TITLE: POLY(3-HYDROXYBUTYRATE-*CO*-3-HYDROXYVALERATE-*C0*-4-HYDROXYVALERATE) PRODUCTION FROM CRUDE GLYCEROL AND LEVULINIC ACID BY *Pandoraea* sp. MA03PRP25

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ABSTRACT:
Polyhydroxyalkanoates (PHAs) are microbial polyesters which are composed of 3-hydroxy fatty acid monomers. Among approximately 150 different constituents of PHAs, the poly(3-hydroxybutyrate) [P(3HB)] is the most common type and extensively studied. The P(3HB) resemble the physical and chemical properties of petroleum-based polypropylene (PP) and so can replace this nonbiodebradable plastics. However, the P(3HB) application range has been limited due to its high crystallinity and low extension to break. These unfavorable characteristics can be improved by co-monomers resulting in appropriate characteristics for thermoplastic processing. Although PHAs are promising candidates to replace petrochemical plastics, they have encountered its main delay in the costs ascribed to carbon sources for bacterial cultivations. Similarly, biodiesel industry has focused their efforts to associate this biofuel production with value-added chemicals in order to support their production process. For this, the utilization of crude glycerol surplus, the main byproduct from biodiesel production, is imperative. Therefore, the main goal of this work was to obtain a terpolymer poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-4-hydroxyvalerate) [P(3HB-*co*-3HV-*co*-4HV)] from crude glycerol and levulinic acid. *Pandoraea* sp. MA03prp25 mutant strain was cultivated in shake flasks in mineral salts medium containing 1 g/L $(NH_4)_2SO_4$ with different crude glycerol concentrations (10, 20, 30, 40 and 50 g/L). Cultivations were carried out at 30 °C for 96 h at 200 rpm. Levulinic acid was added after 24 and 48 h cultivation as 3HV and 4HV precursor at a concentration of 1 g/L. Cells were harvested by centrifugation and freeze-dried. Biomass was determined gravimetrically as cell dry weight (CDW). Freeze-dried cells were subject to methanolysis reaction, whose methyl esters obtained were determined by GC coupled MS analysis for quantitative and qualitative analysis of PHAs. Glycerol and levulinic acid were determined by HPLC analysis. The best polymer production (0.72 g/L and 51.17 % of CDW) was obtained in cultivations with 20 g/L crude glycerol, which was observed a terpolymer comprised of 79.41 mol% of 3HB, 17.70 mol% of 3HV and 2.89 mol% of 4HV. These results showed that the mutant *Pandoraea* sp. MA03prp25 is a promising bacterial strain for the synthesis of a innovative terpolymer from crude glycerol and levulinic acid.

Keywords: polyhydroxyalkanoates, bioplastic, biodiesel, glycerol, terpolymer

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