Biodegradation of Radiolabeled Cellulose Acetate and Cellulose Propionate

RONALD J. KOMAREK,* ROBERT M. GARDNER, CHARLES M. BUCHANAN, and STEVEN GEDON

Eastman Chemical Company, Research Laboratories, Kingsport, Tennessee 37662

SYNOPSIS

Biodegradation of cellulose acetate and cellulose propionate was conclusively established with a naturally derived mixed microbial culture derived from activated sludge and ^{14}C labeled cellulose esters. Radiolabeled cellulose esters were synthesized with either [1-^{14}C]-acetate or [1-^{14}C]-propionate and back hydrolyzed to the desired degree of substitution (DS) ranging from 1.77 to 2.64. Biodegradation was measured in an *in vitro* aerobic culture system that was designed to capture $^{14}CO_2$ produced by the aerobic microbial metabolism of the cellulose esters. Microorganisms were able to extensively degrade cellulose [1-^{14}C]-acetate (CA) with DS ranging from 1.85 to 2.57 over periods of 14–31 days. More than 80% of the original ^{14}C-polymeric carbon was biodegraded to $^{14}CO_2$ for CA substrates with a DS of 1.85. CA polymers with a DS of 2.07 and 2.57 yielded over 60% conversion to $^{14}CO_2$. The amount of biodegradation that was observed with cellulose [1-^{14}C]-propionate with DS of 2.11, 2.44, and 2.64 were lower than the corresponding acetyl ester and ranged from 0.09 to 1.1%. However, cellulose [1-^{14}C]-propionate with a DS of 1.77 and 1.84 underwent very rapid degradation in the mixed culture system, with 70 to over 80% conversion of labeled polymeric carbon metabolized to $^{14}CO_2$ in 29 days. The high level of microbial utilization of carbon from both cellulose esters and its conversion to CO_2 confirms the biodegradability of these polymers and the potential they have for total mineralization in natural microbiologically active environments. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

In order to accurately classify a polymer as biodegradable it must be able to enter the biosphere's carbon cycle, nature's recycling process. The magnitude of the carbon cycle is best exemplified by cellulose, one of the major contributors to the cycle. Approximately 10^{11} tons of this polymer are produced and biodegraded annually.[1] The process of biodegradation is a key facet of the carbon cycle and is responsible for ultimately breaking down the huge quantities of organic compounds to CO_2. Thus, the best strategy for creating products that are capable of entering the carbon cycle is to ensure that the polymeric carbon can be biodegraded. Cellulose esters represent a class of polymers that has the potential to participate in the carbon cycle via microbiologically catalyzed deesterification and decomposition of the resulting cellulose and organic acids. Cellulose acetate is currently used in high volume applications ranging from fiber, to film, to injection molding thermoplastics. It has the physical properties and relatively low material cost that have excluded other biodegradable polymers from being widely accepted in the market place.

Reese[2] presented evidence of esterase activity on soluble cellulose acetate with a low degree of substitution (DS, 0.76 sites esterified per anhydroglucose monomer). A pure culture of *Pestalotiopsis westerdijkii* QM381 was reported to completely utilize this low DS cellulose ester. However, Reese did not find any evidence that the fully substituted cellulose triacetate could be biodegraded. Cantor and Mechalas[3] found evidence of esterase activity on reverse-osmosis membranes composed of cellulose acetate (DS 2.5). Using infrared analysis, up to 50% deacylation was detected on the desalinating surface.

* To whom correspondence should be addressed.

Journal of Applied Polymer Science, Vol. 50, 1739–1746 (1993)
© 1993 John Wiley & Sons, Inc. CCC 0021-8995/93/101739-08

Table I Degree of Substitution (DS), Specific Activity (SA), and Total Radioactivity of Cellulose [1-^{14}C]-Acetates Used in Biodegradation Experiments

Expt. No.	Labeled Polymer	DS (^{1}H NMR)	SA (DPM × 10^3/mg)	Total/Flask (DPM × 10^6)
1	Cellulose [1-^{14}C]-acetate	1.85	10.98	2.65
		2.07	14.10	2.63
		2.07	14.10	2.62
		2.07	14.10	2.66
		2.07	14.10	2.64
		2.57	17.62	2.62
2	Cellulose [1-^{14}C]-acetate	B2.07	14.10	1.33
		B2.07	14.10	1.30
		2.07	14.10	1.34
		2.07	14.10	1.32
		2.57	17.62	1.37
		2.57	17.62	1.36
3	Cellulose [1-^{14}C]-acetate	B2.07	14.10	1.30
		B2.07	14.10	1.30
		2.07	14.15	1.19
		2.07	14.10	1.32
		2.57	17.62	1.29
		2.57	17.62	1.36

B, Biocide (1,2-dibromo-2,4-dicyanobutane).

No reduction in acylation was detected with cellulose triacetate. Ji-Dong Gu et al.[4] recently presented evidence of anaerobic biodegradation of cellulose acetate (DS 1.7) with about 19% weight loss over a 60-day period. Recently, Buchanan et al.[5,6] presented evidence supporting the inherent biodegradability of cellulose acetate with naturally occurring microorganisms in activated sludge and *in vitro* aerobic microbial cultures.

The objective of this report was to establish unequivocally the biodegradability of cellulose acetate, to further investigate the degradative mechanism, and to present experimental results on the biodegradation potential of cellulose propionate. ^{14}C-labeled cellulose esters represented the most direct approach to accomplish these goals. Enriched mixed cultures derived from environmental sources of aerobic microorganisms were used in these studies

Table II Degree of Substitution (DS), Specific Activity (SA), and Total Radioactivity of Cellulose [1-^{14}C]-Propionates Used in Biodegradation Experiments

Expt. No.	Labeled Polymer	DS (^{1}H NMR)	SA (DPM × 10^3/mg)	Total Flask (DPM × 10^6)
4	Cellulose [1-^{14}C]-propionate	2.44[a]	11.71	2.06
		2.64	11.87	2.10
5	Cellulose [1-^{14}C]-propionate	1.77	8.06	1.37
		1.77	8.06	1.39
		1.84	8.34	1.52
		1.84	8.34	1.50
		2.11	9.99	1.47
		2.11	9.99	1.56
		2.44[a]	11.71	1.40
		2.44[a]	11.71	1.41

[a] Contains 0.11 DS of unlabeled acetate.

in order to relate the results to natural biodegradation. This report presents the results of biodegradation experiments with ^{14}C-labeled cellulose esters and describes the inherent biodegradation potential of cellulose acetate and cellulose propionate.

EXPERIMENTAL

Synthesis of Cellulose [1-^{14}C]-Esters

Radiolabeled cellulose esters were synthesized by a previously reported procedure whereby cellulose was reacted with [1-^{14}C]-acetyl chloride or [1-^{14}C]-propionyl chloride in a mixture of trifluoroacetic acid and trifluoroacetic anhydride to produce the fully substituted triesters.[6,7] The resulting cellulose triesters were back hydrolyzed[6,7] to the desired degree of acyl substitution. The DS of each preparation was determined by proton NMR and the radiochemical purity by HPLC.

Radiochemical Purity

^{14}C-Labeled cellulose esters were fractionated by gel permeation chromatography (GPC) using PL-Gel, 100 Å, and PL-Gel, Mixed Bed, columns (Polymer Laboratories). The columns were chosen for their ability to separate the low molecular weight fractions and unreacted [1-^{14}C]-acetate or [1-^{14}C]-propionate that could have been produced during the hydrolysis. The effluent from the columns was collected in a fraction collector and 1-min fractions were counted in an LKB 1217 RACKBETA liquid scintillation counter to determine the radioactive profile of the chromatogram.

In Vitro Culture and Growth Media

The *in vitro* culture system utilized a mixed microbial population that had been adapted to cellulose acetate or cellulose propionate.[6] These cultures were obtained from naturally occurring microorganisms common to most activated sludge wastewater treatment plants and cultured in media containing cellulose esters. The growth media used in these experiments was designed to stimulate active growth and to favor the microbial population utilizing the polymer.[6]

Biodegradation Assay System

Biodegradation experiments were conducted in a culture apparatus that included a mass flow controller for the oxygen flow, a shaking water bath, glass Erlenmeyer culture vessels, CO_2 absorption columns, and a switching system that permitted automatic 4-h collections over a 24-h period. The fermentations were conducted in a 250 mL Erlenmeyer flask with 70 mL of the media and 10 mL of inoculum ($> 10^7$ cells/mL) in each flask. Between 0.6 and 1.2 μCi (84–242 mg) of ^{14}C-labeled cellulose esters (as several particles of precipitate) were added to each flask (Tables I, II). Experimental flasks were incubated at 30°C on a shaking water bath at 150 rpm. A controlled oxygen flow of 15 mL/min was first saturated with water at 30°C, and then bubbled through the fermentation flask. The gases were then passed through methoxy ethylamine (Carobosorb, Packard Instrument Co.) in a jacketed CO_2 absorp-

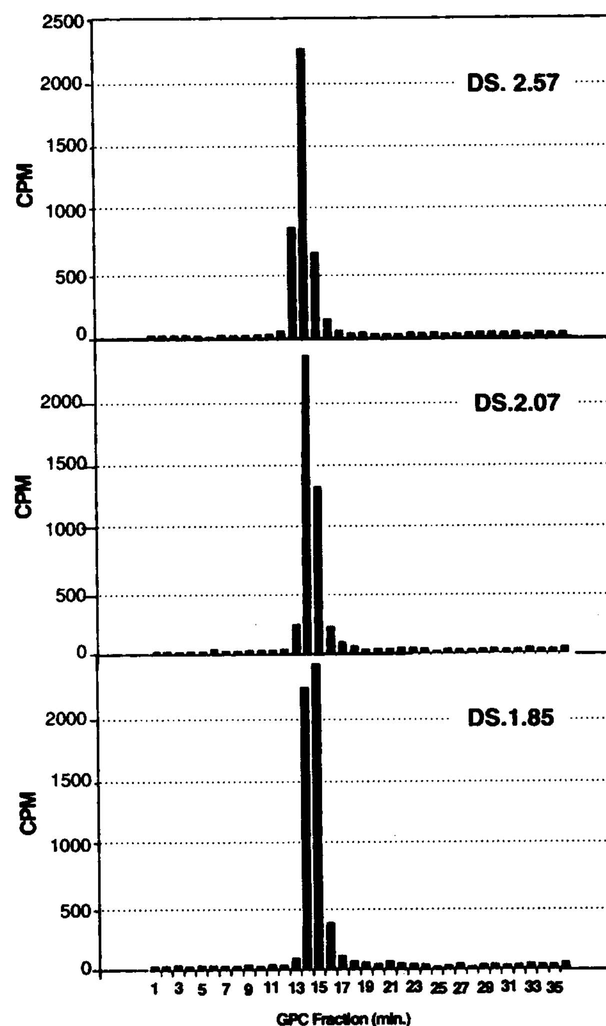
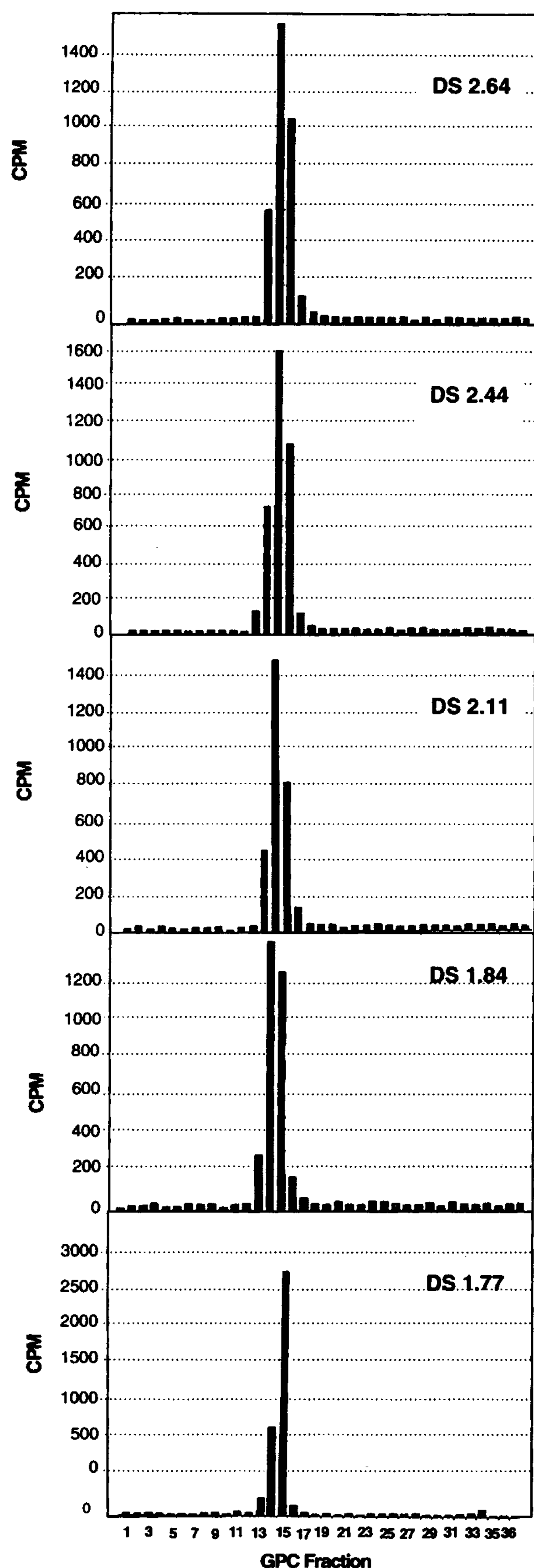


Figure 1 Distribution of radioactivity in fractions collected from an HPLC separation by gel permeation chromatography indicating the radiochemical purity and molecular weight distribution of three preparations of cellulose [1-^{14}C]-acetates (DS 1.85, 2.07, and 2.57).



tion column filled with glass beads and cooled to −2°C. After completion of the collection period, the methoxy ethylamine was drained from the collection column into a scintillation vial, followed by three 4-mL rinses of the liquid scintillation cocktail (Permafluor V, Packard Instrument). Scintillation vials were then counted on an LKB RACKBETA liquid scintillation counter.

Cellulose [1-^{14}C]-Acetate Experiments

Three biodegradation experiments were conducted with cellulose [1-^{14}C]-acetates (Table I). Experiment 1 measured the biodegradation of cellulose [1-^{14}C]-acetates (DS of 1.85, 2.07, and 2.57) by the mixed microbial culture in the biodegradation apparatus over a 14-day period that collected $^{14}CO_2$ every 4 h. Experiment 2 was designed to replicate the biodegradation found in Experiment 1, and in addition, to measure the effect of stopping the biodegradative activity of the microbial population with a biocide (1,2-dibromo-2,4-dicyanobutane). Cellulose [1-^{14}C]-acetate with DSs of 2.07 and 2.57 and controls with DS 2.07 plus 420 mg of biocide were each incubated in the presence of the mixed culture over a period of 22 days. There were two replications per treatment group. Experiment 3 was a complete replicate (except for the inoculum) of Experiment 2. In contrast to Experiment 2, the inoculum was taken from an aged microbial culture that had a very uniform consistency. This consistency permitted a more uniform addition of cells and particulate material to each test flask. The $^{14}CO_2$ evolved from these fermentations was collected over a 31-day period.

Cellulose [1-^{14}C]-Propionate Experiments

Two biodegradation experiments were conducted with cellulose [1-^{14}C]-propionates (Table II). Experiment 4 investigated the biodegradation of cellulose [1-^{14}C]-propionates with DS of 2.44 and 2.67 over a period of 26 days. The inoculum was taken from a mixed culture adapted to cellulose propionate. Experiment 5 measured biodegradation of four cellulose [1-^{14}C]-propionates (DS 1.77, 1.84, 2.11, and 2.44) over a period of 29 days. The inoculum was similar to that used in Experiment 4.

Figure 2 Distribution of radioactivity in fractions collected from an HPLC separation by gel permeation chromatography indicating the radiochemical purity and molecular weight distribution of five preparations of cellulose [1-^{14}C]-propionates (DS 1.77, 1.84, 2.11, 2.44, and 2.64).

RESULTS AND DISCUSSION

A series of radiolabeled acetate and propionate cellulose esters were synthesized with ^{14}C in the carbonyl position (Tables I, II). The cellulose [1-^{14}C]-propionate esters, with DS of 1.77, 1.84, 2.11, and 2.64, were esterified exclusively with [1-^{14}C]-propionate. Cellulose [1-^{14}C]-propionate, DS 2.44, however contained a small amount of unlabeled acetate (DS 0.11). The polymers were prepared with the ^{14}C in the carbonyl carbon to directly address the ability of microorganisms to deacylate the cellulose esters with naturally occurring esterases, or by some other hydrolytic mechanism. Removal of the acyl groups would then expose the cellulose to natural degradation by cellulases.[8]

GPC chromatograms indicated that in all the synthetic preparations the label was associated with a single peak, indicating radiochemical purity. The GPC chromatograms also established that there were no contaminating radioactive reaction products. Only background levels of radiation were detected prior to and after the main peak (Figs. 1, 2). The polymers displayed a relatively narrow polydispersity and no major molecular weight reductions had occurred during the back hydrolysis process (Figs. 1, 2). Even the most extensively hydrolyzed cellulose esters had a significant portion of the molecular weight distribution above 50,000. Proton NMR indicated that the desired spread in DS ranging from 1.77 to 2.64 was achieved by the hydrolysis. Prior work has indicated the hydrolysis results in a randomization of attachment to esterification sites.[7]

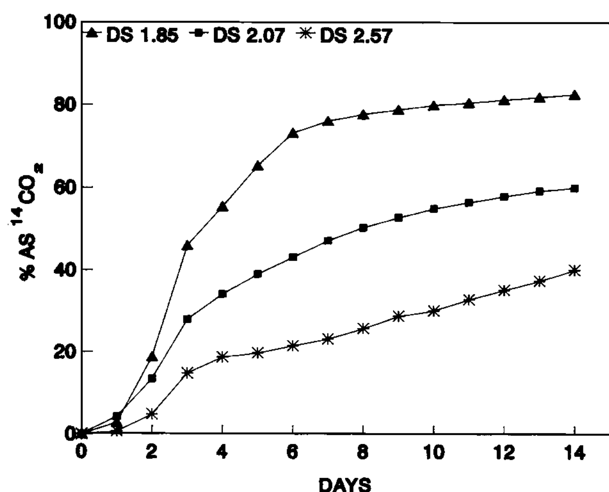


Figure 3 Experiment 1. Biodegradation of cellulose [1-^{14}C]-acetates (DS 1.85, 2.07, and 2.57) as indicated by the percent of labeled carbon metabolized to $^{14}CO_2$ by the mixed microbial population.

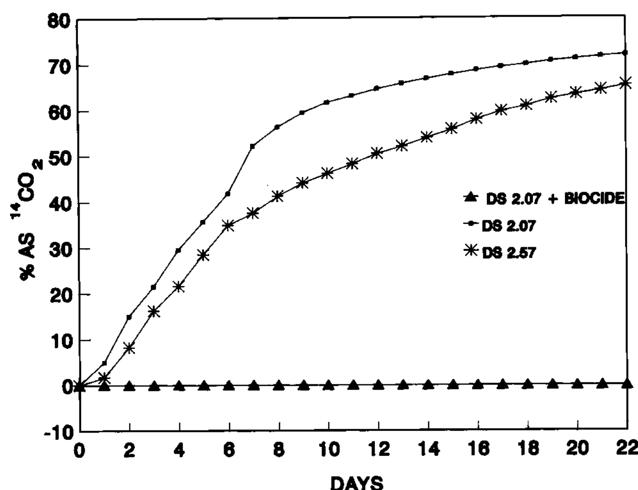


Figure 4 Experiment 2. Biodegradation of cellulose [1-^{14}C]-acetates (DS 2.07, 2.57, and a control of 2.07 + biocide) as indicated by the percent of labeled carbon metabolized to $^{14}CO_2$ by the mixed microbial population. Each curve represents the average of duplicate fermentations.

The results of Experiment 1 with cellulose [1-^{14}C]-acetate having DSs of 1.85, 2.07, and 2.57 are illustrated in Figure 3. Cellulose [1-^{14}C]-acetate with the lowest DS, 1.85, was rapidly degraded and within 14 days over 80% of the labeled polymeric carbon was converted to $^{14}CO_2$. The oxidation of polymeric carbon to CO_2 represents the final biochemical utilization of the ^{14}C; however, some of the label will also be directed toward synthesis of new microbial biomass. Ultimately as metabolic turnover occurs and as cells die, the carbon will eventually undergo complete mineralization to CO_2. After only 14 days, the $^{14}CO_2$ produced from cellulose [1-^{14}C]-acetate (DS 2.57) accounted for 40% of the total starting label, and there was no indication of a reduction in the rate of evolution. The early rate of biodegradation was inversely proportional to the DS. Considering that the time scale of natural biodegradation is measured in years, the relative rates of biodegradation found in this experiment, even for the highest DS, were rapid. In the four replications of the cellulose [1-^{14}C]-acetate (DS 2.07) cultures the percent biodegradation varied from 35 to 82%. This variation appeared to be related to the difficulty of adding a uniform inoculum because of the adherent nature of the active microorganisms attached to particulate material. As this assay demonstrated, the rate of biodegradation could be influenced by inoculum size.

Figure 4 illustrates the biodegradation response found in Experiment 2 with cellulose [1-^{14}C]acetate with DS of 2.07 and 2.57. Due to the variation ex-

perienced in Experiment 1 all treatments were performed in duplicate. A biocide was included as a treatment group in order to determine the effect of stopping all the microbial activity. The biocide was chosen to not interact chemically with cellulose acetate. No $^{14}CO_2$ was produced when biocide was added to the fermentation flask, indicating that the production of $^{14}CO_2$ was associated with the microbial activity and not chemical reactivity with the growth media. The results presented in Figure 4 display the same relationship of DS to rate of biodegradation as was seen in Experiment 1. Experiment 2 was extended 8 more days than Experiment 1. During this period a positive rate of biodegradation continued and after 22 days accounted for 65 and 75% of the labeled carbon for DS 2.57 and 2.07, respectively.

Except for the inoculum, Experiment 3 (Fig. 5) was a complete replicate of Experiment 2. The inoculum was taken from an aged microbial population where the cellulose acetate substrate had been reduced to a fine particle size. This fine suspension with the attached microbes permitted more reproducible inoculations than the younger cultures with larger substrate particles with adherent microorganisms. Excellent replication between duplicate fermentation flasks was found in comparison to previous experiments (Fig. 6). Experiment 3 had a much longer lag period at the start of the experiment

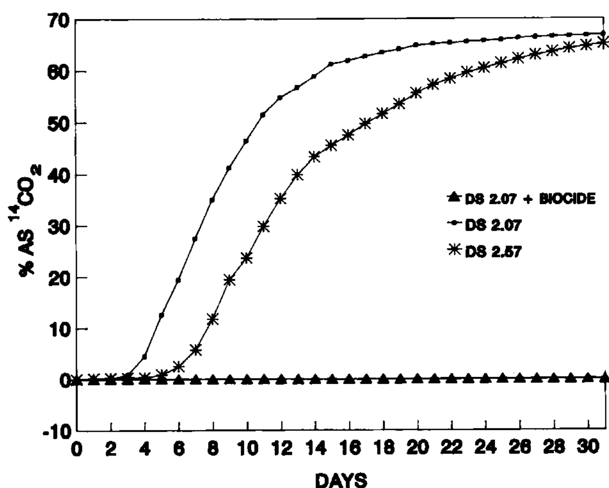
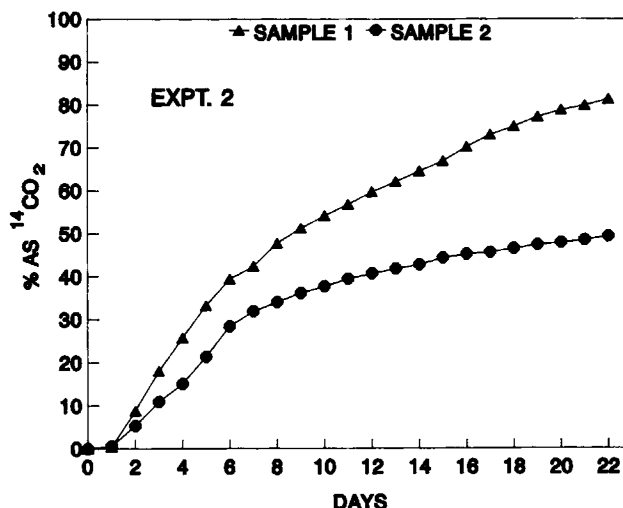


Figure 5 Experiment 3. Biodegradation of cellulose [1-^{14}C]-acetates (DS 2.07, 2.57, and a control of 2.07 + biocide) as indicated by the percent of labeled carbon metabolized to $^{14}CO_2$ by the mixed microbial population. Each curve represents the average of duplicate fermentations. A replicate of Experiment 2 with a more uniform aged inoculum.



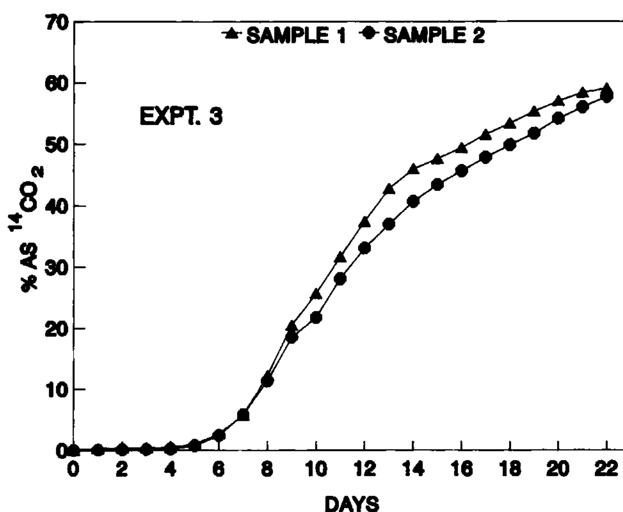


Figure 6 Comparison of the incubation replicates from Experiment 2 and Experiment 3 fermenting cellulose [1-^{14}C]-acetate, DS 2.07. Experiment 3 was inoculated with a more uniform aged inoculum.

than Experiment 2 due to the age of the microbial population. Again the biocide suppressed all microbial activity and no significant amount of $^{14}CO_2$ was measured. A lag period of 3 days for cellulose [1-^{14}C]acetate (DS 2.07) and 6 days for cellulose [1-^{14}C]acetate (DS 2.57) occurred before significant amounts of $^{14}CO_2$ were detected. After this lag period relatively rapid biodegradation rates were observed. Because of the lag period, this experiment was conducted 9 days longer than the previous experiment. After 31 days the percent degradation of cellulose [1-^{14}C]-acetate (DS 2.57) was almost equal to that of cellulose [1-^{14}C]-acetate (DS 2.07) (65 vs. 67%).

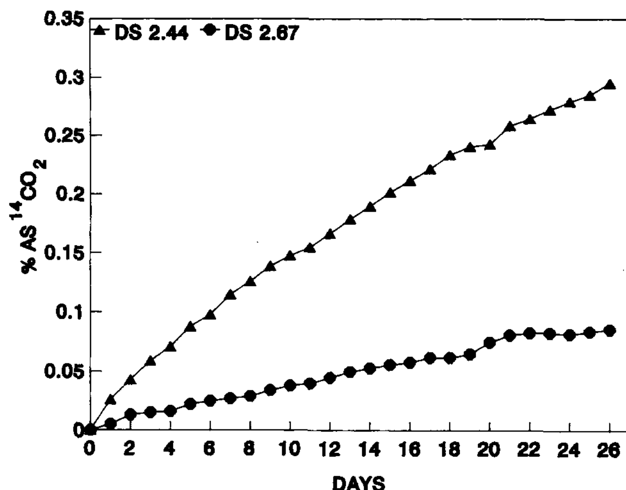


Figure 7 Experiment 4. Biodegradation of cellulose [1-^{14}C]-propionates (DS 2.44 and 2.64) as indicated by the percent of labeled carbon metabolized to $^{14}CO_2$ by the mixed microbial population.

Cellulose [1-^{14}C]-Propionate

Biodegradation of cellulose [1-^{14}C]-propionate was first tested in Experiment 4 with propionyl substitutions of 2.44 and 2.67 (Fig. 7).

With cellulose [1-^{14}C]-propionate (DS 2.67) less than 0.05% of the original starting label was metabolized to $^{14}CO_2$ in 26 days of incubation. Reducing the DS level to 2.44 resulted in a modest increase in biodegradation (to about 0.3%). The accurate measurement of these relatively small differences in biodegradation illustrates the great sensitivity possible with radiolabeled compounds.

In Experiment 5 three preparations of cellulose [1-^{14}C]-propionate with lower DS were tested along with DS 2.44 (Fig. 8). The results of Experiment 4 were essentially replicated in Experiment 5 with 0.28% of the starting label in cellulose [1-^{14}C]-propionate, DS 2.44, converted to $^{14}CO_2$. With cellulose [1-^{14}C]-propionate, DS 2.11, biodegradation was greater than DS 2.44, but only about 1% of the label was found in CO_2 over a 29-day period (Fig. 8B). Surprisingly, cellulose [1-^{14}C]-propionate (DS 1.84) was extensively metabolized with 72% of the label detected as $^{14}CO_2$ (Fig. 8A). Even higher levels of conversion to $^{14}CO_2$ (84%) were obtained with cellulose [1-^{14}C]-propionate with a DS of 1.77. With these high levels of ^{14}C reaching the end product of metabolism (CO_2), most if not all of the remaining label would be expected to be part of the microbial biomass.

The results obtained from these experiments demonstrate the ability of naturally derived microorganisms to deacylate both cellulose acetates and propionates and to oxidize the carbonyl carbon to CO_2 (Table III). The cleavage is presumably the result of microbial esterases present in these natural populations. Stutzenberger and Kahler[9] have shown that cellulolytic enzymes alone (from *Thermomonospora curvata*) do not have the ability to attack cellulose acetate (DS 2.5). The mixed culture however can be expected to have both esterases and cellulases, and therefore be capable of cleaving the ester and degrading both cellulose and the short-chain acid. The high level of microbial utilization of carbon from both cellulose esters and its conversion to CO_2

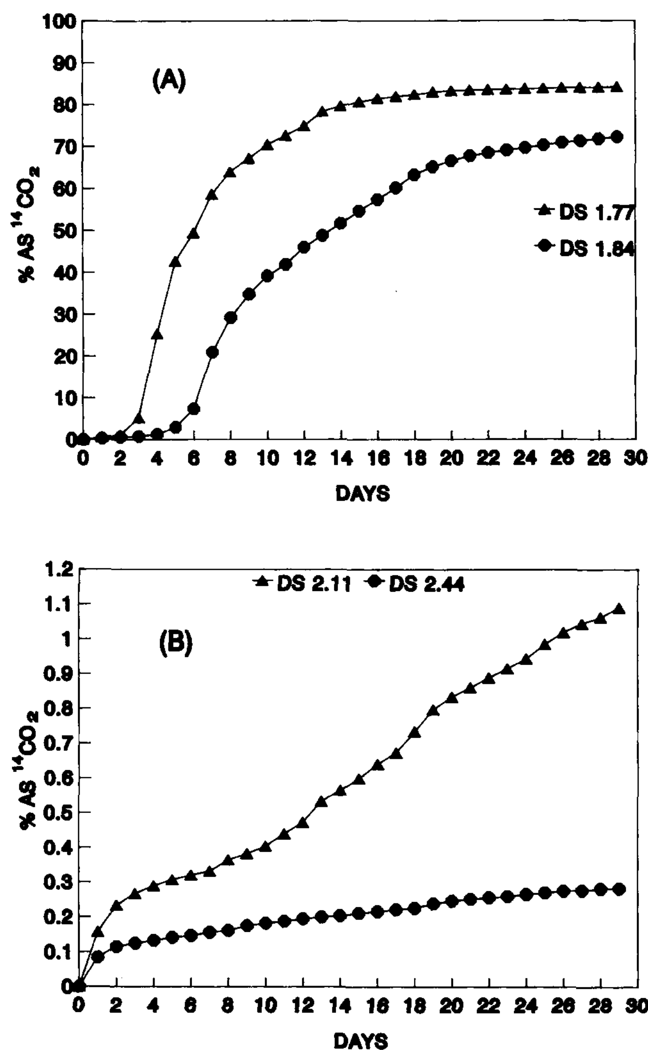


Figure 8 Experiment 5. Biodegradation of cellulose [1-^{14}C]-propionates as indicated by the percent of labeled carbon metabolized to $^{14}CO_2$ by the mixed microbial population. (A) DS 1.77 and 1.84; (B) DS 2.11 and 2.44. Each curve represents the average of duplicate fermentations.

Table III Summary of Biodegradation of Cellulose [1-^{14}C]-Acetates (CA), and Cellulose [1-^{14}C]-Propionates (CP) at 5-Day Intervals Relative to Percent of Carbon Converted to $^{14}CO_2$

			Days of Incubation				
Expt.	Polymer	DS	5	10	15	20	25
1	CA	1.85	73.2	79.9	82.8		
		2.07	32.9	52.4	60.3		
		2.57	19.8	30.1	40.2		
2	CA	2.07	35.8	61.8	67.9	71.2	72.0
		2.57	28.5	46.2	55.8	63.5	65.4
3	CA	2.07	12.6	46.4	61.2	64.8	65.9
		2.57	0.9	23.7	45.5	55.6	61.4
4	CP	2.44[a]	0.09	0.15	0.20	0.24	0.29
		2.64	0.02	0.04	0.06	0.07	0.08
5	CP	1.77	42.7	70.4	80.7	83.3	83.9
		1.84	2.9	39.2	54.7	66.6	70.3
		2.11	0.31	0.40	0.60	0.83	0.99
		2.44[a]	0.14	0.18	0.21	0.25	0.27

[a] Contains 0.11 DS of unlabeled acetate.

confirms the inherent biodegradability of these polymers and the great potential they have for total mineralization in the environment. As exemplified in this study, previous studies that employed either pure cultures or enzyme techniques cannot fully encompass the variety of capabilities that are present in a complex naturally derived mixed microbial population.

The mixed cultures used in these experiments had the advantage of accurate temperature control, a complete balance of nutrients, and thorough oxygenation, plus a prior enrichment by the selective pressure of cellulose esters as a sole carbon source. This permitted the technique to be a relatively rapid predictor of biodegradation. The correlation of these results with the wastewater treatment data, previously reported,[6] indicates that the *in vitro* enrichment culture system merely accelerates the natural process. It does not impart an ability that the cells do not already possess. A limitation of this assay system is that it represents a batch culture process (closed system) with a finite nutrient supply and no vehicle for end product removal, therefore its activity can be expected to plateau after an extended incubation time.

CONCLUSIONS

Cellulose acetates and low DS cellulose propionates are biodegradable polymers and can be expected to be degraded in natural environmental systems such as soil, compost, lakes, rivers, and other natural systems. The rate of biodegradation is sufficient to allow these cellulosic polymers to readily participate in nature's carbon cycle.

The authors acknowledge the technical assistance and contributions of R. Finch, A. Matosky, and D. Dorschel given to this research project.

REFERENCES

1. K. Hess, *Die Chemie der Zellulose und Ihrer Begleiter*, Akademische Verlagsgesellschaft, M. Bitt, Leipzig, 1928, p. 5.
2. E. T. Reese, *Ind. Eng. Chem.*, **49**, 89 (1957).
3. P. A. Cantor and B. J. Mechalas, *J. Polym. Sci.*, **28**, 225 (1969).
4. Ji-Dong Gu, S. P. McCarthy, G. P. Smith, D. Eberiel, and R. A. Gross, *Proc. ACS Div. Polym. Mater. Sci. & Eng.*, **67**, 230 (1992).
5. C. M. Buchanan, R. M. Gardner, R. J. Komarek, S. C. Gedon, and A. W. White, in *Fundamentals of Biodegradable Polymers and Materials*, Technomic Pub. Co., Lancaster, PA, 1993.
6. C. M. Buchanan, R. M. Gardner, and R. J. Komarek, *J. Appl. Polym. Sci.*, **47**, 1709 (1993).
7. C. M. Buchanan, K. J. Edgar, J. A. Hyatt, and A. K. Wilson, *Macromolecules*, **24**, 3050 (1991).
8. J. A. Gascoigne and M. M. Gascoigne, *Biological Degradation of Cellulose*, Butterworths, London, 1960.
9. F. Stutzenberger and G. Kahler, *J. Appl. Bacteriology*, **61**, 225 (1986).

Received December 3, 1992
Accepted April 29, 1993