Characterization of polyhydroxy alkanoates – Biodegradable plastics from marine bacteria

A wide variety of bacteria accumulate polyhydroxy alkanoates (PHAs) as intracellular storage material1-4. Because of their physical and structural properties and amenability to biodegradation, PHAs are considered potential substitutes for petrochemical plastics. PHAs vary in their mechanical properties depending on the composition of the monomeric units5. The medium chain-length PHAs are semicrystalline elastomers with a low melting point (Tm), low tensile strength and high elongation to break6,7, and can be used as biodegradable rubber. Polyhydroxy butyrate (PHB), smallest known PHA displays a similar degree of crystallinity and Tm as polystyrene8, and is stiffer and more brittle than polypropylene9,10; but its copolymerization with hydroxy valerate (HV) monomer units reduces its stiffness and increases its toughness, giving a product with desirable properties for commercial applications11. Polymers may fail in specific applications, simply because they do not possess the necessary strength to carry the designed load or occasional overload10. Hence, it is important to study the mechanical and physical properties of such commercially important polymers before their use in the industry.

Tropical mangrove and marine ecosystems from the mid-west coast of India were screened for promising bacteria, with capability of accumulating high amounts of PHA11. The isolates designated as 61/4, 64/4, 87/4, 182/5, 12/BL, 85/6 and 86/6, which accumulated more than one gram PHA per litre culture broth were studied for physico-chemical factors influencing quantitative yield of PHA (unpublished results). The physical and mechanical properties of the PHA produced by these organisms are presented here.

The selected isolates were grown routinely in 50 ml of E2 mineral medium12 consisting of NaH2PO4, 4H2O, 3.5 g; K2HPO4, 3H2O, 7.5 g; KH2PO4, 3.7 g per litre; MgSO4, 7H2O, 0.17 g, and microelements stock solution, 1 ml containing FeSO4, 7H2O, 2.78 mg; MnCl2, 4H2O, 1.98 mg; CoSO4, 7H2O, 2.81 mg; CaCl2, 2H2O, 1.47 mg; CuCl2-2H2O, 0.17 mg, and ZnSO4, 7H2O, 0.29 mg supplemented with yeast extract 0.04% (w/v), glucose 2% (w/v), for 48 h on an Orbitek shaker at 28°C and 150 rpm. The cells were washed with saline by centrifugation. The PHA extracted from the cell pellet by the hypochlorite method13, was washed with methanol and acetone consecutively and centrifuged at 8000 rpm for 20 min. The polymers were then dissolved in hot...
chloroform (60°C) and the solution poured onto glass trays. The chloroform was allowed to evaporate slowly by placing the trays in the cold room at 4°C. The film of PHA so obtained was used for further analysis.

PHA from the seven cultures was esterified by acidified methanolysis. Thin layer chromatogram (TLC) on silica gel of the methyl esters, run in benzene and ethyl acetate (1:1), visualized with iodine vapours, exhibited two different patterns. Esters of PHA extracted from five isolates, viz. 61/4, 64/4, 87/4, 182/5 and 12/BL exhibited two distinct spots with Rf 0.75 and 0.6. Methyl esters of homopolymers of PHB are known to exhibit two spots, one being that of the ester of 3-hydroxy butyrate, and the other with higher Rf that of the dimeric hydroxy butyrate which is more nonlinear and separates first. The methyl ester of PHA of Rf 0.6 was eluted in benzene with 50% of ethyl acetate on the silica gel column. The IR (Shimadzu FT IR 8101 A) (Figure 1 a and b) and the H NMR (Bruker WT 300 MHz FT NMR) spectra (Figure 1 c and d) of the PHA films and the purified methyl ester recorded in duplicates, confirmed the polymer to be homopolymers of 3-hydroxy butyrate. The TLC profiles of the methyl esters of PHA extracted from isolates 85/6 and 86/6 exhibited four distinct spots with Rf 0.75, 0.6, 0.44 and 0.3. These PHAs were therefore initially thought to be betomers, with 3-hydroxy butyrate as one of the components. Surprisingly, the H NMR spectrum of the methyl esters of the monomer with Rf 0.44 also resembled that recorded for the methyl ester of 3-hydroxy butyrate, except for the signal at 3.5 ppm, of the methyl ester moiety. Re-esterification of the fraction by acidified methanolysis showed a single spot of Rf 0.6, confirming it to be methyl ester of 3-hydroxy butyrate. Hence, the fraction with 0.44 was also confirmed as 3-hydroxy butyric acid, a fraction of the PHA which got hydrolysed but not esterified, possibly because of the high number average molecular weight of the PHA (Table 1). The spot at 0.3 was confirmed to be that of the oligomers of PHA, on co-TLC with the PHA polymer. The C NMR spectrum of PHA (Figure 1 e), recorded four distinct peaks at 20, 40, 67 and 170 ppm, confirming the polymer to be homopolymer of 3-hydroxy butyrate.

Although all the seven polymers were confirmed to be homopolymers of PHB,
Crystallinity and molecular weight have marked effect on the mechanical properties of polymers. On the basis of their molecular weight, the isolates 61/4 and 85/6 were selected for the study. The mechanical measurements were done using PHA bars of 2 cm x 7 cm, with 1.0–1.5 mm thickness on an Instron Corporation series IX Automated Materials Testing System 1.04, with sample rate 10 point/s and cross head speed 20 mm/min, at 27°C with 50% humidity. The mechanical tensile data were calculated as an average of three specimens. The toughness of the polymer is measured in terms of its impact strength, while the flexibility is related to the capacity for elongation to break [1,5,10]. The elongation to break (%) for PHB from isolate 61/4 (Table 2) was found to be much lower than that reported for polyethylene, but higher than that reported for PHB [8,15,18]. Placed in the increasing order in terms of toughness and Young’s modulus, these are polyethylene < 85/6 < 61/4 < polypropylene (Table 2). Since the PHB from 61/4 and 85/6 is tough, it can find applications in load-bearing.

Overall, the PHA extracted from the bacterial isolates in the tropical marine ecosystem possessed varied physical and mechanical properties, thus reflecting the diversity of the ecosystem to harbour such bacteria.

Table 2. Mechanical properties of PHB from isolates 61/4 and 85/6

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Stress at break (MPa)</th>
<th>Young’s modulus (MPa)</th>
<th>Toughness (MPa)</th>
<th>Strain at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>61/4</td>
<td>35.51</td>
<td>1785.00</td>
<td>33.44</td>
<td>11.40</td>
</tr>
<tr>
<td>85/6</td>
<td>30.71</td>
<td>1474.00</td>
<td>25.81</td>
<td>15.41</td>
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