the medium containing an *A. niger* spore suspension was poured into the petri dishes. This procedure allowed the samples to be

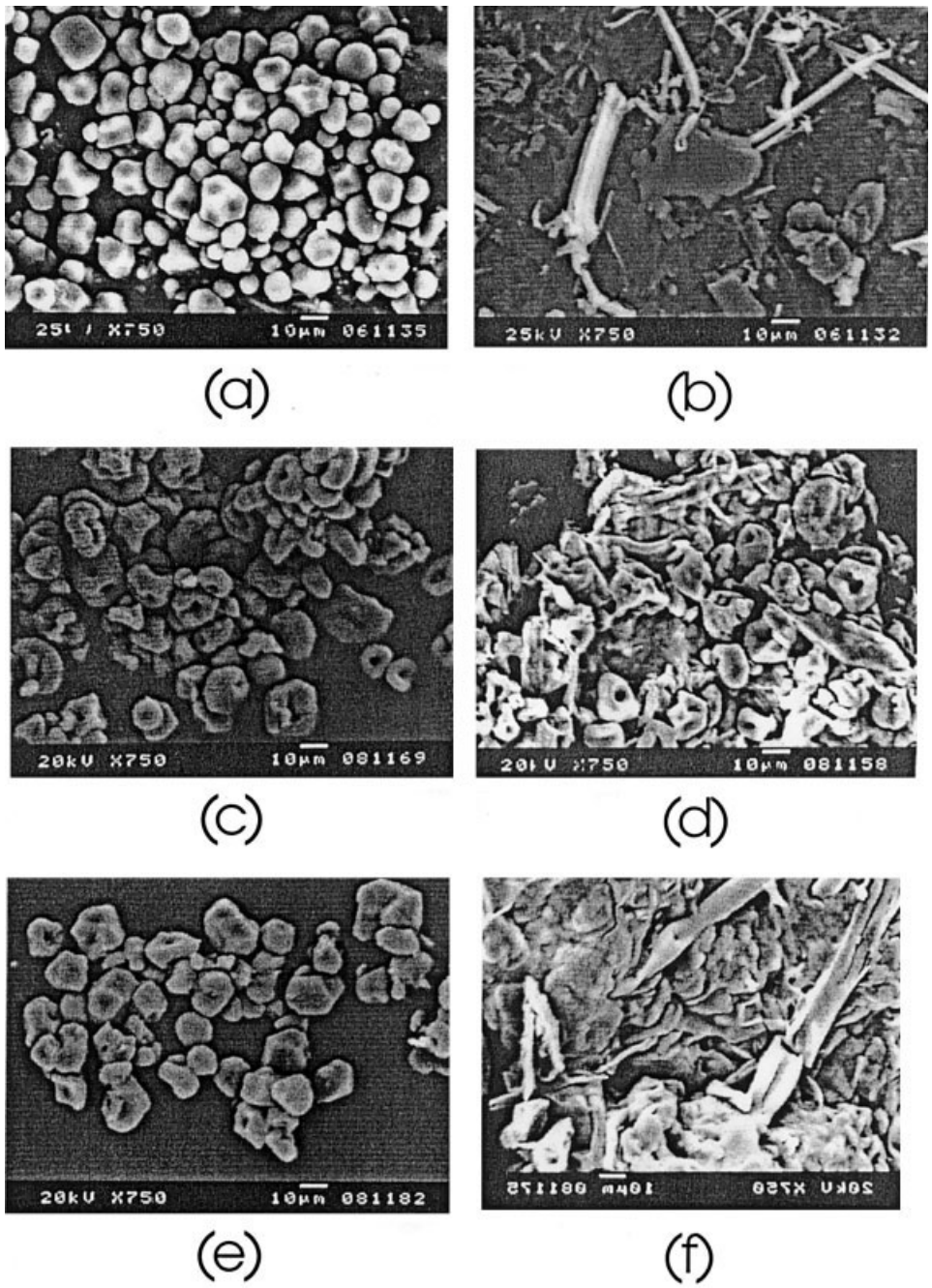

Figure 2 SEM micrographs of starch and grafted starch particles not biodegraded (left) and biodegraded (right) with *A. niger*: (a,b) starch, (c,d) starch-*g*-PMMA, and (e,f) starch-*g*-PBA.

maintained between the two layers of the semisolid medium. The petri dishes were kept at room temperature in the dark. The growth of the fungus was evaluated by visual observation at 4, 8, 12, and 20 days of culturing.

Biodegradation in a liquid medium

One gram of the sample (starch or grafted starch) was placed in a 250-mL Erlenmeyer flask with 50 mL of a mineral salt solution without a carbon source.[23] The suspensions of starch and grafted starch were inoculated with 2 mL of an *A. niger* spore suspension. The flasks were placed on a rotary shaker at room temperature in the dark. Samples were taken every 4 days for the evaluation of the degradation of the starch and grafted starch particles. A 60% (v/v) commercial chlorine solution (50 mL) was added to each flask to dissolve the organic matter. The flasks were shaken for 2 h, and the medium was centrifuged for 40 min at 3500 rpm. The solid phase was dried at room temperature for 4 days and then in an oven for 24 h at 40°C to a constant weight.

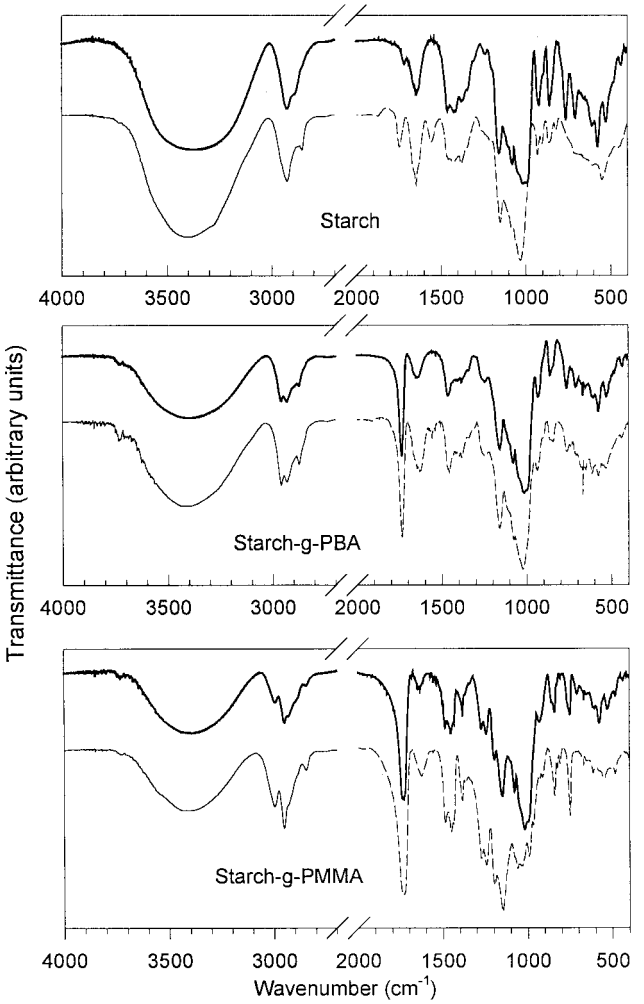

Figure 3 IR spectra of starch and grafted starch: (—) before and (- - -) after biodegradation with *A. niger*.

Characterization of the starch and grafted starch particles

Fourier transform infrared spectra of starch and grafted starch were obtained with a Nicolet 460 Protégé Magna IR spectrophotometer (Madison, WI). The samples were mixed with KBr and pressed to form transparent tablets. The transmission technique was used, and IR spectra were obtained in a 4000–400-cm^{-1} range with a resolution of 4 cm^{-1}. Thermogravimetric analysis (TGA) of the samples was performed with a PerkinElmer TG7 thermogravimetric balance (Shelton, CT), with a heating rate of 10°C min^{-1} and a temperature range of 50–600°C under a nitrogen atmosphere.

For the observation of starch and grafted starch particles before and after biodegradation, a JEOL SEM LV5400 scanning electronic microscope (Peabody, MA) was used; the sample particles were coated by gold with sputtering before the test so that damage would be avoided.

RESULTS AND DISCUSSION

Images of starch and grafted starch before and after biodegradation with *A. niger* in the semisolid medium are shown in Figure 1. The fungi showed fast growth within the first 10 days of culturing when starch was used as a carbon source. At day 5, the fungi covered 90% of the petri dish surface [Fig. 1(c)]. At day 20, no more starch grains were observed in the medium [Fig. 1(e)]. When the fungi were cultured with grafted starch with acrylic polymers, the generation of conidial heads was observed during the first 10 days of culturing [Fig. 1(d)], after which the mycelium growth began [Fig. 1(f)]. The fungi growth delay could be attributed at the polymer grafted onto the starch grain surface, which limited the accessibility of the enzymes to the starch chains. Spore generation and fungi growth delay were longer for PBA-grafted starch; this polymer behaved as a rubber at room temperature and could cover the surface of the grafted starch with better effectiveness, limiting the growth of the fungus. Starch recovery from the semisolid medium for their characterizations was difficult because of the presence of agar, and the growth of the fungi was nonhomogeneous. For these reasons, we decided to carry out the biodegradation of grafted starch and starch grains in the liquid medium.

Microphotographs obtained by scanning electron microscopy (SEM) of starch and grafted starch recovered from the liquid culture medium are shown in Figure 2. The starch grains [Fig. 2(a)] had an irregular shape with size dispersion, varying between 10 and 25 μm. After 20 days of biodegradation, almost all the starch particles [Fig. 2(b)] were degraded, forming small amorphous aggregates. Also, there were some fungus hyphae, which indicated that the treatment with a chlorine solution did not eliminate all the fungi. Because a more effective cleaning process resulted in the partial removal of the sample, causing potential errors in analysis,[19] we only used the treatment with a chlorine solution to remove the fungi. The starch

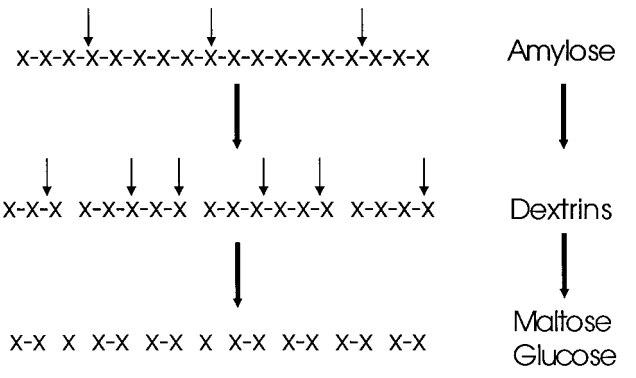

Figure 4 Schematic representation of amylose biodegradation by random attack. -X-X- represents glucose repeating units connected by 1,4-linkages.

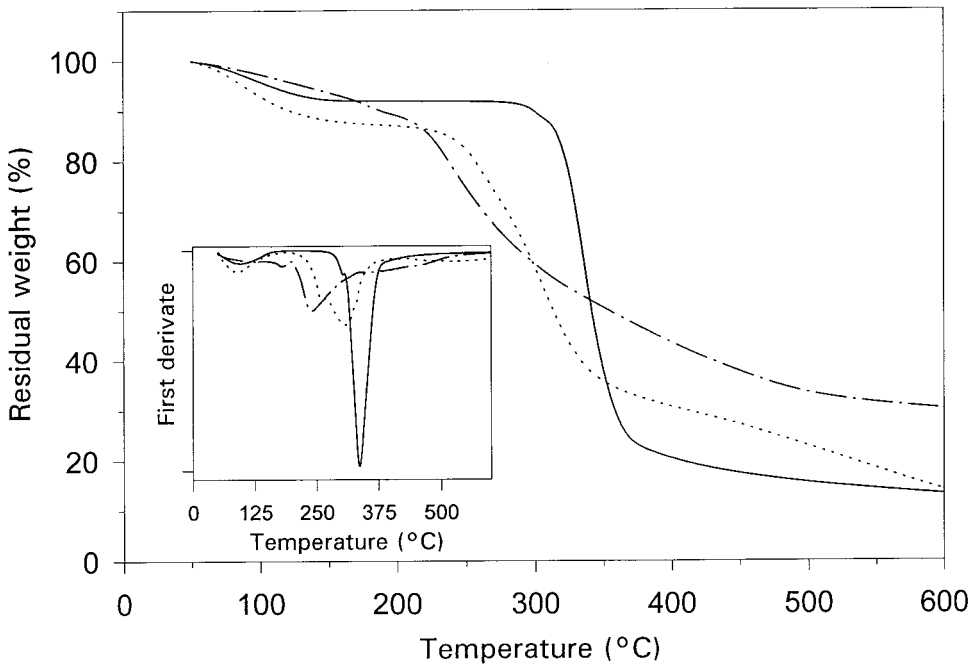

Figure 5 Thermograms of starch: (—) before biodegradation with *A. niger*, (- - -) after 4 days of culturing, and (- · -) after 20 days of culturing. The inset shows the first derivatives of the curves shown in the figure.

grains grafted with PMMA or PBA also presented irregular forms, but with small holes within their structures; this was more evident for the starch-*g*-PMMA grains [Fig. 2(c)]. These holes were produced by starch degradation during the grafting reaction, which was carried out in an acidic medium. The solids recovered from the biodegradation of starch-*g*-PMMA grains [Fig. 2(d)] partially lost their original shape, forming small aggregates. Biodegraded starch-*g*-PBA grains [Fig. 2(f)] were completely deformed, yielding an amorphous mass. Fungal residues were also found in these samples.

IR spectroscopy

Figure 3 shows the IR spectra of starch and grafted starch before and after biodegradation with *A. niger* fungus. The starch spectra presented a broad band in the 3700–3000-cm^{-1} region and a peak at 1650 cm^{-1} corresponding to O—H stretching and bending modes, respectively. A characteristic absorption band due to C—O stretching (C—O—C and C—O—H) appeared at 1150–950 cm^{-1}. The spectra of biodegraded starch showed only a small little difference from the spectrum of the original starch: a small increase in O—H stretching due to the enzymatic degradation products of starch, glucose and maltose.[24,25] It has been reported that starch biodegradation occurs by the hydrolysis of the 1,4-linkages in amylose and amylopectin. This attack is carried out at random points, as shown in Figure 4.

A strong peak at 1740 cm^{-1}, corresponding to the carbonyl group (C═O) stretching, was observed in the grafted starch spectrum, in addition to the characteristic starch peaks. The biodegraded grafted starch (Fig. 3) showed weaker starch bands after degradation by the fungus; the spectra became more like the polymer spectra. This change was more evident in PMMA-grafted samples.

Thermal analysis

Figure 5 shows the thermogravimetry (TG) and differential thermogravimetry (DTG) curves of starch before and after biodegradation by *A. niger*. It can be observed from the TG curves (Fig. 5) that starch presented an initial weight lost of approximately 10% at 100°C because of the humidity of the sample. The main degradation of starch appeared in the 300–360°C range with a 78% weight loss. A residual mass of 13% was obtained at 600°C.

The weight loss of biodegraded starch samples was initiated at lower temperatures than that of the nonbiodegraded starch. Two main zones of thermal decomposition were observed at 220–320 and 320–380°C with weight losses of 50 and 20%, respectively. These changes resulted from the reduction in the size of the starch chain caused by the action of the enzymes produced by the fungus. Therefore, as a part of the hydrocarbonated skeleton of the starch was consumed, its molecular weight decreased, leading to a lower thermal stability of the sample. This could be

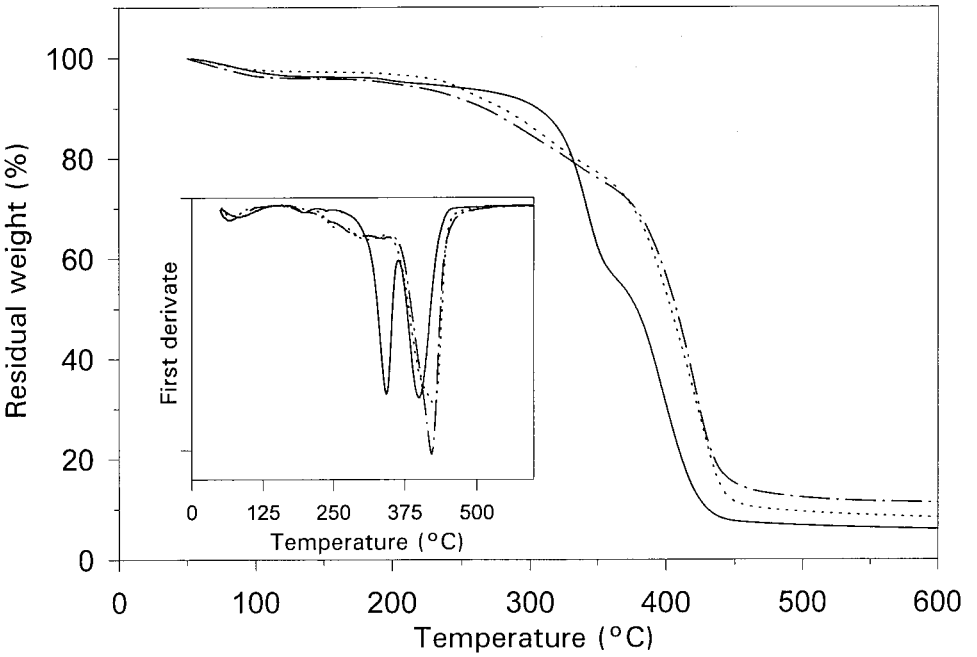

Figure 6 Thermograms of PMMA-grafted starch: (—) before biodegradation with *A. niger*, (- - -) after 4 days of culturing, and (- · -) after 20 days of culturing. The inset shows the first derivatives of the curves shown in the figure.

observed better in the DTG curves (see the inset in Fig. 5), in which the peaks corresponded to the temperature of maximum decomposition (DT_{max}) of the samples. Nondegraded starch presented a DT_{max} value of 335°C, whereas biodegraded starch presented DT_{max} values of 305 and 237°C after 4 and 20 days, respectively. This lower DT_{max} for biodegraded starch could be attributed to a reduction in the molecular weight of starch and a wider molecular weight distribution due to the random attack of the enzyme on the starch molecules resulting in lower thermal stability of the biodegraded starch.

Figures 6 and 7 show the TG and DTG curves of PMMA-grafted starch and PBA-grafted starch before

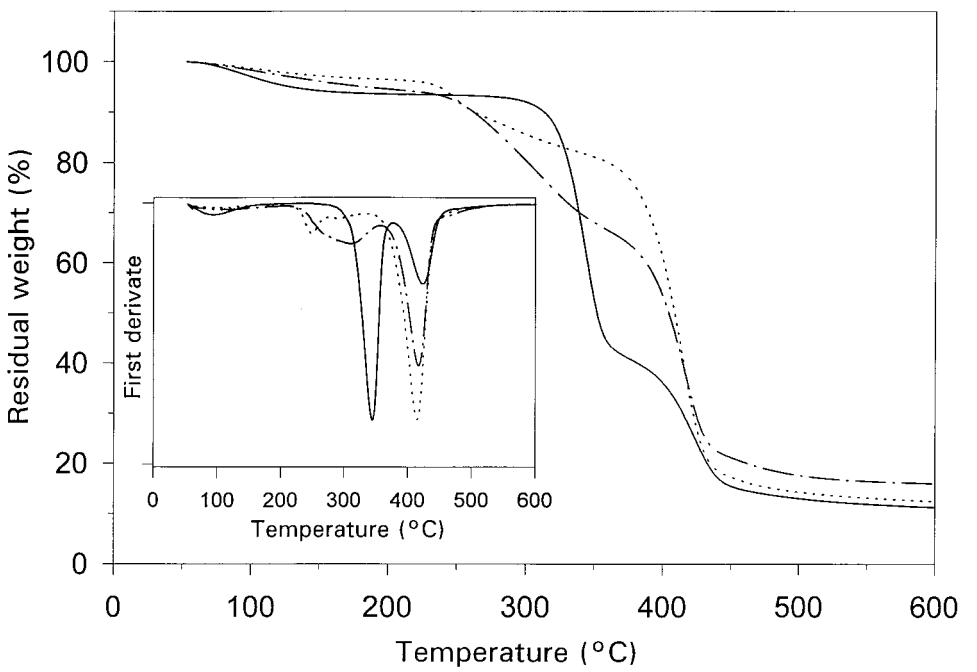

Figure 7 Thermograms of PBA-grafted starch: (—) before biodegradation with *A. niger*, (- - -) after 4 days of culturing, and (- · -) after 20 days of culturing. The inset shows the first derivatives of the curves shown in the figure.

TABLE I
TD_{max} for Grafted Starch Before and After Biodegradation with *A. niger*

Culture time (days)	PMMA grafted starch		PBA-grafted starch	
	DT_{max} I	DT_{max} II	DT_{max} I	DT_{max} II
0	337	397	343	422
4	—	416	251	415
20	—	414	Broad	417

and after biodegradation by *A. niger*. Grafted starch presented two main thermal decomposition zones, the first one for starch and the second for the grafted polymer. This resulted in two peaks in the DTG curves. After biodegradation, DTG curves of PMMA-grafted starch (inset in Fig. 6) showed only a DT_{max} value of 416°C attributable to grafted PMMA, indicating that starch chains were degraded by fungus enzymes. The lower DT_{max} value of PMMA grafted to starch (Table I), compared with that of PMMA obtained after starch degradation, could be attributed to a reduced thermal stability of starch with respect to PMMA, which reduced the thermal stability of grafted PMMA.

The DTG curves (see the inset in Fig. 7) of PBA-grafted starch after 4 days of biodegradation still showed two DT_{max} values, one at 251°C for biodegraded starch and another at 417°C for grafted PBA (Table I). The DTG curves for PBA-grafted starch after 20 days of biodegradation showed a peak at 415°C and a broad peak due to thermal degradation of the mycelium from the fungus, which was not completely removed from the sample, as can be observed in Figure 2(f).

CONCLUSIONS

The grafting reactions modified the form of the starch grain because of degradation by the acidic medium in which the reaction was carried out. This was observed in SEM micrographs. The grafted polymer on the starch grain could delay the fungus growth in a semisolid or liquid medium, but at sufficiently long periods, the starch was completely degraded, and only the grafted polymer was present in the biodegraded samples. The degradation of the starch from grafted starch samples was confirmed by TGA because only a peak corresponding to the DT_{max} value of grafted polymers was observed in the DTG curves of biodegraded PMMA- and PBA-grafted starch.

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