**Production of poly-3-hidroxybutyrate with ultra-high molecular weight by mutant strains of *Azotobacter vinelandii* under microaerophilic conditions.**

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**Highlights**

* The MW of P3HB obtained under oxygen limitation was as high as 25,000 kDa.
* MW of P3HB increased by decreasing the OTRmax of the culture.
* *A. vinelandii* *phb*Z- strain accumulated up to 93 % of intracellular P3HB.

**1. Introduction**

Poly-3-hydroxybutyrate (P3HB) is a biopolymer of the polyhydroxyalkanoate family; this bioplastic is produced in the form of intracellular inclusions as a carbon and energy reserve by the bacterium *Azotobacter vinelandii* [1]. P3HB has characteristics similar to those of plastics derived from the petrochemical industry [2]. Its use has been focused on the biomedicine field, because it is also a totally biodegradable and biocompatible polymer [3].

The thermomechanical properties and biodegradability of P3HB are determined by the molecular weight (MW) of the polymer [4]. It has been reported that the synthesis of the polymer is favored when *A. vinelandii* is cultured under oxygen limitation. In recent studies it has been found that, at low percentages of oxygen saturation (1 % DOT), high molecular weight polymers (between 3,500 and 5,500 kDa) are produced [5].

In this study, two mutant strains of *A. vinelandii*, with the ability to produce high molecular weight P3HB were cultured under microaerophilic conditions in bioreactor by using low agitation rates.

**2. Methods**

Strains of *A. vinelandii* (OP and *phb*Z-) were grown in PY medium, which contains sucrose (20 g L-1), yeast extract (3 g L-1) and peptone (5 g L-1). Batch cultures were carried out in a bioreactor using the OP strain as well as the *phb*Z- strain with a work volume of 2 L in Applikon 3 L bioreactors. Two agitation conditions were used (300 and 500 rpm) with a 1 vvm aeration, controlling pH to 7.2 and without control of the dissolved oxygen tension.

Recovery of P3HB was performed as described previously [5]. The molecular mass analysis was performed by gel permeation chromatography (GPC) using a Shodex K-800 column in an HPLC system (Waters 2695, USA) coupled with a refractive index detector (Waters 2414, USA). The mobile phase was chloroform at 30°C at a flow rate of 0.7 mL min-1. A calibration curve was constructed with polystyrene standards as described in [5]. Samples were dissolved in chloroform at a concentration of 2-3 mg mL-1 and were filtered through a 0.45 μm membranes before being injected into the HPLC.

**3. Results and discussion**

Results of mean molecular weight (MMW) of P3HB obtained from the cultures performed at 300 and 500 rpm are presented in Figure 1. The MMW of P3HB increased significantly in the cultures carried out at OTRmax of 5 mmol L-1 h-1 (22-25,000 kDa) with both strains, with respect to the MMW reached in the cultures developed at 8 and 11 mmol L-1 h-1 obtaining a P3HB between 15,000-16,000 kDa (Figure 1).

The initial accumulation of P3HB in the cells was around 50 % with both strains, as the culture evolved, the accumulation of P3HB increased , reaching up to 93 % with respect to dry cell biomass for *phb*Z- strain and 89 % for the OP strain cultured at high OTRmax (8 and 11 mmol L-1 h-1).

**Figure 1.** Mean molecular weight of P3HB produced by OP and *phb*Z- strain at 500 rpm (OTRmax of 8- 11 mmol L-1 h-1) and 300 rpm (OTRmax of 5 mmol L-1 h-1).

**4. Conclusions**

* Microaerophilic conditions promote the production of P3HB with ultra-high molecular weight in bioreactor cultures using *A. vinelandii* strains OP and *phb*Z-.
* There is an inverse relationship between P3HB accumulation and the molecular weight of the polymer, finding that at high OTRmax both strains accumulate a high percentage of P3HB (90 %), but with a lower molecular weight (15,000 kDa) than that obtained at low OTRmax, where the accumulation was lower, 50 and 70 % respectively with the OP and *phb*Z- strain; however, the molecular weight reached up to 25,000 kDa with the OP strain and 22,000 kDa with the *phb*Z- strain.

**References**

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