Process Performance Models in the Optimization of Multiproduct Protein Production Plants

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Received 20 February 2000; accepted 21 January 2001

Abstract: In this work we propose a model that simultaneously optimizes the process variables and the structure of a multiproduct batch plant for the production of recombinant proteins. The complete model includes process performance models for the unit stages and a posynomial representation for the multiproduct batch plant. Although the constant time and size factor models are the most commonly used to model multiproduct batch processes, process performance models describe these time and size factors as functions of the process variables selected for optimization. These process performance models are expressed as algebraic equations obtained from the analytical integration of simplified mass balances and kinetic expressions that describe each unit operation. They are kept as simple as possible while retaining the influence of the process variables selected to optimize the plant. The resulting mixed-integer nonlinear program simultaneously calculates the plant structure (parallel units in or out of phase, and allocation of intermediate storage tanks), the batch plant decision variables (equipment sizes, batch sizes, and operating times of semicontinuous items), and the process decision variables (e.g., final concentration at selected stages, volumetric ratio of phases in the liquid–liquid extraction). A noteworthy feature of the proposed approach is that the mathematical model for the plant is the same as that used in the constant factor model. The process performance models are handled as extra constraints. A plant consisting of eight stages operating in the single product campaign mode (one fermentation, two microfiltrations, two ultrafiltrations, one homogenization, one liquid–liquid extraction, and one chromatography) for producing four different recombinant proteins by the genetically engineered yeast *Saccharomyces cerevisiae* was modeled and optimized. Using this example, it is shown that the presence of additional degrees of freedom introduced by the process performance models, with respect to a fixed size and time factor model, represents an important development in improving plant design. *Biotechnol Bioeng* **74**: 451–465, 2001.
Keywords: protein production; multiproduct batch plant; process performance models; design; optimization

Correspondence to: J. A. Asenjo
Contract grant sponsors: VITAE; ANTORCHAS
Contract grant numbers: B-11487/6B004; A-13668/1-9

INTRODUCTION

Multipurpose protein production plants have been used in the biotechnology industry for many years as pilot plants; however, more recently, full production plants have been built as multipurpose or multiproduct protein production plants, due mainly to the variety of different products arriving onto the market with wide-ranging demand, that is impossible to predetermine. Thus, many companies have built multipurpose or multiproduct batch plants for the production of recombinant proteins. Although the main host for recombinant proteins for many years has been *E. coli*, the use of yeast cells (*Saccharomyces* and *Pichia*) has grown rapidly. The fact that many recombinant proteins made in yeast can be made to be secreted, thus overcoming the very cumbersome protein renaturation from inclusion bodies, and that yeast allows for at least partial glycosylation is an added bonus.

The design and structural optimization of multiproduct batch plants have been widely investigated in recent years. The aim has been to determine plant configuration and equipment size that minimize capital cost. The usual strategy for solving this problem has been to consider constant values for size and cycle time factors, which can be obtained from laboratory or pilot plant data (Grossmann and Sargent, 1979; Modi and Karimi, 1989; Ravemark, 1995; Ravemark and Rippin, 1998). These values rely only on the product under consideration and no interactions with other products are analyzed.

Another approach is to incorporate process information into the design by predicting the size and time factors through process performance models for the unit stages. These performance models define the size and time factors

as functions of the process variables selected to optimize the plant. The use of process decision variables in the model yields a more detailed representation and approximation of size and time factors, thus allowing a more accurate design of a multiproduct batch plant (Salomone and Iribarren, 1992). Previously, process performance models have only been used for a small number of stages that were all operated in batch mode (Montagna et al., 1994). In this study, we double the number of stages and incorporate composite stages wherein semicontinuous items operate on the material contained in the batch items (e.g., homogenization and crossflow filtration); structural optimization is also carried out.

There is a balance between complexity and level of detail of the individual stages that comprise the process to keep a bound on the size of the optimization model. There is abundant literature that approaches single-product, single-stage cases with quite detailed models (e.g., fermentors such as in Uesbeck et al. [1998], extractors as in Mistry et al. [1996], etc.). Single-product multistage cases resort to simpler dynamic models (e.g., for reaction–evaporation stripping in Salomone et al. [1994], reaction separation networks in Smith and Pantelides [1995], distillation trains in Sharif et al. [1998], particle separation processes in Agena et al. [1998], etc.). Finally, multiproduct, multistage cases (e.g., Bhatia and Biegler, 1996; Montagna et al., 1994) resort to even simpler stage models. In the context of biochemical processes, Groep et al. (2000) recently developed performance models and showed the interactions among the different unit operations for a typical enzyme production process in a plant with fixed topology.

Samsatli and Shah (1996a) addressed a somewhat similar problem by developing a design procedure for a biochemical plant that consists of two subproblems. The first subproblem determines the processing conditions of all unit operations using dynamic optimization with manual branch and bound. The only structural decision at this level concerns the number of fermentors in parallel, and scheduling decisions are aggregated. At a second level, a scheduling problem determines the sequence and timing of operations (Samsatli and Shah, 1996b).

In this study, a plant that consists of eight stages for producing four recombinant proteins by genetically engineered *Saccharomyces cerevisiae* is modeled and optimized. The four recombinant proteins to be produced would be typically proteins for human therapeutic use as the plant would need FDA clearance and approval. However, for practical and desmonstration purposes (since production and purification data was available and the proteins have been or are being cloned in yeast) the four proteins used in this paper are two therapeutic proteins. Human Insulin and Vaccine for Hepatitis B, a food grade protein, Chymosin, and a detergent enzyme, cryophilic Protease. The results obtained, however, are generic for any plant producing four recombinant proteins using yeast (e.g. Proteins A, B, C and D) and could also be extended to recombinant proteins using yeast (e.g. Proteins A, B, C and D) and could also be extended to recombinant proteins being synthesized in *E. coli* (with some modification of the process) or also with additional chromatographic steps. The simplest possible process performance models for size and time factors are introduced while still retaining the influence of the dominant process variables (those suspected to have the largest economic impact on the design). For the design and structural optimization of the multiproduct batch plant a modular model that considers in- and out-of-phase parallel units and allocation of intermediate storage tanks is considered. The results obtained with the process performance models are compared with a more traditional approach that considers constant size and time factors (Montagna et al., 2000).

PROCESS DESCRIPTION

Figure 1 shows the flowsheet of a multiproduct batch plant intended for the production of recombinant proteins. In most process flowsheets for recombinant proteins there are differences, which depend on the specific host used to synthesize the product and on the specific properties of the product and its contaminants that will determine the purification process, as well as its final use. We have recently made an attempt to "standardize" such a process for purposes of generating a generic plant (Montagna et al., 2000). In this process, even a liquid–liquid extraction step was included as an initial separation/purification stage. Such a separation has been very successful in the initial purification of many proteins and enzymes, particularly hydrophobic ones (chymosin and α-amylase) (Hayenga et al., 1991; Schmidt et al., 1994), recombinant ones (Andrews et al.,

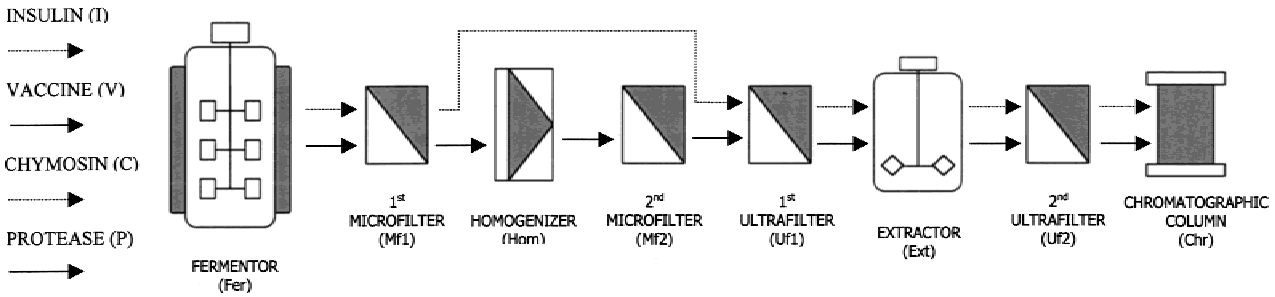

Figure 1. Flowsheet of the batch plant for the production of proteins.

1991; Hart et al., 1994) and recombinant protein particles synthesized in yeast (Andrews et al., 1995). The products involved in the multiproduct batch plant are human insulin and vaccine for hepatitis B, both therapeutic proteins, and chymosin and a cryophilic protease, a food product and a detergent enzyme, all produced by *Saccharomyces cerevisiae*.

All these proteins are produced as the cells grow in the fermentor. Vaccine and protease are considered as being intracellular; hence, for these two products, the first microfilter is used to concentrate the cell suspension, which is then sent to the homogenizer for cell wall disruption to liberate the intracellular proteins. The second microfilter is used to remove the cell debris from the solution of proteins.

The ultrafiltration prior to extraction is designed for concentrating the solutions in order to minimize the extractor volume. In the liquid–liquid extractor, salt concentration (NaCl) is manipulated to first drive the product to a polyethylene-glycol (PEG) phase and again into an aqueous saline solution in the backextraction (Huenupi et al., 1999). In this process, many of the proteins other than the product are removed.

Ultrafiltration is again used for concentrating the solution (in case this is required before the chromatographic step), and finally the last stage is chromatography, during which selective binding is used to separate further the product of interest from other proteins.

Insulin and chymosin are extracellular products. For both products, protein is separated from the cells in the first microfilter, where cells and some of the supernatant liquid stay behind. To reduce the amount of valuable product lost in the retentate, extra water is added to the cell suspension. The filtration operation with make-up water is also called diafiltration and dilutes the protein solution.

The homogenizer and microfilter for cell debris removal are not used when the product is extracellular. Nevertheless, the ultrafilter is necessary to concentrate the dilute solution prior to extraction. The final steps of extraction, ultrafiltration, and chromatography are common to both the extracellular and the intracellular products. Insulin and hepatitis B vaccine are therapeutic proteins for which several additional chromatographic steps are necessary to obtain the necessary purity. Chymosin is a protein for food use and cryophilic protease is used for detergent or for wound debriding. These two products evidently require less purification.

For therapeutic proteins, several chromatographic steps are usually necessary to obtain the required level of purity (Leser and Asenjo, 1992). However, modern techniques of combinatorial chemistry have made it possible, in the case of insulin, to obtain ligands specifically designed to achieve a virtually pure and very high-yield protein in one step (Lowe, 1998).

Insulin and hepatitis vaccine are well-established commercial products. The plant shown in Figure 1 would produce technical-grade products with further purification steps rendering clinical grade. On the other hand, chymosin and the protease are newer products that could be made in a portion of the plant shown in Figure 1. Although there is enough process information on chymosin, cryophilic protease production process is still in a developmental stage and most of the data have been estimated.

Rather than using all available details for each product, first level process performance models allow preliminary estimates on the economic viability of the multiproduct facility such as equipment size required, idle times, and percentage of units used by each product. More important than the details is the consistency of the data used with regard to the demand of each resource by each product.

The general batch process literature describes the design of batch plants through size and time equations. As noted in Salomone and Iribarren (1992), the following constraints hold for batch stages:

$$V_j \geq S_{ij}\, B_i \qquad \forall i, \forall j \qquad (1)$$

$$T_{ij} = T^0_{ij} + T^1_{ij}\, B_i \qquad \forall i, \forall j \qquad (2)$$

where V_j is the size of stage j (m^3), B_i is the batch size for product i (kg product that leaves the last stage), and S_{ij} is the size factor at stage j to produce 1 kg of final product i ($m^3 kg^{-1}$). In Eq. (2), T_{ij} is the time required at stage j to process a batch of product i and T^0_{ij} is a time factor that accounts for a fixed amount of time, whereas T^1_{ij} accounts for time demands that are proportional to the batch size to be processed.

Semicontinuous units use the following expression:

$$R_j = D_{ij}\, \frac{B_i}{\theta_j} \qquad \forall i, \forall j \qquad (3)$$

where R_j is the size of the semicontinuous item j, usually a processing rate as in the case of the homogenizer capacity (m^3/h); however, in the case of the filters, R_j is the filtration area A (m^2). In every case, the sizes are proportional to the batch size B_i (kg) and inversely proportional to the operating time θ_j (h), through a so-called duty factor D_{ij} that must have appropriate units (m^3/kg for the homogenizer, m^2 h/kg for the filters).

In the case of composite stages with a semicontinuous item that processes the material held in a batch item (as in the case of the homogenizer), the modeling approach of Salomone et al. (1994) is followed in this study. The stage is described with Eq. (1) for the batch item size, and the batch processing time T_{ij} includes the fixed amount T^0_{ij} plus the operating time θ_j of the semicontinuous item. Substituting for θ_j from Eq. (3) gives rise to a new expression for the operating time:

$$T_{ij} = T^0_{ij} + T^1_{ij}\, \frac{B_i}{R_j} \qquad \forall i, \forall j \qquad (4)$$

where the time factor T^1_{ij} turns out to be the duty factor.

PROCESS PERFORMANCE MODELS

If the size (duty in the case of semicontinuous units) and time factors S_{ij}, D_{ij}, T^0_{ij}, and T^1_{ij} in Eq. (1)–(4) are constant values, this gives rise to a posynomial model for the process. To obtain these constant factors it is necessary to guess

or estimate a value for every process variable so as to cover the degrees of freedom of the process mass balances. In the present approach, we use simple process performance models still retaining the influence of the process variables that we a priori expect to have the largest impact on the economics of the process, as proposed by Douglas (1988).

Once these variables have been selected, we write the mass balances and kinetic equations that describe each stage by guessing or estimating constant values for every nonselected process variable, except for the process variables that will be further optimized. As a result, we obtain analytical expressions for the size and time factors that will be functions of these process variables.

Conceptually, the mathematical optimization model for the design of the multiproduct batch plant will be the same as that presented by Montagna et al. (2000) if size and time factors were held constant, *plus* the additional constraints that describe these factors as functions of the process variables. As a result, it is expected that the introduction of these new degrees of freedom into the optimization model will provide a better design.

The process variables that have been selected as optimization variables are the biomass concentrations both at the fermentor ($X_{i,\mathrm{fer}}$) and at microfilter 1 ($X_{i,\mathrm{mf1}}$) for all products, the volumetric ratio of diafiltration water to suspension feed at microfilter 1 ($W_{i,\mathrm{mf1}}$) for extracellular insulin and chymosin and at microfilter 2 ($W_{i,\mathrm{mf2}}$) for intracellular vaccine and protease after cell disruption, the number of passes through the homogenizer (NP_i) for intracellular vaccine and protease, and the volumetric ratio (R_i) of PEG to phosphate phases at the extractor for all products. Overall, 18 process variables were selected.

Larger values of $X_{i,\mathrm{fer}}$ increase the batch size for a given fermentor design, but beyond some upper limit the production rate decreases. Larger concentrations may also reduce the cost of the stages downstream of the fermentor.

Process variables $X_{i,\mathrm{mf1}}$, $W_{i,\mathrm{mf1}}$, and $W_{i,\mathrm{mf2}}$ all increase the cost of filtration, but the increase of $X_{i,\mathrm{mf1}}$ reduces the cost of the stages downstream of the filter, whereas more diafiltration water increases product recovery. Larger values of NP_i increase the cost of the homogenizer and increase cell disruption, but also the denaturation of the already-released proteins; hence, there is a value for NP_i that maximizes homogenizer yield.

Increasing R_i increases the cost of the extractor and also the efficiency of the first extraction into the PEG phase. However, it reduces the efficiency of the backextraction into the phosphate phase, because the dilution of NaCl is poor and the partition coefficient is strongly dependent on this dissolved concentration.

In what follows is a description of the process performance models for each stage. Most of the development information was taken from Asenjo (1990) and Belter et al. (1988). We use the following convention in the model equations: C for chymosin; I for insulin; P for cryophilic protease; and V for hepatitis B vaccine.

Fermentor

A logistic kinetic expression, constrained by a maximum biomass concentration, is assumed for cell growth:

$$\frac{dX_{i,\mathrm{fer}}}{dt} = \phi_i X_{i,\mathrm{fer}} \left(1 - \frac{X_{i,\mathrm{fer}}}{X_{i,\max}}\right) \qquad \forall i \qquad (5)$$

We estimate the same kinetic constant, $\phi_i = 0.26315\ \mathrm{h}^{-1}$, and maximum biomass concentration, $X_{i,\max} = 55\ \mathrm{kg/m^3}$, for all products. The batch size relates to the fermentor vessel volume through the biomass concentration:

$$B_i^{\mathrm{fer}} = 0.8\ \mathrm{V_{fer}}\ (X_{i,\mathrm{fer}}\ 0.4\ k_i) \qquad \forall i \qquad (6)$$

Eq. (6) assumes that the batch volume occupies 80% of the vessel; that 40% of biomass is composed of proteins; and that k_i is a ratio (kg of product *i*/kg total proteins) estimated as $k_i = 0.05$, 0.1, 0.15, and 0.2 for insulin, vaccine, chymosin, and protease, respectively. Then, it must be taken into account that the batch size at any stage *j* relates to the batch size exiting the plant through the yields of all stages between this stage *j* and the last stage of the plant through the following expression:

$$B_i = B_1^j \prod_{n=j}^{M} \eta_{in} \qquad \forall i, \forall j \qquad (7)$$

where M is the total number of stages of the plant.

Replacing Eq. (7) into Eq. (6) and using the appropriate value of k_i for each product results in the size factor expressions in Tables I and II. Note that the size factor expressions assume that fermentation and ultrafiltration have an efficiency value of 1, as will be discussed next.

By integrating Eq. (5) between an initial biomass concentration of $0.05\ X_{i,\max}$ and $X_{i,\mathrm{fer}}$ (inoculum seeded amounts to 5% of the fermentor capacity), and adding an estimated downtime of 4 h yields the time expressions for the fermentor in Tables I and II for insulin, vaccine, chymosin, and protease, respectively.

By comparing the time expressions to the general expression [Eq. (2)] there are nonzero values for T_{ij}^0. Moreover, $T_{ij}^1 = 0$; that is, there is no time demand proportional to the batch size, which is typical of operations governed by reaction kinetics (bioreactors, crystallizers, etc.). Note that, if variables $X_{i,\mathrm{fer}}$ were fixed, this would result in a fixed time factor model.

Microfilter 1

This stage consists of three items: a batch retentate holding vessel; the microfilter itself; and a permeate holding vessel (used only by extracellular insulin and chymosin). The size factor for the retentate holding vessel is the same as that of the fermentor.

Considering that the initial condition at the retentate vessel is a batch volume $BV_{i,\mathrm{mf1}}^{\mathrm{in}}$ at a concentration $X_{i,\mathrm{fer}}$, that the final condition is $BV_{i,\mathrm{mf1}}^{\mathrm{ret}}$ at a concentration $X_{i,\mathrm{mf1}}$, and that the biomass concentration in the permeate is zero, mass balances yield the relation between permeate, retentate, and feed volumes.

For the cases that diafiltration follows (extracellular chy-

Table I. Size and time factors for extracellular human insulin and chymosin ($i = \{\mathrm{I,C}\}$).

Fermentor

$$S_{i,\,\mathrm{fer}}\,[\mathrm{m^3/kg}] = \frac{K_i^1}{X_{i,\,\mathrm{fer}}\,\eta_{i,\,\mathrm{mf1}}\,\eta_{i,\mathrm{ext}}\,\eta_{i,\mathrm{chr}}} \qquad K_\mathrm{I}^1 = 62.5 \qquad K_\mathrm{C}^1 = 20.8$$

$$T_{i,\,\mathrm{fer}}\,[\mathrm{h}] = 4 + 3.8\;\ell\mathrm{n}\left[\frac{0.35\,X_{i,\,\mathrm{fer}}}{\left(1 - \dfrac{X_{i,\,\mathrm{fer}}}{55.}\right)}\right]$$

$$\eta_{i,\,\mathrm{fer}} = 1$$

Microfilter 1

$$S_{i,\,\mathrm{mf1}}\,[\mathrm{m^3/kg}] = \frac{K_i^1}{X_{i,\,\mathrm{fer}}\,\eta_{i,\,\mathrm{mf1}}\,\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}} \qquad \text{(retentate vessel)}$$

$$S_{i,\,\mathrm{mf1}}\,[\mathrm{m^3/kg}] = \frac{K_i^1\left(1 - \dfrac{X_{i,\,\mathrm{fer}}}{X_{i,\,\mathrm{mf1}}} + W_{i,\,\mathrm{mf1}}\right)}{X_{i,\,\mathrm{fer}}\,\eta_{i,\,\mathrm{mf1}}\,\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}} \qquad \text{(permeate vessel)}$$

$$T_{i,\,\mathrm{mf1}}\,[\mathrm{h}] = 1.75 + \left[\frac{K_i^2\left(1 - \dfrac{X_{i,\,\mathrm{fer}}}{X_{i,\,\mathrm{mf1}}} + W_{i,\,\mathrm{mf1}}\right)}{X_{i,\,\mathrm{fer}}\,\eta_{i,\,\mathrm{mf1}}\,\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}}\right]\frac{B_i}{A_{\mathrm{mf1}}} \qquad K_\mathrm{I}^2 = 250 \qquad K_\mathrm{C}^2 = 83.5$$

$$\eta_{i,\,\mathrm{mf1}} = 1 - \frac{X_{i,\,\mathrm{fer}}}{X_{i,\,\mathrm{mf1}}}\exp\left(-\frac{W_{i,\,\mathrm{mf1}}\;X_{i,\,\mathrm{mf1}}}{X_{i,\,\mathrm{fer}}}\right)$$

Homogenizer —

Microfilter 2 —

Ultrafilter 1

$$S_{i,\,\mathrm{uf1}}\,[\mathrm{m^3/kg}] = \frac{K_i^1\left(1 - \dfrac{X_{i,\,\mathrm{fer}}}{X_{i,\,\mathrm{mf1}}} + W_{i,\,\mathrm{mf1}}\right)}{X_{i,\,\mathrm{fer}}\,\eta_{i,\,\mathrm{mf1}}\,\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}} \qquad \text{(retentate vessel)}$$

$$T_{i,\,\mathrm{uf1}}\,[\mathrm{h}] = 1 + \frac{K_i^3\left(1 - \dfrac{X_{i,\,\mathrm{fer}}}{X_{i,\,\mathrm{mf1}}} + W_{i,\,\mathrm{mf1}}\right)}{X_{i,\,\mathrm{fer}}\,\eta_{i,\,\mathrm{mf1}}\,\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}}\left[1 - \frac{0.12\,X_{i,\,\mathrm{fer}}\,\eta_{i,\,\mathrm{mf1}}}{50\left(1 - \dfrac{X_{i,\,\mathrm{fer}}}{X_{i,\,\mathrm{mf1}}} + W_{i,\,\mathrm{mf1}}\right)}\right]\frac{B_I}{A_{\mathrm{uf1}}} \qquad \begin{matrix} K_\mathrm{I}^3 = 2500 \\ K_\mathrm{C}^3 = 835 \end{matrix}$$

$$\eta_{i,\,\mathrm{uf1}} = 1$$

Extractor

$$S_{i,\,\mathrm{ext}}\,[\mathrm{m^3/kg}] = \frac{0.15\,(1 + R_i)}{\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}} \qquad \text{(mixer–decanter vessel)}$$

$$T_{i,\,\mathrm{ext}}\,[\mathrm{h}] = 1.8$$

$$\eta_{i,\,\mathrm{ext}} = \frac{K_i^4\,R_i}{(1 + K_i^4 R_i)\left(1 + R_i\,10^{K_i^5\left(\frac{7R_i}{R_i+1} - 5\right)}\right)} \qquad \begin{matrix} K_\mathrm{I}^4 = 31.6 & K_\mathrm{C}^4 = 50.1 \\ K_\mathrm{I}^5 = 0.75 & K_\mathrm{C}^5 = 0.85 \end{matrix}$$

Ultrafilter 2

$$S_{i,\,\mathrm{uf2}}\,[\mathrm{m^3/kg}] = \frac{K_i^6}{\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}} \qquad \begin{matrix} K_\mathrm{I}^6 = 0.15 \\ K_\mathrm{C}^6 = 0.05 \end{matrix} \qquad \text{(retentate vessel)}$$

$$T_{i,\,\mathrm{uf2}}\,[\mathrm{h}] = 0.3 + \left[\frac{K_i^7 - \eta_{i,\,\mathrm{ext}} - \dfrac{K_i^8\,R_i}{(R_i+1)^2}}{\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}}\right]\frac{B_i}{A_{\mathrm{uf2}}} \qquad \begin{matrix} K_\mathrm{I}^7 = 6 & K_\mathrm{I}^8 = 5 \\ K_\mathrm{C}^7 = 2 & K_\mathrm{C}^8 = 1 \end{matrix}$$

$\eta i,uf2 = 1$

Chromatography column

$$S_{i,\,\mathrm{chr}}\,[\mathrm{m^3/kg}] = \frac{0.025\left[\eta_{I,\mathrm{ext}} = \dfrac{K_i^8\,R_i}{(R_i+1)^2}\right]}{\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}} \qquad \text{(vessel)}$$

$$S_{i,\,\mathrm{chr}}\,[\mathrm{m^3/kg}] = \frac{0.1}{\eta_{i,\,\mathrm{chr}}} \qquad \text{(column)}$$

$$T_{i,\,\mathrm{chr}}\,[\mathrm{h}] = 0.375 + \left[\frac{0.0025\left[\eta_{i,\,\mathrm{ext}} + \dfrac{K_I^8\,R_i}{(R_i+1)^2}\right]}{\eta_{i,\,\mathrm{ext}}\,\eta_{i,\,\mathrm{chr}}}\right]\frac{B_i}{V_{\mathrm{chr}}}$$

$$\eta_{i,\,\mathrm{chr}} = 0.95$$

Table II. Size and time factors for intracellular Hepatitis B vaccine and cryophilic protease ($i = \{V,P\}$).

Fermentor

$$S_{i,\text{fer}}\,[\text{m}^3/\text{kg}] = \frac{K_i^9}{X_{i,\text{fer}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad K_V^9 = 31.2 \qquad K_P^9 = 15.6$$

$$T_{i,\text{fer}}\,[\text{h}] = 4 + 3.8\;\ell\text{n}\left[\frac{0.35\,X_{i,\text{fer}}}{\left(1 - \frac{X_{i,\text{fer}}}{55.}\right)}\right]$$

$$\eta_{i,\text{fer}} = 1$$

Microfilter 1

$$S_{i,\text{mf1}}\,[\text{m}^3/\text{kg}] = \frac{K_i^9}{X_{i,\text{fer}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad \text{(retentate vessel)}$$

$$T_{i,\text{mf1}}\,[\text{h}] = 1.25 + \left[\frac{K_i^{10}\left(1 - \frac{X_{i,\text{mf1}}}{X_{i,\text{mf1}}}\right)}{X_{i,\text{fer}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}}\right]\frac{B_i}{A_{\text{mf1}}} \qquad K_V^{10} = 125 \qquad K_P^{10} = 62.5$$

$$\eta_{i,\text{mf1}} = 1$$

Homogenizer

$$S_{i,\text{hom}}\,[\text{m}^3/\text{kg}] = \frac{K_i^9}{X_{i,\text{fer}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\;\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad \text{(vessel)}$$

$$T_{i,\text{hom}}\,[\text{h}] = 1.25 + \left[\frac{K_i^{11}\,\text{NP}_\text{i}}{X_{i,\text{mf1}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}}\right]\frac{B_i}{Cap_{\text{hom}}} \qquad K_V^{11} = 25 \qquad K_P^{11} = 12.5$$

$$\eta_{i,\text{hom}} = [1 - \exp(-1.5\,NP_i)]\quad \exp(-0.03\,NP_i)$$

Microfilter 2

$$S_{i,\text{mf2}}\,[\text{m}^3/\text{kg}] = \frac{\text{K}_\text{i}^9}{X_{i,\text{fer}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad \text{(retentate vessel)}$$

$$S_{i,\text{mf2}}\,[\text{m}^3/\text{kg}] = \frac{K_i^9\,(0.5 + W_{i,\text{mf2}})}{X_{i,\text{mf1}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad \text{(permeate vessel)}$$

$$T_{i,\text{mf2}}\,[\text{h}] = 1.75 + \left[\frac{K_i^{12}\,(0.5 + W_{i,\text{mf2}})}{X_{i,\text{mf1}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}}\right]\frac{B_i}{A_{\text{mf2}}} \qquad K_V^{12} = 250 \qquad K_P^{12} = 125$$

$$\eta_{i,\text{mf2}} = 1 - 0.5\,\exp\,(-2W_{i,\text{mf2}})$$

Ultrafilter 1

$$S_{i,\text{uf1}}\,[\text{m}^3/\text{kg}] = \frac{K_i^9\,(0.5 + W_{i,\text{mf2}})}{X_{i,\text{mf1}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad \text{(retentate vessel)}$$

$$T_{i,\text{uf1}}\,[\text{h}] = 1 + \frac{K_i^{13}\,(0.5 + W_{i,\text{mf2}})}{X_{i,\text{mf1}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \times \qquad K_V^{13} = 1250 \qquad K_P^{13} = 625$$

$$\times\left[1 - \frac{0.24\,X_{i,\text{mf1}}\,\eta_{i,\text{hom}}\,\eta_{i,\text{mf2}}}{50\,(0.5 + W_{i,\text{mf2}})\,\exp(-0.03NP_i)}\right]\frac{B_i}{A_{\text{uf1}}}$$

$$\eta_{i,\text{uf1}} = 1$$

Extractor

$$S_{i,\text{ext}}[\text{m}^3/\text{kg}] = \frac{K_i^{14}\,(1 + R_i)}{\exp(-0.03\,NP_i)\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad K_V^{14} = 0.15 \qquad \text{(mixer–decanter vessel)} \qquad K_P^{14} = 0.075$$

$$T_{i,\text{ext}}\,[\text{h}] = 1.8$$

$$\eta_{i,\text{ext}} = \frac{K_i^{15}\,R_i}{(1 + K_i^{15}\,R_i)\left(1 + R_i\,10^{K_i^{16}\left(\frac{7R_i}{R_i + 1} - 5\right)}\right)} \qquad K_V^{15} = 39.8 \qquad K_V^{16} = 0.8 \qquad K_P^{15} = 25.1 \qquad K_P^{16} = 0.7$$

Ultrafilter 2

$$S_{i,\text{uf2}}\,[\text{m}^3/\text{kg}] = \frac{K_i^{14}}{\exp\,(-0.03\,NP_i)\,\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}} \qquad \text{(retentate vessel)}$$

$$T_{i,\text{uf2}}\,[\text{h}] = 0.3 + \left[\frac{K_i^{17} - \eta_{i,\text{est}} - \frac{K_i^{18}\,R_i}{(R_i + 1)^2}}{\eta_{i,\text{ext}}\,\eta_{i,\text{chr}}\,\exp(-0.03\,NP_i)}\right]\frac{B_i}{A_{\text{uf2}}} \qquad K_V^{17} = 6 \qquad K_V^{18} = 5 \qquad K_P^{17} = 3 \qquad K_P^{18} = 2$$

$$\eta_{i,\text{uf2}} = 1$$

Table II. Continued

Chromatography column	$S_{i,\mathrm{chr}}\,[\mathrm{m^3/kg}] = \dfrac{0.025\left[\eta_{i,\mathrm{ext}} + \dfrac{K_i^{18} R_i}{(R_i+1)^2}\right]}{\eta_{i,\mathrm{ext}}\,\eta_{i,\mathrm{chr}}\exp(-0.03\,NP_i)}$ (vessel) $S_{i,\mathrm{chr}}\,[\mathrm{m^3/kg}] = \dfrac{0.1}{\eta_{i,\mathrm{chr}}}$ (column) $T_{i,\mathrm{chr}}\,[\mathrm{h}] = 0.375 + \left[\dfrac{0.0025\left[\eta_{i,\mathrm{ext}} + \dfrac{K_i^{18} R_i}{(R_i+1)^2}\right]}{\eta_{i,\mathrm{ext}}\,\eta_{i,\mathrm{chr}}\exp(-0.03\,NP_i)}\right]\dfrac{B_i}{V_{\mathrm{chr}}}$ $\eta_{i,\mathrm{chr}} = 0.95$

mosin and insulin), an extra amount permeates through the membrane, which is $W_{i,\mathrm{mf1}}$ times the feed volume.

The time required to perform the filtration is proportional to the permeate volume $BV^{\mathrm{per}}_{i,\mathrm{mf1}}$, inversely proportional to the filter area A_{mf1}, and also inversely proportional to the permeability of the membrane, taken to be 0.2 $\mathrm{m^3/h{\cdot}m^2}$ of the filtration area. Taking into account an estimated downtime of $T^0_{i,\mathrm{mf1}} = 1.25$ h for intracellular vaccine and protease and a longer time of $T^0_{i,\mathrm{mf1}} = 1.75$ h for extracellular insulin and chymosin, both requiring filtration and diafiltration, results in the time expressions in Tables I and II for all products.

The yield for microfilter 1 is $\eta_{i,\mathrm{mf1}} = 1$ for intracellular vaccine and protease because no product is lost through the permeate. In the case of extracellular chymosin and insulin, only a portion of product is recovered during filtration. After filtration there is a final volume, $BV^{\mathrm{ret}}_{i,\mathrm{mf1}}$, in the retentate. This material is at an initial concentration of product, $C_{0,i}$, and diafiltration water at a flow rate of q_i ($\mathrm{m^3/h}$) is added, equal to the permeation capacity of the membrane, so that $BV^{\mathrm{ret}}_{i,\mathrm{mf1}}$ remains constant. The differential mass balance of product is:

$$BV^{ret}_{i,\mathrm{mf1}}\frac{dC_i}{dt} = -q_i \cdot C_i \qquad i = \{\mathrm{C}, I\} \qquad (8)$$

Integrating this equation between an initial concentration, $C_{0,i}$, at $t = 0$ and a final concentration, $C_{f,i}$, at $t = t_i$ renders:

$$\ln\frac{C_{f,i}}{C_{0,i}} = -\frac{q_i \cdot t_i}{BV^{\mathrm{ret}}_{i,\mathrm{mf1}}} = -W_{i,\mathrm{mf1}}\frac{BV^{\mathrm{in}}_{i,\mathrm{mf1}}}{BV^{\mathrm{ret}}_{i,\mathrm{mf1}}} \qquad \mathrm{i} = \{\mathrm{C,I}\} \qquad (9)$$

where $C_{f,i}/C_{0,i}$ is the portion of product i not recovered by diafiltration. Further rearranging produces the yield expressions in Table I. Note that if the process variables were fixed, the yields would be constant, as well as the size and time factors, which depend on these yields. Finally, to keep the global protein balance, we assumed conservatively that 30% of total proteins permeated along with the products of interest.

Homogenizer

The vaccine and protease batches proceed through the homogenizer for cell disruption that must be sized to hold the retentate volume of microfilter 1.

The homogenization time is proportional to the volume fed to the homogenizer, $V_{i,\mathrm{hom}}$ ($\mathrm{m^3}$), and inversely proportional to the homogenizer capacity, Cap_{hom} ($\mathrm{m^3/h}$), plus a constant downtime, $T^0_{i,\mathrm{hom}}$:

$$T_{i,\mathrm{hom}} = T^0_{i,\mathrm{hom}} + \frac{V_{i,\mathrm{hom}}}{Cap_{i,\mathrm{hom}}} \qquad i = \{\mathrm{P,V}\} \qquad (10)$$

The volume fed to the homogenizer is the batch volume in $BV^{\mathrm{in}}_{i,\mathrm{hom}}$ times the number of passes, NP_i, through the homogenizer; adopting the 1.25-h downtime yields the time expressions for the homogenizer shown in Table II. It can be seen that the term inside the brackets is the homogenizer duty factor.

Successive passes through the homogenizer drive the fraction of cells disrupted asymptotically to 1, which is also the fraction of proteins released. The same approach is valid to estimate the fraction of proteins released that are denatured by the homogenizer. The yield of the homogenizer in Table II is the product between the fraction released and the fraction not denatured.

Microfilter 2

Cell debris is separated from vaccine and protease at microfilter 2. No change in batch volume occurs at the homogenizer, so the size factor of the microfilter 2 retentate vessel is the same as that of the homogenizer, as shown in Table II.

Both filtration and diafiltration operations are assigned to this microfiltration stage. Filtration is limited to 50% reduction in the retentate initial volume to avoid operational problems due to the large concentration of solid matter. The permeate vessel size factor considers the extra amount of diafiltration water. The time required to perform the filtration is similar to the one for microfilter 1, but membrane permeability is taken as half that of microfilter 1, because of the smaller pore size required to separate cell debris; a 1.75-h downtime was assumed for this two-step microfiltration.

The yield expression for microfilter 2 in Table II considers that half the product is recovered in the filtration step. Finally, to keep overall protein balance, we assume that 60% of the total proteins of the cell permeate along with the products of interest.

Ultrafilter 1

The purpose of this stage is to remove water up to a limit of total protein concentration, estimated as 50 kg/m^3, in order to reduce, as much as possible, the size requirement of the downstream stages while still avoiding the risk of protein precipitation as NaCl is added to the extractor.

In the case of insulin and chymosin, the size factor for the retentate vessel of ultrafilter 1 is the same as that of the permeated vessel of microfilter 1. The outlet batch volume results from the change in total protein concentration.

The time required to perform the filtration is similar to that of the previous filters. We adopt a smaller permeability of 0.02 m^3/h·m^2 of filtration area, because of the smaller pore size required to separate proteins with respect to separating cell debris. The yield of the ultrafilter is 1; that is, no product is lost at this stage.

In the case of vaccine and protease, the size factor for the retentate vessel of ultrafilter 1 is the same as that of the permeate vessel of microfilter 2. Tables I and II illustrate the proposed expressions.

Extractor

After standardization of the total protein concentration to 50 kg/m^3 at ultrafilter 1, the batch volumes, $BV^{\text{in}}_{i,\text{ext}}$, going into the extractor simplify to:

$$BV^{in}_{i,\text{ext}} = \frac{rpp_i\, B^{\text{uf1}}_i}{50} \qquad \forall i \qquad (11)$$

where rpp_i is the weight ratio of total proteins to product protein, obtained by balance.

The decision variable at this stage is the volumetric ratio of PEG to phosphate phases, R_i, so the size factor for the extractor is:

$$S_{i,\text{ext}} = 1.25 \cdot BV^{\text{in}}_{i,\text{ext}}\,(1 + R_i) \qquad \forall i \qquad (12)$$

Backextraction is assumed to be conducted with an aqueous phase volume identical to the feed volume, thus obtaining the maximum dilution of NaCl that is compatible with the use of the same vessel for the consecutive extraction and backextraction.

The extraction–backextraction model used in this study is a simplification of the rigorous model presented by Mistry et al. (1996). The kinetics for both mixing and phase separation was simplified by assuming that these are completely achieved after 5 min of mixing and 30 min of settling. Adding 10 min for each charge or discharge and considering the sequence of eight operations (charge–mixing–settling–discharge of the phosphate phase–charge of fresh phosphate phase–mixing–settling–discharge) results in a constant time of 1.8 h.

For the mass balances, the PEG–phosphate phase equilibrium was simplified by assuming a constant top phase composition 30% PEG–2.5% phosphate and bottom phase composition 0% PEG–15% phosphate in the range of 2% to 8% NaCl. Make-ups of PEG and phosphate are supposed to set the compositions of these figures at every batch. Loss of product due to nonzero bottom PEG composition or incomplete separation of phases is neglected. The contaminant protein partition coefficient was approximated to 1 over the whole NaCl range.

The partition coefficients of the product proteins were approximated by a straight line in the log K vs. %NaCl plot, pivoting, on 5% NaCl. Data for α-amylase (α) of Figure 7 in Mistry et al. (1996) were taken as reference, resulting in the following gradient:

$$\text{Grad}_\alpha = \frac{\Delta \log K_\alpha}{\Delta \%\text{NaCl}_\alpha} = 0.925 \qquad (13)$$

So, the approximation for the K_i prediction is:

$$\log K_i = \text{Grad}_i\,(\%\text{NaCl}_i - 5) \qquad \forall i \qquad (14)$$

We took all partition coefficients lower than $K_{\alpha\text{-amylase}}$ and present Grad values in the 0.7 to 0.9 range, with the following hydrophobicity for the products: $\text{Grad}_\text{C} = 0.85 > \text{Grad}_\text{V} = 0.80 > \text{Grad}_\text{I} = 0.75 > \text{Grad}_\text{P} = 0.70$. The concentration of products, $X^{\text{in}}_{p,i}$, and contaminants, $X^{\text{in}}_{c,i}$, in the feed are calculated from mass balances as follows:

$$X^{in}_{p,i} + X^{in}_{c,i} = 50 \text{ kg/m}^3 \qquad \forall i \qquad (15)$$

$$X^{in}_{p,i} + X^{in}_{c,i} = rpp_i X^{in}_{p,i} \qquad \forall i \qquad (16)$$

The concentration, $X^{\text{in}}_{p,i}$, includes both denatured and valuable product, which are assumed to distribute with the same partition coefficient. The mass balance and phase equilibrium provide the distribution of components during extraction:

$$X^{\text{PEG}}_i \cdot V^{\text{PEG}}_i + X^{\text{Ph}}_i \cdot V^{\text{Ph}}_i = X^{\text{in}}_{p,i} \cdot BV^{\text{in}}_{i,\text{ext}} \qquad \forall i \qquad (17)$$

$$X^{\text{PEG}}_i = K^e_1 X^{\text{Ph}}_i \qquad \forall i \qquad (18)$$

Taking into account that $V^{\text{PEG}}_i = R_i \cdot V^{\text{Ph}}_i$, the yield of the first extraction can be written as:

$$\eta^e_i = \frac{X^{\text{PEG}}_i\, V^{\text{PEG}}_i}{X^{\text{in}}_i\, BV^{\text{in}}_{i,\text{ext}}} = \frac{K^e_i\, R_i}{(1 + K^e_i\, R_i)} \qquad \forall i \qquad (19)$$

Similarly, for backextraction, one obtains:

$$\eta^b_i = \frac{X^{\text{Ph}}_i\, V^{\text{Ph}}_i}{X^{\text{PEG},e}_i\, V^{\text{PEG}}_i} = \frac{1}{(1 + K^b_i\, R_i)} \qquad \forall i \qquad (20)$$

In the particular case of NaCl, $K^b = 1$. Therefore, $\%\text{NaCl}^{\text{Ph}} = \%\text{NaCl}^{\text{PEG}}$. Taking $\%\text{NaCl}^{\text{PEG},e} = 7\%$, the following expression holds for both phases:

$$\%\text{NaCl}_i = \frac{7\, R_i}{(1 + R_i)} \qquad \forall i \qquad (21)$$

Replacing this concentration into Eq. (14) renders K^b_i for all proteins, while replacing %NaCl = 7 in the same equation provides K^e_i. Multiplying the yields of extraction [Eq. (19)] and backextraction [Eq. (20)] results in the total yield for the extraction stage in Tables I and II.

Table III. Size Factors ST_{ij} for storage tanks.

Between stages	Size Factor equal to size factor of stage
Fermentor—microfilter 1	Microfilter retentate vessel
Microfilter 1—homogenizer (P and V)	Homogenizer
Homogenizer—microfilter 2 (P and V)	Homogenizer
Microfilter 2–ultrafilter 1 (P and V)	Ultrafilter 1 retentate vessel
Microfilter 1—ultrafilter 1 (I and C)	Ultrafilter 1 retentate vessel
Ultrafilter 1—extractor	Ultrafilter 2 retentate vessel
Extractor—ultrafilter 2	Ultrafilter 2 retentate vessel
Ultrafilter 2—chromatographic column	Chromatographic column

Ultrafilter 2

The batch volume into this stage is the same as that into the extractor. The purpose of this stage is to raise the concentration of total proteins to 50 kg/m^3. The amount of total proteins can be estimated through the ratio rpp_i after the extraction, which can be taken from the extractor outlet concentration of products and contaminants. For vaccine and protease it must be taken into account that $X_{p,i}$ represents the concentrations of both denatured and desired product.

The outlet volume can be estimated as:

$$BV^{\text{out}}_{i,\text{uf2}} = \frac{(\text{Total proteins})_i}{50\ \text{kg/m}^3} \qquad \forall i \qquad (22)$$

The batch volume permeated is given by the difference between $BV^{\text{in}}_{i,\text{uf2}}$ and $BV^{\text{out}}_{i,\text{uf2}}$. The time required to perform the filtration is calculated as:

$$T_{i,\text{uf2}} = T^0_{i,\text{uf2}} + \frac{BV^{\text{per}}_{i,\text{uf2}}}{0.02\, A_{\text{uf2}}} \qquad \forall i \qquad (23)$$

We use a downtime of 0.3 h, smaller than that for ultrafilter 1, because batch volumes are lower. The yield of ultrafilter 2 for all products is 1.

Chromatographic Column

We assume that the chromatographic column works at a constant linear velocity (v_{chr}) of 4 m/h and that its packing has a binding capacity (bc_{chr}) of 20 kg/m^3. Just a percentage of this maximum capacity is used so as to avoid excessive product breakthrough. A 50% capacity usage was assumed, leading to $\eta_{i,\text{chr}} = 0.95$.

Size factor is thus given by:

Table IV. Estimates (and initial values) of the process variables.

Product	X_{fer}	X_{mf1}	W_{mf1}	W_{mf2}	NP	R
Insulin	50.0	200.0	1.25	—	—	1.0
Vaccine	50.0	200.0	—	1.50	3.0	1.0
Chymosin	50.0	200.0	1.25	—	—	1.0
Protease	50.0	200.0	—	1.50	3.0	1.0

Table V. Optimal values of the process variables.

Model/product	X_{fer}	X_{mf1}	W_{mf1}	W_{mf2}	NP	R
Process performance, no tanks						
Insulin	46.54	250.0	0.35	—	—	0.636
Vaccine	38.52	250.0	—	1.81	2.36	0.474
Chymosin	38.96	248.7	0.10	—	—	0.634
Protease	31.26	250.0	—	1.75	2.39	0.635
Process performance with tanks						
Insulin	49.35	250.0	0.21	—	—	0.636
Vaccine	45.54	250.0	—	1.31	2.23	0.582
Chymosin	49.12	250.0	0.20	—	—	0.634
Protease	45.54	250.0	—	1.31	2.23	0.582

$$S_{i,\text{chr}} = \frac{1}{0.5 bc_{\text{chr}} \eta_{i,\text{chr}}} = \frac{0.1}{\eta_{i,\text{chr}}} \frac{\text{m}^3}{\text{kg}} \qquad \forall i \qquad (24)$$

A column height of 0.5 m was assumed, which is large enough to allow high resolution and is still compatible with reasonable linear velocities. The cross-sectional area of the column is thus:

$$A_{\text{chr}}[\text{m}^2] = \frac{V_{\text{chr}}[\text{m}^3]}{0.5\ [\text{m}]} = 2\ V_{\text{chr}} \qquad (25)$$

The time required by $BV^{\text{in}}_{i,\text{chr}}$ to pass through the column is:

$$T_{i,\text{chr}}[\text{h}] = \frac{BV^{\text{in}}_{i,\text{chr}}\ [\text{m}^3]}{A_{\text{chr}}[\text{m}^2]\ v_{\text{chr}}[\text{m/h}]} = \frac{BV^{\text{in}}_{i,\text{chr}}}{8\ \text{V}_{\text{chr}}} \qquad \forall i \qquad (26)$$

Elution plus washing–regeneration solution volumes were assumed to amount to three times the column volume, and the linear velocity for these processes to be the same (4 m/h) as for loading. This gives the constant time, $T^0_{i,\text{chr}} = 0.375$ h, which must be added to the time expression in Eq. (26), and this results in the time expressions given in Tables I and II.

DESIGN MODEL FOR THE MULTIPRODUCT PLANT

A general optimization model for the design of multiproduct batch plants operating in the single product campaign mode is now considered. The following assumptions were made:

- The plant consists of a sequence of M batch processing stages, which are used to manufacture P different products.
- At each batch stage j there are M_j units in parallel operating out of phase, and N_j units operating in phase, each with a size V_j.
- Each product i follows the same general processing sequence, with the restriction that only some stages can be skipped.
- A storage tank of size VT_j may be allocated between batch stages j and $j + 1$.
- When an intermediate storage tank is not allocated,

Table VI. Stage volume and plant cost obtained with the four models.

Model	Plant cost	Stage volume							
		Fer	Mf1[a]	Hom[b]	Mf2[a]	Uf1[a]	Ext	Uf2[a]	Chr[b]
Fixed factors no tanks	1,770,418	5.638	5.638	1.409	1.409	11.28	2.614	1.307	0.193
Process performance, no tanks	1,505,326	5.557	5.557	0.856	0.856	6.479	1.454	0.986	0.187
Fixed factors with tanks	920,790	27.81	4.319	1.080	1.080	15.96	0.967	0.484	0.068
Process performance, with tanks	800,138	27.33	4.24	0.773	0.773	7.037	0.747	0.472	0.063

[a]Retentate vessels.
[b]Vessels.

batches are transferred from one stage to the next without delay (i.e., the "zero-wait" policy is considered).

- The production requirements, Q_i, for product i in the time horizon, H, are known.

With these assumptions, the design problem for the multiproduct batch plant is posed so as to minimize the capital cost given by:

$$\text{Min CC} = \sum_{j=1}^{M} M_j N_j a_j V_j^{\alpha_j} + \sum_{j=1}^{M} c_j \, VT_j^{\gamma_j} \tag{27}$$

where a_j, α_j, c_j, and γ_j are appropriate coefficients for each item type in the plant. The first term on the right-hand side of Eq. (27) considers the processing stages. In the case of composite stages, the summation includes all batch and semicontinuous items associated with it. The second term on the right-hand side considers storage tanks.

The batch units are selected so as to contain the sizes required by all products. Thus:

$$V_j = \frac{S_{ij} \, B_i}{N_j} \qquad \forall i, \forall j \tag{28}$$

where S_{ij} is the size factor for product i and stage j, and B_i is the batch size of product i. The size factor equations are obtained from Tables I and II.

The time T_{ij} required to process product i in batch stage j is given by the expressions in Tables I and II. The operation of the plant is characterized by a cycle time, TL_i, for each product, which corresponds to the limiting time for each product (i.e., the time between two consecutive batches). Therefore:

$$TL_i \geq \frac{T_{ij}}{M_j} \qquad \forall i, \forall j \tag{29}$$

Over the time horizon, H, it is required that the plant processes the amounts, Q_i, of each of the P products. Then:

$$\sum_{i=1}^{P} Q_i \, E_i \leq H \tag{30}$$

where E_i is the inverse of the production rate of product i.

The storage tank allocation decouples the process into two subprocesses upstream and downstream of the tank, so independent batch sizes and limiting cycle times for each subprocess are introduced. Therefore, the previous unique B_i is transformed into batch sizes B_i^j defined for product i in stage j. The size of the intermediate storage tank is obtained using the following expression from Modi and Karimi (1989):

$$VT_j \geq ST_{ij} \, (B_i^j + B_i^{j+1}) - F_j \, (1 - y_j) \quad \forall i, \forall j = 1,\ldots M-1 \tag{31}$$

In (31), ST_{ij} is the size factor for an intermediate storage tank in location j for product i. Binary variables, y_j, are used to select tank allocation that assume the value 1 if the tank is placed in position j, or zero otherwise. Table III shows typical values for ST_{ij} used in this study. Constraint (31) assures that the tank is sized only if it exists. F_j is a valid upper bound such that when y_j is 0 (the tank does not exist), the constraint is trivially satisfied. In particular, the cost minimization will drive $VT_j = 0$. When the tank exists ($y_j = 1$), the lower bound of the tank volume holds. The relationship between the values of batches in successive stages

Table VII. Number of units in parallel per stage obtained with the four models.

Model	Phase	Stage							
		Fer	Mf1	Hom	Mf2	Uf1	Ext	Uf2	Chr
Fixed Factors/no tanks	Out	5	1	1	1	1	1	1	1
	In	1	1	1	1	1	1	1	3
Process Performance/no tanks	Out	5	1	1	1	1	1	1	1
	In	1	1	1	1	1	1	1	2
Fixed factors/with tanks	Out	1	1	1	1	1	1	1	1
	In	1	1	1	1	1	1	1	1
Process performance/with tanks	Out	1	1	1	1	1	1	1	1
	In	1	1	1	1	1	1	1	1

Table VIII. Optimal permeate vessel volumes and semicontinuous associated unit sizes.

Model	Permeate vessel volume			Semicontinuous associated unit size				
	Mf1	Mf2	Chr	Mf1	Hom	Mf2	Uf1	Uf2
Fixed factors, no tanks	11.275	2.819	0.451	14.799	0.955	7.411	112.050	7.423
Process Performance, no tanks	6.479	1.981	0.459	9.991	0.663	8.300	70.282	7.676
Fixed factors, with tanks	8.637	2.159	0.121	17.487	1.048	8.758	101.991	8.238
Process Performance, with tanks	4.289	1.398	0.126	9.053	0.678	7.260	50.348	8.153

is modeled using the following expression (Ravemark, 1995):

$$1 + \left(\frac{1}{\Phi} - 1\right) y_j \leq \frac{B_i^j}{B_i^{j+1}} \leq 1 + (\Phi - 1) y_j \qquad \forall i, \forall j = 1, \ldots, M-1 \qquad (32)$$

If the storage tank does not exist between two consecutive stages ($y_j = 0$), then their batch sizes are constrained to be equal. Otherwise ($y_j = 1$), this constraint is relaxed. Φ is a parameter that corresponds to the maximum ratio allowed between two consecutive batch sizes.

Also, the constraints are written so that the same productivity is used in all stages. Taking into account that the relationship between the cycle time and the batch size ($E_i = TL_i/B_i$) is the same in all the subprocesses, the constraints are explicitly written as functions of E_i. Furthermore, there is a set of constraints that correspond to the upper and lower bounds for all variables involved. A more detailed description has been given by Montagna et al. (2000).

The resulting mathematical optimization model is a nonconvex MINLP (mixed integer nonlinear program). The binary variables are for the structural design of the plant, which are intermediate tank allocation and number of units in parallel. The structural decisions of number of batch units in parallel have been represented as constraints that involve the summation of binary variables (Kocis and Grossmann, 1988).

COMPUTATIONAL RESULTS AND DISCUSSION

To compare the design optimization problem with process performance models to the one with fixed size and time factors, we first solved the mathematical model of the batch plant assigning reasonable estimates for the process variables shown in Table IV. We then replaced these values in the equations in Tables I and II, which leads to fixed size and time factors. Furthermore, we used the same values for the process variables as initial estimates and optimized the process variables. Both models have been solved with and without the allocation of intermediate storage tanks. Overall, four design optimization models have been solved:

- *Model I.* Fixed size and time factors with no intermediate storage tanks.
- *Model II.* Process performance models for size and time factors with no intermediate storage tanks.
- *Model III.* Fixed size and time factors with the allocation of intermediate storage.
- *Model IV.* Process performance models for size and time factors with the allocation of intermediate storage.

Models I to IV were solved with the outer approximation–augmented penalty–equality relaxation code DICOPT^{++} (Viswanathan and Grossmann, 1990) included in the GAMS optimization modeling software (Brooke et al., 1996).

The optimal values of process variables obtained with models II and IV are shown in the Table V. Comparing these to the estimated values in Table IV we note that, aside from $X_{i,\mathrm{mf1}}$, they attained different values depending on the product and plant structure. The optimal values for $X_{i,\mathrm{mf1}}$ are at the common upper bound imposed on this variable to avoid handling a semisolid stream of retentate. On the other hand, the increase in the extent of concentration is very effective in reducing the cost of downstream stages, so this places $X_{i,\mathrm{mf1}}$ at its upper bound.

The estimated values for the washing water ratios at the microfilters were in the 1 to 2 range, with a larger value at microfilter 2 because its feed is more concentrated. The optimal values further expand this difference. The estimates for $X_{i,\mathrm{fer}}$, NP_i, and R_i are usual values for these units, while the optimal values departed from them by approximately 50% to optimize the economic trade-offs.

The results obtained using all optimization models are summarized in Tables VI to X. From Table VI it can be seen that the introduction of intermediate storage tanks leads to a considerable reduction in plant cost. This reduction could be obtained using both approaches (fixed factors and process performance models).

The process performance models provide additional cost savings in plant with respect to fixed factors. This reduction is approximately 15% ($1,505,326 vs. $1,770,418) for the cases with no intermediate storage, and 13% ($800,138 vs. $920,790) with tanks. This gain corresponds to the size reduction of some stages (mainly in those batch units with

Table IX. Optimal intermediate storage tank location and volume.

Model	Volume and location of the intermediate tank			
	After Fer	After Mf2	After Uf1	After Uf2
Fixed factor	32.12	2.82	1.21	0.27
Process performance	31.57	1.89	1.00	0.27

Table X. Stage idle times per product obtained with the four models.

Product	Model[a]	Fer	Mf1	Hom	Mf2	Uf1	Ext	Uf2	Chr
Insulin	I	0.0	0.0	—	—	0.01	3.00	2.16	4.37
	II	0.0	0.0	—	—	0.0	2.54	2.02	3.92
	III	0.0	0.0	—	—	0.0	0.0	0.72	0.93
	IV	0.0	0.0	—	—	0.0	0.0	0.76	0.92
Vaccine	I	0.0	2.40	0.0	0.0	3.26	3.00	0.0	4.32
	II	0.0	0.56	0.0	0.03	2.12	1.89	0.0	3.24
	III	0.0	1.73	0.0	0.0	0.0	0.0	0.0	0.27
	IV	0.0	0.51	0.0	0.0	0.0	0.0	0.0	0.25
Chymosin	I	0.0	0.66	—	—	0.0	3.00	3.00	4.33
	II	0.0	0.49	—	—	0.0	1.92	2.43	3.28
	III	0.0	0.0	—	—	0.0	0.0	1.00	0.06
	IV	0.0	0.51	—	—	0.0	0.0	1.05	0.02
Protease	I	0.0	2.95	0.13	0.12	3.28	3.00	0.85	4.29
	II	0.0	0.81	0.0	0.0	1.82	1.46	0.74	2.80
	III	0.0	2.07	0.0	0.0	0.0	0.0	0.23	0.0
	IV	0.0	1.15	0.0	0.0	0.0	0.0	0.26	0.0

[a]I: fixed factors, no tanks; II: process performance, no tanks; III: fixed factors with tanks; IV: process performance with tanks.

semicontinuous items associated). This size reduction is very reasonable, based on the fact that the process performance models optimization set more appropriate values to the process variables than the constant estimated values for the size and time factors.

The optimal plant configurations corresponding to models II and IV are shown in Figures 2 and 3, respectively. From these figures, and the results expressed in Tables VI to X, it is apparent that the introduction of intermediate storage tanks reduces the number of fermentors in parallel (out of phase) from five to one with both approaches (constant factors and process performance). There is also a reduction in the number of chromatographic columns in parallel (in phase), from three to one for fixed factors and from two to one for process performance models (see also Table VII). These two units are the most expensive in the plant. The allocation of intermediate storage tanks allows for a reduction in equipment volume, cost, and idle times. The units

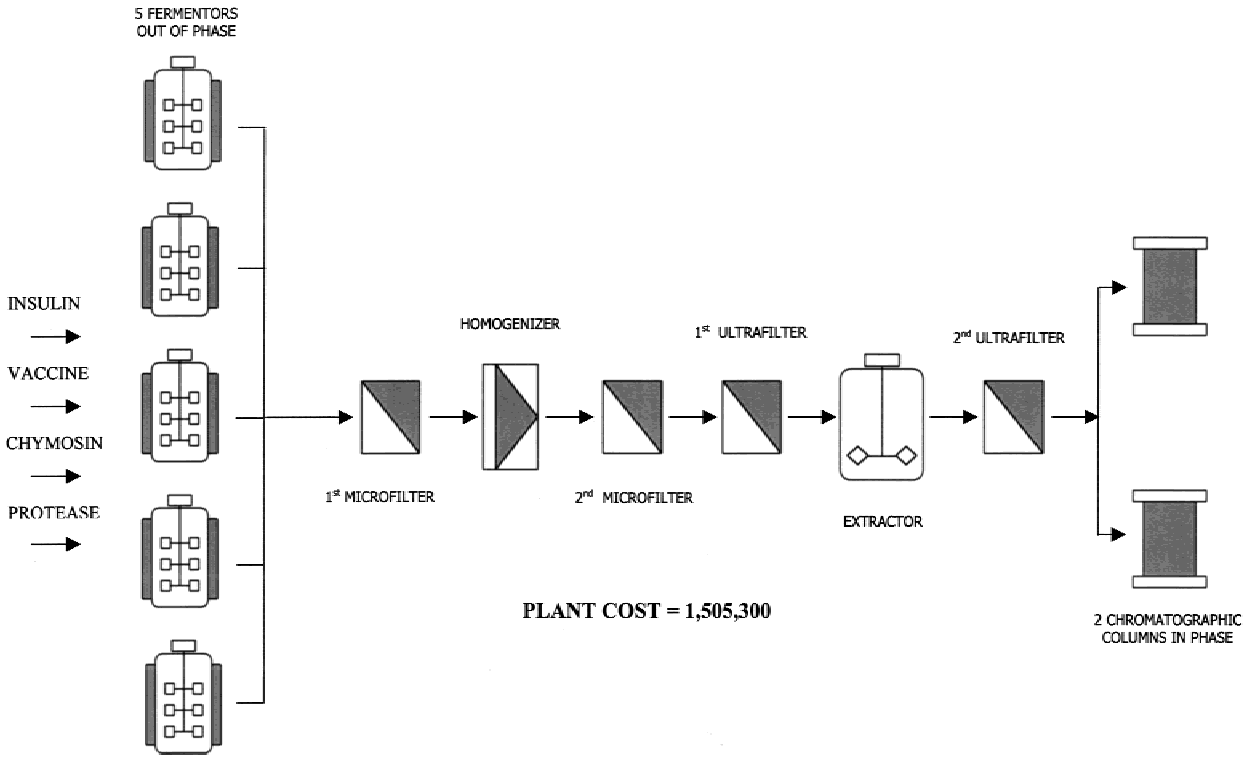

Figure 2. Protein plant design with process performance models without intermediate storage.

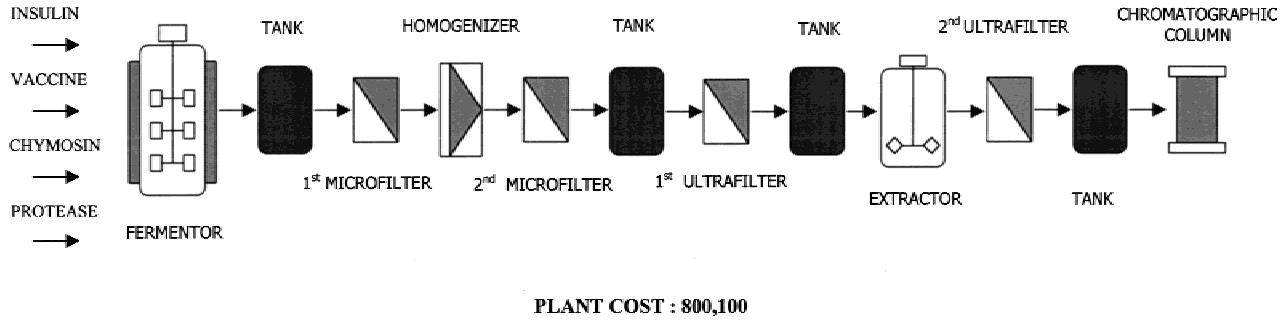

Figure 3. Protein plant design with process performance models and intermediate storage tanks.

operate with reduced size more often, thus better utilizing their capacity.

With respect to the intermediate tank locations, models III and IV allocate storage at the same places. Table IX shows that the tank capacities are very similar in both approaches.

The presence of process performance models allows for further reduction of idle times by adjusting size and time factors and, consequently, better unit capacity. The impact of these models on the idle times is shown in Table I. The table shows the results obtained with the four models presenting differences depending on the product.

First, we notice that the fermentor, which comprises the most expensive stage, has no idle time in any of the models and products. Overall, the models with intermediate tanks (models III and IV) have much smaller idle times than the models that involve only parallel units (models I and II). Finally, in comparing the process performance vs. fixed factors models (e.g., model II vs. model I), in the former we notice that the process variables accommodated themselves at the optimum in such a way that reduced idle times.

The computational performance is summarized in Table XI, which shows model size in terms of number of variables and equations as well as the execution time and number of major iterations to reach the solution with GAMS/DICOPT^{++} (Brooke et al., 1996). From Table XI it can be seen that the addition of the process performance constraints increases the number of variables and equations compared with fixed size and time factor models. The execution time for the design models with process performance constraints is larger by one order of magnitude with respect to the ones with fixed factors, but it takes no longer than 4 CPU minutes for the worst case when using an IBM-PC Pentium 233-MHz platform.

CONCLUSIONS

Process performance models for the optimal design of a multiproduct protein production batch plant have been developed. The model is an MINLP (mixed integer nonlinear program) that considers parallel units in phase and out of phase, as well as the allocation of intermediate storage tanks through 0–1 binary variables. The model also considers semicontinuous and composite units (batch units combined with semicontinuous items assumed here as a single stage).

The performance models are nonlinear and nonconvex and thus more difficult to solve than the fixed factor models, which generate geometric programming design problems. The reported solutions are the best local optima obtained after several different initial points. Note that global optimality is not guaranteed with these models.

It is not trivial to estimate time and size factors for the plant design. This difficulty may be overcome by the proposed process performance models, which predict the size and time factors as a function of process variables. It is more reasonable to estimate values for these process variables than for time and size factors of the stages.

We compared the results when using the performance models to those considering fixed size and time factors. As expected, the results obtained show that the process performance models provide better solutions, because of the extra degrees of freedom that this approach introduces. The cost improvement is on the order of 15% with reduced equipment idle times. As compared with a global solution approach (Bhatia and Biegler, 1996), we expect that the present model will favor the solution of larger problems, because it can eventually be disaggregated into separate moduli. In this sense, process performance moduli may pre-

Table XI. Computational performance of the MINLP problems.

Model	Equations	Variables	Discrete variables	Solution time (seconds)	Major iterations
Fixed factors, no tanks	150	164	80	2	4
Process performance, no tanks	232	253	80	71	5
Fixed factors, with tanks	179	180	87	22	4
Process performance, with tanks	285	297	87	208	4

dict the size and time factors (one for each product) and the multiproduct plant model with fixed factors would optimize the plant structure.

We focused our work on the standard design problem (as presented in Grossmann and Sargent, 1979; Modi and Karimi, 1989; Ravemark, 1995; Ravemark and Rippin, 1998), posed in essentially complete form (e.g., including all available structural optimization decisions), and incorporated its dependence on the process variables through performance models.

The present work represents a step toward adding detail to the multiproduct plant design problem. In doing so, we combined a simple plant model (e.g., single product campaigns disregarding campaign sequence) with simple stage models (e.g., capital costs and level of detail limited to 18 process variables).

With respect to limitations due to the use of simple process models, these are constructed in the context of the hierarchical approach to process design as proposed by Douglas (1988). Models rely on a list of dominant design variables suspected to have the strongest economic impact on the design. So, reasonable values were estimated for all other variables, which become parameters in the simplified models. An important step to be performed afterwards is to check if these assumed values were reasonable, focusing on parameters with the strongest economic impact. To detect them, one needs to perform sensitivity studies. Although sensitivity analysis is beyond the scope of the present study, we may anticipate that the concentration bounds on biomass and proteins have an important economic impact because the process optimization variables tend to be quite close to them at the optimal solutions. Further refinement in selecting these bounds (e.g., by experimental determinations) may prove to be worthwhile.

Furthermore, some pieces of equipment modeled with constant factors might need to be represented with process performance models. For example, in the case of storage after the fermentor, the model assumed that no product is lost. A more detailed model would consider first order product degradation, so that this could discourage storage in favor of duplication. In conclusion, one needs to check the effect of simplified model quality on the design.

With respect to the plant model consideration of single product campaign mode, this assumption is common to all the literature on batch process design, with the exception of Birewar and Grossmann (1989), who assessed a rather simple plant structure (neither intermediate storage nor duplication units). The campaign sequence will certainly affect the overall performance, but its consideration at this level would severely increase model complexity. Thus, campaign sequencing is postponed to an inferior decision level.

Even with these limitations, this is, to our knowledge, the model with the highest level of detail (for plantwide multiproduct processes) available in the literature. Moreover, this approach captures the influence of the variables that establish economic trade-offs between or among stages (Barrera and Evans, 1989) and the interactions among different product requirements. None of these effects can be taken into account with the constant factor model.

J.A. Asenjo thanks Fundacion Andes for donation of all the advanced equipment to the Centre for Biochemical Engineering and Biotechnology, University of Chile and the Millenium Institutes Programme (Project ICM-P99-031F) of MIDEPLAN.

NOMENCLATURE

A	filtration area or cross-section of chromatographic column (m^2)
a_j	cost coefficient for batch (composite) unit in stage j
B_i	batch size of final product i (kg)
B_i^j	batch size of product i in stage j (kg)
$BV_{i,j}$	batch volume of product i in stage j (m^3)
bc	binding capacity of the chromatographic column (kg m^{-3})
C	concentration in diafiltration (C_0, initial; C_f, final) (kg m^{-3})
c_j	cost coefficient for intermediate storage tank allocated in position j
Cap	capacity of the homogenizer (m^3 h^{-1})
D_{ij}	duty factor of semicontinuous item j (size kg^{-1} h)
d_j	cost coefficient for the semicontinuous unit associated with batch unit at stage j
E_i	inverse of the production rate of product i (h kg^{-1})
Grad	gradient of log K vs. %NaCl, defined by Eq. (13)
H	net available production time for all products (h)
K	partition coefficient at the extractor (−)
k_i	ratio of product i in the fermentor (kg product/kg total proteins)
M	number of stages in the plant
M_j	number of batch units in parallel out of phase in stage j
N_j	number of batch units in parallel in phase in stage j
NP_i	number of passes at the homogenizer for product i (−)
P	number of products
q_i	flow rate of diafiltration water of product i (m^3/h)
Q_i	production requirement of product i (kg)
R_i	volumetric ratio of PEG to phosphate phases of product i (−)
R_j	size of semicontinuous item j: A (m^2) or Cap (m^3/h)
rpp_i	ratio (kg total proteins/kg product i)
S_{ij}	size factor of product i in batch item j (size kg^{-1})
ST_{ij}	size factor for product i for intermediate storage tank in location j
T_{ij}	processing time of product i at batch stage j (h)
T_{ij}^0	time factor that accounts for fixed amount of time in T_{ij} (h)
T_{ij}^1	time factor for time proportional to B_l in T_{ij} (h kg^{-1})
TL_i	limiting cycle time of product i (h)
v	linear velocity at the chromatographic column (m h^{-1})
V_j	size of batch item j (m^3)
VT_j	size of intermediate storage tank allocated in position j (m^3)
W	volumetric ratio of diafiltration water to feed at the microfilters (−)
X_{ij}	concentrations of biomass of product i and contaminant proteins in stage j (kg/m^3)
y_j	binary variable that determines the allocation of storage tank in position j

Greek letters

α_j	cost exponent for a batch unit at stage j
γ_j	cost exponent for an intermediate storage tank allocated in position j
ϕ_i	kinetic constant of product i fermentor (h^{-1})
η_{ij}	yield of product i at stage j (−)
θ_j	operating time of semicontinuous item j (h)
Φ	maximum ratio allowed between two consecutive batch sizes (−)

Subscript or superscript

chr	chromatographic column
e	extraction from Ph to PEG phases

ext	extractor
fer	fermentor
hom	homogenizer
in	inlet (stage)
max	maximum concentration of biomass at the fermentor
mf1	microfilter 1
mf2	microfilter 2
out	outlet (stage)
PEG	polyethylene-glycol phase in extractor
Ph	phosphate phase
per	permeate in filters
ret	retentate
uf1	ultrafilter 1
uf2	ultrafilter 2

References

Agena SM, Bogle IDL, Cornish ARH. 1998. Process synthesis for particle separations using centrifuges. Comput Chem Eng 22:351–356.

Andrews BA, Nielsen S, Huang R, Hodgson C, Asenjo JA. 1991. Partitioning, separation and purification of three recombinant proteins in aqueous two-phase systems. Paper presented at the Seventh International Conference on Partitioning in Aqueous Two-Phase Systems, New Orleans, LA, USA.

Andrews BA, Huang RB, Asenjo JA. 1995. Purification of virus like particles from yeast cells using aqueous two-phase systems. Bioseparation 5:105–112.

Asenjo JA. 1990. Separation processes in biotechnology. New York: Marcel Dekker.

Barrera MD, Evans LB. 1989. Optimal design and operation of batch processes. Chem Eng Commun 82:45–66.

Belter PA, Cussler EL, Hu WS. 1988. Bioseparations. Downstream processing for biotechnology. New York: John Wiley & Sons.

Bhatia T, Biegler LT. 1996. Dynamic optimization in the design and scheduling of multiproduct batch plants. Ind Eng Chem Res 35:2234–2246.

Birewar DB, Grossmann IE. Incorporating scheduling in the optimal design of multiproduct batch plants. Comput Chem Eng 13:141–161.

Brooke A, Kendrick D, Meeraus A. 1996. GAMS: A user's guide. San Francisco, CA: GAMS Development Corp.

Douglas JM. 1988. Conceptual design of chemical processes. New York: McGraw-Hill.

Groep ME, Gregory ME, Kershenbaum LS, Bogle IDL. 2000. Performance modeling and simulation of biochemical process sequences with interacting unit operations. Biotechnol Bioeng 67:301–311.

Grossmann IE, Sargent RWH. 1979. Optimum design of multipurpose chemical plants. Ind Eng Chem Proc Des Dev 18:343–348.

Hart RA, Lester P, Reyfsneyder DH, Ogez JR, Builder SE. 1994. Large scale *in situ* isolation of periplasmic IGF-1 from *E. coli*. Biotechnology 12:1113–1119.

Hayenga K, Murphy M, Arnold R, Lorch J, Heinsohn H. 1991. Application of two-phase liquid–liquid extraction to the purification of calf chymosin from *A. awamori.* Presented at the Seventh International Conference on Partitioning in Aqueous Two-Phase Systems, New Orleans, LA, USA.

Huenupi E, Gomez A, Andrews BA, Asenjo JA. 1999. Optimization and design considerations of two-phase continuous protein separation. J Chem Technol Biotechnol 74:256–263.

Kocis GR, Grossmann IE. 1988. Global optimization of nonconvex mixed-integer nonlinear programming (MINLP) problems in process synthesis. Ind Eng Chem Res 27:1407–1421.

Leser EW, Asenjo JA. 1992. Rational design of purification processes for recombinant proteins. J Chromatogr Biomed Appl 584:43–57.

Lowe CR. 1998. New affinity adsorbents for the purification of biopharmaceuticals. Plenary lecture of the IV Iberian Congress on Biotechnology (I Ibero-American Meeting on Biotechnology), Guimaraes, Portugal.

Mistry SL, Kaul A, Merchuk JC, Asenjo JA. 1996. Mathematical modeling and computer simulation of aqueous two phase continuous protein extraction. J Chromatogr A 741:151–163.

Modi AK, Karimi IA. 1989. Design of multiproduct batch processes with finite intermediate storage. Comput Chem Eng 13:127–139.

Montagna JM, Iribarren OA, Galiano FC. 1994. The design of multiproduct batch plants with process performance models. Trans IChemE 72:783–791.

Montagna JM, Vecchietti AR, Iribarren OA, Pinto JM, Asenjo JA. 2000. Optimal design of protein production plants with time and size factor process models. Biotechnol Progr 16:228–237.

Ravemark DE. 1995. Optimization models for design and operation of chemical batch processes. PhD thesis, Swiss Federal Institute of Technology, Zurich, Switzerland.

Ravemark DE, Rippin DWT. 1998. Optimal design of multiproduct batch plant. Comput Chem Eng 22:177–183.

Salomone HE, Iribarren OA. 1992. Posynomial modeling of batch plants. Comput Chem Eng 16:173–184.

Salomone HE, Montagna JM, Iribarren OA. 1994. Dynamic simulations in the design of batch processes. Comput Chem Eng 18:191–204.

Samsatli NJ, Shah N. 1996a. An optimization based design procedure for biochemical processes. Part I: Preliminary design and operation. Trans IChemE 74:221–231.

Samsatli NJ, Shah N. 1996b. An optimization based design procedure for biochemical processes. Part I: Detailed scheduling. Trans IChemE 74:232–242.

Schmidt AS, Ventom AM, Asenjo JA. 1994. Partitioning and purification of a-amylase in aqueous two-phase systems. Enzyme Microb Technol 16:131–142.

Sharif M, Shah N, Pantelides CC. 1998. On the design of multicomponent batch distillation columns. Comput Chem Eng 22(suppl):S69–S76.

Smith EMB, Pantelides CC. 1995. Design of reaction/separation networks using detailed models. Comput Chem Eng 19(suppl):S83–S88.

Uesbeck F, Samsatli NJ, Papageorgiou LG, Shah N. 1998. Robust optimal fermentation operating policies. Comput Chem Eng 22(suppl):S167–S174.

Viswanathan JV, Grossmann IE. 1990. A combined penalty function and outer approximation method for MINLP optimization. Comput Chem Eng 14:769–782.