

Integration of mechanical cell disruption and fluidised bed recovery of G3PDH from unclarified disrupted yeast: a comparative study of the performance of unshielded and polymer shielded dye-ligand chromatography systems

ABSTRACT

The development of a simplified process for the simultaneous disruption and direct selective purification of intracellular proteins from unclarified yeast disruptate has been investigated. The recovery of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) from baker's yeast was selected as a potential demonstration of the generic applicability and practical feasibility of this integrated technique. The application of an adsorbent characterised by high density (UpFront steel-agarose; $\rho = 2.65 \text{ g ml}^{-1}$) facilitated the combining of cell disruption operation (bead milling of 50% w/w of yeast suspension at 7.2 l h⁻¹) with fluidised bed dye-ligand (Cibacron Blue 3GA) adsorption operated immediately downstream of the disrupter. The adoption of a polymer shielded, dye-ligand technique advanced recovery efficiency. It was demonstrated that G3PDH could be recovered with a yield of 67.5% bound activity and a specific activity of 40.2 IU mg⁻¹, after a single step elution with 0.15 M NaCl. The generic application of this approach has been evaluated.

Keyword: Cell disruption; Fluidised bed; Dye-ligand; Intracellular proteins; G3PDH