

Chapter 12

Soil and Marine Biodegradation of Protein–Starch Plastics

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Plastics produced from soy protein and cornstarch were determined to be biodegradable in marine and soil environments. Dialdehyde starch and zein plastics were less readily biodegradable in soil. The plastics were manufactured by extrusion and injection-molding, and by compression-molding the protein/starch mixtures into standard tensile articles. Respirometric studies, measuring CO₂ evolution, showed the ground molded protein/starch plastics to have faster biodegradation rates than that of the individual raw materials. This was attributed to the denatured protein and gelatinized starch of the molded specimens, which were more susceptible to microbial biodegradation.

Over 21.9 billion pounds of plastic materials were discarded in 1992 [1]. The loss of landfill space and a change in the public perception of acceptable waste, waste reduction, and waste elimination has increased interest in biodegradable plastics. It is estimated that the degradable plastics market will exceed 3.2 billion pounds by the year 2000 [2]. Recent research efforts to develop polymeric alternatives to petroleum-based products have centered on biopolymers as starting materials.

Protein and starch are two major biopolymers in crops. Soybean seeds consist of ca. 30–45% protein [3]. Soy proteins are classified as globulins, being soluble in water or salt solutions above or below the pH (4.5) of their isoelectric point [3]. The properties of the protein are largely defined by the higher number of acidic and basic amino acids in the proteins and the disulfide bonds which bind the polypeptide subunits together. The proteins have a molecular weight range of 181,000 to 350,000 [3]. Soy protein isolate contains >90% protein [3].

Corn grains contain ca. 72% starch and 10% protein [4]. Corn starch consists of two polymers, amylose and amylopectin. Amylose is a linear polymer

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of 1,4- α -linked gluco-pyranose units with few branches [5]. Amylopectin is a highly branched polymer defined as α -1,4-glucopyranose chains linked by α -1,6-branches. Normal corn starch typically consists of 28% amylose and 72% amylopectin. Zein is the major protein in corn (ca. 50% of total protein)[4] and is categorized as a prolamin, soluble in aqueous alcohol. Zein is low in ionized amino acids and high in nonpolar amino acids; and the high proportion of hydrophobic side chains results in its hydrophobicity [6,7].

Dialdehyde starch is a unique product produced by the oxidation of native corn starch with periodate. The reaction involves a high degree of specificity, with the periodate cleaving the bond between C2 and C3 of the glucopyranose ring [8]. This bond cleavage generates an aldehyde on each of the two carbons.

Research using vegetable protein in plastics dates to the early part of this century. In the 1930's, formaldehyde was used as a hardening agent with water-plasticized soy protein to produce plastics [9,10]; however, the soy plastics were more hydrophilic and costly than their petroleum-based counterparts. With growing environmental pollution concerns and increasing waste-disposal problems, the use of agricultural biopolymers for plastics is once again being investigated.

Gennadios et al. [11] have shown that vegetable proteins can be used to cast films. Paetau et al. [12,13] demonstrated the effects of molding temperature, acid treatment, crosslinking, and fillers on the mechanical properties and water absorption of compression-molded soy protein. Soy protein isolate (11.7% moisture content) displayed a maximum tensile strength (39 MPa) when compression molded at 140°C [12]. Acid-treatment (HCl, propionic acid, acetic acid) of the soy protein, to the isoelectric point, reduced the 26 h water absorption of the plastics from >100% to 30% and did not effect the tensile strength [12]. Crosslinking with formaldehyde (5% w/w) significantly increased the tensile strength (48 Mpa) and reduced the water absorption (23%) of the molded plastics. Crosslinking with glyoxal or adipic/acetic anhydride decreased the extensibility and tensile strength of the plastics [13]. Incorporating short-fiber cellulose (up to 20% w/w) into the soy protein plastic increased the tensile strength and Young's modulus, but long-fiber cellulose decreased the plastic's mechanical properties [13].

Corn starch has also been compounded with petroleum-based polymers in the production of plastics [14,15,16]. Lim and Jane [17] demonstrate that corn starch and the hydrophobic corn protein, zein, can be cross-linked and molded into a plastic. A modified corn starch, dialdehyde starch, forms crosslinks with zein in compression molding to produce a plastic that has good tensile strength (49 MPa) and is water resistant (2.5% after 24 h soaking)[18].

For many years the terms degradable and biodegradable were used interchangeably. The 1988 ISO 472 definition reads: Biodegradable plastic - A degradable plastic in which the degradation results from the action of naturally-occurring microorganisms such as bacteria, fungi, and algae [19]. Tests for biodegradability have included studies of weight loss, mechanical property changes, O₂ consumption, and CO₂ evolution. Weight loss is of limited use for polymers that fragment during the test. A test of CO₂ evolution is considered a good criterion for mineralization and hence ultimate biodegradation of an organic

molecule [20]. In the test, a portion of the carbon is incorporated into microbial biomass and the percentage of CO₂ produced will never reach 100% of that theoretically to be produced from the total carbon of the substrate. Therefore, regulations, such as those adopted for surfactants, regard chemical substances that produce 60% of their theoretical total mass within a defined time period, (28 days), as "readily biodegradable" [21]. It is assumed the time period for other materials, particularly insoluble materials such as plastics, would need to be longer.

Soy protein isolate, native corn starch, modified starch, and zein have been investigated for manufacturing biodegradable plastics. Various combinations of the protein and starch were molded into shaped specimens by suitable processing methods. The objective of this study was to investigate the biodegradability of soy protein/starch plastics and dialdehyde starch/zein plastics, by respirometry, in simulated soil and marine environments.

Experimental

Materials. Soy protein isolate (PRO-FAM) (Archer Daniels Midland, Decatur, IL), zein (Freeman Industries, Tuckahoe, NY), corn starch (American Maize, Hammond, IN), and polymeric dialdehyde starch (PDS, 90% oxidized starch)(Sigma Chemical Company, St. Louis, MO) were used. Other chemicals were reagent grade and used without further purification.

Preparation of Plastics. Native soy protein isolate was compression molded (45 MPa, 140°C, 10 min) to form standard tensile bars using a Wabash compression molding machine (Wabash Metal Products, Wabash, IN). Mixtures of soy protein isolate (3 parts) and corn starch (2 parts) with glycerol (14%, and 18%) as a plasticizer were mixed in a heavy duty mixer (KitchenAid, St. Joseph, MI). The mixture was equilibrated to the desired moisture content and extruded on a twin-screw extruder (Micro 18 twin screw, American Liestritz Extruder Corp., Somerville, NJ) and pelletized. The pellets were injection molded using a Boy injection-molding machine (Boy 22S, Boy Machine Inc., Berwyn, IL) at 1300 bar to form tensile specimens.

Zein (1 part) was dissolved in 75% aqueous methanol and mixed with dialdehyde starch (3 parts) of various degrees of oxidation (1%, 5%, and 90% oxidation) [22]. After evaporating the methanol in a fume hood, the mixture was dried at 50°C in a forced air oven. The mixture was ground in a cyclone mill (UDY Corp., Fort Collins, CO). The moisture content was adjusted to the desired level in a humidity chamber. Tensile bars were produced using the Wabash compression molding equipment under the same conditions as described in the preceding paragraph.

Biodegradation

Soil/Sand/Composted Manure Environment. A respirometer was designed to measure mineralization of polymers in a soil environment. The basic design of

the system consisted of air conditioning pretraps, the soil reactor, and a CO₂ posttrap. CO₂-free humidified air was generated by bubbling air through four 2-L pretraps containing 1-L of 2N NaOH, 2N NaOH, 2N H₂SO₄, and distilled water, respectively. Air flow, at 15 mL/min, was directed through a leakproof barbed bulkhead fitting in the base of one side of each 500 mL polycarbonate square bottle (Figure 1). Each reactor contained an all-purpose potting soil (Hyponex All-Purpose potting soil, Hyponex Corp., Marysville, OH), sand, and compost and manure (Fertalife Compost and Manure, Hyponex Corp., Marysville, OH) (1:1:1) mixture (200 g total dry weight basis) with a moisture content of 30%. The reactor bottles were kept in a water bath at 25°C. Temperature and water circulation were controlled by an Immersion Circulator Model 70 (Fischer Scientific, Pittsburgh, PA). Gases exited each reactor to the posttrap through the stoppered top. Five replicates (2 g each) of each sample of raw materials and processed plastics and the controls (soil/sand/compost and manure) were analyzed for comparison. Evolved CO₂ gas was trapped in 10 mL NaOH solution and titrated using HCl with a Mettler DL 12 autotitrator (Mettler Instruments, Hightstown, NJ). The degree of polymer biodegradation was calculated as the difference between the average CO₂ value of the reactors with samples and the controls. These values were then converted to percent biodegradation based on the amount of carbon evolved divided by the theoretical carbon amount of each sample. Native soy protein isolate, native corn starch, soy protein plastic, soy protein/starch (3/2, w/w) plastics with 18% glycerol, and the controls were monitored over 456 h.

Simulated Marine Environment. Mineralization of ground soy protein/starch plastic specimens was carried out by using respirometry procedures in a simulated marine environment developed by Allen et al. [23]. Erlenmeyer flasks (250 mL) were used with a working volume of 100 mL. In each flask, 10 mg of sample substrate were used as the sole carbon source. The composition of the defined marine medium was as follows, in g/L: NH₄, 2; MgSO₄, 2; K₂HPO₄, 0.1; KNO₃, 0.5; and "Instant Ocean" salts (Aquarium System Inc., Mentor, OH), 17.5. The media were inoculated with a suspension (100 µL) of eleven marine cultures previously characterized for polymer degradation [24]. A Microoxymax respirometer (Columbus Instruments International Corporation, Columbus, OH) was used for the study. Gas sensors were calibrated using two flasks with 100 mL of 0.1% phosphoric acid to which 90 mg of Na₂SO₃ and 50 mg of Na₂CO₃ were added. Oxygen consumption (sulfite oxidation) and CO₂ evolution for the calibration solutions were monitored along with the sample flasks. A three-minute sampling time was used for each flask, followed by a three-minute purge of the sensor cell. This cycle repeated every three hours. All the flasks were kept in a light-tight incubator at 30°C and agitated with magnetic stirrer bars.

Raw materials and processed plastics were analyzed in parallel for comparison. Samples of ground soy protein plastic, ground soy protein/starch (3/2, w/w) plastics with glycerol (14%), native soy protein isolate, and native corn starch were analyzed along with controls (no substrate) and empty flasks. Triplicate analyses were run for each sample. Moles of CO₂ evolved, derived by

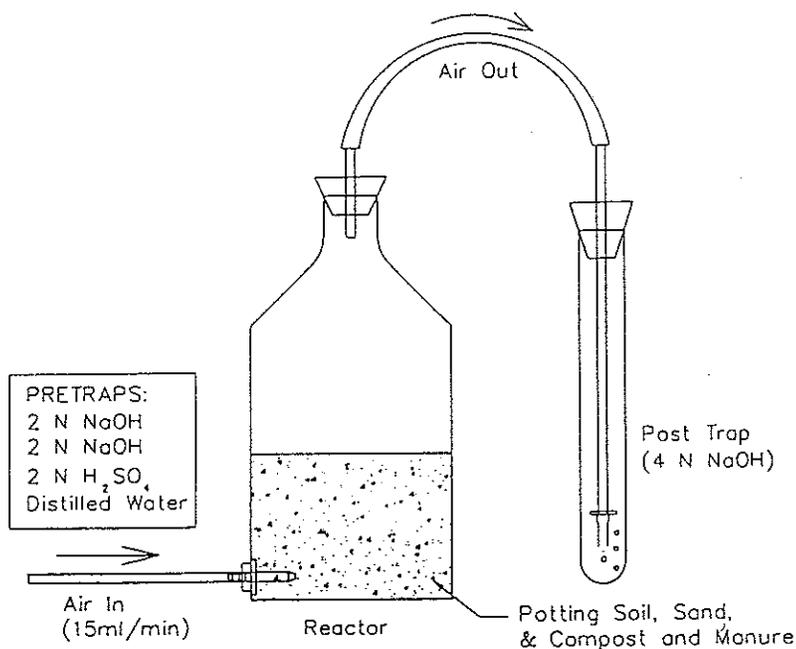


Figure 1. Respirometry reactor showing the details of CO₂-free air entering the bottom of the reactor and exiting to the posttrap through the top.

subtracting CO_2 from controls, were averaged for each sample and divided by the theoretical carbon (ThCO_2) in the sample to calculate percentage mineralization.

Soil/Sand Environment. Biodegradation of the dialdehyde starch and zein plastics was conducted in potting soil and sand (1:1) under the same conditions as described for soil/sand/compost and manure [18]. However, in this set-up both the incoming air and exiting gases passed through the top of a 400 mL glass bottle above the soil line and the water pretrap was placed at beginning rather than the end. The analysis was conducted over 180 days. Corn starch, zein, polymeric dialdehyde starch, and ground samples of plastics made from dialdehyde starch (various degrees of oxidation) and zein (3:1) were studied. Each reactor contained 10 g of sample in 100 g soil.

Results and Discussion

Respirometry using potting soil, sand, and compost and manure demonstrated that the soy, soy plastics, and soy and starch plastics were readily biodegraded (Figure 2). Soy protein isolate and starch (3/2, w/w) (18% glycerol) and compression molded soy protein isolate plastics reached 62% and 60% mineralization, respectively. The percentage of carbon evolved as CO_2 for native soy protein was 58% after 456 h. Corn starch displayed a limited carbon evolution of 28%. The enhanced degradation rates of the plastics (CM Soy, Inj. SCG) were attributed to the molding process, which denatured proteins and gelatinized starch; thus, the material became more susceptible to microbial degradation. Mixtures of protein and starch provided a balanced nitrogen and carbon source for microorganism growth which also enhanced degradation. The slow degradation of the native starch sample was attributed to its granular structure. Native starch is present in a semicrystalline, granular form, which is more resistant to enzymatic and chemical degradation.

After 174 h of simulated marine-environment biodegradation, soy protein/starch injection-molded plastics (14% glycerol) displayed 41% mineralization, and compression molded soy protein showed 37% (Figure 3). Native soy protein isolate displayed 30%, whereas native corn starch displayed only 4% mineralization. The results showed that soy protein/starch plastics were degraded faster than the raw materials from which they were made. The results also showed that the soy protein/starch plastics were promptly degraded in a marine environment. Oxygen consumption (Figure 4) was indicative of respiratory activity of biotics in the reactors. It is evident that the microbial activity was greatest in reactors containing protein-based plastics, which had enhanced nitrogen availability. Oxygen consumption in the reactor containing starch was no greater than the control.

Potting soil and sand as media, without microbial inoculation via compost and manure, was the initial experiment for biodegradation studies and demonstrated a slow rate of substrate degradation (Figure 5). Aeration of the reactor from the top, rather than through the soil, and the arrangement of the pretraps with 2N H_2SO_4 at the end had a dehydrating effect and likely slowed

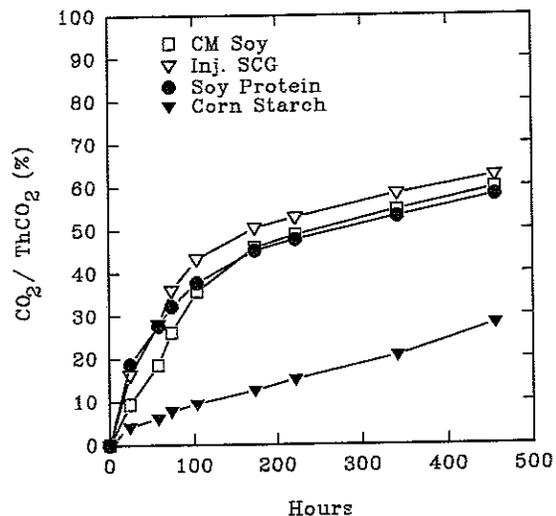


Figure 2. CO_2 evolved represented as percent of theoretical CO_2 each sample could produce in a soil/sand/compost and manure (1/1/1, w/w) environment. Samples are compression molded soy protein (CM Soy), injection molded soy protein/starch (3/2, w/w) with 18% glycerol (Inj. SCG), native soy protein, and corn starch.

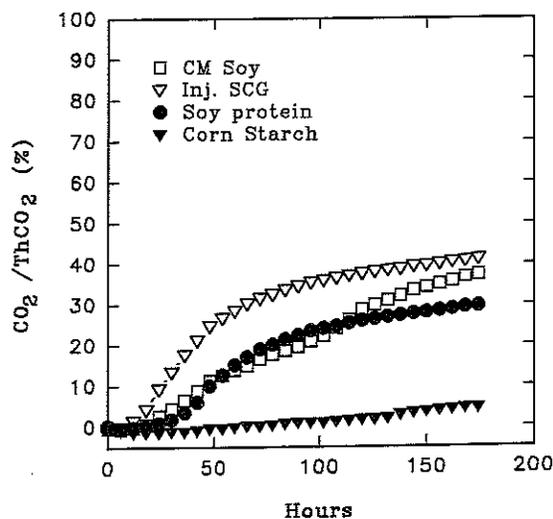


Figure 3. CO_2 evolved represented as percent of theoretical CO_2 each sample could produce in a simulated marine environment. Samples are compression molded soy protein (CM Soy), injection molded soy protein/starch (3/2, w/w) with 14% glycerol (Inj. SCG), native soy protein, and corn starch.

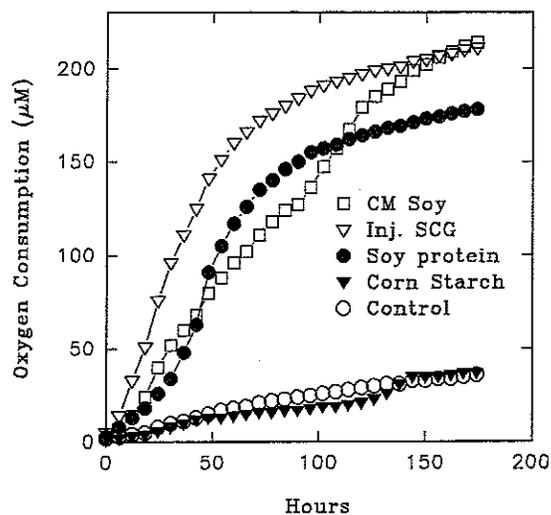


Figure 4. O_2 consumption of samples in simulated marine environment. Legend the same as Figure 3.

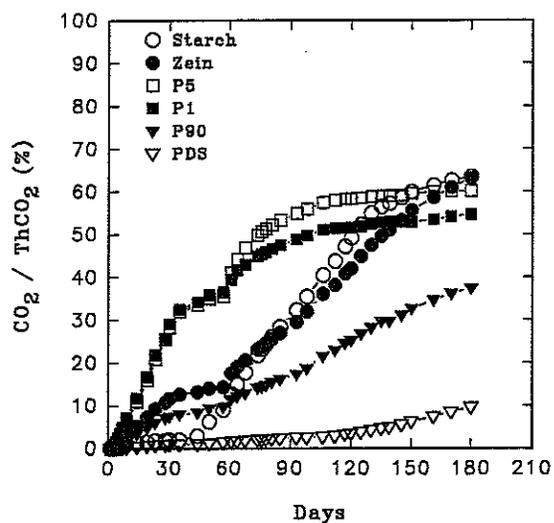


Figure 5. CO_2 evolution from soil/sand respirometer represented as percentage of theoretical CO_2 which a sample could produce. Samples are native corn starch (starch), corn zein protein (zein), 90% oxidized dialdehyde starch (PDS), and dialdehyde starch and zein (3/1, w/w) plastic in which the number following "P" represents the percentage of starch oxidation. (Reproduced with permission from Ref. 18. Copyright 1995 Journal of Environmental Polymer Degradation.)

down mineralization. After 180 days, both corn starch and zein reached about 63% mineralization. Polymeric dialdehyde starch of ca. 90% oxidation showed little mineralization (9.6%). Plastics made from zein and dialdehyde starch of 5% and 1% oxidation showed 60% and 55% mineralization, respectively. The values were fairly close to those of zein and cornstarch. The plastic samples made from zein and polymeric dialdehyde starch (90% oxidation) showed a retarded mineralization of 37%. Degradation curves showed that plastic samples made from zein and dialdehyde starch (1% and 5% oxidation) had much greater initial degradation rates than raw materials (i.e., zein and corn starch). This further demonstrated that processing made the starch and protein more susceptible to microbial attack. The degradation rates, however, slowed down after the first 100 days. Corn starch showed almost no CO₂ evolution during the first 40 days. After the lag period, mineralization of corn starch increased and reached 63% at the end of 180 days. Replacement of this respirometer design with airflow through the bottom and the H₂O pretrap at the end helped maintain a humid environment for faster biodegradation in the other experiments.

Conclusions

In conclusion, plastics made from soy protein and starch mixtures were determined to show "ready biodegradability" in both marine and soil environments under the conditions of these experiments. Plastics made from highly oxidized dialdehyde starch and protein, however, showed retarded biodegradation.

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