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Effects of Natural Polymer Acetylation on the Anaerobic Bioconversion to Methane and Carbon Dioxide

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ABSTRACT

The successful production of novel biodegradable plastic copolymers incorporating both synthetic plastic formulations, such as polystyrene, and naturally occurring biodegradable polymer components, such as cellulose, starch, or xylan, requires stable chemical bonding between these polymers. Modification of the natural polymers through acetylation of the available hydroxyl groups permits the formation of appropriate film-forming plastic copolymers. However, modification of natural polymers has been demonstrated to result in decreased attack by microbial catalysts. For this study, the abundant natural polymers cellulose, starch, and xylan were substituted with acetate to various degrees, and the effect of this modification on the anaerobic biodegradation was assessed using the biochemical methane potential (BMP) protocol. Significant reduction in anaerobic biodegradability resulted with all polymers at substitution levels of between 1.2–1.7. For the xylan acetate series, the trends for anaerobic biodegradation were in good agreement with reduced enzymatic hydyolysis using commercially available xylanase preparations.

Index Entries: Acetylation; cellulose; starch; xylan; anaerobic digestion; xylanase.

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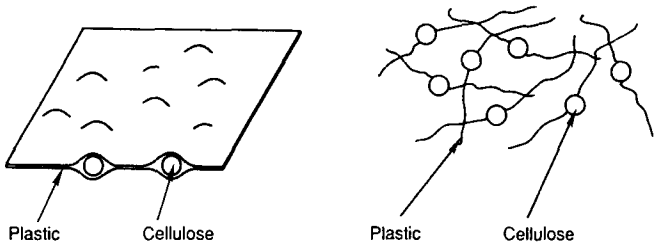

Fig. 1. Conceptual depiction of natural polymer blending (filler) and acetylated natural polymer graft copolymers for the production of biodegradable plastics.

INTRODUCTION

The development of plastics for a variety of useful purposes has historically focused on increasing the durability of formulations to photo-, chemical, and biological degradation (*1*). With an estimated 320 billion pounds of municipal solid wastes (MSW) produced and discarded each year in the United States (*2*), of which a large and growing segment consists of short-term packaging plastics, alternatives to plastic wastes have become an important issue. Plastics currently constitute 7% of the solid waste stream, and are considered relatively inert to the biological (primarily anaerobic) degradation processes occurring naturally in landfills or in biological conversion processes designed to convert wastes to useful fuels or products.

Current interest in biodegradable plastics production and use is the result of the high cost of MSW disposal, the controversy of dumping plastics at sea, the concern for carbon cycling in the environment, and the reduction in unsightly litter. The development of biodegradable plastics includes mixtures (copolymers) of traditional plastic formulations (i.e., polyethylene and polystyrene) with natural polymers, known to be readily biodegradable, such as cellulose or starch. The incorporation of natural polymers should improve the biodegradation of the copolymer as a whole (*3,4*). These natural polymers (cellulose or starch) are renewable and available in great supply and may, therefore, be competitively priced when compared to oil-derived plastic formulations. Since there is no inherent compatibility of natural polymers and standard plastic formulations, such as polyethylene, the addition of natural polymers acts essentially as a filler (Fig. 1). Because of this, the level of natural polymer addition is limited to 6–12% without substantial loss in physical characteristics. However, it has been estimated that greater natural polymer addition levels (30–50%) are required to achieve significant biodegradation of the copolymer (*3*). At these high natural polymer addition rates, the copolymer is difficult to process, and the loss of desirable physical properties is evident.

The modification of natural polymers through substitution of hydroxyl groups with organic acids, such as acetate, has long been shown to produce plastic-like polymers for a variety of uses, including thin films. These acetylated natural polymers have been used successfully at high blending ratios with standard plastic formulations (i.e., polystyrene) to develop graft copolymers (Fig. 1, [*4,5*]). However, increasing the degree of substitution has also been shown to decrease the biodegradability of such modified natural polymers (*6–10*).

The present study evaluates the biodegradability of a representative graft copolymer of acetylated cellulose and polystyrene (50–50%), and further serves to evaluate the effects of acetylation of natural polymers on their anaerobic biodegradation. The effects of natural polymer acetylation on anaerobic biodegradability are also compared with enzymatic hydrolysis using a commercial xylanase enzyme preparation.

MATERIALS AND METHODS

Graft Copolymers

The graft copolymer of cellulose acetate (degree of substitution = 2.4)/polystyrene maleic anhydride (50/50%) was prepared as previously described (*4*) by R. Narayan, Michigan Biotechnology Institute. The material was received as an off-white powder and stored at room temperature. The components utilized in the production of the graft copolymer, that is cellulose acetate (DS 2.4) and polystyrene maleic anhydride, were also obtained from Narayan and were used for anaerobic bioconversion analysis.

Acetylated Natural Polymers

Cellulose Acetates

The development of a series of acetylation levels for cellulose was accomplished by the production of cellulose triacetate followed by controlled deacetylation as previously described (*11*). The acetylation of cellulose was initiated by the pretreatment of native cellulose fibers with aqueous acetic acid (4:1 acid to water) to swell the fibers. Acetylation was initiated by the addition of acetic anhydride, additional acetic acid, and sulfuric acid. The reaction was carried out for 6 h until the mixture changed from a thick slurry to a viscous gum. The reaction was then terminated by the addition of distilled water to convert the remaining acetic anhydride to acetic acid. The mixture was neutralized by the addition of NaOH. The controlled deacetylation was accomplished by timed exposure to 5% sulfuric acid at 80°C. Varying the time of exposure to acid allowed the production of acetylated cellulose in a series of degrees of acetylation. After deacetylation, the cellulose acetate polymers were precipitated in dilute acetic acid and washed repetitively with distilled water to remove remaining acetic acid. The cellulose acetate precipitates were dried and ground to a fine white uniform powder using a cyrogrinder (SPEX, Model 6700).

Starch (Amylose) *Acetates*

The series of amylose acetates were developed through controlled acetylation using high amylose starch (Sigma Chemical Co). The amylose (20 g) was added to formamide (400 mL) in small portions at room temperature under vigorous stirring, and the resulting gel-like mixture was stirred overnight. To initiate acetylation, stoichiometric quantities of acetic anhydride and pyridine were added. Pyridine was added in a 1:1 molar ratio with respect to the acetic anhydride in each case. After stirring the mixtures for 2 d, the slurries were poured into 2 L of deionized water, and the resulting white precipitates were collected using centrifugation. The precipitates of amylose acetate were washed twice with distilled water to remove all traces of residual acetic acid. The starch acetate precipitates were then dried in a vacuum oven at 60°C for 2 d, and the solid cakes were ground to a fine white uniform powder using a cyrogrinder and stored at room temperature.

Xylan Acetates

The series of xylan acetates were prepared by controlled acetylation of native xylan from oat-spelts (Sigma Chemical Co). Xylan was initially swelled by stirring 10% w/v xylan in formamide for 18–24 h. Once sufficiently swelled, appropriate volumes of pyridine and then acetic anhydride were added drop-wise to the slurry. These reaction mixtures were left to react at 24°C for 4–6 h (*12*). To quench the reaction and precipitate the water-insoluble, acetylated xylan fraction, the mixtures were then poured directly into distilled water. These samples were stirred for 0.5–1 h and then filtered through a Whatman GF/C filter to collect the precipitate. The resulting filter cakes were washed twice with distilled water and freeze-dried. Alternatively, the xylan with lower levels of acetylation were dialyzed against distilled water to remove formamide and other low-mol-wt compounds. Insoluble xylan fractions were collected by centrifugation and freeze-dried also. The dried, acetylated xylans were ground to a fine white uniform powder using a cryogrinder and stored at room temperature.

Acetylation Characterization

The degree of acetylation of acetylated-cellulose, amylose, and xylan was determined by the conversion of the esterified acetate to free acid. This was accomplished by the hydrolysis of the acetylated polymers in 64% w/w H_2SO_4 for 2 h at ambient temperature (to assure complete solubilization), followed by dilution with distilled water to 3% H_2SO_4 and a moderate temperature cook (121°C for 1 h) to liberate the acetate and to complete the polymer hydrolysis. Control analysis consisted of several saponifications (1*N* NaOH at 121°C for 1 h) followed by acidification (*13*). The free acetic acid of the subsequent hydrolyzates were analyzed by gas chromatography after acidification of the samples (pH < 2.0) using 0.03*M* oxalic acid as described below for volatile fatty acids in sludge samples.

Anhydrosugars were determined by a modification of the procedure developed at the US Forest Products Laboratory (*14*). Once the respective dry weights and the amounts of free acetic acid and anhydrosugar in the samples were determined, the degree of acetylation (the molar ratio of acetyl gropus to anhydrosugar units) could be calculated.

Anaerobic Degradation Consortium

The anaerobic digestion consortium utilized in bioconversion studies was originally obtained from the anaerobic digestion of municipal sewage (North Metro Water Reclamation Plant, Denver, CO.). This anaerobic consortium was further enriched for microorganisms capable of degrading the components of MSW (including the plastics fraction) through the feeding of a refuse-derived fuel (RDF) fraction of MSW obtained from Future Fuels Inc. (Thief River Falls, MN), as previously described (*15*). This consortium was maintained in laboratory-scale anaerobic digesters with 3.5-L working volumes and semi-continuous stirring (15 min of each 1/2 h). These reactors were constructed and operated as previously described (*16*). The anaerobic reactors were batch-fed daily a 5% w/v processed MSW meal in a nutrient supplement solution. The nutrient supplement solution used in this study was previously described (*15*), and was used to provide optimum levels of nitrogen, phosphate, and minerals. In low-solids continuous stirred tank reactors (CSTR), total biogas production was measured using calibrated water displacement vessels. Effluent from the semi-CSTR reactors was removed and used for inoculum for the BMP assays.

Biochemical Methane Potential (BMP) Assays

The BMP assays were performed as previously described (*17*) to determine the ultimate yields of methane (product) and, therefore, anaerobic bioconversion of the polymer samples. BMP studies were conducted in 155-mL serum bottles incubated at 37°C and mixed using an orbital shaker. Total biogas production was measured using a pressure transducer equipped with a 22-gage needle for penetration into, and subsequent overpressure release from, the serum bottle. BMP studies were conducted using triplicate samples.

BMP and Digester Effluent Analysis

Upon termination of BMP analysis (generally 90 d) and for general performance of seed culture digesters, anaerobic sludge was analyzed for pH and volatile organic acids. Levels of volatile organic acids (C_2-C_5 iso and normal acids) were determined by gas-liquid chromatography (GLC). A Hewlett-Packard Model 5840A gas chromatograph equipped with a flame ionization detector, a Model 7672A autosampler, and a Model 5840A integrator (all from Hewlett-Packard) were used. The chromatograph was equipped with a glass column packed with Supelco 60/80, Carbopack C/0.3%, Carbowax 20M/0.1% H_3PO_4 for separations.

Biogas Analysis

The composition of biogas produced was determined using gas chromatography for methane and carbon dioxide. For this analysis, a Gow-Mac (Model 550) gas chromatograph equipped with a Porapak Q column and a thermal conductivity detector with integrating recorder were used (*15*).

Theoretical Methane Yield

The theoretical methane yield for anaerobic bioconversion of the various polymers tested were calculated as previously described (*17*) from the chemical oxygen demand (COD) values for the respective polymers. The COD was determined as previously described (*18*). The COD assay employed the microdetermination method using the commercially available "twist tube" assay vials (Bioscience, Inc., Bethlehem, PA).

The ratio of actual methane yields obtained in an anaerobic fermentation of a specific polymer to the theoretical methane yield calculated from the COD value for that polymer is a direct reflection of the total anaerobic bioconversion for that substrate.

Enzymatic Polymer Hydrolysis (for Xylan Acetate)

The acetylated xylan acetate series was assayed for enzymatic hydrolysis using a commercial enzyme preparation of NOVO Celluclast 1.5L/ Novozyme SP188 as previously described (*9*). The stock solution was found to have a xylanase activity of 55 IU/mL (1 IU representing that amount of enzyme that releases 1 μmol of xylose equivalents/min from oat-spelts xylan at 50°C). The stock solution was also found to have an acetyl-xylan esterase activity of 12 IU/mL (1 IU representing the amount of enzyme that releases 1 μmol of acetic acid from a 2% w/v slurry of acetylated xylan [degree of acetylation 0.26]/min at 50°C). Both activities were determined at 50°C in 50 m*M* citrate buffer at pH 4.9. The enzymatic hydrolysis of acetylated xylan samples was performed as previously described (*19*), utilizing an appropriate dilution of the stock enzyme solution to obtain an enzyme loading of 23 IU/mL xylanase activity. Xylan acetate hydrolysis was determined gravimetrically, as well as by post-digestion saponification, and the determination of acetic acid and reducing sugars (*19*).

RESULTS

The anaerobic biodegradation of a novel graft copolymer, cellulose acetate/polystyrene maleic anhydride (50/50%), was previously discussed (*20*) and results in an anaerobic conversion of 1.6% (Fig. 2). Additionally,

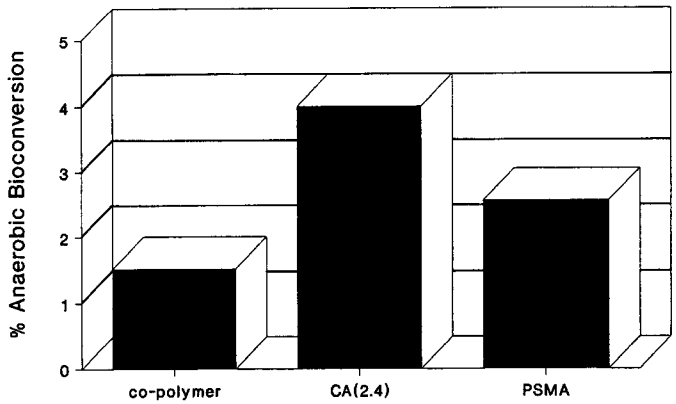

Fig. 2. Anaerobic biodegradation of graft copolymer of cellulose acetate (DS 2.4)/polystyrene maleic anhydride (50/50%) and individual components as determined by BMP analysis. The data represent the average of triplicate determinations. Results represent the cumulative anaerobic conversion over a 95-d incubation period.

the biodegradation occurs only after an extensive and variable lag time (50–80 d). The biodegradation of the individual copolymer components was only slightly higher than that of the copolymer and constituted $< 4\%$ of that theoretically achievable as determined by COD analysis of the polymers. Because of the major impact that acetylation imposed on the normally biodegradable cellulose polymer and because of a scarcity of commercial sources of acetylated polymers, a series of natural polymers of various acetylation levels was prepared as described in Table 1. The anaerobic biodegradation, determined in BMP assays, is shown in Figs. 3, 4, and 5 for cellulose acetate, amylose acetate, and xylan acetate, respectively. Common features were the rapid initiation of bioconversion and the near completeness of digestion within 21 d for the readily biodegradable polymers. Increasing the level of acetylation initially resulted in increases in the lag time before the onset of biodegradation or a less rapid biodegradation once it commenced. A summation of the results of the anaerobic biodegradation for the acetylated series of cellulose, amylose, and xylan for 90–98 d of incubation is shown in Fig. 6. These data indicate a similarity in the level of acetylation, which results in reduced anaerobic biodegradation. In general, an acetylation level of between 1.2–1.7 results in reduced biodegradation. Increasing the acetylation of natural polymers beyond 1.7 results in negligible anaerobic biodegradation.

A comparison of the anaerobic biodegradation obtained for the xylan acetate series by BMP assays with enzymatic hydrolysis utilizing known levels of commercially available xylanase is shown in Fig. 7. A similarity in these data is evident and indicates that even with high loadings of xylanase (and acetyl-xylanase) negligible hydrolysis occurs at acetylation levels > 1.2.

Table 1
Series of Acetylated Polymers Produced for Anaerobic Bioconversion Testing

Polymer	Degree of acetylation	Source
Cellulose	0	Whatman
Cellulose acetate	0.82	Kodak/S. Kelley
Cellulose acetate	0.99	Kodak/C. Buchanan
Cellulose acetate	1.25	Kodak/C. Buchanan
Cellulose acetate	1.65	Kodak/C. Buchanan
Cellulose acetate	1.69	Kodak/S. Kelley
Cellulose acetate	2.11	Kodak/S. Kelley
Cellulose acetate	2.40	Kodak/S. Kelley
Amylose	0	Sigma Chemical Co.
Amylose acetate	0.31	SERI/L. Moens
Amylose acetate	0.44	SERI/L. Moens
Amylose acetate	0.64	SERI/L. Moens
Amylose acetate	0.84	SERI/L. Moens
Amylose acetate	1.48	SERI/L. Moens
Amylose acetate	1.65	SERI/L. Moens
Xylan	0	Sigma Chemical Co.
Xylan acetate	0.39	SERI/D. Mitchell
Xylan acetate	0.96	SERI/D. Mitchell
Xylan acetate	1.04	SERI/D. Mitchell
Xylan acetate	1.20	SERI/D. Mitchell
Xylan acetate	1.71	SERI/D. Mitchell

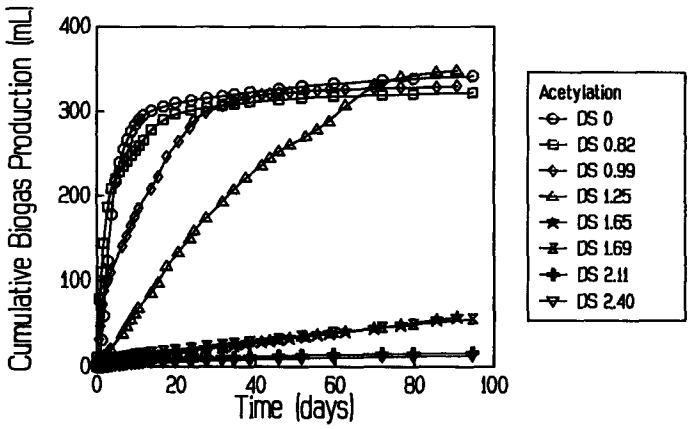

Fig. 3. Anaerobic biodegradation of cellulose acetate polymers of different degrees of acetylation. The BMP assay was performed as described in the Materials and Methods section.

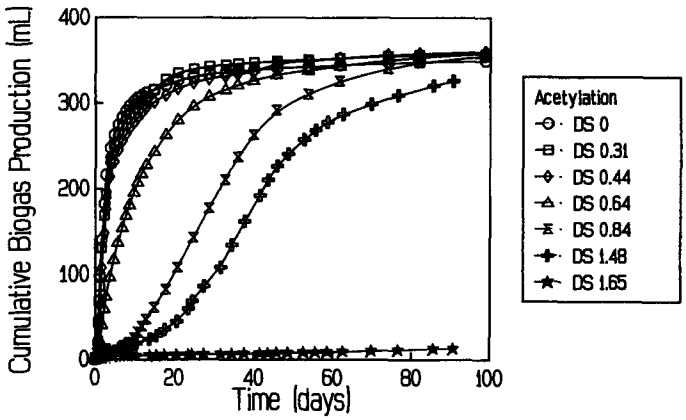

Fig. 4. Anaerobic biodegradation of amylose acetate polymers of different degrees of acetylation.

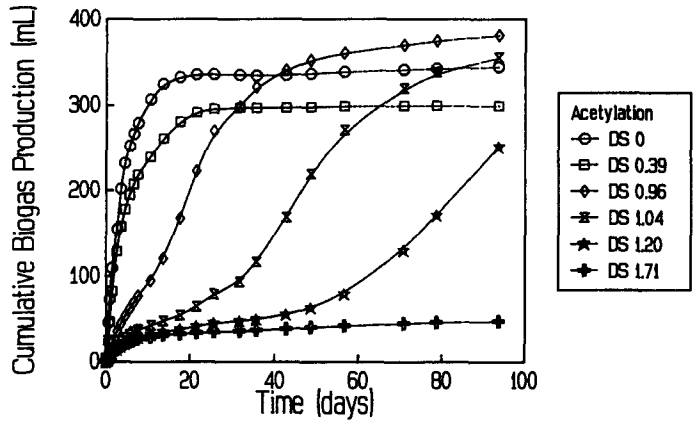

Fig. 5. Anaerobic biodegradation of xylan acetate polymers of different degrees of acetylation.

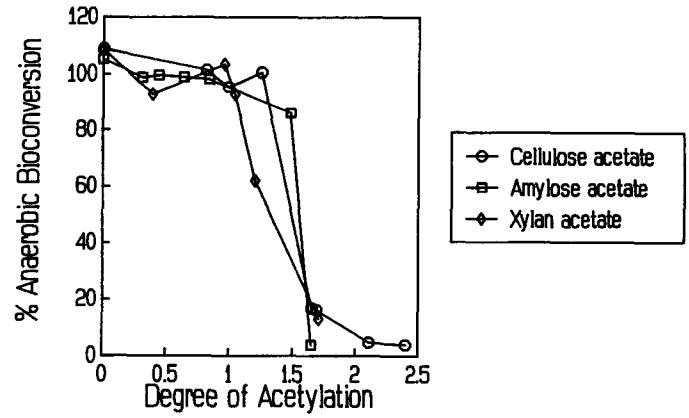

Fig. 6. A summation of the anaerobic bioconversion of the natural polymer acetylation series. Data represent the average of triplicate experimentals. Data represent a total incubation period of 90–98 d.

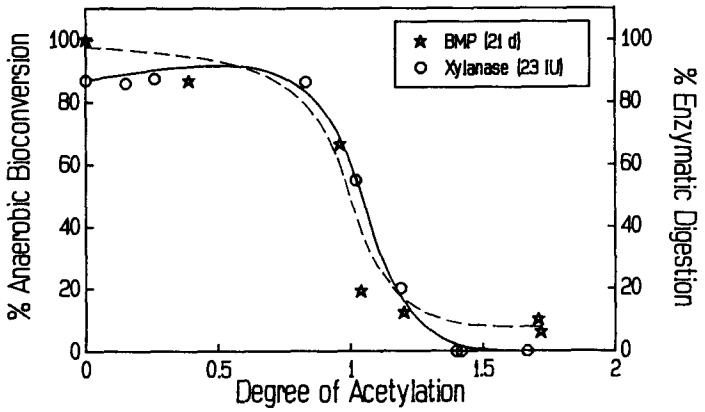

Fig. 7. Comparison of anaerobic biodegradaton and enzymatic hydrolysis for the xylan acetate polymer series. Data for BMP analysis represent the average of triplicate experimentals. Data for xylanase hydrolysis represent the average of duplicate determinations.

DISCUSSION

Plastics account for a $100 billion industry, much of which is represented by short-term use, packaging plastics. The desire to improve the fate of plastics in disposal (in landfills or at sea) and to reduce unsightly litter has prompted the use of natural biodegradable polymers as a blending agent for conventional plastic formulations. Initial "filler" plastics were determined to be less than adequate, since the majority of natural polymers were undegradable by virtue of the plastic polymer coating, and thus inaccessible to microbes and enzymes (*21*). The compatibilizing of natural polymers by acetylation has allowed higher levels of natural polymers to be blended with plastic formulations without substantially reducing the physical properties. However, the commercial availability of acetylated natural polymers is limited to cellulose acetate. The commercial cellulose acetate available is restricted to a degree of substitution of 2.4 to optimize the polymers' solubility in acetone (commonly used as a solvent for spinning fibers). The graft copolymer of cellulose acetate (DS 2.4) and polystyrene maleic anhydride was essentially not anaerobically biodegradable (Fig. 2). The individual components used in copolymer production (i.e., cellulose acetate [DS 2.4] and polystyrene maleic anhydride) were also not substantially anaerobically biodegraded. Since natural polymers, such as cellulose, starch, and xylan, are known to be readily biodegradable, knowledge of the effects of increasing acetylation in biodegradation is imperative to determine the maximum acetylation level of natural polymers that permits effective bioconversion of blended copolymers. Because of the lack of commercial interest in acetylated natural

polymers other than cellulose, an acetylation series of cellulose, starch (amylose), and xylan was developed. There is remarkable similarity in the acetylation level at which a reduction in the anaerobic biodegradation of these natural polymers occurs. Dramatic reductions in bioconversion occur at acetylation levels of between 1.2–1.7 for all polymers tested. A comparison of the biodegradability (BMP) and the xylanase enzyme digestibility of a series of xylan acetates ranging in levels of esterification showed similar sensitivity to acetylation level.

Although the mechanism for reduced anaerobic bioconversion and enzymatic hydrolysis of highly acetylated natural polymers is unknown, it has been speculated that the inhibition may be either the result of the increased hydrophobic nature of acetylated polymers, which reduces wetting and thus enzyme/microbe surface contact, or the steric hindrance presented by substrate acetyl groups, which reduce enzymatic activity.

In summary, for the natural renewable polymers cellulose, starch, and xylan, acetylation levels exceeding 1.7 drastically reduce the potential for anaerobic biodegradation. Therefore, the use of acetylated natural polymers in "biodegradable" copolymer plastic production should focus on lower levels of acetylated polymers than those currently commercially available. Whether the physical properties of copolymers produced with a lower level of acetylation will be appropriate for use in thin films remains to be determined.

ACKNOWLEDGMENTS

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