A heuristic approach to fed-batch optimisation of streptokinase fermentation

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Abstract

Previous studies have shown that the rate of formation of streptokinase, a secondary metabolite, in batch fermentation is proportional to the specific growth rate of the biomass, which in turn is inhibited by its substrate and the primary product (lactic acid). These kinetics suggest the suitability of fed-batch operation to increase the yield of streptokinase. A near-optimal feed policy has been calculated by the chemotaxis algorithm, and it shows a substrate feed rate decreasing nonlinearly and vanishing after 11 hours. This is followed by batch fermentation for a further 8 hours, at the end of which 12% more streptokinase is generated than by purely batch fermentation. Further improvements in productivity are possible.

List of symbols

\( k_d \) decay constant for active cells
\( k_p \) decay constant for streptokinase
\( K_l \) inhibition constant for lactic acid
\( K_S \) inhibition constant for substrate
\( M \) lactic acid concentration
\( P \) streptokinase concentration
\( Q \) substrate feed rate
\( S \) substrate concentration
\( S_{in} \) inlet concentration of substrate
\( t \) time
\( t_b \) end-point of batch fermentation
\( t_f \) end-point of fed-batch fermentation
\( V \) volume of broth in fermenter
\( V_0 \) initial value of \( V \) (at \( t=0 \))
\( V_m \) maximum value of \( V \)
\( X \) total biomass concentration
\( X_a \) concentration of active biomass
\( Y_{MX} \) yield coefficient for lactic acid from biomass
\( Y_{PX} \) yield coefficient for streptokinase from biomass
\( Y_{XS} \) yield coefficient for biomass from substrate

Greek letters

\( \mu \) specific growth rate of biomass
\( \mu_m \) maximum specific growth rate

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1 Introduction

Streptokinase (SK) is a vital and effective drug for the treatment of myocardial infarction. It is a single-chain protein having a molecular weight of about 47,000, and it is synthesised by various strains of hemolytic streptococci. The usefulness of SK stems from its ability to associate with plasmin in the blood and transform it into plasminogen, a proteolytic enzyme that promotes dissolution of fibrin filaments in blood clots, thereby increasing the flow of arterial blood to the heart [1].

There are two important aspects to the fermentative production of SK. One is the choice of a suitable host-plasmid system which expresses the protein at reasonable levels of activity without significant amounts of other products such as deoxyribonucleases and proteases [2]. Apart from attacking SK and reducing its activity, these by-products also increase the cost of recovery of pure SK. Since most natural strains of streptococci produce low yields of SK, a general approach has been to clone the SK gene into another organism, the most popular being Escherichia coli [1, 3, 4]. While this approach has been successful in the laboratory, inherent problems such as structural and segregational instability of recombinant strains [5] have not been fully overcome in large fermenters. Therefore current industrial production of SK continues to be based on natural or mutated strains of streptococci. Their productivity is enhanced to economically viable levels by suitably choosing the fermentation conditions and the operating strategy, which is the second aspect of SK fermentation. However, compared to the molecular biology of SK-producing strains, there is little published information on the fermentation aspects.

The only available model of the kinetics comes from Stuebner et al. [6], which is presented in the next section. They consider the biomass to be composed of ‘active’ and ‘inactive’ cells, both of which grow but only the active cells synthesise SK. As Eq. (5) shows, the rate of formation of SK is proportional to the rate of production of active biomass, which varies linearly with the overall specific growth rate. The model uses a lumped approximation for a complex substrate and identifies lactic acid as the key primary metabolite and SK as the secondary metabolite. The specific growth rate is inhibited by the substrate and by lactic acid, but more strongly by the latter. The nature of the growth rate variation, Eq. (6), indicates that fed-batch operation is likely to generate more SK than batch operation [7, 8]. Since only batch data are available in the literature [6, 9], the present study was done to develop a feeding strategy that improves the yield of SK.
Several methods of optimising fed-batch fermentations have been reported. Constantinides [10], Fishman and Biryukov [11], and San and Stephanopoulos [12] applied Pontryagin's maximum principle to improve penicillin productivity. Takamatsu et al. [13] applied a combination of the maximum principle and Green's theorem to maximise amino acid production and minimise transient time in a continuous fermentation. Wei and coworkers [14] linearised the process model and used the average dynamic gain array [15] to optimise controller settings for fed-batch production of ß-amalyse by two Bacillus subtilis strains. The maximum principle, however, fails in many fed-batch fermentations because the control variable (usually the feed rate) appears linearly in the system equations and/or the performance index to be maximised [7]. This leads to a problem in singular control and the feed policy will consist of singular and nonsingular segments. Algorithms have been proposed to compute these segments, and there are applications to penicillin G [16, 17] and ethanol [18] production.

Both the maximum principle and the singular control algorithms are often computationally expensive. Therefore recent efforts have been directed toward developing algorithms that are fast, simple, easy to implement on-line, and generate solutions which are close enough to the global optimum to economically attractive. One such method is dynamic programming, which Luus [19] applied to penicillin biosynthesis. The performance index was augmented to include the constraints through penalty functions, and a piecewise optimal feed policy was determined. More recently, Montague and Ward [20] applied the chemotaxis algorithm [21] to compute near-optimal feed rates for an industrial fermentation and for citric acid production involving enzymatic and microbial reactions with inhibition. The steps in the chemotaxis algorithm are outlined later. Although not a rigorous optimisation, it is simple, efficient and easy to automate. Moreover, the random nature of the search pattern prevents the optimisation procedure from getting stuck at a local optimum. In the present study this method has been applied to compute the feed strategy that maximises SK activity in fed-batch operation. The results are compared with previous work on batch fermentation.

2 Kinetics and feed rate computation

Stuebner et al. [6] proposed the model given below for the fermentative production of SK in a batch process. Details of the experiments on which the model is based are described elsewhere [6, 21]:

\[ r_x = \frac{dX}{dt} = \mu X_a, \]
\[ r_{xa} = \frac{dX_a}{dt} = (\mu - k_{a}) X_a, \]
\[ r_s = \frac{dS}{dt} = -\mu X / Y_{s}, \]
\[ r_M = \frac{dM}{dt} = Y_{MN} \mu X_a, \]
\[ r_p = \frac{dP}{dt} = Y_{px} dX_a / dt - k_p P. \]

The specific growth rate is described as:

\[ \mu = \mu_m \left( \frac{S}{K_s + S} \right) \left( \frac{K_b^o}{K_b^o + M^o} \right), \]

where \( b \) was empirically determined to be 2.39. Values of the other parameters are listed in Table 1. Stuebner and coworkers solved the model and determined that SK activity increases up to about 19 hours and then declines, in agreement with experimental findings and as expected for a reaction inhibited by the substrate and the product. The peak SK activity was 8780 U ml\(^{-1}\).

To increase SK activity, a proposed general form for the substrate feed variation with time was optimised in the present work by the chemotaxis algorithm. For comparison with results for a batch fermentation, the initial conditions were maintained the same. Using the rate equations (1) to (5), the material balances for fed-batch operation with sterile feed are:

\[ \frac{dX}{dt} = \frac{r_x - Q X}{V}, \]
\[ \frac{dX_a}{dt} = \frac{r_{xa} - Q X_a}{V}, \]
\[ \frac{dS}{dt} = \frac{r_s + V V_0 - S}{V}, \]
\[ \frac{dM}{dt} = \frac{r_M - Q M}{V}, \]
\[ \frac{dP}{dt} = \frac{r_p - Q P}{V}, \]
\[ \frac{dV}{dt} = Q(t). \]

Following Montague and Ward [20], the feed rate may be expressed as a polynomial function of time:

\[ Q = \sum_{j=0}^{n} a_j (t / t_f)^j, \]

where \( t_f \) is the time at which the fermentation is stopped. The steps outlined below are an extended version of their procedure to determine the best \( Q \).

Step 1. Set \( n = 0 \) in Eq. (13).

Step 2. Initialise the parameters \((a_j, j = 1, 2, \ldots, n), t_f \) and \( V_0 \).

Step 3. Integrate Eq. (12), substitute the expressions for \( Q(t) \) and \( V(t) \) in Eqs. (7) to (11), and solve for \( P(t) \) and other variables.

Step 4. Generate a set of Gaussian random increments in the parameters.

Step 5. Implement Step 3 with the new parameters.

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