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Simulation and dynamic optimisation of animal cell culture

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Abstract

In animal cell culture, there are some 25 substrates that both have a significant effect on the culture performance and which can be measured with relative ease. A detailed dynamic simulation for such a culture has been produced and an optimisation policy that use this model to identify ideal media conditions has been developed. This paper describes an extension of that work to include the dynamic optimisation of cultures under fed-batch operation. Two different types of feeding policy were considered – in the first, discrete shots of feed were supplied, while in the second, feed was added continuously. Both policies offered significant improvements in the predicted productivity of the culture - up to 30% that of an experimentally “optimised” batch culture.

Introduction

Animal cell culture is an area of great commercial interest, providing high value-added products, such as therapeutical drugs and diagnostic kits, for use in human and veterinary medicine, as well as in biotechnological research. In a typical culture, there are a substantial number of components which have a significant effect on productivity and of these, the concentration of at least twenty-five (e.g. sugars, amino acids, products) can be measured with relative ease. Given the fact that a typical batch culture runs for at least a week, the experimental optimisation of all of the components in the medium is an enormous task and the development of a rigorous feeding policy for a fed-batch culture is almost impossible.

Fortunately, all animal cells share a common set of metabolic reactions, and the stoichiometry of these reactions has, to a large extent, been identified. These reaction networks can be incorporated into a structured, dynamic model which should be capable of describing any animal cell culture system. Such a model has already been produced and successfully applied to a number of industrially significant systems, including the production of monoclonal antibodies by murine hybridoma cells (Sanderson *et al.*, 1994) and interac-

tion of baculoviruses with insect cells (Sanderson *et al.*, 1995a).

In previous work (Sanderson *et al.*, 1995b), the model that had been tuned to Marquis's (1994) experimental data for a murine hybridoma system was combined with an optimisation algorithm based on Powell's method (Press *et al.*, 1988). This optimiser was used because the objective function hypersurface was found to be particularly uneven, causing gradient-based methods to perform poorly. The orthogonal direction search method, however, was able to find solutions that improved the culture productivity by up to 100%. It has been found by other researchers (Glacken *et al.*, 1989; Bédard *et al.*, 1994; Marquis, 1994; Xie and Wang, 1994) that the productivity of a culture can be further improved by moving from a batch to a fed-batch mode of operation. As an extension to our previous work, therefore, it was decided to optimise two modes of fed-batch operation that can be implemented easily in the laboratory.

Animal cell culture model

The animal cell model that forms the basis of this project has been described in a number of other publi-

cations (Barford *et al.*, 1992; Sanderson *et al.*, 1994) and will be only briefly discussed here. An animal cell culture may contain several million cells per millilitre of broth and in each of these cells, several thousand interacting reactions occur. The intracellular space is divided up into several different regions in which particular reactions occur and components concentrate. The metabolic reactions are, in general, controlled by the availability and activity of enzymes, so the cell is capable of implementing very tight control over its internal reaction network. Similarly, transport across cellular membranes depends on cellular proteins whose effectiveness can be controlled.

Given such a complex system, a number of simplifying assumptions must be made before the process of modelling can be pursued. The bioreactor is treated as a well controlled CSTR, so spatial variations in concentration can be ignored and pH, temperature and oxygen availability within the reactor are held at constant levels. It is also assumed that the viable population is so large that it can be described by a single, "average" cell of a determined volume – the (commonly measured) number of cells per volume of broth can therefore be described in terms of a volume reactor space enclosed by cells. This allows intracellular concentrations to be determined and used in reaction and transport equations. Dead cells are assumed to rupture and their contents come to equilibrium with the medium instantaneously.

Only two intracellular regions are considered in the model – the cytoplasm and the mitochondrial space. While this is a considerable simplification, these two regions are the ones in which the majority of the well-characterised reactions are thought to occur. In describing these reactions, the number of components that are considered is reduced by including only those that occur at branching or joining points in the reaction pathways, which are transported across cellular membranes, or which occur immediately before a recognised metabolic control point. This simplification reduces the number of reactions that are considered from several thousand to around fifty.

(1)

Despite this simplification, all the major metabolites have been described, including sugars, fatty acids, the twenty amino acids and several metabolic products. Among the metabolic pathways considered are glycolysis; glutaminolysis; the TCA cycle; amino and fatty acid degradation and formation; cell and product synthesis; and energy formation and utilisation. Transport of material across cellular membranes accounts for effects such as competitive uptake of amino acids or component exchange across the mitochondrial walls.

The overall model consists of some 90 ordinary differential equations which describe the mass balances of the components in each of the three modelled regions (i.e. the medium, the cytoplasm and the mitochondria). These mass balances are based on the well defined stoichiometry of the underlying reactions. The kinetics of the system are accounted for by a set of 110 algebraic equations that define the rates of reaction and transmembrane transport. These rate equations are less well characterised, and so their forms have been inferred. In general, Michaelis-Menten kinetics have been used: this means that it is implicitly assumed that the levels of cellular enzymes are constant throughout the culture period.

A number of modelling environments have been used to solve this set of mixed differential and algebraic equations. The version of the model described here was developed in both SPEEDUP (AspenTech Inc., Mass.) and gPROMS (Barton and Pantelides, 1994). Both of these packages provides access to robust and efficient solution algorithms and allow the (rather stiff) model to be solved in around 2 minutes on a Pentium 90 PC. It is worth noting that SPEEDUP is clearly a commercial package, that has access to powerful debugging and data viewing tools, while gPROMS is particularly strong at solving problems that involve discontinuities and has an in-built event control language but is still under development and is therefore somewhat less user-friendly.

The profitability (Prof) for the overall culture was calculated using the following function, which describes the worth of a process in terms of net dollars produced per reactor volume per hour of continuous operation (i.e. repeated batches).

$$\text{Prof}(\$/h.l) = \frac{P_{AB} * m_{AB}(t_b) - (P_{in} * X_0 + \sum_i \{P_i * C_{0,i}\}) * V_0 - \sum_j \{P_j * C_{f,j}\} * V_{add}}{(t_b + t_d) * V(t_b)}$$

where

P_{AB}	Price of antibody (\$/mg)
$m_{AB}(t_b)$	Cumulative mass of antibody formed (mg)
P_{in}	Price of inoculum (\$/mg)
X_0	Inoculum cell conc (mg/l)
P_i	Price of i^{th} component in initial medium (\$/mmol)
$C_{0,i}$	Conc. of i^{th} component in initial medium (mM)
V_{add}	Volume of feed supplied (l)
t_b	Current batch time (h)
t_d	Down-time for harvesting, cleaning and setting up reactor (h)
$V(t_b)$	Volume of material in reactor (l)
V_0	Initial culture volume (l)
P_j	Price of j^{th} component in feed (\$/mmol)
$C_{f,j}$	Conc. of j^{th} component in feed (mM)

The cost of the individual components used throughout this work are based on the 1993 Sigma catalogue prices and it was assumed that the down-time for the system was 5 h.

The composition of the solution could be optimised but this would increase the number of free variables significantly. Its composition was not, therefore, allowed to vary; the concentration of the substrates was determined in the following way:

- i) The concentrations of the substrates at the beginning and end of the batch culture were compared.
- ii) The volume of a feed stream containing 200 mM glutamine that would be required to restore the glutamine concentration to its initial level was calculated.
- iii) The concentration of the other components that would be required in this calculated volume to restore those components to their original levels was found.
- iv) The above concentrations were compared to the composition of a commercially available concentrate and the lesser of the two values used. This step is intended to ensure that the solubility limits of the components were not exceeded.

Shot-feeding

The shot-feeding mode of operation was chosen because it is relatively simple to perform in the laboratory (i.e. no pumps or control apparatus are required)

and because it has provided significant experimental improvements in the past (Sanderson *et al.*, 1996). The use of small volumes of highly concentrated feed was also advantageous because it meant that the increase in culture volume was only small – it was not necessary to provide an overflow vessel and the initial culture volume could be close to the bio-reactor's capacity. In addition, our existing optimisation algorithm could easily be adapted to deal with this dynamic optimisation problem. The free variables (initial concentrations, timing and volume of feed) can also be constrained between upper and lower bounds by limiting the size of the steps taken in the orthogonal directions.

The volume and timing of the shots was optimised in conjunction with the initial medium concentrations of glucose and glutamine. The two substrates provide the cell with the majority of its metabolic energy but form byproducts (lactate and ammonia, respectively) that are thought to be inhibitory to cell growth and product formation.

Table 1 shows the results for the base case, batch culture and the batch culture in which only the initial glucose and glutamine concentrations have been optimised. Three shot-feeding policies are also included – two in which a single shot of feed is added and one in which two separate shots are considered. In the first case, a maximum of 10 ml of feed are added per litre of culture; in the second, the maximum volume of feed is 20 ml/l; in the third case, the two shots are both constrained to have a maximum of 10 ml/l.

It can be seen that supplementing the initial culture broth with glutamine significantly improved both the profitability and lifespan of the culture (from 190/l.h to 383/l.h and from 64 h to 106 h, respectively). With the addition of substrates, cells are able to continue growing and synthesising product until either lactate or ammonia formation inhibits growth, or until one of the substrates is depleted. The latter explanation actually holds true according to the model (one of the benefits of using a structured model is that all the culture details are available at every step of the optimisation). From the presented data, it can be observed that the time at which the first shot of feed is added in the fed-batch cultures roughly coincides with the optimal time of harvest for the optimised batch culture. By relieving that limitation through the addition of shots of feed, both the lifespan and the productivity of the culture are therefore improved.

The addition of the single 10 ml/l shot can be seen to further improve both the productivity and lifespan of the culture – the profitability is increased to \$472/l.h

Table 1. Results of the optimisation of the initial medium and the time and volume of feed shots

Comment	[GLN] ₀ μM	[GL] ₀ μM	Volume added		Time of addition		Prof \$/l.h	t _{max} h
			V ₁ ml/l	V ₂ ml/l	t ₁ h	t ₂ h		
Base case	3142	22800					190	64
0 ml/l	4831	24399					383	106
10 ml/l	7026	35005	10.0		99.3		472	143
20 ml/l	6025	28025	20.0		99.0		518	165
2 × 10 ml/l	5730	27509	9.5	10.0	97.5	130	531	175

and the lifespan to 143 h. As is expected, either policy that allows 20 ml/l of feed to be added leads to a higher profitability and lifespan than is the case for 10 ml/l. The additional volume of feed contains nutrients that allow the culture to proceed for a longer period – an increase of about \$45/l.h or 10% is achieved.

A further small improvement can be achieved through the addition of two independent shots of feed – a 2.5% increase in profitability is realised through delaying the addition of the second shot by around 30 h. Under this final feeding policy, the cellular nutrients are at a lower net level and so antibody formation is slightly enhanced because fewer new cells are formed (ie the reactor conditions are such that antibody formation is favoured over growth) and because there is a lower average concentration of glucose and glutamine, which marginally increases the net rate of product synthesis. The best policy, therefore, extended the culture's lifespan from 64 to 175 h and increased the profitability by 280% from 190/l.h to 531/l.h.

Continuous feeding

A second protocol for fed-batch operation that was considered allows a piecewise continuous supplementation rate over a number of discrete operating periods. Such a policy was investigated using the gPROMS version of the model and gCOSOF, an associated dynamic optimisation program under development at Imperial Collage. The culture period was divided into a (user-defined) number of periods. The length of, and flow rate during, each period was determined along with the initial concentrations of glucose and glutamine for the case of 1, 2 and 3 operating periods.

There are a number of features that are common to all of these experiments. The constraint of maximum

allowable volume limits the rate at which feed is added to the reactor. This is expected as it has already been demonstrated that one of the nutrients contained in that mixed stream limits the culture – the optimiser is therefore adding as much of this component as possible.

It can be seen (Table 2) that the objective function is relatively insensitive to the initial glucose concentration, as long as it does not become limiting – additional glucose incurs an insignificant cost according to the objective function used here, and lactate does not inhibit cell growth significantly before substrate limitation occurs.

It should also be noted in passing that gCOSOF uses an infeasible path solution algorithm that has some interesting, and somewhat annoying, features. The most significant of these is that the optimiser may start at a good initial point (for example the 3-step optimiser may start at the solution found for the 2-step case) and move away from that point into the infeasible region. It then has a tendency to re-enter the feasible region at a substantially different point, and perform a local optimisation about this point. This solution is often inferior to the starting point.

The result shown in Table 2 should be similar to those of the shot-feeding policy (Table 1) if the optimal solution is insensitive to when the medium is supplemented: this is found to be the case. The maximum profitability that was observed for continuous feeding was 501.9/l.h, and 531/l.h in the case of shot-feeding. The optimiser seems to be favouring a single control period, during which the feed flowrate was such that the volume constraint was met at the optimal time of harvest.

Table 2. Results of continuous feeding optimisation

Steps	Max Prof \$/l.h)	Harvest at (h)	GLi (mM)	GLNi (mM)	Feeding policy	Step 1 (h)	Step 2 or (μ l/l.h.)	Step 3
1	501.9	180.0	24.68	2.97	duration flow	180.0 111.0		
2	501.8	179.4	24.68	2.98	duration flow	178.4 111.0	1.0 174.0	
3	469.7	143.9	30.44	4.37	duration flow	141.9 137.8	1.0 105.4	1.0 296.2

The time (in hours) and the feed flowrate (in millilitres per hour per litre of initial medium) for each control period.

Conclusions

This paper has described the development of rigorous optimisation policies that are capable of exploiting the potential improvements in profitability through fed-batch operation. A simple and robust optimisation program was used in conjunction with the SPEEDUP model to examine the case in which the initial medium is optimised together with a shot-feeding policy. The aim for this feeding policy was to produce a protocol that could be implemented in the laboratory easily. It was found that by adding two shoots of a mixture of amino acids and glucose, the predicted profitability of a culture could be increased by almost 300% (as compared to a batch case using the improved medium of Marquis (1994)).

A more complicated dynamic optimisation program, gCOSOF, was also used to investigate the case in which medium was continuously added to the culture. This program is closely related to the gPROMS program. It was found that gCOSOF produced results very similar to the shot-feeding optimisation routine, though there were some problems with the stability of the solution.

It can therefore be concluded that there are substantial improvements that can be achieved through the addition of feed to an otherwise batch culture. The fact that the shot-feeding and continuous policies produced similar results suggests that the simple (2-shot) feeding policy is essential identical to the more complex (continuous, multi-level) one. Such a complicated experimental protocol may not, therefore, be necessary.

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