

Effect of Temperature on the Biosynthesis of PHB by *Ralstonia eutropha* in Phosphorus-limiting Fermentation

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ABSTRACT

Abstract Polyhydroxyalkanoate, synthesized by microbes, is one kind of biodegradable plastics. PHB is the representative with the property similar to polypropylene. PHB may partly replace traditional plastics made from petroleum to reduce the threat to the environment. PHB can be accumulated as an intracellular polymer by microbes under the excess of carbon sources and a limitation of certain nutrients. Phosphorus is an important substance related to the energy transport of ATP. When ATP regeneration is restricted due to the lack of inorganic phosphate, the metabolism would channel to PHB synthesis. In this study, *Ralstonia eutropha* was cultivated in batch fermentation with sufficient nitrogen and carbon but phosphorus-limited sources under various temperatures (26, 30 and 35 °C). The microbial growth, PHB production, consumption of glucose, nitrogen and phosphorus, and the metabolic acids secreted by *Ralstonia eutropha* were investigated. At 26 °C, the highest productivities of biomass and PHB were obtained to be 0.44 and 0.35 g/L h at 72 h. The highest coefficients of yield, YX/G, YP/X and YP/G, were 0.42, 0.79 and 0.33, respectively, at 72 h. The most quantity of metabolic acids, 9.78 mg per gram of biomass, secreted by *Ralstonia eutropha* was formic acid at 96 h. The quantity of fumaric acid was the lowest among the detected acids. The succinic, citric and fumaric acids, were 6.66, 4.87 and 0.07 mg per gram of biomass, respectively. At 30 °C, the highest productivities of biomass and PHB were obtained to be 0.74 and 0.56 g/L h, respectively, at 48 h. The highest coefficients of yield, YX/G, YP/X and YP/G, were 0.39, 0.82 and 0.32, respectively, at 96 h. The most quantity of metabolic acids, 3.93 mg per gram of biomass, secreted by *Ralstonia eutropha* was citric acid at 96 h. The quantity of fumaric acid was the lowest among the detected acids. The succinic, formic and fumaric acids were 3.37, 1.62 and 0.02 mg per gram of biomass, respectively. The quantity of formic acid raised gradually at the beginning, but declined obviously in the middle period. At 35 °C, the highest productivities of biomass and PHB were obtained to be 0.63 and 0.50 g/L h at 60 h. The highest coefficients of yield, YX/G, YP/X and YP/G were 0.40, 0.82 and 0.32, respectively, at 96 h. The most quantity of metabolic acids, 8.43 mg per gram of biomass, secreted by *Ralstonia eutropha* was succinic acid at 96 h. The quantity of fumaric acid was the lowest among the detected acids. The formic, citric, malic and fumaric acid were 5.00, 3.24, 0.66 and 0.05 mg per gram of biomass, respectively. The above results indicated that the quantity of metabolic acids secreted by *Ralstonia eutropha* were the highest when cultivated at 26 °C. And therefore, biomass and PHB productivities were somewhat restricted. The quantity of metabolic acids secreted by *Ralstonia eutropha* was the lowest at 30 °C, but biomass and PHB productivities were the highest. The quantity of metabolic acids secreted by *Ralstonia eutropha* was not the lowest at 35 °C. It seemed that the quantity of metabolic acids secreted by microorganism was not directly related to temperature, but the biomass productivity was obviously affected by the quantity of metabolic acid. At 35 °C, malic acid was produced in the later period, it might suggest that the metabolic pathway of microorganism was changed due to a higher temperature. Keywords: PHB, *Ralstonia eutropha*, productivity, coefficient of yield

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