Effect of Biodegradation on Thermal and Crystalline Behavior of Polypropylene–Gelatin Based Copolymers

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ABSTRACT: Gelatin-based graft copolymers of polypropylene (PP), has been synthesized by chemical method using benzoyl peroxide (BPO), as radical initiator. Biodegradation studies of pristine PP and PP-*g*-Gelatin have been carried out by soil burial test in simple soil and soil enriched with nitrogenous content by adding urea. The microbial degradation was substantiated by the direct attack of the microbes on the grafted samples. The rate of degradation by the direct attack was fast in comparison to the degradation in soil burial studies. The biochemical tests performed on the organisms isolated from the soil, identified these organisms as *Bacillus circulans, Kurthia gibsonii*, and *Flavobacterium* sp. which helped biodegradation of PP-*g*-Gelatin samples. The degradation of the grafted samples was further confirmed by carrying out the physical characterization of the original samples and the degraded samples by SEM, XRD, and TGA. The XRD and thermal data indicate an increase in the crystallinity of the degraded samples. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 118: 1476–1488, 2010

Key words: biodegradation; polypropylene; gelatin; soil burial method; microbial studies; thermal degradation

INTRODUCTION

Development of biodegradable polymers is a newly emerging field. A vast number of biodegradable polymers have been synthesized recently and some microorganisms and enzymes capable of degrading them have been identified. In developing countries, environmental pollution by synthetic polymers, such as polyethylene (PE) and polypropylene (PP) has assumed dangerous proportions. As a result, attempts have been made to solve these problems by introducing biodegradability into these polymers. Biodegradation is a process whereby bacteria, fungi, yeasts and their enzymes consume a substance as a food source so that its original form disappears. Under appropriate conditions of moisture, temperature, and oxygen availability, biodegradation is a rapid process. Biodegradation for limited periods is a reasonable target for the complete assimilation and disappearance of an article leaving no toxic or environmentally harmful residue.

Gelatin, an animal protein is a water soluble, biodegradable polymer with extensive industrial, pharmaceutical, and biomedical uses. It can be hydrolyzed by a variety of the proteolytic enzymes to yield its constituent amino acids peptide components.[1] This nonspecificity is a desirable factor in intentional biodegradation. A method was developed to prepare a simple, flexible gelatin film-based artificial skin that could adhere to an open wound and protect it against fluid loss and infection. The films were tough and adhered to open wounds spontaneously.[2] Kuwajima et al.[3] grafted methyl methacrylate onto gelatin by radical initiators and studied these in aqueous solution at temperatures between 60 and 80°C. Kumar et al.[4] prepared gelatin-*g*-poly(ethyl acrylate) in an aqueous medium, using $K_2S_2O_8$ as an initiator. The copolymer was tested for their microbial susceptibility. The effect of γ-sterilization on the biodegradation of polyolefins was studied by Alariqi et al.[5] under composting and fungal culture environments. The changes in functional groups surface morphology and chain scission were characterized by various physical methods. Thermal properties and enzymatic degradation of blends of poly(ε-caprolactone) with gelatin was investigated by Rosa et al.[6] Morancho et al.[7] studied the biodegradability of mixtures of PP and gelatin by colorimetry and thermogravimetric analysis and observed an increase in the thermal stability of the Gelatin units, PP remains unaffected. Specimen in film shape as well as in powder shape was subjected to the biodegradation tests by Yang et al.[8] to investigate dependence of test results on the shape of specimens.

In the present manuscript, we report on the biodegradation studies of PP modified by grafting of gelatin. Biodegradation was done by soil burial test, microbial studies of the soil containing samples in

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Nutrient Agar and Czapec Dox, and periodic hydrolysis of the samples placed for degradation.

EXPERIMENTAL

Materials

Commercial polypropylene (PP) (Thukral Trading Co. Delhi, India) was recrystallized from xylene using methanol and irradiated from Co^{60} source housed in Gamma Chamber-900 (BARC, Trombay, Mumbai, India) at a constant dose rate of 3.40 kGy/h. Gelatin (Merck) and benzoyl peroxide (BPO) (Merck) were used as received. Isolation of bacteria and fungus from soil was made through Nutrient Agar and Czapec Dox enrichment culture.

Graft copolymerization

Preirradiated, dried, and weighed PP (0.200 g) was suspended in a known volume of water (0–25 mL) in a flask placed in a water bath maintained at constant temperature (45–75°C), under constant stirring. To this was added a solution of a definite amount of the polymer, gelatin (0.100–0.250 g) dissolved in a known volume of water (0–25 mL) and a definite amount of the initiator, BPO (0.100–0.300 g). A continuous flow of nitrogen gas free from oxygen was passed through the reaction medium over the period of reaction time (60–210 min). After the stipulated time period, the flask was removed from the water bath and the contents were filtered. The residue containing grafted PP, unreacted gelatin, unreacted PP, and some amount of BPO was washed with benzene to remove unreacted BPO. The left over product was dried at 45°C till constant weight was obtained. The product was referred to as the 'Composite'. Apparent percentage of grafting of the composite was calculated from initial increase in the weight of original PP as follows:

$$\% \text{ Apparent grafting} = \frac{W_c - W_0}{W_0} \times 100$$

where, W_0 and W_c are the weights of the original PP and the composite, respectively.

The composite was then stirred with water for 2 h at 60°C to remove ungrafted gelatin. The reaction mixture was filtered and the residue was dried at 45°C and weighed till constant weight. Percentage of grafting i.e. the percent increase in weight of PP upon grafting (W_g) after complete removal of the unreacted to be grafted polymer gelatin from initial weight of pristine PP (W_0) was determined as above.

To determine the extent of formation of true graft i.e., the actual portions of the PP backbone and to be grafted polymer, gelatin, involved in the formation of the graft copolymer, the graft copolymer was further stirred for 2 h at 60–70°C with *p*-xylene to remove unreacted, PP. The reaction mixture was filtered, residue was dried at 45°C, weighed till constant weight. The product is the 'True graft' and the percentage of true graft was calculated as follows:

$$\% \text{ True grafting} = \frac{W_t - W_{0'}}{W_{0'}} \times 100$$

where W_t is the weight of the grafted PP after water and xylene washing, respectively, removing both unreacted gelatin and unreacted PP and $W_{0'}$ is the weight of PP obtained after hydrolysis.

True graft was hydrolyzed with 6*N* HCl for 4 h. This results in the hydrolysis of grafted gelatin leaving the backbone polymer, PP. The solution was filtered and residue i.e. PP was dried, weighed.

Graft copolymerization of gelatin onto PP was carried out as a function of different reaction variables, such as time of reaction, concentration of BPO, amount of water, temperature and amount of to be grafted polymer, gelatin. The optimum conditions were thus evaluated for achieving the maximum percentage of grafting. The optimum conditions were used to study the effect of amount of gelatin on percent apparent grafting, percent grafting, and percent true grafting measurements.

Maximum percentage of apparent grafting (120%), percent grafting (115%), and percent true grafting (85%) of Gelatin (0.250 g) onto PP (0.200 g) was obtained at optimum conditions of [BPO] = 4.132×10^{-2} mol/L at 70°C in 120 min. using 30 mL of water. Characterization of PP-*g*-Gelatin was carried out through FTIR, thermogravimetric analysis, scanning electron micrographs, swelling, and water retention studies.[9]

Biodegradation studies

Soil burial method[10]

Soil of known moisture content (17.5%) and pH (8.78) was taken in different pots. Soil of known moisture content (17.5%) and pH (8.78) was taken in different pots. Moisture content and pH of at least three samples were determined. For determining moisture content a definite weight of soil was taken in Preweighed dry beaker. These beakers were kept in an oven for 48 h at 80°C. The change in weight was measured as percent moisture content of soil.

For determining pH of the soil, soil suspension was prepared in distilled water in 1 : 5 ratios. The suspension was continuously stirred for an hour and pH measured by the pH meter. The microbial activity of soil was tested using a cotton textile strip (i.e. tea bag), which looses 90% of its tensile strength

within 10 days of exposure to the soil. A weighed amount (0.500 g) of each of the samples i.e. pristine PP, PP-*g*-Gelatin in the form of composite (GC; containing both unreacted PP and gelatin) and true graft (GT; with both unreacted PP and gelatin removed) wrapped separately in loose knitted synthetic net were placed in each pot. Care was taken that the samples were completely buried under the soil beds and kept at room temperature (25–30°C). Samples were removed after a specific number of days for assessment of changes in weight loss.

Percent weight loss determined as a function of number of days was calculated as:

$$\% \text{ Wt. loss} = \frac{W_0 - W}{W_0} \times 100$$

where W_0 is the initial weight in the beginning, W is the weight after a specific number of days i.e. 10, 20, 30, 45, 60, 75, 90, and 120 days.

In a separate study, the soil beds were also supplemented with organic fertilizer (6 g of urea per Kg of soil) to encourage an active microbial flora to check the effect of nitrogenous compounds on the degradation of samples of PP. Finally, the samples can be used to 'bait' microorganisms involved in the degradation process. These microbes can be isolated and characterized by incorporated into the petri dish screen method.

Petri dish screen

The principle of this method[10] involves agar in a petri dish containing no additional carbon source. The agar surface are sprayed or painted with a standardized mixed inoculum of isolated fungi or bacteria. The petri dishes are sealed and incubated at a constant temperature (30°C). The agar surface is then examined at definite interval of time for the amount of growth on its surface and the number of colonies was counted. The more growth on the surface, the more likely it is that the samples is intrinsically able to support growth and if less growth, likelihood that it will fail in service.

The isolation of microorganisms such as bacteria, fungi present in natural soil was made by microbial study of the soil containing the samples for biodegradation. The number of bacteria and fungus in soil was determined by a count of the growth colonies on enrichment culture plates of Nutrient agar (NA) and Czapec Dox (CZ) by Serial dilution method.[11] Three petri plates each of NA and CZ were prepared for measuring microbial count in natural soil. Petriplates along with NA and CZ medium were sterilized in an autoclave for 45 min. at 121°C, allowed to cool to 60°C, and these medium poured into petriplates. Soil containing samples of pristine PP, PP-*g*-P (both composite and true graft), and control soil sample, each of 1 g soil were dissolved in 10 mL of sterilized saline water (89%) using a vortex mixer. They were each then diluted by adding 1 mL of the soil suspensions to 9 mL sterilized saline water and mark it 10^{-1} dilution. From the 10^{-1} dilution sample, 1 mL were removed after shaking test tube and added to next 9 mL sterilized saline water and mark it 10^{-2} dilution. The same procedure was repeated with 10^{-2} dilution to prepare 10^{-3} and so on. From each of the 10^{-2}, 10^{-4}, and 10^{-6} serially diluted solution samples, 100 μL were removed and spread onto NA and CZ plates, after which they are incubated at 30°C for 24/48 h. The number of growth colonies was measured, for 5, 10, 20, 30, 45, 60, 75, and 90 numbers of days. This procedure was continued for same numbers of days to work out the increase and decrease in the number of growth colonies with numbers of days and to observe the growth colonies, which persist over this period of 90 days. After this, these isolated growth colonies were individually grow on other NA plates and maintained by subculturing. Some of the growth colonies were submitted in IMTECH (Institute of Microbial Technology), Chandigarh, for their identification. The growth pattern was observed and the numbers of colony forming units (CFU)[12] were counted.

Direct microbial studies

Direct microbes study of PP and PP-*g*-Gelatin (both composite and true graft) with isolated culture/microbes/cells was studied by using a 20 mL of medium, which was prepared by adding 1.25 g peptone and 0.75 g beef extract in 250 mL of water. The reaction was carried out for 7 days in Orbitek shaker (RPM = 160) at 30°C. After completion of the reaction, the sample was collected by centrifuging it in Remi Cooling Compufuge CPR 23 by setting at 7000 rpm for 10 min. and filtered it very carefully. Sample was washed with water and dried at 40°C until it gives constant reading. Dried sample was weighed and percntage weight loss was calculated as above.

Hydrolysis studies

During the process of degradation by soil burial, hydrolysis of the degrading samples was carried out at a definite interval of time as follows. Samples of PP and PP-*g*-Gelatin (both composite and true graft) (0.500 g) were separately wrapped in synthetic net and buried in soil having good plantation. The samples were removed from the pots at a predetermined time washed to remove the adhered soil and dried at 45°C for 12 h. Dried samples were weighed and hydrolyzed with 20 mL of 6*N* HCl for 4 h at 80°C. After the hydrolysis, solution was filtered the

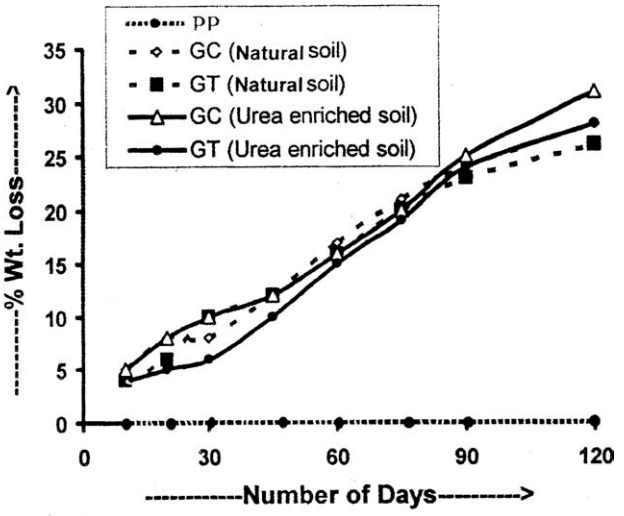

Figure 1 Percent weight loss of PP and PP-*g*-Gelatin samples with number of days by soil burial method.

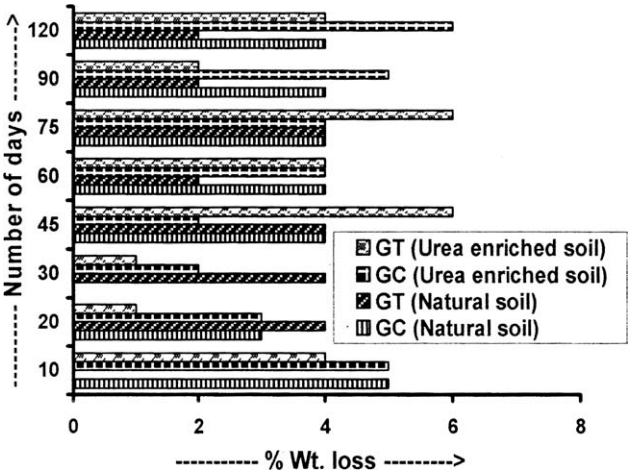

Figure 2 Percent weight loss of PP-*g*-Gelatin samples within consecutive number of days by soil burial method.

residue i.e. PP was dried and weighed. Degradation of PP was calculated from the % of PP left from the grafted samples after hydrolysis as follows.

$$\% \text{ PP left} = \frac{W_0 - W_H}{W_0} \times 100$$

where W_H is the weight of grafted sample left after hydrolysis and W_0 is the initial weight of sample taken.

Characterization of PP and PP grafted samples

Physical characterization of PP and PP grafted samples (pure and degraded) has been done by scanning Electron Microscopy (SEM), X-ray diffraction (XRD), and thermogravimetric analysis (TGA).

RESULTS AND DISCUSSION

Biodegradation studies

Soil burial studies

Biodegradation of gelatin grafted samples (both composite; GC and true graft; GT) of PP buried under the

TABLE I
Enumeration of Bacteria (CFU) from Soil Containing Degradation Samples of PP and PP-*g*-Gelatin

		Soil		PP		Gelatin		GC		GT	
Number of days	Dilution	NA	CZ	NA	CZ	NA	CZ	NA	CZ	NA	CZ
10	10^{-2}	104	94	213	108	697	4 + Whole plate	184	457	125	225
	10^{-4}	29	24	32	7	133	112	23	55	68	137
	10^{-6}	3	–	55	–	30	40	–	–	8	44
20	10^{-2}	112	86	2	1	288	Whole plate	319	292	486	306
	10^{-4}	58	15	2	3	203	310	109	123	205	228
	10^{-6}	24	2	4	4	103	108	17	24	96	54
30	10^{-2}	81	19	20	5	250	264	119	52	82	63
	10^{-4}	54	16	4	1	114	95	52	12	37	41
	10^{-6}	49	3	–	–	53	18	5	3	–	1
45	10^{-2}	75	36	10	12	Whole Plate	3 + Whole plate	165	215	394	286
	10^{-4}	7	1+Pin pointed	3	4	276	215	10	15	78	4
	10^{-6}	3	1+Pin pointed	1	–	68	43	–	–	13	–
60	10^{-2}	168	38	40	7	–	–	54	41	78	81
	10^{-4}	–	–	Whole plate	1	–	–	26	31	52	44
	10^{-6}	–	–	1	–	–	–	15	6	20	11
75	10^{-2}	102	32	44	2	–	–	290	220	360	225
	10^{-4}	45	15	2	–	–	–	198	112	295	29
	10^{-6}	10	–	–	–	–	–	61	35	106	–
90	10^{-2}	151	Whole plate	54	10	–	–	95	83	56	40
	10^{-4}	25	102	12	–	–	–	36	51	12	4
	10^{-6}	22	16	–	–	–	–	4	–	1	–

NA = Nutrient Agar, CZ = Czapec Dox.

TABLE II
Morphological, Physiological, and Biochemical Tests of Identified Microorganisms

Tests	Results		
	I	II	III
Colony morphology			
Configuration	Round	Round	Round
Density	Opaque	Opaque	Translucent
Margin	Wavy	Wavy	Entire
Elevations	Convex	Convex	Convex
Surface	Rough	Rough	Smooth
Density	Opaque	Opaque	Translucent
Pigments	Cream	Slightly Yellowish in colour	Yellowish
Gram's reaction	+ve	+ve	−ve
Shape	Rods	Rods	Rods
Size	Rods thick	Thin rods in chain with fragmentation in to small rods	Moderate rods
Arrangement	Single	Single	Single
Spore			
Endospore	+	+	−
Position	Central		
Shape	Oval		
Sporangia bulging	+		
Motility	+	+	±
Fluorescence (UV)	−	−	−
Growth at temp. (°C)			
4	−	−	−
10	−	−	−
15	+	+	−
25	+	+	−
30	+	+	+
37	+	+	+
42	−	+	−
55	−	−	−
65	−	−	−
Growth at pH			
5.0	±	+	+
5.7	+	+	+
6.8	+	+	+
8.0	+	+	+
9.0	+	+	+
11.0	+	+	+
Growth on NaCl (%)			
2.5	+	+	+
5.0	+	+	+
7.0	±	±	±
8.5	−	−	−
10.0	−	−	−
Growth Under Anaerobic Condition	+	±	±
Biochemical tests			
Growth on MacConkey agar	−	−	−
Indole test	−	−	−
Methyl red test	+	+	−
Voges Proskauer test	±	−	−
Citrate utilization	−	−	±
Gas production from glucose	−	−	−
Casein hydrolysis	+	−	+
Starch hydrolysis	+	−	+
Urea hydrolysis	+	−	−
Nitrate reduction	+	±	−
H_2S production	−	−	−

TABLE II *Continued*

Tests	Results		
	I	II	III
Cytochrome oxidase	+	±	±
Catalase test	+	+	+
Oxidation/fermentation (O/F)	−	−	F
Gelatin hydrolysis	+	−	+
Arginine dihydrolase	+	+	+
Lysine decarboxylase	−	−	−
Ornithine decarboxylase	±	−	−
Tests for acid production from carbohydrates			
Adonitol	−	−	−
Arabinose	+	−	−
Cellobiose	+	+	−
Dextrose	+	+	+
Dulcitol	±	−	±
Fructose	+	+	+
Galactose	+	+	±
Inositol	−	−	−
Lactose	±	−	−
Maltose	±	+	−
Mannitol	+	±	−
Melibiose	±	−	±
Raffinose	±	+	+
Rhamnose	−	+	−
Salicin	+	−	+
Sorbitol	−	−	−
Sucrose	+	±	+
Trehalose	+	+	+
Xylose	+	+	−

S. no.	Strain designation	Identity	MTCC number
1	I	*Bacillus circulars*	8571
2	II	*Kurthia gibsonii*	8572
3	III	*Flavobacterium* sp.	8573

natural soil and urea enriched soil was monitored as a function of number of days and the results are presented in Figure 1. It is observed from the figures that percent weight loss of both the samples increases with increase in the number of days indicating that the samples continuously degrade with increase in the length of time. PP-*g*-Gelatin (composite) showed 31% weight loss, whereas PP-*g*-Gelatin (true graft) showed 28% weight loss in urea enriched soil, which is higher than the weight loss observed in natural soil (28 and 26%, respectively) in 120 days. In comparison to the grafted samples, pristine PP showed 0 wt % loss in 120 days in both soil systems. As more wt % loss is observed in urea enriched soil than in simple soil this indicates that the microorganisms present in urea enriched soil are more numerous than those found in natural soil because of the additional nourishment provided in a urea enriched soil environment.

The degradation study was also under taken to take into account the wt % loss for PP-*g*-Gelatin (both composite and true graft) between every consecutive time period i.e. % weight loss after every 10 days. The loss of weight is calculated by subtracting the weight of the sample on the 10^{th} day from the weight of the sample before 10 days. The results are presented in Figure 2. It is observed that the maximum % weight loss (5%) of PP-*g*-Gelatin (composite) in the first phase of 0–10 days is higher than any weight loss observed for other time periods. In the next 20 days % weight loss decreases to 0% and then again increases to 4% between 50 and 60 days, beyond which it acquires constancy in % weight loss for next periods of time. In urea enriched soil also the initial weight loss of PP-*g*-Gelatin (composite) is 5% in the first 10 days time period which decreases to 2% in 30–40 days time period. Beyond 40 days the % weight loss is found to increase with increasing number of days and attains a maximum % weight loss value of 6% between 90 and 120 days time period. Similar observations of increase and decrease of percent weight loss with increasing number of days was observed for PP-*g*-Gelatin (true graft) both in natural and urea enriched soils. The increase and decrease in the % weight loss with increasing time period is due to the reason that the microorganisms feed upon the sample, grow in colonies and changes the weight statistics.

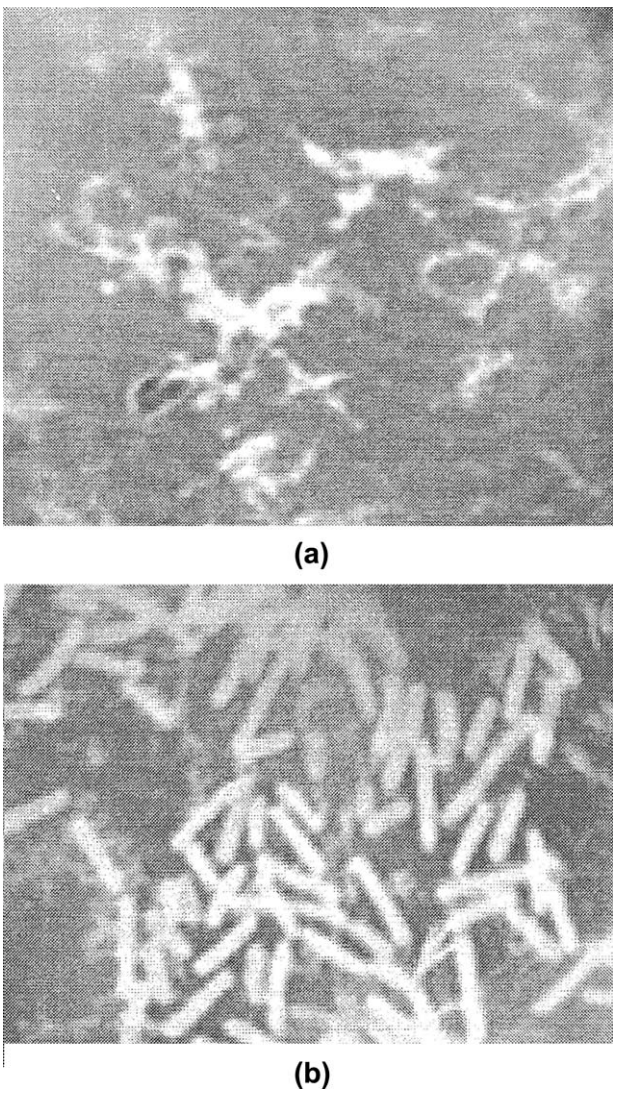

Figure 3 (a,b) Microbes growth pattern under microscope (LEICA DM L52) during microanalysis studies.

Microanalysis

The microanalysis of the soil samples containing PP-*g*-Gelatin samples (both composite and true graft) placed for degradation was carried out in Nutrient Agar and Czapec Dox by serial dilution method.

TABLE III
Biodegradation Study with Isolated Microbes from Soil (for 7 days)

Samples	Percent weight loss					
	Degradation without microbes	Degradation with microbes				
		I	II	III	IV	V
PP	0	5	2	5	1	0
PP-*g*-Gelatin (composite)	30	80	70	80	60	80
PP-*g*-Gelatin (true graft)	15	45	40	30	30	60

TABLE IV
Percent Residue Left of PP from PP-*g*-Gelatin Samples by Hydrolysis Method

Number of days	% Residue left of PP after hydrolysis with 6*N* HCl	
	PP-*g*-Gelatin (composite)	PP-*g*-Gelatin (true graft)
10	72	80
20	70	74
30	56	66
45	50	60
60	48	56
75	47	53
90	47	52
120	45	51

Thick growth of colonies was observed in the soil containing grafted sample than in the soil containing either only pristine PP or no sample. Total counts of different colonies observed during microanalysis are tabulated in Table I. On comparison of the maximum count of colonies in Nutrient Agar and Czapec Dox on particular number of days and the maximum drop in the loss of % weight loss during

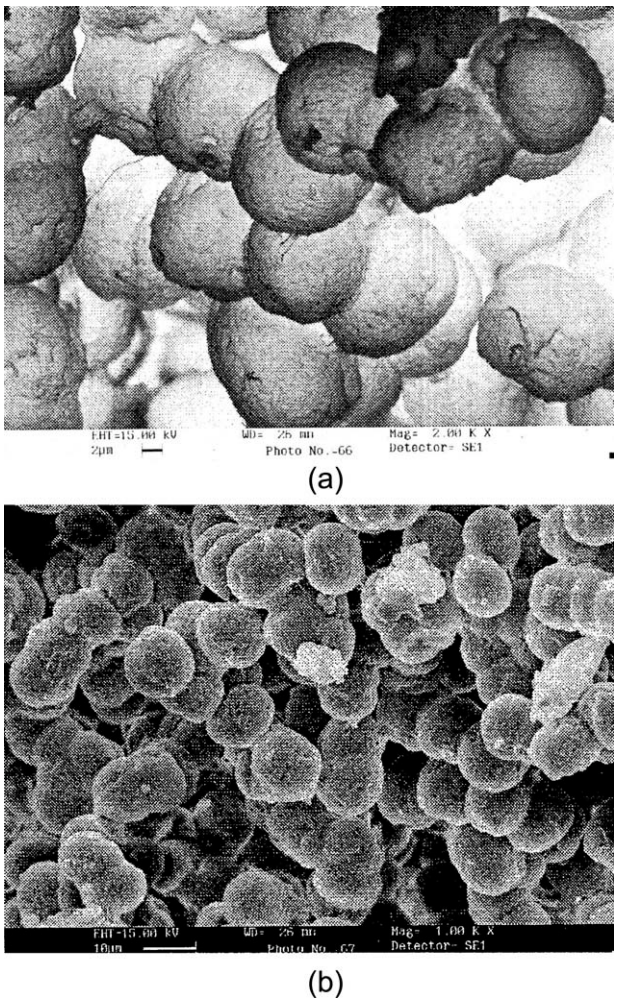

Figure 4 SEMs of pristine PP (a) 2.00KX, (b) 1.00KX.

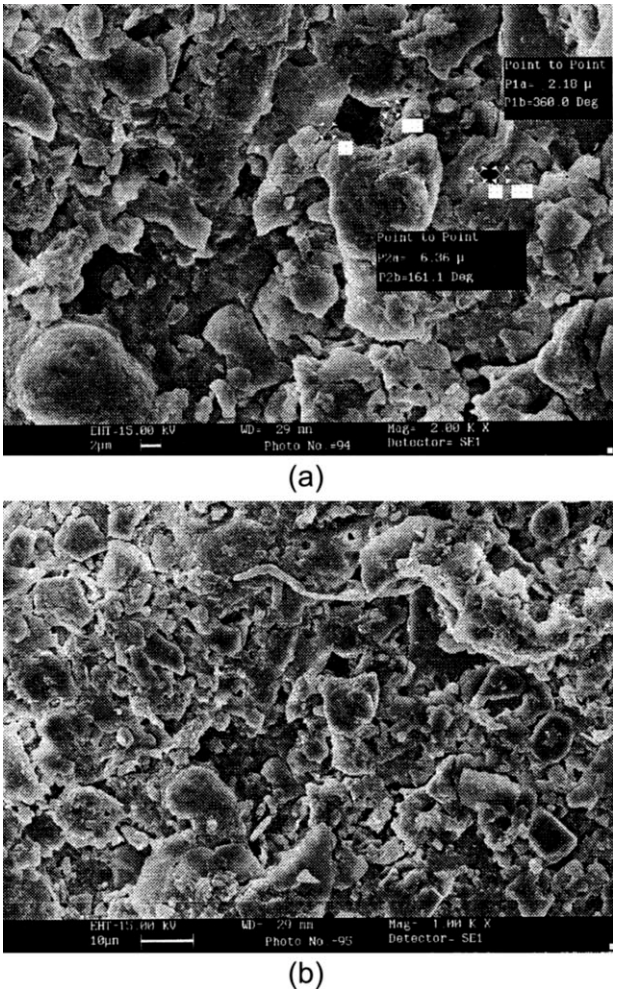

Figure 5 SEMs of pristine PP after soil burial test (120 days) (a) 2.00KX, (b) 1.00KX.

degradation (Fig. 2) it is observed that maximum growths of colonies between 20–30 and 60–90 days corresponds to maximum drop in the weight loss during that period. This implies that the growth of colonies is due to the attack of microorganisms, which feed upon the material their by growing their colonies and simultaneously degrading the material. The cultures (I–V) were isolated for direct attack on the samples. Identification of cultures (I–III) indicates the presence of these organisms and the identification report of cultures (I–III) is given in Table II. On the basis of biochemical tests performed on the organisms (I–III) these are identified as *Bacillus circulans* (MTCC number 8571), *Kurthia gibsonii* (MTCC number 8572), and *Flavobacterium* sp. (MTCC number 8573), respectively. The growth pattern of isolated microbes under microscope (LEICA DM L52) is shown in Figure 3(a,b).

The microbial degradation was further substantiated by the direct attack of the isolated microbes from microanalysis studies of soil on the grafted samples. It was observed that the rate of degradation by direct attack was faster than to the degradation rate in soil burial studies. The results of direct microbial interaction by isolated culture I–V with pristine PP and gelatin grafted PP samples for one week are presented in Table III. It is observed that pristine PP shows no loss in weight when placed in the control sample containing no cells but when it is treated with cells of isolated cultures containing *Bacillus circulans* (I), *Kurthia gibsonii* (II), and *Flavobacterium* sp. (III) % weight loss increases to 5, 2, and 5%, respectively. PP-*g*-Gelatin composite and true graft on the other hand when treated with simple medium containing no cells shows 30 and 15% weight loss. With grafted PP, (composite) on treatment with cultures containing (I), (II), and (III) organisms shows a total % weight loss 80, 70, and 80% while in case true graft the % weight loss observed was 45, 40, and 30%, respectively. However, with culture V maximum 80 and 60% weight loss is observed for composite and true grafted samples of gelatin grafted PP, respectively. The results thus indicate that the presence of microorganisms facilitates degradation.

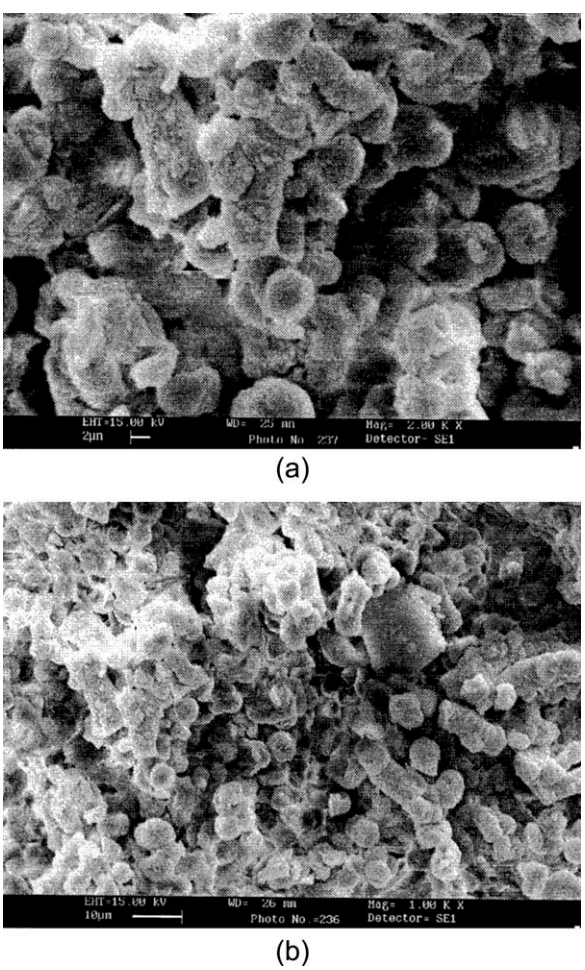

Figure 6 SEMs of PP-*g*-Gelatin (a) 2.00KX, (b) 1.00KX.

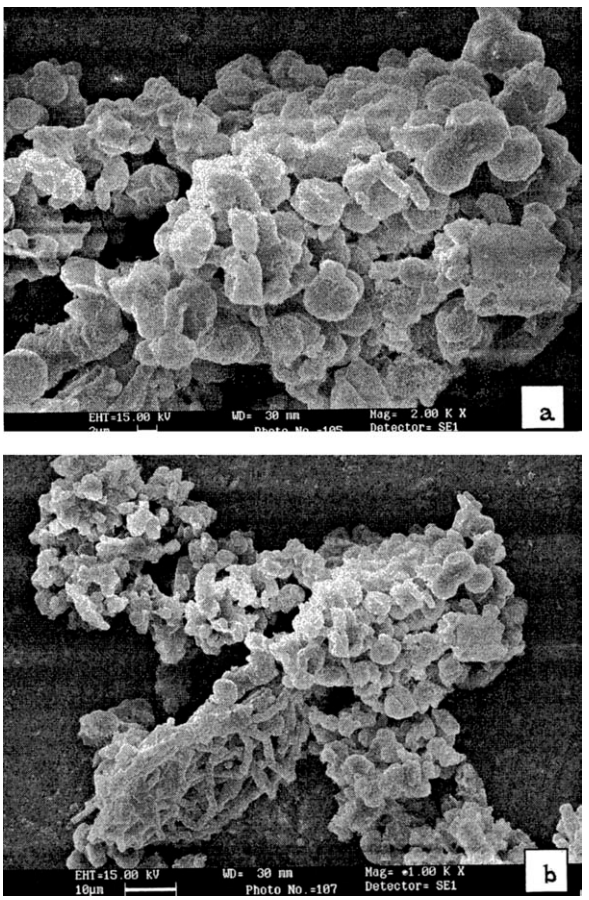

Figure 7 SEMs of PP-*g*-Gelatin after soil burial test (60 days) (a) 2.00KX, (b) 1.00KX.

Hydrolysis studies

The hydrolysis studies of the samples kept for degradation under soil was carried as a function of number of days and the results are presented in Table IV. On perusal of hydrolysis data, it is observed that the amount of % residue left after hydrolysis (i.e. PP) decreases continuously with increasing number of days both in the case of PP-*g*-Gelatin composite and PP-*g*-Gelatin true graft. The amount of the residue left in the respective samples is 45 and 51%. From the hydrolysis studies it is thus evident that nearly 50% of grafted samples (both composite and true graft) degrade during soil burial studies in 120 days. Degradation is triggered due to the presence of the gelatin chains on the PP backbone. After degrading the gelatin chains, degradation approaches PP chains as is evinced from the amount of PP left, which decreases with increasing time of degradation.

Characterization of PP and PP grafted samples

Scanning electron microscopy

Scanning electron microscopy of the pure and grafted samples were taken before and after the soil burial test exposures at different magnification (i.e. 2.00KX and 1.00KX) on LEO vp 435 instrument and are presented in Figures 4–9. Prior to the test exposure the pristine PP (Fig. 4) sample exhibited a relatively continuous morphology of the surface, which do not change even after a soil test exposure for 60 days continued for 120 days in the soil. However, some difference in the surface morphology of PP (Fig. 5) observed may be attributed to some oxidative degradation.

Figures 6–9 shows images of the biodegraded surface of PP-*g*-Gelatin before and after the degradation studies. Before the biodegradability test, the PP-*g*-Gelatin (Fig. 6) was characterized by contiguous surface of PP, in which gelatin appears as being incorporated onto the surface of PP upon grafting. However, after 60 days (Fig. 7) in the natural soil, there were changes in the PP-*g*-Gelatin surface, indicating that the surface of PP-*g*-Gelatin was attacked by the microorganism under soil environment. The biodegraded surface after 120 days (Fig. 8) of soil test exposure presented larger holes and more numerous surface irregularities compared to that of before and after 60 days in the soil burial test. This is due to the degradation that occurred by the microbial attack on

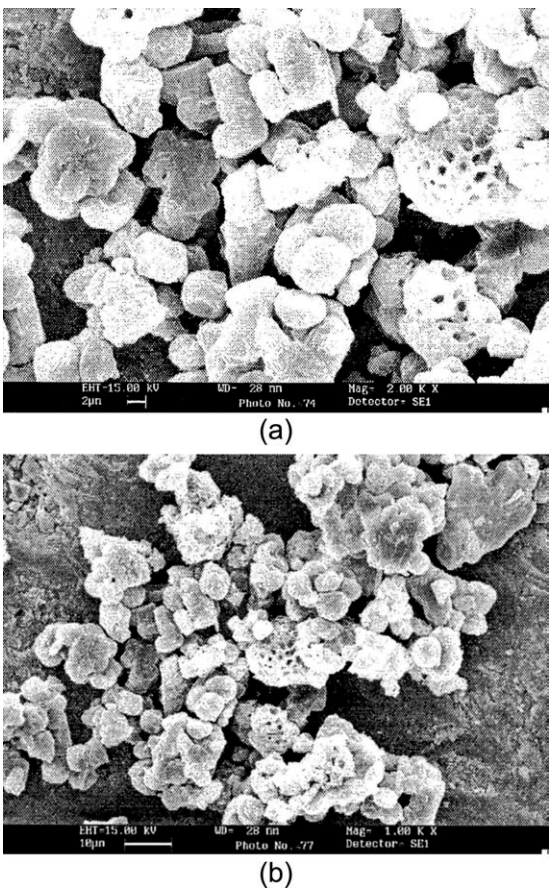

Figure 8 SEMs of PP-*g*-Gelatin after soil burial test (120 days) (a) 2.00KX, (b) 1.00KX.

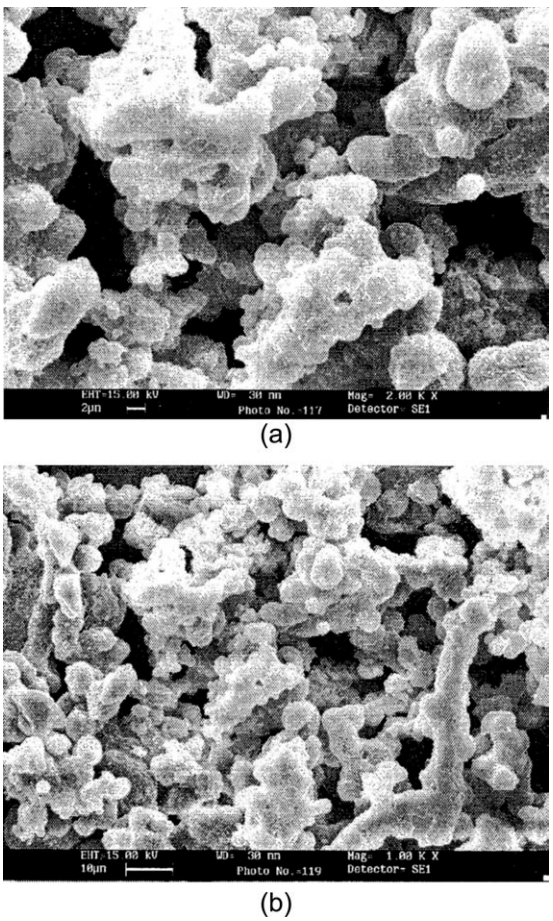

Figure 9 SEMs of PP-*g*-Gelatin after microbes attack of 7 days (a) 2.00KX, (b) 1.00KX.

PP-*g*-Gelatin and is enhanced with increasing time period.

The samples of PP and PP-*g*-Gelatin collected after 7 days of direct microbes study were also examined by their SEM and results are shown in Figure 9. Results indicate that microorganisms introduced into the medium degraded PP-*g*-Gelatin (both composite and true graft) more than by soil burial test as observed from the scanning electron microscopy. This is due to reason that gelatin grafted to PP would be degraded initially thus creating micropores and more space into the PP surface, giving rise to concomitant increase in the rate of degradation of PP surface.

X-ray diffraction studies

X-ray diffraction studies were performed under ambient conditions on X-ray diffractometer (Bruker-D8 advance model; IIT, Roorkee) using Cu target and goniometer scanning range 0–150°. The X-ray diffraction patterns of the pristine PP and degraded PP (after 120 days of biodegradation) and PP-*g*-Gelatin and degraded PP-*g*-Gelatin are presented in

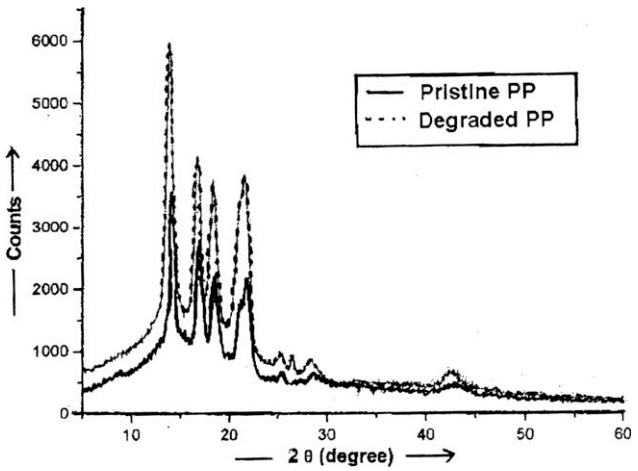

Figure 10 X-ray diffraction spectra of pristine PP before and after soil burial test (120 days).

Figures 10 and 11, respectively. The main four peaks that are observed in the diffraction pattern of all the samples clearly indicate that the samples are semicrystalline in nature. These peaks are compared in the Table V and the following conclusions are drawn.

It is observed from the diffraction pattern of pristine PP (at $2\theta = 14.1$, 16.9, 18.6, and 21.6°) and its degraded sample after 4 months of biodegradation, that the intensity of almost all the peaks is increased. However, in case of degraded PP-*g*-Gelatin, the intensity of almost all the peaks is reduced. The broad peak at 21.6° splits into three peaks, which may be due to redistribution of the molecular chains in precisely different family planes on biodegradation. The peaks at 12.7 and 27.4° also show different behavior as compared to other peaks due to the reorientation or redistribution of the molecular chains on

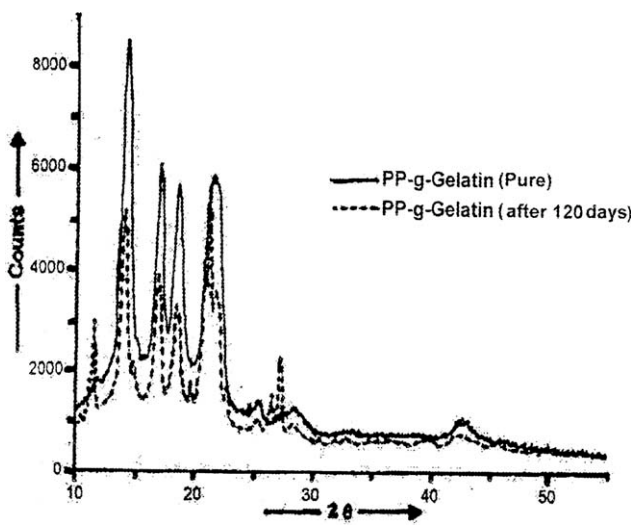

Figure 11 X-ray diffraction spectra of PP-*g*-Gelatin before (pure) and after soil burial test (120 days).

TABLE V
X-Ray Diffraction Data for PP, Degraded, PP-*g*-Gelatin, and Degraded PP-*g*-Gelatin (120 days)

	Pristine PP				Degraded PP (after 120 days)				
Peaks	Center 2θ (deg)	Width *B* (deg)	Height	Crystallite size *L* (A°)	Center 2θ (deg)	Width *B* (deg)	Height	Crystallite size *L* (A°)	Increase/decrease in crystallite size (%)
1	14.1	1.94	3530	40.1	13.9	1.64	5960	47.4	+18.20
2	16.9	1.20	2600	65.1	16.7	1.17	4167	66.7	+2.45
3	18.6	0.89	2118	87.9	18.5	1.05	4743	74.5	−15.24
4	21.6	2.80	2130	28.1	21.4	2.02	3832	38.9	+38.43
Average increase in crystallite size =									+10.96
	PP-*g*-Gelatin (Pure)				Degraded PP-*g*-Gelatin (after 120 days)				
1	14.135	0.84258	7322	95.0	14.017	0.58681	3951.1	136.3	+43.15
2	16.961	0.89040	4758	90.2	16.857	0.53270	2777.9	151.5	+67.96
3	18.596	0.89776	4297	89.6	18.464	0.58713	2348.3	137.0	+52.90
4	21.584	1.5554	5394	51.968	21.287	1.3863	3603.8	58.278	+12.14
Average increase in crystallite size =									+44.04

degradation. The reduction of peak intensities (14.1, 16.9, 39.0°, etc) shows the decrease in the alignment of polymeric chains of particular family. But on the other hand, increase in intensity of two peaks at 12.7 and 27.4° shows that alignment of chain is increasing of that region.

It is further observed from the diffraction pattern of degraded PP-*g*-Gelatin that the decrease in the full width at half maximum (FWHM) occurs which shows an increase in the crystalline nature. The larger the crystal's size of a given component, the sharper is the peaks i.e. decrease in FWHM on the XRD pattern for each crystal plane. Thus, the width of the peak can be related to the crystal size.

The Scherrer equation,[13] relates FWHM (*B*) of an XRD peak to the size of crystallites, *L*, as: $B = K\lambda / L\text{Cos }\theta$, where *B* is in radians, λ is wavelength of X-Ray beam (1.5425A°), *L* (crystallite size) is in A°, and *K* is a constant usually equal to 1. From the above equation, the crystallite sizes corresponding to peaks of the pure and degraded samples were calculated and the results are reported in Table V.

From the Scherrer equation, the crystallite sizes corresponding to peaks of the pure and degraded samples were calculated and the results are reported in Table V. The increase (positive sign) and decrease (negative sign) in the crystallite size[14] of both degraded PP and degraded PP-*g*-Gelatin corresponding to four peaks is listed in Table V. Thus, on the average the crystallite size of degraded PP (+44.04%) and degraded grafted PP (+20.445%) increases on soil exposure to 120 days. The same observation was made during thermal analysis studies of pristine PP and its degraded sample where degraded samples acquire higher temperature than their pristine samples indicating a higher crystalline structure of the degraded sample.[15]

From the above discussion, it is concluded that both the factors i.e. increase in the peak intensities and crystallite size (+ value) favor degradation of PP samples, whereas in case of degraded grafted samples only increase in crystallite size (+ value) is observed and peak intensities decrease upon degradation. This is due to reason that grafted samples undergo redistribution of the molecular chains in different family planes i.e. conversion of some —OH groups into >C=O and —COOH groups on degradation.

The percentage crystallinity (% Crt) and crystalline index (C.I.) of PP-*g*-Gelatin and its degraded sample has also been evaluated by using the following equations reported by Kaith and Kalia[16] and results are presented in Table VI. Crystalline index was determined by using the wide angle X-ray diffraction counts at 2θ angle close to 22 and 18°. The counter reading of peak intensity at 22° corresponds to crystalline material whereas the peak intensity at 18° corresponds to the amorphous material in grafted PP.

$$\%\ \text{Crt} = \frac{I_{22}}{I_{22} - I_{18}} \times 100$$

$$\text{C.I.} = \frac{I_{22} - I_{18}}{I_{22}}$$

where I_{22} and I_{18} are the crystalline and amorphous intensities at 2θ scale close to 22 and 18°, respectively.

It is observed that the degraded PP-*g*-Gelatin sample has higher % Crt and C.I. values (51.83; 0.071) as compared to that of PP-*g*-Gelatin (50.92; 0.036),

TABLE VI
Percent Crystallinity and Crystalline Index of PP-*g*-Gelatin and Degraded PP-*g*-Gelatin (after 120 days)

Samples	2θ = 18°	2θ = 22°	% Cry.	C.I.
PP-*g*-Gelatin	4195	4352	50.92	0.036
PP-g-Gelatin (after 120 days)	2480	2669	51.83	0.071

TABLE VII
Thermogravimetric Analysis of PP and PP Grafted Samples

Sample	IDT (°C)	FDT (°C)	DT (°C) at every 10% weight loss									
			10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Pristine PP	425.6	460.5	414.1	428.8	436.0	440.8	444.5	447.8	451.1	454.7	459.1	470.6
PP-*g*-Gelatin	410.6	463.9	321.3	401.9	418.5	427.6	434.1	439.1	444.1	448.9	454.5	463.9
Pristine PP (after 120 days)	432.5	471.5	421.4	434.3	444.5	446.3	450.1	453.7	457.4	461.3	466.2	485.2
Hydrolyzed product of PP-*g*-Gelatin (after 120 days)	415	462	379.8	409.4	423.0	431.9	438.3	444.5	449.2	454.6	461.2	653.0
PP-*g*-Gelatin (after 120 days)	414.2	464.1	355.1	408.7	423.7	433.1	439.9	445.8	451.2	457.4	466.9	[a]

[a] % residue left = 6.44%.

respectively. Thus an increase of 0.91% in the % Crt behavior of the degraded sample is observed.

Thermogravimetric analysis

The thermogravimetric analysis of pristine PP, PP-*g*-Gelatin and their respective samples kept for degradation undisturbed for 120 days was carried out in nitrogen (200 mL/min.) atmosphere at a rate of 10°C/min on PerkinElmer (Pyris Diamond, IIT Rorkee, India). The initial decomposition temperature (IDT), final decomposition temperature (FDT), and decomposition temperature (DT) at every 10% weight loss for each sample is presented in Table VII and Figure 12.

The TG analysis of PP and PP-*g*-Gelatin kept for degradation undisturbed for 120 days was carried out and the results are presented in Table VII. It was observed from the Table VI that TG data of pristine PP before and after degradation do not vary much. The DT values at every 10% weight loss of pristine PP lies little lower side than degraded PP. However, on comparison of the TG data of the grafted PP before and after degradation it is observed that the DT values at every 10% weight loss of the degraded samples is higher than that of the pure grafted PP. This is due to reason that the crystallinity of PP increases during degradation although the net weight loss during soil burial test is nil. It has been also shown by grafted PP samples, in which percentage residue left after TGA studies was 6.44%. The increase in crystalinity during degradation has also been observed during degradation studies of polybutylene succinate.[15] Further these DT values of the degraded sample beyond 50% weight loss are parallel to those of the DT values of pristine PP. These observations indicate that the degradation begins at the grafted gelatin chains and approaches towards the PP chains.

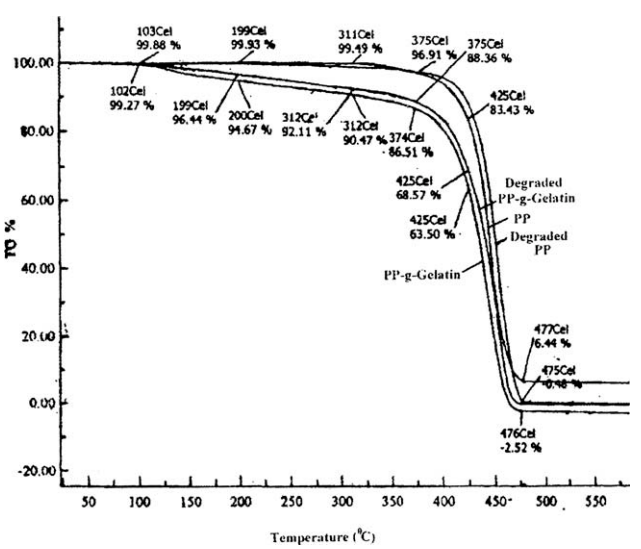

Figure 12 Thermograms of pristine PP and PP-*g*-Gelatin before and after soil burial test (120 days).

CONCLUSION

Polypropylene has been successfully intercross linked with gelatin through chemical method of graft copolymerization. The biodegradation of the graft copolymer was monitored as a function of time during soil burial investigation. The introduction of gelatin moieties into the PP backbone has triggered degradation into the otherwise resistant PP after initial degradation beginning at the gelatin sites. A contrast difference in the topological morphology, the crystallinity, and the thermal behavior between the original and the degraded samples further substantiate the degradable behavior induced into the grafted PP. The XRD and thermal data indicate an increase in the crystallinity of the degraded samples.

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