4.5 SUMMARY AND CONCLUSION

PHB was accumulated intracellularly; the development of an efficient recovery process including cell disruption is indispensable to reduce its total production cost. Extraction and purification of Polyhydroxybutyrate (PHB) by different methods were studied.

- Some of the well known methods of PHB extraction and purification were done using solvents, hypochlorite, dispersions of hypochlorite and solvent, enzymes mixtures of, surfactants and chelating agents. A simple and effective microbial method of extraction of polyhydroxybutyrate from bacterial cells has been developed.

- The hypochlorite concentrations in the aqueous solutions used were 3, 5, 10, and 20 % (vol/vol). After the cell powder was treated at 30 °C for 1 hr, the mixture was centrifuged at 4,000 rpm for 10 min, which resulted in three separate phases. Cells were collected by centrifugation (6000 rpm for 20 min at room temperature) and were washed with sufficient acetone for 10 min. Acetone dried cells were held in 5 volumes of chloroform and left overnight at room temperature.

- PHB containing cells were collected by centrifugation (6000 rpm for 20 min at room temperature). These cells were treated with dispersion of hypochlorite and chloroform in the ratio of 1:1 and kept for digestion for 1 hr at 37 °C.

- Biomass containing PHB was collected by centrifugation (6000 rpm for 20 min at room temperature). Cells were suspended in known quantity of water. 0.6% of triton X 100 and 0.06% of EDTA were added to the cells and pH was adjusted to 13 with 1 N NaOH solution and they were kept at 50 °C for 10 min.
A proteolytic enzyme such as protease (Novozyme) was used for extraction of PHB. In this method lytic enzyme of an actinomycete (Microbispora) culture has been used to lyse *Lysinibacillus sphaericus* BBKGBS6 cells.

*Lysinibacillus sphaericus* BBKGBS6 biomass was thermally inactivated at 80 °C for 10 min and suspended in phosphate buffer. The crude culture filtrate obtained was used at 1 volume level to hydrolyze 3 g/L of thermally inactivated biomass of *Lysinibacillus sphaericus* BBKGBS6. The hydrolysis was carried out at 50 °C for 3 hrs and PHA was isolated using chloroform as described above under growth associated lysis.

Lytic activity was determined by hydrolyzing *Lysinibacillus sphaericus* BBKGBS6 cells of known dry weight (3 mg/ 5 ml) with 48 to 72 hrs old clarified culture filtrate (0.5 ml) of cell lytic culture at 50 °C, pH 6 to 7 for 1 hr. OD was measured at 620 nm and activity was calculated as 1000 X.

*Lysinibacillus sphaericus* BBKGBS6 was grown on the modified PHB production medium (pH 7.0).

The polymer obtained by extraction method described above was used for crotonic assay. The absorbency at 235 nm of the solution was measured in a U-V spectrophotometer. Characterization of the extracted biopolymer was carried out using Infrared- spectroscopy, gas chromatography and differential scanning calorimeter. Scanning electron microscopy of enzyme treated biomass. Scanning electron microscopic analysis of the lytic enzyme treated biomass was carried out.

Dispersions of sodium hypochlorite and chloroform at 50 % sodium hypochlorite in chloroform gave yields similar to that of sodium hypochlorite alone. Samples extracted by
sodium hypochlorite showed (viscosity average) molecular weight of 1400 kDa. The PHB recovery by enzymatic digestion of *Lysinibacillus sphaericus* BBKGBS6 was 90%.

- PHB from *Lysinibacillus sphaericus* BBKGBS6 cells after treatment by surfactants and chelating agents was 69% pure. Samples extracted from ammonium hydroxide showed a molecular weight (viscosity) average of 1300 kDa. Gas chromatographic analysis showed the presence of polyhydroxybutyrate in the enzyme-extracted sample. The DSC melting curves show two peaks. The major peak was at 291.31 °C.

In conclusion, modified method of cell lysis for PHB extraction has been worked out. PHB was released into the broth and was extracted by a minimum quantity of chloroform. PHB extracted by this method was 93 to 97% pure. Crude culture filtrate was also effective in lysing of *Lysinibacillus sphaericus* BBKGBS6 cells. Samples extracted from ammonium hydroxide showed a molecular weight (viscosity) average of 1300 kDa. This suggests that the PHB extracted after the enzyme extraction was pure and free of any associated cell material such as protein. Gas chromatographic analysis showed the presence of polyhydroxybutyrate in the enzyme-extracted sample. Ammonium hydroxide was found to be more effective for extraction of *Lysinibacillus sphaericus* BBKGBS6.