Study of the Biodegradation Behavior of Soy Protein-Grafted Polyethylene by the Soil Burial Method

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ABSTRACT: In this article, we report on the biodegradation of soy-protein-grafted polyethylene, which was successfully synthesized by a graft copolymerization method with benzoyl peroxide as the radical initiator. The biodegradation behavior of the grafted polyethylene was ascertained by a soil burial test. The weight-loss percentage was measured as a function of the number of days, and it was observed that the percentage weight loss increased with increasing number of days. To further substantiate the degradation, microanalysis of the soil containing the samples was carried out. An increase in microorganism colonies was observed with increasing number of days. The hydrolysis of the samples taken from the soil after a specified number of days also corroborated the findings and revealed a continuous loss of weight. The effect of the degradation of the grafted samples on the growth of plants (wheat and soybean) was studied, and we observed that the products of degradation were not harmful to the growth of the plants. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 111: 2460–2467, 2009

Key words: biodegradable; copolymerization; polyamides; polyethylene (PE)

INTRODUCTION

In this era of many astonishing industrial developments, probably no industry has undergone such rapid growth and development as the plastic industry because of the use of plastics in varied applications in almost every sphere of life. However, plastic's lack of biodegradability has caused much concern with regard to environmental pollution. The production of plastic emits substantial amounts of toxic chemicals (e.g., ethylene oxide, benzene, xylene) into the air and water, which can cause cancer and birth defects and can also damage the nervous system. Various attempts have, therefore, been made to develop biodegradable plastics that after use do not harm the environment. Because of the worldwide environmental pollution problem with petroleum polymers, soy protein polymers have been considered as alternatives for biodegradable plastics. In the 1930s, Henry Ford pioneered the use of soy protein for plastics and fibers.[1] The effects of molding temperature and pressure on the properties of soy protein polymers was studied by Mo et al.[2] Tummala et al.[3] modified soy flour with polyester amide to fabricate composites using hemp fibers. The characterization of flax yarn and glutaraldehyde/poly(vinyl alcohol) modified soy protein concentrate composites was done by Chabba and Netravali.[4,5] Biocomposites were synthesized by Tran et al.[6] from chemically modified soy oils and biofibers without additional petroleum-based polymers. These composites were prepared from maleic anhydride and epoxide functionalized soybean oils that were cured in the presence of various biofibers by a flexible amine catalyst. Biodegradable soy protein/polyester blends were made by a reactive extrusion process by Graiver et al.[7] The synergistic effect of combining ultraviolet sunlight and soil-burial treatments on the biodegradation rate of low-density polyethylene (LDPE)/soy protein blends was studied by El Rehim et al.[8] The electronic detection of the enzymatic degradation of soy protein was studied by Star et al.[9] Cláudia et al.[10] studied the *in vitro* degradation behavior of biodegradable soy plastics and carried out thermal treatments with either an isotonic saline solution without enzymatic activity or containing bacterial collagenase. The changes in the weights of the samples during the *in vitro* degradation were studied and compared with the variations in the mechanical properties. The formation of lysinoalanine after the alkaline processing of soya bean meal in relation to the degradability of protein in the rumen was studied by Uchida and Ohshima.[11]

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Because of the biodegradation behavior of soy protein, we grafted polyethylene (PE) with soy protein, and in this article, we report on the biodegradation studies of soy-protein-grafted PE by the soil burial test. The weight loss of the grafted PE kept for soil burial studies was measured, and this was followed by hydrolysis to corroborate the degradation.

EXPERIMENTAL

Materials and methods

Commercial LDPE was obtained as beads from Thukral Trading Co. (Delhi, India). PE was dissolved in *p*-xylene and was precipitated with the addition of methanol. PE was irradiated from a Co^{60} source housed in a Gamma Chamber 900 (BARC, Trombay, Mumbai, India) at a constant dose rate of 3.40 kGy/h. Soy protein and benzoyl peroxide (S.D. Fine Chemicals, Mumbai, India) were used as received.

Synthesis of the graft copolymer

Soy protein was grafted onto preirradiated PE with benzoyl peroxide as the radical initiator. Optimum conditions pertaining to the maximum percentage of grafting were evaluated. The maximum grafting percentage (135%) was obtained at a benzoyl peroxide concentration of 2.15×10^{-2} mol/L grafted at 70°C for 150 min with 0.200 g of PE, 0.300 g of soy protein, and 40 mL of water.

The grafted PE containing both unreacted PE and soy protein was called the PE-*g*-soy protein composite, whereas the graft from which the unreacted soy protein and PE were removed by washing with water and xylene was called the PE-*g*-soy protein true graft.

Biodegradation studies

We studied the biodegradation behavior of PE, the PE composite, and the true graft by ascertaining the loss in weight during the soil burial test.

Soil burial method

Garden soil (1200 g) was placed in different pots. A weighed amount (1 g) of each of the samples, that is, pristine PE and PE-*g*-soy protein (the composite and true graft), wrapped in synthetic net were placed separately in each pot. Care was taken that the samples were completely covered with the soil. The pots were covered with the aluminum foil and kept at room temperature. The weight of all of the samples, PE and the grafted PE, were taken at regular intervals of time (10 days). The percentage weight loss as a function of the number of days was determined as follows:

$$\text{Weight Loss } (\%) = \frac{\text{Initial Weight at the beginning} - \text{Final Weight after 10 days interval}}{\text{Initial Weight at the beginning}} \times 100$$

$$\text{Weight Loss (after every 10 days) } (\%) = \frac{\text{Initial Weight before 10 days} - \text{Final Weight after 10 days}}{\text{Initial Weight before 10 days}} \times 100$$

To study the effect of nitrogenous compounds on the degradation behavior of the samples, similar studies were also done with samples in urea-enriched soil (6 g of urea/kg of soil).

Microanalysis

To corroborate degradation, an assay of the soil containing samples for degradation was studied in Nutrient Agar medium and CzepeckDox medium. The growth of microorganisms such as bacteria and fungi was checked at definite intervals of time. Samples of the soil (1%) containing PE and PE-*g*-soy protein (composite and true graft) were mixed well separately with 10 mL of saline solution. This was diluted through a serial dilution method. Supernatant (1 mL) was added to tube number 1 (from the saline solution containing soil samples) and thoroughly mixed. From tube 1, 1 mL of each was transferred to the second tube and so on to make the further dilutions.

Hydrolysis studies

For hydrolysis studies, a definite weight (0.500 g) of different samples of pristine PE and PE-*g*-soy protein (composite and true graft) wrapped in synthetic net were buried in soil. The samples were taken out

after a definite interval of time (10 days), washed with water to remove the adhered soil, filtered, and dried, and calculated the percentage weight loss as:

$$\text{Weight Loss (\%)} = \frac{\text{Initial Weight at the beginning} - \text{Final Weight (after every 10 days interval)}}{\text{Initial Weight at the beginning}} \times 100$$

The dried sample was weighed and hydrolyzed with 20 mL of 6*N* HCl for 4 h. After the hydrolysis, the residue was dried and weighed.

Growth of plants

To find out whether or not the degradation products from the polymer samples were harmful to the growth of the plants, the growth of soybean plants and wheat plants was checked from the germination stage. The soybean seeds and wheat seeds were placed uniformly in different pots containing the samples for degradation along with a reference pot containing no grafted sample. The plants were allowed to grow in the open for 35 days. The length and height of the roots and shoots, respectively, were measured. For wheat plants, an average of six plants were used to measure the length and height of the roots and shoots, respectively.

RESULTS AND DISCUSSION

Biodegradation studies

Soil burial studies

The soy-protein-grafted PE had pendant chains of soy protein suspended from the PE backbone where the process of degradation began because soy protein is well known for its biodegradability.

The biodegradation of the grafted samples, both the composite and true graft, buried in the soil was monitored as a function of the number of days, and the results are presented in Figure 1. The percentage weight loss due to degradation was determined by the subtraction of the weight of the sample taken out on a particular day (i.e., after every 10 days) from the initial weight, that is, the weight of the sample at the start of the degradation study each time. As shown in Figure 1, the percentage weight loss of both of the samples increased continuously with increasing number of days. Maximum weight losses of 76 and 74% for the composite and true graft samples, respectively, were observed after a period of 4 months, which indicated that the samples continuously degraded with increasing time.

In another set up, the percentage weight loss of the sample was measured every 10 days, but the percentage weight loss of the sample was taken as the weight of the sample on each 10th day minus the preceding weight of the sample before 10 days, and the results are presented in Figure 2. As shown in Figure 2, the composite sample showed an initial decrease in percentage weight loss of 7.86% in the first 20 days. It then increased up to 14.51% over the next 40 days. After a decrease in percentage weight loss in the next 10 days, an increase in percentage weight loss up to 19.04% was observed on the 90th

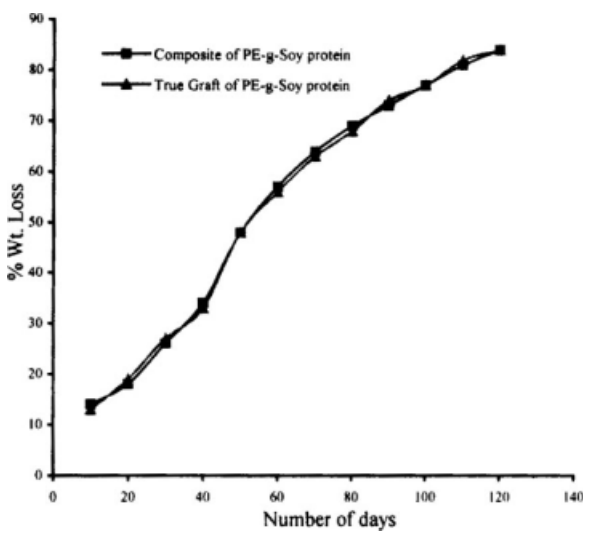

Figure 1 Weight-loss percentage as a function of the number of days in simple soil.

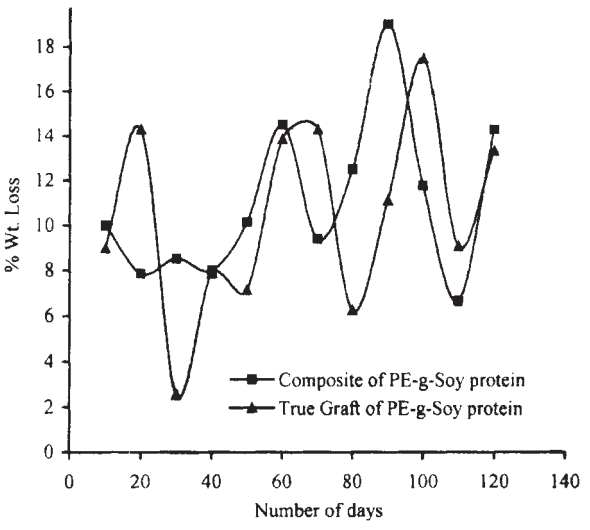

Figure 2 Weight-loss percentage as a function of the number of days in simple soil (weight loss for every 10-days interval).

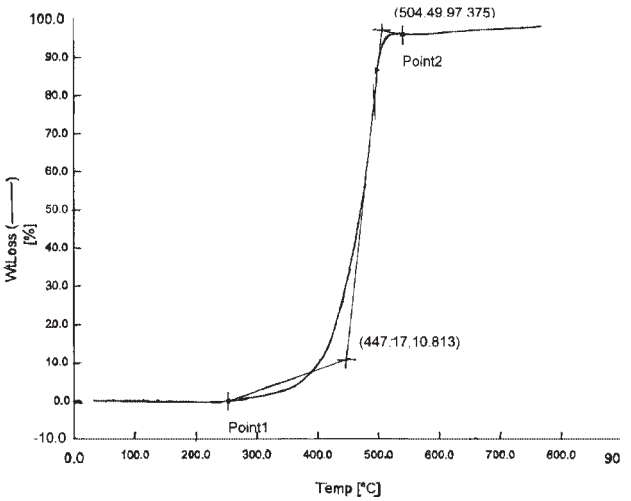

Figure 3 Primary thermogram of PE.

day, which further increased to 14.28% after 120 days. In case of the true graft, the sample showed a 14.28% weight loss in the first 20 days, which further dropped to 2.56% in the next 10-days interval. However, the percentage weight loss increased to 14.51% on the 60th day and decreased to 6.25% for the next two 10-days intervals. Thereafter, the weight loss increased to 17.5% after 100 days. The percentage weight loss showed a decrease of 9.09% and an increase of 13.33% for the next two 10-days intervals, respectively. The decrease in the percentage weight loss was due to the invasion of microorganisms into the substrate and the absorption of moisture by the samples. The microorganisms, when feeding upon the substrate, increased the percentage weight loss of the sample. However, for pristine PE, the percentage weight loss was 0% in all respective degradation studies.

Thermogravimetric analysis of the degraded PE and PE-g-soy protein composite samples. Thermogravimetric

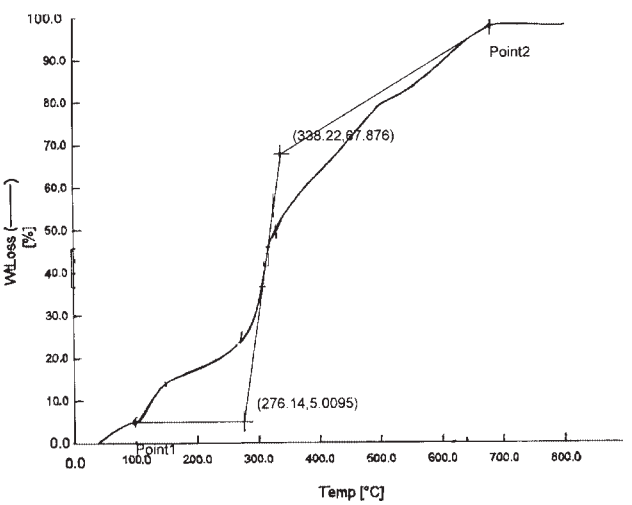

Figure 4 Primary thermogram of PE-*g*-soy protein.

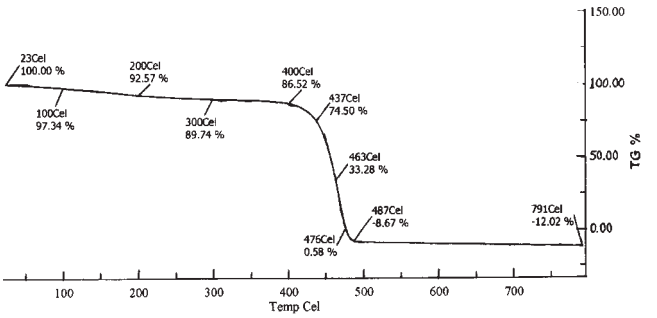

Figure 5 Primary thermogram of degraded PE.

analysis of the PE, PE-*g*-soy protein composite, degraded PE, and degraded PE-*g*-soy protein composite are represented in Figures 3–6, respectively. The initial decomposition temperature (IDT), final decomposition temperature (FDT), and decomposition temperature (DT) values at every 10% weight loss are presented in Table I. As shown in Table I, the IDT, FDT, and DT values at every 10% weight loss of the degraded soy protein-grafted PE and degraded PE are almost the same. However, on comparison of the thermogravimetry (TG) data of the grafted PE before and after degradation, we observed that the DT values of the degraded sample were parallel to those of the pristine PE and degraded PE. These observations indicate that the degradation began at the grafted soy protein chains and approached the PE chains.

Scanning electron micrographs of the degraded PE-g-soy protein composite sample. Scanning electron micrographs of the PE, PE-*g*-soy protein composite, and degraded PE-*g*-soy protein composite at different magnifications (i.e., 2000 and 4000×) are shown in Figures 7(a,b), 8(a,b), and 9(a,b), respectively. Comparing the scanning electron micrograph of the degraded PE-*g*-soy protein composite with the scanning electron micrograph of the PE-*g*-soy protein composite (Fig. 8), we observed that the grafted soy protein, appearing as a thick homogeneous deposit on the PE surface, was reduced to scattered and thin deposits after degradation. These observations,

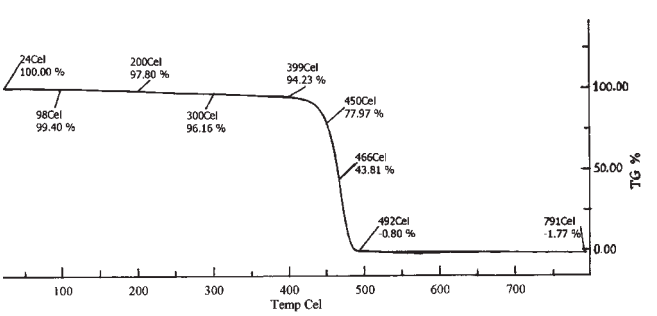

Figure 6 Primary thermogram of degraded PE-*g*-soy protein.

TABLE I
Primary Thermographs of PE, Soy Protein, Grafted PE, and Degraded PE and Grafted PE Samples

Sample	IDT [°C (%)]	FDT [°C (%)]	DT (°C) at every 10% weight loss									Residue left (%)
			10%	20%	30%	40%	50%	60%	70%	80%	90%	
PE	447.17 (10.81)	504.49 (97.37)	404.76	428.56	447.62	457.14	466.67	476.19	480.95	490.48	495.23	1.5
PE (degraded)	425.0	479.16	341.66	425.0	437.5	450.0	454.16	458.3	462.5	466.6	470.83	0
Soy protein	260.86 (9.69)	356.68 (92.06)	200.00	261.90	285.71	300.00	319.05	347.62	447.61	502.38	538.09	5
PE-*g*-soy protein	276.14 (5.009)	338.2 (67.87)	123.81	233.33	290.48	309.52	314.29	371.43	438.09	495.24	595.24	2.5
PE-*g*-soy protein (degraded)	437.5	483.3	437.5	449.3	454.16	458.3	462.5	468.0	470.8	475.0	479.16	0

therefore, symbolize the degradation process during the soil burial studies of the soy protein-grafted PE.

Microanalysis

During the microanalysis studies, we observed that the pure soy protein completely degraded in 10–20 days. The soil containing pristine PE and the soil without any sample showed a very small growth of bacteria, whereas the composite material and the true graft of PE showed a rich growth of bacterial colonies.

The growth of microorganisms as the number of colonies as a function of the number of days in Czapek-Dox media and Nutrient Agar media is presented in Tables II and III, respectively. A careful perusal of Table II reveals that in the case of the PE-*g*-soy protein composite, the growth of colonies increased continuously from 55 (20th day) to 142 (120th day) in a 10^{-2} dilution. For the PE-*g*-soy protein true graft, the growth of colonies showed an increase of 115 in 20 days and decreased to 57 for after 75 days in a 10^{-2} dilution, after which it increased to 105 after 120 days. For a 10^{-4} dilution, the number of colonies showed maximum growth of 75 and 60 in 120 days for the composite and true graft samples, respectively.

However, for PE, maximum growths of colonies of 88 in 30 days and 4 in 120 days were observed in 10^{-2} and 10^{-4} dilutions, respectively.

When the microanalysis was done in Nutrient Agar (Table III), the growth of colonies was lower than that observed in Czapek-Dox media. The maximum growth of colonies for the composites and true graft observed were 165 (in 75 days) and 125 (in 120 days) in a 10^{-2} dilution respectively. For a 10^{-4} dilution, maximum growth of 65 (in 60 days) and 72 (in 120 days) were observed for the composites and true graft, respectively.

However, for PE, maximum growth of colonies of 32 in 30 days and 1 in 10 days were observed in 10^{-2} and 10^{-4} dilutions, respectively.

On comparing the growth of and decrease in the number of colonies with the percentage weight loss as a function of the number of days during the soil burial studies, we observed that the increase and decrease in percentage weight loss were parallel to the growth and decrease in the number of colonies with increasing number of days. These observations implied that the microorganisms attacked the

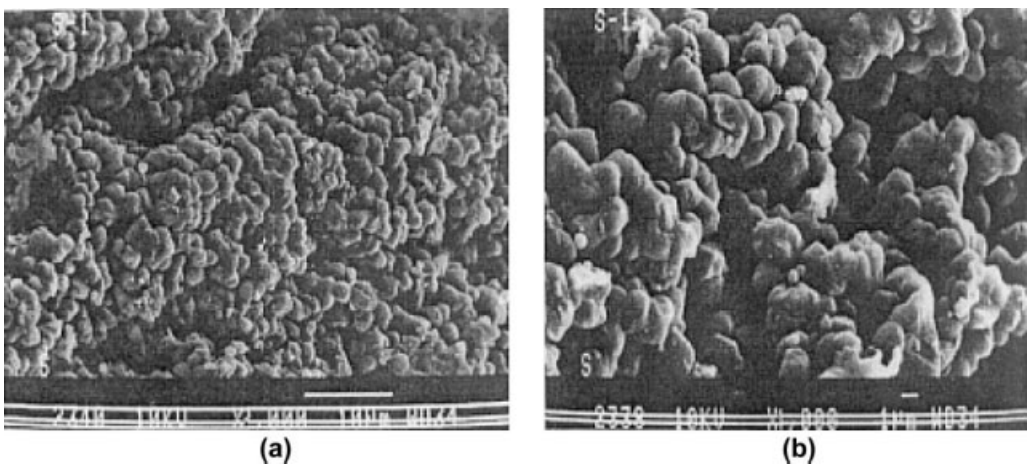

Figure 7 Scanning electron micrographs of PE at magnifications of (a) 2000× and (b) 4000×.

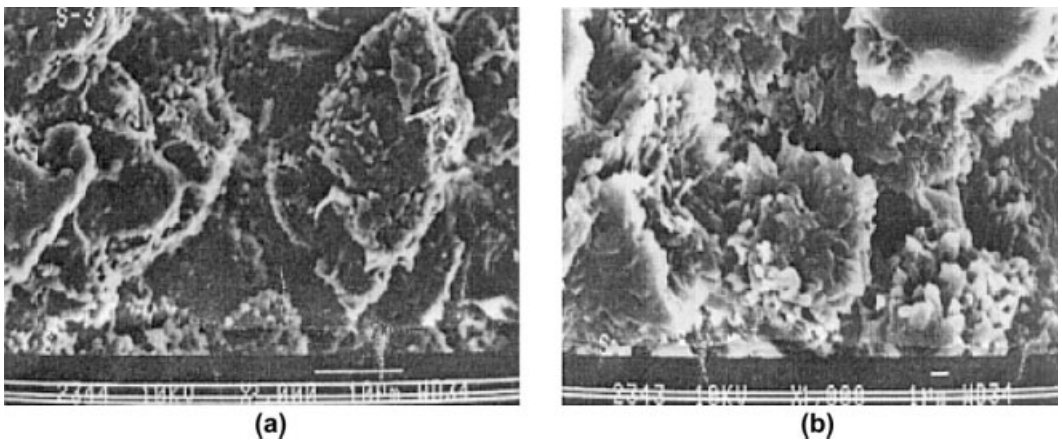

Figure 8 Scanning electron micrographs of the PE-*g*-soy protein composite at magnifications of (a) 2000× and (b) 4000×.

polymer and fed upon, which led to a decrease and increase in the percentage weight loss accordingly.

Hydrolysis studies

The results of the hydrolysis of the PE-*g*-soy protein composite and PE-*g*-soy protein (true graft) placed for degradation in soil are presented in Tables IV and V, respectively. The percentage weight loss increased with increasing number of days. The maximum percentage weight losses for the composite and true grafted sample were 72% (Table IV) and 70% (Table V), respectively, in 120 days. The percentage weight loss was comparable to the percentage weight loss presented as a function of the number of days (Fig. 1) where maximum percentage weight losses for the composite and true graft (76 and 74%) were observed. This further substantiated the observed degradation. The hydrolysis of the sample left PE behind, the amount of which decreased continuously with increasing number of days.

Effect of the degradation of the grafted samples on the growth of the plants

The lengths of the roots and shoots of soybean and wheat plants were measured after 35 days, and the results are presented in Tables VI and VII, respectively. We observed that the growth of the plants was much better in urea-enriched soil. From Table VI, it is clear that the lengths of the roots of plants grown in soil containing the composite and true graft sample were 5.4 and 5.3 cm, respectively, which were higher by 0.2 and 0.1 cm from the reference plant, which had a length of 5.2 cm, in comparison to the plant in the urea-enriched soil, where the length of the root in soil containing the composite sample was 5.6 cm (the same as that of the reference plant) and in soil containing the true graft sample was 5.5 cm (smaller by 0.1 cm than the reference with a 5.6-cm root length). For plants grown in simple soil and urea-enriched soil containing PE, the lengths of the roots measured were 5.1 and 5.5 cm, respectively. The length of the shoot for the plants grown in soil containing the composite and true

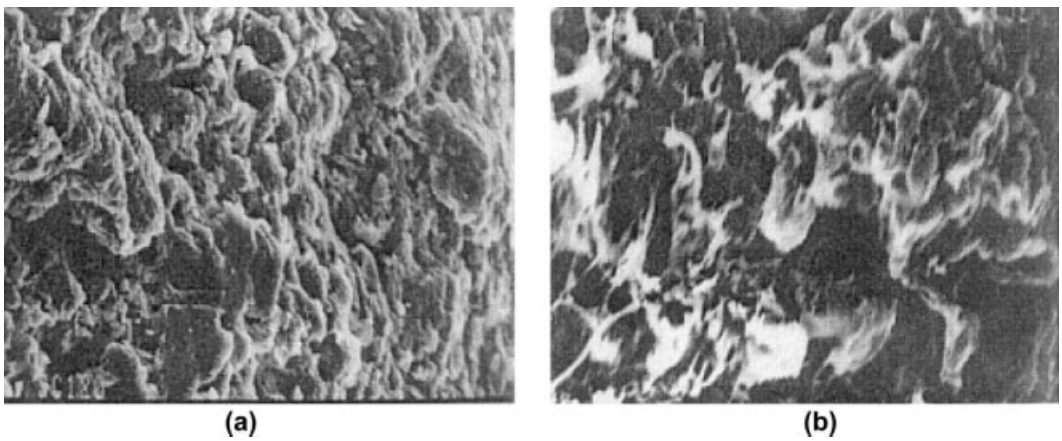

Figure 9 Scanning electron micrographs of the degraded PE-*g*-soy protein composite at magnifications of (a) 2000× and (b) 4000×.

TABLE II
Growth of Microorganisms as a Function of the Number of Days in the Czapek-Dox Medium

Number of days	Dilution	Total number of colonies		
		PE	PE-*g*-soy protein composite	PE-*g*-soy protein true graft
10	10^{-2}	60	86	70
	10^{-4}	5	40	48
20	10^{-2}	70	55	115
	10^{-4}	35	32	52
30	10^{-2}	88	70	55
	10^{-4}	30	30	37
45	10^{-2}	17	110	68
	10^{-4}	10	45	42
60	10^{-2}	26	128	78
	10^{-4}	6	72	48
75	10^{-2}	58	135	57
	10^{-4}	12	62	41
90	10^{-2}	12	101	92
	10^{-4}	8	68	32
120	10^{-2}	16	142	105
	10^{-4}	4	75	60

graft sample was 4.3 cm, which was higher by 0.2 cm from the reference plant, which had a length of 4.1 cm. In the urea-enriched soil containing the composite and true graft, the length of the shoot of the plant was 4.9 cm, which was the same as that of the reference plant (4.9 cm). In case of the simple and urea-enriched soil containing PE, the lengths of the shoots were 4.2 cm (higher than the reference plant) and 4.8 cm (smaller than the reference plant), respectively.

TABLE III
Growth of Microorganisms as a Function of the Number of Days in the Nutrient Agar Medium

Number of days	Dilution	Total number of colonies		
		PE	PE-*g*-soy protein composite	PE-*g*-soy protein true graft
10	10^{-2}	2	72	50
	10^{-4}	1	40	28
20	10^{-2}	23	42	95
	10^{-4}	16	28	32
30	10^{-2}	32	90	45
	10^{-4}	22	32	47
45	10^{-2}	25	98	58
	10^{-4}	18	40	22
60	10^{-2}	27	145	98
	10^{-4}	22	65	45
75	10^{-2}	29	165	52
	10^{-4}	7	69	51
90	10^{-2}	22	88	102
	10^{-4}	15	52	55
120	10^{-2}	8	114	125
	10^{-4}	4	62	72

TABLE IV
Hydrolysis of the PE-*g*-Soy Protein Composite as a Function of the Number of Days of Soil Burial

Number of days	Weight of sample (g)	Weight loss (%)	Weight of PE after hydrolysis (g)
10	0.410	18	0.140
20	0.380	24	0.130
30	0.320	36	0.120
45	0.270	46	0.100
60	0.210	58	0.090
75	0.180	64	0.070
90	0.160	68	0.060
120	0.140	72	0.050

Table VII shows growth measurements of the wheat plant, from which it is clear that for the plants grown in soil containing the composite and true graft, the average lengths of the roots were 6.3 cm (higher by 0.2 cm than the reference plant) and 6.1 cm (the same length as that of reference plant), respectively, and for PE, it was 6.0 cm (smaller by 0.1 cm than the reference plant). For plants grown in the urea-enriched soil containing the composite, true graft, and PE, the average lengths of the roots of the plants were same, that is, 6.7 cm, which was higher by 0.1 cm than that of the reference plant, which had an average root length of 6.6 cm.

The results for shoot measurements show that the average length of the shoot of plants grown in soil containing PE (6.7 cm) was shorter by 0.1 cm, and the average length of the shoot of the plants grown in soil containing the composite was 7.0 cm, which was longer by 0.2 cm than the reference plant, which had an average shoot length of 6.8 cm. For plants grown in soil containing the true graft, the average length of the shoot was same as that of the reference plant. However, plants grown in urea-enriched soil containing the composite showed an increase of 0.2 cm in average length of shoot in comparison to the reference plant, which had a length of 7.2 cm. Plants grown in soil containing PE and true graft

TABLE V
Hydrolysis of the PE-*g*-Soy Protein True Graft as a Function of the Number of Days of Soil Burial

Number of days	Weight of the sample (g)	Weight loss (%)	Weight of PE after hydrolysis (g)
10	0.430	14	0.230
20	0.400	20	0.180
30	0.360	28	0.170
45	0.320	36	0.150
60	0.290	42	0.130
75	0.220	56	0.100
90	0.170	66	0.060
120	0.150	70	0.050

TABLE VI
Growth of Measurements of Soybean Plants Grown in Soil Containing PE and PE Grafted Samples

	Simple soil		Urea-enriched soil	
	Length of root (cm)	Length of shoot (cm)	Length of root (cm)	Length of shoot (cm)
Reference plant without a sample	5.2	4.1	5.6	4.9
PE-*g*-soy protein composite	5.4	4.3	5.6	4.9
PE-*g*-soy protein true graft	5.3	4.3	5.5	4.9
PE	5.1	4.2	5.5	4.8

TABLE VII
Growth of Measurements of Wheat Plants Grown in Soil Containing PE and PE Grafted Samples

	Simple soil		Urea-enriched soil	
	Average l ength of root (cm)	Average length of shoot (cm)	Average length of root (cm)	Average length of shoot (cm)
Reference plant without a sample	6.1	6.8	6.6	7.2
PE-*g*-soy protein composite	6.3	7.0	6.7	7.4
PE-*g*-soy protein true graft	6.1	6.8	6.7	7.2
PE	6.0	6.7	6.7	7.2

did not show any change in the average length of shoot from the reference plant.

Thus, we observed that the growth of the plants was much better in urea-enriched soil. In case of soil containing the grafted samples, the length of the roots and shoots showed slight increases, whereas in urea-enriched soil, not much variation in length of the roots and shoots was observed, which indicated that the presence of the PE or grafted PE samples in soil undergoing the degradation process did not harm the growth of the plants.

CONCLUSIONS

The grafting of a biodegradable polymer, soy protein, induced biodegradability into otherwise stubborn PE. Differences in the topological morphology and thermal behavior between the original and the degraded samples further substantiated the degradation behavior induced into the grafted PE. The synthesis of soy protein-grafted PE will be beneficial in making the best use of PE without the risk of environmental pollution.

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