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Process intensification of fluidized bed dye-ligand adsorption of G3PDH from unclarified disrupted yeast: A case study of the performance of a high-density steel–agarose pellicular adsorbent

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Abstract

The development of a process intensified primary capture step for the direct selective recovery of intracellular proteins from very dense particulate-containing yeast extract has been explored. The purification of glyceraldehyde 3-phosphate dehydrogenase from bakers' yeast was chosen as a potential demonstration of this approach. A high throughput (50%, w/v, yeast extracts at a superficial linear velocity of 450 cm h^{-1}) was achieved by adoption of a high-density adsorbent (UpFront steel–agarose; $\rho = 2.65\ g\,ml^{-1}$) derivatized with selective ligand chemistries (Cibacron Blue 3GA). This should ultimately minimize adsorption time and maximize process efficiency of fluidized bed adsorption.

Keywords: Direct selective recovery; Cibacron Blue 3GA; Fluidized bed; Intracellular proteins; G3PDH

The industrial application of fluidized bed technology has been rather limited by a lack of suitable commercially available adsorbents [1]. The viscosity of process feedstocks has necessitated that they are diluted prior to loading of the fluidized bed contactor. The demand for denser adsorbent particles has recently been widely discussed [2–6]. Various attempts have been explored to increase the density of adsorbents to meet these demands. Thoemmes et al. [7] described the use of a high-density porous material, i.e., silica beads, for fluidized bed adsorption. The combination of light, biocompatible porous materials, i.e., agarose or cellulose, with high-density non-porous inert fillers has also been reported. Gilchrist et al. [8] developed a cellulose based adsorbent particle characterized by the incorporation of titanium dioxide. Morton and Lyddiatt [9] presented the performance of a composite material commercially assembled from silica and dextran (Spherodex). Inert cores having an even higher density, such as zirconia [1,6] and stainless steel [4] have been described.

It has been noted that a number of mass transfer resistances are associated with the adsorption of a protein molecule from a bulk fluid to the internal surface of an adsorbent [10]. The mass transfer of the protein molecule from the bulk fluid to the outer particle surface area (liquid film mass transfer resistance) is the first step. This is followed by the intraparticle diffusion of the molecule within the internal volume of the particle (constrained by pore diffusion resistance). Finally, the chemical interaction of a protein molecule with the binding site (surface reaction resistance or adsorption–desorption kinetics) has an impact on performance.

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Here, Gibson [11] suggested that pellicular adsorbent particles would be suitable for optimized fluidized bed adsorption when considering the pore diffusion resistance step. In conventional porous adsorbent, the achievement of adsorption saturation at available ligands progresses from the surface to the internal surface area of the particle. The porous characteristics of such particles would be expected to have a high intraparticle mass transfer resistance [12,13]. The characteristic of the pellicular structure (i.e., an inert core and an adsorptive coating) is attractive due to the inherent shortened diffusion distances [14]. The development of a pellicular adsorbent particle with increased density has been identified as a very promising strategy for solid phase optimization [1,5]. Jahanshahi et al. [6] have proposed that a dense pellicular adsorbent be employed to facilitate fluidized bed recovery as it exhibits fast adsorption and desorption characteristics.

In the present paper, the adsorption performance of a high-density pellicular adsorbent (UpFront steel–agarose; $\rho = 2.65\ \mathrm{g\ ml^{-1}}$) developed by UpFront Chromatography A/S, Denmark, was explored. The particle density of this adsorbent was increased by means of incorporation of a stainless steel core in agarose. Cibacron Blue 3GA was used as an affinity ligand for the direct selective fluidized bed recovery of an intracellular enzyme, glyceraldehyde 3-phosphate dehydrogenase (G3PDH) from bakers' yeast extract.

Materials and methods

Adsorbent particle and immobilization of dye-ligand Cibacron Blue 3GA

UpFront steel–agarose is a pellicular adsorbent developed by UpFront Chromatography A/S, Denmark. It comprises a non-porous stainless steel core coated with a thin layer of 6%(w/v) porous agarose. The depth of the adsorptive coating was estimated as 40% of the particle radius, i.e., a 60 μm stainless steel core coated with a 20 μm agarose layer [15]. The volume ratio of steel core to agarose was 1:3.5 [15]. The particle density was $2.65\ \mathrm{g\ ml^{-1}}$ and the particle size was in the range of 151–323 μm. Cibacron Blue 3GA was immobilized onto adsorbent particles using the procedures described by Zhang et al. [5].

Effect of superficial velocity and biomass concentration upon G3PDH adsorption performance

The adsorption performances for different feed superficial velocities and biomass concentrations were determined by employing BRG (25 mm i.d.) and UpFront (20 mm i.d.) contactors. Adsorbents were loaded into either fluidized bed contactor corresponding to a common SBH of 20 cm and equilibrated with buffer A (10 mM Tris–HCl, pH 7.5, containing 1 mM EDTA) in fluidized bed mode (>6 SBH volumes). The adsorption performance at selected experimental superficial velocities (300–750 $\mathrm{cm\ h^{-1}}$) was determined using feedstock comprising 15%, w/v, yeast extract at pH 6.1 in the UpFront contactor. The adsorption performance for feedstock comprising different biomass concentrations (15–50%, w/v, original cells, pH 6.1) was performed at a superficial velocity of $450\ \mathrm{cm\ h^{-1}}$ in the BRG contactor (25 mm i.d.). For all experiments, the feedstock was applied to the bed in a single pass operation by means of a peristaltic pump connected to the inlet of the contactor. Samples were taken from the effluent outlet at regular intervals, and the feedstock application was terminated when an adsorbent saturation capacity ($C/C_0 \approx 1$) was achieved. Samples (1 ml) were centrifuged and assayed for enzyme activity [6] and expressed relative to the feed concentration (C_0). Following feedstock application, the adsorbent bed was washed with buffer A in fluidized bed mode until no residual particulates could be detected in the effluent. The adsorbent was re-generated with 3 M KSCN in buffer A and re-equilibrated in buffer A before re-use. Elution steps were not carried out at this study since the prime aim was to optimize the adsorption stage of fluidized bed chromatography.

Results and discussion

The influence of superficial flow velocities (300–750 $\mathrm{cm\ h^{-1}}$) upon adsorption performance was measured by establishing the breakthrough curves in the UpFront contactor (20 mm i.d.) and the results are presented in Fig. 1 and Table 1. Here, it was demonstrated that the impact of superficial flow velocities (300–750 $\mathrm{cm\ h^{-1}}$) upon adsorption performance was not significant. However, there appeared to be an optimum velocity (i.e., $450\ \mathrm{cm\ h^{-1}}$) with regard to the dynamic binding capacity at $C/C_0 = 0.1$ recorded in this study (see Table 1). Restricted film mass transfer at lower interstitial flow velocities (<$450\ \mathrm{cm\ h^{-1}}$) might contribute to such an observation [2]. The film mass transfer step is normally more rapid than the intraparticle diffusion and/or surface reaction steps. However, the former has been found to influence the initial breakthrough point of target molecules [16]. Increasing the superficial flow velocity up to $750\ \mathrm{cm\ h^{-1}}$ results in a significant decrease of dynamic binding capacity. This might indicate that the residence time for diffusion of G3PDH into adsorbent is shortened with increasing flow velocity, which resulted in early breakthrough of enzyme (see Fig. 1). A similar observation was made by Thoemmes et al. [7] when Bioran SP was employed for fluidized bed adsorption of BSA. They suggested that the diffu-