

Towards energy-based dynamic optimization of monoclonal antibody producing GS-NS0 Cultures

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Abstract

Mammalian cell culture systems produce clinically important high-value biologics, such as monoclonal antibodies (mAb). Cell lines transfected with the *Glutamine Synthetase* (GS) gene are amongst the most industrially significant mAb production systems due to the high yields they achieve. Metabolic models of GS culture systems presented thus far take into account only glucose as a growth limiting nutrient, neglecting the fact that in the absence of glutamine in the media, glutamate becomes a necessary dietary component in GS systems. Previously, we have presented the development of a systematic framework for modelling of mammalian cell bioprocesses. Herein, we present, for the first time, the development of a dynamic model describing growth and monoclonal antibody formation in GS-NS0 cell cultures that interlinks cellular growth rate with the availability of both glucose and glutamate. This is the first step, of many, towards the derivation of a dynamic model that interlinks the availability of ATP, through the dietary intake of the cell, to its growth and productivity characteristics. Such a model would facilitate the derivation of an optimal feeding profile, constraining the amount of provided energy through the feed to the required minimal, hence avoiding the excessive feeding of glucose which in turn shifts metabolism towards energy inefficient pathways.

Keywords: Mammalian Cell Modeling, ATP/ADP balance, energy based optimization, monoclonal antibodies, GS-NS0 cell line

1. Introduction

The advancements in molecular biology and analytical techniques over the last 20 years have significantly elevated the biological industry in the economical scale. mAbs alone have a projected market of \$49bn by 2013 (Monoclonal antibodies Report, 2007). mAbs are primarily produced in batch or fed-batch processes, however the control of such processes in the biotechnological industry still remains fundamentally manual. In previous work (Kiparissides *et al.* 2009) we have shown the advantages of using a systematic model development framework from conception to validation and how such a framework paves the way towards model based optimization and control. Moreover we have showcased the benefits of using a hybrid approach to modeling, by coupling structured models describing the assembly and secretion of mAbs to unstructured growth/metabolic models thus reducing computational and experimental costs.

The common denominator of all metabolic models of GS systems thus far in the literature is that they disregard a number of vital metabolites for the growth of cells in culture. More specifically, to the extent of our knowledge, none of the presented models monitors the concentrations of essential amino-acids in the extracellular environment and how their depletion affects growth and mAb productivity. Furthermore studies for the derivation of optimal feeding profiles for fed-batch cultures presented thus far in the

literature merely take into account the cells' needs on glucose. Even though the results are indeed an improvement to heuristic or empirical feeding strategies they lead to an excessive amount of glucose being fed to the culture. According to the work of Xie and Wang (1994) however, the presence of glucose in abundance in the culture media shifts cell metabolism towards more energy inefficient pathways.

There is an imminent need to update existing metabolic models of GS culture systems so that they account for the effects of essential amino acid concentrations and available ATP levels on growth and productivity. A model able to identify the cells' minimal requirements of ATP for proliferation and mAb production could lead to the derivation of a feeding profile that would maximise final antibody titre, whilst supplying the culture with merely the required amount of nutrients. Such an approach would be beneficial in multiple ways. First and foremost by eradicating the excessive presence of glucose fed to the culture, the amount of accumulating lactate will be significantly reduced, allowing for prolonged culture viability. Moreover, when glucose is not fed in excessive concentrations, cellular metabolism is limited to energy efficient pathways (Xie and Wang, 1994).

Herein we present the first step towards an energy orientated model that would allow the derivation of such an optimal feeding profile. We present for the first time, the coupling of cellular growthrate of GS systems with glutamate concentration. This is of great importance, since for GS systems which grow in glutamine free media, glutamate becomes a necessary dietary component and its depletion would inhibit growth.

2. Mathematical model and experimental setup

2.1 Experimental Setup

GS-NS0 cells (kindly provided from Lonza biologics) were cultured in triplicate 1L Erlenmeyer flasks (Corning) with 200mL working volume. The media contained Advanced-DMEM X1 (Invitrogen Ltd.), MEM-Vitamins (Gibco) X2, GS-Supplement (SAFC) X2, Penicillin/Streptomycin (Gibco) X1, 4.5 g/L MSX (Sigma-Aldrich) and 10% Fetal bovine serum (Gibco). Samples were taken on 24h intervals and stored in -20°C prior to analysis.

Metabolite Measurements

1.5mL samples were taken from each flask on 24h intervals and centrifuged at 800 RPM for 5minutes. The supernatant was stored in -20°C prior to analysis. Extracellular Glucose, Glutamate and Lactate concentration were measured using a Nova BioProfile 400 Analyser.

Extracellular Antibody Quantification

The extracellular antibody concentration was determined using a sandwich-based Enzyme- Linked Immunosorbent Assay (ELISA). A 96-well plate was first coated with an anti-human gamma Fc antibody (Jackson immunoresearch, US) in a coating buffer (15 mM Na₂CO₃, 35 mM NaHCO₃, pH 9.6) at a concentration of 2 µg/mL overnight in a 4°C refrigerator. The coating solution was then removed and the wells blocked with a solution consisting of the coating buffer with 0.5% (w/v) casein hammerstein (VWR) for 1 hour at room temperature. Subsequently, the wells were rinsed 6 times with 300 µL of washing solution (PBS with 0.05% Tween). Known standard concentrations of the cB72.3 IgG antibody (kindly provided by Lonza Biologics, UK) and cell free supernatant samples diluted in sample-conjugate buffer (12.1 g/L Tris, 5.84 g/L NaCl, 2.0 g/L Casein Hammerstein (VWR) and 0.2 mL Tween) were added next to the wells

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(100 $\mu\text{L}/\text{well}$) and incubated for 1.5 hours at room temperature on an orbital shaker. Sample-conjugate buffer was also added to at least two wells to serve as negative (background) controls. The standards, samples and sample-conjugate buffer were discarded and the wells washed with the washing solution described above. An antihuman kappa chain Fab antibody fragment conjugated to horseradish peroxidase (Sigma) was then added at a dilution of 1:8000 (in sample-conjugate buffer) to each well (100 $\mu\text{L}/\text{well}$) and incubated for a further 1 hour with shaking at room temperature. After the incubation period, wells were washed with the washing solution before substrate solution (100 $\mu\text{L}/\text{well}$) was added to the wells. The substrate solution consisted of a TMB tablet (1mg/tablet of 3,3',5,5'-Tetramethylbenzidine, Sigma), which was dissolved in 10 mL of 50 mM phosphate-citrate buffer (pH 5.0). Immediately prior to use, 2 μL of 30% (w/v) hydrogen peroxide solution was added to the mixture. The reaction was allowed to proceed in the dark at room temperature for 15 to 30 minutes before being stopped by the addition of 50 μL of 2.5 M H_2SO_4 solution to each well. The OD450 of each well was measured using an ELISA microplate reader (BioTek, US). OD450 values for standards and samples were normalised by subtracting the average OD450 reading of the negative control wells. Each sample was assayed at least in quadruplicates.

2.2 Mathematical model

The proposed model is the first to couple the cellular growth rate of GS systems to glutamate concentration. The model works under the standard operating assumption of perfect mixing within the bioreactor and furthermore assumes the presence of a homogeneous culture of “average” cells. The model will be presented in its batch operation mode in accordance to the experiments described above.

2.2.1 Unstructured metabolic model

The total balances on viable (X_V , cells mL^{-1}) and dead (X_D , cells mL^{-1}) cells is given by:

$$\frac{d(V * X_V)}{dt} = (\mu - k_d) * X_V * V \quad (2.2.1.1)$$

and

$$\frac{d(V * X_D)}{dt} = k_d * X_V * V - k_{lys} * X_D * V \quad (2.2.1.2)$$

respectively, where μ denotes the specific growth rate (h^{-1}) and k_d the specific death rate (h^{-1}). k_{lys} (h^{-1}) is the specific cell lysis rate.

The specific growth rate has been modelled using standard Monod kinetic expressions for the growth affecting nutrients, namely glucose and glutamate and is given by:

$$\mu = \mu_{MAX} \frac{[GLC]}{K_{GLC} + [GLC]} \frac{[GLU]^2}{K_{GLU}^2 + [GLC]^2} \quad (2.2.1.3)$$

Where, [GLC] and [GLU] are the extracellular concentrations of glucose and glutamate respectively, measured in mM and μ_{MAX} denotes the maximum specific growthrate (h^{-1}). The specific death rate has been adapted from the work of deTrembley *et al.* (1992), as presented by Ho (2006) for the same cell line, and is given by:

$$k_d = \frac{K_{d,1}}{\mu_{MAX} - K_{d,T}[LAC]} \quad (2.2.1.4)$$

Where, $K_{d,1}/\mu_{MAX}$ is the minimal specific death rate in the absence of lactate in the media and $K_{d,T}$ ($\text{h}^{-1}\text{mM}^{-1}$) is the death rate associated with the toxicity of lactate ([LAC], mM). The specific lysis rate has been adapted from the work of Ho (2006) and is modelled as a linear function of the specific death rate.

$$k_{lys} = k_{l,1} * k_d - k_{l,2} \quad (2.2.1.5)$$

Where, $k_{l,1}$ (dimensionless) and $k_{l,2}$ (h^{-1}) are the associated constants. The nutrient uptake rates are given by:

$$\frac{dS_i}{dt} = - \left(\frac{\mu}{Y_{X,i}} + m_i \right) * X_V * V \quad (2.2.1.6)$$

Where, S_i denotes nutrient (i) and is measured in mM. $Y_{X,i}$ denotes the yield on biomass when nutrient (i) is consumed by the cells, and m_i (Mm h^{-1}) is the non-growth associated consumption rate of nutrient (i) for housekeeping purposes. Similarly the accumulation of the metabolism's by-products is given by:

$$\frac{dP_j}{dt} = \left(\frac{\mu}{Y_{j,i}} + m_j \right) * X_V * V \quad (2.2.1.7)$$

Where, P_j denotes metabolite (j) and is measured in mM. $Y_{j,i}$ denotes the yield on metabolite (j) from the consumption of nutrient (i), and m_j (mM h^{-1}) is the non-growth associated metabolite accumulation term.

2.2.1 Structured model of covalent mAb assembly

The unstructured metabolic model was coupled to a structured model describing the covalent assembly of the IgG4 antibody that is produced by the GS-NS0 cells studied. According to the work of Percy (1970), IgG4 antibodies are assembled in the endoplasmic reticulum of the cells from heavy and light polypeptide chains following mechanism of equation (2.2.2.1):



The model has been successfully adapted and applied to describe the accumulation mAb in GS-NS0 cultures in previous studies from our group (Ho *et al.*, 2006). For a detailed derivation of the structured model and its complete set of equations the reader should refer to the original work of Ho *et al.* (2006).

3. Results and Discussion

Parameter estimation experiments and model simulations were carried out on an Intel® Core™2 Duo (E4600 – 2.4, 2.39) personal computer with 3.24 GB of RAM memory and all model simulations and parameter estimation experiments were implemented in the advanced process modelling environment gPROMS® (Process Systems Enterprise, 2009). gPROMS is an equation-oriented modelling system used for

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building, validating and executing first-principles models within a flow sheeting framework. The experimental results and model simulations can be seen in figures (1) and (2).

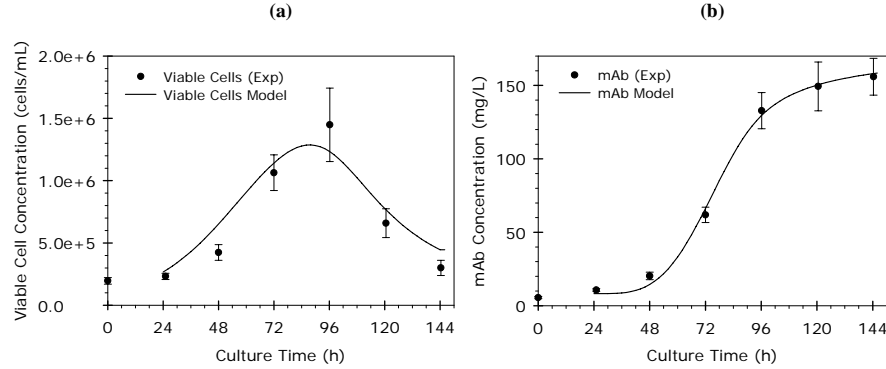


Figure 1: a) Viable Cell concentration and b) mAb concentration over time for batch cultivation of GS-NS0 cells.

During the first 24 h of culture the cells remained completely idle; therefore no effort to model the cellular behavior during that period was made. From that point onwards the cells start growing and a peak in viable cell concentration can be observed (Figure 1) after roughly 96 h of culture time. The model successfully predicts the time point of the peak although it slightly underestimates the actual magnitude of the peak. What is interesting is that after this peak is reached, nutrient uptake is completely halted (Figure 2). Both glucose and glutamate consumption stop abruptly even though both nutrients are still in abundance. This is a significant observation that leads us to the conclusion that neither glucose nor glutamate is the growth limiting substrate. Therefore, in order to derive a truly optimal feeding profile, identification of the growth limiting nutrient is required.

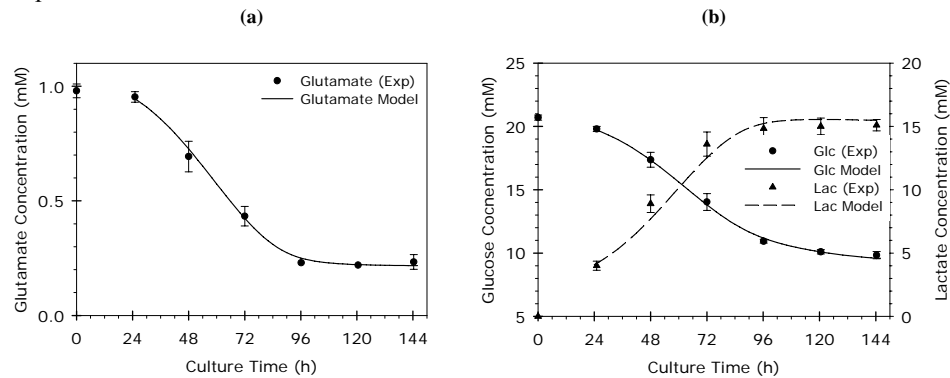


Figure 2: a) Glutamate concentration and b) Glucose & Lactate concentrations over time for batch cultivation of GS-NS0 cells.

In the absence of glutamine from the culture medium, it is synthesized through a GS catalyzed reaction involving glutamate, ATP and a NH_3 group usually provided by asparagine. We therefore presume that growth might be halted at 96 h of culture time due to the depletion of a viable NH_3 source for the production of glutamine. The next

step in our work towards the energy based derivation of an optimal feeding profile is the identification of the growth limiting nutrient and its inclusion in the current model. None the less the model is able to successfully capture the trends observed experimentally and is a solid first step towards the derivation of a dynamic model that successfully captures the most significant elements of GS-NS0 metabolism, facilitating the derivation of a truly optimal feeding profile.

4. Conclusions & Future Work

We have successfully presented, for the first time, a dynamic model that couples both glucose and glutamate concentration to the cellular growth rate of GS-NS0 cultures. Moreover we have identified that neither glucose nor glutamate are the nutrients that limit growth in the particular system, leading to the assumption that one or more of the essential amino acids are depleted after 96h of culture time. Therefore the next step is to include the uptake rates of the most significant essential amino-acids in our model prior to coupling ATP availability to growth and productivity characteristics for the first time. This will enable us to derive optimal feeding profiles for GS-NS0 cultures that maximize mAb titer whilst avoiding the excessive feed of glucose, thus maintaining cellular metabolism in energy efficient pathways and avoiding the overproduction of lactate.

5. Acknowledgements

This work was supported by the European Union with the following projects: a) PROBACTYS (FP6 – NEST-PATHFINDER EU call on Synthetic Biology, Project Number 029104), b) PSYSMO in the framework of the SYSMO initiative (BBSRC - ERA-NET program on the Systems Biology of Microorganisms, Project Number 0133980) and c) TARPOL (FP7 EU – KBBE Coordination Action for SynBio in Environmental Sciences).

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