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The Influence of Bakers' Yeast Cells on Protein Adsorption Performance in Dye-Ligand Expanded Bed Chromatography

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Abstract The influence of whole yeast cells (0~15% w/v) on the protein adsorption performance in dye-ligand chromatography was explored. The adsorption of a model protein, bovine serum albumin (BSA), was selected to demonstrate this approach. The UpFront adsorbent (ρ = 1.5 g/cm^3) derivatised with Cibacron Blue 3GA and a commercially available expanded bed column (20 mm i.d.) from UpFront Chromatography, Denmark, were employed in the batch binding and expanded bed operation. The BSA binding capacity was demonstrated to not be adversely affected by the presence of yeast cells. The dynamic binding capacity of BSA at a C/C_0 = 0.1 biomass concentration of 5, 10, 15% w/v were 9, 8, and 7.5 mg/mL of settled adsorbent, respectively.

Keywords: dye-ligand, Cibacron Blue 3GA, yeast cells; bovine serum albumin, expanded bed

Expanded bed chromatography has potential for the cost-effective recovery of bioproducts from unclarified microbial feedstock, as the prior purification steps, such as centrifugation, filtration and initial purification are eliminated in this integrated technique [1]. However, a robust and chemically stable ligand is an essential prerequisite for the success of this operation. Minimal interaction and/or competition of contaminants (*e.g.*, cells, cell debris, DNA) with the ligand adopted in expanded bed chromatography are desirable. The selected ligand also has to be less sensitive to the ionic strength and pH of typical unclarified feedstocks, including fermentation broth, cells homogenate and mammalian cell cultures [2].

Cibacron Blue 3GA is one of the most extensively explored chlorotriazin dyes adopted as an affinity ligand for the recovery of proteins. For example, it has been demonstrated that the reactive dye can bind specifically to dehydrogenases and kinase [3], as well as non-specifically to a wide range of serum albumins [4]. The overall molecular structure of the dye is similar to those of natural biological compounds including coenzymes, cofactors, substrates and nucleotides [5-7]. This dye ligand offers many advantages including resistance to chemical and biological degradation, large protein binding capacity, easy immobilization to matrix base and relatively high selectivity and/or specificity [8-9]. It has been immobilized successfully onto polysaccharide matrices (*e.g.* Sepharose CL-6B) for the packed bed adsorption of protein products [3,9,10].

The cell/adsorbent interactions in expanded bed chromatography are influenced by the feedstock (*e.g.*, types of microbial and biomass concentration) and adsorbent characteristics (*e.g.*, types of ligand, density and size distribution). Lin *et al.* [11] examined the adsorption of yeast cells onto anion exchangers, and the authors suggested that minimizing the biomass/adsorbent interactions was one of the main strategies for the design of an efficient expanded bed operation. Viloria-Cols *et al.* [12] demonstrated that the interaction of cells with an anion exchanger could be minimized and/or prevented by adopting a polymer shielding technique. Chase and Draeger [13] reported that the adsorption of hIgG to an affinity ligand (*i.e.*, Protein A) was not significantly diminished with increasing the yeast cells content.

In the present paper, the performance of the application of a dye ligand (Cibacron Blue 3GA) as an affinity tool for the expanded bed adsorption of proteins in the presence of microbial cells has been explored. A model protein, bovine serum albumin (BSA) and whole yeast cells (up to 15% w/v) were chosen to demonstrate this approach. The UpFront adsorbent (ρ = 1.5 g/mL) and UpFront expanded bed column (20 mm i.d.), from UpFront Chromatography, Denmark, were employed in this study.

Immobilization of Dye-Ligand Cibacron Blue 3GA on UpFront Adsorbent

The UpFront particle is a pellicullar adsorbent devel-

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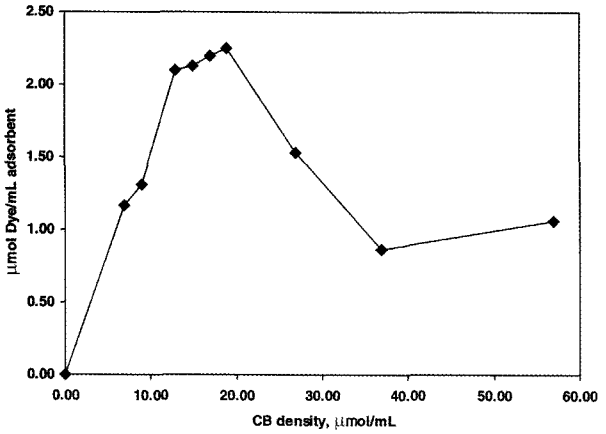

Fig. 1. Immobilisation of Cibacron Blue 3GA onto the Upfront adsorbent.

oped by UpFront Chromatography A/S, Denmark. The adsorbent size was in the range of 151~323 μm. The Cibacron Blue 3GA (Sigma Chemicals Co., St. Louis, MO, USA) was immobilized onto the UpFront adsorbent employing the method described by Dean and Watson [5] and the concentration of immobilized dye-ligand was determined by using an adaptation of the method reported by Chamber [14].

Effect of Dye Concentration upon Batch Binding of BSA

The absorbent immobilized with different concentration of dye (0 to 2.25 μmol/mL) and feedstock, consisting of 1 mg/mL BSA in 50 mM Tris-HCl, pH 7.5, was used in the batch binding study. The mixture of feedstock and adsorbent was rolled on a roller for 4 h at room temperature. The samples were collected for total protein analysis at the end of the incubation period [15]. The bound protein was estimated by mass balance.

Bed Expansion of UpFront Adsorbent

The bed expansion characteristics of the UpFront adsorbent were determined in an UpFront FastLine20 column following the procedure described previously [16]. The UpFront FastLine20 column was a purpose designed column for expanded bed operation. The even flow distribution of the UpFront column was achieved by a magnetic stirrer at the bottom of the device. The UpFront adsorbent (50 mL, equal to 15 cm settled bed height, H_0 in the UpFront column) was loaded into the expanded bed column and the liquid was applied to the column by a peristaltic pump. The expanded bed height (H) was examined visually as a function of flow rate of 0 to 1,000 cm/h and the degree of bed expansion was determined according to the Eq. (1), as follows:

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

Expanded Bed Adsorption of BSA in the Presence of Yeast Cells

Expanded bed adsorption of BSA in the presence of yeast cells (5~15% wet weight per volume, w/v) was performed using the UpFront FastLine20 column at room temperature. The UpFront adsorbent (50 mL, equal to 15 cm settled bed height, H_0 in the UpFront column) was loaded into the expanded bed column. The feedstock (3 mg/mL BSA consisting 5~15% wet weight per volume, w/v of yeast cells) was applied to the contactor at selected flow rates, between 225~450 cm/h, using a peristaltic pump. Samples were taken at regular intervals, and centrifuged prior to the total protein analysis. The bound protein was estimated by mass balance. The application of feedstock into the bed was continued until the desired adsorbent saturation state ($C/C_0 \approx 1$) was obtained. The adsorbent bed was regenerated with 3 M potassium thiocyanate (KSCN) after each experiment.

Immobilisation of Dye-Ligand into UpFront Adsorbent

The reactive dye was immobilized onto the matrix by using a biomolecular nucleophilic substitution reaction, under alkaline conditions. The covalently coupling operation was achieved by the nucleophilic substitution of the hydroxyl groups on the adsorbent with the reactive chlorine in the Cibacron Blue 3GA. It was demonstrated that the immobilized dye concentration in the UpFront adsorbent was increased with increasing initial dye concentration, from 0 to 12 μmol/mL (Fig. 1). This observation was attributed to the higher initial dye concentration (>12 μmol/mL) decreasing the generation of nucleophiles, with a subsequent reduction in the substitution of the hydroxyl groups on the adsorbent with the reactive chlorine of the dye [7]. However, the behavior observed in this study would not preclude to be generic to other types of matrix base under given conditions. The relative dye concentration immobilized on the adsorbent depends on the chemical and physical nature (*e.g.*, agarose contents and pore sizes) of matrix base. For example, Boyer and Hsu [10] demonstrated that the concentration Cibacron Blue 3GA immobilized onto Sepharose CL-6B (adsorbent) increased with increasing initial dye concentration, from 0 to 30 μmol/mL (*i.e.*, a linear relationship).

Effect of Dye-Ligand Concentration on Batch Binding Capacity of BSA

The profile of BSA uptake by the UpFront Cibacron Blue 3GA for various CB coupling concentrations is shown in Fig. 2. The results suggested that the binding of the BSA to the adsorbent increased with increasing dye-ligand concentration, but the increment rate decreased with increasing concentration. Boyer and Hsu [10] studied the kinetics of Bovine serum albumin (BSA) adsorption onto Cibacron Blue 3GA-Sepharose CL-6B, using a lumped kinetic model, and found that the lumped forward rate constant decreased with increasing dye density. The steric effect of increased ligand coverage and pore

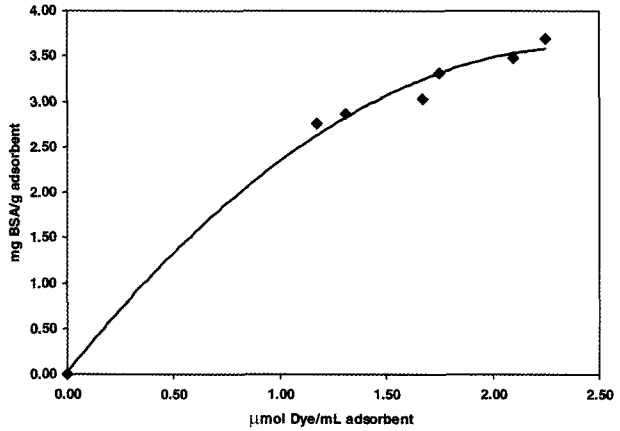

Fig. 2. Effect of dye-ligand concentration upon BSA binding.

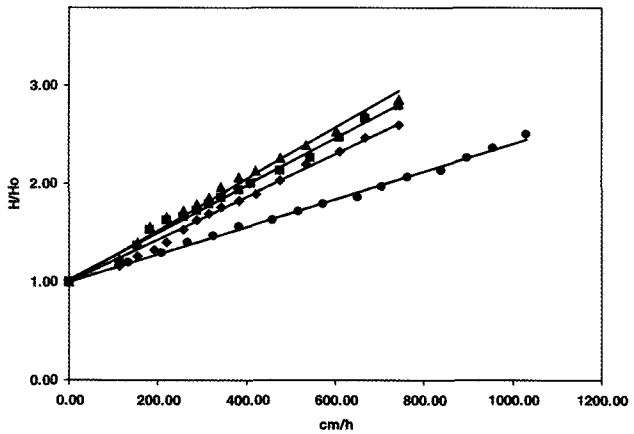

Fig. 3. Bed expansion characteristic of UpFront adsorbent in the presence of biomass. The different biomass concentrations were: 0% (●), 5% (◆), 10% (■), and 15% (▲) w/v, in buffer.

blockage by the adsorbed protein molecules might contribute to such an observation [4]. The increased electrostatic repulsion (*i.e.*, larger electrostatic hindrance effect of BSA) with increasing Cibacron Blue 3GA concentration might also contribute to the reduction in the adsorption rate [9].

Bed Expansion Characteristics and BSA Adsorption Performance of UpFront Cibacron Blue 3GA

The bed expansion of the UpFront adsorbent when fluidised in feedstock containing different biomass concentration is depicted in Fig. 3. The bed expansion characteristics are governed by a number of parameters, including the density and size distribution of the adsorbent, the column design, biomass concentration and feed rate. No significant variation in the degree of bed expansion was demonstrated when the biomass was increased from 5 to 15% (w/v). The adsorbent contributed to this observation due to its relatively higher density.

The expanded bed adsorption of BSA onto the UpFront Cibacron Blue 3GA in the presence of biomass was

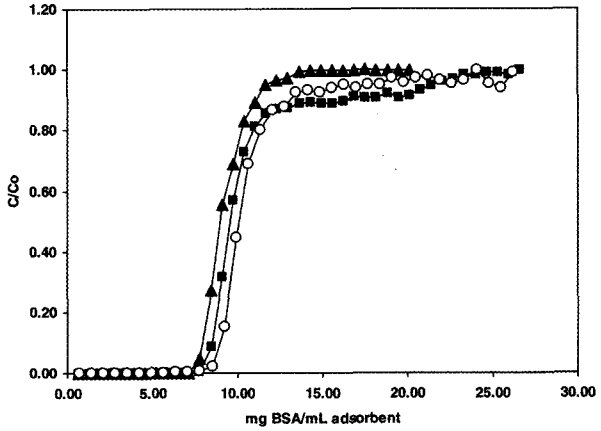

Fig. 4. Effect of the presence of yeast cells on expanded bed adsorption of BSA onto UpFront CB 3GA. The feedstocks (3 mg/mL BSA) comprising 5% w/v (○), 10% w/v (■), and 15% w/v (▲) equivalent wet cell weights, at pH 7.5, were studied.

Table 1. Effect of biomass concentration on the dynamic binding capacity

Biomass concentration		Dynamic binding capacity at C/C_0 = 0.1 (mg BSA/mL adsorbent)
Wet weight per volume (% w/v)	Dry cell weight (g/mL)	
5	0.013	9.0
10	0.028	8.0
15	0.041	7.5

performed in the UpFront contactor (2.0 mm i.d.) and the BSA breakthrough curve is depicted in Fig. 4. As discussed earlier, a dye concentration of 2.1 μmol yielded the optimum BSA binding capacity. Therefore, the UpFront adsorbent immobilised with 2.1 μmol dye-ligand was chosen for the expanded bed adsorption study. The steepness of the BSA breakthrough curve was relatively unaffected by increasing the yeast cells concentration from 5 to 15% (w/v). The dynamic binding capacity of BSA at C/C_0 = 0.1 (Table 1) was reduced by 17% (*i.e.*, 9.0 to 7.5 mg BSA per mL adsorbent) with a 300% increase in the biomass concentration (*i.e.*, from 5 to 15% w/v). This result indicated that there was no significant increase in competition between the BSA and yeast cells for the binding sites (*i.e.* dye ligand) of the adsorbent (Figs. 4 and 5). Fernadez-Lahore *et al.* (2000) examined the influence of whole yeast cells on the protein (*i.e.* BSA and lysozyme) adsorption performance of various adsorbents (ion exchange, metal affinity ligand and hydrophobic interaction). The authors concluded that electrostatic forces dominated the cells/adsorbent interactions and the affinity adsorption system is less sensitive to the yeast cells. The early work of Chase and Draeger [13] and Ling [18] also revealed the cells had little adverse effects on affinity adsorption systems.

In the present paper, it has been demonstrated that the interaction of yeast cells with dye ligands is minimal, and

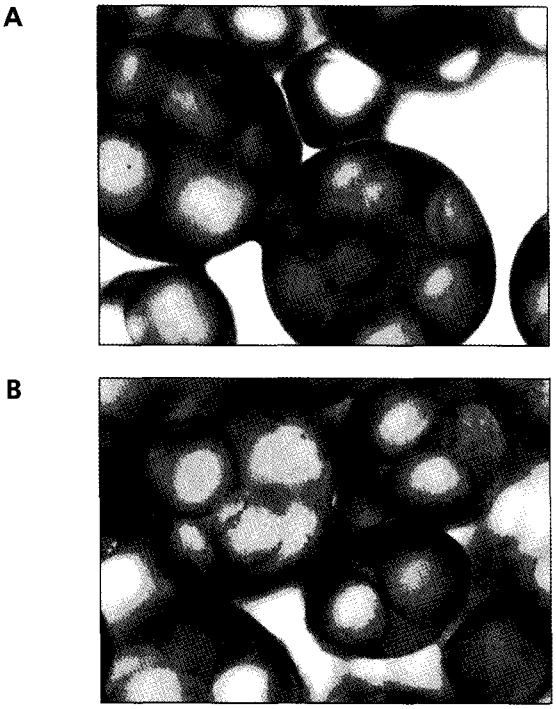

Fig. 5. The UpFront Cibacron Blue 3GA adsorbent in the presence of yeast cells. An inspection of sample of the adsorbent under an optical microscope with magnification × 20 (A) intact adsorbent and (B) adsorbent in the presence of yeast cells.

the binding capacity of the targeted protein (BSA) was not significantly reduced with increasing biomass concentration (0~15% w/v). The dynamic binding capacity of BSA at C/C_o = 0.1 was slightly reduced (<20%) when the biomass concentration was increased from 5 to 15% w/v (300%). The use of Cibacron Blue 3GA as an affinity ligand constitutes an appealing alternative technique for minimising cells/adsorbent interactions in expanded bed chromatography.

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