Biodegradation of Synthetic Polymers. III. The Liberation of $^{14}CO_2$ by Molds Like *Fusarium redolens* from ^{14}C Labeled Pulverized High-Density Polyethylene*

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Synopsis

In model experiments based on aerated cultures of molds like *Fusarium redolens,* ^{14}C liberated from randomly labeled pulverized high-density linear polyethylene (HPDE) with a specific surface of 10 m^2/g appeared mainly in the form of respiratory $^{14}CO_2$. The quantity liberated in this case was slightly greater than that stemming from HDPE film, according to our previous report (0.56% by weight against less than 0.40%). Contaminations could be detected, especially after two years aeration in several culture jars. Two unidentified bacterial strains as well as *Acremonium kiliense, Aspergillus versicolor,* and *Verticillium lecanii* were all thriving on the sparse media and enhanced to some extent the degradative $^{14}CO_2$ liberation, especially in mixed cultures together with *F. redolens.* Repeatedly close coincidence in $^{14}CO_2$ development between experiments with somewhat shifting mixed microbial populations point to preference for test with mixed microbial cultures instead of one single pure culture. Such tests should, however, be based on species more likely to utilize hydrocarbons then the celluloses metabolizers often applied for test of plastics according to several internationally accepted prescriptions. Increased liberation of $^{14}CO_2$ with decreasing particle size indicate that the accessibility of the metabolizable fractions of polyethylene must affect the degree of biodegradation. This is because with an increased surface/volume ratio in the plastic powders of decreasing mesh, more and more structurally hidden low molecular polyethylene material can be released and thus rendered accessible to the enzymes of attacking fungal hyphae. Also the possibility of an increased autooxidative scission of some of the long polymeric chains due to a "mastication effect," as well as an autocatalytic type of oxidative deterioration caused by remnants of the silico-alumina supported CrO_3 primary polymer catalyst must be considered in this connection, especially with regard to the general phenomenon of abiotic aging in the dark which was consistently registered by us.

INTRODUCTION

Mobilization of ^{14}C from randomly labeled high-density (linear) polyethylene (HDPE) by soil, wood rot fungi, and a hyphomycete isolated from soil, indicated a limited but evidently not negligible degradation when samples of polymeric film were exposed to strongly aerobic cultivation for more than two years.[1] On the basis of these results one could not definitely decide the controversial question whether polyethylene is successively biodegradable or is completely withstanding any biological impact, implicit that no physical or chemical cleavage is enhancing the biological effect.

An extension of experiments based on the very sensitive $^{14}CO_2$ recovery

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measurements of converted ^{14}C of the HDPE preparation is thus reported herewith. In the present case, however, labeled HDPE in powder form was used as substrate exposed to attack by fungi, viz., *Fusarium redolens*. This powder is the first polymeric solid product in the industrial processing of polyethylene, preceding the granulate form, the raw material of molding. Hitherto, such powder has only in one single occasion been reported to be the subject of biodegradative experiments.[2] Low-density polyethylene (LDPE) film broken down to powder by photodegradation or thermal degradation and mechanical grinding has, however, been examinated in soil tests.[3-5] Using our technique even comparative experiments with different mesh of powder became feasible.

EXPERIMENTAL

Material and Methods

High-density (linear) polyethylene randomly labeled with ^{14}C was prepared by Unifos Kemi AB, Sweden as described in our preceding publications.[1,4] At variance with conventional market HDPE products, this research batch lacked antioxidative additives.

The antioxidant-free powder generally used in the present and subsequent experiments had a specific surface area of 10 m^2/g. Deviation from this value, if any, will be indicated in the experimental part.

Samples of the HDPE powder were maintained in (a) aerated nutrient medium[1] inoculated with *Fusarium redolens,* or, for control experiments; (b) aerated uninoculated distilled water; and alternatively, (c) aerated uninoculated nutrient medium containing 0.05% $AgNO_3$ as growth inhibitor. In each instance five replicate runs were started.

The isolation, identification, and details of the cultivation of the hyphomycete *F. redolens* have been previously described in detail,[1] as well as the steps taken for microbial control, i.e., to isolate and identify other fungi, appearing in the cultures. All organisms obtained in pure culture were sent for identification to the Central Bureau voor Schimmelcultures, BAARN, Netherlands.

Radioactivity measurements were conducted on the fermented cultivation medium, and on the mycelial mat at the end of each run. The respiratory $^{14}CO_2$ evolved, was measured successively each month with the aid of a Packard Tri-Carb Model 3375 liquid scintillation counter.

RESULTS

As far as the total amount of ^{14}C radioactivity stemming from the randomly labeled high-density (linear) polyethylene is concerned, the present results with polyethylene powder show but a limited increase in strict biodegradation compared with that obtained on HDPE film. It is to be remembered, however, that these experiments were designed as models in order to definitely decide the actual limits of microbial enzymatic impact on antioxidant-free HDPE under strict laboratory conditions. All extraneous physical and chemical impacts were possibly avoided, to isolate those accountable for by the fungal attack.

With HDPE powder on a restrictive medium and inoculated with the hyphomycete *Fusarium redolens* strongly aerated for nearly three years, the catabolic conversion of polymeric alkane ^{14}C to $^{14}CO_2$ followed the same pattern

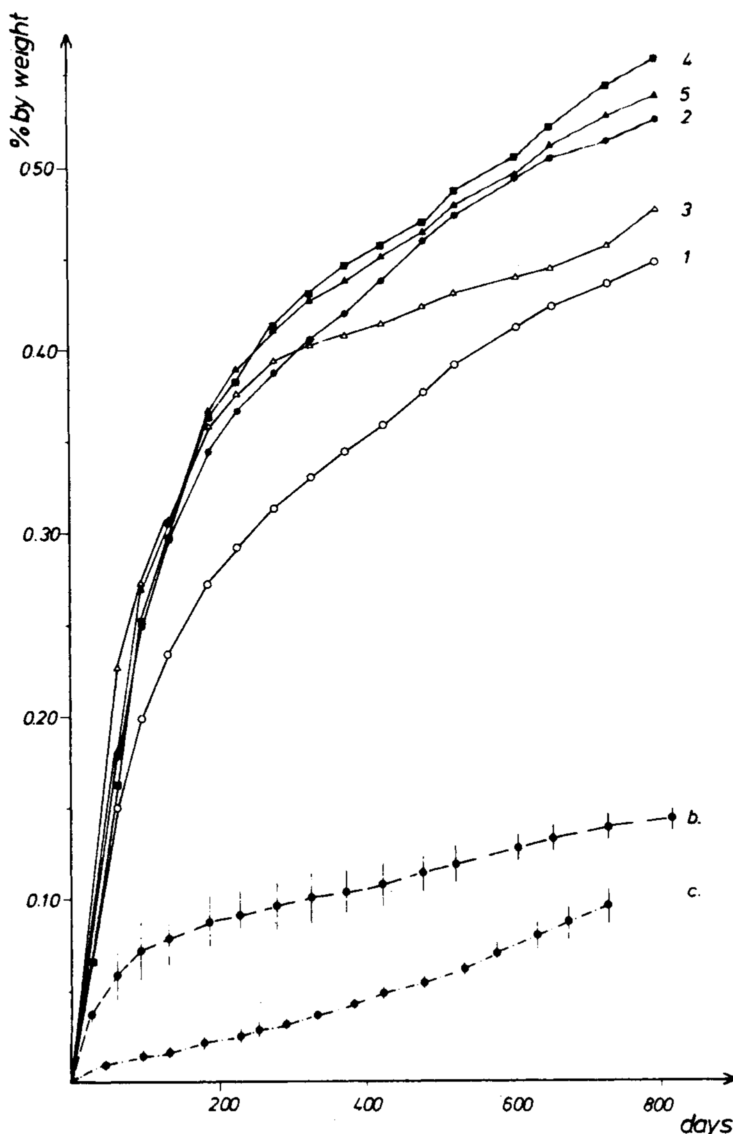

Fig. 1. Recovery of $^{14}CO_2$ from HDPE powder with 10 m^2/g specific surface maintained under aeration in a cultivation medium originally inoculated with *F. redolens* (curves 1–5). The figure includes curves of mean values from controls in uninoculated distilled water (b) (cf. Fig. 2), and in uninoculated cultivation medium containing 0.05% $AgNO_3$, (c) in order to inhibit growth (cf. Fig. 3). Some contaminations were found in jars 2, 4, and 5.

(Fig. 1) as in the case of HDPE film in experimental runs of similar duration (cf. ref. 1). More explicitly, the scintillation readings were always very close to the theoretical points of a parabola of similar slope and curvature as the corresponding empirical one. For the calculation of mean values, the same principles applies as often put into practice in biological experimentation, viz., if less than half of the experimental runs show an aberration from an ideal curve and/or clearcut signs of death of inoculum or other biological aberration, then these runs are not included. Such curves have nevertheless been presented by us for the sake of completeness of the records and as an illustration of the actual events.

Droplets were taken from the media of the experimental runs after one year and after two years of aeration, and spread on nutrient agar plates. This microbial control revealed the presence of infections in more than half of the experiments. These were especially conspicuous after two years of aeration. The infective organisms, two strains of bacteria and five species of molds, are tentatively described as follows (Table I, last column).

The use of mixed cultures in biodegradation tests on textiles (especially uniforms) has been conventional since the First World War and current recommendations for testing the resistence of plastic materials seem to stem from these traditional methods. We present in Table I a compilation of list of fungi recommended in some of the most important internationally quoted biodegradation tests, for comparison with the organisms repeatedly found in our degradation experiments (final column). The sequence of species names in this presentation is, however, differing from that of the original publications[6–8] in order to show more clearly the taxonomic groupings of the different test organisms used in these selected methods.[9] Group A refers to yeasts, group B to Ascomycetes (and more dubious Monilia), while group C comprises of Hyphomycetes among the Deuteromycetes. All the strains found in our biodegradation trials on HDPE belong to the Hyphomycetes, evidently a taxonomic restriction showing where to look in fungal systematics for other potential alkane utilizers.

In the following presentation of the experimental results, those symbols of Table I will be given for each curve in parentheses which corresponds the actual contamination found in each culture jar after the second year of aeration.

In the first $^{14}CO_2$ recovery experiment with HDPE powder (10 m^2/g specific surface) the biotic agent originally inoculated was *Fusarium redolens* (Fig. 1). The picture of microbial recovery was somewhat scattered. For curve No. 1 in this figure [*F. redol.*], curve No. 2: [*F. redol., Bacillus*], No. 3: [*F. redol.*], No. 4: [*F. redol., A. versic.*], and finally for Curve No. 5: [*F. redol., A. versic., Acr. kili.*]. Thus one or two alien microorganisms appeared in three of the five jars in the long runs. Expectedly, the mixed cultures gave the most uniform degradation with slightly higher terminal values than *F. redolens* alone (cf. curve Nos. 1 and 3).

For the explanation of the two lowest curves of Figure 1 should consider first the detailed descriptions for Figures 2 and 3. In Figure 2 the HDPE powder was maintained in uninoculated distilled water. Aeration, temperature and other conditions were the same as for the main experiments. This was true also for the runs pictured in Figure 3 where HDPE was maintained in the uninoculated culture medium fortified with 0.05% $AgNO_3$ as a growth inhibitor in order to provide a second control besides the distilled water test, namely, the effect of the nutrient medium without noticeable growth.

Invasive infections could be found in most flasks despite very limited chances of microbial propagation and only very faint visible signs of change in the jars. The recovery was as follows: Figure 2, jar 6: [sterile]; jar 7: [*F. redol., A. versic., Acr. kili.*]; jar 8: [*A. versic.*]; jar 9: [*Acr. kili., A. versic.*]; and for 10: [*Acr. kili., A. versic.*].

Concerning Figure 3 the findings were: jar 11: [*A. versi., F. redol., V. leca.*]; jar 12: [*Phialophora jeanselmei*]; jar 13: [*A. versic.*], jar 14: [*A. versic.*]; and finally for jar 15: [*Bacillus with creamy colonies*].

The presence of contaminations in these experiments have not led to more significant biodegradation with the possible exception of that in jar 7 of Figure

TABLE I

A Compilation of List of Fungi Recommended Internationally for Biodegradation Tests, Compared with the Organisms Repeatedly Found in our Degradation Experiments (final column)

	Russian proposal USSR-1 1958	AFNOR NF X 41-514 Aout 1961	ISO R 846 1968	ASTM D 1924 1970	Isolated from soil and infections (Albertsson)
(A)[a]		*Sterigmatocystis nigra*			
(B)[b]	*Chaetomium* (1) *globosum*	*Chaetomium globosum*	*Chaetomium globosum*	*Chaetomium globosum*	
				Pullularia (2) *pullulans*	
		Memnoniella (3) *echinata*			
(C)[c]		*Trichoderma Tl*	*Trichoderma viride*	*Trichoderma sp*	
					Acremonium kiliense
	Stachybotyrys atra	*Stachybotrys atra*			
	Penicillium brevicompactum	*Penicillium brevicompactum*			
	Penicillium cyclopium	*Penicillium cyclopium*			
		Penicillium funiculosum	*Penicillium funiculosum*	*Penicillium funiculosum*	
	Paecilomyces varioti	*Paecilomyces varioti*	*Paecilomyces varioti*		
	Aspergillus amstelodami	*Aspergillus amstelodami*			
					Aspergillus versicolor
	Aspergillus niger		*Aspergillus niger*	*Aspergillus niger*	
		Aspergillus flavus			
					Verticillium (4) *lecanii*
		Myrothecium verrucaria			
					Fusarium redolens

[a] A = yeast; [b] B = ascomycetes; [c] C = deuteromycotina (Hyphomycetes); (1) = *pyrenomycete;* (2) = *dematium, monilia;* (3) = (*monilia*); (4) = (*cephalospora*).

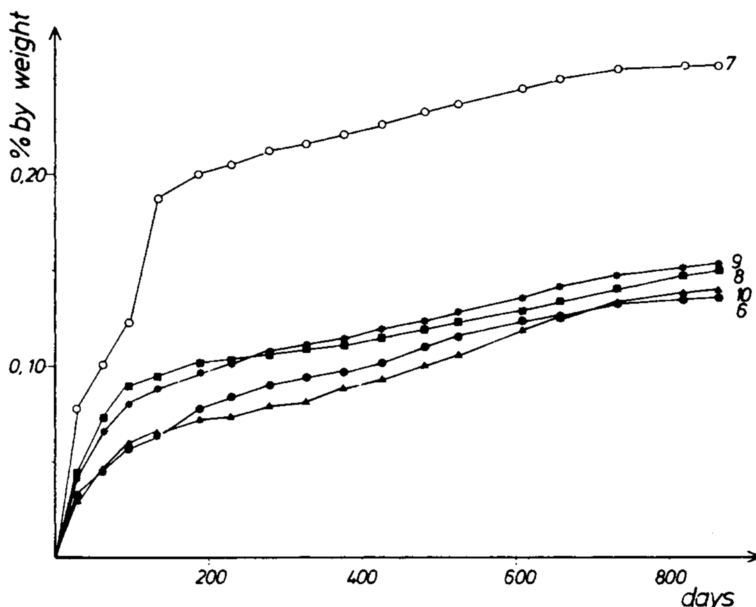

Fig. 2. Recovery of $^{14}CO_2$ from HDPE powder aerated in uninoculated distilled water only. Multiple invasion of molds was found in jar No. 7, while single fungal species could be isolated from jar Nos. 8, 9, and 10. Jar No. 6 was found to be sterile even after two years of aeration.

2, and eventually jar 15 of Figure 3. Consequently, the corresponding two curves were excluded from the calculation of the mean values of the remaining rather congruent curves in both figures. The means obtained from the four typical runs of Figure 2 as well as those from Figure 3 were then plotted in Figure 1 in order to demonstrate the differences between conversion of ^{14}C to $^{14}CO_2$ in actual cultivation on one hand and in restricted cultures on the other hand. These differences are high enough to demonstrate the existence of a biotic conversion or "biodegradation" of HDPE with fungi as *F. redolens*.

Although this limited biodegradation is merely a confirmation of previous findings, the accuracy and the relability of the data obtained must be emphasized. It is less easy to explain the "aging" effect, viz., the slow but definite conversion of ^{14}C to $^{14}CO_2$ in uninoculated culture jars where conditions have not favored microbial growth (cf. Figs. 2 and 3). Even if limited growth and restricted enzymatic conversion can be accepted in the jars where contaminations were found, the existence of the same "aging" effect in the jar where no infection could be found (Fig. 2, No. 6) is pointing to a possibility of the existence of a purely nonenzymatic, abiotic, oxidative autocatalytic degradation of the HDPE preparations tested by us. The peculiarity of this process is that it proceeds in the dark and at as low a temperature as 20°C.

However, in accordance with the early warning of Hawkins,[10] we have been made aware of the fact by Potts (personal communications, 1977) that the CrO_3 catalyst supported on silica-alumina (SiO_2·Al_2O_3) of the Philips Petroleum Company[11] is the same in the Union Carbide Process.[12] In the original process the catalyst is fed continuously as a dry powder in the fluidized-bed reactor and is not removed from the final product. In our case the samples of specific HDPE material were certainly prepared in batches in a pilot plant reactor (cf. ref. 1) but the catalyst was not removed either. Consequently, remnants of the catalyst

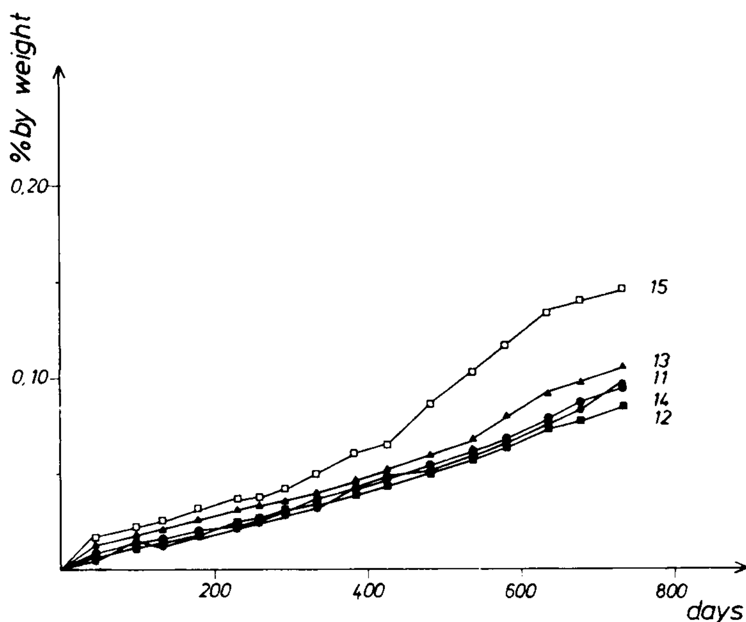

Fig. 3. Recovery of $^{14}CO_2$ from HDPE powder aerated in uninoculated culture medium containing 0.05% $AgNO_3$. Fungal infection was found in two vessels already after one year and in all jars after two years (a Baccilus in jar 15), however, respiratory oxidation was inhibited to a very high degree since the $^{14}CO_2$ recovery remained consistently below the values on distilled water alone.

in the HDPE might exert a new function being deteriorative in the final polymeric product instead of polymerizing the free monomers, through enhancement of autocatalytic oxidative degradation of linear long chains to shorter molecules by oxidative scission. It is, however, outside the scopes of the present work to deal with the quantitative molecular aspects of this possible route, the final steps of which, the abiotic degradation as far as to carbon dioxide, is evidently the less understood part of the whole. It remains to be investigated whether an autooxidative process of the HDPE can be responsible for this limited conversion to CO_2.

At this stage, we present a compilation of different results of the present work compared with mean values of curves published already in a previous paper on HDPE film.[1] The top curve of Figure 4 is thus representing the means of the three most congruent curves obtained with HDPE powder (cf. this publication, Fig. 1), while the second curve is a similar calculation based on four curves obtained previously with HDPE film. Also the curves of powder and film at the foot of the figure are reflecting the means of the four curves of Figure 2, in this work, and of Figure 8 in the previous paper quoted.

This type of comparison of mean values and deviations of typical runs disclose a significantly higher biodegradative conversion of ^{14}C with HDPE powder than with film. Even the "aging" control conversion was slightly elevated. So there is either more degradable material in the powder or else a better access to it.

The validity of the first part of this assumption has not yet been unambiguously demonstrated. Meanwhile, the second part of this assumption obtained a rather good support from the following experiment. Powders of different mesh were produced by passing the original HDPE powder through different sieves. In this way three different batches were obtained with statistical medium values

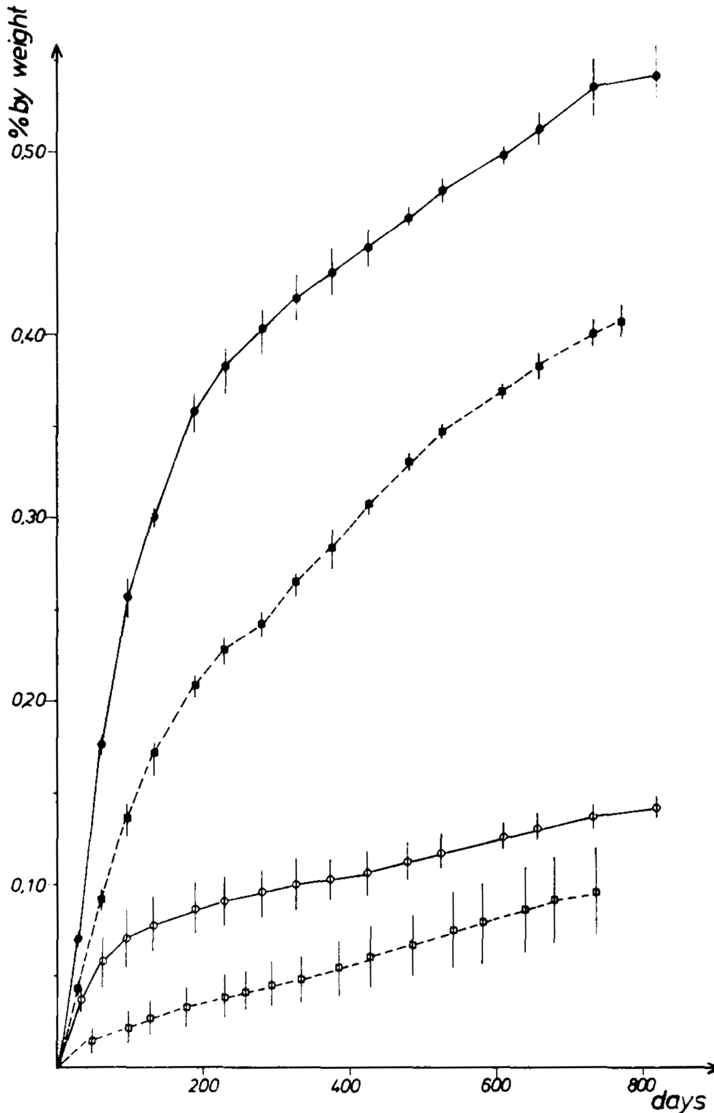

Fig. 4. Comparison of the biodegradation of HDPE film and HDPE powder in nutrient medium originally inoculated with *F. redolens*. The upper curve for powder [●] is a summation of the three most consistent experiments of Figure 1. The curve for film [■] represents mean values of four curves previously published (1, Fig. 5). The lower curves are mean control curves for aeration in uninoculated distilled water with powder [□] and with film [□].

of approximately 8, 9, and 10.5 m^2/g specific surface, respectively. Although these batches differ only in the upper values of rather scattered size distribution curves, the results, when presented in Figure 5 as mean values of duplicate runs, point rather demonstratively to a successive increase in biodegradation with decreasing particle size of the HDPE.

We should be repeatedly reminded that this last experiment was conducted in a somewhat less scrupulous manner than is usual in our work. In other words, clearcut differences between the powder batches of different mesh will be evident only when means are calculated. This has its explanation in the very structure of the HDPE powder samples where different batches were bolted for different

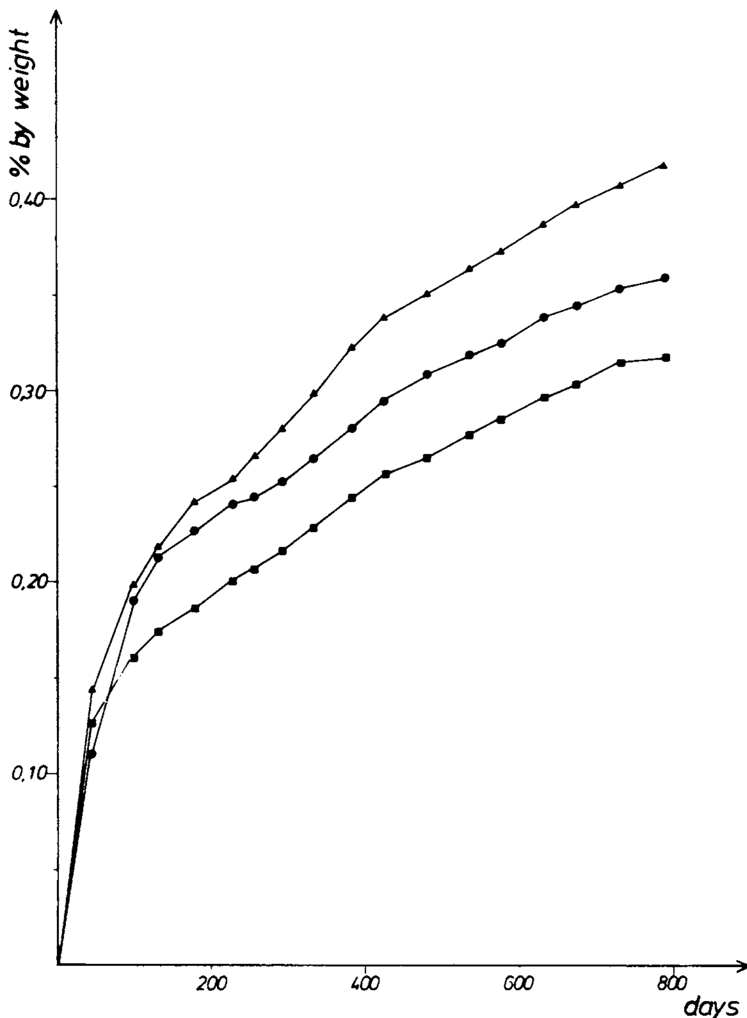

Fig. 5. Recovery of $^{14}CO_2$ from HDPE powder of different particle sizes by cultivated soil (1). Each point represents the mean of duplicate runs. ([■] = 8 g/m^2, [●] = 9 g/m^2 and [▲] = 10.5 g/m^2.)

mesh. Thus each batch comprises of a great variety of particle size but statistically the mesh sizes described above are predominant for each batch. Still—when the readings were summarized the way we did—the gradual correlation between oxidative "biodegradation" and particle size will become rather evident.

Unfortunately, due to the scarcity of the material, we had no control series in these experiments on distilled water only, or with $AgNO_3$ providing aseptic growth inhibition. These curves indicate a seemingly direct correlation between mesh size and "biodegradation" and stimulate a reasoning according to which an easier leaking out of low molecular polymer fractions from the smaller particles in the culture medium will contribute to increased enzymatic conversion of such fractions to final CO_2.

On the other hand, a small but not negligible colinear abiotic degradation of at least two types might also play a role in the biodegradative differences ap-

pearing as function of the surface/volume ratio of the HDPE particles. First, the possible deteriorative oxidative effect of the remnants of the supported CrO_3 silica-alumina catalyst on the long linear polymeric molecules must be taken in account. Second, a possible "mastication effect" must be considered, since such mechanical degradation resulting in chain scission by rupture of carbon-carbon bounds and by formation of free radicals is documented already in the case of polyethylene (cf. refs. 13 and 14).

We can conclude now, on the basis of our results—obtained with very sensitive methodology—that a pure, antioxidant-free, long-chain HDPE primary solid product will not be attacked enzymatically by soil microorganisms in a direct way. Only some part of the extractible waxy material, sheltered in this solid seems to be attacked. Such structure bound or structure hidden accessory material of the same origin and the same basic composition as the main polymeric product accompanies all the solid plastic products, and is generally described as the "extractible" low molecular fraction of the polymer. In our case, this means a mixture of more or less volatile straight chain alkanes, the slow but continuous production of which occurs to be a possibility to consider also in connection with purely a abiotic oxidative "aging" in the dark. The specific microbiological utilization and conversion of such alkanes belong for practical reasons, together with other paraffines, to the field of hydrocarbon (petroleum) microbiology and enzymology.

We have intimated similar thoughts in our previous work, as other authors have done. A decisive standpoint in polyethylene "biodegradation" based on a highly sensitive experimental approach will give us a better start for a prediction concerning the fate of such synthetic materials in natural surroundings. We do not want to question the importance of the macroscopic approaches but instead complete those with tedious radioactivity analyses within rather narrow ranges in order to tackle with the problem of durability of polyethylenes and other synthetic polymers at its very roots.

With regard to our rather sensitive experimental approach to these questions, the summarized values for ^{14}C recovery must be definitely regarded to be far less than the actual total liberation and disappearance of ^{14}C from the labeled HDPE material. Table II shows the total recovery but not the total disappearance of ^{14}C which has not been assessed in the present project. The "aging" effect mentioned previously in connection with Figures 2 and 3 is also apparent here. The cumulative values of this effect are rather high in the case of nutrient medium containing $AgNO_3$, especially when the radioactivity of the medium itself is considered. For this the following explanation is offered.

Silver salts, like those of other heavy metals, are known to be uncompetitive

TABLE II
Recovery of ^{14}C Embedded Randomly in Antioxidant-Free HDPE Powder (Specific Surface 10 m^2/g for this Mesh)

	% ^{14}C in nutrient medium	% ^{14}C in mycelial mat	% ^{14}C recovered as $^{14}CO_2$	% ^{14}C recovered total
(a) Microbial growth (mainly *F. redolens*)	0.05 ± 0.01	0.02 ± 0.01	0.49 ± 0.05	0.54
(b) Destilled water	0.08 ± 0.03	–	0.14 ± 0.01	0.22
(c) $AgNO_3$	0.17 ± 0.02	–	0.10 ± 0.01	0.27

inhibitors even in very low concentration for some sensitive enzymes. In case of invertase (= saccharase = β-D-fructofuranosido-fructo-hydrolase), this is believed to depend on the reaction of silver ions with the coenzyme binding histidine residue.[15] Silver and mercury ions have in variance also been reported to have the exceptional ability of activating the enzyme diphosphoglycerate phosphatase.[16] It is, however, too pretentious for this presentation to offer hypotheses concerning the possible effect of Ag^+ ions on the intricate enzymatic mechanism underlying the empirical phenomenon generally known as the "Pasteur effect."

Still a simple comparison of the values obtained with distilled water and with nutrient medium containing $AgNO_3$, disclose a slightly increased accumulation of nonrespiratory ^{14}C radioactivity appearing in the $AgNO_3$ inhibited nutrient medium. The visible growth of infections in these jars (expecially that plotted in curve No. 15 in Fig. 3) was presumedly sufficient to metabolize minute amounts of ^{14}C-labeled short alkanes. It is therefore suggested that the relatively high ^{14}C activity present in this media is due to an accumulation of the alkane carbon in fungal metabolic products, resulting a shift from respiration to fermentory activity. It is to be pointed out that the restricted amounts of total radioactivity in distilled water alone and in $AgNO_3$ inhibited medium were close to each other and significantly below that of the inoculated experiments. A study of all the nonrespiratory microbial metabolic activities which can be involved in polyethylene biodegradation was, however, beyond the scope of our project.

DISCUSSION

The situation is rather complex when HDPE powder is compared with HDPE film. The film is produced from the powder by heating and moulding, so that it is to be expected during the processing of film, that the amount of low molecular volatile oligomeric material will successively decrease through the alternative or cumulative effects of (a) evaporation, (b) crosslinking, and (c) increased impregnation of the submicroscopic cavities of the crystalline polymer. Controversially, the high-temperature treatment of the powder will also contribute to an increase of the low molecular fraction through oxidative cleavage. This is usually counteracted in industrial moulding processes by the addition of specific antioxidantia.

Our results showed no dramatic differences between the powder and film forms of HDPE, except for some increase in the accessibility of biodegradable material in the case of the powders. On the other hand, the experiment with powders having different mesh sizes presents a convincing indication of the better availability of metabolizable material when the particle size decreases so that the polymeric crystals and spherulites can come in closer contact with the cultivation medium or with the enzymes of the fungal hyphae as such. Unfortunately, no experiments were conducted on HDPE powder with varying mesh size under abiotic "aging" conditions; consequently, no conclusions can be drawn at this time on the effect of particle size on autooxidative processes due to increased access to oxygen—which is self-evident. Neither have the degree of possible autooxidation enhancing effect of remnants of the polymerization catalyst (of chromium type) considered in this connection, since we laid the emphasis on the possible biodegradative effects defined as microbial $^{14}CO_2$ liberation amounting above the level of "aging" effect in the abiotic experiments, which has indeed its importance as well as its complexity of its own.

In an earlier paper[1] we discussed the quantitative aspects of our methodological approach to the problem of biodegradation of HDPE and pointed out the extreme sensibility and accuracy of the $^{14}CO_2$ measurements even in cases of rather limited access to metabolizable alkanes for the test organisms as compared with other approaches (cf. ref. 15). But, if highly sensitive methods can be applied to this field of research, why not increase the chances of biodegradative impact on the polymers by tailoring a test procedure based on alkane utilizers instead of on conventional mixed cultures of fungi originally dedicated to testing the resistance against rotting of appreturized cellulose fibres ASTM D 1924 1970,[6] and ISO R 846 1968.[7]

It must be evident from the compilation presented in Table I that the quoted recommendations for microbial mixed strain cultures for use in biodegradative assay on plastics relate to species used for about 50 years and aimed explicitely for testing the resistance of cotton material (usually uniforms).

Indeed a Canadian research group, well known in this field, has very recently disapproved the use of the ASTM D 1924–63 test as a general method for biodegradability of polymeric materials, of rather similar reasons.[17]

Cellulose or lignin degradation may resemble the degradation of plastics in a general way, but certainly not with respect to enzymological details. Since species differences among microorganisms always imply differences in enzymatic activities, a restriction in a method by applying fungal strains whose enzymatic capacity probably is unrelated to the test for plastics, can hardly be justified. We feel that molds with an established ability to utilize alkanes must be the right tools for such tests, although simultaneous test with the conventional cellulose utilizers might ensure a wider angle for the assay as an extension to alkane utilizers.

At least one more question should be raised in connection with a discussion of the microbiological test methods in biodegradation experiments, and this is the problem of restrictions in the biotic parameters of the test. In other words, the question as to whether one single pure culture, or a coinoculation of several pure cultures, or a random natural population, e.g., in form of a living soil sample should be used in an otherwise carefully controlled laboratory biodegradation test. Hueck[18] believes that adequate proof of a cause-effect relationship can only be provided by studies with pure cultures and proposed the introduction of Koch's postulates also in biodeteriorative investigations. This is, however, in variance with the basic teachings of Winogradsky, Omeliansky, Beijerinck, and so many other ecological microbiologist, as well as with the ASTM and other international recommendations (cf. Table I). Incidentally, when we speak about the use of a *F. redolens* pure culture, we consider this inoculum as a start with a prototype and accept the fact that a mixed population establishes itself in the majority of the cultivation vessels because of invasive contamination. Still, parallel experiments with varying composition of mixed microbial populations showed an astonishing coincidence in almost all cases. The answer to Hueck's apprehensions is probably that the number of variable biological parameters (e.g., exoenzymes, change of pH of the medium, excretion of acids or bases, intercellular endogenous oxidation processes, etc.) outnumber the individual interspecies differences among most of the soil organisms we encountered and identified in our work.

Besides the size and shape variations in the HDPE products, the main feature of this presentation is that we have not followed the traditional international

recommendations but have accepted any invader from air or dirt when performing its best as biodegradative or at least alkane oxidizing agent.

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