Production and Characterization of Biodegradable Terpolymer Poly(3-Hydroxybutyrate-*co*-3-Hydroxyvalerate-*co*-4-Hydroxybutyrate) by *Alcaligenes* sp. A-04

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Polyhydroxyalkanoate (PHA) is one of the most accepted natural polymers produced by bacterial fermentation using agricultural and renewable resources as substrate materials. The major applications of PHA include complete degradation in various environments without generating any toxic compounds. Nowadays, the problem of the depleting petroleum has been recognized and the motivation for utilizing renewable resources leads to a better environment and higher added value for agricultural products. Although the development of PHA production is still at a trial research basis, it already promises long-term potential and suitable alternative materials for the replacement of petrochemical-based plastics.

In this paper, the production of terpolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-4-hydroxybutyrate), P(3HB-*co*-3HV-*co*-4HB), by *Alcaligenes* sp. A-04 was investigated to determine the superior polymer properties over those of poly(3-hydroxybutyrate), P(3HB), and its derivative copolymers. The objective of this study was to produce a large amount of terpolymer with a wide range of 4HB mole fraction units using *Alcaligenes* sp. A-04. Moreover, the terpolymer produced from 1,4-butanediol was also investigated to reduce the production cost of using γ-hydroxybutyrate sodium salt, 4HB-Na, which is more expensive than 1,4-butanediol.

Alcaligenes sp. A-04 was isolated from soil in Thailand and used as a PHA-producing strain. Our preliminary study on the production of terpolymer by *Alcaligenes* sp. A-04 was performed according to the method described in the literature. However, for terpolymer production, there is a need to increase the amounts of butyric acid, valeric acid, and 4HB-Na in the production medium. The inhibition by these compounds of cell growth and terpolymer production has been observed. Therefore, the preculture medium should be improved to obtain a high cell mass and increase terpolymer production and it was modified by supplementing fructose with caution for the high toxicity of valeric acid as observed when it was used as one of the mixed carbon sources for terpolymer. Finally, the highest terpolymer content of 68% (w/w) was produced by *Alcaligenes* sp. A-04 at 60 h. The terpolymer with 93 mol% 4HB mole fraction units was produced when the cultivation time was extended to 96 h. Next, terpolymers with 4HB mole fraction units ranging from 50 to 90 mol% were produced by varying the medium composition and cultivation time. The thermal and mechanical properties of the resulting terpolymers were different from those of the copolymers with a similar mole fraction of monomer units. The terpolymer P(4%3HB-*co*-3%3HV-*co*-93%4HB) showed an elongation of 430%, a toughness of 33 MPa, and Young's modulus of 127 MPa similar to those of low-density polyethylene. The terpolymer P(11%3HB-*co*-34%3HV-*co*-55%4HB) showed Young's Modulus of 618 MPa similar to that of polypropylene.

Table 1. Thermal properties of terpolymer compared with those of copolymers produced by *Alcaligenes* sp. A-04

Terpolymer	Toughness (MPa)	Young's modulus (MPa)	Elongation (%)
P(3HB-*co*-3HV-*co*-4HB)			
10%3HB 40%3HV 50%4HB	0.22	503	4
11%3HB 34%3HV 55%4HB	0.26	618	3
11%3HB 23%3HV 66%4HB	0.32	392	5
12%3HB 12%3HV 76%4HB	0.39	142	9
10%3HB 6%3HV 84%4HB	20	118	300
4%3HB 3%3HV 93%4HB	33	127	430
Plastic bag (HDPE)	62	640	576
Plastic bag (PP)	64	590	435
Plastic bag (LDPE)	15	156	126
UV degradable bag	60	674	384

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