

Biosynthesis of Polyhydroxyalkanoate Blends from Tamarind Seed: Xyloglucan and Polyhydroxybutyrate Precursors

P. Sakthiselvan¹, R. Madhumathi²

¹ Department of Petroleum Engineering, AMET University, Kanathur, Chennai – 603112.

² Departments of Chemical Engineering, A.C.College of Technology, Anna University, Chennai – 600025.

Abstract: Polyhydroxybutyrate (PHB) is biodegradable aliphatic polyester that is produced by a wide range of microorganisms. Basic PHB has relatively high glass transition and melting temperatures. To improve flexibility for potential packaging applications, PHB is synthesized with various co-polymers such as Xyloglucan (XG) leading to a decrease of the glass transitions and melting temperatures. In this work, the brittle Polyhydroxybutyrate (PHB) was blended with Xyloglucan (XG) a hemicelluloses, rich in tamarind seeds, and solvent casted with plasticizer (Polyethylene glycol) PEG which is biodegradable copolymer. Moisture absorption (%) of the blend film was determined. Thus, the results conclude that XG-PHB blend improves the process-ability to create a new type of eco-friendly blend material suitable for potential packaging applications.

Keywords: Polyhydroxybutyrate (PHB), Xyloglucan (XG), Polyethylene glycol (PEG) Blending, Moisture absorption.

I. INTRODUCTION

Utilization of biopolymers from renewable resources in order to replace petroleum derived polymers is of increasing interest both in industry and medical field. The packaging industry is one of the major partners of the global plastic production [1], [2]; where food packaging is the most important application. In packaging applications, the packaging material should possess flexibility, mold ability, optical transparency and gas barrier qualities. Several biopolymers such as Starch, Cellulose, Polyhydroxyalkanoates (PHAs), Chitin, Polyglycolic acids, Polylactides, polysaccharides and their co-polymers and/or blends etc are under study [3]. In order to be applied as alternatives to plastics in many applications. However, only PHAs are of particular interest because they possess thermoplastic characteristics [4], [5]; and resemble synthetic polymers to a larger extent. Some microbial species have been identified to produce Polyhydroxyalkanoate (PHA) family.

This problem could be solved by blending of 3-hydroxybutyrate with other Polyhydroxyalkanoates such as Polyhydroxyvalerate (PHV), polylactic acid (PLA) other biopolymers such as naturally occurring polysaccharides [6]. Major advantage of the copolymers over the PHB homopolymer is that the copolymers have a lower level of crystalline, which makes it tougher and more flexible [7], [8]. Xyloglucan (XG) which is biodegradable polymer comes under the category of hemicelluloses [9]. Xyloglucans (XGs), also called amyloids, are widespread in plants. Xyloglucan is a food grade, starch-like polysaccharide and present in the primary cell walls of dicotyledons and non gramina ceous monocotyledons. The glucose backbone of XG is abundantly substituted with (1, 6)-linked xylopyranose branches that in turn may have further derivatives as (1, 2)-linked galactopyranosyl residues.

To improve blend compatibility a compatibiliser as a third component can be used. Such compatibilisers include glycidyl methacrylate (GMA), poly (ethylene glycol) (PEG), sodium alginate, polyacrylic acid and poly (vinyl acetate) (PVAc) [10]. Polyethylene glycol shows good toxicological safety when it comes to acute and chronic oral toxicity or skin compatibility, supported by absorption/excretion investigations. Therefore, they have been used for many years in cosmetics, foodstuffs and the pharmaceutical industry and are registered in all relevant pharmacopeia's [11], [12].

II. EXPERIMENTAL METHODS

A. Sample Preparation of PHB Film

A PHB producing strain *Bacillus safensis* EBT1 was grown in a production medium containing sugarcane baggase as the carbon source along with yeast extract, peptone and NaCl in 100 mL distilled water. The pH of the production medium was adjusted to 7. Then it was incubated at 45 °C for 48 h. After the incubation period, the microbial cells were collected by centrifugation at 10,000 rpm for 10 min. The pellet collected was washed with acetone and ethanol and re-suspended in equal volume of 4% sodium hypochlorite solution. This solution was incubated for 30 min in room temperature. The whole mixture was centrifuged again and

the supernatant was discarded. The pellet collected was again washed with acetone and ethanol [13]. Thus, the extracted polymer granules were stored for further use.

B. Sample Collection of Blends

Various natural polymers were collected and tested for their film forming abilities. The natural polymers were chosen so as to include a wide range of polysaccharides such as glucose, sucrose, galactose, mannose, xylose, maltose, etc. Natural polymers were chosen instead of synthetic polymers because of their biocompatibility and biodegradability. Also, because of their contribution as an inexpensive substrate in reducing the high production costs of PHB. The Table 1 lists some of the waste natural polymers considered and their sources

TABLE 1
SAMPLE COLLECTION FOR BLENDS

S. No	Area of collection	Plant Source	Sample
1	Thangam flour mill	Tamarindusindica	Tamarind seed polysaccharide
2	From residential areas	Moringa oleifera	Resin
3	Sago industry	Manihot esculenta	Sago waste
4	From agricultural fields	Sorghum bicolor	Sorghum stalk
5	Drought affected regions (Pudhukottai)	Cassia tora, Cassia fistula & other Cassia sp.	Cassia seeds

C. Xyloglucan

Tamarind kernel powder was purchased from Thangam flour mill, Tuticorin. 20 g of Tamarind Kernel powder was added to 200 mL of cold distilled water in order to prepare slurry. The slurry obtained was then poured into 800 mL of boiling distilled water and boiled for 20 min on a water bath. A clear solution was obtained which was then kept undisturbed overnight. This clear solution was then centrifuged at 5000 rpm for 20 min to separate all the foreign matter. Thus, obtained solution is precipitated using excess of absolute alcohol. And the precipitates were collected by centrifugation and were dried at room temperature for 2-3 days and stored for further use [9].

D. Preparation of PHB-PEG-XG Blend

1 g of xyloglucan was dissolved in 100 mL of distilled water and heated for 60 min at 60°C. PEG (400 Da) was added to this solution in different percentages and magnetic stirred for 1h. Table 2 shows the percentage of PEG and the ratios of XG: PHB.

TABLE 2
PERCENTAGE OF PEG AND THE RATIOS OF XG: PHB

XG:PHB	Percentage of PEG
90:10	1 %
80:20	
70:30	
60:40	
50:50	
90:10	2 %
80:20	
70:30	
60:40	
50:50	

This solution was blended with 1% PHB-chloroform solution in different ratios of 45:5, 40:10, 35:15, 30:10 and 25:25 by magnetic stirring overnight. The blended mixtures were then poured onto preheated Petri dishes and the solvents were slowly evaporated at room temperature.

E. Preparation of Copolymers of PHB with Natural Polymers

Instead of using glucose alone as a substrate, various natural polymers were used in conjunction with glucose as a carbon source for the microorganisms to obtain different copolymer formulations of PHB. The procedure adopted for the production of copolymers is the same as described for the preparation of PHB except for the variation of carbon sources. The various natural polymers used along with glucose and their copolymers were formulated.

F. Moisture Absorption

The blend films of 20 mm×20 mm were conditioned at 0 % RH for 24 h. After that the films were weighed and were conditioned in a desiccator containing CaNO₃ saturated solution to obtain a relative humidity of 55 %. The samples were weighed at desired intervals until they reached an equilibrium state²⁰. The Moisture Absorption of the blend films was calculated by following equation (1):

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

Where MA% is Moisture Absorption (%), W_d and W_w are the weights of the sample film before and after conditioning, respectively.

G. Viscometric Studies

The molecular weight of PHB and PHB-XG was determined with a capillary glass type Ostwald dilution viscometer. Intrinsic viscosity was measured at 30±0.5 °C. All the measurements were carried out in chloroform solution. Initially, a 1% solution of the polymer in chloroform was prepared. At least four dilutions viz 0.2, 0.4, 0.6 and 0.8% of polymer stock solutions were prepared before carrying out the actual measurement. Dilution was done using the volumetric expression, V₁N₁=V₂N₂. For the measurement, 25 mL of the solvent was poured into the viscometer. By keeping the upper mark of small reservoir of the viscometer parallel to the eyes, the pure solvent was allowed to flow down to the lower mark and the efflux time in s was noted using a stopwatch. The same procedure was repeated for at least twice to get the average efflux time (t₀) for pure solvent. Similarly, the efflux time of various concentrations of polymer-chloroform system (i.e., t) was noted adopting the same procedure. Specific viscosity was calculated as

$$\eta_{\text{spec}} = (t - t_0) / t_0 \quad (2)$$

Molecular weight was calculated from the Mark-Houwink equation with the following coefficients:

$$[\eta] \text{ In dl/g} = 1.18 \times 10^{-4} \times M^{0.78} \quad (3)$$

To determine the intrinsic viscosity [η], the experimental reduced viscosity values (η_{spec}/C) were plotted against the polymer solution concentration, C

III. RESULTS AND DISCUSSION

A. PHB-XG Film Formation

Film preparation of XG and PHB is often challenging. XG in the present study showed excellent film forming characteristics that could be attributed to its high molar mass of around 1.5 - 2.5 MDa when it is used alone. Despite the high molar mass, XG can still be dissolved in water up to a concentration of 4 %, forming a viscous solution.

However, the preparation was more challenging and extensive mechanical treatment was necessary when XG dissolved in water was blended with PHB-chloroform solution. As both are immiscible solvents a third compound that is PEG was added in order to have a homogenous blend. The blends were casted into films at room temperature. Appearance of films as evaluated by visual observation was found to be opaque, yellowish films with uniform texture. An image of the copolymer film PHB-XG has a better visual appeal than the blend film was obtained. The thickness of the films as measured using a screw gauge at three different points was found to be in the range of 0.2 to 0.5 mm. The films with 90% and 80% XG showed better smooth surface than the blend films containing 70%, 60% and 50% XG. After the blending, considerable phase separation was seen in films containing XG and PHB in the ratio 25:25. And also those blends when casted were very brittle and could not be taken as films from the Petri plate. The addition of compatibiliser that is PEG allowed to form homogenous blend. However, films containing 1g of PEG showed smooth surface and good flexibility but the films with 0.5 g PEG showed good mechanical properties.

B. Moisture Absorption

Water resistant is an important criteria in materials intended for packaging. The Moisture Absorption of XG-PHB blends were shown in Fig 1.

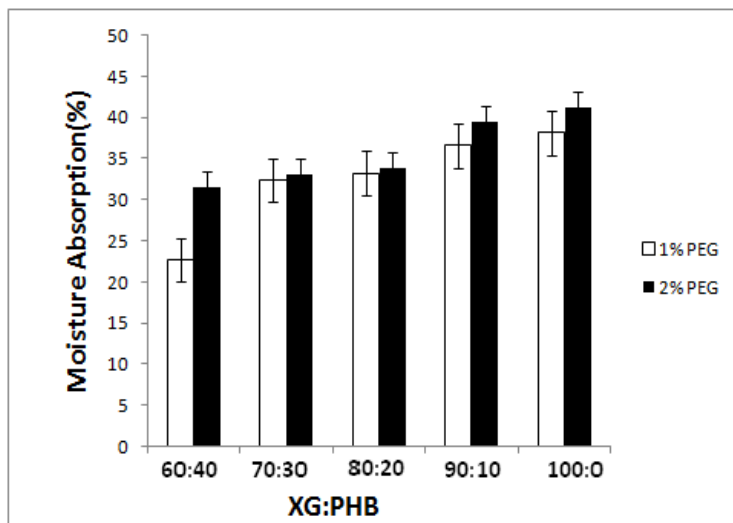


Fig. 1 Moisture Absorption (%) of XG-PHB blends films of different composition

The Figure clearly shows that the moisture uptake increases with decrease in PHB ratio (60:40), (70:30), (80:20), (90:10) and (100:0). This indicates that blending of PHB on XG increases its hydrophobicity. The reason could be that the XG is able to form hydrogen bonds with the hydroxyl group of PHB and this strong structure could reduce the diffusion of water molecules in the material. This may also be due to the high crystalline nature of the PHB since hydrophobicity is directly related to crystalline nature. Thus, these results indicate that the addition of PHB improve the water resistance of the XG matrix. In previous study results [15]; also, same trend of observations have made i.e., a decrease in the water absorbed by polymer blends and biocomposites which were made with the addition of cellulosic substances was observed.

C. Evaluation of Copolymer Films

The copolymer films produced using different combinations of carbon substrates were evaluated for their physico-mechanical properties represented in the Table 3.

TABLE 3
PHYSICO-MECHANICAL PROPERTIES OF FILMS

S.No	Folding endurance	Thickness (mm)	Appearance	Copolymer Formulations
1	3	0.23	Yellowish White, rough	Extracted PHB
2	27	0.366	whitish, flexible & smooth	PHB-co-XG
3	18	0.59	Brownish, Flexible and rough	PHB-co-GM
4	10	0.408	Brownish white, coarse surface	PHB-co-Alginate
5	6	0.574	whitish, smooth and transparent	PHB-co-Xanthan gum

The appearance of the films was mostly white or brown depending on the colour of the natural polymer used as the substrate. The moisture uptake was found to lower for PHB-co-XG films because of the fact that XG is sparingly soluble in hot water and PHB is insoluble in water. While the other natural polymers are highly soluble in water and thus have good water uptake which is not

desirable for a bioplastic copolymer. Besides PHB-co-XG shows good folding endurance and tensile strength, thus shows improved properties compared to PHB alone.

D. Molecular Weight Determination

Each solvent has its own viscosity. Addition of a polymer to a solvent of low viscosity results in sharp increase in its viscosity. The extent of increase in its viscosity depends on the molecular weight and amount of the polymer added. This principle was exploited to determine the molecular weight of the polymer by using the Mark Houwink empirical equation.

In polymeric materials, the rheological behavior and industrial processability is strongly dependent on the molecular weight and structure, and hence examination of molecular weight vs viscosity is necessary. Determination of intrinsic viscosities for PHB and PHB-XG respectively for molecular weight calculation. From the above two eqns. (2) and (3), the molecular weight of the polymer sample was calculated and it was compared with literature values which are listed in Table 4.

Table 4

Compare the molecular weights of extracted polymer, copolymer and other samples with literature values

Reference	(M _w) (x 10 ⁵ Da)	Polymer
This work	1.94	PHB
This work	3.86	PHB-co-XG
[14]	4.7	PHB-co-PHV
[15]	1.394	PHB-co-PHV

The differences in the molecular weight values were attributed to the fact that molecular weight of PHB depends on the organism, substrate used, growth conditions and isolation techniques. The molecular weight of PHB extracted was found to be higher compared to other studies and higher molecular weight attributes the enhanced properties like lower crystallinity and exceeding processability for packaging applications.

IV. CONCLUSIONS

The ultimate aim of this work was to understand the thermal, mechanical, and physical barrier properties of XG - PHB from various manufacturers with different XG content. Biodegradable XG-PHB blend have been successfully prepared by mixing PHB, XG and new type of plasticizer PEG300. The molecular weight of the films was determined and moisture absorption of films was also determined. The results reveal that the blend films XG-PHB showed improved properties when compared to the extracted PHB and extracted XG. As XG is an inexpensive biopolymer, making use of it in improving the physical as well as chemical properties of PHB. Biodegradable XG-PHB blend have a potential for compostable packaging applications.

REFERENCES

- [1] M. P. Arrieta, J. Lopez, S. Ferrandiz, and M.A. Peltzer, "Characterization of PLA– limonene blends for food packaging applications", *Polymer Testing*, vol.32, pp.760–768, 2013.
- [2] R.Preethi, P.Sasikala, and J. Aravind, "Microbial production of polyhydroxyalkanoate (PHA) utilizing fruit waste as a substrate", *Research Biotechnology*, vol. 1, pp.61-69, 2012.
- [3] W. Yu, C. Wei, H. Chin, W. Ho, S. Yi, L. Chi, and J. Murugan, "Screening and evaluation of polyhydroxybutyrate-producing strains from indigenous isolate *Cupriavidus taiwanensis* strains", *International Journal of Molecular Science*, vol.12,pp.252, 2011.
- [4] A.P. Bonartsev, V.L.Myshkina, D.A. Nikolaeva, E.K. Furina, T.A. Makhina, V.A. Livshits, A.P.Boskhomdzhev, E.A. Ivanov, A.L.Iordanskii, and G.A. Bonartseva, "Biosynthesis, biodegradation, and application of poly (3-hydroxybutyrate) and its copolymers - natural polyesters produced by diazotrophic bacteria", *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, vol.1,pp.295-307, 2007,.
- [5] B. Senthil Kumar, and G. Prabhakaran, "Production of PHB (bio plastics) using bio-effluents as substrates by *Alcaligenes eutropha*", *Indian Journal of Biotechnology*, vol.5, pp.76-79, 2006.
- [6] S.Modi, K. Koelling, and Y. Vodovotz, "Assessment of PHB with varying hydroxyvalerate content for potential packaging applications", *European Polymer Journal*, vol. 47,pp.179-186, 2011.
- [7] S.V. Otari, and J. S. Ghosh, "Production and Characterization of the Polymer Polyhydroxybutyrate-co-polyhydroxyvalerate by *Bacillus megaterium* NCIM 2475", *Current Research Journal of Biological Science*, vol.1, pp.23-26, 2009.
- [8] B.J.Wang, Y.J. Zhang, J.Q.Zhang, Q.T.Gou, Z.B. Wang, P. Chen, and Q. Gu, "Crystallization behaviour, thermal and mechanical properties of PHBV/graphene nanosheet composites", *Chinese Journal of Polymer Science*, vol. 31,pp.670-678, 2013.
- [9] G.V. Radha, and N.M. Santosh, "An overview study on tamarind seed polysaccharide as a novel excipient in pharmaceutical formulations", *International*



Journal of Inventions in Pharmaceutical Science, vol. 1, pp.140-148, 2013.

- [10] Z.Peng, "Synthesis and the effect of hydrophobic dodecyl end groups on pH-responsive micellization of poly(acrylic acid) and poly(ethylene glycol) triblock copolymer in aqueous solution", *Iran polymer Journal*, vol. 21, pp.253-261, 2012.
- [11] R.Auras, B. Harte, and S. Selke, "An overview of polylactides as packaging materials", *Macromolecular Bioscience*, vol. 4, pp.835-864, 2004.
- [12] Chang, A., pH- sensitive starch-g-poly (acrylic acid)/sodium alginate hydrogels for controlled release of diclofenac sodium, *Iran polymer Journal*, 2015.
- [13] H.L. John, and A.S. Ralph, "Assay of polyhydroxybutyric acid", *Journal of Bacteriology*, vol. 82, pp. 33-36, 1961.
- [14] S.P. Valappil, S.K. Misra, A.R. Boccaccini, T. Keshavarz, C. Bucke, and I. Roy, "Large-scale production and efficient recovery of PHB with desirable material properties, from the newly characterised *Bacillus cereus* SPV", *Journal of Biotechnology*, vol. 132, pp.251-258, 2007.
- [15] S.O.Kulkarni, P.P. Kanekar, J.P. Jog. S.S. Sarnaik, and S.S. Nilegaonkar, "Production of copolymer, poly (hydroxybutyrate-co-hydroxyvalerate) by *Halomonas campisalis* MCM B-1027 using agro-wastes", *International Journal of Biological macromolecules*, vol. 72, pp.784-789, 2015.