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The Influence of Bakers' Yeast Cells on Protein Adsorption in Anion Exchange Expanded Bed Chromatography

Chow Yen Mei[1], Tey Beng Ti[2], Mohammad Nordin Ibrahim[1], Arbakariya Ariff[3], and Ling Tau Chuan[1]*

[1] Department of Process and Food Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[2] Department of Chemical and Environmental Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3] Department of Bioprocess Technology, Faculty of Biotechnology and Molecular Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

Abstract The adsorption of a model protein bovine serum albumin (BSA) in expanded bed chromatography was undertaken by exploiting a commercially available expanded bed column (20 mm i.d.) from UpFront Chromatography and Streamline DEAE (ρ = 1.2 g/cm^3) from Amersham Pharmacia Biotechnology. The influence of whole yeast cells on the adsorption capacity of column was explored by employing yeast cells in a concentration ranged of 0 to 15% (w/v). Equilibrium isotherms for adsorption of BSA on Streamline DEAE were correlated by using Langmuir equation. The presence of yeast cells resulted in decreased of BSA binding capacity in both batch binding and expanded bed chromatography. Results indicated that the yeast cells act as competitor for proteins to bind to the sites on adsorbents.

Keywords: expanded bed chromatography, yeast cells, bovine serum albumin, binding sites, adsorbent

Adsorptive bioproduct purification is conventionally performed in packed beds, which require clarified feedstock due to the risk of blocking of the bed by particulate materials. Therefore, a few initial separation steps such as centrifugation, microfiltration, concentration and initial fractionation are needed to remove the particulate materials. These multiple and intensive steps may results in the loss of a significant amount of desired product and/or product activity [1] and leads to high operating and capital costs [2,3]. One approach towards developing simplified and cost effective downstream process is the integration of unit operations in the primary capture protocol.

Expanded bed adsorption has been introduced as an integrative technique for the direct recover of desire proteins from particulate-containing feedstock without any prior solid-liquid separation steps (*i.e.* centrifugation and filtration). This approach bears some potential of simplifying traditional protein recovery processing [4,5]. The adsorbent bed is equilibrated in expanded bed mode by applying an upward flow of a suitable buffer. The feedstock application step was carried out at the end of equilibration stage. After feedstock application, the remaining weakly bound suspended solids, *e.g.* cells and cell debris, can be washed off with buffer in expanded bed mode [6]. Finally, the adsorbent bed is subjected to the cleaning-in-place (CIP) step for regeneration of the adsorbent particles. The stability and bed expansion characteristics of adsorbents in expanded beds are influenced by the characteristics of particulate-containing feedstocks, which severely affect both the biochemical and hydrodynamic performance of adsorption.

The present study was undertaken to investigate the effect of yeast cells concentration on BSA adsorption in expanded-bed adsorption chromatography. The Streamline DEAE from Amersham Pharmacia Biotechnology (APB) and expanded bed contactor (20 mm i.d.) from UpFront Chromatography were employed here. The data generated were used to identify the influence of the yeast biomass concentration on the biochemical performance of the anion exchangers.

The batch binding experiments were carried out by mixing 0.5 mL of settled volume of Streamline DEAE (Amersham Pharmacia Biotechnology, Sweden) with 9.5 mL of feedstock (5 to 80 mg/mL of BSA) comprising 0~15% equivalent to original wet cell weight per volume. The reaction bottles were rolled on a roller incubator for 4 h at room temperature. Samples (1 mL) were taken from each vessel and centrifuged at 10,000 rpm prior to BSA assay at the end of the incubation period. The bound BSA was determined based upon mass balances. Bed expansion characteristic of Streamline DEAE in the presence of unclarified feedstock was performed by using the UpFront contactor (20 mm i.d.) at room temperature. UpFront contactor was obtained from UpFront Chroma-

***Corresponding author**
Tel: +60-3-8946-6366 Fax: +60-3-8656-7123
e-mail: ltc555@eng.upm.edu.my

tography A/S, Denmark. The UpFront Chromatography contactor has a novel design of using a magnetic stirrer to evenly distribute incoming fluid. Streamline DEAE (32 mL settled volume, corresponding to 10 cm settled bed height, H_0) was loaded into the contactor. The feedstock consisting of a range of biomass concentration (0~15% wet weight per volume, w/v) was applied to the contactor and the expanded bed height (H) was monitored visually as a function of linear velocity of 0~350 cm/h. The stable bed height was recorded after a stepwise increase of selected linear velocityy. The bed expansion degree of adsorbent was measured by using Eq. (1).

$$\text{Degree of bed expansion (\%)} = (H - H_0) / H_0 \times 100\% \quad (1)$$

For BSA adsorption performance, the feedstock (3 mg/mL BSA comprising 5~15% wet cell weight, pH 7.5) was transported to the adsorbent bed by a peristaltic pump connecting to the inlet at the base of the UpFront contactor. The flow rate was maintained at 250 cm/h during the feedstock application. The samples were collected from the effluent outlet at regular intervals and assayed for BSA concentration after centrifugation at 10,000 rpm (room temperature).

The effect of yeast cells, *S. cerevisiae*, on the equilibrium characteristics of the BSA adsorption was determined by measurement of the adsorption isotherm. The analysis of batch adsorption was based on Langmuir's adsorption isotherm.

$$q = K_c q_m C^* / (1 + K_c C^*) \quad (2)$$

where q is adsorbed phase concentration, q_m is the maximum binding capacity, K_c is an association constant, and C^* is concentration in fluid phase, provides the best fit to adsorption isotherms.

A quantitative assessment of the results was performed by fitting the data to the following rearraged equation of Langmuir isotherm.

$$q_m = q_o C^* / (K_c + C^*) \quad (3)$$

where q_m is the maximum capacity.

The results (Fig. 1 and Table 1) indicated that adsorption capacity of Streamline DEAE is decreased with increase in biomass concentration (0~15% w/v). It was hypothesized that the increase of competition between targeted product (*i.e.* BSA) and impurities (Fig. 2) and effective ionic strength might contribute to such an observation [7]. The adverse effect of yeast cells on protein adsorption capacity of anion exchanger has been reported in the literature [8-10]. The mixed mode (*e.g.* hydrophobic and/or ionic) adsorption mechanics which is less sensitive to ionic strength conditions of feedstock was expected to improve the protein binding capacity [11].

The bed expansion of Streamline DEAE in the presence of biomass (0~15% w/v of yeast in buffer A) versus selected experimental velocity is depicted in Fig. 3. The result demonstrated that the design and configuration of

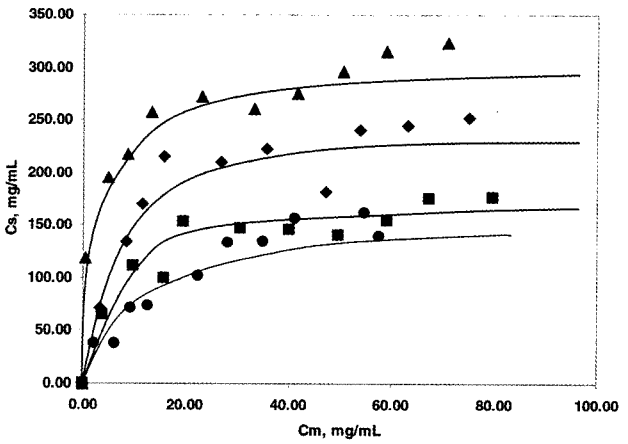

Fig. 1. The adsorption of BSA onto Streamline DEAE in the presence of yeast cells, *S. cerevisae*. Adsorption isotherms were measured based on mass balance. The adsorption of BSA was studied on Streamline DEAE equilibrated with 0.01 M Tris-HCl buffer, pH 7.5. The concentration of cells present in various experiment were: 0% (▲), 5% (♦), 10% (■) and 15% (●).

Table 1. Equilibrium adsorption of BSA on Streamline DEAE in the presence different concentrations of *S. cerevisiae*

Adsorbent	Adsorbate	*S. cerevisiae* concentration (% w/v)	q_m (mg/mL)	K_c (mg/mL)
Streamline DEAE	BSA	0	319.96	2.96
		5	266.64	7.74
		10	181.82	7.96
		15	174.61	23.63

The data obtained was well fitted to Langmuir adsorption isotherms. The maximum capacity of the adsorbent (q_m) and the dissociation constant (K_c) of the adsorbent-protein were determined by using Eq. (2).

Streamline DEAE and UpFront contactor permitted a smooth flow of feedstock containing up to 15% w/v biomass. It was demonstrated that the degree of bed expansion increased significantly with increasing biomass concentration. For example, the estimated bed expansion degree of 5% w/v and 15% w/v biomass were 300% and 500% at linear velocity of 250 cm/h (Fig. 3). The increased of bed expansion degree observed here was attributed to the elevated viscosity (*i.e.* biomass concentration) of process feedstock [12].

The breakthrough curve of BSA adsorption at different biomass concentration (5 to 15% w/v) of expanded bed chromatography is shown in Fig. 4. The binding capacity (Fig. 4) of entire bed appeared to be adversely affected by the presence of higher yeast cells concentrations (*i.e.* from 5 to 15% w/v) and ionic strength condition in the feedstock. The early breakthrough of BSA noted in the expanded bed study might reinforce the phenomenon observed in batch binding experiments (see Figs. 1 and 2). The adsorption performance may be improved by employing adsorb-

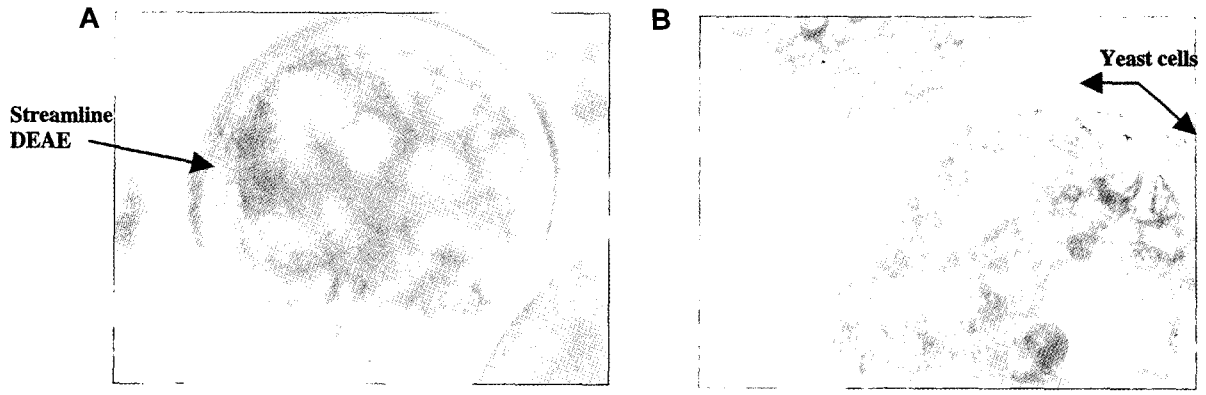

Fig. 2. Binding of yeast cells *S. cerevisiae* on Streamline DEAE. A, Intact Streamline DEAE. B, The adsorption of cells to the solid phase decreased the available charged groups for protein binding and subsequently decreased the equilibrium capacity for the target protein.

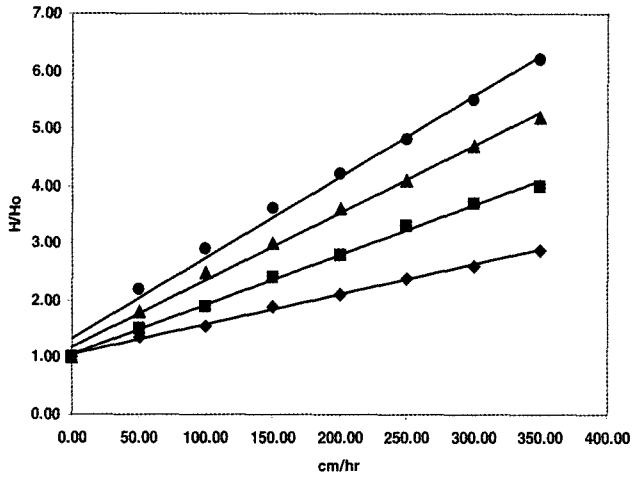

Fig. 3. Relative expansion at different flow velocities of Streamline DEAE matrix in the presence of different biomass concentration. The different concentrations of biomass were: 0% (◆), 5% (■), 10% (▲), 15% (●) (w/v) wet weight per volume in Tris-HCl buffer in a FastLine 20 column.

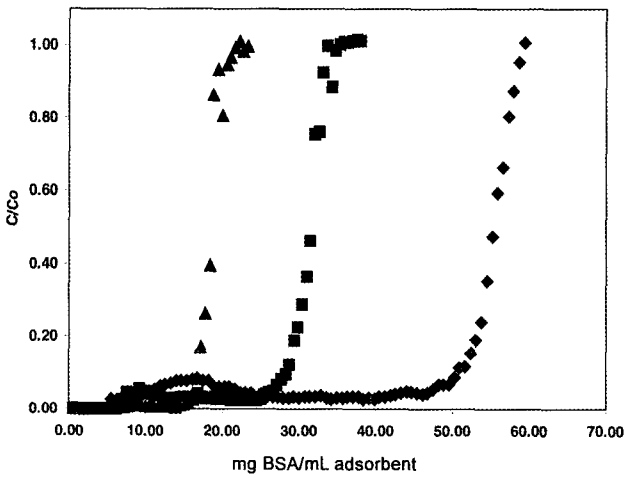

Fig. 4. Effect of the presence of yeast cells on expanded bed adsorption of BSA on Streamline DEAE. The feedstocks (3 mg/mL BSA) comprising of 5% (◆), 10% (■), 15 % (▲) equivalent wet cell weights were investigated herein. The dynamic binding capacity of BSA at C/C_o= 0.1 for various biomass concentration were 50.97 mg/settled mL adsorbent (5% w/v), 28.84 mg/settled mL adsorbent (10% w/v), and 16.0 mg/settled mL adsorbent (15% w/v).

ents characterised by high density and salt-tolerant ligand (*e.g.* Streamline Direct CST I and Streamline Direct HST, ρ = 1.8 g/cm^3, new types of adsorbents which are introduced recently by Amersham Pharmacia Biotechnology). The shielding of anion exchanger with a layer of uncharged hydrophilic polymer in order to reduce/prevent the cell-adsorbent interaction [8] was also expected to improve the adsorption system.

The study demonstrated that expanded bed adsorption exploiting Streamline DEAE and UpFront contactor could successfully capture BSA from feedstock comprising up to 15% w/v biomass. However, the BSA binding capacity achieved in expanded bed adsorption was reduced significantly (*i.e.* 51 to 16 mg/settled mL adsorbent) with increasing of biomass concentration (*i.e.* 5 to 15% w/v). The presence of 15% w/v yeast cells also decreased the batch binding capacity (*i.e.* from 320 to 175 mg BSA/settled mL adsorbent) of the anion exchanger. The increased of ionic strength conditions and binding of contaminant (*i.e.* yeast cells) from feedstock might contribute to such a reduction. The application of adsorbents having a more selective [10] and high ionic strength tolerant ligand was expected to be more suitable for such conditions.

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