# ALGINASE ACTIVITY AS A FUNCTION OF OXYGEN AND ITS RELATIONSHIP WITH ALGINATE MOLECULAR WEIGHT IN *AZOTOBACTER VINELANDII* CULTURES

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**Introduction.** Alginate is a polymer with several industrial applications (1). The molecular weight (MW) is an important characteristic, because determines its applications. Dissolved Oxygen Tension (DOT) determines the MW of alginate produced by *Azotobacter sp*. (1-3). The drop of alginate MW has been related to the extracellular alginase activity (2,3). However, an analysis of the intracellular and extracellular alginase activity during the culture of *A. vinelandii*, as a function of DOT, has not been carried out.

The aim of this study was to analyze the influence of DOT (1 and 5%) on the extracellular and intracellular alginase activity, in order to understand its relationship with the alginate MW.

**Methods.** *Azotobacter vinelandii* ATCC 9046 was used. Cultures were conducted in biorreactors using 2 L of working volume of modified Burk's medium at 29ºC, 500 rpm and pH= 7.2. DOT was accurately controlled by gas blending (nitrogen and oxygen). Alginate MW was analyzed as previously reported (2). Alginase activity was analyzed by spectrophotometric assay (3). For intracellular activity, the periplasm extraction was conducted according to Franklin and Ohman (4).

**Results.** DOT values were established based on the conditions under which the alginate MW was clearly different. At 1% DOT, the polymer MW was considerably higher (1200 ± 235 kDa) than that produced under 5% DOT (42 ± 12 kDa) (figure 1a).

In the cultures developed at 1% DOT, the alginase activity (both intracellular as extracellular) was lower (figure 1b and 1c) with respect to that obtained in cultures at 5% DOT and was present in a basal level during all culture time. The low alginase activity found at 1% DOT was consistent with the higher polymer molecular weight, measured in this condition.

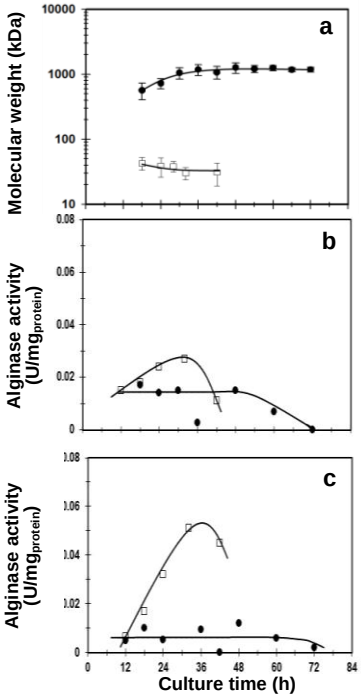


**Fig.1.** Alginate molecular weight (a) and intracellular (b) and extracellular (c) alginase activity in *Azotobacter vinelandii* cultures conducted at 1% (●) and 5% (□) DOT.

In cultures at 5% DOT, the extracellular alginase activity was 5 fold higher (0.052 $U/mg_{protein}$) (figure 1c) with respect to the cultures at 1% DOT. A pronounced increase was observed between 12 and 32 h. This high lyase activity was consistent with the low alginate MW produced at 5% DOT (figure 1a).

**Conclusions.** DOT determines the intracellular and extracellular alginase activity. A low alginase activity was found at 1% DOT, which corresponded with the alginate having high molecular weight.

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