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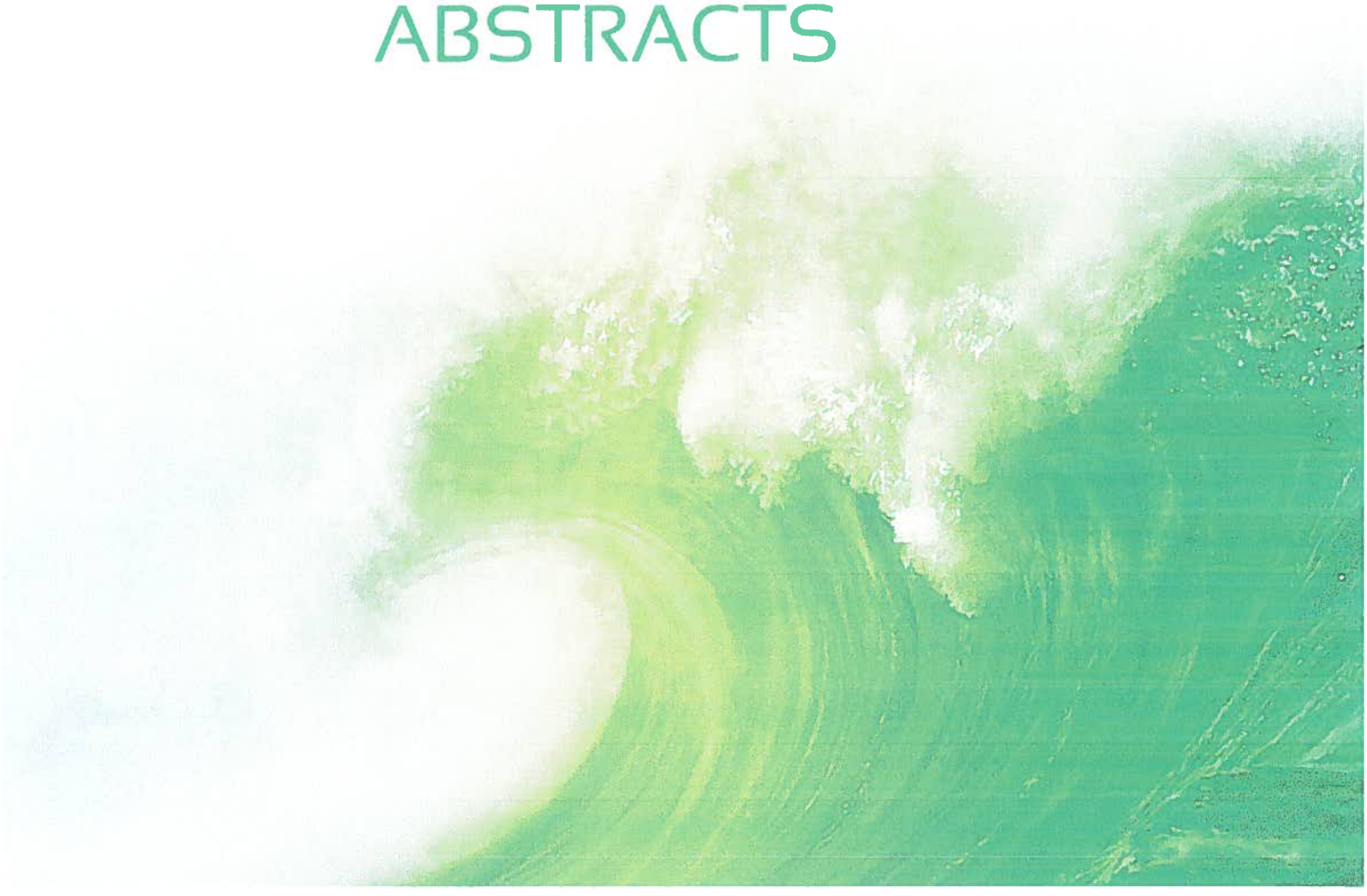
BOOK OF ABSTRACTS



Immobilization of amidase in polymeric matrixes

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Microbial amidase (acylamide amidohydrolase, EC 3.5.1.4) is an intracellular enzyme that catalyses the hydrolysis of amides producing the corresponding acid and ammonium ions. Amidase has a great biotechnological application; it has been used in neuro-biochemistry, plant physiology, medicine, detoxification of industrial effluents containing toxic amides and in food and detergent industry [1,2].
In addition to the unquestionable advantages, there exist a number of practical problems in the use of enzymes. To these belong: the high cost of isolation and purification of enzymes, the instability of their structures once they are isolated from their natural environments, and their sensitivity both to process conditions other than the optimal ones, normally narrow-ranged, and to trace levels of substances that can act as inhibitors. The latter two result in enzymes' short operational lifetimes. Most enzymes operate dissolved in water in homogeneous catalysis systems, which is why they contaminate the product and as a rule cannot be recovered in the active form from reaction mixtures for reuse [3].
In order to overcome these limitations, the enzymes have been immobilized in different supports. Immobilization is achieved by fixing enzymes to or within solid supports, as a result of which heterogeneous immobilized enzyme systems are obtained. By mimicking the natural mode of occurrence in living cells, where enzymes for the most cases are attached to cellular membranes, the systems stabilize the structure of enzymes, hence their activities. Thus, as compared to free enzymes in solution immobilized enzymes are more robust and more resistant to environmental changes. More importantly, the heterogeneity of the immobilized enzyme systems allows easy recovery of enzyme and product, multiple reuses of enzymes, continuous operation of enzymatic processes, rapid termination of reactions and greater variety of bioreactor designs [3].

The aim of this work was to produce and purify a mutant amidase from L10 strain of *P. aeruginosa*, obtained from a wild-type ATCC 8602 and to immobilize the purify enzyme or the produced cells, using poly(vinyl alcohol), chitosan and calcium alginate, as support.

The cultures were performed in an orbital shaker during 32 h at 37ºC, 250 rpm. Cells were collected by centrifugation and broken by sonication. Amidase activity was measured by a direct potentiometric method, using an ion-selective electrode (ISE) to quantify the ammonium released by acetamide hydrolyse, used as subtract (Fig. 1). Purification of the amidase was performed by affinity chromatography on epoxy-activated Sepharose 6B-acetamide column.

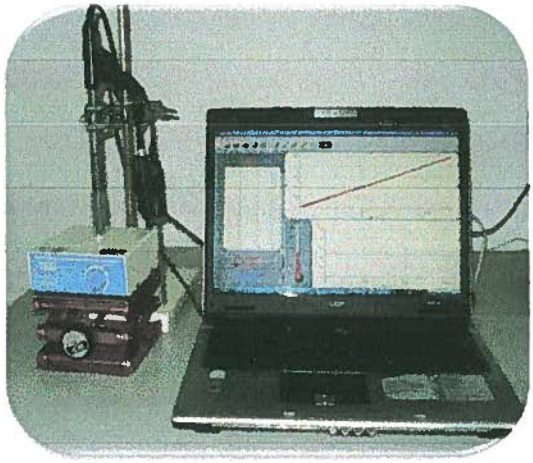

Fig. 1 Acetamide hydrolyse. Amidase activity determination

The PVA membranes were prepared, according to Vidal et al. [4], with different concentrations of PVA (10% and 20% w/w) and cross-linked with

glutaraldehyde.
The alginate membranes were prepared adding sodium alginate to $CaCl_2$.

Table 1 shows the catalytic activity of amidase immobilized in alginate and PVA matrixes in the acetamide hydrolyse. It was observed that the recovery (immobilized cells activity / free cells activity x100) of membrane C is highest. However, PVA/glutaraldehyde membranes offer a more resistent system, allowing membranes re-utilization.
The membranes will be used to develop a biosensor.

Table 1. Activity membranes recovery

Membrane		Recovery (%)*
A	Alginate membranes	17,53
B	PVA membranes (10% w/w) with 50% glutaraldehyde + cells	2,29
C	PVA membrane (20% w/w)+ cells	19,44
D	PVA membrane (10% w/w) +cells	12,37
E	PVA membrane (10% w/w), with HCl and with glutaraldehyde + cells	1,33

*Immobilized cells activity / free cells activity x100

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