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MODEL FORMULATION FOR HYBRIDOMA CULTURES IN BATCH AND FED-BATCH MODE

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Abstract: Metabolic Flux Analysis was performed to identify the significant metabolic reactions for hybridoma cells during batch and fed-batch culture. Correlation analysis yielded the factors that influence biomass growth and productivity and elucidated the nature of the relationship between them. Consequently, an integrated dynamic model expressing the rate of consumption and production of nutrients/metabolites and the biomass growth and decline was developed.

Keywords: hybridoma, metabolic flux, quadratic programming, cross-correlation functions, parameter-estimation, prediction.

1. INTRODUCTION

The large-scale production of monoclonal antibodies (MAb) by mammalian cells in batch and fed-batch culture systems is limited by the unwanted decline in cell viability and reduced productivity that may result from changes in culture conditions. Therefore, it becomes imperative to gain an in-depth knowledge of the factors affecting cell viability and subsequently antibody production. The aim of the present work is to obtain an overall dynamic model that predicts the behaviour of both batch and fed-batch systems as a function of the extra-cellular nutrient/metabolite concentration at any time and utilize this model for optimization of Monoclonal antibody (MAb) production in the future.

Although considerable effort has been made to understand the kinetics of hybridoma growth and metabolism, it has become evident that a model structure is not a priori obvious. A systematic approach based on Metabolic Flux Analysis (MFA) has been proposed by Provost and Bastin (2004) and applied by Gao, *et al.*, (2006) with some modifications for batch production of MAb. MFA permits to calculate values of intracellular fluxes from available extracellular fluxes, some of which are significant and some are negligible. Thus, the original metabolic network can be reduced to contain only the reactions corresponding to the significant fluxes that are then used to formulate a set of fundamental macro- reactions linking the substrates to the products, thus eliminating involvement of intracellular metabolite concentrations in the mathematical model. A set of dynamic mass balances can be devised that involve rate expressions for the consumption/production of substrates/ metabolites.

In previous studies, the MFA analysis has been done for batch operations (Provost and Bastin, 2004). Also, in the development of the MFA and the resulting dynamic model for metabolites, the viable and dead cell concentrations and their corresponding rates of change have not been explicitly modeled. Instead, their experimental values have been used as an input for the metabolites model.

The current work addresses these issues as follows:

1. MFA is applied to a fed batch situation in order to obtain a dynamic model for this

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mode of operation. In this context, this study compares the flux values obtained in batch and fed batch operations and investigates whether the same structure of the dynamic model is applicable to both.

2. The viable and dead cell concentrations are explicitly modeled. Correlation analysis is used to investigate the dependence of growth and death rates on nutrient and product concentrations. Then, the cell concentrations model is coupled to the metabolites dynamic model to give rise to an overall model that utilizes experimental starting values and predicts for all significant system variables, including viable and dead cell concentrations, independent of subsequent experimental values.

2. MATERIALS AND EXPERIMENTAL METHODS

Murine hybridoma 130-8F producing anti-F-glycoprotein monoclonal antibody (MAb) was provided by Sanofi Pasteur Ltd. (Toronto, Canada) and was propagated in D-MEM medium (Gibco 12100) with 2% FBS (JRH 12107-78P). The medium was supplemented with proline (Sigma P-8449), l-asparagine (Sigma A-4159), and l-aspartic acid (Sigma A-4534). Seed cultures were subcultured on a three-day regime. Seed and batch cultures were grown in 250 mL and 500 mL spinners in a CO_2 incubator (Sanyo IR Sensor, 37 °C, 5.0% CO_2). Batch cultures were maintained for at least 7 days with frequent sampling. To avoid ammonia toxicity, fed-batch cultures were maintained under glutamine-limited conditions and the fed-batch operation was effected by injecting defined amounts of glutamine and glucose solution when its concentration inside the spinner was approaching zero.

Viable and total cell concentration were determined by the Trypan Blue Exclusion test using a haemocytometer. Glucose, lactate, glutamine, and glutamate concentration was quantified using YSI Analyser. Ammonia was measured using a Sigma Ammonia Kit (Sigma 171-B). Total Immunoglobulin titre (MAb concentration) was determined using Enzyme Linked ImmunoSorbent Assay (ELISA) with alkaline phosphatase conjugated goat anti-mouse IgG as the primary reagent. The amino acids in de-proteinated medium were derivatized with OPA (o-phthaldehyde and 3-mercaptopropionic acid in borate buffer) followed with FMOC (9-fluorenylmethylchloroformate in acetonitrile) and assayed using High Performance Liquid Chromatography (Hypersil AA-ODS column).

3. METABOLIC FLUX ANALYSIS

Based on published reports (Bonarius, *et al.,* 1995; Gambhir, *et al.*, 2003; Gao, *et al.,* 2006), a simplified metabolic network shown in Figure 1 is constructed. The figure representing the system under study involves m(=30) metabolites and n(=32) fluxes corresponding to 32 reactions. Mass balances for the intracellular and extracellular metabolites can be represented as follows:

$$\frac{d\boldsymbol{\psi}(t)}{dt} = \mathbf{R}X(t) \qquad (1)$$

where $\boldsymbol{\psi}$ is the vector of intracellular metabolite concentrations and t is the culture time. $\mathbf{R}$ is the vector of uptake/production rate of substrates/metabolites. $X(t)$ is the viable cell concentration and is a function of culture time, t.

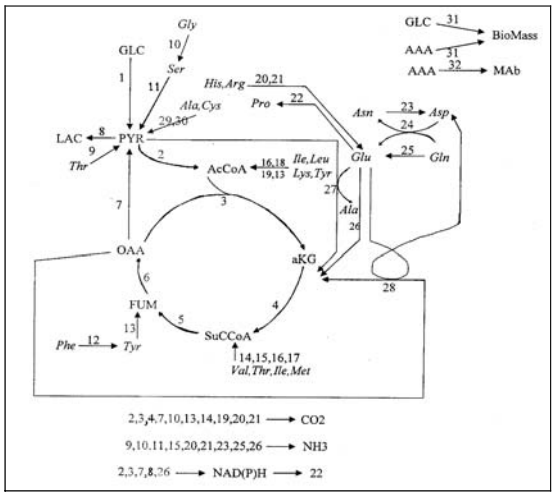

Fig. 1. The simplified metabolic network of Hybridoma cells.

From integration of equation (1) and assuming an average value of R over the integration interval:

$$\int_0^t d\boldsymbol{\psi}(t) = \mathbf{R}\int_0^t dX(t) \qquad (2)$$

$$\Rightarrow \boldsymbol{\psi}_t - \boldsymbol{\psi}_0 = \mathbf{R}(CH_t - CH_0) \qquad (3)$$

CH is referred to as the cumulative volumetric cell hours (Dutton, *et al.*, 1998) and can be mathematically expressed as:

$$CH = \int_0^t X(t)dt \qquad (4)$$

Under the assumption of quasi-steady state, the conversion/ production rate, $R(M)$ of a nutrient/metabolite in a biological system is expressed as (Bonarius, *et al.*, 1996):

$$R(M) = \sum_i \alpha_{i,M} j_i \qquad (5)$$

where R is the uptake or production rate of metabolite M (=1…m) and it is calculated from linear regression between measured concentrations of metabolites and volumetric cell hours (CH) based on equation (3). The need for concentration measurements for intracellular metabolites is eliminated by assuming that the system operates under balanced growth conditions and consequently, the corresponding reaction rates (*1* to *6* according to