

Environmental biodegradation of polyethylene

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Abstract

The degradation of a commercial environmentally degradable polyethylene was investigated in two stages. Firstly by abiotic oxidation in an air oven to simulate the effect of the compost environment and secondly in the presence of selected microorganisms. Initial biofilm formation was followed by fluorescence microscopy and the subsequent growth of bacteria on the surface of the plastic was observed by scanning electron microscopy (SEM). It was observed that microbial growth occurred on the presence of PE samples that had been compression moulded to thick sections but had not been deliberately pre-oxidised. Molecular enlargement and broadening of molecular weight distribution occurred after preheating in air at 60 °C but not at ambient temperatures but colonisation of microorganisms occurred on all samples. Erosion of the film surface was observed in the vicinity of the microorganisms and the decay of oxidation products in the surface of the polymer film was measured by FTIR measurements and was found to be associated with the formation of protein and polysaccharides, attributable to the growth of the microorganisms.

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1. Introduction

It is frequently asserted by environmental pressure groups that the polyolefins cannot biodegrade since the molecular weight must be less than 500 for this to occur. However, it was demonstrated by an interdisciplinary group at the University of Aston over 25 years ago [1,2] that, whilst normal commercial polyethylenes do not biodegrade, polyethylenes formulated with transition metal prooxidants (notably iron complexes), after ageing or weathering by exposure to UV light, support microbial growth. At that time, plastics litter was becoming a major problem and it was suggested by these workers that this provided “for the first time the possibility of a combined photo- and bio-degradation process in which degradation commenced by the former can be completed by the latter, ultimately converting the plastic to useful humus, water and CO₂” [2]. Pre-ageing by either light or heat were shown to be essential abiotic precursors to biodegradation and low molar mass oxidation products are

rapidly bioassimilated by thermophilic microorganisms. Albertsson and co-workers have since shown that a wide range of biodegradable oxidation products are formed in the abiotic peroxidation process [3].

It was shown in our earlier study of a commercial photo-biodegradable polyethylene (Plastor[®]) [4] that low molar mass products are removed from the surface of the polymer by bioerosion without significant effect on the molar mass of the bulk polymer. The biodegradation of photodegradable polyethylene begins at M_w 40,000 and it was concluded that photo-initiated peroxidation is the rate-determining step in the biodegradation of the polyolefins in sunlight.

However, in recent years the emphasis in polymer biodegradation has shifted from simply protecting the environment from unwanted plastics packaging litter to recovering value from the plastics used [5]. In the case of agricultural plastics, once they have fulfilled their purpose, for example as mulching films, they are required to be bioabsorbed into the soil as humic material [5,6] but composting of biodegradable plastics requires a rather different solution. The European Union Waste Framework Directive put forward by the EU in March 1991 defines “recycling/reclamation

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of organic substances” as “spreading on land resulting in benefit to agriculture or ecological improvement including composting and other biological transformation processes” [7]. Rapid conversion to carbon dioxide is not “recovery” since carbon dioxide has a negative influence on the environment. Conversion of degradable polyolefin packaging to cell-biomass on the other hand provides added value to the compost.

It is now recognised that biodegradation can occur by two different mechanisms; namely hydro-biodegradation and oxo-biodegradation [5,6,8]. The former is much more important in the case of hydrolysable natural polymers such as cellulose, starch and polyesters whereas the latter predominates in the case of other natural polymers such as rubber and lignin. Lignin normally requires the presence of enzymes that initiate peroxidation [9]. The synthetic hydrocarbon polymers do not hydrolyse under normal environmental conditions but it was shown in the early studies discussed earlier that, after transition metal catalysed thermal peroxidation, they biodegrade readily in the presence of a variety of thermophilic microorganisms [2]. It was subsequently shown that lignin biodegrading organisms are particularly effective [10].

The purpose of the present study was to attempt to correlate the loss of low molar mass oxidation products from the polymer with the growth of selected microorganisms on the surface of the polymer thermooxidised under conditions close to those experienced in compost. A further objective was to determine the degree of thermal abiotic peroxidation necessary to support microbial colonisation in the polymer surface and to follow the sequence of events that lead ultimately to the formation of cellular biomass.

2. Experimental

2.1. Materials

Degradable polyethylene was a green film (LC) containing TDPA[®] (thickness $36 \pm 2 \mu\text{m}$), from EPI (Europe). For the purpose of the present study a number of superimposed films were compression moulded at 100°C to give sections of polymer (compacted samples) that varied around $550 \mu\text{m}$ in thickness. In this thick system, the extent of oxidation was evaluated using FTIR-spectrophotometry in the photoacoustic mode (Nicolet L800 spectrophotometer equipped with the photoacoustic cell MTEC model 200). It should be noted that this procedure examines only the first 10–15 μm into the surface. After compression moulding, the films showed no evidence of oxidation as indicated by the absence of an FTIR absorption in the region of 1715 cm^{-1} due to carbonyl formation.

2.2. Experimental procedures

2.2.1. Microorganisms

Strains *Rhodococcus rhodochrous* ATCC 29672 (bacterium) and *Cladosporium cladosporoides* ATCC 20251 (fungus) were obtained from American Type Culture Collection. *Nocardia asteroides* GK 911 (bacterium) was isolated previously in our laboratory [11].

2.2.2. Cultures

Polyethylene samples ($3 \times 1 \text{ cm}$) were sterilised by UV exposure at 254 nm, $2 \times 5 \text{ min}$) and inoculated during 30 min with a suspension of each strain. Assays and controls were incubated for 6 months at 27°C and 85% humidity in an environmental cabinet. Cultivations were carried out in petri dishes containing glass beads and a mineral salt medium (MSM) consisting of (g l^{-1}): $\text{MgSO}_4(7\text{H}_2\text{O})$, 0.5; KH_2PO_4 , 0.5; $\text{Na}_2\text{HPO}_4(12\text{H}_2\text{O})$, 2.52; NH_4Cl , 1; CaCl_2 , 0.002; $\text{MnSO}_4(7\text{H}_2\text{O})$, 0.007; $\text{FeSO}_4(7\text{H}_2\text{O})$, 0.001g and $\text{ZnSO}_4(7\text{H}_2\text{O})$, 0.007. After varying times of cultivation, pieces were taken from the petri dishes and treated for microscope examination. For each strain, one of the sample was submitted to a vortex treatment (2 min in a 0.25% sodium hypochlorite solution in water supplemented with 0.1% Tween 80) in order to remove microorganisms from the surface.

2.2.3. Epifluorescence microscopy

The method used was previously published by Chavant et al [12]. Samples were fixed with 2.5% glutaraldehyde in 0.05 M cacodylate buffer (1 h 30m at 4°C), and rinsed with the same buffer. Samples were stained for 3 min with a solution of 0.05% acridine orange, washed twice with demineralised water, dried in air for 1 h, and then examined with an Axioplan 2E microscope coupled with a camera under UV light filtered through a blue filter. The images were analysed with Visiolab 1000 software (Biocom, Les Ulis, France) as grey scale interpretations on the screen. The area covered by the biofilm was converted into a percentage of the total area. These data represent an aggregate of 15 observations for each sample.

2.2.4. Scanning electron microscopy

Samples were fixed with 2.5% glutaraldehyde in 0.05 M cacodylate buffer (1 h 30 min at 4°C), after washing with the same buffer they were post-fixed with osmic acid vapours and dehydrated in graded ethanol (50, 70, 95, 100). The dehydrated samples were sputter-coated with a gold layer (Edwards S150B) and examined with a scanning electron microscope (Cambridge S360).

2.2.5. FTIR spectroscopy

The original film (LC) was examined by transmission FTIR after heating in an air oven at 40, 60 and 80°C or after exposure to photooxidation in an accelerated

weathering machine (SEPAP) and compared with a film stored at 20 °C. Compacted samples were stored at 5 °C (System #1). Others were stored at 20 °C in the laboratory (System #2) and others were heated at 60 °C (System #3) and 80 °C (System #4) for 300 and 200 h, respectively followed by storage at 5 °C. These were examined by photo-acoustic FTIR spectroscopy (PAS).

2.2.6. GPC experiments

GPC experiments were carried out on the samples before (#1–4) and after (#5–13) being subjected to microbial attack using the following microorganisms, which were used in the earlier study [4]; *Nocardia asteroides*(Na), *Rhodococcus rhodochrous* (Rr) and *Cladosporium cladosporioides* (Cc). The incubated samples were then subjected to GPC analysis after removal of the micro organisms by vortex treatment.

3. Results

3.1. FTIR spectral analyses

In Fig. 1, the growth of the carbonyl peak at 1715 cm^{-1} in air at 40 °C is compared as a function of time for both thermooxidised and photooxidised LC films with the control film after storage at 20 °C for the same length of time. It is evident that both the control film and the film heated at 40 °C show only slightly different induction periods (IP) but the heated film oxidises more

rapidly than the control at the end of the IP. The films heated at 60 and 80 °C show a similar but very short IP (≈ 50 h) and there was little to distinguish them. The weathered (SEPAP) sample displayed rapid carbonyl formation from the beginning of UV exposure.

The compacted films were compared with the original sample by PAS FTIR after storage in the dark at 5 °C (sample #1) and 20 °C (sample #2). Typical results are shown in Fig. 2 after 243 days. It is clear that both samples were extensively oxidised, even the one stored at 5 °C. Incubation of the compacted films separately with each of three microorganisms; *N. asteroides* (shown typically in Figs. 3–5), *R. rhodochrous* and *C. cladosporioides* immediately after moulding led to the formation of new absorbances in the region 3000–3600 cm^{-1} , peaking around 3300 cm^{-1} (Fig. 3) and bands at 1655, 1636, 1539 and 1523 cm^{-1} (Fig. 4) which are assigned to proteinic material [13,14]. There is also the growth of an intense band at 1088 cm^{-1} , (Fig. 5) which has been assigned to polysaccharides, the usual metabolites produced by microorganisms, which are major constituents of biofilms [13,14]. The carbonyl concentration remained quasi-stationary and is actually lower than the value that would be anticipated from abiotic thermo-oxidation at 27 °C. The polysaccharide bands (Fig. 5) are seen to increase rapidly while the protein concentration also remained constant in the surface.

Fig. 6 compares the development of transformation products at after 300 h at 60 °C followed by exposure to the three microorganisms for 6 months. The

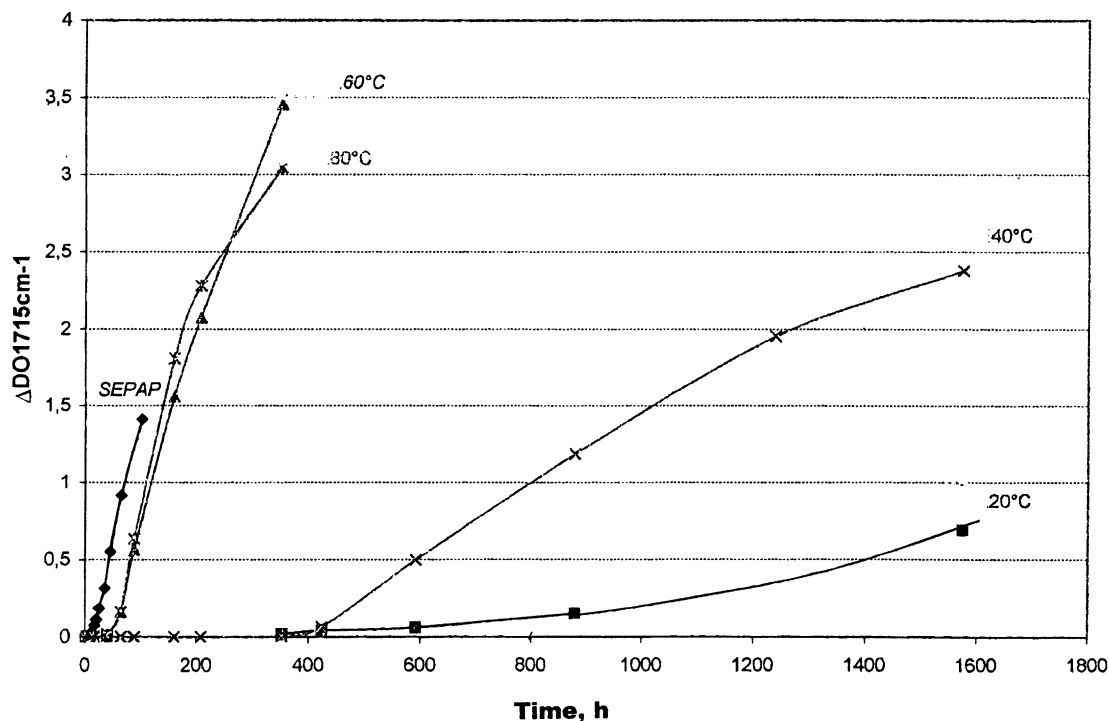


Fig. 1. Development of transmission FTIR band at 1715 cm^{-1} in LC films after heating at 20, 40, 60 and 80 °C in an air oven and in an accelerated weatherometer (SEPAP) at 60 °C.

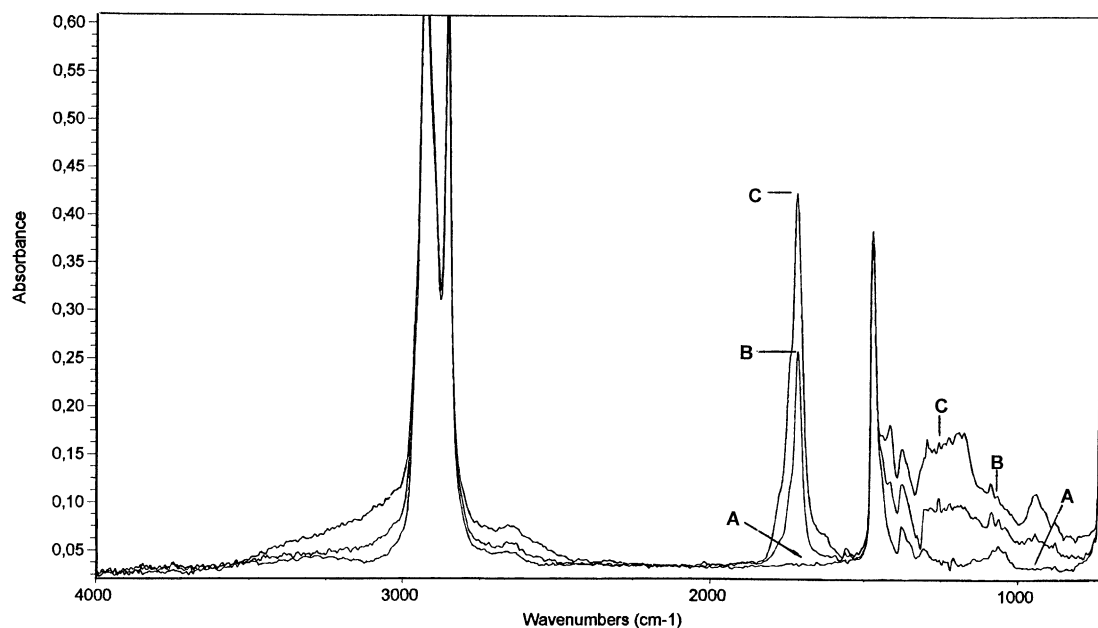


Fig. 2. Transmission FTIR of compacted films after storage. A after compression moulding, B after storage at 5 °C for 243 days and C after storage at 20 °C for 243 days.

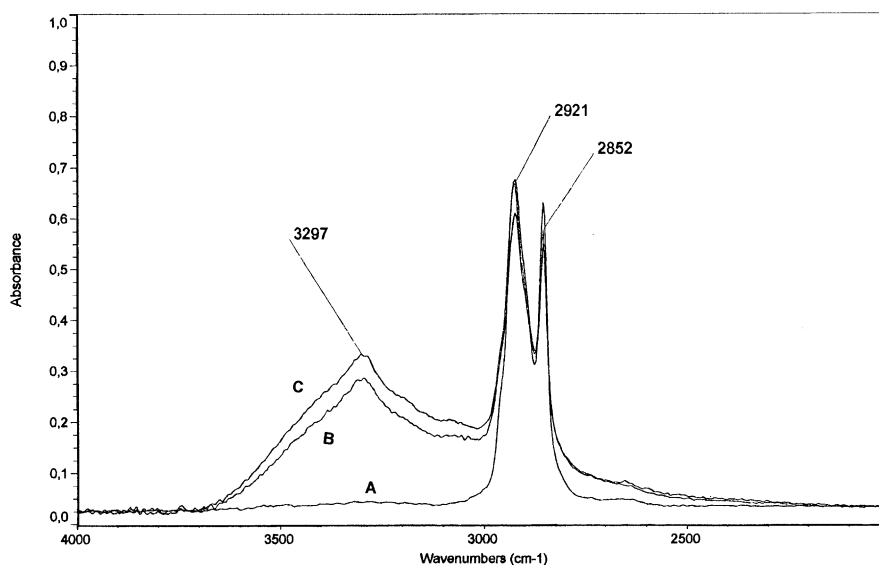


Fig. 3. PAS FTIR spectra of compacted films. A after compression moulding, B after incubation with *N. asteroides* for 4 months and C after incubation with *N. asteroides* for 6 months and 3 weeks.

non-incubated control sample had been stored at 5 °C for 243 h after heating at 60 °C. The carbonyl concentrations were reduced compared to the non-incubated control held at 5 °C. The formation of the polysaccharide band was particularly marked in the case of *Nocardia asteroides* (Na). The corresponding samples heated at 80 °C were similar to those obtained at 60 °C.

3.2. GPC measurements

The GPC molecular weights (M_n and M_w) and the polydispersity (I_p) of the initial, non incubated, compacted

samples #1–4 are shown in Table 1 and the corresponding distribution curves in Fig. 7. It is clear that, with reference to the initial samples, #1, stored at 5 °C for 243 days, the samples stored at 20 °C for the same time, #2, has undergone a very profound downward shift in both M_n and M_w with narrowing of molecular weight distribution. This is characteristic of atmospheric peroxidation processes in which there is no restriction of oxygen access and this is confirmed by the much increased formation of carboxylic acid carbonyl groups at 1714 cm^{-1} . By contrast the samples subjected to air oven aging at 60 °C (#3) and 80 °C (#4) both show an

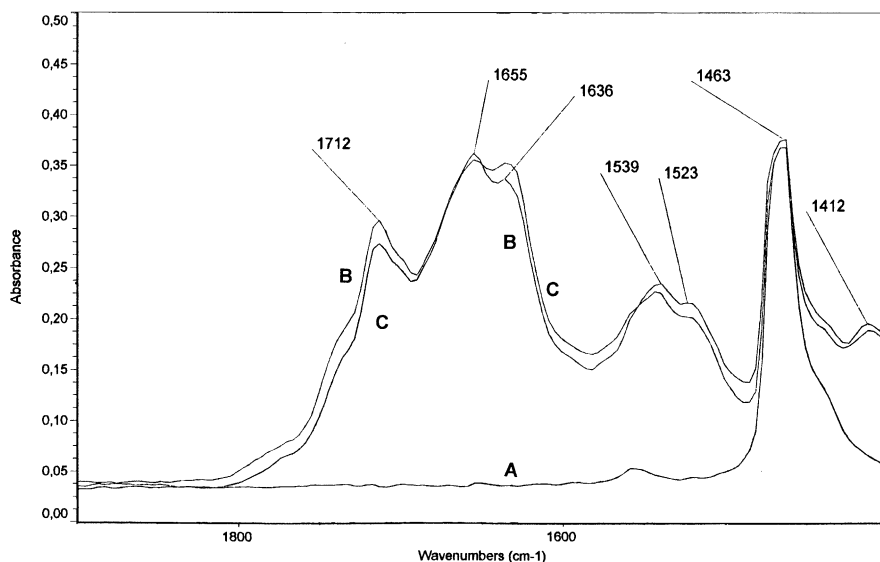


Fig. 4. PAS FTIR spectra of compacted films. Identification as for Fig. 3.

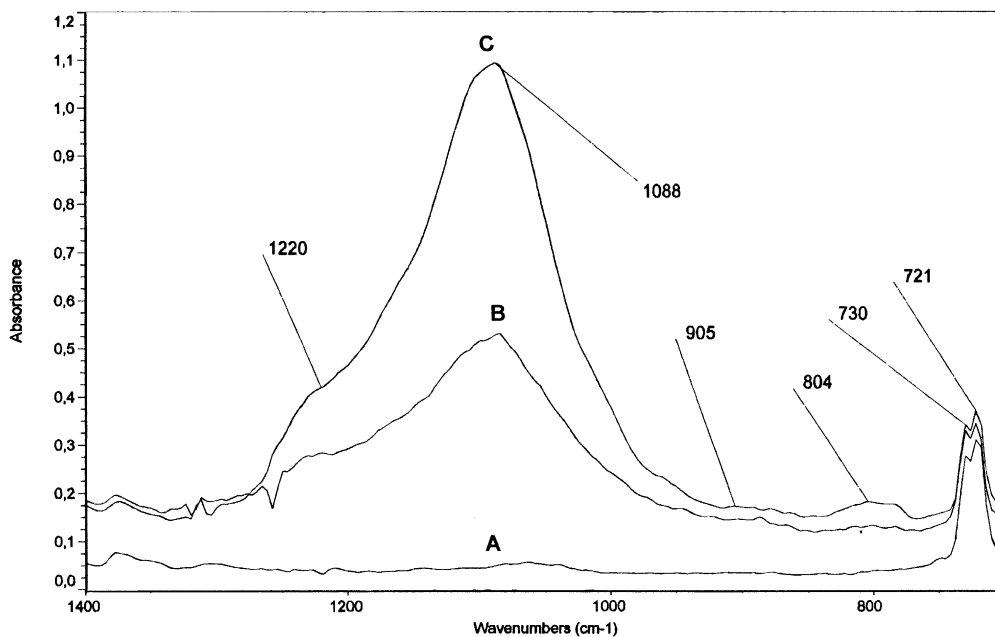


Fig. 5. PAS FTIR spectra of compacted films. Identification as for Fig. 3.

increase in M_n and an associated broadening of molecular weight distribution.

These results imply a concomitant cross-linking and chain-scission at the higher temperatures. Cross-linking is normally associated with a deficiency of oxygen at the reaction site, which leads to the formation of relatively stable peroxidic cross-links (possibly even carbon-carbon crosslinks) due to competition between radicals to react with one another rather than to react with oxygen to give unstable hydroperoxides.

The reactions outlined in Scheme 1 can occur only in thick sections of polymer under conditions where oxygen has been depleted. This is known to occur in the

presence of highly redox-active transition metal ions where the metal ion rapidly converts hydroperoxides to alkoxy radicals. This mechanism is used in the Oxbar™ process to reduce the rate of oxygen transport through packaging materials by scavenging molecular oxygen. However, it does not occur in very thin polymer films particularly at ambient temperatures, when oxygen diffusion to alkyl radicals can compete with their reaction with other radicals.

Non-thermo oxidised samples were inoculated at 27 °C with three different strains *N. asteroides*, *R. rhodochrous* and *C. cladosporoides* (#5–7) and examined by GPC. The surface layers were examined after removal

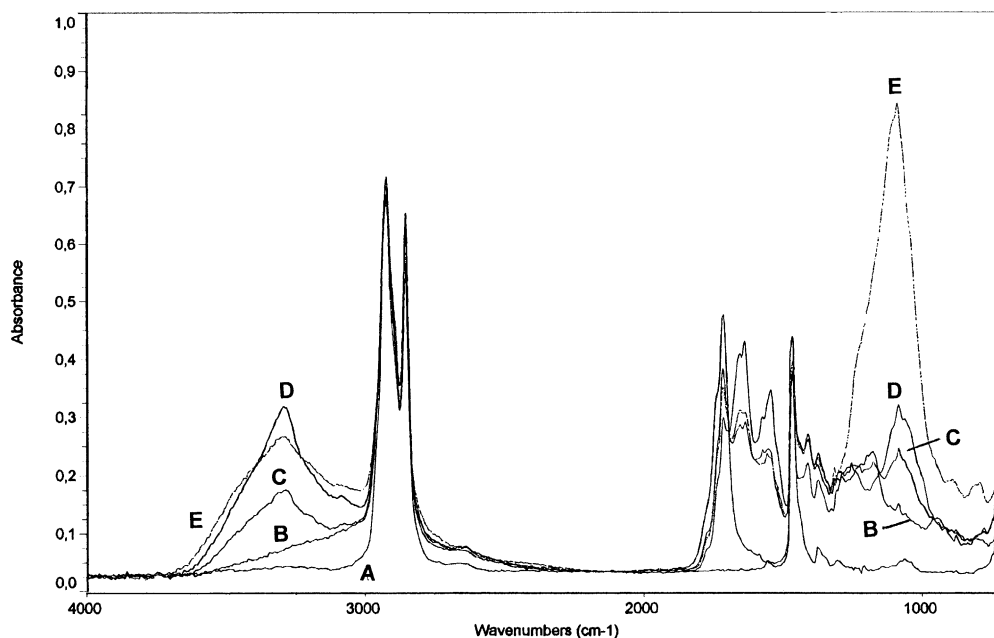


Fig. 6. PAS FTIR spectra of compacted films after heating in an air oven. A after compression moulding, B after storage at 5 °C for 243 days, C after 300 h at 60 °C, followed by incubation with *C. cladosporoides* for 27 weeks, D as for C with *R. rhodochrous*, E as for C with *N. asteroides*.

Table 1
Molecular weight data on degradable polyethylene in thick sections before and after ageing

System	M_w	M_n	I_p
1	62,600	10,300	6.08
2	12,300	3500	3.51
3	153,300	8800	17.42
4	111,700	7300	15.30

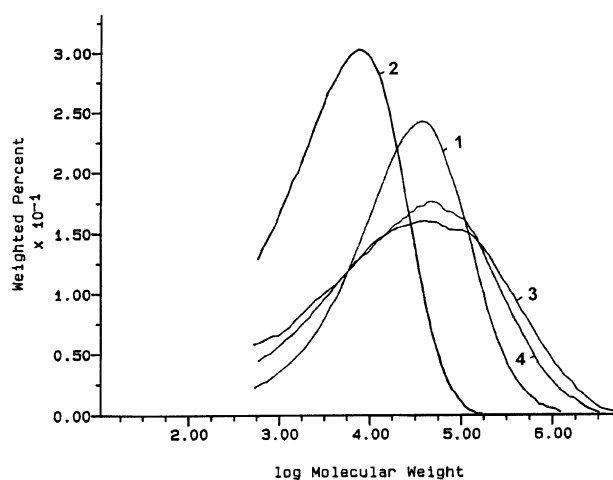
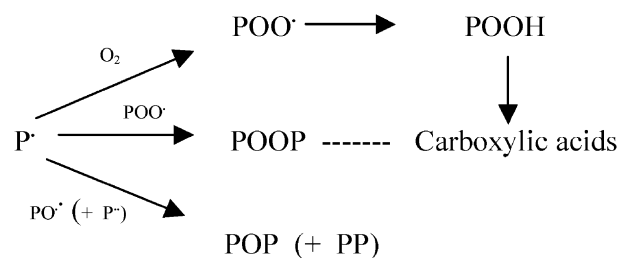


Fig. 7. Molecular weight distribution of compacted samples. 1 After storage at 5 °C for 243 days; 2 stored at 20 °C for 243 days; 3 after heating in an air oven for 300 h at 60 °C, 4 after heating in an air oven for 200h at 80 °C.

by microtoming. The original thickness of the films and the thickness of the surface section are shown in Table 2.

The molecular weight distribution of the film surfaces after incubation for 204 days are shown in Table 3.



Scheme 1. Radical reactions of polyolefins under conditions of normal and restricted oxygen access. PH = polyolefin; POOH = polyolefin hydroperoxide; POP and PP are cross-linked polymers.

Table 2
Characterisation of samples examined by GPC

System	Original film thickness, μm	Thickness of surface analysed by GPC, μm
5	640	100
6	470	110
7	490	90
8	580	140
9	590	135
10	520	180
11	560	190
12	500	200
13	560	190

Incubated and microtomed System #2 samples that had been stored at 20 °C are compared with #5–7 in Fig. 8. They are seen to be almost identical and different from the samples maintained at 5 °C. It must be concluded then that the reduction in molecular weight observed during incubation with the microorganisms is essentially due to abiotic peroxidation.

Table 3
Molecular weight distribution data for samples incubated with three microorganisms for 204 days at 27 °C

System	Micro organism	M_w	M_n	I_p
5	Na.	14,000	3700	3.78
6	Rr	12,700	3600	3.52
7	Cc	14,400	3700	3.89
8	Na	14,500	3600	4.03
9	Rr	14,000	3700	3.81
10	Cc	14,400	3400	4.24
11	Na	14,700	3400	4.32
12	Rr.	11,900	3100	3.84
13	Cc	14,300	3300	4.33

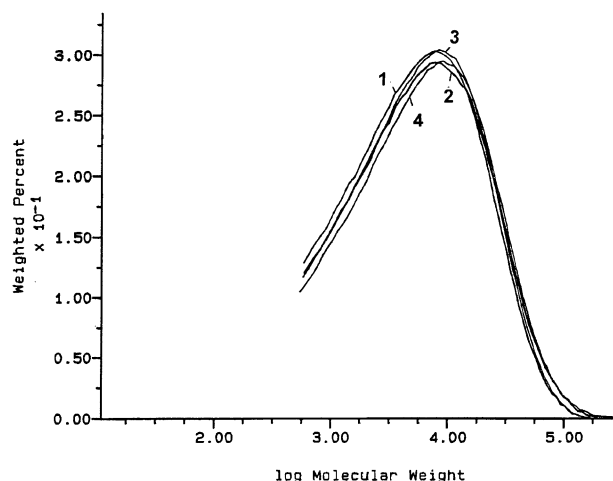


Fig. 8. Molecular weight distribution of compacted samples. 1 Control, after storage at 20 °C for 243 days without incubation (#1); 2 after storage, incubated with *C. cladosporoides* for 27 weeks (#7); 3 after storage, incubated with *R. rhodochrous* (#6) for 27 weeks; 4 after storage, incubated with *N. asteroides* for 27 weeks (#5).

The situation is very different in the case of the samples abiotically peroxidised at 60 °C (#3), which were found to be partially cross-linked. After incubation with the three microorganisms (#8–10), the high molecular weight fraction had been removed and the molecular weight distribution was similar to that of the polymer abiotically oxidised at 20 °C for a similar time. The microbial action does not induce any significant reduction of the molecular weight of PE macromolecules. That is the long-term abiotic thermooxidation that occurred at 27 °C during the bio-assessments accounts for the loss of high molecular weight polymer through chain-scission of macromolecules and crosslinks. The same results were obtained with samples abiotically peroxidised at 80 °C and incubated with the three strains (#11–13) (see Table 3).

3.3. Surface studies by fluorescence microscopy

Images could only be obtained by fluorescence microscopy on compacted films before thermooxidation, since heat treatment of the films caused major alteration in the film surface that interfered with the digitalisation of the fluorescence signals. Fig. 9 shows the progress of colonisation of the surface for *Rhodococcus rhodochrous* (Rr) on compacted films that had not been subjected to thermal ageing. The rates of colonisation are compared for *N. asteroides*, *R. rhodochrous* and *C. cladosporoides* in Fig. 10. Colonisation is rapid and surface coverage is complete for *N. asteroides* and *R. rhodochrous* within 17 weeks. The growth of *C. cladosporoides* is slightly slower.

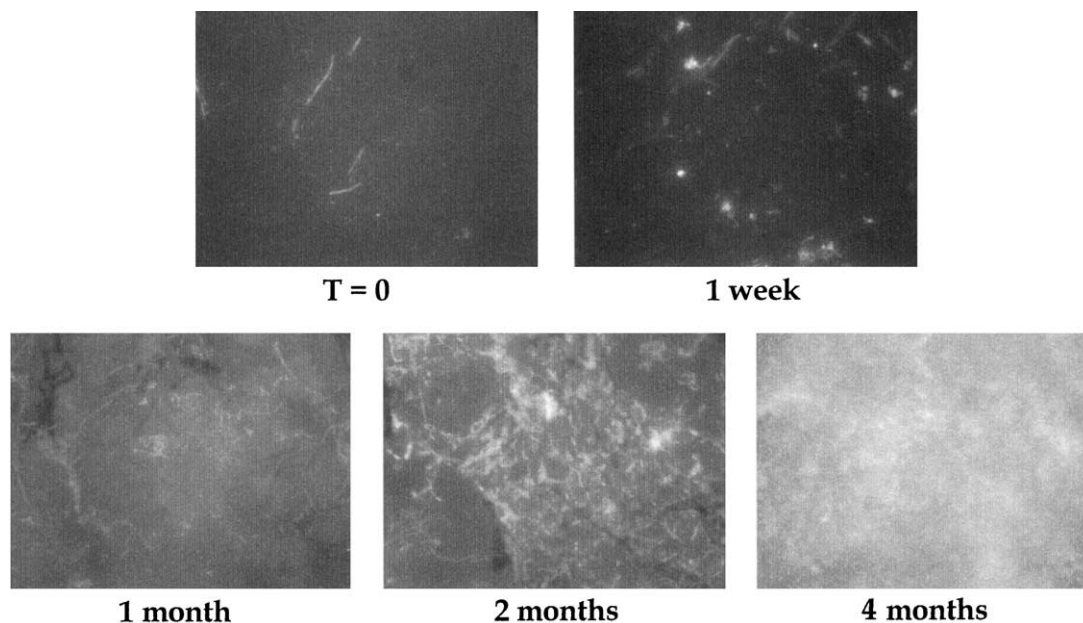


Fig. 9. Fluorescence microscopy scans of non-thermooxidised compacted samples of LC films incubated with *R. rhodochrous* (Rr) for different times.

3.4. Surface studies by scanning electron microscopy (SEM)

As already mentioned, thermal treatment of the compacted films leads to rapid disintegration of the surface. Fig. 11 shows a typical pattern of microbial (*R. rhodochrous*) growth on the top surface of the sample. Erosion of the surface can be observed but interestingly, although microorganisms grow around the fissures resulting from crack propagation, none grow within the fissures suggesting that most of the low molecular weight nutrients migrate to the surface from the (oxidised) layers of the polymer.

Similar results were obtained with *Nocardia asteroides* (Na), Fig. 12, although the morphology of the bacterium was quite different. It should be noted that there was prolific bacterial growth in areas of the film well away from the fissures.

As a cross-reference to the earlier studies on the biodegradation of Plastor, another commercial oxo-biodegradable polyethylene [6] after accelerated weathering, it was shown (Fig. 13) that *R. rhodochrous* propagates very rapidly on the surface of photooxidised PE. *R. rhodochrous* rods can be seen after only 1 month. When the biofilm is removed from the surface of samples, thermooxidised at 60 °C for 300 h and incubated with

R. rhodochrous for 6 months, the surface layers of the polymer were found to be deeply fissured and the surface was flaking due to the activity of bio-promoted peroxidation in the surface. This behaviour, shown in Fig. 14 is typical of bioerosion of polymers and has been observed previously for both hydro-biodegradable [15] and oxo-biodegradable [4] polymers. Surface bioerosion is the primary cause of mass loss from the surface. However, due to the weakness of the flaky surface under vortex treatment, it is not possible to accurately measure mass loss due to bioassimilation alone.

4. Discussion and conclusions

A number of important observations from the present work throw more light on the progression and mechanism of oxo-biodegradation.

It is apparent that very little abiotic peroxidation is necessary to initiate biological attack by the microorganisms used. The difference between the original LC films and the compacted films (#1–4) is that the latter have been compression moulded at 100 °C. Although the compacted films show no evidence of peroxidation as indicated by FTIR, it seems highly likely that this is due to the antioxidant(s) that are essential to allow the

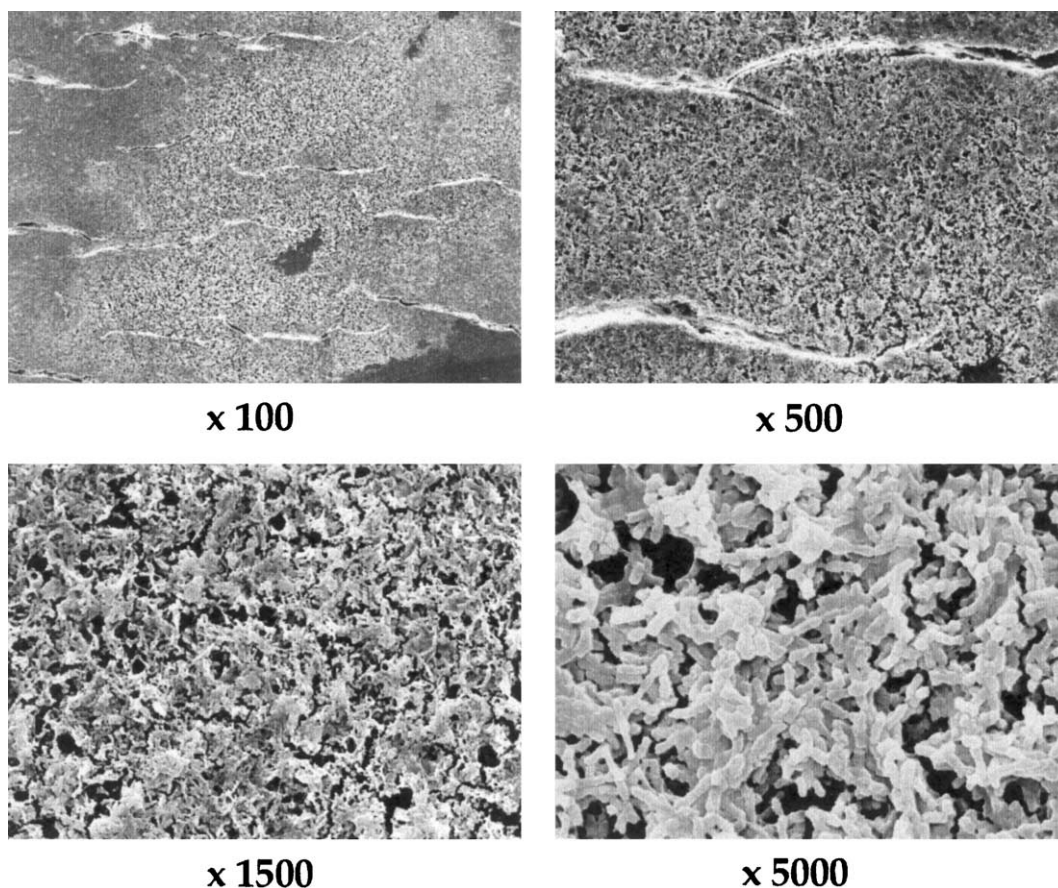


Fig. 12. SEM micrographs of compacted films oven aged for 300 h at 60 °C and incubated for 2 months with *N. asteroides*.

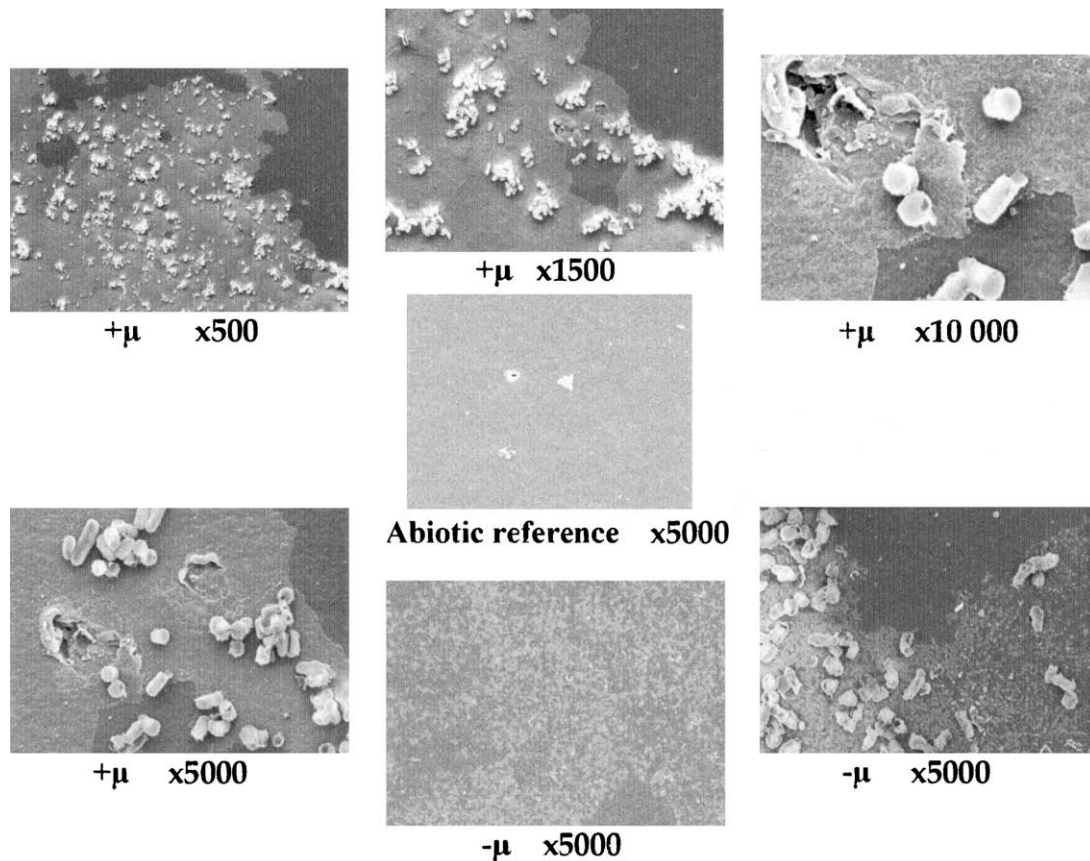


Fig. 13. SEM micrographs of Plastor films after UV exposure in a SEPAP accelerated weatherometer and exposed to *R. rhodochrous* for 1 month. (+μ = uncleaned surface; -μ = vortex cleaned).

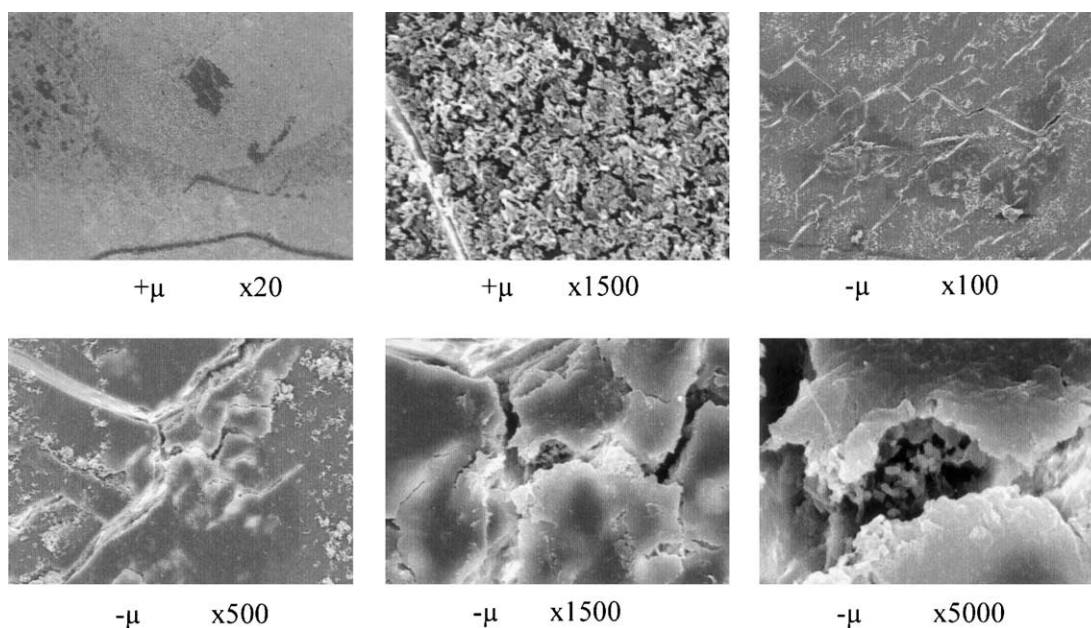


Fig. 14. SEM micrographs of EPI compacted samples after thermooxidation at 60 °C for 300 h and incubated with *R. rhodochrous* for 6 months.

material to be processed [16]. Their initial protective effects are clearly in evidence in the induction times shown in Fig. 1 for the original films. This suggests that the antioxidant(s) added initially are essentially used up in the initial moulding procedure. This results in the very rapid formation of oxidation products in the polymer surface during storage, even at 5 °C and rapid bio-film formation when incubated with micro-organisms (Fig. 10).

Ageing of compacted films (#1–4) results in a reduction in molecular weight in all pre-ageing regimes. It is particularly striking that samples stored at 20 °C show a quite profound reduction in M_n and M_w with a narrowing of molecular weight distribution whereas samples pre-oxidised at higher temperatures showed molecular enlargement. However, after incubation with micro-organisms, the higher molecular weight material disappeared and the distribution became similar to that of the samples oxidised at lower temperatures. It is concluded that under conditions where oxygen diffusion is a limiting factor, for example, in thick sections of polymer, –C–O–O–C– (and possibly but less likely –C–O–C– and –C–C– bonds) may be formed in the polymer at higher temperatures. The peroxidic cross-links dissociate slowly in the biotic environment. It is further concluded that molar mass reduction due to the action of microorganisms does not occur to any significant extent during incubation and that the main role of the microorganisms in this process is to scavenge the low molecular weight oxidation products as they are formed. That is, the abiotic peroxidation process is the rate determining step. This has been proposed previously to explain why degradation products formed as intermediates in oxo-biodegradation are never observed in the vicinity of the polymer [4].

FTIR (PAS) and SEM experiments have shown the presence of a biofilm, which builds up with time. The formation of bio-films has been observed for other Actinomycetes when degrading rubber, including strains from the genus *Gordonia* [14] and *Nocardia* [17]. It is well-known that the synthesis of biofilms by bacteria favours their adhesion to surfaces and helps them to survive under low-nutrient environments and utilise solid substrates [14].

There is clear evidence from photo-acoustic FTIR (PAS), which measures spectral changes in the surface of the polymer, that proteinic materials associated with the growth of microorganisms at the expense of the polymer oxidation products and polysaccharides are both formed in the surface layers of the polymer (Figs. 4–6). SEM evidence confirms that microorganisms build up on the surface of the polymer and after removal of the micro-organisms, the surface (as reported earlier) becomes physically pitted and eroded. The surface of the polymer after biological attack is physically weak and readily disintegrates under mild pressure.

The enzymes involved in this biodegradative process are not known. As suggested in the case of rubber degrading *Gordonia* and *Nocardia* isolates, the polymer-degrading activity could be bound to the cell surface [14] as (1) these strains do not produce extracellular enzymes (which is different from lignin-degrading organisms producing extracellular peroxidases) and (2) they must first oxidatively cleave the high molecular weight polymer into smaller molecules before uptake by the cell.

In the later stages of bioassimilation, the synergistic processes of abiotic and biotic peroxidation continue further by normal transition metal and oxidase enzyme catalysed peroxidation. This continues so long as there is oxygen and microbes in the system. The bio-conversion process has recently been confirmed in biometric studies of CO₂ formation from peroxidised polyethylene using both soil [18] and mature compost [19] inoculate. Over 60% mineralisation has been observed in 6 months [18]. These results are of considerable significance for commercial composting processes, in which degradable plastics are required to disintegrate to small fragments within the composting cycle time and to be subsequently absorbed into the soil environment as nutrients for growing plants [20].

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