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Biodegradation of Degradable Plastic Polyethylene by *Phanerochaete* and *Streptomyces* Species†

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The ability of lignin-degrading microorganisms to attack degradable plastics was investigated in pure shake flask culture studies. The degradable plastic used in this study was produced commercially by using the Archer-Daniels-Midland POLYCLEAN masterbatch and contained pro-oxidant and 6% starch. The known lignin-degrading bacteria *Streptomyces viridosporus* T7A, *S. badius* 252, and *S. setonii* 75Vi2 and fungus *Phanerochaete chrysosporium* were used. Pro-oxidant activity was accelerated by placing a sheet of plastic into a drying oven at 70°C under atmospheric pressure and air for 0, 4, 8, 12, 16, or 20 days. The effect of 2-, 4-, and 8-week longwave UV irradiation at 365 nm on plastic biodegradability was also investigated. For shake flask cultures, plastics were chemically disinfected and incubated-shaken at 125 rpm at 37°C in 0.6% yeast extract medium (pH 7.1) for *Streptomyces* spp. and at 30°C for the fungus in 3% malt extract medium (pH 4.5) for 4 weeks along with an uninoculated control for each treatment. Weight loss data were inconclusive because of cell mass accumulation. For almost every 70°C heat-treated film, the *Streptomyces* spp. demonstrated a further reduction in percent elongation and polyethylene molecular weight average when compared with the corresponding uninoculated control. Significant ($P < 0.05$) reductions were demonstrated for the 4- and 8-day heat-treated films by all three bacteria. Heat-treated films incubated with *P. chrysosporium* consistently demonstrated higher percent elongation and molecular weight average than the corresponding uninoculated controls, but were lower than the corresponding zero controls (heat-treated films without 4-week incubation). The 2- and 4-week UV-treated films showed the greatest biodegradation by all three bacteria. Virtually no degradation by the fungus was observed. To our knowledge, this is the first report demonstrating bacterial degradation of these oxidized polyethylenes in pure culture.

Recalcitrant plastics accumulate in the environment at a rate of 25 million tons per year. The fate of these organic polymers in the environment and the time required for their total mineralization to CO_2 have yet to be fully understood. There is a growing interest in the development of degradable plastics to enhance the biodegradability of the plastics in landfills and composts. One of the most commonly suggested uses for starch-based degradable plastics is for composting of lawn, garden, and shrub litter, which could reduce the volume of material entering the landfills up to 20%. Therefore, we are investigating the ability of litter- or lignocellulose-degrading microorganisms to attack starch-containing degradable plastics in pure culture.

The degradable plastic must still retain all of the physical properties expected by the consumer and, then, when placed in the appropriate environment, degrade more rapidly than conventional disposable plastics. To enhance the degradation of the polyethylene, chemical or photo initiators or both are added to the degradable plastic films. For polyethylene films containing photo- and pro-oxidants, the primary initiators of oxidation are light and temperature, respectively. Both the pro-oxidant and the photo-oxidant produce free radicals on the long polyethylene chain, causing the material to lose some of its physical properties, to become oxidized, and, possibly, to become more accessible to microbial biodegradation (8, 9).

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In this paper, we describe a pure culture system for evaluating the biodegradability of degradable plastic films containing pro-oxidant and 6% starch. Biodegradability was evaluated by weight loss, tensile strength loss, changes in percent elongation, and changes in polyethylene molecular weight distribution. Chemical degradation of the plastic was initiated by a 70°C heat pretreatment or a 365-nm UV irradiation pretreatment of the film. Biological and chemical transformations in the degradable plastic material upon incubation under shaking with aerobic lignin-degrading microorganisms and with uninoculated culture broth were demonstrated.

MATERIALS AND METHODS

Microorganisms. The lignocellulose-degrading microorganisms used were the bacteria *Streptomyces viridosporus* T7A (ATCC 39115), *Streptomyces badius* 252 (ATCC 39117), and *Streptomyces setonii* 75Vi2 (ATCC 39116) and the fungus *Phanerochaete chrysosporium* (ME 446). All cultures were maintained on agar slants at 4°C (13).

The starch-degrading ability of each microorganism was determined by using culture streaks onto starch agar plate containing 1% (wt/vol) native corn starch, 0.05% (wt/vol) yeast extract, and mineral salts solution (13). Each culture was incubated at 37°C for 1 to 2 weeks. Starch utilization was confirmed by flooding the plates with iodine (10). The results demonstrated starch clearing by *S. badius* and *S. viridosporus* only. *P. chrysosporium* and *S. setonii* did grow on the culture plate, but no clearing of the starch was detected.

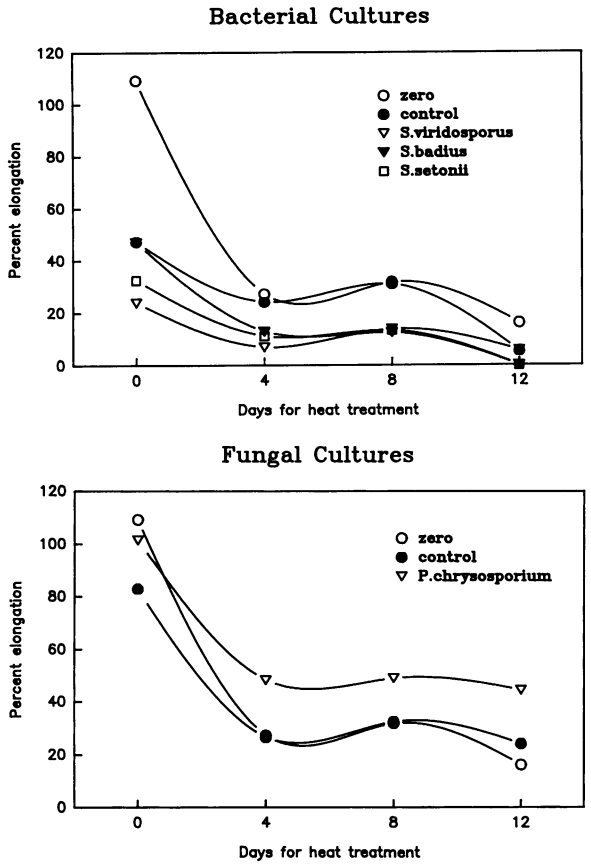

FIG. 1. Change in percent elongation for 0-, 4-, 8-, and 12-day heat-treated (70°C) degradable plastic films after 4 weeks of incubation in culture media, with and without (control) ligninolytic microorganisms. Zero control is heat treated but not incubated. Each data point represents an average of four replicates.

Degradable plastic film pretreatment and culturing. (i) Heat (70°C)-treated films. Archer-Daniels-Midland POLYCLEAN masterbatch degradable plastic films made with linear low-density polyethylene containing pro-oxidant and 6% starch were used. Pro-oxidants are mixtures of transition metals (i.e., Fe, Zn, Ni, and/or Mn) and lipids (i.e., corn or soybean oil) which are compounded into the final polyethylene product at very low levels. Films were commercially prepared according to Archer-Daniels-Midland-recommended specifications. To accelerate the pro-oxidant activity, sheets of degradable plastic were placed in a 70°C forced-air oven, with both sides exposed to air for a maximum of 20 days. Sheets were removed every 4 days. The sheets were then cut, following the same orientation (opposite to machine direction), into strips (4 by 1 in. [10.16 by 2.54 cm]; 0.06 to 0.07 mm thick).

(ii) UV (365 nm)-irradiated films. The same starting films just described were cut into strips (6 by 1 in. [15.4 by 2.54 cm]) and irradiated in a chamber under a long-wave UV lamp (365 nm; 115 V, 60 Hz, 0.6 A, 2/15 W; Spectronics Corp., Westbury, N.Y.) for 2, 4, or 8 weeks, with equal time exposure on both sides of the film. Films were 17 cm from the lamp, with an irradiation area of 39 by 32 cm at 33°C and a relative humidity of 40.5%. The films were then recut into 4 by 1-in. strips after irradiation.

(iii) Chemical disinfection of films. The disinfection proce-

TABLE 1. Weight-average molecular weights ($\bar{M}_w$), number-average molecular weights ($\bar{M}_n$), and polydispersity ($\bar{M}_w/\bar{M}_n$) values for specific 70°C heat-treated degradable plastic with pro-oxidant and 6% starch before and after 4-week shake flask incubation at 37°C with ligninolytic *Streptomyces* spp.[a]

Bacteria	0 days[b]			4 days			8 days			12 days			16 days			20 days		
	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$	$\bar{M}_w$	$\bar{M}_n$	$\bar{M}_w/\bar{M}_n$
Zero[c]	181,379	37,395	4.85	81,107	17,434	4.65	94,528	21,529	4.39	35,127	11,036	3.18	23,958	8,108	2.95	17,429	7,470	2.33
Control[d]	96,828	22,050	4.39	54,947	17,271	3.18	90,891	19,297	3.99	30,909	8,550	3.62	19,033	6,599	2.88	15,723	6,770	2.47
S. viridosporus	88,701	20,738	4.28	36,602[e]	11,717	3.12	29,229[e]	10,753	2.72	25,486	9,158	2.66	17,102	7,579	2.26	17,579	6,739	2.61
S. badius	86,303	24,738	3.49	32,039[e]	10,598	3.02	46,661[e]	12,030	3.80	27,731	8,849	3.14	21,856	8,285	2.64	22,817	7,496	3.04
S. setonii	86,098	24,462	3.52	30,586[e]	10,088	3.03	32,548[e]	11,131	2.92	25,641	8,354	3.58	18,668	8,202	2.28	20,625	7,699	2.68

[a] Values were determined by HT-GPC (see Materials and Methods) and represent averages from four replicate plastic strips each obtained from duplicate HT-GPC runs.
[b] Days of heat treatment at 70°C.
[c] Zero values represent specific heat-treated samples without disinfection or cultural incubation (zero control).
[d] Control values represent specific heat-treated samples that were chemically disinfected and incubated-shaken but were uninoculated (uninoculated control).
[e] Significant difference between the mean $\bar{M}_w$ for the bacteria and the corresponding uninoculated control mean $\bar{M}_w$, with $P < 0.05$.

TABLE 2. Weight-average molecular weights ($\overline{M}_w$), number-average molecular weights ($\overline{M}_n$), and polydispersity ($\overline{M}_w/\overline{M}_n$) values for specific 70°C heat-treated degradable plastic with pro-oxidant and 6% starch before and after 4-week shake flask incubation at 37°C with ligninolytic *P. chrysosporium*[a]

Fungus	0 days[b]			4 days			8 days			12 days			16 days			20 days		
	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
Zero[c]	181,379	37,395	4.85	81,107	17,434	4.65	94,528	21,529	4.39	35,127	11,036	3.18	23,958	8,108	2.95	17,429	7,470	2.33
Control[d]	103,024	26,085	3.95	50,341	15,327	3.28	73,670	17,058	4.21	19,166	8,650	2.22	18,107	7,866	2.30	19,198	8,141	2.36
P. chrysosporium	143,697	34,125	4.21	82,988	19,690	4.21	85,532	20,163	4.24	59,100	16,263	3.63	22,994	8,926	2.58	23,243	8,720	2.67

[a] Values were determined by HT-GPC. All values represent averages of four replicate plastic strips each obtained from duplicate HT-GPC runs.
[b] Days of heat treatment at 70°C.
[c] Zero values represent specific heat-treated samples without disinfection or cultural incubation (zero control).
[d] Control values represent specific heat-treated samples that were chemically disinfected and incubated shaken but were uninoculated (uninoculated control).

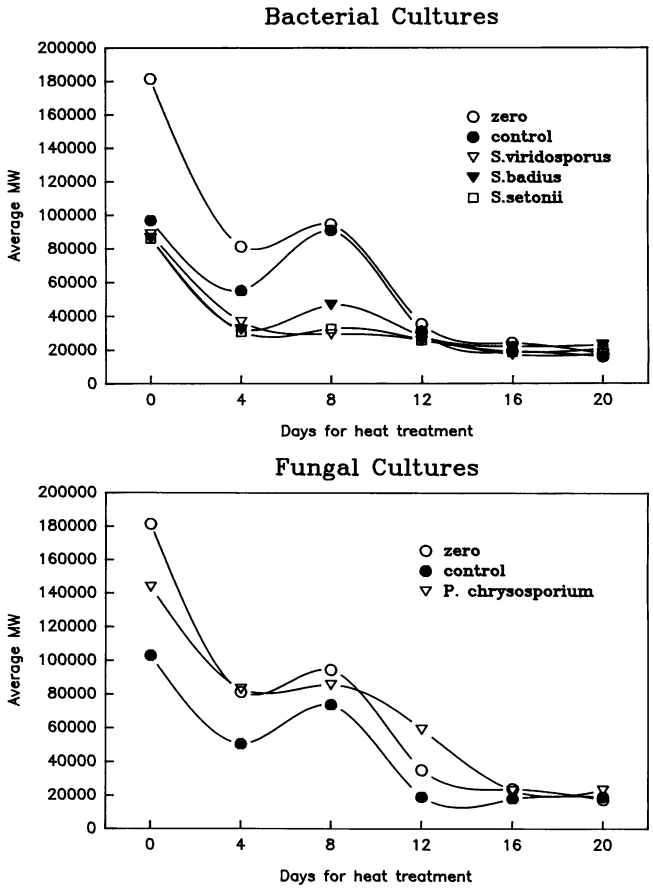

FIG. 2. Weight-average molecular weight ($\overline{M}_w$) for 0-, 4-, 8-, 12-, 16-, and 20-day heat (70°C)-treated degradable plastics films after 4 weeks of incubation in culture media, with and without (control) ligninolytic microorganisms. Zero control is heat treated but not incubated. Each data point represents an average of four replicates.

dure used with each pretreated film consisted of placing the strips into a covered beaker (no more than 15 to 20 strips), adding a fresh solution of universal disinfectant (10) containing 7 ml of Tween 80, 10 ml of bleach, and 983 ml of sterile water, and stirring for 30 to 60 min. Each film was removed with sterile forceps and placed into a covered beaker of sterile water, where it was stirred for 60 min at room temperature. The films were then aseptically transferred into a standing 70% (vol/vol) ethanol solution and left for 30 min. Each film was then placed into a preweighed sterile petri dish. The dishes with films were placed into an incubator at 45 to 50°C to dry overnight, allowed to equilibrate to room temperature, and weighed to ±0.1-mg accuracy; the weight of the film was then determined.

(iv) Film culturing and media. Preweighed disinfected films were aseptically added to sterilized culture medium. The medium contained either 0.6% (wt/vol) yeast extract (Difco Laboratories, Detroit, Mich.) in a nitrogen-free mineral salts solution (5.03 g of Na_2HPO_4, 1.98 g of KH_2PO_4, 0.20 g of $MgSO_4 \cdot 7H_2O$, 0.2 g of NaCl, 0.05 g of $CaCl_2 \cdot 2H_2O$, plus 1 ml of trace element solution [14] per liter of deionized H_2O, pH 7.1 to 7.2) or 3.0% (wt/vol) malt extract (Difco) in deionized water (pH 4.5) for the *Streptomyces* spp. and *P. chrysosporium*, respectively. Films in culture medium were incubated with shaking for 24 h before inoculation to ensure

asepsis. Culture medium was inoculated with spores from a specific ligninolytic microorganism and was incubated with shaking at 125 rpm for 4 weeks at 37 and 30°C for the bacteria and fungus, respectively (7, 15, 16). Four replicates were prepared for each different pretreated film.

(v) Film harvest. Plastic strips were harvested, washed in 70% ethanol to remove as much cell mass from the residual film as possible, dried at 45°C as just described for 24 h, and equilibrated, and the weights were determined. Each of the different films with and without heat or UV pretreatment was compared with the corresponding uncultured material (zero controls) as well as with uninoculated incubated films (uninoculated controls) in either 0.6% yeast extract or 3% malt extract medium.

Test used to evaluate changes in degradable plastics. Biodegradation was followed by weight loss, changes in tensile strength (the stress measured at fracture of the specimen), percent elongation (the extension of the material under load [ASTM D882-83]), and changes in polyethylene molecular weight distribution. The data analyses were determined by SAS program (17) by using an analysis of variance to ascertain differences between corresponding zero control and uninoculated control (chemical degradation) and for differences between the corresponding uninoculated control and each of the microbial treatments (biological degradation). Values with $P < 0.05$ were considered significantly different.

(i) Tensile strength and percent elongation determinations. Changes in tensile strength and percent elongation were determined on an Instron model 1011 at room temperature and 500 mm/min with a 5-cm gap. All samples were equilibrated to 50% relative humidity for at least 40 h preceding analysis (ASTM D882-83, Standard Test Method for Tensile Properties of Thin Plastic Sheeting).

(ii) Polyethylene molecular weight distribution. A Waters model 150-C (Waters/Millipore Co., Milford, Mass.) high-temperature gel permeation high-pressure liquid chromatograph (HT-GPC) was used to determine changes in the molecular weight distribution for the residual polyethylene. Three identical Waters μ-Styragel HT-linear columns, with functional molecular weight (M_w) range of 500 to 8,000,000, were used in series. A mobile phase of 1,2,4-trichlorobenzene (GC/GPC grade; Burdick & Jackson/Bacter Inc., Markeson, Mich.) was used without antioxidant. A flow rate of 1 ml/min and an injection volume of 200 μl were used. Total run time was 55 min per injection, followed by a 5-min equilibration delay. A refractive index detector was used. Injector, columns, and detector were all held at 140°C, and the solvent pump was held at 50°C. A molecular weight calibration curve was constructed based on nine different narrow-molecular-weight distribution polystyrene standards, with peak molecular weights ranging from 2,700 to 2,700,000. Samples were prepared in 1,2,4-trichlorobenzene containing 200 ppm (20 μg/ml) of Santanox R (Monsanto, Akron, Ohio) as antioxidant and contained 0.15% (wt/vol) polyethylene. Initially, 45-mg polyethylene samples were added to amber jars along with 30 ml of 1,2,4-trichlorobenzene with antioxidant. The jars were capped and placed in a 150 to 155°C convection oven for 4 h with occasional swirling. The dissolved samples were transferred to Waters filter vials, manually filtered through the integral, Teflon housed, sintered stainless-steel filter (0.5 μm) and immediately placed into the HT-GPC autosampler at 140°C. Duplicate injections were run from each sample. Maxima 820 computer software (Waters/Millipore Co.), was used to determine weight-average molecular weight ($\overline{M}_w$), number-average molecular weight ($\overline{M}_n$), and polydispersity ($\overline{M}_w/\overline{M}_n$) of the polyethylene samples.

RESULTS

Heat (70°C)-treated films. Weight loss data were inconclusive because of bacterial or fungal cell mass accumulation on the films. Usually, a weight gain was measured, but a slight loss of weight by *S. badius* 252 and *S. setonii* 75Vi2 was detected for 12-day heat-treated films (1.04 and 0.73%, respectively) and for *S. badius* 252 for 16-day heat-treated films (1.02%). Uninoculated-controls generally had a weight gain (average, 2.0%), which is consistent with oxidation of the polyethylene (4, 5) and water absorption by the starch.

The 16- and 20-day heat-treated samples for the inoculated and uninoculated controls were too brittle for tensile strength and percent elongation measurements. Tensile strength values for bacteria and fungus indicate little change compared with the zero control and uninoculated control samples. The only exception was for the 4-day heat-treated samples with *S. viridosporus* T7A, which had a 50% reduction in tensile strength compared with the uninoculated control and zero control. All bacterial strains demonstrated reductions in the percent elongation values with each of the heat treatments, whereas the fungus caused an increase in percent elongation values (Fig. 1). The initial material (zero-time film with no heat treatment), after a 4-week incubation (uninoculated controls) in the bacterial and fungal medium, had 47 and 12% reductions in percent elongation, respectively, when compared with the zero control. Differences in percent elongation between the uninoculated controls and zero controls for 4-, 8-, and 12-day 70°C heat-treated samples were slightly different (range, 0 to 11%). The bacterial cultures generally showed about a 16% reduction (range, 13 to 23%) in percent elongation when compared with the corresponding uninoculated controls. Only the initial material incubated with *S. badius* 252 demonstrated relatively no change compared with the controls. However, fungus-cultured heat-treated films consistently demonstrated an increase in percent elongation (range, 16 to 21%) when compared with the corresponding uninoculated controls (Fig. 1).

The HT-GPC data were used to evaluate changes in molecular weight distribution of the residual polyethylene films for the different microorganisms and physical treatments (Tables 1 and 2). Changes in weight-average molecular weight ($\overline{M}_w$) due to heat (70°C) treatment or corresponding microbial degradation paralleled number-average molecular weight ($\overline{M}_n$) values. During the 70°C heat treatment of the initial degradable film (zero control), the polyethylene $\overline{M}_w$ dropped dramatically after a 4-day heat treatment. This was followed by a slight rise and then a continual reduction. This undulating pattern in $\overline{M}_w$ is attributed to cross-linking between polymer chains (6, 9). The same trend, consisting of a slight rise for the day 8 treated film in $\overline{M}_w$, was observed for the uninoculated controls and for each of the inoculated films (Fig. 2). The $\overline{M}_w$s for both the fungal and bacterial uninoculated controls were consistently lower than for the zero control, with significant ($P < 0.05$) differences being determined between the 0- and 4-day controls. In almost every treatment, the *Streptomyces* spp. effected a reduction in polyethylene weight-average molecular weight, with significant ($P < 0.05$) reduction being determined for the 4- and 8-day heat-treated films (Table 1, Fig. 2). *S. viridosporus* was the overall best, with an average reduction among treatments of 21% (range, 11.8 to 67.8%), but there was no significant ($P < 0.05$) difference among bacterial

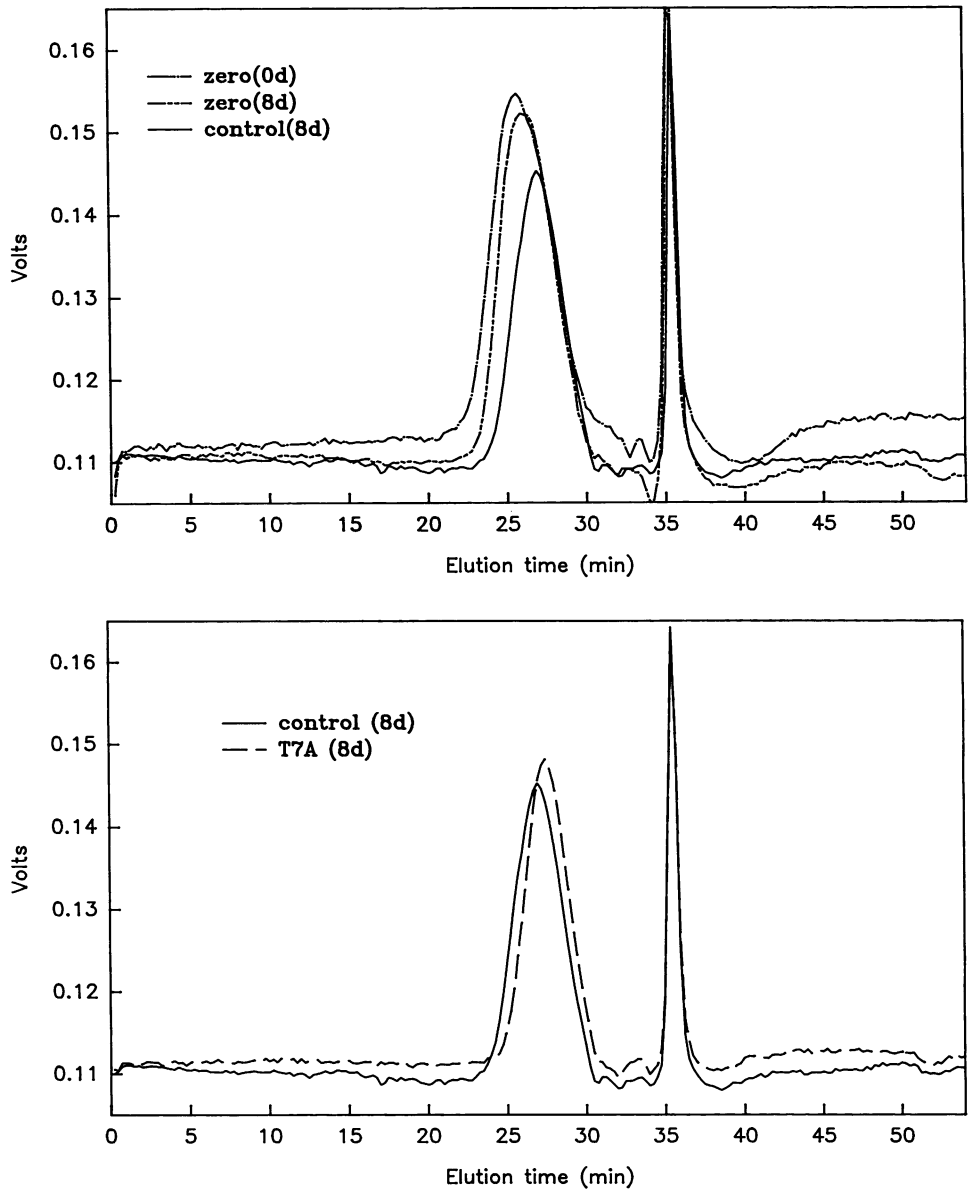

FIG. 3. Comparison of HT-GPC chromatograms for 0- and 8-day heat (70°C)-treated degradable plastics after 4 weeks of incubation with ligninolytic microorganisms. (Top) Zero (0 days) is initial material (zero-time control), zero (8 days) is 8-day heat-treated material, and control is uninoculated 8-day heat-treated material incubated for 4 weeks at 37°C (uninoculated control). (Bottom) Control is 8-day heat-treated uninoculated control, and T7A (8 days) is the 8-day heat-treated film incubated with *S. viridosporus* at 37°C for 4 weeks.

treatments. For the 20-day 70°C heat-treated films, *S. badius*, *S. setonii*, and *P. chrysosporium* showed a slight increase in $\overline{M}_w$, whereas *S. viridosporus* effected no change compared with the control (Tables 1 and 2). Generally, the films incubated with the *Streptomyces* spp. demonstrated a reduction in polydispersity ($\overline{M}_w/\overline{M}_n$), which signifies a narrowing in the overall molecular weight distribution (Table 1). However, this was not the case for the fungus-cultured films (Table 2). Almost all of the fungal treatments demonstrated a higher $\overline{M}_w$ when compared with the corresponding uninoculated controls, with significant ($P < 0.05$) differences for 0-, 12-, and 20-day heat-treated films. This reduction in polydispersity is illustrated by Fig. 3, which displays the actual shift in the HT-GPCs for the initial material zero-time controls, the 8-day heat-treated zero control, and the 8-day uninoculated controls and *S. viridosporus*-cultured films. For each of the controls, the right side of the chromatogram (around 28 min) is essentially the same line, with a serial reduction in both polydispersity ($\overline{M}_w/\overline{M}_n$) and peak height being evident (Fig. 3, top). However, the bacterial film chromatogram not only is narrower than the controls but is shifted completely to the right (Fig. 3, bottom). This shift to the right is indicative of a breakdown of the polyethylene to smaller-chain-length molecules.

UV-irradiated films. Weight loss data were inconclusive because of cell mass accumulation on the films. All percent elongation values abruptly increased after 2 weeks of UV irradiation and dropped after 4 and 8 weeks of treatment

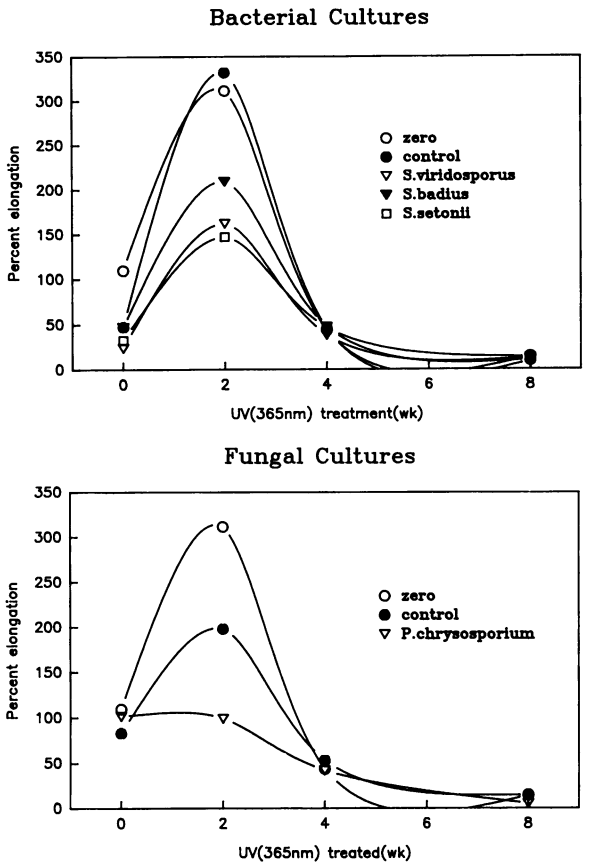

FIG. 4. Change in percent elongation for 0-, 2-, 4-, and 8-week UV (365 nm)-irradiated degradable plastics films after 4 weeks of incubation in culture media, with and without (control) ligninolytic microorganisms. Zero control is heat treated but not incubated. Each data point represents an average of two replicates.

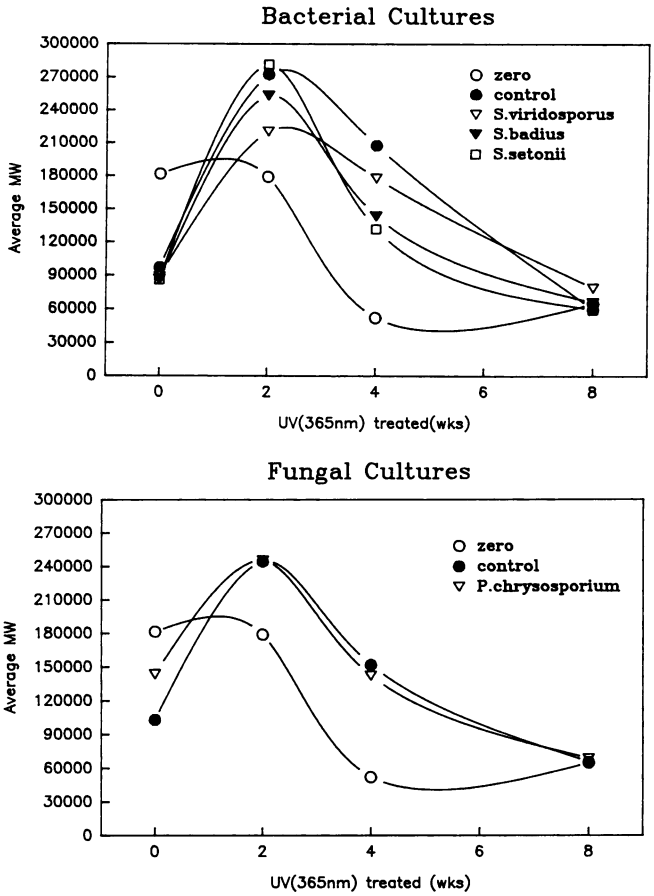

FIG. 5. Weight-average molecular weight ($\overline{M}_w$) for 0-, 2-, 4-, and 8-week UV (365 nm)-irradiated degradable plastics films after 4 weeks of incubation in culture media, with and without (control) ligninolytic microorganisms. Zero control is heat treated but not incubated. Each data point represents an average of two replicates.

(Fig. 4). This increase has been associated with UV-initiated cross-linking between polyethylene chains (6, 9, 11). Generally, the bacterial and fungal strains showed a reduction in percent elongation for the 2-week UV-irradiated films when compared with the zero control and the uninoculated control, but generally no change for the 4- and 8-week UV-treated films (Fig. 4). Furthermore, for the 2-week UV-treated film, the bacterial uninoculated controls demonstrated very little loss compared with the zero control, whereas the fungal control displayed a significant reduction in percent elongation (Fig. 4).

The molecular weight distribution for the UV-treated films paralleled the percent elongation patterns, showing a significant increase in average molecular weight for the 2-week treated film, followed by a steady decrease for the 4- and 8-week UV treatments (Fig. 5). Unexplainably, the zero control $\overline{M}_w$ and $\overline{M}_n$ values were generally lower than those of the uninoculated controls for both the bacterial and fungal media (Tables 3 and 4). Compared with the corresponding uninoculated controls, all three bacterial cultures demonstrated a loss in $\overline{M}_w$ and $\overline{M}_n$ for the 4-week UV-treated films, whereas only *S. viridosporus* and *S. badius* effected a reduction for the 2-week UV-treated films (Table 3). No degradation was displayed by any of the bacterial cultures for the 8-week UV-treated films. The fungus effected essentially no reduction in $\overline{M}_w$ for the UV-treated films. Furthermore, the uninoculated controls and the microbe-incubated 2- and 4-week UV-treated films demonstrated an increase in polydispersity ($\overline{M}_w/\overline{M}_n$), and the 8-week UV-treated zero control, inoculated, and uninoculated control films remained relatively constant (Tables 3 and 4).

DISCUSSION

One of the key advantages of a pure culture biodegradation assay is the ability to identify what portion of the degradation is due to chemical degradation and what can be attributed directly to biological degradation. Pro-oxidant activity due to culture medium, temperature, and shaking is evident by comparing the zero controls with the corresponding uninoculated controls for each of the different heat treatments and different culture media (Tables 1 and 2). These differences could be associated with temperature, dissolved oxygen, medium composition, and pH. There is a higher transition metal concentration in the bacterial medium (see Materials and Methods). Furthermore, for the *P. chrysosporium*-incubated films, fungal growth on the plastic film was extensive and difficult to remove. In all fungal treatments, an increase in percent elongation (average, 20%) and $\overline{M}_w$ (average, 62%) was detected when compared with the corresponding uninoculated controls (Table 2). How-

TABLE 3. Weight-average molecular weights ($\overline{M}_w$), number-average molecular weights ($\overline{M}_n$), and polydispersity ($\overline{M}_w/\overline{M}_n$) values for UV (365 nm)-irradiated degradable plastic with pro-oxidant and 6% starch before and after 4-week shake flask incubation at 37°C with ligninolytic *Streptomyces* spp.[a]

Streptomyces sp.	0 wks[b]			2 wks			4 wks			8 wks		
	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
Zero[c]	181,379	37,395	4.85	178,717	18,564	9.63	51,955	10,448	4.97	64,756	11,046	5.86
Control[d]	96,828	22,050	4.39	272,495	23,650	11.52	207,215	21,203	9.77	59,749	10,643	5.61
S. viridosporus	88,701	20,738	4.28	220,110	21,637	10.17	177,563	20,077	8.84	78,690	12,020	6.55
S. badius	86,303	24,738	3.49	253,212	22,893	11.06	143,292	19,057	7.52	65,583	11,440	5.73
S. setonii	86,098	24,462	3.52	281,397	24,826	11.33	131,524	18,666	7.05	59,374	10,903	5.45

[a] Values were determined by HT-GPC. All values represent averages obtained from duplicate plastic strips each obtained from duplicate HT-GPC runs.
[b] Weeks of UV (365 nm) irradiation.
[c] Zero values represent UV (365 nm)-irradiated sample without disinfection or cultural incubation (zero control).
[d] Control values represent UV (365 nm)-irradiated samples that were chemically disinfected and incubated-shaken but were uninoculated (uninoculated control).

ever, degradation was evident when compared with the corresponding zero control values. This reduction in pro-oxidant activity for the fungal cultures could be due to the fungal mat insulating the film from environmental factors such as additional transition metals in the medium and, possibly, oxygen.

The degradable plastics used in this research are not designed to be photodegradable. However, UV light is a known initiator of polyethylene oxidation, and photo-oxidant activity is enhanced by the addition of transition metals such as cobalt, manganese, nickel, and zinc (8), which are also used as pro-oxidant catalysts. Almost no change in $\overline{M}_w$ was observed for the zero control after 2 weeks of UV irradiation, whereas an average 2.5-fold increase in $\overline{M}_w$ was demonstrated for all incubated films (inoculated and uninoculated) (range, 1.7 to 3.3-fold increase) when compared with zero time samples (Tables 3 and 4). However, the 4-week UV treatment did accelerate bacterial biodegradation when compared with its corresponding control (Table 3).

For the UV-treated films, *S. badius* and *S. setonii* effected the highest percent reductions in $\overline{M}_w$ of 31 and 36%, respectively, for the 4-week UV-treated films, and *S. viridosporus* effected the highest degradation rate (68% reduction in $\overline{M}_w$) for the 8-day heat-treated films, when compared with the corresponding uninoculated controls (Tables 1 and 3). This suggests that the heat and UV treatments both generate very different residual oxidized polyethylene products, which had a direct effect on the biodegradability of the polymer. Generally, the UV-treated films were more recalcitrant than the heat-treated films, which also suggests a difference between the two residual polyethylenes.

Lignocellulose-degrading bacteria, common to composting systems, can biologically cleave the water-insoluble, high-$\overline{M}_w$, chemically oxidized polyethylene residue of degradable plastics. Furthermore, the data indicate that pro-oxidant activity is essential for initiating polyethylene biodegradation. This parallels previous soil degradation research on polyethylene-containing photo-oxidants (4, 5). Also, heat treatment at 70°C and UV treatment at 365 nm did accelerate oxidative activity, making the plastic more rapidly biodegradable by breaking up long polyethylene chains. Previous studies have shown only the low-molecular-weight portion of polyethylene to be biologically degraded (3–6).

The cultural conditions used are considered to be conducive to lignin degradation and the induction of bacterial lignin peroxidases (1, 2, 7, 15, 16). Many of the *P. chrysosporium* culture broths (3% malt extract) became colorless during incubation. Fungal ligninase veratryl alcohol assay (18) revealed that culture flasks with colorless medium had positive ligninase activity while the flasks with no change in medium color were negative (unpublished data). If the bacterial ligninases are involved in polyethylene biodegradation and the *Phanerochaete* lignin peroxidases are not, this would indicate a distinct difference between the two degradation systems, which has not been documented previously.

Using a starch agar assay, we determined that *S. setonii* and *P. chrysosporium* were unable to utilize native corn starch, which is a component of the degradable plastic film. *S. setonii* did demonstrate polyethylene degradation, while *P. chrysosporium* did not. Therefore, the requirement for starch as a cosubstrate in polyethylene biodegradation by microorganisms will need further study. However, the degradation of starch in degradable plastics has already been documented (12). On the other hand, non-plastic-degrading

TABLE 4. Weight-average molecular weights ($\overline{M}_w$), number-average molecular weights ($\overline{M}_n$), and polydispersity ($\overline{M}_w/\overline{M}_n$) values for UV (365 nm)-irradiated degradable plastic with pro-oxidant and 6% starch before and after 4-week shake flask incubation at 30°C with *P. chrysosporium*[a]

Fungus	0 wks[b]			2 wks			4 wks			8 wks		
	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$	$\overline{M}_w$	$\overline{M}_n$	$\overline{M}_w/\overline{M}_n$
Zero[c]	181,379	37,395	4.85	178,717	18,564	9.63	51,955	10,448	4.97	64,756	11,046	5.86
Control[d]	103,024	26,085	3.95	244,419	23,847	10.25	151,735	20,268	7.49	65,461	11,606	5.64
P. chrysosporium	143,697	34,125	4.21	245,391	24,453	10.04	142,082	20,098	7.07	68,558	12,025	5.70

[a] Values were determined by HT-GPC. All values represent averages obtained from duplicate plastic strips each obtained from duplicate HT-GPC runs.
[b] Weeks of UV (365 nm) irradiation.
[c] Zero values represent UV (365 nm)-irradiated sample without disinfection or cultural incubation (zero control).
[d] Control values represent UV (365 nm)-irradiated samples that were chemically disinfected and incubated-shaken but were uninoculated (uninoculated control).

microorganisms, growing solely on the starch, could form an insulating film on the surface similar to *P. chrysosporium*, reducing the pro-oxidant degradation rate. This insulation effect could explain some of the variation in degradable plastic performance that has been observed in field studies. This situation could be corrected by adding additional catalyst (transition metal) to the films and not relying on the immediate environment to provide supplemental catalyst.

Conclusions. From the results presented, we can conclude that there is strong evidence to support reduction in plastic integrity caused by microbial biodegradation of degradable plastics containing pro-oxidants and 6% starch. Furthermore, use of a pure culture system permits the distinction between chemical and biological degradation of these novel materials by providing the necessary controls. It also facilitates the experimental replication needed to obtain statistical evaluations of the data. To our knowledge, this is the first pure culture study to demonstrate that lignin-degrading microorganisms can actually degrade the oxidized polyethylene component of degradable plastics, as indicated by molecular weight reductions.

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