Review

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Batch-to-batch reproducibility of fermentation processes by robust operational design and control

Batch-to-batch reproducibility aims to keep every batch of the fermentation process exactly on track. This can be obtained by two methods: first, which is currently most often applied in industrial fermentation processes, is operating the fermentation processes in the open-loop control mode. This requires robust (i.e., failure-tolerant) fermentation strategies as corrections upon unexpected deviations from the desired path are not intended. The second control method counteracts for distortions in the process state variables by corrective measures using advanced closed-loop or feedback control modes. This is less frequently used as most companies have difficulty in making precise state estimations, which are a prerequisite for feedback control. In this review we discuss various aspects of both techniques that are considered decisive for the success of these control procedures.

Sebastian Schaepe[1], Artur Kuprijanov[1,2], Rimvydas Simutis[2], Andreas Lübbert[1], Michael Pohlscheidt[3], Marco Jenzsch*[3]

[1]Institute for Biochemistry & Biotechnology, Martin-Luther-University Halle-Wittenberg, Germany
[2]Institute of Automation & Control Systems, Kaunas University of Technology, Lithuania
[3]Roche Diagnostics GmbH, Pharma Biotech Production, Nonnenwald 2, 82377 Penzberg, Germany
*Author for correspondence:
E-mail: marco.jenzsch@roche.com

During the early process development phase it is essential to first explore which process design and operational variants are allowed to get the desired product with a sufficiently high efficacy. In later phases of process development one can then exploit this design space or the intervals between the specification limits for optimizing productivity and product quality.

As most biologic products are very complex, all product properties that influence efficacy are extremely difficult, or even impossible, to characterize quantitatively. Thus, in practice, product quality is related to process quality – in other words, the reproducibility of the product formation process during all its phases. This fact led to the still valid phrase: the process is the product [1].

The objective in process optimization then becomes clearer: within the design space, a process design variant must be chosen that leads to the highest productivity and meets the desired batch-to-batch reproducibility. The distortions we primarily must cope with in obtaining batch-to-batch reproducibility are typically: variability in the upstream feedstock or raw materials; in the inoculum size and quality; in the timing of operator inventions; or in sensor calibration. Precise process operation not only in the early upstream processing phase, with respect to media preparation and sensor calibration, but also during the measurements of the at-line variables, are indispensable for keeping the variability low.

As will be shown in this chapter, batch-to-batch reproducibility can be approached in two ways. First, by looking for robust process trajectories, that is, process operational procedures that are insensitive to distortions that may appear within a running cultivation and may change from batch-to-batch. Second, it can be obtained by automatic corrections for deviations between the set points of the controlled variables from their actual values.

When robust trajectories are possible and feasible economically, they allow for a safe open-loop control procedure. Often,

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Key Terms

Batch-to-batch reproducibility: Consistent process behavior with respect to key performance variables such as biomass concentration, cell viability and product titer, as well as product quality based on the design of a production process, robust operational procedures and application of process controls to adjust for potential distortions or deviation that might occur.

Robust process trajectories: Describes the course of a process variable, which allows reproducible process performance even in the case of (minor) distortions.

however, the robust control procedures are not necessarily the most productive variants. Where frobustness is not practical, by technical or economic reasons, the distortions must be combated by corrective measures, that is, feedback control. Conceptually, both variants have clear pros and cons. The practical aspects that influence their performance will be discussed in this paper from a bioprocess engineering perspective.

It is important to note that high batch-to-batch reproducibility of bioprocesses cannot be achieved when the controllers for the basic variables of temperature, pressure, pH, dissolved oxygen (dO), feed flows and reactor weight determining the cells' environment are not working perfectly. This highly underestimated fact will be addressed later in this review.

We will discuss opportunities for improving manufacturing and quality assurance through innovative process development, analysis and control, in line with the Process Analytical Technology (PAT) initiative of the US FDA/European Medicines Agency [2].

Robust cultivation processes

Robust operational procedures are insensitive with respect to unexpected changes in the values of process variables that may appear within a batch or from batch-to-batch. An important example is the inoculum size and its quality, which may be slightly different in every fermentation run. As recognized by many companies, the initial biomass concentration varies even when prepared carefully. Its value is rather low and can usually only be detected by standard biomass concentration measurements with remarkably high errors. In most real cultivations the biomass is initially grown at high specific rates in order to gain high biomass concentrations for the production phase. Typically, the cultures are then operated at lower growth rates for maximized target product formation in the second phase of the cultivation. When initially operated at maximum specific growth rates, small changes in the inoculum biomass may lead to large differences after some hours of cultivation.

This is particularly true for cultivations that are started with a batch phase. Then the cells grow with maximal specific growth rate (μ_{max}) until the initial substrate concentration is nearly exhausted. An initial difference in the biomass set point grows exponentially with time so that after a few hours there is a considerable difference in the desired biomass concentration and possibly also in the desired specific growth rate since the substrate could already have been exhausted. Hence, from the point of view of batch-to-batch reproducibility, an initial batch phase in the cultivation process contributes considerably to variations in sequence of cultivation runs. Figure 1, taken from a commercial monoclonal antibody production, gives a practical example.

The same may apply when the biomass growth phase is already operated in the fed-batch operational mode and the feed rate profiles were computed for a well-defined initial biomass concentration and then adjusted by programmable controllers. Since most companies try to run microbial

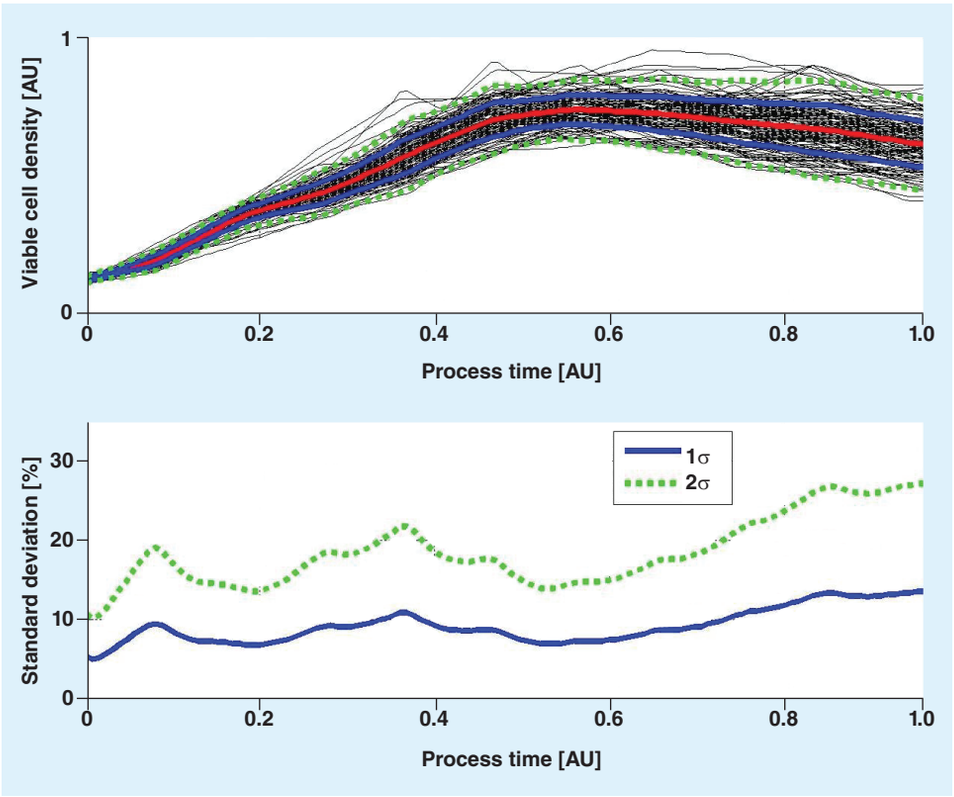

Figure 1. Cell density profiles from 147 batches of a mAb production-scale cell culture process. Since this process is operated in an open-loop control mode, the deviation introduced by inoculation of the bioreactor with different amounts of cells remains during the entire process time.

cultures at maximal pace through the biomass growth phase in order to achieve high productivities, the situations described below appear.

Deviations from the nominal inoculum size to higher biomass values lead to higher initial biomass concentrations, thus the individual cell gets less substrate than desired. Hence, it grows slower until the biomass concentration reaches the value for which the feed profile was computed. When the initial biomass concentration is smaller than the desired value, the cells see more substrate than expected, but cannot grow faster as they are already growing at their maximal speed. Hence, with variations in the inoculum size, the biomass profiles will vary from batch-to-batch.

This divergent behavior can be avoided if the desired specific growth rate in the biomass formation phase is kept below the maximal specific growth rate μ_{max}. Then, if the initial biomass concentration is smaller than desired, the cells can react on the higher substrate concentrations by increasing their specific growth rate until the biomass consumes exactly the amount of substrate that is fed and, thus, the error is compensated for.

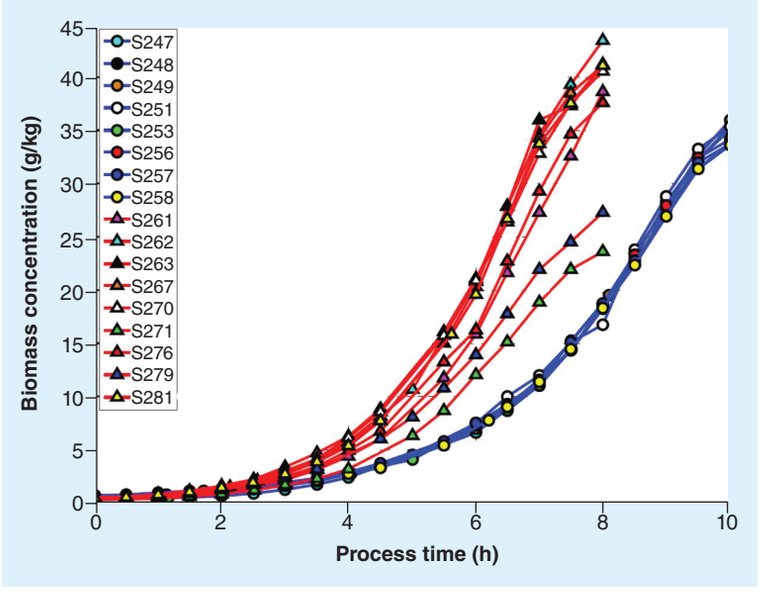

Figure 2. Biomass concentration profiles measured in a set of 17 individual experiments. Feed rate was computed with a fixed X_0 = 0.15 [g/kg] and a μ_{set} = 0.67 [1/h] (Δ) as well as a reduced μ_{set} = 0.5 [1/h] (○). Since 0.67 [1/h] is equal to the maximum growth rate μ_{max}, minor fluctuation in inoculum cell density led to significant changes in biomass concentration profiles (Δ). With a feed profile based on the reduced growth rate very reproducible biomass curves have been obtained (○) when applying the same fluctuation in initial biomass concentration.
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The practical result of this discussion is that high batch-to-batch reproducibility at the end of the biomass growth phase can be obtained by starting the biomass growth phase of the process with a fed-batch operational mode and with an exponential feed rate computed for a specific growth rate μ_{set} that is smaller than μ_{max} for the particular substrate.

An experimental validation of this fact is shown in Figure 2, where a series of biomass concentrations profiles from open-loop controlled fermentations operated with a fixed feeding profile is shown. In these experiments, the initial biomass concentration was systematically changed from batch-to-batch [3].

Obviously, the advantage of running the process at lower specific growth rates must be paid for by some reduction in productivity. The immediate question is how big the reduction in μ_{set} with respect to μ_{max} must be in order to get the focusing effect. This can be solved by numerical simulation of the process. A simple Monte-Carlo simulation allows estimating the resulting biomass concentration trajectories for various assumptions about the variance in the inoculum size. Quite simple dynamical process models can be used for this purpose. Generally, the tighter the distribution of the inoculum size in a production system, the smaller the reduction in the set-point for the specific growth rate in the biomass growth phase of the process and the smaller the loss in productivity.

An example of a result of such a Monte-Carlo simulation is shown in Figure 3. The common probability density distribution $p(X_0)$ for the initial biomass concentration for all trials discussed here is shown in the lower left graph. The time development of a number of cultivations chosen from this distribution and propagated with maximal growth speed $\mu_{set} = \mu_{max}$, are shown in the upper left quadrant. Another set of biomass concentration profiles, started with the same initial conditions, but with a feeding rate corresponding to a smaller set point $\mu_{set} < \mu_{max}$ are shown in the upper right quadrant. Finally, in the lower right plot the two biomass concentration distributions of both sets of experiments at a fermentation time

Key Terms

Open-loop control: Simple type of controller were the process is operated according to a predefined fixed profile of the actuating variable without using any information on the running state of the process. This mode of control is applied in cases where the controlled variables cannot be determined accurately enough within the time intervals required for closed-loop control.

Feedback control: Also known as closed-loop control. In contrast to open-loop control, here the controlled variable is monitored during the running process, which then allows a feedback loop to the actuation variable in case of distortion or any deviation of the controlled variable compared with the desired process trajectory (set point).

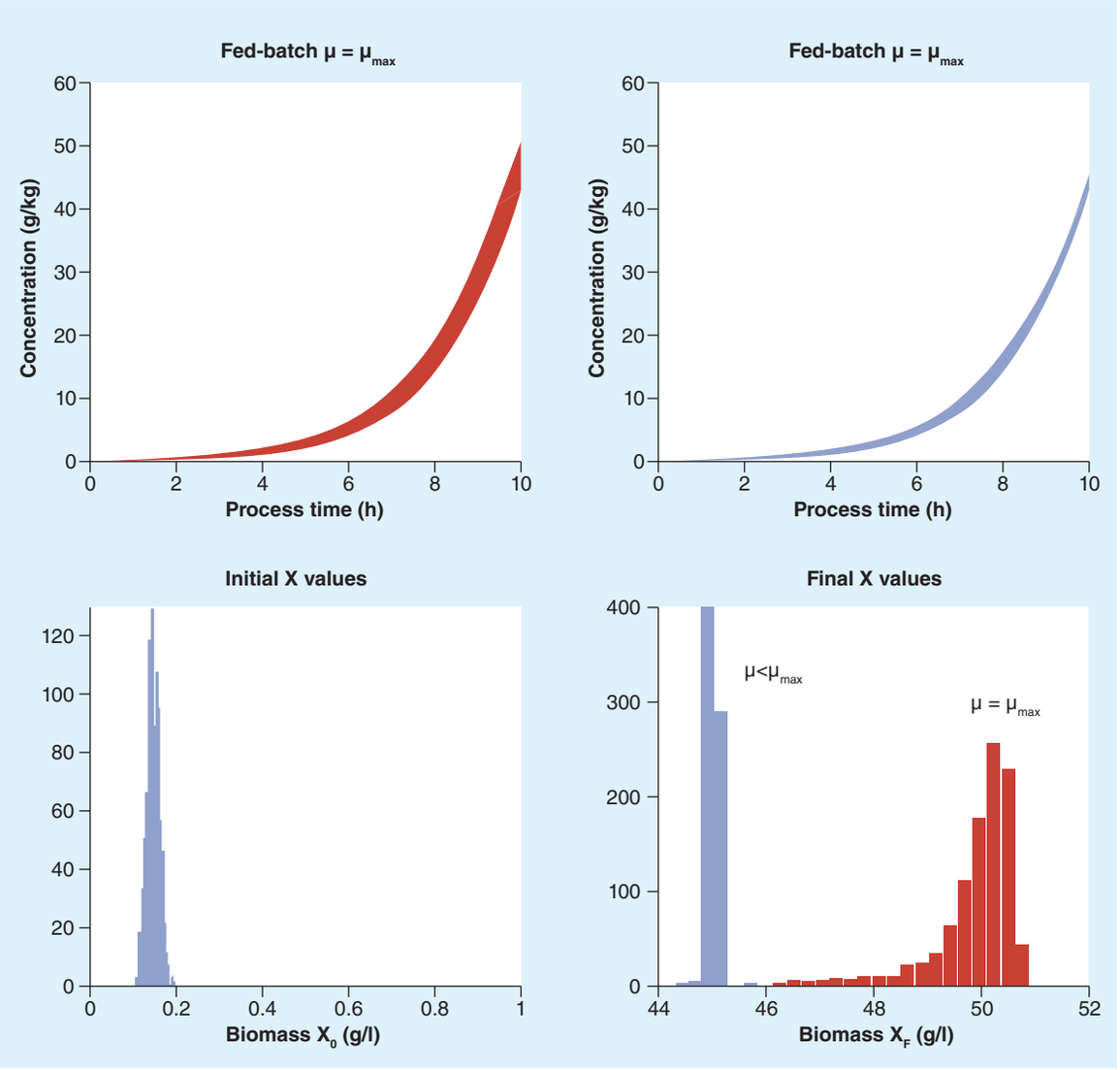

Figure 3. Result of a Monte-Carlo simulation of cultivations operated at maximal specific growth rate $\mu_{set} = \mu_{max}$ (dark red) and at a smaller value of μ_{set} (light blue).

t_F = 10 h are shown. The experiments with $\mu_{set} = \mu_{max}$ depict a broad distribution, the corresponding distribution for $\mu_{set} < \mu_{max}$ is comparably tight around a lower mean value $\bar{X}(t_F)$.

Hence, it is emphasized to start all cultivation processes in the substrate limited fed-batch mode with exponential feeding corresponding to a specific growth rate set-point (μ_{set}) that is slightly smaller than μ_{max}. This seems to be a little bit more complicated than using batch operation, but results in significant improvements with respect to the batch-to-batch reproducibility of the cultivation runs.

Generally, model-supported sensitivity analyses are important to explore the influence of random variations of variables from batch-to-batch, or within a single batch, on the key performance variables such as biomass or product concentration. Monte-Carlo simulations are valuable tools for such analyses, particularly for processes with complex dynamic models where the partial derivatives (i.e., the sensitivities) are not easy to determine analytically.

All such mechanistic approaches require models that accurately describe the dynamic behavior of the process under consideration. Such models can only be developed when measurement data are available from these processes that are accurate enough. Hence, process measurements become very important in this respect.

Process monitoring & supervision

The level of scientific understanding of cultivation processes for producing pharmaceutical products is

characterized by the accuracy of the models that can describe and predict the process performance within its design space. The level of accuracy that can be obtained largely depends on the quality of the measurement data from these cultivation processes since a serious model development and model validation is impossible without accurate data. This was already expressed by the founders of the quality discussion, for example, by Shewhart, who already stated "*knowledge begins and ends in experimental data*" [4].

As a model cannot be guaranteed to be more accurate and precise than the data used for its development and validation, we must first focus our attention on the goodness of the process data. We are primarily interested in the key state variables of the process under consideration. These are the biomass and the product concentration. Both cannot directly be measured on-line at sufficiently high sampling rates with sufficient precision and accuracy. Hence, indirect measurements become necessary for these quantities [5–7].

Particularly in larger reactors where inhomogeneity is not avoidable, global measurements lead to more representative, and thus accurate, results as local probe measurements. In this respect, measurements of the volume fractions of the gas components oxygen and carbon dioxide in the vent line of the reactor are of importance. They can immediately be transformed to the informative rate expressions of oxygen uptake rate (OUR) and carbon dioxide production rate (CPR). Both of these measurements correlate excellently with the biomass concentration and its growth rate.

The use of these signals for biomass estimation was discussed in numerous papers published in the biochemical engineering literature. As Jenzsch *et al.* [8] found for microbial systems and Aehle *et al.* [7] for animal cell cultures, the biomass concentration in cultures can be estimated on-line by several approaches. The smallest root mean squared errors (RMSE) were obtained with artificial neural networks (ANNs), but non-linear relationships between OUR, CPR and the base consumption with the biomass concentration X that are linear in their parameters led to comparably good results; **Figure 4** shows an example. It depicts results from an ANN-based estimator for the packed cell volume (PCV) using on-line process data

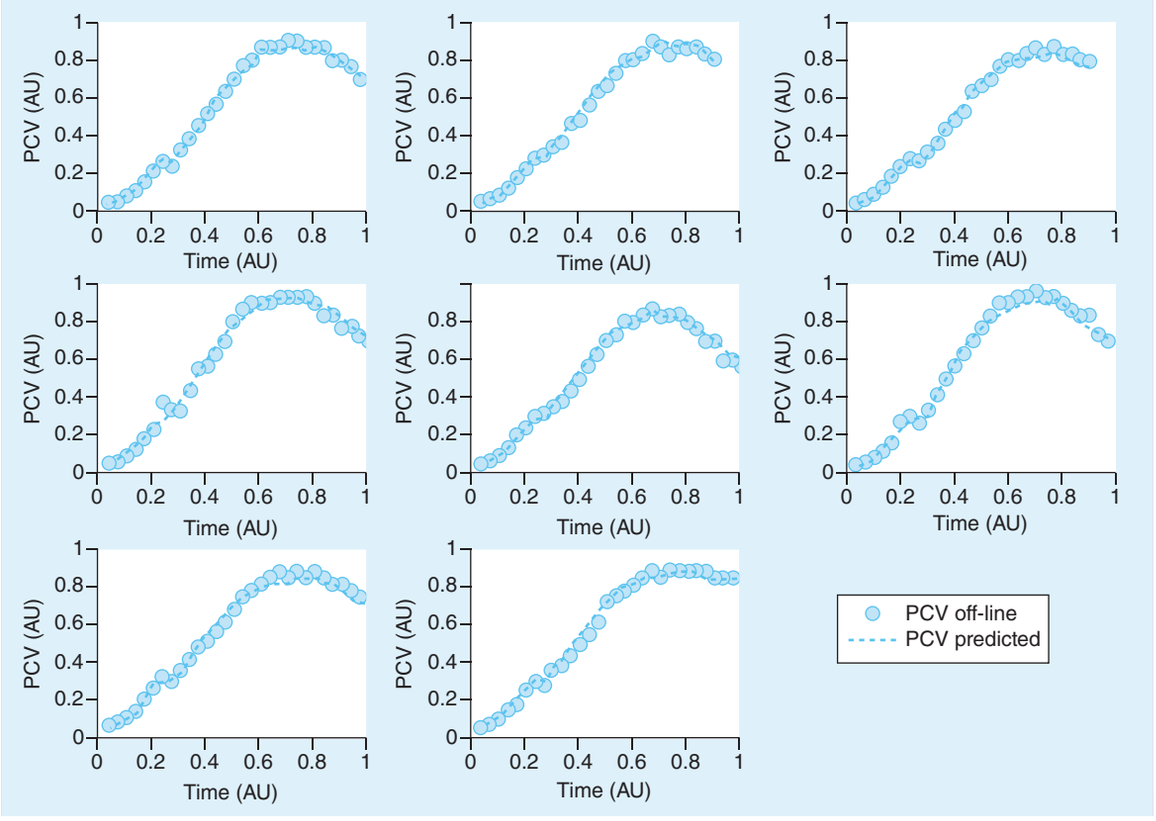

Figure 4. Packed cell volume off-line data (symbols) from eight individual batches of a Chinese hamster ovary recombinant protein production process together with the on-line estimates from an artificial neural networks (lines). Input variables of the neural network are several signals from the dissolved oxygen controller of the bioreactor, as well as base consumption, and reactor weight.
PCV: Packed cell volume.

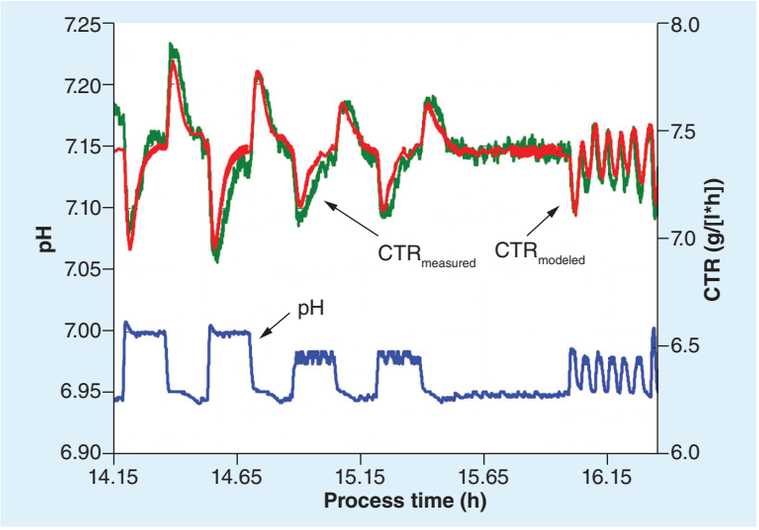

Figure 5. Influence of modulation of the pH-value (blue) in an *Escherichia coli* culture on the carbon dioxide transfer rate. In the first part of this experiment (14.5 < t < 15.65), the pH was modulated by boxcar functions with two different amplitudes (16.1 < t < 16.3) then a sin function was used for that purpose. The green line indicates the on-line measurements of the carbon dioxide transfer rate, the red line a first principle model as described in [9]. Reproduced with permission from [9] © Springer (2008).

from a cell culture production process of eight individual batches. Artificial neural networks are a general approach of representing non-linear relationships between several input and output variables that now count to the established data-driven models in science and engineering. In this example a very simple feed-forward network with a single hidden layer was proven to represent the mappings that are of interest in the case of this specific biomass soft sensor. Input variables used in this specific example were stirring speed, aeration rate and oxygen content in gas supply of the bioreactor, as well as base consumption from pH control. Additionally, reactor volume needed to be considered due to dealing with a fed-batch process.

Instead of discussing the estimators once again, we would like to focus on the precision of the estimates, which depends on the information content in the signals these estimators use for computing the biomass. The information content in a measurement signal is characterized by the signal-to-noise ratio (SNR; i.e., the ratio of the signal power to the noise power in the signal). This is used to characterize the ratio of useful to irrelevant data and is considered a measure of the technical quality of a signal. Generally, the SNR is considered a primary quality index of a signal. In order to get precise information of the current state, we need measurement signals with a high SNR for estimating the state variables.

Key Term

Soft sensor: An estimation algorithm for a quantity that cannot be easily detected on-line. Information from other on-line available variables are utilized to generate an estimation of the quantity of interest. A soft sensor associates sensor hardware and estimation methods based on a software routine.

As an example let us consider the CPR signal from a microbial cultivation, which is not the simplest case to discuss. Again we start with a knowledge- or model-based sensitivity analysis. This shows that the CPR is sensitively influenced by the culture pH. **Figure 5** shows the result of an experiment in which the pH set-point of the pH-controller was varied [9]. In the first hour of the time interval shown in the graph, the step responses in the carbon dioxide transfer rate upon upward and downward steps of two heights are shown. One can immediately determine the time constants of the response dynamics. The response function was modeled using a basic stirred tank model. The modeled signal (the one depicting the smaller noise) is compared with the actual measurements. Both signals are in fair agreement, showing that the influence of pH changes and its dynamics is quite well understood. This can be considered an engineering example of Deming's key requirement for 'understanding variation' [10].

The most important practical result of this experiment with respect to batch-to-batch reproducibility and its modeling is that very small changes of about 0.03 units in the pH value immediately lead to well-resolved fluctuations in the order of 10% of CTR. This essentially says that the usual pH controllers that only compensate for changes in the order of 0.1 pH units do not perform sufficiently well.

The PI controllers with fixed parameters usually installed by fermenter manufacturers for pH control cannot do a much better job. The simplest way of improving these controllers is to adapt the controller parameters automatically to the changing dynamics of the process.

For that purpose a simple but effective technique is required that can be used at production plants without too large tuning expenses. This technique is referred to as the gain scheduling technique [11]. The problem that often prevents using this elegant technique in process industries is finding an appropriate scheduling signal. This, however, is quite easy in cultivation experiments where the CPR signal in microbial or the OUR signal in animal cell cultures [12] do an excellent job **(Figure 6)**. Alternatively, the predetermined substrate feed rate (F) can be used as the gain scheduling variable. This helps in cases where the OUR or CPR signals are heavily distorted or when appropriate analyzers are not available.

A similar problem appears with dO control. Here the action variables used to keep dO on track are the aeration rate and the stirrer speed [13].

Consequently, only the scientifically less attractive expenses in basic fermenter control systems lead to

the desired SNR ratios that allow for an exact monitoring of the cultivation processes. The quality of the on-line CPR signals in *Escherichia coli* cultivations that reflect the dynamics of the fermentation process become immediately clear in Figure 7. The CPR signals are nearly noiseless, that is, their information content is quite high; we will discuss the benefit of this later in this article.

The question of how well the basic controllers can perform in real cultivations also depends on the pace at which a mismatch between the actual value of the controlled variable and it's set point can be eliminated by an appropriate controller action. A feed-forward (gain scheduling) component in the controller helps significantly; however, the delayed response of the fermenter to additions of base solution is another important issue. When batch-to-batch reproducibility is an objective in process design and operation, then not only the controller algorithms, but also the controlled system must be improved accordingly.

In most practical cases it is sufficient to approximate the process around its working point by means of a first-order dynamic system with time delay, in control engineering terms, this is referred to as a FOTD system. In such cases, its controllability primarily depends on two time constants. The first is the time constant τ_T for the dynamics of the reactor's response to changes that influence the value of the controlled variable; the second is the time constant τ_L for signal delays or time lags in the system. Both can easily be estimated from stimulus/response experiments, preferably from step response measurements. The difficulty of controlling a process, and accordingly, of improving the quality of the controlled system, can then be characterized by the controllability ratio (CR) of the process [11], which is defined by CR = Error!

CR can vary between 0 and 1; processes with small CR are easy to control, and their control quality, for example, quantified by the average RMSE from the set point, is high. For pH control, for instance, the dynamic response time, which in production reactors is dominated by the mixing time of the fermenter, is important. Hence, when we are interested in improving pH control we must reduce the mixing time and the time lags (e.g., in the base feeding lines). This can be done by increasing the impeller speed in the tanks, however, also by constructive measures such as the addition of the corrective between the impellers or, counterintuitively, by reducing the number of impellers from the standard three-impeller versions to a two-impeller system without changing the power draw [14]. Both measures allow easily halving the mixing time.

Monitoring fermentations using on-line sensors does not mitigate batch-to-batch variability as is often suggested in the current PAT discussion. This can only be reached by combating the distortions and improving the process operational strategy, for example, choosing robust control trajectories or by direct intervention upon deviations from the specified paths by feedback control.

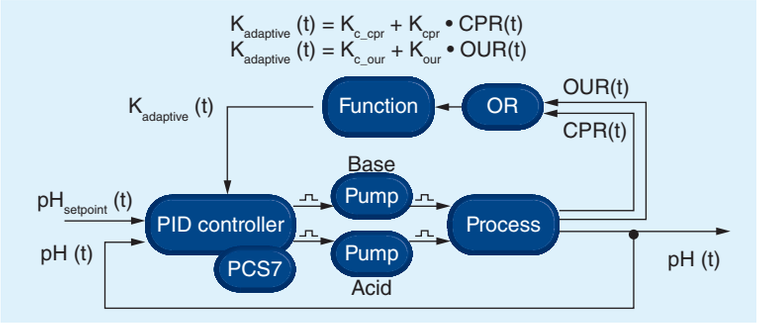

Figure 6. Scheme of a gain scheduling controller for improved pH control in cultivations.
CPR: Carbon dioxide production rate; OUR: Oxygen uptake rate.
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Feedback control

Where robust open-loop control paths cannot be obtained or where such paths are not feasible economically, closed-loop (i.e., feedback control) is the only option for forcing the process to stay on its optimal trajectories.

Particularly with respect to the batch-to-batch reproducibility problem it is worth remembering the fact that an important advantage of feedback controllers is that they are able to compensate for systematic deviations of the process or systematic measurement

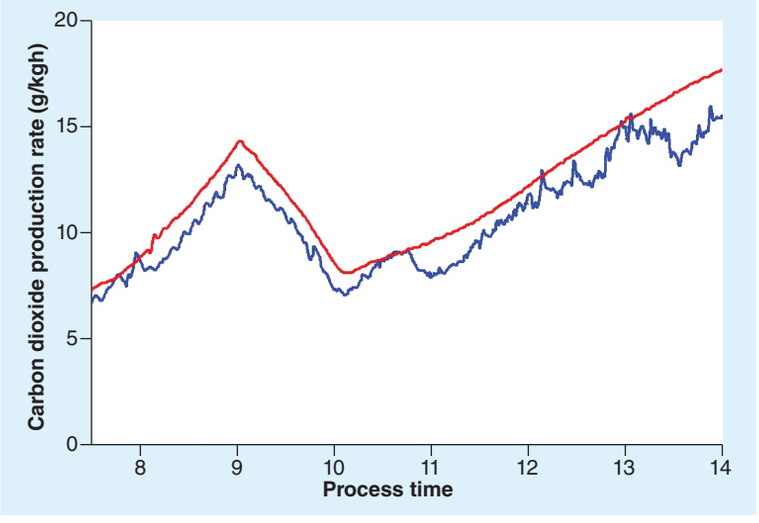

Figure 7. Improvements obtained from experiment one (blue) to experiment two (red) by adaptive pH and dissolved oxygen control in *Escherichia coli* cultivations with respect to the signal-to-noise ratio.
Shows the information content in the carbon dioxide production rate signals.
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errors. In this particular case they can compensate for changes that appear from batch-to-batch, for instance, small changes in the substrate concentration or quality as well as for changes in the growth and product formation ability of the organisms employed. Furthermore, errors that may appear during the calibration procedures of measurement devices can be compensated for as long as they do not change the value of the controlled variable itself.

The problem of which variable should be controlled during a production run can be discussed from various points of view. The most common approach to advanced control published in literature chose the specific biomass growth rate as the controlled variable [15–20], since this is the variable that most closely resembles the physiological state of the cells. The specific growth rate (μ) can be estimated using the estimation techniques mentioned previously.

However, as a simple knowledge-based analysis of this methodology shows, what is good from the physiological perspective might be bad from the process reproducibility point of view: when the production process is tightly following a predefined specific growth rate profile, after every disturbance in the initial biomass concentration, as well as after every deviation of the biomass at later time instances, for instance by errors in probing or temporary growth disturbances, the process will proceed on a different trajectory with respect to the biomass concentration. This will finally lead to a bad batch-to-batch reproducibility, not only with respect to biomass but also concerning product formation.

Hence, from the batch-to-batch reproducibility perspective another controlled variable must be chosen. After the initial discussion of state estimation technologies, the obvious choice would be controlling the biomass concentration directly. This works fine [21], but is dependent on a powerful biomass estimation technique. A well-performing simpler alternative is controlling the respiration rates, or more directly the more or less directly measurable variables: CPR in microbial systems [6] or OUR in animal cell cultures [12]. Further improvements can be achieved when the total cumulative versions of these signals are taken for controlling the process instead of directly taking these rates. This allows elimination of short-term disturbances and increases the SNR of these signals considerably, and thus, as discussed above, increases the precision by which the variable can be provided for control. So, finally, a better control variable is simply the mass of carbon dioxide produced, or the oxygen consumed, so far by the running culture. The set-point profiles for these quantities can be estimated using their non-linear relationships to the specific growth rate μ or directly taken from the best previously obtained experimental results, which are considered to be the 'golden batch'. In microbial cultures, the heat production rate by the cells can alternatively be taken as the control variable since it is proportional to the oxygen uptake rate. This can easily be measured on-line, as shown by Schaepe *et al.* [22].

An example of a set of cultivations controlled in this way is shown in **Figure 8**. The trajectories directly show the performance of the controller.

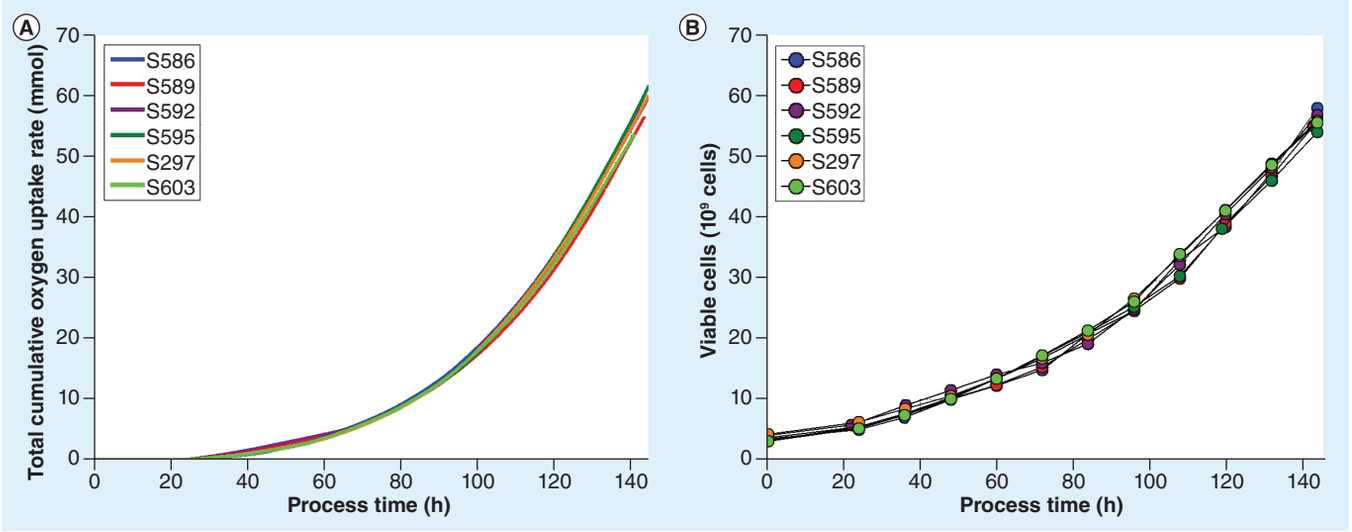

Figure 8. Example of the batch-to-batch reproducibility in a Chinese hamster ovary cell culture obtained by controlling the amount of oxygen [mmol] consumed by the cells, which is equal to the total cumulative oxygen uptake rate. (A) Trajectories of this quantity for six cultivations, **(B)** the corresponding viable cell count profile.
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As shown in Figure 8, the batch-to-batch reproducibility is nearly perfect, not only for the controlled variable, the oxygen amount consumed by the cells (tcOUR) as a function of time, but also for the key state variable the viable cell count.

Where the accumulation of the signals is thought to damp down the signal response too much, the controllers can be supplemented by a second controller that enables changes in the process to be followed more quickly. For that purpose the total carbon dioxide production or the total oxygen consumption rate profiles can be used as a second control variable in a sequence of two simultaneously acting controllers. This works well in both cases, for microbial systems as shown in Figure 9 as well as for animal cell cultures [23].

As shown in Figure 9, differences between different culture batches can no longer be recognized in the plots of the controlled variables, and in the corresponding biomass profiles the differences are within the measurement accuracies.

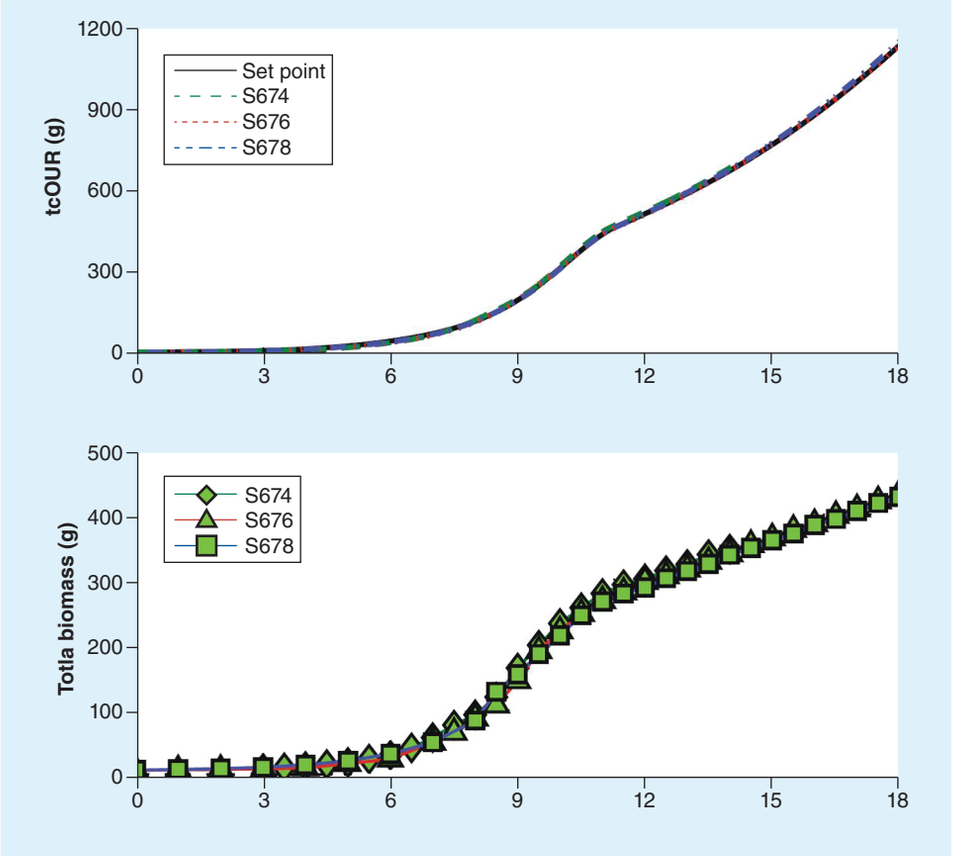

Figure 9. Control of a microbial cultivation system by means of a sequence of two controllers as shown in.
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Discussion

In manufacturing processes for complex biologics, product quality and performance are ensured through the design of effective and efficient protein formation processes [2].

Batch-to-batch reproducibility can be increased by choosing robust trajectories. The insensitivity of the initial biomass growth phase to variations in the inoculum size by running the process with an exponential feeding profile corresponding to a specific growth rate set point smaller than μ_{max} is an example of assuring quality-by-design in the bioprocess engineering sense.

When a process is initially run in the batch operating mode or in the fed-batch mode with the highest possible growth rate, small variations in the inoculum size will lead to considerable scatter in the biomass concentration at the end of the biomass growth phase. These processes can be made robust with respect to fluctuations in the inoculum size by running them entirely in the fed-batch mode with an exponential feed rate profile computed for a specific growth set-point smaller than μ_{max}. The reduction in μ_{set} required depends on the accuracy that can be guaranteed for the inoculum size.

In order to make sure that the processes are running on track, precise and accurate on-line measurements are required. For this purpose, various state estimation techniques are described in the literature. The most important problem with respect to batch-to-batch reproducibility, however, is that the signals of most process variables usually fluctuate considerably. To cope with that problem, one usually applies numerical filtering procedures. However, this is not the most elegant method from a process engineering perspective. The first option for bioprocess engineers should be combating the noise by improving the cultivations accordingly. Here we show in a knowledge-based way that enhancing the performance of the basic low level controllers for pH, dO, pressure, substrate flow rates and temperature is the preferred engineering solution to increase the information content in the on-line signals.

Where robust trajectories cannot be obtained or the process paths that are insensitive to distortions do not lead to sufficiently profitable cultivations, feedback control is the only option. As the dynamics of

cultivation processes are changing with time, adaptive controllers are necessary. Here, gain scheduled controllers are shown to be a simple and very efficient solution. Compared with other process industries it is easy in bioprocess technology to find appropriate scheduling variables. This is demonstrated by examples from microbial as well as animal cell fermentation technology.

The question whether to look for robust open-loop operating strategies or closed-loop control is simple to answer. In most practical cases both techniques should be applied. In the very beginning of almost all cultivations, the measurement signals are usually too noisy to guarantee well-performing feedback control since initially the biomass concentrations are too small to generate sufficiently intensive signals in the on-line variables. In these situations the only choice is running the process in an open-loop control mode. In such situations it is advisable to run the process with an exponential feeding with a set point for the specific biomass growth rate that is smaller than μ_{max}. At later phases of the cultivation process, towards the end of the growth phase, the situation is quite different. Now, the signals are strong enough and can be made available with a low noise level. At this point closed-loop control is the matter of choice as this allows running the process at close to its optimal path without cutting back on anything. Rational or optimal paths for various recombinant protein cultivation processes can be obtained using various probing control methods and algorithms.

Clearly, the ideal case is that the optimal path is at the same time the most robust path, but this usually remains a dream.

Future perspective

Batch-to-batch reproducibility can only be improved by avoiding failures and combating the remaining distortions by control actions in the engineering sense. The main activity has to focus on decreasing process variability since improvements of on-line analytics will enable immediate detection of deviations from the desired paths. FDA's PAT initiative triggered a number of developments that can be used in future. Once reliable and informative on-line data about the key process variables are available, closed-loop control becomes possible. Feedback control will, thus, be the matter of choice in future bioproduction processes. Only where robust trajectories are possible without significant reductions in productivity will the currently preferred open-loop control survive. As shown in this article, this applies to the biomass growth phase in recombinant protein production cultures.

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Executive summary

- Increasing reproducibility of cultivation processes is still an important issue in industrial practice.
- The final process performance with respect to productivity and reproducibility is directly influenced by process operational conditions.
- The influence of control of the basic cultivation variables pH, temperature and pO_2, on reproducibility is widely underestimated.
  - Integral measurements (e.g., using offgas analysis) provide representative global on-line information about the process state. After a few hours fermentation time, the signal-to-noise ratios are quite high.
  - These data allow well-performing feedback control along the desired state variable profiles (i.e., low process variability).
  - Open-loop control results in good process performance, when the processes are run along robust process trajectories.
- Open-loop is well suited for the initial biomass formation phase of the cultures where most measurement signals are rather noisy. Then robust growth profiles can be advised.

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