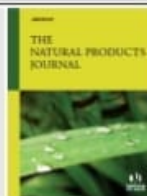


RESEARCH ARTICLE

Characterization of *Nostoc muscorum* NCCU-442 Derived Poly-3-hydroxybutyrate (PHB) and Polyethylene Glycol (PEG) Blends



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Abstract: Background: Poly-3-hydroxybutyrate (PHB) has attracted much consideration as biodegradable biocompatible polymer. This thermoplastic polymer has comparable material properties to polypropylene. Materials with more valuable properties may result from blending, a common practice in polymer science.

Objective: In this paper, blends of PHB (extracted from cyanobacterium *Nostoc muscorum* NCCU-442 with polyethylene glycol (PEG) were investigated for their thermal, tensile, hydrophilic and biodegradation properties.

Methods: Blends were prepared in different proportions of PHB/PEG viz. 100/0, 98/2, 95/5, 90/10, 80/20, and 70/30 (wt %) using solvent casting technique. Morphological properties were investigated by using Scanning Electron Microscopy (SEM). Differential scanning calorimetry and thermogravimetric analysis were done for thermal properties determination whereas the mechanical and hydrophilic properties of the blends were studied by means of an automated material testing system and contact angle analyser respectively. Biodegradability potential of the blended films was tested as percent weight loss by mixed microbial culture within 60 days.

Results: The blends showed good miscibility between PEG and PHB, however increasing concentrations of plasticizer caused morphological alteration as evidenced by SEM micrographs. PEG addition (10 % and above) showed significant alternations in the thermal properties of the blends. Increase in the PEG content increased the elongation at break ratio i.e enhanced the required plasticity of PHB. Rate of microbial facilitated degradation of the blends was greater with increasing PEG concentrations.

Conclusions: Blending with PEG increased the crucial polymeric properties of cyanobacterial PHB.

Keywords: Biodegradable plastics, Polyhydroxybutyrate (PHB), cyanobacteria, Polyethylene Glycol (PEG), plasticizer, material properties.

1. INTRODUCTION

Plastics consisting of organic polymers possess a number of extremely desirable properties viz. high strength-to-weight ratio, thermal properties, electrical insulation, stress resistance, flexibility and durability, resistance to acids, alkalis and solvents. Hence plastics have brought benefits to humanity in many provisions of life. But because of the resistance of these materials to peroxidation and to degradation by water and microorganisms, the detrimental effects of plastics on the environment have become increasingly evident in recent decades [1]. Biodegradable plastics are thus the only way out to combat this problem. Among biodegradable polymers, Polyhydroxyalkanoates (PHAs), a group of biodegradable polymers of biological origin, have gained tremendous impetus in recent years.

These are isotactic, highly crystalline and stiff polymers. Their glass to rubber transition temperature (T_g), melting temperature (T_m), and mechanical properties like Young's modulus and tensile strength are comparable with the isotactic polypropylene. Other properties of PHAs useful for specific applications are resistance to humidity, biocompatibility, piezoelectricity and optical purity [2].

Polyhydroxybutyrate (PHB), the most common representative of polyhydroxyalkanoates (PHAs), is widespread in different taxonomic group of prokaryotes as intracellular storage compounds [3, 4]. PHB production had been reported upto 50% - 80% of the dry cell mass in various wild type and recombinant bacteria under fermentation processes [5]. But commercial production of bacterial PHB is hindered due to high cost for raw material especially carbon source, small production volumes, and high PHB purification costs [6]. This results in bacterial PHB to be 5-10 times more expensive than the petroleum-derived polymers. Under such circumstances, cyanobacterial photosynthetic PHA produc-

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tion offers remarkable advantages which include no need to supplement carbons for growth and their successful cultivation in wastewaters due to their ability to use nitrogen and phosphorus from waste discharges. A large number of cyanobacteria have been shown to produce PHB [7, 8]. Besides this, cyanobacteria are also efficient in heavy metal removal from contaminated water [9, 10]. Therefore, utilization of cyanobacteria for PHB production from heavy metal contaminated wastewater seems highly promising as it has dual advantage of polymer production along with wastewater treatment.

PHB is well-known for its similar mechanical properties as that of polypropylene (PP). Further it offers a better oxygen barrier than PP and good thermal resistance along with resistance to solubility in water. Besides being highly biodegradable, it is biocompatible and has much promising properties for a wide range of applications. However, it bears some drawbacks compared with conventional thermoplastics. PHB have low elongation to break ratio hence it cannot be stretched much before it breaks. Relatively high crystallinity of PHB results in its brittle nature and fairly long degradation time under physiological conditions [11,12]. Poor hydrophilicity restricts its use as tissue engineering material. Thermal processing of PHB is difficult as its melting temperature is near to its thermal degradation temperature.

Blending is a simple and effective approach for obtaining new polymeric materials with improved properties, in which the drawbacks of the parent components can be suppressed [13]. Typically, the physical and mechanical properties of polymer blends can be tuned by choosing the proper starting materials and varying the compositions of the blend and preparation conditions. In order to improve its elongation at break ratio, reduce its crystallinity, and increase the hydrophilicity and to lower its melting temperature for thermal processing, PHB can be blended with plasticizers like polyethylene glycol. Polyethylene glycols (PEG), also known as macrogols, are liquid or solid polymers of the general formula $H(OCH_2CH_2)_n-OH$. It has been found that PEG shows great promise as a plasticizing agent for various polymers like polylactic acid (PLA), polycaprolactone (PCL) including bacterial PHB [14, 15]. Previously, standard bacterial PHB had been blended with PEG to determine the changes in its various properties [16, 17]. To the best of our knowledge, cyanobacterial PHB blends (with any compound) had been studied. Therefore, we investigated the alterations in the thermal, mechanical, hydrophilic and biodegradation properties of cyanobacterial PHB (extracted from *Nostoc muscorum* NCCU-442) after blending with different PEG concentrations.

2. MATERIALS AND METHODS

2.1. Materials

PHB was extracted from *Nostoc muscorum* NCCU-442 under optimised conditions following our previous study in which the cyanobacterial biomass was stirred with magnetic bar in the pretreatment solvent mixture made up of methanol: acetone: water: dimethylformamide [40: 40: 18: 2 (MAD-I)] for 2h to remove the impurities and then followed by continuous chloroform soxhlet extraction for 30 h [7].

Analytical grade chloroform and polyethylene glycol (M_w of 600 g/mol) were purchased from Merck.

2.2. Film Preparation

Solvent casting technique was applied to prepare the PHB and PHB-PEG films [16]. Powdered PHB and PEG samples were dissolved in chloroform in different ratios [100: 0, 90: 10, 80: 20 and 70: 30 (w/v)] to make 15% (w/v) solutions which were stirred (160 rpm) thoroughly at 60 ± 1 °C for 10 min in a sterile sealed vessel. Cooled solutions were poured into sterile, glass petri-dishes, and the solvent was allowed overnight to evaporate at room temperature. Subsequently, in order to remove any residual solvent, the resulting films were maintained at 40 °C under vacuum for 12 hours and finally labelled as below:

PHB : PEG	100:0	98:2	95:5	90:10	80:20	70:30
Label	PHB 100	PHB 98	PHB 95	PHB 90	PHB 80	PHB 70

2.3. Scanning Electron Microscopy

The film surface was observed using a scanning electron microscopy Hitachi S3700 (Cambridge, UK). The samples were mounted on aluminium stubs and coated with a gold film.

2.4. Thermal Analysis

The thermal properties of the polymer films were studied by a DSC and TGA instrument. Thermal stability of the polymer films was investigated by thermogravimetric analysis (TGA) using a Perkin Elmer TGA 4000 under nitrogen atmosphere. 10-15 mg of the samples were placed in an aluminum pan and then heated from 40 to 700 °C with a heating rate of 10 °C min^{-1} . The initial decomposition temperature (T_{onset}) denotes the temperature at 2% weight loss and was determined directly from the thermograms [17].

Differential scanning calorimetry (DSC) was performed in Perkin Elmer DSC 6000 instrument. Approximately, 10-15 mg PHB-PEG blends was exposed to a temperature profile over 0 °C to 200 °C, at a heating rate of 10 °C min^{-1} . Melting temperature (T_m) of the blends was obtained directly from the DSC thermograms. The degree of crystallinity (X_c) of the PHB in the polymer blends is calculated according to following equation

$$X_c = \frac{\Delta H_f \times 100}{\Delta H_o \times w(\text{PHB})}$$

ΔH_f = melting enthalpy of the sample (extracted PHB/ standard PHB) ($J g^{-1}$), ΔH_o = theoretical melting enthalpy of the 100% crystalline PHB which is assumed to be 146.6 $J g^{-1}$ [18] and w is the weight of sample. Indium (melting point =156.61 °C; $\Delta H = 28.54 J g^{-1}$) was used for calibrating the DSC apparatus.

2.5. Mechanical Properties Determination

Material properties of biopolymer films were analysed using a tensile testing instrument (Instron-5543, Norwood, MA, USA) at room temperature. Polymer films were cut into rectangular shape (30 × 15mm) and were clamped using

pneumatic grips of a calibrated tensile testing instrument. They were subjected 5 mm min^{-1} stretching. The tensile strength, extension at break ratio and Young's modulus were computed automatically from the graphical output by the computer connected to the instrument. Means from at least eight samples were determined ($n=8$).

2.6. Hydrophilicity Test

The hydrophilicity of the polymer films was determined by the measurement of contact angle. It was carried out at $28\text{ }^{\circ}\text{C}$ by pendant drop method, using a contact angle measurement apparatus (Phoenix, USA). The static contact angle was measured at contact time $t=30\text{ s}$. Drops of liquid (1.5-2.0 mm diameter) were prepared with a microsyringe and were dropped on the surface of polymer films. Mean of five separate points was taken for each polymer film on the same contact time.

2.7. Biodegradation Studies

A mixed microbial culture obtained from a soil sample was used for the biodegradability test of the polymer films [19]. 100 g of soil was mixed with 500 ml of water and the suspension was decanted and filtered, and the filtrate was collected. Parallely, tryptone (5 g), yeast extract (2.5 g) and NaCl (5 g) were mixed with 500 ml of water to obtain the nutrient media. The soil filtrate and the nutrient media was mixed in 1:2 ratio by volume in which polymer film samples ($3\text{ cm} \times 3\text{ cm}$) were incubated at $28 \pm 2\text{ }^{\circ}\text{C}$. At periodic intervals over an 60-day timescale, samples were removed from the incubation medium, washed with distilled water and dried in a dessicator ($40\text{ }^{\circ}\text{C}$, 24 h) before being weighed.

The degradation rate was determined by the ratio of weight loss to the initial weight as shown below:

$$\text{Degradation rate (\%)} = \left\{ \frac{(W_i - W_o)}{W_o} \right\} 100$$

Where, W_o is the initial weight and W_i is the weight at test days.

2.8. Statistical Analysis

The experimental data are presented as mean \pm SEM of three replicates. All analysis was conducted using Graph-pad Prism Version-6.0 (Graph Pad Software, San Diego, CA, USA). Statistical analysis of the data was done by one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

Blending is a common practice in polymer science to improve unsatisfactory physical properties of the existing polymer and has been applied to PHB in recent years [13,20]. The techniques most commonly adopted to blend polymers are either melt blending (direct mixing of the component polymers in the molten state), or film casting from a common solvent. While the former method tends to mimic industrial processing conditions, the latter is a simple laboratory approach [20]. In this study the latter approach was adopted to study the effect of blending PEG on the physical properties of PHB. The PHB-PEG films were transparent, homogeneous and flexible compared to the brittle, pure PHB film.

3.1. Morphological Studies

Morphological analysis of cyanobacterial PHB-PEG films by scanning electron microscopy (SEM) showed that PHB was completely miscible with PEG and the dispersion of PEG was uniform. Standard bacterial PHB had been determined to be completely miscible with PEG during the preparation of the polymers films [Parra *et al.*, (2006)]. Also the obtained PHB-PEG films were transparent, homogeneous and flexible as obtained for PHB-PEG blends by Rodrigues *et al.* [16]. However, increasing PEG content causes alternations (increase in porosity) in the surface morphology of cyanobacterial PHB-PEG films as determined from the SEM micrographs (Fig. 1). Earlier reports provide proofs for significant increase in PHB film porosity upon PEG blending [Chan *et al.* 2011]. This may be due to PEG molecules which interrupt in the close binding of the PHB molecules leading to formation of pores in the blend films.

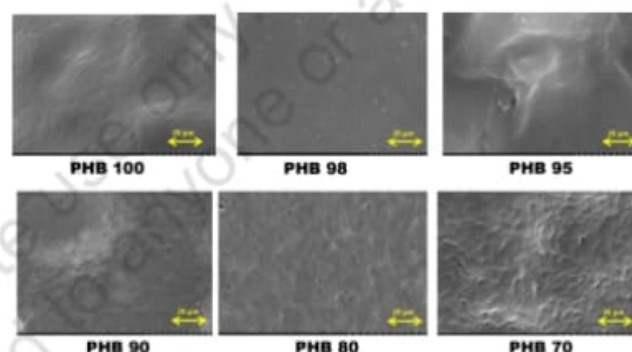


Fig. (1). SEM micrographs of cyanobacterial (*NCCU-442*) PHB - PEG blends.

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3.2. Thermal Analysis

Melting temperature (temperature at which the polymer melts) is an important characteristic parameter. Lower melting temperature than the degradation temperature (temperature at which the polymer undergo degradation) allows the thermal processing of the polymer without the risk of thermal degradation [Van der Walle *et al.*, 2001]. Increasing concentration of PEG decreased the melting temperature (T_m) of the PHB-PEG blends in comparison to PHB100 (Fig. 2 & Table 1). PEG due to its plasticizing property, decreased the intermolecular forces among the adjoining PHB chains due to an intermolecular interaction between PHB-PEG. As a result, this caused a change in free volume that reduced the melting temperature (T_m) of the samples. Another reason for decrease in melting point can be due to thinner lamellae formation of the resulting PHB-PEG blends. According to Yoshie *et al.* [21], thinner lamellae have lower melting temperature. Plasticizer like PEG undergoes crystallization at lower temperature at which the mobility of PHB chains is reduced, therefore resulting in thinner lamellae formation. PEG incorporation into PHB obtained from *Azotobacter chroococcum* 7B also showed a decrease in melting temperature [22].

PEG addition showed significant reduction in the crystallinity of PHB 80 and PHB 70 blend films (Table 1). Reduction in crystallinity had been also reported for PHB-PEG

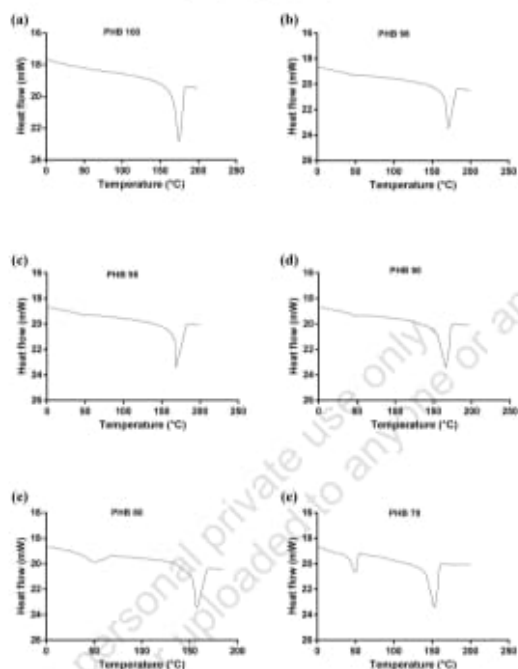


Fig. (2). Differential scanning calorimetry analysis of cyanobacterial (*Nocella muscorum* NCCU-442) PHB -PEG blends. (a) PHB 100, (b) PHB 98, (c) PHB 95, (d) PHB 90, (e) PHB 80 and (f) PHB 70.

Table 1. Thermal analysis of cyanobacterial PHB-PEG blend films.

Sample	T_{melt} (°C)	T_g (°C)	ΔT	Crystallinity
PHB 100	256	171	85	56.78
Blend-I (PHB 98)	233	170	83	55.12
Blend-II (PHB 95)	254	168	86	54.68
Blend-III (PHB 90)	251	165	86	51.59
Blend-IV (PHB 80)	249	158	91	48.17
Blend-V (PHB 70)	248	152	94	45.25

blends [16, 23]. This is because during PHB crystallization, PEG polymer chains remain mobile and move to intra- and interspherulitic regions, which reduces the PHB crystallization rate [24].

The nature and extent of thermal degradation of the PHB-PEG films was observed from their TGA spectra (Fig. 3 & Table I). The thermal degradation of PHB films is known to occur through random chain scission reaction. PHB chains

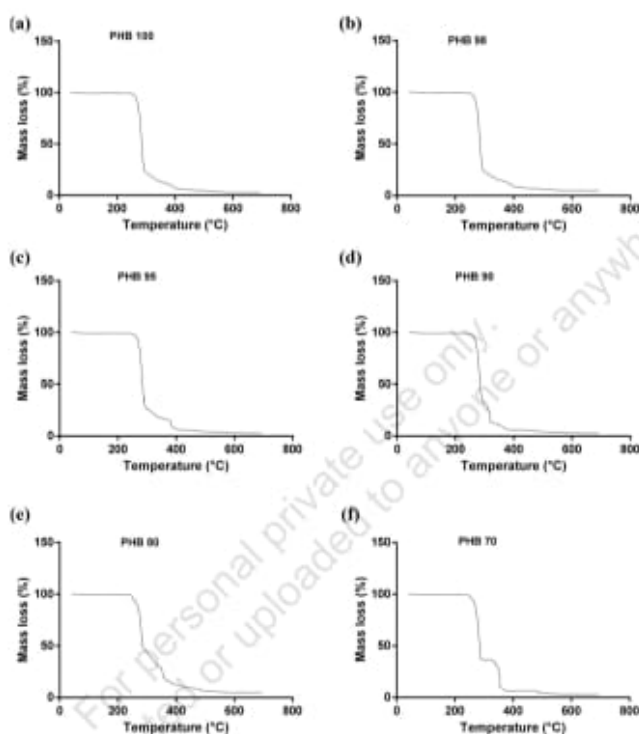


Fig. (3). Thermogravimetric analysis of cyanobacterial (*Nocella muscorum* NCCU-442) PHB -PEG blends. (a) PHB 100, (b) PHB 98, (c) PHB 95, (d) PHB 90, (e) PHB 80 and (f) PHB 70.

are broken down (at the ester groups) to form shorter chains with carboxylic and olefinic terminal groups, however, some kinetically favoured scissions occur near the ends of the macromolecules also [27]. Thus the thermal stability of the polymer films were investigated by determining the initial decomposition temperature (T_{onset}), which is the temperature required to decompose 2% weight loss of the original weight directly from the TGA spectra. The T_{onset} values of these blends though showed a decreasing trend but the decrease was insignificant for the low PEG content blends (upto 10%). This demonstrated a minor effect of PEG on the thermal stability of the blends. Nevertheless, T_{onset} of PHB 80 and PHB 70 showed a displacement upto 249 °C and 246 °C respectively from 256 °C of PHB 100. Parra *et al.* [17] also detected little alteration in the T_{onset} value of bacterial PHB-

PEG polymer blends upto 10% PEG addition and an enhanced decrease in the T_{onset} value on increasing PEG concentration. Hence similar explanation can be deduced for thermal stability of the polymer blends that probably in higher concentrations, the diffusion of the plasticizer to the surface is enhanced by heating and the T_{onset} of the blend decreases.

Though both the T_{onset} and T_{50} showed a decreasing trend with the increase in PEG content but the difference between the T_{onset} and T_{50} (denoted as ΔT in the Table I) was calculated to determine their thermo-processability. Greater the ΔT value of a blend, better its thermo-processability. The addition of PEG caused an increase in ΔT for all blend compositions, with the maximum of 94 for PHB 70.

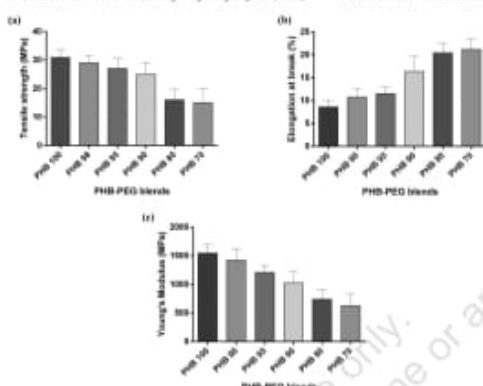


Fig. (4). Mechanical properties determination of cyano-bacterial (*Nostoc muscorum* NCCU-442) PHB-PEG blends. (a) Tensile strength, (b) Elongation to break ratio and (c) Young's modulus.

3.3. Mechanical Properties Determination

Three mechanical properties viz. tensile strength, extension to break ratio and Young's modulus were determined for PHB-PEG films. Tensile strength is the stress (force) required to rupture a sample whereas elongation to break is the strain (deformation) produced on a sample when it ruptures. Young's modulus is the ratio of stress to strain and is the slope of the stress-strain curve (Carráher, 1996). In the present study, tensile strength of the PHB-PEG films decreased with the increase in PEG content (Fig. 4a). Such decrease may result from inhibition of relative motion of the chains or due to reduction in secondary intermolecular bonding between the PHB chains [25]. In fact, PEG increases the free volume between the polymer chains leading to greater chain mobility and film flexibility, thus making the plasticized polymer less resilient and allowing it to deform at a lower force than without the plasticizer. Contrasting to tensile strength, elongation to break ratio was observed to increase with the increase in PEG content (Fig. 4b). Elongation of polymeric materials depends on the mobility of their molecular chains [26]. PEG decreases the intermolecular bonds causing an increase in the PHB chain mobility and thus increases the elongation to break ratio. A reduction in the tensile strength and increase in the elongation to break ratio of standard bacterial PHB has been observed with the increase in the PEG content earlier [17, 27]. Fig. 4(c) shows the variation of Young's modulus for the PHB-PEG films with the addition of plasticizer PEG. It decreased with the increase in PEG content. Due to its plasticizing effect, PEG changed the stiff PHB into flexible films (blended films) which required lower loads to elastically deform resulting in decrease in their Young's modulus values. A progressive reduction in Young's modulus and tensile strength of standard PHB with the addition of PEG had been also observed earlier [28].

3.4. Hydrophilicity Test

Materials with a special affinity for water are known as hydrophilic or water-loving (from the Greek words for water, *hydro*, and love, *philos*). Hydrophilic is defined by the geometry of water on a flat surface, specifically, the angle between a droplet's edge and the surface underneath it. This is called the contact angle. If the droplet spreads, wetting a large area of the surface, then the contact angle is less than 90 degrees and that surface is considered hydrophilic. Besides contact angle, hydrophilicity can be determined by calculating the surface tension (also called surface free energy). Polymers with greater surface tension are more hydrophilic than those having lower surface tension [29]. In the present study, increasing PEG content, decreased the contact angle (Fig. 5a) and increased the surface tension (Fig. 5b) resulting in increase in hydrophilic property of the PHB-PEG blends. Since PEG is a hydrophilic compound, hence increased hydrophilicity of the resulting PHB-PEG blends upon PEG blending is an obvious result. Increased hydrophilicity of PHB-PEG blends with the increase in PEG contents had also been reported in earlier studies [26, 30].

3.5. Biodegradation Studies

Increasing content of the hydrophilic plasticizer PEG increased the rate of biodegradation of the cyano-bacterial PHB-PEG blend films (Fig. 6). The addition of larger amounts of PEG increased the number of polar groups in the samples, and promoted the interaction of these groups with water molecules during hydrolysis that preceded biodegradation. Increasing concentrations of plasticizer also caused increase in porosity on the polymer blend surfaces that favoured increased attachment of the soil microorganisms, thereby accelerating biodegradation. Earlier reports also

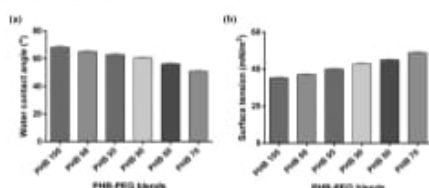


Fig. (5). Hydrophilic properties determination of cyano-bacterial (*Nostoc muscorum* NCCU-442) PHB-PEG blends. (a) Water contact angle and (b) Surface tension.

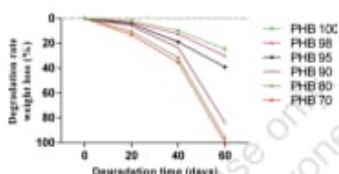


Fig. (6). Biodegradation properties of the cyano-bacterial (*Nostoc muscorum* NCCU-442) PHB-PEG blends.

determined an increased degradation in PHB-PEG films with the increase in PEG contents [17, 30]. Significant degradation of PHB-PEG films had been observed with ascending PEG content only after 10 days, though maximal degradation (upto 50-60% weight loss) occurred after a long period of 80-90 days [27]. Interestingly, in the present study, PHB 100 film showed 24.58 % (weight loss) with mixed microbial culture whereas PHB 70 showed complete degradation after 60 days incubation [31-33].

CONCLUSION

Blending with PEG increased the crucial polymeric properties of cyanobacterial PHB. The PHB-PEG films were transparent and flexible compared to the pure, brittle PHB film. The initial temperatures of decomposition of these films were very close to each other, indicating that the addition of plasticizer (PEG) upto 10% did not alter the thermal stability of the final blends. The plasticizer probably weakened the intermolecular forces between the adjacent polymer chains. Consequently, there was a change in free volume with reduction of the melting temperatures (T_m). Mechanical properties viz; tensile strength and young's modulus showed a decreasing trend whereas the elongation to break ratio increased with addition of PEG, revealing its plasticizing effect. The hydrophilic nature of the PHB-PEG blends improved and also the biodegradation rates were greater with increasing concentrations of the plasticizer (PEG).

LIST OF ABBREVIATIONS

DSC = Differential Scanning Calorimetry
 PEG = Polyethylene Glycol
 PHB = Polyhydroxybutyrate

TGA = Thermogravimetric Analysis
 T_m = Melting Temperature
 T_{onset} = Initial Decomposition Temperature

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

FUNDING

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interests and acknowledge CSIR, New Delhi for providing SRF to first author.

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