

Title: POLYHYDROXYBUTYRATE (PHB) PRODUCTION IN DIFFERENT CULTURE MEDIUM FORMULATIONS BY *AZOSPIRILLUM BRASILENSE* Ab-V5

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Abstract:

The polyhydroxybutyrate (PHB) is an intracellular storage biopolymer synthesized by many microorganisms, including bacteria of the genus *Azospirillum*. These are known for their ability promotes plant growth, and used in the production of commercial inoculants. *Azospirillum* biomass production with high content of PHB can provide greater viability and rihizocompetence of the bacteria in inoculants formulations. Knowing that the intracellular accumulation of PHB is influenced by factors such as culture medium composition and culture age, the aim of this study was to evaluate the production of this biopolymer by *A. brasilense* Ab-V5 using formulations with differences in C/N ratio. *A. brasilense* Ab-V5 was grown in 120 mL Erlenmeyer flasks with the tested formulations (MCA2, C/N = 7.66; MCA4, C/N = 33.4; FORM15, C/N = 8.39) under stirring of 180 rpm and 28 °C for 228 h (10 days), in triplicate. Samples were collected 12 h after inoculation, and then at 24 h intervals until they reached 10 days of cultivation for determination of PHB and microscopic evaluation. At each harvest, the cell culture was centrifuged and the biopolymer extraction was made from dry biomass cells resuspended in sodium hypochlorite solution (5.25% v/v) and incubated for 2 h at 40°C. The mixture was centrifuged followed by successive washes of PHB, and from the dry biopolymer digestion was carried out with concentrated sulfuric acid (30 min; 90 °C). The PHB content was determined in a spectrophotometer at 235 nm and the formation of intracellular granules was visualized with an epifluorescence microscope using Nilo red dye. The production of PHB showed a high variability as a function of culture age and tested formulation. The MCA4 provided a greater accumulation of PHB by the bacterium (1,73 gL⁻¹), and 44% cellular biomass was represented by polymer after 204 h of culture. However, the highest percentage of PHB in *A. brasilense* cells grown in FORM15 and MCA2 medium was 29,44% in 228 h of culture, and 20,21% at 156 h of culture, respectively, confirming the influence of the relationship C/N on the accumulation of PHB. The microscopic observation of PHB granules in cells of *A. brasilense* Ab-V5 confirm the polymer production results during the cultivation. It follows that the C/N culture medium and the culture age influences the intracellular accumulation of PHB by *A. brasilense* Ab-V5, and should be considered for the development of commercial formulations inoculant application.

Key-words: bacterial biomass production, inoculants, rihizocompetence.

Funding Agencies: INCT-FBN; CNPq; Fundação Araucária; PROPPG/UEL.