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# Fermenter balancing for semi-continuous, multi-tank mammalian cell culture processes

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**Abstract**

Process design and scheduling for optimal economic return from semi-continuous, multi-stage mammalian cell culture processes are considered. Heuristics are identified for the optimization of the production phase duration for processes that display linear accumulation of a monoclonal antibody. These heuristics are used to gauge the validity of the commonly accepted process design assumption that harvest titre be maximized irrespective of the fermenter configuration. The heuristics are further tested against performance predictions for non-linear cell culture data. We show that, in general, the choice of production schedules that result in a balanced utilization of fermentation vessels leads to higher economic return and reduced wastage of expensive culture media and consumables. © 1997 Elsevier Science S.A.

*Keywords:* Fermenter scheduling; Antibody production; Bioprocess optimisation

## 1. Introduction

Line balancing [1] and production scheduling methods [2,3] have been shown to develop low cost operating heuristics for manufacturing processes. Such procedures consider the flow of work elements through a train of serial workstations with fixed [4] and variable [5] flow-sheet topologies. Optimal process performance results from correctly assigning discrete, fixed time-span work elements to workstations to minimize the unit manufacturing cost.

It will often be necessary to develop new biological processes that yield optimal performance with a particular plant layout. Unit operation time-spans may then be variables to be defined during development, with regard to product specifications and plant constraints. This is commonly the case for biopharmaceuticals produced by multi-stage culture of mammalian cells and purified through discrete downstream processing workstations in existing plant. Their high value within niche markets has previously detracted from considerations of manufacturing efficiency but high development costs, competing therapies and healthcare reform pressures have encouraged pharmaceutical companies to improve capital utilization [6]. Therefore, it will be important to devise strategies that minimize unit manufacturing costs in the face of regulatory constraints that require process shapes to be frozen at an early stage of development and impede major plant modifications to accommodate new flow-sheet topologies. Thus, heuristics are needed to design processes that can be optimally implemented in existing plant.

Johnson [7] considers the dynamic optimization of fed-batch culture processes, while Samsatli and Shah [8] discuss secondary process scheduling for an intracellular enzyme produced in a single fermenter. We focus here on a different topic, and seek heuristics to optimize the added value from primary therapeutic protein manufacturing by semi-continuous, multi-tank cell culture processes that operate in fixed, existing plant. Through two case-studies, we explore guidelines by which unsatisfactory process operating strategies can be rejected at an early stage of development, avoiding expenditure of time and cost. We show for these cases that those operating strategies which correctly balance fermenter utilization yield optimal economic return and process operability.

### *1.1. Biopharmaceuticals manufacturing procedures*

Generic procedures for primary biopharmaceuticals manufacture by mammalian cell culture are as follows. Cells are first revived from a frozen cell bank vial into a few millilitres of growth medium and then cultivated through a train of progressively larger fermentation vessels until culture in a principal growth vessel is achieved. This vessel may be up to 15 000 l in size and the entire process of cell culture from revival to its inoculation may take in excess of 35–50 days,


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depending on the growth characteristics of the particular cell line [9,10].

The industry has subsequently employed two patterns of product harvesting. Some companies harvest a single batch of product from the cells of each such revival, using either a conventional batch or extended, fed-batch culture process. The principal growth vessel usually serves as the production vessel and, after harvesting, the vessel is cleaned and sterilized to receive cells from the next revival. This approach aims to overcome regulatory concerns over the relationship between product integrity and the duration of cells in culture, because each product harvest is taken from cells of similar age. This argument, and the manufacturing productivity of the approach, rely on precise scheduling to ensure that cells from successive revivals reach the production vessel on demand, because production delays are otherwise incurred. In theory, this schedule might be precisely determined from cell growth studies. In practice, it is difficult to predict, because of the imprecise nature of cell growth, the errors associated with measuring cell densities, and the random (unintentional) occurrence of vessel contamination. (See, for example, Ref. [1] for a discussion of line effectiveness for such paced lines without buffers.)

We address a different pattern of production in which cell culture is sustained in the principal growth vessel through successive 'draw-and-fill' cycles (this vessel is then commonly called a Solera tank, by analogy to traditional fermentation processes). With this approach, a certain proportion of cells are (ideally) fed from the Solera to one of a number of production vessels at the end of a fixed-duration growth cycle. Both vessels are then fully charged with sterile-filtered growth media; the Solera vessel to commence a new growth cycle, and the production vessel to allow the formation of product by either a batch or extended culture process. At the end of the production cycle, which would be of several days duration, the media from the production vessel are harvested so that the product can be extracted and purified through downstream processing. The production vessel is then cleaned and sterilized to be ready to receive a fresh inoculum from the Solera tank at the completion of the Solera cycle.

This approach is more economical in the utilization of capital assets, operating personnel, media and other cell culture consumables; gives higher plant productivities, and can be more precisely scheduled than the method of a single harvest per revival. However, rigorous validation studies are required to demonstrate batch-to-batch product integrity and to establish the maximum permitted duration of cells in culture after revival [11].

### *1.2. Process synthesis*

Fixed costs accrue uniformly in time for facility depreciation, maintenance and labour, while variable costs arise from the use of utilities and the purchase of media, filters and other materials which are largely batch related. The Solera cycle time is determined by biological factors, because it must be of the correct duration to ensure a reliable supply of active and viable cells with sufficient density to inoculate the production vessels. The duration of the production cycle can be chosen from a range of values, provided that it is sufficiently long to ensure an adequate harvest titre, while not being so long that cell viability falls to some low level at which the accumulation of culture contaminants (cell debris, host cell proteins, DNA and enzymes) would pose an intolerable burden on downstream processing.

In practice, plant operability considerations demand that vessel inoculation and turnround be accomplished on a regular basis. Therefore, to avoid irregular shift patterns, production and Solera cycles are normally selected to be an integer number of days. In financial terms, longer production cycle times incur higher fixed costs per batch but, provided product titres increase steadily up to the point of harvest, these higher fixed costs might be mitigated by the higher productivity for fixed variable costs.

The duration of production vessel and Solera cycles must be carefully balanced for a given tank configuration. If a production vessel is not available to receive cells at the end of a Solera cycle, then the Solera contents must be disposed of to a biological kill system, because delays in transferring cells would lead to a loss of culture activity. Such disposals waste costly cell culture materials and incur decontamination costs.

Two classes of optimal process synthesis are evident. For processes that are to be transferred to a new manufacturing facility, the choice of production-phase duration is largely unconstrained, because the number of Solera and production vessels can be chosen to optimize productivity (except where the capital costs of facility construction might impinge on the product business case, which is not considered here). More commonly, processes are developed for transfer to existing facilities when optimal process synthesis is constrained by the available tank configuration, because only a discrete set of production-phase durations precisely matches the seeding pattern from Solera tanks. Because the time allocated for drug development projects is driven largely by 'speed to market', decisions must be made about the duration of the production vessel cell culture cycle at an early stage of development. Various financial and practical considerations must then be weighed on the basis of meagre laboratory data.

We seek here to establish simple heuristics to guide such decisions. In particular, we test the commonly adopted heuristic that the production-phase duration should be chosen to maximize the harvest titre and cell viability, irrespective of tank configuration.

## 2. Characterization of production patterns

To illustrate our arguments, the tank configuration shown in Fig. 1 is considered throughout. With this configuration, a single Solera tank feeds cells to either of two equal-volume production vessels, or to the drain for decontamination and

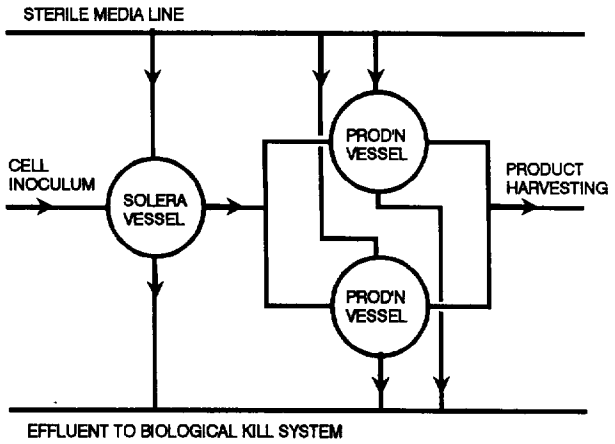


Fig. 1. General arrangement of Solera and two production vessels that have been considered in the case-studies presented here, together with their connections to harvesting and decontamination systems.

disposal in the biological kill system. Solera tank contamination is not considered here and the production vessels are assumed to feed either to a centrifuge for harvesting or to a kill system if contaminated.

Typical production schedules for this configuration are shown schematically in Fig. 2, where transfers between the vessels, to drain or to harvesting, are represented by links between nodes on a time continuous network [12]. Fig. 2 illustrates how a Solera cycle of 2 days (hereafter denoted S2) would be operated with a production cycle of either 3 or 4 days (P3 or P4), allowing a minimum of 1 day for harvesting and turnround of each production vessel. Hereafter, this plant configuration and these production patterns will be denoted by the notation {1S2, 2P3} and {1S2, 2P4} respectively.

Fig. 2 shows that different durations of the production cycle affect the efficiency of utilization of the Solera batches. For the pattern {1S2, 2P3}, the availability of fresh cell inocula from the Solera vessels precisely matches the demand from the production vessels. Every Solera batch is used to seed a production vessel and no Solera batch is disposed of directly to the kill system. Furthermore, no production vessel lies idle for longer than the single day required for clean-down and sterilization. This production pattern results in a balanced utilization of fermenters.

In contrast, for the production pattern {1S2, 2P4}, every third Solera batch must be disposed of to the kill system, because no production vessel is available for seeding when the Solera batch reaches that stage in its cycle of operation (see Fig. 2). When a production vessel does become available to receive a fresh inoculum, no Solera batch is at the correct phase of its culture cycle to provide cells. Production vessels then lie unproductive for two days between batches. This production pattern results in poor balancing of fermenter utilization, and the asynchronous flow of materials is more costly for media and sterilizing filters (for lost Solera batches), and incurs fixed cost penalties while production vessels lie idle. These costs may only be offset if sufficient extra product is produced by the longer production cycle.

Fig. 2 also shows that each production pattern is characterized by a certain time duration after which the sequence of operations is repeated. This repeat duration ($R$) is four days for {1S2, 2P3} and six days for {1S2, 2P4}. During the repeat

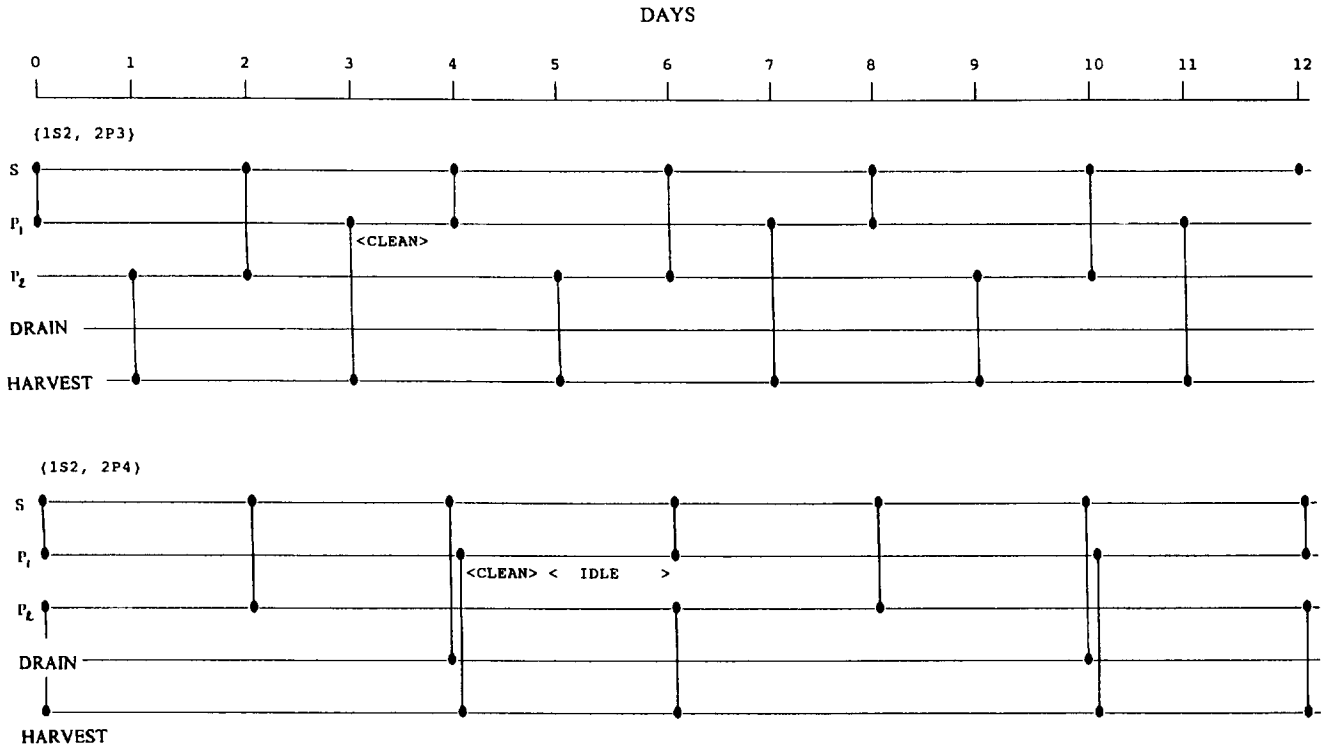


Fig. 2. Network that depicts the schedule of cell transfers between Solera vessel (S) and the production vessels ($P_1$ and $P_2$), and from the cell culture vessels to drain or product harvesting for the typical production patterns {1S2, 2P3} and {1S2, 2P4}.

period, the number of harvests ($h$) is equal to the number of production vessels. If the number of non-productive disposals of the Solera vessel to the kill system within a repeat period is denoted $d$ and the duration of a Solera cycle is $S$, then we have

$$R=(h+d)S \tag{1}$$

Because any pattern must contain at least one production cycle and one vessel turnround cycle, we have

$$R-P\geq T \tag{2}$$

where $P$ is the duration of the production phase and $T$ is the minimum time required to clean and sterilize a production tank ready for the start of a new batch. Operationally, production vessel turnround can be reliably completed in 1 day, so Eqs. (1) and (2) give that

$$(h+d)S-P\geq 1 \tag{3}$$

Both $h$ and $d$ must be integers, as are $S$ and $P$, for practical purposes. Therefore, the constraint of Eq. (3) can be used to calculate the values of $h$ and $d$ that correspond to the minimum value of $R$ for a given production pattern. For illustration, Table 1 gives values of $h$, $d$ and $R$ for a range of typical production patterns. We note that balanced lines have $R-P=1$.

## 3. The linear performance problem

Consider the case where the product of cell culture accumulates linearly with time during the production cycle. This case is considered for analytical expediency to identify putative design heuristics. It is generally artificial, because product accumulation from mammalian cell culture is commonly a non-linear process, but it has an approximate validity for actively growing cultures over relatively short time-spans.

Table 1
Typical values of linear performance parameters for various durations of production phase for the class of schedules {1S2, 2P } and {1S3, 2P }, employing the economic parameters of Table 2

| | $P$ | $d$ | $R$ | $P'$ | $d'$ | $Y'$ | $(R-P)$ |
|---|---|---|---|---|---|---|---|
| {1S2, 2P } | 3 | 0 | 4 | 0.75 | 0 | 25.0 | 1 |
| | 4 | 1 | 6 | 0.67 | 0.17 | 22.3 | 2 |
| | 5 | 1 | 6 | 0.83 | 0.17 | 28.7 | 1 |
| | 6 | 2 | 8 | 0.75 | 0.25 | 25.8 | 2 |
| | 7 | 2 | 8 | 0.88 | 0.25 | 30.8 | 1 |
| | 8 | 3 | 10 | 0.8 | 0.3 | 27.9 | 2 |
| | 9 | 3 | 10 | 0.9 | 0.3 | 31.9 | 1 |
| {1S3, 2P } | 3 | 0 | 6 | 0.5 | 0 | 15.7 | 3 |
| | 4 | 0 | 6 | 0.67 | 0 | 22.3 | 2 |
| | 5 | 0 | 6 | 0.83 | 0 | 29.0 | 1 |
| | 6 | 1 | 9 | 0.67 | 0.11 | 22.7 | 3 |
| | 7 | 1 | 9 | 0.78 | 0.11 | 27.1 | 2 |
| | 8 | 1 | 9 | 0.89 | 0.11 | 31.6 | 1 |
| | 9 | 2 | 12 | 0.75 | 0.17 | 26.2 | 3 |

For example, we have found that a linear pattern approximates the increase in titre of a humanized monoclonal antibody (MAb-A) from a recombinant CHO cell culture in a production vessel over the period from 2–5 days (see Fig. 3). In this particular example, the batch culture was harvested after five days, because cell viability had by then fallen below the target minimum level. If the culture had proceeded for longer, then the antibody accumulation rate would eventually have fallen. In this case, the linear assumption would incorrectly bias in favour of longer production cycles in the analysis that follows.

Let the net total financial gain that accrues from the manufacture of purified bulk product from harvests during the repeat period $R$ be denoted by $Y$; let the internal value of purified bulk product that derives from each harvest be $\alpha$; let the variable cost of primary processing and purification for material from each production batch harvest be $\beta$; let the variable costs of media, materials and decontamination that are incurred by disposal of a Solera batch to the kill system be $\delta$; and let the fixed cost per day of facility operation be $\tau$. To a reasonable approximation, for modelling, we have

$$Y=(\alpha-\beta)h-\delta d-\tau R \tag{4}$$

Assuming that the installed secondary processing capacity is sufficient to process whatever quantity of antibody that can be obtained from a production harvest with equal cost efficiency and yield, then $\alpha$ is proportional to the harvest titre, which is assumed here to be a linear function of the production cycle duration, i.e. we have

$$\alpha=\alpha_0 P \tag{5}$$

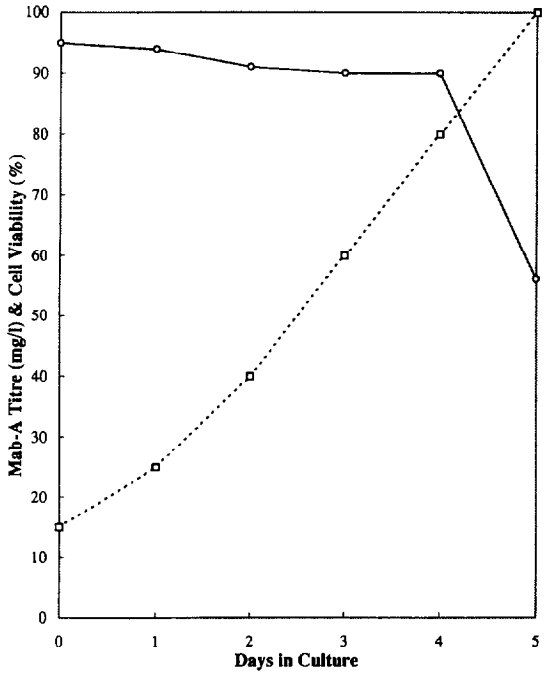


Fig. 3. Accumulation of the monoclonal antibody MAb-A (□) and associated cell viability (○) during batch culture of a recombinant CHO cell line according to the production pattern {1S2, 2P }.

where $\alpha_0$ is the daily rate of accumulation of internal (company) value of purified bulk product which derives from a single production batch harvest.

To a good approximation, each production batch—and the subsequent purification processes—use the same quantity of media, filters and other raw materials, and incur the same costs for quality assurance. Then, the parameters $\beta$, $\delta$ and $\tau$ are not functions of the harvest titre but are fixed per batch. Also, because the repeat periods are of varying duration for different production patterns, the appropriate objective function for performance optimization is the daily average financial gain $Y'(=Y/R)$. Thus, we have

$$Y' = \alpha_0 hP' - \beta h/R - \delta d' - \tau$$

where $P' = P/R$ and $d' = d/R$. From Eq. (1), $1/R = 1/(hS) - d'/h$, so that

$$Y' = \alpha_0 hP' + (\beta - \delta)d' - (\tau + \beta/S) \quad (6)$$

$Y'$ is to be optimized subject to the inequality constraint of Eq. (2), which can be rewritten as

$$1 - P' - 1/(hS) + d'/h \geq 0 \quad (7)$$

In addition, the duration of the production batch is limited by the requirement to maintain cell viability above some critical value. If this duration is denoted $\phi$, then the further constraint is imposed that $P \leq \phi$, or

$$P' \leq \phi[1/(hS) - d'/h] \quad (8)$$

In addition, $P' \geq 0$ and $d' \geq 0$. Inferences about optimal production patterns for a range of production situations can be drawn from this theory.

### 3.1. Case-study: MAb-A production

Consider first the optimization of the objective function $Y'$ in the continuous $(P', d')$ space for the manufacture of MAb-A with a Solera cycle of 2 days and the production pattern {1S2, 2P }. The fixed and variable costs of production for purified monoclonal antibodies in a licensed manufacturing facility have been evaluated, from which the typical values of the economic parameters required to optimize $Y'$ are given in Table 2 (scaled by a factor of $10^4$ for brevity). For these parameters, Eq. (6) can be written as

$$Y' = 40P' + 3d' - 5 \quad (9)$$

Table 2
Scaled values of economic parameters employed for the optimization of the linear MAb-A production and the non-linear production of MAb-B

| | Linear model economic parameters ($\times 10^{-4}$) | Non-linear model economic parameters ($\times 10^{-4}$) |
|---|---|---|
| $\alpha_0$ | £20 per day | £2 $m^{-3}$ $g^{-1}$ |
| $\beta$ | £4 | £4 |
| $\tau$ | £3 per day | £3 per day |
| $\delta$ | £1 | £1 |

For MAb-A production, the maximum permissible value of $\phi$ is 4 (see Fig. 3). Therefore, Eq. (9) must be optimized subject to the constraints of Eqs. (7) and (8), i.e.

$$0.75 - P' + 0.5d' \geq 0 \quad (10)$$

$$1 - 2d' - P' \geq 0 \quad (11)$$

$$P' \geq 0 \quad (12)$$

$$d' \geq 0 \quad (13)$$

The constraints of Eqs. (10)–(13) define a feasible region in the continuous $(P', d')$ space in which $Y'$ is to be optimized (shown as the region ABCD in Fig. 4). Consider the variation of $Y'$ along the lines AB and AC for the economic parameters of Table 2. We have

$$\left(\frac{dY'}{dd'}\right)_{AB} = 23, \quad \left(\frac{dY'}{dd'}\right)_{AC} = -77$$

As $d'$ increases, $Y'$ rises moderately along AB and then falls more rapidly along AC. Therefore, the optimum value of $Y'$ occurs at the vertex A (i.e. when $P' = 0.8$, $d' = 0.1$ and $Y' = 27.3$) at the boundary of the feasible region (as is also predicted by Dantzig's simplex algorithm).

Now consider the operability constraint that the production and Solera cycles be an integer number of days to avoid irregular shift patterns. A comparison of the optimal values of $P'$ and $d'$ with the practical alternative production schedules (i.e. with integer values of $P$ to retain regular shift patterns) shows

{1S2, 2P3}: $P' = 0.75$, $d' = 0$, $Y' = 25.0$

{1S2, 2P4}: $P' = 0.67$, $d' = 0.17$, $Y' = 22.3$

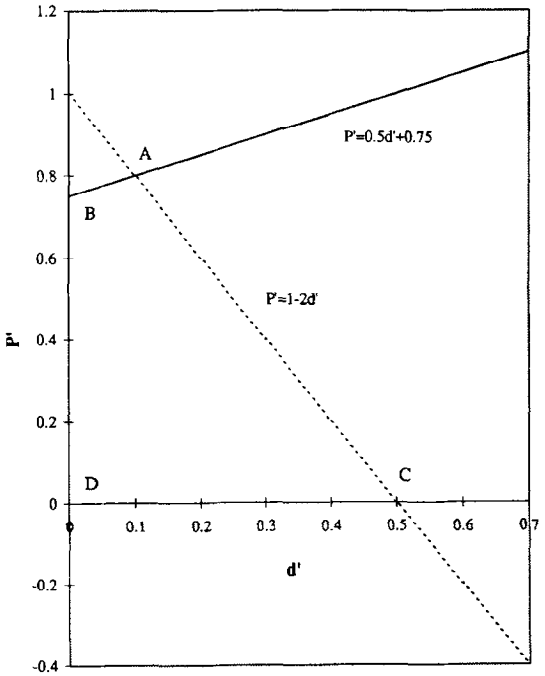


Fig. 4. Feasible region (ABCD) in $(P', d')$ space for the linear optimization of $Y'$ for the class of production patterns {1S2, 2P }, employing the economic parameters of Table 2.

For the economic parameters employed, and with the Solera and production vessel configuration considered here, the production-phase process of duration 3 days provides a higher economic return than does the process of duration 4 days, even though it yields a lower harvest titre. The generally accepted heuristic that the production phase should be selected as long as possible, irrespective of tank configuration, fails for this linear model. Nevertheless, this assumption is the basis for much current research in mammalian cell culture to extend the period over which product can accumulate while maintaining the cell viability and productivity above some critical target level.

Various fed-batch and extended culture techniques have been proposed to increase $\phi$. Their effect is to rotate the boundary line AC (Fig. 4) clockwise about the point C, moving the vertex A to the right along the line AB. Because $Y'$ increases monotonically with both $P'$ and $d'$, its optimal value also increases with increasing $\phi$. From Eqs. (7) and (8), vertex A has coordinates $((1/S-h/(\phi+1), \phi/(\phi+1))$. As $\phi$ tends to infinity, $Y'$ tends to the maximum limiting value of $(\alpha_0 h-\delta/S-\tau)$ or 36.5 for the parameters of Table 2. This time continuous trend is depicted in Fig. 5 for the production pattern {1S2, 2P } (and in Fig. 6 for the pattern {1S3, 2P }).

The practical constraint of regular shift operation, which restricts $P$ and $d$ to integer values, does not allow the trajectory of allowable operation patterns to follow that of $Y'$ for continuous variation of $P$ in Fig. 5, because not all integer values of $P=\phi$ lie at the vertex A. Production patterns for {1S , 2P } and their corresponding values of $Y'$ and $R-P$ for a production phase of up to 9 days are shown in Table 1. Only those practical operating patterns for which $R-P=1$ lie at the vertex A and display local optima in $Y'$ that fall on the optimal trajectory in Fig. 5. Other patterns with $R-P>1$

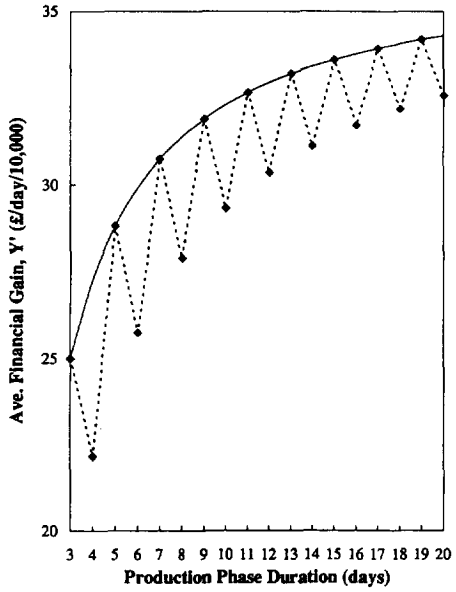


Fig. 5. Variation of $Y'$ for continuous (———) and permissible, discrete (– – –) values of $P$ with the production patterns {1S2, 2P }, indicating the asymptotic trend as $P\rightarrow\infty$.

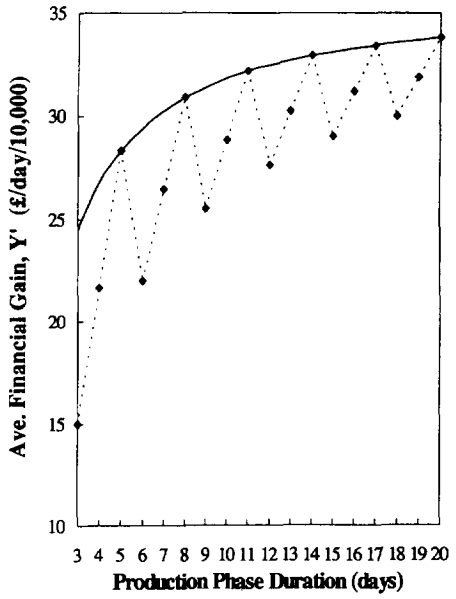


Fig. 6. Variation of $Y'$ for continuous (———) and permissible, discrete (– – –) values of $P$ with the production patterns {1S3, 2P }, indicating the asymptotic trend as $P\rightarrow\infty$.

lie off the optimal trajectory of $Y'$ and, in certain cases, $Y'$ decreases as $\phi$ is increased. This effect is shown more dramatically for the pattern {1S3, 2P } (see Fig. 6), where increasing $\phi$ from 5 to 6 or even 7 days (say) results in a less economic process. Only when $\phi$ is increased from 5 to 8 days is the financial return improved.

### 3.2. *Effect of variable cost changes on optimal production pattern*

The sensitivity of the optimal product pattern to decreases in product value or cell productivity (i.e. a fall in the value $\alpha_0$), or increases in raw materials costs (increases in $\beta$) can be determined. The effect of each of these changes can be visualized in Fig. 4. The optimal production pattern remains close to the vertex A until the gradient of contours of constant $Y'$ becomes parallel to one of the bounding lines AB or AC. At this point, the optimal pattern switches to either vertex B or C, accordingly.

In algebraic terms, Eq. (6) gives that

$$\left(\frac{dP'}{dd'}\right)_{Y'}=\frac{\delta-\beta}{\alpha_0 h} \tag{14}$$

Along AB, we have from Eq. (7) that

$$\left(\frac{dP'}{dd'}\right)=\frac{1}{h}$$

and, along AC, Eq. (8) gives that

$$\left(\frac{dP'}{dd'}\right)=\frac{-\phi}{h}$$

Therefore, the optimal value changes when $\alpha_0=\delta-\beta$ or when $\alpha_0=(\beta-\delta)/\phi$.

Consider changes in the optimal production pattern that result from variations in $\alpha_0$ for values of the other economic parameters in Table 2. This could only occur if $\alpha_0 = -3$ or 0.75. The case of $\alpha_0 = -3$ is impractical, because $\alpha_0$ cannot take a negative value. In practice, $\alpha_0$ might change to 0.75 but such an extreme fall in product value is unrealistic; were it to occur, then the optimal value of $(P', d')$ would move to the vertex C on the line $P' = 0$, corresponding to a negative value of $Y'$. Because such a production process is unrealistic, the {1S2, 2P3} production pattern is optimal for all sensible values of product value (provided raw materials costs are unaltered).

Consider next changes in the parameter $\beta$ or $\delta$. The optimal production pattern would switch to the vertex C (i.e. $P' = 0$) if, for the current product value, the value of the objective coefficient $(\beta - \delta)$ increased to a value of 80. Again, such a dramatic increase in raw materials costs is wholly unrealistic and would result in an uneconomical process.

### 3.3. Robustness of optimal production pattern to batch failures

Consider now the robustness of the optimal process pattern to batch failures that result from contamination of a production vessel. First, suppose that, on failure, the run is aborted, the production vessel contents disposed of to the biological kill system, and the vessel cleaned and sterilized ready for the commencement of the next scheduled production batch (which might not necessarily correspond to the earliest possible next production batch). Suppose that, on average, one in every $\kappa$ such batches fails, irrespective of the duration of the production cycle (a reasonable assumption, because failures commonly result from operator error during tank turnround rather than from chance events during vessel operation).

During $\kappa$ repeat periods, $\kappa h$ production batches are run, of which $h(\kappa - 1)$ succeed and $h$ fail. Only the successful batches are processed to purified bulk product, incurring cost $\beta$ per batch and generating financial return. The failed batches incur costs for media, filters and other consumables that are approximately equal to those incurred in the disposal of Solera tank contents (i.e. $\delta$). In this case, the modified average daily economic gain $(Y'' = Y/\kappa R)$ is given by

$$Y'' = \alpha_0 h\left(\frac{\kappa - 1}{\kappa}\right)P' + (\beta - \delta)\left(\frac{\kappa - 1}{\kappa}\right)d' - \left[\tau + \frac{\beta}{S}\left(\frac{\kappa - 1}{\kappa}\right) + \frac{\delta}{S\kappa}\right] \quad (15)$$

An identical equation to Eq. (14) can be derived from Eq. (15), so the choice of optimal production pattern is unaffected by $\kappa$ or the failure of certain production batches. In other words, the selection of {1S2, 2P3} in preference to {1S2, 2P4} is robust to production batch failures; however, profitability falls. For example, the limiting value of $Y''$ as $\phi$ tends to infinity is now $[\alpha_0 h(\kappa - 1)/\kappa - \delta/S - \tau]$. For the parameters of Table 2 and the production pattern {1S2, 2P3}, the maximum value of $Y''$ is 34.5 for a 5% batch failure rate (compares with 36.5 for no batch failures).

Next, suppose that, on failure, the production batch is aborted and the vessel made ready for immediate inoculation from the first available Solera batch, which might otherwise have been disposed of to effluent. This strategy permits rapid restarting of production and avoids vessel downtime, but is only an option for those production patterns with redundant Solera cycles (i.e. the option would not exist for {1S2, 2P3} but might exist for {1S2, 2P9} with a 60% probability). This adjustment of production schedules does not affect the selection of optimal pattern but corresponds to a phase shift in the schedule. Therefore, the optimal pattern is robust.

## 4. The non-linear performance problem; MAb-B production

The analytical predictions of the linear model are now compared with the behaviour of a non-linear antibody production. Fig. 7 shows the accumulation of a second monoclonal antibody (MAb-B), together with the viable and non-viable cell densities during extended culture of a recombinant NS0 cell line. In these extended cultures, the period for which the cell viability was maintained above the critical level was prolonged to permit the accumulation of higher antibody titres.

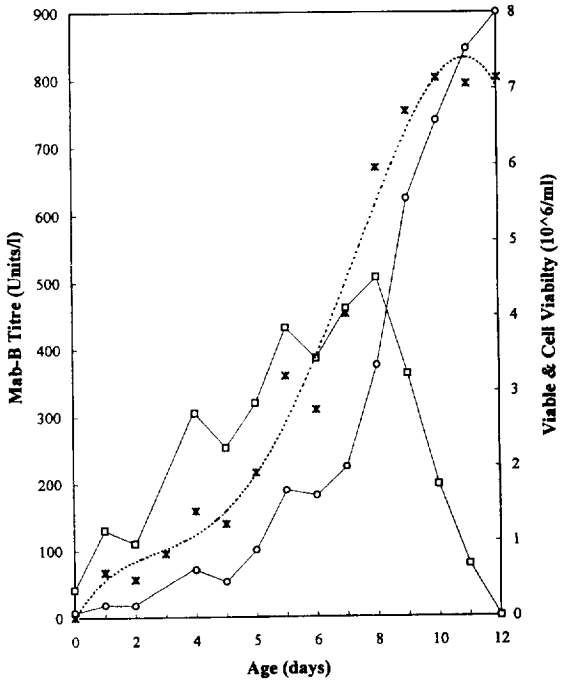


Fig. 7. Accumulation of the monoclonal antibody MAb-B (★), viable cell density (□) and non-viable cell density (○) during extended culture of a recombinant NS0 cell line according to the pattern {1S2, 2P }. The trend of the fourth-order polynomial least-squares fit to the MAb-B titre variation is also indicated (– – –).

Eq. (4) remains valid, while Eqs. (5) and (6) become

$$\alpha = \alpha_0 f(P) \quad (16)$$

$$Y' = \alpha_0 f(P)/S + [\beta - \delta - \alpha_0 f(P)]d' - (\tau + \beta/S) \quad (17)$$

Eq. (17) is to be optimized subject to the constraints of Eqs. (2), (3), (12) and (13). For ease of calculation and to interpolate the data, we correlated the antibody production data in Fig. 7 using a fourth-order polynomial. This provides an analytical form for the concentration of MAb-B as a function of culture duration, i.e.

$$f(P) = \sum_{i=0,4} a_i P^i$$

(see Table 3).

Using the same $\beta$, $\delta$ and $\tau$ values as those in Table 2 (but now with $\alpha_0 = 2$ to correct the units but maintain the same specific value of antibody as that implicit in Table 2 for MAb-A), we have again considered the set of production patterns {1S2, 2P }, for which acceptable sets of $P$, $d$ and $R$ are the same as those given in Table 1, together with the set {1S3, 2P }, for which sets of $P$, $d$ and $R$ are given in Table 5. The corresponding values of $Y'$ for $P(=\phi)$ from 3 to 17 days (Tables 4 and 5) are plotted in Fig. 8.

Consider the {1S2, 2P } pattern, first without restriction on $\phi$. There is initially a marked drop in $Y'$ as $P$ is increased from 3 or 5 days (for which $R-P=1$) to 4 or 6 days ($R-P=2$), which parallels the situation for linear production. After this, the average daily financial gain increases steadily to a sharp maximum at $P=13$ days, which corresponds to the point at which the antibody titre plateaus (Fig. 7). This behaviour reasonably supports the accepted heuristic regarding the choice of production phase length. However, the peak viable cell density occurs at 8 days and the cell viability falls rapidly below an acceptable level immediately thereafter at 9 days. If constraint Eq. (3) is applied with $\phi = 8$ days, then harvesting must occur well short of the maximum $Y'$.

Next, consider the {1S3, 2P } pattern. The trend in $Y'$ is now more erratic with local optima in $Y'$ at 5, 8 and 11 days,

Table 3
Values of the fourth-order polynomial [a] coefficients that describe the temporal variation of MAb-B antibody titre during fed-batch culture

| $a_4$ | $a_3$ | $a_2$ | $a_1$ | $a_0$ |
|---|---|---|---|---|
| −0.167 | 4.689 | −38.238 | 141.18 | −108.56 |

[a] $f(P) = \sum_{i=0,4} a_i P^i$.

Table 4
Values of non-linear performance parameters for various durations of production phase for the class of schedules {1S2, 2P }, employing the economic parameters of Table 2

| $P$ | $d$ | $R$ | $d'$ | $R-P$ | $f(P)$ | $Y'$ |
|---|---|---|---|---|---|---|
| 3 | 0 | 4 | 0 | 1 | 83.93 | 78.93 |
| 4 | 1 | 6 | 0.17 | 2 | 101.75 | 63.33 |
| 5 | 1 | 6 | 0.17 | 1 | 123.27 | 77.68 |
| 6 | 2 | 8 | 0.25 | 2 | 158.60 | 75.05 |
| 7 | 2 | 8 | 0.25 | 1 | 213.88 | 102.69 |
| 8 | 3 | 10 | 0.30 | 2 | 291.20 | 112.38 |
| 9 | 3 | 10 | 0.30 | 1 | 388.69 | 151.38 |
| 10 | 4 | 12 | 0.33 | 2 | 500.44 | 162.81 |
| 11 | 4 | 12 | 0.33 | 1 | 616.56 | 201.52 |
| 12 | 5 | 14 | 0.36 | 2 | 723.16 | 202.69 |
| 13 | 5 | 14 | 0.36 | 1 | 802.32 | 225.30 |
| 14 | 6 | 16 | 0.38 | 2 | 832.14 | 204.16 |
| 15 | 6 | 16 | 0.38 | 1 | 786.72 | 192.80 |
| 16 | 7 | 18 | 0.39 | 2 | 636.13 | 137.53 |
| 17 | 7 | 18 | 0.39 | 1 | 346.47 | 73.16 |

Table 5
Values of non-linear performance parameters for various durations of production phase for the class of schedules {1S3, 2P }, employing the economic parameters of Table 2

| $P$ | $d$ | $R$ | $d'$ | $R-P$ | $f(P)$ | $Y'$ |
|---|---|---|---|---|---|---|
| 3 | 0 | 6 | 0.00 | 3 | 83.93 | 51.62 |
| 4 | 0 | 6 | 0.00 | 2 | 101.75 | 63.50 |
| 5 | 0 | 6 | 0.00 | 1 | 123.27 | 77.84 |
| 6 | 1 | 9 | 0.11 | 3 | 158.60 | 66.49 |
| 7 | 1 | 9 | 0.11 | 2 | 213.88 | 91.06 |
| 8 | 1 | 9 | 0.11 | 1 | 291.20 | 125.42 |
| 9 | 2 | 12 | 0.17 | 3 | 388.69 | 125.73 |
| 10 | 2 | 12 | 0.17 | 2 | 500.44 | 162.98 |
| 11 | 2 | 12 | 0.17 | 1 | 616.56 | 201.69 |
| 12 | 3 | 15 | 0.20 | 3 | 723.16 | 189.11 |
| 13 | 3 | 15 | 0.20 | 2 | 802.32 | 210.22 |
| 14 | 3 | 15 | 0.20 | 1 | 832.14 | 218.17 |
| 15 | 4 | 18 | 0.22 | 3 | 786.72 | 171.16 |
| 16 | 4 | 18 | 0.22 | 2 | 636.13 | 137.70 |
| 17 | 4 | 18 | 0.22 | 1 | 346.47 | 73.33 |

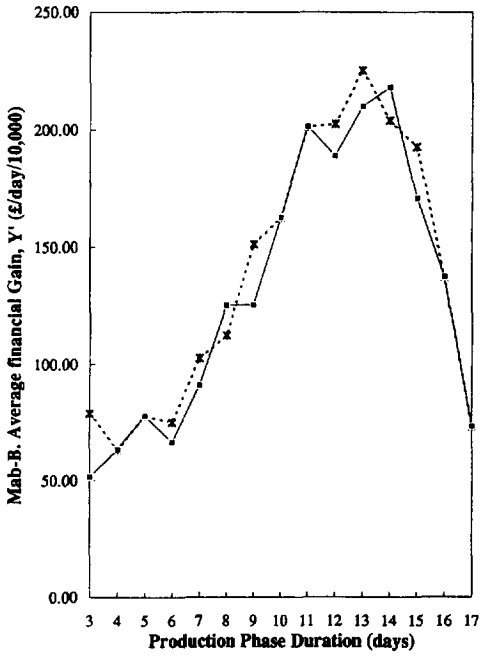


Fig. 8. Values of $Y'$ for the non-linear production of MAb-B according to the production patterns {1S2, 2P } (★) and {1S3, 2P } (■), employing the economic parameters of Table 2.

demonstrating the general failure of the accepted heuristic. Indeed, were harvesting to be conducted at day 9 (for which $R-P=3$) when the constraint on $\phi$ is imposed, then an inferior economic return would result compared with that for harvesting at day 8 ($R-P=1$).

## 5. Discussion and conclusions

The concept of line balancing has been introduced and shown to discriminate between the economic merits of different operating strategies for semi-continuous, multi-tank cell culture processes. Two different patterns of product accumulation were considered. For linear antibody production with a single-day production vessel turnround capability ($T=1$), the case-studies support the fermenter scheduling heuristic that $P$ be selected to be as long as possible, subject to the conditions that $R-P=T$ and $P \leq \phi$. This heuristic results in a balanced utilization of fermenter vessels in which no Solera batch is unduly discarded to effluent and no production vessel lies idle after turnround for want of fresh cell inocula. Furthermore, such a balanced production schedule yields the optimal financial gain, irrespective of reasonable changes in the variable and fixed cost structure, or in the frequency of production batch failures. This result contradicts the generally assumed heuristic that harvest titre should be maximized irrespective of fermenter train balancing, and arises from the operational requirement for fixed shift patterns. The failure of this assumption is readily explained as the incremental financial benefit which results by prolonging production vessel cultures (at the expense of balancing) is offset by the additional costs of discarded Solera batches or unproductive use of capital assets.

The optimum production schedules that we have identified have all been specific to the case of $T=1$. While production vessel turnround in a single day seems reasonable—and is commonly achievable in our experience—longer turnround times have a profound effect on the optimum production pattern. For example, production schedules similar to those in Fig. 2 show that the production pattern {1S2, 2P3}, for which $R-P=1$, is no longer balanced for the case of $T=2$. In this case, one in every three Solera batches must be disposed of to drain, because no production vessel is ready to receive a fresh inoculum. Also, after turnround, each production vessel lies idle for 1 day to await the completion of the next Solera batch. The pattern {1S2, 2P4}, for which $R-P=T$, similarly results in every third Solera batch being disposed of to drain, but this loss is better justified by a longer production phase, yielding a higher harvest titre, and no idle period for production vessels. Thus, the economic return from {1S2, 2P4} is preferable to that from {1S2, 2P3} if $T=2$. This general behaviour of production vessel utilization is repeated for the schedules {1S2, 2P5} and {1S2, 2P6}, although half the Solera batches are now disposed of to drain in each case. Qualitatively, the heuristic $R-P=T$ for optimum production schedules still applies.

For non-linear antibody production, the superiority of balanced fermenter train operation is less marked. The case-study suggests that, for certain tank configurations, when harvesting is carried out during the period of peak antibody accumulation rate (as a result of the constraint on $\phi$), some marginal financial benefit might be obtained by operating an unbalanced, asynchronous fermenter train. Whether this marginal benefit is justified by operability considerations requires detailed analysis to evaluate the efficiency of labour utilization, the cost of raw materials inventory and the risk of Solera or production batch failures for different operating patterns. We believe it likely that the unbalanced line operation would be rejected, because the additional media make-up and tank operations, the increased load to effluent treatment and raw materials inventory required for unduly discarded Solera batches, together with the increased risk of Solera batch failure as a result of operator handling errors would not justify the marginal gain.

## Appendix A. Nomenclature

| | |
|---|---|
| $d$ | number of Solera batch disposals to effluent during the repeat period $R$ |
| $d'$ | $=d/R$ (day$^{-1}$) |
| $h$ | number of production vessel harvests during the repeat period $R$ |
| $P$ | duration of a production vessel cycle (days) |
| $P'$ | $=P/R$ (–) |
| $R$ | duration of the repeat period after which the pattern of Solera and production vessel operation is repeated (days) |
| $S$ | duration of a Solera vessel cycle (days) |
| $T$ | time required to turn round a production vessel on completion of a production batch (days) |
| $Y$ | total financial gain that accrues from the manufacture of purified bulk product from harvests during the repeat period $R$ (£) |
| $Y'$ | average daily financial gain that accrues from the manufacture of purified bulk product from harvests during the repeat period $R$ (£) |

*Greek letters*

| | |
|---|---|
| $\alpha$ | internal (company) value of purified bulk product that derives from a single production batch harvest (£ per batch) |
| $\alpha_0$ | daily rate of accumulation of $\alpha$ for linear productivity of antibody (Eq. (5)) (£ per batch per day) |
| $\alpha_0$ | specific value of antibody for non-linear productivity (Eq. (16)) (£ M$^3$ g$^{-1}$) |
| $\beta$ | variable cost of primary processing and purification for material from each production batch harvest (£) |
| $\delta$ | variable cost of media and materials that is incurred by disposal of a Solera batch to the kill system (£) |
| $\phi$ | maximum duration of a production batch (days) |

$\kappa$ average number of production batches up to, and including, the first vessel contamination

$\tau$ fixed cost per day of facility operation (£ per day)

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