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Starch esters as biodegradable plastics: Effects of ester group chain length and degree of substitution on anaerobic biodegradation

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A novel series of starch esters was prepared, which varied both in degree of substitution (DS) and in ester group chain length (C-2 to C-6). Proton nuclear magnetic resonance–based characterization of esterified starch polymers was determined to be superior to standard saponification digestion (SD) analysis. The effects of starch modification on the anaerobic biodegradation potential for the polymer were assessed using the biochemical methane potential (BMP) protocol. Results indicate that increasing the starch ester group chain length necessitates lower levels of substitution to achieve substantial biologic degradation of the polymer.

Keywords: Starch esters; anaerobic digestion; degree of substitution

Introduction

Historically, petroleum-based plastics have been formulated to increase durability and reduce photo, chemical, and biologic degradation.[1] Popular chemical formulations include polyethylene, polystyrene, and polyurethane. However, recent concern about the persistence of plastic materials in the environment, either as litter or through landfill disposal of municipal solid wastes, has prompted a rethinking of the use of plastic-based products.[2] In several states and communities, legislation has been enacted requiring that biodegradable plastics be used in common short-term applications such as packaging materials.[3]

Modified natural polymers such as cellulose acetate have been used instead of conventional plastics, for various purposes. Often these cellulose acetate films resemble plastics in their hydrophobic nature and strength. Cellulose acetate films are commonly used in applications such as filtration media for industrial and scientific use.

Although public concern is increasing about potential groundwater pollution resulting from landfill disposal of wastes, landfilling will continue to be a major disposal method well into the future because of its relatively low cost. Therefore, the most useful test for biodegradability is based on an anaerobic microbial population.

Previously, we evaluated the effects of increasing the level of acetylation of the natural polymers—cellulose, starch, and xylan—on their inherent anaerobic biodegradation potential.[4] In the current study, we evaluate the effects of increasing ester group chain length and degree of substitution (DS) on the anaerobic biodegradation of starch.

Materials and methods

Preparation of starch esters

Starch (amylose) acetates. The series of starch acetates was developed through controlled acetylation using high amylose starch (Sigma Chemical Co., St. Louis, MO). The starch (20 g) was added to formamide (400 ml) in small portions at room temperature under vigorous stirring. The resulting gellike 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 days, the slurries were poured into 2 l of deionized water. The resulting white precipitates were collected using centrifugation. The precipitated starch acetates 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 days, and a cryogrinder was used

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to grind the solid cakes to a fine, white uniform powder. The powder was then stored at room temperature.

Starch esters. Substituted starch esters were prepared using propionic, butyric, valeric, and hexanoic anhydrides (Sigma) in the same manner as described for starch acetates.

Starch ester characterization

The DS of starch esters was determined by saponification and nulcear magnetic resonance (NMR) spectra.

Saponification analysis. Previously, acid hydrolysis of polymers was used to de-esterify and hydrolyze polymers such as cellulose, starch, and xylan. However, in this study, acid hydrolysis of highly substituted starch polymers was ineffective in hydrolyzing the polymers completely. Therefore, saponification analysis was performed on all the polymers to determine substitution level. Saponification was conducted by adding 1N NaOH to the starch ester, followed by thermal treatment at 121°C for 1 h.[5,6] Following de-esterification and hydrolysis of the starch, the sample was analyzed for free organic acid. Free organic acids were determined by gas chromatography after acidification of the samples (pH <2.0) using 0.5 M sulfuric acid, then analyzed as described below for volatile fatty acids in sludge samples. Because of the extensive destruction of monomeric sugar units during saponification, anhydrosugars were determined by the difference obtained by subtracting the organic acid weight from the initial sample weight. The accuracy of the saponification analysis was confirmed through random acid hydrolysis analysis performed on identical samples within the DS range of 0 to 1.5. Once the respective molar equivalents of free acid and anhydrosugar were determined, the DS (the molar ratio of organic acid groups to anhydrosugar units) could be calculated.

NMR spectra. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were collected for all starch esters following the procedures used by Buchanan *et al.*[7–9] The spectra were run on a Varian Unity 300 operating at 300 MHz for proton. All of the chemical shifts are reported in parts per million (ppm) using tetramethylsilane as an internal standard. The spectra were acquired at 80°C with a spectral window of 4,000 Hz, a delay time of 5 ms, and 64 accumulations. The sample tube size was 5 mm with sample concentrations of 30 mg ml^{-1} in DMSO-d_6. Several drops of trifluoroacetic acid were added to the sample to move the peak because of residual moisture in the sample.

Anaerobic degradation consortium

The anaerobic digestion consortium used in bioconversion studies was originally obtained from a municipal sewage anaerobic digester (North Metro Water Reclamation Plant, Denver, CO). This anaerobic consortium was further enriched for microorganisms capable of degrading the components of municipal solid waste (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 described previously.[10] The consortium was maintained in laboratory-scale anaerobic digesters with 3.5-l working volumes and semicontinuous stirring (15-min intervals). These reactors were constructed and operated as described previously.[11] 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 described previously.[10] It was used to provide optimum levels of nitrogen, phosphate, and minerals. Total biogas production was measured using calibrated water displacement vessels. Effluent from the semicontinuously stirred tank reactors (CSTR) was used as inoculum for the biochemical methane potential (BMP) assays.

Biochemical methane potential assays

The BMP assays were performed in triplicate as described previously[12] to determine the ultimate yields of methane (product) and, therefore, anaerobic bioconversion of the polymer samples.

Anaerobic sludge analysis

Once the BMP analysis was terminated (generally 90 days), anaerobic sludge was analyzed for pH and volatile organic acids, as well as to gauge the general performance of seed culture digesters. 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 68/80, Carbopack C/0.3%, and Carbowax 20 M/0.1% H_3PO_4 for separations.

Biogas analysis

The composition of the biogas produced in BMP assays and seed digesters 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.[10]

Theoretical methane yield

The theoretical methane yield for anaerobic bioconversion of the various polymers tested was calculated as described previously[12] from the chemical oxygen demand (COD) values for the respective polymers. The COD was determined as described previously.[13] The COD assay employed the microdetermination method using the commercially available "twist tube" assay vials (O.I. Corporation, College Station, TX).

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.

Results

Starch ester polymers

Following development of the starch ester polymer series, conventional analysis by acid hydrolysis[5] was followed by independent analysis of sugars and organic acids. However, acid hydrolysis was not effective for the highly substituted starch esters. In addition, acid hydrolysis became less effective as the chain length of the substituting organic acid increased. Alternatively, the saponification method, which uses sodium hydroxide for hydrolysis, was successful at all levels of substitution and at increasing ester group chain lengths. However, saponification resulted in degradation of the free sugars, which made their analysis impossible. Therefore, the level of polymer substitution was determined by calculating the sugar content as a difference between the free organic acid and polymer starting weights.

The level of starch ester substitution may also be determined by ^{1}H-NMR and carbon nuclear magnetic resonance

Table 1 Analysis of degree of substitution of starch esters as determined by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy[a] and saponification digestions (SD)

Anhydride (mol eq.)	Propionate		Butyrate		Valerate		Hexanoate	
	^{1}H-NMR	SD	^{1}H-NMR	SD	^{1}H-NMR	SD	^{1}H-NMR	SD
0.5	0.10	0.13						
1.0	0.30	0.35	0.35	0.47	0.40	0.37	0.40	0.42
1.5	0.55	0.75						
2.0	0.85	1.13	0.95	1.30	0.50	0.72	1.10	0.97
2.5	1.25	1.34						
3.0	1.15	1.57	1.55	1.62	1.60	1.97	1.80	1.10
3.5	1.80	1.68						
4.0	1.95	1.81			1.85	2.57	1.70	2.04
5.0	2.35	2.25	1.95	1.82	1.75	2.80	1.80	2.63

[a]The protons attached to the terminal methyl group are seen as a single peak at 0.93 ppm. The starch backbone protons were integrated over the range of 3.0 to 5.7 ppm

(^{13}C-NMR). Several systematic changes occur in the ^{1}H-NMR spectra of the starch esters as the DS increases. The DS values for each series of starch esters prepared are shown in *Table 1*. For saponification estimates, the DS values were determined by taking the ratio of moles of organic acid to moles of sugar (calculated by difference). For ^{1}H-NMR methods, the DS of the starch esters was determined by taking a ratio of the normalized, integrated intensity of the terminal methyl group (three protons) to the normalized, integrated intensity of the starch backbone protons (seven protons).

In general, the DS of the starch esters increased as the equivalents of added acid anhydride was increased. The DS of the esters appeared to be independent of the length of the aliphatic chain attached to the acid anhydride. Comparison of the saponification and ^{1}H-NMR methods for analyzing the level of substitution of starch esters indicated that the ^{1}H-NMR method was more accurate. The accuracy of both methods was compared for the lower levels of substituted polymers by the acid hydrolysis method. For the lower DS levels, the ^{1}H-NMR method data agreed most closely. The increasing error for saponification analysis may be attributed to the harsh conditions of this method as well as the determination of sugars by difference.

In addition to the analysis of DS, the type of acyl group can also be determined by ^{1}H-NMR. *Figure 1* shows a series of ^{1}H-NMR for starch esters with different acyl groups. Several distinct trends could be seen in ^{1}H-NMR spectra. As the length of the acyl group increased from propionate (C-2) to hexanoate (C-5), new peaks were seen in the ^{1}H-NMR spectrum. These peaks were related to the additional methylene groups present in the acyl side chain.

Anaerobic bioconversion of starch esters

The anaerobic bioconversion potential for the starch ester series was determined using the BMP assay. This assay employs an extended incubation period of 90 days to allow the anaerobic microbial consortium to adapt to the new feedstock and also to allow for complete degradation. The cumulative data are commonly referred to as BMP_{90} data.

The BMP experiment conducted with the starch acetate

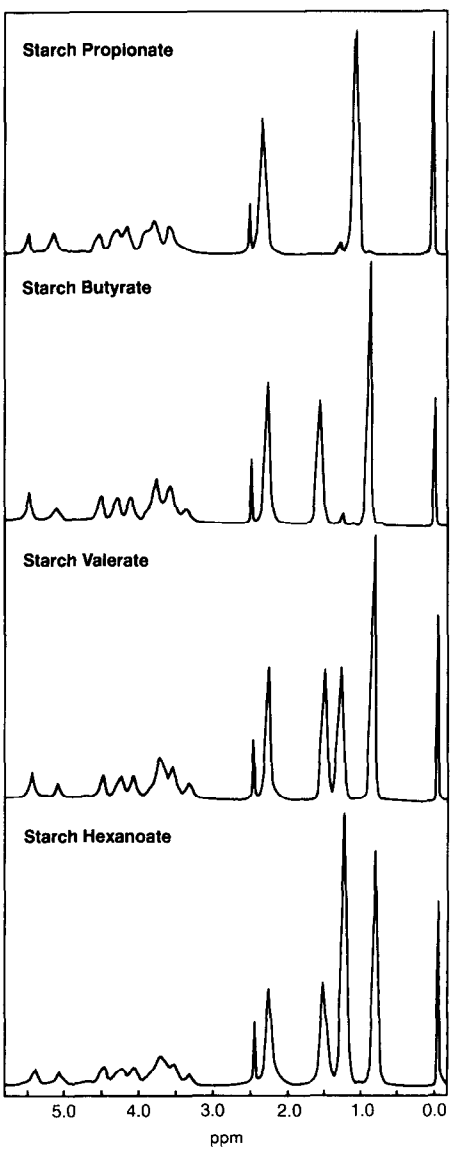

Figure 1 ^{1}H-NMR spectrum of starch esters with acyl groups of varying lengths

series of polymers prepared previously is shown in *Figure 2* for cumulative biogas production. Similar data patterns for anaerobic bioconversion were demonstrated for starch propionate, starch butyrate, starch valerate, and starch hexanoate (data not shown). In general, the data indicated that increases in the DS of the starch polymer resulted in a loss in anaerobic bioconversion potential. For many of the polymer series, the anaerobic bioconversion data followed a logarithmic trend. The BMP_{90} data were summarized for the extent of conversion based on the relationship of added COD content of the polymer, as determined by Owen *et al.*[12] In a previous study, we explored the anaerobic bioconversion of a series of acetylated starch, xylan, and cellulose polymers.[4] The bioconversion data demonstrated a sigmoidal relationship with regard to increasing levels of acetylation for all polymers, as shown in *Figure 3*. The six carbon sugar polymers, starch and cellulose, demonstrated a drop in conversion with acetylation levels of ≥ 1.5. The five carbon xylan polymer demonstrated a loss in conversion at an acetylation level of 1.2. The data generated previously for starch acetate were added to the data for longer chain length organic acid starch esters, as described in *Figure 4*. These data indicated a similar sigmoidal reduction in anaerobic bioconversion as the level of starch substitution was increased. Furthermore, the reduction in anaerobic biodegradation of the starch ester polymer occurred at lower levels of substitution as the ester group chain length was increased.

Discussion

Plastics amount to a $100-billion U.S. industry, much of which is represented by materials for short-term use, such as packaging. The high levels of acetylation required to modify natural polymers such as cellulose, starch, and xylan for use as substitute plastics have been demonstrated to reduce the anaerobic biodegradation potential dramatically.[4] The anaerobic biodegradation of cellulose and starch was essentially inhibited at acetylation levels of 1.5; xylan degradation was inhibited at an acetylation level of 1.2. Furthermore, these anaerobic biodegradation trends were also demonstrated when the acetylated polymers were hydrolyzed using concentrated hydrolytic enzyme preparations.[4,14]

In this study, the production of starch esters through formamide–organic acid anhydride chemistry appeared to be limited to the production of a maximum DS of 2.4 to 2.5. This limitation resulted from the one-step esterification procedure. Complete esterification (DS 3.0) of cellulose with long chain acid anhydrides has been noted to require a several-step esterification procedure.[7]

Analysis of starch esters using ^{1}H-NMR demonstrated improved accuracy over saponification digestions in which the sugar content was determined by difference. In addition, ^{13}C-NMR analysis offers the capability of determining the location of the ester groups around the glucose ring.

In this study, increasing the carbon chain length of the substituting group reduced the anaerobic biodegradation po-

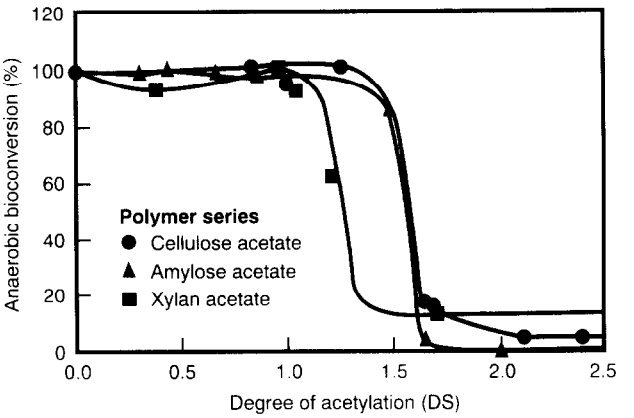

Figure 3 Effects of polymer DS on the anaerobic bioconversion of acetylated cellulose, starch (amylose), and xylan

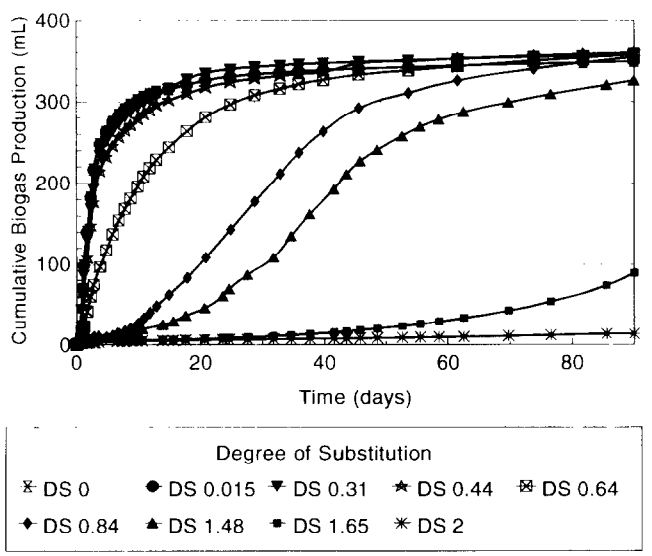

Figure 2 Effects of polymer DS on the anaerobic bioconversion potential for starch acetate

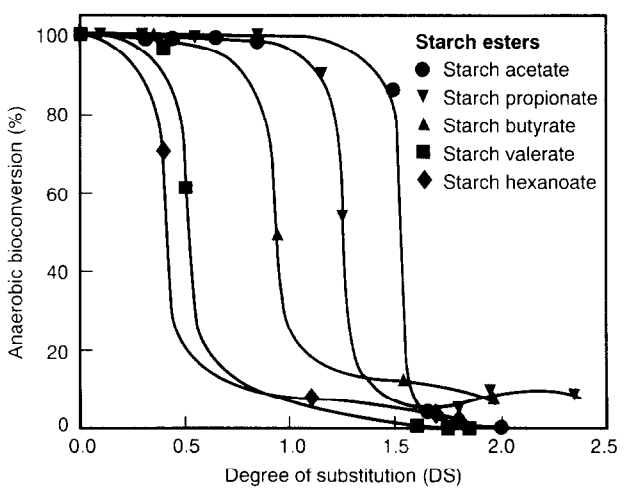

Figure 4 Effects of polymer DS on the anaerobic bioconversion of starch esters ranging from C-2 to C-6

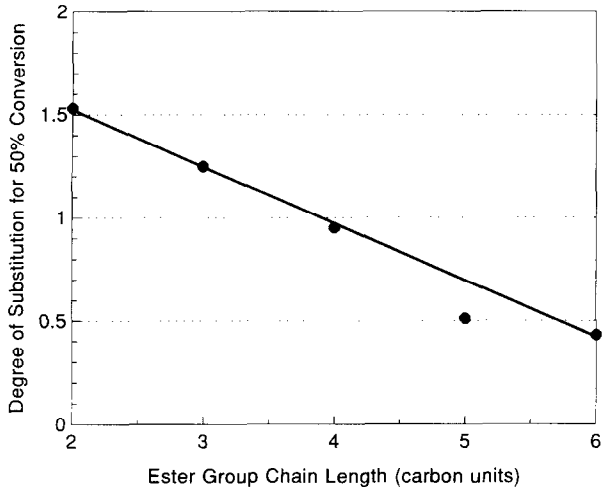

Figure 5 The effect of increasing ester group chain length on the minimum DS for starch esters (which allows for 50% anaerobic conversion)

tential for the resulting starch ester polymer over the C-2 to C-6 range. The loss in anaerobic bioconversion with increasing levels of substitution of the starch polymer followed a sigmoidal trend as demonstrated previously for actylated cellulose, starch, and xylan polymers.[4] *Figure 5* summarizes the effect of increasing the ester group chain length on the anaerobic bioconversion potential of starch esters. The data for the limiting substitution level are represented by the midpoint of the sigmoidal curve from *Figure 4*, which represents a 50% conversion for the BMP_{90} assay. The data indicate that as the ester group chain length is increased, the DS must be decreased if the anaerobic bioconversion potential is to be maintained for natural polymers such as starch. The functionality of such moderately substituted renewable polymers for short-term-use plastics is unknown. Additional research is required to determine the potential use of such moderately substituted starch esters as biodegradable plastics.

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