Biochemical Engineering Journal 93 (2015) 260–273

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Regular Article

An unstructured model of metabolic and temperature dependent cell cycle arrest in hybridoma batch and fed-batch cultures

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ARTICLE INFO

Article history:
Received 4 August 2014
Received in revised form 9 October 2014
Accepted 19 October 2014
Available online 25 October 2014

Keywords:
Cell cycle model
Hypothermia
Temperature control
Cell arrest
Cell cycle specific productivity

ABSTRACT

Cell productivity in fed-batch processes can be increased by cell cycle arrest through mild hypothermia. However, hypothermia can simultaneously reduce cell growth, which is regulated by the cell cycle. Consequently, the time point for the temperature shift is important and requires optimization while considering the cell cycle. An unstructured cell cycle model which includes the distribution of proliferating (G_1, S, G_2/M) and arrested cells (G_0) has been proposed in order to predict the time point of temperature shift in fed-batch cultures. A model development and analysis framework that enables the evaluation of the required model parameters is described. The parameters are estimated from (fed)-batch cultivations, carried out at 37 °C and at 33 °C in order to characterize temperature dependency. The batch cultures are also used to evaluate substrate depletion and fed-batch cultures are used to study the impact of metabolite accumulation on the cell cycle. The reliability of the proposed framework for parameter estimation is validated using a mAb-producing hybridoma cell culture and the model predicts hypothermic transitions within the cell population at different shift time points. In conclusion, this framework can be used to optimize the time point of the temperature shift, which is commonly adjusted in industrial fed-batch processes in order to obtain a good balance between temperature induced growth limitation and cell cycle specific enhanced productivity.

1. Introduction

Fed-batch processes for the production of monoclonal antibodies (mAbs) are well established at industrial scale. The complexity of these proteins, in particular the requirement of post-translational modifications, necessitates the use of mammalian cell culture for their expression. Since the initial use of cell culture technology for mAb production [1], increasing cell productivity [2] has been essential. Several improvements have contributed to the productivity increase including the use of advanced production cell lines such as Chinese Hamster Ovary (CHO), NS0 and other cell lines [3]; optimization of the culture media [4]; and implementation of fed-batch bioprocessing [5]. For instance, decreasing the bioreactor pH set-point can result in increased productivity in multiple mAb producing cell lines [5–7].

Temperature, a process parameter that can be rather easily controlled, has been reported to similarly influence mAb production rate, particularly when applying mild hypothermic conditions. NS0 cells, when grown at 34 °C accumulated in the G_1 phase and achieved higher antibody production rates, while reaching higher maximal viable cell concentration with prolonged cell viability [8]. Similarly, CHO cells showed cell arrest in G_1 with a 1.7-fold increase in specific productivity at 30 °C accompanied by a reduced cell growth rate [9]. The reported effect of temperature is not limited to the G_1 phase, as others have reported cell blockage in the G_2/M phase post temperature shift [10], or even a rapid decrease in the S phase [11]. In addition, Ducommun et al. [12] reported a 6-fold increase in the specific production rate and a stabilization of the viable cell concentration in continuous CHO

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http://dx.doi.org/10.1016/j.bej.2014.10.013
1369-703X/

cell culture with temperature decrease to 32 °C. Similarly, Schatz et al. [13] showed a 14-fold increase in product yield with a temperature shift from 37 °C to 28 °C. Nevertheless, temperature shifts do not always result in increased productivity. Some reports showed a prolonged cell viability [14] or higher maximum viable cell concentration [15] at lower temperatures, accompanied by a lower antibody yield. Others have reported a longer lag phase at 33 °C but comparable cell densities to 37 °C, together with reduced productivity [16]. Moreover, temperature influences the cell cycle, as evident from an increased G_1 phase fraction [17], though this was not linked to a positive effect on mAb titer.

Literature reports on the effects of temperature are variable and the results seem to depend on the cell lines, the expression systems, and the expressed recombinant product [18,19]. To a certain extent, the differences can be attributed to the complex link between the cell cycle, which is at the center of cellular growth, death, and productivity [20–22]. In order to find a good balance between temperature-induced growth limitation (and consequently the integral viable cell density – IVCD [23]) and cell-specific productivity [19], temperature dependency has to be investigated and the time point of the temperature shift needs to be optimized with the aim to increase the final antibody titer.

A quantitative understanding of the effect of temperature can aid bioprocess development to improve product titers and product quality. Even though different temperature conditions and shift strategies are investigated during industrial bioprocess development, they are empirical and lack a systematic, model-based approach. The development of relevant mathematical models can help define optimal temperature profiles and is therefore of practical importance. Early contributions [24,25], examined the temperature effect on the cell cycle by employing and formulating mathematical models. Further developments of cell cycle models to study and analyze conditions that enhanced antibody production have been reported [26–30]. Although model contributions [30] accounted for reduced growth (using DNA inhibitors or by selective inhibition) and negatively associated mAb production, temperature effects were not considered. Temperature was however considered by Fox et al. [31] with the aim to optimize temperature shift times, albeit without considering the cell cycle. Recently, Karra et al. [32] proposed a detailed mass-based population balance model with cell cycle segregation and linked it to an unstructured model including the temperature effect. Unfortunately, the model was validated only during batch culture, which limits its industrial relevance.

Herein, we formulate a temperature-dependent cell cycle model for a mAb producing mammalian cell line. The model is developed for both batch and fed-batch cultures – covering a range of nutrient conditions – while capturing the distribution of cells in different cell cycle phases, including the quiescent state (G_0). The model was validated experimentally and was used to predict the time point for temperature shifts in fed-batch culture. The overall methodology maps the path to systematically develop industrially relevant mathematical models with the aim of optimizing cultivation temperature profiles.

2. Materials and methods

2.1. Cell line and media

HFN 7.1 murine hybridoma cells (ATCC CRL-1606) [33] adapted to chemically defined, protein- and peptide-free culture medium TurboDoma® TP6 (Cell Culture Technologies) were used in this study. The medium was prepared following the vendor's instruction and supplemented with 4.5 g/L D-glucose, 4 mM L-glutamine and 0.1% (w/v) pluronic F-68. Cells were sub-cultured in suspension for 14 days at 37 °C in a humidified incubator containing 5% CO_2 and subsequently used for batch and fed-batch cultivations.

2.2. Shake flask batch culture

HFN 7.1 cells were cultured in triplicate in 1 L Erlenmeyer flasks (Corning) with 240 mL working volume at a seeding cell concentration of 0.6×10^6 cells/mL. At the start of the batch experiments the temperature was set to either 37 °C or 33 °C. Mixing was accomplished using a Stuart SSL1 orbital shaker (Bibby Scientific) at 125 rpm. Viable cell concentration and viability were calculated manually by a dye-exclusion method using Erythrosin-B stain solution (ATCC). Samples were counted using a haemocytometer under the Leica DM-IL inverted phase microscope (Leica). Samples were taken twice a day and centrifuged at 660 rpm for 3 min. The supernatant was stored at −20 °C for metabolic profiling and antibody titer determination. 1×10^6 cells were fixed by drop wise addition of 1 mL of 75% v/v ice-cold ethanol (VWR) for DNA analysis. The fixed cells were stored at −20 °C prior to cell cycle analysis.

2.3. Fed-batch cell culture

Fed-batch cultures were performed in a parallel bioreactor system (DasGip) equipped with online temperature, pH and dissolved oxygen measurement probes (Mettler-Toledo), a pitched-blade impeller and a porous sparger (10 μm). Cells were seeded into the bioreactor at a cell concentration of 0.6×10^6 cells/mL in a working volume of 1 L. Dissolved oxygen tension was controlled at 50% air saturation with a constant airflow of 3 L/h and oxygen on demand strategy. pH set-point was at 7.2 over the whole culture and controlled initially by CO_2 sparging and by base addition (2 mol/L NaOH) depending on the lactate production of the growing cells. Temperature was either set to 37 °C and kept constant throughout the culture or shifted to mild hypothermia 33 °C at three different time points ($t = 0$ h, $t = 30$ h, $t = 76$ h) by changing the controller set-point. The time required for the reactor to reach the new temperature set-point was short (<10 min).

A continuous feed of amino acids (RPMI-1640 50×, Sigma), vitamins (RPMI-1640 100×, Sigma), trace elements (trace element mix 1000×, Bioconcept) and L-glutamine (64 mmol/L) was initiated 30 h after inoculation with a constant pump rate of 2.33 mL/h. D-glucose feeding solution (1 mol/L) was fed from the beginning of the culture to keep glucose at a set-point of 20 mM. The pump rate of the glucose solution was adjusted every 12 h according to the measured viable cell concentration and specific glucose consumption resulting in a semi-continuous feeding mode. Samples were taken twice a day and the viable cell concentration was determined by a CedeX cell counter (Innovatis) using the trypan blue exclusion method [34]. Concentrations of glucose and lactate were measured immediately using a Super GL compact instrument (Hitado). Samples were stored at −20 °C for later analysis of metabolites and IgG_1 concentration. Once a day 2×10^6 cells were fixed by drop wise addition of 2 mL of 75% v/v ice-cold ethanol (ACS). The fixed cells were stored at −20 °C prior to be used in cell cycle analysis.

2.4. Cell cycle analysis

Prior to flow cytometric analysis, fixed cell samples from the batch culture were washed with a rinsing solution with 1% w/v bovine serum albumin (Sigma) and 0.01% w/v sodium azide (Sigma–Aldrich) in phosphate buffered saline (PBS) [35]. DNA staining was performed for 30 min in the dark followed by addition of 1 mL of 50 μg/mL propidium iodide (PI; Sigma, P4170) and 100 μg/mL of ribonuclease A (Sigma, R4875) in PBS at room temperature. Fixed fed-batch cell samples were washed twice with 1% w/v fetal bovine serum (PAN Biotech GmbH) in PBS and re-suspended in 200 μL washing solution. 100 μL of that cell suspension were incubated with 5 μL FITC conjugated Mouse Anti-Human Ki-67 antibody (556026, BD Pharmingen) for 30 min in the dark at room