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A Hybrid Modeling Approach to Accelerate Media and Feed Development

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Abstract

There is a growing interest in the biopharmaceutical industry to improve the understanding of growth kinetics in mammalian cell-based processes, particularly for monoclonal antibody (mAb) production. By applying a first principle or mechanistic models as a new approach, mathematical and complex fundamental relationships can be described within the cell and between the cell and environmental variables, e.g., between a glucose concentration and the growth rate.

While mechanistic models can help understand the process better and determine key parameters like substrate limiting concentration, virtual representations of the process in the form of digital twins will enable the simulation of experiments in silico, reducing the process development workload and time and supporting decision-making.

Here, we present a study that focuses on building a mechanistic model to determine the growth kinetics for a CHO-based process producing a mAb. After fitting the model with calibration data, the model was applied to an independent validation data set. In parallel, an artificial neuronal network approach was used to determine the specific productivity. Both modeling approaches were merged to produce a hybrid model to predict the overall product concentration. This hybrid model would form the basis of the next step: a digital twin.

Introduction

The biopharmaceutical market is a fast-growing market that, in 2020, generated 192.46 billion USD and is expected to grow to a 326.3 billion USD business by 2026.[1] Today, 5 of the top 10 most sold pharmaceuticals are biopharmaceutical products. With a total revenue of 20.4 billion USD, the top-selling drug in 2020 was Humira, a biopharmaceutical from AbbVie.[2]

Despite being such a big business and the complexity of the products, the production processes for biopharmaceuticals are poorly monitored and not perfectly understood. Therefore, the FDA encouraged manufacturers to use more process analytical technology (PAT) tools by issuing the "PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance" in 2004.[3] Although the implementation of PAT is more challenging in the biopharma industry compared to the chemical or petroleum industry,[4] the use of PAT for mAb production is gaining momentum.[5]

While PAT addresses the better controllability of bioprocesses, the second aspect of better process understanding is only partially met with PAT. One way of getting a better understanding can be achieved by first principle or mechanistic models. These models describe mathematically fundamental relationships, e.g., between a glucose concentration and the growth rate.

Some of the discoveries made in this field are more than 100 years old,[6] and the basic relationship between substrate and growth was already described in 1949 by Jacques Monod.[7] It took another 43 years for the first hybrid model and an additional 17 years to publish a biotechnology application for a digital twin.[8] While mechanistic models can help better understand the process and determine key parameters like substrate limiting concentration, digital twins enable the simulation of experiments in silico, reducing the workload and time for process development.

In the present study, we focused on building a mechanistic model to determine the growth kinetics for a CHO-based process producing a mAb. After fitting the model with calibration data, the model was applied to an independent validation data set. In parallel, an artificial neuronal network approach was used to determine the specific productivity. Both modeling approaches were merged to produce a hybrid model to predict the overall product concentration. That hybrid model would be the basis of the next step: a digital twin.

Cell Insights by Umetrics® Studio.

Materials and Methods

Model for Process Simulation (Biosimulation Library)
For this study, an ordinary differential equation (ODE)-based approach was selected using an in-house developed Python library (a version of this bioreactor modeling library can now be found in Cell Insights by Umetrics® Studio). The model is an unstructured, unsegregated model. More information on the different types of models can be found in this review paper.[8] The structure of models behind the library can be broadly divided into three parts:

1. A first-principles cell balance model (growth/death)
2. A hybrid metabolic evolution model
3. A hybrid productivity (titer) model

The first part tracks the population of cells as they move through three phases: live cells, dead cells, and lysed cells. Mathematically, the evolution of the live, dead, and lysed cells is tracked using ODEs as follows:

$$\frac{dx_v}{dt} = \left(u_{eff} - u_d - \frac{F_b}{V}\right) \times x_v$$

$$\frac{dx_d}{dt} = u_d \times x_v - \left(k_l + \frac{F_b}{V}\right) \times x_d$$

$$\frac{dx_1}{dt} = k_l \times x_d - \frac{F_h + F_b}{V} \times x_l$$

where x_v is the viable cell density (VCD), x_d is the dead cell density, and x_l is the lysed cell density (concentration of lysed cells). F_b is the bleed rate, F_h is the harvest rate, and V is the reactor volume. u_{eff}, u_d, and k_l are the effective growth, effective death, and lysing rates, respectively.

The second part focuses on the metabolites. Metabolites are tracked in the model using basic mass balances and piecewise constant (trained from data) metabolic consumption rates. The primary function of metabolites in the model is to consider them as substrates, quadratic factors, or inhibitors. This role might depend on the biology of the given cell line. The cell line used was CHO DG44 expressing a mAb (IgG1).

The third part is a feed-forward neural network with 2 hidden layers (128 and 16 nodes, respectively) and an exponential linear unit (ELU) activation function. Based on batch evolution data of available parameters (VCD, cell viability, metabolites, etc.), the network predicts an average titer production rate (total titer/total biomass) for the batch. It was trained using a node dropout probability of 0.3, L1 loss, and Adam optimizer with a learning rate of 0.001.

Process Analytics
Process analytics were performed during the course of the cultivation process. The VCD and viability were determined using a Vi-CELL™ XR* or Cedex HiRes®*. The titer was determined using an in-house HPLC method. Metabolites, including glucose and lactate, as well as blood gases, metal ions, and osmolality, were determined using a Nova BioProfile® FLEX*. Metabolites were tracked using basic mass balances and piecewise constant (trained from data) metabolic consumption rates. The primary function of metabolites in the model is for inclusion as substrates, quadratic factors, or inhibitors as appropriate for the biology of the given cell line.

*Vi-CELL™ XR is a registered trademark of Beckman & Coulter. Cedex HiRes® is a registered trademark of Roche. Nova BioProfile® FLEX is a registered trademark of Nova Biomedical

Results

Calibration Data

The processes were run in an Ambr®15 system (Sartorius, Göttingen) with an initial working volume of 14 mL. Design of experiments (DoE) approach was used, focusing on feed and media optimization.

These experiments were used to calibrate the model. The varied factors are presented in Table 1.

Table 1: *Factors Varied to Calibrate the Model*

Factor	Low	High
Start media	Media_B1	Media_B2
Feed media	Media_F1	Media_F2
Relative feed amount to initial working volume	3.328%	5.120%

In addition, a third feed (Media_F3) was added as a percentage of either Media_F1 or Media_F2 (1.2%). All feeds were added every other day starting day 1 post-inoculation. To ensure sufficient glucose was present in the cultures; glucose was added on demand when it fell below 4g/L to a target concentration of 8g/L.

The temperature was tightly controlled at 37 °C with a temperature shift when the VCD reached 15×10^6 cells/mL or day 5 for batches. Based on the temperature control of the bioreactor system, a complete set of 12 bioreactors was shifted to 33 °C. The bioreactors used did not have a temperature probe. Consequently, the exact time when the individual bioreactor reached the new target temperature was unknown. This introduces some error into the modeling approach. Table 2 shows all batches included in the calibration model.

Table 2: *Overview of Processes Used for Model Fitting (Calibration Data)*

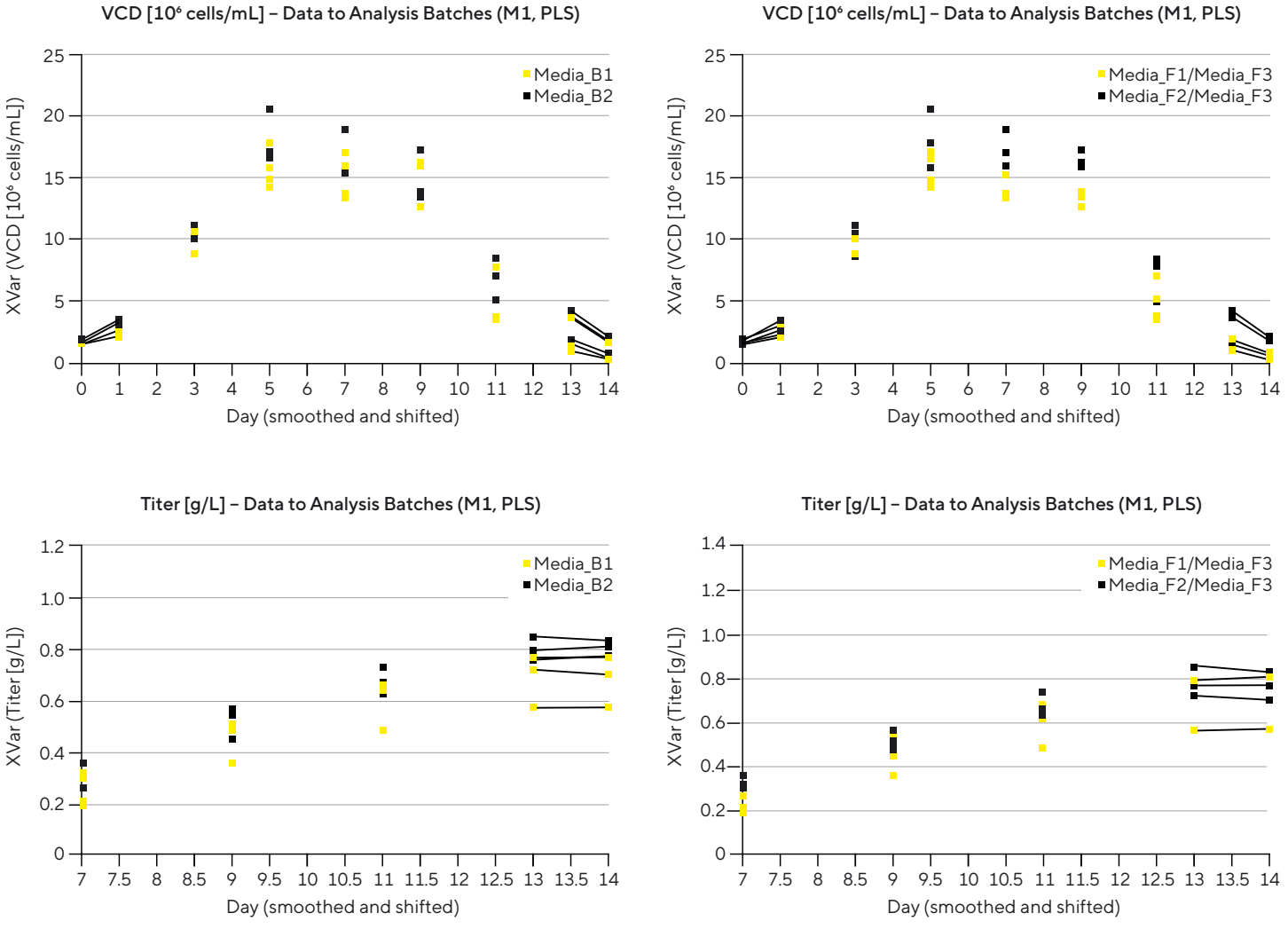
Culture ID	Inhibit Media	Temperature Shift [days]	Inhibit Feed	Feed Media Fractional Reduction	Control | Test
1	1	5	1	0	Control
2	1	5	1	0,15	Test
3	1	5	1	0,25	Test
4	1	5	1	0,35	Test
5	1	5	0	0	Control
6	1	5	0	0,15	Test
7	1	5	0	0,25	Test
8	1	5	0	0,35	Test
9	0	5	1	0	Control
11	0	5	1	0,25	Test
12	0	5	1	0,35	Test
17	1	5	1	0	Control
18	1	5	1	0,15	Test
19	1	5	1	0,25	Test
20	1	5	1	0,35	Test
21	1	5	0	0	Control
22	1	5	0	0,15	Test
23	1	5	0	0,25	Test
24	1	5	0	0,35	Test
25	0	5	1	0	Control
26	0	5	1	0,15	Test
27	0	5	1	0,25	Test
28	0	5	1	0,35	Test
29	0	5	0	0	Control
30	0	5	0	0,15	Test
31	0	5	0	0,25	Test
32	0	5	0	0,35	Test

Data Exploration Calibration

The first part of finding a process model is ensuring the data is fit for purpose. A good model base consists of data with a high assignable variation and little unstructured variation or noise. Effects on the cell culture that are not varied in a meaningful way cannot be easily modeled since they depict one-time events. Therefore, all batches were reviewed and excluded if process deviations occurred during the cell culture run (e.g., issues with CO_2 gas flow). The selection of batches to exclude was made by our collaboration partner Merck. It is important to review the batches before starting the actual modeling work to make sure the models are not biased.

When looking at the overall variation with the data on VCD and titer (Figure 1), it seems that the media has a stronger effect than the feed. This is an important consideration in an, e.g., inhibition effect. The temperature was another strong effect since the VCD stopped increasing after the temperature was shifted to 35 °C at day 5.

Figure 1: *Variation of Control Experiments for Calibration*



Note. Coloring is displayed according to media for left plots and feed for right plots.

Model Result

After representative data was identified, the modeling process began. This is an iterative approach and needs several cycles until a good model is found. Typically, each cycle of model fitting contains either more calibration batches or more model parameters. In this sense, a good model is a model with high accuracy for VCD (measured using the root mean squared error [RMSE]).

The first model build contained only the valid control processes and a rudimentary model structure. The objective of this first model was to establish the baseline values for the maximum growth rate, the death rate, the toxicity, the lysing rate (used to express toxicity by an increase in death rate), and the biomaterial (relative accumulation of a set of unmeasured materials expressed by viable cells that inhibit growth). The inhibition based on biomaterial is the threshold that these unmeasured materials begin to inhibit the growth rate. In fed-batch, the biomaterial hidden state is equivalent to the cell hours integral viable cell density (iVCD).

Once these basic growth parameters were narrowed down, the other calibration processes were included for model fitting. New model parameters to be included were found by searching for processes that differ the most from the true VCD performance and the model prediction. Differences for those processes (e.g., earlier temperature shift or higher start osmolality) were included as new model parameters, and the model re-fitted.

Table 3: *Model Parameter Values After Fitting Based on All Calibration Runs*

Parameter	Role	Type	Value
primary_growth_rate	Growth parameter	Growth parameter	0.705
primary_death_rate	Growth parameter	Growth parameter	0.039
toxicity	Growth parameter	Growth parameter	0.002
lysing_rate	Growth parameter	Growth parameter	2.234
Biomaterial	Growth parameter	Inhibitor	170.942
Inhibit feed	Independent	Inhibitor	0.969
Inhibit Media	Independent	Inhibitor	0.849
Cum_Osmo	Independent	Inhibitor	2696.566
Ammonia	Metabolite	Inhibitor	7.518
Glucose	Metabolite	Substrate	0.248
Temperature	Independent	Quadratic	37 ± 0.787

Note. Used file [20211028_config_sim_all_batches_yaml]

As a first confirmation of the plausibility of the model, we compared some of the relevant model coefficients to literature values. For the growth rate (μ) for CHO cells, Ozturk et al. (2005) valued in the range of 0.3 and 1.4/day.[10] Ritter (2009) reported μ between 0.035/h and 0.040/h, depending on the media selected.[11] In this study, the maximum growth rate was determined to be 0.705/day.

For the temperature influence, Yoon et al. (2005) reported a decrease of the μ from 0.027 to 0.011 when changing the temperature from 37 °C to 32 °C.[12] Bedoya-López et al identified a drop in μ from 0.021 to 0.005 when shifting the temperature from 37 °C to 30 °C.[13] In the presented case, growth starts to decrease when the temperature is lower than 36.2 °C (37 °C – 0.8 °C).

For ammonia, an inhibition was reported starting with 5 mM NH_4Cl and an IC-50 of 33 mM.[14] The final model in this publication shows an ammonia-based inhibition when concentrations exceed 7.5 mM.

The result of the final model is given in Figure 2. The root means squared error for viable cell density across all data points was 20×10^5 cells/mL. Figure 2 shows the VCD profile over 14 days. The fit of the initial growth phase is excellent for all of the cultivations, but the value predicted by the model was less accurate for some batches just after the temperature shift. This was especially true for batches 3, 4, 5, and 28; one reason might be that the exact time-point of the temperature shift is unknown. The temperature is controlled for all 12 bioreactors, and none of the vessels have an individual temperature probe. So when the temperature set-point is changed, it takes a certain time until each and every bioreactor reaches the new target temperature. Since the temperature has a strong effect, a slightly delayed temperature shift (e.g., based on slightly different thermodynamics) will contribute to a model mis-fit (higher true values compared to the model predictions).

In addition, the model was not able to capture the death phase for some batches (e.g., 1 – 8 and 18,19). The reason might be that the processes had different death kinetics that can not be modeled with the current model structure applied in this study. Therefore the model tried to fit average death kinetics. To overcome this problem, the model could be augmented by death-promoting or death-preventive model terms, similar to growth inhibition by a toxic by-product. We tested an ammonia-dependent death promotion and achieved slightly better results for the death phase. The improvement was not yet significant, as a proper optimization algorithm is missing in the HSSM library (data not shown).

Figure 2: *Simulation Result for Calibration Batches (Viable Cell Density Result)*

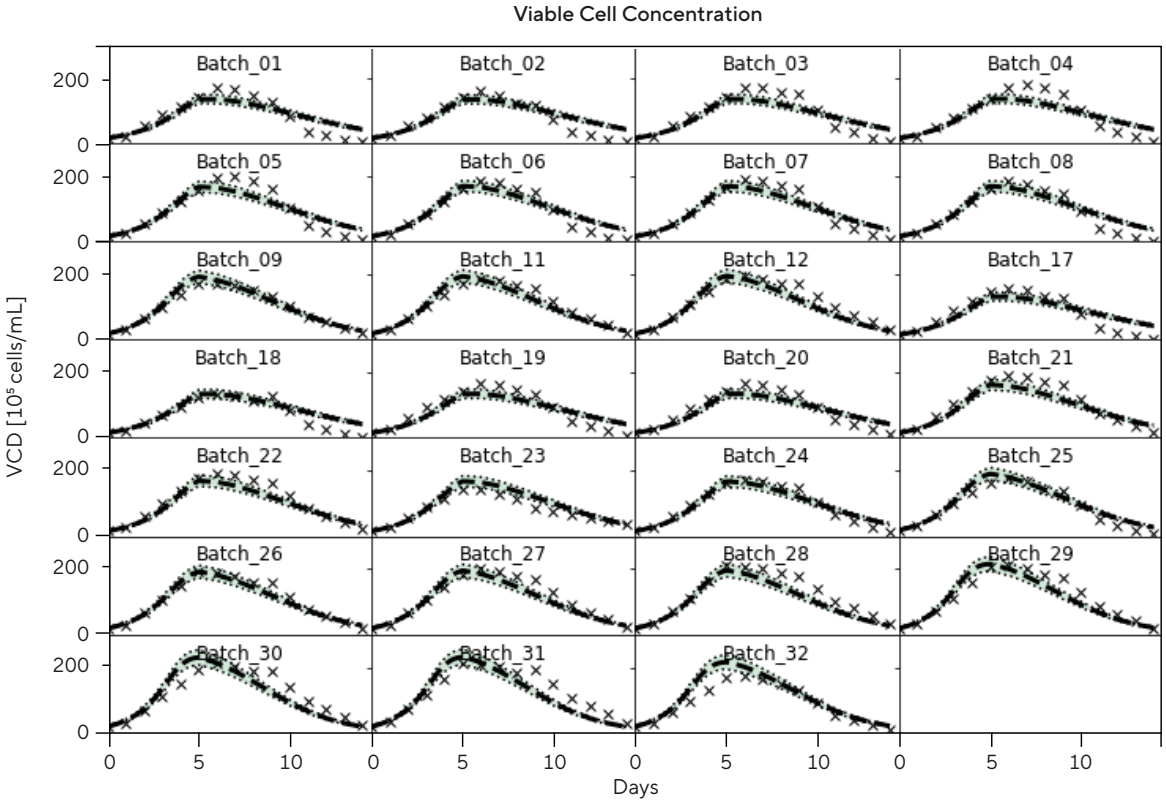
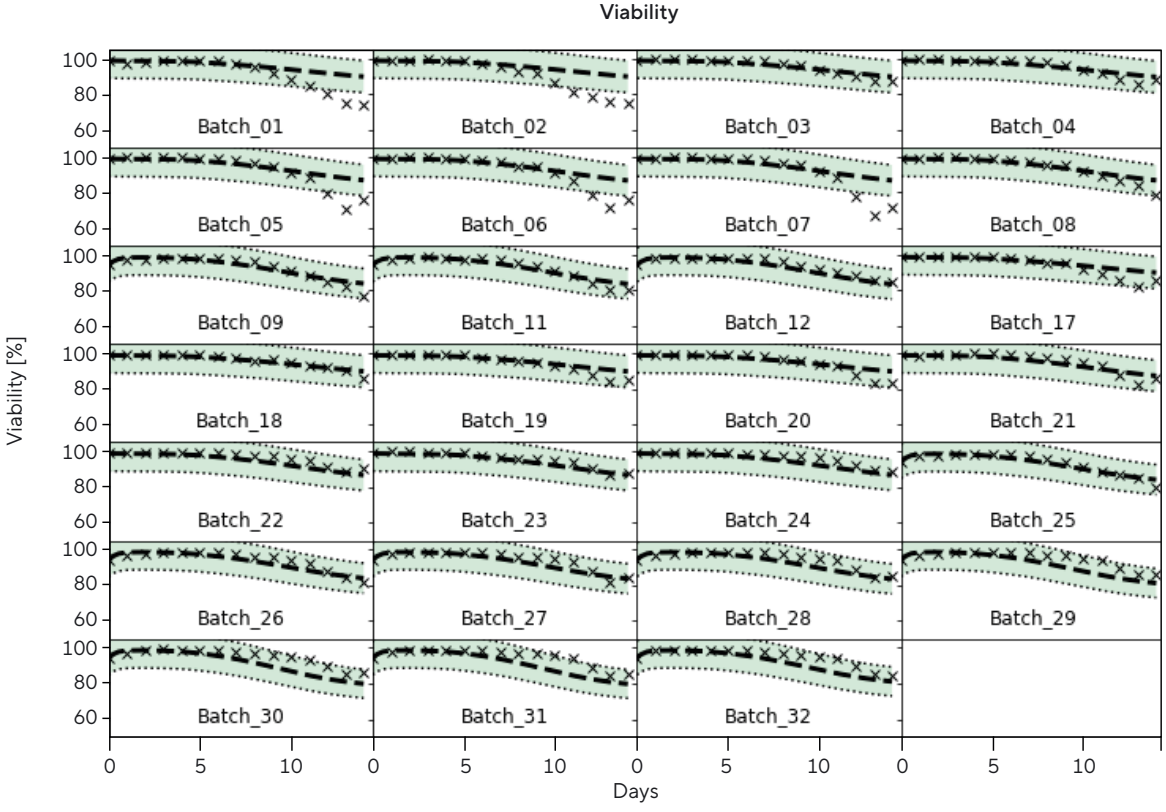


Figure 3: *Simulation Result for Calibration Batches (Viability)*



The main focus of this study was to model the initial growth phase, with more emphasis on the first two-thirds of the process. This decision was made as the initial process phase is typically the foundation for a process resulting in high titer and correct quality. Interestingly, the viability was modeled well in most of the cases (Figure 3).

This could be an indication that if the model is used for simulating new conditions, conditions resulting in lower viability will be captured. In addition, a low VCD at the late stage of the process is considered less critical compared to a low viability.[15] As a consequence, we favored models that better captured the initial process phase and were able to sufficiently monitor the viability. As a next step, the model was applied to the validation data.

Table 4: *Validation Batches*

Primary ID	Inhibit Media	Temperature Shift [days]	Inhibit Feed	Setpoint	Glucose Strategy [g/L]
Validation 01	1	3	0	Setpoint_III	If < 4 or > 8
Validation 02	1	3	0	Setpoint_IV-10%	If < 4 or > 4
Validation 03	1	3	0	Setpoint_IV-8%	If < 4 or > 4
Validation 04	1	3	0	Control	If < 4 or > 8
Validation 05	1	3	1	Setpoint_III	If < 4 or > 8
Validation 06	1	3	1	Setpoint_IV-10%	If < 4 or > 4
Validation 07	1	3	1	Setpoint_IV-8%	If < 4 or > 4
Validation 08	1	3	1	Control	If < 4 or > 8
Validation 09	0	3	0	Setpoint_III	If < 4 or > 8
Validation 10	0	3	0	Setpoint_IV-10%	If < 4 or > 4
Validation 11	0	3	0	Setpoint_IV-8%	If < 4 or > 4
Validation 12	0	3	0	Control	If < 4 or > 8
Validation 13	0	3	1	Setpoint_III	If < 4 or > 8
Validation 14	0	3	1	Setpoint_IV-10%	If < 4 or > 4
Validation 15	0	3	1	Setpoint_IV-8%	If < 4 or > 4
Validation 16	0	3	1	Control	If < 4 or > 8
Validation 25	1	3	0	Setpoint_III	If < 4 or > 8
Validation 26	1	3	0	Setpoint_IV-10%	If < 4 or > 4
Validation 27	1	3	0	Setpoint_IV-8%	If < 4 or > 4
Validation 28	1	3	0	Control	If < 4 or > 8
Validation 29	1	3	1	Setpoint_III	If < 4 or > 8
Validation 30	1	3	1	Setpoint_IV-10%	If < 4 or > 4
Validation 31	1	3	1	Setpoint_IV-8%	If < 4 or > 4
Validation 32	1	3	1	Control	If < 4 or > 8
Validation 33	0	3	0	Setpoint_III	If < 4 or > 8
Validation 34	0	3	0	Setpoint_IV-10%	If < 4 or > 4
Validation 35	0	3	0	Setpoint_IV-8%	If < 4 or > 4
Validation 36	0	3	0	Control	If < 4 or > 8
Validation 37	0	3	1	Setpoint_III	If < 4 or > 8
Validation 38	0	3	1	Setpoint_IV-10%	If < 4 or > 4
Validation 39	0	3	1	Setpoint_IV-8%	If < 4 or > 4
Validation 40	0	3	1	Control	If < 4 or > 8

Validation Data

In a second phase, different feeding strategies were applied to the same core process. Much higher feed amounts were selected to bring the process to the limit. The osmolality values, in particular, were much higher than the calibration data.

Another difference was that the temperature shift was performed on day 3 instead of day 5. This resulted, on average, in lower VCD values and approximately half the product concentration at the end of the process. An overview of the validation batches and the feed profiles for each set point are given in Tables 4 and 5 respectively.

Table 5: *Profile for Different Set Points*

Conditions	Setpoint_III	Setpoint_IV-10%	Setpoint_IV-8%	Controls [%]
Day_0				
Day_1	3,70%	4,86%	4,86%	5,12
Day_2	3,70%	10,00%	8,00%	
Day_3	3,70%	10,00%	8,00%	5,12
Day_4	3,70%	0,00%	0,00%	
Day_5	3,70%	0,00%	0,00%	5,12
Day_6	3,70%	0,00%	0,00%	
Day_7	3,70%	0,00%	0,00%	5,12
Day_8	3,70%	1,10%	1,10%	
Day_9	3,70%	1,37%	1,37%	5,12
Day_10	3,70%	10,00%	8,00%	
Day_11	3,70%	10,00%	8,00%	5,12
Day_12	3,70%	10,00%	8,00%	
Day_13	3,70%	8,53%	8,53%	5,12
Day_14				
F3 Feed percentage		0.60%		

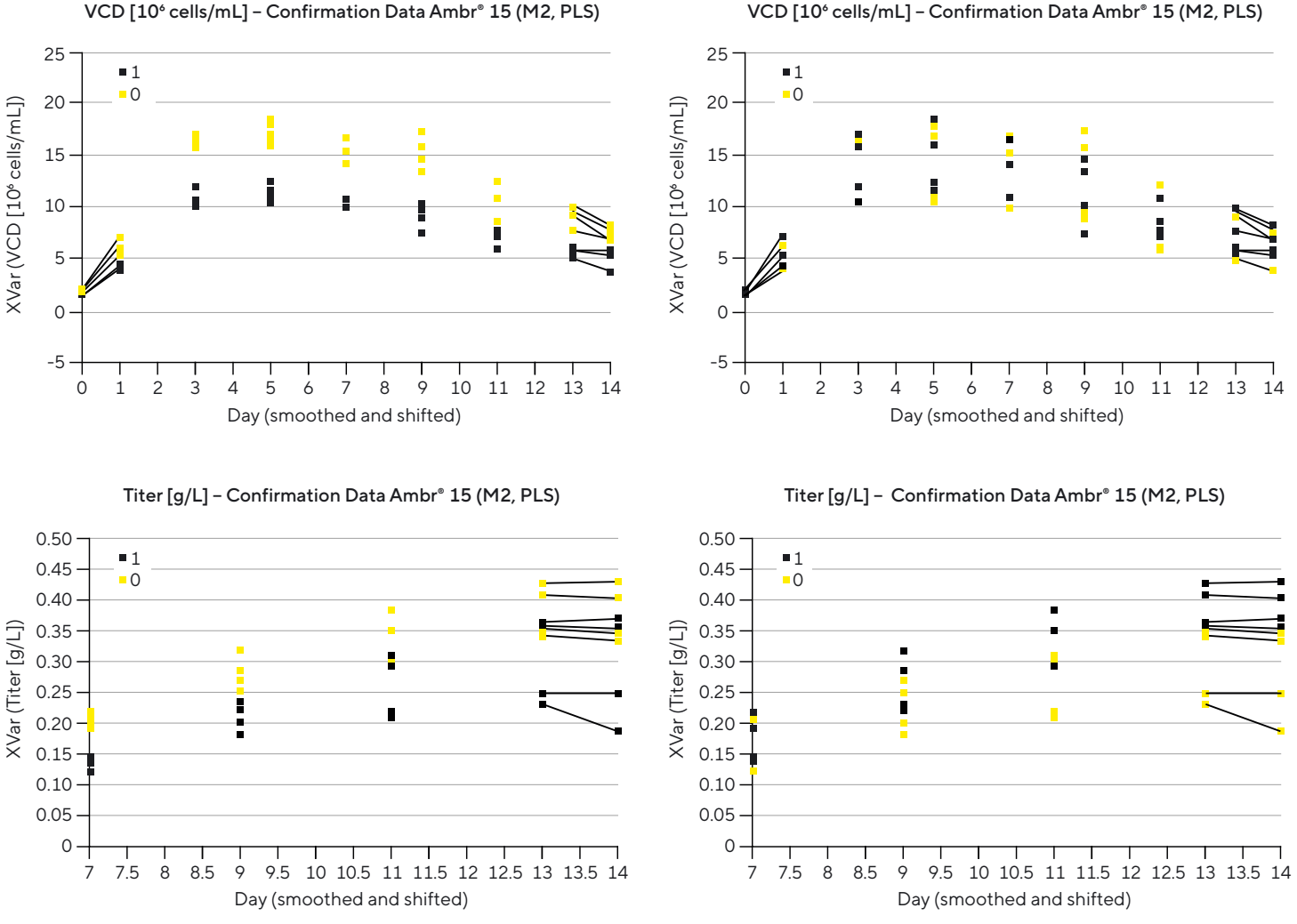
Data Exploration Validation

Like the calibration data, the VCD and titer profiles were assessed. Compared to the calibration, the temperature shift was performed earlier, and consequently, lower VCD values were reached. In addition, the media appear to have a bigger influence on the maximum VCD for the validation data. When it comes to productivity, the validation controls resulted in half the final titer values compared to the controls of the calibration data set (Figure 4).

Validation Result

The results of the validation batches are presented in Figures 5 and 6. Compared to the calibration data, the model predictions were less accurate. This was expected since validation errors tend to be larger than calibration errors, and the processes for the validation batches were run differently. Overall, the model tends to underpredict the VCD for the validation batches, especially those mentioned in Table 6 (note: the last six batches are a replicate of the first six). For most batches, the validation processes grow faster in the first days than the model predicts. All listed batches had an inhibit feed value of 1 and hence should experience growth inhibition according to the model. All control batches with an inhibit feed value of 1 (Validation-08, Validation-16, Validation-32, and Validation-40) were identified as batches that did not fit the model well.

Figure 4: *Control Experiments for Validation Data Set*



Note. Coloring is displayed according to media for left plots and feed for right plots.

When looking at all processes with a inhibit feed value of 1, 6 out of 8 process conditions are listed in Table 6 as not well predicted. These observations can indicate that the inhibition effect of the feed is overestimated by the model. In addition, the model mismatch seems more likely to happen in combination with the media that shows no inhibition. This phenomenon was already partially observed in the data exploration phase, indicating that there was a strong media effect. However, this was not further broken down into a potential interaction between feed and media.

Enhancing the existing calibration data set with the controls of the validation data could improve the results by creating a more realistic estimation of the media or feed effect and providing a broader estimate of the interaction between feed and media. The data of just one set of bioreactors may not be adequate to capture the true effect of the media or feed.

Table 6: *Validation Batched Not Well Predicted During Initial Growth Phase*

Batch	Inhibit Media	Inhibit Feed	Setpoint	Glucose Strategy [g/L]
Validation-05	1	1	Setpoint_III	If < 4 or > 8
Validation-08	1	1	Control	IIf < 4 or > 8
Validation-13	0	1	Setpoint_III	If < 4 or > 8
Validation 14	0	1	Setpoint_IV 10%	If < 4 or > 4
Validation 15	0	1	Setpoint_IV 8%	If < 4 or > 4
Validation 16	0	1	Control	If < 4 or > 8
Validation 29	1	1	Setpoint_III	If < 4 or > 8
Validation 32	1	1	Control	If < 4 or > 8
Validation 37	0	1	Setpoint_III	If < 4 or > 8
Validation 38	0	1	Setpoint_IV 10%	If < 4 or > 4
Validation 39	0	1	Setpoint_IV 8%	If < 4 or > 4
Validation 40	0	1	Control	If < 4 or > 8

Figure 5: *Simulation Result for Validation Batches (Viable Cell Density Result)*

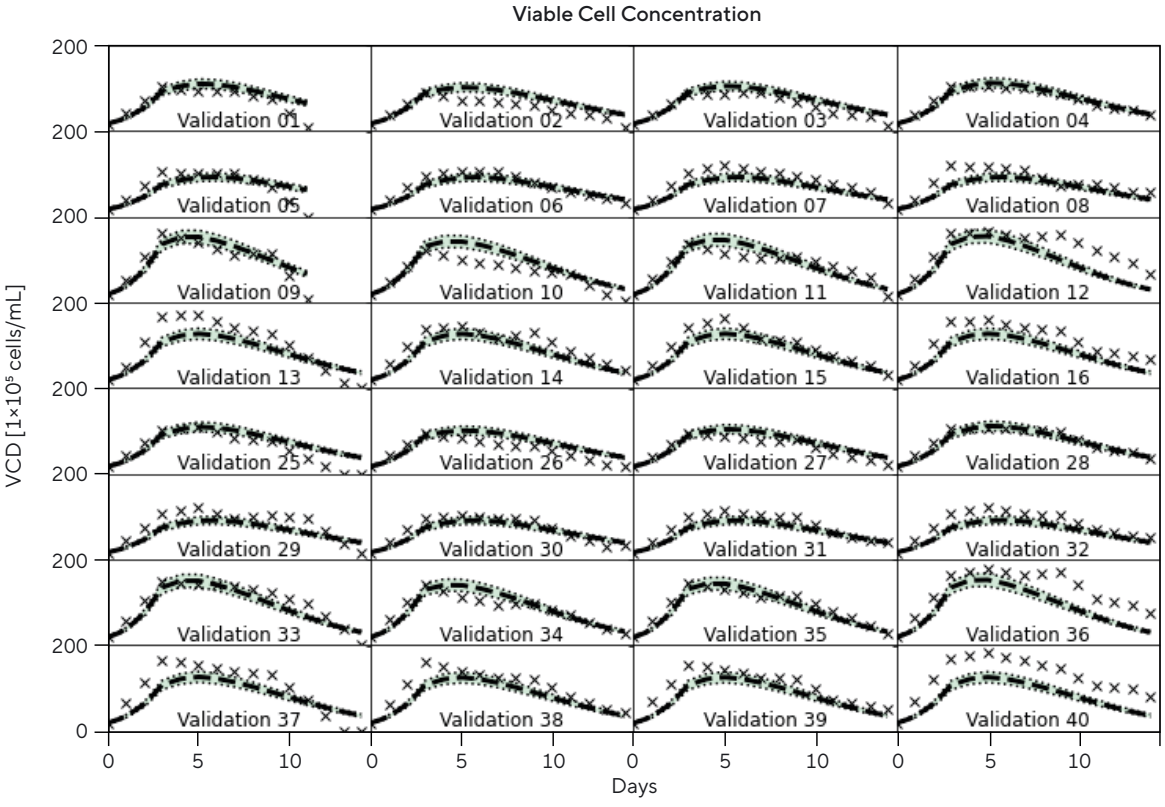
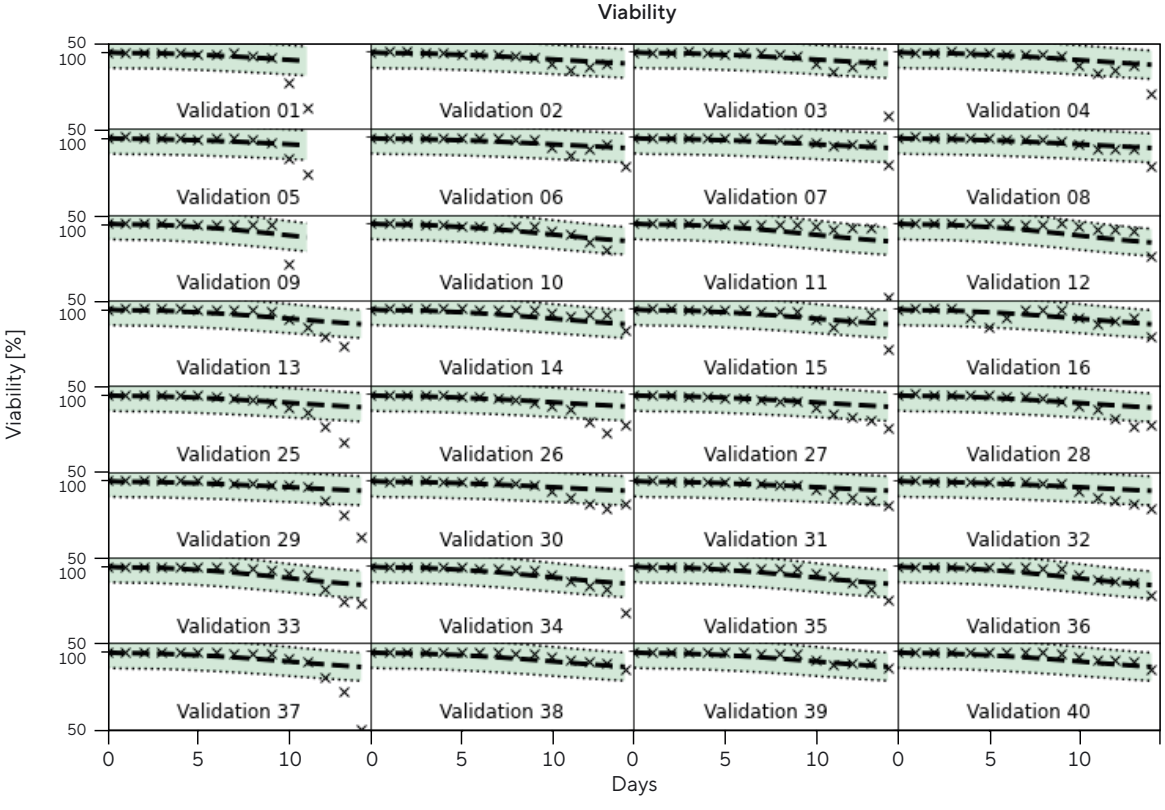


Figure 6: *Simulation Result for Validation Batches (Viability)*



Titer Prediction

The neural network for the titer prediction used data from the mechanistic model (e.g., simulate values, biomaterial) and the inputs for the different cultivations (e.g., the media or feed used). In a first iteration, the cell-specific productivity (qP) at different time points was the focus for prediction. However, the calculation of the qP is dependent on very accurate product quantification with HPLC and the much less accurate VCD determination with a dye exclusion method. Consequently, the calculated qP values were inaccurate or unrealistic when calculated between time points.

Therefore, we decided to use the overall qP per batch which reduced the impact of the inaccurate VCD measurement. Figure 7 shows the results for the calibration data. In general, the trend was followed by the prediction with the neural network. Once the model structure was defined, it was applied to the validation data set, meaning that lower qP values are predicted as such and higher qP as higher predicted values (Figure 8). Considering the qP was lower in the validation data set, that is a good confirmation that the over relations between process performance and qP is stable and well-established with the artificial model.

Figure 7: *Result of qP Values for Calibration Data*

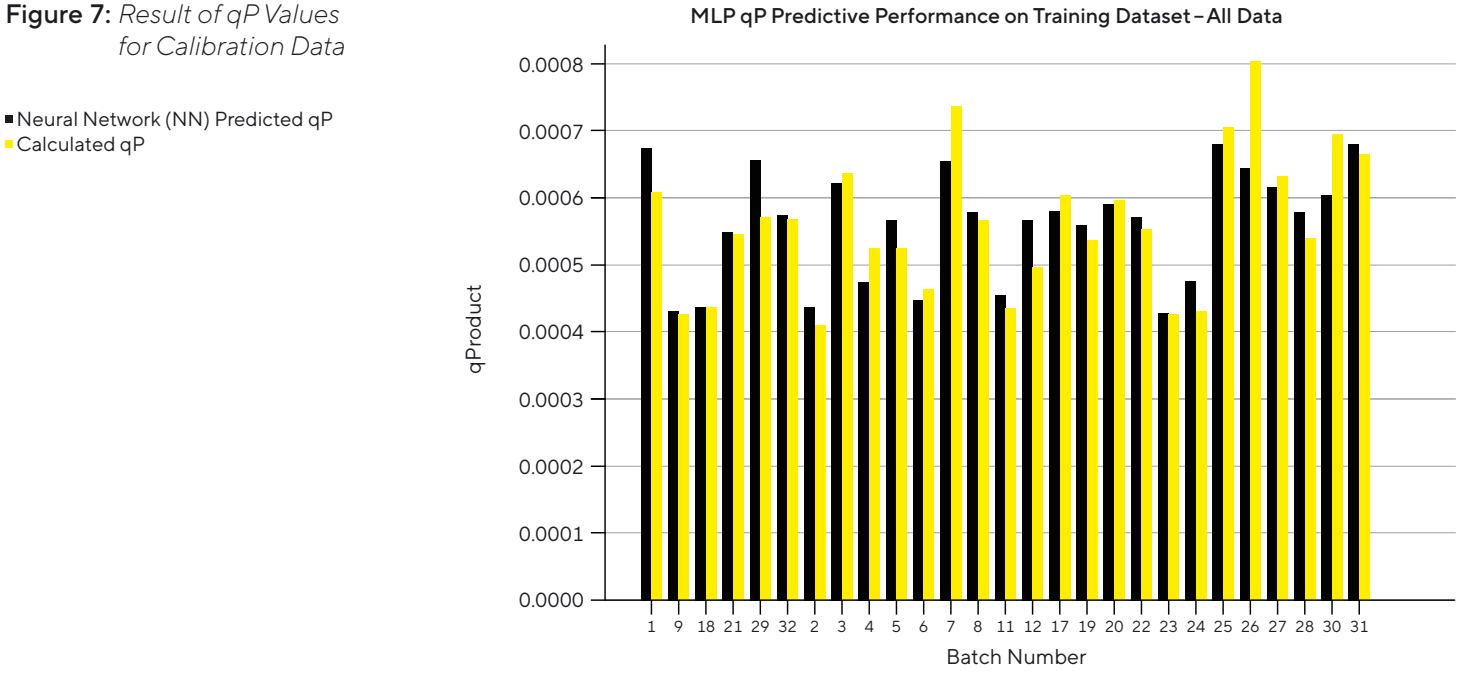
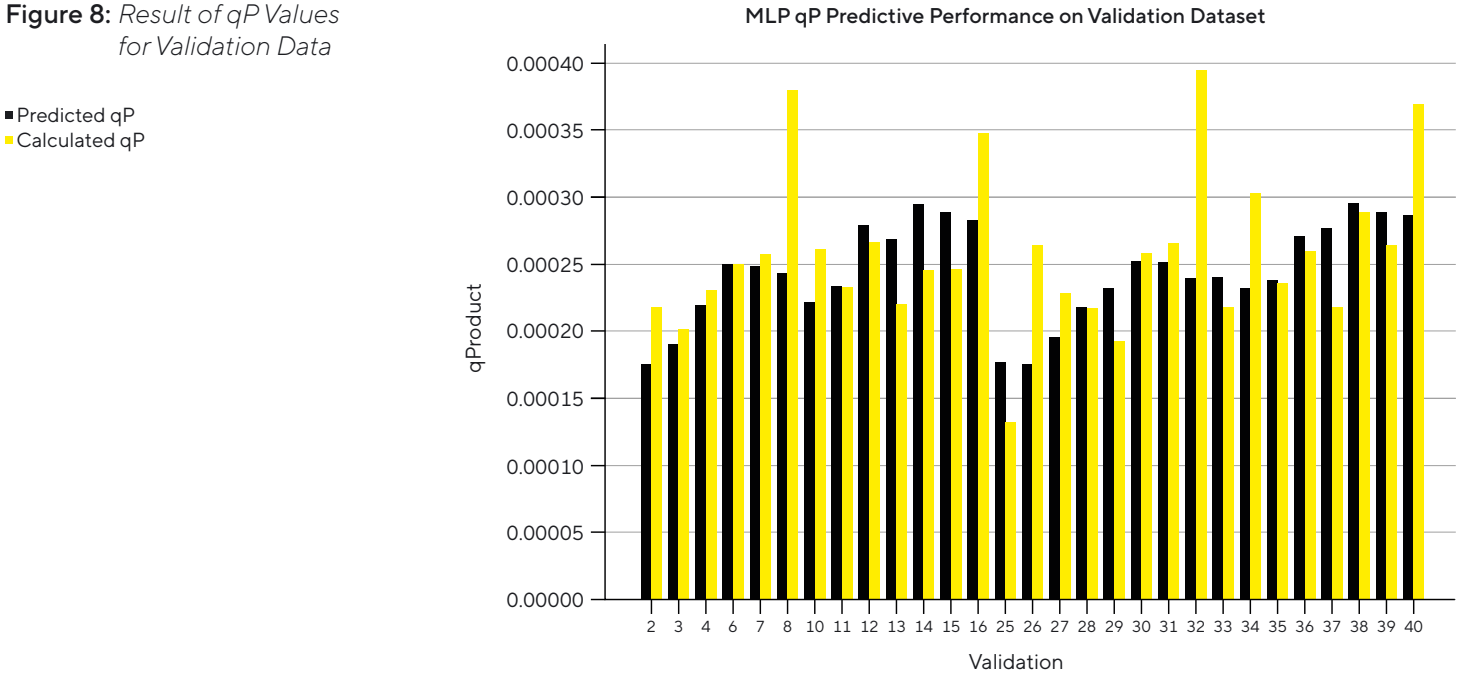


Figure 8: *Result of qP Values for Validation Data*



Conclusion

In this study, an unstructured, unsegregated mechanistic model for a bioprocess producing a mAb was developed. The experiments to build the model were performed with two different media, two different feeds, and four variations in the amount of feed added. The best model provided a good representation of the calibration data. The model was then tested against an independent validation data set containing a much higher feeding volume, resulting in higher osmolality measurements. In addition, the temperature shift was performed two days earlier in the validation experiment. The model predictions were less accurate for the validation set mainly due to a lower feed inhibition effect. Building the model over both data sets can help improve the model's robustness, which would also require a new independent validation data set.

The second phase of the modeling approach was the implementation of an artificial neural network to predict the average specific productivity (qP). The artificial neural network represents a data-driven or statistical model. The data-driven model is the second part of a hybrid model. The result of implementing the data-driven artificial neural network shows that the input from the mechanistic understanding led to a reliable prediction of the qP, even though the qP was generally lower for the validation data set. The next logical step would be to combine both parts to create a digital representation of the process, then run an optimization function to find the best compromise between growth-promoting and productivity-promoting conditions.

To learn more about Cell Insights by Umetrics® Studio, visit
www.sartorius.com/cell-insights

Case Study "Model-based intensification of CHO cell cultures: one-step strategy from fed-batch to perfusion"
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