Biodegradation of Montmorillonite Filled Oxo-Biodegradable Polyethylene

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ABSTRACT: Oxo-biodegradation of polyethylene has been well studied with different pro-oxidants and it has been shown that pro-oxidants have limited role in the oxidation of polyethylene and do not have any role in microbial growth. However, in few recent studies, montmorillonite clay has been reported to promote the growth of microbes by keeping the pH of the environment at levels conducive to growth. In an attempt to improve the overall oxo-biodegradation of polyethylene, montmorillonite nanoclay has been used in this study along with a pro-oxidant. Film samples of oxo-biodegradable polyethylene (OPE) and oxo-biodegradable polyethylene nanocomposite (OPENac) were subjected to abiotic oxidation followed by microbial degradation using microorganism *Pseudomonas aeruginosa*. The progress of degradation was followed by monitoring the chemical changes of the samples using high-temperature gel permeation chromatography (GPC) and infrared spectroscopy (FTIR). The growth of bacteria on the surface of the polymer was monitored using environmental scanning electron microscopy.

GPC data and FTIR results have shown that the abiotic oxidation of polyethylene is influenced significantly by the pro-oxidant but not by nanoclay. But, the changes in molecular weight distribution and FTIR spectra for the biodegraded samples indicate that the growth rate of *P. aeruginosa* on OPENac is significantly greater than that on OPE. It indicates that nanoclay, by providing a favourable environment, helps in the growth of the microorganism and its utilisation of the polymer surface and the bulk of the polymer volume. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 113: 2826–2832, 2009

Key words: biodegradable; clay; degradation; nanocomposites; polyethylene (PE)

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 have directed polymer scientists and microbiologists to find suitable solutions to this problem. Polymer scientists have worked on modifications or blendings 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 catalyse the oxidation of polyethylene and lead to its molecular weight reduction and thereby facilitate its biodegradation. This combination of oxidation and microbial consumption of polyethylene is now widely known as oxo-biodegradation of polyethylene.[6,11,12] Oxo-biodegradation of polyethylene has been widely studied with different pro-oxidants and it has been now established that oxidation products are readily biodegradable.[10] However, biodegradation of polyethylene occurs only on its surface not in the bulk volume, probably due to the inaccessibility of oxidation products to microorganisms.[13] Thus the role of pro-oxidants is limited to the oxidation of polyethylene and not extended to microbial growth.

Montmorillonite clay, which is used to enhance mechanical, thermal and barrier properties of polyethylene, is known to promote the growth of microorganisms by keeping the pH of the environment at levels conducive to their sustained growth.[14] It has been also suggested that the cation exchange capacity and the large surface area of clay are also responsible for the microorganism growth.[14–16] In an attempt to improve the overall oxo-biodegradation of polyethylene, Reddy et al.[17] have used montmorillonite nanoclay along with a pro-oxidant and proved that clay does not affect the oxidation mechanism significantly.

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In the present work, the effect of clay on the biodegradation of oxo-biodegradable polyethylene is investigated. Initial abiotic oxidation corresponding to about four years of composting was carried out as thermo-oxidation at composting temperatures. This was followed by inoculating the polymer samples with *Pseudomonas aeruginosa* in mineral medium up to 6 weeks.

EXPERIMENTAL

Materials and film preparation

The materials used in this investigation are: low density polyethylene (LDD203 film grade) supplied by Qenos, Australia, organo-modified clay (with trade name Cloisite 15A) supplied by Southern Clay Products, USA and a pro-oxidant (proprietary material) for low density polyethylene.

Conventional extrusion was carried out using a Brabender twin screw extruder for the initial blending of polyethylene, pro-oxidant, and nanoclay. An advanced extrusion blown film co-extrusion 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 the film preparation techniques are discussed in Reddy et al.[17] Throughout this article, oxo-biodegradable polyethylene (polyethylene with 2 wt % pro-oxidant) and oxo-biodegradable polyethylene nanocomposite (polyethylene with 2 wt % pro-oxidant and 2 wt % nanoclay) are referred as OPE and OPENac, respectively.

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 minimise further oxidation before FTIR analysis.

Biodegradation procedure

Thermo-oxidised 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 was grown in a minimal synthetic medium containing (per litre 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 (GPC)

High temperature GPC analysis was performed at 140°C using a Waters Alliance GPCV2000 chromatographer, equipped with differential refractive index (DRI) and viscometer 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 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 Mn, weight average molecular weight Mw, and polydispersity index PI of samples were determined from GPC analysis.

Fourier transform infrared (FTIR) 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, defined as the ratio of carbonyl and methylene absorbances, was used to express the concentration levels of carbonyl compounds measured.

Environmental scanning electron microscopy (ESEM)

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 before imaging. A

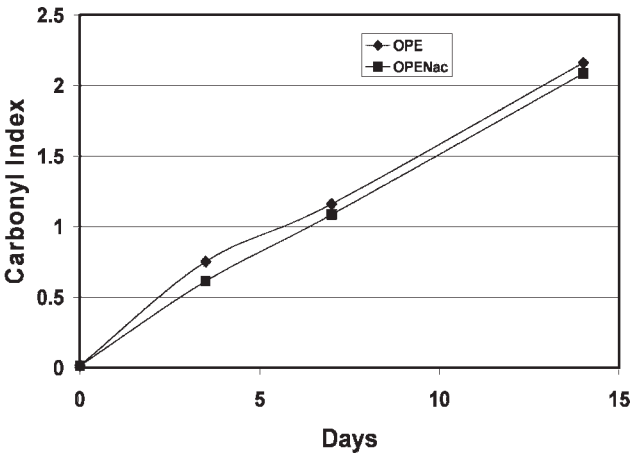

Figure 1 Carbonyl Index (C.I.) of oxo-biodegradable polyethylene and its nanocomposite at 70°C.

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.

RESULTS AND DISCUSSION

Abiotic oxidation

Abiotic oxidation is an important step because microbial degradation in the oxo-biodegradation process takes place only if polyethylene has been oxidised. Drastic reduction in the molecular weight of polyethylene and the subsequent production of low molecular weight carbonyl compounds are the characteristics of abiotic oxidation of oxo-biodegradable polyethylene. FTIR results can be used to study the changes in the molecular weight of polyethylene as was done by many authors.[18–22] In addition to carbonyl absorption spectra, carbonyl index (C.I.), which is determined by the taking the ratio of absorbances 1713 and 1413 cm^{-1}, can be used to indicate the formation of low molecular weight carbonyl compounds.

The changes in the C.I. values with oxidation time for OPE and OPENac are shown in Figure 1. It can be seen that the C.I. values for both OPE and OPENac are greater than zero and increase with time. These results show clearly that both of these materials have undergone significant oxidation during the 2 weeks period. However, it is interesting to note that the rate of oxidation of OPENac is lower than that for OPE throughout the oxidation period. This could be attributed to the reduction in oxygen permeability in OPENac due to the presence of clay. The impact of delamination of clay layers on the tortuous diffusion path formation in the nanocomposites is well known and has been reported by many authors.[23,24]

Many authors have reported that polyethylene molecules are broken into low molecular compounds by chain scission mechanism during both photo-and thermo oxidation.[5,25,26] To verify this fact in the present study, molecular weight data for both OPE and OPENac obtained from GPC analysis are plotted against C.I. in Figure 2. It can be seen that the molecular weight decreases significantly (more than one order of magnitude) with increase in C.I. for both OPE and OPENac. This will be usually accompanied by the production of low molar mass, oxidized fragments, which due to their wettability and functionality will become vulnerable to microorganisms.

The above observations suggest that the pro-oxidant in both OPE and OPENac is responsible for initiating the auto-oxidation of polyethylene matrix. The mechanism responsible for this enhanced oxidation rate is considered to be the production of free radicals which react with molecular oxygen to produce peroxides and hydroperoxides, which subsequently lead to the accelerated oxidation. Based on the results obtained in this work, it can be said that the addition of clay does not alter the oxidation mechanism greatly in OPENac.

Microbial degradation of abiotically oxidised polyethylene nanocomposites

Biofilm formation

Growth of biofilm and microbial activity were investigated using ESEM and FTIR. ESEM micrographs of abiotically oxidised and biodegraded OPE and OPENac samples are shown in Figures 3 and 4, respectively. The micrographs clearly indicate that the growth of biofilm on unaged samples is relatively insignificant. However, it can be seen that *P.*

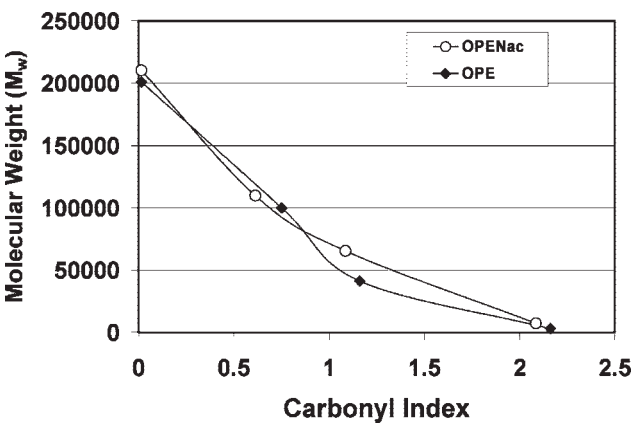

Figure 2 Molecular Weight (M_w) and carbonyl index (C.I.) relationship for OPE and OPENac.

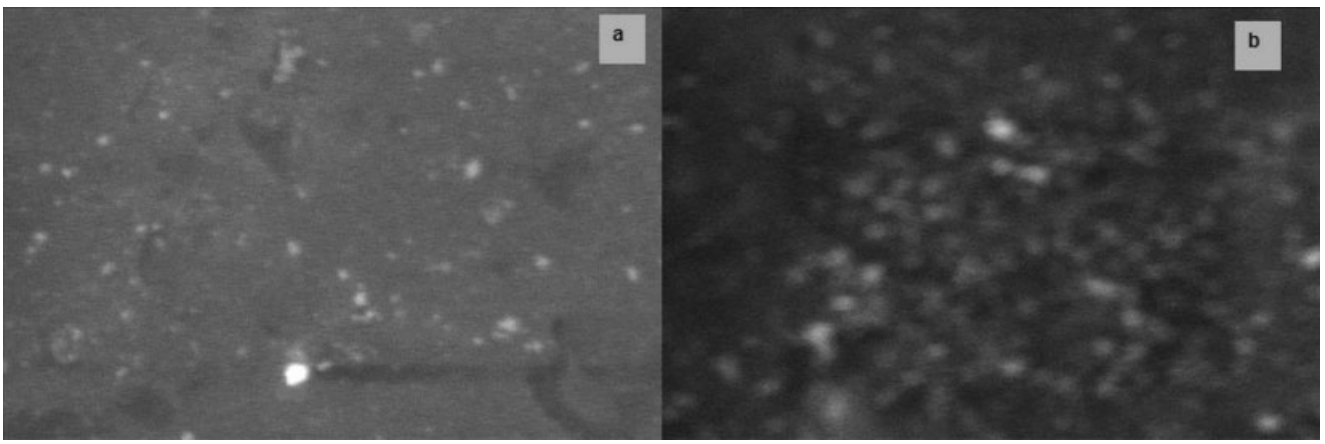

Figure 3 ESEM micrographs of the biofilm formed by *Pseudomonas aeruginosa* on unaged (a) OPE and (b) OPENac.

aeruginosa has effectively colonized a vast proportion of the oxidised samples of both films. These results indicate that oxidized polyethylene samples are greatly bioeroded by *P. aeruginosa*. Similar phenomenon has been reported for both hydro-biodegradable and oxo-biodegradable polymers by Bonhomme et al.[10] It is also interesting to see that the growth of biofilm on OPENac is much greater than that on OPE.

Predominant colonization of *P.aeruginosa* on OPENac samples is also confirmed by FTIR spectra shown in Figure 5. OPENac shows a larger absorbance at 1643 cm^{-1}. The nearby bands on the right, which can be assigned to protein material, confirm that the growth of biofilm on OPENac is greater that on OPE. 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.[27,28] The polysaccharides peaks are observed for both OPE and OPENac but the one for OPE is relatively shorter.

Utilisation of carbonyl compounds

Carbonyl Index (C.I.) values of OPE and OPENac at various stages of oxo-biodegradation process are shown in Figure 6. It can be seen that the rate of carbonyl compounds consumption as indicated by the decrease in C.I. value is more or less same for both OPE and OPENac during the early periods of oxo-biodegradation process. However, the carbonyl consumption rate for OPE is slower during the sixth week. It has been observed from its molecular weight distribution data that microbes could not further perturb the whole of the polymer volume even after 6 weeks.[29] The entire microbial action is on the end chain oxidation products. However, the carbonyl consumption rate for OPENac during the sixth week is greater than that for OPE. This could be probably due to some active action of *P. aeruginosa* in accomplishing further chain cleavage in OPENac or could be due to the presence of clay which makes a difference in the biotic environment. However, the biodegradation pathway may be the same as

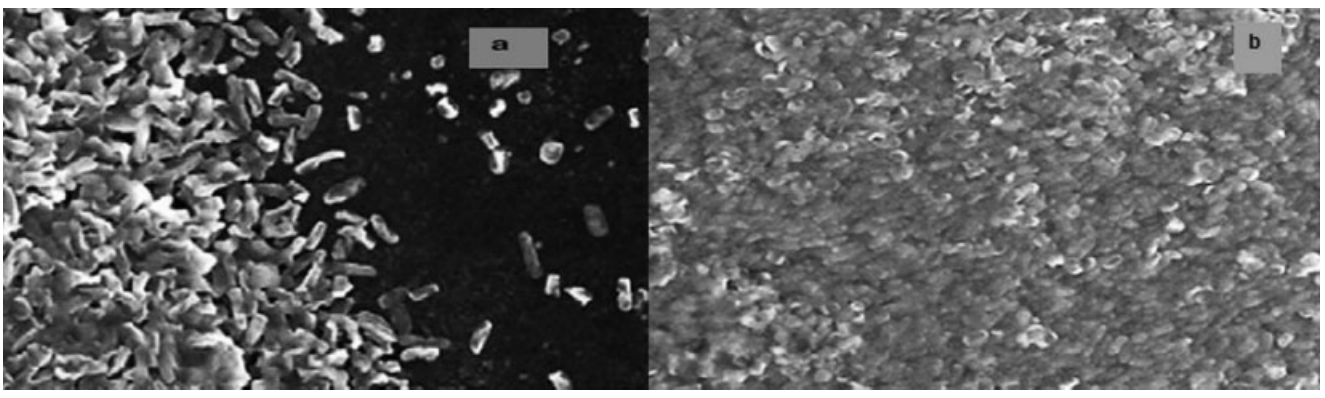

Figure 4 ESEM micrographs of the biofilm formed by *Pseudomonas aeruginosa* on abiotically oxidised (a) OPE and (b) OPENac.

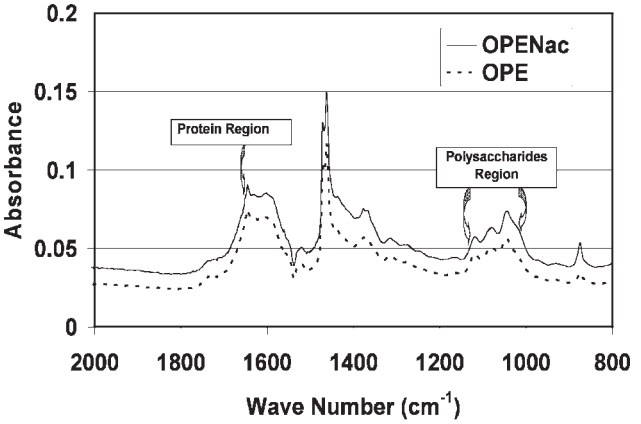

Figure 5 FTIR spectra of the biofilm covered abiotically oxidised polyethylene after 6 weeks of biodegradation.

explained in our previous publication[29] and this is confirmed by the decrease in C.I. which can be attributed to the preferential assimilation of ester/carbonyl compounds formed during abiotic oxidation.

Oxo-biodegradation mechanism in OPENac

An attempt has been made to understand the reason for the relatively enhanced rate of carbonyl consumption in OPENac by analysing its molecular weight data. Figure 7 shows the changes in molecular weight distributions with time for the OPENac. It can be seen that molecular weight distribution varies significantly with time. As expected, the molecular weight distribution curve for OPENac after 2 weeks of abiotic oxidation shifts toward left indicating the formation of low molecular weight compounds. However, after inoculation with *P. aeruginosa*, the molecular weight distribution curve for the abiotically oxidised OPENac narrows down signifying the change occurred especially in high molecular weight fractions. This trend is contrary to that observed for

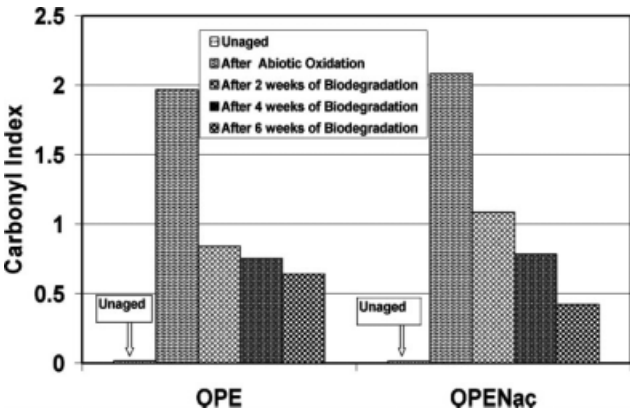

Figure 6 Changes in carbonyl index of OPE and OPENac at various stages of oxo-biodegradation process.

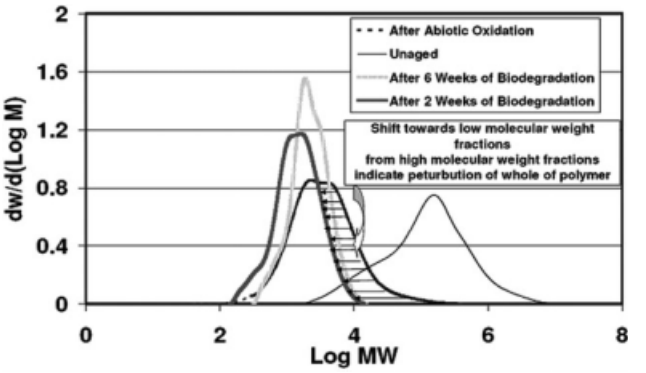

Figure 7 Molecular weight distribution curves of OPENac at various stages of oxo-biodegradation process.

OPE by Reddy et al.[29] and may be due to the perturbation of whole of polymer volume by *P. aeruginosa*. Even in this case, the initial growth of *P. aeruginosa* would have started with the consumption of end chain low molecular weight compounds in abiotically oxidized OPENac. However, after the initial period, it appears that *P. aeruginosa* would have started the perturbation of the whole of polymer volume owing to the difference in biotic environment created by the presence of nanoclay.

Figure 8 clearly shows the difference in the action of *P. aeruginosa* on abiotically oxidised OPE and OPENac in terms of molecular weight distributions. The shaded portion on the plot shows that biodegraded OPE has more higher molecular weight fractions than OPENac. Although the shape of distribution curves for both OPE and OPENac are more or less the same, the fact that biodegraded OPENac has fewer high molecular weight fractions suggest that the clay in the nanocomposite has helped *P. aeruginosa* in metabolising more of high molecular weight fractions. This confirms that the microorganism is able to perturb whole of the polymer volume in the presence of clay.

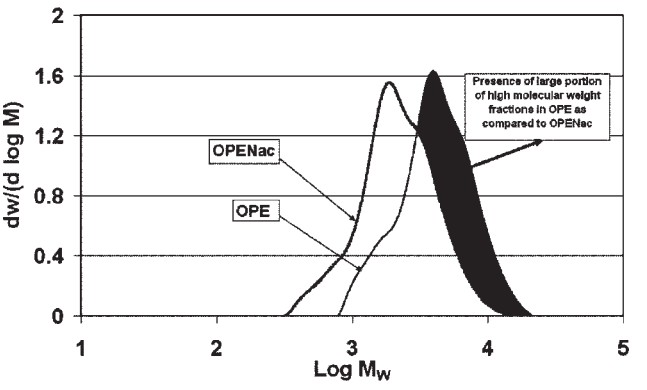

Figure 8 Molecular weight distribution curve of OPE and OPENac after 6 weeks of oxo-biodegradation process.

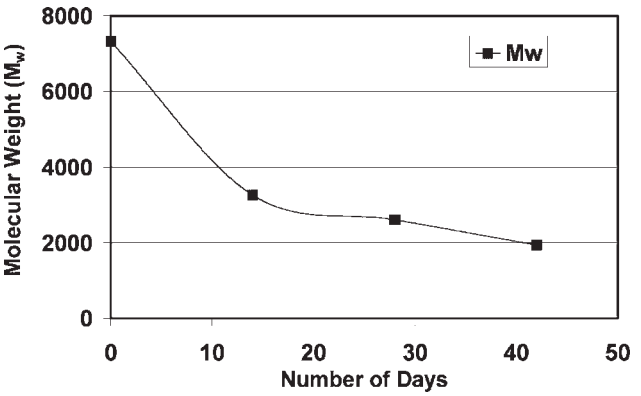

Figure 9 Changes in molecular weight of abiotically oxidised OPENac during microbial degradation.

The above mentioned phenomenon can also be observed in the molecular weight data shown in Figure 9 for abiotically oxidised OPENac. Molecular weight decreases continuously with biodegradation time as expected from molecular weight distribution data. This could be attributed to the attempt from *P. aeruginosa* to access the polymer volume further. The final Mw of 1934 Da after 6 weeks compares well with the results reported by Kawai et al.[30,31] They used PEwax and *P. aeruginosa* and found that the microbes are able to rapidly consume molecules that are even bigger than 1000 Da. Based on this suggestion and results from the present study, it can be said that *P. aeruginosa*, in the presence of clay is able to access the polymer volume completely and therefore can utilise the remaining portions of OPENac.

This difference in degradation pattern between OPE and OPENac may be due to some active action of *P. aeruginosa* in achieving further chain cleavage of the polymer or may be due to the presence of clay which creates a different biotic environment. If it is due to active action of *P. aeruginosa*, then similar trend should have been observed in OPE as well. Since this is not the case, the root cause for the differential and quick degradation of OPENac can only be attributed to the different biotic environment created by nanoclay. The results of some articles published in the literature on biodegradation of thermooxidised OPE in soil and composting units also support this theory.[32–34]

Montmorillonite used in OPENac is expanding 2 : 1 type nanoclay and it supports the growth of soil microorganisms.[14] It also has an ability to mediate the pH of microbial systems by replacing H ions produced during metabolism with basic cations from the exchangeable complex.[15] Stotzky et al.[16] have also proven that montmorillonite effectively maintains pH and helps in the growth of many soil bacteria. They have also observed a strong correlation between the surface area of clay and bacterial growth. When the initial pH is sufficiently high, the bacterium is able to initiate and maintain growth until the accumulation of acidic metabolites reduces the pH to an inhibitory level. But the nanoclay is capable of neutralizing these metabolites and thereby helping to maintain the pH of the ambient solution at adequate levels for bacteria growth. Therefore the bacterium can continue to metabolize until the buffering capacity is exhausted. Based on the above discussion, the presence of clay can be said to support the growth of *P. aeruginosa* and help it to further disturb the polymer. This action seems to be more effective after 2 weeks of biodegradation when further attack on polymer occurs, which consequently perturbs the higher molecular weight fractions.

Biodegradation of polymer in the presence of clay has been reviewed in detail by Kounty et al.[7] Based on their discussions, a mechanism for the biodegradation of polyethylene is proposed as shown in Figure 10. Before the abiotic oxidation, the molecules of polyethylene are relatively larger and therefore they cannot enter into the cells of microorganisms. After oxidation, some of the smaller fragments can be utilised by microorganisms and biodegraded. During this period, some enzymes produced by the microorganisms can help in the assimilation of oxidation products in the O-oxidation pathway. However, this can happen only if the oxidation products are available easily and the microbial activity is maintained. Since both of these requirements are difficult to achieve together, a significant difference in molecular weight distribution patterns is usually not observed before and after biodegradation in most of the studies reported in the literature indicating superficial microbial activity.[10,13] However, in the present case, the presence of nanoclay makes a considerable difference in the biotic environment

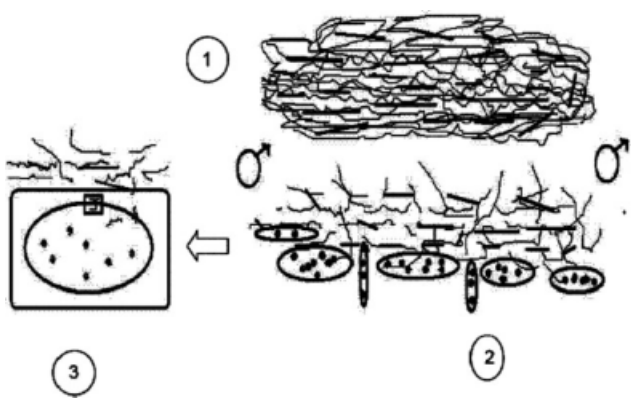

Figure 10 Biodegradation mechanism of polyethylene in the presence of clay. (1) Polyethylene nanocomposite (2) Disintegration of polymer matrix due to oxidation and metabolism by *Pseudomonas aeruginosa* (3) Expanded view of metabolism by *P. aeruginosa*.

and therefore helps in microbial growth which helps in *P. aeruginosa* to act on bulk of the sample.

CONCLUSIONS

Oxo-biodegradation of OPE and OPENac was investigated by subjecting the film samples of these materials to abiotic oxidation and then inoculating them with *P. aeruginosa* for 6 weeks. It has been observed that pro-oxidant in both OPE and OPENac helps in the drastic reduction of molecular weight during the abiotic oxidation stage leading to the production of low molecular weight compounds. However, the presence of clay does not influence the abiotic oxidation mechanism significantly. It has been also found that *P. aeruginosa* is able to utilise the low molecular weight compounds and form biofilm. The changes in molecular weight distribution observed with biodegradation substantiate this argument. However, the difference in molecular weight distributions observed after biodegradation for OPE and OPENac reveals that *P. aeruginosa* acts differently on them. Owing to difference in biotic environment created by the presence of clay, *P. aeruginosa* is able to perturb the whole of the polymer volume for OPENac but not for OPE.

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