Production of poly-3-hydroxybutyrate and oxygen transfer characterization by *Azotobacter vinelandii* in a 30-L bioreactor

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Polyhydroxybutyrate (PHB), is a biopolymer produced by Azotobacter vinelandii. The PHB is a biodegradable thermoplastic material, used in packaging production, drug encapsulation and medical implants. The objective of this work was to develop a PHB production process in 30L bioreactor

Cultures of *A. vinelandii* OP were performed in batches in an Infors HT stirred tank reactor model Techfors-S with 20L of culture medium composed of sucrose as a carbon source and yeast extract as nitrogen source. The culture was developed at 100rpm, 1vvm, 30°C and pH 7.1 controlled with 2N NaOH. The dissolved oxygen tension (DOT) was characterized with a polarographic sensor and the oxygen transfer rate (OTR) was estimated by analyzing O_2 and CO_2 in the gas phase.

The results show that the biomass reached a maximum value of 7.5g L^{-1} after 70h of cultivation. Sucrose was completely consumed, indicating that it was the nutrient limiting growth (figure 1A). The DOT remained at values close to zero during the cell growth phase and the OTR reached a constant maximum value for 30 hours, reaching 10mmol $L^{-1}h^{-1}$ (figure 1B). This OTR behavior is typical of oxygen-limited cultures.

The concentration of PHB was increased during cell growth (figure 1D), until reaching a maximum concentration of 6.0 g L^{-1}. Likewise, the percentage of intracellular accumulation of PHB varied between 65% and 82% between 35 and 50h of culture (figure 1C). Results of PHB like that reported by Millán *et al.*, 2017 with percentages of accumulation of constant PHB over 70%.

$Y_{PHB/S}$ of 0.24g g^{-1} with q_{PHB} of 0.01g $g^{-1}h^{-1}$.

Our results have demonstrated it was possible to develop a PHB production process on a 30L scale getting PHB concentrations of 6g L^{-1}. Furthermore, by characterizing the oxygen transfer it is possible to explain the high percentage of PHB accumulated by *A. vinelandii*.

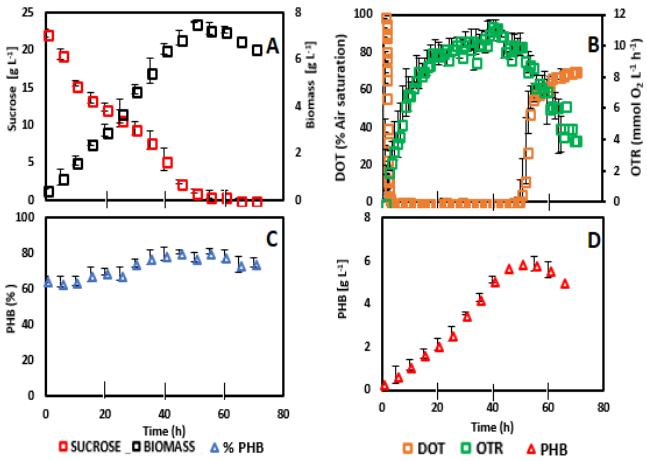


FIGURE 1: **A** Cell growth profile and sucrose consumption. **B** Evolution of DOT and OTR. **C** Profile of intracellular PHB percentage. **D** Evolution of PHB concentration.

Recent Publications

1. Peña C, Castillo T, Garcia A, Millán M, Segura D (2014) Biotechnological strategies to improve production of microbial poly-(3-hydroxybutyrate): a review of recent research work Microbial Biotechnology 7: 278-293
2. Millán M, Segura D, Galindo E, Peña C (2016) Molecular mass of poly-3-hydroxybutyrate (P3HB) produced by Azotobacter vinelandii is determined by the ratio of synthesis and degradation under fixed dissolved oxygen tension. Process Biochemistry 51: 950-958.
3. 3. Millan M, Salazar M, Segura D, Castillo T, Diaz-Barrera A. Peña C (2017) Molecular mass of Poly-3-hydroxybutyrate (P3HB) produced by Azotobacter vinelandii is influenced by the polymer content in the inoculum Journal of Biotechnology, 259, 50-55
4. Díaz-Barrera A, Andler R, Martínez I, Peña C (2016) Poly-3-hydroxybutyrate production by Azotobacter vinelandii strains in batch cultures at different oxygen transfer rates. J Chem Technol Biotechnol 91: 1063–1071.
5. Jiménez, L., Castillo, T., Flores, C., Segura, D., Galindo, E., Peña, C. (2016) Analysis of respiratory activity and carbon usage of a mutant of *Azotobacter vinelandii* impaired in poly-β-hydroxybutyrate synthesis. J Ind Microbiol Biotechnol 43: 1167-1174

Biography

Andrés Pérez is a student of Master of Science of engineering in biochemical engineering of Pontificia Universidad Católica de Valparaíso, Chile, Chile. His profession is a biotechnologist engineer of Concepcion University of Chile. In the first instance I worked obtaining bioactive molecules from marine Actinomycetes of Chile's coasts with potential medical use against multiresistant bacteria and infectious fungi. Then when I entered the magister I was interested in the line of work on cell cultures, in the fermentation and bioprocess, mainly in the production of biopolymer (PHB) with *Azotobacter vinelandii.* I am currently developing the degree thesis.

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