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Influence of γ-dose rate on biodegradation of γ-sterilized biomedical polyolefins

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ABSTRACT

In our previous study, we have investigated the biodegradation of γ-sterilized polyolefins in composting and microbial culture environments at different doses. It was found that the biodegradation increases with the increasing dose of γ-sterilization and time of incubation in compost. It was concluded that the pretreatment of γ-sterilization can accelerate the biodegradation of neat polymer matrix in biotic conditions significantly. The aim of the present study is to study the effect of γ-dose rate on the biodegradation of γ-sterilized polyolefins. Films of isotactic polypropylene, high density polyethylene and ethylene-propylene (EP) copolymer were sterilized under γ-radiation with doses of 10 and 25 kGy. Two different ^{60}Co sources were used with dose rate 600 and 780 Gy h^{-1}. Neat and sterilized samples were incubated in compost and fungal culture environments. The changes in functional groups, surface morphology and intrinsic viscosity in polymer chains were characterized by FT-IR spectroscopy, SEM and viscometric measurements, respectively. It was observed that both γ-degradation and biodegradation processes depend on the dose rate of γ-source. It was found that the biodegradation of γ-sterilized polyolefins in composting and microbial culture environments increases with decreasing the γ-dose rate.

KEYWORDS

Polyolefins;
Sterilization;
γ-dose rate;
Biodegradation;
Composting;
Fungal culture.

INTRODUCTION

Synthetic polymers are ubiquitous in our world, finding diverse applications in many fields because of their useful properties and inexpensive and contribute to enhancement of comfort and quality of life in our modern industrial society. The properties of polymers like durability, resistance to weathering and photo-degradation as well as biological attack and hydrophobicity, have contributed to their skyrocketing utility in different applications. In biomedical field, they have been the choice of materials for medical supplies such as syringes, catheters, vials, blood transfusion bags, dialyzers for blood purification etc. The most commonly validated dose used to sterilize medical devices is 25 KGy[1]. This process is replacing the hazardous and environmentally destructive use of ethylene oxide/CFC mixtures, which are vented into atmosphere after use and the residue of

ethylene oxide is even carcinogenic. However, sterilization of biomedical polymers using γ-radiation is also known to result in physical changes including embrittlement, stiffening, softening, discoloration, odour generation and decreases in molecular weight. During the degradation process of polymers, there is generally a change in physical properties such as the average molecular weight, the molecular distribution, and the mechanical properties[2].

Biomedical polymer wastes are mostly incinerated. Incineration of polymers which needs higher energy input, emits toxic gases and great deal of energy which may damage the furnace. It requires more land-space and finding of acceptable sites for landfilling near urban areas is becoming difficult but, from an economic point of view, plastics to disappear in the soil would be environmentally acceptable method[3]. Bio-recycling / biodegradation can be an alternative to over come the problem of plastic waste disposal. Biodegradation of polyolefins has been another paramount importance in the field of research for few decades. These polyolefins have been found to undergo photo-induced biodegradation[4] and in natural and accelerated composting conditions[5]. In our previous study[6] we have investigated the biodegradation of γ-sterilized polyolefins in composting and microbial culture environments at different doses. It was found that the biodegradation increases with increasing the dose of γ-sterilization and time of incubation in compost. It was concluded that the pretreatment of γ-sterilization can accelerate the biodegradation of neat polymer matrix in biotic conditions significantly.

The relative ranking of polymers according to their radiation resistance is complicated by the marked effect of dose rate[7]. For total γ-sterilization dose, we have found that the low dose rate was more damaging than high rate[8-10]. This effect is not fully understood, but has been discussed by some authors[2,8-11]. In addition, the study of dose rate effects and the comparison of data from different authors are complicated by differences in exposure conditions. There have been no reports made on the effect of γ-dose rate on the biodegradability of polyolefins. Thus, it is worthwhile to study the effect of γ-radiation dose rate on the biodegradability of polyolefins after sterilization. This will show whether the pretreatment of γ-sterilization using different dose rate will effect the biodegradation of polymer matrix in biotic conditions. The present investigation is intended to study the effect of γ-dose rate on the biodegradation of high-density polyethylene (HDPE), polypropylene (PP), and ethylene-propylene (EP) copolymer in composting and microbial culture environments.

EXPERIMENTAL

Materials

Commercial samples of isotactic polypropylene (i-PP) and high-density polyethylene (HDPE), ethylene-propylene copolymer (EP) were obtained from M/s Indian Petrochemicals Corp Ltd., Baroda, India. The molar percentage of ethylene content in copolymer sample (EP) was 4.45. These polymer pallets were purified by dissolving in refluxing xylene under nitrogen atmosphere. The solution was precipitated with cold methanol, filtered, and dried at 50°C in vacuum oven. These samples were assumed to be additive free and designated as purified samples.

Films preparation and γ-radiation

The purified samples were molded into 100 ± 10μm thickness film in aluminum foil between two plates by first heating at 210, 170, and 200°C, for PP, HDPE and EP copolymer, respectively, and holding for 7 min and then increasing the molding pressure to 15000 pounds. The pressure was allowed to fall, the mold were then immediately quenched into a large bath filled with water at 20°C. The films were kept in a well type ^{60}Co for γ-radiation allowing uniform exposure. Two different ^{60}Co sources were used with different dose rate 600 and 780 Gy h^{-1}. The samples were sterilized to the range of sterilization dose (25 KGy) under different ^{60}Co sources at room temperature in presence of air.

Viscosity measurements

Intrinsic viscosities [η], were determined in decalin solution at 135°C. The antioxidant, 0.1 wt % (2, 6-di-tert-butyl-p-cresol) was added to prevent any further degradation. The decalin solution was filtered through a No.3 glass filter at 70°C before dissolving the PP films. The viscosity-average molecular weight (M_v) was calculated from the intrinsic viscosity by using Mark-Houwink equation: $[\eta] = K M^a$. The constant values K

and a were taken from Ref.[12]. The error due to expansion of flask is negligible as preheated flask and pipette (140°C) were used to mix the solvent into an Ubbelohde viscometer.

Incubation in compost

The biodegradability tests were performed in a laboratory scale composer, and the size of the films was 5×5cm. The constitution[4,13] of solid waste mixture (compost) used for biodegradability testing of γ-sterilized samples was as follows (dry weight): 40.8% shredded leaves, 11.4% cow manure/dung, 15.8% newspapers and computer paper, 2% white bread, 7.8% sawdust, 19.2% food waste (dry milk, potato, carrot, banana, and others vegetables) and 3.0% urea. Total dry weight was 5kg. The moisture content was maintained by periodic addition of water. From figure 1, it was observed that the temperature of compost had been slightly higher than atmospheric temperature and the temperature of compost increased to approximately 40 degrees in 3 months, which was kept for one month and then began to drop. The biodegradability was determined by measuring the gravitational weight loss (per surface area in gm/cm^2) on digital balance, Prescisa 205 SCS, Switzerland.

Incubation in culture

The test fungi i.e. *Aspergillus niger* which was cultivated by inoculating in Sabouraud Dextrose Agar (SDA) of pH 6.5 at 28 C for 4 days. The SDA agar was prepared by dissolving 10g of peptone, 40 g of dextrose, 15g agar in one liter of deionized water. The grown spore suspension was used further. The basic salt agar for testing the fungal growth on polymer was prepared by dissolving potassium dihydrogen phosphate (0.70g), magnesium sulfate (0.70g), ammonium nitrate (1.0g), sodium chloride (0.005g), ferrous sulfate (0.002g), manganese sulfate (0.001g) and agar (15.00g) in one liter of deionized water. After the medium was sterilized at 120 ± 5°C for 25 min, the pH was adjusted between 6.5 and 7.0 by addition of a 0.1 N solution of NaOH. For providing the solidified agar layer (depth 4-7mm) basic salt agar was poured into sterilized petri dish. The surface of test specimen was inoculated by spraying the spore suspension. The petri dishes were incubated at 28-30°C after sealed by wax to avoid the contamination for six weeks. The rate of fungal growth was estimated in accordance to ASTM G-2170 where the recorded parameter (S) is the fraction of the surface covered by fungus (S < 10% (1), 10 ≤ S < 30% (2), 30 ≤ S < 60 (3) and S ≥ 60% (4)).

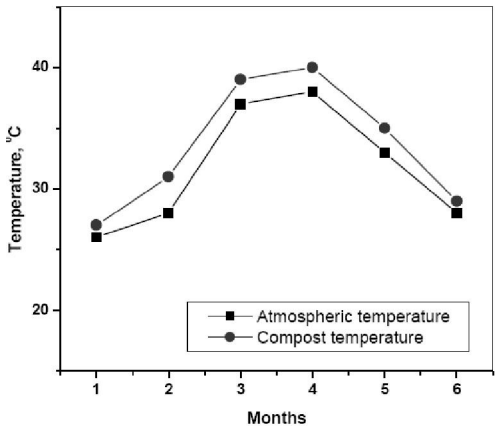

Figure 1 : Temperature profile of composting

FT-IR Spectroscopy

FT-IR (Fourier transform infrared 16 PC spectrometer) was used to characterize the chemical changes caused by γ-radiation in the polymer films, and our interest was mainly focused on the changes in hydroxyl (3700-3100 cm^{-1}) and carbonyl region (1600-1800 cm^{-1}) to follow γ-induced oxidation.

Scanning electron microscopy

The films were placed in stoppered bottles containing osmium tetroxide (2% aqueous) and allowed to stand for 48 h. The films were washed with water and dry ethanol and they were dried under vacuum for 24 h at 50°C. The gold-coated samples were examined under electron microscope (Leica Cambridge Stereoscan 440 model).

RESULTS AND DISCUSSION

γ-degradation

FTIR spectral changes

FTIR Spectroscopy is a conventional technique to follow oxidative degradation by monitoring changes in functional groups and our interest was focused at carbonyl and hydroxyl regions. Figure 2 show the FTIR spectral changes of polymers upon γ-sterilization. It is clearly observed that the increase in absorbance at carbonyl and hydroxyl regions was observed for all the

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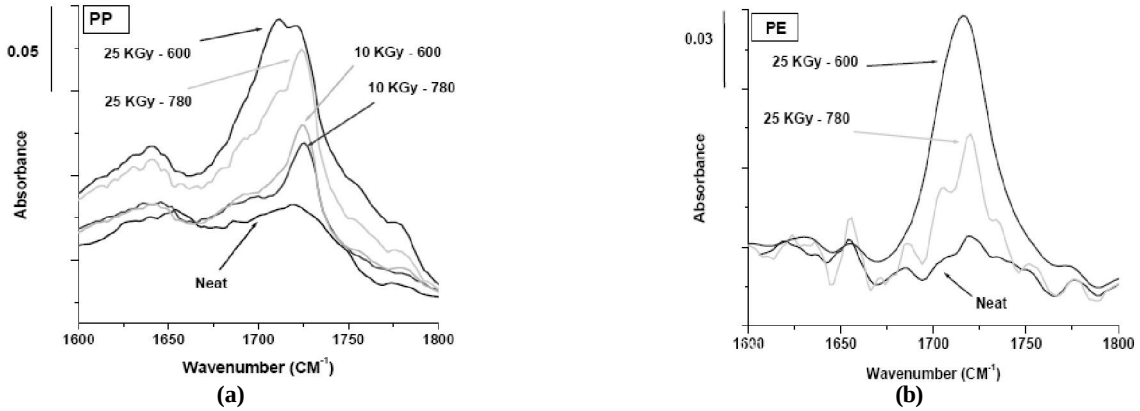
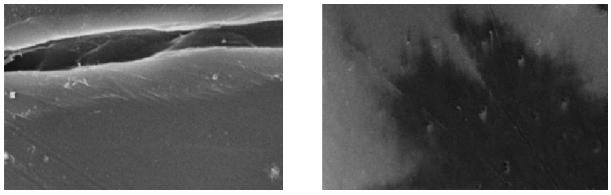
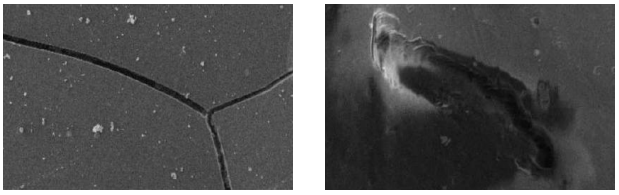


Figure 2 : FTIR spectra of neat and γ-sterilized (a) PP and (b) PE at carbonyl region with different dose rate



(a) (b)

Figure 3 : SEM of γ-sterilized samples (with dose rate 780 Gyh^{-1}) a) PP and b) PE



(a) (b)

Figure 4 : SEM of γ-sterilized samples (with dose rate 600 Gyh^{-1}) a) i-PP and b) PE

sterilized polymer samples. The formation of oxidation products absorbance increases with decreasing γ-dose rate. On the other hand the lower dose rate, the higher degree of oxidation. The appearance of peaks at carbonyl region (1550-1800cm^{-1}) with maximum absorption at 1720cm^{-1} can be attributed to the radio-oxidative degradation of polymers. This also illustrates the presence of γ-lactones at 1795cm^{-1}, peresters at 1774cm^{-1}, peracids at 1750cm^{-1}, carboxylic acids at 1705cm^{-1}, and clear shoulder at 1685cm^{-1} assigned to α, β- unsaturated ketones (-CH = CH-CO-). A week band appears at 1733cm^{-1} assigned to aldehydes. The major band at 1720cm^{-1}, regularly used to monitor the oxidation process, is attributed to ketones. It is clear seen that the concentration of these groups increases with decreasing dose rate of γ-radiation. Among all the polymers, the increase at carbonyl region was observed to be higher for 25 KGy γ-sterilized PP samples.

Variation in viscosity

TABLE 1 shows the changes in intrinsic viscosities of polymers after γ-sterilization. It can be seen that [η] of all the polymers decreased with decreasing the γ-dose rate. The ethylene content has an effect on the molecular weights of pure PP matrix, therefore, [η] of unsterilized EP is higher than that of unsterilized PP. In all the polymers, the decreasing the dose rate of γ-radiation reduces the intrinsic viscosity where γ-sterilized samples with dose rate 600 Gyh^{-1} showed lower [η] values than γ-sterilized with dose rate 780 Gyh^{-1}. The decrease in [η] can be attributed to chain scission or the formation of low molecular weight compounds during sterilization. There may be competition between chain scission and crosslinking, but in our investigation, appreciable crosslinking was not observed in the system, as films remained completely soluble in decaline.

Morphological changes

Figure 3 and 4 show the scanning electron micrographs of γ-sterilized samples of i-PP, and HDPE at different dose rate. It is evident from the micrographs that under γ-sterilization, samples were observed to show deformed/cracked surface. It can be seen that the surface crack of all the polymers increased with decreasing the γ-dose rate indicating that the degradation are more severe for the sterilized samples with lower dose rate. It is well known that this cracked (amorphous) surface will accelerate the degradation further, since the oxidative degradation is initiated at surface of materials. These results supporting to that obtained from FT-IR where the concentration of the oxidation products increased with decreasing the dose rate.

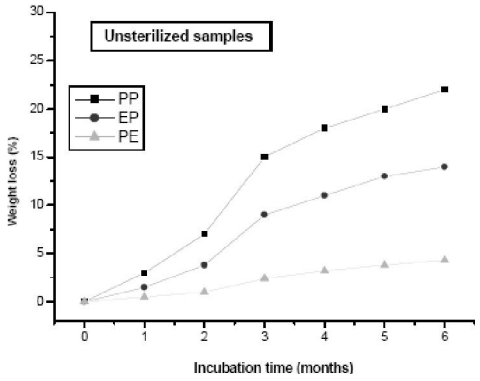


Figure 5 : Weight loss (%) of unsterilized samples

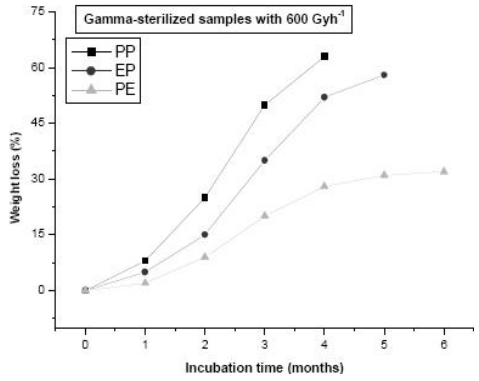


Figure 6 : Weight loss (%) γ-sterilized samples with dose rate 780 Gyh^{-1}

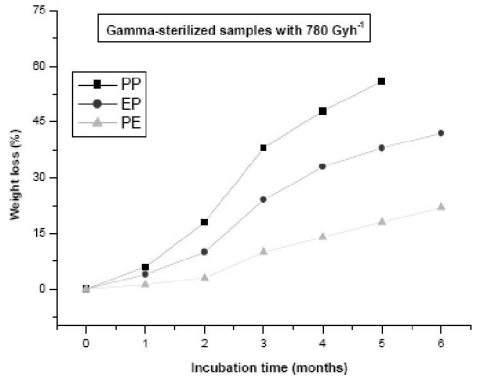


Figure 7 : Weight loss (%) γ-sterilized samples with dose rate 600 Gyh^{-1}

Biodegradation

Composting

Weight loss is one of the most valuable data indicating the actual biodegradation of polymeric material after composting whenever validated by parallel monitoring of the neat respirometric microbial activity bound to the carbon content of the sample under testing. The weight loss of neat and γ-sterilized and compost-buried, polymer samples (~100μm thickness and 5cm×5cm size) was measured monthly during six months of composting by removing, washing the samples with distilled water and ethanol and drying in a vacuum oven at 60-65C until constant weight. Figure 5-7 show the weight loss of unsterilized and γ-sterilized samples with different dose rate (600 and 780 Gy h^{-1}). It is quite obvious that the degradation rate (gravitational weight loss) increased rapidly with decreasing the dose rate of γ-radiation and time of incubation in compost. Among all the polymers, samples of unsterilized HDPE have also shown lower rate of weight loss.

The samples of PP have comparatively shown higher weight loss than that observed for HDPE samples in both γ-sterilized and neat samples indicating the greater susceptibility of i-PP to microorganisms during composting. The HDPE samples were more stable towards the microbial attack than samples of other polymers while γ-sterilized HDPE films with 600 Gy h^{-1} have shown higher weight loss than neat and γ-steril-

TABLE 1 : Changes in intrinsic viscosity during γ-sterilization at different dose rate

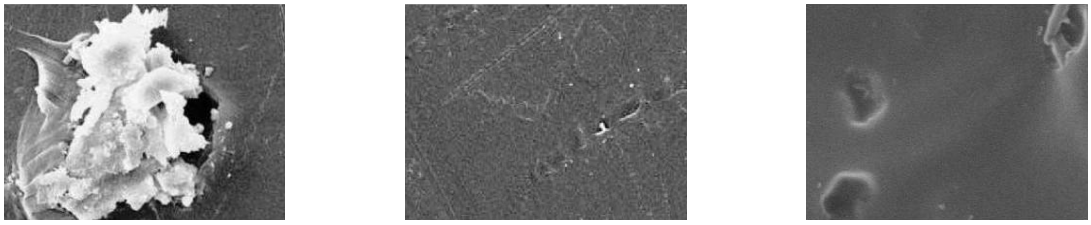
S. No.	Matrix	Neat	Sterilization dose with different dose rate	
			780 Gyh^{-1}	600 Gyh^{-1}
1	HDPE	1.61	0.97	0.85
2	PP	1.83	0.73	0.56
3	EP	2.27	1.05	0.74

TABLE 2 : Visual growth rating of *Aspergillus niger* on polymer films

Weeks	HDPE			i-PP			EP		
	Neat	780 Gyh^{-1}	600 Gyh^{-1}	Neat	780 Gyh^{-1}	600 Gyh^{-1}	Neat	780 Gyh^{-1}	600 Gyh^{-1}
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	1	0	0	1
3	0	0	1	0	1	2	0	1	1
4	0	0	1	1	1	3	0	1	2
5	0	1	2	2	2	3	1	2	3
6	0	2	2	2	3	4	1	2	4

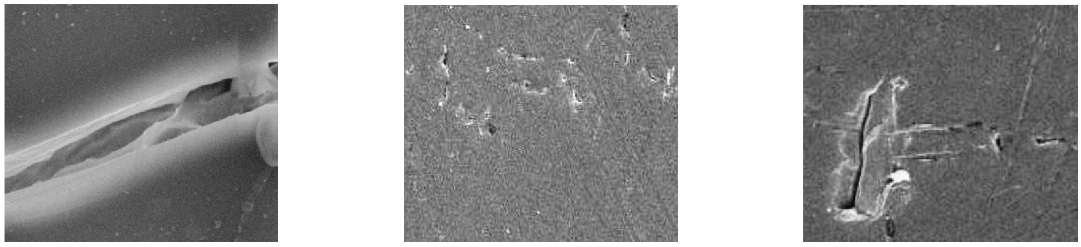
TABLE 3 : Variations in the intrinsic viscosity [η] of γ-sterilized and composted samples

Months	HDPE			i-PP			EP		
	Neat	780 Gyh^{-1}	600 Gyh^{-1}	Neat	780 Gyh^{-1}	600 Gyh^{-1}	Neat	780 Gyh^{-1}	600 Gyh^{-1}
0	1.61	0.97	0.85	1.83	0.73	0.56	2.27	1.05	0.74
2	1.59	0.94	0.74	1.68	0.55	0.35	2.11	0.93	0.54
4	1.57	0.89	0.63	1.50	0.44	0.21	1.94	0.78	0.42
6	1.54	0.81	0.56	1.30	–	–	1.88	0.61	–



(a) (b) (c)

Figure 8 : SEM of γ-sterilized (with dose rate 780 Gyh^{-1} and 4 months composted samples (a) PP, (b) PE, and (c) EP



(a) (b) (c)

Figure 9 : SEM of γ-sterilized (with dose rate 600 Gyh^{-1}) and 4 months composted samples (a) PP (b) PE and (c) E

ized HDPE with 600 Gy h^{-1}. The weight loss (%) of unsterilized samples of EP copolymer is significantly observed after 2 months and EP was more susceptible to biodegradation than HDPE. Of all the polymers, the γ-sterilized samples with 780 Gy h^{-1} have shown the significant weight loss but less than γ-sterilized samples with 600 Gy h^{-1}. After 4 months of composting, γ-sterilized PP samples with 600 Gy h^{-1} were not recoverable from the compost. However, γ-sterilized EP and PP sample with 780 Gy h^{-1} were not recoverable from the compost after 5 months suggesting the biodisintegration of films in composting and the acceleration of biodegradation process due to changing the dose rate of the used γ-radiation. In figure 1, temperature of compost was found to be higher than atmospheric temperature. This may be due to the fact that earlier months of composting, compost itself could increase the temperature because of exothermic microbial degradation[14-16]. In correlation of figure 1 and figure 6-7, it can be seen that the weight loss was lower for initial days and rapidly increases after 2-3 months suggesting that temperature of compost will also have a significant effect on the biodegradation.

Incubation in culture

The visual growth rating[14] test is valuable in assessing the performance of polymer during its use under such conditions. The samples were used as the sole carbon source for a fungus, e.g, *Aspergillus niger*. During incubation with fungal culture, the spores (black spots) as colonies were observed to grow. TABLE 2 represents the data of fungal colonization (visual growth) on the surface of polymer films after 6 weeks of incubation in culture. The absence of any colonization in a controlled petri-dish (without polymer sample) clearly suggest that fungus is using the polymer specimen as a sole source of carbon, as there was complete absence of carbon in nutrient agar. It can be observed that with increasing time of incubation, the fungal colonization was found to increase for all the samples. Among the unsterilized samples, microbial growth was not observed on HDPE while it was seen for PP after 4 weeks. The fungal colonization was relatively lower on the surface of EP than that of PP and higher than HDPE. It was interesting to observe that among all incubated sterilized polymers, the fungal colonization was relatively higher on the surface of γ-sterilized with 600 Gyh^{-1} than γ-sterilized with 780 Gyh^{-1}. Then, it was also seen that the γ-sterilized samples have shown higher fungal colonization than unsterilized and it was increased with decreasing the dose rate. In case of sterilized samples, the coverage on the surface of films was much more for γ-sterilized PP films than others in both cases (either 780 or 600 Gyh^{-1}). The higher colonization on the sterilized samples can be attributed to easy consumption of short chains as energy source by fungus.

FTIR spectral changes

The FT-IR spectral changes after composting of γ-sterilized films with 600 and 780 Gyh^{-1} has shown a

decrease found at carbonyl region may be due to the release of short chain carboxylic acids in the form of degradation products during the biotic step such as in polyethylene, where the carboxyl functionalized short pieces can undergo β-oxidation by co-enzymatic action and the reaction mechanism can be compared with paraffin degradation[15-17]. This decrease was not much significant, since there may be incomplete consumption of carbonyl compounds and may be possibility of continuing oxidation processes (thermal oxidation and aerobic condition). The absence of primary alcohol group in HDPE after treating with NaOH suggests the microbial oxidation of the terminal methyl group of short and long chains.

Variations in viscosity

The variations in intrinsic viscosity [η] of iPP, HDPE and EP films after composting are tabulated in TABLE 3. During composting, with increasing time of incubation, the gradual decrease in intrinsic viscosity was observed for both sterilized and unsterilized samples. This may be due to the microbial consumption of low molecular weight (functional group) compounds that may be randomly present at polymer backbone chain. Under composting, chain scission was observed to be lower at initial days of composting and to increase with increasing time of incubation. Chain scission under composting condition also was observed to be higher but less than that observed under sterilization. It was clearly seen that all sterilized samples with 600 Gyh^{-1} shows lower intrinsic viscosity than that sterilized samples with 780 Gyh^{-1}. The higher surface deterioration of due sterilization with low γ-dose rate and the increase in weight loss and the decrease in intrinsic viscosity of all γ-sterilized samples after composting are evident that the degradation process occurs initially and dominantly on the surface of the films and then it moves into polymer matrix by penetration of microorganisms.

Morphological aspects

Figures 8 and 9, respectively, show the scanning electron micrographs of composted samples after sterilization with different dose rate of γ-radiation. The deformation/deepness of erosion of γ-sterilized samples with 780 Gyh^{-1} was less, which may be due to the lower extent of oxidation in comparison with γ-sterilized samples with 600 Gyh^{-1}. A network of crack formation was observed with an increase in dose of γ-sterilization. Formation of microcracks on the polymer surface is due to chain scission of polymer after γ-irradiation. The cavities on the surface were observed after composting of sterilized samples, suggesting that microorganisms penetrate the polymer matrix during the degradation process. The presence of small and large cavities on the surface may be due to the absence of a uniform distribution of short branches or degradation products generated during sterilization, which are preferable carbon source (food) for microorganisms. Their consumption by microbes results in good erosion on the surface of polymer films.

CONCLUSION

The effect of γ-dose rate on the biodegradation of γ-sterilized polyolefins was studied in composting and microbial culture environments. An increased absorption in carbonyl and hydroxyl regions of FTIR spectra was observed for γ-sterilized samples and it was found to increase with the decrease in the dose rate of γ-sterilization. Significant changes in the carbonyl region were observed for composted samples. In general, a decrease in intrinsic viscosity and increase in chain scission were also observed with the decreasing γ-dose rate and time of incubation in compost. The higher weight losses of γ-sterilized samples with lower intrinsic viscosity suggested that chain scission and radio-oxidized functional groups were important units in the bio-/g-degradation of polymers. The variation in degradation behaviour of polymers (iPP, HDPE and EP copolymer) suggested that the γ-sterilization with different dose rate have significant effects on both γ-degradation and biodegradation. We conclude from this investigation that the pretreatment of γ-sterilization with low dose rates can accelerate the biodegradation of neat polymer matrix in biotic conditions significantly.

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