4th BioProScale Symposium

Bioprocess intensification through Process Analytical Technology (PAT) and Quality by Design (QbD)

6 to 8 April 2016
Berlin – Germany

Three-day symposium about industrial scale bioprocess intensification from process development to large-scale understanding

■ Industrial-scale bioprocess operation

Manufacturing at large scale from process to product, robustness vs. repeatibility, scaleability of operations, single-use techniques, process analytical technologies and process control

■ Scale down approaches and process analytical technologies for advanced process design

Comprehensive process monitoring, non-invasive sensors for all process scales, multiparameter and soft-sensors, addressing inhomogeneities by sensor applications and multiposition sampling, modelling and control approaches, influence of early process steps on later down-stream operations, understanding heterogeneity and population development

■ Quality by design in bioprocess development

High throughput applications, model based DoE, in-line process analytics, data handling and analysis, multivariate data processing, scale down methods, evolutionary strategies for strains and processes, computation based process development

Organisers Technische Universität Berlin – Chair of Bioprocess Engineering & Institute for Biotechnology and Fermentation in Berlin (IfGB)

Location Technische Universität Berlin, Institute for Architecture, Lecture Hall A151
Strasse des 17. Juni 152, 10623 Berlin (Charlottenburg), Germany

Institute for Biotechnology and Fermentation in Berlin (IfGB)
Seestrasse 13, 13353 Berlin, Germany

www.bioproscale-conference.org

Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik
Chair of Bioprocess Engineering

IfGB
Institute for Biotechnology and Fermentation in Berlin

In cooperation with

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Welcome address

Dear Colleagues, Ladies and Gentlemen
Dear Guests and Students

My co-workers and I would like to warmly welcome you to Berlin to the 4th BioProScale Symposium. This symposium is dedicated to 100 years of the fed batch technique, which was initially discovered and developed by Friedrich Hayduck in 1915 in Berlin at the "Verein der Spiritusfabrikanten in Deutschland in Berlin", a predecessor organization of the IfGB.
We also celebrate 40 years of Bioprocess Engineering in Berlin with the appointment of Matthias Reuss as professor for *Bioverfahrenstechnik* at the TU Berlin in 1976, and 30 years of the degree of Biotechnology at our university.

With more than 200 participants, the Bioproscale Symposium has become a broadly accepted important platform for the discussion of industrial-scale bioprocess issues with a focus on cell physiology. The specific character of this symposium is the view on the methodology of characterization and controlling large-scale bioprocesses independent from the specific process, and thus we will have talks ranging from bioenergy to biopharmaceutical production. Important aspects for a better control of industrial scale production are novel process analytical tools, model and softsensor approaches. Keys for the successful implementation of bioprocesses towards a bio-based economy are the comprehensive understanding of scale up phenomena and the availability of effective tools to simulate large scale conditions, and finally to test the robustness of a new process already in a laboratory environment. Currently, we see an increasing acceptance for the idea to consider scale issues already at the early product development stage. This provokes many new developments in the bioreactor field, but also needs parallel progress in sensor and data handling technologies. Integration of this knowledge provides a paradigm change in the way of how to translate biomolecular research into new bioproducts.

We are very delighted to have a wealth of promising lectures in our program and would like to extend our gratitude especially to the speakers who followed our invitation and will share and discuss their expertise with us, as well as to our exhibitors and sponsors, who provided a substantial basis for a pleasant atmosphere. I wish you all a very interesting symposium and a great stay in Berlin!

Professor Dr. Peter Neubauer
Technische Universität Berlin – Chair of Bioprocess Engineering

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About the organisers

Technische Universität Berlin: Department of Biotechnology – Chair of Bioprocess Engineering

The research at the Chair of Bioprocess Engineering at the TU Berlin is directed to the development and application of new methods for faster bioprocess development, including genetic, cultivation, and analytical tools with a special focus on the industrial scale. It aims specifically in understanding the impact of reactor inhomogeneities on the microbial metabolism and adaptation, both affecting process robustness. This knowledge is applied to design molecular biological and process engineering solutions and thus contributes to the understanding and improvement of microbial processes of both fundamental and industrial interests.
By combining state-of-the-art cultivation, sensor and data analysis, automation, and mechanistic modelling technologies with molecular biological and physiological techniques, the activities at the Chair of Bioprocess Engineering contribute to improve the efficiency of bioprocesses and thus to the societal advancement of Industrial Biotechnology.

www.bioprocess.tu-berlin.de

IfGB – Institut für Gärungsgewerbe und Biotechnologie zu Berlin

Founded in 1874, under the umbrella of the Institute of Fermentation and Biotechnology in Berlin (IfGB) fermentation oriented research and education has been conducted for more then 140 years – always in close cooperation with the Technische Universität Berlin (resp. its predesessor institutions). Since 2003 the Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V. is the soule holder of IfGB.

Since 2003, under the brand name IfGB services and training for the
spirits industry and distillers have been offered. Starting in 2009 our service and training programmes will be expanded into the field of biotechnology – again in close cooperation with the Institute of Biotechnology of TU Berlin.

www.ifgb.de

IfGB is a brand of VLB Berlin e.V.
www.vlb-berlin.org

Programme at a glance, Wednesday, 6 April 2016

13:00 Welcome address and introduction
Peter Neubauer (Department of Bioprocess Engineering, TU Berlin, Germany)

13:30 Plenary lecture: Reflections of a 40-years ripening process for modeling of bioreactors – from well mixed towards more reality (L01)
Matthias Reuss (Stuttgart Research Center Systems Biology SRCSB, University of Stuttgart, Stuttgart, Germany)

■ ■ Industrial scale operation

Chair Alvin Nienow (University of Birmingham, Birmingham, UK), Peter Neubauer (TU Berlin, Berlin, Germany)

14:15 Keynote lecture: Continuous improvements in the understanding of large scale aerobic fermentation processes (L02)
Stuart Michael Stocks
(Novozymes, Bagsvaerd, Denmark)

14:45 Keynote lecture: Large-scale production of two molecules of pharmaceutical interest in *Saccharomyces cerevisiae* (L03)
Claus Lattemann (SANOFI, Paris, France)

15:15 Keynote lecture: Microbial GMP manufacturing at large-scale – challenges and considerations (L04)
Torsten Schmidt (Lonza, Visp, Switzerland)

15:45 Sponsor Talk: Bioprocess scale-up from small to large pilot scale using Eppendorf fermentation systems (L05)
Christof Knocke
(Eppendorf Bioprocess Center, Jülich, Germany)

15:55 Coffee break with poster session and exhibition

■ ■ Bioprocess and bioreactor modelling

Chair Christoph Herwig (TU Wien, Vienna, Austria), Nicolas Cruz-Bournazou (TU Berlin, Berlin, Germany)

16:40 Keynote lecture: Hybrid modelling and multi-parametric control of bioprocesses (L06)
Christoph Herwig (TU Wien, Vienna, Austria)

17:00 Industrial case study: Data science investigations to improve bioprocess scale-up (L07)
Patrick Sagmeister (Exputec GmbH, Vienna, Austria)

17:15 Multivariate data- and knowledge-driven tools for robust and efficient cell culture process development (L08)
Michael Sokolov (ETH Zürich, Zürich, Switzerland)

17:30 Implementation of computational fluid dynamics (CFD) as a Quality by Design (QbD) tool for industrial process development (L09)
Thomas Wucherpfennig
(Boehringer Ingelheim Pharma, Biberach, Germany)

17:50 Scale down of industrial scale fermentors using Euler-Lagrange CFD: A penicillin case study (L10)
Cees Haringa
(Delft University of Technology, Delft, The Netherlands)

18:05 A modelling framework for bioreactor simulations connecting physical and biological heterogenities (L11)
Jérôme Morchain (Laboratoire d'Ingénierie des Systèmes Biologiques et des Procédés, Toulouse, France)

18:20 Investigation of scale-up effects on mammalian cell culture process performance and critical quality attributes (L12)
Matthias Brunner (TU Wien, Vienna, Austria)

18:30 Keynote lecture: Modelling populations of microorganisms – The Anaerobic Digestion Model No. 1 as an example (L13)
Krist V. Gernaey (Technical University of Denmark DTU, Lyngby, Denmark)

19:00 Keynote lecture: Need for speed: Tools for enhanced microbial bioprocess development (L14)
Marco Oldiges (Forschungszentrum Jülich, Germany)

19:30 Poster session, Exhibition & Welcome Reception

21:30 End

Programme at a glance, Thursday, 7 April 2016

■ ■ Metabolic adaptation and population dynamics

Chair Marco Oldiges (Forschungszentrum Jülich, Germany), Christian Reitz (TU Berlin, Germany)

9:00 Keynote Lecture: Impacts of large-scale gradients mirrored on metabolic and transcriptional regulation (L15)
Ralf Takors (University of Stuttgart, Germany)

9:30 Rapid feast-famine cycles for studying metabolic response and adaptation representative for large-scale (L16)
Liang Wu
(DSM Biotechnology Center, Delft, The Netherlands)

9:50 Metabolic regulation of carbon metabolism: Key element for robustness to bioreactor inhomogeneities (L17)
Michael Limberg
(Forschungszentrum Jülich, Germany)

10:05 Fermentation process gradient effect on Corynebacterium glutamicum 1945 ΔACT3 PTUF-LDCCOPT producing cadaverine (L18)
Williams Olughu
(Loughborough University, Loughborough, UK)

10:20 Performance loss of Corynebacterium glutamicum cultivations under scale-down conditions (L19)
Anja Lemoine (TU Berlin, Germany)

10:35 Studies on the clavam biosynthetic pathway: A controling step for clavulanic acid production (L20)
Howard Ramirez-Malule
(Universidad de Antioquia UdeA, Medellín, Colombia)

10:50 Sponsor Talk: Single-use online capacitance biomass measurement supporting process scale-up from 10 L to 1000 L in cell culture application (L21)
Stuart Tindal
(Sartorius Stedim Biotech, Göttingen, Germany)

11:00 Coffee break with poster session and exhibition

11:40 Keynote Lecture: Exploiting the heterogeneity of biotic and abiotic phase in order to increase bioprocess robustness: Toward new bioreactor design (L22)
Frank Delvigne (University of Liège, Belgium)

12:10 Multiparameter monitoring of gradients in the liquid phase of fermentation processes (L23)
Anika Bockisch (TU Berlin, Germany)

12:25 Quantification of spatial heterogeneity in large bioreactors (L24)
Anders Nørregaard
(Technical University of Denmark DTU, Lyngby, Denmark)

12:40 Sponsor Talk: Connected in situ measurement system (L25)
Yoann Gasteuil and Céline Vinson (smartINST, Lyon, France)

12:50 Sponsor Talk: Optical sensors for bioprocess development and control (L26)
Gernot John (PreSens Precision Sensing, Regensburg, Germany)

12:55 Lunch break, poster session and exhibition

■ ■ Scale down approaches and process analytical technologies for advanced process design

Chair: Krist Gernaey (Technical University of Denmark DTU, Lyngby, Denmark), Stefan Junne (TU Berlin, Berlin, Germany)

14:00 Keynote Lecture: Bioprocesses under magnification: Learning from single cells about large-scale processes (L27)
Alexander Grünberger
(Forschungszentrum Jülich, Germany)

14:30 Development of a perfusion system in a microbioreactor using sedimentation as a scale down tool for ATF perfusion bioreactors (L28)
Steffen Kreye (Glycotope, Berlin, Germany)

14:45 Keynote Lecture: Issues that are relevant when scaling-up hMSC microcarrier production processes in stirred bioreactor (L29)
Regine Eibl (Zürich University of Applied Sciences ZHAW, Wädenswil, Switzerland)

15:15 Sponsor Talk: Evaluation of a stirred small scale single-use bioreactor for microbial applications (L30)
Thomas Dreher (Sartorius Stedim Biotech, Göttingen, Germany)

15:25 Scalability of cultivations in the 2-dimensional rocking single-use bioreactor CELL-tainer (L31)
Anna-Maria Marbà-Ardébol (TU Berlin, Germany)

15:40 Towards efficient microaerobic processes using engineered Escherichia coli strains (L32)
Alvaro R. Lara and Karim Jaen
(Universidad Autónoma Metropolitana-Cuajimalpa (UAM), México)

16:10 Coffee break with poster session and exhibition

16:50 Keynote Lecture: Novel fluorescence-based on-line capable monitoring of cell cycle distribution and growth rate for mammalian cell cultures (L33)
Grischa Fuge, An-Ping Zeng (TU Hamburg, Harburg, Germany)

17:20 Multiposition sampling, multiparameter monitoring and electrooptical tracking of microbial activity: New tools for better understanding biogas production (L34)
Erich Kielhorn (TU Berlin, Berlin, Germany),
Jörn Beheim-Schwarzbach (IASP, Berlin, Germany)

17:45 In-line photon density wave spectroscopy for bioprocess monitoring (L35)
Roland Hass
(University of Potsdam, Potsdam, Germany)

18:00 Keynote lecture: Stirred, not shaken – Unsolved issues and new problems in scale-down for scale-up (L36)
Alvin W. Nienow
(University of Birmingham, Birmingham, UK)

19:30 Conference Dinner
Restaurant Zillemarkt, Bleibtreustr. 48a, 10623 Berlin
(S Bahnhof Savignyplatz)

Programme at a glance, Friday, 8 April 2016

■ ■ Quality by design in bioprocess development

Chair: Ralf Takors (University of Stuttgart, Stuttgart, Germany), Erich Kielhorn (TU Berlin, Berlin, Germany)

9:00 Keynote lecture: Next generation mAb production: Continuous manufacturing, PAT, and real time release (L37)
Douglas Richardson (Merck, New Jersey, USA)

9:30 Keynote lecture: High-level protein production with Pichia: Case studies of development path from strain generation over bioreactor (L38)
Roland Weis (VTU Technology, Grambach, Austria)

10:00 Keynote lecture: ESETEC 2.0, a new, secretory Escherichia coli strain for the production of complex recombinant proteins at industrial scale (L39)
Guido Seidel (Wacker Biotech, Jena, Germany)

10:30 Different process strategies for the Glutathione overproduction in Saccharomyces cerevisiae (L40)
Martin Senz (Research and Teaching Institute for Brewing in Berlin VLB, Berlin, Germany)

10:50 Coffee break with poster session and exhibition

11:40 Keynote lecture: Parallel small-scale bioreactors with on-line monitoring to prove the consistency of medium ingredients and pre-cultures and to efficiently assess process characteristics (L41)
Jochen Büchs (RWTH Aachen, Germany)

12:10 Towards intensifying design of experiments: An industrial Escherichia coli feasibility study (L42)
Moritz von Stosch (Newcastle University, Newcastle, UK)

12:30 Robot based bioprocess development by automated on-line optimal re-design in parallel bioreactors (L43)
M. Nicolas Cruz-Bournazou (TU Berlin, Berlin, Germany)

12:45 Plenary lecture: From advanced PAT to QbD-compliant quasi-continuous integrated pharmaceutical production (L44)
Reiner Luttmann (Hamburg University of Applied Sciences, Hamburg, Germany)

13:30 Concluding remarks
Peter Neubauer (TU Berlin, Germany)

13:45 End of symposium

Wednesday, 6 April 2016

Opening Session

13:00 Welcome address and introduction

Peter Neubauer

Technische Universität Berlin, Institute for Biotechnology, Chair of Bioprocess Engineering, Berlin, Germany, peter.neubauer@tu-berlin.de

13:30 Plenary lecture: Reflections of a 40-years ripening process for modeling of bioreactors – from well mixed towards more reality (L01)

Matthias Reuss

Stuttgart Research Center Systems Biology (SRCSB), University of Stuttgart, reuss@ibvt.uni-stuttgart.de

The lecture aims at a "Tour de force" through a 40 years period of development of bioreactor modeling. Out of my personal perspective it is obvious to select the year 1976 as a starting point for this retrospective - the year in which I started with the installation of the department of Bioprocess Engineering within the Institute of Biotechnology at the TU Berlin. The models used at that time were based on the well-known textbook assumption of well mixed system applied to single phase and gas-liquid applications. The first milestones for consideration of more realistic mixing conditions at larger scale were tackled with the aid of so called micro-macromixer models for simulating glucose gradients during fed-batch operations of baker´s yeast production in stirred tank reactors. These models were later extended to coupling mass transfer gas-liquid and mixing in highly viscous non-Newtonian fermentation broths. The modeling activities were linked with extensive experimental investigations of recirculation time distributions for single and multiple impeller systems. In the mid of the eighties we started with the application of compartment models, which were later extended to multiphase applications at the University of Stuttgart. We soon realized the limitation of these models a far as the problems of quantitative predictability of the many parameters at different scale of operation is concerned and consequently stepped into the application of CFD. In the beginning of the nineties these applications were performed with the aid of hybrid modeling approaches - an integration of compartment models and CFD. After a period of studying alternatives to the k-E models the tool box of simulating the fluid dynamic conditions in single and multiple flow was coupled to agent-based simulations of the structured biophase via Euler-Lagrange modeling strategies. For the first time it was possible to simulate the lifelines of single cells and populations in large scale operations. The presentation is wrapped up with a discussion of future perspectives of model-based scale down strategies and applications of parallel processing for simulations of very large bioreactors used in bio-industry.

Industrial scale operation

Chair Alvin Nienow (University of Birmingham, Birmingham, UK), Peter Neubauer (TU Berlin, Berlin, Germany)

14:15 Keynote lecture: Continuous improvements in the understanding of large scale aerobic fermentation processes (L02)

Stuart Michael Stocks

Novozymes A/S, Bagsvaerd, Denmark, stus@novozymes.com

Novozymes is a large and growing biotechnology concern headquartered in Denmark, producing tens of thousands of tons of finished enzyme products each year with food, feed and technical applications, as well as a small interest in pharmaceutical production. Scale up and scale down of production processes is a core activity supported by a number of engineers and scientists based in R&D, pilot plants and production facilities located around the globe. We aim to improve our understanding of scale up and scale down, so we can be smarter about how we do it. Much of what we do is confidential, but over the years, in collaboration with the Danish Technical University (DTU), and other institutions, we have been active in pursuit of new knowledge and models which can describe fermentation process performance across a number of scales and have made some progress towards this goal. While this journey is by no means complete, results of work done on agitator geometry, oxygen transfer and modelling of the processes has taken us a good distance; much of this data can now be presented. In addition, there are now some further opportunities to become wiser, namely, the development of low cost off the shelf programmable electronics packages and the maturation of fluorescence spectroscopy gives us an unprecedented opportunity to peer into other wise opaque stainless steel reactors, and validate our progress towards better understanding what we are doing.

14:45 Keynote lecture: Large-scale production of two molecules of pharmaceutical interest in Saccharomyces cerevisiae (L03)

Claus Lattemann

SANOFI - C&BD Biochemistry, Paris, France, claus.lattemann@sanofi.com

Reports on the production of heterologous metabolites in microbial systems have been published recently demonstrating that various compound classes comprising natural and non-natural molecules can be produced in microorganisms. Production of such complex heterologous molecules in microorganisms in large volume fermentation however, requires the coordinated development of production strains and the corresponding fermentation process. Two examples of large volume production of active pharmaceutical ingredients in baker's yeast will be discussed.

15:15 Keynote lecture: Microbial GMP manufacturing at large-scale – challenges and considerations (L04)

Torsten Schmidt

Head of Microbial Manufacturing, Lonza AG, Visp, Switzerland, torsten.schmidt@lonza.com

Microbial processes for production of biopharmaceuticals are characterized by a huge variety of different process steps. Based on the selected host strain and expression system there are multiple different options for processing the target protein. Fermentation titer, step yields and product purity are the typical selection criteria in this process development phase. Lacks in scalability and process robustness are considered with a lower priority. For the first production of early clinical material those potential issues can be easily solved by the flexibility of small-scale facilities and oversizing. When it comes to large-scale other solutions are needed. Based on the design of the facility large-scale plants for late-phase clinical or commercial manufacturing are less flexible. Also Cost of Goods (COGS) have a more important role at large-scale.

15:45 Sponsor Talk: Bioprocess scale-up from small to large pilot scale using Eppendorf fermentation systems (L05)

Ulrike Becken[1], Bin Li[2], Christof Knocke[3], Jens Schiffler[1], Bruno Sommer Ferreira[4], Ma Sha[2]

[1]Eppendorf AG Bioprocess Center, Jülich, Germany, [2]Eppendorf Inc., USA, [3]Vaudaux-Eppendorf AG, Schönenbuch, Switzerland, knocke.c@eppendorf.ch, [4]Biotrend, Portugal

The ultimate goal in bioprocess development is the realization of commercial production. Currently, the scale-up of fermentation processes, which is critical to the success of industrial fermentation for bioproduction, is receiving much attention. Eppendorf fermentation systems cover a wide range of working volumes from less than 1 L to as large as 2,400 L. We investigated their scale-up capabilities from small to pilot scale. Within this presentation, we discuss engineering parameters critical for scale-up, such as vessel and impeller geometry, tip speed, mixing time, oxygen transfer rate (OTR), kLa, and power number. Furthermore, we present recent customer data on the production of the biopolymer polyhydroxy butyrate (PHB) using Bacillus sacchari. Successful scale-up of the process from 2 L to 200 L exemplifies the scale-up capabilities of Eppendorf fermentation systems.

15:55 Coffee break with poster session and exhibition

Bioprocess and bioreactor modelling

Chair Christoph Herwig (TU Wien, Vienna, Austria), Nicolas Cruz-Bournazou (TU Berlin, Berlin, Germany)

16:40 Keynote lecture: Hybrid modelling and multi-parametric control of bioprocesses (L06)

Christoph Herwig

Christian Doppler Laboratory for Mechanistic and Physiological Methods for Improved Bioprocesses, Research Division Biochemical Engineering, Institute of Chemical Engineering, Vienna University of Technology, Vienna, Austria, christoph.herwig@tuwien.ac.at

The goal of bioprocessing is to optimize process variables, such as product quantity and quality in a reproducible, scalable and transferable manner. However, bioprocesses are highly complex. A large number of process parameters and raw material attributes exist, which are highly interactive and may vary from batch to batch. Those interactions need to be understood, and source of variance must be identified and controlled.
While purely data driven correlations, such as chemometric models of spectroscopic data, may be employed for the understanding how process parameters are related to process variables, they can hardly be deployed outside of the calibration space. Currently, mechanistic models, models based on mechanistic links and first principles, are in the focus of development. They are perceived to allow transferability and scalability, because mechanistics can be extrapolated. Moreover, the models deliver a large range of hardly measureable states and physiological parameters.
For implementation of mechanistic models, however, models need to be simplified and linked to process parameters for real time execution. For this, hybrid models, hence links between Data Driven and Mechanistic Models may be a helpful solution.
Moreover, models need to be deployed in the control context: Bioprocesses need to be controlled on the one hand on different parameters simultaneously (e.g. constant precursor concentration and specific growth rate) and on the other hand may have different objective functions (maximum productivity and correct product quality). Hence novel solutions and case studies for multiple input and output controls need to be developed, as they already exist in other market segments.
The current contribution wants to display current solutions and case studies of development and deployment of hybrid models and multi-parametric control of bioprocesses. The following elements can be covered:

- Hybrid model solutions, combinations of data driven and mechanistic models.
- Workflows how mechanistic models can be developed from data driven approaches and vice versa.
- Discussion between explorative (DoE-based) and model based experimental design
- Implementation of hybrid models in the real-time context
- Models as PAT tool: Demonstrations of cases in which models were a solution to measure less
- Observer solutions for real-time parameter optimization
- Multiparametric control and event prediction
- Life Cycle management of models
- Knowledge management using hybrid models

17:00 Industrial case study: Data science investigations to improve bioprocess scale-up (L07)

Patrick Sagmeister[1], Sandra Abad[2], Christoph Herwig[3]

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Scale-up of bioprocesses refers to the transfer of processes from laboratory to pilot and production scale. This is an essential and critical step for the commercialization of any biotechnological product. This step can be accompanied by unexpected deviations impacting on process performance and product quality. Scale-dependent deviations are typically recognized very late in the commercialization of biotechnological products, thereby exhibiting a high economic impact, such as failed batches and reprocessing under GMP regulations. Therefore, it is of the utmost importance to achieve a thorough and science-based understanding in order to make the scale-up of bioprocesses even more robust and predictable.
In the presented industrial case study is demonstrated how the scale-up of an industrial E. coli manufacturing process was improved based on a data science approach, leveraging integrated data from process development, piloting and manufacturing. Firstly, we present how a data science framework was put to practice to identify- and understand scale-dependent changes. Secondly, we discuss how scale-up improvement strategies, including the definition of scale-down models, were derived from the obtained mechanistic insights. Thirdly, we discuss how a systematic analysis of available big data from manufacturing databases can be leveraged to make bioprocess scale-up more predictable and derive clear- and interpretable design guidelines for the design and scale-up of bioprocesses.

17:15 Multivariate data- and knowledge-driven tools for robust and efficient cell culture process development (L08)

Michael Sokolov, Alessandro Butte, Massimo Morbidelli

ETH Zurich, Zurich, Switzerland, alessandro.butte@chem.ethz.ch

In recent years, the European Medicines Agency (EMA) and the United States Food and Drug Administration (FDA) have stressed the need of greater process understanding and quality control in the area of pharmaceutical development, manufacturing and quality assurance, all which is summarized in the Process Analytical Technology (PAT) initiative. In bioprocesses, and particularly in cell cultures, extensive data acquisition and analysis are likely to provide versatile and significant real-time information. Similarly, the Quality by Design (QbD) initiative was launched in order to promote the need for building product quality into the process. In process development, this can be achieved by a deep process understanding combined with a broad investigation of the product quality in order to connect those two domains and ensure robust product quality in an efficient manner. All of these goals go along with very large data sets, which usually feature a high degree of correlated variables. This motivated to develop multivariate approaches, which reduce those high-dimensional problems to the major unique properties of the process as well as the corresponding product.
This work presents several cases studies, where different multivariate data- and knowledge-driven tools were successfully applied at various stages of cell culture process screening and development. In particular, high throughput screening for early process development, product quality prediction at multiple scales during process characterization, process monitoring by Raman spectroscopy as well as hybrid approaches incorporating a deterministic process model backbone will be discussed. All the presented results go well beyond the possibilities offered by commercial tools and provide an important basis for risk reduction and efficiency during process development.

17:30 Implementation of Computational Fluid Dynamics (CFD) as a Quality by Design (QbD) tool for industrial process development (L09)

Thomas Wucherpfennig[1], Erik Hasenfus[1], Johannes Wutz[2], Ralf Takors[2] und M. Berger[1]

[1]Biopharma Process Development, Biberach an der Riss, Germany, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach an der Riss, Germany, thomas.wucherpfennig@boehringer-ingelheim.com, [2]Institute of Biochemical Engineering, University of Stuttgart, Stuttgart, Germany

The demand for complex therapeutic proteins and antibodies for treatment of various diseases has increased continuously in the last decades, prompting the continuous development of new processes. For the development of these novel processes, the Quality by design (QbD) approach has gained popularity since the FDA´s PAT-publication. However, this practice requires a thorough understanding of a product and especially of the manufacturing process and equipment. Tools to further such an equipment understanding e.g. for the simulation of the complex turbulent flows in stirred tank bioreactors have been around for some time. Their application in the biomanufacturing industry, however, has remained scarce due to the demand of computational power, complex underlying mathematics and the high amount of training needed.
In this study it is demonstrated, how the simulation of scale-up criteria like P/V, kLa, or mixing times can facilitate a robust scale-up or be used in a facility fit analysis to determine whether a developed process is suited to the production equipment at various sites.
Furthermore, a specific application example is provided by using CFD methods for the set-up of a process design space for an acceptable range of the volumetric oxygen mass transfer coefficient (kLa). Agitation and aeration are some of the first parameters which are tested during process development; based on these parameters and the filling volume as input parameters an DOE study (MODDE 10, Umetrics, Sweden) was conducted in an aerated 2L stirred benchtop bioreactor. For each experimental setup a two phase Euler-Lagrange CFD model was established and a kLa value determined in silico (Fluent 16.6, ANSYS Inc., Canonsburg, USA). A process design space was established for maintaining a required kLa value of a 6.5±1 h-1 needed to support high density cell culture. At a filling volume of 1.5 L between 0.09 and 0.1 L L-1 min-1 and agitation rates between 197 and 300 rpm are sufficient to maintain a kLa value of 6.5 h-1 with a probability of 95% in the studied bioreactor system. All simulated values were validated experimentally and showed a good fit. The conducted in silico approach could be used in future process characterization studies and save valuable experimental time.

17:50 Scale down of industrial scale fermentors using Euler-Lagrange CFD: A penicillin case study (L10)

Cees Haringa[1], Wenjun Tang[2], Jianye Xia[2], Amit T. Deshmukh[3], Matthias Reuss[4], Joseph J. Heijnen[5], Robert F. Mudde[1], Henk J. Noorman[3,5]

[1]Chemical Engineering department, Delft University of Technology, Delft, The Netherlands, c.haringa@tudelft.nl, [2]East China University of Technology, Shanghai,China, [3]DSM Biotechnology Center, Delft, The Netherlands, [4]Stuttgart Research Center Systems Bioloogy (SRCSB), University of Stuttgart, [5]Biotechnology department, Delft University of Technology, Delft, The Netherlands

Micro-organisms living inside industrial scale fermenters may be exposed to rapid variations in the extracellular environment, for example, variations in the observed substrate concentration. Both the magnitude and frequency of these variations may impact the metabolism of the organism and thereby the industrial viability of the process. To estimate their upfront via scale-down simulation, it is essential to gain insight in the magnitude and frequency at which organisms are exposed to these variations.
Based on pioneering work of Lapin et al. and Delvigne et al., we propose the use of an Euler-Lagrange CFD framework to study these aspects on an industrial scale. The Lagrangian point of view allows to quantify the observed fermentation environment from the point of view of the organism, and analyse the observed 'lifelines' to extract the frequencies and magnitude of environmental variations. From this information it is possible to determine the design parameters for a scale down simulator, as specified by Noorman, and set-up an industrially relevant scale-down simulation.
We present a case study, based on the fermentation of Penicillium chrysogenum, in which we showcase the procedure to extract the relevant information from CFD simulations, and the consecutive design of scale-down simulators based on this information. With this, we show a comprehensive methodology for the design of quantitatively representative scale-down simulators based on CFD data, and how this methodology can be applied in practical situations.
This is a multi-party research project, between DSM-Sinochem Pharmaceuticals, TU Delft, East China University of Science and Technology and Guojia, subsidized by NWO and MoST.

[1] A Lapin et al. Modeling the dynamics of E. coli populations in the three-dimensional turbulent field of a stirred-tank bioreactor, a structured segregated approach. Chem Eng Sci 61(14):4784-4797, 2006. [2] F Delvigne et al. A methodology for the design of scale-down bioreactors by the use of mixing and circulation stochastic models, Biochem Eng J 28(3):256-268, 2006. [3] H J Noorman, An industrial perspective on bioreactor scale-down: what we can learn from combined large-scale bioprocess and model fluid-studies. Biotechnol J, 6(8):934-943, 2011

18:05 A modelling framework for bioreactor simulations connecting physical and biological heterogeneities (L11)

Maxime Pigou[1,2,3] and Jérôme Morchain[1,2,3]

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Large scale gradients of substrate and oxygen are often observed at the reactor scale because of insufficient macromixing and heterogeneous gas-liquid transfer rates. It appears that the most promising approach resides in the coupling of population balance models along with CFD and metabolic approaches. This requires a high level of expertise in separate scientific domains. Moreover the set-up of such high resolution models is very time consuming.
In order to ease the task we have developed a simulation tool, named ADENON, that combines each type of models (hydrodynamics, population balance and metabolic) in a general framework. Rather than using a fully resolved CFD model, hydrodynamics description is based on a compartment approach. A population balance model describing adaptation dynamics is formulated in terms of uptake rate distribution. The population balance approach solves the problematic of accessing individual history, at lower cost than Lagrangian tracking methods, while handling the issue that rises when two populations with different properties are mixed. Finally a metabolic model is invoked to calculate the fate of the substrate inside the cell, by taking into account the cell state and local concentrations. The specificity here lies in the fact that there is no optimization procedure to compute the metabolic fluxes. Altogether, these modelling choices allow for a simultaneous calculation of all aspects of the problem at a reasonable computational cost.
Several examples of dynamic simulation of microbial cultivation in unsteady continuous, multi-stage and large scale bioreactors will be presented and the results compared to available experimental data.

18:20 Investigation of scale-up effects on mammalian cell culture process performance and critical quality attributes (L12)

Matthias Brunner[1,2], Jens Fricke[1,2], Christoph Herwig[1,2]

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In large-scale mammalian processes heterogeneities of critical process parameters are most likely to occur, due to different gassing strategies and elevated mixing times compared to small scale fermentations. Understanding the effect of these inhomogeneities on mammalian cell cultivations is an essential requirement for process scale-up. Furthermore, comprehensive knowledge of the relationship between the process and the products critical quality attributes (CQAs) is necessary to satisfy Quality by Design (QbD) guidelines.
In this context a comprehensive Design of Experiments (DoE) approach including various product quality analytics was performed to investigate the influences and the interactions of scale up relevant parameters as pH, dissolved oxygen tension (pO2) and dissolved carbon dioxide tension (pCO2) on chinese hamster ovary (CHO) cell batch performance and monoclonal antibody (mAb) quality. Through independent control of process pH, pO2 and pCO2 we could derive single process parameter effects and interactions on CHO physiology as well as on mAb charge and glycosylation variants. Finally, process pH was recognized as the key-parameter, exerting strong effects on CHO metabolism and mAb CQAs.
Subsequently, we furthermore investigated the effect of permanent pH-shifts to elevated levels, similar to those present in large-scale due to base addition (pH 7.8 and 9.0). Therefore, one compartment batch fermentations including pH-shifts were conducted and short-term as well as long-term effects of external pH-shifts on intracellular pH and overall CHO metabolism could be derived.

Within this contribution a comprehensive study regarding large-scale heterogeneities on mammalian cell culture process performance and critical quality attributes will be presented. This includes DoE results regarding the effect of critical scale-up parameters on process performance attributes and industry-relevant CQAs. Furthermore, the effect of large-scale pH inhomogeneities on intracellular pH and CHO physiology will be evaluated out of one compartment experiments.

18:30 Keynote lecture: Modelling populations of microorganisms – the Anaerobic Digestion Model No. 1 as an example (L13)

Krist V. Gernaey[1], Ulf Jeppsson[2], Damien Batstone[3] and Xavier Flores-Alsina[1]

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Many industrial scale fermentation processes rely on pure cultures. However, there are exceptions, for example a yogurt production. With the current focus on the production of biofuels and chemicals from renewables, new processes are developed, and there is also increased focus on the potential benefits that can be obtained by using mixed cultures instead of pure cultures. For process design and operation, it is important to develop mathematical models that can describe a process relying on such a mixed culture. From a modelling point of view, and taking a pure culture as a reference, the complexity of modelling a mixed culture is higher, since the co-existence of several populations of microorganisms and their interactions have to be described. In the wastewater treatment field, almost all processes rely on mixed cultures, and mathematical modelling has during the past decades played an important role in building up process knowledge, and in development of novel operation and control strategies. The IWA Anaerobic Digestion Model No. 1 (ADM1) is taken as an example here, and starting from the original model, a series of recently published extensions of the ADM1 are highlighted to functionally upgrade the ADM1 to allow for plant-wide phosphorus (P) removal simulation. The close connection between the P, sulfur (S) and iron (Fe) cycles indeed requires a substantial (and unavoidable) increase in model complexity due to the involved three-phase physico-chemical and biological transformations.

19:00 Keynote lecture: Need for speed: Tools for enhanced microbial bioprocess development (L14)

Johannes Hemmerich, Joachim Koepff, Holger Morschett, Marco Oldiges

Research Center Jülich, Institute of Bio- and Geosciences: IBG-1 Biotechnology, Jülich, Germany, m.oldiges@fz-juelich.de

The biotechnological production of fine and bulk chemicals as well as heterologous protein production using microbial systems is one important field in the growing bioeconomy. One of the important keys to success is the increasing speed of genetic manipulations possible for platform organisms like Escherichia coli or Corynebacterium glutamicum and others. This allows to engineer microbial strains in a fast way and easily provides strain libraries harbouring large biological variance.
However, the capability of detailed phenotyping of such genotype library at well-defined bioprocess level is orders of magnitude slower. Thus, it represents a substantial bottleneck in strain engineering as well as bioprocess development. Especially, this holds true for the challenge of identifying the best performing strain under bioreactor process conditions instead of artificial screening conditions. All this demands for increased experimental throughput in microbial phenotyping at well-defined bioprocess conditions. To fully release the potential of rapid strain and bioprocess characterisation not only the cultivation, but also upstream processing, analytics and data treatment need to be accelerated, in order to provide a valuable integrated tool for microbial strain engineering, synthetic biology, gene-function relationship as well as bioreaction engineering and bioprocess development.
This is illustrated taking microbial application examples with Corynebacterium glutamicum, Streptomyces lividans and Chlorella vulgaris as case studies, showing how developments in miniaturized cultivation technology combined with smart lab automation and data processing facilitates microbial phenotyping and bioprocess development. Such elevated throughput in microbial cultivation technology and bioprocess engineering provide a comprehensive data basis and paves the way to generate improved knowledge about microbial systems in terms of metabolism, regulation and application.

19:30 Poster session & Exhibition & Welcome Reception

Foyer of the Institute for Architecture

21:30 End

Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik

Chair of Bioprocess Engineering

Thursday, 7 April 2016

Metabolic adaptation and population dynamics

Chair Marco Oldiges (Forschungszentrum Jülich, Jülich, Germany), Christian Reitz (TU Berlin, Berlin, Germany)

9:00 Keynote Lecture: Impacts of large-scale gradients mirrored on metabolic and transcriptional regulation (L15)

Michael Löffler, Joana Simen, Ralf Takors

Institute of Biochemical Engineering (IBVT), University of Stuttgart, Stuttgart, Germany, takors@ibvt.uni-stuttgart.de

Industrial workhorses such as Escherichia coli are applied in large scale bioreactors and are thereby exposed to gradients mirroring impacts of hydrostatic pressure, insufficient mixing conditions and spatially segregated cellular activities. Cells circulating in production vessels are exposed to frequently changing micro-environmental conditions that trigger not only metabolic but also transcriptional cellular responses. Yet, cellular performance in large scale is requested to meet expectations of lab scale studies in order to fulfil economic demands (Takors, 2012).
At IBVT a scale-down device consisting of a stirred tank bioreactor and a plug flow reactor was installed for simulating large scale gradients. In contrast to the common approach scale-down experiments were performed under steady-state conditions. Consequently an accurately defined reference scenario was set that allowed the in-depth elucidation of short- and long-term metabolic and transcriptional responses of E. coli on the exposed heterogeneities.
More than 600 genes were found to be up- or down-regulated (threshold 1.5 fold) during the first 110 s in PFR. Distinct patterns of short- and long-term adaptation strategies were deduced with respect to carbon (C-) and nitrogen (N-) gradients. The occurrence of stable subpopulations was observed. Biological motivated criteria for scale-up were formulated.
Takors R. 2012. Scale-up of microbial processes: Impacts, tools and open questions. J. Biotechnol. 160(1-2): 3-9.

9:30 Rapid feast-famine cycles for studying metabolic response and adaptation representative for large-scale (L16)

Liang Wu[1], Lodewijk de Jonge[2], Camilo Suárez Méndez[2], Amit Deshmukh[1], Sef Heijnen[2], Walter van Gulik[2], Aljoscha Wahl[2], Henk Noorman[1,2]

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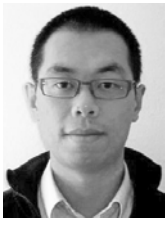

In large-scale bioreactors, microorganisms face continuous changes in their environment. Most prominent are glucose, oxygen and shear rate dynamics, but also pH, temperature and other factors can play a role. In lab-scale research, such dynamics conditions are often not applied, or at best based on qualitative scale-down reasoning. As a result, the performance of cells in large-scale can be disappointing compared to expectations raised from lab tests.
Metabolic mechanisms behind such scale-up losses can be multiple, but relatively little is quantitatively known today. We will report on some recent results from studies with repetitive input cycles, applied at lab-scale, where we investigated the metabolic response and adaptation of fungi and yeast.
A key observation from these feast-famine studies is a high turnover of intracellular storage compounds, associated with ATP futile cycles. Also other regulatory mechanisms are becoming more clearly, gaining valuable insight in scale-up performance and scale-down design requirements.

9:50 Metabolic regulation of carbon metabolism: Key element for robustness to bioreactor inhomogeneities (L17)

Michael Limberg, Tita Aryani, Mathias Joachim, Wolfgang Wiechert, Marco Oldiges

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Performance losses during the scale-up from laboratory into production scale and the associated increase of production costs, imperils the competitiveness of sustainable biotechnological production. One reason for these deficiencies is based on the decreasing mixing quality and the resulting glucose, oxygen and pH gradient formation at the industrial scale. But not all organisms seem to be negatively affected by such inhomogeneous cultivation environments in a similar manner. Especially the industrial platform organism Corynebacterium glutamicum provides an astonishing degree of robustness to oscillations through such gradients. This raises the question which key pathways are necessary for such an ability to adapt and how they are regulated. This potentially can lead to novel targets for the design of even more robust production strains.
This study picks up this question by generating a broad range of oscillating pH, oxygen and substrate supply conditions and investigating their influence on a 1,5-diaminopentane producing Corynebacterium glutamicum strain.
Therefore a two compartment scale-down device composed of two connected stirred tank reactors (STR-STR) was setup. This device provides the possibility of monitoring and controlling the inhomogeneous cultivation parameters in each reactor for investigating the sole influence of one parameter or the combination of several inhomogeneities. During this study one compartment provides well aerated and pH neutral cultivation conditions. The additional compartment was used for the generation of a broad spectrum of adverse growth environments which mimic different sections in large scale bioreactors. These sections are characterized by varying oxygen availability, changing pH and oscillating substrate supply conditions (excess and limitation). Depending on the residence time (up to 3 min) and the combination of disturbing parameters in this compartment the intermediary formation of side products like organic acids as well as growth rate were significantly influenced. By investigating the intracellular transcript and proteome levels the compartment specific activation of different stress response mechanisms as well as regulatory activation and overexpression of NAD+ regenerating pathways under O2-limitation were observed. This matches with oscillating changes of metabolite concentration levels in the glycolysis and pentose phosphate pathway. The study is thus the first step towards a global understanding of biological robustness and predicting their limits.

10:05 Fermentation process gradient effect on Corynembacterium glutamicum 1945 ΔACT3 PTUF-LDCCOPT producing cadaverine (L18)

Williams Olughu[1], Chris Rielly[1], Chris Hewitt[2] and Alvin Nienow[3]

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Typically on scale-up of a bioprocess, it is uneconomical to maintain high specific power inputs and hence the mixing time tends to increase and the local mass transfer coefficients are reduced, with increasing size of the fermenter. An increase in the time scale required for the fermenter system to homogenise increases the likelihood of the presence of physical and chemical gradients, i.e. the substrate, dissolved oxygen concentrations, temperature and pH become more spatially inhomogeneous on a larger scale. As cells circulate within such a large reactor, they respond to the varied environment within the system, which may result in a decline in their productivity or a change in the formation of metabolites.
Here a two-compartment model consisting of a plug flow reactor (PFR) and a stirred tank reactor (STR) was used to investigate how C. glutamicum 1945 Δact3 puf-ldccopt (modified to produce cadaverine) responds to gradients (pH, dissolved O2, nutrient) and varying mixing times, in an attempt to mimic what happens in a large-scale industrial fed-batch process. Glucose and pH controlling agents were introduced to either the PFR or STR reactors to create chemical gradients of substrate and / or pH concentrations in different regions of the flow; the PFR section had volumes of 10 % and 20 % of the STR section. The results showed no decline in biomass productivity in all simulations studied, but in the simulation which was closest to mimicking a large-scale fed-batch environment, a 26 % loss in final cadaverine titre was recorded when the mean residence in the PFR (PFR Ʈmean) was set at 60 s. A further increase in cadaverine loss of 36 % was observed when the PFR Ʈ mean was increased to 120 s. This suggests that as the cells faced an increasingly stressful environment, more energy was redirected to produce stabilising amino acids to maintain homoeostasis, resulting in a loss of cadaverine productivity. For the first time, a dual staining protocol in flow cytometry was developed for C. glutamicum to probe viability at the individual cell level. The flow cytometry analyses showed that cell viability improved in all cases studied, above 95 % at the end of the process, which suggests that the cells became better adapted to their environment as fermentation progressed. Samples taken from the top section of the PFR had the highest concentration of lactate and at dry cell densities above 25 g/L was twice the titre in the STR. This indicates that mixed acid fermentation occurred predominantly in the oxygen-depleted PFR section of this two-compartment model. Comparing this study with other similar works (with E. coli, B. subtilis and S. cerevisiae) confirms that on scale up of biological systems, the degree of response or loss of productivity is organism specific.

10:20 Performance loss of Corynebacterium glutamicum cultivations under scale-down conditions (L19)

Anja Lemoine[1] , Michael Limberg[2], Marco Oldiges[2], Peter Neubauer[1], Stefan Junne[1]

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Scale-down reactors are frequently used to study the impact of oscillations in the laboratory-scale, as they occur in large-scale nutrient limited fed-batch processes. Corynebacterium glutamicum is a widely applied host organism of industrial relevance for large-scale lysine production. Recent experiments in multi-compartment reactors indicated robustness of C. glutamicum against environmental oscillations in oxygen and substrate availability [1, 2]. However, in contrast to industrial application, mineral salt media is mostly used in these experiences.
In this study, we cultivated C. glutamicum with complex media based on molasses, sucrose and corn steep liquor in a three compartment reactor. The set-up of the scale-down experiments were based on computational fluid flow studies performed for an industrial-scale reactor with feed addition in the bottom of the liquid phase. Three distinct zones were mimicked: (i) substrate starvation and oxygen limitation, (ii) substrate limitation and oxygen excess, and (iii) substrate excess and oxygen limitation [3]. In contrast to previous studies, we observed ceased growth and a reduced accumulation of the main product cadaverine after ten hours of feeding, while the substrate uptake of sucrose was reduced by 20%. At the same time, several organic acids, among them lactate and acetate were accumulated, both with a rate of 2 mmolL-1h-1. Proteome analysis showed higher concentration levels of several enzymes, which indicates a general stress response to the inhomogeneous conditions.
In situ determination of the cell size distribution with laser-light back reflection showed agglomeration under oscillatory cultivation conditions. These findings indicate that the morphological and physiological status of cells are both drastically influenced by the unfavorable cultivation conditions as they appear in the late production phase at an elevated cell density.

[1] Kaess F et al. Process inhomogeneity leads to rapid side product turnover in cultivation of Corynebacterium glutamicum. Microb Cell Fact, 2014. 13. [2] Oldiges M et al. Scale-down study of oscillations in oxygen and substrate supply for Corynebacterium glutamicum. New Biotechnol, 2014. 31: S50-S50. [3] Lemoine A et al. Response of Corynebacterium glutamicum exposed to oscillations cultivation conditions in a two- and a novel three compartment scale down bioreactor. Biotechnol Bioengin, 2015. 116(6): 1220-1231.

10:35 Studies on the clavam biosynthetic pathway: a controling step for clavulanic acid production (L20)

Howard Ramirez-Malule[1], Albeiro Restrepo[2], Wilson Cardona[3], Stefan Junne[4], Peter Neubauer[4], Rigoberto Rios-Estepa[1]

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Clavulanic acid (CA), produced by Streptomyces clavuligerus (Sc), is a secondary metabolite with clinical interest due to its potential to inhibit serine β-lactamases; nevertheless, low yields are commonly obtained during cultivation processes. The synthesis of CA is mediated by the so called clavam biosynthetic pathway, for which some steps still remain unclear; thus, improved knowledge about its intricacies would help to eventually identify controlling steps, specifically the steps involved in the conversion of (3S, 5S)-clavaminic acid into (3R, 5R)-clavulanic acid. In this work, a chemostat cultivation of Sc was conducted; acetate accumulation occurred within the time frame in which CA was accumulated. Although the acetyl group is present in β-lactam intermediate compounds such as N-acetyl-glycyl-clavaminic acid (NAG-clavam), it is still unknown how it is incorporated. Here, we propose a reaction mechanism – based on a computational simulation approach – for acetate incorporation during NAG-clavam formation, in CA biosynthesis.

10:50 Sponsor Talk: Single-use online capacitance biomass measurement supporting process scale-up from 10 L to 1000 L in cell culture application (L21)

Jochen Scholz, Stuart Tindal, Sebastian Ruhl

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Online capacitance measurement has already become a well-established PAT tool in biopharmaceutical applications utilizing traditional multi-use bioreactor and fermentation equipment. According to Aspen Report 2013 and Report and Survey of Biopharmaceutical Manufacturing Capacity and Production (2015), over 40% of the bio manufacturing responders use this technology for biomass process monitoring or control.
As this PAT tool emerges in the multi-use process world, simultaneously there is a rapid uptake to single-use (SU) cultivation equipment in process development to production scale (2000L). Consequently, SU capacitance sensors for biomass monitoring have been developed and presented to the community throughout the past years. Recently, BioPAT® ViaMass SU capacitance measurement became commercially available with significant uptake in market.
Capacity adaptation and bioprocess transfer are two key requirements that present significant challenges. During the scale-up process, different types of bioreactors are used. Today, SU cultivation equipment is available with good scalability from small process development to production scale. Here, aligned geometrical aspect ratios and well characterized process parameters are the key for good comparability. In this scale-up context, online biomass capacitance measurement can play a key role to monitor consistent cell growth rate and cultivation performance.
This talk demonstrates the process scalability of online capacitance measurement in a CHO cell culture from 10 L to 1000 L. Additionally, a cross-system comparability of conventional stirred tank and rocking motion cultivation bioreactors is shown. The investigations conclude by showing a clear golden batch trajectory of cell growth and productivity in all scales and both agitation systems. As verification, the online capacitance monitoring was corroborated by multiple offline reference techniques during the frequent sampling of the process.
In conclusion, the SU capacitance technology displays the same capabilities as the multi-use technology and enables the identical process control potential at all scales/systems.

11:00 Coffee break with poster session and exhibition

11:40 Keynote Lecture: Exploiting the heterogeneity of biotic and abiotic phase in order to increase bioprocess robustness: Toward new bioreactor design (L22)

Frank Delvigne

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Cell-to-cell variation related to phenotypic diversification strategies is known to induce variability and lack of predictability in bioprocesses. Actually, the single cell toolbox comprises modelling procedures and advanced experimental devices that could be used to decipher this important phenomenon in process-related conditions.
Modelling procedures are actually focused on the stochasticity associated with the intrinsic component of biological noise, the other components, i.e. extrinsic and intracellular diffusion are often omitted. However, in bioprocess conditions, extrinsic component of noise is of critical importance since it is known that environmental fluctuations are likely to occur in large-scale bioreactors [4]. Alternative modelling procedures are thus needed in order to overcome actual limitations.
The actual single cell experimental toolbox comprises high resolution and high frequency optical devices [5], but needs to be adapted for the monitoring of relevant cultivation devices, such as stirred bioreactor [1-3]. The second limitation of the actual toolbox relies on its dependence of fluorescent markers. This constraints will be illustrated through two biosensing strategies, and alternative approaches comprising sub-population culturing will be proposed.
Alternatively, a better understanding of phenotypic plasticity during bioprocesses can lead to new bioreactor design.

[1] Baert J.t al. 2015. Phenotypic variability in bioprocessing conditions can be tracked on the basis of on-line flow cytometry and fits to a scaling law. Biotechnol J 10, 1316–1325. [2] Brognaux A. et al. 2013. A low-cost, multiplexable, automated flow cytometry procedure for the characterization of microbial stress dynamics in bioreactors. Microb Cell Fact 12. [3] Delvigne F. et al. 2015. Dynamic single-cell analysis of Saccharomyces cerevisiae under process perturbation: comparison of different methods for monitoring the intensity of population heterogeneity. J Chem Technol Biotechnol 90, 314–323. [4] Delvigne F, Goffin P. 2014. Microbial heterogeneity affects bioprocess robustness: Dynamic single cell analysis contribute to understanding microbial populations. Biotechnol J 9, 61–72. [5] Delvigne F. et al. 2014. Metabolic variability in bioprocessing: implications of microbial phenotypic heterogeneity. Trends Biotechnol 32, 608–616.

12:10 Multiparameter monitoring of gradients in the liquid phase of fermentation processes (L23)

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In large scale fermentation like in the brewing process, process conditions as the local power input and fluid flow might be uneven. This would lead to gradients. Since the available sensor technology is not designed for the consideration of such heterogeneities, the knowledge about the magnitude of gradients in bioprocesses is rather low. Off line samples or on line data from devices installed at one single position of the tank are not representative for the whole liquid phase. Computational fluid dynamics is often not able to describe gradient formation, as this depends on the cells' metabolic activity, which even alters during a process.
In order to improve process monitoring and to identify critical reactor zones in a fermentation process, mobile multi-parameter sensor tools have been developed for the in situ and on line monitoring of various process parameters (pH-value, dO2, dCO2, redox potential, conductivity, temperature, pressure) in industrial bioreactors.
The time-dependent and spatial distribution can be correlated to data from off line studies like metabolite concentrations and lipid content measured with HPLC and GC methods as well as the cells' physiological state investigated by flow cytometry. When this data is applied, the impact of gradients on the cellular performance can be described.

12:25 Quantification of spatial heterogeneity in large bioreactors (L24)

Anders Nørregaard[1], Stuart M. Stocks[2], Brian Madsen[3] and Krist V. Gernaey[1]

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Substrate gradients are known to exist in production scale stirred tank bioreactors due to imperfect mixing and differences in the mass transfer rate of oxygen [1,2]. Access to experimental mixing and mass transfer data from large scale is limited, and the results are dependent on the geometry of the vessels and impellers in use. A method for measuring oxygen gradients in situ in a production scale bioreactor with minimal alteration of the vessel is proposed. The method utilizes different types of fiber optical oxygen sensors that are introduced to the bioreactor. The flexibility of the sensors makes the method applicable in the industry and facilitates the collection of experimental data at large scale without disturbing the workflow at the production plant. The setup has been tested in a 1 m3 pilot scale mixing vessel with a model oxygen transfer experiment. Furthermore it has been tested in a pilot scale bioreactor during yeast fermentation in order to investigate the durability of the sensors during heat sterilization cycles as well as fouling from biological growth. The setup is able to quantify differences in the oxygen driving force in the model system, indicating the existence of mass transfer gradients.

[1] Larsson G et al. 1996. Substrate gradients in bioreactors: Origin and consequences. Bioprocess Eng 14(6), 281–289. [2]Oosterhuis N M, Kossen N W 1984. Dissolved oxygen concentration profiles in a production-scale bioreactor. Biotechnol Bioeng 26, 546–550.

12:40 Sponsor Talk: Connected in situ measurement system (L25)

Yoann Gasteuil, Céline Vinson

smartINST 213 Rue de Gerland, Lyon, France, celine.vinson@smartinst.fr

The flow in a mixing tank like a bioreactor depends on several parameters as the reactor geometry, the fluid properties, the liquid volume, the impeller geometry and the rotation speed. Numerical simulations help to optimize the processes, but they usually provide only a crude approximation of the real dynamics.
The smartINST system allows true measurement of the way each control parameter impacts the flow and therefore the mixing and internal reactor process. The system consists of the smartCENTER, which is the data logger, a data analyzer, and communication center with smartPARTs. The smartPARTs are embedded sensors, which freely move around the core of the fluid in order to monitor simultaneously, and in real-time, the evolution of products and processes. A smartPART in the reactor characterizes the flow and provides new possibilities for process optimization, scale-up and scale-down studies and reactor optimization.
The smartPART characterizes the flow thanks to new data like agitation rate and hydrodynamic signature. The agitation rate represents the energy supplied in the fluid. This rate is obtained in real time thanks to an accelerometer embedded in the smartPART. The hydrodynamic signature characterizes flow, providing detailed understanding of it, arising from the agitation rate statistics connected to control parameter settings. It forms a measurable quantitative description of the flow, allowing:

- Flow comparison
- Detection of zones of very strong agitation, which can damage cells
- Detection of dead areas

Moreover, the wireless measurement devices, smartPARTs, measure also at the most relevant place: at the core of the fluid where the reaction is taking place. It can measure pH, turbidity, conductivity and temperature, within the process. This unique feature makes the system extremely reactive and reliable. Having the ability of in-line measurements of these parameters makes the instrument very suited for advanced process design using PAT and QbD approach. Such, it can also contribute to better controlled processes, being the basis of reproducible product quality. During the presentation, we will describe the tools to characterize a flow and detail the benefits for process description, optimization and control.

12:50 Sponsor Talk: Optical Sensors for bioprocess development and control (L26)

Gernot John

PreSens Precision Sensing GmbH, Regensburg, Germany, g.john@presens.de

Starting end of the 90s chemical-optical sensors are now widely used in bioprocess development and production. The same sensor is available for different sizes. Examples and latest developments are shown for O_2, pH and CO_2 sensors.

12:55 Lunch break with poster session and exhibition

Scale down approaches and process analytical technologies for advanced process design

Chair: Krist Gernaey (Technical University of Denmark DTU, Lyngby, Denmark), Stefan Junne (TU Berlin, Berlin, Germany)

14:00 Keynote Lecture: Bioprocesses under magnification: Learning from single cells about large-scale processes (L27)

Alexander Grünberger, Wolfgang Wiechert and Dietrich Kohlheyer

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Our understanding of large-scale bioprocesses is still dominated by an average cell approach. However, as confirmed in recent years, isogenic bacterial populations can be physiologically heterogeneous. This presentation will give a short overview on microfluidic single-cell cultivation (MSCC) and its application in the field of bioprocess development. Microfluidic single-cell bioreactors offer precisely controlled external conditions and allow single-cell analysis at full spatio-temporal resolution. On the example of Corynebacterium glutamicum I will demonstrate how picoliter bioreactors can be used to get a deeper understanding of microbial bioprocesses. Finally, existing bottlenecks and the challenge of the integration of MSCC into the existing bioprocess development workflow will be discussed.

14:30 Development of a perfusion system in a microbioreactor using sedimentation as a scale down tool for ATF perfusion bioreactors (L28)

Steffen Kreye, Rainer Stahn, Antje Danielczyk, Steffen Goletz

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With the biotech industry moving to continuous bioprocessing and focusing increasingly on product quality, perfusion bioreactors are becoming more and more important. Whereas batch or fed-batch bioreactors can be successfully represented in a small scale with sufficient throughput for early stage process development, the 1 L benchtop bioreactor remains the smallest scale down unit for perfusion culture. Here, we demonstrate the development of a sedimentation based perfusion system in the single-use ambr system with good comparability to 1 L ATF perfusion bioreactors when comparing cell growth, viability and product quality, especially glycosylation, for GlycoExpressTM (GEX) and CHO cells. The system is better suited for clone screening, media optimization and the prediction of product quality compared to batch or chemostat culture. Furthermore, data will presented of Glycotope's proprietary GlycoExpressTM technology showing extremely consistent glycosylation of different glycoproteins between batches, batch sizes, reactor sizes, process control strategies, DSP scales and production site.

14:45 Keynote Lecture: Issues that are relevant when scaling-up hMSC microcarrier production processes in stirred bioreactor (L29)

Regine Eibl, Valentin Jossen, Dieter Eibl

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The potential of human mesenchymal stem cells (hMSCs) as agents for allogeneic cell therapies is undoubted. In order to provide the required amounts of clinical grade hMSCs, the availability of efficient scale-up procedures is stringently required. Promising results have been achieved in stirred bioreactors that operate with microcarriers at benchtop- and pilot scale. Usage of Computational Fluid Dynamics (CFD) together with bioengineering data (fluid flow, shear stress, microcarrier distribution) has contributed to an increase in hMSC expansion efficiency. However, formation of large microcarrier-cell-aggregates may result in mass transfer limitations and inhomogeneities in the culture broth.
In our presentation focusing on microcarrier-based stirred bioreactors, scale-up approaches for hMScs from adipose tissue (hADSCS) and bone marrow (hBM-MSCS) are presented. In addition, we discuss an own approach for the 50 L scale. Cell numbers between 1*1010 and 3.6*1010 cells were achieved within 7 days, while maintaining stem cell properties and qualities. Finally, the superiority of the NS1u criterion and the importance of time-dependent microcarrier-cell-aggregate size for rapid and successful scale-up of hMSC expansions are described.

15:15 Sponsor Talk: Evaluation of a stirred small scale single-use bioreactor for microbial applications (L30)

Thomas Dreher[1],Marco Leupold[1], Ute Husemann[1], Mwai Ngibuini[2], Gerhard Greller[1]

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For a successful process transfer, as well as for bioprocess development and optimization a suitable small scale approach is essential to reduce time and costs. To further increase the efficiency of these transfers single-use technology is very attractive. Unfortunately, small scale single-use systems often cannot mimic the conditions of established bioreactors, in terms of oxygen transfer and heat removal, especially, for microbial applications. Consequently, having such a model in small scale is of special interest.
Based mentioned limitations a small scale single-use bioreactor, the ambr® 250 modular (250 mL maximal working volume), was developed and evaluated. The design criteria and geometrical ratios are similar to established bioreactors. Due to integrated pumps, processes with high automation efforts can be realized. A further feature is the integrated exhaust gas analyzer, fulfilling all requirements for PAT. To benchmark the ambr® 250 modular an industrial relevant Escherichia coli high cell density process was performed. The achieved results were compared with a conventional glass bioreactor (UniVessel® glass 5 L), where the same process was carried out.
It was possible to reach a peak cell density of OD600 = 335 (dry cell weight = 120 g/L). No process deviations occurred during the fermentation in terms of oxygen transfer or heat removal. The results are completely comparable to the conventional glass bioreactor. Overall, the ambr® 250 modular is a useful tool for the transfer, development and optimization of industrial bioprocesses. Furthermore, process development cycles can be shortened and the costs are reduced including a high flexibility due to the advantages of the single-use technology.

15:25 Scalability of cultivations in the 2-dimensional rocking single-use bioreactor CELL-tainer (L31)

Anna Maria Marbà-Ardébol, Peter Neubauer, Stefan Junne

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While scale-up of stirred SUBs can follow traditional procedures of conventional STRs, the scaling of rocking SUBs is more demanding. However, these last ones have the advantage that they are more flexible in terms of filling volume due to the absence of stirrers. Moreover, in the case of the 2-D rocking CELL-tainer, the possibility to increase the working volume during the cultivation does not only reduce contamination risks, but also reduces cell stress due to transfer steps. An important parameter for the scalability of microbial aerobic cultivation is also the gas mass transfer coefficient (kLa), which can be maintained from the mL to the 120 L scale.

15:40 Towards efficient microaerobic processes using engineered Escherichia coli strains (L32)

Alvaro R. Lara[1], Karim E. Jaén[2]

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Operational and economic constraints in large-scale bioreactors often result in local or global microaerobic conditions, which lead to less efficient bioprocesses. Escherichia coli (E. coli) adapts to microaerobicity by activating fermentation pathways that accumulate acidic by-products, in detriment of growth rate and biomass yield (YX/S). This will be illustrated with a study showing the effects of constant and oscillating dissolved oxygen (DOT) conditions on the production of plasmid DNA (pDNA). Constant microaerobicity (3 % air sat.) affected the metabolism, but not the pDNA production. In contrast, heterogeneous DOT severely impacted the quality of the produced pDNA. Therefore, the metabolism of E. coli was modified to better cope with constant microaerobicity. For that purpose, genes coding for global regulators like CreB and ArcA, or for fermentative pathways were inactivated. The performance of a wild-type (W3110) and engineered E. coli strains was evaluated in batch cultures at constant low DOT. By combining the partial elimination of fermentation pathways and the expression of the Vitreoscilla hemoglobin (VHb), a 32 % decrease on carbon waste as by-products, 24 % increase on YX/S and 13 % increase of growth rate were obtained. Flux balance analysis of the best strain estimated major differences in the fluxes through the pentose phosphate pathway and tricarboxylic acid cycle as consequence of VHb presence. Overall, our results show that E. coli can be genetically modified to overcome some of the disadvantages of microaerobic growth, which is potentially useful for better bioreactor scale-up and operation.

16:10 Coffee break with poster session and exhibition

16:50 Keynote Lecture: Novel fluorescence-based on-line capable monitoring of cell cycle distribution and growth rate for mammalian cell cultures (L33)

Grischa Fuge, Yaeseong Hong, Uwe Jandt and An-Ping Zeng

Institute of Bioprocess and Biosystems Engineering, TU Hamburg, Germany, grischa.fuge@tuhh.de

A reliable on-line measurement of the cell growth rate is highly desirable but still out of reach for process control in most cases. For mammalian cell culture on-line monitoring of the cell cycle distribution is of particular interest, since it determines the cell growth rate and productivity.
We have developed an on-line capable monitoring technique for the cell cycle distribution of mammalian cell cultures in bioreactors, which is based on two stably expressed *Fluorescent Ubiquitination based Cell Cycle Indicator* (FUCCI) proteins in the cells.
As demonstrated with two CHO cell line derivatives, the detection of the specific fluorescence properties has proven to be a reliable, qualitative read-out for cell growth.

17:20 Multiposition sampling, multiparameter monitoring and electro-optical tracking of microbial activity: New tools for better understanding biogas production (L34)

Jörn Beheim-Schwarzbach[*1], Erich Kielhorn[2], Alexander Angersbach[3], Emma Ritzi[1], Manja Laqua[2], Anna Vaupel[2], Boris Habermann[1], Peter Neubauer[2], Stefan Junne[2]*

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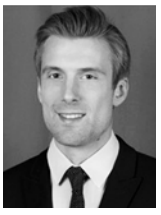

Gradients might appear in the usually laminar mixed biogas fermentation. Therefore, innovative sampling and monitoring tools have been developed to achieve a better understanding of gradients in the liquid phase in order to identify suitable areas for sampling. Microsensors for the determination of temperature, pH-value, redox potential and CO2 concentration have been optimized for long-term use in the culture broth. They are combined to a multi-parameter probe and integrated into portable lance systems that allow the movement of sensors inside the reactor for the detection of gradients in situ.
Since the estimation of the cells' physiological state is difficult, electro-optical monitoring of the polarizability is applied to gain information about cell activity. It is shown that the microbial activity correlates well with process parameters such as the methane formation rate.
The presented tools allow an improved monitoring of the liquid phase and microbial activity in industrial plants for on site process optimization. They contribute to a better understanding and concomitantly to a reduction of operational risks, especially at a flexible feedstock load, or whenever a process runs unstable.

17:45 In-line photon density wave spectroscopy for bioprocess monitoring (L35)

Roland Hass, Oliver Reich

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Highly concentrated bioprocesses cause analytical challenges for process spectroscopy due to the significant amount of light scattering generated by the cultivated biomaterial. Photon Density Wave (PDW) Spectroscopy can independently determine absorption and scattering properties of highly turbid liquid materials. With the help of fiber-optical probes it can be used as novel process analytical technology for bioprocess monitoring.
Besides an introduction into the PDW technology, examples like the mashing process during beer production, the cultivation of Scenedesmus rubescens in non-conventional photo bioreactors and enzymatic milk coagulation will be discussed. It is found that parameters like biomass growth and biomass decomposition or the casein conversion process in fresh milk (hydrolysis, aggregation, gelling) are well monitored.

[1] Vargas Ruiz S et al. Optical monitoring of milk fat phase transition within homogenized fresh milk by Photon Density Wave spectroscopy, Int Dairy J 26, 120–126, 2012. [2] Hass R et al. Industrial applications of Photon Density Wave spectroscopy for in-line particle sizing, Appl Optics 52, 1423–1431, 2013. Also in: Virtual Journal for Biomedical Optics 8. p. 1423–1431, 2013. [3] Hass R et al. Optical monitoring of chemical processes in turbid biogenic liquid dispersions by Photon Density Wave spectroscopy, Anal Bioanal Chem 407, 2791–2802, 2015.

18:00 Keynote lecture: Stirred, not shaken – Unsolved issues and new problems in scale-down for scale-up (L36)

Alvin W. Nienow

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Most bioprocesses are developed at the bench scale (mL to L) and then scaled-up to stirred bioreactors of many 10s or even 100s of cubic metres if the product of the process offers commercial possibilities. Such scale-up has often been based on rather arbitrary rules such as impeller tip speed. However, a better approach is to consider what would be the desired operating conditions at large scale, especially taking into account reasonable operating costs and then devise small scale experiments that simulate the large scale operating conditions-scale-down for scale-up. One of the major differences associated with large scale operation is the increased mixing time which is manifested as temporal and spatial inhomogeneities with respect to pH, nutrients, dO2, etc.; and much of this conference is devoted to devising small scale tests which can give an insight into how these fluctuations impact on cell density, product yield and quality. Other important physical features change with scale, such as Reynolds number and superficial gas velocity. The former of these leads to changes in flow regime so that, for example, at very small scale (ambr15, which is currently very popular for clone selection for therapeutic protein production), the flow becomes transitional rather than turbulent. Alternatively, with rheologically-complex, very high viscosity broths (as found with mycelial fermentations), the flow may be transitional or even laminar at the bench scale but turbulent or nearly so at the commercial. In addition to the above consideration of physical bioreactor scale, there is also an impact of the scale of the biological entity. Such a scale is important when considering the impact of fluid dynamic stress on process performance. A current example of this scale issue is the difference between the culture of animal cells (~15- 18 µm) in free suspension and of stem cells on microcarriers (~ 200 µm). This lecture will give a broad brush airing to all these issues.

19:30 Conference Dinner

Restaurant Zillemarkt, Bleibtreustr. 48a, 10623 Berlin (Charlottenburg)

(S Bahnhof Savignyplatz)

Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik

Chair of Bioprocess Engineering

Friday, 8 April 2016

Quality by design in bioprocess development

Chair: Ralf Takors (University of Stuttgart, Stuttgart, Germany),
Erich Kielhorn (TU Berlin, Berlin, Germany)

9:00 **Keynote lecture: Next generation mAb production – Continuous manufacturing, PAT, and real time release (L37)**

Douglas Richardson, David Pollard, John P. Higgins

Merck, New Jersey, USA, douglas.richardson14@merck.com

Recent success in monoclonal antibody based immuno-oncology therapeutics such as Keytruda have initiated a manufacturing revolution within the Biopharmaceutical industry. Preparation for a wave of immuno-modulatory receptor- focused clinical candidates highlights the need for a more flexible manufacturing and testing network. To support this revolution development efforts focusing on single-use technology, continuous manufacturing, process analytical technology, IT/MVDA, and real time release are being pursued. This presentation will describe the investments, needs, and challenges in next- generation mAb production and testing.

9:30 **Keynote lecture: High-level protein production with Pichia: Case studies of development path from strain generation over bioreactor (L38)**

Roland Weis

VTU Technology GmbH, Grambach, Austria, roland.weis@vtu.com

Protein production processes using Pichia pastoris are increasingly recognized as highly efficient by many industries. Especially for biopharmaceuticals, key-to-success is the rapid and reliable generation of high-level production strains matching protein quantity and quality attributes. A seamless transferability of lab-scale results to more controlled bioreactor conditions is of utmost importance. The application of a versatile promoter library for fine-tuned expression of both, the target gene(s) as well as specific or general auxiliary gene(s) creates a diversity of expression strains that can only be analyzed in depth with a solid high throughput cultivation and screening system.
VTU´s reliable 96-well scale cultivation and screening platform delivers high-level production candidate strains which perform equally well under bioreactor conditions so that time-consuming and laborious intermediate steps like shake flask cultivation in order to select specific strains are obsolete. The creation of novel AOX1-promoter variants with unique expression characteristics in glycerol- or glucose-fed processes, fully devoid of methanol now enable methanol-free high-level protein production, as well.
A large number of model proteins were subjected to this high-throughput screening platform followed by direct transfer into bioreactors, often yielding multi g/L levels of secreted recombinant protein. Strain generation and bioreactor production procedures of some selected examples including growth factors, HSA-fusion proteins as well as enzymes will be disclosed in detail.

10:00 **Keynote lecture: ESETEC 2.0, a new, secretory Escherichia coli strain for the production of complex recombinant proteins at industrial scale (L39)**

Guido Seidel

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Several years ago WACKER BIOTECH developed a unique E. coli strain which is capable to secrete complex proteins directly to the fermentation media. We focused our research on the next wave of biologics so called antibody fragments (FAbs). Over the last two years we redeveloped and engineered our work horse and we have introduced ESETEC 2.0 as an optimised platform for the production of FAbs (mono-, bi- or multispecific) and other complex proteins as soluble proteins secreted to the fermentation media. Therefore cell disruption and/or refolding steps are not necessary to process the product of interest. ESETEC 2.0® combined with our fermentation process is able to reach fermentation titers in the [g/l]-range.
The talk will describe our new engineered and optimised expression system, the industrial fermentation process and we will give some information in our integrated antibody fragment purification scheme. We have developed several integrated processes by using cell line selection with medium throughput screening, aligned with fermentation and purification based on state-of-the-art analytics. Our processes are scalable to industrial levels to supply clinical programs with large amounts of high quality drug substances.

10:30 **Different process strategies for the glutathione overproduction in Saccharomyces cerevisiae (L40)**

Martin Senz[1], Eric Lorenz[2], Maximilian Schmacht[1]

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Glutathione (GSH) plays a key role in protective mechanisms against reactive oxygen species in eukaryotic and prokaryotic organisms. Due to its anti-oxidative character it is widely used in pharmaceutical, cosmetic and food industry. For instance, in bakeries GSH enriched yeast cells can be used as dough modifier having the advantage of no need for declaration in the final product. There are various strategies for the fermentation process of GSH production, the most common being fed-batch procedure with the yeast Saccharomyces cerevisiae. For an efficient production process, factors like medium costs and process robustness at various scales, as well as achieving high cell density with high intracellular GSH content, are essential.
This talk provides a compilation of different process optimization strategies for the production of GSH-enriched yeasts.

In context of quality by design, studies addressing the fermentation format, feeding strategy, medium composition, precursor for GSH transformation and its type of supplementation, are presented. Promising process formats were varied concerning the applied yeast strain and process scale. Therefore, the here shown results offer insights and prospects for current and further process developments in GSH production.

10:50 Coffee break with poster session and exhibition

11:40 Keynote lecture: Parallel small-scale bioreactors with on-line monitoring to prove the consistency of medium ingredients and pre-cultures and to efficiently assess process characteristics (L41)

Jochen Büchs

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Manufacturing of pharmaceutical products strictly has to comply with GMP, PAT and QbD requirements. Therefore, robust and easy to use tools and methodologies are urgently needed to assess the quality of complex medium ingredients and to prove the consistency of pre-cultures. In addition, more process understanding is required to predict the impact of process deviations on product quality. The application of parallel shaken bioreactors systems (200 µL to 20 mL scale) is introduced which allow a fast and efficient assessment of bioprocess characteristics. Many different starting conditions can be chosen and critical components can be pulsed into running cultures to investigate the influence of these variations on product quality.

12:10 Towards intensifying design of experiments: An industrial Escherichia coli feasibility study (L42)

Moritz von Stosch[1], Jan-Martijn Hamelink[2], Rui Oliveira[3]

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The combination of Design of Experiment (DoE) with multivariate data analysis is commonly used in upstream bioprocess development to evaluate the impact of process parameters on the final product titer and, where possible, product quality. However, for an increasing number of process parameters, i.e.: design factors, the number of experiments increases exponentially, reaching numbers, which are infeasible to execute.
This contribution introduces a novel concept, referred to as intensified Design of Experiments (iDoE), i.e.: intra-experiment variation of the design factors. The iDoE was experimentally implemented for an E. coli process in industry and the resulting data used to develop a dynamic hybrid model. The model was validated and evaluated against data from a traditional statistical Design of Experiments (DoE). The results show that the model (which was developed based on the dynamic experiments) can accurately describe 12 experiments of classical DoE. The results suggest that iDoE in combination with dynamic models can be used to significantly reduce the number of experiments and timelines. In addition, the methodology delivers detailed process understanding, thus supporting the implementation of QbD and PAT in process development.

12:30 Robot based bioprocess development by automated on-line optimal re-design in parallel bioreactors (L43)

Mariano Nicolas Cruz Bournazou[1], Tilman Barz[2], Florian Glauche[1], Peter Neubauer[1]

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Modern bio-industry demands a faster and more robust process development. To achieve this, advances in sensor technology, miniaturization, parallelization, and automation need to be supported by proper computer aided tools which allow the optimal use of all existing resources in combination, can predict the behavior of the system under different conditions, and create a path that supports a consistent flow of information between all stages and scales during development.
Due to the inherent complexity of biological systems, simple adaptive models and efficient automatic experimental facilities are required to allow a fast and cheap fitting of the model to small variations (e.g. between similar strains, different operating conditions, changes in substrate composition, etc.).
We present a framework for the online optimal experimental re-design of parallel nonlinear dynamic processes and apply it to the E. coli W3010 fed-batch in eight mini-bioreactors in an automatic liquid handling facility. By this, an "intelligent" automatic facility that can fit simple models to real data in minimal time is available.
This work shows that the re-computation of the optimal setup on the fly, while the automatized experiment is running, is possible. As a result a fifty fold lower average variation coefficient on the parameter estimates (4.83% instead of 235.86%) compared to a sequential Optimal Experimental Design proves the strengths of the approach.

12:45 Plenary lecture: From advanced PAT to QbD-compliant quasi-continuous integrated pharmaceutical production (L44)

Reiner Luttmann

Research Center of Bioprocess Engineering and Analytical Techniques, Hamburg University of Applied Sciences, Germany, reiner.luttmann@haw-hamburg.de

Nowadays dreams of QbD-compliant pharmaceutical production are based on small full continuous operated integrated processes with a global monitoring of biological variables and of course with disposable technology. These goals are far away from today and moreover contradictory.
This contribution describes a way to realize some of these tasks with state of the art equipment and process strategies, explained at a process development of potential Malaria vaccine production with Pichia pastoris.
A first intensification of processing is based on a recursive expression of pharmaceutical proteins and can be achieved by a two-stage upstream strategy, including a repeated cell breeding with a subsequent protein production in a parallel reactor.

These processes were developed in a fully automated manner accompanied by intensive PAT applications. For advanced on-line monitoring of biotechnological variables spectroscopic in-line methods using NIR-, Raman- and 2D-fluorescence-spectrometer and the associated MVDA are applied for detection of cell density and glycerol but also for total secreted proteins and cell internal AOX. Moreover an at-line application of IMAC with HPLC enables the discrimination of secreted proteins and therefore a target protein evaluation.
A QbD-compliant production of pharmaceutical proteins needs a risk analysis and the identification of Critical Process Parameters with appropriate Design- and Control-Spaces. Therefore a fully automated bioreactor plant was developed for optimization of expression conditions. This plant images the sequential production strategy and fulfills the process development boundary conditions of ICH-Q8.
The development of a quasi-continuous operated production plant starts with the procedural integration of all unit operations of an overall process. This in turn results in a two-step upstream and a five-step downstream process, consisting of a 10 l cell breeding and a 30 l protein production reactor, a cell clarification via a separator, a retention of cellular debris using a micro-filtration, and a concentration of the secreted product by ultra-filtration with subsequent buffer exchange through dia-filtration, followed by a final purification using column chromatography.
The three main operations cell breeding, protein production and all downstream operations here run serially over 3 days, but also in parallel with a one-day-offset.
With this strategy, a quasi-continuous process flow is possible. However, a QbD-compliant production process requires a knowledge-based support and monitoring. This includes, for example, an additional optimization of all individual downstream steps using DoE, from which the respective design spaces and control spaces have also to be identified.
A monitoring of the reproducibility of the entire repeated process flow requires the use of an on-line evaluation along with a Golden Batch approach. For this purpose the plant is equipped with corresponding software modules such as SIPAT, SIMCA Q, and SIMCA-online, and also with MATLAB for enhanced data processing and in-silico process development.

13:30 Concluding remarks

Peter Neubauer

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13:45 End of symposium

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Chair of Bioprocess Engineering

Please note:

5th BioProScale Symposium

April 2018, Berlin

Poster abstracts

Metabolic adaptation and population dynamics

Combination of scale-down approach with 13C-labeling experiments (P01)

Pooth Viola[1], Nöh K., Wiechert W., Oldiges M.[2]

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Imperfect mixing induces gradient formation in industrial scale bioreactors. There is evidence that microorganisms adapt their metabolism to such inhomogeneous conditions. Nevertheless, bioprocesses may suffer from reduced profitability as microbial metabolism and its performance are highly dependent on environmental conditions. Thus, scale-down bioreactor systems are used as a powerful tool to mimic gradients during early lab scale optimization.
In order to follow metabolic changes, 13C-labeling experiments serve as basis for metabolic flux analysis. Under dynamic process conditions only very short periods of metabolic steady-state, being a precondition for a carbon labeling experiment, are present. So, for short transient biological changes an isotopically non-stationary carbon labeling experiment (INST-CLE), where a transient label enrichment in the intracellular metabolite pools can be observed, is the preferred experimental approach (Nöh et al. 2007). Currently, there are no 13C metabolome data from scale-down bioreactor experiments available for metabolic flux analysis. Especially fast sampling and further sample processing, as well as the analysis of metabolome data from dynamic scale-down cultivations remain challenging and demand for single bioreactor setup with periodic oscillations. At this point, we started with the batch and fed-batch cultivation of Escherichia coli K12 in a two compartment scale-down bioreactor (STR^2, stirred tank reactor) under oscillating oxygen supply. Due to the oscillation between aerobic and non-aerated bioreactor compartments different growth phenotypes were observed. The additional substrate excess or substrate depletion at fed-batch cultivations revealed impacts on by-product metabolism.
The results of the dynamic conditions of the STR^2 will be compared to a single STR with periodic oxygen limitation during cultivation. The conditions of the STR^2 scale-down bioreactor will be transferred to the single STR with periodic oscillations, because only that type of bioreactor is suitable for INST-CLEs due to the integrated rapid sampling device and substrate pulse unit (Nöh et al. 2007). After the complete establishment of the workflow, the system is to be applied under various limiting conditions targeting industrial production strains. Thereby, a closer look into the metabolic state of the cell under dynamic cultivation conditions is facilitated.

[1] Nöh et al. (2007). J Biotechnol 129(2): 249-267

Bacterial phase diagrams: Analyzing and predicting cell-to-cell heterogeneity of microbial gene expression (P02)

Iska Steffens[1], Alexander Grünberger[1], Dennis Binder[2], Thomas Drepper[2], Wolfgang Wiechert[1], Dietrich Kohlheyer[1]

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Phenotypic heterogeneity represents varying cell behavior of clonal populations and is a major issue in the context of industrial bioprocessing. Population heterogeneity is considered to enhance the fitness of microbial populations in natural environments, but appears to be responsible for failure, reduced control and lower yield in bacterial production processes. Accordingly, novel analytical tools and strategies need to be developed to predict and control bacterial population heterogeneity [1].
In this contribution, we introduce the concept of "Bacterial phase diagrams" (BPDs). Similar to chemical phase diagrams, BPDs can be used to visualize and predict the state/behavior of microbial gene expression (e.g. homogeneous or heterogeneous gene expression) at certain environmental conditions. In order to determine single-cell responses, novel picoliter bioreactor cultivation platforms were used [2,3]. As a proof of principle T7-RNA-Polymerase based EYFP production in E. coli BL21 (DE3) [4] was investigated. This model system generates a non-gradual and partially inhomogeneous induction behavior over a bacterial population. Here we present the manipulation of expression behavior, by means of varying inductor (here IPTG) and repressor concentrations (here glucose). Especially for low amounts of inducer molecules, an inhomogeneous all-or-nothing expression response can be observed. Latest results, potential application and ongoing research directions will be shown.
Our results show that the concept of "bacterial phase diagrams" has the potential to predict bacterial behaviour in gene-expression. This paves the way for an improved understanding of cell-to-cell heterogeneity and could lead to novel strategies of controlling microbial bioprocesses.

[1] Grünberger A et al., 2014, Curr Opin Biotechnol 29:15-23.
[2] Grünberger A et al., 2012, Lab on a Chip, 12(11): 2060-2068.
[3] Grünberger A et al., 2015, Cytometry A, DOI: 10.1002/cyto.a.22779.
[4] Binder D et al., 2014, Integrative Biol 6:755-765.

Chemical evolution of a bacterial proteome (P03)

Christian Schipp[1,2], Robert Kaml[2], Stefan Oehm[2], Michael Georg Hösl[2], Stefan Junne[1], Peter Neubauer[1], Nediljko Budisa[2]

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The introduction of alternative biochemical building blocks to the standardized chemistry of a living cell remains an important milestone in synthetic biology. Changing the fundamental components of live such as amino acids or nucleic acids has mostly detrimental effects on biochemical processes. Therefore, the system must adapt towards the new substrate in an evolving manner rewiring its genetic basis to overcome the structurally unfavorable replacement.
During a long-term evolution experiment (LTEE), a tryptophan auxotroph E. coli strain was forced by a serial-transfer regime in a tryptophan (Trp) limiting environment to adapt towards the non-canonical analog L-beta-(thieno[3,2-b]pyrrolyl)-alanine ([3,2]Tpa). Genomic and proteomic analysis revealed deep changes on genetic and translational level. Some mutations obviously led to the shutdown of E. coli's stringent response, which is a well-known artifact of LTEE. Nevertheless, the replacement of Trp to [3,2]Tpa in more than 20.000 UGG codons yielded in robust growing descendants in minimal glucose medium only containing the surrogate [3,2]Tpa as a tryptophan source.
This study illustrates an approach for the construction of synthetic cells with alternative biochemical building blocks exploiting the flexibility of the genetic code. Furthermore, the experiment will deepen the understanding the evolvability of living cells in respect to non-canonical substrates.

Let there be light – Quantitative analysis of phototrophic cyanobacteria in controlled microfluidic environments (P04)

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Recent biotechnology increasingly focusses on harnessing the photosynthetic machinery of cyanobacteria for the production of energy carriers and chemical building blocks [1]. However, in order to facilitate industrial processes based on bioartificial photosynthesis, the cyanobacterial cell as a living, light-driven catalyst with its complex molecular mechanisms and cellular functions has to be understood in detail by means of single cell analysis [2]. Most surprisingly and despite of the huge potential, single cell studies dealing with the analysis of phototrophic microorganisms are scarce and still limited to feasibility studies without proper physiological characterization. In our contribution we demonstrate how microfluidic technologies can be applied to study single phototrophic organisms and answer questions concerning their biotechnological application at the scale of a single cell.

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LEANPROT: Systems biology platform for the creation of lean-proteome Escherichia coli strains (P05)

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The rapid development of genetic engineering and -omics technologies together with new metabolic modeling tools allows the creation of recombinant chassis with superior characteristics. However, the current strains still contain a number of synthesis and regulatory pathways which have evolved in nature, but which are not needed in a bioprocess and consume energy and metabolic resources and consequently lower the final production yields.
Therefore in the LEANPROT ERA-SysAPP project we consider to develop lean proteome strains based on a systems biology-based strain engineering approach. The proteome is optimized by iterative cycles of modeling and biological experiments which are performed by parallel consideration of different parameters as Design of Experiments (DoE) under well-defined process related environmental conditions. Our approach includes the use of a single cell model, which includes the sequestration of metabolites and energy into the different metabolic routes under consideration of the cell cycle and cell volume dynamics (based on a model by Cooper Helmstetter Donachie). We believe that this new way of approaching the problem of protein production together with state of the art chemical engineering optimization principles could open a new avenue for the creation of superior cell factories.

Bioprocess modelling

Computational analysis of hydrodynamics and light distribution in photo-bioreactors for algae biomass production (P06)

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Microalgae can be directly used in health food or as bio-filters for waste water treatment. They also have numerous commercial applications in cosmetics, aquaculture and chemical industry as a source of highly valuable molecules, e.g., polyunsaturated fatty acids [1]. Moreover, they are increasingly recognized as a promising source for biodiesel production [2]. To realize the full potential of microalgae, optimal operating conditions for their cultivation in photo-bioreactors (PBR) need to be identified in order to maximize productivity, lipid content, and efficiency of photosynthesis. The most important parameters affecting PBR performance are reactor shape, light intensity distribution, algae growth and other metabolic properties.
The presented study aims at optimizing these parameters using Computational Fluid Dynamics (CFD) simulations with the COMSOL Multiphysics software. Specifically, flat panel photo-bioreactors with turbulent mixing due to air sparging and one-sided lighting are studied. First, flow profiles of both liquid and gas phases are computed using the Euler-Euler approach for analyzing the air sparging and detecting potential dead zones. Then, light intensity distributions are calculated inside different PBR types, based on absorption and light scattering by algae and gas bubbles. Subsequently, the paths of individual algae are traced, and the environmental conditions they are exposed to, are recorded over time, in particular aeration and light intensity. Results of the above described simulation stages will be presented and discussed.

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Nutrient distribution in perfusion based picoliter bioreactors (P07)

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Cell-to-cell heterogeneity has been found to have the potential to significantly influence biotechnological processes. Microfluidic devices offer new opportunities for research and optimization of microorganisms on single-cell level, which can give further insights in underlying causes and resulting effects of heterogeneity [1]. A recently developed microfluidic platform allows cultivation of several hundred microcolonies, each consisting of up to several hundred cells, in separate cultivation chambers on one device. Data, e.g. growth rates, can be generated by automated time-lapse microscopy with spatiotemporal resolution on single-cell level [2]. Especially perfusion based cultivation chamber types are expected to offer good environmental control with regard to the cultivation medium, i.e. constant substrate and low product and byproduct concentration. Due to length-scales in the micrometer range and corresponding small volumes of several picoliters per reaction chamber it is currently impossible to directly measure those concentrations and their gradients within the devices. Therefore the microfluidic device employed in [2] was modeled and simulated using COMSOL Multiphysics. The liquid velocity field and the mass transfer within the supply channels and cultivation chambers were calculated to gain insight in the spatial distribution of supplied nu-

trients and metabolic products secreted by the cultivated bacteria. The goal was to identify potential substrate limitations or product accumulations within the cultivation device and the resulting inhomogeneity experienced by single cells. The metabolic uptake and production rates, colony size, and growth medium composition were varied covering a wide range of operating conditions [3]. This lays the foundation for further studies and the optimization of existing picoliter bioreactor systems.

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Combination of modeling and environmental life cycle assessment approach for demand driven biogas production (P08)

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A suitable strategy to even the fluctuating energy provision by some renewable energy sources is a flexible operation of biogas plants. Nevertheless, this process needs to remain stable, profitable and environmentally friendly. In order to identify reasonable and suitable operation points, process modelling is combined with an LCA. In this proof-of-concept study, a biogas process was simulated, which flexibly generates energy from the co-digestion of maize, grass and cattle manure, while emitting the lowest amount of greenhouse gas emissions. The program was structured in Matlab to simulate any biogas process based on the AMOCO model and combined with equations that provide climate change, acidification and eutrophication potentials of the whole production system based on ReCiPe midpoint v.1.06 methodology. The simulation was optimized based on real data of biogas plants and existing literature research.

Effect of light modeling on the prediction of microalgae growth (P09)

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The prediction of growth of microalgae requires accurate modeling of light propagation in photobioreactors. Although Lamberts Law provides a simple way to approximate light attenuation, it does not capture any scattering effects and, in consequence, growth predictions deviate from experimental results. To reach higher level of accuracy, numerical simulation of light distribution in photobioreactors may serve as an alternative. In the present contribution, the effects of light modeling on predictions of microalgae growth are investigated by numerical simulations.

Modelling dissolved oxygen and glucose gradients in pulse-based fed batch culture of Escherichia coli (P10)

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Local addition of substrate and air, coupled with insufficient mixing in industrial scale fed-batch cultures result in the occurrence of zones throughout the bioreactor. E. coli cells that are intermittently exposed to these zones react metabolically by producing acetate [1], and physiologically by accumulating non-canonical amino acids (NCAA) in both cellular and recombinant proteins [2]. In the current work, we develop a dynamical mechanistic model to predict the response of an E. coli strain for recombinant protein production to perturbations in glucose feed and oxygen transfer in fed-batch culture. The model is validated with experimental data from pulse-based fed-batch cultures of E. coli. The simulations show a good agreement with the experimental data and provide a framework for model-based process monitoring and optimisation of fermentation strategies.

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Bioprocess scale-up and scale-down

How to successfully scale up a microcarrier based expansion process of human mesenchymal stem cells from adipose tissue (P11)

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Human mesenchymal stem cells isolated from adipose tissue (hASCs) are of increasing interest for clinical applications. Due to their good clinical efficacy and tolerability, they can be used for allogeneic therapies (e.g. acute myocardial infarction). However, the required number of therapeutically active hASCs for allogenic applications is in the region of a trillion cells per year. Therefore, alternatives to 2-dimensional planar cultivation systems, which are typically used to propagate them, and suitable scale up strategies for the expansion procedure are urgently required. Promising alternatives to traditional systems are stirred and wave-mixed single-use bioreactors. In these dynamically mixed systems, the required growth surface for the adherent cells is provided by microcarriers (MCs) that are suspended in the bioreactor. However, this raises the question of how a MC-based expansion process for hASCs can be scaled up.
A successful scale up approach for a MC-based production process is presented for adipose derived stromal/stem cells in a stirred (35L; pilot scale) and a wave-mixed (2L; benchtop scale) single-use bioreactor. To determine the optimum medium-microcarrier-combination, extensive screening studies based on the experimental design were performed in spinner flasks. In addition, experimental and numerical investigations with a spinner flask, a BIOSTAT STR 50L and a BIOSTAT CultiBag RM 2L were also carried out. This allowed the optimum

cultivation conditions to be predicted, while keeping the shear stress levels low. The scale up approach resulted in peak cell densities of 2.9x105 hASCs mL-1 (1.015x1010 hASCs) in the BIOSTAT STR 50L and in 1.9x105 hASC mL-1 (2.85x108 hASCs) in the BIOSTAT CultiBag RM 2L (proof-of-concept cultivation). During the cultivation the cells maintained their stem cell properties and surface markers.

Scalability of multiple strain co-fermentation (P12)

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During scale-up of fermentation processes, scale dependent changes can have a significant impact on the process. Variation in process parameter such as pH and temperature, which can be easily investigated in laboratory scale, may show to be insignificant, compared to changes in media preparation or process time.
Scale-up of fermentation processes were multiple strains are co-fermented can be especially challenging, as each strain can have different sensibility to process changes. This may lead to a changed strain balance and the interactions between the different strains may be affected. Identifying the significant parameters to control and investigate may therefor prove complex.
In this study, the effect of changes in the media preparation between laboratory and production scale fermentations of a multiple strain lactic acid bacteria culture is investigated. Furthermore, it is demonstrated, how the scale of the reactor itself may impact the culture composition, and which challenges can arise when transferring models from laboratory scale to an industrial setting.

Impact of dissolved oxygen tension on plasmid DNA production by Escherichia coli (P13)

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Despite the growing potential of plasmid DNA (pDNA) as a therapeutic agent, little is known about the impact of large-scale conditions on pDNA production. There are a few reports on the effect of dissolved oxygen tension (DOT) heterogeneities on pDNA production, however, the results are not clear. In the present contribution, we investigated the effect of constant aerobic (DOT = 30 % air sat.), microaerobic (DOT = 3 %) and oscillatory oxygen availability on the production of a pDNA vaccine model (pVAX1) by E. coli W3110 *recA*$^-$ in 1 L batch bioreactor cultures. DOT oscillations were created by stirrer speed shifts from 100 to 1200 RPM every 10 min that caused DOT oscillations with decreasing amplitude from 0 - 90% air sat. Oscillatory conditions did not affect the specific growth rate, but resulted in a decrease of biomass yield on glucose of ca. 24 %, a higher accumulation of fermentation by-products and a 30 % increase of the respiratory quotient (RQ), compared to constant microaerobic conditions. This can be attributed to the lower O_2 uptake under such conditions. DOT oscillations caused periodic changes of the respiratory activity, as evidenced by off-gas analyses. Interestingly, temperature oscillations were also observed in response to DOT changes. pDNA yield, titer and productivity were not significantly influenced by the different DOT conditions. Under constant aerobic and microaerobic conditions, the pDNA supercoiled fraction (SCF) remained close to 90 % throughout the cultures, and the DNA fidelity (analyzed by Illumina sequencing technology) was close to 100 %. In contrast, under DOT oscillations, SCF dropped to 82 % and showed more heterogeneity as revealed by agarose gel and chip microfluidics electrophoresis. Overall, our results show that despite cellular behavior is strongly influenced by the DOT, pDNA production is affected mainly on its quality attribute (SCF), rather than in productivity, by DOT heterogeneities.

Increasing efficiency of baculovirus-based r-protein productions in orbitally shaken single-use bioreactors (P14)

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Insect cells used in conjunction with the baculovirus-expression vector system provide a suitable production system for the development and manufacturing of recombinant protein products such as virus-like particle vaccines. In order to speed up vaccine development and production, and to react more quickly to pandemic scenarios, new strategies for efficient baculovirus-based production technologies need to be investigated. Among these is the implementation of single-use (SU) bioreactors, which have been proven as cost-effective (48% less capital cost), and ready-to-use systems without the time-intensive cleaning and sterilization processes required by stainless steel bioreactors. Further time- and cost-savings can be achieved by using the titerless infected-cell preservation and scale-up (TIPS) method as an alternative to the classical 2-phase production process. The TIPS method enables a large-scale protein expression in 100 L bioreactors with cryopreserved, baculovirus-infected insect cells (Spodoptera frugiperda Sf9) in one scale-up step. Its major advantages include the elimination of titering virus stock supernatants, and the stable storage of highly concentrated recombinant baculoviruses enclosed in the insect cells.
In this study, a combination of the TIPS method with SU bioreactors was investigated with the aim of increasing the efficiency of baculovirus-based production processes in orbitally shaken bioreactors from mL- to L-scale. After successful establishment of the TIPS method by infecting Sf9 cells with the recombinant baculovirus Autographa californica multicapside nucleopolyhedrovirus (AcMNPV), a model protein, the secreted alkaline phosphatase (rSEAP), was expressed in 125 mL shake flasks. Subsequently, the production process was transferred to 3 L shake flasks and to the orbitally shaken SU bioreactor SB10-X. The rSEAP activities obtained were comparable in all three orbitally shaken systems (152 – 173 U mL-1). In fact, they were up to 40 % higher than those described for stirred and wave-mixed SU bioreactors using the classical 2-phase production process.

Performance loss of Corynebacterium glutamicum cultivations under scale-down conditions (P15)

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See L19

Simulating large scale conditions in a scale-down bioreactor: Impacts on cell physiology of recombinant Escherichia coli (P16)

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Escherichia coli is an important production strain for industrial and pharmaceutical recombinant protein production in large-scale fed-batch bioprocesses. Limited mixing capacities lead to the development of gradients concerning the nutrient and oxygen availability when a critical level in scale and cell density is reached. E. coli responds to high substrate concentrations in combination with oxygen limitation with an increased production of metabolites based on pyruvate via overflow metabolism and mixed-acid fermentation. Furthermore, an increased flux into the branched-chain amino acid pathway can be seen leading to an increased production also including non-canonical amino acids like norvaline [ ]. Non-canonical amino acids can be incorporated into proteins, e.g. norvaline as a substitute for leucine. Also, methionine is known to be exchanged by norleucine [ ]. These exchanges may have effects on the production and quality of a recombinant protein. To better understand the impact of heterogeneous oscillating conditions on the physiological state of the cells and quality of the produced recombinant protein, experiments in a scale-down multi-compartment reactor system based on [ ] are performed and in addition compared to glucose-pulsed-based stirred tank reactor cultivations.
Metabolome analysis from samples of cultivations of a recombinant E. coli K-12 W3110 strain overexpressing a leucine-rich recombinant protein shows an accumulation of metabolites deriving from pyruvate (acetate and lactate) as well as an increased production of non-canonical branched-chain amino acids under scale-down conditions. Furthermore, we see increased incorporation of the non-canonical amino acids norvaline, norleucine and β-methyl-norleucine when oscillating cultivation conditions are applied, influencing the quality of the recombinant product. These results have implications for the design of molecular and engineering approaches for developing robust processes aiming towards a high quality of the desired protein product.

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Bioprocess development

Tools enabling Real-Time Raman identification of biofilm fouling (P17)

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This poster describes a proof-of-concept for a compact real-time surface-enhanced Raman spectroscopy (SERS)-online sensing approach for detection of bacteria in drinking water membrane filtration. In this study we created a custom-designed flow-cell that mimics a cross-flow membrane filtration system. This enables one to measure changes in surface-foulants, such as Brevundimonas dimiuta (BD) bacteria and adenine, under conditions that are similar to conventional membrane filtration systems. For measurements we used a common portable Raman-spectrometer with a laboratory Raman-probe mounted into a specifically designed flow-cell with an optical window and the laser-beam focused onto specially developed gold nanoparticle (Au NP) SERS-sensing area on filter-membranes. This allowed real-time detection of low concentrations of surface-foulants immediately after inoculation into an ultra-pure water reservoir under pressure-driven filtration conditions with a limit of detection (LOD) at around 10-7 M. We compared these online results with static measurements from an offline, sample-taking approach, using a confocal Raman-laboratory-microscope. The developed gold nanoparticle (Au NP) SERS-sensing-area on the membranes proved to be stable over a long period of surface fouling investigations and to suppress the strong interfering Raman-signal originating from the composition layer of most filtration membranes.

Bioprocess optimization using culture transfer in multi-reactor system (P18)

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The physiology of producing organism is known to affect the outcome of production processes, thus physiology must be well controlled in optimization studies. Continuous cultivation fixes the physiological state of the organism, but is time and material consuming. We have developed a system combining the high throughput of batch cultivations with the controlled environment of continuous ones. In this system microorganisms are cultivated in one bioreactor followed by culture distribution in a network of receiver bioreactors and continuation of independent steady state experiments therein - effectively the physiological state of the culture is multiplied. In addition to starting experiments from precisely established physiology the novel system is very suitable for the production of recombinant proteins or other re-search and production applications which entail physiology disruption, as growth and production units are decoupled. In current study the efficiency analysis of the system is presented, proof of concept experimental data of recombinant protein production optimization is included.

Bio-Butanol production with Clostridium acetobutylicum in a multi-stage continuous process (P19)

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Finiteness of fossil hydrocarbon resources and environmental pollution resulting from the use of these resources make production of renewable energy sources necessary. Well known products in this area are ethanol and butanol. Compared to ethanol, butanol has higher energy content and can be blended with gasoline. In addition, butanol acts as a bulk raw material in the chemical industry.
The OPTISOLV project consortium consists of industrial and academic partners from Italy and Germany. Within the framework of the project, new continuous processes for solvent production with Clostridium acetobutylicum are being developed. Experimental investigation supported by mathematical modelling is used to evaluate feasible and productive continuous bioreactor configurations. While in the widely used batch processes, the two metabolic phases of C.

acetobutylicum – acetogenesis and solventogenesis – follow each other in time, in a multistage continuous bioreactor system they are spatially separated. A change of metabolic states throughout the multi-stage bioreactor system allows their characterization. Multistage processes can be operated with both suspended cells (cells are traveling through reactors and are exposed to environmental changes) and immobilized cells (constant environment of the cells).
In our group, we focus on modeling the dynamics of microbial metabolic network and its regulation as well as on the generation of data from continuous cultivation experiments. We also attempt optimizing the bio-butanol production process in a cascade of continuous stirred tank reactors (CCSTR) using model predictions. Several optimization possibilities are eligible like an adding/removal of reactor stages, change of residence times in the stages by reactor volume adaptation and recirculation or feeding between the stages. Currently our system achieves a final butanol concentration of 11 gBUT L-1 with a volumetric production rate of 1 gBUT (Lh)-1.
Mathematical modeling of the suspended cells system is still a challenge. The agent-based model, developed in our group, includes simulation of chemical concentrations in each bioreactor stage and biological conversion rates of biomass in each metabolic state. Agents represent subpopulations with proper physiological properties. Moving these agents according to the rules of residence time distributions, these subpopulations are changing in size and are moving to new environments. The performance of each reactor stage will then be calculated from the mean metabolic reactions of the subpopulations.

Improving mammalian cell culture process development by combining model-based simulations with DoE tools (P20)

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Bioprocesses involving mammalian cells are most frequently based on optimized feeding profiles within a fed-batch. Statistical Design of Experiment (DoE) tools are widely used for this kind of process optimization because of their simple structure and easy handling. Limitations occur with respect to the high number and duration of experiments during process development. Even if high-throughput systems can perform these numbers of experiments in parallel, the heuristic restriction of boundaries results in stepwise iterations with multiple runs. This is time-consuming, cost-intensive and further complicates the path from process development to process establishment. The use of DoE tools in combination with an appropriate growth model might be a valuable method for evaluating fed-batch strategies and decreasing the experimental space required for statistical optimization methods in silico before experiments are carried out. To prove this concept, a model was used to describe the dynamics of cell metabolism of CHO-XM-111 cells in a two-step growth process with medium exchange followed by a fed-batch. Model parameters were fitted to averaged data from three parallel shake flask cultivations, and model predictions were used to decrease the experimental space and the number of cultivation parameters in silico. Therefore, the concentration of L-glutamine (feed), constant feed rate, point in time for the medium exchange, and the start of feeding were identified as critical process parameters. At first, simulations were applied to minimize experimental space. Therefore, the point in time for medium exchange and the starting point for feeding were determined from a batch simulation at a minimal L-glutamine concentration of 0.1 mmol L-1. Secondly, to increase the total cell number in a fed-batch, the L-glutamine concentration and constant feed rate were optimized by simulated DoE. In total, two simulated and iterative response surface plots were evaluated. Instead of performing multiple experiments to generate data for response surface plots, data gained from simulation was used. Finally, one appropriate combination of cultivation parameters was tested and compared with the initial model.
The method shown is suitable for the generation of deeper process understanding, e.g. the linkage of different process parameters to quality attributes. Furthermore, cultivation strategies for mammalian cell lines can be compared and evaluated. This resulted in a significant reduction in the number of experiments required during process development and process establishment.

Advanced pre-cultivation method for recombinant Escherichia coli cells (P21)

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E. coli cultures are widely used for recombinant protein production. Cultivation conditions play an important role in final protein yield and correct protein folding. The quality of the starter culture affects the recombinant protein production process, however there do not exist any standards for controlled pre-cultures. The EnBase technology, based on fed-batch principle, is widely applied for enhanced recombinant protein production. Here we investigate; whether EnBase based EnPresso media could also provide benefits for starter cultures. This study, at the example of a process for production of a thermophilic nucleoside phosphorylase in E. coli shows that EnPresso B medium based cultures, which can be directly inoculated from a frozen stock culture, provide a reproducible and robust starter culture for protein production processes.

Enhanced plasmid DNA production by enzyme controlled glucose release and an engineered Escherichia coli (P22)

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To evaluate the combination of a culture medium employing glucoamylase-mediated glucose release from a gluco-polysaccharide and an E. coli strain engineered in its glucose transport system for improving plasmid DNA (pDNA) production.
The production of pDNA was tested using E. coli DH5 grown in shake-flasks and the recently developed VH33 D(recA deoR)-engineered strain, which utilizes glucose more efficiently than wild type strains. Three glucoamylase concentrations for releasing glucose from the polysaccharide carbon source were used: 1, 2 and 3 U/L. Both strains reached similar cell densities ranging from 5 to 8.8 g/L under the different conditions. The highest pDNA yields onbiomass (YpDNA/X) for both strains were obtained when 3 U enzyme/L were used. Under these conditions, 35 ± 3 mgof pDNA l-1 were produced by DH5a after 24 h of culture. Under the same conditions, the engineered strain produced 66 ± 1 mgpDNA/L after 20 h. pDNA supercoiled fractions were close to 80 % for both strains.
The pDNA concentration achieved by the engineered E. coli was 89 % higher than that of DH5a. The combination of the engineered strain and enzyme-controlled glucose release is an attractive alternative for pDNA production in shake-flasks.

Enzymatic production of nucleotide analogues for click chemistry-based applications (P23)

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The use of enzymes as efficient and sustainable biocatalysts is especially interesting in those fields of industrial biotechnology that are currently dominated by chemical production processes. One of those fields is the area of molecular biology where modified nucleotides are increasingly used for labeling of PCR products and the production of fluorescent probes for fluorescence in situ hybridization (FISH) applications. Recently the application of copper catalyzed click chemistry is gaining more and more interest for those applications using 5-ethynyl dUTP, C8-alkyne-dUTP or C8-alkyne-dCTP as substrates.
In the presented study we are showing the enzymatic production of phosphorylated building blocks using 5-ethynyl-deoxyuridine as a model compound. The heterologous expression of all involved enzymes was optimized. Finally, the biocatalysts were successfully produced as his- or GST-tagged proteins in LB or EnPresso®1 medium and purified by affinity chromatography.
The production of 5-Ethynyl dUMP was achieved by using deoxynucleotide kinase2 with conversion rates between 50 and 100 %. The activities of guanylate kinase (GUK), CMP-UMP kinase (CMPK) and a pyruvate kinase (PK) were already shown with natural substrates. NDPs and NTPs were formed with yields of 30 to 70 %. The activities towards 5-Ethynyl dUMP remain to be investigated for those enzymes.

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Biological activity of halogenated cladribine and fludarabine derivatives (P24)

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Years after the approval of cytarabine and edoxudine as anticancer and antiviral agents, respectively, in 1969, nucleosides analogues still hold their place as an important class of medicinal compounds. The development of new molecules is focused on the improvement of drug properties with regard to: long term toxicity, resistance and bioavailability. In the present project, it is the objective to find the best conditions for the production of at least 25 different purine nucleoside analogues using thermophilic enzymes in the scale of 100 mg with purity ≥ 99%. The structures are confirmed using different spectroscopic analyses (MS/NMR). Finally different biological tests will be carried out. During the in vitro biological tests, it's intended to analyze the virostatic activity and to perform cytotoxic assays accounting for both solid tumors and hematologic malignancies.

Reconstructing the biosynthetic machinery of Ruminococcin A, a lanthipeptide identified from a strictly anaerobic Gram positive bacteria, in Escherichia coli (P25)

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Ruminococcin A (RumA) is a trypsin-dependent lanthipetide that is produced by the strict anaerobic Gram-positive bacterium Ruminococcus gnavus E1, isolated from human intestinal microbiome. The biosynthesis pathway of RumA involves, amongst others, the modifying enzyme (RumM) which catalytically installs three thioether crosslinks and an α,β-unsaturated amino, didehydrobutyrine into its core structure. The enhanced activity of the peptide against pathogenic clostridia further makes it a plausible target for the treatment of human infections and applications in livestock. Due to the nature of R. gnavus E1, standard cultivation strategies are obviously not optimal for large-scale production and subsequent pharmaceutical commercialization of RumA. In this work, we aimed at engineering the biosynthetic route of RumA in E. coli to enhance the development of a bioprocess scheme for a viable industrial production. We isolated the genes for the structural peptide (rumA) and the modifying enzyme (rumM) from R. gnavus E1 and co-expressed them in E. coli under separate regulatory mechanisms in order to balance expression in a way to guarantee a minimal load for the cell. We also investigated the behavior of the engineered strain with respect to the toxicity of the target product to the host. Our results show that active RumA is produced in E. coli which presents a very good basis for scale-up optimization. To our knowledge, this is the first time that a lanthipeptide from a strictly anaerobic Gram-positive microbe is heterologously expressed in a Gram-negative host.

Platform technologies for automated bioprocess development (P26)

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Bioprocess development is time and cost intensive due to the large design space to be investigated. Laboratory automation combined with experimental design strategies offer new ways to increase experimental throughput. However, often key parameters such are selected from screening experiments which do not resemble the conditions at production scale. At the chair of Bioprocess Engineering of the TU Berlin, a laboratory of the future concept has been established which features automated high-throughput cultivation systems combined with fed-batch growth media, chemooptical sensors and a modular database for data evaluation and modelling. The platform technologies were applied for of recombinant protein production process development from microbial hosts.

Novel bioprocess types based on CHO lysate cell-free protein synthesis systems (P27)

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Nowadays, different types of bioprocesses are available for the production of pharmaceutically relevant target proteins. In general, host organisms used during fermentation processes can be divided into four groups that comprise E.coli, yeast, insect cells and mammalian cells. The selection of suitable organisms depends on the protein production structure of the desired protein product process itself. Human proteins in particular play an essential role in therapeutical applications. Due to their complex structure, posttranslational modifications and elaborate folding procedures, these types of proteins need special production requirements and synthesis milieus. Therefore the most favorable systems for the production of functionally active human protein are based on cultured mammalian cells.
Currently, Chinese Hamster Ovary (CHO) cells are one of the most important and well-known mammalian cell lines used for industrial production. CHO cell fermentation processes were already implemented in industrial production during the 1980s. Over the last decades, the system was continuously developed and provides various possibilities that could be adapted for specific applications.
Besides the conventional in vivo protein production, alternative bioprocess modes, so called cell-free protein synthesis platforms were developed. Cell-free protein synthesis systems enable protein production in an open manner using cell lysates. This procedure provides the advantage that milieu conditions can be easily adapted to each type of protein, by for example adjusting redox conditions or addition of chaperons. Additionally, the protein production process in the cell-free system is faster compare to in vivo processes. Cell-free systems harbor the entire functionally active translational machinery, including ribosomes, translation factors and tRNAs. Prokaryotic cell-free systems enable high protein production rates, but they are limited in protein folding and modifications. For human target proteins and so called "difficult-to-express" proteins, cell-free systems are available that are based on eukaryotic lysates. Eukaryotic cell-free systems derived from Sf21 cells and mammalian cell lines harbor endogenous microsomal structures due to a mild cell disruption and lysate preparation procedure. Endogenous microsomes, obtained from the endoplasmatic reticulum, enable to implement post-translational modifications and directly integrate membrane proteins into their appropriate lipid environment.
To close the gap between conventional lab scale cell-free protein synthesis and in vivo protein production processes, we are developing cell-free production processes based on CHO cell lysates. CHO cell-free protein synthesis is implemented in two reactions modes which distinguish themselves by reaction conditions, protein yield and process costs. The "simply-to-perform" batch mode, a one pot reaction, provides protein yields up to 50 µg/ml. Synthesis of various proteins could be demonstrated in batch mode including integral membrane proteins, e.g. GPCRs, ion channels and glyco proteins as well as cytosolic proteins. Additionally, the possibility to perform cell-free synthesis in the presence of non-canonical amino acid was shown by incorporation into target protein. In this manner, new opportunities will be provided that enable for example specific fluorescence labeling of proteins. Besides the mentioned batch mode a novel, dialysis process mode is developed for cell-free protein synthesis. A compartmentalization of the process reaction into a reaction chamber and an additional feeding chamber leads to a prolonged reaction time, thereby increasing the total protein yield by removing inhibitory byproducts and delivering energy components along with the diffusion gradient. Different process optimization strategies for the dialysis mode enhance protein yields up to 10 times yields compared to batch based synthesis. With regard to the presented results our technology offers an enormous potential in terms of a novel production process for "difficult to express" proteins and their subsequent functional analysis.

Enhancement of lipopeptide yield by integrated bioprocess development (P28)

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Lipopeptide are surface active compounds and can be used as biosurfactants in many industrial areas including all application fields of chemical surfactants such as agriculture, food production and cosmetics and also in special fields like bioremediation. Moreover, they have antibiotic activity that allows to use the lipopeptides as plant protection agents. Currently relatively low yield and high-cost downstream processing make the production process of lipopeptide economically inefficient.
In this project a bioprocess for production of the lipopeptid fengycin, starting from strain screening, via optimization of media and cultivation conditions to downstream processing, using an in-situ product removal (ISPR) strategy by foam fractionation, was developed.
It was shown that using a cost-effective cultivation medium based on potato fruit juice (side–product from starch production), optimized cultivation conditions such as low temperature and oxygen-limitation and also by applying the integrated foam fractionation system, a five-fold increase of fengycin yield was achieved. At the end of the bioprocess development, concentration of fengycin in the cultivation media of more than 1gL-1could be obtained.

Docosahexaenoic acid (DHA) production of new strain of Indonesian Thraustochytrid Aurantiochytrium sp. LR52 in single use TubeSpin 600 system (P29)

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Thraustochytrids Aurantiochytrium sp. are unique marine microorganism in term of docosahexaenoic acid (DHA) production because they grow fast, produce high cell density, high proportions of lipid and DHA content. DHA has already proven as important fatty acid for infant development, maintenance of normal brain function, visual acuity, and prevention of neurological disorders and inflammatory diseases in adult. The application of DHA-producing Aurantiochytrium is rapidly expanding for food additive. In addition, dried Aurantiochytrium has GRAS status for use as feed to broiler chickens, laying hens, and aquaculture. The genus Aurantiochytrium sp. (before 2007 is Schizochytrium) belonging to the Labyrinthulomycetes of the kingdom Chromophyta (Straminipila). Aurantiochytrium sp. produced overall DHA productivity more than twice higher than another established species from dinoflagellate Crypthecodinium cohnii whereas the cultivation time of Aurantiochytrium was shorter than that of C. cohnii. In addition, Aurantiochytrium also showed a lesser incidence of unwanted microbial contamination than occurred in various Crypthecodinium cohnii fermentations. This has attracted many researchers and industry to screen new strain of Aurantiochytrium and optimize

the fermentation performance. Recently, we have isolated a strain of Aurantiochytrium from Indonesian mangrove habitat, namely LR52. Cultivation of Aurantiochytrium sp. LR52 in TubeSpin 600 with low artificial salt water (ASW) concentration of 3.6 gL-1 yielded dried biomass and DHA content more than 63 and 18 gL-1 , respectively in 6 days. The DHA production rate of LR52 is one of the highest value reported for any DHA producing microorganisms in shake flask scale.

Process analytical technologies

Spectroscopic bioprocess monitoring and control of CHO cell cultivations (P30)

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Process analytical technology (PAT), an initiative of the American Food and Drug Administration (FDA), provides tools for holistic, knowledge-based and risk avoiding control of manufacturing processes in chemical and pharmaceutical industry. One tool is online monitoring of critical process parameters (CPP) by spectroscopic methods combined with multivariate data analysis [1, 2]. This is particularly important for cost-intensive manufacturing of biopharmaceuticals to enable an early process fault detection and real time product release.
Mammalian cell cultivation (CHO-K1 cell line) in 15 l stirred tank reactor is chosen as a benchmark process for monitoring and control. Monitoring of apoptosis is carried out by UV/Vis spectroscopy. By non-invasive MIR spectroscopy substrate and metabolite concentrations (glucose and lactate) could be monitored during the cultivation. The results of spectroscopic inline measurement and consequential developed partial least squares regression models (PLS) are shown exemplarily. Based on the online glucose prediction an automated feed strategy is built-on to prevent an early cell death by substrate limitation. The process strategy is charged from batch to fed-batch process by this application and higher cell counts were realized.

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Mathematical model of adherent vero cell growth and poliovirus production in animal component free medium (P31)

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Sabin–IPV (or sIPV, inactivated polio vaccine based on attenuated Sabin strains) is anticipated to replace the oral polio vaccine (OPV) for the endgame in polio eradication [1, 2]. Optimization of sIPV production will lead to a better economically feasible vaccine [3]. The aim of our study was to develop a descriptive mathematical model able to capture dynamics of adherent Vero cell growth and poliovirus infection kinetics in animal component free medium to further support sIPV process optimizations.
To be able to monitor Vero cell growth closely, the experimental bioreactor set up included a Fogale biomass probe and a cell free sampling device for online metabolite analyses using a Nova BioProfile FLEX. Online biomass data corresponded well with the offline measured cell concentrations, also the on- and offline main metabolites (glucose, lactate, glutamine, glutamate, ammonia) were comparable (Fig 1). The developed model predicts the cell density, metabolites profiles and viral yields in time.
The viral growth model implied that the multiplicity of infection (MOI) and the time of infection (TOI) do not affect maximal poliovirus yields. However, they do affect the process time, which may be reduced by selecting a low TOI and a high MOI. Additionally, we observed a correlation between viral titers and D-antigen, a measure for immunogenicity, of Sabin poliovirus type 1.
The developed model is adequate for further studies of the cell metabolism and infection kinetics and may be used to identify control strategies to increase viral productivity. Increased viral yields can potentially reduce the costs of polio vaccines with could have large implications on public health. In addition, the biomass probe and the online metabolite analyzer may be used in combination with the model to monitor the cell and virus growth in a process analytical application (PAT).

Multiparameter monitoring of gradients in the liquid phase of fermentation processes (P32)

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Multiposition Sampling, Multiparameter Monitoring and Electrooptical Tracking of Microbial Activity: New Tools for better understanding biogas production (P33)

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Online-monitoring tools in batch and (semi-)perfusion cultivations for vaccine production (P34)

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The increasing availability of online-monitoring tools enables a better understanding and control of animal cell culture-based processes. However, real-time process monitoring of cell concentrations, nutrients and other parameters is still being limited in large-scale vaccine manufacturing. Nevertheless, for further intensification of vaccine production, i.e. the establishment of high-cell-density (HCD) perfusion processes, online-monitoring is of crucial importance. In the present work, the suitability of devices for online-monitoring of pH and dissolved oxygen concentration in shake flasks, as well as of glucose, lactate and cell concentrations in 1 L bioreactors for virus production processes was tested.
High cell concentrations (~50×106 cells/mL) were achieved either through medium renewal by periodic centrifugation (semi-perfusion) in 125 and 250 mL shake flasks or by using a hollow fiber-based (ATF or TFF) perfusion system for 1 L bioreactors. For shake flask cultivations, oxygen concentration and pH value were monitored during cell proliferation and MVA virus propagation in suspension AGE1.CR.pIX cells using the

SFR® system (Presens). In bioreactors, the concentrations of glucose and lactate were measured online during perfusion cultivation of AGE1.CR.pIX and batch cultivation of BHK-21 cells using a BioPAT® Trace probe (Sartorius). Additionally, viable and total cell densities were monitored online using the Incyte and Dencytee probes (Hamilton), respectively, during the propagation of MVA and influenza virus A/PR/8/34 (H1N1) in AGE1.CR.pIX cells, and yellow fever virus (YFV 17D) in adherent Vero and BHK-21 cells.
The SFR® system showed that pH values of 7.2 to 7.4 and dissolved oxygen concentrations above 86% can be maintained while cultivating AGE1.CR.pIX cells up to 45×106 cells/mL in shake flasks under a semi-perfusion regime. Interestingly, a reduced oxygen uptake rate of the cells could be observed when delaying the medium renewal. This seemed to correlate with the beginning of lactate consumption usually observed in batch processes when glucose concentrations drop below ~2.5 g/L and lactate concentrations increase above ~2.0 g/L. During the bioreactor cultivations, the BioPAT® Trace readouts correlated well with offline glucose and lactate measurements from a BioProfile® 100 Plus and allowed periodic adjustments of perfusion rates to keep optimal glucose concentrations. However, particularly during BHK-21 cultivations, other key metabolites rather than glucose also seemed to play an important role for optimal cell growth.
Concerning the total and viable biomass, online-measurements obtained with the Dencytee and Incyte probes during the cell growth phase of AGE1.CR.pIX, BHK-21 and Vero cells correlated fairly well with offline analyses carried out with a Vi-CELL® system (Beckman Coulter). As expected, the online monitoring of cell concentration was problematic for cultivations of BHK-21 cells at higher cell concentrations and after infection with YFV due to changes in cell physiology as well as cell morphology. Towards late-stage infection time points, measurements were not reliable. However, during MVA virus propagation in AGE1.CR.pIX cells, a correlation with offline analysis was observed even at late-stage infection times. Here, cell lysis during virus propagation could be followed by the frequency and capacitance readouts of the Incyte probe. In addition, it was observed that stepwise addition of large amounts of base to adjust the pH value, and the increase in gas sparging could interfere with the estimation of the viable and the total cell concentrations, respectively.
The SFR® offers reliable and valuable information of pH values and dissolved oxygen concentrations for characterization of cell lines in shake flasks. Although the BioPAT® measurements could be used for manual perfusion control based on glucose concentrations, additional specific nutrient requirements of each cell line should be taken into account and monitored if necessary. Online data obtained from the Incyte and Dencytee sensors can be useful to monitor cultivations, and for manual and automatic control of perfusion cultures. The Incyte also enables frequency and capacitance measurements that may support process control during virus production.

A PAT tool for real-time determination of spore quality in filamentous fungi bioprocesses (P35)

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Spore inoculum quality in filamentous bioprocesses is a critical parameter associated with viable spore concentration and spore germination [1]. It influences pellet morphology and, consequently, process performance [2]. An essential step before inoculation is the determination of the viable spore concentration, in order to apply quality control and decrease batch-to-batch variability. The state-of-the-art method to investigate this variable is tedious, associated with significant inherent bias, and not applicable in real time. Therefore, it is not usable as a process analytical technology (PAT). PAT aims to design, analyse and control pharmaceutical production in order to ensure product quality [3].
Monitoring of spore germination is not only necessary to investigate further spore quality attributes but can also be used as validation of the beforehand mentioned method. Quality attributes connected to spore germination are not only the amount of germinating spores but also how long spores need for germination and whether all spores germinate at the same time or not. Those elements were so far monitored using image analysis [4], which is hampered by complex medium background often observed in filamentous bioprocesses [5].
The method presented here is based on the combination of viability staining and large-particle flow cytometry which enables measurements in real-time and hence aims to be applicable as PAT tool. It is compatible with the complex medium background, and allows the quantification of metabolically active spores. Furthermore, spore swelling as a previous step of spore germination and germination itself may be monitored. A distinction of germinated spores from not germinated spores was based on a logistic regression using multiparameteric data from forward scatter and green fluorescence channel.
In an industrial bioprocess with filamentous fungi, a good correlation to CFU counts was found. The morphological parameters as spore swelling and spore germination as well as metabolic activity were followed over the initial process phase with close temporal resolution. The validation of the method to measure spore germination showed an error of spore classification of less than 5%. Differences in spore germination for various spore inocula ages and spore inoculum concentrations were monitored. Not all spores showing metabolic activity germinated. The amount of spores germinated was found to be dependent on the quality of the spore inoculum.
This combination of viability staining and flow cytometry is a promising tool to not only determine spore inoculum quality before batch inoculuation but also to at-line monitor spore swelling and spore germination in order to react in real-time on deviations and hence decrease batch-to-batch variability. Therefore the method could be applicable for the implementation as PAT tool in filamentous bioprocesses [6].

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Scalability of cultivations in the 2-dimensional rocking single-use bioreactor CELL-tainer (P36)

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Analysis of the single-cell size distribution as a process analytical tool in bioprocesses (P37)

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At line and on line microscopy is a promising technique to gain valuable data about population heterogeneity and even substrate conversion and product synthesis, as these steps have an impact on the cell size in various bioprocesses, e.g. cellular growth, intracellular lipid accumulation, etc. In this study, real-time monitoring of the single cell size distribution is investigated for the quantification of intracellular accumulation of the polyunsaturated fatty acid docosahexaenoic acid in the heterotrophic microalgae Crypthecodinium cohnii. The novel photo-optical microscopy probe of SOPAT was applied in situ and the performance was compared to at line 3-dimensional holographic interferometric microscopy (Ovizio), which provided also data about the surface structure, and traditional flow cytometry. It was proven that both, the at line and the in situ microscopy, are well-suited for the prediction of the fatty acid accumulation in cells, and thus can be applied as a process analytical tool. Further studies investigated the impact of oscillating cultivation conditions on the population heterogeneity in Saccharomyces cerevisiae cultures. Both tools are suitable to reduce times in process development and optimization.

Electrooptical monitoring of polarizability in Lactobacillus plantarum batch and fed-batch fermentations (P38)

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The degree of polarizability of cells depends particularly on the cells' physiological state [1] and is related to their viability: cells with high metabolic activity and intact energy household possess a larger polarizability [2]. The anisotropy of polarizability (AP) can be monitored on line applying electrooptical methods [2] with the help of automated sample preparation. In previous reports, we showed that the monitoring of the AP of rod-shaped bacteria in batch cultivations allowed the prediction of metabolic synthesis rates [3, 4].
The objective of our research is the investigation of whether different growth conditions and variable feed rates in anaerobic L. plantarum ATCC 8014 batch and fed-batch cultivations influence the AP, aiming to identify suitable cultivation conditions. The effects of substrate pulses, temperature variation, pH shifts and presence of oxygen have been investigated concerning influences on the AP. In all cases, the growth phase could be divided in several stages, based on AP spectra of four frequencies (200, 400 and 900 kHz and 2.1 MHz).
The monitoring of this parameter enable a deeper insight into the actual physiological state of cells in real time and without any staining or fluorescent markers, as used in flow cytometry. Finally, the automated measurement approach allows its application as a process analytical tool.

Simulation of biomass transport and deposition in expanded bed adsorption chromatography (P39)

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Integrative approaches in downstream bioprocess play an important role in developing cost effective purification process for bio-molecules with increased recovery [1, 2]. Expanded bed adsorption (EBA) is one of such integrated unit operations in protein purification with established hands on application in research as well as industrial sectors. However, EBA has been evidenced to suffer from the biomass interaction in an unclarified feedstock. Hence, understanding the rationale behind the biomass adsorbent interaction forms the basis of research while using EBA related approaches. Additionally, investigating the potential strategies to reduce this interaction form a challenging scientific question that has to be addressed which has gained current research interest in this area [3]. Present study consists of results obtained from biomass deposition experiments on expanded bed using Yeast and CHO cells as a model organism and Streamline DEAE and Fastline MabDirect Protein A as model adsorbents. Process was modelled in order to extract quantitative information such as, attachment efficiency (α), single-collector contact efficiency (η), particle deposition rate coefficient (kd sec-1), attachment rate ka1 [1/s] and detachment rate kd1 [1/s]. These parameters are compared with process modification by changing mobile phase properties or adsorbent surface properties.

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On-line state estimation applied to 550L filamentous fungal fermentations (P40)

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State estimation allows online prediction of state variables which are not able to be measured on-line by use of available online data. For bioprocesses, key states of interest include biomass concentration and product concentration which are seldom available as direct measurements [2]. This lack of knowledge of the current states in the system leads to challenges for process monitoring and control. There is therefore an interest in state estimation methods, in order to model these key process states based on available on-line measurements [1].
In this work a first principle model is applied to 550L scale filamentous fungal fermentation processes operated at Novozymes A/S. The model comprises of an online parameter estimation block, coupled to a physical model of the system. The parameter estimation block utilizes on-line off gas measurements and nitrogen source addition in order to model changing reaction rates in the system. Based on process stoichiometry, the current rates of product and biomass formation are then identified [3]. This parameter estimate is then used as an input to a dynamic mechanistic process model, which describes the mass transfer capabilities of the system based on the operating conditions, including stirrer speed, aeration rate and headspace pressure [4], [5]. The full model therefore provides insight into the current metabolic state of the system, and the oxygen transfer capacity in real-time. This process model is successfully applied on-line to eight new pilot scale batches as a state estimator in order to predict the biomass and product concentration, from robust, available on-line measurements. Such state estimators are valuable tools for online process monitoring, and control strategy development for on-line process control and optimization.

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Influence of agitation intensity on the morphology, rheology and productivity of Trichoderma reesei in fed-batch fermentations (P41)

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Filamentous microorganisms are widely used as hosts in the production of industrial enzymes. Nevertheless, they have major disadvantages, due to the unavoidable oxygen transfer limitations they present. This is a consequence of the high viscosities of the media they develop, which is related to high biomass concentrations, growth rate and morphology. An obvious strategy to overcome this problem is to increase agitation power. However, the question arises whether the fragmentation or morphological changes, which occur due to shear, have an influence on productivity in that case.
In this work, we determined the influence of agitation intensity on the morphology, rheology and protein production capability of Trichoderma reesei RUT-C30 in bench scale fermenters. Eight fed-batch fermentations were conducted in 2L bioreactors at different agitation speeds and with two different glucose concentrations in the medium during the batch phase. The fermentations were fed at very slow feed rates ensuring that no oxygen limitations conditions were encountered; lactose was used as carbon source in the fed-batch phase. All the fermentations received the same ratio of fed-carbon /initial glucose concentration. The fermentations were conducted at four different agitations speeds (600, 1000, 1400 and 1800 rpm).
For the fermentations ran at very high agitation speeds, higher CPR (carbon dioxide production rates) were observed for both media concentrations; the increased agitation speed also resulted in lower biomass concentrations. This suggests that the fungus is respiring at higher rates when the agitation speed increases to sustain the effort caused by the high shear rate, which is in agreement with [1]. However, the yields for extracellular protein over total fed carbon were not significantly affected. The morphology and rheology were considerably affected, i.e. higher fragmentation was observed at the higher agitation speeds – as expected – which led to lower viscosities.
This study has allowed us to conclude that agitation intensity can be manipulated in order to reduce oxygen transfer limitations and improve bulk mixing. It can also be used to reduce viscosity by affecting the morphology without affecting enzyme production. This is in agreement with the results from [2].

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