Polymer Compatibility and Biodegradation of Starch–Poly(ethylene-*co*-acrylic acid)–Polyethylene Blends*

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SYNOPSIS

X-ray diffraction, CP/MAS C-13 NMR, DSC, FTIR and fluorescence microscopy have been used to study the structure, compatibility, and morphology of films made from starch, poly(ethylene-*co*-acrylic acid) (EAA), and polyethylene (PE) before and after exposure to a mixture of highly amylolytic bacteria. The components of starch, amylose and amylopectin, interact with EAA via the formation of V-type inclusion complexes and hydrogen bonds. PE appears to be immiscible with the starch–EAA complex, with each forming sheetlike domains. The amylopectin in the films is susceptible to digestion by the bacterial consortium while the crystalline EAA–amylose complex is resistant. Digestion begins at the film surface and then proceeds inwards with sheetlike areas of starch removed. The good compatibility between starch and EAA as well as migration of EAA to the film surface explains the resistance of such films to digestion by conventional amylases.

INTRODUCTION

Films containing starch, polyethylene (PE), and poly(ethylene-*co*-acrylic acid) (EAA) have been developed by Otey[1,2] and more recently by Swanson[3] for potential use as biodegradable crop mulches. Little is known, however, regarding the structure, component interactions and morphology of these films and how these may affect biodegradability. Although conventional amylases alone do not digest these films, Gould et al.[4] have shown recently that a consortium of bacteria (LD76) can metabolize much of the starch in the starch–EAA–PE plastic. A large decrease in tensile strength accompanies the loss of starch.

Solvent extraction,[5] X-ray diffraction,[6] optical rotation,[7] CP/MAS solid state NMR,[6] and FTIR[6] techniques have been utilized to characterize the structure of model complexes of amylose and amylopectin with EAA in solution and in the solid state. These experiments demonstrated that amylose and amylopectin form stable V-type inclusion complexes with EAA. Similar V-type complexes are formed from mixtures of amylose and amylopectin with fatty acids and monoglycerides.[8–12] The latter occupy the hydrophobic interior of the hollow starch 6_1 helix. EAA–amylose complexes were highly crystalline, stable at temperatures over 100°C, and highly resistant to α-amylase digestion. EAA–amylopectin complexes were poorly crystalline, partially disrupted at 90°C, and susceptible to amylolytic attack. EAA carboxyl groups formed extensive hydrogen bonds with starch hydroxyls.

In this study, starch/EAA/PE films digested with LD76 were characterized as a function of digestion time using a variety of methods. X-ray diffraction, CP/MAS C-13 NMR, and FTIR techniques were used to characterize starch structure and interactions with EAA. DSC was used to further

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evaluate compatibility between polymeric components. Fluorescent microscopy of films having fluorescent probe molecules covalently linked to starch and EAA was used to assess film morphology. Relationships between film structure and susceptibility to biodegradation are discussed.

EXPERIMENTAL

Plastics

Corn starch was Buffalo 3401. Low density polyethylene was Chemplex 3404B. EAA (20 wt % acrylic acid) was Primacor 5981 (Dow Chemical). Formulations containing 40% starch, 25% EAA, 25% PE, and 10% urea (dry weight basis) were compounded using the semidry method of Otey.[2] Films were blown with a Brabender Plasticorder extruder as described previously.[2]

Microbial Digestion

These procedures have been described in detail elsewhere.[4] Briefly, films were first sterilized by soaking in 3% hydrogen peroxide solution followed by rinsing in water. This washing also removed urea. A proprietary mixed bacterial culture (LD76) was grown in 250 mL flasks containing plastic film strips (0.75 g) and 100 mL of 35 m*M* phosphate buffer (pH 7.3) supplemented with minerals, vitamins, yeast extract, and ammonium chloride. Flasks were incubated aerobically at 28°C and were agitated with a gyrorotary shaker at 90 rpm. After the desired time, film strips were washed with water and air-dried.

Analytical Methods

Fluorescence microscopy studies were conducted on pieces of film cut from the interior of the strip and on cross sections. Sections approximately 10 μm thick were cut from paraffin embedded samples using an American Optical 820 microtome whereas sections 0.5 μm thick were cut from acrylic resin embedded specimens using a Sorvall Porter-Blum MT-2 Ultramicrotome. Starch hydroxyl and EAA carboxyl groups present on the films were reacted with the fluorescent probes 5-(4,6-dichlorotriazinyl)aminofluorescein (DTAF) and 4-(diazomethyl)-7-methoxycoumarin (DMMC) (Molecular Probes Inc.), respectively, using published procedures.[13,14] Starch plastic film ($\sim$ 1 mg) or several thin sections were incubated in 100 μL of 1% DTAF in 50 m*M* NaOH (pH $\sim$ 10.5) for 5 min at 25°C and then washed with 10 m*M* NaOH and water to remove unreacted DTAF. Films were wet mounted on slides with 50 m*M* sodium phosphate buffer, pH 9. EAA and PE films were unreactive with DTAF. Starch plastic film (1 mg) was added to 200 μL of 1% DMMC in acetonitrile and heated in a covered test tube at 60°C for 3 h. Unreacted DMMC was removed by washing with acetonitrile. Starch and PE films showed no reaction with DMMC. Films were examined with a Zeiss Universal Research Microscope equipped with a Hg lamp. For DTAF excitation filter I (350–500 nm) and emission filter 50 (< 500 nm cut off) were used whereas for DMMC filters IIII (300–400 nm) and 41 (< 410 nm cut off) were used. Photographs were taken with Kodak Ektachrome 160 color slide film.

X-ray diffraction experiments were performed by Oneida Research Services, Inc., Whitesboro, NY, using a Siemens D500 APD with CuKα radiation.

CP/MAS C-13 NMR experiments were performed using a Bruker MSL-300 spectrometer. Pulse widths, contact times, and delay times were 5 μs, 2 ms, and 5 s, respectively. Samples were spun in zirconia rotors at approximately 3500 or 4500 Hz.

FTIR experiments were conducted using an Analect RFX-75 spectrometer. Spectra were obtained at 4 cm^{-1} resolution and were an average of 32 scans. Samples (1 mg) were pulverized by vigorous shaking in a stainless steel vial with two steel balls using a Wig-L-Bug amalgamator (Crescent Dental Manufacturing, Lyons, IL). KBr (300 mg) was added to the vial and the shaking was continued. Vials were cooled with liquid nitrogen prior to shaking. Pellets were pressed at 24,000 psi for 1–2 min.

DSC experiments were performed using a Perkin-Elmer DSC7. Four to 8 mg of pulverized sample were sealed inside airtight Al pans. Heating and cooling rates were 10°C/min. Samples were first heated to 130°C and then cooled to 30°C to erase previous thermal histories.

RESULTS

An X-ray powder diffraction scan for a starch–EAA–PE film incubated in growth media for 35 days (control film) is shown in Figure 1(A). The broad peaks at about 12° and 19° 2θ are characteristic of the crystalline EAA/amylose V-type inclusion complex.[6] They are rather weak here since corn starch contains only about 25% amylose. In comparison, the EAA/amylopectin complex and amorphous amylopectin have very broad peaks with maxima near 18° and 17° 2θ, respectively (data not shown). Similar X-ray data for amorphous starch

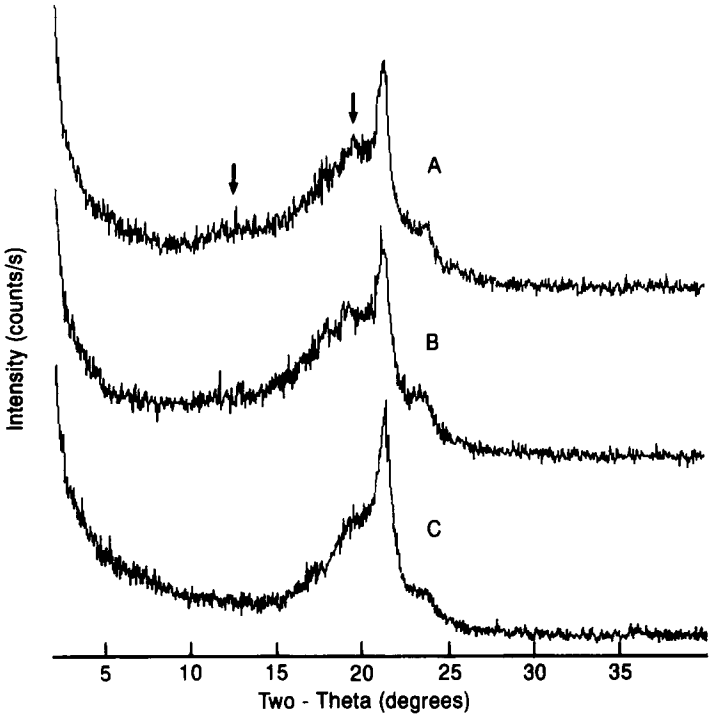

Figure 1 X-ray diffractometer scans of starch–EAA–PE films exposed to culture media [control sample (A)], LD76 for 14 days (B) and 35 days (C).

(maximum at $2\theta = 18°$) has been published previously.[15] The sharp peaks at 21.3° and 23.8° 2θ are the 110 and 200 reflections from the crystalline domains of polyethylene. No peaks characteristic of retrograded (native B-type) starch, normally seen at 5.6°, 15.0°, and 17.0° 2θ, are evident.

Peaks at 12° and 19° 2θ are less evident in films exposed to LD76 [Figs. 1(B) and 1(C)]. Since the NMR results show that the starch remaining is indeed the V-helical complex (see below), the decrease in the X-ray diffraction maxima could be due to a reduction in size of the crystallites, leading to a further broadening of the peaks.

CP/MAS C-13 NMR spectra of pulverized control, 14-day and 35-day LD76-treated films are shown in Figure 2. The broad resonance at 90–105 ppm is due to the C1 carbon of the glucose residues of amylose and amylopectin. Intensities at 103 and 95 ppm reflect contributions from crystalline V-type structures and disordered, amorphous starch, respectively.[6] Using the equation % V-structure = 100 $(0.34\text{-}I_{95}/I_{103})/0.34$, where 0.34 is the value of I_{95}/I_{103} for amorphous amylose, one can estimate that roughly 20, 40, and ~ 100% of the starch was in the V-complex form in the control, 14-day LD76, and 35-day LD76 films, respectively. Note that previous FTIR studies have shown that films treated for 14 and 35 days or longer have approximately 60 and 18% of the starch remaining, respectively.[4] Since our previous NMR studies demonstrated that the highly crystalline EAA/amylose complex gave a single peak at 103 ppm whereas the EAA/amylopectin complex showed a spectrum similar to amorphous amylose,[6] the starch in the film which remains resistant to LD76 digestion is likely the EAA/amylose complex.

FTIR spectra for control, 14-day and 35-day LD76-treated films as well as pure EAA are shown in Figure 3. The spectral contribution from starch has been subtracted and spectra were normalized to the intensity of the C—H stretching mode of EAA. The vibration at 2674 cm^{-1} represents O—H stretching in a hydrogen-bonded carboxyl dimer.[16] This peak is very small for the control film, likely due to formation of starch–EAA hydrogen bonds in place of the EAA carboxyl dimers. As starch is digested away by bacteria, this peak increases toward the value for pure EAA. These results indicate that EAA is mixed with starch on a molecular scale and that LD76 cultures are able to penetrate and digest the amylopectin present.

DSC data for the melting and recrystallization of the crystalline domains of EAA (peak 1) and PE (peak 2) in control and LD76 treated films are given

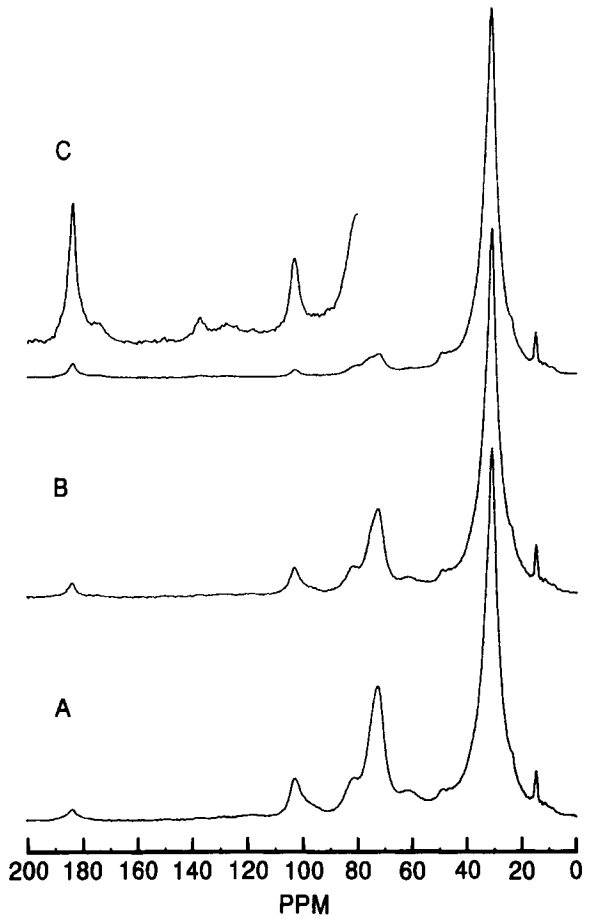

Figure 2 CP/MAS C-13 NMR spectra of starch–EAA–PE films exposed to culture media (control sample (A)], LD76 for 14 days (B) and 35 days (C).

in Table I. Similar data for pure EAA and PE are shown for comparison. Enthalpies are given in terms of J/g of pure component. Samples of PE and EAA used were 27 and 9% crystalline, respectively, based on measured enthalpies of crystallization and a value of ΔH = 276 J/g for purely crystalline PE. There is little difference in melting temperature (T_m), enthalpy of melting (ΔH_m), crystallization temperature (T_c), or enthalpy of crystallization (ΔH_c), for PE in starch–EAA–PE films and pure PE, suggesting that PE exists as a separate phase, interacting little with starch or EAA. Values of ΔH_m and ΔH_c for EAA in the starch–EAA–PE film are only about half that for pure EAA, indicating that part of the hydrocarbon portion of EAA is interacting strongly with starch. This is consistent with the inclusion of part of the EAA hydrocarbon chain inside the starch V-helix. As starch is removed by the bacterial cultures, ΔH_c decreases somewhat towards the value for pure EAA. A sample of the control film was incubated with 50% water overnight and then subjected to DSC analysis to determine whether native crystalline or retrograded (recrystallized) amylopectin was present. No endotherms were evident at < 120°C, suggesting that all amylopectin had been gelatinized during processing and that recrystallization was inhibited, likely due to interaction with EAA.

A micrograph of the planar surface of a control film after reaction with DTAF is shown in Figure 4(A). The freshly cut edge of the film shows very bright fluorescence due to starch–DTAF whereas the

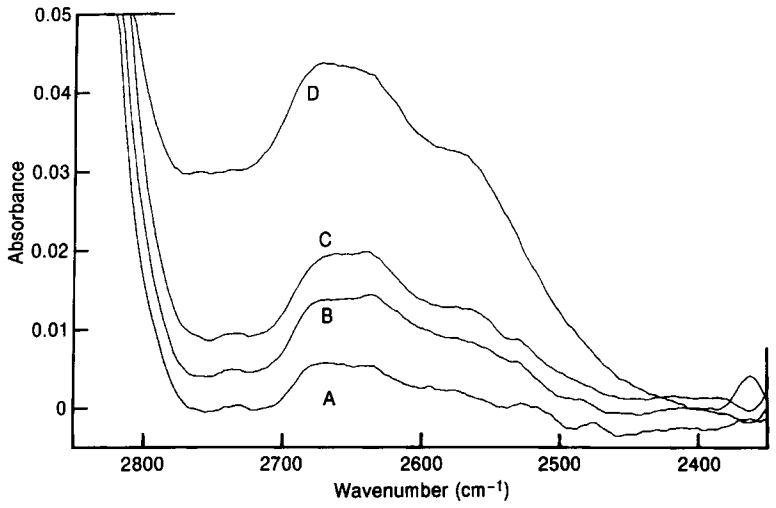

Figure 3 FTIR spectra of starch–EAA–PE films exposed to culture media [control sample (A)], LD76 for 14 days (B), LD76 for 35 days (C), and pure EAA (D). The spectral contribution from starch has been subtracted and spectra were normalized to the intensity of the C—H stretching mode of EAA at 2852 cm^{-1}.

Table I Thermal Data for EAA, PE, and Starch–EAA–PE Films[a]

	Heating				Cooling			
	Peak 1		Peak 2		Peak 2		Peak 1	
Sample	T_m	ΔH_m	T_m	ΔH_m	T_c	ΔH_c	T_c	ΔH_c
EAA	86.2	13 ± 1					64.1	−25.4 ± 0.5
PE			112.2	82 ± 5	100.5	−74.6 ± 1		
Control	83.4	6	112.0	66	98.2	−71.3	62.4	−12.6
14 day	83.3	6	110.9	72	98.2	−71.2	63.9	−13.1
35 day	85.4	7	111.1	69	97.9	−69.0	63.6	−15.3

Temperatures (T) and enthalpies (ΔH) are given in units of °C and J/g of pure component. c and m refer to crystallization and melting processes.

surface shows dim fluorescence arranged in patchy "clouds" ~ 50 μm in size. This suggests a thin surface layer composed largely of EAA or PE was present. A DMMC-control film showed bright surface fluorescence similar to a pure EAA film, suggesting that the surface layer is largely EAA. A micrograph of a 10 μm thick cross section of the DTAF-control film, shown in Figure 5(A), has rather uniform fluorescence across its entire width, indicating that the surface EAA layer must be quite thin (< 1 μm). A micrograph of the same image using white (tungsten) light (Fig. 6) gave a higher resolution image, showing distinct thin sheets of yellow (white in the black and white photograph shown) starch–DTAF separated by dark, colorless sheets and spheres. Based on our knowledge of the compatibility between starch and EAA, the former probably represents the starch–EAA phase whereas the latter is likely PE. A similar image was seen for the thinner 0.5 μm cross section.

A micrograph of the planar surface of a film exposed to LD76 for 14 days and then reacted with DTAF is shown in Figure 4(B). The cut edge is brightly fluorescent but the surface is virtually dark. Thus LD76 bacteria have digested most of the starch from the surface, leaving some of the internal starch intact. A cross section of the same film, shown in Figure 5(B), has brightly fluorescent starch sheets in the interior of the film, 5–10 μm in width, separated by dark areas. It is likely that bacterial enzymes, beginning at the surface, digest away the starch in the starch/EAA phase until a PE sheet is reached and then require additional time to circumvent the PE "maze" to reach the hidden starch.

Films exposed to LD76 for 28 days and reacted with DTAF show no starch fluorescence at cut edges and some dim surface fluorescence [Fig. 4(C)]. Some of this fluorescence may be due to bacterial surface and extracellular polysaccharides. Some very dim patchy fluorescence is seen in cross sections only after very long exposures (40 min) [Fig. 5(C)]. The intensity of the fluorescence observed visually is much less than that predicted based on the starch content (16% of control), suggesting that the amylose/EAA V-complex is unreactive with DTAF.

DISCUSSION

These results have shown that films made with starch, EAA, PE, water, and ammonium hydroxide contain a compatible starch–EAA phase and a largely incompatible PE phase. EAA interacts with amylose and amylopectin via the formation of V-type inclusion complexes and hydrogen bonds. This is consistent with our previous studies of model complexes of EAA with amylose and amylopectin.[6,7] Part of the hydrocarbon portion of EAA does not interact with starch, however, and thus coats the starch with a hydrophobic layer. Also, it has been shown previously that EAA added to amylose or amylopectin in excess of a ratio of 0.5/1 begins to form separate domains.[6,7] The films described here had an EAA/starch ratio of 0.62/1 and thus the excess EAA can further coat the starch as well as migrate to the film surface.

The good starch–EAA compatibility seen here would seem to explain the resistance of the starch–EAA–PE films to digestion with common amylases.[5] Steric interference of EAA molecules neighboring the starch would likely inhibit such large (several nanometers) sized molecules from binding to and cleaving starch. In addition, amylases would not be able to permeate to the interior of the film due to its very low swelling in water. Previous studies have shown very low permeation rates of small solutes

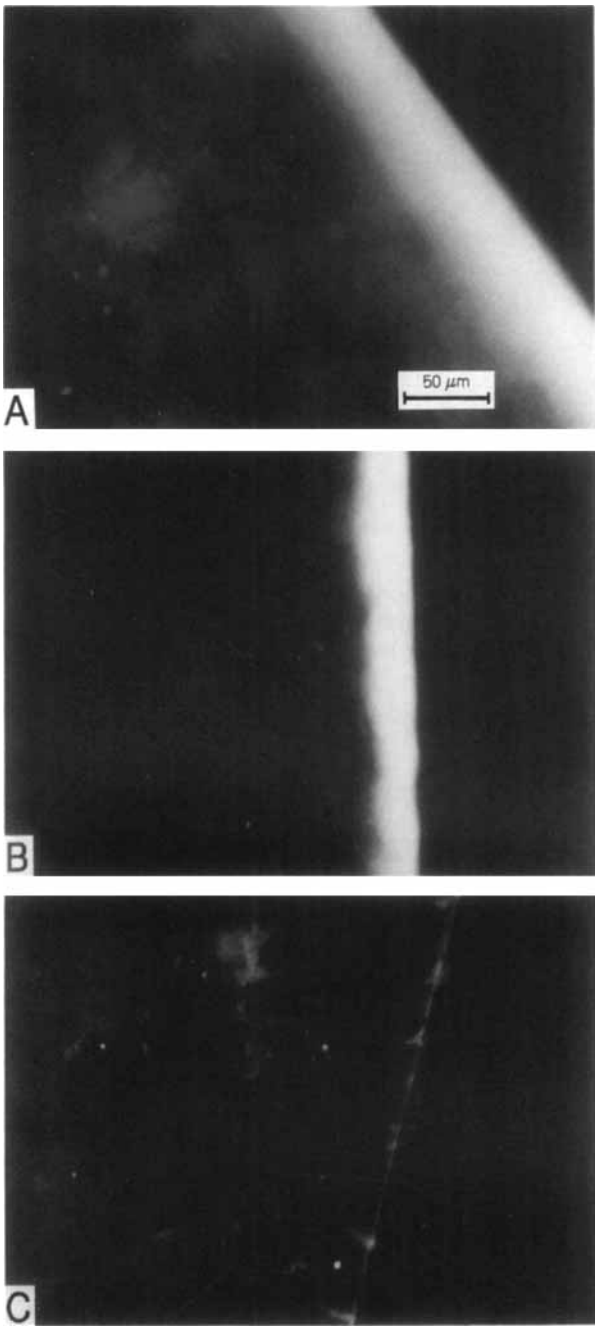

Figure 4 Fluorescence micrographs of planar surfaces of starch–EAA–PE films exposed to culture media [control sample (A)], LD76 for 14 days (B) and 35 days (C) after derivatization with DTAF. Magnification is 125×. Exposure times were 30 s.

through films prepared with ammonium hydroxide.[17] Water, however, permeates these films rather quickly as determined by FTIR studies of films exchanged with D_2O (data not shown). Layers of PE add a further barrier to penetration of enzymes in the aqueous phase. Increased compatibility in blends of degradable polyesters with nondegradable polymers has also been shown to decrease the extent of polyester degradation.[18,19]

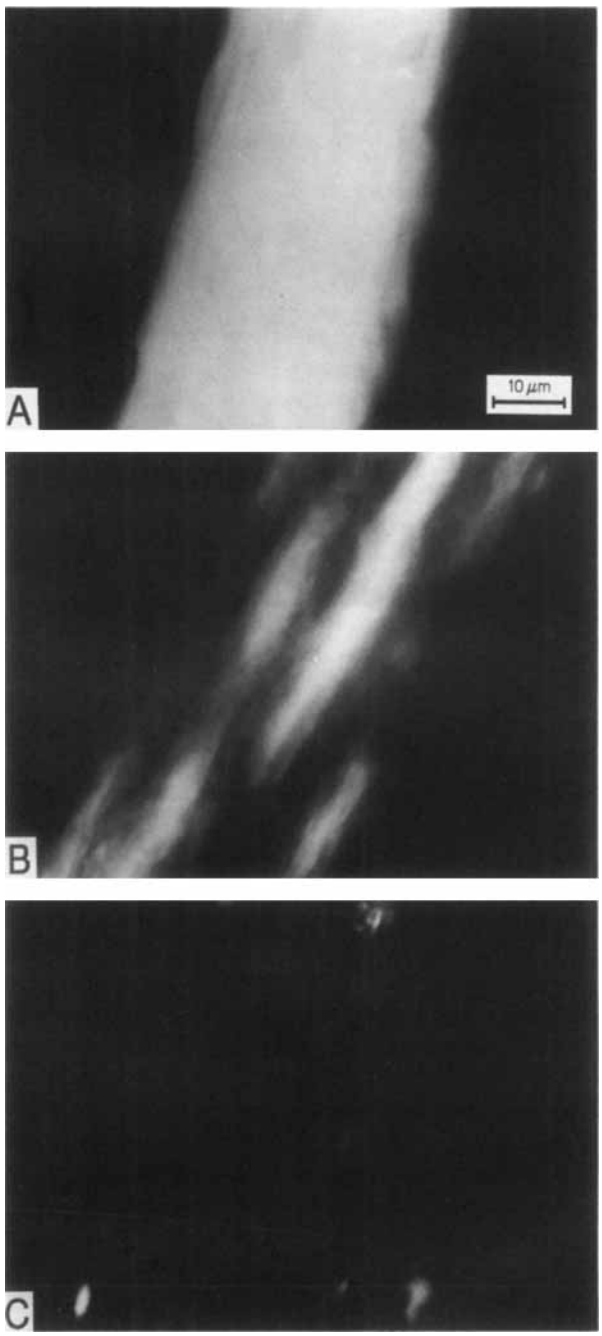

Figure 5 Fluorescence micrographs of 10 μm thick, paraffin-embedded cross sections of starch–EAA–PE films exposed to culture media [control sample (A)], LD76 for 14 days (B) and 35 days (C) after derivatization with DTAF. Magnification is 492×. Exposure times were 2 min (A, B) and 40 min (C).

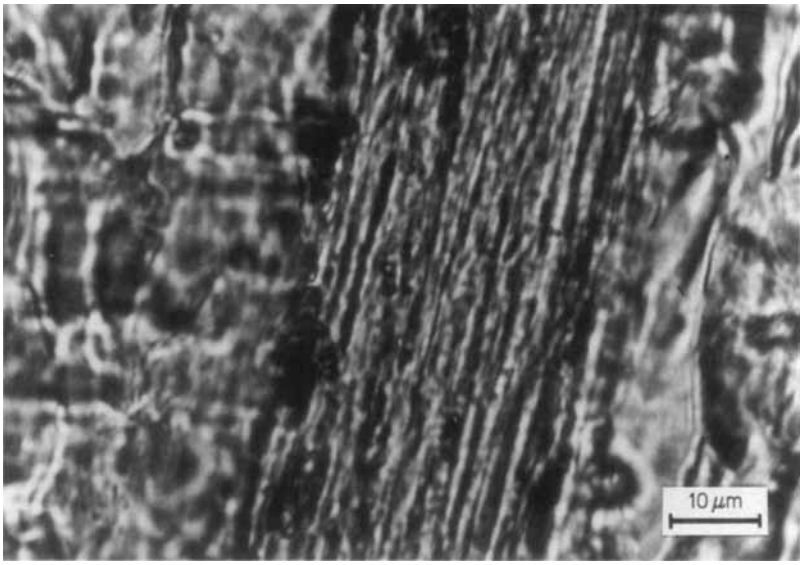

Figure 6 Micrograph of the same image as in Figure 5(A) except using tungsten (white) light illumination.

Enzymes and perhaps other molecules present in cultures of LD76 bacteria are apparently able to dissect the amylopectin away from the EAA, hydrolyse the amylopectin, and then diffuse through the digested area of the film to access more of the starch–EAA domain. The highly crystalline EAA–amylose complex remains resistant to enzymatic attack. Work is currently underway to identify the components of the cultures responsible for this unique behavior.[20] It has also been reported recently that surfactants such as triton X-100 greatly increase the rate and extent of amylase digestion of starch in starch/EAA films.[21] Such surfactants may loosen the starch–polymer interface or serve as phase transfer agents.

This study has demonstrated some of the problems and potential of developing biodegradable polymers containing starch and polyethylene. The continuous nature of the gelatinized starch phase allows access of all the starch to microorganisms. This contrasts with films made with granular starch where many granules are not accessible due to encapsulation with PE. The continuous nature of the gelatinized starch phase leads to a stiffer, more brittle film, however. Use of a compatibilizing block copolymer which is a more effective gelatinized starch–PE interfacial agent than EAA should help reduce domain size and reduce the formation of amylase resistant complexes. This should result in improved biodegradability and mechanical properties.

REFERENCES

1. F. H. Otey, R. P. Westhoff, and W. M. Doane, *Ind. Eng. Chem. Prod. Res. Dev.*, **19,** 592 (1980).
2. F. H. Otey, R. P. Westhoff, and W. M. Doane, *Ind. Eng. Chem. Prod. Res. Dev.*, **26,** 1659 (1987).
3. C. L. Swanson, R. P. Westhoff, and W. M. Doane, in *Proceedings Corn Utilization Conference II,* National Corn Growers Assoc., St. Louis, 1988.
4. J. M. Gould, S. H. Gordon, L. B. Dexter, and C. L. Swanson, *Am. Chem. Soc. Sympo. Ser.*, **433,** chap. 7 (1990).
5. G. F. Fanta, C. L. Swanson, and W. M. Doane, *J. Appl. Polym. Sci.*, **40,** 811–822 (1990).
6. R. L. Shogren, A. R. Thompson, R. V. Greene, S. H. Gordon, and G. Cote, *J. Appl. Polym. Sci.*, **47,** 2279–2286 (1991).
7. R. L. Shogren, R. V. Greene, and Y. V. Wu, *J. Appl. Polym. Sci.*, **42,** 1701–1709 (1991).
8. F. F. Mikus, R. M. Hixon, and R. E. Rundle, *J. Am. Chem. Soc.*, **68,** 1115–1123 (1946).
9. A. C. Eliasson and N. Krog, *J. Cereal Sci.*, **3,** 239–248 (1985).
10. L. V. Batres and P. J. White, *J. Am. Oil. Chem. Soc.*, **63,** 1537–1540 (1986).
11. C. G. Biliaderis and H. D. Seneviratne, *Carbohyd. Polym.*, **13,** 185–206 (1990).
12. M. Gudmundsson and A. C. Eliasson, *Carbohyd. Polym.*, **13,** 295–315 (1990).
13. A. N. DeBelder and K. Granath, *Carbohyd. Res.*, **30,** 375–378 (1973).
14. A. Takadate, T. Tahara, H. Fujino, and S. Goya, *Chem. Pharm. Bull.*, **30,** 4120–4125 (1982).

15. C. Mercier, R. Charbonniere, J. Grebaut and J. F. de la Gueriviere, *Cereal Chem.*, **57,** 4 (1980).
16. G. C. Pimentel and A. L. McClellan, *The Hydrogen Bond,* Freeman, San Francisco, 1960, p. 83.
17. F. H. Otey and R. P. Westoff, *Ind. Eng. Chem. Prod. Res. Dev.*, **23,** 284–287 (1984).
18. H. M. Dollinger and S. P. Sawan, *Polym. Prepr.*, **31,** 429–430 (1990).
19. P. Dave, R. A. Gross, C. Brucato, S. Wong, and S. P. McCarthy, *Proceedings of 199th ACS National Meeting, Part II, Division of Polymer Materials Science and Engineering,* Boston, MA, 1990, Abstract No. 47.
20. A. Burgess-Cassler, S. H. Imam, and J. M. Gould, *Appl. Environ. Microbiol.*, **57,** 612–614 (1991).
21. P. Allenza, J. Schollmeyer, and R. P. Rohrbach, in *Proceedings Corn Utilization Conference III,* National Corn Growers Assoc., St. Louis, 1990.

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