STABILITY STUDIES OF JACK BEAN UREASE IMMOBILIZED IN CALCIUM ALGINATE BEADS.

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Introduction. Urease is a nickel-based metalloenzyme (urea amydohydrolase, E. C. 3.5.1.5) that hydrolyze urea to NH_3 and CO_2. Jack bean urease is the most studied and the best known among the ureases (1). However, their stability is limited in native form. Several methods of enzyme immobilization have been known, each of them have their own limitations. Enzyme immobilization onto matrices which are non-toxic, cheap and biodegradable, like alginates, have utmost importance in food, biomedical, cosmetics and pharmaceutical applications (2).
The aim of this study was to evaluate the stability of the urease immobilized in calcium alginate beads. Also, we evaluated the possibility to use this biocatalyst as tool to assess the bioavailability of nutrients in agriculture applications.

Methods. Jack bean urease (0.7 mg/mL) was mixed in sodium alginate (3.5%) in 25 mM Tris-acetate (pH 7.5) and dropped into $CaCl_2$ (400mM) solution. Beads were collected and stored in the same buffer at 4°C (3). The enzyme activity was measured with Nessler´s reagent at 460 nm. The temperature stability was determinate in 30°-80°C range *vs* free enzyme. The reusability was tested by checking the activity at different time intervals. The time stability of immobilized enzyme in saline medium (Hoagland solution) with different urea concentration (7.5 mM and 15 mM) was evaluated.

Results. The immobilization process showed a 43% of efficiency. The thermostability was increase after the urease immobilization and retained more than 60% of residual activity between 30°-70°C. However, the beads retained 20% of activity after 4-5 uses and were soft and clumsy. The urease-alginate beads retained more than 37% of their residual activity in Hoagland solution at both urea concentrations. These bead surfaces were affected after seven days of incubation and were corroborate by SEM (Figure 1).

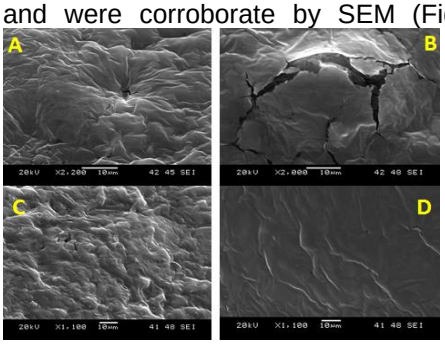

Figure 1. Morphological changes on external surface of Immobilized enzyme: A (7.5mM urea); B (15 mM urea) and Internal surface: C (7.5mM urea); D (15 mM urea).

Conclusions. The immobilization process increased the stability of urease upon different denaturalized conditions. These results could increase the application of theses biocatalysts in agriculture.

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References.

1. Marzadori, C., Miletti, S., Gessa, C.. Ciurli, S. (1998). *Soil Biol. Biochem.* 30 (12): 1485-1490.
2. Kumar, S., Dwevedi, A., Kayastha, A. (2009). *J. of Molec. Cat.B: Enzymatic* 58: 138-145.
3. Talat, M., Prakash, O., Hasan, S. (2009). *Bioresource Technology* 100: 4462-4467.