

Evaluation of a Metabolic Flux Model for CHO Cells to Design Highly Effective Fermentation Processes



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1. Introduction

The demand to develop highly productive processes in shortened timelines requires a shift from extensive testing procedures in bioreactor systems to a multi cell parameter analysis and intelligent prediction of optimal process design using a sophisticated model. We are in the process to develop and optimize such kind of model. This model was applied to a CHO-DG44 clone producing a monoclonal antibody for the first time.

2. Modelling assumptions

Metabolic Flux Analysis (MFA) uses quasi-stationary phases of cellular growth to resolve the flux distribution of metabolite usage. Within one phase, e.g. exponential growth or under hypothermic conditions, the cell specific growth rate, substrate consumption and production rate is set constant. Therefore it is assumed that pathway intermediates, s_{int} , do not accumulate and enzymes operate at steady state.

$$\frac{dX}{dt} = \mu \cdot X \quad (1) \quad \frac{ds_{ext}}{dt} = v_{s_{ext}} \cdot X \quad (2) \quad \frac{ds_{int}}{dt} = 0 = N \cdot v_{s_{int}} \quad (3)$$

For a network defined by a stoichiometric matrix N , uptake rates, $v_{s_{ext}}$, and under the quasi-stationary assumption, the internal fluxes, $v_{s_{int}}$, can be solved by a least-squares approximation. In our case, we used the tool CellNetAnalyzer [1].

3. Model structure

The underlying network structure is taken from a metabolic network which was originally developed for MDCK cells [2]. Besides commonly modelled substrates like glucose and lactate, the model also considers amino acids.

4. Rate determination and model extension

In order to determine uptake rates, batch experiments were carried out under varied culture conditions such as temperature shifting and different nutrient concentrations. This is exemplary shown in Fig. 1 for a change in nutrient uptake and release of Aspartate and Glutamate before and after shifting from 36.5 °C to 33 °C. The slope of the green and red curve thereby represents the specific rate within one phase.

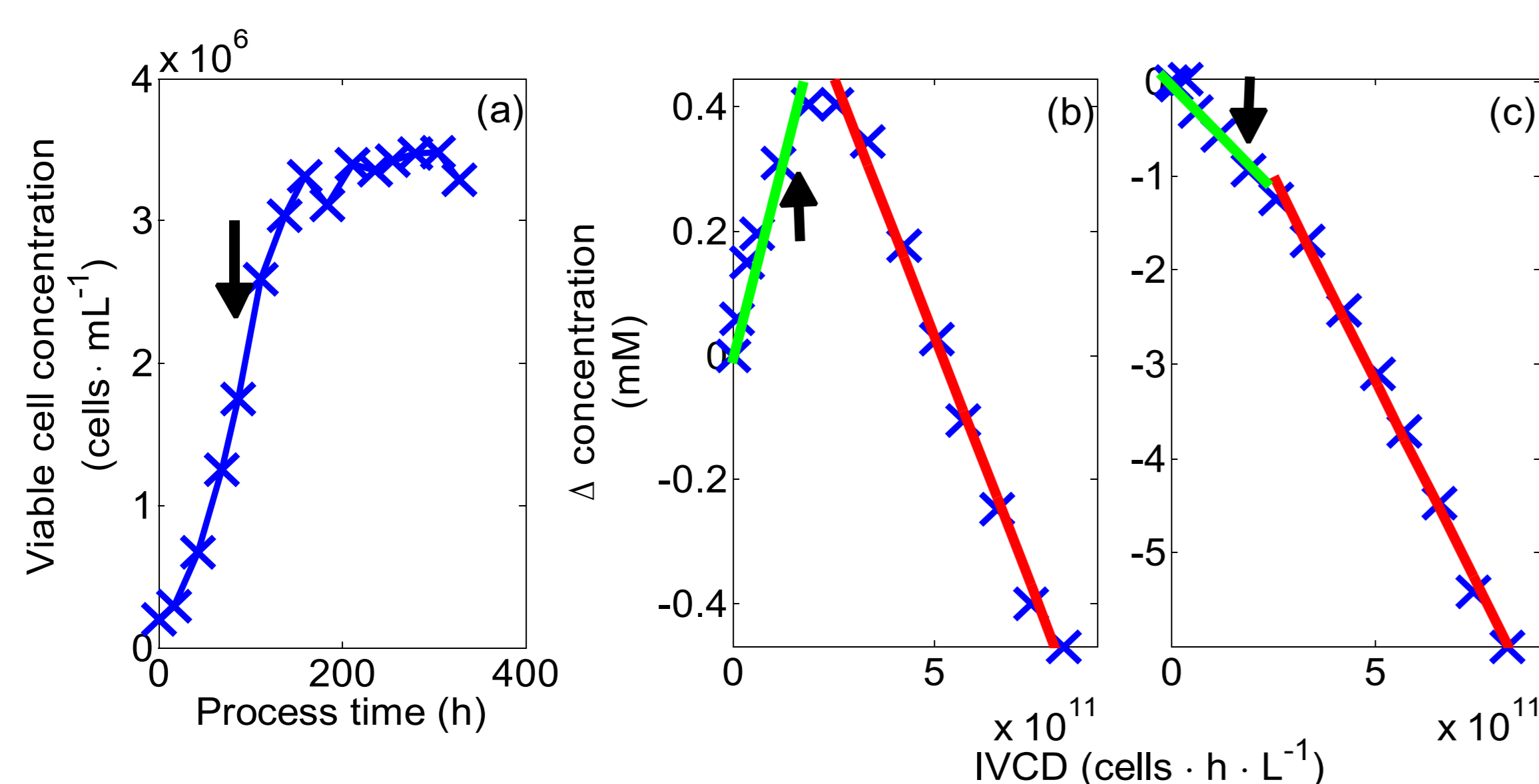


Fig. 1: Determination of substrate consumption and uptake rates before and after temperature shifting (indicated by an arrow) from 36.5 °C to 33 °C in one exemplary batch experiment. (a) viable cell concentration, (b) Aspartate, (c) Glutamate.

The overall growth behaviour is described by a multi-phase model which switches between characteristic growth phases under different nutrient concentrations and process temperatures, cf. [3].

The flux distribution for each phase is then calculated individually. To describe the whole process the individual phases are linked with switching functions, ϕ_i . The status of the switching functions (0 or 1) is defined by measured values of temperature, oxygen uptake rate and glutamine concentration. The correlation between certain parameter values and the status of the switching function was determined in previous experiments.

The model structure yields to equations (4) - (6) to be able to describe a fed-batch process:

$$\frac{dX}{dt} = \left(\mu - \frac{F}{V} \right) \cdot X \quad (4) \quad \frac{ds_{ext}}{dt} = v_{s_{ext}} \cdot X + (s_{Feed} - s_{ext}) \cdot \frac{F}{V} \quad (5)$$

with: $\mu = \phi_1 \cdot \mu_1 + \phi_2 \cdot \mu_2 + \dots + \phi_i \cdot \mu_i$ $v_{s_{ext}} = \phi_1 \cdot v_{s_{ext1}} + \phi_2 \cdot v_{s_{ext2}} + \dots + \phi_i \cdot v_{s_{exti}}$

$$\phi_i = f(T, OUR, Gln), \quad \phi_i \in \{0, 1\} \quad (6)$$

5. Use within a fed-batch process

5.1. Model integration

The model is integrated into a framework to optimize and control the feeding with two feed solutions composed of either amino acids or energy-sources. For the fed-batch process online and offline data were available. Offline process data were fed daily. Furthermore data acquired during previous runs were used as well to acknowledge amino acid consumption. A genetic algorithm was applied to calculate optimal feeding profiles for a 32 h prediction horizon. This 32 h interval was further divided into distinct feeding events every four hours to adjust nutrient concentrations smoothly.

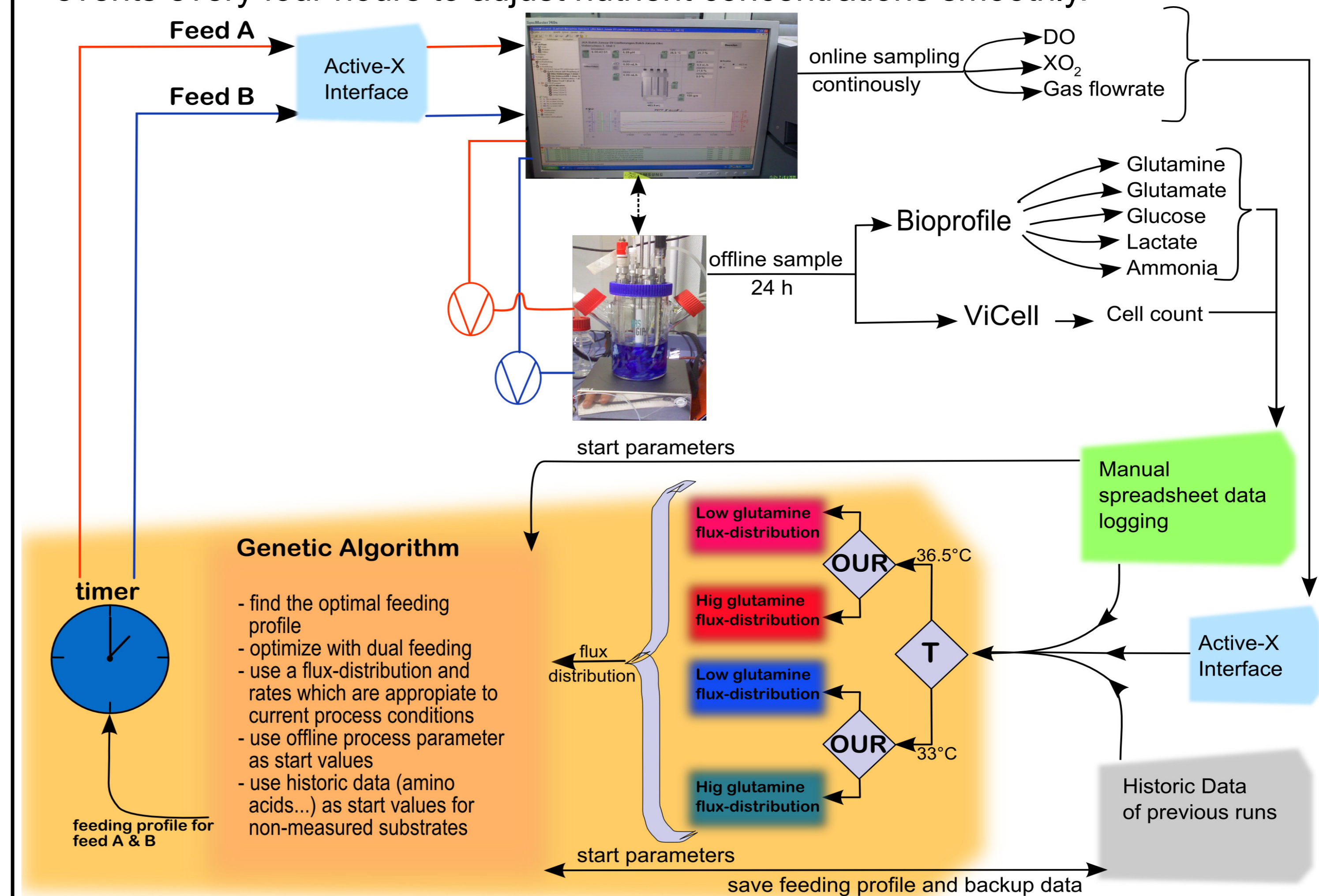


Fig. 2: Schematic representation of model integration into a fed-batch process for which on- and offline data is available.

5.2 Wet-lab application

The generic fed-batch process was performed at 36.5 °C fixed. One model-controlled process was run at 36.5 °C fixed as well, a second model-controlled process was shifted to 33 °C after six days. The termination criterion for all three runs was set to 50% viability.

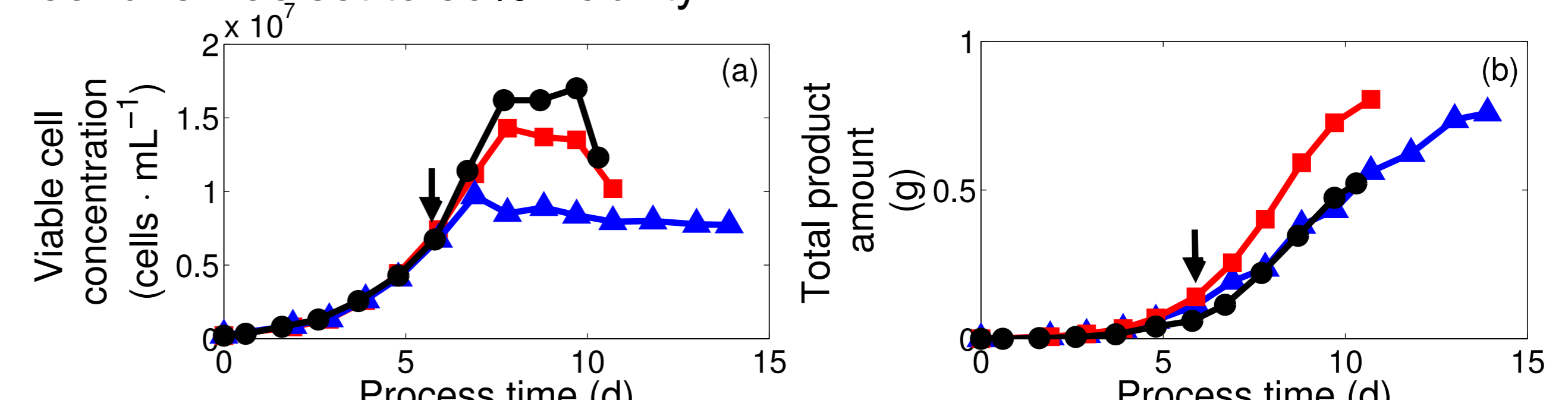


Fig. 3: Experimental results for a generic, manual feeding strategy (●) and the use of model at strictly 36.5 °C (■); as well as model-aided feeding with temperature shifting to 33 °C (▲). Experiments were carried in 1-L-scale CSTR systems. The shifting time point is indicated by an arrow. (a) viable cell concentration, (b) total product amount.

The model-based feeding did not change cell growth but showed an increase in cell specific productivity resulting in a higher total mAb amount (153%) at 36.5 °C (titer of ≈1.5 g/L) compared to the generic process within the same period.

A temperature shift down to 33 °C combined with the optimized feeding model leads to a steady state phase of at least one week (still running) with the potential of even higher antibody amount at the end of the process due to the longer cultivation time. The current product concentration (≈1.7 g/L) exceeds the final result of the generic process (≈1.3 g/L) by 30%.

6. Conclusions

The model-based process control fits with cellular needs and leads depending on other conditions (e.g. temperature) either to higher cell specific productivity or prolonged cultivation time. Both ways increase the final product amount substantially as shown in our case study.

7. Future directions

The use of MFA offers a tool for a better understanding of cellular needs during cultivation and to design an optimal feed composition. Further, we aim to use the model to quickly design robust processes for different clones expressing different products and to apply this strategy to other cell substrates, especially ProBioGen's proprietary AGE1 cell line family.

More extensive results and analysis will be available in [4].

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