MEAN MOLECULAR MASS AND MECHANICAL PROPERTIES OF POLY(3-HYDROXYBUTYRATE) SYNTHESIZED BY *Azotobacter vinelandii* MUTANT STRAIN (OPN) IN SHAKEN FLASK CULTIVATIONS

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Introduction. Poly-β-hydroxybutyrate (PHB) is a storage polymer produced by numerous bacteria, including *Azotobacter vinelandii*. PHB has mechanical properties similar to conventional plastics such as polypropylene or polyethylene with the advantage of being fully biodegradable [1]. The mean molecular mass (MMM) of PHB determines its elastic behavior, as well as its mechanical resistance [2]. For commercial purposes, PHB requires suitable MMM for their final applications. The aim of this study was to evaluate the influence of oxygen supply (in shaken flasks cultivations) on the mechanical properties of PHB synthesized by *A. vinelandii* mutant strain OPN which is a PHB overproducer [3].

Methods. Two aeration conditions were evaluated, by changing the filling volume in shaken flasks cultivations. The high aeration condition was achieved in 500 mL Erlenmeyer flasks with 100 mL of filling volume, and the low aeration condition was achieved with 200 mL of filling volume. OPN strain was grown on PY medium at 29 °C, 200 rpm during 60 h. The MMM analyses were performed by gel permeation chromatography using chloroform as mobile phase. Mechanical properties (Young's modulus) were evaluated from the elastic region of the stress strain curves [4]. In addition, biomass, protein and PHB concentration were measured.

Results. The specific growth rate (μ) for cultivations at high aeration condition was 0.076 h^{-1} with a maximum protein concentration of 1.5 g L^{-1}. In contrast, under low aeration the μ was 0.066 h^{-1} with 1 g L^{-1} of protein. At the end of cultivations (60 h), the PHB concentration was of 4.2 g L^{-1} under the high aeration condition, compared to 2.2 g L^{-1} obtained under low aeration. The MMM of the polymer was higher in the cultivations grown under low aeration (3,671 kDa) than the MMM of PHB obtained at the high aeration condition (Figure 1).

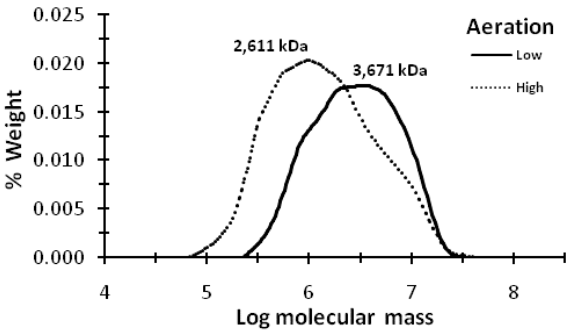

Fig.1 Molecular mass distribution of PHB produced by the OPN mutant of *A. vinelandii* cultured at high and low aeration at 60 h.

These results suggest that PHB biosynthesis and/or polymerization could be regulated by oxygen availability. Also, the uniaxial tensile tests on as-cast films showed that the Young's modulus increased as the molecular weight increased, ranging from 180 to 560 MPa (Table 1).

Table 1. Mean molecular mass and mechanical properties of PHB synthesized by OPN mutant of *A. vinelandii*.

	Mutant strain OPN	
	High aeration	Low aeration
MMM (kDa)	2,611 ± 171	3,671 ± 271
Polydispersity	3.1 ±0.3	2.7 ±0.3
Young´s Modulus (MPa)	180	560

Conclusions. The MMM and in turn the mechanical properties of PHB synthesized by the OPN strain is affected by the oxygen availability in shaken flask cultivations.

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