ABSTRACT

Polyhydroxyalkonates a family of polyesters is accumulated as granules in the cells of bacteria was significantly depended on the ratio of carbon and nitrogen in the culture medium. In this present work, PHB was produced from the Bacteria isolated from dump yard soil. The organism capable of producing PHB was confirmed using the appearance of black granules against pink background in Sudan black staining and appearance of orange fluorescence by staining with Nile blue A. The isolated strain (*Pseudomonas putida*) and the positive control strain *Rhizobium leguminosarum* was subjected for the production of PHB using the kitchen waste, oil waste and the artificial PHA medium as the source medium. The produced compound appeared as thin plastic sheets which preliminarily indicated PHB. The Rf value and the color appeared in TLC plates indicated positively the presence of PHB. By evaluating the functional groups C=O, -OH, CH\textsubscript{2}- using FTIR and the no. of H and C atoms using NMR, the isolated compound was confirmed to be PHB.

The isolated and the standard PHB was subjected to degradation using the isolated strains from dump yard and garden soil. From the garden soil *Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas fluorescence* was isolated and confirmed and from the dump yard the organisms *Pseudomonas fluorescence, Pseudomonas aeruginosa, Alcaligenes faecalis, Acinetobacter sp, Thiospaera sp* was isolated and identified. All the strains when subjected to degradation was noticed that 80% of degradation was produced using the *Bacillus subtilis*. The thickness of the films was also reduced; the appearance of the films was also different from the control with lots of holes and cracks. Further in the supernatant of the degradation medium the presence of soluble monomers *i.e.*) butyrate was identified using FTIR,
which supported the event of degradation. Finally, the remained films was viewed using SEM, to see the presence of unorganized morphology, holes and cracks of smaller to larger size which is absent in the control, further strongly supported degradation.

During degradation, the culture supernatant was subjected for PHB depolymerase isolation, purification and enzyme activity. The highest activity of 0.5U/ml was recorded for the enzyme after purification than only 0.5U/ml for crude supernatant. This indicated the specificity of enzyme towards the substrate and proved that the agent responsible for degradation is PHB depolymerase.

For application microspheres was constructed using PHB. Different sized spheres were viewed using SEM. During construction of microspheres the probiotic bacteria *Lactobacillus* was encapsulated and the encapsulation was confirmed by the growth of the organism in the Nutrient broth inoculated with microspheres. Also the rate of release of bacteria from the microspheres was evaluated in different pH of which, the release was longer (7days) in physiological pH and acidic pH (maintained in the intestine) than the water medium. Hence, it is proved that PHB microspheres can be used for the sustained drug delivery with no side effects.