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Isolation, purification and characterization of Polyhydroxybutyrate from *Vibrio mimicus*

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Keywords

Polyhydroxybutyrate,
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Bioplastic,
PHA,
Vibrio mimicus.

Abstract

This study was on isolation, purification and characterization of PHB from *Vibrio mimicus* strain isolated from sediment samples collected from Vellar estuary, Parangipettai. PHB produced from the cells that were grown at mass scale level under optimized growth parameters were used in the present study. The FT-IR analysis showed the presence of CH_2-CH_3-C=0 methyl esters in the extract and it also confirmed that the isolated product as PHB. Regarding degradation in the present work the complete bioplastic degradation i.e. 100% degradation was observed in 49 days at 35^oC, followed by 94% and 88% degradation at 30^oC and 25^oC respectively in 49 days of incubation whereas the least degradation was observed at 40^oC.

Introduction

Poly 3-hydroxybutyric acid (PHB) is the most common natural microbial (polyhydroxyalkanoate) PHA. In some microbial species, accumulation of PHA occurs during the presence of excess carbon and a limitation of nitrogen sources (Verlinden *et al.,* 2007). PHAs produced in response to stressful conditions serve as energy storage molecules to be utilized when common energy sources are absent (Solaiman and Ashby, 2005). The family of PHAs include several polymeric esters such polyhydroxybutyrates, polyhydroxybutyrate co-hydroxyvalerates (PHBV), polyhydroxybutyrate co-hydroxyhexanoate (PHBHx) and polyhydroxybutyrate co-hydroxyoctonoate (PHBO).

Bioplastics are made from biological materials which meet the need of keeping the huge advantages of conventional plastics by keeping away the harmful and serious environmental effects of it. Conventional plastics provide functionality that cannot be easily or economically replaced by other materials. Most of the plastics and plastic containing materials last for thousands of years.

Any physical or chemical change in polymer are due to environmental factors such as light, heat, moisture, chemical conditions and biological activity such as by microorganisms is termed as degradation of plastic. PHA is biodegradable and biocompatible, which is regarded as potentially useful polyester replacing petroleum-derived thermoplastics. PHB, is the well known member of the PHA series of polyesters, accumulates in many bacterial species as a carbon or energy storage material similar to sugar. PHB is readily biodegradable. Synthetic plastics provide a range of utilities in the civilization of mankind, at the same time the accumulation of these non degradable plastics in the environment is a menacing drawback increasing day by day. Hence the objective of the present study was isolation, purification and characterization of Polyhydroxybutyrate (PHB) produced from marine isolate *Vibrio mimicus*.

Materials and Methods

Isolation and purification of PHB granules

From our previous study cells which were growth optimized and produced at mass scale level was used for this purification and characterization study. The strain was isolated from soil sample collected from Vellar estuary, Parangipettai and it was identified as *Vibrio mimicus*. Cells containing the polymer were harvested from culture broth by centrifugation at 5,000 rpm for 20min at 4^{o}C and the cells pellet was washed once with sterile distilled water and suspended in 30% alkaline hypochlorite solution. For one volume of concentrated cells, three volumes of hypochlorite solution was added and digested for 90 min. at 37^{o}C. After centrifugation, pellet was dialyzed against distilled water for 24hrs. The pellet was repeatedly washed with distilled water and acetone. This partially purified pellet was dissolved in small volume of chloroform; with the insoluble remains being discarded by centrifugation. The polymer was precipitated from the concentrated solution with 10 volume of ethanol and the resulted material was poured in a pre-weighted glass tray and after complete evaporation, weight of PHB produced was estimated.

Alkaline hypochloride preparation

To 200 ml of distilled water 20g of bleaching powder and 5g of sodium bicarbonate were added and mixed thoroughly after which the solution was filtered using Whatman No. 1 filter paper and the pH was adjusted to 9.6.

FT-IR Analysis

The purified sample was first dried in an oven at 60^{o}C for 4hrs. After removing the moisture content, the samples were grown into fine powder. The IR spectrum of the PHB film was recorded with Perkin-Elmer model 297 IR Spectrophotometer. A thin film was scanned between 600 and 4000 wave number 9cm-1) at a speed of 1 micron/min., and with a programmed split opening 2X and air as reference. Infrared spectral analysis of biological material was utilized to investigate their chemical constituents. These are recognized even when the amount of material available is very small.

Degradation studies

Preparation of polyester sheet

The PHB was produced by *V. mimicus* was extracted using solvent extraction method and this was used for the preparation of the polyester sheet. 1.0 g of powdered PHB was dissolved in 100 ml of chloroform. The PHB solution was then poured (0.25 mm thickness) into an open, flat glass-tray and was allowed to evaporate slowly at 28-30^{o}C to form a film and it was used for the degradation studies.

Bioplastic degradation

Sediment samples were collected from Vellar estuary, Parangipettai was tested for degradation studies. The pre-weighed test pieces were buried into freshly collected sediment samples in wide-mouth jars. The mouth of the jars was kept open and incubated at four different temperatures under laboratory conditions (i.e.) 25, 30, 35 and 40^{o}C. Moisture content of samples was maintained by adding sterile distilled water. Degradation of PHB was measured by the loss of weight after definite period of incubation as per the method described by Manna and Paul (2000). For each temperature 3 different jars (i.e.) triplicates were maintained. 12 such sets were maintained for incubation and each set was assessed at an interval of 1 week. Based on the results the experiment was terminated after 7 weeks in most cases. % of degradation was estimated using the formula given below.

$$\frac{\{\text{Weight of the initial film} - \text{Weight of the film after degradation}\} \times 100}{\text{Weight of the initial film}}$$

Results and Discussion

Characterization of any product is important as it identifies whether the correct target product is produced. In the present investigation FTIR was used to characterize PHB produced. From the mass scale medium bioplastic was extracted using chloroform and it was kept for drying (Fig. 1). Partial purification of PHB was carried out using dialysis (Fig. 2). The IR spectrum of the PHB film was recorded with Perkin-Elmer model 297 IR Spectrophotometer and the results revealed different chemical groups (Fig. 3).

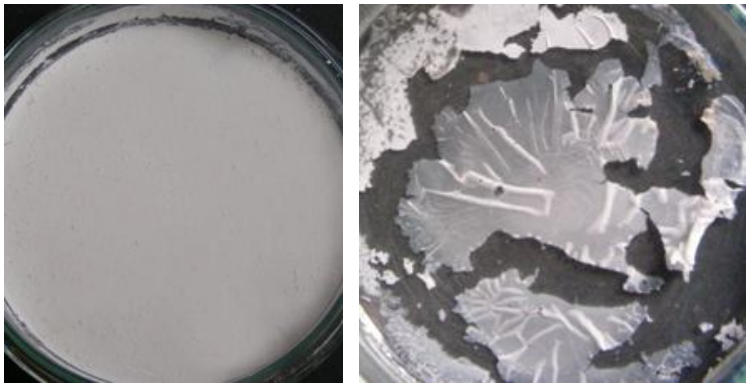

Fig. 1: Extraction of PHB

Fig. 2: Purification using Dialysis

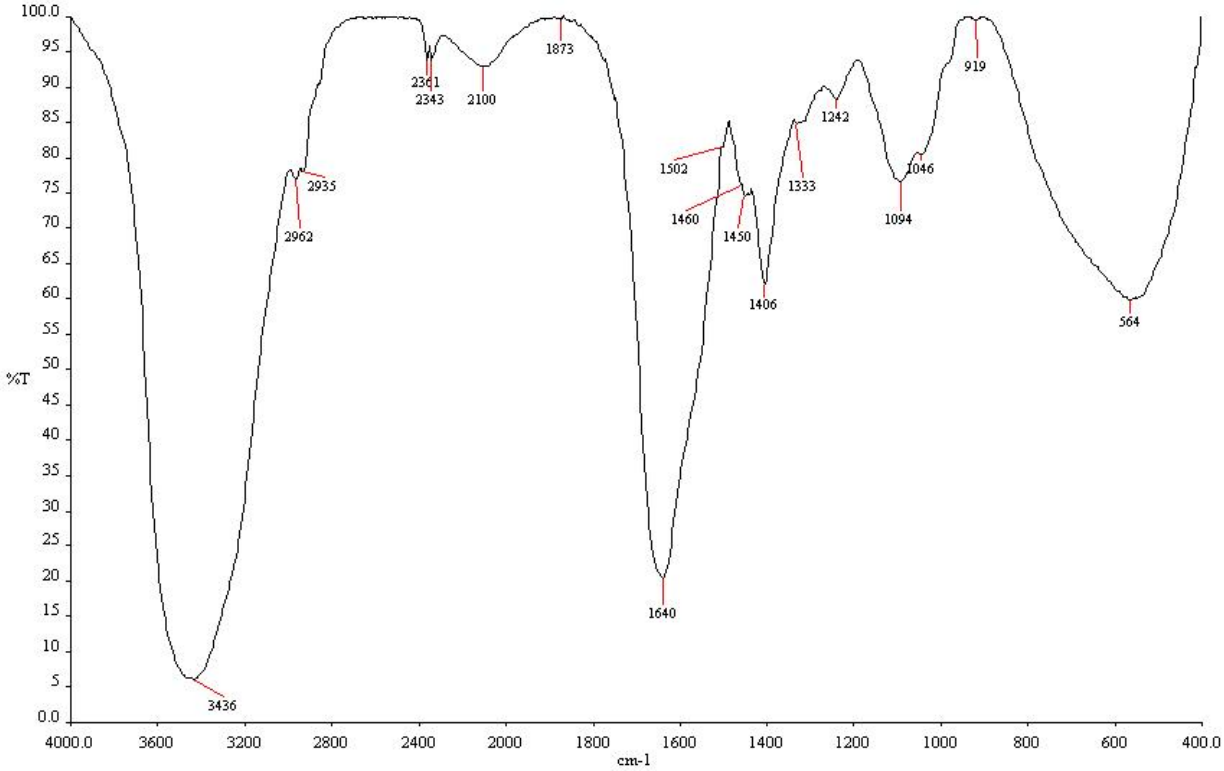

Fig. 3: FT-IR analysis of PHB

In the present study FT-IR spectral analysis revealed the following result (Fig. 3). The FT- IR analysis showed the presence of CH_2- CH_3-C=0 methyl esters in the extract. The wave numbers in the IR spectra showed presence of different chemical groups among them wave number 3372 represents OH. Similarly, 2936 represent O-H, whereas 1502 corresponds to N-H bend and 1460to CH_2 and CH_3 bend. 1406 and 1450 also denote CH_2 bending, 1333, 1046 and 1094 showed the presence of O-C stretch, 1242 and 564 signifies C=O (H-bonded) stretch, 919 to C-H and CH_2 bend. The IR spectrum was confirmed with the other published reports and thus confirmed as PHB. Otary and Ghosh (2009) extracted PHB from *B. megaterium* observed a large peak at 2956 cm^{-1} which represented the CH_2 groups and a peak at 1251 cm^{-1} which represented by C-O by using FT-IR analysis. Similarly, Shamala *et al.,* 2003 observed intense absorption FT-IR spectra typical to PHA of C=O and C-O stretching groups respectively with extracted PHB from *Bacillus spp*. Misra *et al.,* 2000 recommended IR spectra of the intact cells with positive absorption at 1724 cm^{-1} could be used as a tool to screen the PHB producing organisms. Thus the present study not only gave a solution for PHB production but also a permanent alternate solution for petroleum based plastics. The researcher may further continue the characterization of desirable properties of the PHB produced so that a desirable product may become as an outcome.

Fig. 4: Bioplastic degradation

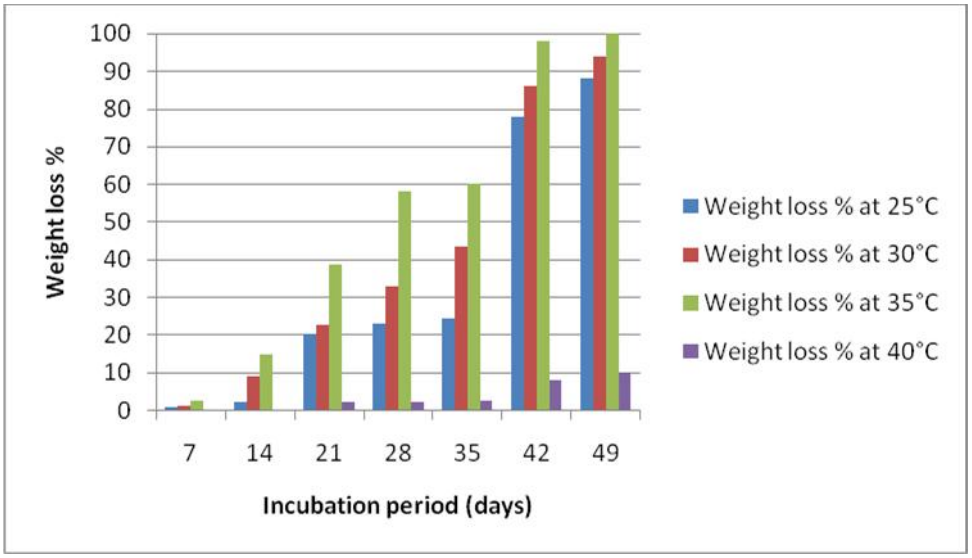

Fig. 5: Estimation of PHB degradation in the soil from Vellar estuary; Parangipettai

According to Budwill *et al.*, 1992, PHB was transformed to methane and CO_2 when a methanogenic bacterium isolated from sewage was used. In another study PHB was fermented to acetate and butyrate by strains isolated from different environments such as estuarine, fresh water lake and polluted pond sediments and they also opined that the rate obviously the rate limiting step in the total degradation of PHB (Janssen and Schink, 1993). Sterile PHB granules autoclaved in the medium did not slow any degradation. Similar observation also made in the present investigation also (data not shown). These studies indicated that microbial degradation is the only method through which environmental depletion is possible.

According to Gu (2003) various environmental parameters such as temperature, pH water and other culture conditions may affect the rage of degradation. However in the present study, as only the temperature was found to be a predominant factor in the degradation process; that factor alone was concentrated.

Regarding degradation of bioplastic, in the present investigation complete PHB degradation i.e. 100% degradation was observed in 49 days at 35^{o}C, followed by 94% and 88% degradation at 30^{o}C and 25^{o}C respectively in 49 days of incubation whereas the least degradation was observed at 40^{o}C (Fig. 4 & 5). In a thermophilic bacterium *Brevibacillus borstelensis* strain 707 it took 30 days at 50^{o}C which resulted in 11 and 30% respectively using gravimetric and molecular weight analysis. Through only FT-IR it was observed that photooxidized polyethylene revealed a reduction in carbonyl groups after bacterial treatment. Rajandas *et al.*, 2012 observed 61% and 50.5% degradation respectively using *Microbacterium paraoxydans* and *Pseudomonas aeruginosa*. Mahidiyah and Mukti (2013) observed a maximum of 17% degradation by the most potential strain kept at 37^{o}C, 130 rpm and 30 days of incubation.

References

Budwill, K., P.M. Fedorak, W.J. Page, 1992. Methanogenic degradation of poly (3-hydroxyalkanoates). *Appl. Environ. Microbiol.*, 58:1398-1401.

Gu, J. D., 2003. Microbiological deterioration and degradation of synthetic polymeric material: recent research advances. *Int. Biodeterior. Biodegrad.*, 52:69-91.

Janssen, P.H. and B. Schink, 1993. Pathway of anaerobic poly-β-hydroxybutyrate degradation by *Ilyobacter delafieldii*. Biodegradation 4: 179. doi:10.1007/ BF00695120.

Mahdiyah, D. and B.H. Mukti, 2013. Isolation of polyethylene plastic degrading-bacteria. *Biosci. Int.*, 2(3): 29-32.

Manna, A and A.K. Paul, 2000. Degradation of microbial polyester poly(3-hydroxybutyrate) in environmental samples and in culture. *Biodegradation.*, 11**:** 323–329.

Misra, A.K., M.S. Thakur, P. Srinivas and N.G. Karanth, 2000. Screening of poly- β-hydroxybutyrate-producing microorganisms using Fourier transform infrared spectroscopy. *Biotechnol. let.*, 22; 1217-1219.

Otari, S.V. and J.S. Ghosh, 2009. Production and characterization of the polymer polyhydroxy Butyrate-copolyhydroxy valerate by *Bacillus megaterium* NCIM 2475. *Curr. Res. J. Biol. Sci.*, 1(2): 23-26.

Rajandas, H., S. Parimannan, K. Sathasivam, M. Ravichandran and L.S. Yin, 2012. Novel FTIR-ATR spectroscopy based technique for the estimation of low-density polyethylene biodegradation. *Polym. Test.*, 31:1094–1099.

Shamala, T. R., A. Chandrashekar, S. V. N. Vijayendra and L. Kshama, 2003. Identification of polyhydroxyalkanoates (PHA) producing *Bacillus spp.* Using the polymerase chain reaction (PCR). *J. appl. Microbial.*, 94:369-374.

Solaiman, D. K and Ashby, R. D. 2005. Rapid genetic characterization of poly hydroxyl alkanoate synthase and its applications. Biomacromolecules, 6: 532 – 537.

Verlinden, R. A. J., D. J. Hill, M. A. Kenward, C. D. Williams and I. Radecka, 2007. Bacterial synthesis of biodegradable polyhydroxyalkanoates. *J. Appl. Microbiol.*, 102:1437-1449.

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