

DEGRADATION OF A PLASTIC—POLY EPSILON-CAPROLACTONE—BY HYPHOMYCETES

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SUMMARY

The Hyphomycetes, *Aspergillus puniceus*, *A. ustus* and *Fusarium solani*, were isolated from enriched greenhouse soil, and shown to be capable of using the polyester, poly ϵ -caprolactone, as their sole carbon source. Possible synergistic and successional effects were studied. Statistical evaluation showed that *F. solani* was initially the most effective decomposer of the plastic, but a spontaneous adaptive mutant of *A. ustus*, which arose during the study, exhibited even higher activity. The monomer, ϵ -caprolactone, could also be used as sole carbon source by *F. solani* and the *A. ustus* mutant.

Natural polymers like cellulose, lignin, keratin and chitin are very stable substances which do not break down when exposed to environmental extremes of temperature, radiation and moisture. Vertebrates, which need these abundant compounds as carbon sources, have not evolved enzymes with which to digest cellulose and lignin, but must rely on bacteria and fungi to decompose these substances.

Man, seeking as usual to improve on nature, is producing synthetic polymers in great profusion: about forty families of these giant molecules are now in widespread use. Synthetic polymers are organic materials, in many cases very similar to natural polymers. This means they could be susceptible to biodegradation. However, it is widely believed that these substances are nonbiodegradable.

The majority of man-made polymers appear to resist biological attack almost indefinitely, even when buried in soil. Most plastics are formulated with fillers, plasticizers, heat stabilizers, U/V absorbers, antioxidants, and antimicrobial agents, as well as the polymer. Yeager (1968) postulated the ideal vinyl chloride formulation as including a mobile but nonspewing (not causing a sticky or oily surface), biologically inert plasticizer, an ultra-violet screening agent to prevent depolymerization and cross-linkage of the resin, a stabilizer to prevent heat breakdown, and an antimicrobial agent to prevent growth of surface organisms. Microbial degradation of some components of the mix is quite common, but decomposition of the polymer itself is not. Lignin is as complex as

many synthetics, yet it is decomposed by microorganisms, probably because they were present when lignin was being evolved, and were able to adapt to its gradually increasing complexity in equally gradual steps.

Many natural organic substrates are decomposed extremely slowly, and it is hard to believe that any test using one, five, or even 100 of the possible thousands of saprobic microbes, over a 3-wk period, can be said to establish the biodegradability or otherwise of a plastic—yet much of the literature makes just such a claim. Polymer engineers and chemists have sought a stable product above all else, and as a result most workers have been primarily concerned with preventing any possibility of biological deterioration. In short, they have been more interested in preservation than in decomposition. The present study was an attempt to redress the balance.

Long term soil burial tests and use of a broad spectrum of microorganisms have resulted in various interpretations. Connally (1971) found that after 8 yr soil burial plastics had undergone some physical changes but he concluded that these changes could be caused by nonbiological as well as biological factors. This was supported by investigations carried out in the U. S. Army Natick Laboratories (Kaplan, 1975) which suggested that, because most plastics have not been shown to be completely reduced to CO_2 and H_2O , they are not biodegradable. This view would also virtually deny the decomposability of some plant material, because residues of plants remain in the soil for extended periods of time. A pine needle will withstand the at first successive then later combined attacks of fungi and meiofauna for 8–9 yr before it becomes unrecognizable (Kendrick, 1959), with residual 'humus' possibly accumulating over long periods of time (Alexander, 1971). However, Kaplan (1975) accepts a lesser definition of biodegradation: ". . . the microbially mediated disappearance of . . . polymers," and this is the definition we used. Kestel'man et al. (1972) showed that physical changes in plastic materials were caused by the action of fungi, and supported the view that because there was change in the molecular structure, the physical changes were also due to the activities of fungi.

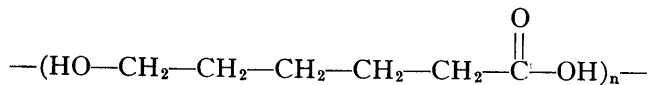
The conflicting results obtained by different authors arose both from the different formulations of the plastics, and the different groups of organisms used. Sometimes these organisms had actually been found associated with the material to be tested, either by direct or indirect enrichment techniques. In these cases materials otherwise thought to be nonsusceptible have been found to be degraded. Using an enrichment technique, Klausmeier and Jones (1961, cited in Osmon et al., 1969) demonstrated that they could isolate fungi capable of degrading almost

all plasticizers, many of which had been previously reported (Berk et al., 1957) to be inert. Mills and Eggins (1974), also using an enrichment technique, were able to show that isolated thermophilic fungi could grow on plasticizers previously reported as unable to support growth. Fukumara (1966a, b) isolated an epsilon-caprolactam-utilizing bacterium from the effluent of a nylon-6 manufacturing plant, and showed that adaptive enzymes caused the degradation of the nylon-6 monomer. Using sewage as a source of microorganisms, Pahren and Bloodgood (1961) showed that of five vinyl compounds—acrylic acid, methacrylic acid, vinyl acetate, methyl-methacrylate, and styrene—none was completely resistant to biological oxidation. Poly-methylmethacrylate has been reported to resist fungal attack (Brown, 1946; Awao, 1971), but Kestel'man et al. (1972) found that it lost its firmness under the influence of *Aspergillus awamori* Nakazawa, which was not included in Awao's test. Polystyrene was reported by Brown (1946) as being resistant to biodegradation, and Heap and Morrell (1968) found that although polystyrene was overgrown by fungi, there was no physical change in the compound. Clearly, circumstances alter cases, and generalization becomes difficult if not impossible.

The picture that emerges from the literature is that synthetic polymers are usually recalcitrant—essentially nonbiodegradable—with exceptions such as ester-based polyurethane and susceptible additives in formulated plastic materials. Where any degradation occurs it appears that decomposition would be very slow (Nykvist, 1974).

The need for a biodegradable plastic, particularly in the packaging industry, has initiated a new surge of investigation. As the existing plastics seem to be either nonbiodegradable or degraded very slowly indeed, two alternatives remain: new biodegradable or self-destructing plastics must be developed, or organisms must be found which can attack existing polymers. Some new polymers undergo controlled photodegradation to small particles, thus quickly reducing the litter problem, while the residue is thought by some authors to undergo complete decomposition over a much longer period of time (Nykvist, 1974; Jones et al., 1974; Guillet et al., 1974).

Union Carbide produces a plastic, called poly epsilon-caprolactone, which is an aliphatic polyester made by the ring-opening of epsilon-caprolactone followed by polymerization. Its formula is as follows:



and its molecular weight is approximately 40,000. This polymer was found, in soil burial tests, to be biodegradable (Potts et al., 1972). We decided to use this compound for more detailed and specific studies of the breakdown process.

METHODS AND RESULTS

1. *Soil burial of polyester.*—Circular wafers of the pure polyester (40 mm diam \times 2 mm thick) were buried in standard greenhouse soil (1 part sand: 1 part peat: 2 parts loam, unsterilized) which had been found to be 'viable' for cellulose-degrading organisms by Federal Test Method #5762 (1968)—more than 50% loss of tensile strength in cotton duck buried for 10 da.

Before burial the wafers were white and smooth. After 20-da soil burial the surface appeared rough with bright yellow raised areas and dark pits. The wafer showed only superficial damage, still retaining its original form and returning to its original shape after bending.

In the infrared absorption spectrum derived by the KBr technique a peak at 1,700 cm^{-1} was due to the C=O bond, that at 1,100–1,200, to the -O- bond. The large peak at 700 was caused by skeletal vibrations in the polymer. These all but disappeared after exposure to the soil. The peak that remained at around 1,400 may have been due to -CH-deformation, but this would be expected to move to 1,400 plus after the loss of the ketone group. It is hard to know what links would be formed by the remaining -CH- chains. The removal of these groups, and of the peak for the skeletal vibrations, suggested that this sample could no longer be called a plastic, but mycelial contamination may have interfered with these results.

Some plastic wafers were left in the soil and observed at intervals over a period of 1 yr. After this time the surface was deeply corroded and some of the 2-mm-thick discs had become perforated (FIG. 1, arrows). Although the original form remained, the wafers had lost their plasticity and were easily broken. Gross fungal mycelium and propagules could be seen producing yellow, green, and black discoloration on the discs. A coelomycete, *Pyrenochaeta acicola* (Lév.) Sacc., was isolated in pure culture from pycnidia on the surface of one of these discs, and was retained for subsequent experimentation.

2. *Weight loss of polyester discs after soil burial.*—In order to make rapid estimates of the susceptibility of the poly epsilon-caprolactone to fungal attack, we decided to use much smaller and thinner discs. Since

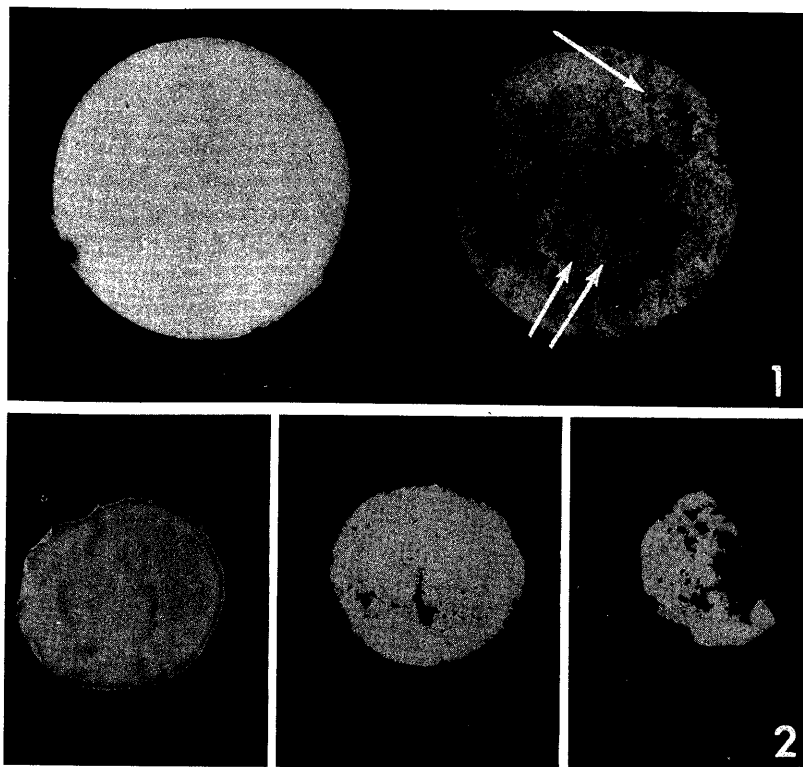


FIG. 1. Plastic wafers before and after 1 yr in soil; arrows indicate perforations in the 2-mm-thick disc after soil burial. FIG. 2. Left to right: plastic discs before soil burial, after 2-wk soil burial, and a fragment recovered after 3-wk soil burial.

the experimental polymer was received from Union Carbide as small cylindrical pellets, we decided to make discs from single pellets, and the following procedure was devised.

A sheet of "Look" nonstick, ovenproof plastic film was spread on a pyroceram (Corningware) hotplate set at 127–135 C (measured by PTC surface thermometer). Single pellets of the plastic were placed on this sheet and allowed to melt then covered with a second sheet of "Look." The clear golden droplets were gently and carefully compressed into a thin layer by means of a broad, flat, stainless steel spatula. The plastic sandwich was then removed and allowed to cool. After the polyester had set, the two layers of "Look" were easily peeled away, freeing the polyester discs, which were now approximately 7 mm in diam \times 0.06

mm thick. Each disc weighed approximately 11 mg. An accurate initial weighing of the discs to the nearest 0.001 mg was made with a Cahn Electro-Balance Model G2, then they were buried and incubated for periods of 1, 2 and 3 wk. When discs were disinterred, adhering soil and surface mycelium were gently removed in repeated changes of water, then the discs were dried at 40 C for 2 da and weighed. It was found experimentally that discs always attained constant weight after this period of drying. The average weight loss of 10 discs after 1 wk of soil burial was 4.07%. After 2 wk of burial the average loss was 9.65%. After 3 wk in the soil only fragments of the discs remained. Since it was impossible to recover all particles, a weight loss was not calculated. The appearance of the discs before and after 2 wk soil burial, and of a fragment recovered after 3 wk, is shown in FIG. 2.

It is clear that the small discs were rapidly broken down in the soil employed for this experiment. We now set out to discover the identities of the organisms responsible for that breakdown.

3. *Isolation of fungi from polyester discs following soil burial.*—Considering the first experiment as a form of enrichment technique, we proceeded to bait the soil from that experiment with polyester discs, 20 mm diam \times 2 mm thick, which were buried at a depth of 2.5 cm in moist soil and maintained at 30 C. Discs were disinterred after 2 wk, and surface soil and debris removed by an initial washing in sterile distilled water. Ten subsequent washings were performed, each involving 5 min of agitation in 50 ml sterile distilled water. One-ml aliquots were taken from each washing, diluted 1:100, and 0.5-ml aliquots of the dilution spread on malt extract agar plates. Further discs were removed from the soil after 3, 4, 5 and 6 wk, and the washing and inoculation process repeated.

The fungi isolated were assessed about 1 wk after inoculation. Five distinct aspergilli were obtained, but three showed marked abnormalities in conidium formation, and could not be identified. Three isolates were retained for further experiments, and cultures deposited in DAOM, as follows: *Aspergillus puniceus* Kwon & Fennell (DAOM 145422), *Aspergillus ustus* (Bainier) Thom & Church (DAOM 145423), and *Fusarium solani* (Mart.) Sacc. (DAOM 145172) which was isolated only at wk 6. Two other isolates, *Pyrenochaeta acicola* (Lév.) Sacc., which had been isolated directly from pycnidia observed on plastic discs, and *Fusarium solani* (DAOM 144846) originally isolated from *Solanum tuberosum* tubers and supplied by the Canadian National Collection of Fungus Cultures, Ottawa, were also used in subsequent experiments.

TABLE I
 AVERAGE PERCENT WEIGHT LOSS OF POLYESTER DISCS INOCULATED WITH
Aspergillus puniceus AND *A. ustus* IN WATER AND MINERALS

	Polyester + water + fungus		Polyester + water + minerals + fungus	Control polyester + minerals	
	3 wk	6 wk	3 wk	3 wk	6 wk
<i>A. puniceus</i>	0.36	1.51	2.9	Negligible	Negligible
<i>A. ustus</i>	0.51	1.31	2.6	Negligible	Negligible

4. *Fungal utilization of polyester as sole carbon source, with and without minerals.*—Five 11-mg plastic discs were placed in each of 10 sterile Petri dishes containing 25 ml sterile distilled water. Five dishes were inoculated with *A. puniceus* and five with *A. ustus*, then all were incubated at 30 C. Percent weight loss was determined after 3 and 6 wk.

A parallel set of 10 dishes was set up, identical to the foregoing except that each dish contained 25 ml of a sterile mineral solution instead of the water. The minerals used were those found in Difco Czapek-Dox agar, as follows: NaNO₃, 3.0 g; K₂HPO₄, 1.0 g; MgSO₄·7H₂O, 0.5 g; KCl, 0.5 g; FeSO₄, 0.01 g; H₂O, 1,000 ml; adjusted to pH 6.5.

TABLE I shows that the presence of minerals increased the degradative ability of the fungi. The average percent weight loss of discs in mineral solution after 3 wk was over six times as great as that in distilled water. The mineral solution was used in all subsequent experiments.

This experiment also suggested that neither of the aspergilli alone, under the given conditions, brought about as much weight loss as did soil burial. Since the conditions of the two experiments were not the same, this result could not be made the basis for a general statement on the relative efficiency of multiple soil organisms versus single species in attacking the polyester (but see below).

5. *Incubation of plastic with individual Fungi Imperfecti.*—This experiment was designed to investigate the extent to which poly epsilon-caprolactone could be degraded by each of the test fungi over a period of time, and to establish whether or not individual species of fungi could reduce the plastic to a powder. Fifty Petri dishes each containing five weighed discs were sterilized, and 25 ml of sterile mineral solution were added to each dish. Ten dishes were inoculated with *A. puniceus*, ten with *A. ustus*, ten with *F. solani* (soil isolate), and ten with *F. solani* (potato

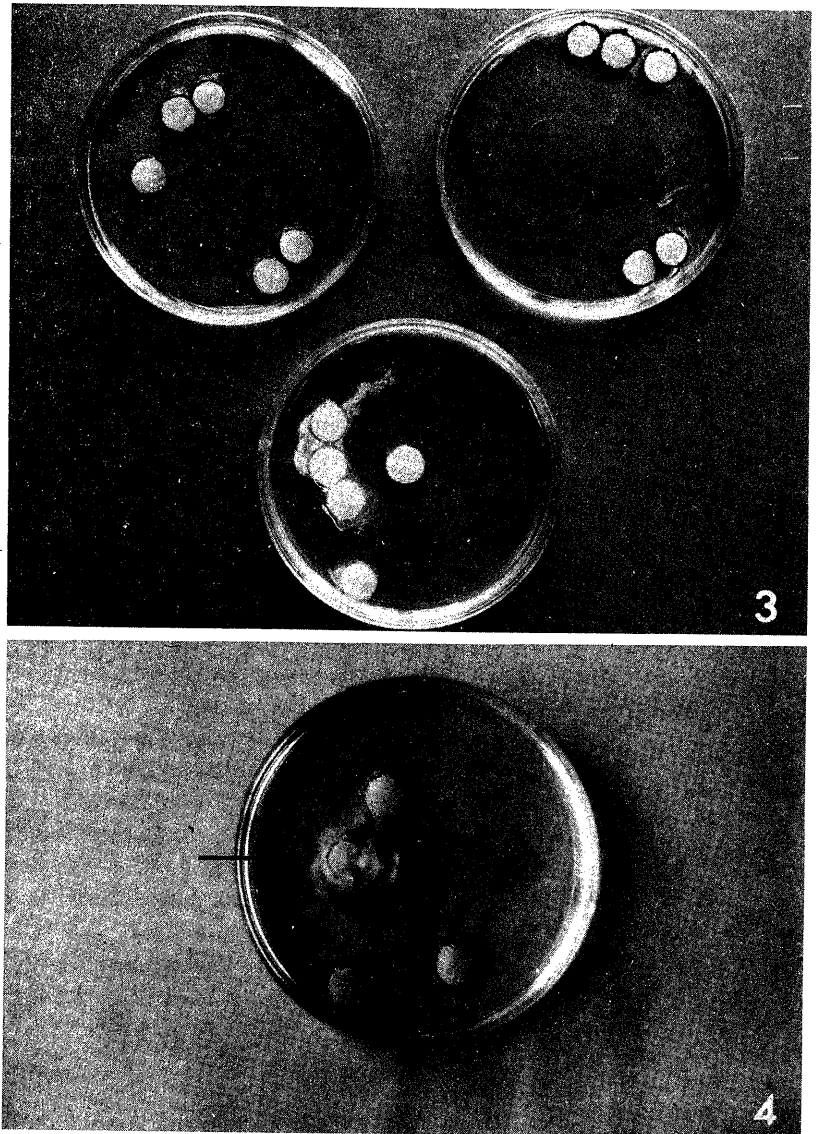


FIG. 3. Two-wk growth of fungi on polyester discs in mineral solution: upper left, *Aspergillus puniceus*; upper right, *A. ustus*; bottom, *Fusarium solani*.
FIG. 4. Appearance of adaptive mutant in *Aspergillus ustus* (arrow) on polyester discs, at 7 wk.

TABLE II
WEIGHT LOSS OF POLYESTER DISCS EXPOSED TO INDIVIDUAL HYPHOMYCETES

Inoculum	% weight loss after					10 wk
	2 wk	3 wk	4 wk	5 wk	8 wk	
<i>Aspergillus puniceus</i>	4.78	19.9*	4.1	4.9	6.0	Unable to weigh
<i>A. ustus</i>	3.46	3.9	3.98	4.65	7.87	
<i>Fusarium solani</i> (soil)	13.47	25.6	29.05	43.8	40.1	
<i>F. solani</i> (potato)	5.19	10.7	16.96	27.4	35.8	
Blank	0.29	0.15	0.42	0.39	0.14	

* This aberrant result, as well as certain irregularities in previous experiments, may have been due to undetected adaptive changes in the fungi concerned.

isolate). Ten dishes received no fungal inoculum and served as controls. The dishes were incubated at 30 C and percent weight loss of the discs was estimated by harvesting dishes at intervals.

Mycelium became visible on all discs during the first wk; during the second wk both aspergilli sporulated around the edges of the discs (FIG. 3). After 2 wk, *F. solani* had covered most of the discs and had extended into the surrounding liquid.

By the third wk, because the discs had become fragile, it became necessary to remove the *Fusarium* mycelium from the plastic by a flotation technique. The discs were repeatedly immersed in distilled water, whereupon the gross mycelium floated off and the plastic could be recovered, dried and weighed. The results of this experiment are presented in TABLE II. The single most obvious conclusion from this experiment is that both isolates of *Fusarium solani* degraded the polyester much more efficiently than did either of the other fungi involved.

In both *A. puniceus* and *A. ustus* mycelial growth became more extensive, and sporulation was reduced, at about the sixth wk. By the tenth wk the discs were thickly covered with white mycelium which extended for some distance into the liquid; no particles of plastic could be recovered for weighing. This change in appearance of the fungi was associated with a rapid degradation of the plastic discs.

A subsequent inoculation of sterile discs with *A. puniceus* and *A. ustus* again resulted in the appearance of a white colony on the *A. ustus* plate at 7 wk (FIG. 4). This strain was isolated on Czapek-Dox agar and has been deposited in the National Mycological Herbarium and Culture Collection at Ottawa (DAOM 147395). It is apparently a mutant of *A. ustus* which possesses augmented ability to degrade the polyester.

6. *Possible synergistic effects among fungi degrading polyester.*—In experiment 3, the *Aspergillus* species were the first to be isolated in washings derived from buried discs at 2, 3 and 4 wk exposure; *F. solani* appeared only at the sixth wk, and *P. acicola* was isolated only directly from plastic discs. This suggested a successional or synergistic effect among the fungi. To test the synergism hypothesis we did an experiment in which sets of five polyester discs in sterile mineral solution were inoculated with all possible combinations of four fungi, *Aspergillus puniceus* (P), *Aspergillus ustus* (U), *Fusarium solani* (F), and *Pyrenochaeta acicola* (A). The results are given in TABLE III.

These results show a possible synergistic effect when *A. puniceus*, *A. ustus*, and *F. solani* were all present. Almost all combinations which included *F. solani* produced more than 15% weight loss after 3 wk, but the really spectacular weight loss occurred when the two aspergilli were present with the *Fusarium*. It was clear that further experiments would be required before we could make a definite statement about synergism (see 7 and 8). *Pyrenochaeta acicola* seemed to have little activity, and was omitted from subsequent experiments.

7. *Possible successional effects among fungi degrading polyester.*—Although the previous experiment suggested that synergistic interaction might have occurred, there was also the possibility that degradation of the plastic might be most rapid and complete when mediated by a succes-

TABLE III
PERCENT WEIGHT LOSS OF PLASTIC WITH VARIOUS COMBINATIONS OF FUNGI

Inoculated with	% weight loss (average of five discs)	
	2 wk	3 wk
P	3.34	6.93
U	6.31	10.2
F	4.38	15.84
A	1.1	1.05
P + U	5.98	13.04
P + F	6.045	23.9*
P + A	3.3	9.56
U + F	6.6	14.47
U + A	7.8	15.1
F + A	6.3	Contaminated
P + F + A	4.89	16.3
U + F + A	9.3	15.7
P + U + F	7.67	39.2*
P + U + F + A	6.89	33.5*
Blank	1.5	0.73

* These specimens were floated onto weighed filter paper, dried and weighed, since it was impossible to separate the fungal growth from the remains of the plastic.

sion of fungi. To test this hypothesis, we subjected the plastic to the action of the three principal fungi separately and in various sequences. The method used was to sterilize the plastic and to kill each fungus after it had been incubated with the plastic for 2 wk, without at the same time changing the chemical composition of the system or rendering conditions inimical to the growth of subsequent inocula.

Discs were weighed, then sterilized with β propiolactone (Matheson, Coleman and Bell, Hydrarlic Acid (β -Lactone), as outlined by Gauger et al. (1967)). About 3 ml of β -propiolactone were poured onto a filter paper at the bottom of a 6-liter glass vacuum desiccator, and a similar amount of water was poured onto an adjacent filter paper to maintain a high humidity in the chamber. The discs were placed in an open Petri dish within the desiccator and the cover of the desiccator replaced. The chamber was aspirated for 5 min, and its contents left exposed to the vapor for 2 h. The stopcock was then opened and the discs left in the container for a further 24 h, allowing the sterilant to disperse.

An experiment, in which discs were inoculated with the three test

TABLE IV
SUCCESSIVE INOCULATION OF PLASTIC DISCS

Inoculum	Time	Harvested (H) or sterilized (S)	Inoculum	Time	Harvested (H) or sterilized (S)	Inoculum	Time	Harvested (H) or sterilized (S)	% weight loss
P*	2 wk	H							3.08
P	2 wk	S	U	2 wk	H				5.49
P	2 wk	S	F	2 wk	H				19.23
P	2 wk	S	U	2 wk	S	F	2 wk	H	6.42
P	2 wk	S	F	2 wk	S	U	2 wk	H	42.14
U	2 wk	H							3.1
U	2 wk	S	P	2 wk	H				4.48
U	2 wk	S	F	2 wk	H				18.52
U	2 wk	S	P	2 wk	S	F	2 wk	H	14.36
U	2 wk	S	F	2 wk	S	P	2 wk	H	24.59
F	2 wk	H							24.41
F	2 wk	S	P	2 wk	H				Crumbled
F	2 wk	S	U	2 wk	H				21.65
F	2 wk	S	P	2 wk	S	U	2 wk	H	17.23
F	2 wk	S	U	2 wk	S	P	2 wk	H	22.19
Blank	2 wk	H							0.49
Blank	2 wk	S		2 wk	H				0.50
Blank	2 wk	S		2 wk	S		2 wk	H	0.29

* P = *Aspergillus puniceus*, U = *A. ustus*, F = *Fusarium solani*.

fungi and incubated for 3 wk, washed, and sterilized as outlined above, then reincubated for 3 wk, showed that sterilization was effective, since there was no growth of fungi subsequent to the sterilization. There was no evidence that the sterilant reacted with the plastic, and since subsequent inocula grew well, we assumed that no sterilant remained on or in the discs.

As in earlier experiments, five 11-mg discs were used in each petri dish. The inoculation and sterilization protocols used, and the percent weight losses recorded, are shown in TABLE IV.

It was again apparent that *F. solani* as sole inoculum degraded the polyester very effectively, and that subsequent inoculations of one or both aspergilli changed the figures very little. The only combination that improved upon *F. solani* alone was *A. puniceus* → *F. solani* → *A. ustus*, and it is difficult to understand how *A. ustus* could double the weight loss produced by *A. puniceus* → *F. solani*. This result may have been due to the appearance of an adaptive mutant of *A. ustus*, reported elsewhere in this study. It was apparent that if the two aspergilli were inoculated first, they tended to reduce the subsequent effectiveness of *F. solani*; and that individually or successively, the aspergilli alone were relatively inefficient degraders of the plastic. There may have been a build-up of staling compounds inimical to the *Fusarium* inoculum.

8. *Possible synergistic effects among fungi degrading polymer—randomized complete block design.*—Earlier experiments (#6, 7) explored the possibility of synergistic or antagonistic effects between *A. puniceus*, *A. ustus*, and *F. solani*, but were inconclusive. Therefore we did a factorial experiment using the three fungi singly, and in all possible combinations, plus a control without fungi, in a randomized complete block design.

Difficulty had been experienced in weighing the polyester remaining at the end of earlier experiments. The process of freeing the discs of fungal growth often resulted in loss of small particles of the plastic. To eliminate this source of inaccuracy in the present experiment, we decided to obtain the dry weight of each entire experimental module before and after the experiment. Five Petri dishes were dried and weighed on a Mettler H54 balance. Aliquots of 25 ml mineral solution were added to each dish, the dishes were freeze-dried for 24 h, then removed to a desiccator where they were kept until constant weight was attained. The difference between the first and final weighings represented the dry weight of the minerals. In this way we found that 25 ml of mineral solution contained an average of 0.08957 ± 0.00317 g minerals. This figure was added to the dried weight of each dish used in the actual

TABLE V
WEIGHT LOSS OF PLASTIC EXPOSED TO VARIOUS TREATMENTS
(RANDOMIZED COMPLETE BLOCK DESIGN)

Treatment	Average change in wt after 3 wk (mg)
P ₀ U ₀ F ₀ *	0.882
P ₁ U ₀ F ₀	0.859
P ₀ U ₁ F ₀	-0.895
P ₀ U ₀ F ₁	-7.86
P ₁ U ₁ F ₀	0.674
P ₁ U ₀ F ₁	-8.812
P ₀ U ₁ F ₁	-10.062
P ₁ U ₁ F ₁	-7.028

LSD (0.01) = 2.699

* P = *Aspergillus puniceus*, U = *A. ustus*, F = *Fusarium solani*.

experiment described below, leaving the weight of the discs plus fungi as the only variable.

Petri dishes and polyester discs were dried, weighed, and sterilized as in previous experiments. Five discs were used in each plate. Next, 25 ml of mineral solution was added to each plate, and the eight treatments shown in TABLE V were set up and disposed in a randomized complete block design in a laboratory incubator with five shelves (blocks).

All treatments were harvested after 3 wk, and no attempt was made to separate the discs from the fungal growth: each entire experimental unit—dish + minerals + plastic discs + fungal growth—was freeze-dried for 24 h then removed to a desiccator until constant weight was attained. Any weight loss recorded was now assumed to represent volatile metabolites or volatile decomposition products of the polymer. The total weight loss of the plastic alone would clearly be higher—considerably higher in cases with profuse fungal growth—since material incorporated into the fungal biomass could not be separately recorded. The results are shown in TABLE V.

Analysis of variance showed that in toto, the treatments resulted in a highly significant difference at the 1% level. Inspection of individual results revealed that the differences were due to the presence of *Fusarium* alone, this fungus showing a highly significant difference at the 1% level. No significant difference was shown for *A. puniceus* and *A. ustus*. The only other significant difference shown using LSD is an apparent inhibitory effect when *A. puniceus* is added to the *A. ustus*—*Fusarium* combination.

TABLE VI
 ORTHOGONAL COMPARISON OF PLASTIC DEGRADATION BY *Fusarium solani*
 AND *Aspergillus ustus* MUTANT

Treatment	Average change in wt after 3 wk (mg)
<i>A. ustus</i> mutant	-12.919
<i>F. solani</i>	- 2.638
Control	2.1714

LSD (0.01) = 5.689.

LSD (0.05) = 4.058.

9. *Orthogonal comparison of plastic degradation by F. solani and A. ustus mutant.*—The mutant form of *A. ustus* isolated from experiment 7 was compared with *F. solani* and a control in a completely randomized design. We already knew that *F. solani* degraded the polyester more efficiently than any other fungus we had so far tested. Changes in weight were monitored as in experiment 8, and the experimental period remained at 3 wk. The results are shown in TABLE VI.

The degradation of poly epsilon-caprolactone by the *A. ustus* mutant was highly significant, at the 1% level, when compared to the control. Experiment 8 showed *F. solani* to be an efficient decomposer of the polyester. In the present comparison, the *A. ustus* mutant decomposed the polyester with even greater efficiency than *F. solani*. It was thus apparent that certain Hyphomycetes have the ability to degrade the polymer, since it was the only carbon source available to them.

Two possible mechanisms might explain the losses in dry weight of the plastic. (I) The polymer may have been completely degraded and metabolized by the fungus, and its basic substances emitted as carbon dioxide and water vapor. (II) The polymer may have been broken down only partially, perhaps to the monomer, which may be volatile.

10. *Volatility of ε-caprolactone (monomer) under experimental conditions.*—Five Petri plates, each containing 0.05 ml of ε-caprolactone monomer¹, an amount comparable to that present in the polyester of the five discs employed in earlier experiments, were weighed, maintained at 30 C for 3 wk, then re-weighed. The average initial weight of ε-caprolactone was 51.5 mg, and after 3 wk the average loss was 29.85 mg, or 57.96%. It is clear that the monomer has an appreciable vapor pressure at the ex-

¹ A clear, colorless, waterlike liquid of M.W. 114, density 1.03, and boiling point 96–97 C.

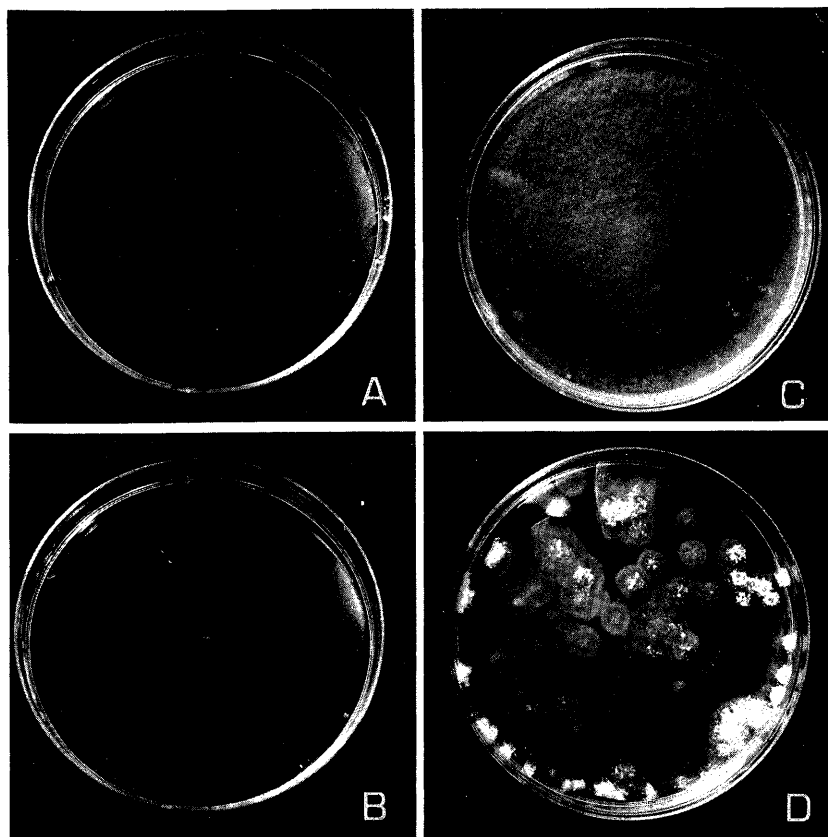


FIG. 5. Comparison between *Fusarium solani* and *Aspergillus ustus* mutant after 2-wk incubation with ϵ -caprolactone monomer: A, *F. solani* + mineral solution only (control); B, *A. ustus* + mineral solution only (control); C, *F. solani* + mineral solution + ϵ -caprolactone; D, *A. ustus* + mineral solution + ϵ -caprolactone.

perimental temperature, though it would hardly be classed as a volatile substance.

Since the end-products of experiments 8 and 9 were freeze-dried to establish a final weight, we set up five further dishes containing monomer to measure the weight loss occasioned by freeze-drying over a period of 24 h. This time the average loss of monomer was 33.24 mg, or 64.54%.

Our final experiment was done to establish whether the fungus can metabolize the monomer as sole carbon source.

11. *Monomer ϵ -caprolactone as sole carbon source for *F. solani* and *A. ustus* mutant.*—Four Petri plates containing 25 ml of mineral solution and 0.05 ml of ϵ -caprolactone were prepared. Two were inoculated with *F. solani* and two with *A. ustus* mutant. Control plates containing only 25 ml of mineral solution and either *F. solani* or *A. ustus* mutant inoculum were also prepared. All were incubated for 2 wk at 30 C. At the end of this period the control plates showed scant growth (FIGS. 5a and b), whereas the plates containing ϵ -caprolactone inoculated with *F. solani* (FIG. 5c) and *A. ustus* (FIG. 5d) showed profuse growth of the fungi.

It is clear that ϵ -caprolactone monomer can function as sole carbon source for the two Hyphomycetes.

CONCLUSIONS

(1) Poly epsilon-caprolactone was inert under the experimental conditions in the absence of test organisms.

(2) Poly epsilon-caprolactone can function as sole carbon source for the Hyphomycetes *Fusarium solani* and a mutant strain of *Aspergillus ustus*.

(3) Although we do not know the nature of the breakdown products formed in this system, we do know that the fungi can utilize the monomer as sole carbon source, and that the monomer is also slightly volatile.

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LITERATURE CITED

- Alexander, M. 1971. *Microbial ecology*. John Wiley and Sons, Toronto. 511 p.
- Awao, T., K. Komagata, I. Yashimura, and K. Mitsugi. 1971. Deterioration of synthetic resins by fungi. *J. Ferment. Technol.* 49: 188-194.
- Berk, S., H. Ebert, and L. Teitell. 1957. Utilization of plasticizers and related organic compounds by fungi. *Industr. Engin. Chem.* 49: 1115-1124.
- Brown, A. E. 1946. The problem of fungal growth. *Modern Plast.* 2: 189-195; cont. 254-256.
- Conally, R. A. 1971. Soil burial of materials and structures. Pp. 168-178. In: *Biodeterioration of materials*. Vol. 2. Eds., A. H. Walters and E. H. Heuck-Van Der Plas. Elsevier, New York.

- Federal Test Method Standards.** 1968. Plastics: Method of Testing No. 191. Viability Tests. Method 5762.
- Fukumara, T.** 1966a. Bacterial breakdown of ϵ -caprolactum and its cyclic oligomers. *Plant Cell Physiol.* 7: 93-101.
- . 1966b. Splitting of ϵ -caprolactam and other lactams by bacteria. *Plant Cell Physiol.* 7: 105-114.
- Gauger, G. W., B. C. Spradlin, J. L. Easterday, D. T. Knuth, R. D. Davidson and G. H. Litchfield.** 1967. Microbial deterioration in electronic components. I. Selected components and materials of construction. *Developm. Industr. Microbiol.* 8: 372-416.
- Guillet, J. E., T. W. Regulski and T. B. Mcanoney.** 1974. Biodegradability of photodegraded polymers. II. Tracer studies of bio-oxidation of ecolyte PS polystyrene. *Environ. Sci. & Technol.* 8: 923-925.
- Heap, W. M., and S. H. Morrell.** 1968. Microbial deterioration of rubber and plastics. *J. Appl. Chem.* 18: 189-194.
- Jones, P. H., D. Prasad, M. Heskin, M. H. Morgan, and J. E. Guillet.** 1974. Biodegradation of photoperiod polymers. *Environ. Sci. & Technol.* 8: 919-923.
- Kaplan, A. M.** 1975. Microbial decomposition of synthetic polymer materials. Pp. 535-545. In: *Proceedings of the first intersectoral congress of IAMS. Vol. 2—Developmental microbiology, ecology.* Ed., T. Hasegawa. Science Council of Japan. Tokyo.
- Kendrick, W. B.** 1959. The time factor in the decomposition of coniferous leaf litter. *Canad. J. Bot.* 37: 907-912.
- Kestel'man, V. N., V. K. Yarovenko, and E. I. Mel'nilova.** 1972. The corrosion of polymeric materials under conditions of the micro-biological synthesis of enzymes. *Int. Biodet. Bull.* 8: 15-19.
- Mills, J., and H. O. W. Eggins.** 1974. The biodeterioration of certain plasticizers by thermophilic fungi. *Int. Biodet. Bull.* 10: 39-44.
- Nykvist, N. B.** 1974. Biodegradation of low-density polyethylene. *Plast. & Poly.* 42(161): 195-199.
- Osmon, J. L., R. E. Klausmeier, and E. I. Jamison.** 1970. The ability of selected yeast cultures to degrade plasticized polyvinyl systems. *Developm. Industr. Microbiol.* 11: 447-452.
- Pahren, H. R., and D. E. Bloodgood.** 1961. Biological oxidation of several vinyl compounds. *J. Water. Pollut. Control Fed.* 33: 233-238.
- Potts, J. E., R. A. Clendinning, and W. B. Ackart.** 1972. *An investigation of the biodegradability of packaging plastics.* Environmental Protection Technology Series. U. S. Environmental Protection Agency, Washington, D. C. 90 p.
- Yeager, C. C.** 1968. Deterioration of vinyl resin systems—special considerations. *Developm. Industr. Microbiol.* 9: 222-241.

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