Advances in Fermentation Process Design for Recombinant Protein Production

Dissertation

Zur Erlangung des Doktorgrades der Ingenieurwissenschaften (Dr. Ing.)

des Zentrums für Ingenieurwissenschaften

der Martin-Luther-Universität
Halle-Wittenberg,

vorgelegt von
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Datum der Verteidigung: 16.12.2014
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Contents

Introduction

Current Advances in Tools Improving Bioreactor Performance

Abstract

Improving the performance of their bioreactors is a continuous distinguished task of process engineers for the entire lifetime of their processes. In this paper new developments which help to improve the performance of bioreactors for recombinant protein formation are reviewed. In order to judge the performance of a cultivation process an important prerequisite is accurately and easily monitoring the key quantities of their processes. Recently, the well-established monitoring techniques, got competition from new, so-called kernel methods, which are more precise as they need a smaller number of free model parameters. In the field of dynamic estimators, “Extended Kalman Filters” are being replaced by “Unscented Kalman Filters” which simplify this sophisticated technique as they do not need Jacobian matrices. They use the full process model instead of linear approximations. Process supervision, optimization and control examples are given from microbial fermentations as well as from animal cell cultures. Optimization the operation of the fermentations, an extremely prominent task, is discussed in case of *E. coli* cultivations where the product appears in soluble form as well as in the form of inclusion bodies. In order to keep the process on its optimal path at a significant batch-to-batch reproducibility, open loop control along robust trajectories should be used for the initial biomass growth phase. Later, during the product formation phase, closed loop feed forward feedback control is the technique of choice to keep the process on its optimal trajectory. All procedures mentioned can be implemented in modern industrial automation systems. Advances in such bioreactor control systems develop towards virtual plants, which simultaneously allow simulating the fermentation process. They allow for improved controller developments, online process supervision, and can also be used for a more realistic training of the personnel similarly to flight simulators for pilot training.

This chapter has been published in *Current Biotechnology*:

Sebastian Schaepe, Artur Kuprijanov, Christian Sieblist, Marco Jenzsch, Rimvydas Simutis, Andreas Lübbert, Current Advances in Tools Improving Bioreactor Performance, 2014, (3)133-144

Chapter 2

Avoiding overfeeding in high cell density fed-batch cultures of *E.coli* during the production of heterologous proteins

Abstract

Heterologous protein production often causes a significant metabolic burden in *E.coli* cells which manifests itself in a substantial decrease in their physiological characteristics such as the maximal specific growth rate on a given substrate, the maximal substrate uptake rate as well as the maximal specific oxygen uptake rate. In high-cell-density cultures, the substrate feed rate must be adapted to this changing capabilities of the cells in order to avoid overfeeding and thus the formation of by-products that inhibit the cell performance further. This requires the precise knowledge about the changes in these specific rates, particularly during the product formation phase. In order to precisely investigate the time profile of the critical specific substrate uptake rate σ_{crit} of microorganisms, i.e. the maximal rate at which the cells can fully oxidize their substrate, a new online tracking technique is presented. The feed rate F is modulated in such a way that the specific substrate uptake rate σ is linearly raised towards its critical value σ_{crit}. When this is reached the feed rate is automatically reduced and the procedure is repeated. In this way the method automatically follows the changing time profile of σ_{crit} during the entire cultivation and avoids significant acetate formation rates. This procedure considerably increases the identifiability of σ_{crit}. The high precision of the technique also results from replacing the pO_2 measurements that seem to suggest themselves for monitoring maximal oxygen uptake rate, by measuring the total oxygen consumption rate tOUR, which is available at a much higher signal-to-noise ratio and is not as prone to distortions. An important advantage of measuring tOUR is that it allows keeping pO_2 controlled at its optimal value. The applicability of the new tracking technology is demonstrated at E.coli cultures. The resulting $\sigma_{crit}(t)$ profile allows determining the substrate feed rate profile and other key variables that can be used for advanced feedback control in protein production processes. The technique can be considered a PAT tool and is well suited to industrial fermentations.

This chapter has been accepted for published in *Journal of Biotechnology*

Sebastian Schaepe, Artur Kuprijanov, Rimvydas Simutis, Andreas Lübbert, Avoiding overfeeding in high cell density fed-batch cultures of *E.coli* during the production of heterologous proteins, 2014, doi: 10.1016/j.jbiotec.2014.09.002.

Chapter 3

Data-based optimization of protein production processes

Abstract

While data-based modeling is possible in various ways, data-based optimization has not been previously described. Here we present such an optimization technique. It is based on dynamic programming principles and uses data directly from exploratory experiments where the influence of the adjustable variables u were tested at various values. Instead of formulating the performance index J as a function of time t within a cultivation process it is formulated as a function of the biomass x. The advantage of this representation is that in most biochemical production processes J(x) only depends of the vector u of the adjustable variables. This given, mathematical programming techniques allow determining the desired optimal paths uopt(x) from the x-derivatives of J(x). The resulting uopt(x) can easily be transformed back to the u(t) profiles that can then be used in an improved fermentation run. The optimization technique can easily be explained graphically. With numerical experiments the feasibility of the method is demonstrated. Then, two optimization runs for recombinant protein formations in E. coli are discussed and experimental validation results are presented.

This chapter has been published in *Biotechnology Letters*:

Sebastian Schaepe, Donatas Levisauskas, Rimvydas Simutis, Andreas Lübbert, Data-based optimization of protein production processes, 2014, (36) 929-935

Chapter 4

k_La of stirred tank bioreactors revisited

Abstract

By means of improved feedback control k_La measurements become possible at a precision and reproducibility that now allow a closer look at the influences of power input and aeration rate on the oxygen mass transfer. These measurements are performed online during running fermentations without a notable impact on the biochemical conversion processes. A closer inspection of the mass transfer during cultivations showed that at least the number of impellers influences mass transfer and mixing: On the laboratory scale, two hollow blade impellers clearly showed a larger k_La than the usually employed three impeller versions when operated at the same agitation power and aeration rate. Hollow blade impellers are preferable under most operational conditions because of their perfect gas handling capacity. Mixing time studies showed that these two impeller systems are also preferable with respect to mixing. Further-more the widths of the baffle bars depict a significant influence on the k_La. All this clearly supports the fact that it is not only the integral power density that finally determines k_La.

This chapter has been published in *Journal of Biotechnology*:

Sebastian Schaepe, Artur Kuprijanov, Christian Sieblist, Marco Jenzsch, Rimvydas Simutis, Andreas Lübbert, k_La of stirred tank bioreactors revisited, 2013, 168(4) 576-583

Chapter 5

Simple control of fed-batch processes for recombinant protein production with *E. coli*

Abstract

A very simple but effective process control technique is proposed that leads to a high batch-to-batch reproducibility with respect to biomass concentration as well as the specific biomass growth rate profiles in *E.coli* fermentations performed during recombinant protein production. It makes use of the well-established temperature controllers in currently used fermenters, but takes its information from the difference between the controlled culture temperature T_{cult} and the temperature T_{coolin} of the coolant fed to the fermenter's cooling jacket as adjusted by the fermenter temperature controller. For process control purposes this measured difference is corrected regarding stirrer influences and cumulated before it is used as a new process control variable. As a spin-off of this control, it becomes possible to estimate online the oxygen mass transfer rates and the corresponding k_La values during the real cultivation process.

This chapter has been published in *Biotechnology Letters*:

Sebastian Schaepe, Artur Kuprijanov, Mathias Aehle, Rimvydas Simutis, Andreas Lübbert, Simple control of fed-batch processes for recombinant protein production with *E. coli* (2011) 33:1781–1788

Summary

The development of fermentation processes in industrial environment runs under tight timelines and minimized use of resources. Rational and evidence based methods can scarcely be found in practice. The associated risks and costs of suboptimal processes are hardly to estimate around the entire process lifecycle. That's one reason for the outstanding importance of bioprocess development. Increasingly, biotechnological production processes are in competition to classical chemical production processes with an optimization history of several decades. These facts call for serious efforts in the field of fermentation development.

Within this thesis concrete methods were developed to achieve a substantial improvement within bioprocess development and bioreactor characterization. The first objective was to generate process data of high quality with high information content (high signal-to-noise ratio). This was the prerequisite for model building and validation and finally resulted in a maximized information retrieval from experiments. Consequently, adequate actions were applied and the success was documented at practical examples. This is a direct response to the industrial need of a streamlined and knowledge based bioprocess development.

For a rapid and reliable assessment of the performance of microorganisms, the specific oxygen uptake rate q_o was used. The time course of q_o and the corresponding specific substrate uptake rate can now be monitored using a newly developed method based on offgas concentration measurements of oxygen. The global information base and the entirely automated evaluation of the measurement data were substantially improved compared to existing methods. The dynamic respiratory characteristics as well as the influence of the product formation on the respiratory rates can be measured reliably. For the first time microbial systems have been investigated which underlie metabolic burden during product formation with a dynamic response method. The dynamic impact of product formation can precisely be monitored. Consequently, the process design space to be considered within a process optimization is narrowed down. Furthermore, valuable data are obtained from the transition process to overflow metabolism. The variety of information was so far not available in this form and quality. Within this method only well-established process measurement equipment was used. It can be implemented within professional automation systems and proves the capability for industrial use.

If the productivity optima are not located around the borderline measured with the dynamic response method, the following data based method can be applied. If process data are available the straightforward engineering approach are model based optimization methods, which require expert knowledge in the field of model identification, parameter estimation and numerical optimization. These skills are not a prerequisite with the new developed method. For the first time a data based optimization method was developed, that is not based on a statistical approach. In particular it performs well when extensive amounts of data are available. Systematic errors within model identification are excluded with this method. The possibility of graphical interpretation of the course of action opens this approach to a broad spectrum of users. The simplicity of the optimization routine makes this method capable for a wide field of application. The applicability of this approach was demonstrated with a comparison of a traditional approach.

In the context of comprehensive bioprocess development, the process design orientated optimization cannot be done without investigating the needed mass transfer capacities of bioreactor systems. The mass transfer coefficient of oxygen plays often a crucial role while designing reaction profiles for fermentation processes. Nearly all published mass transfer measurements are still performed in oversimplified model media, which should reflect the behavior of real biosuspensions. Derived information are only transferable with many limitations and they are connected with major risks. In

detail, the dynamic changing physical-chemical properties of biosuspensions are hardly to map into model media. To tackle this question, a method was developed, allowing for mass transfer studies with variation of aeration rate and power input during fermentation without disturbing the process itself with respect to biomass growth and product formation. By means of these data the bioreactor design was optimized. The efficacy was validated within a series of experiments. It was shown, that a two stage stirrer system has a higher k_La value than a three stage stirrer system at constant power input. The method is fully automated in a process control system and transferable to production scale bioreactors.

While low variability of product profiles within process development is a prerequisite to distinguish between effects and measurement noise, in biopharmaceutical production environment there are regulatory needs to ensure product quality. Minimized deviations in final product profiles are at the same time the cost minimum for subsequent downstream processes. The question now is how to control the variability. Measurement information with global information content, that means they are independent of local inhomogeneous conditions, have an outstanding meaning for monitoring and consequently controlling the variability. The only available global measures are offgas concentrations and temperature based heat measures. For process environments, where offgas analysis is not applicable or available, heat measurements essentially provide the same information. It can also be used as a backup if the offgas analysis fails. The reconstitution of offgas signals from the heat balance measurements was successfully shown.

It was shown within this thesis, that simple temperature difference measurements in the reactor and the reactor jacket can be exploited to control a biomass and product profile along a predefined profile. The heat evolving during stirring is compensated with a calibration function. The needed data were experimentally measured. The metabolic heat portion is directly connected to the oxygen uptake rate. This rate itself is proportional to the growth rate and therefore also to the product formation rate. Temperature sensors are available in practically all production plants. The expense for implementing this process control strategy is therefore lowered.

Publications

Peer Reviewed International Journal Articles

1. Schaepe S, Kuprijanov A, Simutis R, Lübbert A (2014) **Avoiding overfeeding in high cell density fed-batch cultures of *E.coli* during the production of heterologous proteins.** Accepted for publication in Journal of Biotechnology, doi: 10.1016/j.jbiotec.2014.09.002

2. Schaepe S, Levisauskas D, Simutis R, Lübbert A (2014) **Data-based optimization of protein production processes**. Biotechnology Letters, 36(5) 929-935

3. Schaepe S, Kuprijanov A, Sieblist C, Jenzsch M, Simutis R, Lübbert A (2014) **Current Advances in Tools Improving Bioreactor Performance**, Current Biotechnology, 3 133-144

4. Schaepe S, Kuprijanov A, Sieblist C, Jenzsch M, Simutis R, Lübbert A (2013) **k_La of stirred tank bioreactors revisited**. Journal of Biotechnology. 168(4): 576-583

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13. Aehle M, Schaepe S, Kuprijanov A, Simutis R, Lübbert A (2011) **Simple and efficient control of CHO cell cultures.** Journal of Biotechnology 153 (1-2):56-61.

14. Kuprijanov A, Schaepe S, Sieblist C, Gnoth S, Simutis R, Lübbert A. (2008) **Variability Control in Fermentations: Meeting the Challenges Raised by FDA´s PAT Initiative**. Bioforum Europe 9:38-41

15. Simon C, Schaepe S, Breunig K, Lilie H (2013) **Production of Polyomavirus-like particles in a Klgal80 Knockout Strain of the Yeast *Kluyveromyces lactis***. Preparative Biochemistry and Biotechnology 43 (2):217-235.

Patents

Kuprijanov A, Lübbert A, Pfeiffer B, Schaepe S, Simutis R (2012) **Regeleinrichtung für eine Regelung des Sauerstoffpartialdrucks in einem Bioreaktor**. Internationale (EU, US, JP, u.a.) Patentanmeldungsnummer: WO/2012/065631. Veröffentlichungsdatum 24.05.2012.

Presentations on scientific conferences

1. **Tracking the metabolic load of *E.coli* cells during recombinant protein production**. 2014, The 10th European Symposium on Biochemical Engineering Science (ESBES), Lille, France

2. **Mass Transfer in Stirred Tank Bioreactors Monitored Online**. 2012, The 9th European Symposium on Biochemical Engineering Science (ESBES), Istanbul, Turkey

3. **Are classical correlations suitable for high performance lab scale bioreactors ?**, 2012, 2nd BioProScale Symposium, Berlin

4. **Process Development for High Performance Fermentation Processes with *Escherichia coli***, 2011, 1st European Congress of Applied Biotechnology, Berlin

5. **Professional Control of Recombinant Protein Production Processes**, 2010, The 8th European Symposium on Biochemical Engineering Science (ESBES), Bologna, Italy

6. **Rapid Process Development Method for High Performance recombinant protein production processes in *Escherichia coli***, 2009, 1st BioProScale Symposium, Berlin

7. **Variability Control in Fermentations**, 2008, EAPB Science to Market Conference, Hannover

Curriculum Vitae

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School Education

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Erklärung

Hiermit erkläre ich an Eides statt, dass ich die vorliegende Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Hiermit erkläre ich weiterhin, dass ich mich mit der vorliegenden Arbeit erstmals um die Erlangung des Doktorgrades bewerbe. Die Arbeit wurde noch keinem anderen Promotionsausschuss vorgelegt.

Ludwigshafen(Rhein), 04.10.2014 Sebastian Schaepe