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The Operation Performance of an Expanded Bed Contactor Characterised by Mechanical Stirring

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Abstract. The performance of an expanded bed contactor (UpFront i.d. 20 mm) characterised by mechanical stirring flow distribution in the adsorption of intracellular proteins from concentrated unclarified yeast extract was investigated. High density pellicular adsorbent (UpFront steel-agarose; ρ = 2.65 g ml^{-1}) derivatized with selective ligand chemistries (Cibacron Blue 3GA) was adopted in this study. The adsorption of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) from bakers' yeast was chosen as a demonstration of this approach. It was demonstrated that a high biomass throughput adsorption operation (25 % ww/v of yeast extract) was achieved in this contactor design.

Keywords. **expanded bed contactor; mechanical stirring; flow distribution; yeast extract; G3PDH**

INTRODUCTION

The design and configuration of contactor and adsorbent density are key parameters which govern expanded bed adsorption performance (Thoemmes, 1997). An efficient distribution of incoming feedstock over the full cross sectional of the contactor is needed to achieve a stable expanded bed. An uneven flow distribution will contributed to the formation of channelling and dead water zones (Figure 1). Several distributor configurations have been adopted to variously distribute incoming feedstock, including multi-hole plate, glass beads and mechanical stirrers.

The minimum fluidisation velocity (U_{mf}) and the terminal settling velocity (U_t) defined the operational window of fluidisation that could be operated in expanded bed adsorption (Figure 2). A number of parameters, including particle characteristics and process feedstock properties may influence these velocity values. The terminal settling velocity (U_t) of a single particle can be estimated using Stokes law (Eqn. 1). i.e.:

$$U_t = \frac{(\rho_p - \rho_l) . d^2_p . g}{18 . \mu} \quad (1)$$

Here, the terminal settling velocity of a single particle (U_t) is proportional to the density difference between the particle (ρ_p) and process liquid (ρ_l), the square of the particle diameter (d_p) and inversely proportional to the viscosity of the process fluid (μ). However, adsorbent particles in fluidised beds may interact with each other due to adhesion and cannot be simply treated as individual particles. The Stokes equation clearly reflects that the magnitude of the terminal settling velocity of a particle is variously dependent upon viscosity of the process fluid, the particle density and the particle diameter.

In order to process a highly viscous feedstock at a high flow rate, the terminal settling velocity must be increased by increasing the particle diameter and/or the density of the

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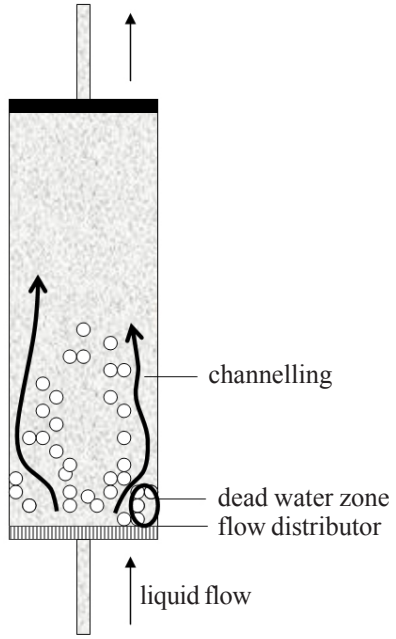

Figure 1. Diagram showing an uneven flow distribution of incoming feedstock. An uneven flow distribution of incoming feedstock will contribute to the formation of channelling and dead water zones in fluidised beds. Various types of devices including plates, mesh, glass beads and mechanical stirrer have been used as flow distributors.

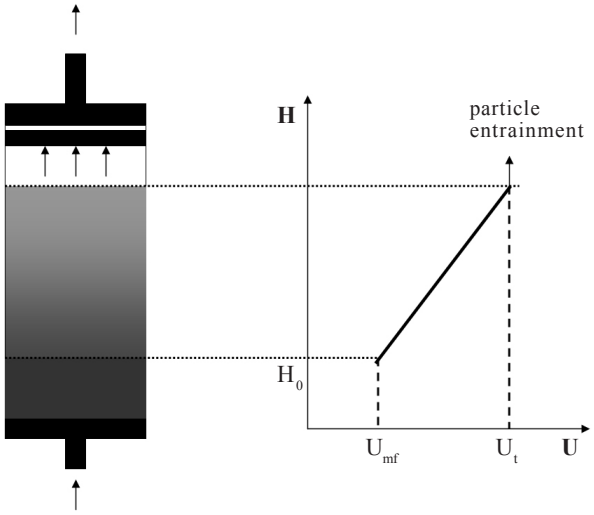

Figure 2. The operational velocity range of an expanded bed system. The adsorbent particles start to fluidise when the upward flow velocity is greater than the minimum fluidisation velocity (U_{mf}). The adsorbent particles are entrained and carried out from the fluidised bed if the velocity is increased above the terminal settling velocity (U_t). The operational velocity range of a fluidised bed system ranges from U_{mf} to U_t. H_0 is adsorbent settled bed height and H is bed height.

particle (Eqn. 1). However, increasing the particle diameter will inevitably increase the diffusion resistance to the achievement of adsorption equilibrium within the adsorbent particles. As a result, the adsorption and desorption performance of the target product by such a particle will be diminished. Wells *et al.* (1987) demonstrated that the use of large particles (160 – 1000 μm) enabled a fluidised bed system to be operated at very high superficial flow velocities of 1440 – 5400 cm h^{-1} with 20 – 100 % degree of bed expansion, although the adsorption performance suffered from diffusional limitations. McCreath *et al.* (1994) developed perfluoropolymer particles as an alternative method of increasing density. They demonstrated that the use of such a high density adsorbent (2.2 g ml^{-1}) allowed the use of comparatively small particles (50 – 80 μm) at a rather moderate fluid velocity of 120 cm h^{-1}.

In this paper, the performance of an expanded bed contactor characterised by mechanical stirrer (UpFront) in processing of highly concentrated yeast extract was investigated. In the early work of Zafirakos and Lihme (1999), the hydrodynamic performance of UpFront contactors (20 mm and 50 mm i.d.) has been explored by exploiting UpFront glass-agarose pellicular adsorbents (1.3 –1.5 g ml^{-1}). It was demonstrated that consistent fluidisation characteristics (£ 20 % variation in theoretical plate number) were achieved in the contactors operated at different scales (20 mm and 50 mm i.d). However, it is not clear how well the stirred distribution concept of such a contactor will perform when dealing with a highly concentrated yeast disruptate using a relatively high density adsorbent (ñ = 2.65 g/mL). Therefore, the feasibility of such a distribution configuration in handling the yeast extract at 25 % (w/v) original cells was investigated.

Adsorbent particle and immobilisation of dye-ligand Cibacron Blue 3GA. UpFront steel-agarose is a pellicular matrix which comprises of 6 % (w/v) porous agarose and a non-porous stainless steel core. It was obtained from UpFront Chromatography A/S, Denmark. The adsorbent has a density of 2.65 g/mL and the particle size is in between 151 and 323 μm. The adsorbent particles were derivatised with Cibacron Blue 3GA as described by Zhang (1999).

Expanded bed contactor. The flow distribution mechanism of UpFront (20 mm i.d.) contactor was achieved by a mechanical stirrer fitted at the base of the contactor. It was developed by UpFront Chromatography A/S, Denmark. The top of the contactor was fitted with a movable plunger. The plunger was used to adjust the operational bed height of expanded bed column. The feedstock was transferred to the UpFront colimn using a peristaltic pump (Watson-Marlowe Company Ltd, UK) at the selected experimental linear velocity of 0 – 2300 cm/h.

Bed expansion characteristics of UpFront adsorbent in expanded bed contactor. The bed expansion characteristics of UpFront adsorbent were determined in the UpFront contactor. Adsorbent particles were loaded into the contactor, corresponding to a settled bed height (SBH or H_0) of 15 cm. The bed height (H) was monitored visually as a function of superficial velocity of buffer A (10 mM Tris/HCl, pH 7.5 containing 1 mM EDTA). The bed was allowed to stabilise (*i.e.* no back-mixing or mixing of the adsorbent particles) with a period of 12 – 15 minutes after a stepwise increase of flow velocity and the stable bed height (H) was recorded. The bed expansion degree was measured according to Eqn. 2. The results were expressed as an average of two reading (duplicates) with an estimated error of 5 %.

$$\text{Degree of expansion (\%)} = \frac{H - H_0}{H_0} \times 100\ \% \qquad (2)$$

Adsorption performances of UpFront Cibacron Blue 3GA adsorbent in UpFront contactor. The adsorption performance of UpFront (20 mm i.d.) contactor was determined by establishing enzyme breakthrough curves in the form of C/C_0 versus the G3PDH loaded per settled ml of adsorbent. Adsorbents were loaded into the contactor to give a settled bed height (SBH) of 20 cm and equilibrated with 10 mM Tris/HCl, pH 7.5 containing 1 mM EDTA (buffer A) in expanded bed mode. The adsorption performance was established using feedstock comprising 25 % equivalent wet cell weight pre-wet milling (w/v) at pH 6.1. The feedstock was previously disrupted by bead milling at 50 % w/v and was diluted to the equivalent of 25 % original wet cell weight pre-wet milling. The feedstock was applied to the bed by a peristaltic pump connected to the inlet of the contactor at a superficial velocity of 450 cm/h. Samples (1 ml) were taken from the effluent outlet at regular intervals and were assayed for enzyme activity (Jahanshai *et al.*, 2002; Ling and Lyddiatt, 2005a). A mixture solution consists of 50 µl of sodium arsenate solution, 100 µl of NAD^+ solution, 790 µl of bicine buffer and 10 µl of enzyme sample solution was incubated for 3 minutes (25˚C). The enzymatic reaction was begun by the addition of substrate (50µl of the D-G3P solution) into the solution. The conversion of NAD^+ to NADH was recorded by a spectrophotometer (Pharmacia Ultrospec III) at 340 nm. The G3PDH activity unit was expressed as the number of µmoles of NADH produced (25˚C, pH 8.5) per minute in 1 ml of solution (IU/ml). The results were expressed as an average of duplicates reading with an estimated error of 15 %.

The result was expressed as C/C_0, where C_0 is original activity and C is the enzyme activity in samples. Feedstock application was terminated when near-saturation of the adsorbent capacity ($C/C_0 \approx 0.8$) was achieved. After the feedstock application stage, the bed was washed with buffer A in expanded bed mode. The adsorbent was regenerated with 3 M KSCN in buffer A.

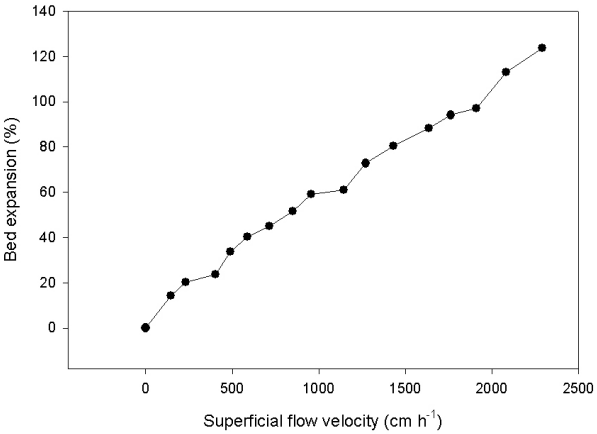

Figure 3. The bed expansion of UpFront Cibacron Blue 3GA as a function of superficial flow velocity of buffer A. Adsorbent particles (UpFront Cibacron Blue 3GA), corresponding to a settled bed height (SBH) of 15 cm were loaded into UpFront contactor. The degree of bed expansion was monitored visually as a function of increasing superficial velocity of buffer A.

Bed expansion behaviour of UpFront Cibacron Blue 3GA. The performance of UpFront contactor (20 mm i.d.) has been explored by exploiting Macrosorb K6AX (another expanded bed adsorbent, ñ = 1.2 g/mL) (Ling and Lyddiatt, 2004). It was demonstrated that the design and configuration of the device permit a smooth flow through of buffer A and 15% (w/v) yeast extract. In this part of study, the operation performance of the contactor exploiting high density adsorbent and buffer A was explored and discussed.

The degree of bed expansion of UpFront Cibacron Blue 3GA (ñ = 2.65 g/mL) in the UpFront (20 mm i.d.) contactor was monitored visually as a function of increasing superficial flow velocity of buffer A (0 – 2300 cm/h) and the curve is depicted in Figure 3. The adsorbent particles are expected to be entrained and carried out from the column if the velocity is increased above 2300 cm/h. A smooth bed expansion behaviour of UpFront Cibacron Blue 3GA was achieved in the UpFront (20 mm i.d.) contactor. This result might indicate that the flow distributors of the UpFront contactor allowed a smooth flow of buffer A through the contactor. It has been reported an even distribution of incoming fluid over the full cross sectional area of contactors contributed to smooth and good bed expansion behaviour of adsorbent (Luca *et al.*, 1994). The air bubbles present in the buffer A could flow through the UpFront contactor smoothly without disturbing the expanded bed. The problem of air bubbles and particulates clogging of conventional expanded

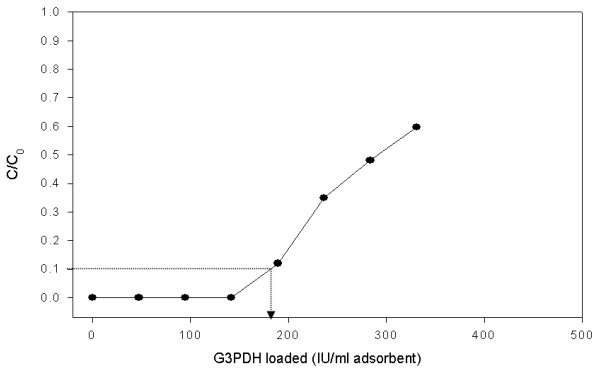

Figure 4. G3PDH adsorption performance in the UpFront contactor. The feedstock was previously disrupted by bead milling at 50 % w/v and was diluted to the equivalent of 25 % original wet cell weight pre-wet milling. The feedstock was applied to the expanded bed contactor at a constant linear velocity of 450 cm/h. Samples were centrifuged and assayed for enzyme activity which was expressed as C/C_0, where C_0 = original activity and C = enzyme activity in samples.

bed contactor which flow distribution achieved by mesh and/or plate (Ling and Lyddiatt, 2004) was avoided here. Zafirakos and Lihme (1999) have reported that the pore size of conventional flow distributors limited the performance of flow distribution of incoming feedstock.

G3PDH adsorption performance of UpFront Cibacron Blue 3GA adsorbent in UpFront contactor. The study of biochemical characterisation of UpFront contactor (20 mm *i.d.*) contactor has been explored using 15% (w/v) yeast extract and UpFront steel-agarose adsorbent (Ling and Lyddiatt, 2005b). It was demonstrated that the performance (*i.e.* G3PDH adsorption to the adsorbent) of such contactor was performed well. However, the application of the UpFront contactor to the processing of higher biomass concentration of bakers' yeast disruptate has not yet been explored. Therefore, the previous work (Ling and Lyddiatt, 2005b) was extended in order to study the performance of such a contactor operated to capture G3PDH by adsorption to UpFront adsorbent from 25% w/v yeast disruptate.

The adsorption performance of the expanded bed contactor in UpFront contactor was studied by establishing enzyme breakthrough curve. The breakthrough curve of G3PDH adsorption from yeast disruptate achieved by the contactor was plotted in the form of C/C_0 versus the G3PDH loaded per settled ml of adsorbent (Figure 4). The estimated dynamic binding capacity (at $C/C_0 = 0.1$, where C = breakthrough sample activity and C_0 = original activity in feedstock) achieved by the UpFront contactor (20 mm *i.d.*) was 181 IU/settled mL adsorbent. The dynamic capacity in the UpFront contactor was comparable with that in the BRG

Table 1. Summary of G3PDH performance in the UpFront contactor.

Total initial feedstock enzyme activity	48064 IU
Total breakthrough enzyme activity	19225.6 IU
Breakthrough time	14 min
Settled bed height (H_0)	20 cm
Expanded bed height (H)	28.4 cm
Superficial velocity	450 cm/h
Degree of bed expansion	42%
Dynamic binding capacity at $C/C_0 = 0.1$	181.2

contactor (an expanded bed contactor designed within Biochemical Recovery Group of The University of Birmingham). The flow distribution of BRG contactor (Ling and Lyddiatt, 2005b) was achieved by using glass beads (3.5 – 4.5 mm). The mechanical stirrer of the UpFront contactor distributed the feedstock over the cross section of the contactor thus reducing any channelling effect during feedstock application. Visual inspection of the inlet of UpFront contactor revealed no clogging with air bubbles and particulate materials. The result obtained here indicated that the flow distributor enable the smooth processing of 25% (w/v) yeast disruptate.

The performance of UpFront contactor was evaluated by establishing bed expansion and enzyme breakthrough curves. It was demonstrated that design of the contactor could be used in expanded bed applications involving highly concentrated yeast extract (25 % w/v original yeast cells).

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REFERENCES

Jahanshahi, M., Sun, Y., Santos, E., Pacek, A., Franco, T. T., Nienow A., and Lyddiatt, A . 2002. Operational intensification by direct product sequestration from cell disruptates: Application of a pellicular adsorbent in a mechanically integrated disruption-fluidised bed adsorption process. *Biotechnology and Bioengineering* 80: 201-212.

Ling, T. C., Jahanshahi, M., and Lyddiatt, A. 2004. Fluidised/Expanded bed dye-ligand recovery of intracellular enzymes from unclarified disrupted yeast: Performance evaluation of kiesleguhr-agarose composites. *Journal of Technical Engineering* 2: 119–128.

Ling, T. C., and Lyddiatt, A. 2005a. 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. *Journal of Biotechnology* 119: 436 – 448.

Ling, T. C., and Lyddiatt, A. 2005b. 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. *Protein Expression and Purification.* 42: 160–165.

Luca, L. D., Hellenbroich, N. J., Hocker, T., and Chase, H. A. 1994. A study of the expansion characteristics and transient behaviour of expanded beds of adsorbent particles suitable for bioseparations. *Bioseparation* 4: 311–318.

McCreath, G. E., Chase, H. A., and Lowe, C. R. 1994. Novel affinity separations based on perfluorocarbon emulsions - Use of a perfluorocarbon affinity emulsion for the direct extraction of Glucose-6-phosphate dehydrogenase from baker's yeast. *Journal of Chromatography* 659: 275–287.

Thoemmes, J. 1997. Fluidized bed adsorption as a primary recovery step in protein purification. *Advance Biochemical Engineering and Biotechnology* 58: 185–230.

Wells, C. M., and Lyddiatt, A. 1987. Liquid fluidised bed adsorption. pp 217 – 224. In: M. S. Verral, and M. J. Hudson (Eds.), *Separation for Biotechnology*. Ellis-Horwood, Chichester, UK.

Zafirakos, E., and Lihme, A. 1999. EBA columns waith a distribution system based on local stirring. *Bioseparation* 8: 85–91.

Zhang, Z. 1999. *Magnetically stabilized Fluidized Bed Adsorption in The Recovery of Protein Products from Particulate Feedstocks*, Ph.D. Thesis. The University of Birmingham, UK.