# Microbial Degradation

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MOST WIDELY USED PLASTIC materials developed in the past 50 years are characteristically durable and inert in the presence of microbes. This stability is important to long-term performance. However, in many applications, only short-term performance is required before the material is discarded, as in the fast food and packaging industries. It is considered advantageous for the discarded plastic to degrade when exposed to microbes. Other applications of plastics actually require degradability. Thus, an interesting challenge currently being presented to polymer scientists is to develop or modify plastics that possess the properties required for their service life, but which have the capability of degrading in a timely and safe manner.

The degradation of plastics under the action of bacteria and fungi has received renewed interest in recent years because of land shortage problems in solid-waste management, litter accumulation, and environmental problems on land and sea. Agricultural plastics applications, such as mulch films, seedling pots, and binding twine, have increased significantly, making biodegradation a desirable function of the plastic, to minimize disposal and soil pollution problems. Many U.S. states and foreign countries have enacted or are considering legislation that requires disposable plastics to be degradable.

In 1986, $20 \times 10^9$ kg ($45 \times 10^9$ lb) of plastics were sold in the United States, 75% of which was used in long-life applications and 25% of which was used for short-term packaging applications. By 1987, the latter use had increased to about $6 \times 10^9$ kg ($13 \times 10^9$ lb) and is anticipated to approach $60 \times 10^9$ kg ($130 \times 10^9$ lb) by 2000 A.D. Most plastics used in packaging, for films, containers, bottles, coatings, closures, and so on, consist of high- and low-density polyethylenes (31% and 33%, respectively), which do not degrade by microbial action. The use of other plastics is polystyrene (PS), 11%; polypropylene (PP), 9%; polyethylene terephthalate (PET), 7%; polyvinyl chloride (PVC), 5%; and others, 4%.

Plastics are being used at a rapid rate as the material advantages of these lightweight, sturdy materials become obvious. Most widely used alkane-derived plastics are poor in biodegradability and may have lifetimes of hundreds of years when buried in typical solid-waste sites. Although these plastics contain monomeric units of composition analogous to those of low molecular weight hydrocarbons, the large chain length and high molecular weight of plastics hinder degradation by microbes.

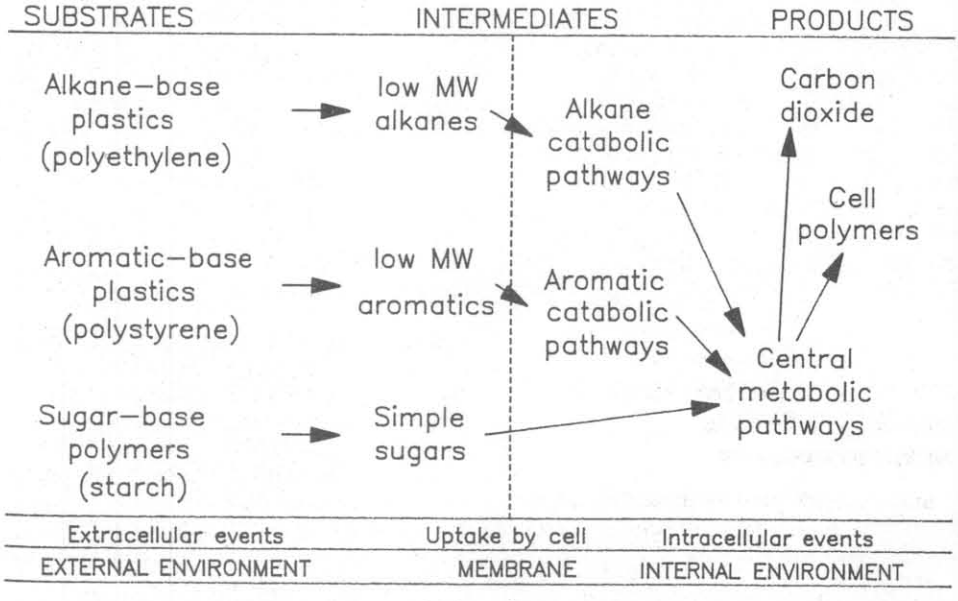


**Fig. 1** Pathways for polymer biodegradation. The existence of extracellular enzymes that depolymerize polyethylene or polystyrene has not been documented.

## Biodegradation Mechanisms

Plastics remain relatively immune to microbial attack as long as their molecular weight remains high. A review of the biodegradability of plastics or lack thereof has been conducted by Potts (Ref 1). Many plastics, such as polyethylene (PE), PP, and PS, do not support microbial growth. Low molecular weight hydrocarbons, however, can be degraded by microbes. They are taken up by microbial cells, "activated" by attachment to coenzyme-A, and converted to cellular metabolites within the microbial cell, as shown in Fig. 1. However, these processes do not function well (if at all) in an extracellular environment, and the plastic molecules are too large to enter the cell. This problem does not arise with natural molecules, such as starch and cellulose, because conversion to low molecular weight components by enzyme reaction occurs outside the microbial cell. Photochemical or chemical reactions may decrease molecular weight to the point that microbial attack can proceed.

Upper limits of molecular weight, beyond which uptake and intracellular degradation do not occur, have not been established for all alkane-derived materials. Very slow degradation of paraffins, PE glycols, and linear alkyl benzene sulfonates occurs when the length of the polymer chain exceeds 24 to 30 carbon atoms (Ref 2-4). It could be concluded from these amply documented results that alkane-base plastics with molecular weights exceeding 400 to 500 daltons (that is, greater than 30 carbon atoms) must be degraded to smaller molecules by photochemical, chemical, or other biological means before biodegradation as we have defined it can proceed. For comparative purposes, low-density PE with a molecular

weight average of $\overline{M}_w$ = 150 000 contains about 11 000 carbon atoms. Decreasing molecules of this size to biologically acceptable dimensions requires extensive destruction of the PE matrix. As will be discussed later, this destruction can be partly accomplished in blends of PE and biodegradable natural polymers by the action of macroorganisms, such as arthropods, millipedes, crickets, slugs, roaches, and snails.

Efforts to construct plastics that fall within the logistical limits discussed above were described by a number of groups during a 1987 symposium on degradable plastics (Ref 5). The ethylene/carbon monoxide copolymer (E/CO) described by Harlan and Nicholas in Ref 5 provides a good example of the difficulties involved in attempting to make large molecules such as those of PE biodegradable. Insertion of carbon monoxide in the chain permits chain scission by a Norrish-type reaction in a photochemical process. It was found that E/CO polymers with 2.5% CO linkages lost about 98% of their original elongation after 40 h of sunlamp exposure. However, after 650 h of exposure, the samples that originally had a $\overline{M}_w$ = 618 700 and number-average molecular weight ($\overline{M}_n$) = 45 000 had photolytic products with $\overline{M}_w$ = 15 000 and $\overline{M}_n$ = 7300. It is likely that the photolysis products described by this group will degrade very slowly unless some other means of further reducing molecular weight can be found. Other authors in the same symposium reported similar photodecay rates and specifically stated (in Ref 5) that E/CO polymers were not biodegradable after ultraviolet (UV) light exposure and soil burial.

The most widely used plastics, that is, PE, PP, PS, polyurethane (PUR), PVC, and polyacrylic, are structurally substituted macroalkanes. These materials are homologues of smaller alkanes, such as motor fuels, motor oils, and surfactants. In discussing biodegradation of plastics, it is worthwhile to consider the definitions and means of measurement of biodegradation and biodeterioration when these terms are applied to smaller alkanes and derivatives such as surfactants. Because these definitions are central to the development of biodegradable plastics (or composite materials containing plastics), they are discussed below.

## Biodeterioration and Biodegradation Definitions

Swisher has discussed biodegradation in depth from the perspective of surfactants (Ref 4). He proposed that primary degradation occurs when the molecule has been oxidized or otherwise altered by bacterial (microbial) action such that characteristic properties are no longer evident, or such that the molecule no longer responds to analytical procedures more or less specific to the detection of the original surfactant. This definition carries two conditions: first, that the molecule itself be chemically altered to an unspecified degree, and, second, that the alterations result in the loss of the utilitarian characteristics of the material.

Applying these concepts to plastics, biodeterioration can be defined as the biologically mediated loss of utilitarian or physical characteristics of a plastic or a hybrid material containing plastics or plastics as a major component. Thus, this definition includes physical disintegration or cosmetic disfigurement of a material but does not require extensive changes in the chemical structure of the components. This definition intentionally avoids criteria based on percentage loss of characteristic properties, or on the time required to attain a specified percentage loss, because the specific application of the material establishes the boundaries of acceptable or unacceptable deterioration rates. This definition describes the end result of many tests conducted according to ASTM G 21 (Ref 6) and reported as evidence of biodegradability of plastic materials.

Biodegradation is defined, for small molecules, as the complete conversion of the surfactant molecules to carbon dioxide, water, inorganic salts, and products associated with the normal metabolic processes of the bacteria (Ref 4). When applied to plastics, the definition of biodegradation may be stated as the conversion of all constituents of a plastic or hybrid material containing plastics to carbon dioxide, inorganic salts, microbial cellular components, and miscellaneous by-products characteristically formed from natural materials.

With this definition, biodegradable plastics are materials whose physical integrity and useful material properties are lost upon contact with microbial and/or invertebrate activity in a natural environment for a limited period of time. The natural environment, whether in laboratory or field tests, with soil or aquatic systems, must be conducive to degradation of readily decayed natural materials, such as carbohydrates, lipids, and proteins. Deterioration is therefore an inevitable consequence of biodegradation because the very chemical structures that composed the original material no longer exist.

## Biodeterioration and Biodegradation Measurements

**Biodeterioration** involves cosmetic blemishing and/or loss of useful properties, which means that the ASTM procedures for determining the resistance of synthetic polymers to fungi and plastics to bacteria (Ref 6, 7) may be applicable in evaluating these effects. Method 2 of Ref 6, in which the material is buried in agar, is preferable to method 1, in which nonporous materials are used. With method 1, in which the film is placed on the agar surface, diffusion of nutrients from the agar through a nonporous film is likely to restrict microbial growth on the top of the film. Water availability on the usually hydrophobic film surface is also an impediment to surface growth.

A procedure based on microscope slide trapping techniques (Ref 8) seems preferable to the agar plate method. Because the organisms used in the ASTM procedure are a mere fraction of the microorganisms likely to make contact with films under use conditions, techniques that allow exposure to soil and/or natural waters are more likely to provide a more realistic assessment of biodeterioration potential. Trapping is a relatively simple and rapid visual technique that does not require extensive microbiological experience. The test is conducted by placing moist soil in a covered container that allows moisture control and gas exchange with the atmosphere. The test film is placed on the surface of the moist soil and covered with a glass microscope slide. The entire assembly is incubated at an appropriate temperature, and microbial colonization can be assessed as described in ANSI/ASTM D 3274 (Ref 9).

This procedure, developed for assessing paint film blemishing, provides good illustrations of graded degrees of blemishing. In their experiments, the authors (Ref 8) use filter paper (cellulose) as a positive control to ensure incubation conditions that are conducive to microbial activity; they also use low-density PE film as a test material on which microbial colonization and disfigurement are minimal. Blemishing of filter paper occurs within a couple of weeks under good conditions, that is, moist soil and temperatures of 20 to 30 °C (70 to 85 °F).

In most cases, polymer films and associated microorganisms adhere to the slides and can be recovered and stained with conventional bacteriological dyes. Incubation in aqueous systems is possible by attaching the short edges of a test film to a microscope slide with an appropriate waterproof adhesive. Growth on the adhesive components may occur, but its origin from the adhesive application site will be obvious.

It should be noted that loss of film integrity with this technique cannot be considered to be persuasive evidence of biodegradability because smaller products can be formed that are neither visible nor subject to degradation. This situation is analogous to some extended photodegradation experiments in which polymer disintegration by ultraviolet radiation yields products that are smaller than the parent materials but are still chemically identifiable as polymer components.

**Measurements of Biodegradation.** Biodegradation involves loss of structural characteristics and mass (total carbon) of a material, as carbon dioxide and water-soluble components are formed. These changes are frequently accompanied by discoloration resulting from extensive growth of pigmented microorganisms. However, discoloration and visible microorganism growth may occur on films in the absence of any changes in the plastic material. This growth is supported by plasticizers and other low molecular weight components that may be mixed with the plastic itself. This type of growth is explicitly discussed in Ref 6, but many investigators may not recognize the difference between biodisfigurement on the surface of a plastic and actual decay of that plastic by biodegradation mechanisms throughout the material.

Biodegradation involves the conversion process and the chemical mechanisms whereby carbon from a biodegrading material is distributed among the products. Guillet, *et al.* (Ref 10) reported evolution of $[^{14}C]$—$CO_2$ from $^{14}C$-labeled, photodegraded PS added to soil. Release of $[^{14}C]$—$CO_2$ was measured for 8 weeks, at which time less than 0.2% of total $^{14}C$ had been evolved as $CO_2$. The authors conclude that complete biodegradation would eventually occur. However, the amount of $^{14}C$ evolved is much less than the theoretical maximum, and one cannot be assured that the process would go to completion. If the polymer were fully degraded, release of $[^{14}C]$—$CO_2$ would be about 50 to 60% (although the percentage is time dependent) of the total $^{14}C$, with about 40 to 50% of carbon as microbial cell material under aerobic conditions. These percentages are based on the release of carbon from natural materials, such as simple sugars, organic acids, and numerous biopolymers (Ref 11-13).

Although the authors of Ref 10 concluded that release of any $^{14}C$ as $[^{14}C]$—$CO_2$ was evidence of biodegradability, the authors of this article suggest from analysis of the data that only 1% or less of the photodegradation products was biodegradable.

Lack of release of the expected amount of carbon as $CO_2$ may be attributed to one of two major causes: Either the material is not being degraded, or it is being incompletely degraded to form nondegradable compounds of lower molecular weight than the parent material. In the latter case, the material may lose some structural integrity and could be judged biodegradable when assessed by methods such as weight loss or depolymerization, but would not necessarily be judged biodegradable if assessed quantitatively by $CO_2$ evolution, $O_2$ uptake, or microbial growth.

The decay of natural rubber described by Tsuchii and coworkers (Ref 14) provides a good example of full decay. With natural rubber, a 100% weight loss of starting material and formation of cell protein equivalent to 27% of the initial rubber weight was found. Because the protein is about 50% of the cell dry weight, the actual cell mass was about two times the protein values, or about 54% of the substrate mass. With cells containing 50% carbon, about 27% of the substrate mass was recovered as cells. Adding 27%, as cells, to 10%, as $CHCl_3$-soluble products, yields 37% of the initial mass. This value is reasonably close to the cell yield expected upon full degradation (the remaining carbon presumably having been lost as $CO_2$), and justifies the conclusion that the natural rubber under investigation was biodegradable.

Huang (Ref 15) provides the most recent extensive review on biodegradable polymers, with an emphasis on degradation studies with pure enzymes or pure microbial cultures. His discussion implies that ASTM procedures involving the formation of microbial cell mass, $CO_2$ production, $O_2$ consumption, material weight loss, and changes in structural characteristics may be used interchangeably to assess degradation. Frequently, in situations other than pure culture, most of these techniques are technically difficult and may yield inconclusive or misleading results. It should be emphasized that most of these techniques involve quantitative procedures with predictable end results. Data obtained with these methods can be used to compare test plastics with other materials that are known to be biodegradable.

A special point needs to be made in regard to the quantitative nature of testing because of the potentially misleading perception that any evidence of a microbial process implies material biodegradability. For example, when interpreting data obtained by Ref 6, the growth is measured as the percentage area covered by fungi without considering whether 100% fungal coverage represents a loss of any of the initial material mass. Although Ref 6 explicitly makes this point, it can be overlooked in practice. For a variety of reasons, the authors of this article propose reexamination of materials if degradability was assessed only by visual procedures (for example, fungal growth on surface) or if the mass balance of starting material and products was not established. On the other hand, it is highly likely that materials that have been interpreted as being nondegradable by ASTM methods are indeed nondegradable, unless their degradation is catalyzed by bacteria or environments not prescribed by the ASTM tests.

## Experimental Example

**Fungal Attack in Cellophane and Amylose Films.** Carr, *et al.* (Ref 16, 17) compared the fungal attack in cellophane (0.025 mm, or 1 mil) and amylose films (0.10 mm, or 4 mil). Fungal colonies were isolated from soil by using a selective solid medium. The samples were incubated at 23 °C (75 °F) on an agar medium for the prescribed length of time and then sterilized in ethylene oxide gas to stop the degradation process. The degraded material was analyzed by x-ray diffraction, infrared spectroscopy, and mechanical property tests. The degree of crystallinity, as measured by x-ray diffraction, was found to increase during the degradation by fungi. This is consistent with the preferential growth of the fungus through the amorphous regions of these semicrystalline polymers. Attack on amylase films by cell-free enzymes, either alpha or beta amylase, resulted in degradation of both the amorphous and the crystalline regions, with preferential degradation of the amorphous regions.

The ultimate and yield tensile stress of the amylose films declined during the degradation process, and the tensile modulus increased as the material became embrittled. Changes in dynamic mechanical properties of cellophane films were measured as a function of fungal degradation. After 16 days of degradation, the storage tensile modulus declined by about 40% over most of the temperature range studied, and considerable broadening occurred in the beta and gamma tan δ peaks. These results were interpreted in terms of the damage caused by cellulase enzymes that are released from a fungal overgrowth and become localized in the noncrystalline regions.

**Starch-base polyethylene films** were formulated by Otey, *et al.* (Ref 18, 19) and consisted of up to 40% starch, urea, ammonia, and various portions of low-density PE (LDPE) and poly(ethylene coacrylic acid) (EAA). The EAA acted as a compatibilizer, forming a complex between the starch and the PE in the presence of ammonia. The resulting blend could be cast or blown into films, and had physical properties approaching those of LDPE.

The ASTM G 21 method (Ref 6) was used to determine fungi resistance. The mixed fungal spore suspension consisted of *Aspergillus niger, Penicillium funiculosum, Trichoderma* sp., and *Pullularia pullulans*. Films containing 30% or more starch could develop a complete mold coverage. Samples were also placed on outdoor soil with their ends buried in the soil.

This experiment was designed to evaluate starch-base plastics as agricultural mulch film. Samples with 90% by weight starch were badly torn within 1 to 3 days after water soaking, primarily because of embrittlement and shrinkage upon drying. Heavy mold growth occurred within 3 to 5 days on the buried portion of these samples. Similar deterioration was observed for samples containing 50% and 70% starch, but not until about 5 to 7 days of exposure. Samples

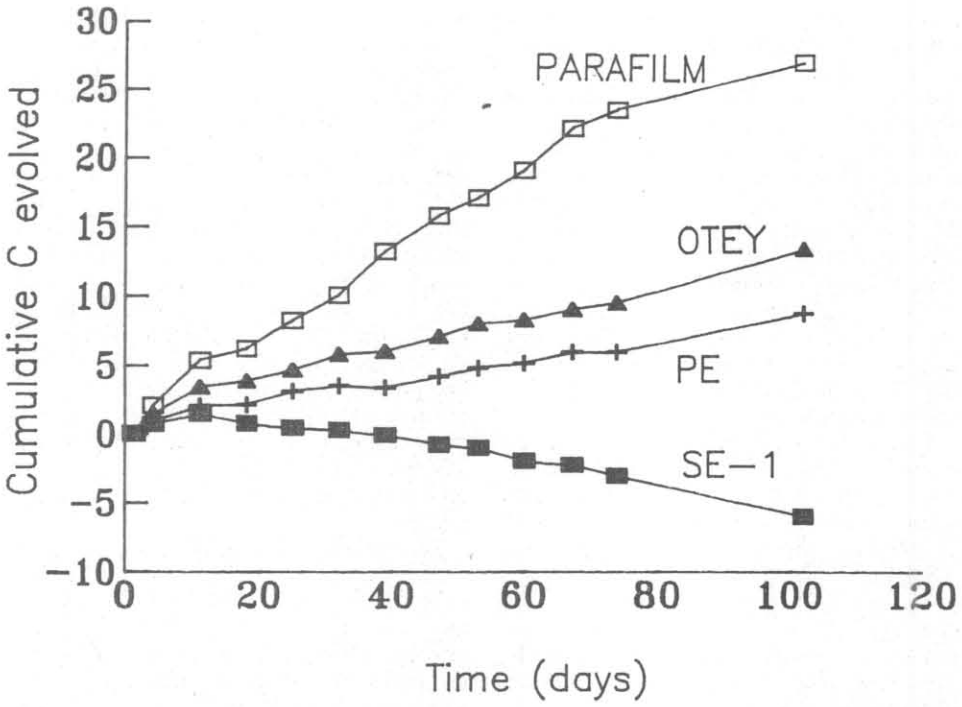


**Fig. 2** Release of carbon dioxide from soil-buried films. The materials are SE-1, commercial starch-extended film with 6% starch, 94% polyethylene; Otey, 40% starch, 25% polyethylene, 25% ethylene acrylic acid; PE, commercial polyethylene film; and Parafilm (R). Moist soil (100 g) and film (50 mg) were placed in Erlenmeyer flasks with a central vial containing KOH as the $CO_2$ trapping agent. The flask assemblies were described in Ref 26 and are suitable for nonradioactive materials when they are added in small quantities to soil. Carbon dioxide evolution was determined by titration of residual alkali. Each point is the mean value of three replicate flasks per soil; standard deviations were ±10% of the mean value for each data point. *y*-axis: summation of mg carbon evolved over the experimental period.

with 30% and 40% starch remained flexible and intact for at least 30 days. Deterioration of the latter films was primarily attributed to microbial attack, as evidenced by heavy mold growth, rather than to embrittlement and shrinkage. The rate of biodegradation of these films could be controlled by varying the amount of starch and by using a fungicide such as paraformaldehyde. Plasticizers could also be added, causing a significant increase in both the percentage elongation-at-break and the rate of fungal attack. These films were also found to be photodegradable, such that the use of carbon black ultraviolet stabilizer was recommended in cases in which prolonged exposure to sunshine is anticipated.

**Films With Modified Starch Additives.** The St. Lawrence Starch Company produces a master batch resin that can be mixed with PEs to produce films consisting of 6 to 15% starch (Ref 5). Degradation most likely proceeds by two interactive mechanisms. The starch is present in the polymer as phase-separated granules and is attacked by fungi and bacteria. This biodisfigurement process weakens the polymer matrix and increases the surface area of the plastic. It is hypothesized that unsaturated oils in the blend act as an autoxidant to form peroxides by reacting with metal salts in the soil or seawater, and the peroxides decompose to form free radicals that attack the polymer chain. The resulting breakdown of the polymer chain weakens the material and reduces the molecular weight to further enhance microbial attack. Related studies by Griffin (Ref 5), who originated this technique, demonstrate that the ultimate tensile strength of a modified LDPE film in a compost heap at 70 °C (160 °F) decreases appreciably after about 5 days. Other companies, such as Archer-Daniels-Midland Company, are using this technology to produce plastic films with a biodegradable component.

**PHBV-Biodegradable Plastic.** ICI Americas Inc. is currently working on the concept of a natural thermoplastic polymer derived from bacteria known as poly(3-hydroxybutyrate-valerate), or PHBV (Ref 5). The naturally occurring soil bacterium *Alcaligenes eutrophus* is grown aseptically in conventional fermenters on a sugar feedstock, usually glucose derived from corn or wheat starch. By controlling the culture conditions, the bacterium can be made to accumulate up to 80% of its dry matter as poly(3-hydroxybutyrate), or PHB. The polymer exists as discrete granules within the cell. The composition of the PHB polyester can be influenced by means of the nutrition of the organism, leading to the incorporation of 3-hydroxyvalerate into the polymer. The resulting material consists of the random copolymer PHBV. The homopolymer PHB is a highly crystalline, somewhat brittle material that melts at 176 °C (350 °F) and has properties similar to those of PP. PHBV containing 25% valerate melts at 138 °C (280 °F) and is much more ductile.

ICI Americas Inc. has demonstrated that this material can be blow molded, injection molded, and processed like conventional plastics. Because it is biologically synthesized in the bacterial cell, PHBV is completely biodegradable. Fungi, in particular, colonize the surface of PHBV in soils and secrete enzymes that digest the plastic. The products of this digestion are absorbed and metabolized, yielding carbon dioxide and water. The rate of biodegradation of a typical PHBV device in garden soil is given as 3 μm (0.012 mil) of thickness per week. Applications of this material are being considered in surgical and orthopedic devices, slow-release systems involving drugs and herbicides, personal hygiene products, and packaging applications. The 1987 cost of PHBV, which was about $33/kg ($15/lb) from pilot-scale plants, may have limited the extent of application of this biodegradable plastic in typical high-volume, low-cost packaging markets.

**Biodisintegration and Biodegradation Studies of Plastic-Starch Blends.** Cole, Wool, *et al.* (Ref 20-22) investigated the degradability of cornstarch-base PEs developed by Otey *et al.* (Ref 18, 19), as described in the section "Starch-Base Polyethylene Films" in this article. Such materials are also being researched and developed by Agri-Tech Industries. The blown-film material (75 μm, or 3 mils) consisted of 40% starch, 10% urea, 25% LDPE, and 25% EAA. Biodegradation of the starch component was studied by monitoring carbon dioxide evolution in loam clay (relatively high in organic matter and very fertile) and sandy soil (low in clay and organic matter). Pure LDPE films were used as controls in similar soils. Although differences in the rate of carbon dioxide evolution from these soils were found, all the starch was removed within about 90 days; the fastest rate (20 days) was in the fine, sandy soil. Typical results for one soil are shown in Fig. 2.

Invertebrate attack on films is more selective than microbial decay. Films made by the Otey formulation are consumed by a range of invertebrates, but these organisms would not consume the pure LDPE films or films containing about 6% starch made by U.S. and Canadian companies. When films are consumed, the remaining plastic material is highly subdivided and is likely to be more susceptible to chemical or microbial attack than intact films would be.

The accessibility of the starch in these plastic-starch blends was determined by enzyme digestion experiments using alpha and beta amylase and amyloglucosidase. Polymer strips were immersed in an appropriately pH-buffered solution for each en-

zyme. A single enzyme type was added to each tube and incubated at 20 °C (70 °F). Merthiolate was added to the tubes to prevent degradation of the products by microbial contaminants. The strips were transferred to fresh buffer and enzyme solutions at different times, and the solutions were analyzed for starch breakdown products (reducing sugars). Hydrolysis of starch in the blends ranged from 11% to greater than 90%, depending on the enzyme used and the blending technique. It was found that only the alpha amylase was able to degrade the starch in the blend. There was neither a release of reducing sugar with the beta amylase or the amyloglucosidase, nor a loss of starch into the solution in the absence of amylase. The total amount of reducing sugar released after 48 h of incubation represents more than 90% of the theoretical yield from the starch in the polymer film.

Changes in the physical characteristics of the above microbial and enzyme degraded films have been analyzed by Wool, *et al.* using percolation models (Ref 21, 22). Accessibility of biodegradable components, such as starch, can be treated as a conductivity (scalar) percolation problem (Ref 23). The most important consequence of this approach is that the biodegradable component must exceed the percolation threshold (~30% by volume) to permit continuous invasion of the fungi or bacteria throughout the bulk of the material. Percolation of fungi on a square lattice is shown in Fig. 3. The observed reduction of modulus, fracture stress, and elongation-to-break of the plastic-starch blends during degradation can be modelled using elasticity (vector) percolation (Ref 24, 25). Plastic materials containing starch additives with a volume fraction appreciably lower than 30% are not expected to undergo biodisintegration by fungal action alone. Such materials may, however, develop a surface growth of fungi, which is a biodisfigurement process that should not be mistaken for biodegradability.

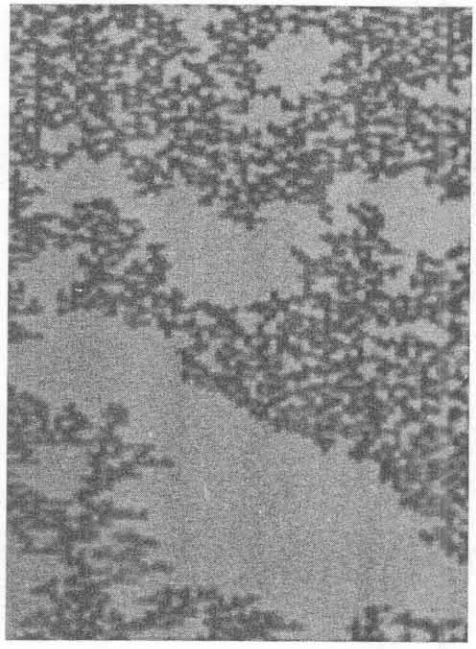

**Fig. 3** Computer simulation of fungal invasion in a two-dimensional matrix containing randomly distributed biodegradable fraction at the percolation threshold

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