Optimization of fed-batch culture of hybridoma cells using dynamic programming: single and multi feed cases

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Abstract. The optimization of fed-batch culture of hybridoma cells is accomplished on a mathematical model using dynamic programming. Optimal feed trajectories are found using a seventh order model for a single feed stream containing both glucose and glutamine and for two separate feed streams of glucose and glutamine. Compared to a constant feed rate, optimal trajectories can improve the final MAb concentration by 11% for the single feed case and by 20% for the multifeed case. Higher MAb concentrations can be expected for fed-batch optimization with feed enriched in nutrients.

1 Introduction

From an industrial point of view, fed-batch operation is becoming more attractive as a cultivation strategy for mammalian cells. Fed-batch mode can substantially increase the final product concentration over batch operation while keeping the simplicity of operation and of quality control to comply with FDA regulations. Fed-batch being a semi-continuous operation, an approved stock of medium is used throughout the culture. Genetic drift and contamination problems are also minimized since the culture lasts about twice as long as a regular batch culture.

Significant increases in final MAb titer obtained from hybridoma fed-batch cultures compared to batch cultures have been reported for feed stream of the same composition as the inoculum medium [1] and for an enriched feed stream [2, 3]. However, few studies have been reported on the optimization of this mode of operation for hybridoma cell cultures, mainly because of the lack of mathematical models describing adequately their growth and production kinetics.

The optimization of feeding trajectories is a classical control problem which has been studied for microbial systems. Techniques based on Pontryagin's Maximum Principle were used to optimize penicillin production [4, 5], and biphasic growth of Saccharomyces carlsbergensis [6]. In all these cases, the mathematical model used consisted of four ordinary differential equations obtained from the mass balances on the biomass, the limiting substrate, the fermentation product and the reactor volume.

For hybridoma cultures, more state variables are required to describe the culture since the cells grow on two main substrates, glucose and glutamine, and release toxic products, lactate and ammonia, in addition to the desired metabolite. This leads to a seventh order model for fed-batch operation, which is difficult to optimize using Pontryagin's Maximum Principle. If reducing the order of the system [7] is not desired, then dynamic programming can be used to determine optimal trajectories of such high-order systems. Indeed, recent modifications to conventional dynamic programming were introduced by de Tremblay and Luus [8] to allow handling of high-dimensional systems.

The present work addresses the use of dynamic programming to optimize fed-batch culture of hybridoma cells with respect to feeding trajectories, initial volume and final culture time. The mathematical model describing the kinetics of hybridoma cells was developed in a previous work [1] and is briefly summarized here. The algorithm for the single feed case is presented and then extended to the multivariable case where separate feeds of glucose and glutamine are optimized independently of each other. Numerical results obtained for both cases are presented and compared with batch and constant feed rate fed-batch operation.

2 Problem formulation

The kinetic model used for the optimization study was developed from batch and fed-batch hybridoma culture results. This model is a seventh order model where both glucose and glutamine concentrations are used to describe the specific growth rate expression \( \mu \). The cells death rate \( k_d \) is governed by lactate, ammonia and glutamine concentrations. The specific MAb production rate, \( q_{MAb} \), is predicted using a variable yield coefficient model related to the physiological state of the culture through the specific growth rate. The mass balance equations for the system in fed-batch mode are:

\[
\frac{dX_v}{dt} = (\mu - k_d) X_v - \frac{F}{V} X_v
\]
Table 1. Kinetic expressions

\[ \mu = \mu_{\text{max}} \left[ \frac{Glc}{K_{\text{Glc}} + Glc} \right] \left[ \frac{Gln}{K_{\text{Gln}} + Gln} \right] \]

\[ k_d = k_{d_{\text{max}}} \left( \frac{\mu - k_{d_{\text{max}}}}{Lac} \right) + \left( \frac{\mu - k_{d_{\text{ammon}}}}{Ammon} \right) k_{d_{\text{ammon}}} \frac{k_{d_{\text{Gln}}}}{K_{\text{Gln}} + Gln} \]

\[ q_{\text{Glc}} = \frac{\mu}{Y_{\text{Glc/V}} + \mu} \]

\[ q_{\text{Gln}} = \frac{\mu + \text{g}_{\text{gln}} \left( \frac{Glc}{K_{\text{Gln}} + Gln} \right)}{Y_{\text{Gln/V}} q_{\text{Gln}}} \]

\[ q_{\text{Ammon}} = \frac{\text{Ammon}}{q_{\text{Gln}} \text{g}_{\text{Gln}}} \]

\[ q_{\text{MAb}} = \frac{\text{g}_{\text{MAb}}}{k_{\mu} + \mu} \]

\[ dX_{\text{Glc}} \frac{dt}{dt} = (Glc_{\text{in}} - Glc) \frac{F}{V} - q_{\text{Glc}} X_{\text{v}} \]  

\[ dX_{\text{Gln}} \frac{dt}{dt} = (Gln_{\text{in}} - Glnc) \frac{F}{V} - q_{\text{Gln}} X_{\text{v}} \]

\[ dX_{\text{Lac}} \frac{dt}{dt} = q_{\text{Lac}} X_{\text{v}} - \frac{F}{V} \text{Lac} \]

\[ dX_{\text{Ammon}} \frac{dt}{dt} = q_{\text{Ammon}} X_{\text{v}} - \frac{F}{V} \text{Ammon} \]

\[ dX_{\text{MAb}} \frac{dt}{dt} = q_{\text{MAb}} X_{\text{v}} - \frac{F}{V} \text{MAb} \]

\[ dV \frac{dt}{dt} = F \]

where \( X_{\text{v}}, \text{Glc, Gln, Lac, Ammon} \) and \( \text{MAb} \) are respectively the concentrations in viable cells, glucose, glutamine, lactate, ammonia and monoclonal antibodies. \( V \) is the fermentor volume and \( F \) the volumetric feed rate. \( \text{Glc}_{\text{in}} \) and \( \text{Gln}_{\text{in}} \) are the concentrations of glucose and glutamine in the feed stream. The mathematical expressions for the specific rates, \( \mu, k_d, q_{\text{Glc}}, q_{\text{Gln}}, q_{\text{Lac}}, q_{\text{Ammon}} \) and \( q_{\text{MAb}} \) are presented in Table 1 and the parameters' values in Table 2. This system of non-linear differential equations can be condensed into a matrix form as:

\[ \dot{X} = f(X, u) \] with \( X(t_0) = X_0 \)

where \( X \) is the vector of the state variables, and \( u \) the control variable \( F \). The system initial conditions are given by the vector \( X_0 \). The system is also subjected to physical constraints on the inlet flow rate and the final culture volume:

\[ 0 = F_{\text{min}} \leq F \leq F_{\text{max}} \]

\[ V(t) \leq V_{\text{max}} \]

The multifeed case which involves two separate feeds \( F_1 \) and \( F_2 \) for glucose and glutamine respectively is reformulated as follows:

\[ \frac{dX_{\text{Glc}}}{dt} = (Glc_{\text{in}} - Glc) \frac{F}{V} - \frac{F_1 + F_2}{F} X_{\text{v}} \]

\[ \frac{dX_{\text{Gln}}}{dt} = (Gln_{\text{in}} - Glnc) \frac{F}{V} - \frac{F_1 + F_2}{F} X_{\text{v}} \]

\[ \frac{dX_{\text{Lac}}}{dt} = q_{\text{Lac}} X_{\text{v}} - \frac{F_1 + F_2}{V} \text{Lac} \]

\[ \frac{dX_{\text{Ammon}}}{dt} = q_{\text{Ammon}} X_{\text{v}} - \frac{F_1 + F_2}{V} \text{Ammon} \]

\[ \frac{dX_{\text{MAb}}}{dt} = q_{\text{MAb}} X_{\text{v}} - \frac{F_1 + F_2}{V} \text{MAb} \]

\[ \frac{dV}{dt} = F_1 + F_2 \]

In this case, \( u \) becomes a control vector with two elements \( F_1 \) and \( F_2 \).

The optimal control problem is to determine how the inlet vector \( u \) should vary with time in order to maximize a performance criterion \( \phi \), for a set of initial conditions \( X_0 \) and a final culture time \( t_f \):

\[ I^* [X_0, t_f] = \frac{\text{Max}}{u(t)} \int_{t_r}^{t_f} \phi [X(t)] \, dt \]

In this study, the criterion to be maximized is the total amount of monoclonal antibody obtained at the end of the fed-batch culture:

\[ I^* [X_0, t_f] = \frac{\text{Max}}{F(t)} [\text{MAb}(t) \cdot V(t)] \]

The constraints on the control variable and the culture volume are:

\[ 0.01/d \leq F \leq 0.51/d \]

\[ V(t_f) \leq 2.01 \]

\[ \mu_{\text{max}} = 1.09 \text{ d}^{-1} \]

\[ k_{d_{\text{max}}} = 0.69 \text{ d}^{-1} \]

\[ Y_{\text{Glc/V}} = 1.09 \times 10^9 \text{ cells/mmol} \]

\[ Y_{\text{Gln/V}} = 3.8 \times 10^8 \text{ cells/mmol} \]

\[ m_{\text{Glc}} = 0.17 \text{ mmol} \cdot 10^{-8} \text{ cells} \cdot \text{d}^{-1} \]

\[ k_{\text{Glc}} = 19.0 \text{ mM} \]

\[ K_{\text{Glc}} = 1.0 \text{ mM} \]

\[ K_{\text{Gln}} = 0.3 \text{ mM} \]

\[ x_0 = 2.57 \text{ mg} \cdot 10^{-8} \text{ cells} \cdot \text{d}^{-1} \]

\[ \beta = 0.02 \text{ d}^{-1} \]

\[ k_{d_{\text{ammon}}} = 0.69 \text{ d}^{-1} \]

\[ k_{d_{\text{Gln}}} = 0.01 \text{ d}^{-1} \text{ mM}^{-1} \]

\[ K_{\text{Ammon}} = 0.02 \text{ mM} \]

\[ Y_{\text{Glc/V}} = 2.57 \text{ mg} \cdot 10^{-8} \text{ cells} \cdot \text{d}^{-1} \]

\[ Y_{\text{Gln/V}} = 0.3 \text{ mg} \cdot 10^{-8} \text{ cells} \cdot \text{d}^{-1} \]

\[ Y_{\text{MAb/V}} = 0.02 \text{ mmol/mmol} \]

\[ Y_{\text{MAb/V}} = 0.85 \text{ mmol/mmol} \]