IMPROVEMENT OF POLY-3-HYDROXYBUTYRATE (PHB) PRODUCTION BY AN *Azotobacter vinelandii* MUTANT ALTERED IN PHB REGULATION USING A FED-BATCH FERMENTATION PROCESS

Andrés, García[1]; Daniel Segura[2]; Guadalupe Espín[2]; Enrique Galindo[1] and Carlos Peña[1];
[1]Departamento de Ingeniería Celular y Biocatálisis and [2]Microbiología Molecular, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Morelos, México, Apdo. Post. 510-3, Cuernavaca, 62250, Morelos, México; E-mail: agromero@ibt.unam.mx

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Introduction. Poly-3-hydroxybutyrate (PHB) is produced by several bacterial species, including *Azotobacter vinelandii.* It is accumulated as an intracellular carbon and energy reserve material (1). Recently, PHB has acquired importance because it is a biodegradable and biocompatible thermoplastic that can be used in a wide variety of products (1). *A. vinelandii* can accumulate PHB up to 80 % of its dry weight; however, the volumetric polymer production in bioreactors is low. Therefore, several strategies have been proposed to increase the productivity of the process. One strategy has been the design of *A. vinelandii* mutant strains such as the OPNA strain that overproduces PHB, coupled with bioengineering strategies, such as the use of fed-batch cultivations (2).
The aim of the present study was to improve the PHB production by the *A. vinelandii* mutant strain OPNA and using an exponential fed-batch fermentation process (EFBP) with nutrient pulses.

Methods. *A. vinelandii* mutant strain OPNA, which carries mutations inactivating the genes coding for IIA-PTSNtr and RsmA/RsmZY (proteins that negatively regulate the PHB synthesis), was grown in EFBP at 4 % DOT, and using PY medium. After this stage, PHB production was improved using the EFBP with nutrient pulses (sucrose and yeast extract). Biomass, residual sucrose and protein were determined as before (3) and PHB analysis was performed according to Karr et al. (4).

Results. A maximal specific growth rate (μ) of 0.16 h^{-1} and a PHB production of 3.3 g L^{-1} were reached in batch cultivations conducted at 4 % DOT. When the mutant OPNA was grown in the EFBP, the biomass and PHB increased up to 3 fold in comparison to the values obtained from the batch cultures. These values were further improved, when the EFBP was coupled with nutrient pulses of sucrose and yeast extract, reaching a maximal PHB concentration of 30 g L^{-1} after 60 h of cultivation. In both culture systems, PHB content was up to 80 % (Fig. 1). As it is shown in Fig. 2, this value of PHB concentration was 10-fold higher than that obtained in batch cultivations.

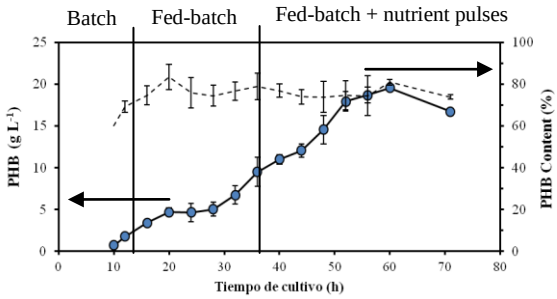

Fig.1 Volumetric production and PHB content of cultures of *A. vinelandii* OPNA at 4 % DOT in EFBP cultures with nutrient pulses.

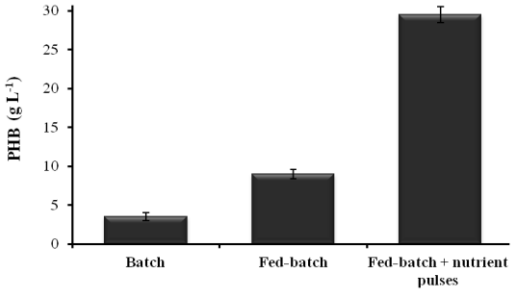

Fig.2 PHB volumetric production in three cultivation systems, using the *A. vinelandii* OPNA strain.

Conclusions. The use of *A. vinelandii* mutant strain OPNA, which overproduces PHB, in combination with multi-stage fermentation processes, is a feasible strategy to optimize PHB production at industrial level.

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