Microbial and Oxidative Effects in Degradation of Polyethene

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Synopsis

Biodegradative conversion of ^{14}C present in high-density (linear) polyethene (HDPE) film to respiratory $^{14}CO_2$ during a two-year aerated cultivation with soil or with *Fusarium redolens* dropped from 0.36% by weight to less than 0.16% by weight when the HDPE film was deprived from most of its low molecular components by extraction with cyclohexane. Decrease of $^{14}CO_2$ production after extraction could be observed in different abiotic aging cultures. This is direct evidence for a primary utilization of the short-chain oligomeric fraction of the main crystalline material. The extractable oligomeric fraction of HDPE was analyzed by gel permeation chromatography (GPC), and $\overline{M}_n$ 1049, 1088, and 1297 were found in untreated, aged, and biodegraded material, respectively, indicating that microbes can oxidize somewhat longer polyolefin chains than abiotic forces do during aging. The limited degradation of HDPE confined to extractable material is comparable to the degradation of straight-chain *n*-alkanes and presumably proceeds according to a similar mechanism. Such material (*n*-alkanes) can exist in the interstitial spaces between the crystalline lamellae as fringed micelles which infiltrate these cavities as amorphous clusters but are also produced to some extent during aging and weathering. Protection of HDPE by antioxidant (a sterically hindered phenol) resulted in an inhibition of microbiological catabolism of ^{14}C to $^{14}CO_2$. Aging was also suppressed in this way, indicating that although remnants of the supported CrO_3 polymerization catalyst are responsible for a slight but cumulative abiotic oxidation of the unprotected polymer, this effect will be counteracted too by the antioxidative additive. As biological degradation is superimposed on the chemistry of aging, a mutual synergism between the two effects is feasible.

INTRODUCTION

Mineralization and biospheric recycling of synthetic polymers, especially inert ones, are challenges to be met with a great number of uniformly conducted experiments and observations extended to several years. When starting the present sequence of long-term runs, we first studied the biodegradability of low-density (branched) polyethene (LDPE) films, with or without the addition of a photochemical oxidation accelerator and after an exposure to ultraviolet irradiation of varying wavelength.[1] This was done partly to challenge inherent restrictions of the initial work of Nykvist.[2] Realizing both the difficulties and the importance of the problem in general, we shifted over to the study of the somewhat less inert high-density (linear) polyethene (HDPE) as film[3] and also in the form of HDPE powder since the high surface/volume ratio here offers an increased chance for environmental impact to occur.[4] Also the effect of incorporating a biodegradable long-chain alkane (dotriacontane) as additive to HDPE film was studied and was found not to affect the basic inertness of the polymer.[5] A preliminary draft of the results of these investigations was presented for open debate as a thesis which also contained a collection of manuscripts in publication.[6]

It was reasoned in our previous presentations, but never finally proved, that

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$^{14}CO_2$ evolved in microbial degradation experiments or in abiotic aging procedures (as measured by its scintillation), must invariably stem from the polymeric material. It was also assumed that this radioactivity was derived from the mobilization and conversion of ^{14}C inherent in rather low molecular weight fractions of the polymer, as a result of a microbial extracellular enzymatic alkane catabolism.

The present work, aimed to prove these assumptions, was taken mainly from ref. 6. Reports have also been submitted elsewhere on continued experiments with LDPE, which correlate the results with those already obtained with HDPE.[7,8]

EXPERIMENTAL

HDPE film was prepared usually without antioxidant or any other additive except when stated otherwise, and was randomly labeled with ^{14}C by mixing about 1 ppm (1 ^{14}C per 1.16×10^6 ^{12}C) of marked monomer with the unmarked bulk of monomer. The labeled HDPE was supplied specifically for this research by Unifos Kemi AB, Sweden (cf. ref. 3 for further details), similarly to the ^{14}C-labeled HDPE protected by antioxidant, as well as other specific preparations used here or in previous parts of this series.

Films were 0.02 mm thick unless otherwise specified and were prepared by molding from HDPE powder with a 10 m^2/g surface/mass ratio. An HDPE film with a conventional antioxidant as additive was also produced for use in some of the experiments. The antioxidant was a sterically hindered phenol similar to butylated hydroxytoluene. Also a special HDPE film, deprived of its low molecular weight components, has been prepared from the HDPE film without additives by extraction with cyclohexane in a Soxhlet apparatus for 24 hr in an inert atmosphere (N_2).

The extraction described above was also performed on biodegraded samples already used in some of the earlier two-year runs, the primary results of which have been reported previously.[3] Molecular weight distribution patterns in extracts from samples that had been maintained for two years in nutrient solution inoculated with soil or containing 0.05% $AgNO_3$ instead were compared with those in extracts from untreated HDPE with the aid of gel filtration.

Gel permeation chromatography (GPC) (or gel filtration) was applied in modified form as described by Holmström and Sörvik,[9] and the results were calculated as indicated in Figure 2 and Table II. A Waters Associates GPC model 200 chromatograph operating at 135°C with 1,2,4-trichlorobenzene as solvent was used. The column combination consisted of five Styragel columns with permeabilities ranging from 10^3 to 10^7 Å.*

Techniques for biodegradative attack, through maintenance of the test samples under continuous aeration in separate jars containing a nutrient medium inoculated either with cultivated soil or with *Fusarium redolens*, the main test organism in our experiment, were described in detail previously, as was also the capture of $^{14}CO_2$ in KOH solution and its monthly assessment in a liquid scintillation counter, Packard Tri-Carb model 3375.

* This work was performed by the Polymer Group, Department of Organic Chemistry, Chalmers University of Technology and University of Gothenburg, Gothenburg, Sweden, under the supervision of Dr. Arne Holmström.

RESULTS

Comparison of Resistance to Degradation of Unextracted HDPE with that of HDPE Deprived of Its Low Molecular Weight Fraction

The notion that the rather limited metabolization of the highly resistant polymers by microorganisms must be accounted for by the selective assimilation of rather low molecular weight oligomeric fractions of the synthetic materials in question was advanced by several research workers in the field of plastic deterioration research. Jen-Hao and Schwartz[10] reasoned so in the early sixties, as did Hueck[11,12] and others later on. In a continued screening of commercially available plastics with the aid of the biodegradative standard procedure designated as ASTM D-1924-63[13] using a mixed culture of four mold strains, Potts et al.[14] concluded that high molecular weight polyethene is not biodegradable. On the other hand, *n*-alkanes not exceeding a molecular weight of 500, with *n*-dotriacontane (n-$C_{32}H_{66}$) as the heaviest representative of this group of olefins, will be metabolized by the test organisms. The chain fragments of the linear HDPE belonging to the oligomeric fraction (below MW 1000) of the polymer were assumed to be basically identical with the aforementioned low molecular weight *n*-alkane homologues.[4,5]

Potts et al.[15] also paraphrased the boundaries of this limited biodegradation of polyethenes by extending and confirming the findings of Barua[16] through testing a series of higher alkanes of both linear and branched types and showing that only the normal ones supported growth of the ASTM test organisms within the above molecular weight limits (n-$C_{32}H_{66}$). These "higher alkanes" are simply lower oligomers of the polyethene material according to our definition.[4,5] As a consequence of the inertness of the branched alkanes one might conclude that if branched LDPE showed any activity in the ASTM test, then this would be attributed to the breaking down of different additives in the waxy plastics. Our ^{14}C tracing experiments so far mainly confirmed the general conclusions of Potts and his collaborators at some basic points, although our deductions were based on more or less indirect evidence.

A fairly direct attempt, intended to cast new light on this problem, is shown in Figure 1, where an aerobic biodegradative attack at 25°C in the dark on HDPE film (prepared without antioxidant) inoculated heavily with cultivated soil and maintained aerobically in a nutrient medium gave the typical declining paraboloid curve within the range of about 0.5% of the total polymeric material in a two-year run. This was already well documented in our earlier articles from the scintillation measurements of trapped $^{14}CO_2$ evolved from the ^{14}C randomly intermingled with the HDPE material. When, however, such HDPE film preparation was first deprived of its amorphous low molecular weight oligomeric fraction by extraction of the film with cyclohexane and then exposed to biodegradative impact by soil, the evolution of $^{14}CO_2$ hardly reached 0.16% by weight of the test material in a two-year test, thus giving further support to the assumptions outlined above and prevalent in this field of research.

Nevertheless, the secondary results of this experiment revealed that soil still liberated about twice as much $^{14}CO_2$ from the cyclohexane-extracted HDPE film, as the amounts found after the aging of extracted HDPE film in distilled water only. These secondary controls—the extracted HDPE films aging in water—gave off some $^{14}CO_2$ in a cumulative manner, but always less than the amounts

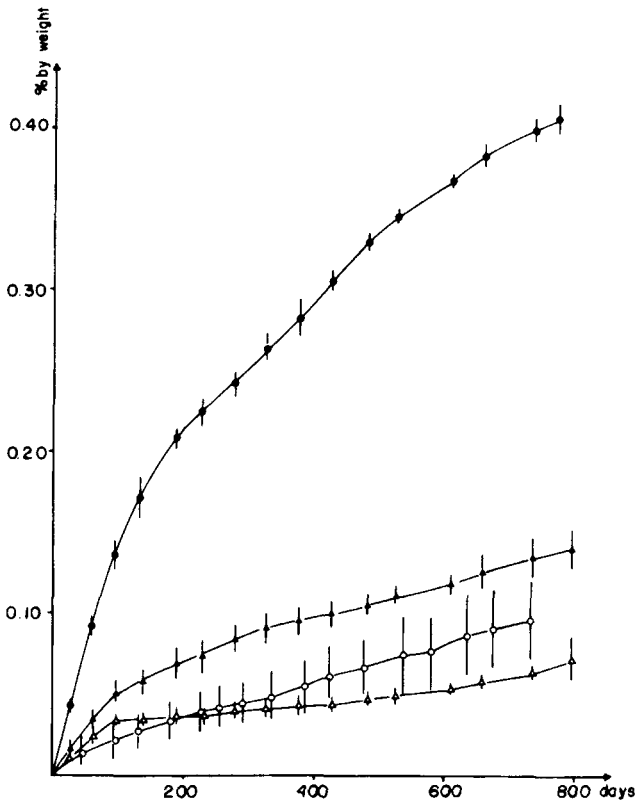

Fig. 1. Long-time aerated maintenance (days on abscissa) of antioxidant-free HDPE film in nutrient medium inoculated with soil (A in Table I) (●); the same but with film first extracted with cyclohexane (B in Table I) (▲); film aerated in distilled water only (C in Table I) (○); the same but after the low MW material was first extracted from the film (D in Table I) (△). Ordinate indicates $^{14}CO_2$ scintillation counts expressed as percentage of total HDPE material present. Each curve represents averages of consecutive readings from three to ten parallel vessels (see Table I).

liberated from the unextracted film aging in water only. Before drawing further conclusions from these experiments, however, some complementary facts should be considered.

Selected data of final readings taken from a similar experimental series are presented in Table I. Here, soil degradation of extracted HDPE film was again compared with that of the unextracted one. Instead of aerating in distilled water alone, however, the two types of HDPE films were kept in uninoculated nutrient media which had been rendered aseptic by the addition of 0.05% silver nitrate. Average percentages of extracted low molecular weight material from the HDPE samples as well as final pH values are also presented in this compilation. It should be recognized, however, that these final readings were reached in our conventional way, i.e., from monthly radioactivity measurements, obtaining a succession of values which were as consistently increasing with time as in any of the long-time run curves published earlier by us. The distribution of $^{14}CO_2$

TABLE I
Comparison of Untreated HDPE Film with That Deprived of Its Low Molecular Fraction by Extraction with Cyclohexane[a]

Designation	Samples and treatment	Number of parallel samples	% Extracted low MW material	% ^{14}C recovered as $^{14}CO_2$	% ^{14}C in nutrient medium	Sum of ^{14}C recovered	^{14}C in nutrient medium as % of total radioactivity recovered	pH in nutrient medium
A	HDPE in nutrient medium inoculated with soil	10	—	0.364 ± 0.030	0.021 ± 0.006	0.385 ± 0.031	5.6 ± 1.5	5.96 ± 0.81
B	(HDPE ext.) in nutrient medium inoculated with soil	4	2.56 ± 0.52	0.157 ± 0.010	0.004 ± 0.001	0.161 ± 0.010	2.4 ± 0.8	4.91 ± 0.31
C	HDPE in uninoculated nutrient medium (with $AgNO_3$)	3	—	0.116 ± 0.017	0.083 ± 0.006	0.200 ± 0.021	41.9 ± 2.9	8.35 ± 0.62
D	(HDPE ext.) in uninoculated nutrient medium (with $AgNO_3$)	5	2.52 ± 0.47	0.091 ± 0.027	0.029 ± 0.009	0.120 ± 0.031	24.9 ± 6.5	7.83 ± 0.34

[a] Radiometric measurements were calculated as percent recovery of the total weight of the test material, after two years of aerobic maintenance in the dark at 25°C in nutrient medium inoculated with soil, or kept aseptically by addition of 0.05% $AgNO_3$.

recovery values also clearly showed the same tardy but cumulatively increasing trend evident in Figure 1.

Not much can be commented on the relatively low percentage by weight (about 2.5%) of oligomeric material extracted from HDPE by boiling cyclohexane. This low gain is an empirical fact accounted for by the high crystallinity of the HDPE material and by the method of extraction we used. By analogy, it can be recalled that Adams and Goodrich[17] eluted 1.98% polyethene with isopropanol, which is essentially the same order of magnitude considering the more polar solvent. Much higher values, averaging 9%, were reported by Griffin[18] when Soxhlet extraction was performed after the plastic material was exposed to composting. No extraction results prior to composting were disclosed here so that no direct comparison with our values is feasible. On the other hand, the data of Table I allow us to make further inferences which are not self-evident from Figure 1 alone. Thus, it can be seen that the total ^{14}C recovery, the sum $^{14}CO_2$ and ^{14}C found to be present in the nutrient medium, was higher when HDPE was aged alone with $AgNO_3$ than in the case when extracted HDPE was attacked by the soil (rows B and C in Table I).

An another peculiar result of these experiments, a comparison of row D (the extracted HDPE film kept abiotically in $AgNO_3$) and of row C (the same but unextracted) reveals a significant accumulation of radioactivity in the aseptic medium, while the level of abiotic $^{14}CO_2$ liberation is much the same for the extracted and the unextracted films. An explanation of the presence of this unusually high ^{14}C content in the aqueous nutrient medium is that it stems from low molecular weight water-immissible HDPE, fractions evidently peaching out as greasy floccules from the crystalline HDPE material and thus became subjected to a slow nonenzymatic oxidative autocatalytic conversion to CO_2.[4]

Finally, a comparison of rows B and A (where the HDPE film was exposed to soil degradative attack) indicates that the differences in $^{14}CO_2$ recovery between the cyclohexane-extracted polymer (row B) and the unextracted one (row A) are as significant as those in Figure 1. It can also be recognized from these data that the amount of the soluble ^{14}C in the culture fluid is rather low, especially if expressed as percentage of the total radioactivity recovered (column 5 of Table I). This indicates that in the biodegradative or, more explicitly, enzymatic conversion of polymeric ^{14}C to $^{14}CO_2$, the oligomeric fraction originating from the solid HDPE and migrating to the aqueous phase is a useful substrate for the catabolic enzymes. One can also raise the question why polymeric ^{14}C goes mainly to $^{14}CO_2$ instead of staying at least partly in the medium as organic acids, bases, alcohols, or other metabolites produced by the attacking microorganisms. We have already discussed this aspect[3] and mentioned the role of a possible shifting of a Pasteur effect to the respiratory side because of the strong aeration. This does not, however, explain the abiotic mineralization of polymeric carbon to CO_2 in samples aging in water. Both questions, however, still await a rigorous elucidation of the underlying molecular mechanisms of both nonenzymatic and enzymatic CO_2 liberation. Yet our observation of low ^{14}C dissolved in the medium compared with $^{14}CO_2$ evolvement is evidently in agreement with the findings of Achammer et al.,[19] who showed that H_2O and CO_2 were indeed the principal volatile products recovered after degradation of polystyrene and other polyolefins.[19–21]

Molecular Weight Distribution in Extracts from HDPE

Molecular weight distribution measurements were considered as a suitable complementary study of the substances that might be attacked by microorganisms in polyethene. We thus applied gel permeation chromatographic techniques (GPC) used mostly for molecular weight determination attempts for large molecules such as those measured here. This molecular sieve technique gave values of 12,000 for $\overline{M}_n$ and 93,000 for $\overline{M}_w$ with the HDPE sample ($\rho = 962$). The sample used most often had an exceptionally high value of $M_n = 250{,}000$ and was therefore beyond the capacity of the GPC method. We could find no significant difference between the HDPE film samples before and after long-term exposure to microbial and/or soil attack. Considering the low biodegradation percentage, this failure was to be expected.

Working with unstabilized polypropylene powder—another inert polymer—Adams and Goodrich[17] showed that the $\overline{M}_w$ value decreased from 230,000 to less than 50,000 in a torque rheometer if sheared to the melting temperature. We have, however, not exploited this type of approach since the labeled HDPE material used by us was not sacrified after two years of work but was kept for experimentation for some years to come.

Instead, another approach to this question was possible by examining the material extracted with cyclohexane from antioxidant-free HDPE film (1) before the experimental run (E in Table II); (2) after two years of aerated aseptical maintenance in a nutrient medium (by addition of $AgNO_3$) (F in Table II); and (3) after two years of aerated maintenance in a medium inoculated with cultivated soil (G in Table II).

About 3% low molecular weight material was extracted totally in a Soxhlet apparatus from each type of HDPE sample and measured by GPC. The curves thus obtained show rather clearly that the MW maximum is shifted from 1800 in the extract obtained from the untreated HDPE film (E) to as much as 2400 in that from the film in the aseptic medium and to 3,000 from the HDPE exposed to long-term attack by soil organisms. For further details Figure 2 and Table II should be considered.

We suggest the following explanation for this difference in molecular weight distribution. The extract obtained from the untreated HDPE film (E) contains mainly short-chain oligomers of HDPE and also still shorter chain scission products of the same origin, corresponding to higher n-alkanes. Long-term aeration in a liquid medium under aseptic conditions (with 0.05% $AgNO_3$) leads evidently to some restricted autocatalytic oxidative degradation of the antioxidant-free HDPE. Since this affects mainly the shortest chain members of the extractible low-molecular-weight fraction, the GPC values of the extract in F shows significantly higher molecular weight than in E.

Finally, in the biodegradative attempt with soil (inoculated on the nutrient medium, G in Fig. 2), a combined effect of autocatalytic oxidation and impact of soil microorganisms on the plastic film works synergistically with the result that still some portion of the low molecular weight extract will be mineralized, leaving a slightly higher molecular weight portion (G).

The question arises why microorganisms of the soil should utilize still higher molecular weight alkanes than the hydrocarbons in the fraction oxidized abiotically. The answer is that among alkane utilizers most species prefer alkanes with medium or higher chain lengths (C_8–C_{12}) and will often be inert with the

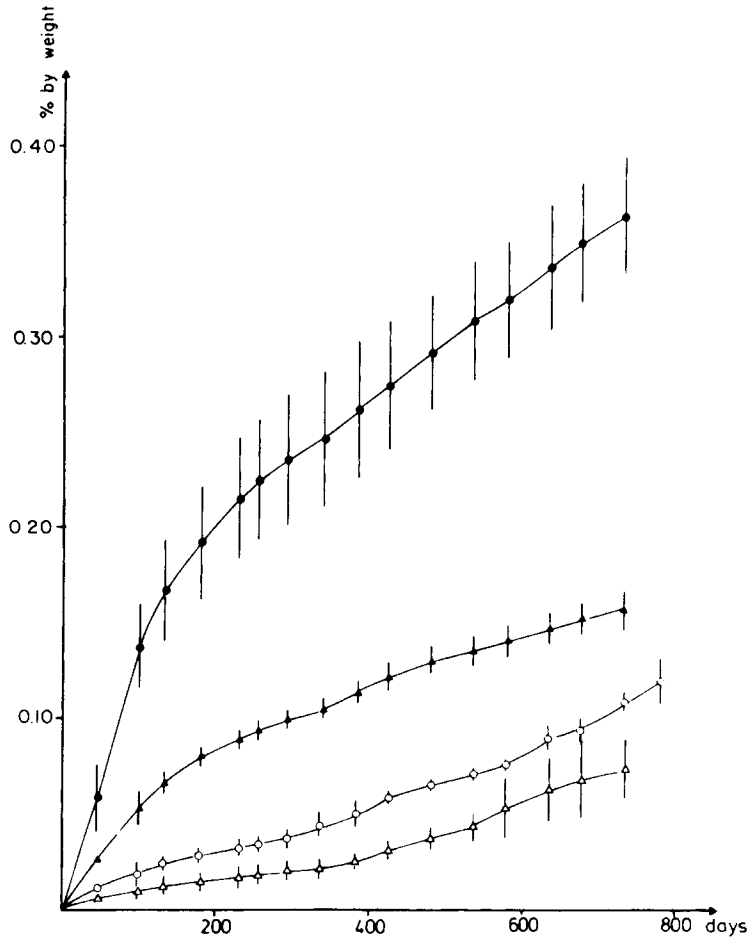

Fig. 2. GPC measurements of (E) cyclohexane extract from unextracted HDPE film; (F) the same but aerated aseptically for two years in a medium containing 0.05% $AgNO_3$; and (G) as before, but in a nutrient medium inoculated with soil (see Table II).

shorter ones ($<C_8$).[22] This problem is, however, rather complex and needs further elucidation, not in the least with respect to the fact that simultaneously with the oxidative degradation of the low molecular weight fraction of HDPE, some further mobilization of extractible shorter chain material must occur since the percent extraction is somewhat greater after some conversion to CO_2 (F and G in Table II) instead of being less than in the extract of the untreated HDPE film (E).

A recent report of Iyoda[23] on GPC measurements of polyethenes under outdoor long-term degradation is not at variance with our results since, according to this report, the molecular weight (total) of the samples decreased with a "narrowing of distribution width in soluble parts," indicating scission of long molecular chains rendering low molecular weight fractions available to mineralization. Still, a direct comparison of this work with ours is hardly possible, since the quality of polyethenes was different (e.g., in respect to their antioxidant content). Also our investigations were focused merely on the extractible fractions.

TABLE II

Degradative Release of $^{14}CO_2$ from HDPE Film in Correlation to GPC Measurements on the Cyclohexane Extracts of the Same Fractions[a]

Designation	Samples and treatment	Earlier recovered material: Evolved as $^{14}CO_2$, %	Earlier recovered material: In the nutrient medium, %	Earlier recovered material: Sum of ^{14}C, %	Extracted low MW material, %	Low MW fraction: $\bar{M}_n$	Low MW fraction: $\bar{M}_w$	Low MW fraction: $\bar{M}_z$	Low MW fraction: $\bar{M}_w/\bar{M}_n$
E	HDPE stored in the laboratory	—	—	—	3.02	1049	2272	4123	2.17
F	HDPE two years in uninoculated nutrient medium (with $AgNO_3$)	0.12	0.08	0.20	3.36	1088	2602	4522	2.39
G	HDPE two years in nutrient medium inoculated with soil	0.36	0.02	0.38	3.29	1297	2875	5127	2.22

[a] Symbols as in Fig. 2.

Retardation of HDPE Degradation by Antioxidant and the Nature of the Slow Abiotic Oxidative Deterioration

Although it was profitable indeed to work with antioxidant-free inert HDPE material with respect to its possible biodegradation, one must still realize that despite the advantages of small important changes detected, the results are not directly applicable to commercial HDPE plastics which always contain various additives, including some kind of antioxidant. Consequently, experiments were designed to compensate for this discrepancy by obtaining ^{14}C-labeled material from the same source (Unifos, Stenungsund) but with conventional amounts (0.1%) of a sterically hindered phenol added as antioxidant according to the patent-protected processing of this company.

In comparative tests with HDPE film with and without an antioxidative additive, the biotic degradation process with antioxidant was greatly inhibited to values almost as low as those of the antioxidant-free abiotic aging (Fig. 3 and Table III, H and J compared with K). On the other hand, the abiotic aging proper (K) was also significantly hindered by the antioxidant (L).

The inhibitory action of the antioxidant is slightly more pronounced when the total recovered ^{14}C is compared in each instance. Seen from this angle, the fungal degradation of HDPE film containing antioxidant was lower than the abiotic aging of the antioxidant-free polymeric film (J and K in Table III).

In an early, though comprehensive, technical paper dealing with the thermal oxidation of polyethylene, especially coordination-polymerized Ziegler polyethylene, Grieveson et al.[24] compared pilot plant preparations containing antioxidants with those deprived of such an additive. In this respect, the quoted work is a forerunner of our work. However, because of the basic difference between their alkyl aluminum titanium chloride type and the supported chromium oxide catalysts in our case, and because of the temperature differences, the results are not directly comparable. Still, their finding that the rate of absorption depends mainly on the surface area of the polymer, especially if the film is thicker than 0.5 mm, has a direct relation to our work. We might in the present case give priority instead to another and probably much more significant effect, namely, the catalytic oxidative degradation caused by the remnants of the polymerization catalyst in the HDPE film. As mentioned above in connection with the work

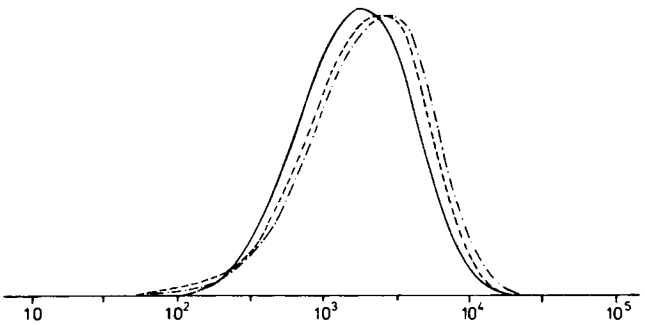

Fig. 3. Degradative attempt with *Fusarium redolens* in conditions comparable with those of Fig. 1; HDPE film without (●) and with antioxidant (H and J in Table III) (▲). Uninoculated aging in distilled water; HDPE without (○) and with antioxidant (K and L in Table III). Each curve represents the average of consecutive readings from three to five parallel vessels (see Table III).

TABLE II
Permeation Data for Hydrocarbon Gases in FEP[a]

Hydrocarbon	Present study			Britt (ref. 11)		Pasternak et al. (ref. 10)	
	E_P	P_0	$P_{90°C} \times 10^{10}$	E_P	$P_{90°C} \times 10^{10}$	E_P	$P_{90°C} \times 10^{10}$
Methane	8.37	1.45×10^{-4}	13.30			8.31	10.96
Ethane	8.80	1.62×10^{-4}	8.30	9.03	6.91	8.74	6.32
Propane	10.48	8.40×10^{-4}	4.14	9.87	3.34	10.29	3.23
n-Butane	12.94	1.78×10^{-2}	2.90	11.56	3.02		
Isobutane	16.10	0.56	1.14				
Propane in unannealed film	11.71	8.12×10^{-3}	7.54				

[a] E_P in kcal/g mole; P_0 and $P_{90°C}$ in [cm^3(STP) cm]/(cm^2 sec cm Hg).

of Grieveson et al., the catalyst used for our preparation was a supported chromium oxide one, $CrO_3(SiO_2 \cdot Al_2O_3)$.[25,26] We have pointed out earlier[4] that according to the Union Carbide Unifos process, the catalyst was not removed from the HDPE product after termination of the polymerization process. Potts has outlined the consequences to be expected from the remnants of the catalyst in the final polymeric product (personal communication 1977, also ref. 27). This type of catalyst is effective in the decomposition of hydroperoxides—the primary cause of degradative scission—and will also evidently function as such in HDPE film, although the surface/volume ratio here is not so favorable for the uptake of some form of oxygen as it was for fine-mesh powder.[3] The ambiguous role of the supported chromium oxide catalyst, being both an oxidative polymerizer and a deteriorating agent in the same material, is certainly not unique. Metal soaps and oxide stabilizers such as zinc oxide converted to zinc chloride in poly(vinyl chloride) will also function as efficient degradation catalysts.[28]

The problem of the behavior of the catalyst residues cannot, however, be solved within the scope of the present work, since some of our results are contradictory to theoretical expectations. For example, we found that the abiotic aging process was of similar magnitude for both powder and film. Furthermore, this aging was cumulative in time as an autocatalytic process rather than a reaction strictly dependent on the presence of some catalyst which must necessarily be exhausted or poisoned in a period as long as that covered by our experiment. It should be remembered that we tacitly assumed that the catalyst residue active in degradation is indeed that part of the chromium oxide in the HDPE which retained its catalytic ability after the completion of the polymerization process.

Bond breakage owing to transition state formation of ^{14}C is a known effect, but its relevance to our work with HDPE might be limited because of the low proportion of about 1 ppm ^{14}C (randomly distributed in the labeled HDPE), as will be shown in our forthcoming paper on infrared spectroscopic and gel permeation chromatographic investigations applied to this material.

Finally, the incorporation of a potent antioxidant in the polymer should effectively counteract the essentially inhibitory effect of the chromium oxide residue. This was, however, not the case, as is evident in Figure 3 and Table III, since the abiotic aging in the HDPE film was only partially retarded by the presence of the antioxidant and was still as progressively cumulative over a two-year period as the inhibited one.

Relevant Microbiological Aspects

Because of the controversy between the schools of Potts,[29] Wiles and coworkers,[30] and others working with LDPE who consider it to be very long-lasting (at least in anaerobic conditions in the dark) and degradational believers such as Wallhäuser,[31] Nykvist,[2] Guillet,[32] and Griffin,[18] we are obliged to emphasize some pragmatic differences in experimentation compared with that of the former group who usually apply the ASTM recommendations which were criticized by us.[4] Especially noteworthy are the facts that the conditions in our work were strongly aerobic and that we measured the release of marked C atoms from the polymer with a rather sensitive method in a range that was far below the effectiveness of the visual rating of microbial growth, if any, and for a much longer period of time than the usual three to five weeks used by the above workers.

Also, the chances for microbial growth and the methods for its recognition were quite different, as discussed earlier.[3,4] In contrast to the Czapek–Dox medium we used both soil inoculum and inoculation with a pure culture of *Fusarium redolens* isolated from soil cultivations for the maintenance of polyethene test samples in a specified nutrient medium that was enriched with B vitamins as growth factors. The progression of actual microbial growth was not measured, but in all our experiments (including the apparently abiotic maintenance on distilled water only, or in cultivation medium containing 0.05% $AgNO_3$) the presence of living microorganisms in the cultures was controlled by outplanting after the first and second year of aerated maintenance.

At least one, and often several, strains of the limited number of fungal species found in these experiments was recovered in each of the culture vessels, despite the long cultivation periods and the scarce media. Besides, two or three strains of bacteria with slightly colored colonies were occasionally found. The lower fungi, or molds, isolated from the soil cultures or infected cultivations were identified as *Acremonium kiliense, Aspergillus versicolor, Verticillium lecanii,* and *Fusarium redolens.* Exceptionally, the presence of some of the following organisms could also be detected: *Aspergillus ustus, Fusarium oxysporum, Pullularia pullulans, Cladosporium* sp., *Penicillium* sp., and *Trichoderma* sp.

On the basis of available chronological records on the occurrence and distribution of these strains in the individual culture vessels, we can give a summary of the situation with reference to Tables I and III instead of the full details of the records. We claim that in each set of the experimental series (each row in the tables) the presence of fungal strains in the individual parallel cultivation jars was random, and no regularity could be observed in different sets (rows) either.

Consequently, the final average pH values in each set of experiments (rows in the tables) were developed rather independently from the microfungi growing therein. On the other hand, some bacterial growth was always found in the jars with the pH above neutral. We could not, however, at this stage of the work decide whether this fact represents the cause or the effect.

Correlating the $^{14}CO_2$ recovery to the changes of the pH in the medium, we can conclude that an increase to slight alkalinity was not accompanied by dramatic changes in gas evolution. Nevertheless, it must be emphasized that in the particular experimental series where microbial growth was severely suppressed by $AgNO_3$ or by a lack of nutrients (distilled water only) and the pH became alkaline, the amount of ^{14}C in the medium was relatively high. This difference is rather evident if the scintillation present in the nutrient medium is expressed as percent of total radioactivity recovered (24.6–41.9%). This greatly surpassed the low percentage found in the experiments with fungal growth (2.4–12.3%) (in Fig. 1, curves (○) and (△) corresponding to Table I, rows C and D; as well as curves in Fig. 3 with the same symbols as in Fig. 1, and rows K and L in Table III).

These observations again focus the attention on the question of how well the aging process can be separated from the impact of living organisms. The moderate action of microorganisms appears to be a successive enhancement of the autoxidation in a synergistic way. Similar to our findings reported earlier, we could observe also in the present case that invasive contaminations resulted in

the recovery of the most of the strains quoted above as a mixed multispecies population in every jar with distilled water only (sometimes with medium containing $AgNO_3$ for that matter), although visible growth in these vessels was very poor.

It might be worth emphasizing that, in the actual soil or in the *F. redolens* cultivation experiments proper, not only a few surviving cells but some visible mycelial development as well were found after one or two years of aeration. This should, however, not necessarily be interpreted as an indication of the utilization of the polyethene itself as a carbon source, since besides the low molecular polyolefin material of the *n*-alkane type, the cultivation medium also contained an actual carbon source in the form of 0.5% ammonium tartrate.[3]

DISCUSSION

It is important to realize that biodegration can in practice never be entirely separated from the purely physical and chemical—mostly autoxidative—progressive aging, found always as an unavoidable slow but cumulative background effect. One might describe biodegradation rather as an ability of any living organism to continously accelerate the aging process or enhance the abiotic degradations of specific biotic ways by breaking down long polymeric molecules to shorter ones through biophysical (e.g., cracking by hyphal turgor), biochemical (e.g., excretion of organic acids), or, what is mostly considered as biodegradation *in sensu stricto,* through specific enzymatic actions, leading to the complete mineralization or recycling in nature of the elemental components of the polymer in question.

An extension of this viewpoint must take into consideration the fact that similar effects on substances accessory to the main polymer can also cause observable deterioration of the mechanical properties of a molded polymer but only in a discontinous manner. Macroscopically as well as microscopically observable mechanical "deterioration" of the physical structure of a molded polymer would thus be the result of any of the physical, chemical, or biological impacts either individually or jointly, in a synergistic way (oxidation, heat, radicals, radiations, acids, solvents, enzymes, etc). As a borderline case, "corrosion" is to be considered as a restricted physical deterioration of the smooth surfaces of such polymers.

The crucial point in the "biodegradation" of inert polyethenes is that a true enzymatic degradation of a long, straight olefin chain dependent on the enzymatic cleavage of the C–C bond should not be expected here, since such an endoenzyme (*n*-alkane C–C endohydrolase) does not seem to occur in nature.* Consequently in a typical case with polyethenes, none of the additives can ever stimulate the production of a long-chain splitting degradative enzyme by so-called "enzymatic induction" since such induction is possible only exclusively in the specific case when an existing type of enzyme, or rather its corresponding genetic information (codon), is present in the cells of an attacking organism. It should also be remembered that natural biopolymers (polysaccharides, proteins) are usually

* To support this statement, we quote Florkin[33] on enzymes: "3.7. There are very few carbon–carbon hydrolases acting on carbon–carbon bond; they mostly catalyze the hydrolysis of 3-Oxocarboxylic acids (*viz* 3.7.1.1. Oxaloacetase, 3.7.1.2. Fumarylacetoactase, 3.7.1.3. Kynureninase, 3.7.1.4. Phlorotin hydrolase)."

degraded stepwise by quite different types of enzymes splitting first to oligomeric derivatives, after which other enzymes further split them to dimeric or monomeric products. Indeed, the same reasoning must be followed when considering the value of long-term biodegradation experiments with microorganisms with respect to expected enzymatic "adaptation." Thus, induction of an enzyme yet unknown in nature should not be expected, while other effects exerted by microbes might be additional to the purely physical and chemical environmental effects and thus enhance the progress of degradation as a whole.

In regard to the final result of deterioration or biodegradation, the ways of such clear-cut enzymatic processes might be easily overshadowed. From a purely enzymatic viewpoint—if we restrict the concept of biodegradation to such terms—it is of no direct importance whether the microbial cells or hyphae can penetrate the interstitial spaces in the macromolecular structure of the polymer (as it is in the case of sugar-containing polymers) if these cells do not possess the necessary enzymes for breaking the C–C bond endogenously within the long aliphatic molecular chains. On the other hand, such penetration might in a mechanical respect be of great importance in the deterioration of a plastic material because of its biophysical or biochemical action, as exemplified in the beginning of this discussion. If substances excreted by a mold significantly accelerate the autoxidative scission of a high polymer and thus contribute to the metabolization of low molecular products by other microbes (e.g., bacteria), then the summed effect will still be a tardy but definite mineralization. This can be exemplified by a very recent report of Suzuki[34] who studied the induction of a kind of oxidizing enzyme in a *Pseudomonas* sp. by poly(vinyl alcohol) (PVA). The enzyme so induced resulted in the production of hydrogen peroxide, followed by the appearance of methyl ketones in this water-soluble synthetic PVA.

Nevertheless, for any reliable valid prediction of the durability of an "inert" synthetic polymer in use, one must emphasize that it will be possible to construct a global picture on the fate of these plastics in natural surroundings and to make predictions about the possible time schedule of the relevant destructive processes only if all the minor events of degradation and deterioration as well as their additive synergetic interaction can be taken into account.

No doubt, any prognostication on the fate of plastics such as polyethene is one of the main and most difficult demands put on this type of research. In particular, there is also a deep industrial and commercial interest in the basic molecular mechanisms of degradation and in the rates of degradation. In the present work we tried to contribute to a sequence of inquiries concerning a better definition of parameters which are relevant for the prediction of the time of degradation of HDPE in a natural surrounding.

The following general conclusions could be drawn from our present and preceding work:

(1) Average chain length straight HDPE molecules above MW 1000 are inert to microbial utilization because of the lack of suitable enzymes that split the C–C bond.[1,3]

(2) Shorter oligomers corresponding to higher n-alkanes, although degradable in a manner similar to other paraffins, will presumably be first converted to ketones and then degrade in form of free CO_2.[4,5]

(3) Antioxidants cannot entirely stop aging or biodegradation, but might retard it temporarily until becoming inactivated by successive oxidation.

(4) Oxidation products of lower oligomers appear in the HDPE plastics because of an abiotic autoxidation also in the dark, partly accounted for by the remnants of the original polymer catalysts if present.

(5) The shortest chain fragments (lower n-alkanes) or HDPE will be mineralized abiotically in the process defined as aging, which is merely an autocatalytic pathway.[3-5]

(6) Occasional oxidation and scission in the long polymeric chains leading to the production of new short oligomers from the bulk must also evidently take place during the aging process.[1,3-5]

(7) The cycles of both aging and biodegradation are thus complementary, or rather synergetic with each other, in supporting substrates for each other and furthering a slow release of fresh low molecular weight material from the high molecular weight bulk in both cases.[1,3,4]

Once the rate of all reactions in this cycle can be established, we shall attempt to revise a prediction formula, presented at the very beginning of our work on these problems.[1] At present we can only conclude that a rather tardy degradation process is taking place in HDPE films of the type studied by us the progessions of which can, however, neither be avoided nor arrested.

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