The Performance of Anion Exchange Expanded Bed Adsorption Chromatography on the Recovery of G6PDH from Unclarified Feedstock with High Biomass Concentration

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Abstract The bed stability of Streamline DEAE (ρ = 1.2 g/mL) in a 20 mm (i.d.) glass expanded bed contactor, and its performance on the recovery of glucose 6-phosphate dehydrogenase (G6PDH) from unclarified yeast homogenate were investigated. A residence time distribution study showed that a stable expanded bed was achieved. The theoretical plate and Bodenstein numbers determined were 25 and 53, respectively. A recovery yield of 87% and purification factor of 4.1 were achieved in the operation using 5% (w/v) biomass concentration feedstock. The performance of the anion exchange EBAC was still considerable good at a biomass concentration as high as 15% (w/v).

Keywords: expanded bed adsorption, G6PDH, biomass, anion exchange, yeast

Ion exchange chromatography is an established technique employed in the separation of almost any charged molecules [1]. The wide applicability, high capacity, simplicity and controllability of the method are the main reasons for its popularity. Diethyl aminoethyl (DEAE), carboxymethyl (CM), quaternary ammonium (Q), and sulphopropyl (SP) are examples of functional groups used in ion exchangers. The choice of exchanger group is dependent upon the net surface charge and the pH stability range of the targeted protein (Fig. 1). Desorption of the bound protein from the adsorbent can be achieved by adjustment of ionic strength and/or pH of elution buffer, so reducing the binding strength of the target protein to the ion exchanger.

Expanded bed adsorption chromatography (EBAC) is a direct purification method applied to recover proteins from particulate containing crude feedstock such as *Escherichia coli* fermentation broth [2] and homogenate [3], and mammalian cell culture broth [4]. The wide range of commercially available adsorbents such as Streamline, Hyper D, and UpFront, has popularized the application of this direct purification method [3,5,6]. In this study, the bed stability and performance of anion exchange EBAC in recovery of intracellular enzymes from unclarified yeast feedstock (5 to 20%, w/v) were assessed. The anion exchanger Streamline DEAE (Amersham Biosciences AB, Uppsala, Sweden) was chosen as an adsorbent and the UpFront column (20 mm i.d.; UpFront Chromatography A/S, Copenhagen, Denmark) was adopted as an expanded bed contactor. A residence time distribution (RTD) study using acetone (1%, v/v) as a tracer was performed to study the bed stability of the Streamline DEAE expanded bed in the UpFront column. The intracellular enzyme, glucose 6-phosphate dehydrogenase (G6PDH) was selected as the model protein to evaluate this approach.

Bakers' yeast used in this study was obtained from Mauri Fermentation Sdn Bhd (Balakong, Malaysia). The yeast cells were disrupted using a Dyno bead mill (Willy A. Bachofen AG Maschinefabrik CH-4005, Basel, Switzerland) to release the intracellular G6PDH as previously described [7].

Streamline DEAE adsorbent was loaded into the UpFront column. The RTD study was carried out according to the method described by Barnfield *et al.* [8] and Chow *et al.* [5]. The adsorbent bed was monitored using a UV-M detector (Amersham Bioscience AB) and acetone solution (1%, v/v) was applied to the bed (positive step input) when the UV reading was stable. The acetone solution was replaced with buffer A (50 mM Tris-HCl, pH 7.5) when the UV reading reached the maximum concentration (negative step input). The bed voidage could be estimated using Eq. 1:

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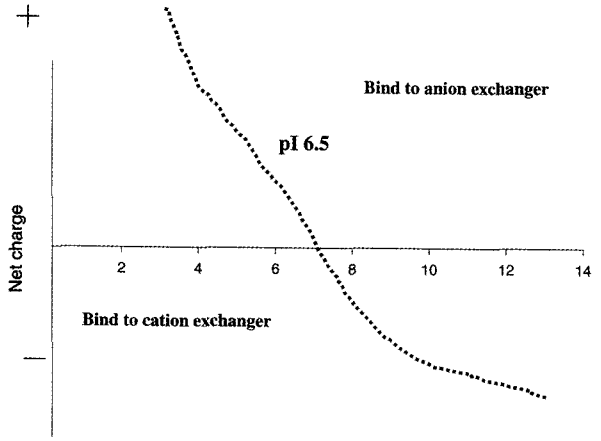

Fig. 1. The pH range at which G6PDH binds to the anion/cation exchanger. G6PDH is characterized by an isoelectric point of 6.5 where the net charge of the molecule at this point is zero. The enzyme will not bind strongly to either anion or cation exchangers at this point.

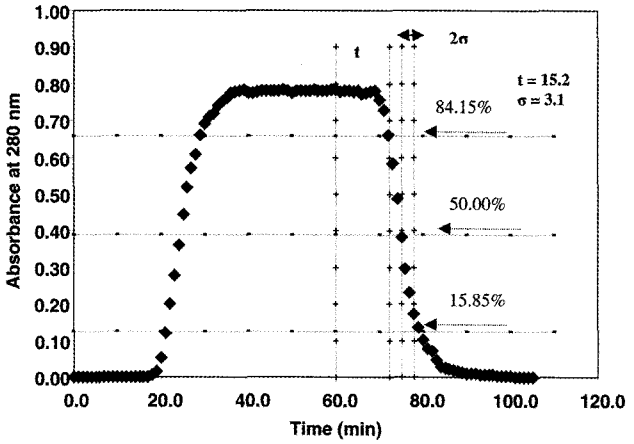

Fig. 2. Residence time distribution (RTD) study of the Streamline DEAE adsorbent. The standard deviation was measured as half the distance between the points 15.85 and 84.15% of the maximum acetone concentration.

$$\frac{H}{H_o} = \frac{(1-\varepsilon_0)}{(1-\varepsilon)} \tag{1}$$

where ε_0 is the settled-bed voidage, and H/H_0 is the ratio of the expanded and sediment bed height. The settled-bed voidage, ε_0, was assumed to be 0.4 [9]. The theoretical plate number (N) and height equivalent to a theoretical plate (HETP) were calculated according to Eqs. 2 and 3:

$$N = t_m^{\,2} / \sigma^2 \tag{2}$$

$$HETP = L/N \tag{3}$$

where t_m is the mean residence time, σ is the standard deviation, and L is the height of the expanded bed. The variance (σ^2) was used to give σ_θ^2 (Eq. 4) and to estimate the Bodenstein number, B_0 (Eq. 5) [10], which is the ratio of convection to dispersion mass transport as defined in Eq. 6 [11].

$$\sigma_\theta^{\,2} = \sigma^2 / t_m^{\,2} \tag{4}$$

$$\sigma_\theta^{\,2} = 2/B_o + 8/B_o^{\,2} \tag{5}$$

$$B_o = uH/\varepsilon D_{ax} \tag{6}$$

where u is the superficial fluidizing velocity, ε is the bed voidage and D_{ax} is axial dispersion coefficient.

EBA chromatography purification of G6PDH from unclarified feedstock was performed using the UpFront contactor (20 mm i.d.) (UpFront Chromatography A/S) at room temperature. Streamline DEAE (54 mL corresponding to 15 cm settled bed height) was loaded into the column and equilibrated with binding buffer (50 mM Tris-HCl, pH 7.5) for 4 h (>6 settled bed volumes). The unclarified feedstock, which had a biomass concentration in the range of 5 to 20% (w/v), was applied to the expanded bed at a selected superficial velocity in a single pass operation with a peristaltic pump. The adsorbent bed was then washed with buffer until no residue could be observed in the effluent samples. The adsorbed G6PDH was recovered from the adsorbent using a 3 step elution operation. The first step was carried out with elution buffer No. 1 (0.05 M NaCl, 50 mM Tris-HCl, pH 7.5), followed by elution buffer No. 2 (0.5 M NaCl, 50 mM Tris-HCl, pH 7.5), and finally with elution buffer No. 3 (1.0 M NaCl, 50 mM Tris-HCl, pH 7.5). The eluent was collected every minute, and assayed for G6PDH activity and total protein concentration. Total protein concentration was quantified by Bradford assay [12]. The G6PDH activity was measured spectrophotometrically using glucose 6 phosphate as substrate as described in Chow *et al.* [7].

The RTD study conducted at a linear flow velocity of 250 cm/h found that the theoretical plate and Bodenstein numbers for Streamline DEAE adsorbent expanded bed in the UpFront column were 24 and 52, respectively (Fig. 2, Table 1). The axial dispersion coefficient, D_{ax}, estimated for this anion exchange adsorbent was 5.80×10^{-6} m^2/s (Table 1), a value very closed to that reported by Thömmes *et al.* [13]. The D_{ax} values of the expanded bed reported by Thömmes *et al.* [13] are in the range of 7.4×10^{-6} to 9.2×10^{-6} m^2/s at flow velocities of 200~300 cm/h. These findings indicated that a stable expanded bed with low axial mixing was achieved in this UpFront expanded bed contactor. The Streamline adsorbent with a wide distribution of size (100~300 μm) has contributed to the low liquid/solid dispersion within the column [14].

The performance of the Streamline DEAE EBAC on the recovery of G6PDH from unclarified yeast cell homogenate using various biomass concentrations [5 to 20% (w/v)] was investigated in this study. In order to stabilize the bed expansion, the flow rate of feedstock was

Table 1. Residence time distribution study of Streamline DEAE. The values of theoretical plate, Bodenstein number, and axial dispersion coefficient are the average of the values from two RTD tests

Column volume (mL)	116.2
Linear flow rate (cm/h)	250
Sedimented height (cm)	15
Expanded bed height (cm)	31
Bed voidage, ε	0.71
Theoretical residence time, t (min)	8.9
Mean residence time, t_m (min)	15.2
Standard deviation, σ	3.1
Number of theoretical plates, N	24
Height equivalent to a theoretical plate (HETP) (cm)	1.3
Bodenstein number	52
Axial dispersion coefficient, D_{ax} (m^2/s)	5.8×10^{-6}

The mean residence time is a function of the distance between the maximum acetone concentrations (MAC) to 50% of the MAC [8] using the RTD test. The theoretical residence time is the hydrodynamic residence time calculated from the applied liquid flow rate.

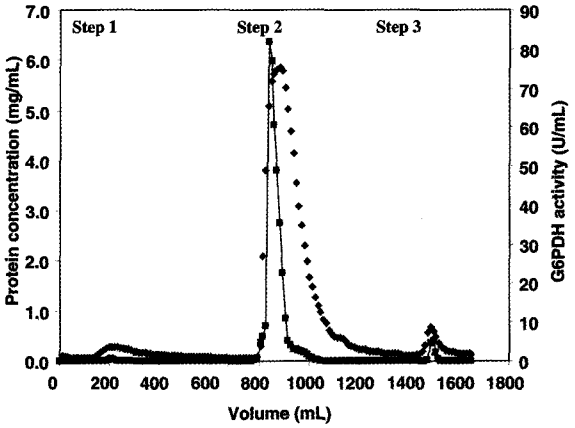

Fig. 3. The elution profile of anion exchange EBA chromatography purification of G6PDH from unclarified bakers' yeast cell homogenate consisting 15% (w/v) biomass. The adsorbed G6PDH was recovered from the adsorbent using a 3 step elution operation. The first step was carried out with elution buffer No. 1 (0.05 M NaCl, 50 mM Tris-HCl, pH 7.5), followed by elution buffer No. 2 (0.5 M NaCl, 50 mM Tris-HCl, pH 7.5), and finally with elution buffer No. 3 (1.0 M NaCl, 50 mM Tris-HCl, pH 7.5). (◆) - Total protein content, (■) - G6PDH activity.

reduced as the biomass concentration of the feedstock was increased. This strategy allowed the stabilization of bed expansion in the presence of cell homogenate as the biomass concentration of feedstock increased up to 15% (w/v). However, as the biomass concentration of the feedstock increased to 20% (w/v), visual inspection of the adsorbent beds revealed that channeling and over expansion of the adsorbent bed had occurred. As a result, the specific activity of the protein recovered from the feedstock containing 20% (w/v) biomass was reduced to about 75% of that of feedstock containing 5% (w/v) biomass. Similarly, the purification factor of the protein recovered from the 20% (w/v) biomass feedstock was only about 66% of that of 5% (w/v) biomass feedstock.

A representative elution profile for the anion exchange EBA chromatography purification of G6PDH with feedstock consisting of 15% (w/v) biomass is depicted in Fig. 3. The result showed that most of the bound enzyme was eluted in the second elution step using buffer containing 0.5 M NaCl. Table 2 shows the summary of the recovery performance from feedstock of various biomass concentrations. A very high recovery yield of G6PDH (87%) was achieved in this anion exchange EBA operation from the feedstock consisting 5% (w/v) biomass. The recovery yield was only slightly decreased to 80% when the biomass concentration of the feedstock was increased to 20% (w/v). In fact, the amount of G6PDH recovered from feedstock containing 20% (w/v) biomass was 3 times higher than that recovered from 5% (w/v) biomass feedstock. These results showed that there is a very good interaction between the streamline DEAE and G6PDH, and the binding capacity of streamline DEAE for G6PDH is still not saturated even at a biomass concentration of feedstock of 20% (w/v). This indicates that there is potential for further improvement of the throughput of this operation by the adoption of a high-density adsorbent. Increasing the density of adsorbent can offset the adsorbent bed instability caused by the increase of fluid viscosity with high biomass concentrations.

The present study demonstrated that the performance of the Streamline DEAE EBAC was good even at biomass concentration as high as 15% (w/v). However, as the biomass concentration was increased to 20% (w/v), the specific activity of the G6PDH recovered was reduced. The purification factor of the process using 20% (w/v) biomass was 34% lower than that using 5% (w/v) biomass. The increased interference of negatively charged

Table 2. Summary of the recovery performance of an anion exchange EBA chromatography purification of G6PDH from feedstock with various biomass concentrations

Biomass concentration, % (w/v)	Flow rate, cm/h	Initial G6PDH activity, U	Initial G6PDH specific activity, U/mg	Recovered G6PDH activity, U	Recovered G6PDH specific activity, U/mg	Yield of G6PDH, %	Purification factor
5	248.30	2892.51	1.05	2524.30	4.28	87.3	4.1
10	210.00	4400.64	1.14	3707.23	4.05	84.0	3.6
15	210.00	6880.00	1.12	5700.01	3.82	83.0	3.4
20	164.20	9024.07	1.17	7217.69	3.21	80.0	2.7

components (*e.g.*, cell debris, cells, and DNA fragments) and ionic strength associated with increased biomass concentrations diminished the recovery performance of anion exchange EBA chromatography. Indeed, it has been reported that the adsorption of biomass and cell debris on anion exchangers has a significant adverse effect on the adsorption of the target protein [15]. Several strategies such as the application of adsorbent with a more selective ligand [16] and the use of mixed-mode adsorbent [17,18] have been suggested to overcome the adverse effects of the interaction between biomass and adsorbent. The mixed mode hydrophobic/ionic matrices used by Lu *et al.* [18] showed very little interaction between the biomass of *Bacillus subtilis* and adsorbent even though a very high ionic strength feedstock was used. The use of a polymer shielding technique demonstrated by Viloria-Cols *et al.* [19] is another option for the reduction of the binding of biomass to the adsorbent. The application of adsorbent of high density and with salt tolerant ligand (*e.g.*, Streamline Direct CST I and Streamline Direct HST, ρ = 1.8 g/mL) may also facilitate the adsorption of protein products in high biomass concentration feedstock.

We therefore conclude that Streamline DEAE is suitable for expanded bed adsorption operation since a stable expanded bed was achieved as demonstrated from the RTD study. Direct purification of G6PDH from unclarified yeast homogenate showed that the performance of the Streamline DEAE EBAC was good even at biomass concentrations as high as 15% (w/v). A recovery yield of 87% and purification factor of 4.1 were achieved in this expanded bed adsorption operation from the feedstock consisting of 5% (w/v) biomass concentration.

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