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Biodegradability of gelatin–PF resin blends by soil burial method

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Cured gelatin–phenol formaldehyde (PF) resin blends have been tested for their biodegradability under soil burial test conditions. Over a period of two weeks and at an ambient temperature of 27°C, the blends were found to be significantly resistant to biodegradation in the test soil up to a composition of 7:3 gelatin to resin ratio. At higher gelatin contents, the blends suffered extensive degradation. Scanning electron micrographs of the blend films after the tests showed extensive bacterial growth. Inorganic inclusions, presumably calcium salts, that are inherently present in gelatin are believed to support bacterial growth and biodegradation of the blends. © 1998 Elsevier Science Limited. All rights reserved

1 INTRODUCTION

Polymers of natural origin are considered to be easily biodegradable and gelatin, a natural protein is no exception. Gelatin is susceptible to hydrolysis by a variety of microbial proteases. Suitable modification of gelatin is therefore highly relevant to have purposeful and effective use of this natural polymer. Chemical modification of natural polymers serves the twofold purpose of utilizing renewable, naturally derived products as replacements for synthetic petroleum-based polymers and as biodegradable compositions which can be tailored for slower or faster rates of degradation depending upon usage conditions and mode of disposal of the used material. There are reports[1–3] that gelatin film or foam crosslinked with formaldehyde (to decrease its solubility) are used in the biomedical field as a bioabsorbable agent in the temporary substitution of tissues. A very conventional approach to modify the biodegradability of natural polymers is by grafting.[4,5] Grafting leaves the main chain of the polymer intact and imparts new properties associated with the grafted side chains. Chemical modification of gelatin by phenol formaldehyde resin (PF) can be a very pragmatic approach to impart an acceptable resistance to microbial attack under indoor hydrothermal usage conditions. PF resin is a potential crosslinker of gelatin and other protein polymers, and the free phenol functionalities in the resin are long known for their bactericidal/bacterostatic activities.

In this article, we report a systematic biodegradation study on cured gelatin–PF resin blends in normal environmental conditions by soil burial methods. The gelatin–PF resin blends have been shown to be totally biodegradable[6] in respect of only the gelatin component by specific strains of a psychrotrophic bacterium, *Aeromonas hydrophila*, PC5 and LA8. It will be interesting to see as to how and to what extent the gelatin–PF resin blends are biodegraded by nonspecific strains of microorganisms under ambient soil burial conditions.

2 MATERIALS AND METHODS

2.1 Soil

The pot experiments were conducted on the lateritic soil[7] of the Indian Institute of Technology, Kharagpur which was sand loam in texture having low organic carbon (0.34%), phosphorus (0.06%) and nitrogen (0.42%), and a pH 5.9. It is classified as Kharagpur sandy loam, typic acrorthox. The soil constituents are clay (15.21%), silt (23.37%) and sand (62.42%).

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2.2 Substrate

Cured gelatin–resin blends of various weight ratios were used as substrates. Preparation of the blends and their curing have been reported in our earlier communication.[6]

2.3 Preparation of soil

The soil collected from the IIT, Kharagpur campus was first dried in the sun for 2 days. The dry soil was ground to powder after removal of coarse aggregates and stones by hand picking and loose sifting.

2.4 Preparation of test medium

About 100 g of the processed soil were taken in a 250 ml beaker. About 150 ml of water was added slowly with mechanical stirring and the mixture allowed to stand for a few hours. The water thus fully penetrated into the soil. Separate test media were prepared in the same way for each blend composition to be tested for biodegradation.

2.5 Test method

In typical tests, the cured gelatin–resin blend to be tested for biodegradation was dried at 50°C under vacuum to constant weight. An accurately weighed amount of the dry sample was buried completely in the wet soil of the test medium and left for 2 weeks. Test beakers were maintained at a constant temperature of 27°C with daily addition of water to replenish any loss on evaporation. The test samples were occasionally checked for any visual changes. After two weeks, the test samples were taken out carefully from the wet soil and washed repeatedly with water to remove the soil adhered onto the surface of the films. The films were then dried at 50°C under reduced pressure to constant weight. The percent degradation and gelatin-content in the blends were then calculated using the two equations

% Degradation of blends = $100\,(W_0 - W_d)/W_0$ and % Degradation of gelatin content in the blends = $100\,(W_0 - W_d)/W_g$

where, W_0 = intial weight of the dry film,
W_d = weight of the degraded film,
W_g = gelatin content in the particular specimen of the blend.

2.6 Morphology of biodegraded films

Morphologies of the degraded films after biodegradation under soil burial condition were obtained in a scanning electron microscope, JEOL, JSM-840 at various magnifications.

3 RESULTS AND DISCUSSION

3.1 Physical appearance of degraded blends

Each of the degraded blends was checked for transparency, development of cracks and pits, as also for discolorations or any change in color at various stages of degradation. At higher gelatin proportion (e.g. 6:4 and 7:3), the films developed an intense dark brown color with extensive pitting all over the surface. They all, however, retained their shapes but with rough and undulating surfaces. In some cases, the films disintegrated into pieces which could not be salvaged from the wet soil. Complete degradation of films was observed with the blends of gelatin to resin ratio of 8:2. At lower gelatin contents (e.g. 1:9 to 5:5 gelatin to resin ratio), the degraded films largely retained their shapes, size, surface smoothness, transparency, and color, although some minor pitting could be observed at a blend composition of 5:5. Development of intense color in the blends of higher gelatin content is indicative of strong bacterial attack.

3.2 Gravimetric assay of biodegradation

Table 1 summarizes the gravimetric trend in the soil burial degradation of cured gelatin–resin blends. It is seen from the data in Table 1 and Fig. 1 that the extent of degradation is low but increases

Table 1. Degradation of cured gelatin–resin blends by soil burial method

Blend compositions (gelatin:resin, w/w)	Weight loss[a] $100\,(W_0 - W_d)/W_1$ (W% on blend)	Weight loss[a] $(W_0 - W_d)/W_g$ (W% on gelatin content)
0:10	0.0	0.0
1:9	0.29	2.9
2:8	0.66	3.3
3:7	2.16	7.2
4:6	3.13	7.8
5:5	4.58	9.16
6:4	17.75	29.6
7:3	25.5	36.4
8:2	100	100.0–

[a]Temparature = 27°C; pH = 5.9; time = 2 weeks.

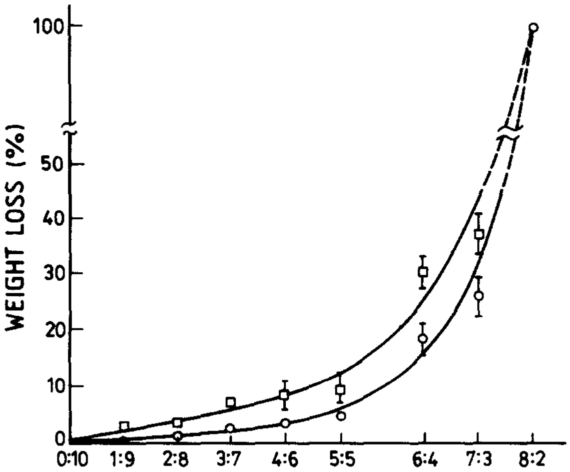

Fig. 1. Effect of composition on the biodegradation of gelatin–PF resin blends in soil burial tests. Temperature, 27°C; pH, 5.9; time, 2 weeks. Percent weight loss: □ on gelatin; ○ on blend.

almost linearly with increasing gelatin content up to a gelatin to resin ratio of 5:5. Beyond this gelatin content, the extent of degradation was substantially higher. The reported values of about 30 and 37% weight loss (of gelatin content) at gelatin to resin ratios of 6:4 and 7:3, respectively, are only the lower estimates since some fine soil particles were observed to be strongly adhered onto the surface of the swollen films. The observed total degradation of the blend at 8:2 composition in the stipulated period of 2 weeks does not necessarily imply that the components of the blends have undergone total biochemical degradation. In fact the neat resin has been shown to suffer little degradation. Also, the biodegradation of the gelatin content of the blends may not be complete within the test period since they could not be salvaged from the wet soil because of their disintegration into small fragments and thus remained mixed up with the soil.

3.3 Morphology of biodegraded films

Scanning electron micrographs of the degraded blends provide some valuable information about the mode of biodegradation and the distribution of gelatin and resin phases in the control blends. Figure 2 is the micrograph of a degraded blend at 5:5 gelatin to resin ratio, at a magnification of 1000×. Some important features of the mocrographs are worth noting.

First, inorganic residues, presumably calcium salts, are seen deeply embedded in the matrix of the blends (top right, bottom right, middle left and bottom middle parts of Fig. 2). These inorganic inclusions are all surrounded by pits, suggestive of an enhanced rate of biodegradation around them. This enhanced rate may be attributed either to any stress concentration around the inclusions or a possible synergism to biodegradation created locally by the inclusions. The inclusions as well might have provided nutrition to the bacteria. Second, surface of the degraded blend at the lower magnification of 1000×, although it appears undulated, is relatively smooth. This points to a quite high degree of apparent homogeneity of the blend components and generally high susceptibility of the blend to biodegradation at this composition (5:5 gelatin to resin ratio). Third, colonies of bacteria are seen scattered specifically around the inorganic inclusions which provide support to a possible synergistic role to biodegradation played by the inorganic residues. An enlarged view (7500×) of one such colony is presented in Fig. 3. At a magnification of 5000× of the degraded 5:5 blend, the apparently smooth degraded surface unveils typical fibrillar morphology (Fig. 4). The colonies are seen

Fig. 2. Surface morphology (1000×) of a biodegraded gelatin–PF resin blends (5:5). Inorganic inclusions are seen at top right, bottom right, middle left and bottom middle. Colonies of bacteria are scattered around the inorganic inclusions.

Fig. 3. An enlarged view (7500×) of the colonies of bacteria on a biodegraded gelatin–PF resin blend (5:5).

Fig. 4. Fibrillar morphology of biodegraded gelatin–PF resin (5:5) surface at 5000×. Fibrils are of assorted length and diameter reminiscent of gelatin distribution pattern in the blend.

to be adhered onto the surface (lower middle part) with fibrils of varying length and diameter aligned along a definite direction. The fibrils are basically undegraded cured PF resin and its morphology is reminiscent of the distribution pattern of gelatin in the blend which has been consumed during biodegradation. This distribution pattern of gelatin in the matrix is exactly the same as the one reported by Slonimskii[8]. Slonimskii *et al.* observed distinct fibrils of diameter of about 0.1 μm formed during gelling of gelatin from aqueous solution at a concentration as low as 10%.

Figures 5–7 illustrate the surface morphology of a blend containing a higher proportion of gelatin (6:4 gelatin to resin ratio) at magnifications of 1000, 5000, and 7500×, respectively. In Fig. 5 extensive micropittings can be seen at the right half of the micrograph. Fibrilar morphology is still prominent in some areas of the degraded blend, as can be seen in Fig. 6. At a higher magnification of 7500× (Fig. 7), a highly irregular surface with extensive pitting is very much apparent. The features clearly suggest that gelatin seggregates when the homogeneous aqueous solutions of gelatin and resole were slowly evaporated to form the films, and is deposited in the form of fibrils. Resole component remains attached to the fibrils and gets crosslinked during curing of the blends.

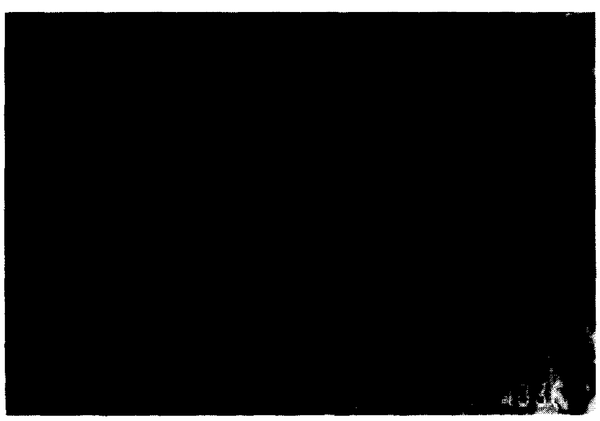

Fig. 5. Surface morphology (1000×) of a biodegraded gelatin–PF resin blend (6:4). Extensive micropitting can be seen in the right half.

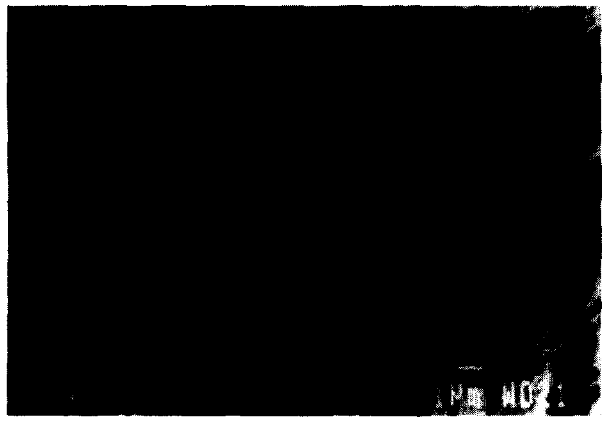

Fig. 6. Prominent fibrillar morphology (5000×) seen on a biodegraded gelatin–PF resin blend (6:4).

Fig. 7. Surface morphology (7500×) of a biodegraded gelatin–PF resin blend (6:4) showing extensive micropitting.

4 GENERAL REMARKS AND CONCLUSION

The results of the soil burial degradation test of the cured gelatin–resin blends clearly show that chemical modification of gelatin by condensation with phenolic resin can greatly enhance its stability against biodegradation, and thereby significantly improve its application potential. By proper choice of composition, it is possible to have blends of high environmental stability under normal indoor usage conditions, and also to impart acceptable and almost total biodegradability when disposed off as waste into soil.

On the mode of biodegradation of the blends, it is not possible to say anything in the absence of specific information on the types and abundance of various active microorganisms in the test soil. However, the results of biodegradation tests possibly indicate that the proven biocidal activity of the phenol functionality is largely retained by cured phenolic resin. This is reflected in its total resistance to biodegradability under present test conditions, as also in its ability to impart high stability to bound gelatin in the blends of higher resin content against biodegradability. It is to be noted that gelatin itself is highly biodegradable even under the mildest hydrothermal environments.

One interesting observation is that the extent of biodegradation bears a parallel correlation with the water swellability of the cured blends under the given test conditions. Degradation is greater when water swellability is greater, i.e. at higher gelatin to resin ratios. At lower gelatin to resin ratios, water swellability is low; so also is the extent of biodegradation.

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