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Measurement of the Biodegradation of Starch-Based Materials by Enzymatic Methods and Composting

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The aim of this study was to evaluate the suitability of in vitro enzymatic methods for assaying the biodegradability of new starch-based biopolymers. The materials studied included commercial starch-based materials and thermoplastic starch films prepared by extrusion from glycerol and native potato starch, native barley starch, or crosslinked amylomaize starch. Enzymatic hydrolysis was performed using excess *Bacillus licheniformis* α-amylase and *Aspergillus niger* glucoamylase at 37°C and 80°C. The degree of degradation was determined by measuring the dissolved carbohydrates and the weight loss of the samples. Biodegradation was also determined by incubating the samples in a compost environment and measuring the weight loss after composting. The results indicated that the enzymatic method is a rapid means of obtaining preliminary information about the biodegradability of starch-based materials. Other methods are needed to investigate more accurately the extent of biodegradability, especially in the case of complex materials in which starch is blended with other polymers.

KEY WORDS: Composting; starch-based biopolymers; enzymatic degradation; biodegradation.

INTRODUCTION

Starch is a renewable and abundant biopolymer which can be used as a raw material in the manufacture of biodegradable thermoplastics. Several reviews concerning starch-based plastics have recently been published [1, 2]. In the first applications granular starch was used as a filler in polyolefins [3]. Starch granules are uniformly dispersed in the polyethylene (PE) matrix without chemical interaction [1]. Several composites have been prepared containing modified starch, and some hydrophilic synthetic polymers using starch as a major component [4, 5]. In these materials, starch can form a continuous phase rather than being present as a particulate filler [6].

The biodegradation of starch-containing polymer blends depends on the starch processing method, as well as on the biodegradability of other components. At very low starch concentrations, only surface starch would be accessible to direct attack by microorganisms [2]. When low-density polyethylene films with varying granular starch concentrations of up to 9% were tested in different environments, part of the starch always remained undegraded and disintegration of the polyethylene matrix was claimed to be necessary for total starch removal [7]. Starch removal can be greatly increased when prooxidants are added to composites [7, 8]. It has been claimed that the concentration of starch should be higher than 40% before it can act as an enhancer of the degradation of PE-starch blends without the aid of prooxidant additives [9, 10].

In the most recent products starch is the only polymer and it is converted to thermoplastic material under the effects of heat and mechanical energy in a closed vessel, usually an extruder, in the presence of plasticizers such as water or low molecular weight polyols [11, 12]. Thermoplastic starches typically contain 70–90% starch, the rest being plasticizer and/or other degradable additives. They are claimed to be biodegradable, compatible with natural fibers and polar polymers, and of reasonable strength without the need for synthetic polymers [13]. Published information concerning the bio-

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degradation of this kind of products has hitherto been rather limited.

Biodegradation can be defined as degradation caused by microorganisms and their enzymes in aerobic or anaerobic conditions. The first testing methods for determining biodegradation of polymers were recently published by standardization committees such as ASTM (American Society for Testing and Materials). The main elements in biodegradability testing are the incubation of the sample under conditions conducive to microbial attack, and the evaluation of degradation; e.g., by measuring changes in sample weight or in certain physical parameters. The testing can be performed in the field or by laboratory methods [14]. Several studies have been carried out to evaluate biodegradation by incubating samples in a compost environment [15, 16, 17]. One dilemma is how to define the time limits for biodegradation; within what time period should the material be completely degraded?

The time needed to perform the biodegradation test can be reduced by using specific enzyme assays. The ability of amylolytic enzymes to degrade starch composites can be limited [18, 19, 20]. According to Wool and Cole [21], however, as much as 90% of the starch from composite films was solubilized in enzymatic hydrolysis. Sung and Nikolov [10] reported that levels of starch degradation in enzymatic hydrolysis ranged between 10% and 50% of the initial starch content, depending on the extent of polyethylene degradation.

The aim of this study was to evaluate the suitability of in vitro enzymatic methods for assaying the biodegradability of new starch-based biopolymers. The biodegradation of these materials was also measured by incubating the samples in a compost environment.

MATERIALS AND METHODS

Materials

Mater-Bi films (ZFO2U, ZFO3U, and AFO5H) were obtained from Novamont North America. Biopac was obtained from the Austrian Biologische Verpackungssysteme GmbH and Biopur from Biotec GmbH & Co. KG. Barley starch (moisture 10.0%) was obtained from the Raisio group (Raisio, Finland) and potato starch (moisture 13.7%) from Hämeen Peruna Oy (Hämeenlinna, Finland). Crosslinked amylomaize starch (moisture 13.8%) was obtained from the National Starch & Chemical Company (Neustadt, Germany). Cellulose-based sausage casing was obtained from Visko Oy (Finland) and low-density polyethylene (LDPE) came from Amerplast (Finland).

Spezyme AA 20 *Bacillus licheniformis* α-amylase was obtained from Genencor International Europe Ltd. (Finland) and had an activity of 1634 U/mL as determined by the MegaZyme Ceralpha method [22]. *Aspergillus niger* glucoamylase was from Boehringer Mannheim (Germany). Its activity was 2.2 U/mg as determined by MegaZyme method [23].

Plasticization

Thermoplastic starch films were prepared from native potato starch (film 1), native barley starch (film 2) and crosslinked amylomaize starch (film 3) by extrusion. The mixture of starch, water and 85% glycerol in the ratio of 45/20/35 (w-%) was fed into a Bersdorff NZ-33 × 25D corotating twin-screw extruder. The temperatures of the sequential warming zones in the extruder were 170/170/170/170/170/170/120/120°C for all starches. The extrudate was cut into small pieces and was further processed in a single-screw Berstorff Plasticord extruder for the production of film. The temperatures of the four different zones in the extruder were 105/160/170/135°C for the native potato starch, 105/160/170/152°C for the native barley starch and 130/180/180/182°C for the crosslinked amylomaize starch.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed at 37°C and 80°C. A total of 100 mg of the sample was incubated in 10 mL 0.1 *M* acetate buffer, pH 5, containing 250 U/g_{sample} α-amylase, 880 U/g_{sample} glucoamylase and 0.02% sodium azide to prevent microbial growth. Commercial samples were ground cryogenically by Retsch ultra centrifugal mill ZM1 (Germany) using a sieve with 0.5-mm hole. From the termoplastic starch films, a piece of sample was taken and used in enzymatic hydrolysis. Incubations without enzymes (only sample) and without the sample (only enzymes) were carried out as controls. The incubations were carried out in test tubes shaken vigorously in a water bath. The test tubes were withdrawn at predetermined time intervals and centrifuged or filtered if necessary. The supernatants were collected for further analysis.

Total carbohydrates (% of dry weight) in the hydrolysis supernatants were assayed by the phenol-sulfuric acid method [24] using glucose as standard. The weight loss of the samples after hydrolysis was determined after drying the samples at 45°C for 24 h.

Composting Experiments

The composting experiments were carried out in an insulated commercial composter bin (Sepe, Suomen kompostointipalvelu Ky, Helsinki, Finland) filled with biowaste consisting mainly of vegetables and fruit rejected from grocery stores. A mixture of bark and wood chips was added for aerating the biowaste. Square sheets of test material (2.5 × 3.5 cm) were attached to the steel frame which was buried in the biowaste. The film samples were attached to the steel frame so that both sides of the sample were directly exposed to the compost biowaste. Four replicates of each sample were incubated in the compost environment for either 49 days (experiment I) or 70 days (experiment II). Cellulose based sausage casing was used as a positive (compostable) and polyethylene (LDPE) as a negative control (noncompostable).

During composting the temperature, pH, moisture, oxygen and carbon dioxide concentrations were monitored. Oxygen and carbon dioxide concentrations were determined by a Servomex 570A oxygen analyzer and Servomex PA 404 gas analyzer (Servomex Company, Crowborough, Sussex, England). The composts were turned every seven to nine days and about 100 g compost was removed for further analysis. The moisture content of the compost was determined after drying at 105°C for 24 h and weighing. pH was measured after adding 20 mL distilled water to 10 g of the compost.

The degree of degradation was determined by measuring the weight loss of the samples at the end of the experiment. The samples were washed with distilled water and dried at 45°C for 48 h before weighing.

DSC Measurements

Differential scanning calorimetry was performed in a Mettler TA thermal analyzer with a DSC 30S cell. A total of 1–2 mg of samples were weighed out in a standard aluminum pan. The sealed pans were scanned at a rate of 10°C/min from 0°C to 250°C, from 250°C to 0°C and again from 0°C to 250°C. Each sample was run in duplicate before and after enzymatic hydrolysis and composting.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis

The aim of the study was to evaluate the suitability of in vitro enzymatic methods for assaying the biodegradability of starch-based materials. As a comparison the hydrolysis of native granular barley and potato starches and crosslinked amylomaize starch was also studied. Native barley starch was hydrolyzed completely in 6 h at 37°C but native potato starch and crosslinked amylomaize starch were more resistant to enzymatic hydrolysis (Fig. 1). Native potato starch has been shown to be relatively resistant to degradation by α-amylase compared to many other native starches [25, 26]. At 80°C native potato starch was also hydrolyzed completely in 1 h.

As expected, the thermoplastic starch-glycerol films were hydrolyzed more rapidly than the granular native starches at 37°C (Fig. 1). Figure 1 indicates only 70% degradation because the remaining part up to 100% is glycerol. The reason for this is probably the disruption of the granular starch during extrusion and the partial degradation of the starch into smaller components. This was also confirmed by HPLC analysis (data not shown). When the starch films were immersed in the acetate buffer solution, the glycerol component dissolved almost immediately. The films were completely hydrolyzed in approximately six hours (Fig. 1). In the incubations without enzymes, the amount of carbohydrates solubilized from the thermoplastic starch films was larger than that from the native starches (Fig. 3). The thermoplastic starch-glycerol films were very hydrophilic, absorbing water more easily than native starches. This has been suggested to be due to the amorphous

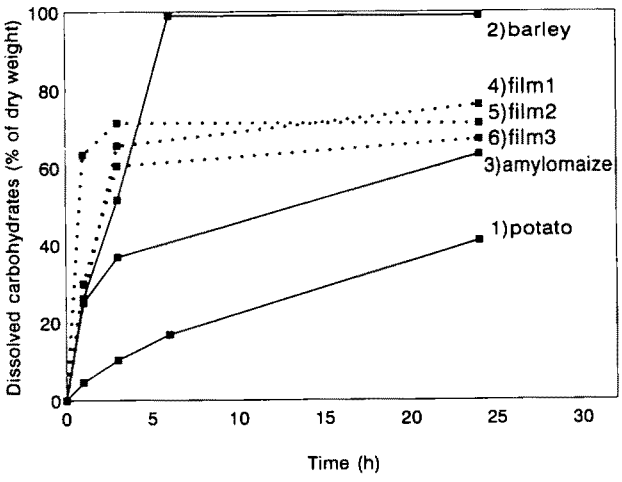

Fig. 1. Enzymatic hydrolysis of thermoplastic starch-glycerol films and the corresponding raw-materials: (1) native potato starch, (2) native barley starch, (3) crosslinked amylomaize starch, (4) film 1 (potato starch), (5) film 2 (barley starch), and (6) film 3 (crosslinked amylomaize starch). Temperature 37°C, acetate buffer pH 5.

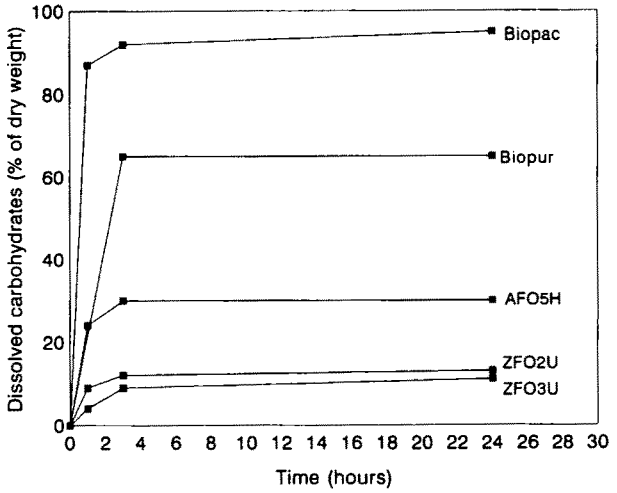

Fig. 2. Enzymatic hydrolysis of the commercial materials studied. Temperature 37°C, acetate buffer pH 5.

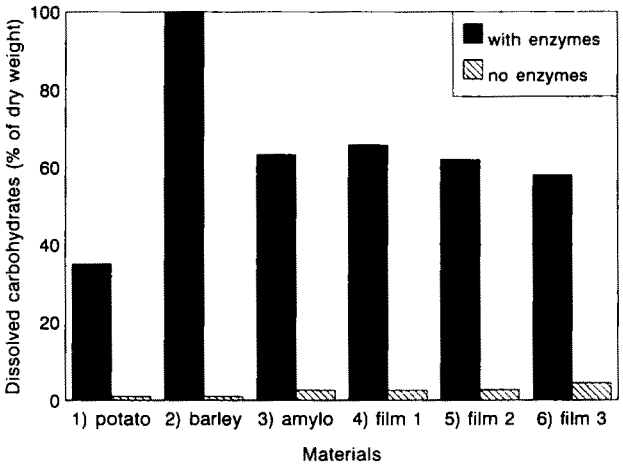

Fig. 3. The amount of carbohydrates solubilized by hydrolysis from the thermoplastic starch-glycerol films and the corresponding raw-materials: (1) native potato starch, (2) native barley starch, (3) crosslinked amylomaize starch, (4) film 1 (potato starch), (5) film 2 (barley starch), and (6) film 3 (crosslinked amylomaize starch). Temperature 37°C, acetate buffer pH 5.

structure of the plasticized starch compared to the partly crystalline structure of native starches [12].

Commercial materials and thermoplastic starch-glycerol films were not hydrolyzed to the larger extent at 80°C compared to the hydrolysis at 37°C. The hydrolysis proceeded faster at the higher temperature, and the differences in the rate of hydrolysis were, therefore, more easily observed at the lower temperature.

Solubilization of the carbohydrates in all commercial samples occurred during the first 3 h of hydrolysis at 37°C (Fig. 2). The addition of fresh enzymes did not increase the extent of hydrolysis from the values shown in Fig. 2. Without enzymes the amount of carbohydrates solubilized from the commercial samples was small except in the case of Biopac (Fig. 4). During enzymatic hydrolysis Biopac and Biopur were hydrolyzed extensively and the amounts of solubilized carbohydrates were 98% and 65% of the dry weight, respectively. Biopac is manufactured by a drying process in which a mixture containing starch and water is shaped in heated moulds [27]. Biopur is made from foamed starches and can be pressed into usable shapes like packaging trays and egg cartons [28].

The solubilization of carbohydrates from Mater-Bi films was low. After enzymatic hydrolysis the amount of solubilized carbohydrates was only 15–30% of the dry weight (Fig. 4). The enzymes did not have a significant effect on the weight loss of Mater-Bi films ZFO2U and ZFO3U during hydrolysis (Table I). With Mater-Bi AFO5H, however, the weight loss increased from 23% to 51% when enzymes were used in the hydrolysis. The differences in degradation between Mater-Bi films can

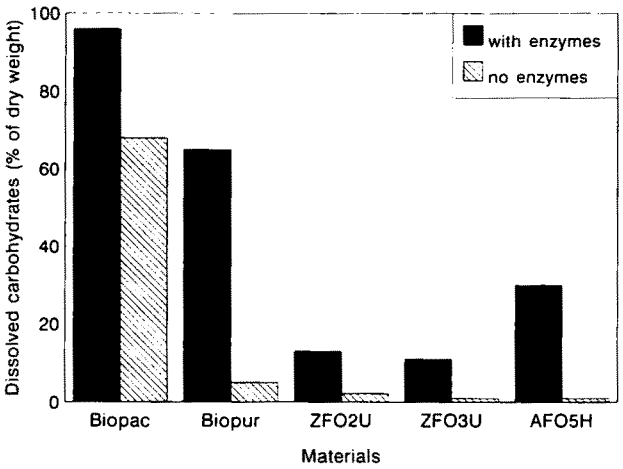

Fig. 4. The amount of carbohydrates solubilized by hydrolysis from the commercial materials studied. Temperature 37°C, acetate buffer pH 5.

Table I. Weight Loss of Mater-Bi Films (%) After Enzymatic Hydrolysis

Sample	Weight loss (%) with enzymes	No enzymes
Mater-Bi ZFO2U	29	23
Mater-Bi ZFO3U	17	14
Mater-Bi AFO5H	51	23

Table II. Melting Temperatures (T_m) and Melting Enthalpies (ΔH_m) of Mater-Bi ZFO2U in DSC Analysis Before and After Enzymatic Hydrolysis/Composting[a]

Sample	T_{m1} (°C)[b]	ΔH_{m1} (J/g)[b]	T_{m2} (°C)[b]	ΔH_{m2} (J/g)[b]
Mater-Bi ZFO2U				
before	61.8	36.2	136.4	6.2
after hydrolysis	58.5	51.6	155.9	7.6
after composting	144.3	7.8	191.3	42.3

[a] Values taken from the first heating period.
[b] Subscripts 1 and 2 refer to the first and second endotherm peaks.

be explained by different microstructures of these films, which is claimed to have a fundamental role in determining the biodegradation rate of this class of products [29]. Starch-based Mater-Bi can be processed by conventional plastic technologies such as film blowing and thermoforming [30]. Mater-Bi films are available in several grades which differ by structure and synthetic component used. For example Mater-Bi AFO5H contains about 60% starch and natural additives and 40% vinyl-alcohol/ethylene copolymer [29]. In the biodegradation tests the shape and the form of the sample can have a very considerable effect on the results. In the case of hydrophobic polymers hydrolysis could be enhanced by using surfactants [18]. In the present study Mater-Bi films were ground before enzymatic hydrolysis in order to achieve a good contact between the tested material and enzymes.

The weight loss of Mater-Bi films in enzymatic hydrolysis was larger than the amount of solubilized carbohydrates, thus indicating the solubilization of other components besides starch. The selective hydrolysis and solubilization was also observed in the differential scanning calorimetry (DSC) analysis before and after the enzymatic test (Table II). The melting enthalpy of the major endotherm (based on dry weight of the total sample) increased by a factor corresponding to the weight loss during enzymatic hydrolysis, thus indicating accumulation of this component in the hydrolysis residue. For example the major endotherm of the Mater-Bi ZFO2U had a peak at 62°C which was reversible during cooling and reheating (Table II).

Composting

The composting test was based on naturally occurring composting reactions and no external heating or ventilation was used. The purpose of the composting experiment was to obtain a measure of the degradation of these materials within a given time, and not to test whether they were fully compostable or biodegradable.

To ensure that the composting proceeded as expected some parameters such as temperature, pH, moisture, and oxygen and carbon dioxide concentrations were measured. During the composting experiments the temperature increased very rapidly to 57–70°C, and then decreased gradually to the temperature of the surrounding air (Fig. 5). After one week of composting, the pH increased to pH 8 from the initial value of pH 5. After the thermophilic stage of composting, the pH began to drop but remained above pH 6. The temperature and pH curves (Fig. 5) were very typical for natural composting [31]. The moisture content of the compost remained above 75% during the composting experiments. Because of the effective natural ventilation of the composter bin, the relative CO_2 concentration inside the biowaste was low during the composting experiments. The maximum carbon dioxide level, 5–10%, was reached at the same time as maximum temperature.

It is very important to use control samples for monitoring the composting process. Cellulose-based sausage casing was 100% degraded in both experiments, indicating that composting proceeded as expected. Polyethylene films did not show any signs of degradation. The thermoplastic starch-glycerol films prepared from different starches were degraded completely after one week of composting, and Biopac was also fully degraded after 17 days (Table III). The weight loss of Biopur was 65%, and its thickness was reduced, but no holes were formed after 49 days of composting. The weight losses of Mater-Bi films were 40–45% and they became very brittle after 70 days. Several holes were also formed in

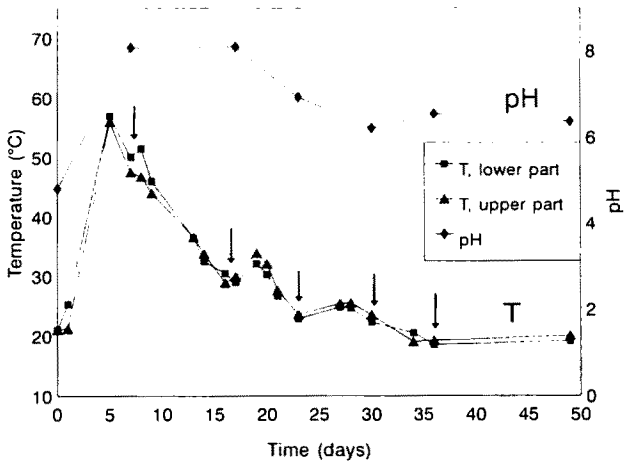

Fig. 5. Temperature and pH during composting experiment I. Turning of the compost biowaste indicated by arrow.

Table III. Weight Loss of the Materials (%) After Composting

Materials	Weight loss (%)	
	Experiment I (49 d)	Experiment II (70 d)
Film 1 (native potato starch)	100	*
Film 2 (native barley starch)	100	*
Film 3 (crosslinked amylomaize)	100	*
Biopac	100	100
Biopur	65	*
Mater-Bi ZFO2U	45	*
Mater-Bi ZFO3U	32	44
Mater-Bi AFO5H	30	40

*not determined

Mater-Bi films ZFO2U and ZFO3U. It was difficult to remove all impurities from the samples, especially from the films, which may have caused some error in the weight loss measurements.

The weight losses of the samples were larger in the compost experiment after 49 days than in the enzymatic hydrolysis except in the case of Mater-Bi AFO5H. The reason for the larger weight losses is nonspecific degradation of the samples in the compost environment compared to the highly specific enzymatic hydrolysis. DSC analysis also indicated a different degradation pattern for the Mater-Bi films in the compost environment. The major endotherm peak of the Mater-Bi ZFO2U at 62°C disappeared after composting, and a new endotherm at higher temperature appeared (Table II).

CONCLUSIONS

The conditions in the compost environment are ideal for degradation mainly because of the mixed microbial population and the broad diversity of the enzymes secreted. One disadvantage, however, is that the composting test is time consuming. It is also difficult to control the composting parameters and the reproducibility of the results is poorer than that with the enzymatic test method. The advantages of the enzymatic method are evident: the short time needed to perform the test and the large number of samples that can easily be tested. The enzymatic method used represents a rapid means of obtaining preliminary information about the biodegradability of starch-based materials. However, other methods are needed to evaluate true biodegradability, especially in the case of complex materials in which starch is blended with other polymers.

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