5 Characterization of PHB using instrumental analysis

5.1 Preamble

Characterization of any product is important as it identifies whether the correct target product is produced along with details on quantity of the product as well as purity of the same. In the present investigation FTIR, NMR and DSC were used to characterize PHB produced.

5.2 Materials and Methods

5.2.1 Fourier transforms infrared spectroscopy (FT-IR)

The purified samples were first dried in an oven at 60 degree for 4 hrs. After removing the moisture content, the samples were grown into a fine powder. The IR spectrum of the PHB film was recorded with a Perkin-Elmer model 297 IR Spectrophotometer. A thin film was prepared from the chloroform solution and scanned between 600 and 4000 wave number (cm\(^{-1}\)) at a speed of 1 micron /min, and with a programmed slit 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 (Edward et al., 1958 and Glasser, 1961).

5.2.2 Nuclear magnetic resonance spectroscopy (NMR)

The polymer was suspended in spectrochem grade deuterochloroform (CDCl\(_3\)). The \(^1\)H NMR spectra of sample was obtained at 400 MHz using a model Bruker Advance 400 NMR spectrometer. The \(^{13}\)C NMR spectral analysis was performed at 80 MHz. Sample was dissolved in chloroform (1 mg/ ml\(^{-1}\) solvent) that was employed for each analysis.

5.2.3 Differential scanning calorimetry (DSC) and Differential thermo gravimetric analysis (DTA)

The thermal properties of samples were determined by using a Perkin–Elmer DSC-7 calorimeter. Approximately 10 mg of sample were used for the analysis. The samples were heated from -25\(^{\circ}\)C to 190\(^{\circ}\)C at a rate of 10\(^{\circ}\)C min\(^{-1}\). The first and second cooling runs were carried out at rates of -190\(^{\circ}\)C min\(^{-1}\) and -10\(^{\circ}\)C min\(^{-1}\), respectively (Fabiane et., al., 2007). This thermal analyzer was determined
under N₂ flow of 20 ml min⁻¹. From the first and second heating runs, mass changing melting temperature (Tm) was determined from the ratio of the melting enthalpy of the sample (ΔHm) (Jianchun et al., 2003 and Gogolewski et al., 1993). Thermo gravimetric analysis was made in a thermo gravimetric analyzer operating with N₂ flow of 20 ml/min and scan rate of 10⁰C/min in the range of 30 to 60⁰C.

5.3 Results and discussion

![FT-IR spectrum of pure PHB](image)

**Fig.34 FT-IR spectrum of pure PHB**

The FT-IR analysis of pure PHB, isolated from the strain *A. eutrophus* revealed that the absorption band occurred at 3430 cm⁻¹ representing the O-H bending. The peak at 2946 cm⁻¹ shows the strong –CH₂ stretching groups. The medium-strong C=H bond occurred at 2359 cm⁻¹ and 2342 cm⁻¹. A medium-weak C=O stretching bond occurred at 2166 cm⁻¹. A medium-weak C=C stretching bond occurred at 2099 cm⁻¹. A medium-strong CH₃ stretching bond was occurred at 1455 cm⁻¹. A medium
strong C-H bond was occurred at 1467 cm\(^{-1}\). A medium strong C-H bond was found at 1360 cm\(^{-1}\). An aliphatic asymmetrical O-H bond was observed at 1342 cm\(^{-1}\). A medium O-H bond was occurred at 1280 cm\(^{-1}\). Sulphate groups were occurred at 114 cm\(^{-1}\). A medium weak C-O stretching bond was occurred 1112 cm\(^{-1}\). Aromatic phosphates were occurred at 962 cm\(^{-1}\). Pal and Paul (2002) extracted PHB from *Azotobacter chroococcum* and analysed by FT-IR. The peaks at the wave numbers at 3440, 2920-290, 1720 and 1240-1370 cm\(^{-1}\) strongly represented the presence of O-H bending, two bands of C-H stretch, strong absorption band of aliphatic carbonyl C=O of \(RCOR\) and C-H band of aliphatic compound respectively. The FT-IR analysis of a PHB film was done by Rohini *et al.*, 2006 in *Bacillus thuringiensis* and their results revealed the two absorption bands at 1280 cm\(^{-1}\) and 1735 cm\(^{-1}\) corresponded to the presence of C=O and C-O stretching groups, respectively. Otary and Ghosh (2009) extracted PHB from *B. megaterium* and made a FT-IR analysis. They observed a large peak at 2956 cm\(^{-1}\) which represented the CH\(_2\) groups. Also they observed a peak at 1732 cm\(^{-1}\) which showed the presence of C=O and a peak at 1251 cm\(^{-1}\) which represented by C-O. Senthilkumar and Prabakaran (2006) analysed PHB obtained from *A. eutrophus*. Their IR spectrum of the compound was recorded in the range of 100-4000 cm\(^{-1}\) and it showed characteristic bands for the groups like CH, C=O and C-O. The methane (CH) group gave a strong band in the range of 1360-1416 and 2914-3047 cm\(^{-1}\). The carbonyl group (C=O) gave a strong band in the range of 1636-1673. Misra *et al.*, 2000 suggested that the 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. Shamala *et al.*, 2003 extracted PHB from a *Bacillus spp* and subjected to FT-IR spectroscopy. There was some intense absorption spectra typical to PHA viz., C=O and C-O stretching groups respectively.

5.3.1.1 NMR analysis

NMR analysis was used to determine quality of PHB structural composition. The \(^1\)H and \(^{13}\)C NMR spectra obtained from PHB sample produced from *A.eutrophus*. 
5.3.1.2 $^1$H NMR analysis

Fig. 35 $^1$H NMR spectrum of pure PHB
The $^1$H NMR spectral analysis demonstrated the presence of 3, 2 and 1 protons at chemical shifts 1.2, 2.4-2.6 and 5.3 respectively representing CH$_3$, CH$_2$ and CH groups. The molecular composition of the polymer as indicated by chemical shifts, generates a structure of (CH$_2$-CH) backbone and assigned the presence of (CH$_3$) group. As an evidence of this finding the work done by Rohini et al., 2006 can be equated. They identified the polymer with the spectrum which revealed the presence of three groups of signals characteristic of PHB by the doublet at 1.3ppm attributed to the methyl group coupled to one proton; and the spectrum of the quadruplet at 2.57ppm the methylene group adjacent to an asymmetric carbon atom bearing a single proton and the multiplet at 5.28ppm to the methylene group. Yu and Marchessault, 2000 identified CH$_3$ at 1.25ppm, CH$_2$ at 2.42ppm. Pal and Paul (2002) identified the chemical shifts 1.2, 2.4-2.6 and 5.3 representing the CH$_3$ and CH$_2$ and CH groups. The results of the present study, exactly matched with above mentioned studies, which confirmed the product as PHB.

5.3.1.3 $^{13}$C NMR analysis and spectral analysis of pure PHB
<table>
<thead>
<tr>
<th>C atom</th>
<th>PHB sample</th>
<th>Commercial PHB</th>
<th>PHB</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td>19.76</td>
<td>19.81</td>
<td>19.65</td>
</tr>
<tr>
<td>CH₂</td>
<td>40.80</td>
<td>40.72</td>
<td>40.66</td>
</tr>
<tr>
<td>CH</td>
<td>67.62</td>
<td>67.34</td>
<td>67.48</td>
</tr>
<tr>
<td>C=O</td>
<td>169.15</td>
<td>169.48</td>
<td>169.03</td>
</tr>
</tbody>
</table>

The results obtained in the present investigation was comparable with the commercial PHB previously referred by Chaijamrus and Udpuay, 2008. The spectrum revealed the presence of three groups of signals characteristic group of homopolymer. The spectrum was found to match perfectly with each other. The peaks observed in the spectra coincided with the different types of carbon atoms presented in the PHB structure, [-O-.CH-(CH₃)-CH₂(C=O)]ₙ. The chemical shift signals of $^{13}$C NMR spectrum obtained in the present work and the commercial PHB were in agreement with those obtained by Fabiane et al., 2007. Pal and Paul, 2002 characterized the polymer on the chemical shifts at 19.74, 40.84, 67.64 and 169.07ppm which assigned to the presence of CH₃ and CH₂ and CH and C=O groups respectively. The results of $^{13}$C NMR also reaffirmed the product as PHB.
5.3.1.4  Differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA)

Fig. 37 Differential scanning calorimetry analysis

Fig. 38 Thermogravimetric analysis

He DSC curve showed that the a melting temperature of the polymer might be 166.68°C. The polymer was degraded rapidly at 269.90°C with a peak at 260-270°C. The following works showed values closer to the present study. The melting temperature of PHB of (166°C) in the present study was nearer to the reports for PHB obtained from B. cereus (170°C) (Labuzek et al.,
1994) and *B. circulans* (173°C) (Roza et al., 1996). Gunaratne et al., 2004 and Desouky et al., 2007 showed that the melting point of PHB extracted from yeast ranged from 152.84°C to 168.33°C. Thermal degradation occurred around 230.07 and 269.90°C with a single weight loss step. Khan et al., 1995 determined the melting temperature of the polymer between 173°C and 176°C which showed a lower value compared to the present study. Pal and Paul (2002) results the melting temperature of PHB as 174.73°C while the thermal degradation at 250°C which were comparable to the results obtained in the present study. Thus characterization of PHB produced in the present study not only confirmed the purity of the product also showed the expected qualities of a plastic.

### 6 DEGRADATION OF POLY-β-HYDROXYBUTYRATE AND POLYMER BLEND

#### 6.1 Preamble

Polymeric materials (plastics) are now used in all sectors of life as very durable products with tailor-made properties. During the past decade the intense use of modern plastics, combined with their