5.5 SUMMARY AND CONCLUSION

The present study deals with to characterize the biopolymer and to investigate their chemical, thermal and mechanical properties. Characterization of PHB was done by Fourier transforms infrared spectroscopy (FTIR), Gas chromatography mass spectrophotometer (GCMS) and Nuclear magnetic resonance (NMR) and extracted from *Lysinibacillus sphaericus* BBKGBS6.

- PHB for characterization was obtained by solvent extraction in a soxhlet apparatus. PHB was recovered from chloroform solution by precipitation with hexane five volumes of the polymer. PHB precipitated as a white cottony mass.

- Analysis was done by Fourier transform infrared spectroscopy (model no.4700, Nicolet). The scanning conditions were a spectra range of 4000-400 cm\(^{-1}\). GC analysis of PHB was carried out by the method described by Brandl method. Nuclear magnetic resonance (NMR) analysis of PHB sample was done at sophisticated instruments facility, Indian institute of Science, Bangalore, India. \(^1\)H and \(^13\)C NMR spectra were recorded using purified samples.

- The X-ray diffraction was done by using X-ray powder crystallography. Differential scanning calorimetric (DSC) experiments was performed using Universal V4.5A TA Instruments, (m.p.156.61 °C; \(\Delta H = 28.54 \text{ J/g}\)). Thermo gravimetric analysis (TGA) was performed on a Shimadzu instrument (Japan).

- The molecular weight of PHB was estimated based on viscosity measurement to give a viscosity average molecular weight. The average molecular weight of the biopolymer PHB was 1300 kDa. Films were prepared by as per the solvent casting method (Savenkova *et al.*, 2000).
• Tensile strength of PHB film was carried out according to ASTMD 882 using universal testing machine (Model Lx 5, LYOD ISNT). Water vapor transmission rate of PHB film was measured as per ASTME 96-95 and carried out according to the desiccant method. Oxygen transmission rate of PHB film was measured as per ASTM D-1434-66.

• The present results indicated the presence and levels of crotonic acid and confirmed the presence of polyhydroxybutyrate in the sample by U-V spectrophotometer from Lysinibacillus sphaericus BBKGS6. The FTIR spectra obtained showed characteristic absorption bonds for esters and the presence of C=O and C-O were obtained at 1724 cm\(^{-1}\) and 1281 cm\(^{-1}\) respectively. Apart from this a peak at 1377 cm\(^{-1}\) was seen which is due to the CH\(_3\) or methyl bending. Peaks due to methyl stretching were also observed at 2975 cm\(^{-1}\) and 2926 cm\(^{-1}\). CH\(_2\) or methylene group was observed at 1450 cm\(^{-1}\) and methine or CH peak was at 3434 cm\(^{-1}\).

• GCMS confirmed the presence of another polymer or a copolymer, which eluted out at higher retention time. The butyrate methyl ester eluted at 9.5 min and 12.81 min. The major molecular fragmentation were, showed m/z 115 (C\(_5\)H\(_9\)O\(_3\)^\(^+\)), m/z 105 (C\(_4\)H\(_7\)O\(_3\)^\(^+\)), m/z 85 (C\(_4\)H\(_7\)O\(_2\)^\(^+\)), m/z 78 (C\(_3\)H\(_6\)O\(_2\)^\(^+\)) m/z 63 (C\(_2\)H\(_3\)O\(_2\)^\(^+\)), m/z 43 (C\(_2\)H\(_3\)O), m/z 57 (C\(_2\)H\(_3\)O\(_2\)) (C\(_3\)H\(_7\)O\(_2\)^\(^+\)), m/z 71 (C\(_3\)H\(_7\)O\(_2\)^\(^+\)). The other fragment ions seen as a β-cleavage reaction to methyl esters was m/z 74. The results confirm that the PHB extracted from the sample contains 3-hydroxy functional group and the presence of methyl esters of hydroxybutyrate.

• The absorbance in ppm is 169.143, 67.929, 40.124 and 22.199 for carbonyl, methine, and methylene. The \(^1\)H NMR spectrum of PHB showed three groups of signals characteristic of PHB indicated the presence of polyhydroxybutyrate in the polymer. X- ray diffraction data showed that the extracted biopolymer was optically active compound.
• The glass transition temperature of the PHB was 140 °C, melting temperature was 291.31 °C and amorphous temperature was 176.08 °C. The enthalpy of melting (ΔHf) extracted PHB was 56.42 J/g. The crystallinity of the PHB was 56.49%. The extracted PHB showed endothermal peaks in between 140 and 300 °C using DSC. The decomposition temperature at a 10% level determined by thermo gravimetric analysis (TGA) for pure PHB in ScCO₂ at 70 °C and 22 MPa was 293.32.

• Molecular weight was measured by viscometry and the viscosity average molecular weight of the extracted biopolymer PHB was 1310 k Da. Tensile strength of the film was observed 28.23 MPa. The maximum load taken was 18.5 N and the tensile strength at this load was 41.1 MPa. The extension to break was 1.2%. The water vapour permeability of the present film was observed 29 g/m²/day at 38 °C and 90 % RH. Oxygen transmission rate of PHB film was calculated and observed as 472.36 (cc/m²/day/atm at 27 °C and RH 65%).

In conclusion, the biopolymer obtained from *Lysinibacillus sphaericus* BBKGBS6 can be efficiently developed into a packaging film. Further, inputs based on knowledge of polymer chemistry and food packaging can improve the film and can be developed as a food packaging material.