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Pure-Culture and Enzymatic Assay for Starch–Polyethylene Degradable Plastic Biodegradation with *Streptomyces* Species[1]

Anthony L. Pometto III,[2,3] Kenneth E. Johnson,[2] and Meera Kim[2]

Eleven starch–polyethylene degradable plastic films were prepared from masterbatches from Archer Daniels Midland Inc. (ADM), EcoStar Inc. (SLS), and Fully Compounded Plastic Inc. The biodegradability of initial and 70°C heat-treated materials was determined using a pure-culture assay with *Streptomyces badius* 252, *S. setonii* 75Vi2, or *S. viridosporus* T7A or without bacterial culture (control). Films were treated with 10-fold *S. setonii* culture concentrates and compared with inactive enzyme controls. Changes in each films mechanical property, molecular weight distribution, and Fourier-transformed infrared spectrum (FT-IR) were determined, and results were evaluated for significant differences by analysis of variance. Cell mass accumulation on each film was quite pronounced. In pure-culture studies, biodegradation was demonstrated for ADM-7 and SLS-2 initial films and for ADM-6 heat-treated films, whereas after 3-week treatment with active *S. setonii* culture concentrates (enzyme assay), reductions in mechanical properties and changes in FT-IR spectrum were illustrated by all the films except SLS-2. Thus the absence of biofilm formation on the film surface permitted enzymatic attack of the materials. Furthermore, inhibition of chemical oxidative degradation in the pure-culture assay was demonstrated for ADM-11, SLS-5, and SLS-10 initial materials and for ADM-4, ADM-7, SLS-8, and SLS-10 heat-treated films. These data suggest that biological and chemical degradation were directly affected by the reduction in oxygen tension on the plastic film surface due to cell mass accumulation. This same phenomenon could be the cause for slow degradation rates in nature.

KEY WORDS: Biodegradation; chemical degradation; *Streptomyces*; starch–polyethylene; degradable plastics.

INTRODUCTION

There is a growing interest in the development of degradable plastics to enhance the biodegradability of plastic products in landfills and compost sites. One type of these novel materials is starch–polyethylene degradable plastic. Degradable plastic must retain all the mechanical properties expected by the consumer and then, when placed in the appropriate environment, degrade more rapidly than conventional disposable plastics. To enhance degradation of polyethylene, chemical and/or photoinitiators are added to the degradable plastic films. For polyethylene films containing pro- and/or photooxidants, the primary initiators of oxidation are oxygen plus temperature or light, respectively. Both the prooxidant and the photooxidant produce free radicals on the long polyethylene chain, which react with oxygen and cause the material to lose some of its mechanical properties, to become oxidized, to prompt molecular weight reductions, and to become more accessible to microbial biodegradation [1–8].

The bottleneck facing this new industry, however, is test methods. The American Society for Testing and Materials is working to develop standard methods for evaluating the degradation performance of these mate-

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[2] Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011.

[3] To whom correspondence should be addressed.

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rials. One suggested method is the use of pure-culture assay, which has the advantage of being reproducible from lab to lab and permits the distinction between degradation due to chemical or photodegradation and degradation due to biological activity. Recently, Lee *et al.* [8] demonstrated the ability of lignin-degrading *Streptomyces viridosporus* T7A, *S. badius* 252, and *S. setonii* 75Vi2 to attack heat-treated polyethylene in degradable plastics in pure shake-flask culture studies. Biological degradation of polyethylene was further demonstrated by a treatment with bacterial extracellular culture concentrates prepared from these three cultures [9]. In this study, we used a modification of these procedures to evaluate the biodegradability of 11 starch–polyethylene degradable plastic films.

MATERIALS AND METHODS

Microorganisms

The lignocellulose-degrading *Streptomyces viridosporus* T7A (ATCC 39115), *Streptomyces badius* 252 (ATCC 39117), and *Streptomyces setonii* 75Vi2 (ATCC 39116) were used. All cultures were maintained on agar slants at 4°C [10]. *S. badius* and *S. viridosporus* can degrade starch, whereas *S. setonii* does not [8].

Degradable Plastic Films

Petoskey Plastic, Inc. (Petoskey, MI), prepared each material via blown extrusion, according to each company's specifications. Masterbatches containing corn starch, plus prooxidant, which consists of lipid (vegetable oil), and a specific transition metal(s) were provided by Archer Daniels Midland Inc., (Decatur, IL), EcoStar (Buffalo, NY), and Fully Compounded Plastic (Decatur, IL). These masterbatches were then mixed with specific lots of polyethylene to produce each film material (Table I). The starch concentration for each material was confirmed according to the method of Fratzke *et al.* [11]. The transition metal concentration of iron, manganese, and copper was determined by combusting each sample to ash and determining each specific metal concentration via atomic absorption spectroscopy [12]. Thermal degradation properties for each film at 70°C for 20 days were determined by measuring changes in polyethylene weight-average molecular weight ($\bar{M}_w$) by high-temperature gel-permeation chromatography (HT-GPC) [8, 12] (Fig. 1). Films were 70°C heat-treated to the desired $\bar{M}_w$ of 120,000 to 180,000.

Table I. Transition Metal Composition and Starch Concentration Present in Each Starch–Polyethylene Degradable Plastic[a]

Plastic type	Copper (ppm)	Iron (ppm)	Manganese (ppm)	% starch
Archer Daniels Midland Inc.				
ADM-3	3	13	140	6
ADM-4	2	64	135	6
ADM-6	2	14	91	6
ADM-7	2	230	74	6
ADM-11	1	300	113	6
EcoStar Inc.				
SLS-2	51	203	1	9
SLS-5	43	153	1	5
SLS-8	4	19	3	9
SLS-8_p	63	3	210	9
SLS-10	63	4	214	6
Fully Compounded Plastics Inc.				
FCP	2	184	100	6

[a]Transition metal levels were determined via atomic absorption of each plastic film ash [12]. Starch content was confirmed using the chemical assay developed by Fratzke *et al.* [11].

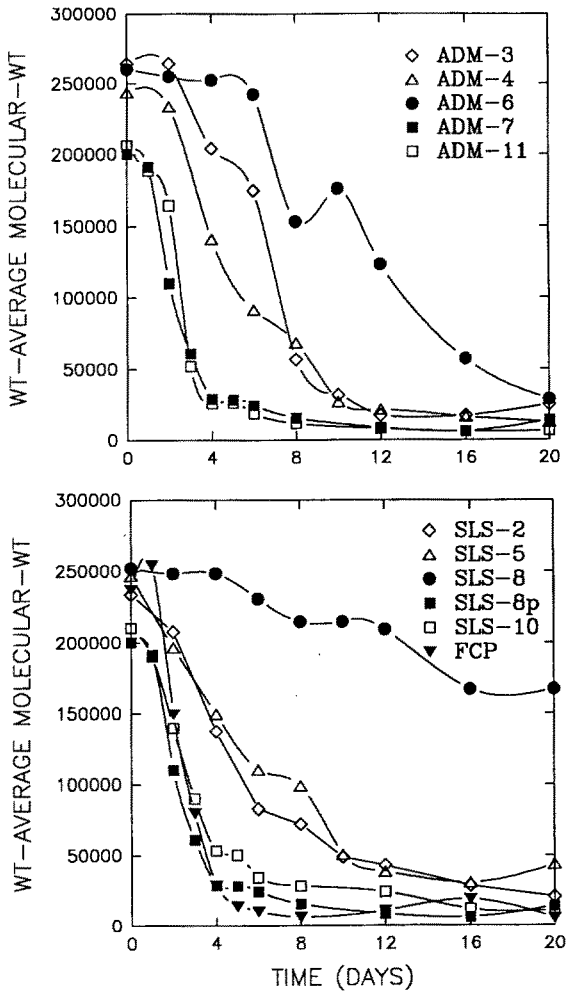

Fig. 1. Changes in weight-average molecular weights for each degradable plastic heat-treated (70°C) for 20 days.

Pure-Culture Biodegradation Assay

A modified disinfection procedure from Lee *et al.* [8] was used. For each initial and heat-treated degradable plastic film, up to 21 strips cut in machine direction 2.5 × 15.2 cm (0.06–0.07 mm thick) were placed in a covered 450-ml sterile specimen jar with a stir bar filled with 70% ethanol. Films were stirred for at least 4 h at room temperature. All transfers were performed in a bioguard hood with sterile forceps. All 21 films were washed in sterile water and aseptically added to a 1-L flask fitted with a cotton plug containing 300 ml of sterile 0.6% (w/v) yeast extract (Difco Laboratories, Detroit, MI) medium [8] plus 8 ml of sterile antibiotic solution (5000 U penicillin, 5 mg streptomycin, and 10 mg neomycin per ml of 0.9% NaCl solution) (Sigma Chemical Co., St. Louis, MO). The culture flask with films was incubated by shaking (125 rpm) at 37°C overnight (≥ 15 h) to encourage spore germination and vegetative cell destruction. Films were placed into a covered beaker containing a fresh solution of universal disinfectant [13] (8 ml of filter-sterilized Tween-80, 20 ml of bleach, and 983 ml of sterile water) and stirred for 60–120 min at room temperature. Films were aseptically washed in sterile water for at least 60 min at room temperature, individually rinsed in 95% ethanol, transferred into a specimen jar containing fresh 70% (v/v) ethanol solution, and stirred for at least 60 min. Each film was separately and aseptically placed into a sterile petri dish and dried overnight at 45–50°C. Each film was added to a 250-ml Erlenmeyer flask fitted with a cotton plug containing 100 ml of sterile 0.6% (w/v) yeast extract medium [8] and subsequently incubated with shaking (125 rpm) at 37°C for 24 h. Properly disinfected films (no turbidity after 24-h incubation) were inoculated with specific *Streptomyces* spores. Controls were uninoculated-incubated, disinfected films. Each control and bacterial culture flask was done in replicates of four for each film. After a 4-week incubation with shaking (125 rpm) at 37°C, films were washed in 70% (w/v) ethanol, and their level of cell mass accumulation on the film was noted. Films were placed in petri dishes and dried at 45°C overnight. Changes in each film's mechanical properties and polyethylene molecular weight distributions were determined.

Extracellular Enzyme Assay

Preparation of Streptomyces setonii *Culture Concentrate.* A 50-L culture of *S. setonii* 75Vi2 was prepared in a Braun U-50 fermentor (Allentown, PA) operated at 37°C (agitation at 300 rpm, dissolved oxygen controlled to 80% saturation and antifoam controlled). A 0.6% (w/v) yeast extract medium with a modified mineral salt solution (0.5 g of Na_2HPO_4, 0.198 g of KH_2PO_4, 0.20 g of $MgSO_4-7H_2O$, 0.2 g of NaCl, 0.05 g of $CaCl_2-2H_2O$, plus 1 ml of trace-element solution [14] per liter of deionized H_2O, pH 7.1 to 7.2) was used. The bacteria were incubated until a pH ≥ 8.0 was achieved. The reactor was harvested into a 50-L polypropylene carboy and stored refrigerated (4°C) overnight to allow the filamentous bacteria to settle to the bottom. The cell-free culture broth was maintained at 4°C throughout the concentration process, and the clear top layer was pumped from the carboy. The residual medium at the carboy bottom with cells was recovered by centrifugation, and the cell mass was discarded. To ensure complete cell-mass removal, this 50 L of cell-free culture broth was filtered via hollow-fiber filtration unit with a cutoff of 1 μm (Amicon Corp., Danvers, MA). This filtrate was then concentrated to 4 L via hollow-fiber filtration unit with a 10,000 molecular weight cutoff.

Degradable Film Preparation. Each film was cut into strips (2.5 × 15.2 cm) in machine direction and coded by a specific cut-design in the film. Two strips from each initial film were placed into a fresh solution of 70% (w/v) ethanol with stirring at room temperature for at least 4 h. Films were washed in sterile water and added to 300 ml of sterile 0.6% (w/v) yeast extract medium [8] with antibiotic solutions and incubated with shaking (125 rpm) overnight at 37°C. A duplicate set of films was prepared simultaneously.

Enzyme Treatment. Two 2-L Erlenmeyer flask were autoclaved for 15 min at 121°C connected to a Fisher (Fisher Scientific, Pittsburgh, PA) stainless-steel sanitary holder (142 mm with a 10-L capacity). This sterile filtration unit was fitted with a 0.5-μm glass prefilter and a 0.22-μm nylon filter from Micron Separation Inc. (Westboro, MA). A 1-L portion of the culture concentrate was filter-sterilized into each 2-L flask. Each flask was aseptically cotton plugged. One flask was autoclaved for 15 min at 121°C (inactive enzyme), and upon cooling, 20 ml of antibiotic solution was added. To the filter-sterilized flask (active enzyme), 20 ml of antibiotic solution was added. One set of these films was then aseptically transferred to each flask and incubated with shaking (125 rpm) for 3 weeks. Twenty milliliters of antibiotic solution was added each week. Changes in each film's mechanical properties and Fourier-transformed infrared spectrum were determined at termination.

Test Used to Evaluate Changes in Degradable Plastics

Mechanical Properties of Films. Changes in tensile strength (stress at fracture of the specimen), percentage of elongation (extension of material under load), and strain energy (overall specimen toughness) were determined on an Instron Model 4502 Universal Tester (Instron Corporation, Canton, MA). Analysis was performed at room temperature and at 500 mm/min with a 5-cm gap. All samples were equilibrated to 50% relative humidity for at least 40 h before analysis (ASTM D882-83, Standard Test Method for Tensile Properties of Thin Plastic Sheeting).

Polyethylene Molecular Weight Distribution. A Waters Model 150-C (Waters/Millipore Co., Milford, MA) high-temperature gel-permeation high-pressure liquid chromatograph (HT-GPC) was used to determine changes in the molecular weight distribution for polyethylene. The procedure of Lee *et al.* [8] was used for sample preparation and chromatographic evaluation with a mobile phase of 1,2,4-trichlorobenzene, a flow rate of 1 ml/min, and an injection volume of 200 μl. During each run, a set of polystyrene molecular weight standards (4016, 53,500, and 610,000 MW) was included. Maxima 820 computer software (Waters/Millipore Co., Milford, MA) was used to determine the weight-average molecular weight ($\overline{M}_w$) and number-average molecular weight ($\overline{M}_n$) of the polyethylene samples.

Fourier-Transform Infrared Spectroscopy (FT-IR) Analysis of Extracellular Bacterial Enzyme-Treated Films. The FT-IR spectrometer used was a Bruker Instruments (Billerica, MA) Model IR 113V controlled by Bruker IFS version 12/87 software. Polyethylene films were affixed directly to standard FT-IR sample plates, which were made from a 1-mm-thick aluminum plate. Wrinkles in the mounted films were avoided. The spectrum from 600 to 4000 cm^{-1} was performed for each sample. Spectrum peak analysis was performed by dividing the peak area integration for the hydroxyl region (870 to 1190) by the methylene peak (1470 to 1485 cm^{-1}).

Statistical Analysis

The data were analyzed using an analysis of variance (ANOVA) to ascertain differences between films incubated with bacteria compared to their corresponding uninoculated control by SAS program [15]. Data from the Universal Tester and HT-GPC were evaluated, and values with $P < 0.05$ were considered significantly different.

RESULTS AND DISCUSSION

Pure-Culture Assay

Cell mass accumulation on each film in shake-flask culture was very extensive on almost every plastic film. High levels of bacterial attachment to these plastic films were observed in this study. Changes in each film's mechanical properties and molecular weight distributions for each degradable plastic are presented in Table II and

Table II. Mechanical Properties and Molecular Weight Distribution of Each Degradable Plastic Before and After Pure-Culture Assay for Biodegradability of Initial and Heat-Treated Materials[a]

Test measurement	Zero time	Uninoculated-Incubated control	*S. badius* 252	*S. setonii* 75Vi2	*S. viridosporus* T7A
		Film Type ADM-3			
		Initial material			
Tensile strength (kg/mm^2)	2.07	1.61*	1.81	1.70*	1.76
Percentage elongation	654	678	698	606	710
Strain-energy (kg · mm)	541	518	552	447	557
Weight-average molecular number ($\overline{M}_n$)	65,390	33,420*	33,120*	29,870*	32,250*
Weight-average molecular weight ($\overline{M}_w$)	224,220	216,160	209,375	200,950*	209,810
		Heat-treated			
Tensile strength (kg/mm^2)	1.47	0.98*	1.22	0.64*	1.11
Percentage elongation	589	324*	472	201*	447
Strain-energy (kg · mm)	422	218*	336	129*	304
Weight-average molecular number ($\overline{M}_n$)	48,760	50,130	58,100*·**	51,690	52,000
Weight-average molecular weight ($\overline{M}_w$)	148,880	170,220*	194,020*·**	163,350	182,995*

Table II. Continued

Test measurement	Zero time	Uninoculated-Incubated control	*S. badius* 252	*S. setonii* 75Vi2	*S. viridosporus* T7A
Film Type ADM-4					
Initial material					
Tensile strength (kg/mm^2)	1.97	1.63	1.67	1.74	1.65
Percentage elongation	661	542*	531*	586	524*
Strain-energy (kg · mm)	516	417	414	431	413
Weight-average molecular number ($\overline{M}_n$)	70,810	56,280*	53,210*	49,695*	55,380*
Weight-average molecular weight ($\overline{M}_w$)	253,230	243,025	239,645	245,545	242,520
Heat-treated					
Tensile strength (kg/mm^2)	1.43	0.76*	0.93*	0.91*	0.73*
Percentage elongation	535	10*	21*	9*	56***
Strain-energy (kg · mm)	376	2*	10*	3*	31*
Weight-average molecular number ($\overline{M}_n$)	40,020	22,630*	28.810*·**	27,230*	32,420*·**
Weight-average molecular weight ($\overline{M}_w$)	129,620	75,535*	87,480*	80,680*	98,350*·**
Film Type ADM-6					
Initial materials					
Tensile strength (kg/mm^2)	1.15	1.86*	1.99*	1.87*	1.80*
Percentage elongation	448	615*	663*	632*	584*
Strain-energy (kg · mm)	254	486*	548*	509*	438*
Weight-average molecular number ($\overline{M}_n$)	63,830	50,170	60,290	59,530	64,897
Weight-average molecular weight ($\overline{M}_w$)	235,000	248,800	250,350	252,845	251,720
Heat-treated					
Tensile strength (kg/mm^2)	1.82	1.45*	1.26*	1.11*·**	1.14*·**
Percentage elongation	639	540*	491*	449*·**	453*·**
Strain-energy (kg · mm)	498	381*	332*	293*·**	297*·**
Weight-average molecular number ($\overline{M}_n$)	47,925	35,570*	43,140**	37.130*	36,780*
Weight-average molecular weight ($\overline{M}_w$)	140,050	123,350	126,910	120,600	127,490
Film Type ADM-7					
Initial material					
Tensile strength (kg/mm^2)	2.00	2.11	1.32*·**	1.41*·**	1.16*·**
Percentage elongation	632	635	466*·**	549	449*·**
Strain-energy (kg · mm)	510	547	323*·**	395	296*·**
Weight-average molecular number ($\overline{M}_n$)	67,680	61,660	67,610	59,130*	65,220
Weight-average molecular weight ($\overline{M}_w$)	235,110	231,000	218,650	235,080	239,700**
Heat-treated					
Tensile strength (kg/mm^2)	0.63	0.83	0.83	1.02*	0.91
Percentage elongation	137	8.1*	7.4*	13.6*	63.8*·**
Strain-energy (kf · mm)	61.5	3.9*	2.2*	6.3*	45.6**
Weight-average molecular number ($\overline{M}_n$)	31,840	19,480*	26,880*·**	24,620*·**	30,460**
Weight-average molecular weight ($\overline{M}_w$)	91,300	62,620*	88,260**	72,200*	92,910**
Film Type ADM-11					
Initial material					
Tensile strength (kg/mm^2)	1.67	1.11*	1.50**	1.74**	1.62**
Percentage elongation	578	498	568	615**	620**
Strain-energy (kg · mm)	417	284**	383	455**	457**
Weight-average molecular number ($\overline{M}_n$)	65,240	68,230	52,130	57,220	56,450
Weight-average molecular weight ($\overline{M}_w$)	227,200	220,710	260,360	207,610	222,880

Table II. Continued

Test measurement	Zero time	Uninoculated-Incubated control	*S. badius* 252	*S. setonii* 75Vi2	*S. viridosporus* T7A
		Film Type ADM-11			
		Heat-treated			
Tensile strength (kg/mm^2)	0.96	0.60	0.85	0.81	0.81
Percentage elongation	10	7	6	9	8
Strain-energy (kg · mm)	4	1*	2*	2	1*
Weight-average molecular number ($\overline{M}_n$)	28,380	16,135*	19,170*	18,830*	18,670*
Weight-average molecular weight ($\overline{M}_w$)	82,175	54,950*	65,450	61,410*	57,825*
		Film Type SLS-2			
		Initial material			
Tensile strength (kg/mm^2)	0.93	1.24*	1.32*	1.52*	1.54*
Percentage elongation	456	527	543	573	600*
Strain-energy (kg · mm)	261	298	325	360	387
Weight-average molecular number ($\overline{M}_n$)	52,385	50,940	49,060	40,100*·**	54,340
Weight-average molecular weight ($\overline{M}_w$)	235,045	185,420*	213,055	210,000	224,290**
		Film Type SLS-5			
		Initial material			
Tensile strength (kg/mm^2)	2.26	1.81*	1.60*	1.41*·**	1.54*
Percentage elongation	625	610	583	529*	522*
Strain-energy (kg · mm)	580	474	441	346*	364*
Weight-average molecular number ($\overline{M}_n$)	65,570	50,860	56,690	52,850*	50,390
Weight-average molecular weight ($\overline{M}_w$)	252,930	183,320*	237,930**	229,930*·**	213,940*·**
		Heat-treated			
Tensile strength (kg/mm^2)	1.59	1.44	1.60	1.49	1.58
Percentage elongation	509	515	534	555	560
Strain-energy (kg · mm)	365	349	391	401	413
Weight-average molecular number ($\overline{M}_n$)	64,520	61,360	64,290	64,700	66,140
Weight-average molecular weight ($\overline{M}_w$)	230,660	212,020	235,700**	232,320	229,310
		Film Type SLS-8			
		Initial material			
Tensile strength (kg/mm^2)	1.07	1.25	1.07	1.34	1.06
Percentage elongation	655	648	563	660	515
Strain-energy (kg · mm)	320	395	343	435	300
Weight-average molecular number ($\overline{M}_n$)	81,670	74,850	79,950	68,630	76,440
Weight-average molecular weight ($\overline{M}_w$)	265,680	271,770	265,510	228,830	270,630
		Heat-treated			
Tensile strength (kg/mm^2)	0.64	0.54	0.85**	0.46	0.41*
Percentage elongation	285	82*	593*·**	67*	113*
Strain-energy (kg · mm)	161	43*	342*·**	35*	62*
Weight-average molecular number ($\overline{M}_n$)	52,880	60,280	56,700	60,280	62,240*
Weight-average molecular weight ($\overline{M}_w$)	170,360	176,610	164,190	177,960	180,370
		Film Type SLS-8$_p$			
		Initial material			
Tensile strength (kg/mm^2)	1.61	1.49	2.25*·**	1.61	1.35
Percentage elongation	617	526	657	629	563
Strain-energy (kg · mm)	435	379	615**	467	388
Weight-average molecular number ($\overline{M}_n$)	64,750	49,140*	47,680*	53,300	45,850*
Weight-average molecular weight ($\overline{M}_w$)	233,210	201,900	191,740*	201,920*	195,710*

Table II. Continued

Test measurement	Zero time	Uninoculated-Incubated control	*S. badius* 252	*S. setonii* 75Vi2	*S. viridosporus* T7A
		Film Type SLS-8$_p$			
		Heat-treated			
Tensile strength (kg/mm^2)	1.64	1.15*	0.96*	0.98*	0.86*,**
Percentage elongation	587	394*	449*	447*	334*
Strain-energy (kg · mm)	437	282*	278*	270*	208*
Weight-average molecular number ($\overline{M}_n$)	43,480	34,310	40,435	38,790	41,730
Weight-average molecular weight ($\overline{M}_w$)	132,790	94,280*	109,005	119,340	118,270
		Film Type SLS-10			
		Initial material			
Tensile strength (kg/mm^2)	1.93	1.37*	1.92**	1.91**	1.12*
Percentage elongation	694	560*	596	628	494*
Strain-energy (kg mm)	567	348*	491	506**	329*
Weight-average molecular number ($\overline{M}_n$)	64,070	64,670	54,020	43,070	56,350
Weight-average molecular weight ($\overline{M}_w$)	225,805	185,060	242,635	213,700	252,680**
		Heat-treated			
Tensile strength (kg/mm^2)	1.36	0.85*	0.91*	0.65*	0.75*
Percentage elongation	520	19*	30*	153*,**	307*,**
Strain-energy (kg · mm)	359	8*	18*	90*	148*
Weight-average molecular number ($\overline{M}_n$)	48,700	27,960*	30,780*	37,050*,**	40,340*,**
Weight-average molecular weight ($\overline{M}_w$)	150,260	81,530*	91,180*	100,290*	122,160**
		Film Type FCP			
		Initial material			
Tensile strength (kg/mm^2)	2.38	2.14	2.52	2.27	2.43
Percentage elongation	390	414	427	368	421
Strain-energy (kg · mm)	249	245	284	226	242
Weight-average molecular number ($\overline{M}_n$)	67,560	50,460*	54,480	58,250	54,400*
Weight-average molecular weight ($\overline{M}_w$)	250,605	200,850*	228,670	236,520	230,920
		Heat-treated			
Tensile strength (kg/mm^2)	2.31	1.68	1.67	1.28	1.06*
Percentage elongation	418	331	348	327	244*
Strain-energy (kg · mm)	267	185	189	182	137
Weight-average molecular number ($\overline{M}_n$)	58,820	61,930	65,050	64,165	67,190*
Weight-average molecular weight ($\overline{M}_w$)	199,670	249,360*	230,830*	239,010*	246,720*

[a] Mechanical properties were determined with an Instron Universal Tester, and molecular weight distribution was determined using a high-temperature gel-permeation chromatograph (Waters' G-150). All values are the means of four replicates.

*Significantly different ($P < 0.05$) from the mean of the corresponding zero-time value.

**Significantly different ($P < 0.05$) from the mean of the corresponding control value.

summarized in Table III. It was very difficult to heat-treat each of the films to a $\overline{M}_w$ of 120,000 to 180,000. The heat-treated film had a $\overline{M}_w$ average of 147,000, with a range of 82,000 to 230,000, and it was impossible to produce a stable heat-treated SLS-2 film.

Biodegradation was detected when significant reductions ($P < 0.05$) in film mechanical properties and molecular weight distributions were observed for films incubated with *Streptomycetes* compared to their corresponding uninoculated-control films. Biodegradation was detected for initial films ADM-7 and heat-treated films ADM-6, but only some biodegradation was detected for SLS-2 (Tables II and III). ADM-6 is the same degradable film used by Lee *et al.* [8], and these results almost match their previous findings by demonstrating biodegradation for heat-treated films but not for initial materials. In this study, however, biodegradation was not detected for heat-treated films by HT-GPC.

Inhibition of chemical oxidative degradation for these degradable films was observed when the uninoc-

Table III. Summary of Pure-Culture Biodegradation Results Compared with The Corresponding Uninoculated Control Flasks

	Initial materials		Heat-treated materials	
Plastic type	Mechanical properties	Molecular weight distribution	Mechanical properties	Molecular weight distribution
ADM-3	NC[a]	NC	NC	NC
ADM-4	NC	NC	NC	Inhibition
ADM-6	NC	NC	Biodegradation	NC
ADN-7	Biodegradation[b]	NC	NC	Inhibition
ADM-11	Inhibition[c]	NC	NC	NC
SLS-2	NC	Some biodegradation	ND[d]	ND
SLS-5	NC	Inhibition	NC	NC
SLS-8	NC	NC	Some inhibition	NC
$SLS\text{-}8_p$	NC	NC	NC	NC
SLS-10	Some inhibition	NC	NC	Some inhibition
FCP	NC	NC	NC	NC

[a] No change.
[b] Biodegradation is when significant reductions in properties were present compared to the corresponding control.
[c] Inhibition of chemical degradation is when there were significantly high values in properties compared with the control.
[d] Not determined.

ulated-control films demonstrated a significant reduction in film properties compared with their corresponding bacterial inoculated culture flask. This inhibition of chemical degradation was demonstrated for initial materials ADM-11 and SLS-5, with some inhibition for SLS-10 and for heat-treated materials ADM-4 and ADM-7 and some inhibition for SLS-8 and SLS-10 (Tables II and III). This observation was also made by Lee *et al.* [8] when films were incubated with *Phanerochaete chrysosporium*, which also accumulated large amounts of cell mass on the film surface. Therefore, significant inhibition of thermal-oxidative degradation for the film with *Streptomyces* biofilm attachment suggests a corresponding reduction in oxygen tension at the film surface. This reduction in oxygen tension produces a corresponding reduction in chemical oxidative degradation. Finally, the majority of the results was scattered, with significant changes in mechanical properties rarely corresponding to changes in molecular weight distributions. This observation is very unusual because changes in mechanical properties almost always parallel some change in molecular weight distribution [6].

Extracellular Enzyme Assay

S. setonii 75Vi2 extracellular culture concentrate treatment of initial films did demonstrate reduction in the film's mechanical properties except for SLS-2 (Table IV). Previously, Pometto *et al.* [9] demonstrated extracellular activity on heat-treated ADM-6 film illustrating enzymatic activity on the initial materials. Furthermore, all films illustrated an increase in the hydroxyl region of the FT-IR spectrum except for SLS-8, which is the same region of the FT-IR spectrum that starch absorbs (*S. setonii* does not produce an amylase) [8]. Pometto *et al.* [9] previously demonstrated a substantial increase in this region when films were treated three times with *S. setonii* culture concentrate.

These data suggest that bacterial cell mass accumulation on the film surface resulted in an inhibition of biological and chemical degradation. This finding would also indicate an enzymatic requirement for oxygen at the film surface for degradation to take place. Therefore, this method needs to be modified to reduce film formation without affecting bacterial growth in the culture flask. Some suggested changes would be to increase the shaker speed from 125 to 250 rpm, or perhaps the addition of a nonionic detergent such as Tween-80 might also reduce biofilm formation. Additionally, the isolation of nonfilamentous or non-film-forming bacteria, which degrade these films, could be used in the assay. Finally, observations in laboratory tests need to be confirmed in field studies.

In a 12-month compost study using the same films, polyethylene degradation was observed for all the materials, with significant degradation being observed for

Table IV. Changes in Mechanical Properties and FT-IR Spectrum for Each Film After Treatment with *S. setonii* Extracellular Culture Concentrate

	Tensile strength (kg/mm^2)		% elongation		Strain-energy (kg · mm)		FT-IR ratio[a]	
Plastic film	Inactive enzyme	Active enzyme	Inactive enzyme	Active enzyme	Inactive enzyme	Active enzyme	Inactive enzyme	Active enzyme
ADM-3	2.21	1.59	629	559	562	397	3.6	4.7
ADM-4	2.70	1.90	668	529	667	428	3.6	4.6
ADM-6	2.79	2.19	678	592	682	516	4.3	5.2
ADM-7	2.26	1.98	582	605	518	473	2.2	3.3
ADM-11	2.51	2.03	715	616	653	530	2.5	3.6
SLS-2	1.80	1.97	518	611	395	472	4.6	5.8
SLS-5	2.66	2.53	626	589	635	554	2.8	3.8
SLS-8	1.45	1.15	639	602	389	320	5.0	3.2
$SLS\text{-}8_p$	2.42	2.10	742	697	654	563	2.2	6.3
SLS-10	1.86	1.23	595	431	410	289	2.2	3.4
FCP	2.49	1.71	365	430	341	219	5.3	6.3

[a] Calculated by dividing the peak area integration for the hydroxyl region (870 to 1190) by the methylene peak (1470 to 1485 cm^{-1}).

ADM-3, ADM-4, ADM-7, ADM-11, and FCP [16]. These results agree with the concentrated enzyme assay but not the pure-culture assay in this study. Johnson *et al.* [16] suggested that reductions in oxygen tension at the film surface in the compost environment might be responsible for slower degradation rates. This pure-culture study supports that conclusion. Therefore, the use of bacterial enzyme concentrate, reduced biofilm-forming microorganisms, or conditions in the pure-culture assay are valuable laboratory assays for determining degradable films biodegradability.

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