Biodegradation of Oxo-Biodegradable Polyethylene

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ABSTRACT: Biodegradation of polyethylene and oxo-biodegradable polyethylene films was studied in this work. Abiotic oxidation, which is the first stage of oxo-biodegradation, was carried out for a period corresponding to 4 years of thermo-oxidation at composting temperatures. The oxidation was followed by biodegradation, which was achieved by inoculating the microorganism *Pseudomonas aeruginosa* on polyethylene film in mineral medium and monitoring its degradation. The changes in the molecular weight of polyethylene and the concentration of oxidation products were monitored by size exclusion chromatography and Fourier transform infrared (FTIR) spectroscopy, respectively. It has been found that the initial abiotic oxidation helps to reduce the molecular weight of oxo-biodegradable polyethylene and form easily biodegradable product fractions. In the microbial degradation stage, *P. aeruginosa* is found to form biofilm on polymer film indicating its growth. Molecular weight distribution data for biodegraded oxo-biodegradable polyethylene have shown that *P. aeruginosa* is able to utilize the low-molecular weight fractions produced during oxidation. However, it is not able to perturb the whole of the polymer volume as indicated by the narrowing of the polymer molecular weight distribution curve toward higher molecular fractions. The decrease in the carbonyl index, which indicates the concentration of carbonyl compounds, with time also indicates the progress of biodegradation. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 1426–1432, 2009

Key words: polyethylene; oxo-biodegradation; *Pseudomonas aeruginosa*; abiotic oxidation

INTRODUCTION

Polyethylene accounts for nearly 40% of the plastics used in packaging applications worldwide. Short-term use and long-term functionality of these packaging materials have created the problem of their disposal. Increased environmental concern and awareness has directed polymer scientists and microbiologists to find the solutions for this problem. Polymer scientists have worked on modifications or blends of polyethylene to increase its biodegradability, i.e., to decrease its long-term functionality,[1–6] whereas microbiologists isolated microbial strains that would directly consume polyethylene for their growth.[7–10]

Among many approaches used to induce biodegradation in polyethylene, use of pro-oxidants has been gaining more popularity recently. Pro-oxidants are transition metal ion complexes, which catalyze the oxidation of polyethylene and lead to its molecular weight reduction and thereby, facilitate biodegradation. This combination of oxidation and microbial consumption of polyethylene is now widely known as oxo-biodegradation of polyethylene.[6,11–13] Oxo-biodegradation of polyethylene has been very well studied with different pro-oxidants, and it has been now established that oxidation products are readily biodegradable.[10] This study has been carried out to complement the past studies and elucidate the action of soil microorganisms such as *Pseudomonas aeruginosa* on oxidized fragments. The key focus is to evaluate whether the microorganism is able to act on the bulk of the polymer volume or just it has superficial action on the polymer surface by consuming the oxidation products.

Oxo-biodegradation of polyethylene and oxo-biodegradable polyethylene (polyethylene with pro-oxidant) was studied in this work. Initial abiotic oxidation was carried out for a period corresponding to about 4 years of thermo-oxidation at composting temperatures. This was followed by inoculating the polymer with *P. aeruginosa* in mineral medium and carrying out the biodegradation up to 6 weeks.

EXPERIMENTAL

Materials and film preparation

The materials used in this investigation are low density polyethylene (LDD203 film grade) supplied by

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TABLE I
Mechanical Properties of PE and OPE Before Abiotic Oxidation

Material	Elongation at break (%)	Tensile strength (MPa)
PE	318.3	21.5
OPE	299.2	20.8

Qenos, Australia, and a pro-oxidant (proprietary material) for low density polyethylene. The composition of oxo-biodegradable polyethylene is 98 wt % low density polyethylene and 2 wt % pro-oxidant. Throughout the article, polyethylene and oxo-biodegradable polyethylene are referred as PE and OPE, respectively.

Conventional extrusion was carried out using a Brabender twin screw extruder for initial blending of polyethylene and pro-oxidant. An advanced extrusion blown film coextrusion assembly (Strand Plast Maskiner, Sweden) was used to prepare the films. The film thickness during the processing was found to vary between 60 and 80 μm. More details on the extrusion and film preparation are discussed in Reddy et al.[14]

Mechanical properties of PE and OPE films measured soon after their preparation (Table I) indicate that the addition of pro-oxidant has no major influence on PE during the processing stage.

Abiotic oxidation (thermo-oxidation)

Thermo-oxidation of polyethylene was studied using an oven ageing test. This test involved ageing of films at selected temperatures. Polyethylene films were cut into 20 mm × 120 mm strips and placed in 40-mL glass vials with loosely screwed caps and subjected to heat treatment in the oven for a period of 14 days at 50–70°C. The aged films were sealed and stored at −10°C to minimize further oxidation before Fourier transform infrared (FTIR) analysis.

Biodegradation procedure

Thermo-oxidized PE and OPE samples were subjected to biodegradation using the *P. aeruginosa* strain, which was obtained from our University culture collection. Bacterial cultures were maintained on nutrient broth or nutrient agar (Difco). Unless otherwise specified, liquid cultures (100 mL) were incubated in flasks (250 mL) held at 30°C on a rotary shaker operating at a speed of 150 rpm.

Media

Bacterial strains assayed for their ability to utilize polyethylene as the sole source of carbon and energy were grown in a mineral synthetic medium containing (per liter of distilled water): 1.0 g NH_4NO_3, 0.2 g $MgSO_4{\cdot}7H_2O$, 1.0 g K_2HPO_4, 0.1 g $CaCl_2{\cdot}2H_2O$, 0.15 g KCl, 0.1 g yeast extract (Difco), and 1.0 mg of each of the following microelements: $FeSO_4{\cdot}6H_2O$, $ZnSO_4{\cdot}7H_2O$, and $MnSO_4$.

Gel permeation chromatography

High temperature GPC analysis was performed at 140°C using a Waters Alliance GPCV2000 chromatographer, equipped with differential refractive index (DRI) and viscosity detectors. 1,2,4-Trichlorobenzene (TCB) was used as the solvent with a flow rate of 1.00 mL/min. The system of three Styragel® HT (4, 5, and 6) columns was calibrated with 10 narrow polystyrene standards, with average molecular weight ranging from 1000 to 5,000,000. PE and OPE samples were dissolved TCB and then filtered through 0.5-μm polytetrafluoroethylene (PTFE) filter to remove solid particles. Universal calibration was applied and the chromatograms were processed using the Millennium® software. Number–average molecular weight, M_n, weight–average molecular weight, M_w, and polydispersity index, PI, of samples were determined from GPC analysis.

Fourier transform infrared spectroscopy

The extent of oxidation was determined by measuring the levels of ester carbonyl (1735 cm^{-1}) and ketone carbonyl (1715 cm^{-1}) absorbance using FTIR spectroscopy. A Perkin-Elmer 2000 infrared spectrometer was used to measure the absorbance levels of carbonyl groups. The spectra were obtained using attenuated total reflectance (ATR). During the analysis, the surface of the film samples was in contact with a Zn-Se crystal that has a 45° angle of incidence. Interferograms were obtained from 32 scans. The scanning range was from 4000 to 1300 cm^{-1}. Before the actual analysis, background spectra were obtained without samples in the chamber. Carbonyl index (C.I.), defined as the ratio of carbonyl and methylene absorbances, was used to express the concentration levels of carbonyl compounds measured.

Environmental scanning electron microscopy

The biofilms were imaged on a Peltier stage (5°C) in a FEI Quanta ESEM (Philips Electron Optics, Eindhoven, The Netherlands) operated in wet mode (∼ 4 Torr) at an accelerating voltage of 10 kV. Specimens were not conductively coated prior to imaging. A random-number-based scheme was used to select fields of view when acquiring the biofilm images. Images (five per biofilm) used for assessing biofilm surface morphology and cell sizes were acquired at magnification of ×2500.

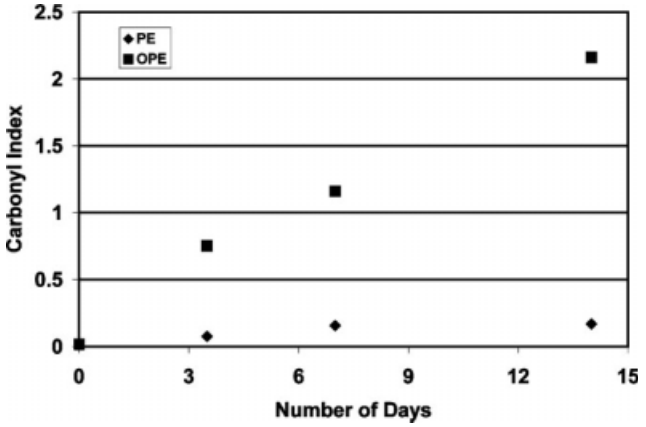

Figure 1 Carbonyl index of abiotically oxidized PE and OPE, Temperature = 70°C, Duration = 14 days.

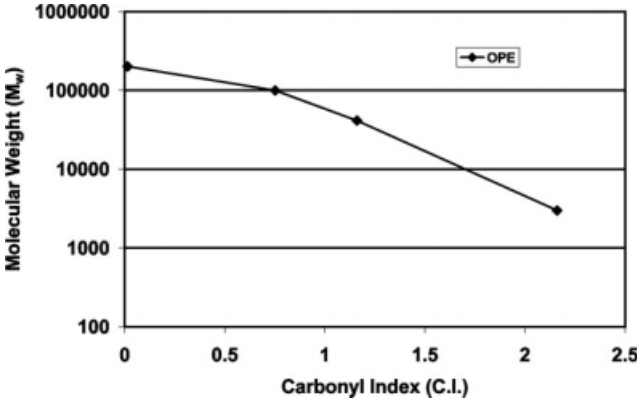

Figure 2 Molecular weight (M_w) and carbonyl index (C.I.) relationship for OPE.

RESULTS AND DISCUSSION

Abiotic oxidation

Abiotic oxidation of PE and OPE was conducted at temperatures 50–70°C to mimic the composting process. Also, as discussed previously, the reduction in molecular weight and the formation of carbonyl compounds due to abiotic oxidation were monitored.

The carbonyl groups usually account for most of the products of abiotic oxidation of polyethylene, and it has become a norm to use their concentration, as determined by the C.I. to monitor the progress of oxidation.[15] C. I. of both PE and OPE is shown in Figure 1. It can be seen that C. I. for OPE increases with oxidation time, whereas that for PE exhibits negligible increase. This indicates that the oxidation rate of OPE is substantially higher than that for PE. These results suggest that the pro-oxidant has played a major role in the oxidation of OPE.

The relationship between M_w and C.I. of OPE is shown in Figure 2. This decreasing trend of M_w with increase in CI agrees with the statistical chain scission mechanism suggested by many authors in the photo and thermal degradations of polyolefins.[16–18] It can be seen from Figure 2 that the molecular weight decreases substantially, i.e., more than one order of magnitude as the oxidation of OPE proceeds. This is usually accompanied by the production of low molar mass, oxidized fragments, which because of their wettability and functionality will be vulnerable to microorganisms.

Microbial degradation of abiotically oxidized polyethylene

Biofilm formation

During the microbial degradation stage, most of the abiotic oxidation products, (i.e., low-molecular weight compounds) will be utilized by the microbes.

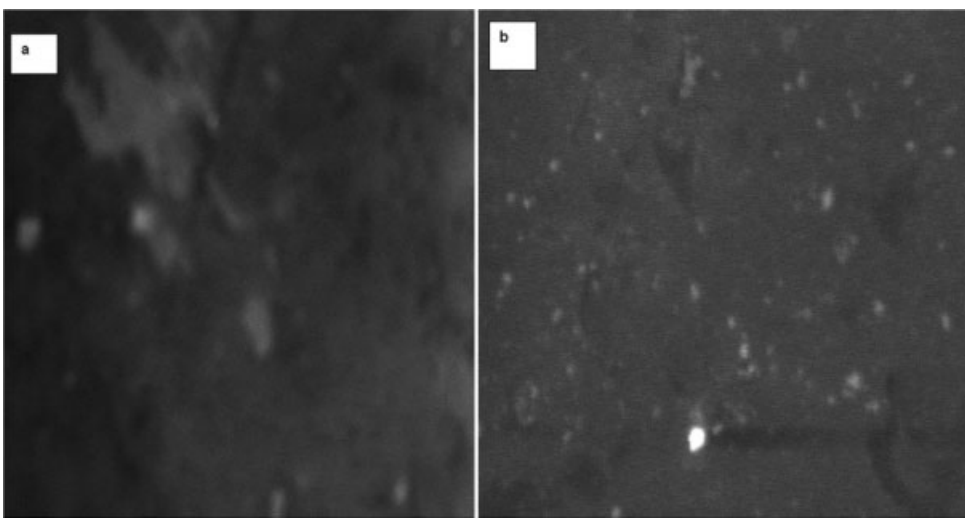

Figure 3 ESEM micrographs of the biofilm formed by *Pseudomonas aeruginosa* on unaged (a) PE and (b) OPE.

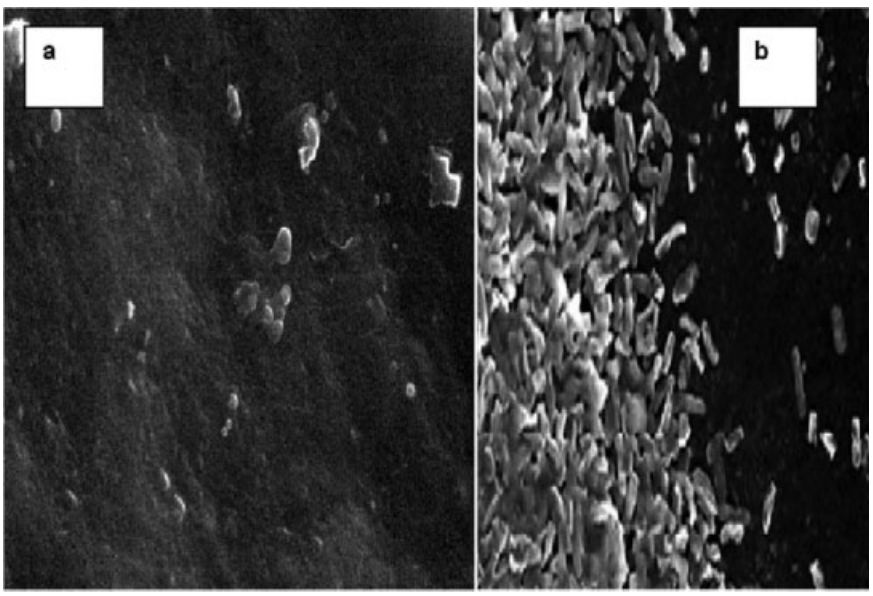

Figure 4 ESEM micrographs of the biofilm formed by *Pseudomonas aeruginosa* on abiotically oxidized (a) PE and (b) OPE.

Also, the most important aspect during the biodegradation is the sustained growth of microorganisms during the entire process.[8,19] One can monitor this growth by investigating the biofilm formation on the polymer surface.

As mentioned earlier in this article, growth of biofilm and microbial activity were investigated using both ESEM and FTIR spectroscopy. ESEM micrographs of unaged and aged polyethylene samples are shown in Figures 3 and 4, respectively. These micrographs clearly indicate that *P. aeruginosa* has effectively colonized the abiotically oxidized (aged) OPE film when compared with its unaged film. On the other hand, the extent of growth of the microorganism in the aged PE sample is found to be relatively low. This indicates that the bioerosion of OPE by *P. aeruginosa* is relatively significant. Similar phenomenon has also been reported for hydrobiodegradable and oxo-biodegradable polymers by Bonhomme et al.[10]

The existence of the biofilm on both abiotically oxidized PE and OPE is independently confirmed further by the FTIR spectra shown in Figure 5. The bands at 1643 cm^{-1} and the nearby bands on the right can be assigned to protein material. The peaks in this region of the spectra indicate the presence of significant amount of protein. The broad bands peaking at 1133 and 993 cm^{-1} show the presence of polysaccharides, the usual metabolites produced by microorganisms, which are the major constituents of the biofilm.[20,21]

Molecular weight changes

Biodegradation is described as the biological process by which potentially toxic compounds are transformed into nontoxic ones.[22] It is important to prove that the microbial growth is due to the utilization of the nontoxic oxidation products. This will help in establishing the mechanism for microbial degradation of polyethylene.

Figures 6 and 7 show the changes in molecular weight distributions and molecular weight, respectively, with time for the OPE. It can be clearly seen from Figure 6 that there is a significant change in molecular weight distribution with the passage of time. The molecular weight distribution curve for OPE after 2 weeks of abiotic oxidation shifts toward left indicating the formation of low-molecular weight compounds. This change is usually accompanied by a drastic reduction in the molecular weight of OPE. After inoculation with *P. aeruginosa*, the molecular weight distribution curve for the abiotically oxidized OPE shifts toward the right indicating that the low-molecular weight fragments present in the oxidation products are being eliminated or utilized.

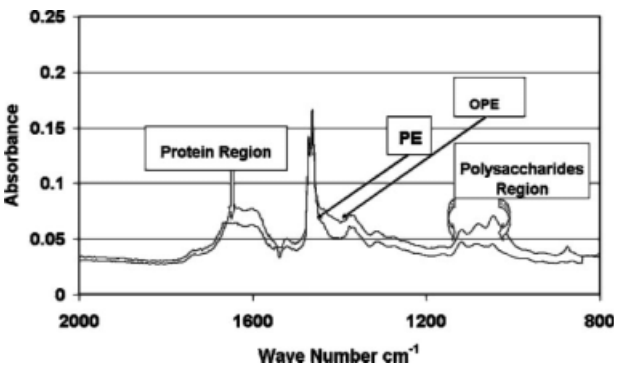

Figure 5 FTIR spectra of the biofilm covered abiotically oxidized PE and OPE after 42 days of incubation.

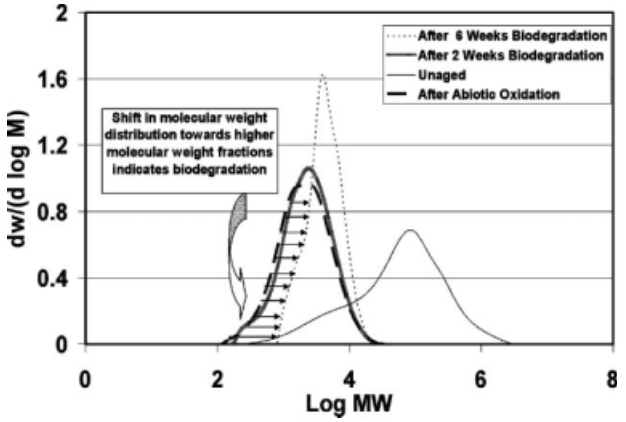

Figure 6 Molecular weight distribution curves of OPE at various stages of oxo-biodegradation process.

Initially (after 2 weeks), *P. aeruginosa* is just able to utilize a minor fraction of the oxidation products shifting the molecular weight distribution curve slightly toward higher fractions. After 6 weeks, a major fraction of oxidation products is utilized leading to a notable shift toward the right hand side of the molecular weight scale. These results indicate that *P. aeruginosa* is just able to utilize chain-end low-molecular weight compounds and unable to perturb high-molecular weight fractions. It is also clear that the action of microorganisms is only on the surface of the polymer.

The aforementioned phenomenon can also be observed in the molecular weight data shown in Figure 7 for abiotically oxidized OPE. Molecular weight (M_w) increase reaches a peak value after 42 days, as expected from the molecular weight distribution data. The small reduction observed during the fourth week (after 28 days), which has been confirmed by repeating the measurement three times, may possibly be due to the attempt from *P. aeruginosa* to access the polymer volume further. However, increase in average molecular weight after 42 days

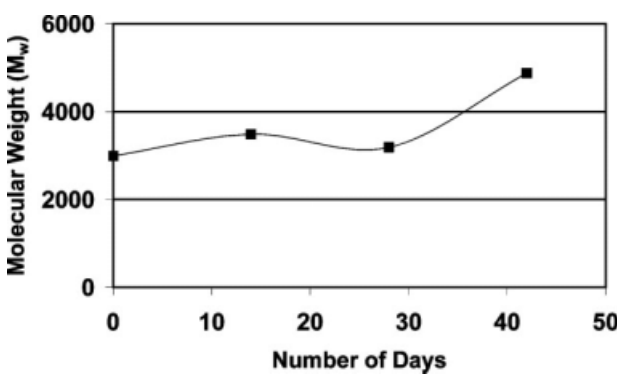

Figure 7 Changes in molecular weight for abiotically oxidized OPE during microbial degradation stage.

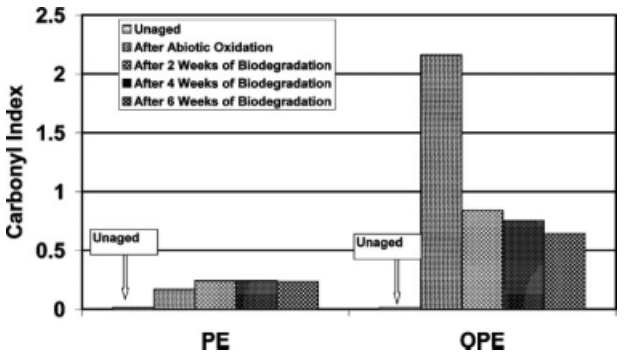

Figure 8 Changes in carbonyl index of PE and OPE at various stages of oxo-biodegradation process.

indicates that *P. aeruginosa* cannot access polymer any further.

These results are significant as they reveal that biodegradation is mainly because of the consumption of pro-oxidant aided oxidation products. The shift toward high molecular weight during biodegradation also suggests that pro-oxidant has ceased its action during the abiotic oxidation stage and is not helping the biodegradation.

The aforementioned results substantiate the oxo-biodegradation theory proposed by Koutny et al.,[7] which suggests that an increase in the abiotic oxidation levels and consequent decrease in the average molecular weight to under 5000 Da are required for achieving significant biodegradation in a reasonable time period. The behavior observed with the additive employed in this study is consistent with the theory described earlier. It has been proven that if the oxidized polyethylene has a molecular weight less than 5000 Da, a significant fraction of it will be in the range of 1000–2000 Da and this fraction can be rapidly biodegraded.[19,23] The vacancies produced due to biodegradation can then cause swelling and relaxation of the whole material structure, which will facilitate diffusion of water and soluble compounds inside and thereby, substantially accelerating the biodegradation. This theory lends support to the prior conclusion that the microbe *P. aeruginosa* utilizes the low-molecular weight fraction produced in abiotic oxidation of OPE in this work.

Carbonyl compounds consumption

C.I. values of PE and OPE at various stages of oxo-biodegradation process are shown in Figure 8. It can be seen that C.I. value for OPE decreases with the time indicating that the concentration of carbonyl compounds for OPE has decreased significantly after the biodegradation process. The rate of decrease of C.I. in PE is lower than that of OPE indicating lower levels of biodegradation in PE. The decrease in C.I. of OPE can be attributed to the preferential

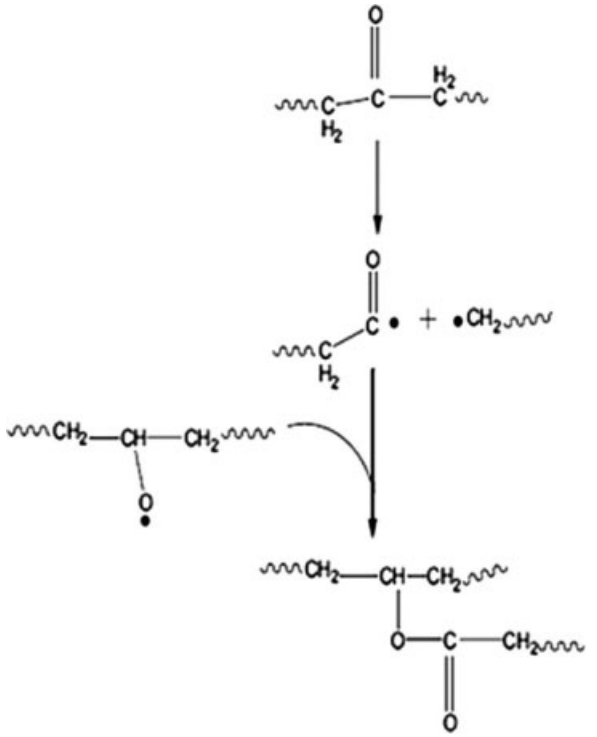

Figure 9 Ester formation during abiotic oxidation of polyethylene.[24]

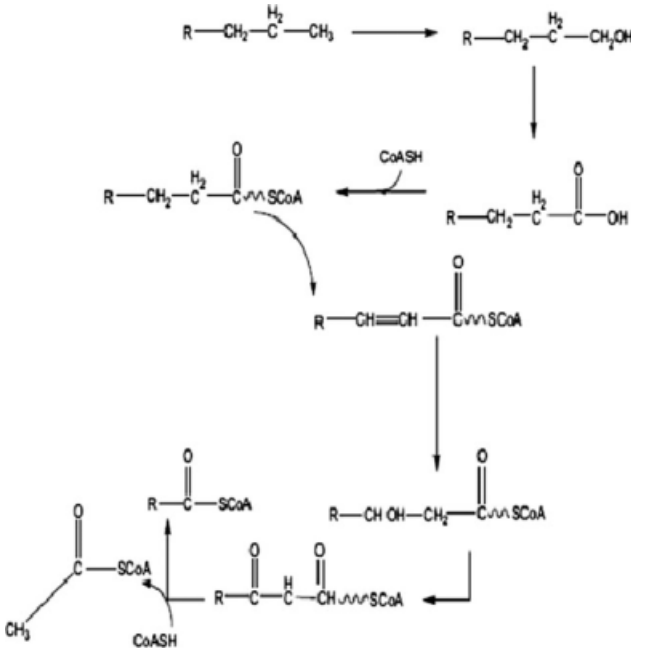

Figure 10 Biodegradation pathway for paraffin.[27]

microbial assimilation of ester/carbonyl compounds formed during abiotic oxidation. The pathway for the formation of ester carbonyl compounds during abiotic oxidation as proposed by Albertsson et al.[6] is shown in Figure 9. The C.I. results as well as the molecular weight data from this work therefore substantiate the previous findings in the literature[8,9,25,26] where thermo-oxidation has led to the formation of ester and keto carbonyl compounds. This insight also helps in understanding the mechanism of biodegradation of polyethylene. The low-molecular weight carbonyl compounds formed during the oxidation will be similar to paraffins. Hence, comparisons can be made on the biodegradation of paraffins and that of oxidized low-molecular weight products.[6] The biodegradation pathway for paraffin is shown in Figure 10.

The biodegradation of paraffin starts with the oxidation of the alkane chain to form ketone and then to carboxylic acid that later undergoes B-oxidation, which is the oxidative breakdown of acids into acetyl-coenzyme A (SCoA) by repeated oxidation at the β-carbon atom. Drawing the parallels between the paraffin biodegradation and low molecular weight carbonyl compounds biodegradation, a mechanism for polyethylene biodegradation was presented by Albertsson et al.[6,24] (Fig. 11).

Oxo-biodegradation of polyethylene involves initial oxidation followed by microbial degradation. During initial abiotic step, oxidation of the polymer chain occurs because of the oxygen present in the atmosphere leading to the formation of carbonyl groups and decrease in molecular weight. During

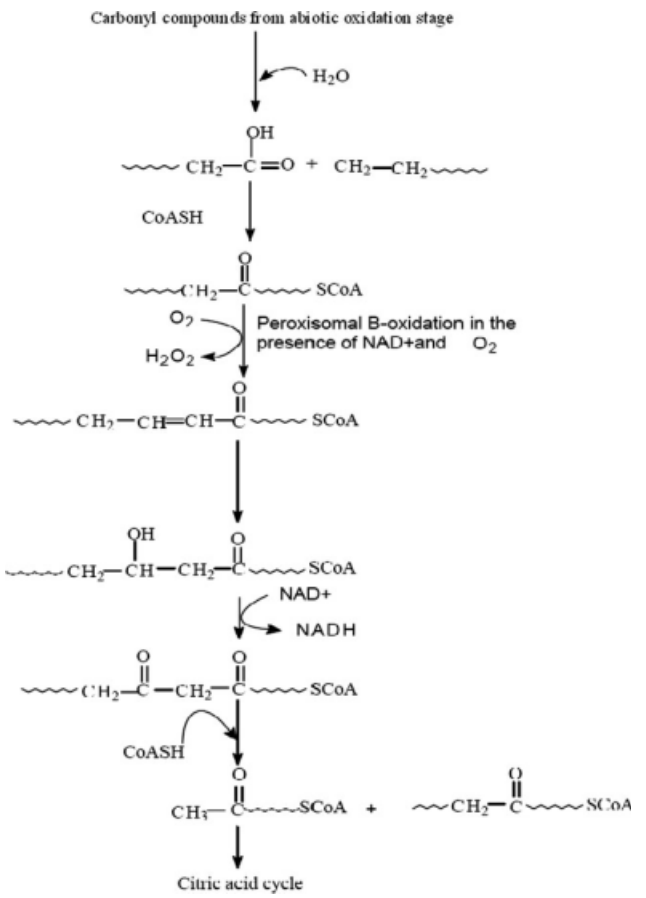

Figure 11 Biodegradation mechanism of polyethylene.[6,24]

microbial degradation, a decrease in the number of carbonyl groups occurs. This leads to formation of carboxylic acids, which react with coenzyme A (CoA) and remove two carbon fragments, forming acetyl-coenzyme. This enters the citric acid cycle and produces carbon dioxide and water as the final degradation products.[6,25] Embrittlement and hydrophilicity due to the introduction of carbonyl groups further promotes the biodegradation of the polymer.

CONCLUSIONS

Oxo-biodegradation of polyethylene and polyethylene loaded with pro-oxidant film was investigated by inoculating abiotically oxidized samples with *P. aeruginosa* for 6 weeks. It was observed that pro-oxidant aids in the drastic reduction of molecular weight of OPE during abiotic oxidation stage yielding low molecular weight compounds. *P. aeruginosa* is able to utilize these low molecular weight compounds and form biofilm. Also, changes in molecular weight distribution observed with biodegradation substantiate this argument. However, the high-molecular weight region in the distribution exhibits little changes indicating that *P. aeruginosa* is just able to utilize the end chain products and unable to perturb the whole of the polymer volume.

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