PHA copolymer synthesis and its characterization.

Bhavsar Nirav[1*], Patel Jatin[1], Raol B.V.[3], Tandel Hirak[1], Ray Sanket[2], Jha Rupesh[1], Vaidya Yati[1], Bhatt Ishita[1]

[1] Shri A.N.Patel P.G.Institute of Science and research, Anand, Gujarat.India

[2] Naran Lala college of Professional and applied sciences, Navsari, Gujarat.India

[3] Shri.P.H.G. Municipal arts and science college, Kalol, Gujarat, India

Abstract:

PHA occurs naturally in microorganisms when amount of nutrient in environment is low and carbon source is high. Known strain *P.phragmitetus* ERC-8 was used for PHA production in Bushnell haas (BH) medium (pH 7.0) with 3% glycerol. After incubation period, to extract out the PHA from biomass solvent extraction method was used. The amount of extracted PHA was 0.1205 g/L. Extracted PHA has the low processability, to improve the processability of PHA was blended with other synthetic bio-degradable polymer such as PLA and naturally occurring polymers like gelatin, agar and starch. Instrumental analysis, such s FTIR and NMR was used for the characterization of PHA and copolymers of PHA. Thermal analysis was used for determination of melting temperature and degradation temperature of PHA and copolymer of PHA. Copolymer of PHA showed the higher melting temperature then pure PHA. By incorporating the PLA into the PHA polymer can increases the processability of PHA polymer. Copolymers with higher melting temperature and higher processability are produced by copolymerization.

Keywords: Polyhydroxyalkanoates (PHA), Polylactic acid (PLA), Blending/polymerization, *P.phragmitetus* ERC-8.

Introduction:
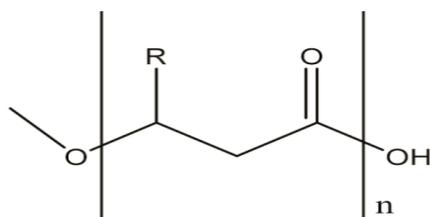
General structure of PHA given below:

Figure.1) general structure of PHA

The properties of PHA can be modified in a relatively wide range. Physical blending is the mixing of polymeric materials in the melt state with no chemical reaction taking place. It is a convenient way to create new materials with desired combination of properties (Xhang, L.,Xiong, 1997). Blending can be carried out by using conventional machinery, i.e. no expensive investment is necessary, which is an important aspect of industry. PHA and other natural polymer copolymerized by the graft copolymerization or block copolymerization. PHA and their copolymer are used for specialty packaging, surgical fixation, controlled drug delivery, tissue culture & tissue engineering, agricultural and veterinary and detergent application (kaneka, 2009).

Materials and method:

Production of PHA in Luria-bertani broth and biphasic growth condition:

P.fragmitatus was inoculated in Luria-bertani broth and incubated at 30°C for 24-48 hours on rotary shaker at 120 rpm. After incubation LB medium transferred to bushnell haas (BH) medium (nitrogen deficient) (pH 7.0±7.2) containing 3% gycerol and incubated at 30°C at 120 rpm for 48 hours.

Extraction of PHA:

After 48 hours of incubation, 50 ml of broth was collected and centrifuged at 7000 rpm for 15 minutes in pre-weighed centrifuge tubes. The supernatant was discarded and the centrifuge tube with pellet post weighed; dried into oven for overnight. Dried pellet was suspended into the 10 ml chloroform and placed over night for digestion on rotary shaker for overnight at 120 rpm. After digestion the chloroform filtered through whatman filter paper no.1 and obtained filtrate placed into the oven for drying. Pre-weigh and post-weigh are compared and weigh of the produced PHA obtained.

Copolymerization/Blending of different polymers:

PHA polymer produced by the microorganisms was blended/copolymerized with other bio-polymer (e.g. Polylactic acid (PLA), Agar-agar, Gelatin, starch). For blending two different biopolymer were taken in ratio of 50:50 in capillary tube then placed inside the paraffin oil for melting of the bio-polymer; when melting occurred at specific temperature two polymer were mixed together by blending. By blending of the bio-polymers copolymer were produced e.g. PHA-Gelatin, PLA-Gelatin, PHA-Agar, Agar-Gelatin, PLA-Agar, PHA-PLA.

Characterization of copolymer:

Products produced by the blending/copolymerizing were characterized by the FTIR, NMR, TGA, and DSC. Functional group and the structure of the copolymer were identified by this instrumental method.

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR can be used to study the various functional groups and interactions present in biopolymers.The purified samples were first dried in an oven. After removing the moisture content, the samples were grown into a fine film. The IR spectrum of the PHA film was recorded with a Perkin-Elmer spectrum version 10.03.06 IR spectrophotometer. A thin film scanned between 450 and 4000 wave number (cm-1) at a speed of 1 micron/min.

NMR spectroscopy:

NMR spectroscopic technique relaxation times and their dependence on temperature and magnetic field have proved to be a useful source of information for molecular structure, phase change, conformational exchange, solubility, and diffusion of biopolymers.The polymer was suspended in spectrochem grade deuterochloroform ($CDCL_3$). The ^1H NMR spectra of sample were obtained at 400 MHz using a model Avance ш FT-NMR. Sample was dissolved in chloroform (1 mg/ ml^{-1}solvent) that was employed for each analysis.

Thermal analysis (TA):

Thermal analysis (TA) method has become an indispensable analytical technique for the characterization of polymers. The characterization technique provides relevant information about thermal and chemical stability, phase transition temperatures and kinetics, rheology and molecular relaxation times of the polymers. It provides information regarding the relation between the polymers chemical structure and their physical properties. In conventional thermal analysis techniques, the method usually involves the monitoring of material properties as a function of temperature. Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TGA) are used to study the thermal behavior of biopolymers.

Differential Scanning Calorimetry (DSC):

Differential scanning calorimetry (DSC) was performed using a METTLER TOLEDO DSC from Mettler Toledo, calibrated with indium standard. The samples weighting between 1.4 and 8.22 mg were cut in square plates, packed in aluminum pans and placed in the DSC cell. Samples were first heated from ambient temperature to 250℃ at a heating rate of 10℃/min, in order to erase any previous thermal history. Then, the samples were cooled to ambient temperature and heated again to 250℃ at a rate of 10℃/min.

Thermo gravimetric analysis (TGA):

Thermogravimetric analysis (TGA) is used to measure the change in weight during heating and thus studies the thermal stability and composition of the sample. Samples of PHA and blend of PHA of are 2.45 and

6.06mg in mass, were prepared in the form of powder or thin film. TGA tests were performed in a METTLER TOLEDO TGA under nitrogen at a heating rate of 10°C /min from room temperature to 200°C.

Results:

Production of PHB from the known strain:

Identified strain of *Pannionibacter phargmitetus*ERC-8 transferred in to the production medium (Bushnell haas) containing 3% glycerol was incubated at 30^0C, 120 rpm for 96 hours. After 96 hours of incubation period extraction method for PHA was performed to extracte out the PHA from bacterial cell. After the extraction of PHA by solvent extraction method, obtained PHA was about 0.1205 g/L.

Figure.2) film of extracted PHA by solvent extraction

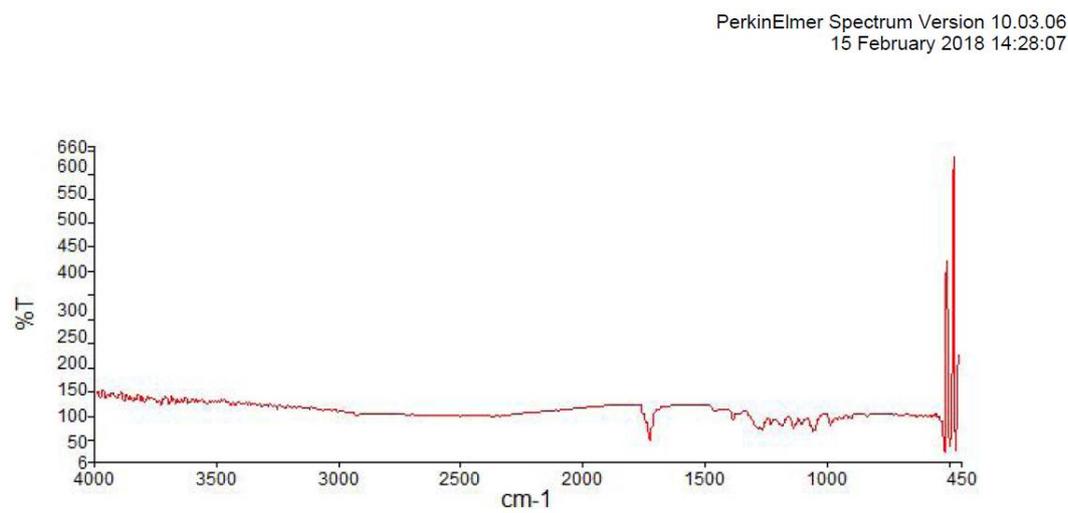## Characterization of PHA by instrumental method:

FTIR analysis:

Figure.3) FTIR of PHA polymer

The FTIR spectrum (fig.3), shows a sharp band at 1718 cm-1, corresponding to the ester carbonyl (C=O) group & another band at 1279 cm-1, corresponding to the -CH group. A series of bands between 1000 and 1300 cm-1 shows stretching of the C-O bond of ester group. The bends at 2969 & 2927 cm-1 indicate the alkyl-CH_3 group and bond at 1377 cm-1 corresponds to a symetric bending -CH_3 group. The band at 1452 cm-1 is related to the asymmetric bending of -CH_2 or -CH_3. Bend at 3434 cm-1 is related to -OH group. There was some intense absorption spectra typical to PHA, C=O & C-O stretching group respectively. From this spectrum determined structure is PHA.

NMR analysis:

NMR analysis was used to determine the quality of PHA structural composition. ^{1}H-NMR spectrum of isolated PHA, indicated signals characteristics of PHA. (Fig.4), spectral analysis of both demonstrated the presence of 3, 2 & 1 proton at chemical shift 1.18, 2.2-2.5 & 5.1 respectively showing CH_3, CH_2 and CH groups. Molecular composition of polymer indicated by the chemical shift generates a structure of (CH_2-CH) backbone assigned the presence of CH3.
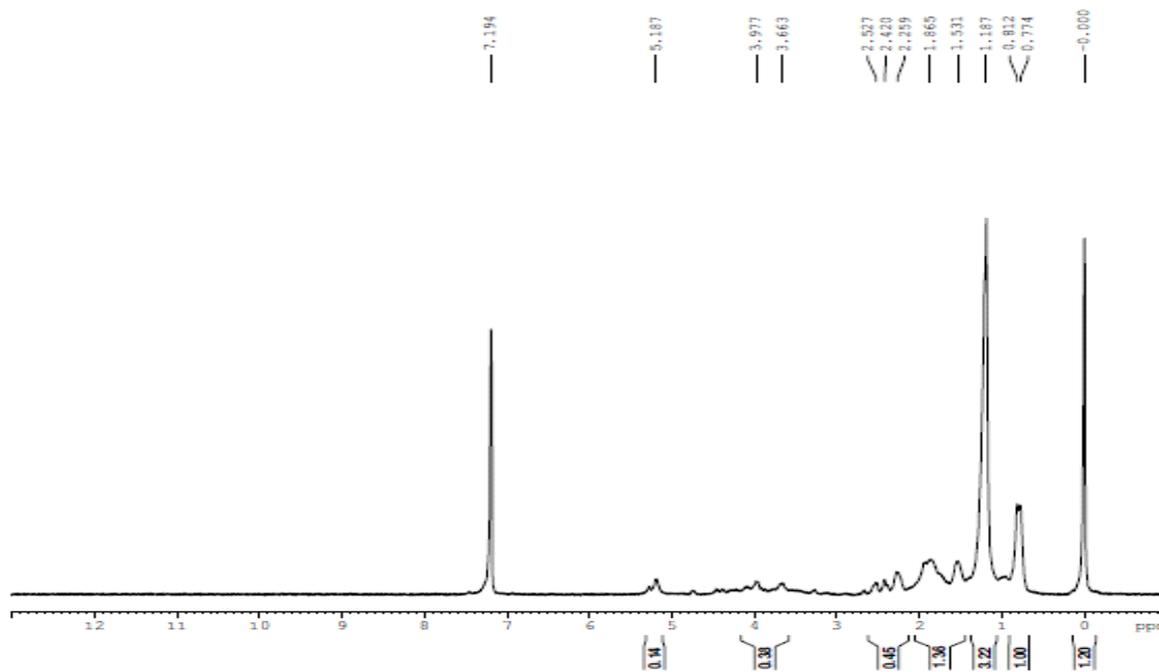
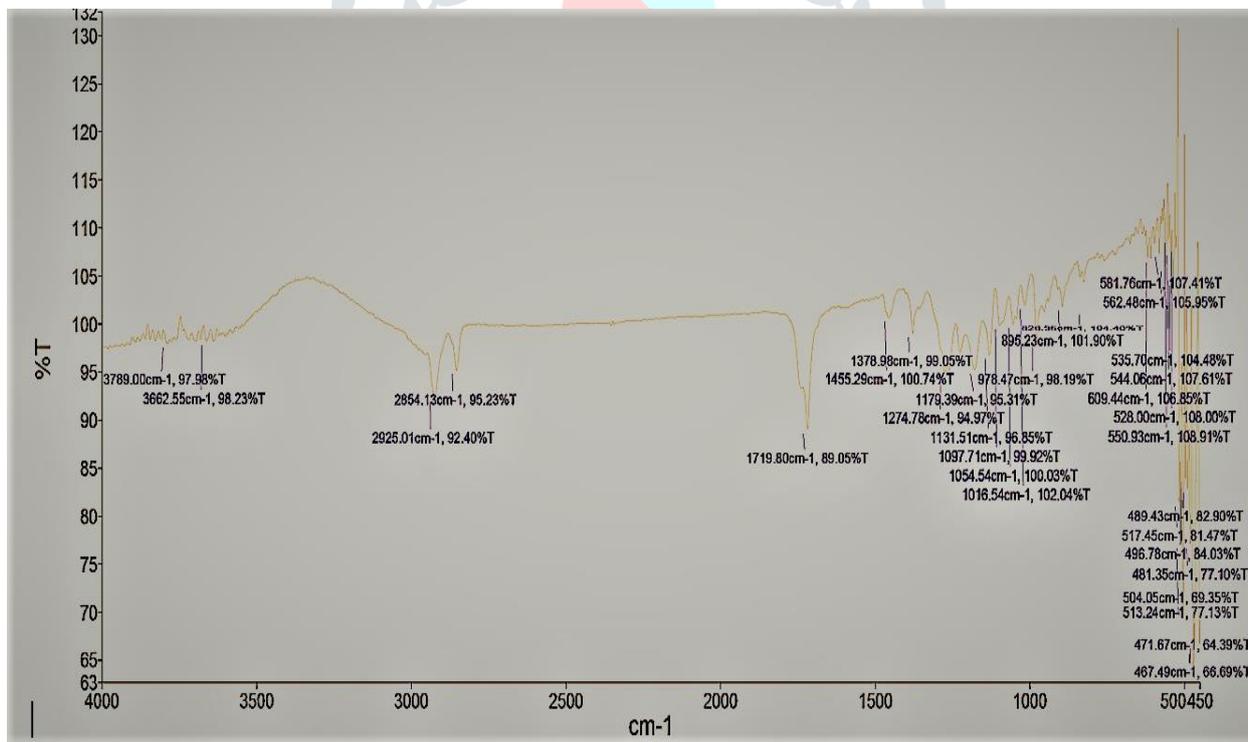Figure.4) ^{1}H-NMR analysis of PHA

FTIR analysis of copolymers:

Figure.5) FTIR of PHA-PLA

FTIR of PHA-PLA (fig.5) shows a sharp band at 1719 cm-1, corresponding to aldehyde -C=O. Another bend at 1455 & 1378, corresponding to CH_3 group.1274, 1179, 1131, 1097, 1054 & 1016 cm-1 stretch of C-O bond of ester group. 2925, 2854 cm-1 are related to -C-H aldehyde group.

Thermal analysis:

DSC analysis:

The DSC scans for heating, cooling, and reheating stages, obtained according to the procedure described above are shown in Figure.6 & 7.
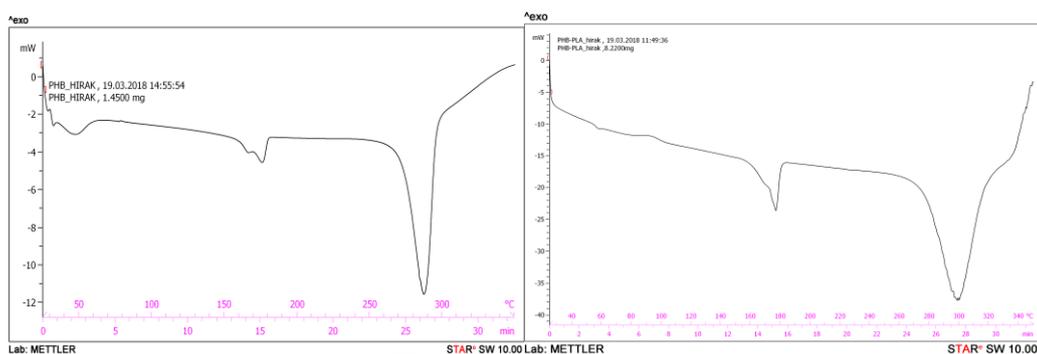

Figure.6.DSC analysis of PHA   Figure 7. DSC analysis of PHA-PLA

The glass transition temperature of PHA at around 48-50^{0}C. The melting temperature peak at around 165-175^{0}C corresponds to crystallization of PHA. The highest temperature peak at 285-290^{0}C where the polymer rapidly degraded(melting of crystallized PHA).The glass transition temperature of PHA-PLA at 50^{0}C. The melting temperature peak at 175-180^{0}C which corresponds to crystallization of PHA and melting of PLA. The highest temperature peak at 300^{0}C where rapid degradation occur. Blending of PHA-PLA at higher temperature led to the lower melting temperature of PHA-PLA blend, it is caused by transesterification.

TGA analysis:

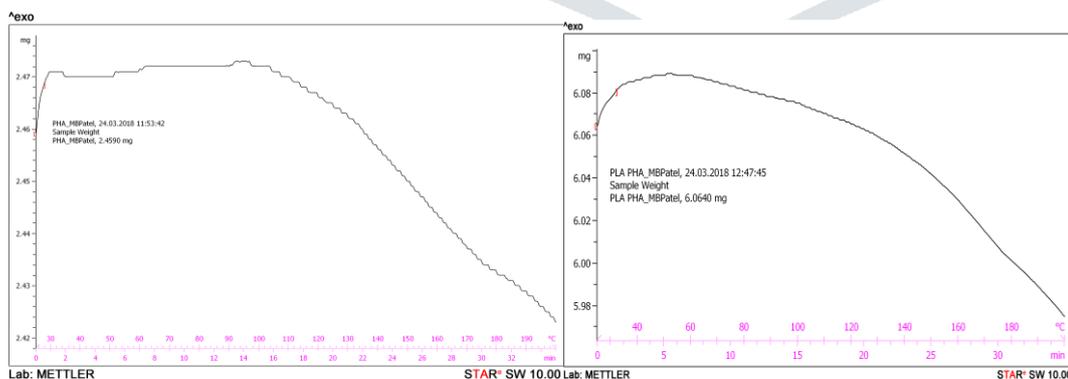Figure.8 & 9 shows the thermal gravimetric (TG) curves from degradation of PHA & PHA-PLA.

Figure.8) TGA of PHA   Figure.9) TGA of PHA-PLA

Thermal degradation occurred around 198^{0}C with a single weight loss step. Thus characterization of PHA produced in this study confirmed the product and showed the expected quality of plastic.Thermal

degradation occurs above 200^{0}C with a single weight loss step. Thus the PHA-PLA blend shows the higher melting temperature.

Conclusion:

Pure PHA polymers have low melting temperature. To improve the quality of PHA polymer can be increased by blending PHA polymer with synthetic biodegradable polymer such as PLA.

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