

## Production and Characterization of the Polymer Polyhydroxy Butyrate-co-polyhydroxy Valerate by *Bacillus Megaterium* NCIM 2475

S.V. Otari and J.S. Ghosh

Department of Microbiology, Shivaji University, Kolhapur 416004. M.S. India

**Abstract:** Polyhydroxyalkanoates (PHAs) are biodegradable polyesters which are synthesized by many bacteria. They are accumulated intracellularly as carbon and energy reserves under certain conditions like, in the presence of excess carbon source. The oldest known such polymer is poly hydroxy butyrate (PHB). However, commercial exploitation of such polymers have not been seriously attempted due to several reasons and one such reason is that PHB is not flexible as expected. It has also been pointed out that if PHB is blended suitably with certain other PHAs like polyhydroxy valerates (PHV) then the resultant material is more strong and flexible than PHB. The present investigation is aimed at production of PHV along with PHB from *Bacillus megaterium* NCIM 2475 and the results show that this was made up of PHB-co-PHV when grown in presence of sucrose.

**Key words:** *Bacillus megaterium*, PHB, PHV, Sucrose, co-polymer

### INTRODUCTION

Microbial polyesters, which contain hydroxyacyl monomers are, termed as polyhydroxyalkanoates (PHA), which are nothing but intracellular carbon reserves. However, of all PHAs, polyhydroxy butyrate (PHB) has been recognized as the main polyester and all other PHAs are co-polymers to this. These reserve polyesters have been the subjects of research of many workers before, with several objectives like the mode of formation etc. In the 1960s and 1970s, synthesis of medium chain length poly- $\beta$ -hydroxyalkanoates (mcl-PHAs) by bacteria was reported, but the evidence presented failed to elicit much interest. The first report of such heteropolymer was by Wallen and Rohwedder in 1974, who found these in chloroform extract of activated sludge. Later DeSmet *et al.* (1983) reported that *Pseudomonas oleovorans* accumulated polyhydroxy octanoates. Further it was reported by Lageveen in 1986 that *Alcaligenes eutrophus* was also producing these polyesters but again when grown on hydrocarbons. The situation changed in the following decade and PHAs composed of a large variety of monomeric units were described. At least 92 different monomers are now known to be present in natural PHAs (Steinbuechel and Valentin, 1995). The microbial PHA is a family of polyesters synthesized by a wide variety of bacteria as intracellular carbon and energy storage materials (Anderson and Dawes 1990; Doi 1990).

In the mean time, tremendous interest was being developed on production of chemical polymers using alkanes and other organic substances. These were not just affordable but also recyclable. Soon these polymers were in great demand both from industries and households, which finally led to its accumulation in the environment

due to the non biodegradable nature, leading to alteration of the native biodiversity, which was threatening the very existence of human beings along with other living forms. This was largely observed in many developing nations. There was a need to change the perception towards the research on these microbial polyesters and that was the beginning of studying the commercial feasibility of using these polyesters to replace at least partly if not wholly, the use of chemical polymers. A wide variety of PHA copolymers have been isolated from bacteria, like many cyanobacteria found in different environmental samples, including marine sediments. These polyesters accumulated as a result of limiting bacterial growth and supplying an excess amount of a carbon source (Jackson and Dawes 1976; Bertrand *et al.*, 1990; Dawes 1990; Labuzek *et al.*, 1994). These consists of short chain-length PHA with C3 to C5 monomer units. The best known example of the PHA family is the homopolymer PHB. Prokaryotic organisms are known to produce PHB amounting to as much as 80% of their cellular dry weight (Doi 1990). The biopolymer PHB, is a biodegradable and biocompatible thermoplastic with an isotactic structure and a high degree of crystallinity (approximately 80%). Thus physical properties of PHB are quite similar to polypropylene. The properties like crystallization, and tensile strength, depend on molecular weight, which are influenced by the strain of microorganism employed, growth conditions, and the purity of the sample obtained. However, there are certain disadvantages of use of PHB due to its tendency to be brittle. This problem could be solved by the synthesis of copolymers of 3-hydroxybutyrate and other hydroxyalkanoates with a relatively low molecular weight and melting point (de Koning 1995; Scandola 1995; Fukui and Doi 1997).

One such co-polymer used was polyhydroxy valerate (PHV). Major advantage of the PHB-PHV copolymer over the PHB homopolymer is that the copolymer has a lower flexural modulus or level of crystallinity, which makes it tougher and more flexible. Materials with high PHV content tend to be soft and tough, while materials with low PHV content are hard and brittle. High molecular weight of PHB is more useful and desirable for industrial applications. The molecular weight of PHB can be reduced during the polymer processing step (Bourque *et al.*, 1992).

In addition to the costs of maintaining pure cultures and the high costs of organic substrates, polymer recovery process is another factor that contributes to the high overall cost of PHA production. Several workers in this field have tried to isolate the genes responsible for such PHA in bacteria (Shamala *et al.*, 2003) and have tried to transfer these in an eukaryote like yeast which can grow on a far less expensive substrate and yet produce these PHAs in significant quantity (Ashraf *et al.*, 2007, Desouky and Haleem, 2009). In this study, an attempt has been made to explore the possibility of reducing the cost of substrate by using less expensive substrate like sucrose which is easily available as molasses from the sugar industry.

## MATERIALS AND METHODS

The *Bacillus megaterium* NCIM 2475 culture was grown in liquid medium containing 2% sucrose along with  $MgSO_4$ :0.4%,  $CaCl_2$ :0.11%,  $Na_2HPO_4$ :3.7%,  $(NH_4)_2SO_4$ :0.200%,  $K_2HPO_4$ :2.0% and Ammonium ferric citrate:0.600%. The flasks were incubated on shaker at 30°C for 72-96 hrs with an agitation rate of 125 rpm. First of all the growth pattern of this microorganism was recorded as shown in figure 1.

Polymer granules were harvested as per the method of Williamson and Wilkinson, 1958. The quantitation of PHAs was carried out by method of Kansiz *et al.*, 2000. This was then analyzed by FTIR, NMR and UV spectroscopy. The results are shown in following Figures 2, 3 and 4.

## RESULTS AND DISCUSSION

It can be seen from the Figure 1, that *Bacillus megaterium* NCIM 2475, entered the idiophase of growth from 8hrs onward and it was further noted that this idiophase continues upto 100 hrs. The polyester granules appeared inside the cell from 8<sup>th</sup> hour of growth and was best obtained around 96 hrs.

The UV absorption spectrum from Figure 2, shows 2 distinct absorption peaks; one around 230nm and the other around 290nm. The major peak, was however, at 290nm. This indicates that polyester accumulating inside the cell must be of at least 2 types.

The FTIR spectroscopic results from Figure 3, shows that the large peak at 2956.97 which represents the  $-CH_2$

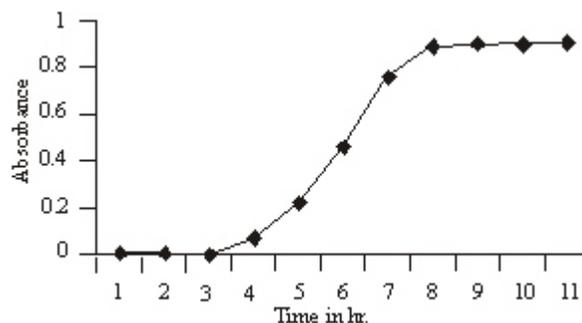


Fig. 1: The growth pattern of *Bacillus megaterium* NCIM 2475 on the above mentioned medium was obtained using the same specified incubation time and other conditions as specified

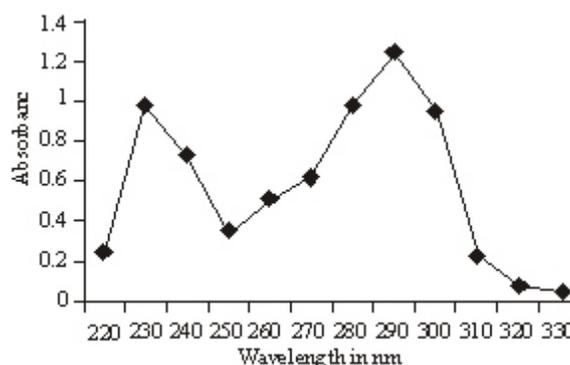


Fig 2 : UV Spectroscopy was done between the range of 220nm and 330nm

groups, followed by a peak at 1732.13 which shows the presence of  $-C=O$  and then a distinct peak at 1251.84 which is the  $-C-O$ . Now this peak should have been at 1280 which would have indicated that the  $-C-O$  is in stretched form which would have meant that the polyesters are all of 3-hydroxy alkanates. Since it was not so, further analysis was required to investigate whether there were other hydroxy alkanates present. This led to investigate the H-NMR spectroscopic data of the compound.

The results from Figure 4 showed that the compound was a polyester of 3-hydroxy butyrate with 3-hydroxy valerate and some amount of 5-hydroxy valerate. The quantitative analysis showed that PHB constituted nearly 10% of the dry weight of the cells, whereas PHVs were barely 2% of the dry weight of the cells at the end of 96<sup>th</sup> hour of incubation. On the other hand this combination of polyesters was not rigid like PHB alone and could be easily folded and shaped.

It is well known that the cells would produce depolymerase enzymes to breakdown PHB which is a reserve energy source. However, it still remains a puzzle, as to why the cells would produce PHVs as reserve energy source.

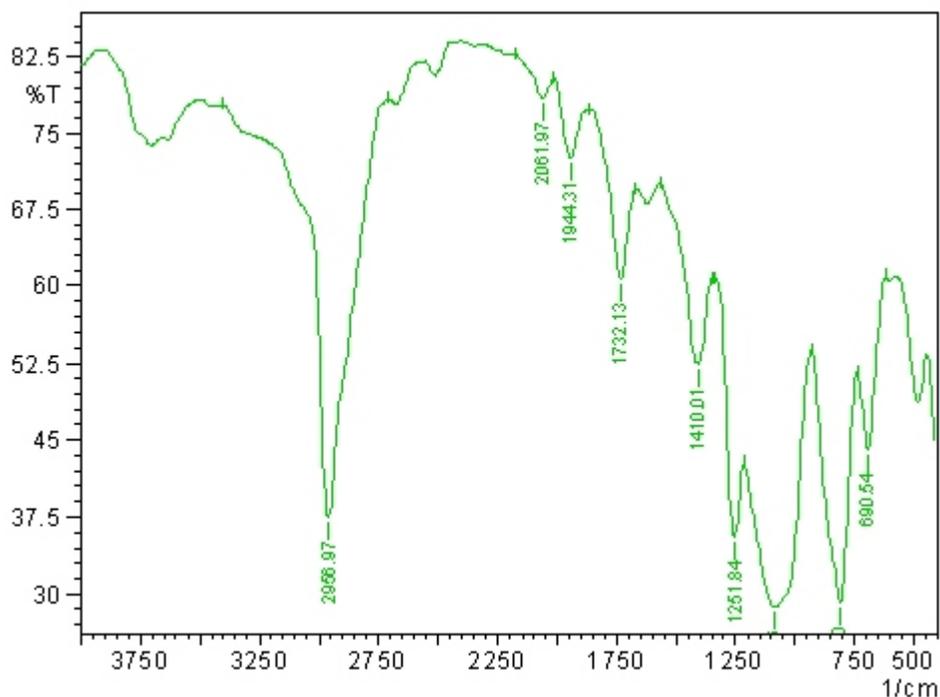


Fig 3: Fourier transform infra red (FTIR) spectroscopic results of compound

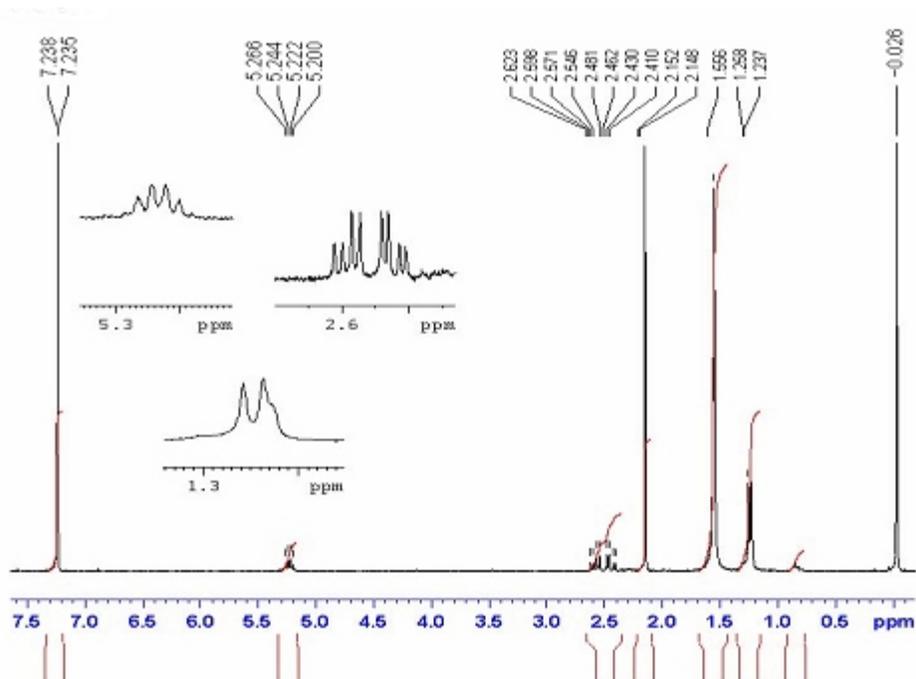


Figure 4: NMR spectra of the compound

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