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RESEARCH ARTICLE

Copolymerization of Lactic Acid for Cost-effective PLA Synthesis and Studies on Its Improved Characteristics

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Abstract The present study involves synthesis of polylactic acid (PLA) using purified lactic acid from fermented broth of Jackal jujube (*Zizyphus oenophlia*). A polyphenolic compound, humic acid (HA) of biological origin was incorporated to the PLA in order to reinforce the PLA chain without compromising its biodegradability and biocompatibility. Under optimized conditions of polymerization, modified L-PLA yield improved up to 93%. The molecular weight was found to be 6.4×10^5. Different physicochemical properties of the polymer were explored for its further application in different fields. Incorporation of intermolecular bond between PLA and HA was confirmed by FT-IR spectroscopy technique. Addition of HA not only reduced the crystallinity of PLA, but also had increased flexibility and elasticity to much greater extent. The results showed that, apart from enhancing the physicochemical properties of PLA, the process also had reduced the production cost of the polymer, while mitigating the demands of environmental protection agencies.

Keywords: polylactic acid, fermented broth, humic acid, toughening, biodegradability, crystallinity

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Introduction

Biopolymers are the class of polymers which have varied application ranging from packaging industries to biomedical sectors. Increased public awareness during last few decades to prevent environmental pollution caused by petrochemical plastics lead to serious research on biodegradable materials. Biopolymers from renewable resources received a great attention from different perspective of material science, both ecologically and biomedically (1), due to the prevailing concerns about the disposal of conventional plastics and the increasing costs associated with fossil fuel-based products (2). Among many biopolymers, polylactic acid (PLA), thermoplastic high-strength polyester, has been explored for different purposes from industrial packaging field to bioabsorbable medical devices (3). It is prepared from renewable resources through combining the knowledge of both biotechnology and chemistry, making it highly biocompatible and biodegradable (4). There are different processes for the synthesis of PLA like direct condensation or ring-opening polymerization of lactic acid (5).

In spite of numerous advantages, its innate brittleness, low toughness, lack of reactive side chains, prolonged degradation period, and intrinsic hydrophobic property has imposed constraints for its applications (6). Thus, different physical and chemical modifications were employed for alleviating the innate properties of PLA. Generally, methods such as copolymerization, blending, incorporation of chemicals, plasma treatment, etc, were executed in order to enhance its property (6-8). During copolymerization, the process initializes simultaneously as the polymerization reaction takes place. Whereas, the processes like blending, incorporations, etc, starts after PLA formation. Thus, they might have increased the reaction time and affect its production cost, in a way reduce its applicability. Therefore, extensive research works were conducted on copolymerization

using various polymers such as polyetherurethane, polyethyleneoxide, polycaprolactone, starch, and other polymers (9-12).

For retaining the biodegradability, PLA copolymerization with degradable polymers is currently gaining high attention. Research using plasticizers such as polyethylene glycol (PEG), polyvinyl acetate, etc, successfully overcome brittleness and widened the application of PLA (13,14).

Humic substances originated from microorganisms, plants, soil, etc, involves physical, chemical, and microbiological transformation of biomolecules. They have a complex structure having functional groups of carboxylic, phenolic, carbonyl, hydroxyl, amine, amide, aliphatic moieties, etc. Being the most explored group of humic substances, their specific properties enable their application in industry, agriculture, environment, and biomedicine (15). None of the study has investigated for using humic acid (HA) as a component to improve the property of polymer. Due to their polyfunctionality, they might interact with other biopolymers as well as with low molecular weight compounds forming complex structure.

The present study aims on the study of synthesis of PLA from purified lactic acid produced from fermented broth of Jackal jujube (*Zizyphus oenophlia*) (16). Jackal jujube is widely found in the subtropical regions of the world having huge amount of starch content. Characterization of the produced PLA was done for its establishment as a plausible biomaterial in the medical field. Effectiveness of HA on the production of high quality PLA was evaluated. Characterization explained interesting findings to understand the structural and chemical change that occurs with the newly formed polyester.

Materials and Methods

Materials The L-lactic acid (90%, LA) was obtained from the fermented broth of Jackal jujube (*Zizyphus oenophlia*) which was purified for its further application. Humic acid (HA) was obtained from Microbial and Downstream Processing Laboratory, IIT Kharagpur. The solvents, chloroform and acetone are HPLC grade and purchased from Merck and used without further purification.

Fermentation method of Jakal jujube and its purification The fermentation was carried out by supplementing the powder of fruits of Jackal jujube with MRS medium at pH 6.5. After sterilization, it was incubated with *Lactobacillus amylophilus* GV6 at 35ºC for 4 days. After fermentation, purification of the LA from crude fermented broth was performed using simultaneous anion and cation exchange chromatographic technique.

Process of polymerization and its optimization Purified LA obtained from fermented broth of Jackal jujube was added to the flask and boiled at 100ºC to remove the water. HA was added and reacted for 1 h. The process was executed on a magnetic stirrer. Synthesis was performed by direct polycondensation (DP) reaction at specific temperature and reaction time. At the end of the reaction, the samples were allowed to cool to room temperature. The samples were then dissolved with acetone and precipitated with water in order to remove unreacted LA and other water soluble compounds from PLA. Different parameters were optimized during the polymerization process. The concentration of HA was varied from 0.005-0.5% to study its effect on polymerization reaction. After selecting a particular concentration, the temperature of the polymerization reaction was varied from 100-180ºC and the reaction time from 6-20 h. The selection was done on the basis of yield and molecular weight obtained.

Molecular weight determination The intrinsic viscosity (η) was determined in an Ubbelohde viscometer with $CHCl_3$ as the solvent at 25ºC. Molecular weight was calculated according to the Mark-Houwink equation:

$$[\eta]=5.45\times10^{-4}\,M^{0.75} \quad (1)$$

where, M is the average molecular weight.

Circular dichroism (CD) The specific optical rotation of the polymers was measured in chloroform at a concentration of 1 g/dL at 25ºC using a circular dichroism spectropolarimeter (J-810; Jasco, Tokyo, Japan) within a range of 200-290 nm wavelength.

Fourier transform-infrared (FT-IR) spectroscopy study FT-IR spectra were collected with a Nexus-870 FT-IR (Thermo Nicolet Corporation, Madison, WI, USA) spectrometer. Potassium bromide was used as the background. The spectra were recorded from 500 to 4,000/cm. Solid powder samples were grounded with solid KBr in mortar pestle and a thin pellet was made with a die using a hydraulic press. Such pellets were placed in the FT-IR beam for analysis.

X-ray diffraction (XRD) analysis XRD study was carried out using Panalytical High Resolution (Philips PW 3040/60; Buehler Ltd., Lake Bluff, IL, USA) with Cu-Kα radiation. The diffractogram was scanned in the ranges from 8-50º angle at a scan rate of 1º/min.

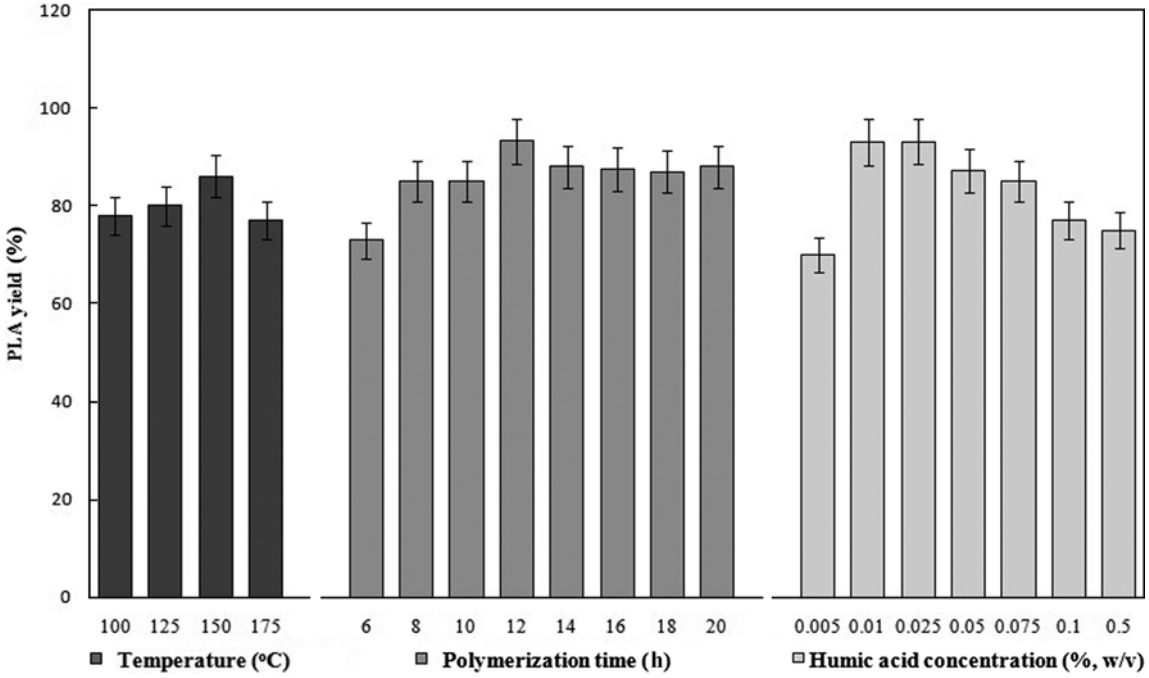

Fig. 1. PLA yield with different process condition (temperature, reaction time, and humic acid concentration).

Results and Discussion

Method of polymerization and its optimization HA being a complex polyphenolic macromolecule have aromatic, hydrophobic core to which a wide range of hydrophilic side chains remain attached. These reactive free functional groups were used to incorporate cross linking with the hydroxyl and carboxyl groups of LA. Thus, the presence of HA was thought to increase the cross linking property of the polymer which in turn might improve other properties like reactivity, flexibility, hydrophilicity, etc. Initial experiments for polymerization with HA showed better results in terms of both yield and molecular weight (16). So, optimization of the process parameters was done to get the best possible condition for the synthesis to take place. When the concentration of HA was varied from 0.005-0.5%, it affected the texture, yield, and molecular weight of the polyester. HA below 0.01% concentration level, gave a sticky solid mass having very lower molecular weight. On raising the concentration, the color of the polymer turned brownish, lowering the molecular weight. In case selection of the temperature for polymerization, it has been varied from 100 to 175°C. At 150°C it showed maximum polymerization with yield of 86%. The corresponding molecular weight was found to be 3.6×10^5. Whereas, the studies regarding selection of total reaction time required for polymerization revealed that, at 12 h maximum polymerization with the corresponding yield of 93% took place (Fig. 1). The molecular weight at this condition was found to be 6.4×10^5 which is quite high confirming its stability and application in different fields.

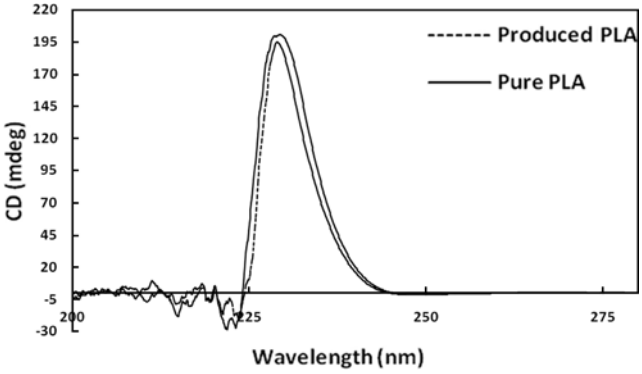

Fig. 2. Circular dichrosim (CD) spectra for produced PLA and pure PLA at a concentration of 1 g/dL dissolve in chloroform at 25°C.

The current study for lactic acid polymerization using HA showed much better results in terms of yield and molecular weight compared to that using other conventional catalysts. In our earlier published results (16), 8 different catalysts belonging to the group of metal, binary, acid, and polyphenolic catalysts were compared for both yield and molecular weight. Yield was found to increase from 66.08 to 80-85% upon use of HA in place of stannous octoate as polymerizing agent. Upon optimization of the process parameters, the yield with HA further got increased upto 93%.

CD The CD spectrum of produced PLA dissolve in

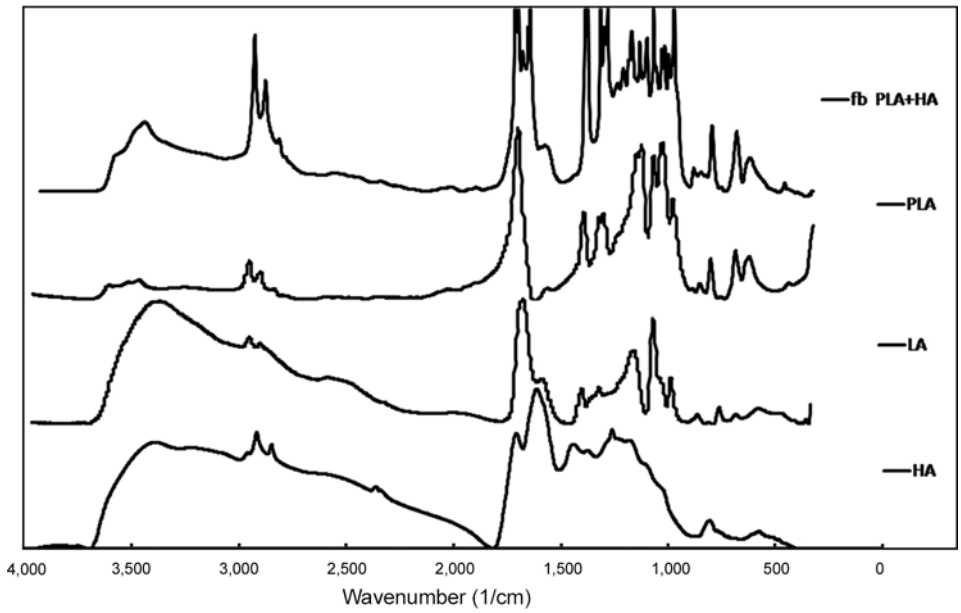

Fig. 3. FT-IR spectra of produced PLA with humic acid (fb PLA+HA), pure PLA (PLA), lactic acid (LA), and humic acid (HA).

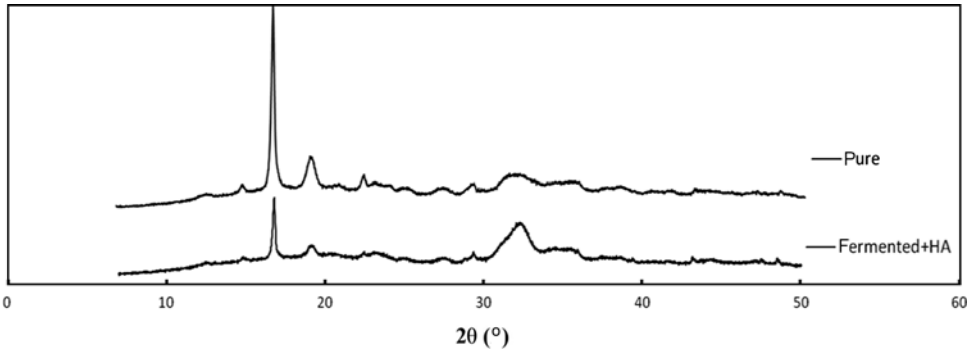

Fig. 4. XRD analysis of pure and fermented broth PLA showing characteristic peaks.

chloroform shows positive peak at 228.6 nm which matched well with the pure PLA, which shows peak at 228.4 nm (Fig. 2). The presence of positive peak confirms the formation of L-PLA (2).

FT-IR analysis FT-IR analyses were carried out to determine the functional groups of the products. Figure 3 shows FT-IR spectrum of LA, HA, pure PLA, and PLA formed with purified LA from fermented broth of Jackal jujube and HA. LA shows typical absorption peaks at 1,380/cm for C-H bending, 1,730/cm for C=O stretching frequency, 1,210 and 1,120/cm for C-O stretching frequency and 1,031/cm for C-O-C stretching vibration (18). On the other hand, HA shows characteristic peak at 3,456/cm for O-H stretching, asymmetrical, and symmetrical stretching of methylene CH_2 peak at 2,922/cm, a very sharp and strong peak at 1,623/cm was for C=O stretching of amide group and 1,546/cm for C=N stretching (17). Both the PLA (pure and produced) showed similar characteristic peaks along with a few extra peaks in case of produced PLA as described below. The presence of typical absorption peaks near 1,778/cm attributed for strong stretching band associated with formation of ester bonds. Its shift to higher intensity in the produced PLA from the pure PLA was explained by the conjugation of single bonded oxygen atom (19) which might have incorporated from the HA. Symmetrical and asymmetrical valance vibration of CH_3 were found in the produced PLA at 1,460 and 1,564/cm with higher intensity peaks as compared with those from lactic acid found in 1,380/cm suggesting the arrangement of molecules in the PLA chain (20). Also, peak of 1,600/cm advocated the inclusion of aromatic C=C bond from the HA (17). Apart from this, a broad band near 3,500/cm corresponds to the presence of OH stretching frequency. The incorporation of OH group from the HA confirms the copolymerization process (18) without hampering the original characteristic features of the PLA.

XRD analysis A polymer can be considered partly crystalline and partly amorphous. The crystalline domains

act as a reinforcing grid, like the iron framework in concrete, and improve the performance over a wide range of temperature. However, too much crystallinity causes brittleness. The crystalline parts give sharp narrow and long diffraction peaks whereas, the amorphous components give short and broad peaks. The graph shows that fermented broth PLA shows the characteristic peaks at 16 and 32° which first confirms the formation of PLA as compared to the pure PLA (21,22) (Fig. 4). Further, it can be said that as compared to the pure PLA, fermented broth PLA with HA has shown a reduction in peak height at 16° which confirmed reduction in its crystallinity. Reduced crystallinity resulted in its improved elasticity.

In summary, it is possible to synthesize polymer from purified LA obtained from Jackal jujube and HA simply by employing directly as starting materials by the process of direct polycondensation reaction. Optimization of different condition for polymer synthesis was performed. The structure and properties of the produced polymer were investigated by different techniques. FT-IR spectroscopy proved the presence of intermolecular bonding between the free functional groups of HA with that of PLA which improves its flexibility. XRD confirms its reduced crystallinity while improving its flexibility. Thus, the PLA produced from Jackal jujube found to be a competent candidate in the biotechnological field of polymer industry.

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