Biosynthesis of Poly(hydroxybutyrate) and its Properties

Moon Shik Kim, Soo-Min PARK
Pusan National Univ. Dept. of Text. Eng.

Microorganisms capable of accumulating poly-β-hydroxybutyric acid (PHB) were isolated from soil by enrichment culture technique. *Alcaligenes* FL-027 was used in this study.

To investigate the optimal conditions for the PHB accumulation, we divided the process into two stages; the first stage for the growth of the cell at 30 ℃ and pH 7.0 in nutrient-rich medium containing 8.0g/l of fructose and 3.0g/l of $(NH_4)_2SO_4$, equivalent to C/N ratio 5.04; the second stage for the PHB accumulation at 30℃ and pH 6.5 in nutrient-deficiency medium containing 8.0g/l of fructose and 0.25g/l of $(NH_4)_2SO_4$, equivalent to C/N ratio 60. PHB accumulation was stimulated by deficiency of nutrients such as NH_4^+, Ca^{2+} and SO_4^{2-} in medium. Among them, NH_4^+ deficiency was chosen because of its effectiveness.

We found that cell growth inhibited by fructose in batch culture. The cells were harvested after 48h, and washed with water and acetone, and then extracted several times by chloroform. The PHB mixture was precipitated with methanol, and filtered the sediment with filter paper after dried. The purified PHB was identified as homopolymer of 3-hydroxybutyric acid by using IR, ^{1}H-NMR, ^{13}C-NMR and DSC.

The ^{1}H and ^{13}C NMR analysis of PHB sample were carried out on a BRÜKER AM 300 spectrometer. Chemical shift assignments for each well-characterized proton resonance (CH_3(1.26-1.28), CH_2(2.42-2.63) and CH(5.21-5.27)) appear in 300MHz ^{1}H NMR spectra.

Specific enhencements in the intencities of carbonyl carbon resonance (169.35) and methine carbon resonance(69.467) were exhibited in 75MHz ^{13}C NMR spectra.

The CH stretching and -COO- absorbance bands by IR analysis are appeared at 2960cm^{-1} and 1730cm^{-1}, respectively.

The maximum point of the endotherm peak in DSC curve is appeared at 174.24℃.