Poly-β-hydroxybutyrate Biodegradation using environmental isolates

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Abstract

Poly-β-hydroxybutyrate is a natural polymer that has the potential to replace traditional polymeric materials. This study was aimed to investigate and observe the extent of biodegradation of poly-β-hydroxybutyrate. PHB was produced by growing *Bacillus cereus* and then PHB films were prepared by employing solvent casting method. Biodegradability of PHB was observed through CO_2 determination produced during degradation process. In addition to CO_2 evolution, scanning electron microscopy and FTIR spectroscopy were employed to determine the morphological changes and crystallinity changes in the PHB during degradation process. During degradation process 1.97 g/L of CO_2 was produced and 26.7 % degradation was observed. SEM showed formation of grooves, pits and cracking on PHB films. FTIR spectra of PHB films before and after test showed formation of some new bonds between 1300/cm to 1700/cm region due to breakage of C-O bonds. The study on the biodegradation of poly-β-hydroxybutyrate have increased our knowledge of PHB biodegradation under study conditions and suggests that this material could be applicable to single-use applications.

Introduction

Synthetic plastics have replaced other materials in many application due to their many technical advantages such as light weight, resistance to corrosion and low temperature processing (Colwill *et al.*, 2010; Davis and Song, 2006). However these materials are inert in nature and remain unaffected in environment when disposed of and this has attracted more public and media attention. These materials can be used for power generation but may result in hazardous emissions (Hopewell *et al.*, 2009). To overcome these problems associated with these plastics, public interested is being shifted towards more eco-friendly and sustainable products e.g. polyhydroxyalkanoates (PHA), polysaccharides and polyphosphates, that are more attuned to human lifestyle (Lee, 1996; Lütke-Eversloh *et al.*, 2001; Kadouri *et al.*, 2005). Polyhydroxy butyrate is the most promising subclass of PHAs due to its mechanical similarity with conventional plastics (Steinbuchel and Valentin, 1995). PHB is stored as granules in microbial cytoplasm and is used by microorganisms as energy source so is degradable in microbially active environment under aerobic conditions. Rate of degradation can be affected by a number of factors including

morphology, surface area, temperature, moisture level, pH and presence of other nutrients (Williams and Peoples, 1996; Lee, 1996).

Biodegradability of PHB has been reported in previous studies and since the pioneering works of Chowdhury (1963) and Delafield (1965), several *in situ* and *in vitro* studies of microbial degradation of PHB have been performed in different natural environments such as soil (Grima *et al.*, 2001), compost (Gilmore *et al.*, 1992; Ruka *et al.*, 2015), natural water (Doi *et al.*, 1992), sludges (Briese *et al.*, 1994) and marine environments as well as under laboratory (*in vitro*) conditions (Manna *et al.*, 1999).

There are different methods used to determine the degradability of such materials both in terms of degree and rate of degradation. Rate and extent of degradation can be assessed by obtaining metrics from degradation experiments. In the previous studies various analytic techniques e.g. weight loss measurement, clear zone formation, and evolution of carbon dioxide, scanning electron microscopy and FTIR spectra (Pagga *et al.*, 2001; Zhao *et al.*, 2006). In previous studies weight loss measurement was considered as a sign of biodegradation but problems usually arise due to moisture absorption and difficulty in recovering the material due to excessive disintegration of material. Hence evolution of carbon dioxide is commonly used to determine the conversion of carbon backbone of polymer to carbon dioxide (Phukon *et al.*, 2012).

A proof of complete biodegradability of bioplastics is a prerequisite for the safe application of bioplastics and for this an assessment of biodegradability of material is required. So degradability of PHB films was measured through carbon dioxide evolution in conjunction with the scanning electron microscopy and FTIR spectroscopy.

Experimental

Bacterial Strain: PHB producing bacterial strain of *Bacillus cereus* was provided by Institute of Industrial Biotechnology, Government college university, Lahore, Pakistan.

PHB production

PHB was produced by growing *Bacillus cereus* in mineral salt medium, carbon source and trace element solution was added separately to medium. The composition of the medium was as follows (g/L); Glucose, 20; $(NH_4)_2SO_4$, 2.5; KH_2PO_4, 1.5; Na_2HPO_4, 3.5; $MgSO_4.7H_2O$, 0.2; 1 ml/L of trace element solution containing chemicals (g/L); $ZnSO_4.7H_2O$, 0.246; $MnSO_4.H_2O$, 0.169; $CoCl_2.6H_2O$, 0.237; H_3BO_3, 0.061; $CuSO_4.5H_2O$, 0.249; $NiCl_2.6H_2O$, 0.237; $NaMoO_4.2H_2O$, 0.442; $CaCl_2.2H_2O$, 0.147; $FeSO_4.7H_2O$, 0.278 and KI, 0.166 was added to medium. The medium was sterilized at 121°C for 20 minutes. After sterilization carbon source was added to the medium at 2% (w/v) ratio and then inoculated with 2% (v/v) pre-culture. The flasks were then incubated at 30°C in a rotary shaking incubator at 200 rpm for 48 hours. At the end of the incubation period PHB was extracted from cell mass by employing a suitable method.

Biodegradation Studies

Films of PHB were prepared by employing solvent casting method containing 1% PHB and chloroform was used as solvent. After preparation films were cut into 1cm × 1cm pieces and were exposed to degrading conditions. PHB films were added to the mineral salt medium bottles containing no other carbon source. About 5% (v/v) bacterial consortium was added to the medium. The bottles were placed on a magnetic stirrer at room temperature. After incubation period, change in mass of biomass/ml and change in CO_2 produced was performed. Theoretical amount of CO_2 and percent degradation was determined through following formulas;

$$ThCO_2 = m \times wc \times \frac{44}{12}$$

Where,

m is the mass of material in grams

w_c is the carbon content of the test material

44 and 12 are the molecular and atomic mases of CO_2 and carbon, respectively.

$$\% \text{ Degradation} = \frac{\text{mg CO2 Produced}}{\text{ThCO2} \times \text{mg test substance added}} \times 100$$

Scanning Electron Microscopy (SEM):

To determine the morphological changes in PHB films scanning electron microscopy was performed before and after test. To observe the changes SEM images of PHB films taken before and after the test was compared.

Fourier Transform-Infrared Spectroscopy

Fourier transform Infra-Red (IR) Spectroscopy was performed to evaluate the functional group changes in PHB used, occurred as a result of biodegradation process in liquid media. FTIR was performed on PHB films before test and FTIR fragments obtained after test. Spectra were performed at a range of 400-4000 cm^{-1} and resolution at 4 cm^{-1}.

Results and Discussion

Sturm test was used to evaluate the biodegradability of polymer materials (Calmon *et al.*, (2000). There are various modifications of this test that have been used for the estimation of polymer biodegradation through measuring CO_2 evolution (Pagga *et al.*, 1998). In this study we employed this method to estimate the biodegradation of PHB. In this test CO_2 evolution was determined gravimetrically by culturing isolated microorganism in MSM medium containing PHB and 1.97 g/L of CO_2 was produced. Percent degradation of the polymer was also determined i.e. 26.87. Sturm test has been employed by many researchers for investigating the mineralization level,

percent polymer degradation or to just determine the mass of CO_2 produced (Kim and Lenz, 2001; Pagga *et al.*, 2001; Ruka *et al.*, 2015).

Scanning electron microscopy was used by researchers to investigate the morphological changes in the surface of polymer films as a result of microbial attack (Sang *et al.*, 2002). In our study, comparison of scanning electron micrographs of PHB films performed before and after performing degradation test indicated clear evidence of PHB degradation in the form of pits, roughening of surface, erosion and cavities. El-Hadi *et al.* (2002) also observed holes and cracks in PHB films after degradation test. Gómez and Michel (2013) and Madbouly *et al.* (2014) also observed different morphological changes in PHA films after performing degradation test.

Poly (β-hydroxybutyrate) films were analyzed through FTIR spectra before and after performing Sturm test and this analysis revealed the bands at 1132, 1282, 1379, 1718 and 2920/cm before test. Bands at 1282 and 1718/cm indicated the –CH group and ester carbonyl group (C=O), characteristics of PHB. The band at 2920/cm indicates aliphatic C-H group backbone of polymer and peak at 1132/cm corresponds to the symmetric stretching vibrations of C-O-O (Ruka *et al.*, 2015). The band observed at 711 and 715 cm^{-1} indicated C-H deformation of CH_3 stretching. Peaks observed by performing FTIR analysis of PHB after Sturm test showed peaks at 1053, 1132, 1183, 1278, 1379, 1720, and 2976/cm. Peaks appeared at 1379, 2934 and 2977/cm indicate CH_3, $-CH_2$, and –CH groups (Wei *et al.*, 2015). Band observed at 1228/cm shows conformational change in helical chains.

Peak observed at 2934/cm after degradation test indicates formation of some new C-H bonds and peaks appeared between 1300/cm to 1700/cm indicates formation of some new C=H bonds due to breakage of C-H bonds. New peaks appeared at 2976 and 2997/cm after Sturm test. Bands at 1379/cm and 1720/cm represents crystallinity of PHB and these bands lowered after degradation (Wei *et al.*, 2015).

Conclusion

Production of carbon dioxide from Sturm test indicated presence of biodegradation process and percent degradation was also determined. Scanning electron microscopy and FTIR spectra also supported results of Sturm test. Change in morphology of PHB films was evident from the SEM images indicating the presence of biodegradation process. The study on the biodegradation of poly-β-hydroxybutyrate have increased our knowledge of microorganisms able to degrade PHB and their extent of biodegradation under study conditions. Extensive research and complete understanding of the biodegradation of bioplastics is required to find a better mechanism to degrade plastics.

Figure Legends

Figure 1. Scanning Electron Micrograph of PHB films at different resolutions (a) before test (b) after test.

Figure 2. FTIR Spectra of PHB films before and after test.

Figures.

Figure 1.

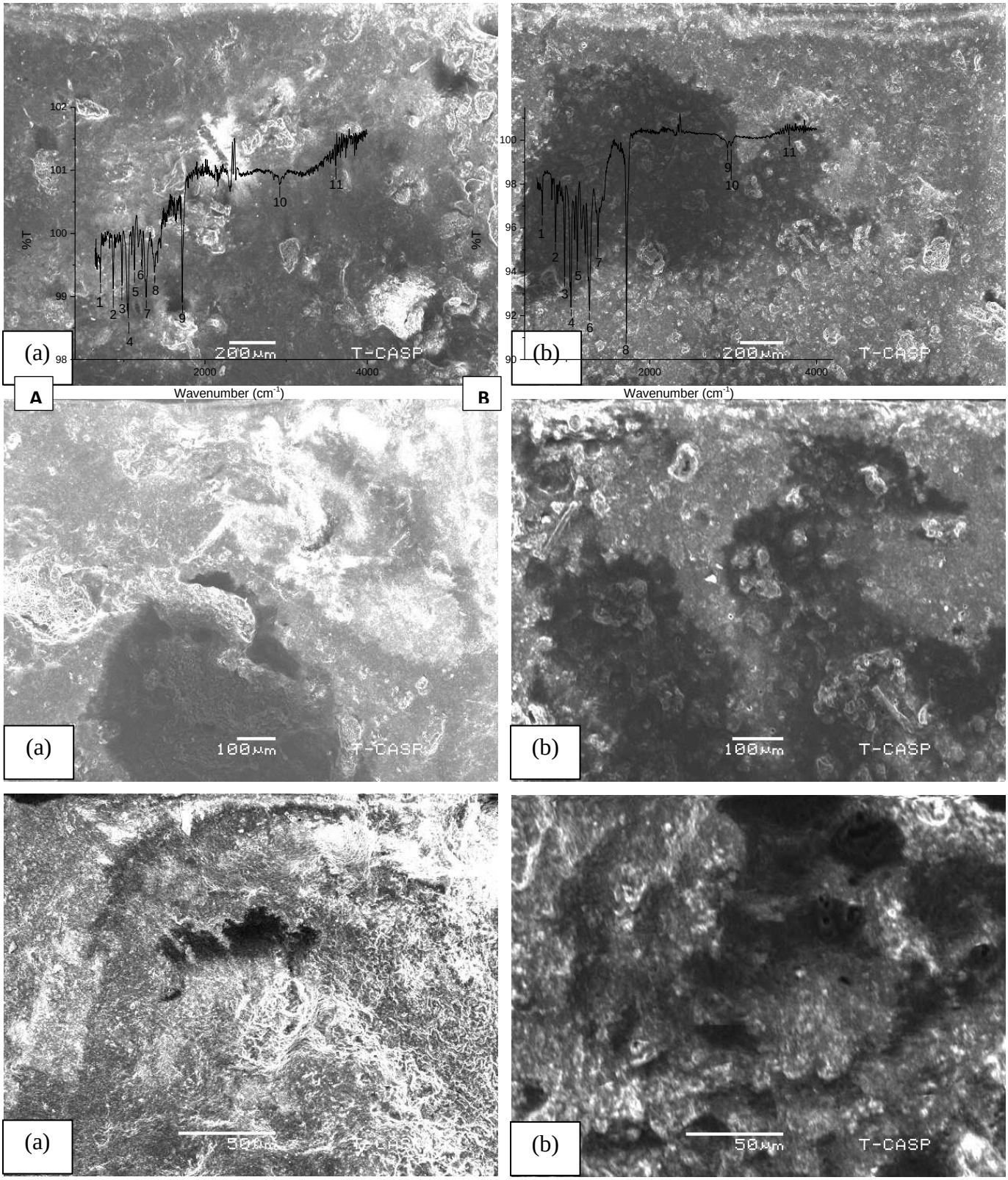

Figure 2.

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