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# Direct enzyme adsorption from an unclarified microbial feedstock using suspended bed chromatography

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## Abstract

Suspended bed chromatography (SBC) is a new hybrid technique concomitantly benefiting from batch adsorption, the process advantages of an enclosed system, and its compatibility with established commercial chromatographic contactors and adsorbents. SBC was evaluated in the anion-exchange capture and chromatographic fractionation of native glyceraldehyde-3-phosphate dehydrogenase (G3PDH) from the complex mixture of molecular and particulate moieties that constitute wet-milled bakers' yeast. Method scouting established operating conditions exploiting Whatman Express-Ion Exchanger Q at pH 7.5 and a disrupted biomass concentration equivalent to 3.5% (wet mass/v original intact cells). Partially purified G3PDH was recovered directly from the yeast disruptate in a scaled-down process developed at 1/756 process scale. This was used to establish operating parameters to facilitate process scale-up to exploit a 44 cm I.D. Millipore IsoPak column, 18 kg (swollen mass) of Express-Ion Q anion-exchange cellulose and 275 l of 3.5% (wet w/v) bakers' yeast disruptate. The generic utility of SBC was demonstrated for direct product adsorption from feedstocks characterised by a modest content of bioparticulates (equivalent to <4% (wet w/v) disrupted cells). Analyses illustrated an enrichment of G3PDH in respect of enzyme concentration and significant reduction in product turbidity and Pico-Green reactivity (correlated with double stranded (ds) DNA content). The application niche for this new approach to primary protein recovery is discussed with particular reference to the downstream processing of coarsely clarified whole broths, cell disruptates and biological extracts.
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## 1. Introduction

The downstream processing of commercially important biopolymers including proteins, peptides, nucleic acids and carbohydrates from various sources is of industrial significance. Conventionally, low-pressure chromatographic processes mandatorily require clarified feedstreams. Such separations are typically carried out using batch or column contactors and are well described elsewhere [1,2]. How-

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ever, as the need for intensified and integrated processes has grown (discussed in Ref. [3]) there has been a drive to position the chromatographic steps earlier in the operational train and thereby reduce the complexity of clarification-related processes, together with the cycle time and the overall operating costs.

One approach to achieve these objectives is the direct adsorption of the target from a particulate-containing feedstream. Over recent years the technique of expanded bed chromatography has emerged to address this very issue. By expanding an adsorbent bed with upward flow of the feedstream, bioparticulates (cells, cell debris, organelles etc.) can pass relatively unimpeded through the enhanced bed voidage without seriously constraining the adsorption of target products to the stationary phase [4]. This approach has been well documented for various biopolymer purifications including monoclonal antibodies [5], enzymes including xylanase [6], plasmid DNA [7] and other bioactive molecules such as endostatin [8]. The technique demands the use of customised dense adsorbents in a specialised contactor requiring relatively sophisticated operating protocols (variously discussed in Refs. [3,4,9,10]).

We have previously reported the technique of suspended bed chromatography (SBC; [11,12]) in which chromatographic adsorbents and columns designed for packed bed operations may be used in alternative protocols. Briefly, adsorption is carried out in batch mode, and the resulting adsorbent suspension is filter collected/clarified in a conventional fixed bed contactor for washing and elution. This approach is enabled by the availability of pump-packed column chromatography systems [13] and has been described in detail elsewhere [11]. Using clarified hen egg-white feedstock, we have reported that process-scale, anion-exchange chromatographic recovery of ovalbumin from this feed is similar under suspended bed conditions to that achievable in conventional fixed bed operations [14]. The SBC adsorption process may be additionally modelled and mechanistically predicted using theoretical analyses [12].

In the present study we have extended the application of SBC to assess the possibility of the purification of a protein from an unclarified feedstream. Our experimental system involved the isolation of glyceraldehyde-3-phosphate dehydrogenase (G3PDH) from wet-milled bakers' yeast [15,16]. G3PDH is an intracellular enzyme of $M_r \simeq 140{,}000$ and isoelectric point ($pI$)$\simeq$6.1 [15] that can be chromatographically purified exploiting anion-exchange (DEAE, quaternary ammonium (QA) etc.) or pseudo-affinity (Cibacron Blue) ligand chemistries [15,16]. The yeast enzyme has recently been exploited as a generic model for studies of direct product sequestration (DPS) from microbial extracts wherein a bead mill and fluidised bed adsorber were mechanically connected to achieve near simultaneous cell disruption and affinity adsorption of G3PDH with minimal exposure to the hostile environment of the disruptate (discussed in Refs. [16–18]).

A feature of this form of DPS is the harnessing of fluidised bed technology to service the *continuous* operation of a cell disrupter such as a bead mill or homogeniser [3]. However, it was the objective of the present study to exploit the documented G3PDH production system [16–18] as the experimental vehicle for a study of the generic applicability of SBC in primary protein recoveries operated at bona fide process scale. Although essentially a *batch* process, SBC could in principle be beneficially applied to the primary capture of biomolecular products from unclarified fermentation broths, cell disruptates and biological extracts *provided* that the inherent concentration of suspended solids did not compromise the characteristic adsorption, washing and desorption procedures associated with this new technology.

## 2. Experimental

### *2.1. Materials*

Express-Ion D (DEAE-Cellulose) and Express-Ion Q (QA-Cellulose) were obtained from Whatman (Maidstone, UK). An IsoPak column (50 cm×44 cm I.D.) and associated recirculating slurry preparation station equipped with a 450-l vessel and laboratory columns (208 cm×1.6 cm I.D.) were obtained from Millipore (Stonehouse, UK). Purified rabbit G3PDH was obtained from Sigma (Poole, UK). All other chemicals were of analytical reagent grade. Frozen

bakers' yeast was obtained from a local supplier in Burton-on-Trent, UK.

### 2.2. Feedstock preparation

Bakers' yeast (50 kg) was thawed overnight in 0.01 *M* Tris–HCl buffer (pH 7.5), containing 0.001 *M* EDTA (100 l). Bakers' yeast suspension was wet milled (Dyno Mill, Type KDL; Willi A Bachofen, Switzerland) at a rate of 15 l/h at 18 °C, to give a stock disruptate of 30% (wet w/v) described (in the manner of *all* disruptates in this work) in terms of the original wet mass of cells. This stock feedstock was diluted with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA to yield feedstreams characterised by selected biomass concentrations for subsequent SBC evaluation.

### 2.3. Method scouting

Two anion-exchange celluloses manufactured by Whatman were evaluated in this study. Express-Ion D and Q were equilibrated with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA (referred to herein as equilibration buffer). The comparative efficacy of Express-Ion D or Q (1 g) was determined by suspending each adsorbent in G3PDH solution (9 ml; partially purified by pseudo-affinity adsorption [17]) containing a total of 600 units of enzyme activity.

After 3 h of agitation at room temperature on a blood mixer, enzyme adsorption was found to be complete and the concentration of unbound G3PDH was determined relative to the starting concentration by enzyme assay [15–17]. The adsorptive performance of Express-Ion D and Q was further characterised with feedstocks representative of a spectrum of pH values. Adsorbents were equilibrated with 0.01 *M* Tris, 0.001 *M* EDTA and adjusted to pH 6.1, 7.5, 8.2 or 8.7 using HCl. Express-Ion D or Q (1 g) was agitated at room temperature on a blood mixer for 3 h in 15% (wet w/v) bakers' yeast disruptate (9 ml) that was either clarified by prior centrifugation or used directly in the unclarified state.

The impact of biomass concentration upon the performance of Express-Ion Q was determined as follows. Bulk adsorbent was equilibrated with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA, and 1 g (swollen mass) aliquots were suspended in 9 ml of 3.5%, 7.5% or 15% (wet w/v equilibration buffer) bakers' yeast disruptate and gently agitated on a blood mixer at room temperature for 40 min. The adsorbent was washed with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA and batch eluted with 0.5 *M* NaCl in 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA. A similar study was conducted using solely 3.5% (wet w/v) biomass in order to examine the impact upon desorption efficiency of 0.1, 0.2, 0.375, 0.5, 0.75 and 1 *M* NaCl in equilibration buffer.

### 2.4. Scale-down laboratory studies

Stock disruptate (30%, wet w/v) was serially diluted to reach a minimum of 3.5% (wet w/v) biomass loading using 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA. Express-Ion Q (32 ml settled volume), pre-equilibrated with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA, was contacted with the various dilutions of yeast disruptate (384 ml) and mixed gently on a blood mixer for 35 min to achieve adsorption. The yeast disruptate–Express-Ion Q suspension (416 ml) was poured into an extended chromatography column (208 cm×1.6 cm I.D.; 418 ml total volume) fitted with 10 μm mesh bed supports, and the G3PDH-depleted effluent was sampled and pumped to waste through the lower contactor outlet. Once the adsorbent slurry had drained and consolidated into a fixed bed, the upper flow adapter of the laboratory device was lowered into contact with the adsorbent and the bed was adjusted to a height of 16 cm. The bed was washed with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA (150 ml) until the ultraviolet absorbance at 280 nm approached baseline values. Bound proteins were isocratically eluted with 0.15 *M* NaCl in 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA (200 ml) at flow-rates of 150 to 300 cm/h. Samples were taken at fixed volume intervals and analysed for G3PDH activity, total protein, DNA and turbidity.

### 2.5. Process-scale suspended bed chromatography

Express-Ion Q (18 kg swollen mass) was equilibrated with 0.01 *M* Tris–HCl buffer (pH 7.5)

containing 0.001 *M* EDTA and suspended in 3.5% (wet w/v) yeast disruptate feedstock (275 l). The ion exchanger was maintained in suspension by continuous recirculation of the slurry through the IsoPak slurry preparation unit at a flow-rate of 30–40 l/min. Adsorption was continued by contacting in suspended bed mode for 45 min.

Prior to the process study, the height of the 44 cm I.D. IsoPak column was pre-set to 13 cm (ca. 19.8 l). Following the adsorption stage, the adsorbent/disruptate suspension was pumped into the column in upflow from the slurry tank at a pressure of ca. 22.5 p.s.i. (1 p.s.i.=6894.76 Pa) according to the column manufacturer's guidelines. During this stage of column packing, depleted feed was sampled and exhausted to waste. The packed bed of Express-Ion Q (13 cm×44 cm I.D.) and trapped biomass had a volume of ca. 19.8 l. Loosely associated materials were removed by washing with 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA (70 l). Predominantly electrostatically bound materials were desorbed isocratically using 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA and 0.5 *M* NaCl (116 l). All procedures were carried out in upflow at room temperature (15–20 °C). Flow-rate was maintained at 158 cm/h during the column operations.

Following suspended bed chromatography the IsoPak column was pump un-packed without disassembly.

### 2.6. Assays

Pooled fractions at various stages of chromatography were assayed for total protein content and G3PDH activity according to standard procedures [16,17], turbidity by measurement of absorbance at 650 nm [17], dsDNA content using a standard PicoGreen assay [19] and molecular purity by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) profile [17].

## 3. Results and discussion

### 3.1. Experimental strategies

In previous work [11,12,14] we reported the utility of the IsoPak column system with non-regenerated microgranular Whatman ion-exchange celluloses not only in conventional packed column mode [14], but in an alternative hybrid strategy of batch adsorption–column desorption termed suspended bed chromatography (SBC) [11,12]. A key practical feature of SBC is the adoption of a commercially established IsoPak chromatography column [13] and associated slurry-packing unit. Together these facilitate adsorbent contacting, collection, washing, product desorption and adsorbent recovery/disposal. Equally important is the availability of physically and chemically robust adsorbents such as those represented by the Express-Ion Q and D range studied herein.

In process development, method scouting and scaled-down experiments provide valuable information regarding parameter optimisation without the use of process-scale facilities, equipment and reagent volumes. We have reported the advantages and occasional unpredictabilities of such approaches [20,21], that are pursued herein to ensure the probability of success of SBC when operated at process scale.

### 3.2. Scouting experiments

The reported molecular mass (140,000) and isoelectric point (p*I*≃pH 6.1) for G3PDH [15], together with previous experience with conventional chromatography of the enzyme in buffers at pH 7.5 [16,17], recommended anion-exchange adsorbents as the starting point for an SBC study. Whatman Express-Ion D and Q celluloses, respectively bearing tertiary and quaternary amine chemistries, were demonstrated to both readily adsorb G3PDH from partially purified preparations of enzyme generated by pseudo-affinity chromatography of clarified yeast disruptates [17].

Subsequently, the effectiveness of Express-Ion D and Q in adsorbing G3PDH from a crude feedstock comprising centrifugally *clarified* bakers' yeast disruptate derived from 15% (wet w/v) biomass prepared in 0.01 *M* Tris–HCl buffer was assessed over the pH range pH 6.1 to 8.7. The equilibrium adsorption data are summarised in Table 1. G3PDH binding capacity was shown to increase with values of pH between 7.5 and 8.7 for both adsorbents. This was anticipated given the reported p*I* for G3PDH [15], although it was interesting to note the greatest

Table 1
Impact of pH upon the adsorption of G3PDH from clarified and unclarified bakers' yeast disruptate using Express-Ion D and Q

| pH | G3PDH adsorption (U/ml settled adsorbent) | | | |
|---|---|---|---|---|
| | Express-Ion D | | Express-Ion Q | |
| | Clarified | Unclarified | Clarified | Unclarified |
| 6.1 | 229 | – | 266 | 243 |
| 7.5 | 109 | 371 | 84 | 314 |
| 8.2 | 175 | 10 | 90 | 18 |
| 8.7 | 193 | 107 | 104 | 107 |

Adsorbent (1 g pre-equilibrated) was contacted at room temperature with a common feedstock of 9 ml of clarified or unclarified 15% (wet w/v) yeast disruptate for 3 h, whereupon G3PDH depletion was measured.

adsorption at pH 6.1. This was unanticipated, but may be attributed to the additive contribution of protein–adsorbent and protein–protein interactions at the point of minimal solubility for this large, tetrameric enzyme ($M_r$ 140,000) [15]. We have reported similar unpredictability of adsorption of a monoclonal antibody to the cation-exchange cellulose Express-Ion S with varying pH and buffer composition [21].

The experiment above was repeated for 15% (wet w/v) *unclarified* yeast disruptate, and the data are compared in Table 1. This is more difficult to interpret than that from the clarified feed, but does show significant influences of both pH and the presence of biomass. Based on this limited initial screen, pH 7.5 was selected for the development of an SBC process utilising Express-Ion Q as the adsorbent. Although Express-Ion D offered some capacity advantage, Express-Ion Q was selected on the grounds of continuity with previous work [11,14].

The impact of biomass concentration (3.5 to 15%, wet w/v) upon the direct batch adsorption of G3PDH from unclarified bakers' yeast disruptate at pH 7.5 using Express-Ion Q is summarised in Table 2. The data demonstrate that enzyme adsorption was greatest from 3.5% (wet w/v) biomass following a 40-min contacting period. We attribute this to the combined influences of the level of suspended solids and the low, inherent conductivity of the disruptate. The data also demonstrate incomplete desorption of bound enzyme using 0.5 *M* NaCl in all but the highest biomass challenge (15% wet w/v). The latter may be attributed to weakened binding of the enzyme in the presence of the complex competition offered by the crude feedstream. Incomplete desorption may reflect the mix of electrostatic and hydrophobic interactions which account for adsorption, and may be exacerbated by the concentration of eluent. Optimised desorption conditions were assessed in experiments where common aliquots of Express-Ion Q were challenged in parallel with 3.5% (wet w/v) yeast disruptate and, following washing, variously eluted with concentrations of 0.1 to 1 *M* NaCl in equilibration buffer. The G3PDH recovery data are summarised in Table 3 and indicate acceptable desorption (>75% bound adsorbate) in the concentration range 0.1 to 0.5 *M* NaCl.

### 3.3. Scaled-down suspended bed chromatography

In summary, the scouting studies recommended the use of 3.5% (wet w/v) yeast disruptate in 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA as feedstock, Express-Ion Q as adsorbent and NaCl concentrations ≤0.5 *M* as eluent in further process development. A primary issue was establishing a credible scaled-down contactor that would reproduce

Table 2
Influence of biomass concentration upon the adsorption of G3PDH to Express-Ion Q at pH 7.5

| Biomass (% wet w/v) | Conductivity (mS) | G3PDH total activity (units) | | |
|---|---|---|---|---|
| | | Feedstream | Adsorbed | Desorbed |
| 3.5 | 1.49 | 191 | 101 | 66 |
| 7.5 | 2.3 | 416 | 90 | 45 |
| 15.0 | 3.3 | 760 | 32 | 35 |

Adsorbent (1 g pre-equilibrated) was contacted at room temperature for 40 min with a common feedstock of unclarified yeast disruptate diluted in equilibration buffer to pre-set concentrations from a stock 30% (wet w/v) yeast disruptate. G3PDH activities were determined in samples of the feedstream, adsorbent breakthrough and 0.5 *M* NaCl eluate.

Table 3
Influence of salt concentration upon desorption of G3PDH from Express-Ion Q following loading from bakers' yeast disruptate

| NaCl concentration ($M$) | Desorption efficiency (% of adsorbed G3PDH) |
|---|---|
| 0.1 | 80 |
| 0.2 | 77 |
| 0.375 | 81 |
| 0.5 | 82 |
| 0.75 | 72 |
| 1.0 | 52 |

Discrete aliquots of adsorbent (1 g pre-equilibrated) were contacted with a common feedstock of 9 ml unclarified 3.5% (wet w/v) yeast disruptate for 40 min at room temperature. After washing in equilibration buffer, adsorbents were batch eluted in various salt concentrations in the same buffer. G3PDH recoveries were determined by assay of feedstock and eluates and expressed as a percentage of bound material post-wash.

a similar packed bed height and linear operating velocity to that planned for the process-scale SBC. Based on previous studies [11,14] an ideal packed bed height for Express-Ion Q appeared to be 15–20 cm at both laboratory and process-scales. The IsoPak column unit available for this work had an internal diameter (I.D.) of 44 cm and, when operated in suspended bed mode, the entire adsorbent–disruptate slurry would necessarily be pumped upwards into the chromatography column. This was planned as a single, continuous operation (as recommended for column packing) such that the upper bed support would retain the adsorbent and some biomass, whilst permitting a large proportion of debris to pass to waste in the packing effluent.

In order to achieve a best approximation of a scaled-down IsoPak system, a 1.6 cm I.D. laboratory chromatography column system (from Millipore) was adapted with extension tubes to offer a total height of 208 cm. This could accommodate an initial slurry volume of ~418 ml which, with an experimental volume ratio of 12:1 (disruptate:adsorbent), would yield a packed bed height of about 16 cm for the Express-Ion Q plus retained biomass.

In order to achieve a credible process mimic of the IsoPak system, the disruptate:adsorbent slurry was introduced into the scaled-down laboratory column and consolidated (albeit by downward flow) adopting those recommended slurry-packing procedures which are applicable to axial flow column systems [14,22]. Since the adsorption stage of SBC is a simple batch operation, this was readily scaled down to a stirred vessel loaded with 32 ml settled volume of Express-Ion Q and 384 ml 3.5% (wet w/v) yeast disruptate. Following a 35-min contact time, the entire, well-mixed slurry was poured by single action into the top of the 418-ml column unit and the bed consolidated by pumping the enzyme-depleted feed to waste through the bottom outlet. Fluid flow was maintained throughout the column assembly and the enzyme-depleted feed emerged through the bottom bed support as a coloured, highly turbid suspension indicating that the consolidating packed bed of adsorbent was an inefficient depth filter. Solids retention in the bed will depend upon the volume ratio of the slurry, and the speed of bed packing and consolidation. The final, darkly coloured packed bed supported typical chromatographic flow-rates (100 to 300 cm/h) throughout the wash and elution (achieved with 0.15 $M$ NaCl) stages operated with down-flow in this purification. Protein masses and enzyme activities, estimated by appropriate analyses of disruptate, breakthrough, wash and elution pools, are summarised in Table 4. The overall recovery of G3PDH was low (i.e. 11% of that in the disruptate challenge), but this was also true of the recovery expressed relative to estimates of the enzyme bound after washing. This suggests that the elution conditions would benefit from further study and optimisation. Nevertheless, the scaled-down experiment demonstrated the potential utility of SBC to the extent that a process-scale study of direct G3PDH adsorption from unclarified yeast disruptates was initiated.

### 3.4. Process-scale SBC

Three separate process scale operations with SBC were undertaken. Typically in each run, 275 l of 3.5% (w/v) yeast disruptate were contacted for 45 min in an appropriate stirred tank (450 l) with 18 kg of pre-equilibrated Express-Ion Q at room temperature. The suspension was then pumped through the packing port in the base of the 44 cm I.D. IsoPak column unit such that a packed bed consolidated against the 20 μm mesh of the top bed support. Both adsorbent and enzyme-depleted feed were darkly coloured and the latter highly turbid, as

Table 4
Protein and enzyme concentrations during scaled-down SBC of G3PDH operated with Express-Ion Q

| Process stage | Volume (ml) | Total protein (mg) | | G3PDH activity (total units) | |
|---|---|---|---|---|---|
| | | Mobile phase | Retained[a] on Express-Ion Q | Mobile phase | Retained[a] on Express-Ion Q |
| Yeast disruptate | 384 | 523 | – | 8640 | – |
| Non-bound material | 384 | 315 | 208 | 1771 | 6869 |
| Wash | 150 | 24 | 184 | 226 | 6643 |
| Elution | 200 | 140 | 44 | 955 | 5688 |

The scaled-down SBC was packed with 418 ml of 12:1 (v/v) slurry of 3.5% (wet w/v) yeast disruptate and pre-equilibrated Express-Ion Q previously incubated for 35 min at room temperature. A packed bed was consolidated, washed with equilibration buffer and eluted in 0.15 *M* NaCl. Total protein and G3PDH content were determined at various stages of the process.

[a] Retained material bound to the adsorbent was estimated from difference analyses after closing the mass and activity balances for the full process.

per our previous observations in the scale-down experiments. The total volume of adsorbent could be readily accommodated in the chromatography column to yield a packed bed volume estimated at 19.8 l and a bed height of about 13 cm. The bed was washed with 3.5 bed volumes of equilibration buffer, before being eluted with a step increase of ionic strength introduced as a total of six bed volumes of 0.5 *M* NaCl in equilibration buffer.

When assessing the effectiveness of SBC it was essential to consider parameters other than the bioactivity conventionally associated with protein purification. These included the clearance of fine debris and nucleic acid in a hybrid operation potentially achieving solids removal, product concentration and purification in a single step. Estimates of protein masses, enzyme activities, together with turbidities and dsDNA content of disruptate, breakthrough, wash and elution pools are thus summarised for a typical SBC process in Table 5 and the corresponding elution profile is presented in Fig. 1. The data demonstrate an estimated 33% recovery of G3PDH activity from the feed together with 42% of the total protein. The recovery of enzyme activity in the 0.5 *M* NaCl eluate, relative to that remaining bound after the wash, was 59% (w/w) which represents an improvement on the performance when using 0.15 *M* NaCl in the scaled down experiments (refer to Table 4) and closer to the batch performance in the scouting experiments (Table 2).

The data in Table 5 represent a modest performance, however 79% (w/w) of the purified

Table 5
Protein and enzyme activity balances for SBC conducted for the process-scale capture of G3PDH operated with Express-Ion Q

| Process stage[a] | Volume (l) | Total protein (g) | | G3PDH activity (units$\times 10^{-3}$) | | Turbidity ($A_{650}$) | Pico-Green (mg dsDNA equivalents) |
|---|---|---|---|---|---|---|---|
| | | Mobile phase | Retained on Express-Ion Q[b] | Mobile phase | Retained on Express-Ion Q[b] | | |
| Yeast disruptate | 275 | 430 | – | 4812 | – | 0.51 | 4785 |
| Non-bound material | 275 | 209 | – | 2261 | – | 0.42 | 3245 |
| Wash | 70 | 19 | 202 | 442 | 2109 | 0.10 | 224 |
| Elution | 116 | 182 | 20 | 1582 | 527 | 0.02 | 2756 |
| G3PDH peak | 8 | 156 | 46 | 1244 | 865 | 0.01 | 62 |

[a] The process-scale SBC was packed with ~300 l of 12:1 slurry of 3.5% (wet w/v) yeast disruptate and pre-equilibrated Express-Ion Q previously incubated for 45 min at room temperature. A packed bed (19.8 l) was consolidated, washed with equilibration buffer and eluted in 0.5 *M* NaCl. Total protein content, G3PDH activity, turbidity and Pico-Green reactivity were determined at various stages of the chromatography.

[b] Retained material bound to the adsorbent was estimated from difference analyses after closing the mass and activity balances for the full process.

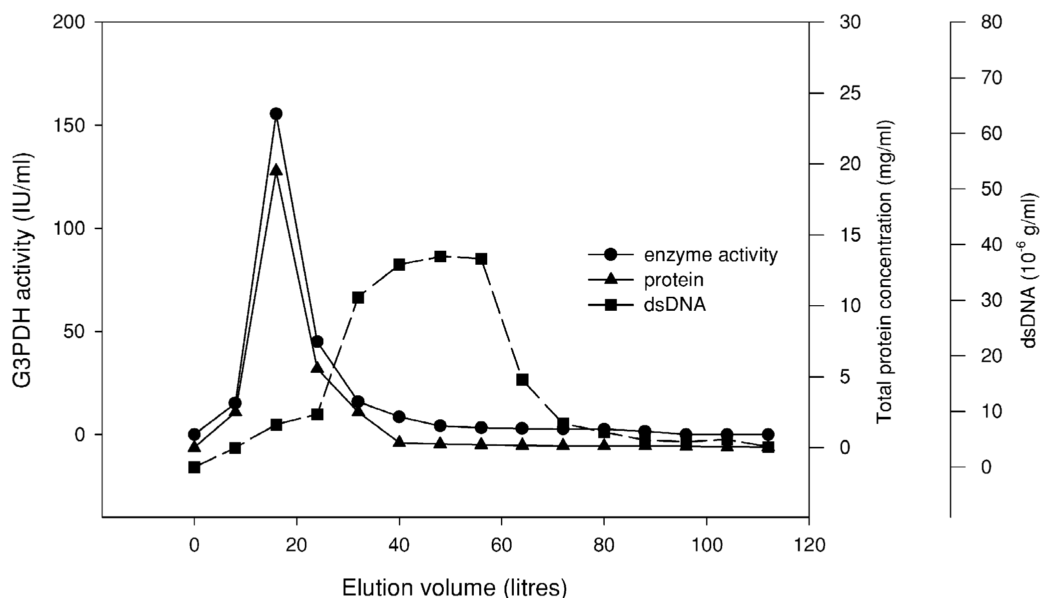


Fig. 1. Elution profile of process-scale SBC conducted for the recovery of G3PDH from crude yeast disruptates. Express-Ion Q was loaded with G3PDH by SBC using an IsoPak column (13 cm×44 cm I.D.) as described in Section 2.5 and the footnote to Table 5. Desorption was achieved in six bed volumes of 0.5 *M* NaCl in 0.01 *M* Tris–HCl buffer (pH 7.5) containing 0.001 *M* EDTA. Total protein content, G3PDH activity and Pico-Green reactivity (correlated with dsDNA content) was determined in eluate samples following suspended bed adsorption.

G3PDH was recovered in an 8-l elution pool volume (refer to Fig. 1) equivalent to a 97% (v/v) reduction in process volume. The turbidity of the eluted peak was reduced by 98% (w/w) compared to the feedstream whilst the Pico-Green reactivity (attributed principally to dsDNA) reduced by 99% (w/w) relative to the disruptate. These are all key purification benefits and parameters not normally recorded for conventional single-step ion-exchange chromatography, since they are generally reduced by additional upstream procedures (e.g. centrifugation, microfiltration, precipitation or alternative adsorption procedures). When assessing SBC or equivalent direct adsorption methodologies such as fluidised bed adsorption, these additional parameters should be included as part of the cost–benefit analysis. The eluted peak of G3PDH was only partially purified as illustrated by the SDS–PAGE analysis in Fig. 2. However, by virtue of the clarification and concentration achieved by SBC, the pool could be further purified by subsequent steps of gradient ion-exchange or dye ligand chromatography as previously reported [15–18].

Following a full cycle of SBC, the used Express-Ion Q was pumped out of the column. The adsorbent was visibly discoloured and an overnight treatment with 0.5 *M* NaOH failed to fully displace the foulants and so facilitate conventional regeneration processes. While a more stringent clean-in-place (CIP) regime could perhaps be developed, the simplicity of SBC and its inherent circumvention of costly clarification procedures, recommends the adoption of a single-use/disposal strategy as the preferred and most cost-effective process option.

## 4. Conclusions

In this initial feasibility study, we have demonstrated the practicality of exploiting a commercial IsoPak Process Chromatography Unit and the ion-exchange cellulose Express-Ion Q for the direct adsorption and partial purification of an intracellular enzyme (G3PDH) from crude yeast cell disruptates in a novel hybrid operation, SBC. A detailed comparative study of SBC and fluidised/expanded bed procedures, sequential operation of centrifugal or membrane clarification, and packed bed chromatog-

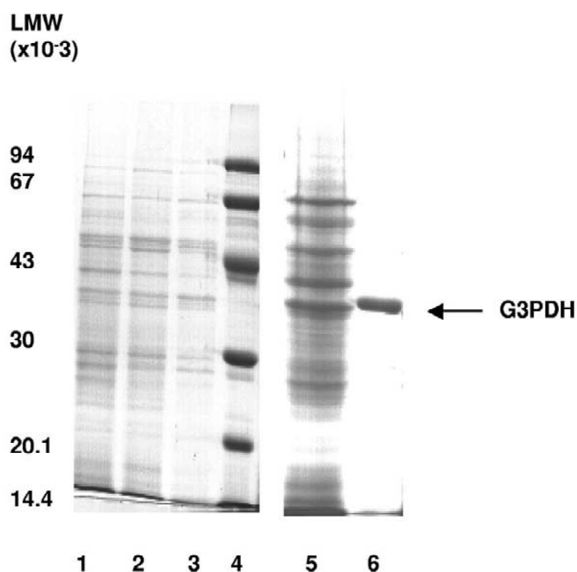


Fig. 2. SDS–PAGE analyses of various fractions taken from the process scale SBC capture of G3PDH from unclarified yeast disruptate. The SBC process was operated as described in Section 2.5 and recorded in the legends to Table 5 and Fig. 1. SDS–PAGE analysis was conducted according to referenced methods [17]. Lanes: 1, the crude disruptate (~15 μg total protein); 2, pooled breakthrough (~9 μg total protein); 3, pooled washings (~3 μg total protein); 4, low $M_r$ markers from Sigma (~14 μg total protein); 5, 8 l elution peak (~78 μg total protein) and 6, standard G3PDH from Sigma (~5 μg total protein). Low $M_r$ standard markers were characterised by sub-unit masses in SDS–PAGE as follows: phosphorylase B (94,000), bovine serum albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), soy bean trypsin inhibitor (20,000) and lactalbumin (14,000). Standard G3PDH had a native molecular mass of 140,000, and a subunit mass in SDS–PAGE of 35,000.

raphy would provide an assessment of the relative merits of each strategy. However, the preliminary studies reported herein indicate that SBC may prove a less technically challenging alternative to such operations. Circumstances where bioproduct recovery is required from particulate feedstocks having modest levels of solids (<5% wet w/v biomass), which may arise in animal cell culture, coarsely clarified microbial broths or disruptates, or crude biological extracts, would appear to be ideally suited to SBC processing. The modest capture and recovery performances recorded in this preliminary exploratory study could readily be improved by decreasing the feedstock:adsorbent ratio (which should concomitantly increase the biomass tolerance) and fine-tuning the conditions of pH and ionic strength at adsorption, washing and desorption. Many important questions regarding the complex physical and biochemical impact of trapped biomass in the chromatographic process must be answered if SBC operations are to be confidently designed, operated, validated and more widely adopted. However, we conclude that SBC offers an additional technique for the bioprocess engineer, and should be seriously considered as an option when pursuing process intensification and integration.

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