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Degradation and Mineralization of Cellulose Acetate in Simulated Thermophilic Compost Environments*

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Residual cellulose acetate (CA) films with initial degree of substitution (DS) values of 1.7 and 2.5 (CA DS-1.7 and DS-2.5) were recovered from a simulated thermophilic compost exposure and characterized by gel permeation chromatography (GPC), proton nuclear magnetic resonance (^{1}H NMR), and scanning electron microscopy (SEM) to determine changes in polymer molecular weight and DS and to study microbial colonization and surface morphology, respectively. During the aerobic degradation of CA DS-1.7 and CA DS-2.5 films exposed for 7 and 18 days, respectively, the number-average molecular weight (M_n) of residual polymer decreased by 30.4% on day 5 and 20.3% on day 16, respectively. Furthermore, a decrease in the degree of substitution from 1.69 to 1.27 (4-day exposure) and from 2.51 to 2.18 (12-day exposure) was observed for the respective CA samples. In contrast, CA films (DS-1.7 and DS-2.5) which were exposed to abiotic control vessels for identical time periods showed no significant changes in M_n and DS. SEM photographs of CA (DS-1.7 and DS-2.5) film surfaces after compost exposures revealed severe erosion and corresponding microbial colonization. Similar exposure times for CA films in abiotic control vessels resulted in only minor changes in surface characteristics by SEM observations. The conversion of CA DS-1.7 and DS-2.5 to CO_2 was monitored by respirometry. In these studies, powdered CA was placed in a predigested compost matrix which was maintained at 53°C and 60% moisture content throughout the incubation period. A lag phase of 10- and 25-day duration for CA DS-1.7 and DS-2.5, respectively, was observed, after which the rate of degradation increased rapidly. Mineralization of exposed CA DS-1.7 and DS-2.5 powders reported as the percentage theoretical CO_2 recovered reached 72.4 and 77.6% in 24 and 60 days, respectively. The results of this study demonstrated that microbial degradation of CA films exposed to aerobic thermophilic laboratory-scale compost reactors not only results in film weight loss but also causes severe film pitting and a corresponding decrease in chain M_n and degree of substitution for the residual material. Furthermore, conversions to greater than 70% of the theoretical recovered CO_2 for CA (DS 1.7 and 2.5) substrates indicate high degrees of CA mineralization.

KEY WORDS: Cellulose acetate; degree of substitution; polymer degradation; polymer mineralization; municipal solid waste; surface colonization; respirometry; biodegradation testing; molecular weight.

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INTRODUCTION

Polysaccharide esters represent an exciting broad class of materials derived from nature. The polysaccharide component may be obtained directly from agricultural products such as corn (starch) and wood (cellulose). Alternatively, agricultural materials may be used in polysaccharide production by microbial fermentation or enzyme catalyzed conversion [1, 2]. The ester component of polysaccharide esters can have a highly variable structure and degree of substitution (DS). Of course, this variability in polysaccharide and side-chain ester structure and substitution allows the polymer scientist to tailor the corresponding material properties of polysaccharide esters for a number of applications. Cellulose esters, most notably cellulose acetates (CA), represent a subset of polysaccharide esters that are used for textile fibers, plastics, film, sheeting, membrane, and lacquers [3]. A description of the relationship among cellulose ester molecular weight, DS, and structure of the side-chain esters to the use of the corresponding materials for specific applications was presented elsewhere [3].

Early work which describes investigations of CA biodegradability was carried out by Reese [4], who noted that approximately half of the cellulolytic organisms investigated utilized cellobiose octaacetate as a carbon source, but only a few were capable of liberating detectable amounts of enzymes. Interestingly, the enzyme responsible for cellobiose octaacetate deacetylation (cellobiose octaacetylesterase) not only was produced when using cellobiose octaacetate as the substrate, but also was formed on water-soluble CA with a DS of 0.76 [4]. One fungal species, *Pestalotiopsis westerdijkii* QM 381 (formerly *Pestalotia palmarum*), was particularly useful for the formation of an exocellular cellobiose octaacetatease [4]. This microorganism was also found to produce this enzyme on water-soluble CA (DS 0.76) and the enzyme was less active than on cellobiose octaacetate [4]. Many years later, Downing *et al.* used *P. westerdijkii* to predigest water-soluble CA forming reducing sugars and acetate prior to converting these intermediate products to ethanol [5]. Reese [4] also exposed cellulose triacetate (the exact DS was not reported by the author) to a number of microorganisms which degraded both cellobiose octaacetate and water-soluble CA and found no growth over a 4-week incubation period. It should be noted that in this work, no effort was made to isolate microorganisms that were capable of degrading CA with a DS value higher than 0.76. Furthermore, it was not clear whether deacetylation occurred prior to, concurrently with, or after water-soluble CA main chain cleavage events. However, the work by Reese, carried out 36 years prior to this publication, surely must be considered a pioneering effort that evoked a number of questions which were not addressed until recently.

Interestingly, in birchwood xylan approximately 70% of the xylose units are acetylated [6]. Therefore, in nature there exists a substantially acetylated biodegradable polysaccharide ester. Luthi *et al.* [7] obtained an acetylxylan esterase from *Caldocellum sacchardyticum* expressed in *Escherichia coli* strain RR28 that showed no activity on xylan and cleaved acetate from acetylated xylan. Unfortunately, these workers did not determine the degree of substitution of the acetylated xylan which was used in their study. Work by Lee *et al.* [8] showed that the yeast *Rhodotorula mucilaginosa* produced an acetyl xylan esterase as an exocellular product when induced by triacetin. Furthermore, *R. mucilaginosa* did not produce a xylan-degrading enzyme and, thus, was free of xylanolytic activity. The acetylxylan esterase from *R. mucilaginosa* did not degrade a water-insoluble CA substrate where, once again, the DS of the CA was not reported. In contrast to *R. mucilaginosa*, the fungus *Trichoderma reesi* can produce two specific and several nonspecific xylanases as well as at least two acetyl esterases [9]. This fungus actively degrades acetylxylan polymer chains which contain 10%, by weight, acetyl groups, to produce xylose, arabinose, xylobiose, and acetate [9].

Work in our laboratory has been carried out to investigate the biodegradability of CA films with DS values of 1.7 and 2.5 (CA DS-1.7 and DS-2.5) when exposed to biologically active in-laboratory composting test vessels maintained at approximately 53°C [10–14]. It was found that the CA DS-1.7 and DS-2.5 films (thickness values, ~0.013 to 0.025 and 0.051 mm, respectively) had completely disappeared (were not visually observed in the test waste mixture) by the end of 7- and 18-day exposure time periods, respectively. Control poisoned reactors showed little CA weight loss over similar exposure time periods so that it may be concluded that film erosion during the composting exposures was due, at least in part, to biologically mediated degradative mechanisms. A 30-day exposure of CA DS-1.7 film samples (0.013- to 0.025-mm thickness) to an anaerobic methanogenic microcosm developed from a sludge inoculum resulted in almost-complete weight loss of the film, while exposure of these same films in poisoned control vessels resulted in negligible film weight loss [10]. Once again, it appears that CA DS-1.7 is degraded by a process which is biologically mediated. Furthermore, we have isolated a number of CA degrading pseudomonads [11, 14] and fungal strains [15].

Recently, Buchanan *et al.* [16] investigated the biodegradation potential of CA using an enrichment culture that was initiated with an activated sludge inoculum. Exposure of CA films and fibers (DS-1.7 and DS-2.5) to this enrichment culture resulted in extensive degradation. Recovered CA DS 2.5 fibers showed a decrease in DS from an initial value of approximately 2.5 to approximately 2.0 as well as a narrowing of the molecular weight distribution M_w/M_n. The biodegradability of CA DS-1.7 and DS-2.5 was further demonstrated using an aerated wastewater treatment basin exposure. Moreover, the biodegradability of CA DS-1.6 and DS-1.85 by the sludge-derived enrichment culture was investigated by exposure of samples which contain ^{14}C-labeled acetyl substituents. Sixty-eight percent recovery of the acetyl substituents in the form of CO_2 was reported using this approach. The latter experiment is, of course, excellent evidence that CA with a DS of 1.85 is indeed mineralizable.

In this paper, changes in the molecular weight, DS, and surface colonization of CA DS-1.7 and DS-2.5 films which were exposed to laboratory-scale aerobic thermophilic composting bioreactors are reported. Furthermore, predigested compost derived from a synthetic municipal solid waste mixture without prior exposure to polymer was used as a support of microbial growth on CA substrates to determine the rate of CO_2 evolution. Thus, respirometric studies on CA were carried out by placing powdered CA in contact with predigested compost which was maintained at 60% moisture and 53°C. The temperature, percentage moisture, and solid support used in this study was intended to simulate some features of a thermophilic composting system. Thus, CA was exposed to thermophilic aerobic simulated compost environments to investigate the biodegradation potential of this family of polymers.

MATERIALS AND METHODS

Degradation Testing in Laboratory-Scale Compost Reactors

CA DS-1.7 films were made by solution casting onto Teflon from either acetone–H_2O (1:1 ratio, v/v; 17% CA DS-1.7, wt/v) or acetone (15% CA DS-2.5, w/v). Powdered CA DS-2.5 and granule (about 2 mm in diameter) CA DS-1.7 (Eastman Kodak Company, Kingsport, TN) were used to prepare the polymer solutions. After removal of residual solvent in a vacuum oven, the films were aged for at least 2 weeks prior use.

A simulated *in-laboratory* compost procedure was developed and used to study the degradability of shaped polymeric materials [10]. In this procedure, a synthetic municipal solid waste mixture was used that contains important components present in the municipal solid waste stream and an appropriate chemical composition so that an active microbial population proliferates [10]. All bioreactors were maintained at 53°C and a 60% moisture content and aerated at 100 ml/min. Abiotic reactors were initiated simultaneously to assess whether chemical degradation of the polymeric materials occurs under the test conditions. A detailed description of the bioreactor configuration and the operational procedure has been published elsewhere [10]. During the degradation testing, residual polymer films were recovered from the composting and abiotic test vessels at desired sampling time intervals. The recovered materials were cleaned by immersion in deionized water to remove the coarse particles on film surface and gentle blotting with 70% isopropylalcohol-moistened Kimwipes EX-L (Kimberly-Clark, Fisher Scientific, PA), dried in a vacuum oven at 30°C and reduced pressure, and then weighed. These films were analyzed for changes in their molecular weight, their degree of substitution, and the microbial colonization on the polymer surface.

Characterization of Partially Degraded Polymer Films

Molecular Weight. The molecular weights of CA DS-1.7 and DS-2.5 films prior to and after exposure in bioreactor and abiotic test vessels were measured by gel permeation chromatography (GPC) on a chromatographic system consisting of a Waters Model 510 pump, a Waters Model 410 refractive index detector, and a Waters Model 730 data module. Three KD gel columns (300 × 8.0 mm, Shodex, Showa Denko, Shoko, Co., Japan; particle size, 5 mm; pore size, 10^5 and 10^3 Å) were placed in series and operated at a flow rate of 1.0 ml/min. The sample concentration was 10 mg/ml and the injection volume was 50 μl. The analysis was performed using dimethylformamide (DMF; Aldrich Co., Milwaukee, WI) containing 0.1% (w/v) LiBr as the eluant. Molecular weights were calculated relative to polystyrene standards without further corrections.

Proton Nuclear Magnetic Resonance (NMR)

The ^1H NMR spectra of residual and unexposed samples were recorded on a Bruker WP-270 SY spectrometer at 270 MHz to determine the polymer DS values. The parameters employed were as follows: 1–1.5% (w/w.) polymer in d_6-DMSO (Aldrich Co.); temperature, 80°C; pulse width, 0.5 μs; receiver delay, 2 s; and

transients, 600–800. The degree of acetylation was determined according to the procedure of Goodlett *et al.* [17]. Free hydroxyl groups were first completely acetylated by reaction with d_3-acetylchloride at room temperature for 72 h. The degree of acetylation was obtained by spectral integration by comparing the ratio of the acetyl ^{1}H NMR signals at approximately 2 ppm to the multiple backbone signals at between 3.6 and 5.3 ppm.

Scanning Electron Microscopy (SEM)

SEM of the polymer films was performed without coating the films or any other surface treatment using an ISIIC-130 SEM.

Respirometry Setup

The respirometric system used in assessing the mineralization of polymer carbon to CO_2 consisted of an air pump, which served as an air source; an air pretreatment system to sterilize, remove CO_2, and control the flow rate; test vessels which contained predigested compost; and post test vessel trapping of the CO_2 produced. The predigested compost was obtained by a 42-day maturation period of a defined synthetic waste mixture [10] and was used to supply the indigenous microbial population and also to serve as a physiological and nutritional support. For each polymer sample analyzed a total of six vessels were set up where three vessels were amended with 1.0 g of the polymer powder (particle size was between 300 and 400 μm using mesh screens) and the other three served as background controls to measure the basal CO_2 production from the indigenous microbial population. The test vessels were maintained at 53°C and 60% moisture throughout the analysis. The difference in CO_2 production between the test vessels with and those without the polymer sample was attributed to the degradation of the polymer sample. Produced CO_2 was trapped by reaction with a NaOH solution. The amount of NaOH left unreacted was quantitated by titration with HCl to pH 9.0 using a Mettler DL 12 (Mettler Instrument Co., Hightstown, NJ). A more detailed description of the methodology has been published elsewhere [18].

RESULTS AND DISCUSSION

Characterization of Degraded Films

Extensive erosion at the surface of CA DS-1.7 films was observed by SEM after exposure times of 5 days in the composting reactors. Figure 1 shows SEM photographs of CA DS-1.7 after a 5-day compost exposure (Fig. 1a), after a 5-day incubation in an abiotic control reactor (Fig. 1b) and of an unexposed film (Fig. 1c). In all of these cases (including the unexposed film) the recovered films were cleaned by immersion in deionized water and isopropylalcohol-moistened Kimwipes (see Materials and Methods) to remove the adhered particles, microbes, and other substances. The comparatively large changes in surface morphology seen in Fig. 1a relative to Figs. 1b and c are supportive evidence that the degradation of CA DS-1.7 in composting test vessels is due in large part to biomediated processes. This is also in agreement with the determination that CA DS-1.7 showed comparatively little weight loss after an exposure time of up to 7 days in the abiotic control vessels [10]. A similar SEM investigation was conducted for exposed and control CA DS-2.5 films. Figure 2 shows SEM photographs of CA DS-2.5 after a 15-day compost exposure (Fig. 2a) and after a 15-day incubation in an abiotic control reactor (Fig. 2b) and of an unexposed film (Fig. 2c). These films also were cleaned as above to remove microbial and other attachments. Figure 2a shows severe surface erosion, whereas Figs. 2b and c appear relatively unchanged. This is once again supportive evidence that CA DS-2.5 film erosion and associated weight loss [10] observed after compost test exposures occur due to biomediated mechanisms.

SEM photographs were also taken without cleaning the surface of CA DS-1.7 and DS-2.5 films after 5- and 15-day exposure times in the composting test reactors (see Figs. 1d and 2d, respectively). In this way, microbial colonization onto the respective film surfaces was also observed. The predominant microorganisms colonized on the CA DS-1.7 film surfaces resemble most closely the filamentous actinomyce or hyphal fungus (see Fig. 1d). In contrast, the most pervasive microorganisms on the CA DS-2.5 exposed films are most likely bacterial species. This suggests that a wide array of microorganisms are capable of functioning either alone or in combination with other microbes to effect CA biodegradation. Indeed, a number of bacteria and fungi species have been isolated in this laboratory for their ability to use CA as a sole carbon and energy source [14, 15].

GPC analyses was carried out on CA samples recovered from composting exposures to determine effects on polymer molecular weight (see Materials and Methods). Since the molecular weights were determined by GPC on CA samples with nonidentical repeat unit composition (see Table I), without correction for polymer

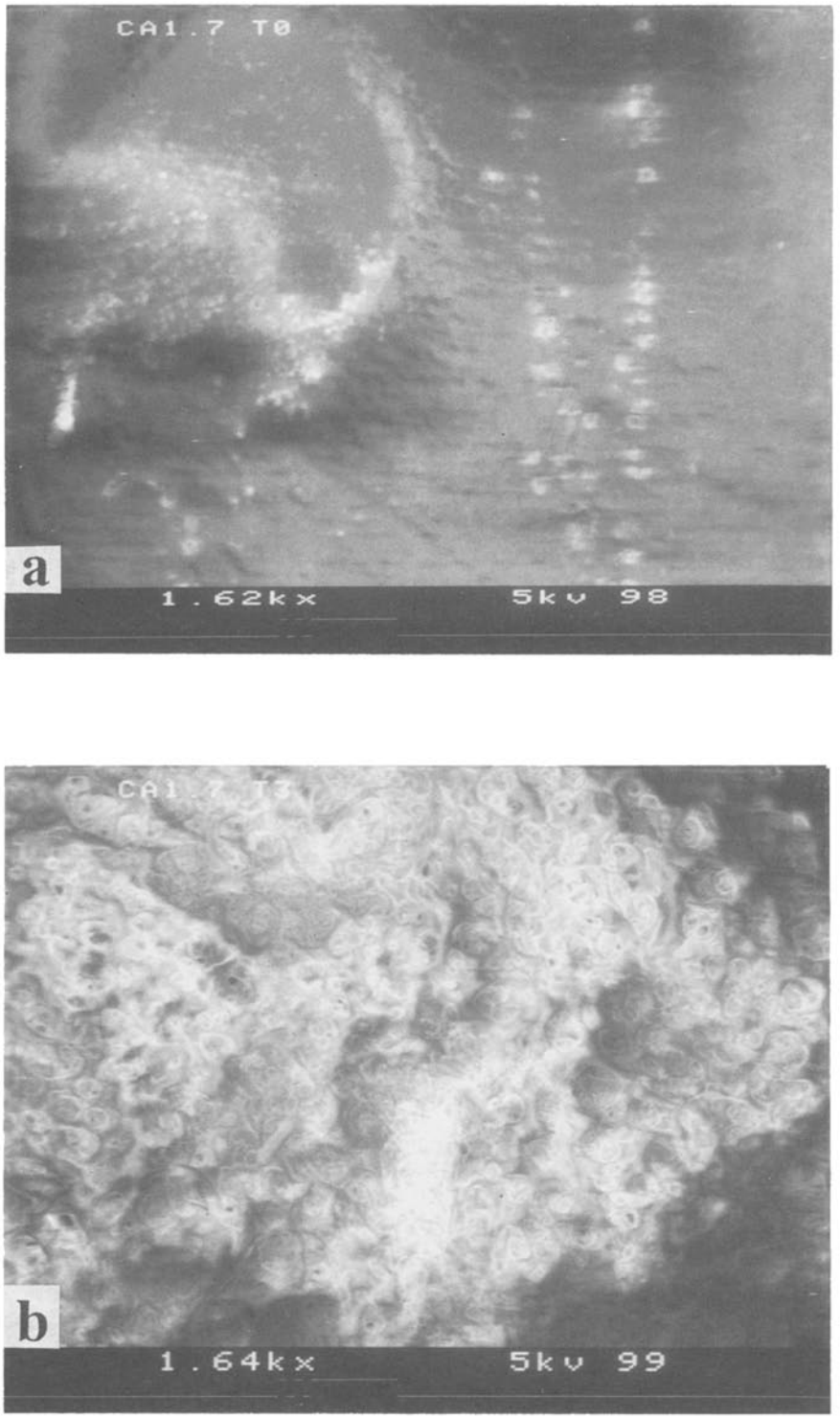

Fig. 1. SEM photographs of CA DS-1.7 films (a) not exposed, (b) after a 5-day exposure in a biologically active test vessel with cleaning of the residual film surface, (c) after a 7-day exposure in an abiotic reactor with cleaning of the residual film surface, and (d) after a 5-day exposure in a biological active test vessel without cleaning of the recovered film surface. (a) ×1620, (b) ×1640, and (c, d) ×1650.

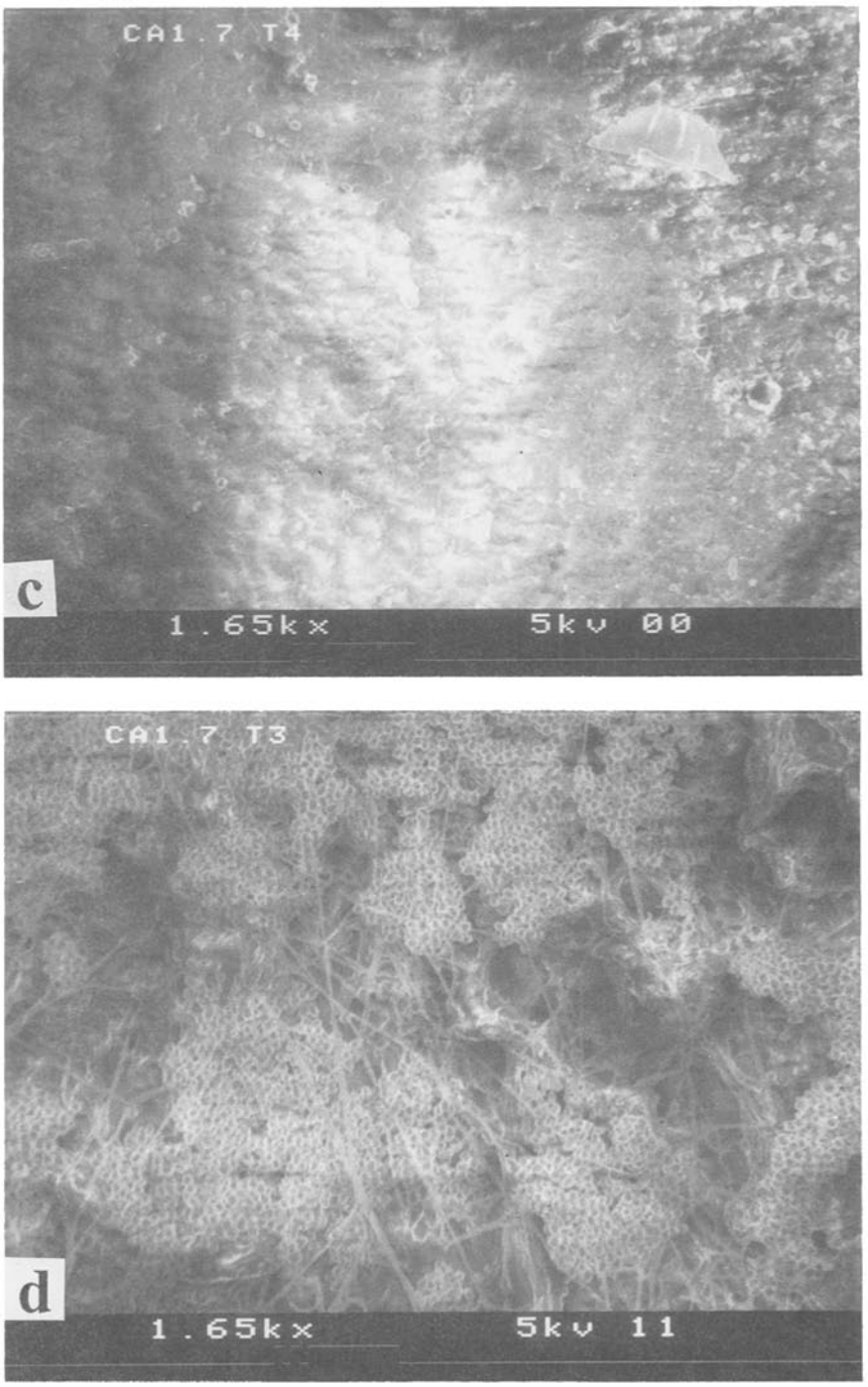

Fig. 1. Continued.

hydrodynamic volume effects, definitive conclusions as to molecular weight changes cannot be made herein and are considered apparent changes for the present discussion. The GPC results shown in Table I indicate that the M_n and M_w values for both CA DS-1.7 and CA DS-2.5 decreased significantly for extended composting exposure times. In contrast, exposures of both CA DS-1.7 and CA DS-2.5 in poisoned reactors showed no measurable events of chain cleavage (see Table I). Therefore, the CA chain glycosidic linkages show a good sta-

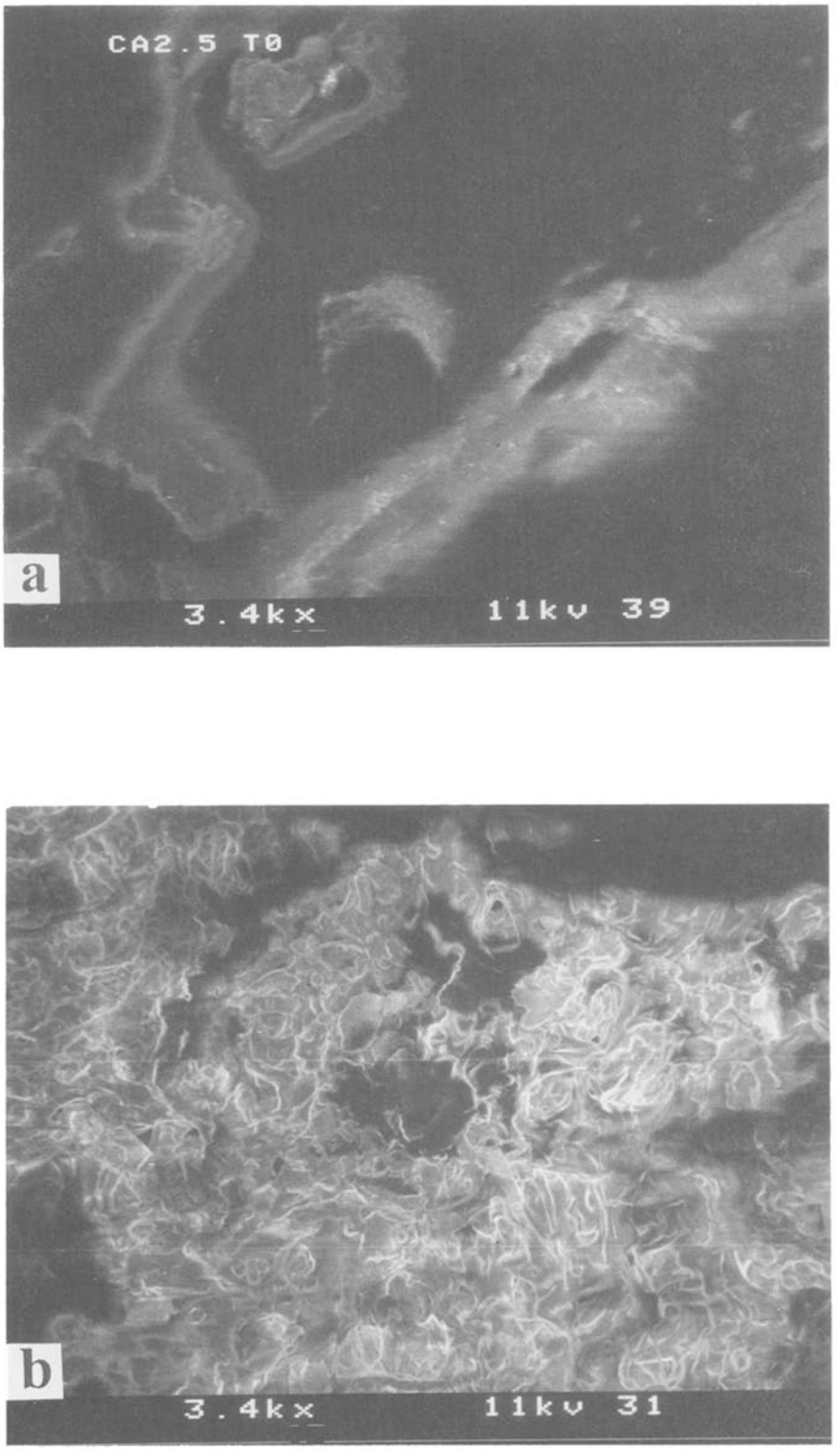

Fig. 2. SEM photographs of CA DS-2.5 films (a) not exposed, (b) after a 15-day exposure in a biologically active composting bioreactor with cleaning of the residual film surface, (c) after an 18-day exposure in an abiotic reactor with cleaning of the recovered film surface, and (d) after a 15-day exposure in a biological active bioreactor without cleaning of the residual film surface. ×3400.

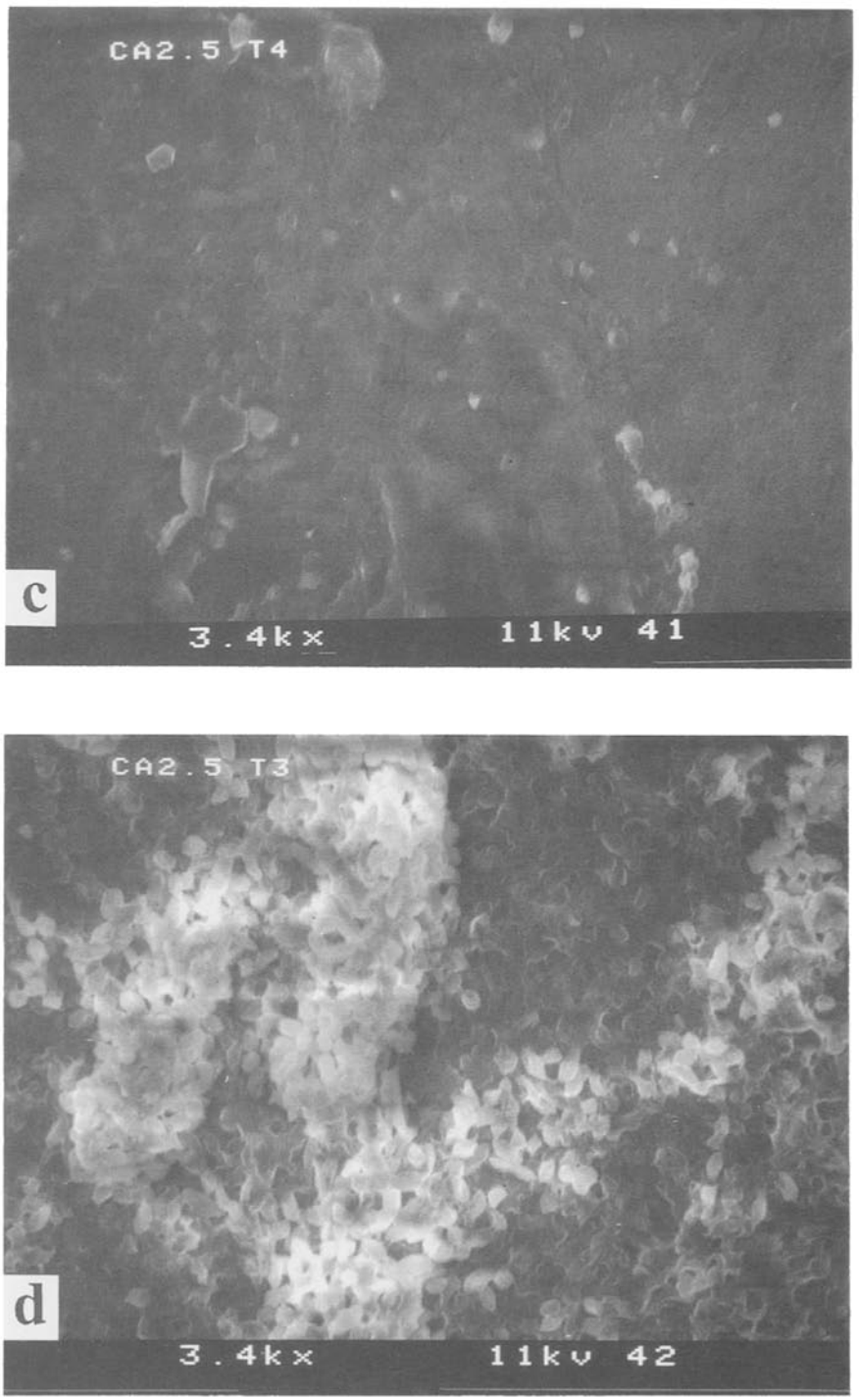

Fig. 2. Continued.

bility to chemical hydrolysis so that the apparent decreases in CA molecular weight are likely due to biocatalyzed processes. Molecular weight loss attributable to bioactivity was also reported by Buchanan *et al.* [16] for exposures of CA DS-2.5 fiber and ground powder to an enrichment culture containing a mixed microbial population. It is also of interest to note that M_w/M_n values changed little for CA samples which were extensively eroded. This is explained by the occurrence of random endo-cleavage events and/or the production of

Table I. Effect of Compost Exposures on Film Weight Loss, Residual CA Molecular Weight, and Degree of Substitution

Exposed CA sample	Test vessel	Exposure time (days)	Weight loss (%)[a]	Recovered film DS[b]	M_n ($\times 10^5$)[c]	M_w ($\times 10^5$)[c]	M_w/M_n[c]
DS-1.7	—[d]	0	—	1.69	1.48	2.03	1.37
DS-1.7	Active	4	35.1 ± 2.7	1.27	1.25	1.66	1.33
DS-1.7	Active	5	49.8 ± 3.4	n.d.[e]	1.03	1.38	1.34
DS-1.7	Poisoned[f]	7	0.8 ± 2.9	1.67	1.46	2.07	1.42
DS-2.5	—[d]	0	—	2.51	2.36	3.35	1.42
DS-2.5	Active	12	39.8 ± 4.0	2.18	2.21	3.29	1.49
DS-2.5	Active	16	45.8 ± 5.1	n.d.	1.88	2.58	1.37
DS-2.5	Poisoned[f]	20	0.9 ± 2.4	2.50	2.35	3.46	1.47

[a] Data taken from Ref. 10.
[b] Determined by ^{1}H NMR spectroscopy (see Materials and Methods).
[c] Determined by GPC measurements (see Materials and Methods).
[d] Not exposed.
[e] Not determined.
[f] Bioactivity was suppressed by the addition of KCN (see Ref. 10).

low molecular weight degradation products by chain-end-cleavage mechanisms that rapidly diffuse away from the residual materials. Such low molecular weight products diffusing from the film are likely rapidly assimilated by colonizing microorganisms. The DS values obtained from ^{1}H NMR analyses (see Materials and Methods) for CA DS-1.7 and DS-2.5 samples which were not exposed, exposed to the composting environment, and exposed in abiotic control test vessels are shown in Table I. It was observed that the DS of recovered CA DS-1.7 and DS-2.5 samples was significantly decreased upon exposures in composting reactors (see Table I). However, the measured DS values of the CA samples that were exposed for comparatively longer time periods in abiotic control test vessels remained identical to that of the unexposed samples. This result, in combination with the molecular weight measurements recorded for CA exposed in composting and abiotic test vessels, indicates that biological degradation of CA is not initiated by initial chemical deacetylation or glycosidic bond cleavage. In other words, biological mechanisms are responsible for the observed weight loss in the composting exposures.

Mineralization of Cellulose Acetates

Mineralization of a polymeric material by microorganisms under aerobic conditions produces CO_2, H_2O, microbial biomass, and humus. Therefore, a respirometric technique was used to quantitate the evolved CO_2 due to polymer biodegradation. The method was summarized above (see Materials and Methods) and was described in detail elsewhere [18]. The low basal level of CO_2 production in test vessels which did not contain the polymer substrate (approximately 12–15 mg CO_2-C per day) was found to be linear as a function of time [18] and was subtracted from that produced in polymer amended vessels to obtain the total CO_2 and the corresponding percentage of the theoretical CO_2 formed as a result of substrate mineralization (see Figs. 3 and 4). Figure 3 shows that exposure of CA DS-1.7 to the predigested compost matrix produced 72.4% of its theoretical carbon as CO_2 after a total incubation period of 24 days. In comparison, the total time required for CA DS-2.5 to reach the plateau value of 77.6% substrate carbon conversion to CO_2 was 60 days (see Fig. 4). Of additional interest, the rate of CA DS-1.7 mineralization increased rapidly after only a 10-day lag period, whereas the lag period prior to rapid CA DS-2.5 mineralization was considerably longer (approximately 25 days). In agreement with these results, both the lag period prior to film weight loss and the total time for film disappearance were notably longer for CA DS-2.5 relative to CA DS-1.7 (see Ref. 10 and above). Indeed, it is not at all surprising that CA of a relatively higher degree of substitution shows relatively slower rates of biodegradation (see discussion below). However, most importantly, the results shown in Fig. 4 suggest that CA with a degree of acetylation of up to 2.5 is mineralized to a high degree under the appropriate environmental exposure conditions.

CA Biodegradation Mechanistic Considerations

Cellulose is the most abundant organic material on Earth and its biodegradation has been the subject of nu-

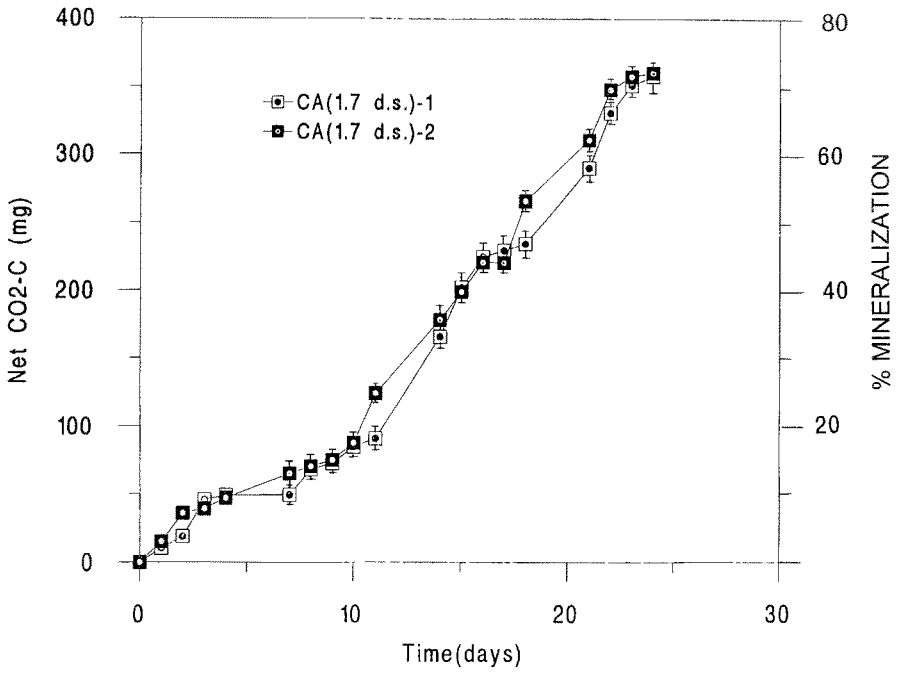

Fig. 3. Respirometric mineralization of powdered CA DS-1.7 in a matured synthetic MSW compost matrix under aerobic thermophilic conditions.

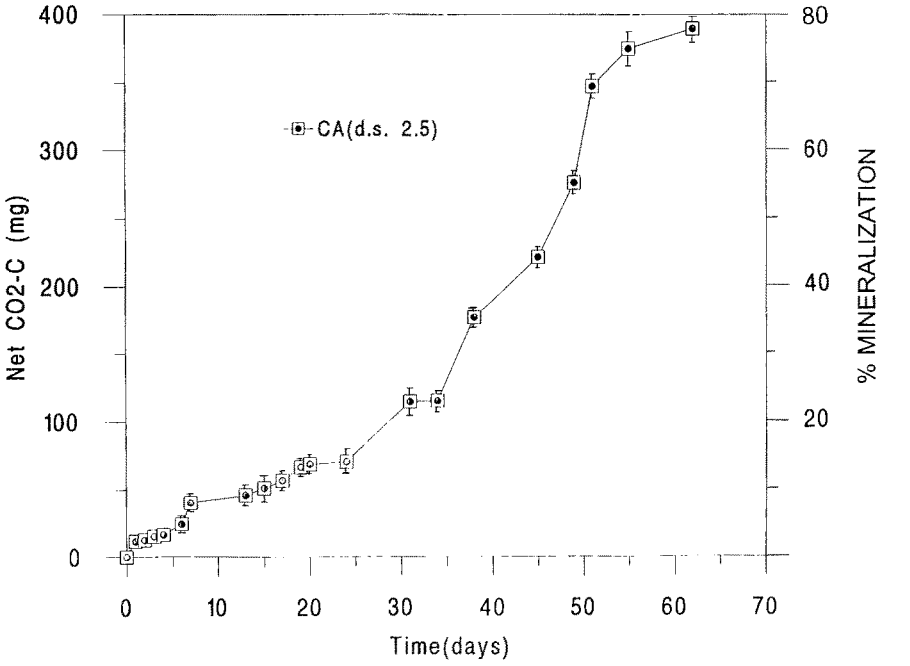

Fig. 4. Respirometric mineralization of powdered CA DS-2.5 in a matured synthetic MSW compost matrix under aerobic thermophilic conditions.

merous studies [19]. Xylan is also highly abundant in nature and is described as a β-1,4-linked D-xylose heteropolymer which can be highly substituted with arabinosyl, acetyl, uronyl, and arabinose side groups [20]. It is well-known that the complete mineralization of cellulose and naturally acetylated xylan requires the presence of multiple enzymes which act in a cooperative manner [19, 20].

In the biodegradation of xylan containing side-chain acetyl groups, it has been discussed above that deacetyl esterases have been identified which function to remove side-chain acetyl groups from the polymer chain [20]. Furthermore, it has been shown by a number of workers that CA polymers which have DS values of more than 0.76 are not degraded by cellulase enzyme systems [4]. From the above, it is highly likely that the biodegradation of CA with a DS greater than approximately 0.8 requires the presence of both deacetylase and cellulase enzyme systems. Therefore, it is hypothesized that deacetylase enzymes are required to provide chains or chain segments with DS values of less than approximately 0.7. Once this critical degree of deacetylation is reached, cellulase enzymes cause events of chain cleavage which are accelerated by further chain deacetylation. A schematic of this is shown in Figure 5. It should be noted that the critical value of deacetylation required for glycosidic bond cleavage by cellulase enzymes has not as yet been firmly established for a range of cellulase enzyme systems.

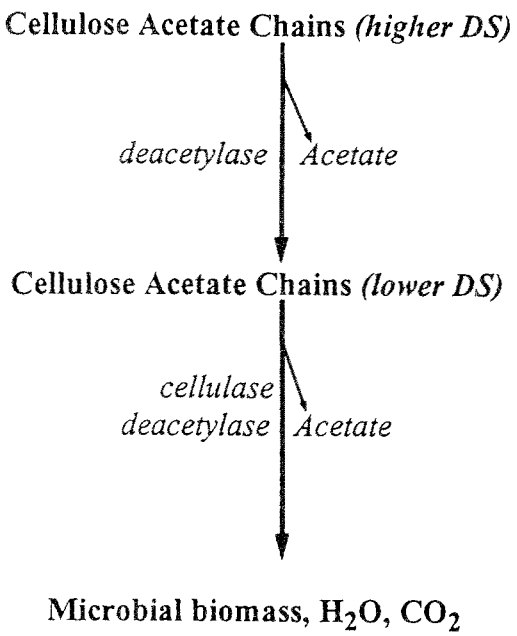

Fig. 5. Proposed scheme for CA biodegradation.

Work is in progress in our laboratory using CA degrading microbial isolates to obtain detailed information on the mechanistic features of CA biodegradation.

ACKNOWLEDGMENTS

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