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MATHEMATICAL MODELLING OF THE CONTINUOUS AFFINITY-RECYCLE EXTRACTION PURIFICATION TECHNIQUE

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SUMMARY

Continuous affinity-recycle extraction (CARE), a continuous protein purification unit operation, has been designed to address design and optimization criteria relevant for process scale chromatographic separation of proteins. The development and application of a mathematical model describing purification in the CARE process are described. The model incorporates adsorption–desorption kinetics into material balance equations describing the operation of two well-mixed reactors operating with recycle. An accurate mathematical model of CARE has aided in its development as a new unit operation for protein purification, in the assessment of its performance tradeoffs, and in its optimization.

INTRODUCTION

As the biotechnology industry undergoes a transition from research to product commercialization, cost reductions in process development and large-scale protein purification are emerging as key determinants to commercial success. Techniques used today for purification are mainly chromatographic in nature and employ equipment and material derived directly from the laboratory/bench scale. With these roots, it is common to find process chromatograms and adsorbents being evaluated based on resolution alone, with little regard to recovery or throughput. Process-scale chromatographic purification of proteins requires a different set of design and optimization criteria than those used for laboratory/research work. For example, final purity is a constraint and not an objective. The ultimate objective is minimum cost of a purified product that meet specifications which, in turn, implies maximal recovery and throughput. A different approach to the selection and design of unit operations for manufacturing, is to first consider the entire process at the largest scale, and then scale-down to an intermediate scale which can simulate, with confidence, the larger scales.

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Due to the similarities in the physico-chemical properties of proteins found in typical fermentation or cell culture broth, very high levels of purity (required for most current commercial applications) can only be achieved by using a series of steps, each incrementally purifying the product via different separation mechanisms. This entire sequence of steps is often termed Downstream processing (DSP). DSP of a crude fermentation broth typically produces the final product with a very high purity but a correspondingly low recovery yield. In general, an average of 10–20% product loss per separation step is encountered; hence, the final recovery of a process with six DSP steps can be as low as 30%. This places a great impetus on integration of DSP steps in order to achieve the same purification with much higher overall recovery.

Protein purification is most often effected by chromatographic techniques. Adsorptive chromatography, which includes ion-exchange, affinity, reversed-phase and hydrophobic interaction chromatography, accounts for a large portion of the preparative chromatography applications. Traditionally, adsorptive chromatography is carried out using a fixed bed of adsorbent particles (*i.e.* column chromatography). While for small molecules, the importance of column length (*i.e.* number of theoretical plates) on resolution is well characterized, for macromolecules experimental evidence suggests a far lesser need for a large number of plates. Early reports of this observation showed that in surface mediated separations, columns of less than 5 cm long have 80% of the resolving power of 30-cm columns[1,2]. Among the adsorptive techniques, affinity chromatography, which uses biospecific interactions to purify the desired protein from a mixture, has been termed an "on–off" process[3], and is little more than solid–liquid extraction, a common unit operation in the chemical process industries. As such, a fixed bed is but one of alternative contactors which have been employed in other applications, such as: moving beds, simulated moving beds, counter current stirred contractors, etc.

An alternative to fixed bed affinity chromatography was recently proposed as a means of overcoming some of its operational limitations[4]. Continuous affinity-recycle extraction (CARE) was shown to allow continuous separation of an intracellular protein from a crude cell lysate following cell disruption without pre-clarification steps; the approach uses conventional chromatographic media. A schematic of the CARE system is shown in Fig. 1. CARE operates as follows. The sample is fed continuously to the adsorption stage where it contacts the adsorbent beads containing the affinity ligand. The desired product adsorbs while contaminants are washed out with wash buffer. The beads, with the adsorbed product, are then pumped to the desorbing stage where the addition of the desorbing buffer causes the detachment of the product from the affinity matrix. The bare beads are then recycled to the adsorption stage, while the product is removed with the desorbing buffer stream. Both vessels are well agitated; the sorbent is retained within the two vessels and the recycle loop by macroporous filters. The system can be operated continuously at steady state.

Initial experiments, where the enzyme β-galactosidase was recovered from a turbid liquor of lysed cells with no clarification (*i.e.* no debris removal), confirmed the technical feasibility of CARE. From an initial purity of 0.5%, a continuous product stream of 14% pure β-galactosidase was produced with 70% recovery[4]. An important advantage of CARE over conventional approaches is the early introduction of an affinity-based technique in a DSP train, and the omission of several steps which

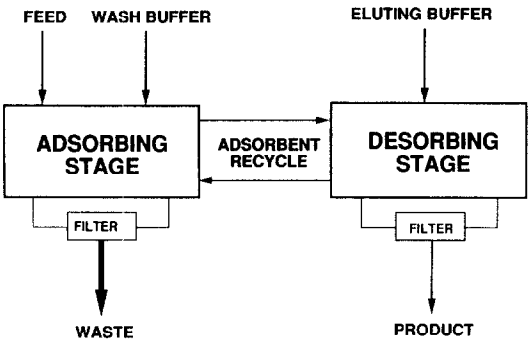

Fig. 1. Schematic of the CARE process.

would otherwise be required prior to the use of a fixed bed. This, in turn, can translate into higher overall product recoveries and lower cost of purification.

In addition to its performance advantages, the CARE technique is readily characterized mathematically. An accurate mathematical model of CARE has aided in its development as a new unit operation for protein purification, in the implementation of computer control for its continuous operation, and in its optimization. This paper describes the mathematical analysis of CARE, and various uses of the model.

MODEL FORMULATION

The strategy to model the CARE system is to mathematically describe the adsorption and desorption processes simultaneously with a material balance. The sorption rate parameters are estimated in batch experiments independent of purification in the CARE system. The methodology used to derive the rate parameters is described below as Microscopic formulation of the model. Descriptions of the sorption processes are then incorporated into a set of material balance equations describing the operation of two well-mixed vessels operating with recycle. This later section is described as a Macroscopic formulation. Purification performance then is predicted, by specifying flow-rate and feed-stream composition data.

Microscopic formulation

The literature is replete with mathematical models describing adsorption of solutes to porous, solid-phase supports[5–11]. A mathematical description must combine equations for the various mass transfer steps (film diffusion, internal pore diffusion) as well as the biochemical adsorption step. In general, one wishes to solve the equations for the decrease in solute concentration in the bulk solution, surrounding the porous support material, as a function of time.

The mathematical formulation employed here is a simple, lumped-parameter model[7]. This model does not explicitly distinguish between mass transport and intrinsic biochemical binding kinetics. However, as shown in this paper, this model describes the experimental system well.

Generalized adsorption model. This model is based on the isothermal sorption of a single solute onto porous particles, suspended in a well-mixed vessel. The bulk liquid

has a solute concentration, $c(t)$. The particles are spherical, with radius, R. The total volume is v, with liquid volume αv and adsorbent volume $(1 - \alpha)v$. The sorbate concentration in the particle is $q_i(r,t)$, where r is the radial position within the particle, and the solute concentration within the pore liquid is $c_i(r,t)$.

The mass balance for the adsorber is

$$\alpha\frac{dc}{dt} + (1-\alpha)\frac{ds}{dt} = 0 \tag{1}$$

where s is the average solute concentration in the particle, which includes solute adsorbed to ligands at the pore surface as well as solute within the pore liquid. The two terms in eqn. 1 account for depletion of solute from the bulk liquid and solute uptake within the particles.

The rate of solute uptake within the particle is equated to the flux of solute into the pores, which is driven by a diffusive process described by Fick's Law:

$$\frac{ds}{dt} = 3\left(\frac{N_0}{R}\right) = 3\left(\frac{D_i}{R}\right)\left(\frac{dc_i}{dt}\right)_{r=R} \tag{2}$$

where D_i is the effective particle diffusion coefficient, and the quantity $3/R$ is the surface area per unit volume of particles.

The particle mass balance relates the solute diffusing into the pore with sorbate adsorbing at the pore surface.

$$D_i\left(\frac{d^2c_i}{dr^2}\right) + \frac{2}{r}\left(\frac{dc_i}{dr}\right) - \beta\left(\frac{dc_i}{dt}\right) - (1-\alpha)\frac{dq_i}{dt} = 0 \tag{3}$$

The four terms in eqn. 3 represent the flux of solute into the pores, the depletion of solute in the pore liquid, and the adsorption of sorbate onto the pore surface, respectively.

The concentration of solute in the particle pores and in the bulk liquid is:

$$k(c - c_i)_{r=R} = D_i\left(\frac{dc_i}{dr}\right)_{r=R} \tag{4}$$

Finally, the rate of binding for affinity adsorption is commonly described by the following equation:

$$\frac{dq}{dt} = k_f(Q_{max} - q)c - k_r q \tag{5}$$

Adsorption is second order in the forward direction and first order in the reverse direction. This rate equation corresponds to a Langmuir isotherm at equilibrium.

$$q_0 = \frac{Q_{max}Kc}{(1 + Kc)} \tag{6}$$

In general, one wishes to solve for the decrease in solute concentration in the bulk solution as a function of time $c(t)$. An analytical solution to the equations developed above does not exist, hence one must resort to numerical techniques. Alternatively, one can lump all resistances to adsorption into a single parameter yielding a simplified and analytically solvable equation set.

Simplified lumped parameter adsorption model. The equations describing adsorption to porous solid phase supports, shown above, distinguish among the various resistances to adsorption. These resistances are: solute diffusion through a thin stagnant film surrounding the adsorbent particles, diffusion within the pores of the solid support, and the biochemical adsorption step itself.

These three resistances have been combined into the biochemical adsorption forward rate constant (k_f) with eqn. 5 representing the adsorption process. The solution for the bulk liquid concentration as a function of time is:

$$c(t) = \frac{[2c_0(N-b) + N^2 - b^2]D + 2c_0(b+N) + b^2 - N^2}{[4c_0 + 2b + 2N]D - 4c_0 - 2b + 2N} \tag{7}$$

where $b = Q_{max}(1-\alpha) + 1/K - c_0$; $N = \sqrt{(b^2 + 4c_0/K)}$; and $D = \exp(Nk_f t)$.

This form of the solution to the adsorption equations was chosen for incorporation into the CARE model, because of its simplicity, both in number of required input parameters, and in its incorporation into a material-balance description of the CARE process. This solution requires the input of three adsorption parameters: two equilibrium and one kinetic.

Batch adsorption experiments were conducted with varying initial β-galactosidase concentration; bulk-liquid enzyme concentration (c) was measured as a function of time[12]. Rather than using equilibrium adsorption experiments to independently estimate the equilibrium adsorption parameters, batch adsorption rate data were fitted to eqn. 7 through non-linear regression yielding estimates for all three adsorption parameters. The estimated forward reaction rate constant (k_f) is not necessarily the true, intrinsic reaction rate constant; it is a parameter in which all resistances to adsorption, mass transfer and biochemical binding, have been incorporated. Similarly, the two estimated equilibrium parameters do not necessarily correctly predict the equilibrium adsorption isotherm, yet when used in conjunction with the rate constant yield good model agreement with experimental data.

The fit of the lumped parameter model to experimentally determined adsorption profiles is shown in Fig. 2. Good model agreement is obtained for both low (200 U/ml gel) and high (7000 U/ml gel) adsorbent loading. Although the three adsorption parameters result from an empirical fit to the data, this simple model predicts experimental adsorption data over a wide rage of adsorbent loading conditions.

Investigation of adsorption mechanism. Although, the adsorption of β-galactosidase to *p*-aminobenzyl-1-thio-β-D-galactopyranoside (PABTG)-Agarose has been successfully described using a lumped parameter approximation, this approach does not shed light on the mechanism of adsorption. One would anticipate that the rate of internal pore diffusion would control adsorption[5,13-15] since the affinity adsorbent is porous and fairly large (100 μm diameter). In addition, β-galactosidase is a large protein (mol.wt. *ca.* 460 000)[7]; its diffusion coefficient in bulk solution is small ($3 \cdot 10^{-8}$ cm^2/s)[16], and one would anticipate the effective diffusivity inside the pores to

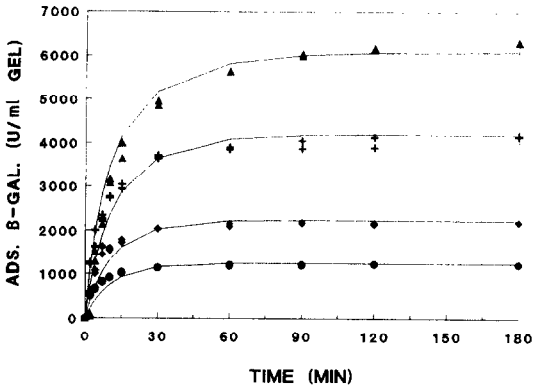

Fig. 2. Batch adsorption of β-galactosidase to PABTG-Agarose. Fit of lumped parameter adsorption model. Adsorption parameters: Q_{max} = 7100 U/ml; K = 0.57 ml/U; k_f = 0.0013 ml/U/min. Experiments performed in a batch vessel containing a total of 50 ml liquid volume; 0.5 ml adsorbent gel contacted with varying initial β-galactosidase concentrations ranging from 10 to 100 U/ml; samples were withdrawn periodically and the decrease in bulk β-galactosidase activity over time was determined. Initial β-galactosidase concentrations are: 13 U/ml: ●; 24 U/ml: ◆; 46 U/ml: +; 75 U/ml: ▲.

be even lower due to hindered diffusion. Finally, most affinity interactions are inherently fast, *e.g.* relative to internal pore diffusion, and this is expected to be the case for the β-galactosidase affinity system employed here.

It was postulated previously that β-galactosidase, does not fully enter into the pores of the affinity support during adsorption[17] and adsorbs at the surface and entrance region to the pores, thus blocking further entry of molecules. As a consequence, it was felt that internal pore diffusion did not play a major role in determining adsorption rates since β-galactosidase was not penetrating into the pore.

In an attempt to verify this hypothesis, an experiment was performed where β-galactosidase was covalently immobilized, via cyanogen bromide activation[18] to Sepharose 4B. In this manner, the β-galactosidase molecule was immobilized in a position that could potentially block pore access as was believed to occur during adsorption of β-galactosidase to PABTG-Agarose. It was anticipated that the accessible volume fraction and possibly the effective diffusivity would decrease relative to unsubstituted Sepharose 4B.

A known volume of adsorbent gel was introduced into a solution of β-galactosidase of concentration c_0. At periodic intervals, samples were withdrawn and the β-galactosidase was determined by measurement of enzymatic activity (Fig. 3). β-Galactosidase concentration in the bulk fluid, from which it was sampled, decreased rapidly and then leveled off once the enzyme diffused into the interior of the adsorbent gel. The volume fraction of the gel accessible to β-galactosidase was calculated using eqn. 8.

$$\beta = \alpha\left(\frac{c_0 - c_f}{c_f}\right) \tag{8}$$

where $\alpha = (V_{bulk}/V_{gel})$.

As shown in Fig. 3, unsubstituted Sepharose, cyanogen bromide activated and blocked Sepharose (using ethanolamine) and Sepharose to which β-galactosidase had

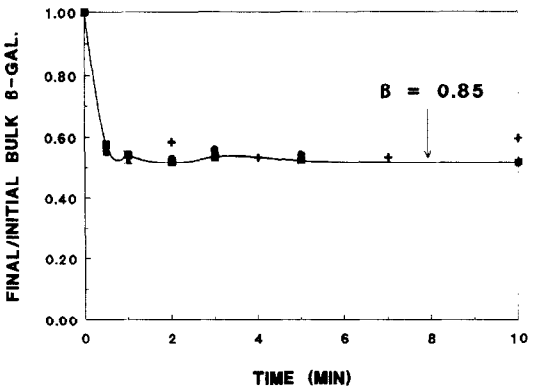

Fig. 3. Estimation of internal pore accessibility. Diffusion of β-galactosidase in Sepharose 4B. Base: + and ▲; CNBr activated: ●; immobilized β-galactosidase: ■. Experiments performed in 50-ml batch vessel; 50 ml of a 50% Sepharose gel suspension was contacted with an initial β-galactosidase concentration of 75 U/ml; samples were withdrawn periodically and the decrease in bulk β-galactosidase activity over time was determined.

been attached all behaved the same. The estimated value of β was 0.85, indicating, that β-galactosidase has access to the bulk of Sepharose 4B's internal volume. In summary, β-galactosidase has access to the interior of the adsorbent particle, transport to the interior is governed by a diffusive process, characterized by a small diffusion coefficient, the adsorbent particle itself, is large, and most enzyme–inhibitor interactions are inherently fast. Thus it is likely that internal pore diffusion limits the overall adsorption rate.

Finally, since adsorption is conducted in a well-mixed vessel, the boundary layer thickness, and hence external film diffusion resistance, should be minimal. In an attempt to validate this assumption, batch adsorption experiments[4], at varying agitation rates, were conducted in one of the CARE reactors. The results for β-galactosidase adsorption are shown in Fig. 4. There were no significant differences in the adsorption profiles, suggesting external film diffusion is fast relative to internal pore diffusion.

Desorption process. Desorption, of β-galactosidase, is accomplished by the introduction of borate ions, and is not associated with the pH change from 7 to 9. It has been shown that borate is a specific eluent for β-galactosidase, and since the ion concentration is orders of magnitude greater than the enzyme's (at pH 9), desorption from the ligand is not an equilibrium process[19]; rather desorption goes to completion. It is assumed that desorption is diffusion controlled in a similar manner to adsorption.

Given these assumptions, desorption of β-galactosidase from the affinity support is a much faster process than adsorption. During adsorption, the driving force, which is the difference between the bulk concentration and the pore liquid concentration in equilibrium with adsorbed enzyme, is typically low; on the order of 10 U/ml for the rate experiments. As enzyme adsorbs to the affinity ligand, the bulk enzyme concentration decreases and the equilibrium pore liquid concentration increases. As a result, the driving force for adsorption decreases and remains small over the entire time course of adsorption. In contrast, during desorption, the initial driving force is proportional to the adsorbed enzyme concentration which is typically

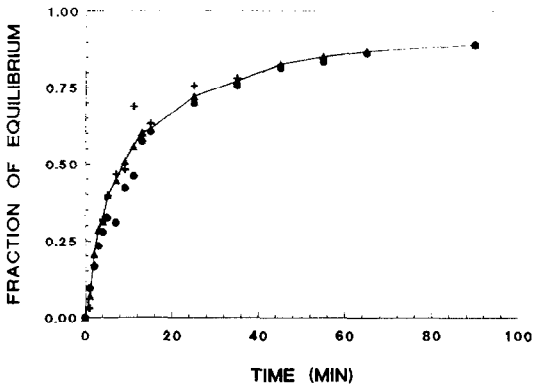

Fig. 4. Evaluation of external mass transfer. Adsorption at varying agitation rate. Experiments performed in a batch vessel containing a total of 50 ml liquid volume; 0.5 ml adsorbent gel contacted with an initial β-galactosidase concentrations of 20 U/ml; the vessels were agitated at varying rate (rpm) in a temperature controlled (25°C) shaker bath; samples were withdrawn periodically and the decrease in bulk β-galactosidase activity over time was determined. ●, 150; ▲, 250; +, 280 rpm.

on the order of 1000 U/ml. Both experiment and theory confirm that desorption is complete within one min[19]. Thus, desorption is described as taking place both instantaneously and completely.

Macroscopic formulation

The equations describing sorption kinetics, developed above, form the basis of a mathematical model of the CARE process. Sorption kinetics are incorporated into a set of material balance equations, describing the conservation of total mass within the process as described below. The model, although developed for the β-galactosidase affinity purification system, is generalizable to any system where sorption kinetics can be mathematically described, non-specific adsorption is minimal and enzyme activity is maintained throughout the time course of the separation.

Adsorption kinetics (at pH 7) can be modeled by eqn. 5, while desorption (at pH 9) is assumed nearly instantaneous and complete based on evidence described above. The macroporous filters, used to retain the adsorbent, offer little resistance to flow[19], thus the two stages are modelled as continuous, well-mixed vessels. The model treats

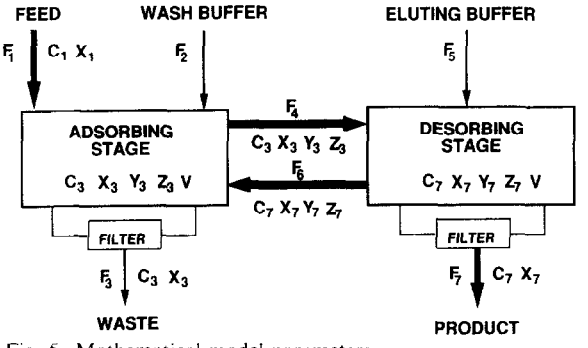

Fig. 5. Mathematical model parameters.

the feed material as a protein mixture and ignores non-proteinaceous contaminants.

The CARE model was developed with the parameters shown in Fig. 5. There are seven flow-rates (F_1–F_7), three free (unbound) enzyme concentrations (X_1, X_3, X_7), two bound enzyme levels (Z_3, Z_7) and three contaminant concentrations (C_1, C_3, C_7). This paper considers the case where the waste steam flow-rate (F_3) equals the sum of the feed (F_1) and wash (F_2) flow-rates; similarly, the eluting buffer flow-rate (F_5) is set equal to the product stream flow-rate (F_7), while the adsorbent recycle flow-rates (F_4 and F_6) are kept equal. Finally the adsorbent concentration in each vessel (Y_3 and Y_7) are kept constant and equal.

With these specifications, a steady state solution describing the CARE system can be derived. The material balances for the free enzyme (2 equations), the bound enzyme (2 equations) and the contaminant protein (2 equations) are coupled to eqn. 5 which describes the rate of product adsorption in the first stage.

Material balances for total enzyme (free and bound) are:

$$F_1X_1 = F_3X_3 + F_7X_7 \tag{9}$$

$$F_1X_1 + F_6X_7 = (F_3 + F_4)X_3 + \left(\frac{V}{V_e}\right)F_4Z_3Y_3 \tag{10}$$

Accumulation of bound enzyme in the adsorption reactor is given by:

$$\frac{dZ_3}{dt}VY_3 = \left(\frac{V}{V_e}\right)F_4Z_3Y_3 \tag{11}$$

Adsorption kinetics (eqn. 5) are incorporated into eqn. 11

$$\left[k_fX_3(Q_{max} - Z_3) - \left(\frac{k_f}{K}\right)Z_3\right]VY_3 = \left(\frac{V}{V_e}\right)F_4Z_3Y_3 \tag{12}$$

Material balances for contaminants are:

$$F_1C_1 + F_6C_7 = (F_3 + F_4)C_3 \tag{13}$$

$$F_4C_3 = (F_6 + F_7)C_7 \tag{14}$$

The set of equations described above suffice to completely specify CARE operation. The equations can be solved either explicitly or iteratively depending on how the problem is defined. There are four sets of parameters and variables that must be specified or predicted by the model. They are: F_1, C_1, X_1, V, (V/V_e); the adsorption parameters, k_f, K, Q_{max} (determined from independent batch adsorption experiments); operating variables (or controllable variables) F_2, F_4, F_5, Y; and, the performance variables, purification factor (PF), recovery yield (REC) and concentration factor (CF). The system's performance variables are defined as:

$$PF = \left(\frac{X_7}{X_1}\right)\left(\frac{C_7}{C_1}\right)^{-1} \tag{15}$$

$$\text{REC} = \left(\frac{X_7 F_7}{X_1 F_1}\right) \tag{16}$$

$$\text{CF} = \left(\frac{X_7}{X_1}\right) \tag{17}$$

The steady-state solution described above is useful in the design and optimization of CARE. In order to model the start-up period and predict system dynamics (*e.g.* for feedback control), the same equation set can be solved numerically (4th order Runge-Kutta method) for unsteady state operation.

MODEL USES

The purpose of the mathematical description of CARE, described above, is to help elucidate relations between system performance and the operating and design variables. By investigating these relations and the tradeoffs among the performance variables, one can gain the insight necessary to incorporate CARE into a protein recovery sequence. In this section three model applications are described: *e.g.* for design, parametric sensitivity, and optimization.

Design

The model may be used to design and specify the operating variables of CARE to achieve a desired performance. For given feed conditions (enzyme level, contaminant concentration and flow-rate), a desired level of final purity, recovery and concentration can be achieved by proper selection of flow-rates and amount of adsorbent. For example, the CARE model was solved for a base case with the performance measures specified as: PF = 30, CF = 5, REC = 90% and feed conditions being: F_1 = 1 ml/min, X_1 = 100 U/ml (*ca.* 0.2 mg β-galactosidase) and C_1 = 10 mg/ml. An iterative solution of the equation set yields a set of operating conditions to achieve the specified performance (Fig. 6).

Surprisingly, the model predicts that for a specified level of adsorbent, above a minimum value, there can be two sets of wash (F_2) and bead recycle (F_4) flow-rates which satisfy the performance constraints. For the base case in this example, CARE, operated with 12.5 ml of affinity adsorbent beads in the adsorption stage, and F_4 = 0.1 ml/min and F_2 = 1.2 ml/min, is a unique solution. If one uses more beads, an additional degree of freedom is gained so that according to the model, a combination of high wash and bead recirculation flow-rates can give identical performance to a case with low flow-rates.

In order to maintain constant system performance, with the addition of more adsorbent, two approaches can be used. If the adsorbent recycle flow-rate is decreased, the amount of regenerated gel being returned to the adsorption reactor, per unit time, decreases and thus the amount of β-galactosidase recovered from the feed would decrease. At the same time, the residence time of the adsorbent in the adsorption reactor increases, and thus, specific adsorbent loading increases. In this manner, the recovery yield can be matched to what it was before the increase in the amount of adsorbent. Since the recycle flow-rate is lower, the wash flow-rate must be lowered in order to keep the same purification factor.

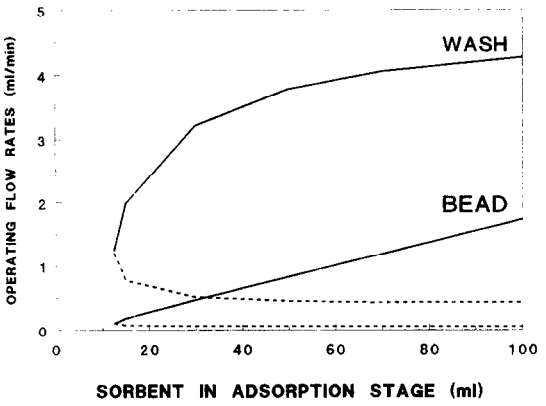

Fig. 6. Operating conditions which satisfy a specified performance. (———) Low loading, dynamic. (– – –) high loading, rear equilibrium.

A second mode of operation is the reverse approach of the first. Both the adsorbent recycle and wash flow-rates are increased. The higher wash flow-rate dilutes the β-galactosidase concentration in the adsorption reactor, hence lowering the driving force for adsorption. Since the adsorption rate decreases, the adsorbent recycle rate is increased to maintain the same level of recovery from the incoming feed. The low flow-rate case allows nearly maximal (equilibrium) loading of the sorbent, whereas the high flow-rate case involves very low (dynamic operation) sorbent loading. Realistically, the high flow-rate case underutilizes the media, uses excess wash buffer and requires high bead recycle rates which may subject the sorbent to excessive mechanical action.

The minimum level of sorbent required to achieve a given performance is a useful design criterion in comparing deviations from the base case. Fig. 7 shows the effect of varying the concentration, purification and recovery, on the minimum level of sorbent required. Typically, the cost of sorbent is an important factor in the economics of an affinity purification step. Hence, the increase in the "price" paid to achieve a higher performance in each case, is to be expected.

Sensitivity

The CARE model may also be used to evaluate a fixed design, and assess the effect of changes in operating variables on system performance. This sensitivity analysis provides further insight into the tradeoffs inherent in the CARE system.

Fig. 8 shows the sensitivity of recovery yield, to changes in three operating variables: wash, recycle and eluting buffer flow-rates. Similar profiles can be generated for purification and concentration factors and serve to illustrate the complex set of tradeoffs among the performance variables.

An alternative approach to sensitivity analysis involves changing only one of the operating-variable flow-rates and following the sensitivity to all three performance criteria simultaneously; Figs. 9–11 show the results of this analysis. In Fig. 9, the arrow points in the direction of increasing bead recirculation rate. When all other variables are kept constant, this change is seen to give rise to an increase in concentration factor, a corresponding linear increase in recovery, but a decrease in purification factor.

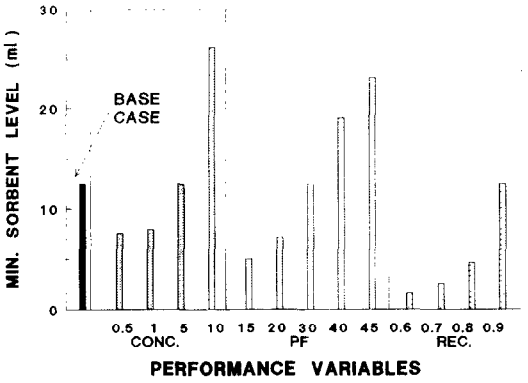

Fig. 7. Minimal amount adsorbent required.

A similar analysis is shown in Fig. 10 where the arrows points in the direction of decreasing elution buffer flow-rate, which in turn causes an increase in concentration factor but little change in recovery or purification factor. Finally, Fig. 11 shows the sensitivity to decreasing wash flow-rate which causes a sharp decrease in purification factor, an increase in recovery along with a small increase of concentration factor.

The sensitivity approach in using the CARE mathematical model results in the following generalized rules where the feed composition and flow-rate are held constant: to increase the purification factor, one must increase the ratio of adsorption reactor throughput relative to bead recirculation rate (*e.g.* increasing the wash flow-rate and/or decrease the bead recirculation rate). Concentration of the product can be achieved by decreasing the ratio of desorbing buffer flow-rate relative to the feed flow-rate. Finally, recovery is increased most effectively by decreasing the wash flow-rate and/or increasing bead recirculation. The existence of tradeoffs in performance suggest an opportunity for system optimization, once suitable objective functions are determined.

Optimization

A predictive mathematical model of the CARE process provides the opportunity

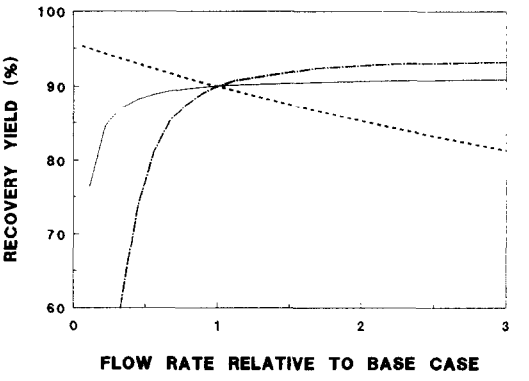

Fig. 8. Sensitivity of recovery yield to operating variables. ——, Borate, – –, wash; - - - -, bead.

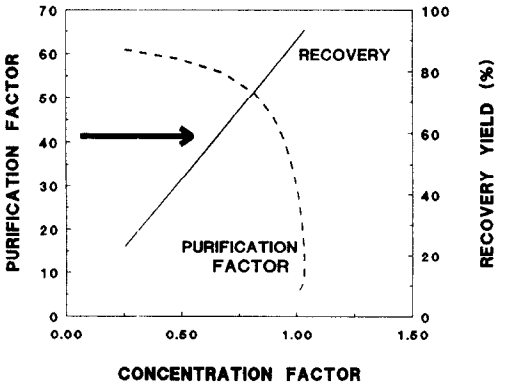

Fig. 9. Sensitivity of system performance to increasing bead recirculation rate.

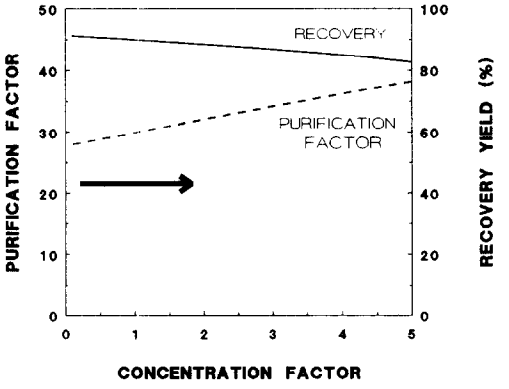

Fig. 10. Sensitivity of system performance to decreasing elution buffer flow.

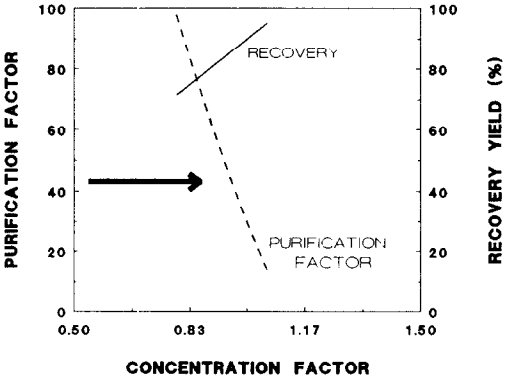

Fig. 11. Sensitivity of system performance to decreasing wash buffer flow.

for optimization. Optimization requires definition of an objective function. The optimum performance of CARE operating as a single step, is described here. In a broader sense, an entire DSP sequence, in which CARE has been incorporated, can be optimized to minimize cost for a fixed amount of product. Such work is in progress and will be the subject of subsequent publications.

Given the large number of "degrees of freedom" (three) in the CARE system as well as the many ways of measuring unit performance (PF, REC, CF), optimization needs to be coupled with the setting of system constraints. For example, one can maximize the purification factor while constraining recovery yield and concentration factor within certain boundaries. Scheme 1, illustrates this optimization strategy and two optimization cases have been considered. The first maximizes purification factor with the constraints of a minimum feed throughput rate of 10 ml/min, 70% minimum recovery yield and a maximum two-fold dilution of product. The second example shows a maximization of feed throughput constrained by a minimum 70% recovery yield, maximum 5-fold dilution and minimum 10-fold purification. The operating conditions required to achieve optimum results are shown in Scheme 1. These examples demonstrate the operational flexibility inherent in the CARE system's design.

These examples in addition to the sensitivity analysis discussed in an earlier section of this paper, provide the basis for the formulation of the following rule: system throughput can be increased by relaxing performance constraints (PF, REC, CF). In fact, any of the four performance measures can be increased by decreasing the constraints on one of the other three performance variables. A unique feature of the CARE system relative to packed bed adsorption is the ability to control unit performance. Control of CARE allows its optimization and continued operation at optimal levels despite variations in feed composition.

MODEL VALIDATION

The formulation of a mathematical model is strengthened after it has been experimentally validated. During the CARE model formulation stage, two key flow-rate ratios were found to influence unit performance. The ratio of the input flow to the adsorption contactor, relative to the adsorbent recycle flow governs the

CASE 1	CASE 2
OBJECTIVE: MAXIMIZE PF	OBJECTIVE: MAXIMIZE THROUGHPUT
CONSTRAINTS:	CONSTRAINTS:
1. Throughput > 10 ml/min	1. PF > 10
2. Recovery Yield > 70%	2. Recovery Yield > 70%
3. CF > 0.5	3. CF > 0.2
PERFORMANCE	**PERFORMANCE**
PF REC CF	PF REC CF
118 70% 0.5	10 70% 0.2
OPERATING FLOW RATES	**OPERATING FLOW RATES**
FEED WASH ELUTE RECYCLE	FEED WASH ELUTE RECYCLE
10 65.8 14.0 0.466	147 0 514 11.3

Scheme 1. Optimization examples.

purification. For a given feed flow-rate, an increase in the wash flow-rate dilutes the reactor contaminant concentration, and hence, the quantity of contaminants transported with the recycle stream to the desorption reactor. Similarly, decreasing the bead recycle flow-rate, increases the adsorbent reactor residence time. As a result, the amount of β-galactosidase adsorbed per unit of sorbent increases (assuming that equilibrium adsorption has not been reached), increasing the ratio of β-galactosidase to contaminants in the bead recycle stream. The ratio of feed to elution buffer flow-rates, the second important flow-rate ratio, determines whether product concentration or dilution occurs.

A qualitative assessment of the mathematical model was undertaken by a series of experiments designed to modify unit performance from a base case run. The results are shown in Table I. The two pertinent flow-rate ratios are normalized to the value in the base case. Steady-state performance is shown for each case. In order to improve the recovery yield, the amount of feed to the system was decreased. In a similar fashion, to improve purification factor, the first flow-rate ratio (feed + wash)/(gel recycle) was increased. Finally, in order to increase the concentration factor, the feed to eluting buffer flow-rate ratio was increased.

The results from an experiment conducted to investigate start-up dynamics serves to validate the quantitative aspects of the model. Fig. 12 shows the results from this experiment where adsorbent in the adsorption reactor was initially devoid of β-galactosidase; experimental conditions are listed in the figure caption. The enzyme concentration in the product stream slowly increases and approaches a steady state level after approximately 18 h of operation. This long start-up period is due to the time required to saturate the adsorbent as well as, the slow adsorbent recycle flow-rate between the two reactors. The waste stream enzyme concentration increases over time and levels off after approximately 10 h reflecting adsorbent saturation.

The solid lines in Fig. 12 indicate enzyme concentration predicted by the model. Recall that the model predictions are based on adsorption parameters (Q_{max}, K,k_f) obtained in independent batch adsorption experiments combined with flow-rates used in this experiment. Good model agreement is shown for both the dynamic and steady state stages of operation, and for β-galactosidase concentration in both the product and waste. Model predictions, of both β-galactosidase and contaminating protein

TABLE I

QUALITATIVE VALIDATION ON THE CARE MODEL

Experiment	*Ratio of flow-rates*		*Performance*		
	$\frac{(Feed + wash)}{(Gel\ recycle)}$	$\frac{(Feed)}{(Elute)}$	*PF*	*CF*[a]	*REC.* (%)
Base case	1	1	18	0.09	72
High recovery	1	0.15	13	0.02	77
High purification	5	0.77	31	0.04	50
High concentration	1.2	3.2	14	0.18	40

[a] A preconcentrated *E. coli* homogenate feed was used for these experiments. The concentration factors relative to the original homogenate are 0.9, 0.2, 0.4 and 1.8, respectively.

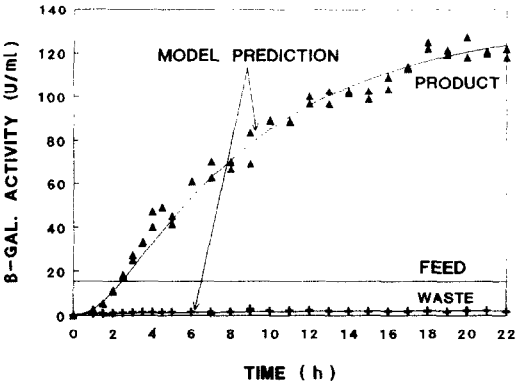

Fig. 12. Start-up dynamics in the CARE system. Reactor volume, 75 ml; gel volume fraction, 0.2; V_s/V, 1.05; F_1, 6.2 ml/min; F_2, 0; F_4, 0.18 ml/min; F_5, 0.60 ml/min, X_1, 15.4 U/ml; C_1, 0.35 mg/ml.

concentrations are compared with experimental results in Table II. Predictions of β-galactosidase concentrations in the various process streams, are matched closely by experimental results. However, their is an apparent contradiction between prediction and measurement of contaminant protein concentrations.

The protein concentration in the waste stream was found to be similar to that of the feed, indicating near total removal of incoming contaminating protein (*e.g.* β-galactosidase is a small portion of feed protein). However, it was not possible to experimentally validate the model prediction for protein in the product stream. Measurement of total protein concentration, using the standard Biorad dye reagent assay, accounts for all proteinaceous components, including the contribution due to β-galactosidase. The contaminant protein concentration reported in Table II, was

TABLE II

MODEL *VS.* EXPERIMENTAL RESULTS FOR START-UP EXPERIMENT

β-Galactosidase sp.act. (U/mg)	*Contaminant protein (mg/ml)*					
	Feed		*Waste*		*Product*	
	Model	*Experimental*	*Model*	*Experimental*	*Model*	*Experimental*
900	0.35	0.35	0.35	0.37	0.078	0.23
600	0.34	0.34	0.34	0.37	0.076	0.16
420	0.33	0.33	0.33	0.37	0.073	0.075
	β-Galactosidase (U/ml)					
	Feed		*Waste*		*Product*	
	Model	*Experimental*	*Model*	*Experimental*	*Model*	*Experimental*
900	15.4	15.4	2.3	2.0	124	122
600	15.4	15.4	2.3	2.0	124	122
420	15.4	15.4	2.3	2.0	124	122

estimated by subtracting the contribution of β-galactosidase to total protein from the measured total protein concentration. In order to perform this calculation, the β-galactosidase specific activity must be known. Values of specific activity ranging from 600 to 900 U/mg protein are reported for purified β-galactosidase preparations, obtained from Sigma.

Model predictions, when contrasted to experimental results in Table II, for Sigma's range of specific activity, show a poor fit. This poor fit can be accounted for in several ways. If their is a certain level of non-specific adsorption of contaminating proteins to PABTG-Agarose, contaminant carry-over between the two reactors would be greater than predicted by the model, and thus account for the discrepany between the predicted and measured contaminant protein concentration in the product stream. However, electrophoretic gels [native polyacrylamide gel electrophoresis (PAGE), not shown] of the components that adsorb, and are subsequantly eluted from the adsorbent, show a single predominant band, corresponding to β-galactosidase. The significant level of non-specific adsorption of contaminants, that would be required to account for the apparent discrepancy with model predictions, was not detected.

An alternate, and more likely explanation, is that a portion of the β-galactosidase in the feed is not enzymatically active. Further, if the non-active component can adsorb to the affinity adsorbent, the resulting β-galactosidase specific activity would be lower than 600 U/mg, and thus, the contribution of β-galactosidase to the measured total protein would increase. The results listed in Table II, show that for a specific activity of 420 U/mg protein, for β-galactosidase, model predictions match experimental results. Although contaminant protein concentrations, and hence purification factors, cannot be reported with confidence, electrophoretic gels (native PAGE, not shown), have confirmed the high purity of the product stream, as predicted by the mathematical model.

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NOMENCLATURE

c	bulk solute concentration
c_i	pore solute concentration
c_f	final bulk liquid concentration in diffusivity experiments
c_0	feed concentration
C_1	contaminant concentration in CARE feed stream
C_3	contaminant concentration in CARE waste stream
C_7	contaminant concentration in CARE product stream
CF	concentration factor

D_i	effective particle diffusion coefficient
F_1	flow-rate of CARE feed stream
F_2	flow-rate of CARE wash stream
F_3	flow-rate of CARE waste stream
F_4	flow-rate of CARE gel recycle stream
F_5	flow-rate of CARE elution buffer stream
F_6	flow-rate of CARE gel recycle stream
F_7	flow-rate of CARE product stream
K	adsorption equilibrium constant
k	fluid film mass transfer coefficient
k_f	forward reaction rate constant
k_r	reverse reaction rate constant
N_0	flux of solute into particle
PF	purification factor
q	average particle sorbate concentration
q_i	particle local sorbate concentration
q_0	sorbate concentration in equilibrium with c_0
Q_{max}	maximum sorbate concentration
R	sorbent particle radius
REC	recovery yield
s	average concentration in particle (including pore liquid)
t	time
v	volume
V	CARE reactor volume
V_e	CARE reactor volume external to retaining screen
V_{bulk}	fluid volume excluding gel volume
V_{gel}	gel volume
X_1	solute concentration in CARE feed stream
X_3	solute concentration in CARE waste stream
X_7	solute concentration in CARE product stream
Y_3	gel volume fraction in adsorption reactor
Y_7	gel volume fraction in desorption reactor
Z_3	particle sorbate concentration in adsorption reactor
Z_7	particle sorbate concentration in desorption reactor
α	adsorbent volume fraction
β	accessible particle volume fraction

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