Activity Comparison of Free and Alginate-Immobilized Papain Using an Azocasein Assay

Jakkula, Baruni (School: The Mississippi School for Mathematics and Science)

Papain enzyme is used in various industries such as food processing, medicine, environmental treatment and other applications but the high cost limits its industrial utility. A common way in scientific literature is that trapping papain in calcium-alginate beads makes it more stable and reusable. Thus, this study compared the activity of free and immobilized papain. The activity of free papain was measured using an azocasein assay as different substrate concentrations and a Michaelis-Menten analysis confirmed a high affinity for the substrate, with a calculated Km of 0.037% and a Vmax of 0.071 Abs/min. These parameters established the enzyme was active and a 0.2% concentration provided near-saturating conditions. Papain was then immobilized with alginate to form beads in first phosphate buffer and then MOPS buffer at pH 7.0. The beads with phosphate buffer had an apparent increase in activity from 100% to 411% by the third cycle. This was identified as a structural failure as the phosphate sequestered the calcium ions, causing the beads to brake apart early and leak papain enzyme into the bulk solution. Contrastingly, while MOPS buffer beads remained intact, the catalytic activity plummeted to 18% by the second reuse and reached near-zero by the fourth. Ultimately, this study shows that while alginate entrapment preserves bead structure in non-chelating buffers, it fails to provide a feasible system for papain reuse. Stability and reusability of enzymes are separate problems. These finding suggest that simple alginate entrapment may not be the best, and industries may must look beyond for viable reusing enzymes.

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