Dynamic Simulation and Visualisation of pH-Modulated Fed-batch Fermentation for mAb Production from CHO Cell Cultures

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Dynamic simulation and visualisation of pH-modulated fed-batch fermentation for mAb production from CHO cell cultures

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Abstract

Monoclonal antibodies (mAbs) are therapeutic proteins used for treating cancer, autoimmune diseases and many other critical ailments, thus constituting essential biopharmaceutical products in global healthcare. The mAb market turnover is predicted to significantly increase, with numerous new products and processes being developed each year. Current mAb processes often rely on Chinese Hamster Ovary (CHO) cell cultures, which are commonly implemented in batch and fed-batch modes, with some demonstrations of continuous/perfusion cultures in pursuit of much leaner manufacturing. Modelling and simulation can allow optimisation of different design and operating parameters towards achieving the most promising process configurations. The dynamic model for mAb fed-batch production from CHO cell cultures employed in this study describes cell growth and death, mAb production and culture volume as a function of time and pH, allowing for systematic simulation in order to elucidate promising dynamic pH modulations. Dynamic pH and state profiles for fed-batch production of mAbs from CHO cells via systematic simulation are presented. Comparisons of attained productivities, resulting concentration profiles and culture volumes are visualised and compared in order to quantitatively elucidate trade-offs in mAb production with a view to manufacturing.

Keywords: Dynamic simulation; monoclonal antibodies (mAbs); fed-batch production; Chinese Hamster Ovary (CHO) cells; pH modulation; comparative evaluation.

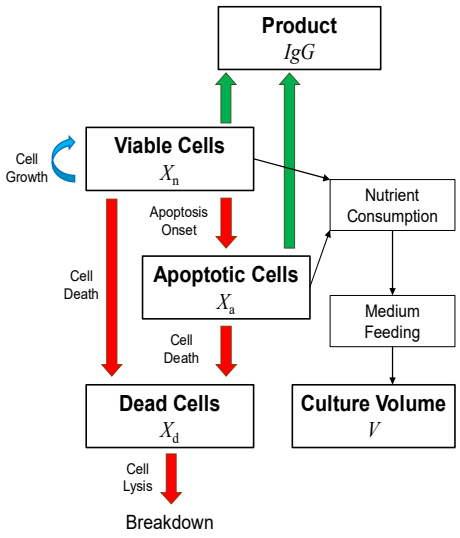
1. Introduction

Monoclonal antibodies (mAbs) represent approximately 50% of the rapidly growing biopharmaceuticals market and are important Drug Substances (DS) for their applications in cancer treatment, autoimmune diseases and many other therapies (Grilo and Mantalaris, 2019). The production of mAbs is most commonly implemented via fermentation of Chinese Hamster Ovary (CHO) cells in either batch, fed-batch or perfusion modes (Shukla et al., 2017), with the latter being pushed towards continuous mode by numerous recent research efforts towards leaner manufacturing campaigns (Schofield, 2019; Papathanasiou et al., 2019). Fed-batch mode is the industrial standard mode of operation for mAb manufacturing due to the ability to tune the nutrient dosing policy throughout the batch runtime (Bunnak et al., 2016). Optimisation of manipulation trajectories during fed-batch operation pinpoints promising process improvements over current experimental demonstrations, and foster manufacturing (Dafnomilis et al., 2019).

Dynamic simulation and optimisation of fermentation operations in bioprocessing is a useful tool in screening promising operating policies while circumventing time and financial investments in laborious experimental campaigns (Shirahata et al. 2019). A recently published dynamic model for the fed-batch production of mAb from a CHO cell culture utilised pH manipulation in order to control fermentation process performance (Hogiri et al., 2018); implementation of the model for optimisation subject to different process constraints can highlight improved pH control policies and trade-offs in performance vs. product quality. This study implements the published dynamic model for mAb production from CHO cells via pH variation for systematic comparative evaluation of different dynamic pH modulations to meet different production objectives. Dynamic pH profiles for fed-batch production of mAbs from CHO cells via systematic simulation are presented. Comparisons of productivities, concentration profiles, and culture volumes are visualised and compared to quantitatively elucidate trade-offs in mAb production.

2. Dynamic model for mAb production from CHO cells

The dynamic model for pH-dependent production of mAb from CHO cells is presented in Fig. 1 (Hogiri et al., 2018). The model describes the production of the mAb immunoglobulin G (IgG) from a CHO cell culture, where X_n, X_a and X_d are concentrations of viable, apoptotic and dead cells, respectively. The mAb is produced from viable (X_n) and apoptotic (X_a) cells, which consume nutrient and dictate the amount of dosing required vs. operation time, t, which affects the culture volume, V. The kinetic model equations are also presented in Fig. 1 (Eqs. 1–6). Here, ρ_1 and ρ_2 are production rate constants and g_1 and g_2 are production kinetic orders from viable and apoptotic cells, respectively. Parameters v_0, v_1, v_2, v_3 and v_4 describe cell growth, apoptosis onset, apoptotic cell death, viable cell death and cell lysis rates, respectively; μ and μ_{max} are specific cell growth rate as a function of cell concentration and its maximum value, respectively and x_{max} = maximum total cell density. Parameters x_{max}, μ_{max}, k_1, k_2, k_3, k_4, r_1, r_2 and r_3 are functions of $pH(t)$, which can be found in the literature (Hogiri et al., 2018).



State Trajectories

$$\frac{dIgG}{dt} = \rho_1(pH - 6.4)^{g_1}X_n + \rho_2(pH - 6.4)^{g_2}X_a \quad (1)$$

$$\frac{dX_n}{dt} = v_0 - v_1 - v_3 = \mu X_n - k_1 X_n \left(\frac{X_a}{V}\right)^{\alpha} - k_3 X_n \quad (2)$$

$$\frac{dX_a}{dt} = v_1 - v_2 = k_1 X_n \left(\frac{X_a}{V}\right)^{\alpha} - k_2 X_a \quad (3)$$

$$\frac{dX_d}{dt} = v_2 + v_3 - v_4 = k_2 X_a + k_3 X_n - k_4 X_d \quad (4)$$

$$\frac{dV}{dt} = r_1 v_0 + r_2 X_n + r_3 X_a = r_1 \mu X_n + r_2 X_n + r_3 X_a \quad (5)$$

$$\mu = \mu_{max}\left(1 - \frac{X_n + X_a + X_d}{x_{max} V}\right) \quad (6)$$

Figure 1: Dynamic model for mAb (IgG) production = $f(pH)$ (Hogiri et al., 2018).

3. Dynamic simulation of static pH operation

Dynamic simulation of the published model for mAb production is performed for varying $pH(t)$ = constant, as per the published experimental results (Hogiri et al., 2018); the dynamic model is valid within the range $6.6 \leq pH(t) \leq 7.2$ and thus constant values of $pH(t) = \{6.6, 6.8, 7.0, 7.2\}$ are considered for a maximum batch duration of $t_f = 350$ hr. As $pH(t)$ is increased, the final amount of mAb produced increases and then decreases beyond some intermediate pH value. Production of mAb is slow at first and then increases around $t = 50$ hr for all considered pH values. As pH is increased, dead cell concentrations increase. Viable cell concentrations reach a peak at different points during the batch run and then decrease; as pH is increased from 6.6 to 7.0, the time at which this is reached becomes later and the value and final viable cell concentration are higher, whereas for pH = 7.2, these values occur earlier and lower, respectively. Specific growth rates decrease and then increase to a plateau over the batch duration with final values decreasing with increasing pH. Culture volumes vary with cell densities, which both increase with pH.

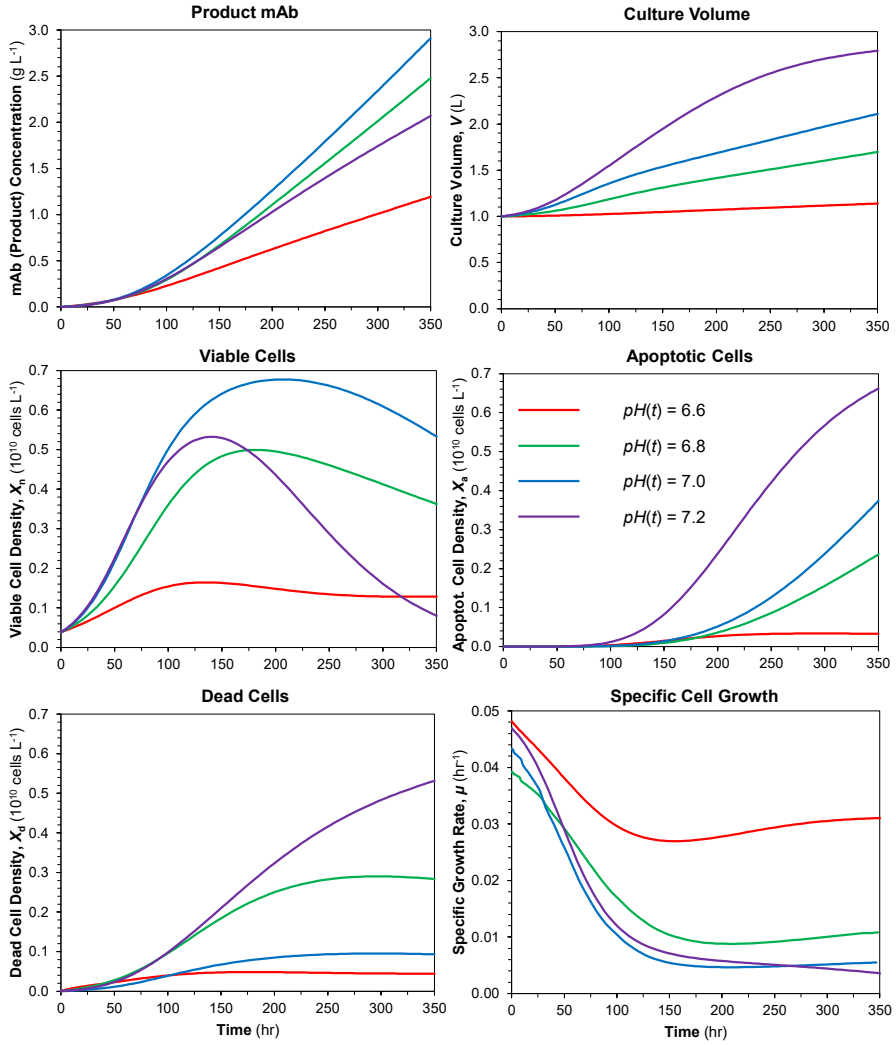


Figure 2: Dynamic simulation of different constant $pH(t)$ profiles on state trajectories.

4. Dynamic pH manipulation: process performances and trade-offs

From the previous static simulations, it is shown that there are trade-offs between variables in the considered system. The model can be used to investigate the effect of dynamic pH profile implementation on different production objectives. Previous work investigated dynamic manipulation to maximise mAb production (Hogiri et al., 2018), but did not consider effects on other variables, such as cell concentrations and culture volume, which can inform the design of more efficient processes, e.g., implementing recycle. Here, we consider the effect of dynamic pH on different process variables and then visualise production trade-offs. The considered pH profiles assume initial $pH(t = 0) = 6.8$, as per experimental demonstrations, and $\Delta pH = \{0.0, +0.2\}$ within each 24 hr period; the maximum possible batch duration is 384 hr. All considered limitations are in accordance with literature demonstrations (Hogiri et al., 2018). The number of time domain discretisation elements, $N = 16$; thus the number of considered pH profiles = 2^{16}.

Fig. 3 shows the considered pH profile attaining the highest final mAb concentration (IgG ($t_f = 384$ hr) = 3.35 g L^{-1}) and corresponding state variables. A gradual increase from the initial pH ($t = 0$) = 6.8 to pH ($t = 168$ hr) attains the maximum final mAb concentration of those pH profiles considered. The best pH profile of those considered lies in the middle of the applicable pH range, i.e. above the lower bound, ($pH_L = 6.6$) and below the upper ($pH_U = 7.2$). The likely reason for pH plateauing at this time is the decrease of viable cells producing mAb thereafter, which implies a concurrent increasing quantity of dead cells. Investigating the region of attainable production performance over the range of possible implemented pH manipulations gives an indication of where process improvement can be achieved, with excessive cell death (reduction in viable cell concentration) thus avoided.

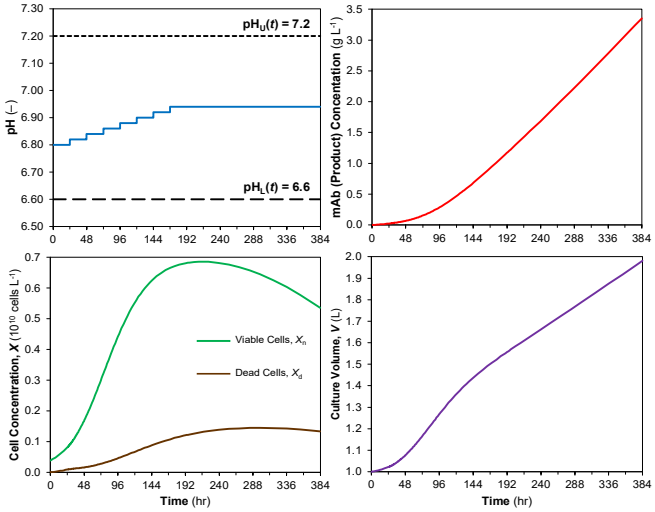


Figure 3: Manipulation (pH) and state profiles attaining maximum mAb concentration.

Fig. 4 visualises process variables vs. batch time for all pH profiles considered. While batch time increases productivity, the culture volume increases; although the considered scale is relatively small (initial volume, V ($t = 0$) = 1 L), increasing culture volumes at larger scales of operation may be undesirable due to the larger material handling requirements post-batch as well as the higher capital expenditures associated with larger equipment. With ongoing batch duration, viable cell concentrations begin to decrease as dead cell quantities increase; this makes potential cell recycling/separation less feasible.

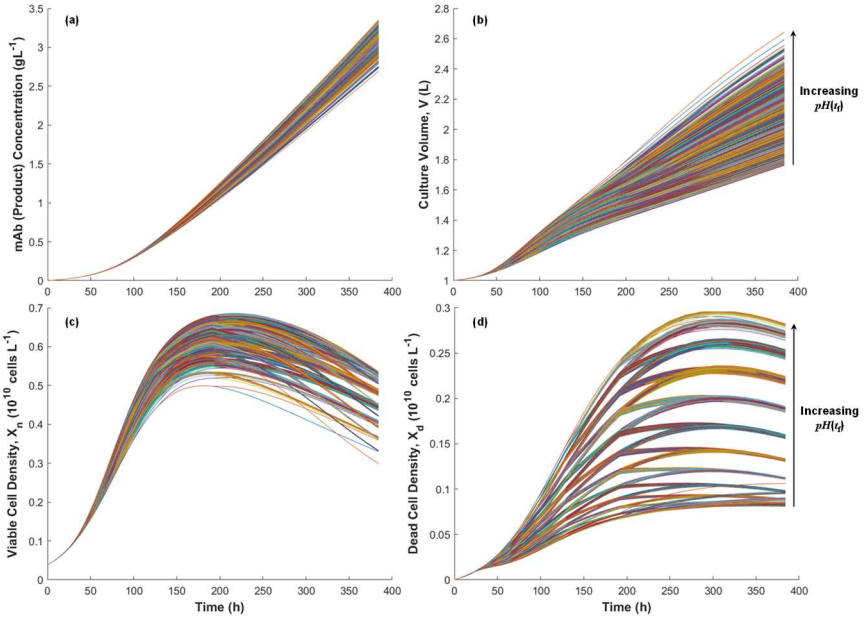


Figure 4: Trade-offs: (a) Product, (b) Volume, (c) Viable cells, (d) Dead cells vs. time.

Fig. 5 shows trade-offs between variables for all pH profiles. Banding is observed due to the constraints on the pH. Increasing productivity leads to lower viable and higher dead cell densities. There is a trade-off between viable and dead cell densities that must be considered if deciding to recycle. The considered pH profiles are limited in their pH step size, temporal discretisation, initial pH and their piecewise constant variation; piecewise linear variation is also possible. Formulation of a dynamic multiobjective optimisation problem of pH for optimal production will further elucidate productivity benefits.

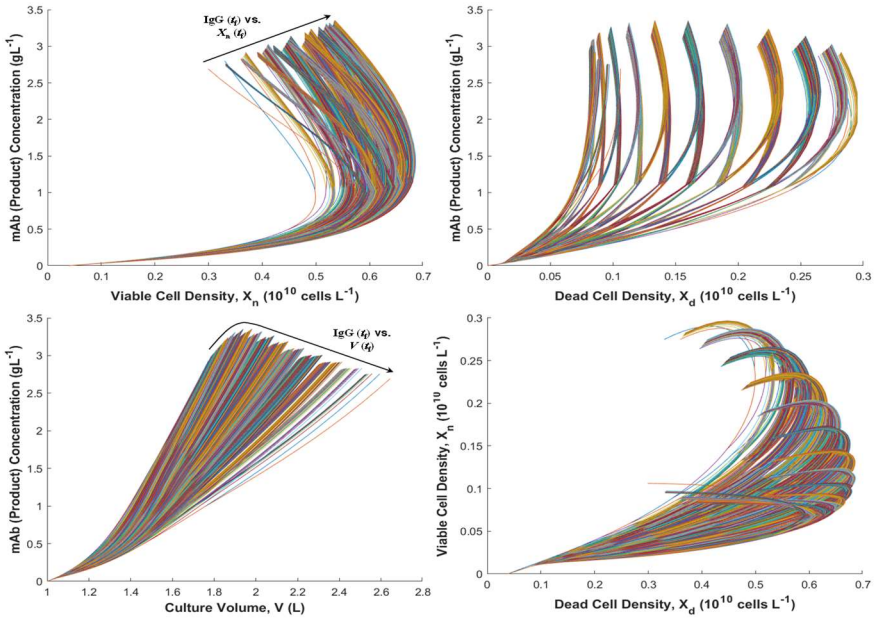


Figure 5: Pareto fronts exhibiting different trade-offs between different process variables.

5. Conclusions

The dynamic modelling and simulation of mAb production from CHO cell cultures can elucidate potential improvements in biopharmaceutical production compared to static pH culture conditions in terms of productivity, cell viability, culture volume and total batch duration. This study has visualised and compared trade-offs between mAb production, cell concentrations and culture volume. The pH manipulation attaining maximum productivity steadily increases until halfway through the batch and then remains steady for the remaining half. This manipulation profile lies in the middle of the applicable pH range of the model. Consideration of the effects of different pH manipulations on other state variables is also important for efficient process design. Trade-offs between mAb productivity and viable as well as dead cell concentrations elucidate the need for dynamic optimisation of pH variation, in order to design biopharma processes of high efficiency.

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