

Research Article

Production and Characterization of Polymer Blends and Assessment of Biodegradation

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Polyhydroxybutyrate (PHB), a microbial polyester well known for its high glass transition and melting temperatures, is found in the intracellular granule within microbes. Herein, we have attempted to synthesize PHB along with xyloglucan (XG) as a copolymer to lower glass transition and melting temperature. Fourier transform infrared spectroscopic (FTIR) and Scanning Electron Microscopic (SEM) analyses were used to investigate the characteristics of blend films. We found that the addition of XG resulted in increased moisture absorption of the blend films. XG-PHB blend of ratio 80:20 showed the highest tensile strength and was subjected to thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) analysis. The results revealed that the XG-PHB blend has a lower melting point (121.3°C) and lesser crystalline degree (17.26%) than PHB. TGA results showed that blend film was more thermally stable than PHB. An effort to understand and analyze microorganisms that can produce and biodegrade copolymers such as PHB-XG is the main effort of this study which unfolds “new hope” in the field of biodegradable alternatives to plastics.

1. Introduction

Polyhydroxyalkanoates (PHAs) are biological macromolecules that are usually seen as aggregates in microbial cells as granules [1]. Owing to the environmental concerns associated with plastics, many countries look forward to alternative solutions such as biodegradable plastics [2, 3]. Thus, it becomes inevitable for us to enhance our manufacturing methods, raw material selection, recycling, and waste con-

version to acceptable forms to avoid introducing any material waste into the environment that nature cannot handle. Bioplastics are employed in numerous investigations, as disposable commodities, and as packing material for their robust and elastic nature [4]. Polyhydroxybutyrate (PHB) is the most common polymer among the different PHAs as it closely resembles polypropylene (tensile strength 40 MPa) [5]. Compared to moisture-sensitive polymers, PHB's properties like higher resistance to hydrolysis and insolubility in

water make them an ideal candidate for polymer research. However, brittleness and relatively low impact resistance of PHB limit its practical applications [4]. Further, the melting temperature and processing temperature of PHB lie between 170 and 190°C which offers a very narrow processing window [6]. To address these issues, various polymer blends are explored throughout the world. Herein, we have incorporated PHB with xyloglucan (XG) which is insoluble in alcohol but dissolves or swells in water [7]. XG has a uniform chemical structure and is amorphous with a molecular weight of more than 100 kDa. XG is more advantageous than starch due to its ductility, low water uptake, and high molecular weight. However, XG has a limitation of high-water absorption capacity suggesting that PEG 300 and PEG B are compatible with PHB [8] with their higher water solubility, which makes them appropriate for a wide range of applications. Microorganisms can break down microbial products and use them as a carbon source and safely convert them to CO₂, biomass, and water through biodegradation. Microbial attack starts where the carbonyl group is available, and therefore, ester linkages are important in biodegradability. Water does not enzymatically hydrolyze polyesters, although enzymatic hydrolysis speeds up degradation [9]. Tamarind powder is a waste product and is cheaper, with higher mechanical properties with higher percentage of elongation. In the present study, PHB, XG, and a new type of plasticizer, PEG 400, were combined to prepare a biodegradable XG-PHB blend in the present study. Infrared spectroscopic analysis, UTM (Universal Testing Machine), scanning electron microscopy (SEM), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) studies were used to characterize PHB and its blends. Further, the prepared blends were subjected to microbial degradation for their suitability for industrial applications.

2. Materials and Methods

2.1. PHB Production. *Bacillus safensis* EBT1 was cultured in a production medium containing 2% glucose, 0.5% yeast extract, 1% peptone, and 1% NaCl in 100 mL distilled water. The pH of the production medium was maintained at 7 and then incubated for 48 h at 45°C. After incubation, microbial cells were collected by centrifugation at 10,000 rpm for 15 min, and the pellet was washed twice with acetone and ethanol before being resuspended in 4% sodium hypochlorite solution [10]. After washing, the obtained polymer granules were stored for future use. The PHB assay was performed according to Law and Slepecky, 1961 technique.

2.2. Preparation of XG and PHB-PEG-XG Blend. Tamarind kernel powder was obtained from the Thangam flour mill in Tuticorin, India. 20 g of tamarind kernel powder was mixed with 200 mL of cold distilled water and then with 800 mL of boiling distilled water. The sample was kept boiling in a water bath for 20 min. It was left overnight to obtain a clear solution. The obtained clear solution was centrifuged at 5000 rpm for 20 min, and absolute alcohol was used to precipitate the resulting solution. The precipitates (xyloglu-

can) were centrifuged and dried at room temperature for 3 days and stored for further use.

1 g of xyloglucan was dissolved in 100 mL of distilled water and heated at 60°C for 60 min. PEG (400 Daltons) was added in various percentages to this solution and magnetic stirred for 1 h. The solution was blended using 1% PHB-chloroform solution in different ratios (%) of 45:5, 40:10, 35:15, 30:10, and 25:25. The blended mixtures (PHB-PEG-XG blend) were then transferred to warmed Petri dishes at room temperature to evaporate the solvents.

2.3. Characterization of PHB-PEG-XG Blends. To identify the functional groups, present in the formation of blends, infrared spectroscopic (FTIR) analysis was performed using PerkinElmer Spectrum RX I (RX-1) US. The film samples were pelletized after being mixed with KBr [11], and spectra were recorded in the transmission mode (4000 to 600 cm⁻¹) with a resolution of 4 cm⁻¹ [12]. The thickness of the patch was measured using a micrometre at different spots on the film with a least count of 0.01 mm, and an average was taken.

The folding endurance was determined manually. Before testing, the films were conditioned for 24 h at 55% relative humidity and 25–30°C. A strip of film was cut and folded evenly in the same spot until it broke. The exact value of folding endurance was calculated by counting the number of times the film could be folded in the same spot without breaking [13].

The film's Young's modulus (E), tensile strength (TS), and percentage of elongation at break (EB%) were measured using Universal Testing Machine INSTRON 3369. The experiment was carried out in rectangular probes (50 mm × 10 mm) with an initial grip separation of 50 mm. The stress-strain curves from films of each composition were used to determine the results [14].

For uptake study, films were in deionized water, and surface water was blotted away with wipes; hydrated samples were picked up and weighed at regular time intervals. The weight difference of the film before and after swelling was then used to calculate the water content.

The percentage of water uptake was calculated using the following:

$$\text{WU}(\%) = \frac{W_w - W_d}{W_d} \times 100, \quad (1)$$

where WU% is water uptake (%) and W_d and W_w are the weights of the sample film before and after being immersed in water, respectively [15, 16].

TGA and DSC analyses were conducted using a Pyris 6 DSC (Perkin Elmer, US) that was calibrated with indium (m.p.156.61°C; $\Delta H = 28.54$ J/g). 10 mg of film samples was packed in an aluminium plate and heated in a nitrogen atmosphere from 20 to 200°C. The samples were cooled to 20°C after one minute of annealing. DSC endothermic peaks were used to measure the melting (T_m) and glass transition temperature T_g .

The films after the consortium test were analyzed gravimetrically through weight loss calculation given in

$$\text{Weight loss (\%)} = \frac{W_i - W_f}{W_i} \times 100, \quad (2)$$

where W_i is the initial weight of the film sample and W_f is the treated weight of the film after being exposed to the consortium, and the rate per day was calculated using

$$\text{Rate/day} = \frac{\text{Weight loss (\%)}}{\text{Number of days}}. \quad (3)$$

To study the surface morphological characteristics, polymer blends were subjected to scanning electron microscopic analysis using Gemini SEM 300 (Carl Zeiss, Germany).

2.4. Biodegradation of PHB and Blends Using Consortium and Pure Cultures. Minimal media were prepared and inoculated with 2% sewage sludge from a dairy and food processing industry sewage treatment plant (STP) in Nesapakkam, Chennai, as well as pure culture of *Rhodocyclus purpureus* EBT1 and consortium (sludge) from dairy industry sludge. Films of PHB and its blends were previously weighed and sterilized using sodium hypochlorite solution for 10 s. Further, the films were thoroughly washed in distilled water and incubated at 30°C for 7 days. Film retention was calculated using collected samples.

2.5. Biodegradation of PHB at Various Time Intervals Using Consortium and Pure Cultures. 10 flasks with minimal media were filled with known quantities of sterilized PHB-PEG-XG blends. The pH of the medium was adjusted to 7.0. Five flasks were inoculated with 2% sewage sludge from the sewage treatment plant (STP) in Nesapakkam, Chennai, while the other five flasks were inoculated with pure culture of *Rhodocyclus purpureus* EBT5. They were incubated at 30°C. Retention was determined after samples were obtained every 5 days (5-35 days).

3. Results and Discussion

3.1. PHB-XG Film Formation. Figure 1 shows the image of the blended film. The films with 90% and 80% of XG showed smoother surfaces than films containing 70%, 60%, and 50% of XG. We believe that the evaporation of solvent could play a major role in significant phase separation in films containing XG and PHB in a 50:50 ratio after blending. The use of PEG as a compatibiliser facilitated the homogenous formation of blend films. Films containing 1 g of PEG had a smooth surface and good flexibility compared to films with 0.5 g PEG.

3.2. Structural Property of PHB, XG, and PHB-XG Blend. FTIR analysis was used to study the possible functional groups present in the formation of blend films as shown in Figures 2(a)–2(c). The peak (3408.99 cm^{-1}) indicates that PHB has only fewer hydroxyl groups. In Figure 2(c), the peak near 3800 cm^{-1} indicates the strong -OH stretching bond originating by terminal OH groups from XG [17].



FIGURE 1: Image of blended film.

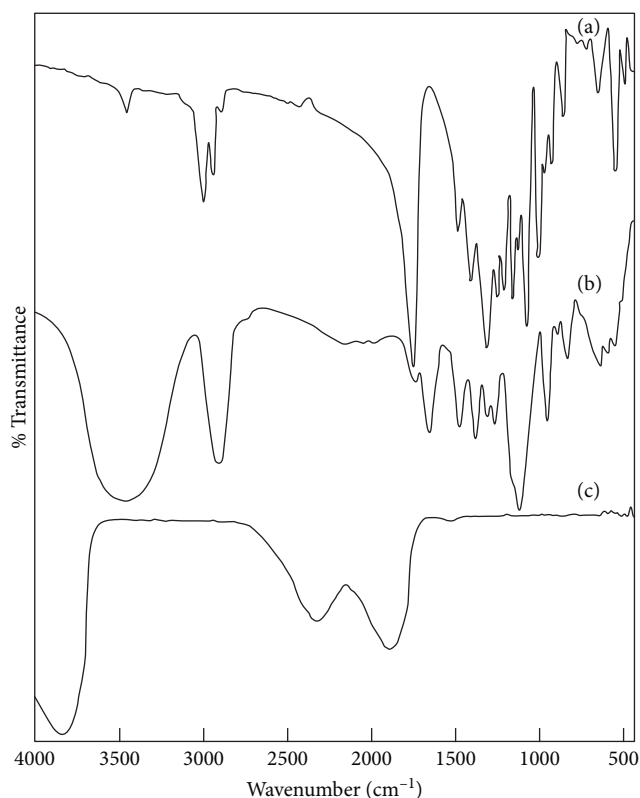


FIGURE 2: Infrared spectrum obtained for (a) PHB, (b) XG-PHB blend (80:20), and (c) XG..

From Figure 2(b), it can be clearly seen that there was a shift of the broad absorption band from the 3800 cm^{-1} region to 3500 cm^{-1} , and it clearly confirms strong hydrogen bonding between PHB and XG. Likewise, 2908.15 cm^{-1} corresponds to C-H stretching showing a characteristic broad absorption peak of PHB. 1722.68 cm^{-1} and 1648.53 indicate the weaker C=O stretching bond of the conjugated carbonyl group of PHB and C=C stretching, respectively, while the peak at 1102 cm^{-1} accounts for C-O stretching (strong bond) (Figures 2(a) and 2(b)) [18].

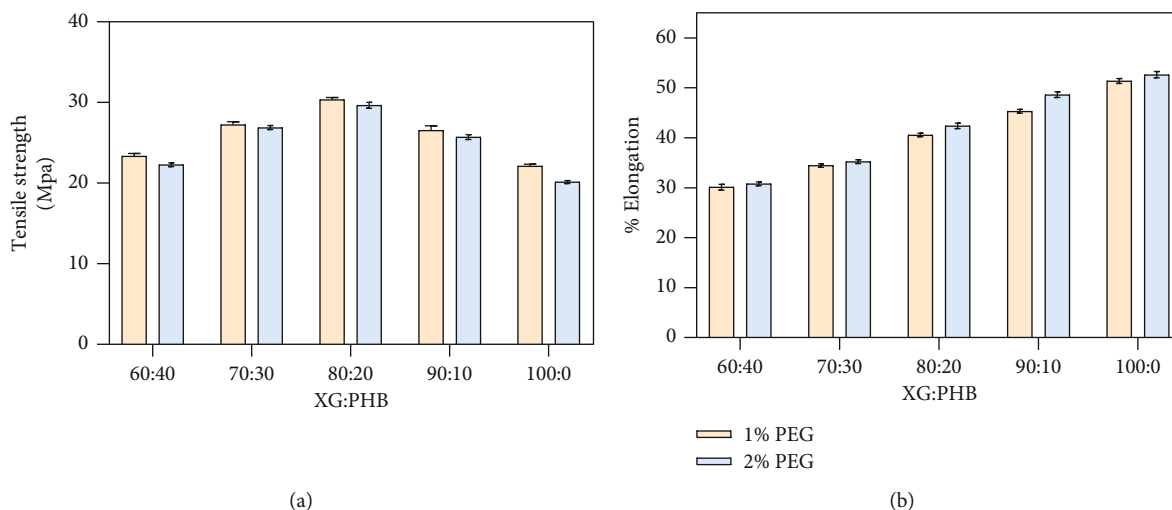


FIGURE 3: (a) Tensile strength and (b) % elongation of XG-PHB blend films of different compositions.

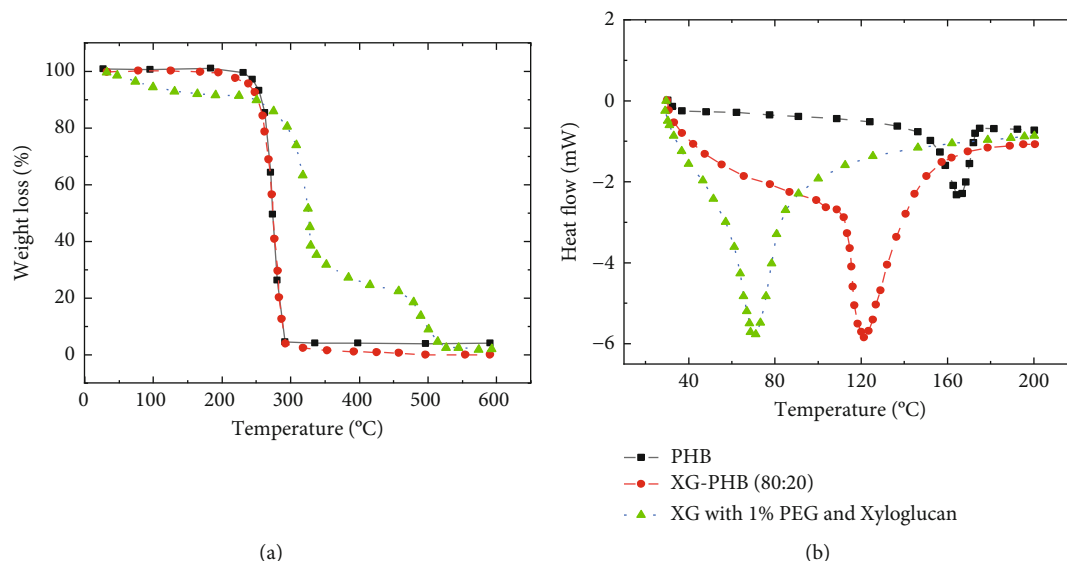


FIGURE 4: (a) TGA and (b) DSC thermographs of PHB, XG-PHB (80:20), and XG with 1% PEG and xyloglucan.

3.3. Mechanical Properties of PHB, XG, and PHB-XG Blend.

The films XG and PHB with 1% PEG had a thickness of 0.082 and 0.087, respectively. All other formulations (XG and PHB) containing 2% PEG resulted in a slight increase in the film thickness. The calculated standard deviation values are all minimal, indicating that the proposed films were all of the same thickness. All the films had a folding endurance of more than 300. Even after being folded over 300 times, the films showed no signs of cracking. Regardless of the polymers used, all the films demonstrated good folding durability, assuring good flexibility. The films subjected for packaging applications are subjected to elongation during the packaging operation; therefore, tensile strength and elongation characteristics come into play. Mechanical tests were conducted using a Universal Testing Machine to investigate the mechanical properties of the XG matrix after the

addition of PHB and plasticizers. The tensile strength of XG films can reach above 50 MPa at 0% relative humidity [19]. The films were stored at room temperature and exposed to the relative humidity of the surrounding. Figure 3 represents the tensile strength and percentage of elongation. The high percent of elongation is interesting although for brittle polymers this parameter is highly sensitive to specimen size and surface smoothness. The ductility of the XG-PHB increases (strain-to-failure increases), while the strength decreases.

The tensile strength increased to 80:20 ratio of XG-PHB and then decreased. This may be due to the swelling of XG molecules because of higher moisture absorption in the XG-PHB blend of ratio 90:10 and XG film alone. The significant interaction between them via the creation of hydrogen bonds is the major reason for the increased tensile strength

of the blended films. The low values of tensile strength and % elongation in 60:40 and 70:30 blends were due to the imperfect homogeneity of PHB distribution in the XG polymer matrix. Thus, this study optimized the ratio (80:20) of XG-PHB blend films as tensile strength for packaging applications. The blend films containing 1% PEG showed good mechanical properties than 2% PEG films. Thus, low PEG concentration on the produced film shows more flexibility, i.e., it caused a reduction in the tensile strength and increase in the elongation. These trends were most likely caused by the reduction in interactions between the biopolymer chains, which is consistent with earlier research on blends of PLA and PHB. The films containing 1% PEG had smoother surfaces than 2% PEG films.

Regarding the thermal stability of PHB, its degradation temperature is closer to its melting point which limits its utilization in practice. Therefore, a study on the thermal stability of PHB, XG, and its blend is necessary. Thermogravimetric analysis at a temperature ranging from 30 to 500°C was performed to examine the thermal stability of PHB, XG, and XG-PHB (80:20), and their TGA curves are shown in Figure 4(a). The data analysis for PHB, XG, and XG-PHB is summarized in Table 1. In both polymers, weight loss happens in a one-step process, i.e., the PHB and XG-PHB blend showed similar thermograms. PHB degradation proceeds with a maximum decomposition temperature at 294.8°C. Similar studies were reported [18], indicating ester cleavage of the PHB component. Degradation of PHB starts at 220°C, and 95.3% of weight loss was observed at a temperature of 294.8°C. However, in XG-PHB, film degradation starts at 186°C which corresponds to the loss of moisture content, and it exceeds up to 310°C, with a weight loss of 96.3%. It shows three-step degradation; the first step implies the loss of water content; the second and third stages correspond to weight loss of 64 and 97%, respectively. Thus, from these results, it can be concluded that the XG-PHB blend was more thermally stable than PHB and XG. Previous experiments with blends of PHB also proposed that the blends showed better stability than PHB and reported that PEG when added with PHB improved the polymer's properties [18].

The DSC thermograms were obtained for the extracted PHB, XG, and XG-PHB blend film of composition 80:20. The data of analysis for PHB, XG, and XG-PHB are shown in Table 2, and the thermogram is shown in Figure 4(b). The melting points of PHB, XG, and XG-PHB blend were 165.5, 75, and 121°C, respectively. This shift in the melting point indicates that there is an interaction between the polymers, i.e., PHB and XG. The interaction between the PHB and XG with the addition of PEG would lead to a better plasticizing effect. The change in melting point from 165.5 to 121°C is more advantageous and could attribute to a broad processing window in processing equipment. The results confirmed that the crystalline degree for extracted XG-PHB blend (17.26%) is lower when compared with extracted PHB (48%). The addition of PEG with blended XG-PHB led to a decrease in the crystalline nature of PHB. The lower degree of crystalline would lead to a higher degradation rate of degradable polymers. However, as XG, its crystalline

TABLE 1: TGA data for PHB and blend films.

Polymer	Initial temperature T_i (°C)	Maximum temperature T_{max} (°C)	Weight loss at T_{max} (%)
PHB	220.7	294.8	95.3
XG-PHB (80:20)	186.3	310.6	96.3
XG	55	530	97

TABLE 2: DSC data for PHB and blend films.

Polymer	Melting temperature T_m (°C)	Melting enthalpy ΔH_f (J/g)	Degree of crystallinity X_c (%)
XG	75	16.9	9.09
PHB	165.2	70.3	48
XG-PHB (80:20)	121.3	25.2	17.3

degree is increased from 9.09 to 17.26% [20] as a result of PHB's ability to act as a nucleating agent, allowing XG to recrystallize. DSC analysis also showed that the melting temperature of PHB was greatly affected by the addition of biopolymer XG. Better interaction between the XG matrix and the PHB crystals resulted in lower crystalline degree and lower T_m of blends, which reduced PHB crystallization and resulted in more ordered chains and perfect homogenous blend. This is similar to the reports on PHB and several blends using PLA, thermoplastic starch, PEG, and starch [21, 22]. Their results showed a decrease in the crystalline degree in the blends, but there was no shift in the melting point which indicates that there is no interaction between the blending polymers.

3.4. SEM Profile of PHB-PEG-XG Blend. Film surface morphology was observed by SEM at 50x, and micrographs are shown in Figure 5. The film composition is of 50:50 and 60:40 ratio of XG and PHB. Figures 5(a) and 5(b) show rough surfaces due to the nonhomogenous distribution of PHB particles in the XG matrix. The projection of the particles was very evident in Figure 5(a) which is associated with a high crystalline degree of PHB. On the other hand, smooth surfaces were observed in the films with composition of 70:30, 80:20, and 90:10 (Figures 5(c)–5(e)). This shows that the PHB particles were dispersed homogeneously in the amorphous XG phase. Thus, the result showed that PHB and XG were incompatible at the ratios of 50:50 and 60:40 and are compatible at a higher ratio of the XG matrix. It is evident that XG-PHB blends with an 80:20 ratio had some distinct characteristics, possessing stronger plasticizing interaction results in flawless film without phase separation (Figure 5(d)).

3.5. Biodegradation of PHB Blends Using Pure Culture and Consortium. PHB-PEG-XG showed maximum degradation after 7 days of incubation with sludge and pure cultures,

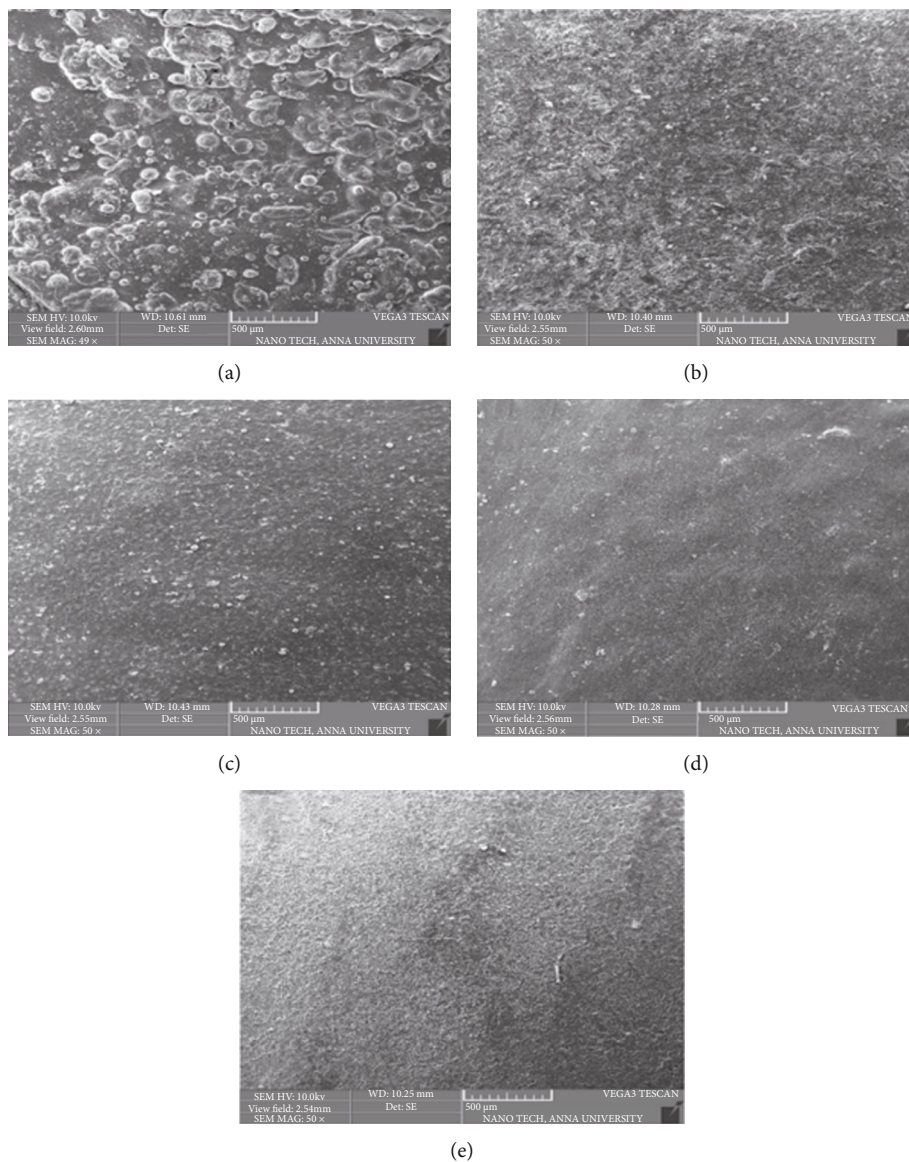


FIGURE 5: SEM images of XG-PHB blend of different ratios (a) 50 : 50, (b) 60 : 40, (c) 70 : 30, (d) 80 : 20, and (e) 90 : 10 with 1% PEG.

TABLE 3: Biodegradation profile of different PHB blends with pure culture and consortium.

Blends	Film retention (%)	
	Pure culture (mg)	Consortium (mg)
PHB (sigma)	100	92
PHBV (sigma)	97	80
PHB (extracted)	94	72
PHB-PEG-XG	90	50

followed by extracted PHB. In the study, pure PHB film exhibited no signs of deterioration. PHB blends were degraded better than pure PHB film as shown in Table 3. The PHB blend exhibited more degradation based on the result obtained, so more research was done using PHB-PEG-XG. Similar results were obtained in the degradation

TABLE 4: Effect of incubation time on biodegradation of PHB-PEG-XG blend with pure culture and consortium.

Incubation time (days)	Film retention (%)	
	Pure culture (mg)	Consortium (mg)
5	90	50
10	88	48
15	82	40
20	79	39
25	77	32
30	72	27
35	70	20

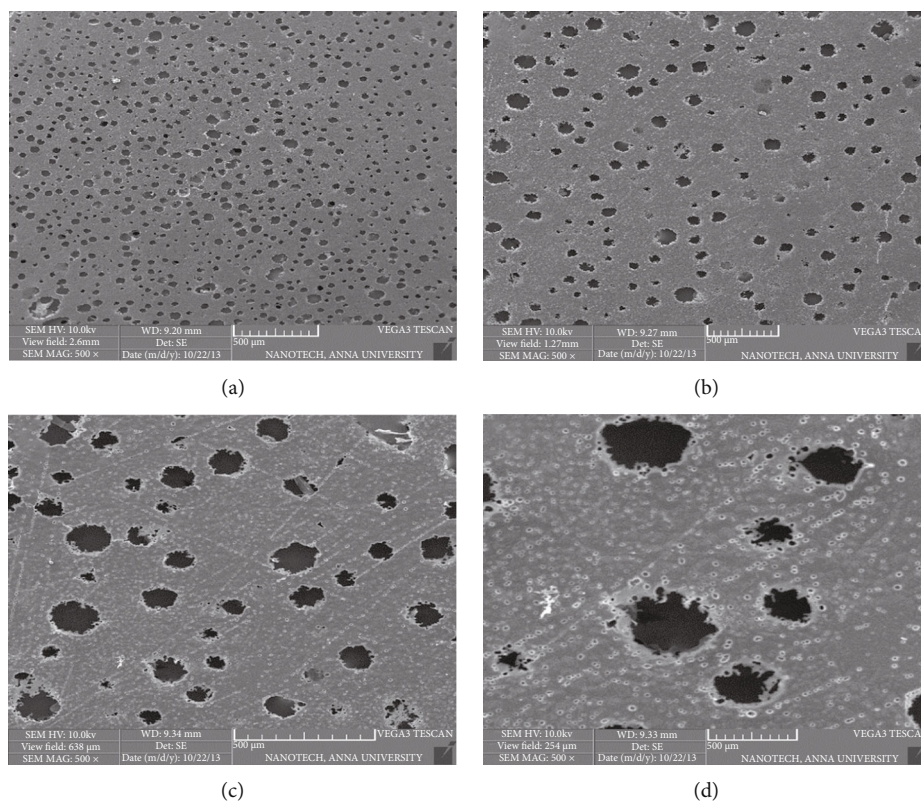


FIGURE 6: SEM images of biodegraded PHB-PEG-XG blend after various incubation times: (a) 10 days, (b) 15 days, (c) 20 days, and (d) 25 days with mixed culture of *Bacillus halodurans* EBT3 and *Rhodocyclus purpureus* EBT5.

of polyester blends containing PHB [19]. Studies also suggest that PHB degrades naturally and completely to carbon dioxide and water in the natural environment by the enzymatic action of microbes.

3.6. Biodegradation of PHB-PEG-XG Blend at Various Time Intervals Using Consortium and Pure Culture. Comparison of the PHB degradation capabilities of consortium and pure culture of *Bacillus halodurans* EBT3 and *Rhodocyclus purpureus* EBT5 was performed. After 35 days of incubation, PHB film incubated with consortium retained 20%, whereas the one incubated with pure culture retained 70% (Table 4). Blended polymers were used to improve aeration and promote plant growth and help. Some of them are used for biomedical applications owing to their sustained release such as an antitumor, antipsychotic, and anti-inflammatory agent [23, 24].

3.7. SEM Profile of Biodegraded PHB-PEG-XG Blends. SEM micrographs of native and biodegraded PHB-PEG-XG blends were taken after treatment with pure and consortium culture. Figure 6 shows that the native blend has a smooth and uniform surface upon treatment, whereas biodegraded PHB-PEG-XG blends had a rough surface and possess numerous pores as a result of biodegradation resulting in rough and porous with several visible fibrous pores. Results indicate that compared to pure culture, biodegradation using consortium seems to be more effective. Similar obser-

vations were previously reported using a soil model community [25].

4. Conclusion

In conclusion, PHB, XG, and a new type of plasticizer PEG300 were successfully mixed to prepare a biodegradable XG-PHB blend. Tensile strength of XG was improved upon the addition of PHB showing that the XG-PHB blend has a lower melting point (121.3°C) and crystalline degree (17.26%) than PHB but higher melting and crystalline degree than that of XG. TGA analysis showed that the XG-PHB blend was more thermally stable than PHB. Due to the enhanced crystal phase and the surface water resistance, the rate of disintegration was found to be higher for XG-PHB blends than that of XG counterparts. Further, PHB blend biodegradation studies indicate that consortium-based degradation was found to be more effective than pure cultures. These results suggest that the combination of XG-PHB blend (80 : 20) with the addition of polyethylene glycol (PEG) could be a better prospect for transparent flexible films for biodegradable food packaging applications.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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