Polymer Bulletin 60, 677–688 (2008)
DOI 10.1007/s00289-008-0903-0

Polymer Bulletin

Effect of Morphology on the Biodegradation of Thermoplastic Starch in LDPE/TPS Blends

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Received: 22 June 2007 / Revised version: 17 November 2007 / Accepted: 16 January 2008
Published online: 30 January 2008 – © Springer-Verlag 2008

Summary

In this study, thermoplastic starch (TPS) was mixed with low density polyethylene with different melt flow indexes in a one-step extrusion process to produce LDPE/TPS blends varied from 32% to 62% by weight of TPS. The influence of starch content and LDPE viscosity on morphology, biodegradation and tensile properties of LDPE/TPS blends were evaluated. Starch continuity and biodegradability were studied by hydrolytic, enzymatic and bacterial degradation. The LDPE viscosity had a considerable effect on the morphology and the connectivity of the starch particles. Evaluation of hydrolytic extraction showed that blends having TPS content above 50 wt% possessed a full connectivity. Studies of biodegradation indicated that the bacterial attack on starch resulted in weight loss of TPS of 92%, 39% and 22%, for PE1/TPS having 62% and 32% TPS, and PE2/TPS (31% TPS), respectively. Comparatively, the weight loss was more significant at 100%, 66% and 31% by hydrolytic extraction. Differences between these two techniques were discussed in terms of the accessibility of starch domains to microorganisms. Tensile properties (ε_b and E) decreased with increasing exposure time to activated sludge. Changes in tensile properties were highly dependent on the biodegradation rate. PE1/TPS blends having 32% starch remained ductile after 45 days of exposure to bacterial attack.

1. Introduction

Starch is a natural carbohydrate storage polymer accumulated in the form intracellular granules by plants. It is composed of linear polysaccharides called amylose and branched polysaccharides called amylopectin [1]. Starch has been considered as an excellent candidate to partially substitute synthetic polymer in packaging, agricultural mulch and other low-cost applications due to its abundance, biodegradability and low cost [2]. In order to destructurize granular starch, moisture, heat and/or shear must be

applied [3]. Once starch molecules are separated, a suitable plasticizer can be used to produce a thermoplastic material. The so-called thermoplastic starch (TPS) can be processed using similar processing equipment as that used with synthetic polymers. TPS has been melt blended with synthetic polymers to produce partially biodegradable materials, which shows high ductility similar to certain commercial plastics [4]. Several works have been devoted to study blends of starch with non biodegradable polymers such as polyethylene (LDPE). Some of these works have reported that LDPE blends prepared with TPS showed a higher biodegradation extent than those prepared with granular starch. This difference was explained on the basis of a more homogeneous distribution of TPS in the blend, which allowed a larger amount of starch to be available to microorganism [5-8]. Despite these work, none has focused on the evaluation of the relationship between morphology and biodegradability of these partially biodegradable systems. The present study analyzes the influence of morphology on the biodegradability of LDPE/TPS blends.

2. Experimental

2.1 Materials

Two commercial LDPE resins, LDPE133a (PE1, MFI = 0.35 g/10 min) and LDPE993 (PE2, MFI = 25 g/10 min) were supplied by Dow Chemical Co. Corn starch (with approximately 8.3% of humidity) was obtained from Arancia Corn Products (Guadalajara, Jalisco, México). Distilled water and glycerol (Proquisa S.A, Saltillo, Coahuila, Mexico) were used to gelatinize and plasticize starch.

2.2 LDPE/TPS blends preparation

In a previous work [9], TPS with 36% of glycerol was mixed with polyethylene (PE1 and PE2) in a one-step extrusion process. TPS composition in blends was varied from 31 to 62% wt. Processing conditions were similar to those used by Favis and coworkers [10] and Rodriguez *et al.* [11], but in this case the twin-screw extruder (TSE) was a Werner and Pfleiderer ZSK30 and the single-screw extruder (SSE) was a Killion T-100. TPS ribbons were extruded through a 3 x 40 mm rectangular die.

2.3 Morphological Analysis

Specimens of LDPE/TPS ribbons were cut in the longitudinal direction using a cryogenical ultramicrotome EMFC LEICA Ultracut. Once microtomed, TPS fraction was extracted with 6N HCl at 60°C for 48 hours. Samples were then washed several times with distilled water and dried in a vacuum oven at 60°C for 24 hours. Dried samples were coated with a gold-palladium alloy and observed with a JSM-820 scanning electron microscope (SEM).

2.4 Hydrolytic degradation

LDPE/TPS ribbons were cryogenically milled to obtain samples of around 3 mm in diameter. In order to evaluate the accessibility of starch domains in those samples, hydrolytic degradation was carried out in a solution of HCl 6N at 60°C for 72 hours. Extracted samples were thoroughly washed several times with distilled water and

dried at 60°C in a vacuum oven for 24 hours. The extraction was determined and expressed as percent continuity. Percent continuity was determined evaluating the weight loss of TPS with respect all the TPS content in the blend once exposed with HCl 6N.

2.5 Enzymatic degradation

Enzymatic hydrolysis was performed in milled samples using the enzymatic cocktails Liquozyme Supra 2.2X (Novozymes), a liquid mixture of α-amylase (*Bacillus licheniformis* (EC. 3.2.1.1)) and Dextrozyme DX 1.5X (Novozymes), a balanced mix of glucoamylase (*Aspergillus* (EC 3.2.1.3) and pullulanase (EC 3.2.1.41)). Incubations were carried out in 50 ml of a 0.2 *M* acetate buffer (pH 5.0) containing 35-40 ppm of $CaCl_2$ at 37°C for 72 hours. 100μl of Merthiolate were also added to prevent microbial growth. After 72 hours, 50 mg of EDTA was added to inactivate the enzymes. The samples were then filtered to separate the solids and the extent of degradation was determined by weight loss of TPS.

2.6 Biodegradation conditions

Milled fractions were exposed for 45 days to activated sludge in a wastewater treatment facility of MAGNA FORMEX, an automotive company located in Ramos Arizpe, Coahuila, Mexico. Exposed samples were then taken out from the medium, washed several times with distilled water and dried at 60°C under vacuum for 24 hours. The extent of biodegradation was calculated from the difference between the initial weight of the sample and the weight after drying and reported as the weight loss of the TPS content in the blend. It is important to note that this weight loss includes partially degraded starch molecules that were dissolved by the medium.

2.7 Tensile testing

LDPE/TPS ribbons were cut in the axial direction to obtain types V tensile specimens and biodegraded in activated sludge. After washing and drying, tensile samples were tested at 15 mm/min on a 4301 universal Instron machine equipped with a 500 N loadcell and data acquisition system. The average values of Young's modulus and elongation at break were calculated from at least 10 measurements.

3. Results and discussions

3.1 Effect of TPS content on morphology of LDPE/TPS blends

Morphology of LDPE/TPS blends was evaluated as a function of starch content and LDPE viscosity. The effect of TPS concentration on the morphology of PE1/TPS blends can be observed in Figure 1. At 32 wt% of TPS, the PE1/TPS blend displays a discrete morphology consisting of a large population of small round particles dispersed around some larger deformed TPS particles (Figure 1a). At 42% of TPS, coalescence is evidenced by the reduction of the amount of small particles and the increase of large elongated particles (Figure 1b). Further increasing the TPS concentration reduces the number of spherical particles smaller than 10 μm and increases the number and size of the larger elliptical particles, which for 51% of TPS

form elongated structures with length of 300 μm or larger due to particle-particle coalescence (Figure 1c). At the highest TPS loading (62 wt %), TPS particles are larger than 1 mm which allows the morphological interconnection in a macroscopical level (Figure 1d).

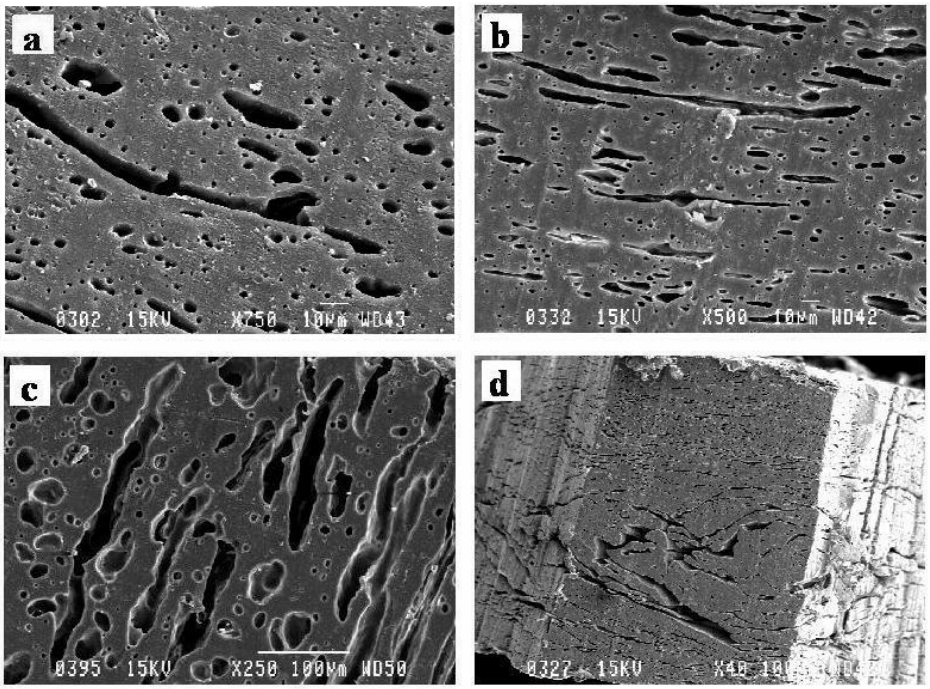

Figure 1. Effect of TPS concentration on morphology of cryogenically microtomed PE1/TPS blends: (a) 32 %, (b) 42 %, (c) 51 % and (d) 62 % by weight of TPS.

3.2 Influence of LDPE viscosity on morphology

Morphology of the LDPE/TPS blends is highly dependent on the viscosity ratios between TPS and PE1 and PE2 (Figure 2). The apparent viscosity (η_a), at shear rate (γ_a) ~ 100 s^{-1}, of PE1, TPS and PE2 were 13100, 4400, 2700 Pa·s, respectively. Consequently, the viscosity ratio, p, for TPS/PE1 and TPS/PE2 blends are 0.33 and 1.63, respectively. As mentioned previously, blends having 32% TPS and p = 0.33 are mainly composed of a large amount of starch domains with particle sizes below 3 μm and just a few of large elongated particles (Figure 2a). Conversely, blends having 31% TPS and p = 1.63 show a small amount of particles below 10 mm and lots of large spherical particles above 30 μm (Figure 2c). At TPS concentration above 50%, blends having p = 0.33 show the effect of coalescence which results in a reduced amount of small particles and the increment of the number and length of large elongated particles (Figure 2b). On the other hand, the influence of coalescence is different in blends having p = 1.63. In this case, the number of particles increases reducing the interparticle distance with the absence of elongated particles (Figure 2d). The results

attest to the fact that low viscosity PE2 does not transfer enough shear stress to deform TPS particles [12].

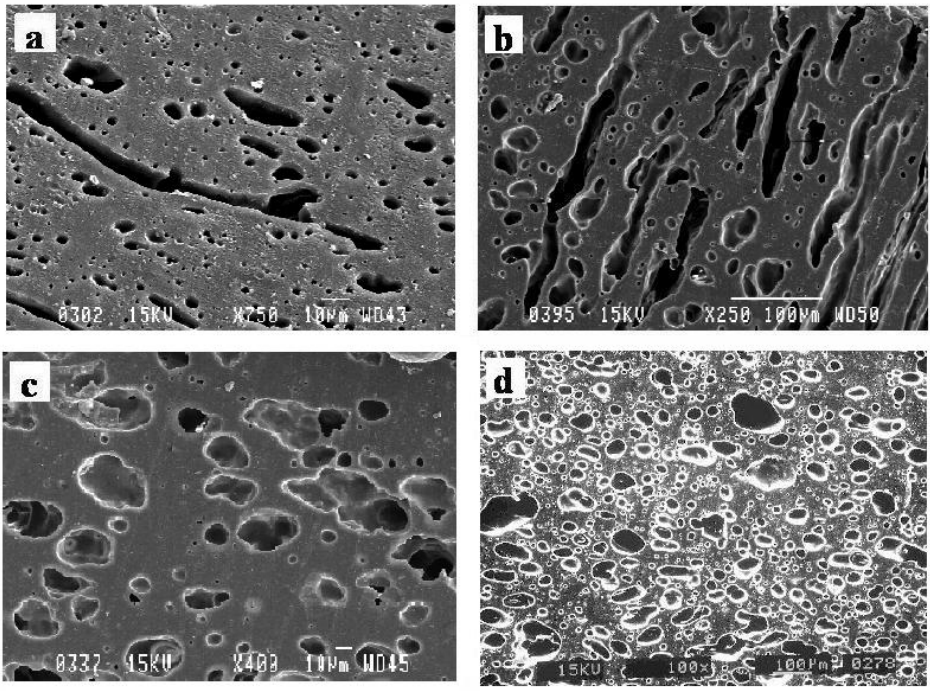

Figure 2. Influence of LDPE viscosity on morphology of cryogenically microtomed LDPE/TPS blends. PE1/TPS: (a) 32 wt *%* TPS and (b) 51 wt% TPS. PE2/TPS: (c) 31 wt% TPS and (d) 52 wt% TPS.

3.3 Hydrolytic degradation of LDPE/TPS blends

It is well known that acid hydrolysis of starch involves the random cleavage of glycoside bonds producing from oligosaccharides fractions to glucose units [13]. In order to quantitatively determine the extent of continuity of TPS blends, samples were exposed to hydrolytic extraction. Figure 3 shows the percent continuity of starch as a function of TPS content for PE1/TPS and PE2/TPS blends. In both cases there is a monotonic increase in continuity as the concentration of TPS increases. At concentration of 43% or lower, blend morphology plays an important role on percent continuity of LDPE/TPS blends. Blends depicting elongated particles show higher percent continuity at comparative concentrations than those displaying spherical morphology. For instance, PE1/TPS blends containing 32% TPS have 66 continuity while PE2/TPS blends composing of 31% TPS have only 38% continuity. Above 50% TPS, at almost 100% continuity, blend morphology does not make any significant difference. At 62 wt% TPS the percent continuity of starch domains reaches 100% and the starch phase could be completely extracted. This is indicative of the full connectivity of starch particles through the entirely sample (Figure 3). The use of hydrolytic degradation as previous technique to biodegradation studies could be an

important tool to predict enzymatic and bacterial biodegradation. These studies are discussed in the following sections.

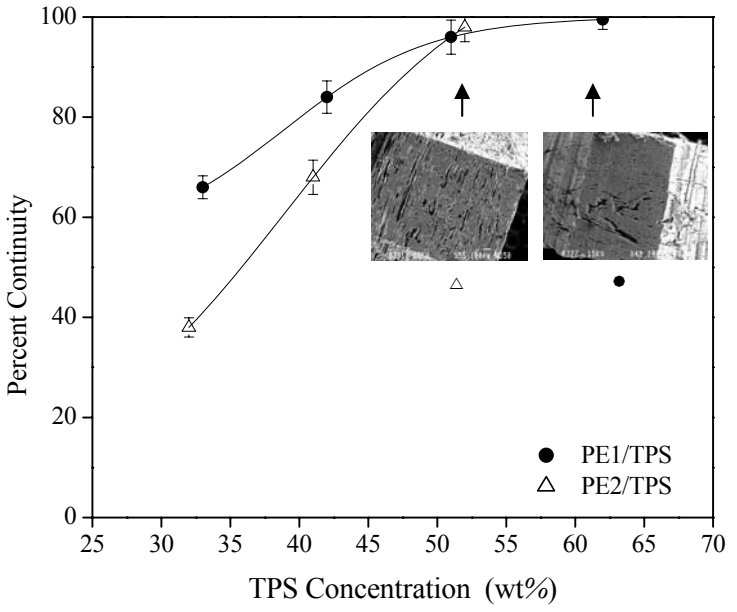

Figure 3. Accessibility of starch domains LDPE/TPS blends exposed in solution of HCl 6N for 72 hours.

3.4 Enzymatic degradation of LDPE/TPS blends

Numerous studies have been done to investigate the enzymatic hydrolysis of starch-based materials. These works involve blends system with synthetic polymers like LDPE [14], ethylene vinyl acetate (EVA) [15-16] and polycaprolactone (PCL) [17]. The kinetic of enzymatic degradation of TPS and LDPE/TPS blends is shown in Figure 4. Amylase from the enzymatic cocktail triggers the cleavage of 1-4 acetal link while glucoamylase attacks the 1-6 links of amylopectin [18], which results in starch solubilization and, consequently, weight loss. The extent of enzymatic degradation of starch is depended on TPS concentration. As expected, raw TPS is completely degraded during the first 36 hours. Blends of PE1/TPS having 62% and 32% and PE2/TPS (69:31) result in weight losses of TPS of 97%, 65% and 32%, respectively at 72 hours. Therefore, weight loss percent is related to the total amount of TPS in the blends. Percolation theory is concerned with the connectivity of one component (in our case, TPS) randomly dispersed in another [19]. Peanansky showed that below an apparent percolation threshold of 30% by volume (40 wt%) of granular starch, only small amounts were accessible for removal. Granular starches are compact particles, such as those observed in the PE2/TPS blends. Fiber-like particles observed in PE1/TPS blends could be responsible for a lower apparent percolation threshold in this system and, consequently, higher enzymatic degradation values [20]. Extent of enzymatic degradation of LDPE/TPS blends is very similar to that obtained by acid hydrolysis.

On the other hand, TPS enzymatic degradation rate is depended on starch concentration and the accessibility of starch domains as is in the case of LDPE/TPS blends. TPS is almost insoluble in cold water. When TPS is exposed to cold water, it swells and glycerol and low molecular fractions become soluble, but the specimen shape remains intact. Enzymatic hydrolysis of insoluble polymers is known to be affected by the mode of interaction between the enzymes and the polymeric chains and typically involves four steps: (i) enzyme diffusion from the bulk solution to the solid surface, (ii) enzyme adsorption on the substrate, resulting in the formation of enzyme-substrate complex, (iii) catalysis of the hydrolysis reaction, and (iv) diffusion of the hydrolyzed fraction from the solid substrate to the solution [21]. Blends with high loadings of TPS show an enzymatic degradation rate as fast as that of the raw TPS during the first 3 hours of exposure. This is probably due to the large amount of TPS observed on the surface of LDPE/TPS blends (Figure 1d). Similarly, blends containing about 30% of TPS have less starch available on the surface (Figure 2a and 2c) and, consequently, the initial enzymatic degradation rate is slower than the others. As the soluble degradation products of TPS diffuse out of the sample, the number of active enzyme units available for starch degradation decreases resulting in a reduction of degradation rate. TPS is completely degraded in 36 hours, whereas PE1/TPS having 62% and 32% TPS and PE2/TPS compounded with 31% TPS reach their maximum degradation in 72 hours. Conversely, the 69:31 PE2/TPS stabilizes at a short period of about 20 hours, whereas the 68:32 PE1/TPS blends reaches its plateau at 48 hours. This is likely due to the connectivity of PE2/TPS (69:31) blend of starch from the surface; therefore the path of the enzyme is less obstructive.

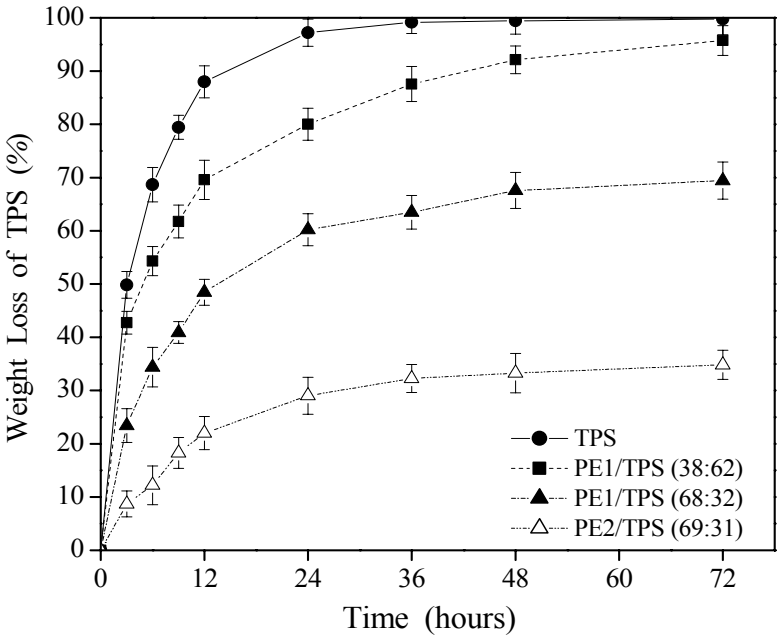

Figure 4. Enzymatic degradation kinetic expressed as weight loss for raw TPS (●), PE1/TPS blends: (■) 62 wt % TPS, (▲) 32 wt % TPS and PE2/TPS blends with (△) 31 wt % TPS as a function of incubation time.

3.5 Microbial biodegradation

Weight loss as a function of time is the most useful method employed to monitor biodegradation [22-26]. Figure 5 shows the weight loss of LDPE/TPS blends exposed to activated sludge as a function of degradation time. As expected, raw PE1 remains unchanged after 45 days. On the contrary, raw TPS is completely consumed within 21 days of exposure. For the LDPE/TPS blends, the maximum biodegradation extent is observed at times longer than the raw TPS. If TPS particles are present only on the surface, and not interconnected with particles inside the LDPE/TPS blends, then it could be expected that starch domains would be completely biodegraded like the raw TPS. Percent continuity observed in Figure 3 shows that TPS particles are interconnected one to another. At TPS concentration of about 30%, interconnection increases when the morphology of starch domains changes from spherical (PE2/TPS blend) to fiber-like particles (PE1/TPS blend). The extent of biodegradation of TPS at 45 days of extraction for PE1/TPS blends at 62%, 32% of TPS and PE2/TPS (69:31) were 92%, 39% and 22%, respectively. However, when the maximum biological extraction is compared with the maximum enzymatic degradation, important difference is noticeable, especially in blends with *ca.* 30 wt% TPS.

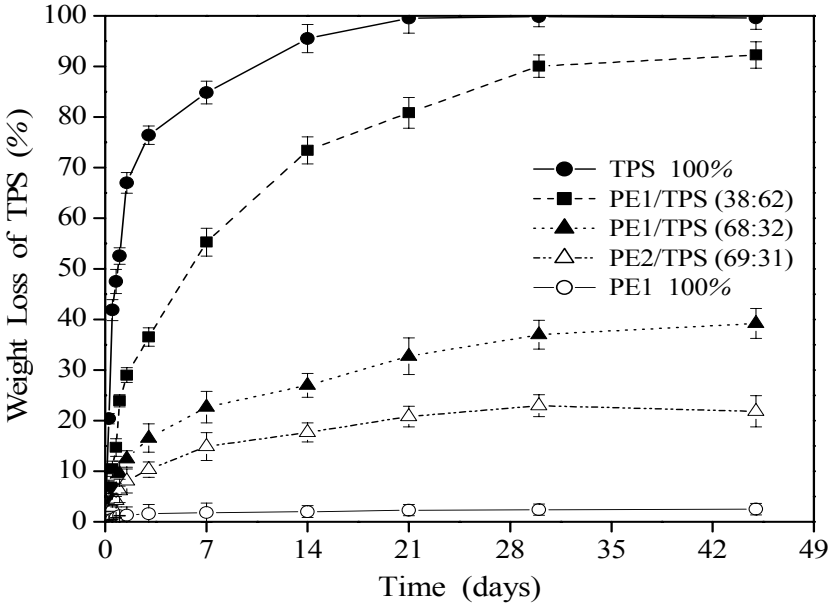

Figure 5. Bacterial biodegradation kinetic expressed as weight loss for TPS (●), PE1/TPS blends with: (■) 62 wt% TPS, (▲) 32 wt% TPS and PE2/TPS blends (△) 31 wt% TPS during exposure in activated sludge.

Kinetic of biodegradation of TPS and LDPE/TPS blends shows two stages (Table 1). In all cases, there is a fast weight loss during the first 1.5 days, followed by another stage where biodegradation rate decreases progressively. The fast stage could be related to the combined effect of biodegradation and diffusion of glycerol and low molecular starch fractions out of the sample. Diffusion of water soluble components can be accelerated by starch swelling, as observed in raw TPS, during the first 6 hr.

Weight loss during this period is almost 4 times faster than the following 30 hr. In the case of LDPE/TPS blends, starch swelling is limited by polyethylene matrix, which results in longer diffusion time. Decrease of biodegradation rate observed after 3 days could be explained by the lower degradability of TPS domains that remain in the material.

From comparison of the three degradation techniques, it can be inferred that some phenomenon is taking place during the bacterial degradation of LDPE/TPS blends. Weight losses for acid hydrolysis and biodegradation were 100% and 92%, 66 and 39%, and 38% and 22%, respectively for PE1/TPS (38:62), PE1/TPS (68:32), and PE2/TPS (69:31). In the case of PE1/TPS (38:62), the difference can be neglected due to the possibility of bacterial waste accumulation inside polyethylene cavities. At around 30% TPS, however, differences are more prominent. This could be related to other phenomena. Figure 2a shows that pores on PE1 matrix left after TPS extraction are below 1 μm, while those observed on PE2 ranged between 3 to 10 μm. On the other hand, different microorganisms have a length between 0.4 and 14 μm and width of 0.2 to 12 μm [27]. In the case of blends having about 30% TPS, it is possible that microorganisms or their colonies can restrict starch diffusion by obstructing the polyethylene pores to result in a significant reduction of the final extent of biodegradation.

Table 1. Biodegradation rate for TPS and LDPE/TPS blends as a function of exposure time in activated sludge.

	dC/dt (g/l.days)			
Time (days)	TPS	PE1/TPS (38:62)	PE1/TPS (68:32)	PE2/TPS (69:31)
0.25	81.6	27.6	5.9	6.4
0.5	85.8	14.4	11.6	5.2
0.75	22.6	16.9	7.7	5.2
1.5	26.0	19.1	8.2	5.2
3	6.3	5.0	2.7	1.5
7	2.1	4.7	1.5	1.1
14	1.5	2.6	0.8	0.5
21	0.6	1.1	0.6	0.4
30	0.3	1.0	0.5	0.2

3.6 Mechanical properties

Favis *et al.* [10] and Rodriguez and coworkers [11] studied the role of the morphology on the mechanical properties of LDPE/TPS blends. They observed that elongation at break (ε_b) and Young's modulus (E) of LDPE/TPS blends decreased as TPS content increased. They also demonstrated that the reduction of ε_b and E was more dramatic in the case of blends having spherical morphology than those composed by fiber-like particles. LDPE/TPS blends prepared in this work showed a similar reduction of ε_b and E as TPS content is increased. ε_b and E for the PE1/TPS (68:32) blend drop 10% and 20%, respectively, while for the PE1/TPS (38:62) blend, they drop a drastic 85% and 65%, respectively. As mentioned previously, blends prepared with PE1 show some elongated particles as opposed to those prepared with PE2 which are mainly spherical (Figure 2). As observed in previous work [10-11], LDPE/TPS blends having

spherical morphology resulted in larger reduction of ε_b and E than those showing elongated particles. This result is verified by our work that the PE1/TPS (68:32) blend has ε_b = 270% and E = 137 MPa whereas PE2/TPS (69:31) blend has ε_b =150% and E = 123 MPa.

LDPE/TPS blends are exposed to activated sludge in order to evaluate the effect of biodegradation on tensile properties (Figures 6 and 7). Tensile properties of raw PE1 remain almost unaffected by exposure to activated sludge. On the contrary, ε_b and E of LDPE/TPS blends display a significant decrease during the first 21 days. The largest effect of biodegradation on ε_b is observed in blends containing about 30% TPS. After 21 days, there is a dramatic reduction from 250% to about 100% for blends prepared with PE1, while for those prepared with PE2 ε_b drops from 150% to 60%. In both cases, ε_b remains almost constant after 21 days of exposure. For PE1/TPS blend having 62% TPS, ε_b decreases gradually from 48% to 10% after 45 days. Reduction of ε_b could be related to the formation of holes on the lateral surfaces of tensile samples exposed to activated sludge. These holes initiate failures that can eventually result in premature fracture. As biodegradation prolongs, holes grow inside tensile samples resulting in lower ductility of LDPE/TPS blends. It is important to note that PE1/TPS blends having 32 wt% TPS remain ductile after 45 days of biodegradation.

E of blends containing *ca.* 30 wt% TPS shows a similar trend as ε_b. It shows a dramatic reduction during the first 21 days, then for the PE1/TPS (68:32) blend, E drops from 136 to 80 MPa and it decreases from 123 to 58 MPa for the PE2/TPS blend (Figure 7). PE1/TPS (38:62) blend shows a reduction from 63 to 18 MPa during the first 14 days. Further biodegradation produces a gradual decrease in E up to 8 MPa at 45 days. It seems that tensile properties of biodegraded LDPE/TPS blends are directly controlled by biodegradation rate.

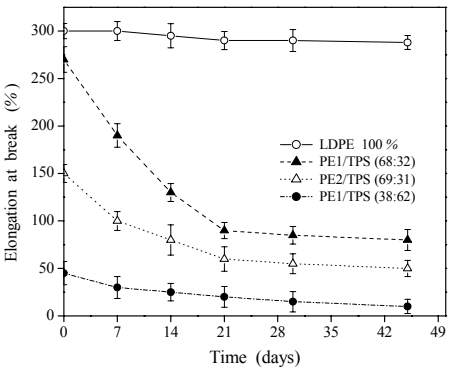

Figure 6. Evolution of ε_b of LDPE and LDPE/TPS blends during biodegradation.

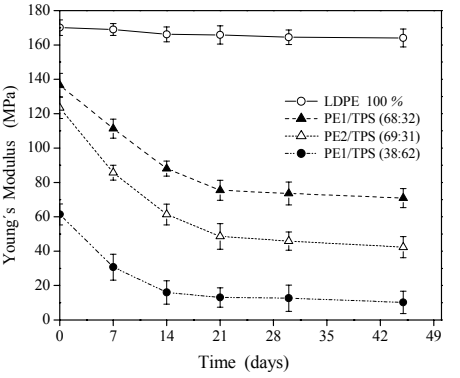

Figure 7. Evolution of E of LDPE and LDPE/TPS blends during biodegradation.

4. Conclusions

In this work, a relationship between morphology and biodegradation of LDPE/TPS blends is evaluated. Morphology of LDPE/TPS blends is evidently affected by both

TPS concentration and LDPE viscosity. Percent continuity of the blends is monitored by means of hydrolytic degradation, from which the results show that at TPS concentration below 50%, it is depended on LDPE viscosity and above that value it is independent. Enzymatic degradation is a technique that is closer to the actual biodegradation than acid hydrolysis but we have demonstrated both to have an excellent correlation. However, a correlation of these two techniques with bacterial biodegradation is difficult because of the accumulative deposit of bacteria through empty pores left by the loss of TPS. This difference is more pronounced for the two blends we investigated which contain *ca.* 30% TPS. In these two blends, the extent of bacterial biodegradation was 39% and 22%, respectively which are less than 60% of the available TPS, as demonstrated by hydrolytic degradation. Tensile properties are affected by biodegradation due to the formation of holes on the lateral surfaces of tensile specimens, which behave as fracture propagation sites. Reduction of tensile properties is proportional to the extent of biodegradation. It is important to remark that blends prepared with PE1 and 32% TPS remain ductile after 45 days of biodegradation.

Acknowledgements. This work was supported by the Mexican Council of Science and Technology (CONACyT), Project SEP-2004-C01-46045. Mr. Tena Salcido (CONACyT 176076) thanks CIQA by the support offered during his PhD studies. Authors are grateful also to the Chemical Engineering Department of the École Polytechnique de Montreal for helpful provided in SEM photographs. Also, the company MAGNA FORMEX is acknowledged for their kind permission to use the wastewater treatment facilities of its plant.

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