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Preparation of poly(lactic acid) from *Prosopis juliflora* and incorporation of chitosan for packaging applications

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The biodegradable polylactic acid (PLA) materials, environmentally friendly alternatives to petroleum-derived plastics, and fermentative production of optically pure lactic acid from cheap raw materials have aroused interest among researchers in the recent years due to its high potential for packaging applications. In this study, we have experimented for the lactic acid production using *Lactobacillus delbrueckii* MTCC 911 with *Prosopis juliflora* as a substrate for fermentation. As a result, 38.23 g/L of lactic acid was produced. Modified ring-opening polymerization with direct polycondensation method was followed to convert lactic acid into polylactic acid, and membrane prepared with 0.25 mm thickness having PLA/chitosan 60/40 composition shows better results with a tensile strength of 17.809 MPa and an elongation at break of 300.11%. The oxygen transmission rate results show low permeability of 1614.21 (cm^3/(m^2·day·atm)). Compatible PLA/chitosan membrane so produced by solvent casting shows good thermal stablity and less permeability to oxygen and increased mechanical properties. This was evident from the instrumental analysis of Fourier-transform infrared spectroscopy, thermogravimetry/derivative thermogravimetry, scanning electron microscope, and high-performance liquid chromatography results.

[**Key words:** Biomembrane; Biopolymer; Polylactic acid; Lactic acid; *Prosopis juliflora*]

Biopolymers yield high interest in the field of packaging and pollutant eradication. The composite membrane such as polylactic acid (PLA)-blended chitosan is an attractive route for producing new polymeric materials with tailored properties without having to synthesize completely new materials. Other advantages of polymer blending are versatility, simplicity, and cost-effectiveness. The physical and mechanical properties of the blends are very much dependent on the state of mix and miscibility between the constituent components. Cheap raw materials such as starch, cellulose, molasses, and whey have been used for the production of lactic acid by fermentation (1). Starchy materials like sorghum, wheat, potato, rice, and barley, and cellulosic materials like corn cob, waste paper, and wood have also been used for lactic acid production (2). In the family of renewable source-based polymeric materials, starch is an inexpensive, abundant natural resource and increasingly used as a packaging material (3). Besides starch, inexpensive and abundant source, such as carbohydrates can also be used as the substrate for the production of lactic acid, which offers a new way of producing lactic acid by microorganisms. Many polymers such as polyhydroxybutyrate (PHB), lactic acid were produced using fermentation of cassava starch, mango kernels, mango peel waste, and tamarind seed; PLA can be obtained from lactic acid produced by chemical synthesis or by fermentation. The aim of this study was to find an alternative substrate as the replacement for the existing substrate used for the production of lactic acid. *Prosopis juliflora*, commonly known as mesquite seed, is considered as an agricultural weed in India. By substituting starch from *P. juliflora*, a range of new applications is possible due to the characteristics of starch availability, biodegradability, low cost, and easy chemical and physical modifications.

The author's previous work (3) was on the production and characterization of cellulose-reinforced starch films with high thermal stability and barrier properties; however, when used in packaging, the material is subject to degradation and lacks hydrophobic property. This research article will overcome these disadvantages by using PLA blended with chitosan as the food packaging material synthesized from inexpensive agricultural resources. Currently, more research works were performed on the materials prepared using starch, because of their biodegradable and renewable properties (4–6). Mesquite is a common name for many plants in the genus *Prosopis* that consists of more than 40 species of small leguminous trees. It grows as a small shrub in shallow soils or as tall as 15 m in deep soil with adequate moisture and forms a rounded canopy nearly as wide with multiple trunks. The multitude of branches with bipinnate leaflets of light green to blue hue color cast a light to deep shade depending on the species. A flat pod of beans can grow from 5 to 15 cm long. The dried whole pod is edible and ground into flour, which is used to make bread in Africa and

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deserted places (7). Mesquite plants/trees are considered as weeds in agricultural land and they are used as firewood in India (Tamil Nadu). They can be easily planted in large numbers in poor and low-rainfall lands. Without changing the vegetation, it will vastly improve the microclimate and convert the dry areas into green and highly productive forests, even during extremely dry climate. It will largely solve the problem of lack of fodder during the annual dry season and during the prolonged droughts. It is the most suitable tree for urban forestry and for municipal forests in the cities of the semi-arid region (7,8).

PLA is usually obtained by polycondensation of D- or L-lactic acid or by ring-opening polymerization (ROP) of lactide, a cyclic dimer of lactic acid. Two optical forms exist D-lactide and L-lactide. The natural isomer is L-lactide and the synthetic blend is DL-lactide. PLA is a biodegradable aliphatic polyester derived from renewable resources (9). It has extensive applications in the biomedical fields, such as surgical sutures, bone fixation, drug delivery, and tissue engineering (10). PLA is a hydrophobic polymer due to the presence of $-CH_3$ side groups. It is considered as a food-grade plastic in packaging industries. The basic building block of PLA is the lactic acid; it is a simple chiral molecule that exists in two enantiomeric, optically active forms, namely L- and D-lactic acid. It can be obtained by fermentative or chemical synthesis. The petrochemical scheme of monomer production was prevalent until about 1990. Today, the most popular route is fermentation, in which sugars and starches are converted into lactic acid by bacterial fermentation using an optimized strain of *Lactobacillus* sp. (11). There are two important methods for PLA synthesis: direct polycondensation of lactic acid and ROP of the lactic acid cyclic dimmer, known as lactide. In direct condensation, the solvent is subjected to higher reaction times. The resulting polymer is a material of low to intermediate molecular weight. ROP of the lactide needs catalyst but results in PLA with controlled molecular weight. Depending on the monomer used and by controlling the reaction conditions, it is possible to control the ratio and sequence of D- and L-lactic acid units of the final polymer (12). Polymers from natural sources have been reported to blend with chitosan for the development of functional films. Starch, with low cost, wide availability, and biodegradability, is one of the most important renewable polysaccharides derived from plants.

Chitosan blended with starch has good film-forming capacities, reduced bacterial adhesion on the packaging, great antioxidant activity, and improved water vapor barrier property, which proved to be promising characteristics for effective packaging. Chitosan/biopolymer films, with combinational properties, were widely researched for its applications in food packaging. These biopolymers such as polysaccharides, proteins, extracts, organic acids are biodegradable, nontoxic, and biocompatible (13). This research work is based on the fermentation of mesquite seed starch, which is not widely used as a fermentative source. The novelty of the work relies in the substrate selection and the conversion of the product lactic acid into PLA, which is further blended with chitosan for producing effective packaging membranes.

MATERIALS AND METHODS

Raw materials Mesquite (*P. juliflora*) pods were collected from the surrounding local agricultural fields of Chidambaram, India. *Lactobacillus delbrueckii* MTCC 911 was purchased from IMTECH (CSIR) Chandigarh, India. Chitosan and all the chemicals used in this study were procured from SRL Media, Maharashtra, India (analytical grade).

Extraction and characterization of *P. juliflora* (mesquite) pods The process of synthesizing mesquite seed starch involves roasting followed by pulverizing. The mesquite seeds were roasted in an oil-fired roaster and decorticated to remove skin. Seeds were then broken into smaller pieces using a grinder. These broken pieces are then finally pulverized to make starch powder. Mesquite seed starch has been defatted using methanol at 65°C for 10 h followed by *n*-hexane at 69°C for another 10 h in a Soxhlet extractor. The defatted starch was blended with 70% ethanol and 0.1 M NaOH for 5 min in each solvent, respectively. The filtrate was centrifuged at 11,000 $\times g$ for 15 min. The starch powder residue thus obtained was mixed with water, refiltered twice using 200 mesh screen and Whatmann analytical grade no. 5 filter paper with 2.5-μm pore size, and then washed successively with 0.1 M NaOH and deionized water. Finally, the starch powder was dried in an oven at 50°C till it reaches constant weight. Prepared starch has been packed and stored in polythene-lined gunny bags at −5°C for further studies. Total starch content was analyzed using the modified method of Association of Analytical Communities (14). However, 3,5-dinitrosalicylic acid, popularly known as DNSA, was chosen as a reagent instead of glucose oxidase–peroxidase–aminoantipyrine buffer mixture as mentioned in the official method. The protein and ash content were determined by AOCS Official Methods (15,16). The particle size distribution of starch was measured, in water, with a laser diffraction system (H1140; Sympatec Inc., Pennington, NJ, USA). The micrographs were obtained through scanning electron microscope with EDS (Hitachi-S3400N, Hitachi, Tokyo, Japan) at the Department of Mechanical Engineering, Anna University, Chennai. The starch grains were scattered on the surface of a double-sided tape which is attached to a stub and coated with gold. The images were taken at an accelerating potential of 10 kV.

Optimization for the production of lactic acid from *P. juliflora* pods *L. delbrueckii* MTCC 911 stock culture was maintained in de Man, Rogosa, and Sharpe (MRS) growth medium. The MRS medium had the following composition (g/L): glucose (20.0), peptone (10.0), yeast extract (5.0), meat extract (10.0), sodium acetate (5.0), ammonium citrate (2.0), K_2HPO_4 (5.0), $MgSO_4 \cdot 7H_2O$ (0.1), and $MnSO_4 \cdot 4H_2O$ (0.05). The pH was adjusted to 6.0 prior to sterilization at 121°C for 15 min. The inoculum was prepared through the transference of 2% of stock culture to Erlenmeyer flasks containing growth medium (MRS). The incubation temperature was maintained at 35 ± 1°C for 18 h at 150 rpm.

Substrate preparation and fermentation Carbohydrates present in the form of starch, celluloses, hemicelluloses, and fibers are not suitable for bacterial diet. Therefore, they were converted into soluble sugars by hydrolyzing with mineral acid (sulfuric acid). The mesquite seed powder solution was prepared at different concentrations (5%, 10%, and 15%) to which 1 mL of 20% H_2SO_4 was added to hydrolyze the carbohydrates. The acidified solution of the sample was heated in a boiling water bath for 20 min. After hydrolyzation, the solution was completely dried to produce hydrolyzed mesquite seed powder (substrate). Different concentrations of the substrates were prepared using mineral salt media (M9) in distilled water. The pH of the medium was adjusted to 6.0 with 4.0 M KOH prior to sterilization. To the above solution, 2% of yeast extract and 1% of peptone were added and made up to 1000 mL with distilled water. The medium containing varying substrates concentration (5%, 10%, and 15%) was inoculated with 2 mL of *L. delbrueckii* culture (4×10^8 CFU/mL) and anaerobically maintained at 37°C.

All the culture flasks of the 1000 mL volume containing 500 mL fermented medium was done in triplicates. The sample culture was collected at different time intervals (24–120 h) and centrifuged in a high-speed centrifuge at 4830 $\times g$ for 10 min to collect the supernatant (crude lactic acid) for further use.

Extraction and purification of the culture filtrate Samples were withdrawn at equal time intervals of incubation period from 24 to 120 h and treated with 1 M H_2SO_4 to release the lactic acid from the medium as it is formed as calcium lactate with a buffering agent, $CaCO_3$ (17,18). Lactic acid was extracted out from the medium and the extract was purified and decolorized using activated carbon or charcoal. A colorless solution was obtained which was diluted to the required level using double distilled water, then added to ethyl acetate in the ratio 1:2, sonicated for 30 min, separated using a separating funnel, and then finally distillated to obtain pure lactic acid. This lactic acid was then confirmed by instrumental analysis.

Analytical methods The total reducible sugar was determined by the DNSA method and the absorbance of the sample was read at 540 nm using a double beam UV–Vis spectrophotometer. The lactic acid content of the fermented samples was determined using the colorimetric method according to the method described (19). An aliquot (0.5 mL) of the final clear solution obtained from above was placed in a screw-capped tube of 16 × 150 mm borosilicate tubes. Thereafter, 3 mL of concentrated H_2SO_4 was added and the tubes were heated to boiling for 10 min. On cooling, 50 μL of $CuSO_4$ reagent and 100 μL of *para*-phenyl phenol (1.5% *para*-phenyl phenol dissolved in 95% ethanol) were added. The tubes were then incubated at 30°C for 30 min until the precipitate was dissolved and the absorbance reading of the resulting solution was taken at a wavelength of 570 nm.

Detection of lactic acid by high-performance liquid chromatography High-performance liquid chromatography (HPLC) was employed to analyze the lactic acid present in the fermentation broth [C-18 HS reverse phase HPLC column water X-Bridge C-18 (250 × 4.6 mm) 5 μm particle size maintained at 40°C by a column heater]. The detector used was Waters 2489 (UV/Vis) detector and 515 isocratic pump. Mobile phase A: 1.0 mL of *ortho*-phosphoric acid in 1000 m; mobile phase B: acetonitrile (pH 2.5) at flow rate of 0.8 mL/min. Samples for HPLC were prepared by centrifuging the samples at 3354 $\times g$ for 10 min in a centrifuge, and the resulting supernatant was filtered through a 0.2-μm polyether sulfone (PES) membrane. For all the analyses, 20 μL of

diluted sample was injected. Concentrations of lactic acid were determined by comparison of area of sample with a standard curve generated using L (+) lactic acid (99%) stalk solutions.

Conversion of lactic acid to PLA The lactic acid produced from the fermented medium was subjected to ROP with modified one-step polycondensation method and converted into a biopolymer called PLA (Fig. 1). The lactic acid obtained was mixed with 400 mL of xylene and 0.01% weight of stannous chloride was added as a catalyst to initiate the reaction. The reaction was carried out at 120°C for 3 h at an inert atmosphere by sparging with nitrogen gas where the water was removed completely by condensation and then the temperature was raised to 138°C for 8–24 h. The reaction was carried out till the composition of the reaction mixture was reduced to half of the initial volume. Then the temperature was maintained at around 145°C to remove excess xylene. The obtained product was then washed with methanol of volume five times and the precipitate obtained was dried and obtained as a white powder. The white powder obtained was PLA, a biodegradable polymer, which was analyzed and confirmed using Fourier-transform infrared spectroscopy (FTIR).

Characterization of lactic acid and PLA using FTIR FTIR was used to determine the functional groups, chemical bonds, and the chemical nature of the sample. The FTIR analysis was performed using Perkin-Elmer Co. (Waltham, MA, USA) model spectrum with samples dispersed in the pellets of KBr. The lactic acid and produced PLA were analyzed. The spectral measurements were carried out in an absorbance range of 400–4000 cm^{-1} with plain KBr pellets as the background reference.

Molecular weight determination with matrix assisted laser desorption/ionization-time of flight mass spectrometry Matrix assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF) spectra were acquired using a Bruker Daltonics, Bremen, Germany Flex analysis using chloroform as solvent and scan range was about 1600–3950 *m/z*; the PLA sample was analyzed at Central Leather Research Institute-Centre for Analysis, Testing, Evaluation and Reporting Services (CLRI-CATERS) (Chennai, India).

Conventional PLA/chitosan conventional membrane preparation The biomembrane was prepared using PLA and chitosan through the solvent casting method. Initially, Chitosan and PLA were separately prepared at a concentration of 1% w/w, using chloroform as the solvent. Slight stirring was used to expedite the dissolution and to homogenize the solutions. Blend films of different compositions (i.e., the weight ratios between chitosan and PLA of 100:0, 80:20, 60:40, 50:50, 40:60, 20:80, and 0:100) were prepared by casting a mixture of the solutions of a specified composition on a Teflon dish. Each cast film was left to dry at room temperature for 24 h and later at room temperature in a vacuum for another 48 h. Then, they were analyzed for characterizing their mechanical and thermal properties.

Thermal analysis Thermogravimetry/derivative thermogravimetry analyses were performed for the PLA/chitosan membrane using TA Instruments STD Q500 (New Castle, DE, USA). Samples of about 10 (±3) mg were placed into alumina pans. The samples were heated at a rate of 5°C/min from ambient temperature to 800°C in a nitrogen atmosphere with a sample flow of 60 mL/min and a balance flow of 40 mL/min, to avoid the production of unwanted by-products in the presence of oxygen during analysis. The experiments were conducted in duplicate.

Mechanical tests The tensile strength of the PLA/chitosan membrane was investigated using Universal Testing Machine (UTM, H10KS; Salfords, Tinius Olsen, UK) according to the American Society for Testing and Materials (ASTM) standard method (20). The samples had a dimension of 150 mm × 25 mm × 0.048 mm with a gauge length of 25 mm at a cross head speed of 10 mm/min. The tensile strength was expressed in MPa.

Oxygen permeability The oxygen transmission rate (OTR) of the membrane was calculated by Oxtran oxygen permeability tester (MOCON, Minneapolis, MN, USA) at 23°C under the condition of 0% relative humidity at 1 atm according to the ASTM standard method (21). The specimens were conditioned at ambient conditions. The oxygen permeability was calculated using Eq. 1.

$$\text{Oxygen permeability} = \frac{\text{OTR}}{\text{Film thickness} \times \text{O}_2 \text{ partial pressure}} \quad (1)$$

Water vapor permeability test Water vapor permeability (WVP) test was performed according to ASTM standard method (22). Film samples were used to seal a hole on the top of a plastic cell. The plastic cell, containing distilled water, was loaded into a desiccator. The desiccator was placed into a chamber at 25°C for 24 h. At least three weights of the cell were taken during the 24 h of storage. WVP was calculated with Eq. 2.

$$\text{WVP} = \frac{\Delta m \cdot l}{A \cdot \Delta t \cdot \Delta p} \quad (2)$$

where $\Delta m/\Delta t$ is the weight of moisture loss per unit of time (g/h), A is the film area exposed to moisture transfer (m^2), l is the film thickness (m), Δp is the water vapor pressure difference between the two sides of the film (Pa).

$$\Delta P = (\Delta RH/100)\, P_{\text{vap sat}} \quad (4)$$

Linear regression was used to estimate the slope of the g/h plot. Δp was calculated according Eq. 3, where $P_{\text{vap sat}}$ is the saturated vapor pressure of pure water and equals to 3160 Pa at 25°C and ΔRH is the relative humidity gradient between the cell and the surroundings.

Biodegradation of PLA/chitosan membrane Soil burial degradation test was carried out from 1 to 8 weeks, the PLA/chitosan membranes were weighed precisely and then incubated in the pot size of 23 × 18 cm, filled with vermi compost and soil in the ratio of 20:80, and finally was incubated at 37°C for 8 weeks. The membranes were taken in the dimension of 3 cm × 2 cm. The materials were cleaned, dehydrated, and weighed to calculate the weight loss rate (WLR).

$$\text{WLR} = (\text{Wo} - \text{W})/\text{Wo} \times 100\% \quad (3)$$

The morphology of the soil treated and the control samples were analyzed using scanning microscope.

RESULTS AND DISCUSSION

Extraction and characterization of starch from mesquite (*P. juliflora*) pods The proximate composition of mesquite starch has been analyzed; the major initial components such as moisture, total ash, total fat, protein, and carbohydrate values were 10.19, 4.83, 1.9, 13.1, and 70.0 g/100 g, respectively. Its total energy value was found to be 349.5 kcal. The total starch percentage shows 23% and 35% total sugar content of the seed. In a previous study on starch concentration in *Prosopis* mesocarp (the middle layer of the pod), it contains high or low concentrations of starch (23,24). The

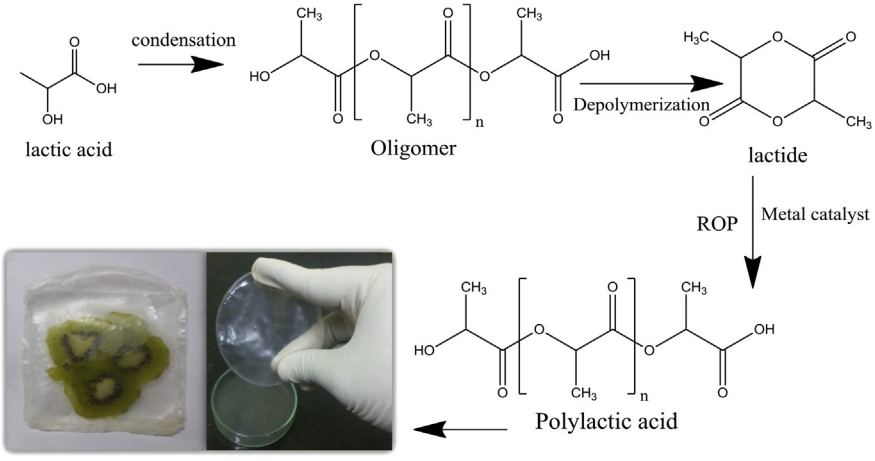

FIG. 1. Ring-opening polymerization method for the conversion of lactic acid into polylactic acid.

proximal composition results shows that, it is rich in carbohydrate and energy value, carbohydrate is comparatively high which can be converted in to simple sugars and then to biopolymer by the fermentation method. Fig. 2A–C shows the raw *P. juliflora* pods, powdered seeds before preprocessing, and defatted samples stored in sealed bags for analysis and further experimentation. Particle size distribution analyses of four different particle size fractions can be observed in Fig. 2B. About 70–80% of the granules were observed in the size ranges from 60 to 90 mm and 90–130 mm. The remaining 20–30% of them were in the range of 0–30 and 30–60 mm. Particle sizes ranging from 3 to 8, 3 to 24, 2 to 35, and 5 to 70 mm have been reported for rice, maize, wheat, and potato starches, respectively (2). The SEM of *P. juliflora* starch revealed a rough and porous surface of irregular shape (Fig. 2C). Multiple layers observed in each granule correlate with the previously observed results (25,26). Pods along with the cotyledons grounded well show the diameter of the granules which ranged between 4 and 130 m (for 500 granules).

Optimization of lactic acid production using mesquite seed as substrates After hydrolysis of the starch from mesquite seed by 20% H_2SO_4, the substrates were prepared on varying concentration of 5%, 10%, and 15%. The optimization study (Fig. 3A–C) implies that in 5% substrate concentration the glucose produced was utilized by the bacterium at the initial stage and the cell biomass showing increased number from 24 to 48 h having reducing sugar of 38–21.01 g/L with lactic acid of 12.34 g/L and biomass of 42.11 g/L. The substrate was utilized in the initial lag and log phase and consumption patterns were stationary during 72–120 h having reducing sugar of 18–13.68 g/L, lactic acid production of 15–16.02 g/L, and biomass of 57.35 g/L, further no progress in the production of lactic acid. This implies the sugars were utilized at the log phase and it attains stationary phase immediately due to the depletion of the substrate in the medium (Fig. 3A). The substrate concentration of 10% increases the lactic acid production from 3.22 to 35.07 g/L and the biomass value from 18.25 to 82.35 g/L. The concentration of reducing sugars was also gradually depleted from 62.74 to 11.89 g/L by the bacterium. A maximum of 38.23 g/L of lactic acid was produced in 96 h. For 72–96 h, there was a steady increase in the production of biomass and lactic acid (Fig. 3B). At 15% concentration, the medium was over concentrated with the substrate color and the lactic acid production was limited to the maximum of 8.27 g/L at 72 h having the biomass of 28.24 g/L. This may be due to the increased concentration of the substrate which was not suitable for the bacteria to produce the biopolymer (Fig. 3C). Throughout the optimization study, the pH was maintained at 6 (27). L-Lactic acid of 3.476 g per 5 g of the substrate was produced using rice straw at 37°C using *L. casei* (28). The samples of crude lactic acid were analyzed via HPLC against standard L-lactic acid and the same peak was obtained with respect to the standard at the retention time of 3.27, which confirms the presence of lactic acid in the fermentation medium.

Extraction and purification of lactic acid from mesquite seed starch Incubated medium shows an initial pH of 5 and an optimum temperature of 37°C. The culture medium of 96 h was centrifuged at 6574 $\times g$ for 10 min to remove cells and the supernatant was collected for further analysis. The obtained supernatant was treated with calcium hydroxide and was added to the fermented culture medium to neutralize the acid produced, maintaining the pH around 5–6, and producing a calcium salt of the acid, the calcium lactate (17) followed by precipitation method with mineral acid precipitation reaction (29) and decolorized using activated carbon for 3–5 h and filtered. The decolorized sample was added to ethyl acetate (1:2) and sonicated for 30 min using a sonicator and the organic phase was obtained using a separating funnel. The obtained organic phase was distillated using a distillation column and the pure sample was obtained. FTIR shows the presence of functional groups of lactic acid (Fig. 4), and the strong C=O peak ranges were observed at 1728 cm^{-1}. The O–H bond in the acid group was absorbed between 2500 and 3300 cm^{-1} and the C–H groups were absorbed in the range of 2850–3000 cm^{-1}. The above said peak ranges can be observed.

Characterization of PLA produced from the lactic acid by direct polycondensation method Lactic acid so produced was purified and concentration up to 85% purity was taken for the conversion of PLA process. First, the reaction vessel was totally vacuumed with only nitrogen gas. The obtained lactic acid sample of 200 mL was mixed with 200 mL of xylene, and 0.01% weight of stannous chloride was added as a catalyst to initiate the reaction. The reaction was carried out at 120°C for 3 h at an inert atmosphere

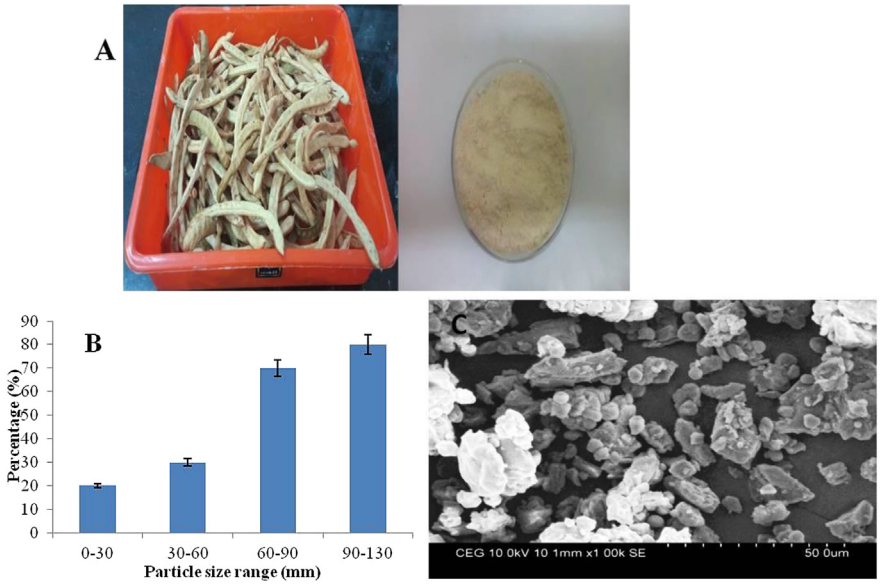

FIG. 2. (A) *Prosopis juliflora* pods collected from in and around village (Tamil Nadu) and grounded and powder. (B) Particle size distribution of the mesquite pods. (C) Scanning electron microscope picture depicting seeds and starch granules of mesquite pods. Length of scale bar: 50.0 μm.

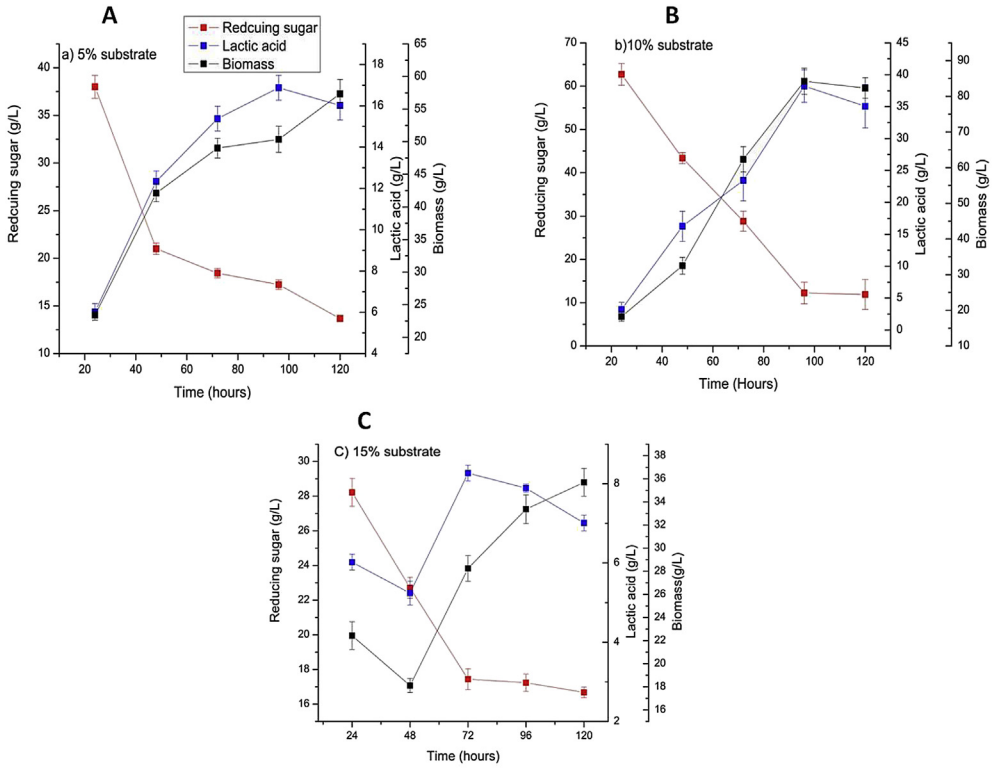

FIG. 3. (A) Five percent of substrate concentration on the effect of reducing sugar concentration on lactic acid and biomass production. (B) Ten percent of substrate concentration on the effect of reducing sugar concentration on lactic acid and biomass production. (C) Fifteen percent of substrate concentration on the effect of reducing sugar concentration on lactic acid and biomass production.

by sparging in nitrogen gas where the water was removed completely by condensation and then the temperature was raised to 138°C for 8–24 h. The reaction was carried out till the composition of the reaction mixture was reduced to half the volume of the initial volume. Then the temperature was maintained at around 145°C to remove the excess of xylene. The obtained product was then washed with the five times the volume with methanol and the precipitate obtained was dried and obtained as a white powder. The white powder obtained was PLA, a biodegradable polymer, and was analyzed and confirmed using FTIR (Fig. 5A), which shows that the O–H bond in the acid group was gradually reduced between 2500 and 3300 cm^{-1} and the polymer characteristic peak of 1750 cm^{-1}

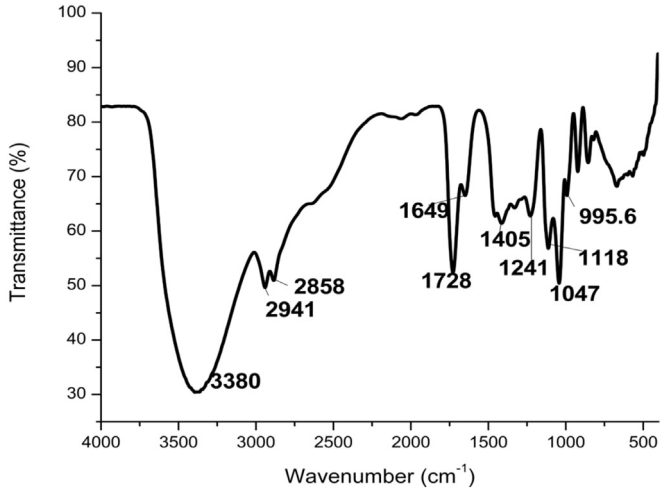

FIG. 4. FTIR showing the functional groups of lactic acid from mesquite seed sample.

strongly appeared. Dean stark apparatus collects the water from the sample and there by polycondensation completed after 48 h. The white powder obtained after methanol wash was used for the confirmation of PLA with FTIR. Fig. 5 shows the presence of PLA after the conversion process. It has lesser O–H group and a strong C=O group in the peak range of 1730.5 cm^{-1}. This confirms the presence of PLA upon conversion. Broad OH group from produced lactic acid (Fig. 5) was reduced and the polymer produced similar results and condensation of polymerization was carried out at 138°C for 48 h; at this time, it is necessary to understand the better activity of $SnCl_2 \cdot 2H_2O$ for polymerization (12). The yield of PLA produced was 54.5 g for 85 g of lactic acid by direct polycondensation method with reference to the study by Gozan et al. (30). The MALDI-TOF mass spectrometry shows a series of intense molecular ion peaks ranging from 1700 to 3950 Da (Fig. 6). Once subtracted the molecular weight of sodium ions, a series of peaks which correspond to multiples of 72 Da, the molecular weight of the lactoyl repeating unit, is clearly identified in the spectra. From these data, it is found that PLA molecules having highest molecular weight of ~2065 Da ($n < 29$, where n is the number of lactic acid units). This is in agreement with Kowalski et al. (31) and Duda and Penczek (32) who observed the existence of macrocyclic PLA as one of the side products of L,L-lactide ROP at 80°C.

Production and characterization of conventional PLA/chitosan composite membrane The composite membrane with chitosan and PLA of 100/0, 80/20, 60/40, 50/50, 40/60, 20/80, and 0/100 was prepared by casting a mixture of the solutions of a specified composition on a Teflon dish. Each cast film was let dry at room temperature for 1 day and later at room temperature in a vacuum for another 2 days (Table 1). All the composite PLA/chitosan samples were tested for its mechanical property.

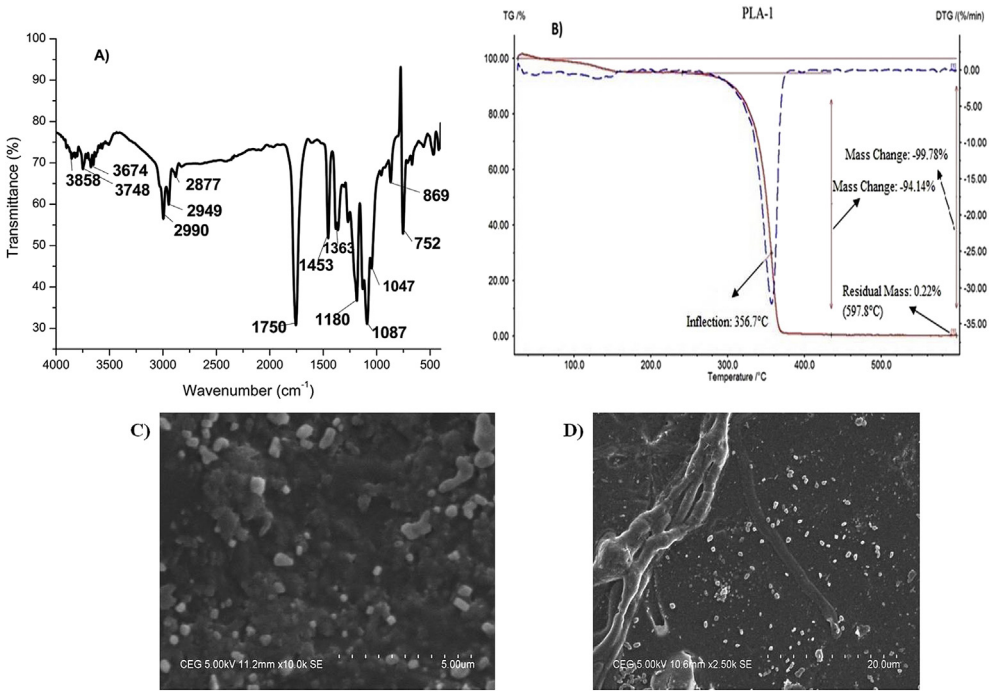

FIG. 5. (A) FTIR of produced polylactic acid (PLA) from lactic acid by direct condensation method. (B) TG—DTG of produced PLA showing thermal stability and gelatinization temperature. (C) Scanning micrographs of produced PLA from mesquite seed. Length of scale bar: 5.0 μm. (D) Scanning micrograph of PLA/chitosan membrane. Length of scale bar: 20.0 μm.

Mechanical tests Tensile strength and elongation results of PLA/chitosan 60/40 composition show better results having a tensile strength of 17.809 MPa with an elongation at break of 300.11%. Although other combination shows an increase in tensile strength, it would be a problem in membrane preparation. High tensile strength is difficult for bending, and hence 60/40 composition gave better results. Both PLA and chitosan are biodegradable and suitable for packaging applications. Because of the high cost of PLA, adding chitosan appears to be a possible approach to develop a cost-effective PLA blends (33).

Thermal analysis The thermal gravimetric curves of PLA/chitosan membrane (Fig. 5B) which clearly describes the initial

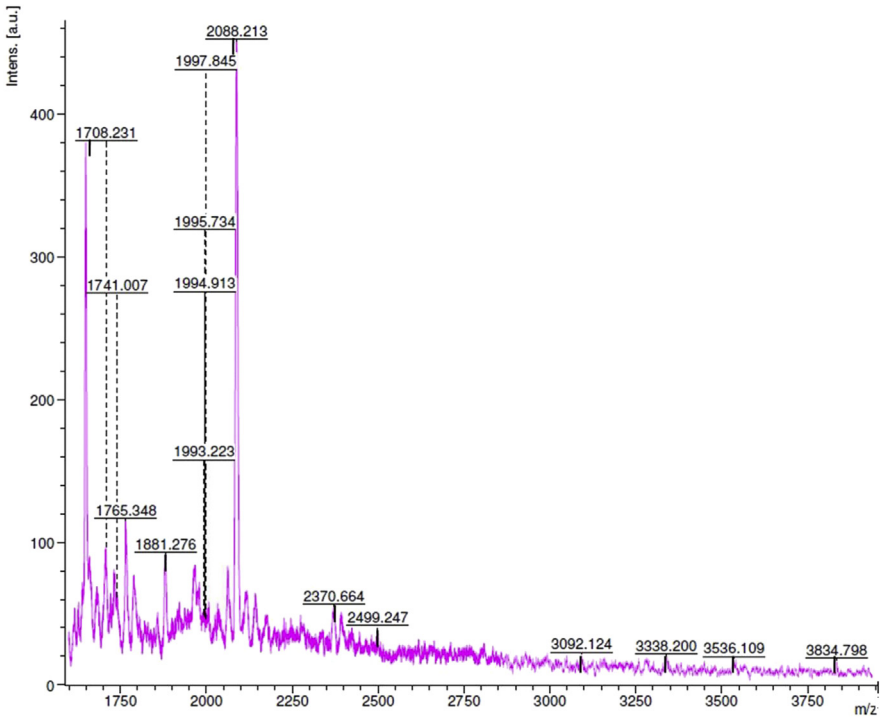

FIG. 6. MALDI-TOF mass spectrometry of polylactic acid.

TABLE 1. Mechanical property of the polylactic acid/chitosan membrane with varying combination.

PLA/chitosan	Tensile strength (MPa)	Elongation (%)	Young's modulus (MPa)
100/0	24.682	261.32	9.41
80/20	20.111	280.13	11.12
60/40	17.809	300.11	5.9
50/50	22.048	222.10	9.9
40/60	26.132	170.11	15.3
28/80	28.223	83.12	33.3
0/100	300.21	72.00	41.6

weight loss of around 5% (w/w) below 150°C may attribute to residual moisture content evaporation (3). Major weight loss of around 94.14% (w/w) was noted between 250°C and 375°C, with major weight loss around 356.7°C. This major weight loss may attribute to the degradation of PLA/chitosan membrane. Residual weight of PLA/chitosan membrane after degradation was only found to be around 0.22% (w/w), this indicates complete degradation of PLA/chitosan membrane without any residual impurities. This complete degradation may attribute to purity of produced PLA from mesquite seed starch.

Morphology analysis The scanning micrograph of PLA (Fig. 5C) shows the regular scattering of PLA in granules and polymer formation. Fig. 5D shows the PLA composed over chitosan; however, slight cracks can be seen due to the film formation by solvent casting, and PLA matrix embedded with chitosan in repeated lamination can be seen and comparatively smooth formation. A packaging material up to 0.25 mm in thickness was generally considered a film, above which it would fall under the category of sheets. The average thickness of the film was determined using the micrometer which was observed as 0.048 mm. Thus, this composite film was considered a film which could be ideal for packaging.

Oxygen permeability The OTR of the PLA/chitosan membrane of 0.25-mm thickness shows 1614.21 ($cm^3/(m^2 \cdot day \cdot atm)$). Lower OTR number explains amount less oxygen is less to through pass the PLA film. As a result, lower OTR number in PLA film shows good barrier properties from oxygen penetration (34,35). It clears, temperature 15°C results a best temperature condition of PLA film to prevent oxygen permeation (36).

Water vapor permeability WVP of optimized PLA/chitosan membrane was found to be 1.83×10^{-10}, 1.12×10^{-10}, and 0.81×10^{-10} g/Pa·h·m for film thickness of 0.25, 0.38, and 0.47 mm, respectively. Increase in thickness of the membrane found to reduce its WVP properties. Low WVP of the PLA/chitosan membrane may be due to the rich hydrophobicity of the PLA. This PLA/chitosan composite film can be used in the food packaging as well as in the medical field for fracture fixation.

Biodegradation of PLA/chitosan membrane Soil burial degradation test was carried out for the PLA/chitosan control sample incubated in open air and another PLA/chitosan membrane of size 3 cm × 2 cm was incubated in the pot containing vermicompost and soil in the ratio of 20:80. In Fig. 7, WLR percentage increases with increase in the weeks incubated in the soil. The control sample shows gradual increase from 0.8% to 4%, whereas the sample which incubated in soil shows WLR percentage from 3.6% to 8.7%. This implies that the hydrophilic property of the chitosan has been modified with the PLA incorporation, which shows increased WLR percentage of 8.7%. There by, this PLA/chitosan membrane can withstand in room temperature for more than 8 weeks, whereas under soil burial method the degradation took only 8 complete weeks, which is evident from Fig. 8A–C. Fig. 8A shows the freshly prepared control film and the sample; Fig. 8B shows the membrane incubated in open air; and Fig. 8C shows the increased porosity and membrane morphology show dilated pores and swellings which is evident for the biodegradation of the PLA/chitosan membrane.

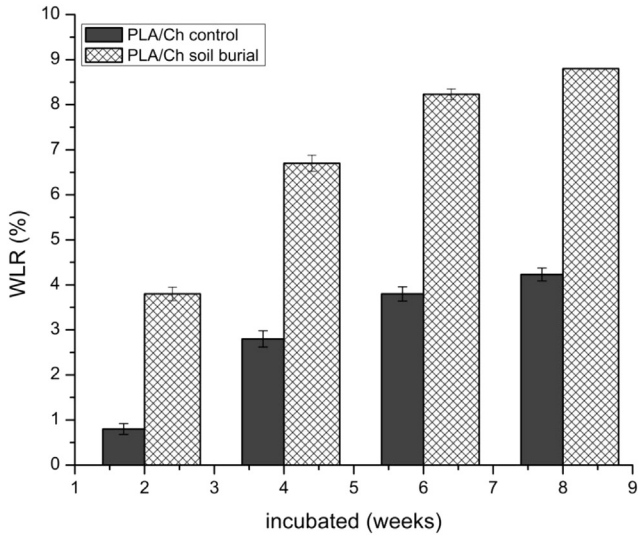

FIG. 7. Biodegradation of polylactic acid/chitosan membrane control versus soil-incubated sample.

DISCUSSION

The amount of starch in *Prosopis* varies based on its species, using it as a food product is still under discussion. Because for the very first time it has been used as a substrate for fermentation. Recent nutrient studies from Santiago del Estero, Argentina, on *Prosopis* flour found the presence of 7% proteins (no gluten), 2% lipids, 3% ash (total mineral), 59% sugar, and 26% fiber (24). Predominantly, this *Prosopis* pods used as a food for fodder.

Choosing natural edible resources such as potato, pea, wheat, and corn for the fermentation procedure can cause food crises in the future; moreover, the costs are comparatively high. *P. juliflora* pods are unused and unwanted product in the agricultural field, as it grows predominantly and covers most of the farming land and has no market value in India. As it is a xerophyte, it adapts to many soil types under a wide range of moisture conditions, which is the cost advantage for choosing it as a substrate for fermentation. This data was referred from the Agroforestree Database (http://www.worldagroforestry.org/output/agroforestree-database).

This research work was focusing about *P. juliflora*, because it is a weed in agricultural fields as well as an invasive weed in certain parts of Asian countries. Selecting a waste weed for the fermentation process is the added advantage for the production of lactic acid, as it contains enough amount of starch for the bacteria to breakdown into sugar, whereas other sources of substrates need preprocessing such as enzyme treatment for the solubilization of starch. Furthermore, the lactic acid is suitable for the PLA conversion.

The reason for choosing *L. delbrueckii* to ferment the natural substrate, based on the literature, which states that attempts have also been made to produce lactic acid from sugarcane molasses as substrate (29). A study by Oh et al. (37) stated that when barley and wheat were used as substrates, even without additional nutrients, lactic acid production amounted to 0.8 g/L h. Substrate concentration of 10% gave the best yield of lactic acid (38.23 g/L), which implies the medium containing 5% substrate starves for the carbohydrate concentration after reaching the log phase. At 15% substrate concentration, the medium was precipitated and the

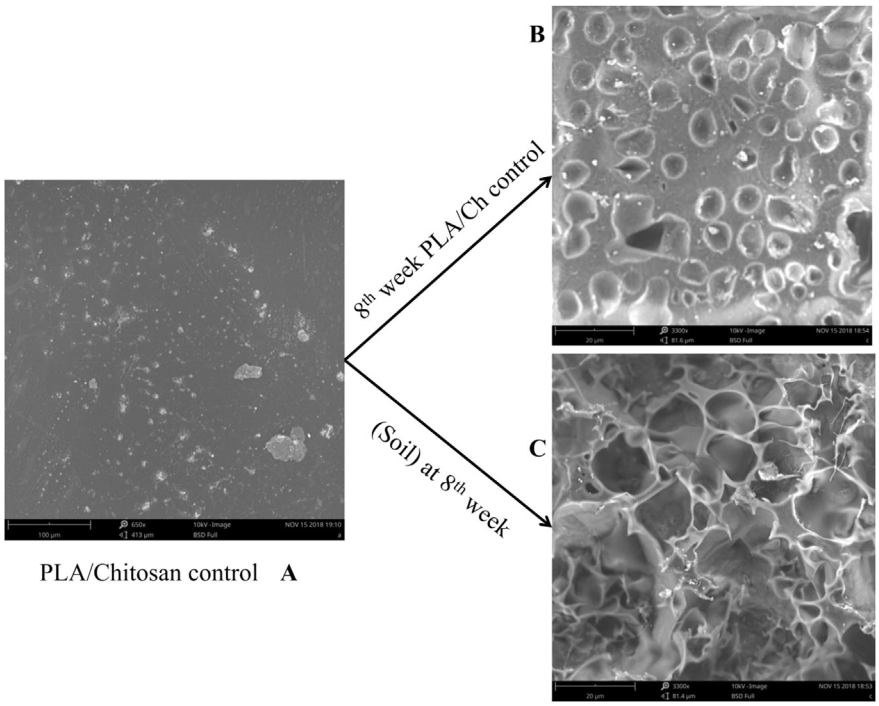

FIG. 8. The scanning electron microscopic images of polylactic acid (PLA)/chitosan membrane subjected to open air and soil burial degradation after 8 weeks of incubation at 37°C. (A) Control PLA/chitosan freshly prepared. Length of scale bar: 100 μm. (B) PLA/chitosan sample incubated in open air. Length of scale bar: 20 μm. (C) PLA/chitosan membrane after 8 weeks. Length of scale bar: 20 μm (soil burial method).

production of the secondary metabolites may cease further production of lactic acid.

Lactic acid was extracted from the fermented medium by precipitation method (18,38). Furthermore, the FTIR results confirmed the presence of C–H functional groups at 2850–3000 cm^{-1}. The direct polycondensation method was used for the conversion of lactic acid into polylactide using $SnCl_2 \cdot 2H_2O$ as the catalyst. The Dean stark apparatus connected to the three-necked round bottom flask collects the water from the sample and there by polycondensation completed after 48 h. The FTIR of the PLA confirmed the presence of functional group of the polymer at 1730.5 cm^{-1}, and the collected water from the sample was evident in the FTIR-reduced O–H functional group. The first novel bioplastic material was synthesized using a waste and cheap raw material mesquite starch as substrate. Chosen *L. delbrueckii* in the culture medium; of the three different carbon concentrations, 10% substrate gave the maximum lactic acid production of 38.23 g/L at 96 h by precipitation method. The UV-Scanning, HPLC, and FTIR results confirm the presence of L-lactic acid. In the conversion of PLA by polycondensation method, a new method was adopted for the effective conversion of lactic acid into PLA. The MALDI-TOFF results shows the PLA molecules having highest molecular weight of ~2065 Da ($n < 29$, where n is the number of lactic acid units). Similar research in which only direct condensation was used, that states one step method to produce PLA, the difficulty in removing water from the reaction medium results in low molecular weight polymer, thus limiting its application. The typical industrial operating condition of ROP for the polymer production is 180°C. At such a high temperature, a significant decrease of polymer molecular weight has been observed (39).

PLA with high molecular weight can be obtained, when reaction pressure was reduced (40). By further optimization of the reacting pressure and the temperature, high molecular weight polymer can be produced. This research articles proves the possibility of obtaining bioplastic such as PLA from the agricultural weed (*P. juliflora*). The membrane PLA/chitosan 60/40 with 0.25-mm thickness having a tensile strength of 17.809 MPa with an elongation at break of 300.11% and an OTR of 1614.21 ($cm^3/(m^2$ day atm)).

The biodegradation of the PLA/chitosan was after 8 weeks of treatment the control and samples results were characterized using scanning electron microscopic images shows major fractions and increased porosity of the membrane and thereby weight reduction in the produced membrane from the controlled one. Fig. 8A shows the PLA/chitosan control membrane, which was kept in the room temperature. Small pores were visible in the sample (20 μm magnification) which was incubated in open atmospheric condition (Fig. 8B). The sample which was incubated in the vermicompost soil shows major difference on the morphology, pores were dilated and major fractions tends to appear on the sample (Fig. 8C). However, the control sample of the soil burial test which had the same composition did not show any difference in degradation. Thus, the study infers that the vermicompost:soil ratio 20:80 is favorable for the biodegradation of the membrane under 8 weeks. The WLR of the composite materials changed slowly compared to that of the pure PLA/chitosan control. This is because chitosan is alkaline, and it can neutralize the acidic product of the PLA, so the self-catalysis is restrained to some degree (41).

This composite bioplastic can be used as a packaging material for any kind of food based on the permeability and humidity conditions. Our continuation of the previous work was successful by achieving the hydrophobic packaging by selecting cheap substrate as raw material; in future, we will do further research using non agriculture renewable resources for the bioplastic production.

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