Immobilization of yeast invertase by gel entrapment

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Immobilization of yeast invertase in calcium alginate is well known. The present work describes the feasibility of gel entrapment of yeast invertase using strontium, barium, calcium-strontium, calcium-barium and strontium-barium alginates.

Keywords: immobilization, yeast invertase, gel entrapment, alginates

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Industrial enzymes are widely immobilized on various natural and synthetic organic polymers and inorganic materials. The use of free enzymes in industrial application has been limited, mainly due to high cost of enzymes, their instability and irrecoverabilty. Using immobilized enzymes, which lead to greater product purity, cleaner processes and economic operational costs, circumvents these limitations of free enzymes. Enzyme immobilization includes adsorption, entrapment, cross-linking and covalent binding. Gel entrapment of enzymes is widely used due to its simplicity and operationally convenient workup. Yeast invertase, a thermally stable glycoprotein, has been used in various immobilized forms in food industry to manufacture invert syrup at room temperature and optimum pH (4.5-6).

Entrapment of yeast invertase in calcium alginate gel has been reported. Present communication reports entrapment and enzyme activities of yeast invertase in terms of Michaelis-Menten constant (Km) and pH optima at room temperature for strontium alginate or barium alginate or their mixed alginates.

Sodium alginate, sucrose and all other chemicals were procured from S D Fine Chemicals or Qualigens. Bakers yeast was procured from a local chemist's shop.

Dry yeast (2 g) was suspended in about 10 ml of 0.1 M Na₂CO₃ solution and incubated for 24 hrs at 40°C. The extracted enzyme was then centrifuged to remove the dead cells and diluted 1:10 times before using it for inversion of sucrose. The enzyme preparation thus prepared was preserved in a refrigerator and used.

A mixture of 10 ml of the enzyme and 25 ml of 3.6% sodium alginate solution was reacted with 4% CaCl₂ solution to get enzyme entrapped beads, which were repeatedly washed with distilled water and then suspended in it for determination of enzyme activity. Similarly, strontium alginate and barium alginate beads were prepared. The mixed beads of any of the above two metals were also prepared using 1:1 aqueous solutions of respective metal chlorides. The enzyme entrapped calcium alginate beads so obtained were subjected to activity studies such as determining Michaelis-Menten constant (Km) at optimum pH and at room temperature using 0.06 M sucrose solution.

Km value of the invertase in solution phase was determined to be 0.04 M at pH 4.5 at room temperature (Fig. 1A). To cross check the pH optima, the authors carried out the same determination of Km using 0.1 M citrate-phosphate buffer at different pH levels and observed that the pH optimum was at 6.0 (Fig. 2A). Therefore, the Km value at pH 6.0 was also determined and found to be the same as that at pH 4.5.

In the immobilized systems, calcium alginate beads entrapping invertase showed a pH optimum in the range 4.0 to 5.5 and Km value of 0.0212 M (Fig. 1B & 2B). After 5 days, the Km value of the same beads was found to be 0.0086 M indicating considerable loss of activity. The beads were suspended in 0.1 M citrate-phosphate buffer of optimum pH to determine its activity and reusability.

In strontium alginate entrapped invertase, a sharp pH optimum at 4.0 and Km value of 0.0158 M on the first day and 0.0218 M on the fifth day were observed. In barium alginate entrapped enzyme, a pH optimum at 4.5 and Km value of 0.0277 M on the first day and 0.0192 M on the fifth day were observed. It was distinctive that the calcium alginate system showed considerable decrease in Km value on the fifth day,
whereas in strontium and barium alginate systems the decrease in Km value for the same period was negligible.

In Ca-Sr mixed alginate system, the entrapped enzyme showed two pH optima at 4.0 and 7.0 with Km values of 0.027 M and 0.018 M, respectively, in which a higher activity was observed at pH 7.0. In Sr-Ba mixed alginate system the entrapped enzyme showed two pH optima at 4.0 and 6.5 with Km values of 0.0094 M and 0.0117 M respectively, in which a higher activity was observed at pH 4.0. In the Sr-Ba mixed alginate and in Sr-alginate systems, the Km values namely 0.0094 M and 0.0158 M, respectively were found to be comparable at pH 4.0. It can be seen that the pH optimum 4.0 characteristic of strontium-alginate, predominates in both the mixed and individual systems.

In Ca-Ba mixed alginate system, the entrapped enzyme showed two pH optima at 5.0 and 6.5 with Km values of 0.0077 M and 0.0294 M, respectively, in which a higher activity was observed at pH 6.5. In Ca-Ba alginate system, the activity of the entrapped enzyme was considerably lower at the higher pH optimum. It is noteworthy that although the Ca-Ba system has two pH optima, the Km value at pH 5.0 compared closely with that of calcium alginate system.

Unlike calcium alginate, barium alginate gels have been shown to have better mechanical compression affecting the pore size and the permeability of the sucrose molecule to the inner core of the beads.
leading to higher Km value\textsuperscript{5}. This resulted in decreased binding and activity between the enzyme and sucrose and hence, the observed higher Km values in Ca-Ba alginate enzyme-entrapped system.

The major limitations of Ca-alginate gels were their destabilization and subsequent solubilization. Hence it may be stated that the Sr-Ba mixed alginate system and Sr-alginate system have displayed better properties in terms of mechanical strength of beads, swelling characteristics and enzyme activity (lowest Km value) at experimental pH conditions and room temperature.

References