Chapter 7

Improving the Batch-to-Batch Reproducibility of Microbial Cultures During Recombinant Protein Production by Regulation of the Total Carbon Dioxide Production

Abstract. Batch-to-batch reproducibility of fermentation processes performed during the manufacturing processes of biologics can be increased by operating the cultures at feed rate profiles that are robust against typically arising disturbances. Remaining randomly appearing deviations from the desired path should be suppressed by automatically manipulating the feed rate. With respect to the cells' physiology it is best guiding the cultivations along an optimal profile of the specific biomass growth rate $\mu(t)$. However, there are two problems that speak for investigating alternatives: Upon severe disturbances that may happen during the fermentation, the biomass concentration $X$ may significantly deviate from its desired value, then $\mu$-control leads to a diminished batch-to-batch reproducibility. Secondly, the specific growth rate cannot easily be estimated online to a favourably high accuracy. The alternative discussed here solves both problems by keeping the process at the corresponding total cumulative carbon dioxide production-profile: It is robust against distortions in $X$ and the controlled variable can accurately be measured directly and online during cultivations of all relevant sizes. As compared to the fermentation practice currently used in industry, the experimental results, presented at the example of a recombinant protein production with $E. coli$ cells, show that CPR-based corrections lead to a considerably improved batch-to-batch reproducibility.

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1 INTRODUCTION

With its PAT initiative (FDA 2004), FDA focused attention towards process supervision and regulation in manufacturing of biologics. Biologics are complex products where product quality is indispensably coupled with the quality of the manufacturing process. Apparently small changes in the operation of the processes can cause significant differences in the product’s clinical efficacies. Hence, the production of a biologic is approved by authorities only together with clearly defined constraints on its manufacturing process. Consequently, the batch-to-batch reproducibility is of primary interest in manufacturing therapeutic proteins. From the engineering point of view there are two challenges in meeting this requirement: First of all, within the given constraints, the operational procedure most robust with respect to the possible process disturbances must be found. And, secondly, while running the process along this desired path, the remaining randomly appearing deviations must be eliminated by automatic corrections at the adjustable variables, for instance, the substrate feed rate.

With respect to batch-to-batch reproducibility, production processes for recombinant proteins are lagging behind most other industrial processes. From the technological point of view, the most important reason is the accuracy and reliably by which the actual states of the complex cultivation processes can be determined online.

From the economical point of view, therapeutic production processes must be developed within short periods and afterwards the gross process layouts are fixed. Since minimizing time-to-market is the overriding objective in this period, the companies avoid any technical complications in the process. Their main fear is a costly delay of the approval through discussions on new technology with the authorities. This situation considerably changed with the paradigm change in the FDA towards a new risk-based inspection procedure. An essential part of the new approach is FDA’s PAT initiative (FDA 2004).

With PAT, the agency concretely stimulates improving pharmaceutical and biologics manufacturing processes with respect to real time automated process monitoring and control. The way proposed is a more rigorous science-based approach to manufacturing since it is generally understood that better understanding leads to more efficient process control, lower process variability, thus higher product quality and finally patient safety. For biologics it is particularly important to keep the processes under control early in the product synthesis process (e.g., DePalma 2004). This message was understood in industry and all major producers nominated persons responsible for PAT affairs.

The first question to be solved is about the strategy of manipulating the process in order to improving batch-to-batch reproducibility. In manufacturing recombinant therapeutic proteins, the primary objective is to produce a given mass \( m_p \) of protein within a more or less well defined production time \( t_p \). From the product mass balance around the culture we obtain

\[
m_p = \int_{0}^{t_p} \pi \cdot x \cdot dt
\]

where \( \pi \) is the specific product formation rate and \( x \) the total biomass. Both arguments of the integral are functions of time, but more importantly, both are primarily dependent on the specific biomass growth rate \( \mu \) in most industrial production systems. The growth rate that a
specific medium supports determines the physiological state of the cells, and particularly, the cell’s protein-synthesizing machinery that is important to recombinant protein production is also under growth rate control (Neidhardt et al. 1990). Hence, the variable that rules the final outcome of the process is the specific biomass growth rate $\mu$. Consequently, this variable should be kept under tight control (e.g. Pirt 1975, Shioya 1992, Levisauskas et al. 1996, Jenzsch et al. 2005, and 2006a).

The number of production reactors in biotechnology where process control by manipulating the feed rate profile is realized is negligible. It seems to be a relatively big step to install process control in this industry. Many of the control procedures that were proposed in literature are rather complex do not really downsize the activation barrier. Thus, simple, easy-to-use regulation procedures are required. The main problem, however, remains that $\pi$, $\mu$ and even $x$-based control suffer from the fact that they cannot accurately enough be measured directly with sensors that can be installed at a production fermenter.

In this paper we address the problem of designing a robust strategy of increasing the batch-to-batch reproducibility. From an underlying profile of the specific biomass growth rate we develop the profile of the total cumulative carbon dioxide production rate and show that guiding the process along this trajectory leads to a robust process behavior. We show experimentally at the example of a recombinant protein expressed by an E.coli strain that this procedure works perfectly. Further we show that the batch-to-batch reproducibility can be improved even more by automatically correcting random deviations of the process from this path.

2 EXPERIMENTAL

Strain and cultivation technique

All experiments were performed with E.coli BL21(DE3) as the host cell. The recombinant target protein was coded on the plasmid pET 28a and expressed under the control of the T7 promoter after induction with isopropyl-thiogalactopyranosid (IPTG). The strain was resistant against kanamycin. The product appears as inclusion body within the cytoplasm.

The cultivations were performed in mineral salt medium (Jenzsch et al. 2006d) with glucose as main substrate. It was fed at a concentration of 600 [g/kg].

All the experiments were performed within BBI Sartorius System’s BIOSTAT® C 15-L-bioreactor with 8 [L] working volume. The fermenter was equipped with 3 standard 6-blade Rushton turbines that could be operated at up to 1400 [rpm]. The aeration rate could be increased up to 24 [SLpm]. Aeration rate and then stirrer speed were increased one after the other in order to keep the dissolved oxygen concentration at 25 [%] of its saturation value. In order to suppress foam formation, increase oxygen solubility and reduce the risk of contamination the fermenter head pressure was kept at 0.5 [bar] above the ambient pressure in the laboratory.

The fermentations were operated in the fed-batch mode from the early beginning with an initial volume of 5 [L] at pH 7 and a temperature of 35 [°C]. All fermentations were started in the night by automatic transfer of the inoculum from a refrigerator into the reactor. Substrate feeding starts immediately after inoculation with a fixed exponential profile. According to Jenzsch et al. (2006c), the specific growth rate setpoint was chosen to 0.5 [1/h]. After the biomass concentration reached values of about 35 [g/kg], the culture was induced with
1 [mM] IPTG. From the 7th fermentation hour on, the growth rate was kept under feedback control along a tcCPR-profile that was derived from a predefined profiles of the specific growth rate and the biomass x. The corresponding profile of the substrate feed rate was determined from the profiles of μ and x. This was taken as reference feeding profile F_ref for all the fermentations described.

Temperature was measured with a Pt-100, pH with an Ingold-pH-probe, pO₂ with an Ingold pO₂-Clark-electrode, CO₂ in the vent line with MAIHAK®’s Unor 610, and O₂ there with MAIHAK®’s Oxor 610. Further, the total ammonia consumption during pH control was recorded by means of a balance beneath the base reservoir. All these quantities were measured online. Additionally, enhanced foam levels could be detected with a foam sensor and, if the critical level was reached, a silicone antifoaming emulsion (ROTH®) was added.

Biomass concentrations were measured offline (via the optical density at 600 [nm]) with a Shimadzu® photo-spectrometer (UV-2102PC). In preceding experiments, a correlation was established between these values and the biomass dry weights which were determined with the standard drying and weighting technique. Glucose was determined enzymatically with a YSI 2700 Select Bioanalyzer. The product was measured with SDS PAGE after separation of the inclusion bodies and their solubilisation.

**Following total CPR-trajectories**

Apart from the difficulty of making available online values of μ, in a μ-controlled fermentation a further problem is the instability of that approach with respect to variations in the initial total biomass amount x₀. As depicted in Figure 1, an initial deviation in x will be amplified when a fixed μ profile will be applied. Hence, merely keeping the process at a fixed μ(t) leads to an unfavourable batch-to-batch reproducibility.
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This problem can be solved by relating the $\mu$ profile to a CPR(t)-profile and running CPR along the resulting trajectory by means of feedforward/feedback control. This variable that is tightly related to the specific growth rate and can easily be measured online. Instead of the carbon dioxide production rate CPR, one can also use the corresponding total CPR signal $t\text{CPR}=\text{CPR}\cdot W$, where $W(t)$ is the corresponding culture mass signal, or even better, the accumulative signal $t\text{CPR}$ of the total carbon dioxide production rate.

Usually a simple relationship in terms of a Luedeking-Piret approach (Luedeking and Piret 1959a, b) is used to relate both quantities $\mu$ and CPR. Here we show that a slightly extended analogue of a Luedeking-Piret-type relationship can be used to relate total CPR to the specific biomass growth rate $\mu$ and total biomass $x$:

$$t\text{CPR} = (Y_{CX} \cdot \mu + m_C) \cdot x \cdot K_{\text{ind}}$$  \hspace{1cm} (2)

where the scalar factor $K_{\text{ind}}$ is 1.0 during the biomass formation phase and $K_{\text{ind}} > 1$ during the protein formation rate. This extension is reflecting the experimentally observed fact that more CO$_2$ is formed per volume, time and biomass during the product formation phase.

Equation (2) we can be rewritten in the following way:

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**Figure 1.** Simple simulation of fed batch processes running with a fixed growth rate profile (dashed line) showing the effect of an initial variance in the total biomass concentration $x$ (full lines). The variance becomes amplified with time.
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\[
\mu = \frac{t_{\text{CPR}}}{x \cdot Y_{\text{CX}}} - \frac{m_C}{Y_{\text{CX}}} \tag{3}
\]

Using the balance equation for the total biomass

\[
\frac{dx}{dt} = \mu \cdot x \tag{4}
\]

the parameters of the equation (2) can simply be identified by means of a numerical fit using the experimental data for x and tCPR measured in a couple of experiments. The fit can be performed with any optimization routine. In Figure 2 an example is shown where the function \(x(t,t_{\text{CPR}})\) was fitted to a typical experimental data record using Matlab®’s routine `lsqcurvefit` (The MathWorks, Inc.).

![Graphs showing total biomass and total CPR](image)

**Figure 2.** Fit of equation (2) to experimental data obtained in 4 experiments in which a recombinant protein was produced in *E.* coli. Full lines are the online measured total carbon dioxide production rates used to estimate (dashed lines) the total biomass (○).

This simple linear model results in a fairly good fit with a root mean square error in the total biomass of RMSE = 7.5 [g] corresponding to about 3 [%] of the final value. The parameters of equation (3) obtained in the fit are:
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\[
\begin{align*}
Y_{CX} &= 0.712 \quad [\text{g/g}], \\
m_C &= 0.136 \quad [1/\text{h}], \\
K_{\text{ind}} &= 1.384 \quad [-] \quad \text{for } t > t_{\text{ind}}.
\end{align*}
\]

An appropriate algorithm for guiding the fermentation along a total CPR profile is:

(i) Decide on an optimal \( \mu \) profile for the cultivation (using experts’ knowledge or a numerical optimization based on a mathematical model of the process)

(ii) Choose an appropriate inoculation size (initial amount of total biomass \( x \))

(iii) Estimate the total CPR profile using equations (2) and (4)

(iv) Integrate the total CPR profile to get the cumulative total CPR setpoint trajectory

(v) Run the cultivation along the resulting tcCPR profile by means of feedforward/feedback control, manipulating the substrate feed.

The proposed method leads to a self-tuning, i.e. a robust process behavior. This is shown in Figure 3. When the initial amount of total biomass is not on target, the specific growth rate will be adapted automatically. When the inoculated biomass is too high, the cells will produce more CO\(_2\) than expected; hence the controller will reduce the feed rate and thus substrate concentration. Consequently, the biomass growth rate will become smaller. If on the other hand the initial amount of total biomass is smaller than expected, the cells will produce less CO\(_2\) and the controller will increase the feeding rate. This will lead to an increase in the biomass growth rate. After a few hours, the specific growth rate is on target simply by controlling the total cumulative CPR to its corresponding profile.

![Figure 3. Simple simulation showing the self-stabilizing effect of controlling the total cumulative CPR in a fed-batch cultivation of a recombinant therapeutic protein production process. The simulated seed biomass varies between 0.65 and 1.35 [g].](image-url)
As shown in the coarse simulation study depicted in Figure 3, the adaptation appears in the first few hours. The essential effect of the adaptation, the reduction in the variance of the biomass trajectories, appears during the protein production phase, i.e. in the second half of the entire process time. Now the essential problem is how to formulate the relationship between the specific growth rate $\mu$, total biomass $x$ and the total carbon dioxide production rate $tcCPR$.

**Nonlinear approach based on artificial neural networks**

As the metabolic details relating biomass growth to carbon dioxide production and vice versa are quite complicated and are changing upon induction of the product formation, a straightforward approach is to employ an artificial neural network to describe the relationship.

What we need is a network that connects the carbon dioxide production rate CPR with the specific biomass growth rate $\mu$. In other words we need a data driven model that is able to determine $\mu$ from the measurement data.

CPR can be measured online. However, to substantiate the measurement information it is desirable to make use of further measurements that are related to the growth rate as input data for such a network. A straightforward choice is the biomass $x$ from which it was already shown that it can be estimated online quite reliably (Jenzsch et al. 2006b). As a third input variable, $t_{ind}$, the induction time can be used. The reason for taking $t_{ind}$ as a further input is that the carbon dioxide production is larger in the product formation phase as already mentioned in the discussion of the linear model. That's the inputs of the artificial neural network. For network training, however, one also needs the corresponding values of the specific growth rate $\mu$ at network output. As $\mu$ cannot be measured directly, it is necessary to develop methods for ANN training using available data, for instance the biomass measurement data.

The idea is to train the network indirectly using the residuals between the measured biomass values $x_{data}$ and those computed with the $\mu$-network via the following equation defining $\mu$:

$$\mu = \frac{1}{x} \frac{dx}{dt}$$

which is used in the form of equation (4)

We choose a simple feedforward artificial neural network (ANN) with a single hidden layer that describes $\mu$ as a function of online input data and network weights $w$.

$$\mu_{NET} = ANN(Data, w)$$

As mentioned above, $Data$ is composed of the total CPR data, the total biomass $x$ and the induction time $t_{ind}$.

$$Data = [tcCPR, x_{on}, t_{ind}]^T$$
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tCPR can directly be derived from the online-measured CPR signal, and $t_{\text{ind}}$ is an adjustable variable. $x_{\text{on}}$, could be taken from indirect measurements routines. Here, however, it is easier to estimate $x_{\text{on}}(t)$ online directly by integrating the differential equation (4) as will be shown later.

In order to train the network, we minimize the root-mean-square of the residuals

$$res = x_{\text{data}} - x_{\text{NET}}$$  \hspace{1cm} (8)

i.e. the performance measure $J$

$$J = res^T \cdot res$$  \hspace{1cm} (9)

by searching for the best weight matrices $w$ for the artificial neural network (6). This search can be performed with any parameter optimization or nonlinear function fitting procedure. However, because of the large number of elements in $w$, it is of advantage to make use of routines that can make use of the sensitivity of the $\mu$ with respect to the biomass $x$ and the sensitivity of the biomass to the network weights $w$. The routine used here was Matlab®’s lsqcurvefit.

In order to determine $res$ we must formulate a function computing $x_{\text{NET}}$ from the actual network output (using the weights $w$) corresponding to a given measured biomass $x_{\text{data}}$.

$$x_{\text{NET}} = x_{\text{function}}(w, x_{\text{data}})$$  \hspace{1cm} (10)

In simple feedforward artificial neural networks with a single hidden layer, $w$ is composed of two partial matrices: $w_1$ and $w_2$, connecting input with hidden and hidden with output layer.

With given weights, $\mu_{\text{NET}}$ can be determined for each online input data vector Data (7).

$$h_1 = \tanh(w_1 \cdot Data)$$  \hspace{1cm} (11)

where $h_1$ is an auxiliary vector whose elements are the responses of the nodes of the hidden layer on the Data at the input-nodes. The network output $\mu_{\text{NET}}$ is then taken as

$$\mu_{\text{NET}} = w_2 \cdot h_1$$  \hspace{1cm} (12)

This $\mu_{\text{NET}}$ can be used recursively in an Euler integration loop to solve equation (4):
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\[ x_{NET}(i) = x_{NET}(i-1) \cdot (1 + dt \cdot \mu_{NET}(i-1)) \]  

(13)

With the initial value \( x_{NET}(1) = x_0 \), the amount of biomass after inoculation, equation (4) can easily be solved numerically.

As the online data are available with rather small time increments \( dt \), the missing biomass at \( t=t_{i-1} \) can be approximated by the last network estimate:

\[ \text{Data}(i-1) = \left[ tCPR(i-1), x_{NET}(i-1), t_{ind}(i-1) \right]^T \]  

(14)

In order to determine the Jacobian matrix, needed to support a gradient search for the best \( w \) in case of a given data record, we again make use of differential equation (4) and differentiate the equation partially with respect to \( w \):

\[ \frac{\partial}{\partial w} \frac{dx}{dt} = \frac{\partial}{\partial w} \cdot \mu(x, w) \cdot x \]  

(15)

\[ \frac{d}{dt} \frac{\partial x}{\partial w} = \left( x \cdot \frac{\partial \mu}{\partial x} + \mu \right) \frac{\partial x}{\partial w} + x \cdot \frac{\partial \mu}{\partial w} \]  

(16)

This is an ordinary differential equation in \( S = \frac{\partial x}{\partial w} \), the so-called sensitivities, that can be solved with the initial conditions

\[ \frac{\partial x(t = 0)}{\partial w} = S(t = 0) = 0 \]  

(17)

as there is no influence of \( w \) on \( x \) in the beginning. Hence this approach is termed the sensitivity equation approach.

However, in order to numerically compute the solution of equation (16) we need expressions for the other sensitivities \( \frac{\partial \mu}{\partial x} \) and \( \frac{\partial \mu}{\partial w} \).

The first expression is one component of the sensitivity of the network output with respect to changes in its input. This output/input-sensitivity \( S_{IO} \) of a simple feedforward network can be determined from equations (11) and (12). Because of the special choice of the activation function (11) of the network nodes, a rather simple expression appears:
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\[ S_{io} = w_1^T \cdot \left( w_2^T \cdot \left( 1 - h_1 \cdot h_1 \right) \right) \]  \hspace{1cm} (18)

We only need the sensitivity with respect to biomass concentration \( X \)

\[ \frac{\partial \mu}{\partial X} = S_{io} \]  \hspace{1cm} (2)

(19)

The other component \( \frac{\partial \mu}{\partial W} = S_{ow} \) is the sensitivity of the network output with respect to the network parameters, the weights. In the networks with single hidden layer, this is according to equations (11) and (12)

\[ \frac{\partial \mu_{net}}{\partial w_2} = h_1 \quad \text{and} \quad \frac{\partial \mu_{net}}{\partial w_1} = w_2^T \cdot \left( 1 - h_1 \cdot h_1 \right) \cdot Data = h_2 \]  \hspace{1cm} (20)

Combining both parts of equation (20) yields

\[ \frac{\partial \mu_{net}}{\partial W} = \begin{bmatrix} h_2 \\ h_1 \end{bmatrix} \]  \hspace{1cm} (21)

The quantities \( \frac{\partial \mu}{\partial X}, \frac{\partial \mu}{\partial W} \) and \( \frac{\partial X}{\partial W} \) can directly be determined within the Euler loop (13) for integrating \( \frac{dx_{NET}}{dt} \).

So far we assumed that the network weights \( W \) were already given. In practice, they must be determined by means of a curve fitting procedure. We used Matlab’s lwqcurvelfit for this purpose. This function was used in the mode where the Jacobian can be exploited.

Using the trained neural net relationship for \( \mu \) in equation (4), the total biomass \( x(t) \) can be derived much more accurately from the measured total carbon dioxide production rate signal. This is shown in Figure 4 for the same set of experimental data that were used to generate Figure 2.
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Figure 4. Total biomass trajectories (dashed lines) derived from the total carbon dioxide production rate signals (full lines) by means of the artificial neural network described in the text. The symbols (○) are the offline measured total biomass values.

The agreement between the computed x values and the biomass measurement data is clearly better than in the case depicted in Figure 2. The root mean square error with respect to the total biomass is now 1.4 [g] which is about 0.5 [%] of the final biomass value or a fifth of the RMSE resulting from the more simple linear model based on Luedeking and Piret’s approach.

In order to determine a the setpoint profile for a tcCPR-controller for a given μ(t)-profile, we must use a model that maps in the reverse direction. For that purpose we took a simple feedforward artificial neural network with a single hidden layer trained on the μ-data from the network described above together with the corresponding biomass x(t) and tcCPR(t) data.

Control algorithm

The control can then be performed with a very simple adaptive control algorithm comprising the following steps:

(i) Calculation of total cumulative carbon dioxide production rate tcCPR

\[ tcCPR(t) = \int_{0}^{t} CPR(t) \cdot W(t) \cdot dt \]

(22)
(ii) Computing the deviation of measured tcCPR from the set point tcCPR$_{set}$

\[ \Delta tcCPR = tcCPR_{set} - tcCPR_{meas} \]  \hspace{1cm} (23)

(iii) Determination of the correction $\gamma$ for the manipulated variable $F$

\[ \gamma = 1 + k_1 \cdot \Delta tcCPR + k_2 \cdot \int_{t_s}^{t} \Delta tcCPR \cdot dt ; \quad 0.7 \leq \gamma \leq 1.3 \]  \hspace{1cm} (24)

(iv) Calculation of current feed rate $F$

\[ F = \gamma \cdot F_{ref} \]  \hspace{1cm} (25)

![Figure 5. Total cumulative CPR profiles of six tcCPR-controlled fed batch fermentation runs.](image)

As the CPR and thus the total cumulative signal as well are directly available without any noticeable time delay, the flow rates $F$ can be adjusted immediately. As can be seen in Figure 5, where the results of 6 successively cultivations are shown, the tcCPR profiles can
nearly ideally be kept on track. Again, the relative deviations show, that the deviations from the mean tcCPR-profile are kept below 1 [%] within one hour after the controller was switched on (Figure 6).

![Figure 6. Relative deviations of the cumulative total carbon dioxide produced from their means.](image)

Thus, the tcCPR-control is perfect. However, the questions are: what are the corresponding total biomass profiles, and how do they deviate from their mean. The answers are given in Figures 7 and 8. In the beginning the measurement errors are large with respect to the current biomass values; hence the fluctuations appear to be very high. Nevertheless the trajectories in Figure 8 finally remain within the ±5 [%] margins. This confirms that we do not only keep the controlled variable on target but more importantly one of the key state variable, the biomass x.
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Figure 7. Total biomass profiles of six tcCPR-controlled fed batch fermentation runs.

Figure 8. Relative deviations between the corresponding trajectories for the total biomass $x$ from their mean.
The next question is whether or not the corresponding specific growth rate profile is really the one initially taken to determine the \( x \) and tcCPR profiles. Figure 9 depicts the \( \mu \) estimates computed with the full model, described by Gnoth et al. (2006).

![Figure 9](Image)

**Figure 9.** Specific growth rate profiles of six tcCPR-controlled fed batch fermentation runs.

Figure 10 finally depicts the measured product concentrations for the tcCPR-controlled experiments. The divergence in the protein data is slightly higher than those in the biomass profiles. However, one should keep in mind that protein measurement accuracies are drastically lower than the accuracies in the other measurement data. The estimated relative error of the measurements is \( \pm 15\% \). All the data shown in Figure 10 stay well within these error bounds around the mean profile. One of the cultivations was performed without inducing the protein formation. This was done in order to see whether the promoter is really tight and in order to get an impression on the metabolic load of the cells by foreign protein formation.
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Figure 10. Concentration profiles of target protein in six tcCPR-controlled fed batch fermentation runs together with an average drawn only over the records from the induced fermentations. The error bars showing the confidential interval of ± 15 [%] resulting from the technique of protein analysis.

3 DISCUSSION

As in most cases the physiology of the cells and particularly the protein formation is mainly influenced by the specific growth rate of the cells (Neidhardt et al. 1990), it is straightforward to guide a protein production process along an optimal $\mu(t)$ profile. As was shown, such $\mu$-controlled fermentation processes depict problems in the batch-to-batch reproducibility. Hence, alternative variables must be monitored and manipulated in order to make sure that the process is tightly reproduced in each batch.

Choosing the total cumulative CPR-profile derived from a given profile of the specific growth rate $\mu(t)$ has several important practical advantages. It is first of all robust as compared to the biomass concentration profiles. Following a tcCPR-profile automatically corrects for deviations from the biomass concentration profile. The second important point is that tcCPR can quite accurately be measured online. CPR as measured via the usually applied offgas analysis is a global quantity that does not depict such heavily fluctuating signals as any variable locally measured with a probe within the multiphase flow of the culture. Finally, a global measurement it is more representative than a locally measured variable. This property is favourable in our models that all assume homogeneity or ideal stirred tank conditions in bioreactors.
As the results depicted in Figure 5 show, the batch-to-batch reproducibility with respect to the tcCPR is very good. Additionally, the robustness of the cultivation strategy is also surprisingly good as shown in Figure 3. All deviations within the decisive product formation phase after induction could be kept below ±1[%]. Even the biomass profiles were simultaneously found to follow closely the profiles observed in the previous runs. And, last but not least, the product titers were observed to be very reproducible.

Hence, keeping the cultures operated during recombinant protein production on predefined tcCPR profiles, that are derived from the desired profiles of the specific growth rate $\mu(t)$ is a very good strategy for keeping fed batch processes on the optimal track.

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