

PL 5 - Encapsulation of D-Hydantoinase and D-Carboamylase from a Crude Cell Extract of *Agrobacterium radiobacter* in Alginate-Chitosan Beads. Effect on the Environment Variables

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The application of D-amino acids in the synthesis of chemicals has a great interest for the industry. D-p-hydroxyphenylglycine (p-HPG) is a lateral chain of β -lactam antibiotics. This molecule can be produced in a hydantoin-transforming reaction starting from D-hydroxyphenylhydantoin (pHPH) by means of two enzymes (D-hydantoinase EC 3.5.2.2 and D-carboamylase EC 3.5.1).

One of the problems associated with the use of the D-hydantoin-transforming reaction in the industry is the low thermostability and sensibility to the oxidative process of the enzyme D-carboamylase.

Encapsulation of enzymes in alginate-chitosan beads is characterised by the very mild conditions in which the immobilisation procedure is carried out and by its low cost and ease of use. Moreover, it is possible to immobilise several enzymes at the same time.

A crude cell extract of *Agrobacterium rb* was encapsulated in alginate-chitosan beads by using two methods of encapsulation. In the first one, calcium-alginate beads were covered by chitosan (V-I) while in the second one alginate-chitosan beads were prepared in one step (V-II).

The effect of the encapsulation on the optimum pH and temperature as well as on the stability of the biocatalysts was studied and compared with the soluble extract. The optimum pH was shifted to a more basic one in both biocatalysts and wide pH stability was observed. The optimum temperatures were 50°C and 55°C, respectively. Both biocatalysts showed lower temperature stability than the soluble extract.