Aerobic Biodegradation of Cellulose Acetate

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SYNOPSIS

Two separate assay systems were used to evaluate the biodegradation potential of cellulose acetate: an *in vitro* enrichment cultivation technique (closed batch system), and a system in which cellulose diacetate (CDA) films were suspended in a wastewater treatment system (open continuous feed system). The *in vitro* assay employed a stable enrichment culture, which was initiated by inoculating a basal salts medium containing cellulose acetate with 5% (v/v) activated sludge. Microscopic examination revealed extensive degradation of CDA (DS = 2.5) fibers after 2–3 weeks of incubation. Characterization of the CA fibers recovered from inoculated flasks demonstrated a lower average degree of substitution and a change in the mol wt profiles. *In vitro* enrichments with CDA (DS = 1.7) films were able to degrade > 80% of the films in 4–5 days. Cellulose acetate (DS = 2.5) films required 10–12 days for extensive degradation. Films prepared from cellulose triacetate remained essentially unchanged after 28 days in the *in vitro* assay. The wastewater treatment assay was less active than the *in vitro* enrichment system. For example, approximately 27 days were required for 70% degradation of CDA (DS = 1.7) films to occur while CDA (DS = 2.5) films required approximately 10 weeks before significant degradation was obtained. Supporting evidence for the biodegradation potential of cellulose acetate was obtained through the conversion of cellulose [1-^{14}C]-acetate to $^{14}CO_2$ in the *in vitro* assay. The results of this work demonstrate that cellulose acetate fibers and films are potentially biodegradable and that the rate of biodegradation is highly dependent on the degree of substitution. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

The technical difficulties associated with designing biodegradable polymers are extremely complex. Biodegradable polymers must be both cost effective and have acceptable performance characteristics, typical of common synthetic polymers and, at the same time, they must be nonpersistent in the environment. Viewed from current technology, these requirements are often mutually exclusive. Based on this viewpoint, one of the most attractive potential materials in terms of cost, material applications, and, perhaps, environmental persistence are cellulose esters. Cellulose is derived from renewable resources and is widely known to be degradable in the environment. Cellulose esters are used in many applications, such as plastics, fibers, and films. Relative to polyolefins and some polyesters, cellulose esters are more expensive. However, relative to other materials that might be used as a nonpersistent material in the environment, cellulose esters are relatively inexpensive. What is unknown is the fate of cellulose esters in the environment.

The biodegradation of cellulose ethers has been studied extensively[1] and it is known that cellulose ethers with a degree of substitution (DS) of less than 1 will degrade due to attack of microorganisms at the unsubstituted residues of the polymers; the ether linkages to the cellulose backbone are considered resistant to microbial attack. In contrast, there have been conflicting reports concerning the biodegradation potential of cellulose esters. Stutzenberger and Kahler[2] have reported that cellulose acetate (CA) is a poor substrate, because of its extreme recalcitrance to microbial attack. However, Reese[3] has isolated cellulolytic filtrates, which deacetylated soluble CA (DS = 0.76) and insoluble cellobiose octaacetate. Furthermore, Cantor and Mechalas[4] have

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demonstrated that CA reverse-osmosis membranes with a DS of 2.5 suffer losses in semipermeability due to microbial attack. These reports suggest that the synergistic action of esterase and cellulase producing microorganisms act in concert to degrade CA. One possible mechanistic pathway would involve attack by cellulase enzymes on the unsubstituted residues in the polymer backbone. Enzymatic cleavage of the acetyls by esterases (or simple chemical hydrolysis) would serve to expose additional unsubstituted residues, which could also be digested by the action of cellulase enzymes. The combined action of the esterase and cellulase enzymes would serve eventually to degrade completely CA in the environment.

We have defined biodegradable materials as materials that are comprised of components which, by microbial catalyzed degradation, are reduced in film or fiber strength by reduction in polymer size to monomers or short chains, which are then assimilated by the microbes. In an aerobic environment, these monomers or small chains are ultimately oxidized to CO_2, H_2O, and new cell biomass, while in an anaerobic environment, oxidation ultimately results in CO_2, H_2, acetate, methane, and cell biomass. The term biodegradation has an important and inherent underlying assumption that direct physical contact must be established between the polymeric material and an active microbial population and/or its enzymes. Successful biodegradation requires that certain minimal physical and chemical requirements be met, such as suitable pH, temperature, oxygen concentration, proper nutrients, and moisture level. Thus, biodegradation is not an universal stipulation that applies equally in all situations and under all environments.

In this report, we will describe our initial work in accessing the biodegradation potential of CA under aerobic conditions. The CA was examined as both a fiber and as a film using a mixed culture isolated from activated sludge or by suspending films into a wastewater treatment basin. We also report our initial results using cellulose [1-^{14}C]-acetate in an aerobic *in vitro* assay.

EXPERIMENTAL

Materials

Cellulose triacetate (CTA), having a DS of 2.95 and cellulose diacetates, having a DS of 2.5 and 1.7 (CDA), are commercial materials and were obtained from Eastman Chemical Company. Cellulose acetate of small fiber size was obtained by ball milling of a 2% aqueous suspension of CDA (DS = 2.5) for 48 h. Films were obtained from a 20% solution (w/v) of CTA in $CHCl_3$, a 20% solution (w/v) of CDA (DS = 2.5) in acetone, or from a 20% solution (w/v) of CDA (DS = 1.7) in 1/1 acetone/water. The films were cast by drawing a film on a glass plate using a 30 mil draw blade. After air drying, the films were cut into 15.2 cm × 1.3 cm strips and dried at 60°C under vacuum for 12–24 h before weighing and measuring film thickness (typically 4.5–5.5 mils).

Carbon 14-labelled cellulose acetate was prepared according to the general procedure described by Buchanan et al.[5] The following is representative of a typical experiment: Cellulose (5.02 g) was treated with 9.4 mL (83 μCi) of [1-^{14}C]-acetyl chloride and 13.1 mL of trifluoroacetic anhydride in 55 mL of trifluoroacetic acid at 5°C for 65 min. The reaction temperature was raised to 25°C for 4 h and finally to 50°C for 1 h. The product was isolated by precipitation into water, followed by extensive washing and drying, which provided 8.34 g of cellulose [1-^{14}C]-triacetate, having a specific activity of 8.02 μCi/g.

Cellulose [1-^{14}C]-triacetate (2.12 g) was dissolved in 42 mL of acetic acid and was heated to 50°C before a solution of 6.26 mL of water, containing 50 mg of H_2SO_4, was added to the reaction mixture. After 24.5 h, the reaction was precipitated into water, washed, and dried, which provided 1.3 g of cellulose [1-^{14}C]-diacetate, having a DS of 1.85 with a specific activity of 4.9 μCi/g.

All other reagents were commercial and were used as received.

Inoculum Source

Fresh composite samples of activated sludge were obtained from the O3 aeration basins in the Tennessee Eastman Chemical Company (Kingsport, TN, USA) wastewater treatment plant. This system has a design capacity of receiving 25 million gallons of waste per day with a biochemical oxygen demand (BOD) concentration of up to 200,000 lbs. per day. The major waste components consisted largely of methanol, ethanol, isopropyl alcohol, acetone, acetic acid, butyric acid, and propionic acid. The sludge operating temperatures typically varied between 32°C to 40°C. In addition, a dissolved oxygen concentration of 2.0 to 3.0 ppm and a pH of 7.1 were maintained to insure maximal degradation rates. Activated sludge from this plant also served as the starting inoculum for the *in vitro* enrichment system used in this work.

Basal Enrichment Media

Cellulose acetate degrading enrichments were initiated in a basal salts medium containing the following ingredients per liter: 50 mL of Pfennig's macro-mineral solution, 1.0 mL of Pfennig's trace element solution, 0.1% (w/v) Difco yeast extract, 2 mM Na_2SO_4, 10 mM NH_4Cl, which supplemented the ammonia levels provided by Pfennig's macro-mineral solution, 0.05% (w/v) cellobiose, 0.05% (w/v) NaOAc. This solution was adjusted to pH 7.0 and a final volume of 945 mL before being autoclaved at 121°C at 15 psi for 15 min. (Autoclaving was shown to inhibit degradation rates by altering the morphology of the CA. As a consequence, the CA substrate was always added with no special considerations for maintaining aseptic conditions.) After cooling to room temperature, 50 mL of sterile 1 M phosphate buffer and 5 mL of a complex vitamin solution, which had been filtered through a 0.2 μm filter, were added. A stable population was obtained by serially transferring the initial inoculum (5% v/v) to a basal salt media containing glucose or cellobiose, acetate, and cellulose diacetate (DS = 2.5).

Fiber Biodegradation

In a typical experiment, 0.1 g of cryogenically ground CDA (DS = 2.5, the average fiber size was 500 μm $\times$ 20 μm) was added to a flask containing 200 mL of basal media, vitamins, and buffer before inoculating the flask (5% v/v) with a stable mixed population enrichment. The flask was placed in a New Brunswick incubator and held at 30°C and 250 rpm for the appropriate period. The contents of the flask were poured into 940 mL of a 2/1 solution of MeOH/$CHCl_3$. The solution was allowed to stand for *ca.* 4 h before adding an additional 700 mL of $CHCl_3$. The layers were separated and the organic layer was extracted with 2 100 mL portions of 9/1 $CHCl_3$/MeOH before drying the combined organics with 4A molecular sieves. Thin layer chromatography (TLC)[6] showed that the aqueous layer did not contain any CA or smaller oligomers. The organic solvents were removed *in vacuo* and the resulting solids were extracted with 4/1 hexane/$CHCl_3$ to removed soluble lipid and protein. Later, we found that the lipid and protein could be removed much more effectively by treating with a neutral detergent solution (*vide infra*).

Film Biodegradation (Enrichment Assay)

The basal growth medium described above was employed in all *in vitro* film degradation studies. This *in vitro* assay represented a batch cultivation system (closed system); hence, this system was capable of supporting normal cell metabolism and division for only a limited time frame. A realistic time limit of 28 days was imposed on all *in vitro* assays to insure that the system maintained its activity.

The test cellulosic film was added to a 500 mL Erlenmeyer flask, containing 200 mL of the basal medium, 10 mL of a 1 M phosphate buffer, 1 mL of the vitamin mixture, and 20 mg of CA powder (DS = 2.5) before inoculating (5% v/v) with a stable mixed population enrichment. The flask was placed in a New Brunswick incubator and was held at 30°C and 250 rpm for the appropriate period. Initially, the films were often observed to turn cloudy and to be coated with a yellow affinity substance, which is an indication of microbial activity.[7] The films were

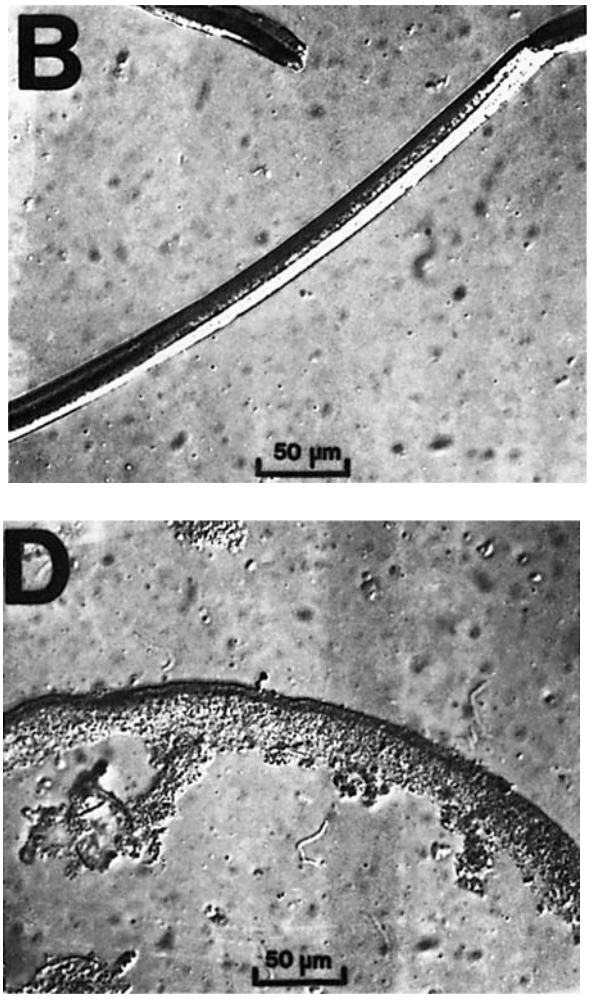

Figure 1 (a) Slide B: a cellulose acetate (DS = 2.5) fiber taken from a control sample. (b) Slide D: a cellulose acetate (DS = 2.5) fiber taken from an inoculated sample.

Table I ***In Vitro*** **Assay of Cellulose Diacetate (DS = 2.5) Fibers**

Entry[a]	DS (^{1}H NMR)	GPC (10^4) M_n	M_w	M_z	M_w/M_n	M_z/M_n	Recovered Fiber
Trials 1 and 2[b]							
Test	1.95	2.5	2.9	3.6	1.23	1.48	—
Control	2.45	1.9	3.3	5.4	1.80	2.90	—
CDA	2.50	1.2	2.8	4.8	2.23	3.85	—
Trial 3[c]							
Control	2.46	1.9	3.8	6.7	2.02	3.63	100 mg
Test	2.44	1.9	3.3	5.0	1.73	2.61	17 mg

[a] The incubation time for each experimental trial was 14–21 days.
[b] Each trial had a total of one control and two test samples.
[c] This trial consisted of one control and four test samples.

harvested by pouring the media through a Buchner funnel. The pieces were collected and washed extensively with water. The film pieces were suspended in a neutral detergent solution (30 g sodium lauryl sulfate, 18.6 g EDTA, 6.8 g sodium borate, 4.6 g Na_2HPO_3, and 10 mL 2-ethoxyethanol dissolved in 1 L of H_2O) at 100°C for 30–60 min before washing extensively with water. The films were placed in a vacuum oven at 40°C until dry before weighing. In each experiment, control experiments were conducted, in which the films were subjected to the same experimental protocol except for inoculation with the microbes. A variety of antibiotics and other antimicrobial agents were used to prevent microbial growth in the control flasks. Addition of high levels of antibiotics and/or arsenic were only able to control microbial growth for short periods of time. After much experimentation, it was found that the addition of 0.1% (w/v) sodium dodecyl sulfate or 1,2-dibromo-2,4-dicyanobutane served as suitable antimicrobials and did not pose any chemical incompatibility with cellulosic films. After harvesting, the weight of the film was then measured and, if the size of the fragments were sufficient, the film thickness and mechanical properties were also measured.

Film Biodegradation (Wastewater Treatment Assay)

Fifteen to twenty numbered cylinders containing one CA test film each were attached to a stainless steel cable and suspended in Tennessee Eastman's AD02 aeration basin. The cylinders' walls had numerous holes, *ca.* 6 mm in diameter, to allow free passage of water to the films. This system represents a continuous feed system, similar to a chemostat, since a fresh supply of nutrients is added continuously while waste products that shut down fermentations are removed. The sludge operating temperatures typically varied between 32°C to 40°C. In addition, a dissolved oxygen concentration of 2.0 to 3.0 ppm and a pH of 7.1 were maintained to insure maximal degradation rates. After the appropriate time, the films were recovered and were treated as described above for the *in vitro* system.

Biodegradation of Cellulose [1-^{14}C]-Acetate

Two fermentation flasks were prepared with identical materials except that a biocide (1,2-dibromo-2,4-dicyanobutane) was added to one flask to stop all bacterial growth and that sample thus functions as a control. The flasks were placed in a water bath held at 30°C and O_2 was bubbled through the fermentation media. Carbon dioxide was absorbed on a scrubbing column containing methoxy ethylamine cooled to −2°C. The column was flushed every 4 h into a scintillation vial and the $^{14}CO_2$ was counted in a liquid scintillation counter.

Analysis

Proton NMR analysis and gel permeation chromatography analysis were made by previously described methods.[5]

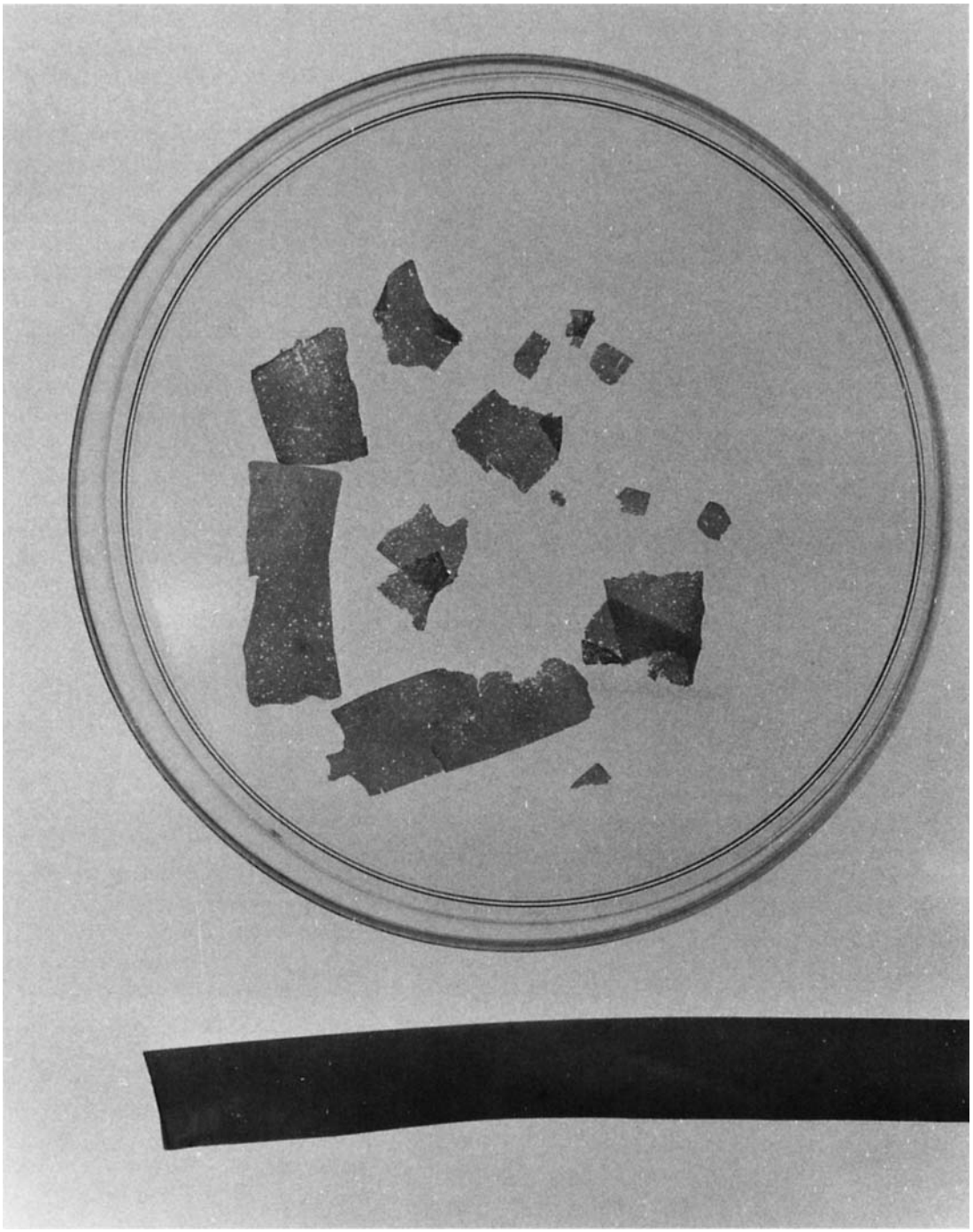

Figure 2 (a) Intact cellulose acetate (DS = 1.7) control film. (b) Film pieces of a cellulose acetate film (DS = 1.7) after 5 days in the *in vitro* enrichment system.

Statistical Analysis

An analysis of variance was performed on all data in accordance with the General Linear Method (GLM) of SAS.[8] This statistical test was combined with Duncan's multiple-range test to determine all main effects for the treatment means.

RESULTS AND DISCUSSION

Our initial experimental approach in studying the biodegradation of CA was via inoculation of cryogenically ground fibers of CDA (DS = 2.5). Our reasoning was that ground fibers would offer more

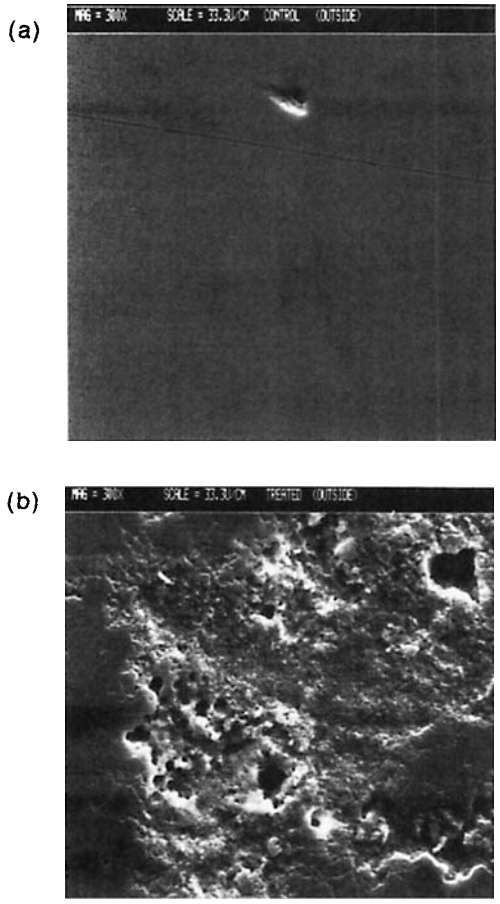

Figure 3 SEM photographs (300×) of (a) the outer, smooth surface of a cellulose acetate (DS = 1.7) film and (b) the outer, smooth surface of a cellulose acetate (DS = 1.7) film after 4 days in the *in vitro* enrichment system.

surface area which should enhance the rate of biodegradation. Isolation and characterization of the fibers remaining after an appropriate period of time of exposure to the microbes would offer information about the action of these microorganisms on the polymer. As we have learned, there are a number of factors that make this approach difficult. First, we found that we could not routinely monitor the course of the reactions by analyzing for endproducts (e.g., cellobiose) by conventional techniques, such as TLC,[6] even though we knew that the CA was degrading (*vide infra*). Evidently, random cleavage of the polymer or loss of acetyl was rate limiting, so that the endproducts were removed before a significant concentration was achieved. In addition, recovery of a small amount of fiber (typically 10–20 mg) from a large reaction volume proved to be tedious at best. Finally, analysis of the remaining CA was complicated by the difficulty we encountered in removing protein and lipid, which had a high affinity for the CA. Nevertheless, the first evidence for biodegradation of CA and the complexity of the mechanism(s) involved in the biodegradation of CA came from these fiber studies. Figures 1(a) and 1(b) are photographs of CA fibers taken from a control flask and from an inoculated flask, respectively, where the CA served as the sole carbon source for the bacteria. Clearly, the CA fibers from the inoculated flask have attached growth and are capable of substaining growth of a stable population. Table I gives a summary of the data for three separate experiments involving the CDA (DS = 2.5) fiber. In the first two sets of experiments, the DS for the inoculated samples was lowered by an average of 0.55, while the DS of the control sample was not significantly affected. Comparison of the GPC data of the inoculated samples to that of the control and starting material shows that M_z of the inoculated samples was decreased while the M_n increased, which is reflected by the narrowing of the polydispersities (M_w/

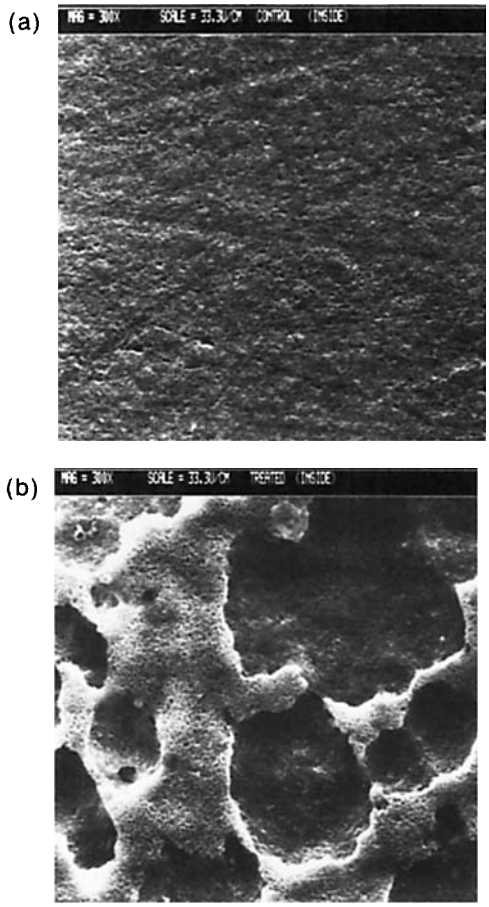

Figure 4 SEM photographs (300×) of (a) the inner, rough surface of a cellulose acetate (DS = 1.7) film and (b) the inner, rough surface of a cellulose acetate (DS = 1.7) film after 4 days in the *in vitro* enrichment system.

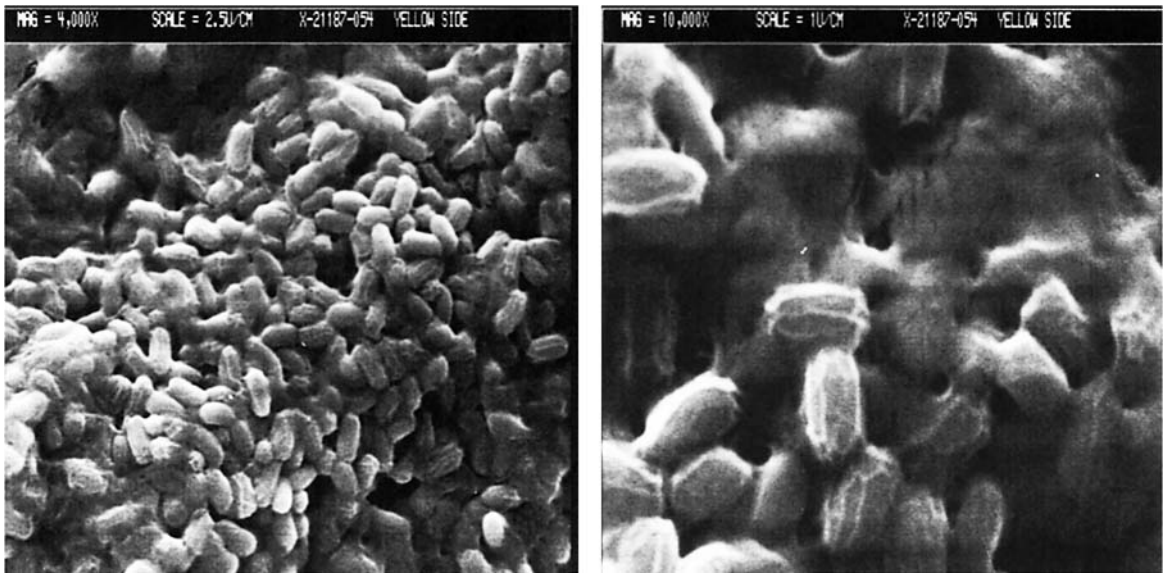

Figure 5 SEM photographs (4000× and 10,000×) of the inner, rough surface of a cellulose acetate (DS = 1.7) film after 4 days in the *in vitro* enrichment system from which the bacteria have not been washed.

M_n, M_z/M_n). Hence, this data suggested that we were observing both deacetylation of the CA and random cleavage of the CA to a smaller chain size. The GPC data for the second set of experiments were consistent with the observations made for the first set of experiments while, surprisingly, little change in DS of the recovered fiber was observed. In the first two sets of experiments, we noticed that we were recovering much less material than we were starting with. In order to determine the amount of material we were recovering, we used very carefully weighed (100 mg) amounts of starting material in the third trial. As expected, the average amount of CA recovered was 17% (vs 100% in the control). The data for the three sets of experiments were pooled and analyzed by Duncan's test, which showed that fiber recovery and polydispersitity of the recovered fiber were significantly different for the control and inoculated test groups at a 95% confidence level.

Table II *In Vitro* Assay Involving Cellulose Acetate (DS = 1.7) Films

Film	Original Weight (mg)	Final Weight (mg)	% Weight Loss
Trial 1 (4 days)[a]			
Control	210	199	5
Test	185	18	92
Trial 2 (5 days)[b]			
Control	191	180	5
Test	179	4	98
Trial 3 (6 days)[c]			
Control	176	174	2
Test	173	18	91

[a] This trial consisted of 3 control and 3 test films. The control flasks received antibiotics to control microbial contamination.
[b] This trial consisted of 2 control and 2 test films. The control flask received sodium arsenate to control microbial contamination.
[c] This trial consisted of 3 control and 2 test films. The control flasks received antibiotics to control microbial contamination.

To measure the biodegradation of CA films, films of known weight and thickness were subjected to either an *in vitro* assay or to a wastewater treatment assay. The loss in film thickness and weight of each film was then determined and, if films of sufficient integrity were recovered, the tensile properties of the films were measured. As with the fiber studies, control experiments were also conducted with the *in vitro* assay. Figure 2 shows the remains of a CDA film (DS = 1.7) after 5 days in the *in vitro* assay along with a control film. As can be seen, the test film is broken into small pieces and is frayed and filled with small holes. Figures 3 and 4 show scanning electron microscopy (SEM) photographs of a CA film (DS = 1.7) before and after 4 days in the *in vitro* assay. Figure 3(a) shows the outer, smooth-surface of the film, which is a result of being in con-

Table III Wastewater Treatment Asasy of Cellulose Acetate (DS = 1.7) Films

	Original Weight (mg)	Final Weight (mg)	% Weight Loss	Original Thickness	Final Thickness	% Thickness Loss
21 Days[a]	219	175	21	6.08	5.41	11.0
27 Days[b]	183	50	76	6.03	4.45	24.7

[a] The values represent the average for 4 test films.
[b] The values represent the average for 10 test films, except for the final thickness where only 9 films were recovered of sufficient size.

tact with the draw blade, while Figure 3(b) shows the same film surface after being treated in the *in vitro* assay. Figure 4(a) shows the inner, rough side of the film, which was in contact with the surface upon which the film was cast, while Figure 4(b) shows the same film surface after being treated in the *in vitro* assay. Both Figures 3(b) and 4(b) show extensive pitting of the film surfaces, but it is evident that the rough side of the film is more extensively pitted than the smooth side. This is consistent with visual observations of one side of the film (the rough side) becoming coated with a yellow substance.[7] We believe that this is due to more surface area on the rough (inner) side for attachment of the bacteria. Figure 5 shows the inner (rough) side, respectively, of a CA film (DS = 1.7), from which the bacteria have not been washed. These photographs show the attached microbes in the cavities in which the degradation is occurring.

Table II shows the data for three trials, which lasted from 4–6 days, using the *in vitro* assay involving films prepared from CDA with a DS of 1.7. In all three trials, we observed average weight losses of 91–98% for the test films and only 2–5% for the control films. Analyses by Duncan's test showed that the weight loss between the two groups was statistically significant at the 99% ($p < 0.01$) confidence level. Due to extensive degradation of these films, we were unable to measure mechanical properties.

Films prepared from CDA, with a DS of 1.7, were also subjected to our wastewater treatment assay for 21 and 27 days (Table III). The films, tested after 21 days, show an average weight loss of 21%, while the films tested after 27 days show an average weight loss of 76%. The large loss in film weight between days 21 and 27 is typical. Generally, an induction period is observed during which we believe microbial attachment is occurring. When the bacteria are attached and enough degradation has occurred to expose more surface area, the rate of biodegradation increases.

Having satisfied ourselves that films prepared from CA with a DS of 1.7 were indeed biodegradable in our assays, we turned to films made from more highly substituted CDA (DS = 2.5). Table IV gives a summary of the data for a trial that lasted 11 days using the *in vitro* assay. The data in Table IV illustrates that substantial losses in film weight, film thickness, and tensile strength were observed ($p < 0.05$). According to our expectation, this CDA (DS = 2.5) is also degradable in film form, but at a slower rate than the films prepared from the CA with a DS of 1.7.

As in the *in vitro* assay, the CA of DS = 2.5 was found to degrade at a slower rate in the wastewater treatment assay relative to the lower DS material. Ten weeks were required in this trial before significant changes in the films were observed. For trials of this experimental duration, operational fluctuations in the wastewater basin became critical to the course of the experiment. Nevertheless, we found the film weight loss (Fig. 6), as well as the percent

Table IV *In Vitro* Assay Involving Cellulose Acetate (DS = 2.5) Films

Film[a]	Original Weight (mg)	Final Weight (mg)	% Weight Loss	Thickness Change (mil)	Tensile Strength (10^3 psi)
Control	144	144	0	+0.43	10.9
Test	162	54	67	−0.98	3.9

[a] Each value is the average for 4 control and 4 test films.

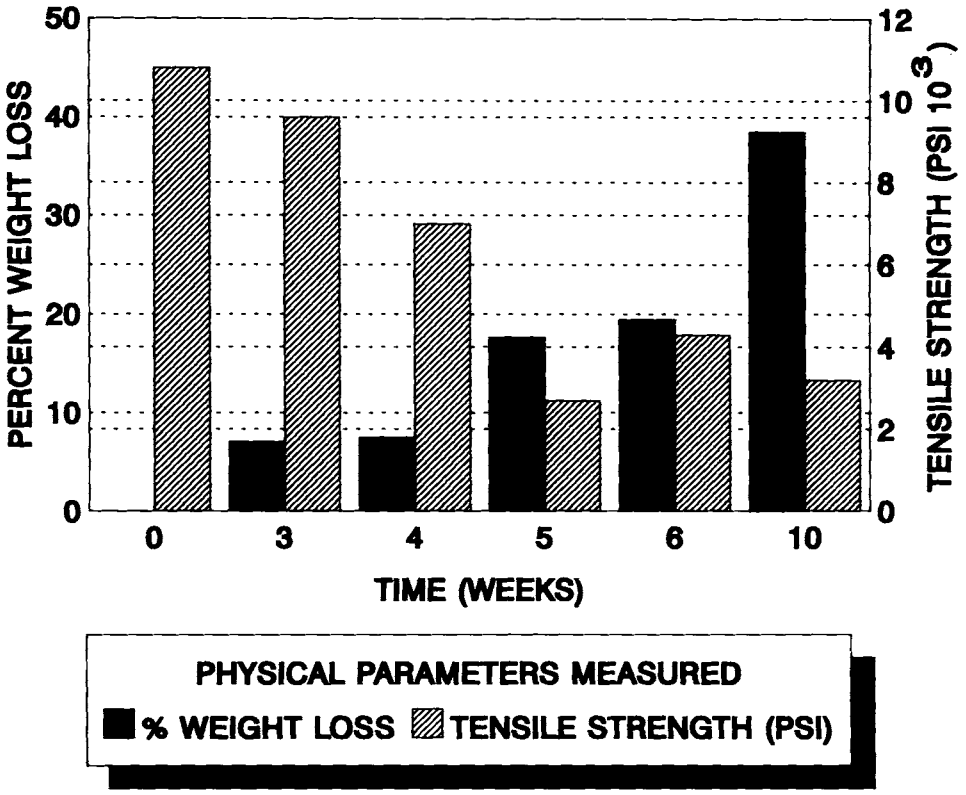

Figure 6 Changes in the film weight and tensile strength for films of CDA (DS = 2.5) in the wastewater treatment assay.

change in tensile strength (70% decrease), to be significant at the 95% confidence level when comparing time 0 with 10 weeks.

We next examined the biodegradability of CTA (DS = 2.95) in the *in vitro* assay. After 28 days, no significant weight loss of the films was observed

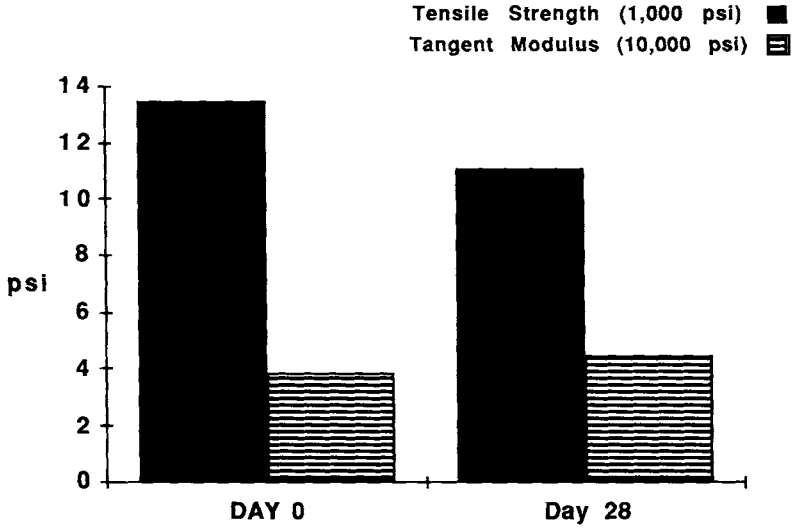

Figure 7 The tensile strength and tangent modulus of CTA (DS = 2.95) films at day 0 and after 28 days in the *in vitro* assay.

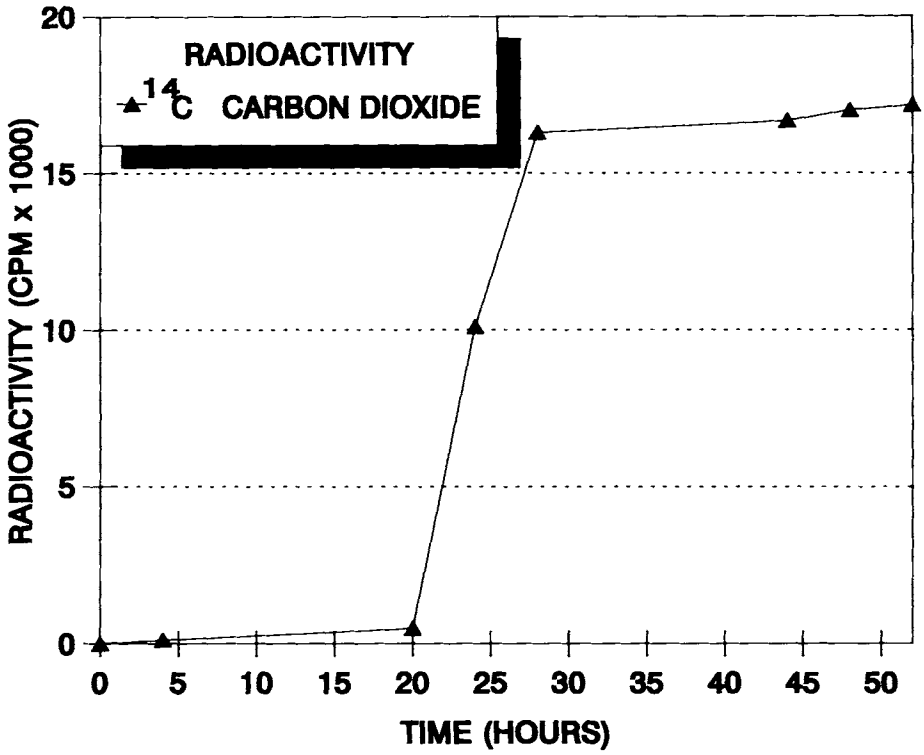

Figure 8 Microbial production of $^{14}CO_2$ from cellulose [1-^{14}C]-acetate (DS = 1.6).

(0.6% weight loss). Proton NMR also showed no change in the DS. Furthermore, the CTA films showed virtually no change in tensile strength and in tangent modulus (Fig. 7). These observations indicate that CTA is highly resistant to microbial attack.

The classical method for evaluating a substrate in microbial degradation is to document the transfer of carbon from the substrate to metabolic end products, such as CO_2 or cell biomass. Preferably, substrate labeled at a strategic point with carbon 14 is utilized in these studies, since the flow of labeled carbon is easily followed. For this study, we elected to use cellulose acetate, in which the carbonyl carbon of the acetyl substitutent was labeled with carbon 14. Since it is known that cellulose is easily degraded by microorganisms, we felt that if we could initially demonstrate deacetylation of the cellulose acetate and conversion of acetate to $^{14}CO_2$, then we could provide better evidence for the biodegradability of cellulose acetate. The required cellulose [1-^{14}C]-acetate was obtained by treating cellulose with [1-^{14}C]-acetyl chloride in trifluoroacetic acid, which yielded cellulose [1-^{14}C]-triacetate. Two different portions of the cellulose [1-^{14}C]-triacetate were subjected to aqueous hydrolysis, which yielded two cellulose [1-^{14}C]-acetates with a DS of 1.6 and 1.85 and a specific activity of 0.13 and 4.9 μCi/g, respectively. Fiber samples of the cellulose [1-^{14}C]-acetate, having a DS of 1.6, were then subjected to the *in vitro* assay (as well as a control sample) and the evolved $^{14}CO_2$ was collected at 4 h intervals. Figure 8 shows that between 20–25 h, 45% of the label was collected as $^{14}CO_2$; no $^{14}CO_2$ was collected from the control flask. The cellulose [1-^{14}C]-acetate with, a DS of 1.85, was converted to a film and subjected to the *in vitro* assay. (Because of the low activity of the initial inoculation, the sample was reinoculated at 175 h.) As Figure 9 demonstrates, around 68% of the ^{14}C label was collected between 200 and 400 h while a control sample showed no loss of $^{14}CO_2$. These initial experiments were intended simply to demonstrate biodegradability and, although the difference in rates between the two cellulose [1-^{14}C]-acetates are in part due to the difference in the form of the substrate and DS, the experiments do not provide sufficient data for obtaining meaningful relative rate data under these fermentation conditions. These types of questions will be addressed in detailed experiments involving labelled cellulose acetates, as well as other cellulose esters. The experiments are in progress and will be reported in future publications.

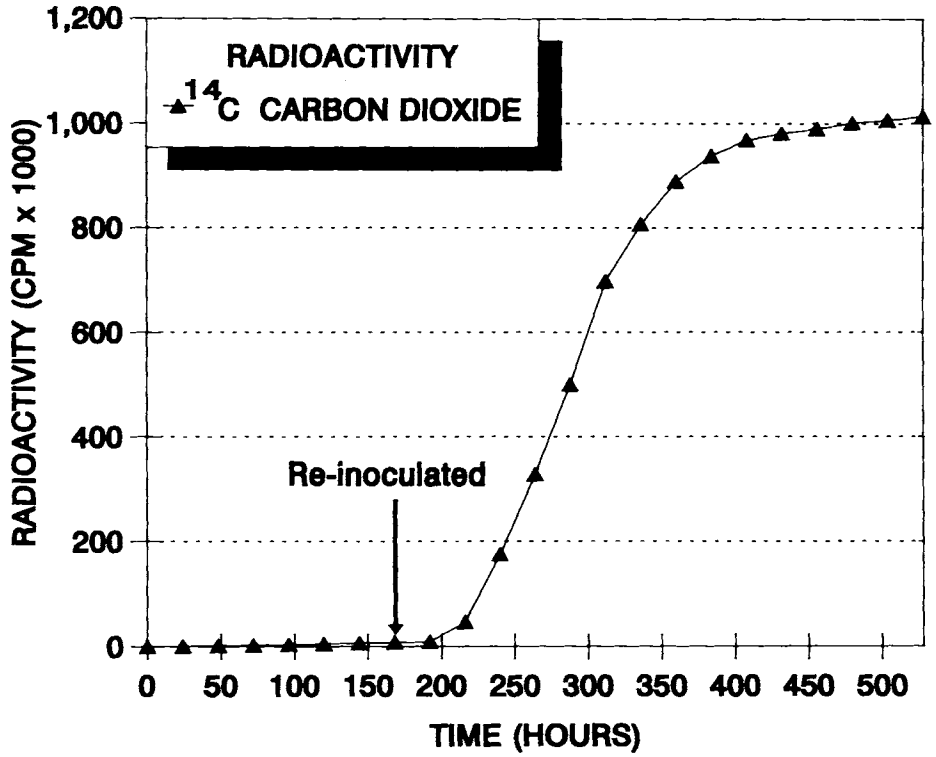

Figure 9 Microbial production of $^{14}CO_2$ from cellulose [1-^{14}C]-acetate (DS = 1.85).

CONCLUSIONS

The work presented in this report demonstrates the potential for aerobic biodegradation of cellulose acetate in the environment. The *in vitro* assay is an active treatment regime under optimal conditions, which accelerated degradation of the polymer and which proved to be useful in accessing the potential for biodegradation of cellulose acetate. In general, the *in vitro* assay proved to be operationally simple and yielded information about the potential for biodegradation of a cellulose acetate in a short time frame. Furthermore, the information obtained from the *in vitro* assay could be substantiated by slower techniques, such as the wastewater treatment assay. By employing radiochemical techniques in this work and showing carbon flow, we have provided good evidence that some cellulose acetates can indeed be degraded by microorganisms. The results are not intended to be accurate models for estimating biodegradation rates in landfills or of surface litter. The work, however, does show that cellulose acetate is potentially degradable in the environment and that the rate of biodegradation is strongly influenced by the degree of substitution. Additional work in this area is in progress and will be reported in future publications.

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