Biodegradation of Gelatin Graft Copolymers. IV

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Synopsis

Gelatin graft copolymers of different compositions were tested for microbial susceptibility in a synthetic medium with pure cultures of *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Serratia marcescens*. The percent weight losses were recorded over 6 weeks of incubation period in nitrogen-free and nitrogen-rich media. The relationship between [log(rate)] during the first week of the test period and composition of the grafted samples showed a linear behavior. There was no difference in the aggressivity of these bacterial strains. Nitrogen analysis data and pH measurements of the media seem to reinforce our earlier observations. Soil burial tests also indicate degradation of polymer samples under natural weathering conditions. This article also summarizes the salient features of our series of investigations.

INTRODUCTION

Chemical modification of natural polymers by grafting serves the twofold purpose of utilizing renewable, naturally derived products such as polysaccharides and proteins as replacements for synthetic, petroleum-based polymers and as biodegradable compositions which can be tailored for slower or faster rates of biodegradation.[1] We have reported earlier the biodegradative behavior of various gelatin graft copolymers employing both mixed bacterial inoculum and pure cultures.[2-7] This article discusses further results of microbiological testing and soil burial tests and concludes with a summary of results of biodegradation tests as evidenced by our investigations.

EXPERIMENTAL

The experimental methods employed in the present investigation are reported in a preceding article[7] and in our earlier publications.[2-6] All the microbiological testing procedures are in conformity with ASTM guidelines.[8]

Soil burial tests were carried out according to the procedure used by Potts et al.[9] All the tests were done indoors in red clay flower pots. The soil used was a mixture of equal parts of garden top soil, peat moss, and builders sand. The mixing was accomplished by proper blending. The pots containing the soil and the samples were kept in a cabinet. The soil was kept constantly

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wet with water. The laboratory temperature was around 28–30°C during the daytime and 20–22°C in the nighttime. The samples were compression moulded samples of 1 mm thickness. Numbers attached to nylon filament enabled each sample to have its own identify, and thus the weight loss in each case was determined. Excess soil was removed from the sample by gentle washing with water, and the samples were dried overnight in a vacuum oven prior to weighing.

Three samples of gelatin–ethyl acrylate copolymers with grafting efficiencies of 33.3% (8.5% N), 60.9% (7.1% N), and 84.1% (5.6% N) designated as GEA, GEB, and GEC, respectively, were selected for soil burial tests. Gelatin–methyl acrylate copolymer samples with grafting efficiencies of 23.05% (8.51% N), 70.18% (6.8% N), and 79.80% (5.52% N) designated as GMA, GMB, and GMC, respectively, were selected for microbiological and soil burial tests. In the case of gelatin–acrylonitrile system, only one sample containing 50% of protein by weight was tested. Each sample was prepared in large quantities by the procedures described earlier[2-7] in one batch and used in all the experiments to maintain the same compositions.

RESULTS AND DISCUSSION

As in our earlier reports, we have followed the percent weight loss of the incubated samples as the index for biodegradation. The percent weight loss–time curves for gelatin–methyl acrylate copolymers inoculated with the pure cultures of *Pseudomonas aeruginosa, Bacillus subtilis,* and *Serratia marcescens* are shown in Figures 1–3. These results reinforce our earlier observations that the larger the number of grafting sites and the efficiency of grafting, the slower will be the rate of biodegradation. The extent of biodegradation is more in the case of samples inoculated with pure cultures compared to the effect of mixed inoculum under the same conditions.[7]

Figures 4–6 illustrate the rates of biodegradation [log/(rate)] of these gelatin-*g*-PMA samples during the first week of the test period plotted against the percentage of PMA is almost linear during the first week. The extrapolation of these results seem to indicate the possibility of rate of degradation becoming negligable, when the percentage of synthetic polymer is approximately 95%. This is in full conformity with the reported stability of carbon chain polymers to bacterial attack.[10]

Figure 7 shows that gelatin-*g*-PNA exhibits greater microbial resistance compared to a gelatin-*g*-PMA or gelatin-*g*-PEA of approximately similar compositions. This could be due to the presence of cyano groups which may retard or inhibit enzymatic action.

The pH of the culture media also remained stationary at 7.6, 7.3, and 7.8 in the case of *Pseudomonas sp., Bacillus sp.*, and *Serratia sp.*, respectively (Fig. 8). A greater increase in pH of the culture medium inoculated with the mixed bacterial inoculum might be responsible for slower rates of degradation we observed in those cases.

Nitrogen analysis data of GEA, GEB, and GEC, before and after testing with the bacterial strains, is presented in Tables I–III. The loss in the nitrogen content of the samples indicates the utilization of the gelatin portion of the molecule by these bacterial strains.

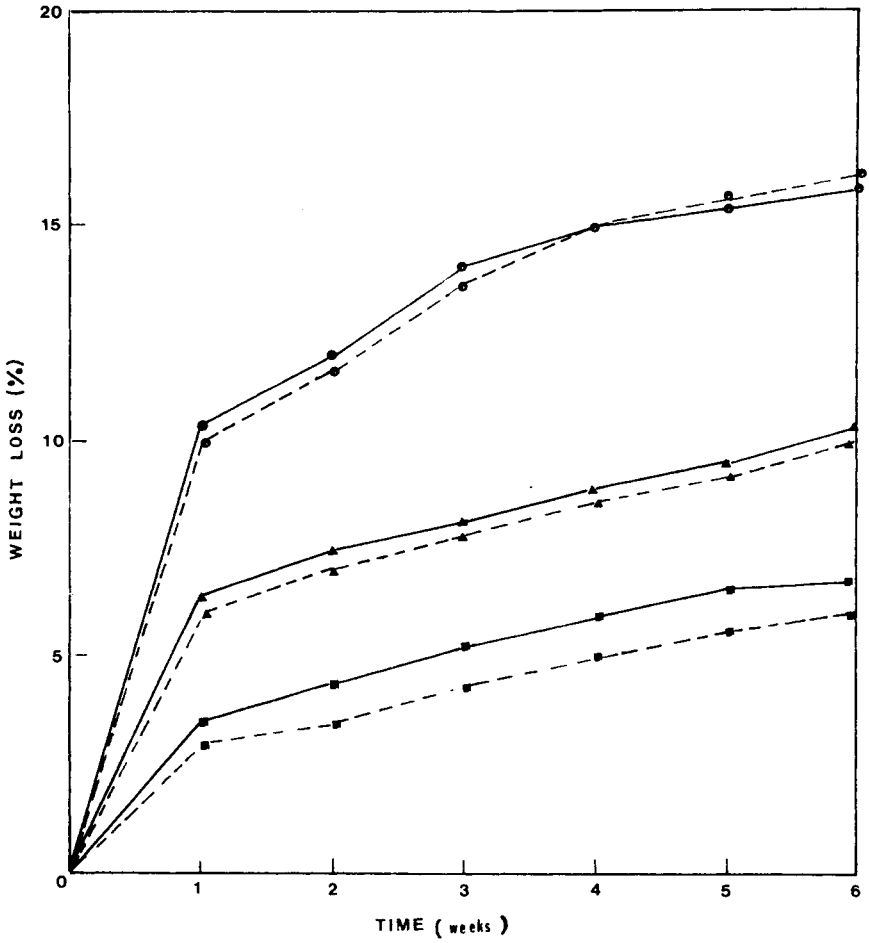

Fig. 1. Weight loss-time curves of gelatin-*g*-poly (methyl acrylate) samples incubated wtih *Pseudomonas Aeruginosa* in nitrogen-rich (- - - -) and nitrogen-free (——) media: (●) GMA; (▲) GMB; (■) GMC.

Soil burial tests to evaluate the resistance of plastics are described in a number of standards and recommendations. Walchli and Zinkernagel[11] compared these methods and results of soil burial tests to assess the resistance of plastics to microbes. Different standard methods were developed which were originally used for testing textiles and which were later on adopted for testing plastics. These methods differed considerably, e.g., type of soil (composition moisture content, pH, C/N ratio), natural flora of microorganisms or subsequent inoculation of previously sterilized soil; number, shape, pretreatment, and installation of the test specimens and sterile controls.

We have followed the procedure reported by Potts et al. in our investigations. Strips of gelatin-*g*-PEA and gelatin-*g*-PMA copolymers were buried in soil for months. The results are shown in Tables IV and V. GEA and GMA showed weight losses in the range of 9–11% after 90 days of testing. GMB and GEB showed only 5–7% weight loss after the same test period.

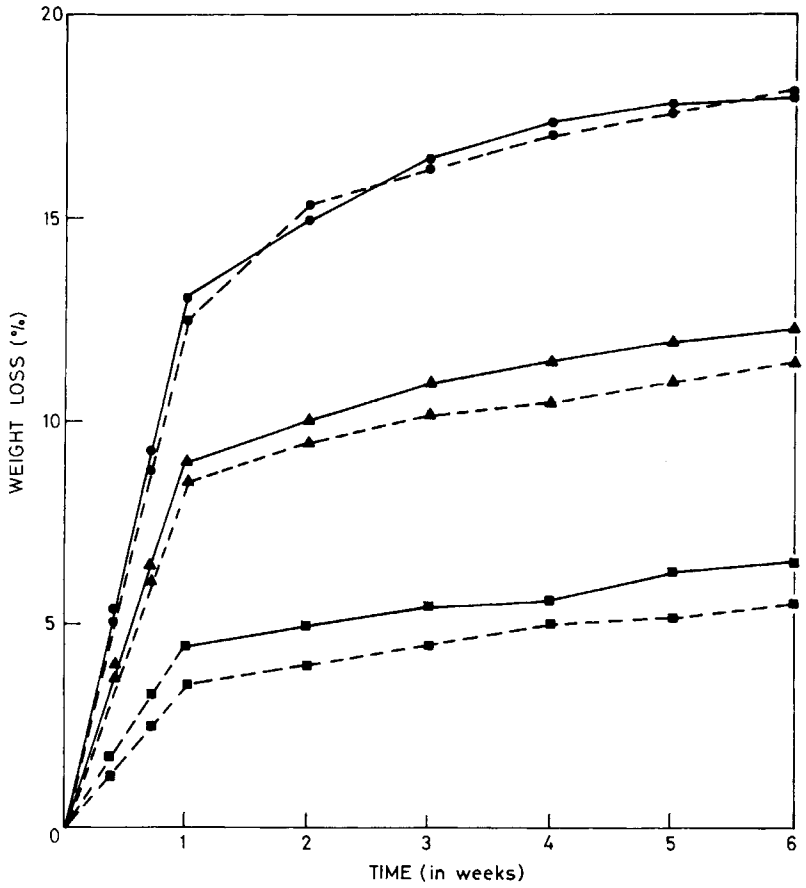

Fig. 2. Weight loss–time curves of gelatin-*g*-poly(methyl acrylate) samples incubated with *Serratia marcescens* in nitrogen-rich (----) and nitrogen-free (—) media: (●) GMA; (▲) GMB; (■) GMC.

GMC and GEC, which contained the least amount of protein, were totally resistant to enzymatic attack. The slower degradation observed in the soil, compared to laboratory testing, may be attributed to the availability of other carbon sources in the soil. In soil, many microbes live in close proximity, and they interact in a unique way which is in marked contrast to the behavior of pure cultures studies in the laboratory.

CONCLUSIONS

To conclude, we wish to report the summary of the salient features of biodegradative behavior of gelatin graft copolymers. The characteristic feature of grafting is the small number of grafted chains per gelatin molecule and their high molecular weights. Gelatin–ethyl acrylate copolymers do exhibit the values of tensile strength and percent elongation comparable to LDPE film which is commonly used as agricultural mulch. Though these samples exhibit high initial strengths and elongations, their strength was found to decrease rapidly upon exposure to moist environment. The rapid decrease in strength should not adversely affect a mulch film, since high

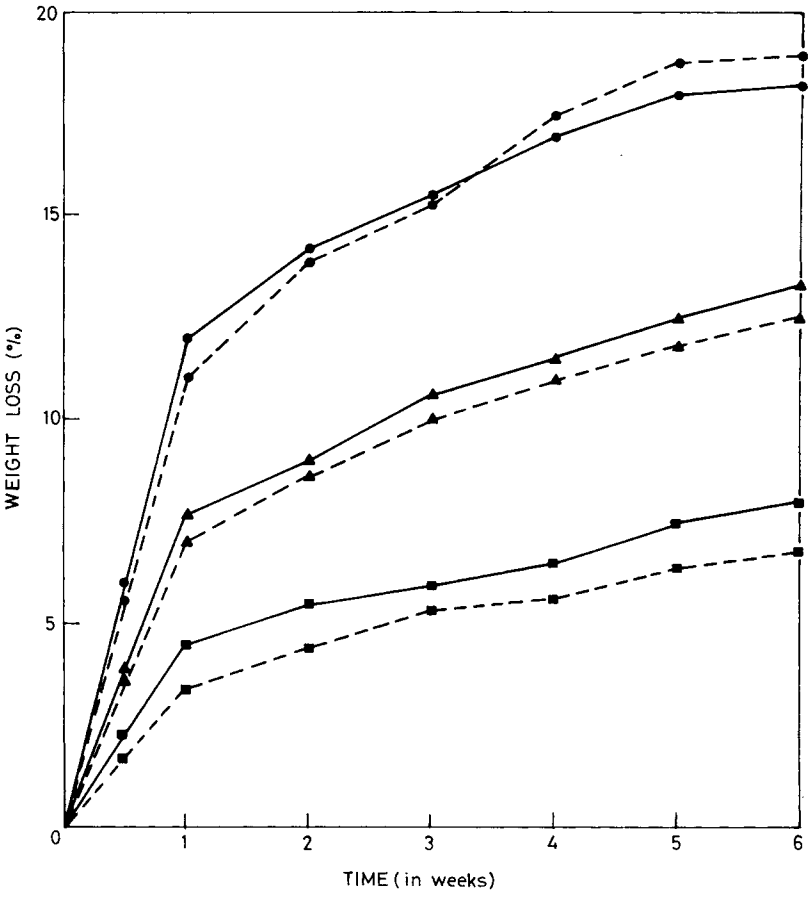

Fig. 3. Weight loss–time curves of gelatin-*g*-poly(methyl acrylate) samples incubated with *Bacillus subtilis* in nitrogen-rich (—) and nitrogen-free (—) media: (●) GMA; (▲) GMB; (■) GMC.

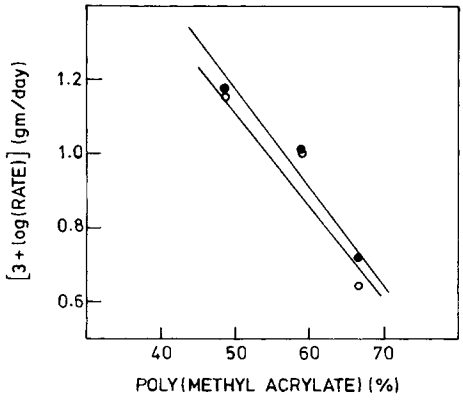

Fig. 4. Rate of biodegradation–Percent of PMA of gelatin-*g*-poly(methyl acrylate) samples (in the first week) incubated with mixed bacterial inoculum in nitrogen-free (●) and nitrogen-rich (○) media.

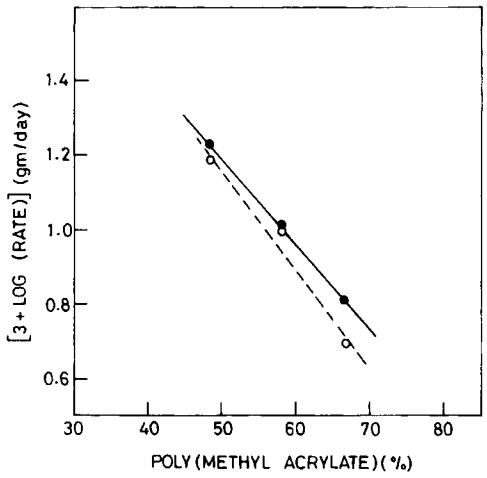

Fig. 5. Rate of biodegradation–percentage of poly(methyl acrylate) of gelatin-*g*-poly(methyl acrylate) samples in the first week incubated with *Bacillus subtilis* in nitrogen-free (●) and nitrogen-rich (○) media.

strength is important only during the application. These films may have applications as biodegradable mulches.

We have selected the bacterial strains which are commonly found in the soil as the test organisms, and loss in weight of the samples was followed periodically and percent weight loss recorded. The results indicate that the rate of biodegradation decreased with the increase in the efficiency of grafting. In all the cases, there was an initial rapid weight loss during the first week of the incubation period, followed by slow but steady disappearance of the substrate. In this respect there was no notable difference in the activity of mixed bacterial inoculum and pure cultures. Another noticeable

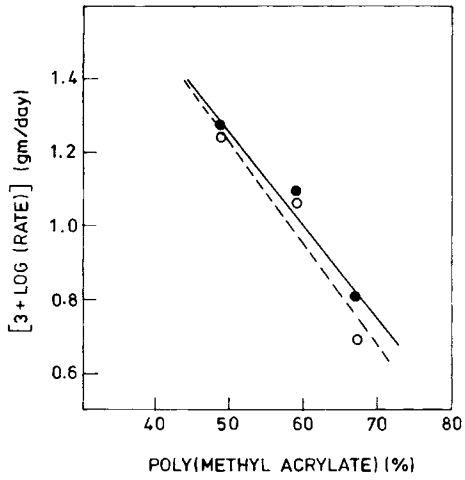

Fig. 6. Rate of biodegradation–percentage of poly(methyl acrylate) of gelatin-*g*-poly(methyl acrylate) samples in the first week, incubated with *Serratia marcescens* in nitrogen-free (●) and nitrogen-rich (○) media.

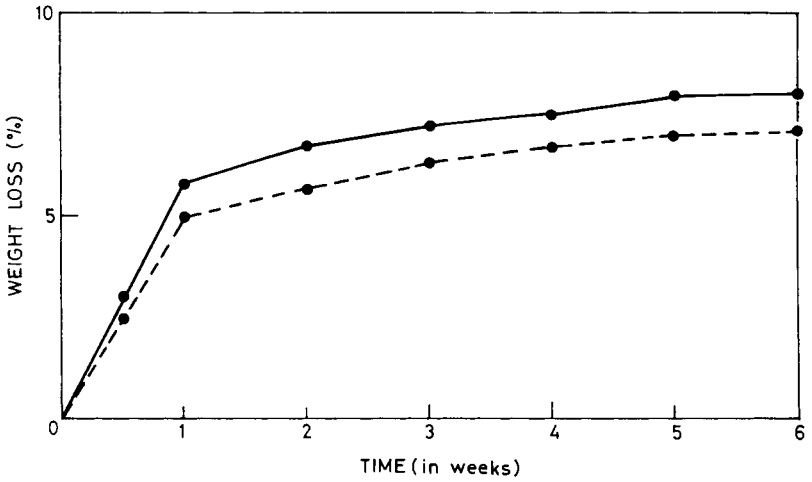

Fig. 7. Weight loss–time curves of gelatin-*g*-PAN (50% PAN) incubated with *Serratia marcescens* in nitrogen-rich (—) and nitrogen-free (—) media.

feature discernible from these results is that there was no essential difference in the aggressivity of the different test organisms. Mixed bacterial inoculum was less effective compared to the pure cultures. It was also noted that when the same samples were used as the only source of both carbon and nitrogen there was marginal but definate increase in the utilization of the polymer.

The relationship between [log(rate)] of gelatin graft copolymer and the percentage of the grafted branches was linear during the first week of the test period. It is expected that such rate–composition plots can be used to predict the rate of degradation of a graft copolymer sample of known composition under given conditions. Comparison of the molecular weights of the grafted branches, before and after testing, did not show any change indicating the total resistance of the grafted branches. Growth tests carried out over a long period might offer the organisms the possibility of adopting

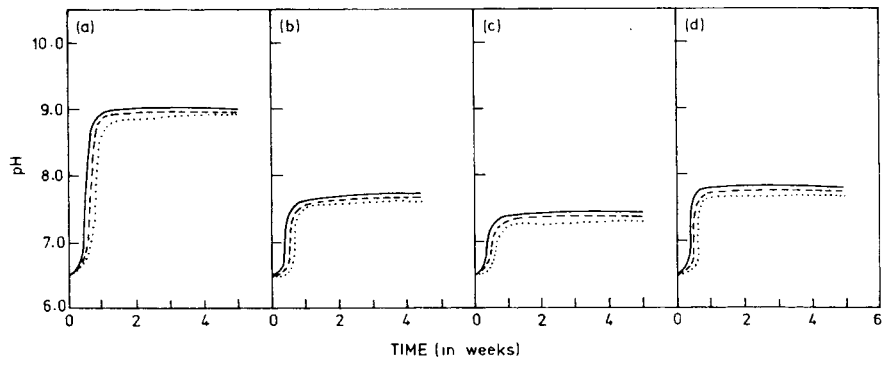

Fig. 8. pH of the culture media vs. time plots of gelatin-*g*-poly(methyl acrylate) samples incubated with: (a) the mixed inoculum of *Pseudomonas aeruginosa, Bacillus subtilis,* and *Serratia marcescens;* (b) *Pseudomonas aeruginosa;* (c) *Bacillus subtilis;* and (d) *Serratia marcescens.* (—) GMA; (—) GMB, (···) GMC.

TABLE I
Total Nitrogen Content of Gelatin–Methyl Acrylate Copolymer Samples Incubated with *Pseudomonas Aeruginosa*, before and after Testing

Sample	Initial nitrogen (%)	After 15 days (nitrogen %)		After 45 days (nitrogen %)	
		N(+) medium	N(−) medium	N(+) medium	N(−) medium
GMA	8.51	6.02	5.38	4.0	4.15
GMB	6.80	5.12	5.22	3.31	3.59
GMC	5.52	3.90	3.81	3.42	3.18

TABLE II
Total Nitrogen Content of Gelatin–Methyl Acrylate Copolymer Samples Incubated with *Bacillus Subtilis*, before and after Testing

Sample	nitrogen (%)	After 15 days (nitrogen %)		After 45 days (nitrogen %)	
		N(+) medium	N(−) medium	N(+) medium	N(−) medium
GMA	8.51	6.1	5.92	4.0	3.6
GMB	6.80	5.3	4.3	4.1	3.43
GMC	5.52	3.75	3.33	3.05	2.75

TABLE III
Total Nitrogen Content of Gelatin–Methyl Acrylate Copolymer Samples Incubated with *Serratia Marcescens*, before and after Testing

Sample	Initial nitrogen (%)	After 15 days (nitrogen %)		After 45 days (nitrogen %)	
		N(+) medium	N(−) medium	N(+) medium	N(−) medium
GMA	8.51	5.8	5.2	4.2	4.31
GMB	6.80	4.9	4.7	3.8	3.8
GMC	5.52	3.6	3.6	3.0	3.2

TABLE IV
Results of Soil Burial Tests of Gelatin-*g*-Poly(methyl Acrylate) Samples

Sample	Efficiency of grafting (%)	Weight loss (%)	
		After 1 month	After 3 months
GMA	23.05	4.2	10.1
GMB	70.18	1.4	5.8
GMC	79.80	—	—

TABLE V
Results of Soil Burial Tests of Gelatin-*g*-Poly(ethyl Acrylate) Samples

Sample	Efficiency of grafting (%)	Weight loss (%)	
		After 1 month	After 3 months
GMA	33.3	3.5	9.5
GMB	60.9	1.5	6.2
GMC	84.1	—	—

themselves and may provide more affirmative answers to biodegradbility.

But gelatin-*g*-PAN exhibited considerably greater resistance to enzymatic attack compared to other acrylate copolymer systems. It seems to suggest the possibility of the effect of nature of grafted branches on the degradability of a biodegradable backbone.

The growth–time curves are of exponential nature. There was a period of lag before the growth began exponentially. The lag period was less in nitrogen-free medium as compared to nitrogen-rich medium. Here again, there was no essential difference in the aggressivity of different strains. The pH of the culture medium turned alkaline in all the cases and remained stationary. In the case of tubes incubated with mixed bacterial inoculum pH increase was higher and it might be responsible for lower rates of biodegradation. Nitrogen analysis data and scanning electron micrographs, before and after testing, also supplement above observations. Soil burial tests also indicate significant losses although to a smaller degree.

The observed phenomenan of rapid weight loss during the first week and the concurrent cooperative changes such as exponential increase in the bacterial population and pH of the culture medium "superimpose" well and indicate rapid and early utilization of the polymer samples. In all these cases probably only the backbone undergoes degradation leaving behind a porous and mechanically weakened residue. However, the increased surface area of the porous material is expected to enhance its biodegradability.

We are investigating the possibility of grafting conventional monomers with the incorporation of photosensitive groups, e.g., copolymerization with alkyl vinyl ketones, to render these polymers both photo- and biodegradable.

In any case, a time-limited investigation such as the present attempt cannot clarify all the questions related to the complexity and variability of the interrelationships between the microorganisms and the synthetic materials. Efforts are being made to utilize the laboratory and field testing data to predict the effective life of a plastic in a given usage environment.

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