

Design and Development of Poly Lactic Acid (PLA)/Mango Seed Kernel Starch Composites Films

Ramya K. V.¹, Aayushi Wawre², Manoj Kumar¹ and Radhashyam Giri^{3*}

¹Centre for Bio-Polymer Science and Technology (Unit of CIPET), Kochi, Kerala, India

²Department of Plastics Technology, Central Institute of Plastic Engineering and Technology-IPT, Ahmedabad, Gujarat, India

³Department of Plastics Technology, Central Institute of Plastics Engineering and Technology-IPT, Bhubaneswar, Odisha, India

*Corresponding author: giripolymer@gmail.com

Abstract: PLA is biodegradable and bioactive thermoplastic aliphatic polyester derived from renewable sources and mango seed kernel starch also being a biodegradable and nutritive material; an eco friendly, biodegradable bio compound was prepared by using PLA and mango seed kernel starch. The compound was made having varying composition of unmodified mango seed kernel starch and modified mango seed kernel starch viz. 5%, 10%, 15% in PLA using solvent casting method. Addition of starch and modified starch resulted in reduced transparency. Whereas, the water absorption, moisture absorption and tensile strength increased with addition of unmodified starch but reduced when modified starch was added. The soil burial test revealed that PLA virgin film remained as such but the addition of starch improved the degradation of PLA films. The DSC scans showed that there was no significant change in the glass transition temperature (T_g), melting temperature (T_m) and crystallization temperature (T_c) of PLA films and starch based PLA films. These properties and the biodegradable nature of the films make it useful for packaging in food industry, agriculture, medical field etc.

Keywords: Poly Lactic Acid (PLA), Mango seed kernel starch, Solvent casting.

I. INTRODUCTION

Recently, because of worries about the disposal of plastics, polymer researchers have been emphatically urged to develop some new biodegradable polymer composite materials from sustainable assets. Be that as it may, inquire about on biodegradable polymers as composite frameworks are constrained in correlation with examining on thermoplastics and thermoset polymers due to the moderately poor accessibility and a high cost of biodegradable polymers. The study concentrated more because of normal fillers on the mechanical properties of petroleum based polymers as rather than utilizing biopolymer

like PLA as a matrix. Numerous favorable circumstances are related to the utilization of regular fillers, including low cost, availability, low density, high specific properties and absence of deposits upon incineration. Consequently, the combination of natural fillers with PLA offers a response to keeping up the sustainable development of economical and environmental innovation [1].

It is well known that synthetic polymers have been generally utilized as a part of each field of human movement amid a decades ago, i.e. post-Staudinger times. These artificial macromolecular substances are generally obtained from petroleum and the majority of the traditional ones are viewed as non-degradable. But, the petroleum assets are restricted and the increasing use of non-biodegradable polymers has caused genuine ecological issues. Also, the non-biodegradable polymers are not appropriate for temporary utilization, for example, sutures. Subsequently, the polymer materials which are degradable and additionally biodegradable have being given careful consideration since 1970s. Both synthetic and natural polymers that contain hydrolytically or enzymatically labile bonds or gatherings are degradable. The benefits of synthetic polymers are self-evident, including predictable properties, batch to batch consistency and can be custom fitted effortlessly. Despite this, they are very costly. This reminds focus on natural polymers, which are intrinsically biodegradable and can be a promising possibility to meet distinctive necessities. Among the natural polymers, starch (Fig. 1) is of intrigue. It is recovered from carbon dioxide and water by photosynthesis in plants. Attributable to its total biodegradability, ease and sustainability, starch is considered as a promising contender for creating sustainable materials. In perspective of this, starch has been getting developing consideration since 1970s. Numerous endeavors have been applied to create starch-based polymers for conserving the petrochemical assets, lessening ecological effect and seeking more applications [14].

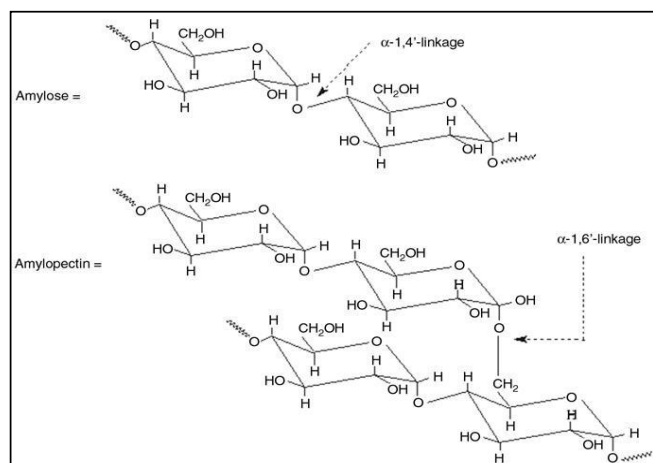


Fig. 1: Structure of Starch

II. EXPERIMENTAL

A. Materials

Poly Lactic Acid (PLA) of grade Ingeo™ Biopolymer 3052D was provided by Nature Works. Mango starch was obtained from naturally available Mango seed kernel (Fig. 2). Other chemicals utilized include, Sodium Hydroxide, Acetic acid, Sodium Hydrogen Sulphite and Chloroform which were utilized from the institute.

B. Methodology

The mango seeds were collected and sun dried for two days. Then the mango seed kernel was manually separated from the hull and cut into small pieces of 5-10 mm size. A sample of mango seed kernel and hull is shown in the figure. 10 g of raw kernel sample was weighed. To this 80 ml of sodium hydrogen sulphite solution was added. The temperature of the process was maintained at 50 °C for 24 hours. Also the content was agitated at an interval of 1 hour with the help of glass rod. After 24 hours it was washed with distilled water and was kept for drying in an oven. Dried samples were kept for further analysis. After drying, a little amount of obtained mass was taken in a test tube and a few drops of KI solution were added. A bluish violet colour was obtained. This indicates the presence of starch. The obtained natural powder was soaked in 1% aqueous sodium hydroxide solution for 24 hours at room temperature to remove impurities and waxy substances. This above process is also known as “mercerization” process. Then it was washed with 1% acetic acid solution and distilled water to neutralize excess sodium hydroxide and the natural powder was dried again in an oven with air circulation for 16 hours at 50 °C. NaOH treatment is used on natural powder for removal of Lignin and Hemi cellulose. Rough surface can also be obtained by NaOH, so it can easily be bonded with polymer.



Fig. 2: Mango Shell

III. FILM PREPARATION

A. Preparation of Virgin PLA Film

PLA films were prepared using chloroform as a dissolving agent. It was dissolved slowly with continuous stirring using a magnetic stirrer. After complete dissolution films were casted in a glass mould and kept for drying at 23±2 °C for few hours. Dried films were conditioned and stored for further studies.

B. Preparation of PLA/Unmodified and PLA/Modified Starch Films

Chloroform was used as the dissolving agent. PLA and starch were taken in different compositions as in the below table. First starch was taken in a small beaker; chloroform was added. It was sonicated for 15 min for proper dispersion of starch in chloroform. PLA was dissolved in chloroform. To this add the starch solution and is again stirred. After complete dissolution films were casted in a glass mould and kept for drying at 23±2 °C for few hours. Dried films were conditioned and stored for further studies. PLA/modified starch films were also prepared by taking modified mango starch as the filler and followed the same procedure as above.

C. Composition

TABLE I: COMPOSITION

Sr. no.	Starch %	PLA %	Batch Name for Unmodified Film	Batch Name for Unmodified Film
1	0	100	PLA	PLA
2	5	95	PLUMM5	PLMM5
3	10	90	PLUMM10	PLMM10
4	15	85	PLUMM15	PLMM15

IV. CHARACTERIZATION OF FILM

The film was characterized in order to evaluate its ability to pack the food products. Film thickness, water absorption,

moisture content of the film, Tensile Strength (TS), Water Vapour Permeability (WVP), transparency, thermal property, and film morphology studies were carried out.

A. Thermo Gravimetric Analysis (TGA)

TGA is a method of thermal analysis in which changes in physical and chemical properties of materials are measured as a function of increasing temperature (with constant heating rate), or as a function of time. Micro-thermo balance measures any changes in the mass of the sample, whether due to adsorption of oxygen, thermal degradation, oxidation, or other heterogeneous reactions.

B. Transparency

The transparency of the film was measured with opacity tester (opacity tester, Elemech Pneumatic). 4×10 cm² films were cut from the sample and opacity was tested according to ASTM D 1003. Five replicates were made in each film. Results were expressed in percentage.

C. Water Absorption

Water absorption of the film was evaluated by immersing dry films of 2×2 cm into 50 ml distilled water at 37 °C. Water absorption time is noted using stop watch. The test was carried out for 24 hrs and the insoluble film was observed for increase in dimension after gently blotting the surface with Whatman filter paper. Water absorption was calculated using the following equation:

$$\text{Water Content(\%)} = \frac{(\text{Initial dry film weight} - \text{Final dry film weight})}{\text{Initial weight of the film}} \times 100$$

D. Moisture Content

To determine the moisture content of films, approximately 2×2 cm² of film pieces were dried at 105 °C for 17 hrs. The weight loss of the sample was determined, and moisture content was calculated as the percentage of water removed from the system. Moisture content of 2 replica of each film sample was estimated.

$$\text{Moisture Content (\%)} = \frac{(\text{Initial weight of the film} - \text{Final weight of the film})}{\text{Initial weight of the film}} \times 100$$

E. Tensile Strength and Elongation at Break

The tensile strength and elongation at break of PLA starch composite films was done using Lloyd instruments UK, model TA plus according to ASTM D882-02 (2002) standard. The 50×15 mm² of film pieces were stored under the temperature

of 23 °C with 50% relative humidity for at least 24 hrs. Samples were cut in length wise and cross direction. For tensile strength test, appropriate load range and grip separation were selected, then placed the test specimen in the grip of testing machine. Extensometer measured the load versus extension. The testing speed was at 500 mm/min with a load cell of 500 N. The distance between the two anchorages was 50 mm. The following formula was used in calculating the Tensile strength and elongation.

$$\text{Tensile Strength} = \frac{\text{Maximum Load} \times \text{Thickness}}{\text{Width}}$$

$$\text{Elongation of Break} = \frac{\text{Final length} - \text{Initial weight}}{\text{Initial length}} \times 100$$

F. Soil Burial Test

Garden soil was taken in different pots. A weighed amount of each samples were placed in the soil. Before placed in the soil the samples were weighed. Then after 7 days take them out and again weighed. Again they were placed in the soil for another 7 days and after that they are weighed.

G. Differential Scanning Calorimetry (DSC)

Thermal property of films was determined using a differential scanning calorimeter (DSC, Q20 V24.11 Buid 124). Before analysis, films were conditioned in a desiccators containing dried silica gel for 2 day at room temperature (23-25 °C) to obtain dehydrated films. Aliquots of approximately 10mg pre-dried samples were placed into aluminum pans, sealed and scanned over the range of -25 to 30 °C with a heating rate of 10 °C/min for PLA starch films; whereas, -50 °C to 150 °C with a heating rate of 10 °C/min for composite and bi-layer films. The empty aluminum pan was used as a reference.

H. Thermogravimetry Analysis (TGA)

Thermogravimetry Analysis (TGA) was used to determine material thermal stability and its fraction of volatile components by monitoring the weight loss of the sample when heated in a chosen atmosphere. Thermal degradation of mango starch was found.

V. RESULTS AND DISCUSSIONS

A. Thermogravimetry Analysis (TGA)

Weight % and derivative weight change of extracted mango starch is shown in Fig. 4. Mango starch showed about four transitions; the first transition at 117 °C and 257 °C may be due to the evaporation of water and other impurities present. The second transition at 411 °C is the onset degradation temperature of starch and it fully degrades at 450 °C. The decomposition temperatures are shown in Fig. 3 and Fig. 4.

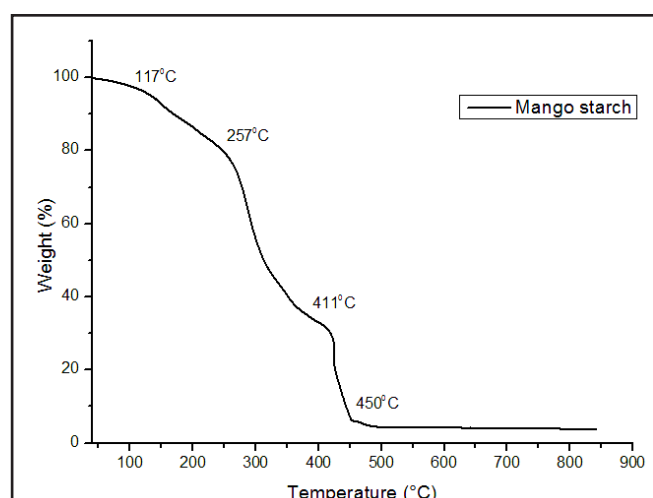


Fig. 3: Percentage Weight Loss of Mango Starch

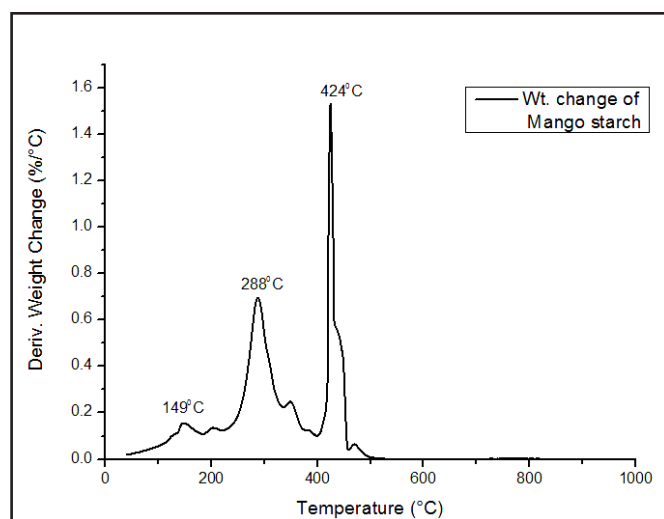


Fig. 4: Derivative Weight Change of Mango Starch

B. Transparency

Transparency of virgin PLA and PLA/starch composite films were tested. Transparencies of the films were obtained as shown in Table II. From the table we can find that the transparency of composite films decreased. Modified starch composite showed lower transparency than unmodified starch composite films. The addition of starch led to decrease in transparency.

TABLE II: TRANSPARENCY OF PLA AND COMPOSITE FILMS

Film	Transparency (%)
PLA	75.00
PLUMM5	71.12
PLUMM10	71.00
PLUMM15	70.69
PLMM5	70.02
PLMM10	69.56
PLMM15	68.98

C. Water Absorption

Water absorption of virgin PLA and PLA/starch composites were examined. Water absorption values of these samples are shown in the table given below.

TABLE III: WATER ABSORPTION OF PLA AND COMPOSITE FILMS

Film	% of Water Absorption
PLA	0.22
PLUMM5	0.29
PLUMM10	0.38
PLUMM15	0.39
PLMM5	0.32
PLMM10	0.26
PLMM15	0.22

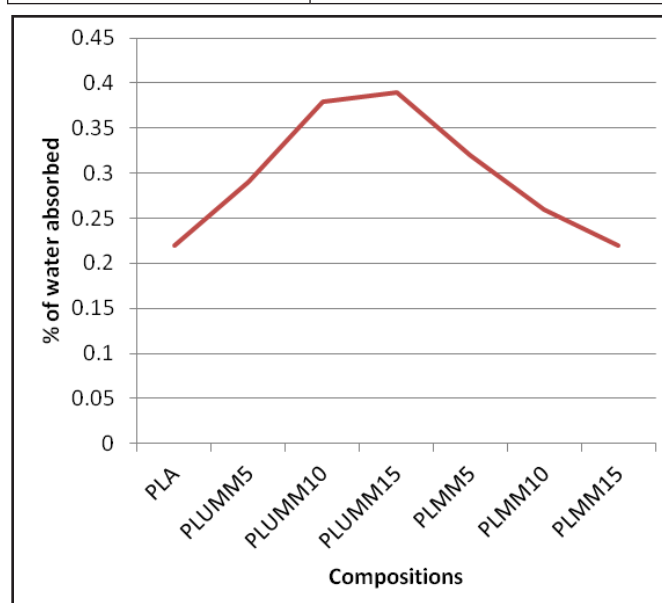


Fig. 5: Percentage Water Absorbed by Composite Films

PLA film containing mango starch has higher water uptake than pure PLA film. Water uptake increases with increasing immersion time and starch content. This finding is due to the hydrophilic character of natural starch, which is responsible for the water absorption in the composite. Therefore, a higher starch content led to a higher amount of water being absorbed. As the percentage of starch increases water absorption increases. Compared to unmodified starch film modified starch film absorbs less water. So the modified starch film is better than unmodified one shown in Fig. 5.

D. Moisture Absorption

The moisture absorption test was also conducted for the samples. The results are given below.

TABLE IV: MOISTURE ABSORPTION OF PLA AND COMPOSITE FILMS

Film	Initial Weight (Grams)	Final Weight (Grams)	% of Moisture Absorbed
PLA	0.0528	0.0499	5.49242424
PLUMM5	0.0781	0.0736	5.76184379
PLUMM10	0.0281	0.0263	6.040569395
PLUMM15	0.0435	0.0404	7.126436782
PLMM5	0.0601	0.0556	7.487521
PLMM10	0.0484	0.0454	6.198347
PLMM15	0.0704	0.0663	5.823864

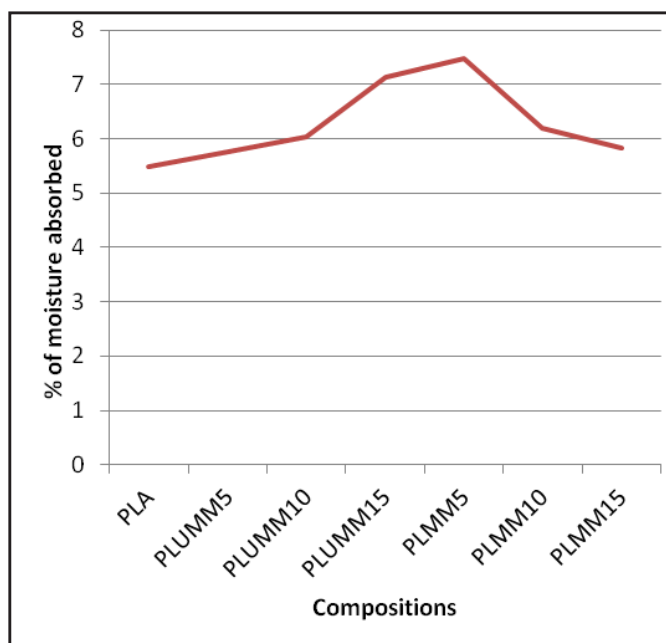


Fig. 6: Percentage Moisture Absorbed by Composite Films

The rate of moisture absorption increases as the starch content increases. Samples containing starch showed higher values of the moisture content in their respective composites. This behavior is associated with the hygroscopic nature of starch compared with PLA.

As compared with the unmodified starch films, the modified ones have less moisture absorption. So we can say that the modified starch films shows better properties than the unmodified ones. But we can also note that as compared with the virgin PLA films, the modified starch films have higher moisture absorption shown in Fig. 6.

E. Tensile Strength

The tensile strength and the elongation of the PLA and PLA/starch composites were tested and shown in Fig. 7. From the Table V given below, we get the results.

TABLE V: TENSILE STRENGTH OF PLA AND COMPOSITE FILMS

Code	PLA	Mango	Tensile Strength (MPa)	% Elongation
PLA	100	0	34.7	3.8
PLUMM5	95	5	33.9	2.55
PLUMM10	90	10	34.2	2.77
PLUMM15	85	15	32.1	3.1
PLMM5	95	5	34.5	2.9
PLMM10	90	10	33.4	3.5
PLMM15	85	15	32.9	2.86

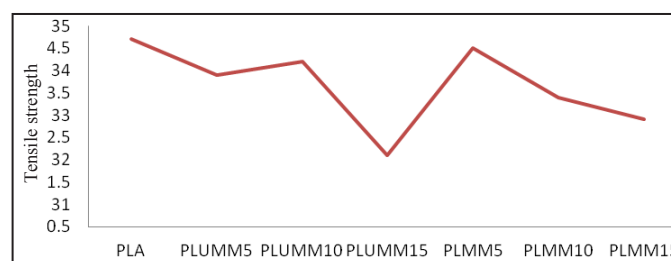


Fig. 7: Tensile Strength of Net PLA and Composite Films

Tensile strength of PLA and starch composites is illustrated as there is decrease in the tensile strength with the addition of starch. Based on the result obtained, it can be observed that samples having a higher content of starch typically give a lower strength. This is because having a higher starch content generally gives a relatively weak PLA and starch adhesion. As the starch content increases it also decreases the effective cross section area of the continuous phase and the PLA matrix becomes discontinuous resulting in decrease in strength.

Like the tensile strength of the films, the % elongation of films is lower with the addition of starch to it. The maximum tensile strength is given by the virgin PLA film. i.e. 34.7. The PLMM5 film shows almost the similar tensile strength of 34.5.

F. Soil Burial Test

The PLA and PLA/starch composites are introduced to soil burial test for a total of 14 days. The values obtained from the result was given in the below Table VI and Fig. 8.

TABLE VI: SOIL BURIAL TEST FOR PLA AND COMPOSITE FILMS

Film	Initial Weight (Grams)	After 7 Days (Grams)	After 14 Days (Grams)
PLA	0.0723	0.0723	0.0720
PLUMM5	0.074	0.0720	0.0701
PLUMM10	0.0699	0.0697	0.0689
PLUMM15	0.073	0.0698	0.0679
PLMM5	0.0715	0.0707	0.0620
PLMM10	0.0719	0.0712	0.0639
PLMM15	0.0725	0.0720	0.0618

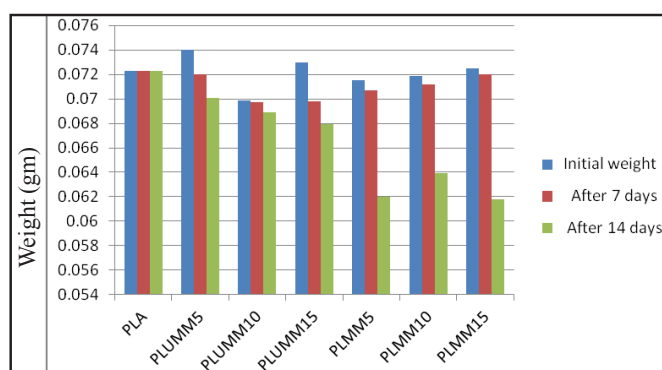


Fig. 8: Soil Burial Test of Net PLA and Composite Films

Biodegradation of PLA is generally slow in ambient temperature in soil. The slow degradation rates of PLA are due to its high molecular weight. For PLA to degrade and disintegrate quickly, it will need to be placed under high temperature and humidity, as in active compost. Biodegradation of the starch begins by hollowing out the polymer matrix. This is associated with the loss of mechanical properties, increased permeability and greater surface to volume ratio and this will facilitate further abiotic degradation process. With increasing starch content, the degradation rate increased. This is because starch is easily degraded in soil. Because poor interfacial adhesion between the PLA matrix and Starch would increase the surface area of starch, and thereby, facilitate its degradation during exposure to microorganisms in soil. Pure PLA did not show any degradation.

G. Differential Scanning Calorimetry (DSC)

The percentage of crystalline PLA in the composites was examined using DSC. The thermograms of PLA and PLA/starch composites are given below and also the thermal characteristics of the samples are shown in Fig. 9.

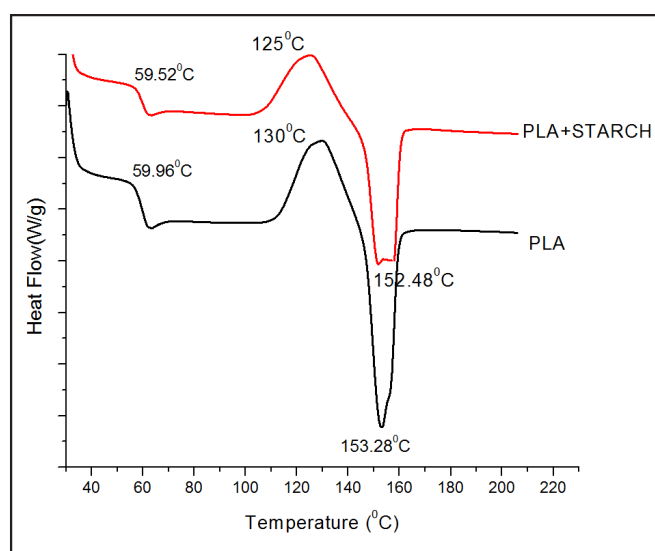


Fig. 9: DSC of Neat PLA and PLA/Starch Film

From the graph it is obtained that the, PLA starch film also showed three thermal transition as in virgin PLA film. With the addition of starch there is no drastic change in the T_g and T_m of PLA film. But the crystallization temperature of PLA film is decreased with the addition of starch. The crystallization temperature of PLA film is 153 °C and it is decreased to 125 °C for the PLA starch film. This reduction in temperature may be due to the amorphous nature of starch.

TABLE VII: DSC FOR PLA AND COMPOSITE FILMS

Sample	T _g (°C)	T _c (°C)	T _m (°C)
PLA	59.96	130	153.28
PLA + Starch	59.52	125	152.48

VI. CONCLUSIONS

In this study, we develop starch based bio composite materials and studied the properties of these films. Biocomposites were prepared with the help of PLA and mango starch using the method of solvent casting. Mechanical properties of the composites such as tensile strength, elongation at break, and toughness decreased with increase in starch content, whereas the Young's modulus increased. However, there was no significant effect on yield strength. Water uptake increased with increase in starch content and was highest when the composites were completely immersed in water. It is well known that the degradation of PLA is very slow in soil. On the other hand, starch is easily biodegraded by microorganisms such as fungi and bacteria in soil. So, the starch is used as filler in the

composite would increase the degradation rate of the composite. When compared with the modified starch/PLA composites, the unmodified starch/PLA composites have better degradation. The hydrophilic character of natural starch is responsible for the water absorption in the composite. There for a higher starch content led to a higher amount of water being absorbed. As the percentage of starch increases, water absorption increases. Based on this study, it can be summarized that neat PLA are generally slow to degrade in soil or in other words it do not show any degradation in the soil. So we can say that, as the starch content increases, the tensile strength of the film decreases. The addition of mango starch would give a weak adhesion against the PLA surface and a larger surface area for degradation. Thus, resulting in decreases of tensile strength and elasticity. Other than the presence of mango starch, the increment in the amount of mango starch added in the samples were also observed to decrease the tensile strength and elasticity as it would give a weaker adhesion against the PLA surface.

ACKNOWLEDGEMENT

The Opportunity given by Centre for Bio-Polymer Science and Technology Eloor, Udyogamandal P.O., Kochi – 683501 (CBPST) (A Unit of CIPET), Kochi, Kerala, India, Dept. of Chemicals & Petrochemicals, Ministry of Chemicals & Fertilizers, Govt. of India and Majlis Arts and Science College Puramannur, Majlis Nagar, Malappuram Dist., Puramannur, Kerala, India, to carry out this piece of work, is gratefully acknowledged.

REFERENCES

- [1] D. Hull, and T. W. Clyne, *An Introduction to Composite Materials*, Cambridge University Press, Cambridge, 1996.
- [2] J. John and S. Thomas, "Bio fibres and Biocomposites," *Carbohydrate Polymers*, vol. 71, no. 3, pp. 343-364, 2008.
- [3] S. V. Prasad, "Natural-fibre-based composites," in *Concise Encyclopaedia of Composite Material*, by Kelly A. (ed.) Pergamon, New York, pp. 197-199, 1989.
- [4] F. L. Mathews, and R. D. Rawlings, *Composite Materials: Engineering and Science*, Chapman & Hall, London, 1994.
- [5] Hanselka, "Fibre composites of raw renewable materials for the ecological lightweight design," *Materialwiss Werkst*, vol. 29, pp. 300-311, 1998.
- [6] Ivens, H. Bos, and I. Verpoest, "The applicability of natural fibres as reinforcement for polymer composites," *In EC Symposium at the International Agricultural Centre Renewable Biproducs: Industrial Outlets and Research for the 21st Century*, Wageningen, The Netherlands, 1997.
- [7] J. E. Potts, R. A. Clendinning, W. B. Ackart, and W. D. Niegisch, "The biodegradability of synthetic polymers," in J. Guillet (ed.) *Polymers and Ecological Problems*, Polymer Science and Technology, vol. 3, Springer, US, pp. 61-79, 1973.
- [8] R. Simpson, and S. Selke, "Composite materials from recycled multilayer polypropylene bottles and wood fibers," *Polymer Preprints (American Chemical Society, Division of Polymer Chemistry)*, vol. 32, pp. 148-149, 1991.
- [9] P. K. Mallick, "Fiber-reinforced composites: Materials, manufacturing, and design," (3rd ed.), *Fiber Reinforced Composites*, Marcel Dekker, New York, 1993.
- [10] Satyanarayana, A. Kulkarni, K. Sukumaran, S. Pillai, P. Cherian, and P. Rohatgi, "Performance of banana fabric-polyester resin composites," *In Proceedings of the International Conference on Composite Structures*, London, Applied Science, I. H. Marshall, (ed.), pp. 535-548, 1983.
- [11] Ramesh, J. R. Mitchell, and S. E. Harding, "Amylose content of rice starch," *Starch*, vol. 51, no. 8-9, pp. 311-313, 1999.
- [12] R. F. T. Stepto, "Understanding the processing of thermoplastic starch," *Macromolecular Symposia*, vol. 245-246, no. 1, pp. 571-577, 2006.
- [13] Primarini, and Y. Ohta, "Some enzyme properties of raw starch digesting amylases from *streptomyces* sp.," *Starch*, vol. 52, no. 4, pp. 28-32, 2000.
- [14] S. N. Maiti, and K. Singh, "Influence of wood flour on the mechanical properties of polyethylene," *Journal of Applied Polylmer Science*, vol. 32, no. 3, pp. 4285-4289, 1986.
- [15] Schwach, and L. Avérous, "Starch-based biodegradable blends: Morphology and interface properties," *Polymer International*, vol. 53, no. 12, pp. 2115-2124, 2004.
- [16] R. A. Wallace, J. L. King, and G. P. Sanders, "Biology-The science of life," Goodyear Publishing Company, California, 1981.
- [17] Avérous, C. Fringant, and L. Moro, "Plasticized starch-cellulose interactions in polysaccharide composites," *Polymer*, vol. 42, no. 15, pp. 6565-6572, 2001.
- [18] J. Choi, C. H. Kim, J. K. Park, "Synthesis and characterization of starch-g-polycaprolactone copolymer," *Macromolecules*, vol. 32, no. 22, pp. 7402-7408, 1999.
- [19] R. Auras, B. Harte, S. Selke, and R. Hernandez, "Mechanical, physical, and barrier properties of poly(lactide) films," *Journal of Plastic Film Sheet*, vol. 19, no. 2, pp. 123-135, 2003.

- [20] J. C. Bogaert, and P. Coszach, "Poly (lactic acids): A potential solution to plastic waste dilemma," *Macromolecular Symposia*, vol. 153, no. 1, pp. 287-303, 2000.
- [21] C. Way, D. Y. Wu, D. Cram, K. Dean, and E. Palombo, "Processing stability and biodegradation of Polylactic Acid (PLA) composites reinforced with cotton linters or maple hardwood fibres," *Journal of Polymers and the Environment*, vol. 21, no. 1, pp. 54-70, 2013.
- [22] Li, and M. A. Huneault, "Comparison of sorbitol and glycerol as plasticizers for thermoplastic starch in TPS/PLA blends," *Journal of Applied Polymer Science*, vol. 119, no. 4, pp. 2439-2448, 2011.
- [23] W. Jang, B. Y. Shin, T. J. Lee, and R. Narayan, "Thermal properties and morphology of biodegradable PLA/starch compatibilised blends," *Journal of Industrial and Engineering Chemistry*, vol. 13, no. 3, pp. 457-464, 2007.
- [24] J. Leadprathom, S. Suttireuengwong, P. Threepopnatkul, and M. Seadan, "Compatibilized polylactic acid/thermoplastic starch by reactive blend," *Journal of Metals, Materials and Minerals*, vol. 20, no. 3, pp. 87-90, 2010.
- [25] K. Sriroth, R. Chollakup, K. Piyachomkwan, and C. G. Oates, "Biodegradable plastics from cassava starch in Thailand," pp. 538-541, 2000.
- [26] Y. Tokiwa, and B. P. Calabia, "Biodegradability and biodegradation of poly(lactide)," *Applied Microbiology and Biotechnology*, vol. 72, no. 2, pp. 244-245, 2006.
- [27] Y. Tokiwa, B. P. Calabia, C. U. Ugwa, and S. Aiba, "Biodegradability of plastics," *International Journal of Molecular Sciences*, vol. 10, no. 9, pp. 3723-3732, 2009.