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Technische Universität Berlin
Bioverfahrenstechnik

IfGB
Institut für Gärungsgewerbe und Biotechnologie zu Berlin

5th BioProScale Symposium

Innovative scale up and scale down for bioprocess intensification

20 TO 22 MARCH 2018, BERLIN, GERMANY

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

+ Industrial scale biotechnology
+ Bioprocess development and automation
+ Bioprocesses for a circular economy
+ Single use bioreactors and applications
+ Biotechnology in Latin America
+ Microsensors for bioprocesses
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5th BioProScale Symposium

Booth B2

Welcome address

Dear Colleagues, Ladies and Gentlemen

My co-workers and I would like to warmly welcome you to Berlin to the 5th BioProScale Symposium.

Over the years, the biannual Bioproscale Symposium has become probably the main conference for industrial scale bioprocessing, scale-up/scale-down, and process development. Despite the overall growing number of conferences it has been attractive due to its focus in the general methodology of process development and characterization in the interdisciplinary field of chemical engineering and microbial physiology. Also we consider new developments in our field, such as circular economy, new sensors, single use systems, as well as digital and automation concepts in bioprocess development.

One of the really nice and important characteristics of this symposium is the focus on the bioprocess independent on whether it is a pharmaceutical, white industrial, or a bioenergy process. All these processes are connected by similar technologies and engineering principles. Thus specific needs in one of the different areas may lead to developments which also are beneficial for the others.

This time we got so many requests from academic and industrial scientists that we took the chance to plan the presentations in two independent streams. Although this means that each of us only can visit half of the presentations, this also gives us the opportunity to allow more young scientists to present their work, and a higher flexibility and broadness to the subject and at the same time gives more time for relaxing discussions in the coffee breaks. We hope that you will like this concept and will enjoy the symposium.

We are very glad that we had the chance to select over 70 presentations for the program, aside from many poster presentations 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.

This time we are proud to host you in the beautiful Langenbeck-Virchow house, one of the nicest conference locations in Berlin. This representative building close to the Charité is the main seat of two important medical societies, the Berlin Medical Society and the German Society of Surgery.

Finally, personally I would like to thank the VLB for supporting us again with their extraordinary expertise in the organization of international conferences, my colleges from the Chair of Bioprocess Engineering for their very enthusiastic help in all the small issues, and the members of the Scientific Advisory Board for their advises and practical support as Chairs.

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) and the organizing committee

Scientific advisory board

Mario Birkholz (IHP Frankfurt/Oder, Germany)
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Stuart Stocks (LEO Pharma, Denmark)
Ralf Takors (University of Stuttgart, Germany)

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 in Berlin for more than 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 the IfGB. 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 have been expanded also into the field of applied biotechnology.

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TUESDAY, 20 MARCH 2018

OPENING SESSION

Historical Lecture Hall (2nd floor)

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

13:20 **Plenary talk: Accelerated process development: How cell culture automation takes upstream process development to the next level (L01)**
Carsten Musmann, Roche Diagnostics GmbH, Penzberg, Germany

14:05 *Short break*

SESSION 1A: INDUSTRIAL SCALE BIOTECHNOLOGY

Chair Sjef Cornelissen / Peter Neubauer

Historical Lecture Hall (2nd floor)

Abstracts see page 12-14

14:15 **Keynote talk: From pilot to production: scaling industrial fermentation processes for enzyme production (L02)**
Sjef Cornelissen, Novozymes A/S, Copenhagen, Danmark

14:45 **Investigation of population heterogeneity caused by insufficient mixing in large-scale bioreactors (L03)**
Alexander Niess, University of Stuttgart, Germany

15:05 **Response of Yarrowia lipolytica to physico-chemical oscillations: Understating the cross-link between glucose availability and morphology (L04)**
Asma Timoumi, Université de Toulouse, France

15:25 **Metabolic diversity of 1,4-butanediol producing E. coli with respect to oxygen oscillations (L05)**
Viola Pooth, Forschungszentrum Jülich, Germany

15:45 **Industry talk**

15:50 *Coffee break and poster session and exhibition*

SESSION 1B: BIOPROCESS DEVELOPMENT

Chair Marco Oldiges / Matthias Gimpel

Room Bernhard von Langenbeck (1st floor)

Abstracts see page 14-16

14:15 **Keynote talk: Design of novel Pichia pastoris promoters with high performance in methanol-free fed batch (L06)**
Brigitte Gasser, BOKU Wien, Austria

14:45 **Effect of the different strain production kinetics in Bioprocess Development for recombinant protein production with Pichia pastoris (L07)**
Xavier Garcia-Ortega, Universitat Autònoma de Barcelona, Spain

15:05 **Model-based prediction of temperature and pH shift to increase volumetric productivity of a CHO cell fed-batch process (L08)**
Katrin Paul, TU Wien, Austria

15:25 **Stable animal cell growth in perfusion processes: Using on-line permittivity sensors to control cell-specific perfusion rates (L09)**
Alexander Nikolay, Max Planck Institute for Dynamics of Complex Technical Systems Magdeburg, Germany

15:45 **Industry talk**

15:50 *Coffee break and poster session and exhibition*

SESSION 2A: INDUSTRIAL SCALE BIOTECHNOLOGY

Chair Sjef Cornelissen / Peter Neubauer

Historical Lecture Hall (2nd floor)

Abstracts see page 16-19

16:35 **Keynote talk: Bioprocess optimization through yeast-derived nutrients selection is critical for probiotics industrial manufacturing (L10)**
Alain Sourabié, Procelys (Lesaffre Group), Maisons-Alfort, France

17:05 **Pragmatic scale-down approaches in downstream processes for shortening innovation cycles in industrial biotechnology (L11)**
Sven Hansen, Evonik Technology and Infrastructure GmbH, Germany

SESSION 2B: SPECIAL SESSION ITN RAPID BIOPROCESS DEVELOPMENT

Chair Timo Hardiman / Stefan Junne

Room Bernhard von Langenbeck (1st floor)

Abstracts see page 20-22

16:35 **Mechanistic models for design, monitoring, and control (L17)**
Krist Gernaey, Technical University of Denmark, Denmark

17:05 **Scaling down further: Model-based study of scale-up effects in minibioreactors (L18)**
Emmanuel Anane, TU Berlin, Germany

TUESDAY, 20 MARCH 2018

17:25	**Flexible biotechnology production – Considering variable upstream options (including transgenic plants and cell based) and innovative downstream Technology (L12)** *Dirk Steinhäuser, Glatt Ingenieurtechnik GmbH, Germany*	**Bioprocess scale-down strategy based on a novel automated CFD based compartmentalization technique (L19)** *Gisela Nadal-Rey, Technical University of Denmark, Denmark*
17:45	**Integrated product and process development to produce a cold-water protease for liquid laundry products (L13)** *Antony Calabria, DuPont*	**pH gradients in a 700 L lactic acid bacteria cultivation: Validation of computational fluid dynamic and compartment modelling (L20)** *Robert Spann, Technical University of Denmark, Denmark*
18:05	**Total process integration of upstream and downstream processing of monoclonal antibodies (L14)** *Martin Kornecki, Clausthal University of Technology, Germany*	**Cocci chain length distribution of Streptococcus thermophilus: Suitable gradient-induced stress indicator and scaling parameter (L21)** *Klaus Pellicer Alborch, TU Berlin, Germany*
18:25	**Industrial scale-up of the production of low cost poly(3-hydroxybutyrate-co-4-hydroxybutyrate) in Halomonas bluephagenesis TD40 based on "blue-biotechnology" via cell-growth adapted fermentation process optimization (L15)** *Jianwen Ye, Tsinghua University Beijing, China*	**A CFD model for the investigation of the sublimation phenomena during freeze-drying of lactic acid starter cultures (L22)** *Teresa Melo de Carvalho, Technical University of Denmark, Denmark*
18:45	**Mixing and fluid dynamics characteristics in conventional and single use bioreactors for improved design and scalability (L16)** *Martina Micheletti, University College London, UK*	**Moving bioprocessing into the cloud and towards big data – the first steps (L23)** *Eric Abellan, Infors AG*
19:05	**Industry talk**	**Industry talk**
19:10	**Industry talk**	**Industry talk**

EVENING PROGRAMME

19:15	**Poster session, Exhibition and Welcome Reception** *at the convention site*
21:30	*End of the day*

WEDNESDAY, 21 MARCH 2018

PLENARY TALK

Historical Lecture Hall (2nd floor)

9:00 **Metabolic engineering and systems biology: Driving the microbial chassis into the future (L24)**
Chris Brigham, Wentworth Institute of Technology, Boston, USA

9:45 *Short break*

SESSION 3A: BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair Joachim Venus / Sebastian Riedel

Historical Lecture Hall (2nd floor)

Abstracts see page 24

9:55 **Keynote talk: Process and product development, scale-up and implementation of applications for the use of by-products (L25)**
Thomas Grimm, ANiMOX GmbH, Berlin, Germany

10:25 **Polyhydroxyalkanoate production from biogenic waste streams (L26)**
Sebastian Riedel, TU Berlin, Germany

10:45 **Enabling of the industrial processing of P(HB-co-HHx) by compounding and reduction of recrystallization (L27)**
Christoph Hein, Fraunhofer IPK, Germany

11:05 **Industry talk**

11:10 *Coffee break and poster session and exhibition*

SESSION 3B: SINGLE USE BIOREACTORS AND APPLICATIONS

Chair Stuart Stocks / Anna Maria Marbà

Room Bernhard von Langenbeck (1st floor)

Abstracts see page 25-26

9:55 **Keynote talk: Application of single-use bioreactors beyond common cell line cultivation (L28)**
Nico Oosterhuis, Celltainer Biotech BV, The Netherlands

10:25 **Analysis of mixing behaviour and microcarrier suspension in a rocking single use bioreactor with in situ imaging methods (L29)**
Robert Panckow, TU Berlin, Germany

10:45 **Multiphase CFD simulations for supporting microcarrier-based human mesenchymal stem cell rocess development (L30)**
Valentin Jossen, Zurich University of Applied Sciences Zurich, Switzerland

11:05 **Industry talk**

11.10 *Coffee break and poster session and exhibition*

SESSION 4A: BIOTECHNOLOGY IN LATIN AMERICA

Chair Daniela Almeida / Juan Antonio

Historical Lecture Hall (2nd floor)

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11:15 **Keynote talk: Genome-scale metabolic reconstruction of Streptomyces clavuligerus as a tool for the identification of novel strain improvement (L31)**
Rigoberto Rios-Estepa, Universidad de Antioquia, Medellin, Colombia

13:25 **Obtaining an empirical model for kLa in stirred tank bioreactors of 7.5 and 80 L (L32)**
Luisa Fernanda Rojas, Universidad de Antioquia, Medellin, Colombia

12:45 **Anti malaria and anti cancer compounds production through plant cell culture in bioreactors (L33)**
Fernando Orozco-Sánchez, Universidad Nacional de Colombia, Colombia

13:05 **Tba (L34)**
tba

13:25 *Lunch break and poster session and exhibition*

SESSION 4B: SINGLE USE BIOREACTORS AND APPLICATIONS

Chair Regine Eibl / Stefan Junne

Bernhard von Langenbeck Hall (1st floor)

Abstracts see page 27-28

11:15 **Keynote talk: Single-use and sustainability: Continued studies emphasizing LCA tools (L35)**
William Whitford, GE Healthcare

13:25 **Numerical analysis of hydrodynamics in a standard stainless steel and a single-use bioreactor by means of an Euler-Lagrange approach: Comparison of flow, pH and mechanical stress (L36)**
Angélique Delafosse, Université de Liège, Belgium

12:45 **Rocking Aspergillus: Controlled cultivation of Aspergillus niger in a wave-mixed bioreactor for the production of secondary metabolites (L37)**
Tutku Kurt, TU Berlin, Germany

13:05 **Utilization latest state-of-art of disposable bioprocesses for production of high quality recombinant proteins (L38)**
Juozas Šiurkus, Thermo Fisher Scientific, Vilnius, Lithuania

13:25 *Lunch break and poster session and exhibition*

WEDNESDAY, 21 MARCH 2018

SESSION 5A: BIOTECHNOLOGY IN LATIN AMERICA

Chair Rigoberto Rios / Anika Bockisch

Historical Lecture Hall (2nd floor)

Abstracts see page 29-30

14:55 **Keynote talk: High yield plasmid DNA production under oxygen limitation using microaerobically induced replication (L39)**
Alvaro Lara, Universidad Autónoma Metropolitana-Cuajimalpa, Mexico

15:25 **Relationship between morphology and clavulanic acid production by Streptomyces clavuligerus in a single-use rocking bioreactor (L40)**
Howard Ramirez-Malule, Universidad del Valle, Cali, Colombia

15:45 **Experiences during the construction and operation of a pilot scale anaerobic digester plant for fruit waste treatment in Ecuador (L41)**
Daniela Almeida Streitwieser, Universidad San Francisco de Quito, Ecuador

16:05 **BIOGASTIGER®-System – Turning global organic waste streams into smart and clean energy (L42)**
Pierre Haider, FWE GmbH, Marktredwitz, Germany / Karen Guerrero, FWE GmbH, Quito Ecuador

16:25 *Coffee break and poster session and exhibition*

SESSION 5B: MICROSENSORS FOR BIOPROCESSES

Chair Mario Birkholz / Anders Henriksen

Bernhard von Langenbeck Hall (1st floor)

Abstracts see page 30-31

14:55 **Keynote talk: An autosampling micro-analyzer – Functions, performance, and prospects (L43)**
Gerhard Jobst, Jobst Technologies GmbH, Freiburg, Germany

15:25 **Development of a portable multi-parameter biosensor set-up for monitoring of biogas processes (L44)**
Johanna Pilas, FH Aachen, Germany

15:45 **Miniaturized spherical sensor probes for bioreactors (L45)**
Tim Lauterbach, TU Dresden, Germany

16:05 **Studies on the metabolic activity of microorganisms utilizing light-addressable potentiometric sensors (LAPS) (L46)**
Shahriar Dantism, FH Aachen, Germany

16:25 *Coffee break and poster session and exhibition*

SESSION 6A: BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair Saija Rasi / Sebastian Riedel

Historical Lecture Hall (2nd floor)

Abstracts see page 32-33

16:55 **Keynote talk: Seeing the scaling up- and down of biorefinery (L47)**
Jyri Maunuksela, Natural Resources Institute Finland, Jyväskylä, Finland

17:25 **Supplementing carbon sources with residues (L48)**
Friedrich Streffer, LXP Group GmbH, Berlin, Germany

17:45 **From waste to value: Green chemical production in mixed microbial cultures (L49)**
Helena Junicke, Technical University of Denmark, Lyngby, Denmark

18:05 **Keynote talk: Feedstock tolerance of continuous mode fermentation using agri-food residues (L50)**
Joachim Venus, ATB Potsdam, Germany

SESSION 6B: MICROSENSORS FOR BIOPROCESSES

Chair Mario Birkholz / Anders Henriksen

Historical Lecture Hall (2nd floor)

Abstracts see page 33-34

16:55 **Keynote talk: Standards and guidelines for microfluidic interconnections, building blocks and verification testing (L51)**
Henne van Heeren, enablingMNT -the Netherlands-, The Netherlands

17:25 **"Pipe based bioreactors" as tool for miniaturization and numbering up of bioprocesses (L52)**
Stefan Wiedemeier, ibaHeiligenstadt, Germany

17:45 **Droplet-based microfluidics and mass spectrometry: Detection of metabolites in nano-/picoliter reactors (L53)**
Konstantin Wink, Universität Leipzig, Germany

18:05 **Keynote talk: Minimalistic impedance instrumentation for process monitoring (L54)**
Uwe Pliquett, iba-Heiligenstadt, Germany

EVENING PROGRAMME

19:30 **Conference Dinner**
Brauhaus Lemke am Alexanderplatz

22:30 *End of the day*

THURSDAY, 22 MARCH 2018

OPENING TALK

Historical Lecture Hall (2nd floor)

9:00 **Industrial scale process optimization for manufacturing of scFv-class antibody (L55)**
Guido Seidel, Wacker Biotech GmbH, Jena, Germany

9:45 *Short break*

SESSION 7A: PROCESS ANALYTICAL TECHNOLOGIES

Chair Alain Sourabié / Anika Bockisch

Historical Lecture Hall (2nd floor)

Abstracts see page 35-36

9:55 **Keynote talk: Controlling microbial population at a single cell resolution: From cybergenetics to bio-process engineering (L56)**
Frank Delvigne, University of Liège, Belgium

10:25 **Budding index, a real-time parameter for monitoring (L57)**
Anna Maria Marbà Ardébol, TU Berlin, Germany

10:45 **In-line determination of spectral light distribution and biomass production in photobioreactors by fibre-optical methods: Spatially distributed light sensing and photon density wave spectroscopy (L58)**
Stephanie Schönfelder, University of Potsdam. Germany

11:05 *Coffee break and poster session and exhibition*

SESSION 7B: AUTOMATED BIOPROCESS DEVELOPMENT

Chair Krist Gerney / Nico Cruz

Room Bernhard von Langenbeck (1st floor)

Abstracts see page 36-37

9:55 **Multi-feed simultaneous saccharification and fermentation: Model-based development of high gravity lignocellulose-based bioprocesses (L59)**
Carl Johan Franzén, Chalmers University of Technology, Sweden

10:25 **Entering the next dimension: Microfluidic single-cell analysis of bacterial interaction (L60)**
Alina Burmeister, Forschungszentrum Jülich, Germany

10:45 **Using integrated process modelling towards understanding process variation across unit operations (L61)**
Christopher Taylor, Exputec GmbH, Vienna, Austria

11.05 *Coffee break and poster session and exhibition*

SESSION 8A: PROCESS ANALYTICAL TECHNOLOGIES

Chair Peter Götz / Anna Maria Marbà-Ardébol

Historical Lecture Hall (2nd floor)

Abstracts see page 37-39

11:55 **Tandem talk: Laser-based in situ back reflection analysis of particle sizes during feedstock pre-treatment by multi capture signal interpretation (L62)**
Stefan Junne, TU Berlin, Germany &
Herrmann B. Schwartz, Sequip GmbH Düsseldorf, Germany

12:20 **Comparison of vibrational spectroscopic techniques for PAT in industrial biotechnology (L63)**
Edo Becker, Keit Spectrometers

12:40 **In situ Raman spectroscopy as a promising PAT tool for monitoring of macro and micro-heterogeneity of antibody glycosylation (L64)**
Meng-Yao Li, LRGP, France

13:00 **SERS and Time-Gated (TG-SERS) Raman spectroscopy as advanced PAT tools for monitoring of lactic acid bacteria fermentations (L65)**
Martin Kögler, VTT, Oulu, Finland

13:20 *Lunch break and poster session and exhibition*

SESSION 8B: AUTOMATED BIOPROCESS DEVELOPMENT

Chair Carl Johan Franzén / Florian Glauche

Room Bernhard von Langenbeck (1st floor)

Abstracts see page 39-40

11:50 **Keynote talk: Beyond miniaturization and parallelization: Standard and tailor-made automated workflows for smart microbial bioprocessing (L66)**
Marco Oldiges, Forschungszentrum Jülich, Germany

12:20 **Model based design of experiments for high throughput bioprocess development (L67)**
Mariano Nicolas Cruz Bournazou, TU Berlin, Germany

12:40 **Mastering the digital transformation challenge in biopharmaceutical processing (L68)**
Michael Sokolov, ETH Zürich, Switzerland

13:00 **From automated genetic element screening to automated early stage bioprocess development in miniaturized stirred-tank reactors (L69)**
Nils Janzen, Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria

13:20 *Lunch break and poster session and exhibition*

THURSDAY, 22 MARCH 2018

SESSION 9A: BIOPROCESS DEVELOPMENT

Chair Frank Delvigne / Mattias Gimpel

Historical Lecture Hall (2nd floor)

Abstracts see page 40-42

14:20	**Keynote talk: Taking the microbial perspective: Computational fluid dynamics for bioprocesses design, operation and optimization (L70)** *Cees Haringa, DSM Biotechnology Center, Delft, The Netherlands*
14:50	**CFD-based strategy to optimize the impeller design for mesenchymal stem cells cultures in bioreactors (L71)** *Céline Loubière, Université de Lorraine, Metz, France*
15:10	**CHO cell-free protein synthesis for mammalian protein production and future bioprocess development (L72)** *Lena Thoring, Fraunhofer IZI-BB, Potsdam, Germany*
15:30	**Reactors for electrobiotechnology: Upgrading bioreactors for bioelectrosynthesis (L73)** *Luis Rosa, Helmholtz Zentrum für Umweltforschung, Germany*
15:50	**Keynote talk: Opportunities and challenges of model-based control of pellet growth in industrial-scale penicillin fermentation (L74)** *Timo Hardiman, Sandoz, Kundl, Austria*
16:20	**Closing remarks** *Peter Neubauer, TU Berlin*
16:30	*End of conference*

TUESDAY, 20 MARCH 2018

Opening Session (Historical lecture Hall, 2nd floor)

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

13:20 Plenary talk: Accelerated process development: How cell culture automation takes upstream process development to the next level (L01)

Carsten Musmann

Roche Diagnostics GmbH, Pharma Biotech Production and Development, Nonnenwald 2, 82377 Penzberg, carsten.musmann@roche.com

In this talk it will be presented how our in-house developed, fully automated multiwell plate (MWP) based screening system for suspension cell culture takes upstream process development to the next level. The development, the applications and the benefits of this cell culture system will be demonstrated. The system itself is characterized by a fully automated process workflow with integrated analytical instrumentation. It uses shaken 24 well plates as bioreactors which can be run in batch and fed-batch mode with a capacity of up to 576 reactors in parallel. Because the results of the multiwell plates are predictive for the bioreactor scale, the system was established as a routine instrument in upstream process development. The cell culture automation system is completed by an in-house developed and unique Process Control System (PCS). It enables the planning and evaluation of the screening experiments as well as the fully automated data handling.

In this talk an overview about several applications in late stage process development of using cell culture automation in contrast to standard bioreactor systems will be presented. In addition, the future trends to increase efficiency will be shown.All examples will show the potential of cell culture automation as a routine tool in process development.

14:05 Short break

Session 1A: Industrial scale biotechnology (Historical lecture hall, 2nd floor)

Chair Sjef Cornelissen / Peter Neubauer

14:15 Keynote talk: From pilot to production: scaling industrial fermentation processes for enzyme production (L02)

Sjef Cornelissen

Novozymes A/S, Copenhagen, Denmark, sjcf@novozymes.com

Novozymes is the world's largest producer of industrial enzymes. Our products help our customers to save energy, water and raw materials, to reduce waste and emissions, and to make everyday products more sustainable. Novozymes' enzymes are often manufactured using large scale aerobic fermentation processes. Scale-up and intensification of these processes are important activities within the company and there's an ongoing effort to improve these by increasing process knowledge. We do this by learning from our processes when scaling up to different sites around the globe and facing challenges that accompany this, for example, dealing with differences in equipment, utilities, raw materials etc. We also increase our process understanding by making models and validating them using real process data and to take control of our processes by developing and using new control strategies. For modelling and control work we often work together with universities and data resulting from such collaborations can be published. A major collaboration partner is the Technical University of Denmark (DTU).

This talk covers some of the challenges encountered when scaling up processes for the production of industrial enzymes. A couple of recent developments from modelling and control projects will also be discussed.

14:45 Investigation of population heterogeneity caused by insufficient mixing in large-scale bioreactors (L03)

Alexander Niess, Michael Löffler, Joana Simen, Ralf Takors

Institute of Biochemical Engineering, University of Stuttgart, Germany, Alexander.Niess@ibvt.uni-stuttgart.de

Bioproduction in large-scale bioreactors is often accompanied by a decreased product yield. This effect is due to substrate gradients formed by inefficient mixing of the culture broth and the spatial addition of substrate feed. Cells travelling between zones of different substrate availability inherently undergo stress response. The resulting transcriptional and translational response is costly and decreases product yields. For this study, a coupled stirred tank-plug flow reactor (STR-PFR) was used to investigate the impact of substrate gradients on the cultivation [1]. Simulation of transcription and translation for a whole bioreactor population inherently comes with a high computational demand. However, a detailed model including all mechanisms is not always preferable, due to the effort and numerical instability of such models. To cover these aspects, we developed a transcription and translation

model with a varying level of detail. By incorporating the key mechanisms, a suitable and stable model was developed for the given scenario. Furthermore the approach provides a fast and efficient simulation that allow the coupling of this single cell model with a process model for the given scale-down bioreactor. Only the coupling of both models allows a successful prediction of the observed short and long term transcriptional dynamics in a nitrogen-limited chemostat [2]. Detailed analysis of the population distribution revealed that over 60 % of the cells permanently undergo stress response. Furthermore, the simulations indicated that the population reaches a transcriptional steady state within ten minutes, whereas the translational steady state takes more than 15 hours to reach stable levels.

References: [1] Löffler M, et al., (2016), Metab Eng, 38: 73–85.[2] Simen JD, et al., (2017). Microb Biotechnol, 10: 858-872.

15:05 Response of Yarrowia lipolytica to physico-chemical oscillations: understating the cross-link between glucose availability and morphology (L04)

Asma Timoumi[1], Julie Lesage[1], Stéphanie Cenard[1], Eric Lombard[1], Stéphane E. Guillouet[1], Carole Molina-Jouve[1], Luc Fillaudeau[1] and Nathalie Gorret[1]

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Microbial populations may exhibit unpredictable behavior when shifting from laboratory scale to industrial conditions. A thorough understanding of the intricate interplay between the external parameters and cell physiology is thus highly needed for an intensified bioprocess [1, 2]. The present study focused on the effects of pH and dissolved oxygen (DO) fluctuations, typically encountered in large-scale bioreactors [3-5], on the physiology of the oleaginous yeast Yarrowia lipolytica. In this regard, transient responses of the cells to different perturbation profiles (frequency, duration) were examined using batch and continuous cultivation systems. The resulting behavior was then characterized at the metabolic (kinetics of growth and metabolite production), morphological (single cell analysis with flow cytometry) and transcriptional (RNA-seq approach) levels. This study showed no significant effect of the various conditions of pH and oxygenation on the kinetics, yields and viability of Y. lipolytica. Particularly, a slight accumulation of citric acid and a transient arrest of growth were detected during the temporary anoxic periods of the batch runs. Interestingly, our results revealed a dependence of the morphological appearance of the yeast on the residual glucose levels within the medium. Indeed, mycelia were only formed in glucose-excess environments (≥ 0.75 g L-1). Though, yeast-like subpopulations predominated under glucose limited conditions (≤ 8 mg L-1) for both types of stressors (pH and DO). Single-use micro-bioreactor experiments (continuous mode) with controlled glucose feeding strategies were then proposed to elucidate the cross-effect between glucose availability and dimorphism in Y. lipolytica.

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15:25 Metabolic diversity of 1,4-Butanediol producing E. coli with respect to oxygen oscillations (L05)

Viola Pooth[1], Kathrin van Gaalen[1], Frederik Bernhofen[1], Wolfgang Wiechert[1], Marco Oldiges[1]

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Regarding industrial application of microorganisms the investigation of dynamic process conditions is indispensable, since large scale bioprocesses are often affected by technical limitations leading to gradient formation in bioreactors. Hence, large scale processes challenge the robustness of microbial metabolism and physiology to overcome these inherent environmental changes. As a consequence, bioprocesses can suffer from reduced profitability as microbial metabolism and its performance are highly dependent on environmental conditions [1].

Focussing on the industrial production strain Escherichia coli ECKh 422, metabolic changes under various inhomogeneous conditions were investigated using a scale-down approach with compartment specific omics analysis. E. coli ECKh 422 is used for production of 1,4 Butanediol (1,4 BDO) from central metabolic intermediates via a heterologous biosynthetic pathway [2]. The 1,4 BDO production demands for microaerobic conditions, which adds a further level of complexity in terms of oxygen availability in large scale processes. When mimicking a microaerobic production process in a two-compartment scale-down bioreactor, the main STR compartment was microaerobic and the second one simulating inhomogeneity was operated either aerobically or anaerobically. The extracellular process data showed a clear phenotype in the scale-down system by reduction of product yield by 22 up to 77 %. Furthermore, the sensitive response to fluctuations of oxygen availability was displayed by omics data, which revealed intracellular adaptations with more than eightfold up- or downregulated proteins in the central carbon metabolism. Thereby, the genotype tailored for heterologous 1,4 BDO production under microaerobic conditions seems to restrict the options for metabolic robustness of the strain. Finally, central carbon metabolism, TCA cycle, glyoxylate metabolism and 1,4-BDO production were mostly affected by aerobic gradients.

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15:45 Industry Talk

15:50 Coffee break with poster session and exhibition

Session 1B: Bioprocess development (Bernhard von Langenbeck Hall, 1st floor)

Chair Marco Oldiges / Matthias Gimpel

14:15 Keynote talk: Design of novel *Pichia pastoris* promoters with high performance in methanol-free fed batch (L06)

Brigitte Gasser[1,2] Roland Prielhofer[1,2], Michaela Reichinger[3], Nina Wagner[3], Katrien Claes[3], Christoph Kiziak[3], Diethard Mattanovich[1,2],

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[3]Lonza AG, Visp, Switzerland

Protein production in Pichia pastoris often applies methanol-induced gene promoters such as PAOX1 to drive the expression of the target gene. The use of methanol has major drawbacks, so there is a demand for alternative promoters with good induction properties independent of methanol such as the PGTH1 promoter which we identified by a transcriptomics study based on simulation of a typical fed batch culture [1]. In order to further increase the potential of this promoter, we investigated its regulation in more detail by screening of promoter variants harbouring deletions and mutations. Thereby we could identify the main regulatory region and important transcription factor binding sites of PGTH1. We also created a PGTH1 variant, called PG1-3, with greatly enhanced induction properties compared to the wild type promoter.

Model based process engineering could successfully be implemented for PG1-3 to outperform the PAOX1-driven production in a simple feed regime, and to establish a speed fermentation with high titers after only two days total fermentation time.

[1] Prielhofer, R.; Maurer, M.; Klein, J.; Wenger, J.; Kiziak, C.; Gasser, B.; Mattanovich, D., Induction without methanol: novel regulated promoters enable high-level expression in Pichia pastoris. Microb Cell Fact 2013, 12:5.

14:45 Effect of the different strain production kinetics in bioprocess development for recombinant protein production with Pichia pastoris (L07)

Xavier Garcia-Ortega, Miguel Angel Nieto-Taype, Javier Garrigós-Martínez, José Luis Montesinos-Seguí, Francisco Valero Barranco

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Currently, the yeast Pichia pastoris is considered one of the most versatile and effective workhorse for recombinant protein production, including both biopharmaceuticals and industrial enzymes. The novel Bioprocess Engineering tools to develop and optimize this fermentation-based bioprocesses are moving from standard protocols towards rational conceptual approaches based on the particular features of the specific cell factory used and its physiology [1].

For fed-batch cultivations, the most used mode for recombinant protein production processes, the implementation of strategies that aims to achieve a specific growth rate by controlling the substrate addition in carbon-limiting conditions becomes a simple but interesting approach to adapt the operational strategy to the microbial physiology. In this sense, the concept "production kinetics", understood as the relationship between specific growth and production rates, is usually taken as a crucial parameter for the production strategies design and development [2].

When constructing novel producer strains, different factors such the promoter used or the gene dosage can affect importantly to the production kinetics. Currently, due to limited physiological knowledge, this relationship can not be theoretically predicted. Thus, to develop a process for a new strain of unknown behavior, an empirical determination of this relationship by means of basic physiological strain characterization is essential.

In the present work, two alternative P. pastoris producer strains of Candida rugosa lipase 1 with different gene dosage were compared obtaining significant differences in the production kinetics pattern. Therefore, leading also to important differences when determining the optimum strategy in carbon-limited fed-batch for each strain.

[1] Looser, V. et al., (2015),. Biotechnol Adv, 33: 1177–1193. [2] Garcia-Ortega, X et al.,(2013). Biochem Eng J, 79: 172–181

15:05 Model-based prediction of temperature and pH shift to increase volumetric productivity of a chinese hamster ovary cell fed-batch process (L08)

Katrin Paul[1,2], Vignesh Rajamanickam[1,2], Christoph Herwig[1,2]

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The expiration of patents on many top selling monoclonal antibodies, an increasing number of biosimilars will enter the European market. An advantage of biosimilars over original biologics lies in their reduced developmental costs and market price [1], leading to increased competition for manufacturers to optimize their production processes. One conventionally applied tool for the improvement of Chinese Hamster Ovary (CHO) processes is the decrease of temperature and pH to maximize product formation. Timing those shifts well is essential to gain the maximal benefits of both culture conditions. To identify the perfect timing for both shifts, multiple experiments need to be conducted. A decrease in the number of needed experiments can be achieved by using a model which can predict the product formation under aforementioned culture conditions.

The model from Amribt et al. [2], which describes Hybridoma metabolism, was adapted to meet the requirement. Since the influence of pH and temperature on cell culture performance is difficult to describe with mechanistic equations, different model parameter sets were generated for each condition. By switching from one model parameter set to another, different points of transition for temperature and pH shift could be assessed. The model predictions were validated with a cultivation, showing the ability of the model to predict product formation under different culture conditions. By following this approach process development can be carried out rationally rather than by trial and error, which is in alignment with Quality by Design (QbD) principles.

[1] Schiestl, M. et al., (2017), Drug Des Devel Ther, 11: 1509-1515. [2] Amribt, Z et al., (2013), .Biochem Eng J, 70: 196-209.

15:25 Stable animal cell growth in perfusion processes: Using on-line permittivity sensors to control cell-specific perfusion rates (L09)

Alexander Nikolay[1], Yvonne Genzel[1], Udo Reichl[1,2]

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Bioprocess intensification resounds throughout all areas of bio-production. In the field of animal cell culture, this comprises perfusion bioreactor systems aiming at increased cell concentrations. The challenge with advanced perfusion processes is an optimal process control. Most perfusion cultivations are based on fixed or adjusted reactor volume exchanges per day resulting in low reproducibility when cell growth alters or feeding rate switches are not well timed. Our approach focuses on the cellular demand for nutrients and feedback control of cell-specific perfusion rates (CSPR) using total viable cell volume (VCV) measurements.

We demonstrate the precise monitoring of EB66® (Valneva) cell growth using an on-line permittivity sensor (Incyte, Hamilton) in a bioreactor equipped with an alternating tangential flow (ATF2, Repligen) perfusion unit. A linear correlation of 'permittivity' to off-line measured 'total VCV' was found for cell concentrations in ranges of 3×105 –1.5×108 cells/mL. The on-line permittivity signal was transferred to an analog output box (Hamilton), which was connected to a pump system adjusting the substrate feeding. Based on on-line measurements of the total VCV, a fixed CSPR was obtained over the whole cultivation period without further interventions. For a virus production with EB66® cells, volumetric productivities could be maximized by factor three.

Taken together, we can maintain CSPR based on on-line measurement of the total VCV using permittivity sensors. The present approach can support metabolic flux analyses under quasi stationary process conditions, helps to standardize animal cell perfusion cultures for future applications and contributes to the improvement of product quality and quantity

15:45 Industry talk

15:50 Coffee break with poster session and exhibition

■ ■ Session 2A: Industrial scale biotechnology (Historical lecture hall, 2nd floor)

Chair Sjef Cornelissen / Peter Neubauer

16:35 Keynote talk: Bioprocess optimization through yeast-derived nutrients selection is critical for probiotics industrial manufacturing (L10)

Alain Sourabié

Procelys (Lesaffre Group), Maisons-Alfort, France, alain.sourabie@biospringer.com

Probiotics are microorganisms that exhibit a beneficial effect on the host health. After early researches and clinical studies the most challenging question remains how to manufacture them in a way to preserve cells fitness and activity. Probiotics are well-known for their complex growth requirements and especially the human microbiome origin strains. Related bioprocesses can be optimized by numerous ways and notably by culture media definition and nutrients selection. Widely used in culture media, yeast extracts and peptones (YE) contain a blend of unique nutrients e.g., peptides, amino acids, microelements and prebiotics.

As the global leader in YE Procelys provides high performance, animal-free ingredients for the probiotics industry. Anticipating the increasing demand for high quality and consistent nutrients, Procelys Labs have developed bioperformance assays to investigate YE influence on probiotic strains industrial manufacturing. This talk will present the results of the study aiming at evaluating the ability of selected YE to boost probiotic strains growth, viability and vitality. Experiments were performed at small scale and in bioreactors and the viable cells count and vitality measurements were carried out using flow cytometry and classical plating.

The use of selected YE allows reaching highly active biomass while maintaining cell fitness. Besides our results demonstrate an accurate nutrients selection procedure is pivotal for producing highly active probiotic strains. The NuCel® YE range specifically designed by Procelys, is the most efficient solution to ensure probiotics strains optimal growth and improve cells physiological state, while compliant with all relevant Food/Pharma regulations and requirements.

17:05 Pragmatic scale-down approaches in downstream processes for shortening innovation cycles in industrial biotechnology (L11)

Sven Hansen, Wilfried Blümke

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The classical approach for launching a new product in the white biotech industry is divided into two steps: First the process development is done in lab or even in pilot scale and afterwards, especially in downstream processing, a dedicated plant is designed and constructed according to the findings in the process development phase. Here, the process scaling follows only one direction, from small to large scale, and can operate with the complete freedom of the available technologies on the market. However, by following this procedure the design and construction of the plant take a considerable time.

Therefore, one way of shortening innovation cycles in biotechnological industry is to avoid long engineering projects by launching new products in already existing production plants, which usually have been designed for different products with different physical and chemical characteristics. When applying this approach reliable scale-down methods with the focus on the key parameters of the plant are necessary. With these methods the process can be adapted in the lab in a way that it can be scaled-up into the given plant. A concrete example from an industrial process will be presented to illustrate how pragmatic methods for scale-down and scale-up help to realize the production of a new product in existing production plants and, thereby, reducing the time to market.

17:25 Flexible biotechnology production – considering variable upstream options (including transgenic plants and cell based) and innovative downstream technology (L12)

Dirk Steinhäuser

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Time-to-Market is a key requirement for new pharmaceutical products and as well for the increase of capacity of existing production facilities. Fast and reliable scale-up methods from laboratory to pilot scale and the further transfer to commercial production are essential for the successful launch and implementation of new pharmaceutical products. Pre-designed modular plant concepts enable fast and flexible API production under defined pharmaceutical (GMP) conditions.

The synthesis of biotechnologically produced active pharmaceutical ingredients (API's) such as insulin, hormones, vaccines or antibodies requires efficient and stable cell lines as well as defined optimized process conditions. The presentation will show and compare variable upstream options (including transgenic plants and cell based) as well as innovative downstream technology referencing to different project examples. Production plants are primarily determined by their core process tasks, which are embedded in a suitable facility, complying with the specific requirements defined for pharmaceuticals, biotechnology and GMP. Pre-designed plant modules, layout typicals and facility structures are applied to accomplish rapid and 'first-time-right' plant conceptualization. The specific modular approach for both process and facility will be outlined with the help of visual examples for the different modular levels and project objectives. Conventional as well as single-use disposable systems will be discussed regarding different aspects, including fulfillment of basic requirements, critical parameters, flexibility, schedule and cost aspects. Key success factors and selected challenges encountered in different project examples will be discussed. Conclusions and 'lessons learnt' will be shared. Recommendations for users, producers and engineers will be given and discussed.

[1] Steinhäuser D., (2017), Biotechnology on the Silk Road, Manuf Chem, pg. 42-43.

17:45 Integrated product and process development to produce a cold-water protease for liquid laundry products (L13)

Anthony Calabria

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DuPont Industrial Biosciences discovers, develops, and manufactures bio-materials and bio-solutions using innovative enzymes, microbes, peptides and performance proteins to help improve the performance, productivity, and environmental footprints of our customers' products. These products are utilized in numerous markets including animal nutrition, food, detergents, textiles, carpets, personal care, and biofuels. A bioactive product example is the cold water laundry protease, which was developed with P&G and awarded the 2014 Sustainable Bio Award for Bio-Based Product Innovation of the Year. This novel protease enables the development of a laundry detergent that at 16°C matches the cleaning performance at 32°C. It was developed using a systems approach to protein engineering that screened for beneficial attributes in the production process, detergent stability, and laundry performance. Integrated process development was performed to identify favorable conditions in fermentation, product recovery and formulations. The result is a production process carried out at large-scale for one of the most widely used engineered enzymes in the world.

1. 2014 Sustainable Bio Award for Bio-Based Product Innovation of the Year 2. 2015 DuPont Sustainable Growth Excellence Awards

18:05 Total process integration of upstream and downstream processing of monoclonal antibodies (L14)

Martin Kornecki and Jochen Strube

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Optimizations in upstream processing concepts have led to increasing product titers and raised impurity profiles during the manufacturing of biopharmaceuticals, e.g. monoclonal antibodies [1–3]. Variations in composition of the complex cultivation broth represent challenges in the downstream processing of biotechnological produced proteins. Furthermore, existing challenges in process engineering rise, since regulatory agencies demand higher product quality as well as an advanced process understanding.

This talk will address a total process integration of upstream and downstream. The aqueous two phase liquid liquid extraction [4] in combination with a chromatographic operation, i.e. iCCC [5], is being used as alternative manufacturing route. The classification of host cell proteins into "The Good, the Bad and the Ugly" based on their physicochemical properties (i.e. isoelectric point, molecular weight and hydrophobicity) leads to an enhanced process understanding by characterizing the process separation efficiency [6].

Furthermore, bioprocess engineering will probably focus in regulated industries on quality by design and process analytical technology mechanisms, in order to design, analyze and control manufacturing processes [7]. The portfolio of online measurements can be extended by adding supplementary analytical methods such as infrared and Raman spectroscopy as well as off-gas analysis, preferably online. For this purpose, multivariate data analysis is being used in order to correlate spectral data with process variables.

The combination of chemometrics with a macroscopic kinetic model can in addition be used for the determination of model parameters or variables. This online determination of process related data shall lead to an improved process control by knowledge-based and statistical methods, which ultimately guarantees process robustness.

[1] Jain, E.& Kumar, A., (2008), Biotechnol Adv, 26: 46–72. [2] Shukla, A.A. & Thömmes, J., (2010), Trends Biotechnol, 28: 253–261 [3] Gronemeyer, P. et al., (2014), Bioengineering, 1: 188–212.[4] Schmidt, A. et al., (2017), Antibodies, 6: 21. [5] Zobel, S. et al., (2014), Ind Eng Chem Res, 53: 9169–9185. [6] Kornecki, M. et al., (2017), Antibodies, 6: 13. [7] Hinz, D.C.(2006), Anal Bioanal Chem, 384: 1036–1042

18:25 Industrial Scale-up of the Production of low cost poly (3-hydroxybutyrate-co-4-hydroxybutyrate) in Halomonas bluephagenesis TD40 Based on "blue-biotechnology" via cell-growth adapted fermentation process Optimization (L15)

Jianwen Ye

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The production of poly(3-hydroxybutyrate-co-4-hydroxybutyrate) (P(3HB-co-4HB)), was successfully scaled-up in a 5-m3 pilot bioreactor for continuous industrialized manufacturing using Halomonas bluephagenesis TD40 under non-sterile open fed-batch conditions with glucose, γ-butyrolactone and corn steep liquid (CSL) as substrates. Based on a cultivation study in shake flasks and cell growth characterization in a 7.5-L fermenter, a feeding strategy for glucose and γ-butyrolactone was designed to achieve a high yield of P(3HB-co-4HB) in 36h in 5-m3 fermenter. With the help of theoretical scale-up calculations, the pilot-scale fermentation test was very smooth and resulted in up to 99.5 g/L cell dry weight (CDW) with 60.4% P(3HB-co-mol13.5%4HB) accumulation. Moreover, an even higher PHA content of 74.12% was achieved by decreasing the use of CSL. Finally, stable and continuous manufacturing of low-cost P(3HB-co-4HB) was achieved by directly coupling the fermentation with the downstream extraction and drying process, which indicated the possibility that truly competitive low-cost production of P(3HB-co-4HB) will be realized in the very near future. In this study, an economic evaluation of the PHA powder manufacturing process was carried out based on the pilot-scale test. In this evaluation, the cost of PHA production on a scale of 5000 tons with a 4HB fraction and purity of 13-15 mol% and 90%, using the surfactant-hypochlorite digestion process, was only $3.47/kg. Furthermore, the addition of waste gluconate as carbon source would further reduce the cost by about 30%, which might potentially bring it under $2.5/kg.

18:45 Mixing and fluid dynamics characteristics in conventional and single use bioreactors for improved design and scalability (L16)

Martina Micheletti

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The pharmaceutical industry is at the forefront of the production of antibodies using mammalian cell-based cultures, with single-use technologies gaining prominence in the manufacturing process. Since the development of the first rocked bag bioreactor in 1997, other novel designs have been developed such as orbitally shaken, two and three dimensionally rocked, pneumatically driven, in addition to inflated cylindrical stirred bags. These have the potential to address new applications like expansion of adult stem cells for allogeneic therapies approaches by providing sufficient mixing while controlling maximum shear stress levels. Rigorous fluid dynamics studies are needed to understand the flow behaviour at different operating conditions, be able to determine meaningful dimensionless characteristics and establish robust scaling laws. A combination of different advanced analytical techniques were used to determine mixing and velocity characteristics and the validity of the approach is demonstrated by three case studies looking at different bioreactor flows relevant to bioprocessing.

In this work, a number of advanced analytical and laser diagnostics techniques were employed to conduct extensive and rigorous engineering studies in different bioreactor types. Phase-resolved Particle Image Velocimetry (PIV) and high frequency visual fluid tracking were used to investigate the flow pattern, mixing characteristics and shear stresses. A mixing time methodology, based on image processing and integrated with advanced post-processing techniques, was optimised and used to quantify the mixing efficiency. A flow visualization technique was developed for accurate evaluation of the minimum speed required for solid off-bottom suspension and for complete homogenisation. The data collected was used to define dimensionless scaling correlations towards better bioreactor design and better definition of operating conditions ranges.

19:05 Industry Talk

19:10 Industry Talk

Evening Programme

19:15 Poster Session, Exhibition and Welcome Reception at the conference venue

21:30 End

Session 2B: Special Session ITN Rapid Bioprocess Development

(Bernhard von Langenbeck Hall, 1st floor)

Chair Timo Hardiman / Stefan Junne

16:35 Mechanistic models for design, monitoring, and control (L17)

Krist V. Gernaey[1], Lisa Mears[2], Christian Bach[1], Daria Semenova[1], Patrick Bürger[1,3], Xavier Flores-Alsina[1] and Ulrich Krühne[1]

[1]Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical, Engineering, Technical University of Denmark (DTU), [2]Novozymes A/S, [3]Department of Particle Technology, Brandenburg University of Technology Cottbus-Senftenberg

Mechanistic models have the drawback that they require a significant investment of time and resources before a validated mathematical model is available, and this has traditionally been used as one of the arguments to discourage mechanistic modelling studies. However, we believe that application of such mechanistic models to multiple stages of fermentation process development and operation can anyhow make this investment highly valuable for industry and academia: In industry, an established fermentation model may be adapted for application to different stages of fermentation process development: planning, process design, monitoring, and control. Although a longer development time is required, this wide range of applications makes these models a highly valuable tool for fermentation research and development. In a research environment, collaboration is important, and developing mechanistic models provides a platform for knowledge sharing and consolidation of existing process understanding.

The presentation will be built around a number of recent case study examples, where each case study serves the purpose of illustrating a specific aspect of the mathematical modelling of bio-based production processes: Antibiotic production with Streptomyces coelicolor, with focus on including the improved prediction of phosphorus concentration dynamics in the process by considering precipitation and polyphosphate formation, as a result of the implementation of a generic physicochemical modelling framework. Mechanistic modelling of electrochemical biosensors, where the model is used to unravel the exact mechanism driving the electrochemical reactions, and to identify critical system parameters affecting the biosensor response. The latter information can then be used in the search for improved device designs. Computational Fluid Dynamics (CFD), generally used as a tool for the description of reactor hydrodynamics, but will here be presented as a modelling framework forming the basis for the development of compartment and scale-down models. Use of mathematical models to support the development of monitoring and control strategies of a Trichoderma reesei fermentation process

In conclusion, the practical usefulness of mathematical modelling is illustrated on the basis of several case studies, and aspects such as model modularity and model reuse are highlighted in view of achieving an efficient and streamlined model-building process.

17:05 Scaling down further: model-based study of scale-up effects in minibioreactors (L18)

Emmanuel Anane[1], Benjamin Haby[1], Sebastian Hans[1], Florian Glauche[1], Nicolas Cruz Bournazou[1], Peter Neubauer[1]

[1]Chair of Bioprocess Engineering, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany; anane@tu-berlin.de

The formation of concentration gradients in large scale bioreactors has attracted much attention in the bioprocess research community in the past three decades, due to the negative impacts of such gradients on process efficiency [1]. Conventionally, the impact of these so-called scale-up effects are studied in scale-down bioreactors in the laboratory [2]. In the same period, there has been a phenomenal increase in the use of high throughput (HT) miniaturized experimental platforms for strain screening and bioprocess development [3]. Yet another field of much progress is the use of mechanistic models for bioprocess development and control. However, these tools and concepts have mainly developed in parallel, with little or no interaction among them.

In this work, we combine the concept of mechanistic modelling with scale-down and HT systems to form a single, versatile platform for studying the response of strains to scale-up effects at the screening phase. As a demonstration, the HT-Scale-down platform was used to study the misincorporation of non-canonical amino acids (ncBCAA) into recombinant mini-proinsulin produced in E. coli using 24 parallel fed-batch cultivations in 2mag® minibioreactors. This misincorporation is a response of E. coli to oscillating glucose and dissolved oxygen concentrations in large scale fed-batch culture [4]. The results showed that in cultivations where the cells were subjected to model-derived glucose and dissolved oxygen gradients, there was a marked increase in the production and misincorporation of ncBCAA into the recombinant product, which significantly undermines the product quality. Thus, the platform offers the opportunity to combine robustness testing with strain screening for faster and more efficient bioprocess development.

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17:25 Bioprocess scale-down strategy based on a novel automated CFD based compartmentalization technique (L19)

Gisela Nadal-Rey[1], Christian Bach[1], Mads O. Albaek[2], Sjef Cornelissen[2], Jakob K. Huusom[1], Anna E. Lantz[3], Ulrich Krühne[1], Krist. V. Gernaey[1]

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Scale-down theory is a potential approach for mimicking large-scale processes in lab scale units in order to facilitate process design, characterization and optimization. Moving across scales is a challenging task, which requires a deep understanding of the process behavior, its limitations and bottlenecks under various operating conditions. Computational Fluid Dynamics (CFD) is a powerful tool to improve our comprehension in terms of the dominating phenomena such as mixing behavior, reaction kinetics and the resulting concentration gradients for e.g. oxygen, glucose, pH and temperature. This possibility provides a remarkable potential to develop representative scale-down designs.

In this work, a scale-down strategy is presented through using a novel automated CFD based compartmentalization technique. The developed compartmentalization method is a useful tool to identify and characterize a network of homogeneous regions (compartments) within an investigated volume with respect to one or a set of target process parameters. The heterogeneity within a large-scale reactor is simulated in a set of connected miniaturized reactors; where each individual reactor and the interchange flows are determined based on the extracted compartmental map from the initial scale.

This strategy was successfully applied in a microbial fed-batch production-scale fermentation process, shown in Figure 1. In this case, the compartmental map, volumes and the exchange fluxes were extracted by considering oxygen and glucose profiles in a 100m3 reactor volume. Finally, the generated map was updated with respect to the lab-scale limitations including the number and volume of connecting reactors, operating flow regime and pumping capacity to set the interchange flows.

17:45 pH gradients in a 700 L lactic acid bacteria cultivation: experimental validation of computational fluid dynamic and compartment modelling (L20)

Robert Spann[1], David Kold[2], Christophe Roca[2], Anna Eliasson Lantz[3], Ulrich Krühne[1], Krist V. Gernaey[1], Gürkan Sin[1],

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pH gradients that occurred during a Streptococcus thermophilus fermentation in a 700 L bioreactor were measured and predicted using multi-position pH measurements, computational fluid dynamic modelling, and compartment modelling. Heterogeneous culture conditions, such as the pH, might have a negative effect on the productivity of microorganisms in large-scale bioreactors. Prediction of gradients is therefore needed to improve biotechnological processes. To this end, we applied a validated computational fluid dynamic (CFD) model, and a six-compartment model of the 700 L bioreactor to simulate a pH controlled lactic acid bacteria (LAB) batch fermentation. A bio-kinetic model, describing the LAB growth and lactic acid production, and a pH correlation were incorporated into the CFD model (1.6 million nodes), and solved in ANSYS CFX 17.1.

The six-compartment model was designed based on the steady-state velocity profile of the CFD model, implemented in MATLAB 2017a, and then solved together with the kinetic model and a mixed weak acid/base model. Both approaches quantitatively predicted the formation of pH gradients in the range between 5.9 and 6.2, which were also measured at six vertically distributed positions during a fermentation. The compartment simulation is – depending on the application – a valuable alternative to the CFD simulation, since the computational CPU time was 2 s for the compartment model compared to 4 days on 20 CPU cores for the CFD simulation. Future studies are needed to further investigate the compartment model in order to take advantage of its promising potential e.g. for risk-based monitoring and control applications.

18:05 The cocci chain length distribution in *Streptococcus thermophilus* cultivations: A suitable gradient-induced stress indicator and scaling parameter (L21)

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Limitations in the power input together with increased mixing times cause the appearance of heterogeneities in production scale bioreactors. In lactic acid fermentation, an uneven distribution of the pH value occurs due to cellular acid production and external base addition. In order to study the impact of these gradients, pulse feeding experiments as well as two- and three-compartment scale down bioreactors were applied [1], thus aiming to mimic large scale production conditions.

The objective of the presented research was to investigate the effects of these oscillating environment on the chain length formation of Streptococcus thermophilus and the consequences concerning growth, substrate conversion, product formation and population heterogeneity. Therefore, automated microscopic image-based chain detection was applied to monitor and compare the chain length distribution throughout the course of cultivations under optimal as well as inhomogeneous pH values.

It was observed that S. thermophilus form chains of varying length and of different cocci shapes depending on the growth conditions [2]. On the one hand, a heterogeneous cocci chain length distribution, predominant under pH-oscillating environments, influences negatively cell growth. On the other hand, a more homogenous distribution with predominant diplococcal morphology is usually observed under optimal conditions. This chain length corresponds to the natural division of Streptococcus [3], whilst 3 and 4 cocci chains may be

expected due to a combination of cocci division and separation throughout ideal growth. Therefore, prediction of the process performance becomes feasible from an early time point on, thus allowing to optimize the process (in this case the mixing times) or adjusting scale down conditions while using the population heterogeneity as criterion to mimic the large scale appropriately.

Acknowledgments: The authors gratefully acknowledge the financial support from the EU-Horizon 2020 Marie Skłodowska-Curie ITN project BIORAPID (no. 643056) as well as the contribution of BioNukleo GmbH to the software development.

[1] Lemoine, A. et al., (2015), Biotech Bioeng, 112: 1220-1231. [2] Zapun, A. et al., (2008), FEMS Microbiol Rev, 32: 345-360. [3] Layec S. et al., (2009), Mol Microbiol, 71: 1205-1217

18:25 A CFD model for the investigation of the sublimation phenomena during freeze-drying of lactic acid starter cultures (L22)

Teresa Melo de Carvalho[1,2], Michelle M. Madsen[1], Anders Clausen[1], Krist V. Gernaey[2], Ulrich Krühne[2]

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Freeze-drying is a complex process that involves simultaneous heat and mass transfer to remove water from a wet product. This technique is commonly used in the pharmaceutical, food and biotechnology industries for the increase of shelf life and stability.

This process is both dependent on the physical form of the material to be dried and on the physical parameters and characteristics of the freeze dryer itself. Physical parameters of a laboratory/pilot freeze dryer are typically very different from production scale dryers, and therefore up and downscaling of the freeze-drying process is not trivial. Furthermore, since the main studies reported in the literature have been done for the pharmaceutical industry, the available information about the drying in bulk freeze-dryers in open trays, mainly used in the food industry, is limited. In this kind of freeze-dryers particles have irregular shapes and a random distribution in the trays which makes it difficult to create a model that can be translated to all kinds of particles.

Local temperature and moisture content in the product are essential for the design of efficient freeze-drying cycles. Consequently a fundamental understanding of water vapor flows during drying in a freeze-dryer is essential for the construction of an accurate model. The present study investigates the sublimation phenomena during freeze drying based on Computational Fluid Dynamics (CFD) techniques. The established model represents a good starting basis to determine the effect of different operating conditions during the freeze-drying of pellets in open trays.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie actions grant agreement No. 643056 (BioRapid). The authors gratefully acknowledge this financial support.

18:45 Moving bioprocessing into the cloud and towards big data – the first steps (L23)

Eric Abellan, Daniel Egger

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Storage handling and exchange of data have been hot topics in life sciences for years. While the discussions have often been focused on genomics and proteomics, bioprocess experts are now asking for solutions.

Therefore, the new generation of bioprocess software must fulfil the ever-growing requirements in this field. It should not only be able to import multiple offline data formats, but aggregate data from all devices in real time using standard protocols (e.g. OPC) or proprietary protocols. Furthermore, an interface capable of exchanging data with various 3rd party software is critical to save time and reduce errors.The adoption of new software technologies finally enables the development of performant, user-friendly solutions. The days of heterogenetic, unorganized datasets are over and big amounts of data from various bioprocess hardware or software can be handled easily in one central database.

By making bioprocess big data available and searchable in a fast way, an unlimited potential of information and knowledge is unleashed. New information technology lets us visualize bioprocess data and navigate the data jungle.

19:05 Industry talk

19:10 Industry talk

19:15 Poster session, exhibition and Welcome Reception at the conference venue

21:30 End

WEDNESDAY, 21 MARCH 2018

Plenary Talk (Historical lecture hall, 2nd floor)

9:00 **Metabolic engineering and systems biology: driving the microbial chassis into the future (L24)**

Christopher Brigham

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There is continued interest in sustainable productions of commodity products like plastics and fuels. With the advent of molecular biology and expansion of the genetic engineering toolkit, we can manipulate microbial strains to make them more efficient at producing value added compounds from a variety of carbon sources. In the scientific literature, many groups have devised methods of engineering biocatalysts to increase productivity of a value-added compound. However, a significant percentage of these methods simply involve adding or removing genes in a host organism, followed up by very little physiological or biochemical analysis. With the advances in molecular biology and genetic engineering technologies, there are multiple methods that can be employed, alone and in concert, to optimize industrial microbial strains.

The method of optimization to be used depends on the molecule being produced and the microbial chassis used to produce it. In the era of computation, big data and "omics" technologies, the microbial strain engineer can utilize genomic, transcriptomic, proteomic and metabolomic data to inform the construction of an optimal biocatalyst. Indeed, a rigorous understanding of the biology and physiology of an engineered industrial microbe is essential for efficient production strain engineering. Regardless of whether the production system is native or heterologous in origin, productivity can be increased by using the various biological and biochemical data to inform efforts on how to "streamline" the host. Analyzing carbon flux, metabolic control and key component interactions can provide important insight into engineering host metabolic pathways to work seamlessly for high-yield production of value-added molecules. Microbial polyhydroxyalkanoate (PHA) production represents an ideal teaching tool for studying metabolic engineering in microorganisms. Since PHA synthesis is directly tied into central metabolism, we can achieve an understanding of how polymer synthesis fits in with the basic metabolic processes of the cell. Furthermore, since the central metabolic pathways of most industrially relevant organisms are well-characterized, these metabolic engineering efforts represent an opportunity to (a) increase metabolic capabilities in native hosts and (b) streamline the construction of heterologous hosts to act as microbial chassis for biopolymers production. Using the PHA production model, it has been shown that productivity can be increased several fold without the need for significant scale up of the operation. In theory, this could be realized for other bioprocesses, as well.

9:45 Short break

Session 3A: Bioprocesses for a circular economy (Historical lecture hall, 2nd floor)

Chair Joachim Venus / Sebastian Riedel

9:55 Keynote talk: Process and product development, scale-up and implementation of applications for the use of by-products (L25)

Thomas Grimm[1]

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By-product streams are a good basis for the development of a bio-based industry. A favorable source for proteins are protein-containing by-products from agriculture (plant by-products from plant oil production) and food processing [1]. To extract these materials, ANiMOX develops processes to produce Protein hydrolysates and produces samples up to the kilo scale for the industrial tests with this material.

The patented hydrothermal hydrolysis of by-products developed by ANiMOX [2] allows, also in combination with enzymes, the separation of the contained substance groups' fat, protein and insoluble inorganic components in three phases. The protein hydrolysate obtained after phase separation is a complex mixture of water-soluble peptides and amino acids, which can be processed to dry powder or to liquid concentrate as required. The animal materials obtained have a protein content of more than 90 % relative to the dry substance and are largely free from fat and minerals. Vegetable materials, extracted from agriculture by-products, have a protein content up to 70 %.

Using the example of three development projects, for the technical use of rape protein [3], for the production of products for the food industry from animal residues and for the use of residual fats for the fermentation of PHA, extraction development and application will be presented.

[1.] Winter et al. (2015), BIOspektrum. 21 (2): 228-229 [2.] EP 1835816 B1. ANiMOX GmbH. Pr.: DE 102004063258 23.12.04. – Method for producing protein hydrolysates [3.] Fetzer et al. (2018), Industrial Crops and Products, 112, 236-246 [4.] Riedel et al. (2015), Journal of Biotechnology, 214, 119-127

10:25 Polyhydroxyalkanoate production from biogenic waste streams (L26)

Sebastian L. Riedel[1], *Ewelina Buziuk*[1], *Peter Neubauer*[1], *Stefan Junne*[1]

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Polyhydroxyalkanoate (PHA), fully biodegradable and biocompatible polyesters, are promising alternatives to petroleum-based plastics. Ralstonia eutropha, the model organism for PHA production, can store carbon up to 90% PHA of its cell dry weight under stress or nutrient limiting conditions. However, high production costs, are preventing their use as bulk material. Inexpensive carbon feedstocks can accelerate the commercialization of PHA. This study focuses on several biogenic waste streams as waste animal fats and waste fat emulgates with a high content on free fatty acids as carbon feedstocks. Besides being inexpensive, the low quality makes them very unattractive for other applications, e.g. the biodiesel production. However, the high melting temperatures >55 °C made it difficult to handle, and R. eutropha cells were unable to emulsify the feedstocks under cultivation conditions, and therefore did not grow. An emulsification strategy, which does not involve mechanical pretreatment or any emulsification agents, since this would not be scalable and would increase production costs was developed for these feedstocks. The cultivation process was scaled-up to 100 L with a total yield of 4 kg PHA. The wild type of R. eutropha was used for PHB production and a recombinant R. eutropha strain was used to produce the PHA copolymer poly(hydroxybutyrate-co-hydroxyhexanoate) (P(HB-co-HHx)). An increasing HHx concentration in the polymer directly correlates to an enhancement of the polymer properties as melting temperature, crystallinity and flexibility. Additionally, cultivation conditions for microwell plates were developed, which allows to perform 24 parallel cultivations for an accelerated bioprocess development in the future.

[1] Riedel et al. (2015), Journal of Biotechnology, 214(20):119-127

10:45 Enabling of the industrial processing of P(HB-co-HHx) by compounding and reduction of recrystallization (L27)

Christoph Hein[1], *Eckart Uhlmann*[1,2], *Julian Polte*[1]

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The processing of P(HB-co-HHX) in industrial scale is limited due to a recrystallization times of several hours. In particular, the processing of P(HB-co-HHX) raw material by injection molding is nearly impossible because of the disturbance of the thermo-dynamic equilibrium and the thermal degradation between cycles.

To improve the processability of the examined polymer, a comprehensive study on the correlation between polymer structure and thermodynamic behavior was performed. By the use of nuclear magnetic resonance analysis (NMR), differential scanning calorimetry (DSC) and gas chromatography the connection between molecular structure, HHX content and characteristic temperatures (melting temperature TM, glass transition temperature TG, solidification temperature TS) was established. By the compounding of the raw material with a specific chain extending additive, the recrystallization of P(HB-co-HHX) was significantly accelerated and reduced to an acceptable level for the processing by injection molding. Using a specifically designed injection molding tool and a micro injection molding machine tool, the replication of samples of P(HB-co-HHX) could be performed with cycle-times tC ≤ 10 s.

Session 3B: Single use bioreactors and applications (Bernhard von Langenbeck Hall, 1st floor)

Chair Stuart Stocks / Anna Maria Marbà

9:55 Key note talk: Cultivation in the 2-dimensional rocking single-use bioreactor CELL-tainer® (L28)

Nico Oosterhuis[1], Peter Neubauer[2], Stefan Junne[2]

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The application of single-use bioreactors (SUB) for microbial cultivations, especially of reactor designs beyond the traditional stirred tank, is usually regarded as crucial. Especially the usually low gas mass transfer coefficients are insufficient, however this is not true for 2-dimensionally rocking motion bioreactors like the CELL-tainer. Volumetric gas mass transfer coefficients (kLa-values) of 600 h-1 are achievable, which allowed bacterial fed-batch cultivations up to a cell density of 50 gL-1 at growth rates of 0.3 h-1 w/o any oxygen blending [1]. One major asset in this respect are the low maximum shear forces in comparison to stirred tank reactors. This feature might be beneficial when shear sensitive microbes or cell lines with a demand for low mixing times are cultivated, like marine phototrophic and heterotrophic microalgae, filamentous organisms or for the application of micro-carriers based cell culture.

Although rocking systems are not as good described as stirred systems in terms of traditional engineering parameters, the consideration of physiological and morphological states of cells in order to choose the best-suited system for an individual purpose compensates this. In this study, the advantages of a 2-D rocking bioreactor concept is shown from the mL to the 120 L scale at the example of the marine heterotrophic microalgae Cryptecodinium cohnii for the purpose of optimizing the production of the polyunsatured fatty acid docosahexaenoic acid [2]. In a phototrophic version of the CELL-tainer, green microalgae were cultivated, either under pure phototrophic or mixotrophic conditions. The system, if applied with the so-called expansion channels, can be used for seed train and scale up w/o changing the reactor, as the liquid volume can vary by two orders of magnitude. Coupling of such a reactor concept to continuous cultivation, e.g. perfusion, can circumvent limitations due to volume size and a large footprint in production scale, while maintaining the advantages of a considerable high power input at low shear forces.

[1] Junne, S. et al. (2013), Chemie-Ingenieur-Technik, 85(1–2), pp. 57–66. doi:10.1002/cite.201200149
[2] Hillig F. (2014), Eng Life Sci 2014, 14(3), p. 254-263

10:25 Analysis of mixing behaviour and microcarrier suspension in a rocking single use bioreactor with in situ imaging methods (L29)

Robert Panckow[1], Lutz Böhm[1], Michael Muthig[2], Jörn Emmerich[2], Matthias Kraume[1]

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The practical high yield culture (several million cells per ml) of anchorage-dependent cells by microcarrier culture cover working volumes ranging from a few millilitres up to a scale of several m3. Applications include the production of large quantities of cells, viruses and recombinant cell products (e.g. interferon, enzymes, nucleic acids, hormones). Appropriate mixing is necessary in these bioprocesses, but is usually introducing cell damaging shear stress by the flow field. Furthermore, cell stress may be caused by particle-particle collisions or damage by the stirrer or the wall and significantly decreases the production rate. An efficient mixing system which gives even suspension with gentle stirring and does not generate high shear forces results in a homogeneous culture environment and is most suited for microcarrier culture. [1]

All experiments were conducted in a CELL-tainer® 20L which patented two-dimensional motion concept combines rotatory and translatory movement. The operating parameters working volume V, rocking rate k and rocking angle ϕ were varied. Microcarrier types of two different suppliers (Carroucell, GE Healthcare) have been investigated. An in-situ working analysis method with high spatial and temporal resolution is selected to detect the suspended microcarriers by measuring their number and size distribution. [2, 3] The photo-optical SOPAT measurement technique for particle sizing is capable of acquiring raw data (two-dimensional images) of the dispersed phase (here: microcarriers) during the process and measure the sizes and shape by means of automated image processing followed by an analysis.The experiments clearly showed that a better description and understanding of the suspension behaviour in the investigated rocking bioreactor could be achieved by in-situ photo-optical measurement. An increase of the detected particle number for higher power inputs was observed and the operating conditions needed for a successful mixing to keep the microcarriers in suspension were identified.

Due to the high sensitivity of the measurement system, a precise differentiation of operating points was possible. Since the measurement technique could not only detect the required microcarriers, but also was able to distinguish between different dispersed phases, for example disturbing bubbles, it demonstrates its capabilities as valuable tool for particle analysis.

[1] GE Healthcare Life Sciences: Microcarrier Cell Culture, Principles and Methods, 2013, Handbook 18-1140-62
[2] Panckow et al.(2015), Chem. Eng. Technol. 38 (11) 2011-2016. [3] Maaß et al. (2012), Comput. Chem. Eng. 45 27–37

10:45 Multiphase CFD simulations for supporting MC-based hMSC process development (L30)

Valentin Jossen, Regine Eibl, Dieter Eibl

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Nico Multiphase CFD simulations forsupportingMC-based hMSC process developmentValentin Jossen, Regine Eibl,Dieter Eibl, Zurich University of Applied Sciences, Institute of Chemistry and BiotechnologyEmail: valentin.jossen@zhaw.chHuman mesenchymal stem cell (hMSC)-based therapies are of increasing interest in the field of regenerative medicine,as the large number of ongoing clinical trials (up to 190; 2017) illustrates. In order to produce the required number of hMSCs for one clinical dose (up to trillion cells), cost-effective and scalable production equipment is an indispensablerequirement. Economic considerations have shown that only stirred, single-use bioreactors in combination with microcarriers (MCs) are able to meet these demands. Even though the MC-technology has beenused for decades for the production of therapeutic proteins,challenges still exist, especially when used in combination with hMSCs,which are assumed to be more shear sensitive than conventional cell lines. This fact can be explainedby a) particle-particle and particle-impeller interactions, b) the formation of cell-microcarrier aggregatesand c) insufficient mixing conditions.Therefore, it is important that the systems andprocess conditions are characterized earlyonin process development. Normally, stirred bioreactors are characterized by traditional biochemical engineering methods and parameters. However, Computational Fluid Dynamics(CFD) hasbeen shown to be avaluable additional tool for this task. Using CFD,itis possible to consider the MCs as a secondary phase,based on Euler-Euleror Euler-Lagrangeapproach. Following a brief introduction to CFD, we aim to highlight how multiphase CFDsimulations can be used to increase knowledgeof MC-based hMSCs expansions. For this purpose, Euler-Lagrangesimulations will be presentedthat were used to calculate local particle related forces,based ona particle tracking approach.Furthermore,their effect on the culture over a defined culture period wasderivedfor the first time.

11:55 Keynote-talk: Genome-scale metabolic reconstruction of *Streptomyces clavuligerus* as a tool for the identification of novel strain improvement strategies (L31)

Rigoberto Rios-Estepa[1], León F. Toro[1], Laura Pinilla-Mendoza[1], Claudio Avignone-Rossa[2]

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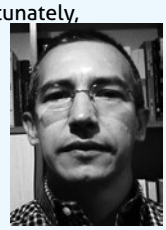

Streptomyces clavuligerus is a gram-positive bacterium, widely used in the production of clavulanic acid (CA); unfortunately, low titers are commonly obtained. The metabolic capabilities of microorganisms can be studied by combining experimental and mathematical tools. In this work, an expanded and updated genome-scale metabolic model of Streptomyces clavuligerus was reconstructed; the model included 1021 genes and 1494 biochemical reactions; genomic-reaction information was curated and new features related to clavam metabolism and to the biomass synthesis equation were incorporated.

Flux Balance Analysis showed that limiting concentrations of phosphate and an excess of ammonia accumulation are unfavorable for growth and clavulanic acid biosynthesis, consistent with known in vivo reports. The evaluation of different objective functions for flux balance analysis showed that maximization of ATP yields the best predictions for the cellular behavior in continuous cultures, while the maximization of growth rate provides better predictions for batch cultures. Through gene essentiality analysis, 130 essential genes were found

using a limited in silico media, while 100 essential genes were identified in amino acid supplemented media. Finally, a strain design was carried out to identify candidate genes to be overexpressed or knocked-out so as to maximize antibiotic biosynthesis while keeping those that are essential for cell growth and maintenance; potential metabolic engineering targets, identified in this study, have not been tested experimentally.

11:05 Industry talk

11:10 Coffee break, poster session and exhibition

■ ■ Session 4A: Biotechnology in Latin America (Historical lecture hall, 2nd floor)

Chair Daniela Almeida / Juan Antonio

12:25 Obtaining an empirical model for kLa in stirred tank bioreactors of 7.5 and 80 (L32)

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Plant cell culture has been carried out in several types of geometries, such as bubble columns, Air-Lift Bioreactors, Rotating Drums and Stirred Tank Bioreactors (STB), the latter being the most used. STB has been specially designed because individual cells are distributed homogeneously in the culture medium, avoiding sedimentation and controlling the size of cell aggregates. Additionally, is possible to maintain uniform temperature throughout the culture [1]. This geometry requires the additional adaptation of a sparger, which maintains the minimum oxygen levels required by the cells within the culture. The above generates different streams of flow inside the bioreactor, as a function of the speed of gas entry and stirring, being required to evaluate its effect on the performance of the cells inside the bioreactor, so one of the scaling criteria used in these systems is the kLa [2]. In the present work, the behavior of kLa was evaluated, in bioreactors of 7.5 and 80 L, in order to establish empirical correlations for similar impeller-sparger configurations in both work scales and thus study the effect of oxygen transfer, based on this criterion. These studies are based on the evaluation of the effect of kLa on cell growth of T. cacao in stirred tank bioreactor, previously carried out [3], where it is observed that for kLa values close to 27 h-1, better growth and production of polyphenols are obtained at laboratory scale.

[1] Furusaky, S.; Takeda, T. 2017. Bioreactors for Plant Cell Culture. Reference Module in Life Sciences. Elsevier. [2] Morchain, J. 2017. Numerical Tools for Scaling Up Biorreactors. Current Developments in Biotechnology and Bioengineering. 495-523. [3] Rojas, L.; Restrepo, M; Flórez, C.; Gallego, A.; Ríos R.; Atehortúa, L. 2014. Producción de Metabolitos del Cacao por Métodos Biotecnológicos. En: Artículos. International Conference on Food Innovation 2014. 3° Edition. EDUNER.

12:45 Anti malaria and anti cancer compounds production through plant cell culture in bioreactors (L33)

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Plant cell culture constitutes an important alternative for the production of secondary metabolites and heterologous protein. Our research group is producing antiplasmodial and anticancer compounds using plant cell culture. Plasmodium falciparum is a parasite responsible of malaria, a disease causing of thousands of deaths every year. The treatment is very difficult because the parasites develop resistance to conventional drugs. A possible alternative are the compounds produced by neem tree (Azadirachta indica). The in vitro antiplasmodial activity of the extract and some fractions from A. indica cell culture has been evaluated on chloroquine-resistant P. falciparum (FCR3) strains. The activity showed by the extract was promising (IC50 6.14 µg/ml). On the other hand, borojó (Borojoa patinoi) - an arboreal species native to Colombia, is used ancestrally for wound healing, pulmonary and diuretic problems, and hypertension control. In our research, an in vitro culture of borojo cells was established. MTT test showed the cytotoxic effect of crude extracts of the cells on Jurkat and MCF7 tumor cell lines. The extracts obtained with hexane and ethyl acetate at 150 µg / ml reduced cancer cells viability between 45 and 57%. Selectivity towards tumor cells was observed, presenting a greater effect in cancer cells. With these results, it could be possible to profile as future alternative to control malaria and to treat some kinds of cancer, some drugs using extracts from A. indica and B. patinoi cell culture, respectively.

13:05 Tba (L34)

tba

13:25 Lunch break, poster session and exhibition

Session 4B: Single use Bioreactors and Application (Bernhard von Langenbeck Hall, 1st floor)

Chair Regine Eibl / Stefan Junne

11:55 Single-use and sustainability: Continued studies emphasizing LCA tools (L35)

William Whitford, GE Healthcare

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Life cycle assessments (LCA) are the best way of comparing the environmental impacts of producing biologicals using traditional, single-use or hybrid process technology. A comparison of these platforms across the full process train including upstream and downstream operations will be presented. This comprehensive study using monoclonal antibody as the model product includes the effect of regional differences and various end-of-life disposal options.

12:25 Numerical analysis of hydrodynamics in a standard stainless steel and a single-use bioreactor by means of an Euler-Lagrange approach: Comparison of flow, pH and mechanical stress (L36)

Angélique Delafosse, Sébastien Calvo, Dominique Toye

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In recent years, single-use bioreactors (SUB) have been increasingly used for cell culture in pharmaceutical industry. However, as far as we known, no comparison between single-use bioreactors and standard reusable ones has been done, at least not at an industrial scale. So, the aim of this work is to critically compare hydrodynamics generated inside a single-use Cultibag STR (SUB) to the one in a standard reusable stainless-steel bioreactor (SSB). The operating conditions (working volume V = 1 3 and power input P/V = 1 W/m3), as well as the comparison criteria (flow structure, pH and mechanical stress), have been chosen in relation to conditions typically used in industrial adherent cell cultures.

The flow structure and the spatial distribution of mechanical stress were characterized using CFD (ANSYS Fluent, standard k-epsilon model). A CFD-based compartment model [1] was then used to compare mixing and pH spatial distribution after an alkali addition. Finally, the trajectories of several particles was simulated with a stochastic model [2] to obtain circulation and residence time distributions in zones of high pH and high mechanical stress.

Significant differences in terms of hydrodynamics are observed between the two bioreactors. Because of the absence of baffles, the flow in the single-use STR is highly tangential compared to the SSB. As a consequence, the mixing time in the STR is 2.5 times longer. Spatial distributions of pH or mechanical stress differ between the two bioreactors, as well as the exposure frequency and duration to high values of pH or mechanical stress.

[1] Delafosse A. et al., (2014), Chem Eng Sci, 106: 76-85.]2.] Delafosse A. et al., (2015), Chem Eng Sci, 134: 457-466.

12:45 Rocking Aspergillus: Controlled cultivation of Aspergillus niger in a wave-mixed bioreactor for the production of secondary metabolites (L37)

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Background: Filamentous fungi including Aspergillus niger are cell factories for the production of organic acids, proteins and bioactive compounds. Usually, stirred-tank reactors (STRs) are used to produce these compounds in an industrial environment. However, agitation via mechanical stirring poses high shear forces thus affecting fungal physiology and macromorphology. The impact of these shear forces and interconnections between form and function are hardly accessible, since a reduction of the stirrer speed would result in a much lower gas mass transfer. Thus, a comparison would always include effects due to oxygen limitation. In this study, the two-dimensional rocking-motion bioreactor (RMB) CELL-tainer® was used to investigate the impact of low shear forces on the growth, macromorphology and antibiotic product formation of A. niger for the first time. This becomes feasible as a sufficient gas mass transfer is achievable in both, the STR and RMB.

Results: We investigated the impact of hydrodynamic conditions on A. niger cultivated at a scale of 5L in the CELL-tainer CT20 (Cell-tainer Biotech) and in the STR (with a stirring speed of 750 rpm). Two different A. niger strains were analysed, which heterologously produce the commercial drug enniatin B. Both strains expressed the *esyn1* gene encoding a non-ribosomal peptide synthetase ESYN under control of the inducible Tet-on system, but differed in their dependence on feeding with the precursors D-2-hydroxyvaleric acid and L-valine. Cultivations of A. niger under low shear forces resulted in the formation of large pellets, which were heterogeneous in size (diameter 300 – 800 µm). Such pellet formation was not observable during STR cultivations. By adding microtalc particles, however, we were able to reduce the pellet size and pellet heterogeneity (diameter 50 – 150 µm). This reduction had an effect on the enniatin production, which increased in parallel to a reduced pellet size. Overall, enniatin B titres of about 1.5 – 2.3 g/L were achieved in the CELL-tainer system with pellet formation, which is about 30-50% of the titres achieved under STR conditions w/o pellet formation.

Conclusions: This is the first report studying the potential use of 2-dimensional rocking-motion for the cultivation of A. niger and the investigation of the impact of low shear forces under controlled conditions. A clear dependency between pellet formation and product yield was observable. However, pellet formation is the standard form for industrial large-scale cultivation to avoid high viscosities and large gradient formation. Thus, although final enniatin yields are not competitive yet with titres achieved under STR conditions, wave-mixed cultivations could open up new avenues for the cultivation of shear-sensitive mutant strains as well as high cell-density cultivations by taking advantage of the large surface area to provide sufficient oxygen to highly viscous culture broth.

13:05 Utilization latest state-of-art of disposable bioprocesses for production of high quality recombinant proteins (L38)

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Approach of Single-use bioproduction process (SUBP) becoming widely accepted by biomanufacturers due to lower cost of development, implementation, operation and required capital investments. It enables to drastically reduce the risks of cross-contamination, ensure continuity from early development through manufacturing, flexibility of scaling up/down and sustainability. During our research we have addressed the major aspects of development of disposable bioprocess, especially scale up/down and transferability of microbial fermentation from conventional steel-tank conditions to Single Use. Furthermore, we have utilized major advantages of SUBP for the unique niche of manufacturing of DNA-free PCR enzymes. We have developed new closed bioproduction system based on integrated single-use technology including UPS and DST to drastically minimize the risk of DNA contamination inherent to the conventional manufacturing process. To help ensure conformance to strict purity requirements, we subject our DNA-free PCR reagents to stringent quality tests to verify that products are free of contaminating bacterial, human, and plasmid DNA.

13:25 Lunch break, poster session and exhibition

Session 5A: Biotechnology in Latin America (Historical lecture hall, 2nd floor)

Chair Rigoberto Rios / Anika Bockisch

14:55 Keynote talk: High yield plasmid DNA production under oxygen limitation using microaerobically induced replication (L39)

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With the aim of increasing plasmid DNA (pDNA) production under oxygen limitation, a self-inducible replication system was created. An extra copy of the gene coding for rnaII, which is a positive control molecule for pMB1-derived replicons, was placed under control of the lac or trp promoters and cloned in plasmid pUC18. The modified plasmid pUC18-Ptrc rnaII resulted in a strong overexpression of rnaII which in turn triggered the plasmid copy number in more than the double of that of pUC18. Based on this, a microaerobically-inducible plasmid was created by inserting an extra copy of rnaII under control of the microaerobic promoter from the Vitreoscilla hemoglobin (Pvgb). Such plasmid was tested in fed-batch cultures of the strain W3110 recA- in which dissolved oxygen was depleted for nearly 6 h. Upon oxygen depletion, rnaII was efficiently induced and pDNA titer increased steadily for pUC18- Pvgb rnaII, reaching nearly 400 mg/L. In contrast, only 200 mg/L of the unmodified pUC18 were obtained. In order to improve cellular performance under oxygen limitations, an engineered strain W3110 recA- vgb+, which expresses the Vitreoscilla hemoglobin encoded in the chromosome, was used. The amount of pUC18 produced by W3110 recA- vgb+ under oxygen limitation doubled that of W3110 recA-. However, when pUC18- Pvgb rnaII was used, the engineered strain produced only 20 mg/L. Moreover, the size of the obtained plasmid was strongly shortened. Plasmid sequencing revealed that an important fraction of the origin of replication was lost. These results demonstrate the feasibility of microaerobically-induced pDNA production, and that the performance of genetic constructions depend on the strain used. Furthermore, unexpected changes in plasmid fidelity can arise when using genetically modified strains.

15:25 Relationship between morphology and clavulanic acid production by *Streptomyces clavuligerus* in a single-use reactor CELL-tainer (L40)

Howard Ramirez-Malule[1], Stefan Junne[2], Peter Neubaue[r2], Rigoberto Rios-Estepa[3]

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Clavulanic acid (CA) is a potent β-lactamase inhibitor produced by Streptomyces clavuligerus (S. clavuligerus) [1]. CA is frequently used in combination with β-lactam antibiotic to treat infections caused by β-lactamase-producing bacteria. However, low yields of CA are usually obtained in submerged cultures [2]. Since S. clavuligerus is a filamentous bacterium, CA production is probably influenced by morphology in submerged cultivations, which is also a common phenomenon in other actinomycetes and filamentous fungi [3–5]. Besides, S. clavuligerus could be catalogued as shear sensitive [6]. In this study, morphology, tricarboxylic acid cycle intermediates and CA production were investigated/monitored during fed-batch of S. clavuligerus with the single-use bioreactor CELL-tainer®. Succinate, pyruvate, malate, oxaloacetate, acetate and CA were accumulated simultaneously under phosphate-limited conditions. The morphological changes were examined with a standard microscope (40x magnification). A putative relationship between morphology and CA production was also observed. Thus, morphological changes in S. clavuligerus could eventually be used as fed-batch monitoring tools for CA production.

[1] Brown, A. et al. (1976), J. Antibiot. (Tokyo). 29, 668–669 [2] Ramirez-Malule, H. et al (2016), J. Theor. Biol. 395, 40–50 [3] Yin, P. et al. (2008), J. Chinese Inst. Chem. Eng. 39, 609–615 [4] Singh, K. P. et al. (2012), J. Ind. Microbiol. Biotechnol. 39, 27–35 [5] Paul, G. et al. (1999), Biochem. Eng. J. 3, 121–129 [6] Pinto, L. S. et al. (2004), Bioprocess Biosyst. Eng. 26, 177–184

15:45 Experiences during the construction, ramp up and operation of a pilot scale anaerobic digestion plant for fruit waste treatment in Ecuador (L41)

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In 2010 a project proposal for the design and construction of a fully automated pilot scale anaerobic digestion plant won the call for the implementation of innovative technologies InnovaEcuador. The call was funded by the Ecuadorian government through the Ministry of production, employment and competitiveness (MCPEC). The proposal as well as the process and plant design was prepared by the Institute for Development of Alternative Energy and Materials IDEMA. Though the beneficiary of the project was a local fruit processing industry, which generated ca. 4000 kg of fruit waste per week. The design established that the organic waste should be pre-treated and fed into the anaerobic digester 7 days per week with an estimated biogas production of 600 m^3 per month, representing an energy production of 3600 kWh per month or a reduction of electric energy costs of 250 USD/month. Despite the energy production, given the energetic, legislative and productive situation of Ecuador the main product of interest is not the biogas, but the organic fertilizer obtained by the anaerobic digestion process, known as *biol.*

In 2012 the pilot plant was operational and the ramp up took place successfully. Personnel from IDEMA – USFQ initiated its operated, stabilized it and accompanied its functionality for 12 additional months. In 2013 the plant was handed out to the company. After four years of regular operation an evaluation of the project and of the anaerobic digestion process has been performed. The experience and results of the final evaluation will be presented in this study.

[1] Daniela Almeida Streitwieser (2017), Bioresource Technology 241, 985–992 [2] Streitwieser et. al (2015), The 9th International Conference in Chemical Kinetics, Abstract Book, ISBN 978-90-9029-133-8, 205-209 [3] Streitwieser et al. (2010), Müll und Abfall, 08, 379 – 382 [4] Streitwieser et al. (2010), Avances en Ciencia e Ingeniería, USFQ, Vol. 2; C11 – C16

16:05 BIOGASTIGER®-System – Turning global organic waste streams into smart and clean energy (L42)

Pierre Haider[1], *Karen Guerrero*[2]

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Where humans live, an enormous amount of biogenic residues (particularly manure, household waste, residues such as straw) are originated by agriculture, animal husbandry, food production and their utilization. The often uncontrolled rotting/composting leads to a high output of climate-wrecking gases. 10 - 12% of anthropogenic greenhouse gas emissions are caused by agriculture. The agricultural share of global methane (CH_4) emissions is 50% due to beef cattle and rice farming.

Without appropriate counteractive measures, the emission values will continue to increase. According to forecasts, an increase in emissions of CH4 by approx. 60 % and N_2O by 35 - 60 % is expected by 2030. The share of climate wrecking gases such as CO_2 also increases. It is about 50% higher than that in a biogas plant.

BIOGASTIGER® is a standardized industrial certified modular biogas plant which is suitable both for power-network-stabilizing "on-grid" application to generate power and heat on demand and for establishing an "off-grid" energy supply. By means of a standardized conception, the BIOGASTIGER®-System makes it possible to use biogenic potential of organic materials to generate energy in such regions where until now only biomass depositing or composting has been possible.

In-situ process-surveillance, online controlled from far distances is an inevitable part of a reliable and efficient concept of a next generation industrial standard "biogas-machine" like the BIOGASTIGER®.

16:25 Coffee break, poster session and exhibition

■ ■ Session 5B: Microsensors for bioprocesses (Bernhard von Langenbeck Hall, 1st floor)

Chair Mario Birkholz / Anders Henriksen

14:55 Key note talk: An autosampling micro-analyzer. Functions, performance, and prospects (L43)

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The yet unmet desire for the automatic monitoring of culture parameters like glucose, ammonium, glutamine, and lactate concentrations becomes feasible with an emerging micro auto-analyzer. Enabled by micro bio/chemical sensor-arrays and reliable micro-pumps, functionalities of usual table top biochemistry analyzers are provided within a package, small enough to be intimately attached to a sampling micro-canula. Aspirated micro-liter volumes of un-filtered broth are analyzed without any dilution avoiding concerns with respect to unwanted sample alterations by filtering or dilution.

This presentation will describe the function and performance of the first-generation analyzer for glucose and lactate monitoring and furthermore will give an outlook of use-scenarios and further analytes and features with regard to subsequent analyzer generations.

15:25 Development of a portable multi-parameter biosensor set-up for monitoring of biogas processes (L44)

Johanna Pilas[1,2], *Thorsten Selmer*[1], *Michael Keusgen*[2], *Michael J. Schöning*[1,3]

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The monitoring of biogas processes is crucial for efficient conversion of organic material to methane. However, analysis of several key parameters, such as the concentration of different organic acids and fatty acids, conventionally requires complex analytical methods like high pressure liquid chromatography or gas chromatography. In this work, an electrochemical multi-analyte biosensor is presented that enables simultaneous detection of four intermediates, namely formate, ethanol, D- and L-lactate. The biosensor-chip (14 × 14 mm2) features five platinum working electrodes, each with a diameter of 2 mm, and a counter electrode (area 40.5 mm2) [1]. For amperometric detection a bi-enzymatic system is used, which consists of a nicotinamide adenine dinucleotide (NAD+)-dependent dehydrogenase in combination with a diaphorase from Clostridium kluyveri. Each working electrode is modified by chemical immobilization of diaphorase and a different dehydrogenase. In this way, an electrochemical detection principle is realized for simultaneous measurement of different analytes at the same working potential of +0.3 V vs. Ag/AgCl (oxidation of enzymatically produced ferrocyanide).

For facile application of the multi-parameter biosensor, a compact and portable measurement device was developed which enables on-site monitoring of organic acids and alcohols. The successful application of the biosensor set-up is demonstrated in real samples, such as maize silage, inoculum and fermentation sludge from different biogas plants.

[1] Pilas, J. et al., (2017), Electrochimica Acta, 251: 256-262

15:45 Miniaturized spherical sensor probes for bioreactors (L45)

Tim Lauterbach[1], Felix Lenk[1], Thomas Walther[1], Michael Grösel[2], Stephan Lenk[2], Tassilo Gernandt[3], Rüdiger Moll[3], Franziska Seidel[4], Dietmar Brunner[4], Tobias Lüke[5], Christian Hedayat[5], Maik-Julian Büker[5]

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Standard sensor probes for biotechnological purposes capture environmental conditions only in one spot of the reactor. While those measurements depict accurately the conditions in the bioreactor for homogeneously mixed processes, heterogeneities in not ideally mixed reactors cannot be detected. Furthermore, the integration of such rod-shaped, wired probes pose a problem when it comes to reactors in which sensor ports are not provided due to design limitations (e.g. shaking flasks, tube or flat panel bioreactors etc.).

We propose a new approach in alternative to state of the art sensors for biotechnology processes, the so called Sens-o-Spheres. Omitting the cable connection while condensing the whole sensor geometry to a small sphere which is only 8 mm in diameter allows a flexible usage in very different reactor types. The re-usable sphere consists of a data and a charging antenna, a micro-controller for the functional coordination, a rechargeable battery and all within a laser welded encapsulation for the use in biotechnological processes. Onto this sensor platform a temperature sensor is mounted. This spherical measurement device moves passively through the reactor volume and transmits the recorded measuring values wirelessly via radio frequency waves to a receiver.[1]

Here we present data using the Sens-o-Sphere system under laboratory conditions namely in shaking flask cultivations and in lab-scale bioreactors. Even under such harsh conditions as a bacterial cultivation in a stirred tank reactor the mobile sensors maintain a stable radio signal which is consistent with the standard probes.

[1] Lüke, T. & Lauterbach, T., (2017), TechnoPharm, Bd. 7, Nr. 5, S. 254–259

16:05 Studies on the metabolic activity of microorganisms utilizing light-addressable potentiometric sensors (LAPS) (L46)

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Real-time monitoring of the metabolic activity of microorganisms can avoid complex process disturbances at an early stage in different bioprocess applications. For instance, process downtimes in biogas plants caused by metabolically inactive microorganisms can induce irreversible hindrances and cost-intensive interventions, which can be prevented by utilizing an efficient monitoring system. Short response times, small size, mass fabrication and solid-state nature of field-effect-based potentiometric sensors such as LAPS (light-addressable potentiometric sensors) are promising candidates enabling a low-cost and precise monitoring system. LAPS provide a spatially resolved concentration detection of an analyte solution and can record 2D-chemical images of concentration changes of (bio-)chemical species on its flat surfaces [1]. In this work, a LAPS-based multi-chamber measuring system with 16 laser-diode modules was developed. The differential LAPS measurement principle was implemented to eliminate external influences such as temperature fluctuations, pH value variations and sensor signal drifts. Simultaneous measurements were facilitated by applying multi-chamber structures measuring the acidification rates of microorganisms, like Corynebacterium glutamicum and Escherichia coli K12 to study their cellular metabolization after glucose uptake. With the knowledge about the acidification behaviour of each microorganism on the sensor surface in a multivariate analysis, a signal-response pattern can be realized. In this way, a correlation between the sensor signal and the status of the biogas process can be determined.

[1] Dantism S. et al., (2017), Electrochimica Acta 246: 234-241

16:25 Coffee break, poster session and exhibition

Session 6A: Bioprocesses for a circular economy (Historical lecture hall, 2nd floor)

Chair Saija Rasi / Sebastian Riedel

16:55 Keynote talk: Seeing the Scaling up- and down of biorefinery (L47)

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Side products formerly called as waste streams contain lots of interesting chemistry. Instead of side products we should regards these chemical as pre-chemistry, side-chemistry and after process chemistry. All these streams can be controlled by process parameters and raw materials. Why are we not doing this in large scale? It is basically a simple thing - we do not know how valuable these streams are, we don't want to risk the quality of the main product and after all companies does not have resources to study these streams and markets and nonexistent for this kind of products. We have to take small steps to right direction and keep on developing.

There is a huge potential of different raw material for biorefineries in Finland. The amount of potential biomass is approximately 54 million t/a. This includes different organic waste and side-streams and agricultural biomass. When forest-based materials are included, the possibilities increase remarkably. Anaerobic digestion (AD) is an effective and well-known process for the treatment of biodegradable waste, when both energy and fertilizers are produced. The forest based raw materials are usually not seen as raw materials for AD because of high lignin content but different new biorefinery concepts have increased the interest to include AD in process chain. For example the biorefineries can utilize AD originated volatile fatty acids (VFA's) in their own process. Including these processing chains to forest biorefinery, more options could raise also in farm scale where processing options for both, agricultural and forest based materials are needed.

[1] Marttinen, S. et al. (2015), Research report no 1.1.3-4. BEST project final report. www.bestfinalreport.fi [2] Tampio, E. et al. (2017), 2nd Bioeconomy Congress, 12-13th September 2017, University of Hohenheim, Stuttgart, Germany, Book of abstracts

17:25 Supplementing carbon sources with residues (L48)

Friedrich Streffer[1]
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Oil is the basis of modern life, ranging from energy production to production of basic chemicals. Thanks to highly efficient processes for extracting and refining crude oil is nowadays utilized economically, but our oil reserves are finite. Therefore, great efforts are made to realize concepts for supplementing oil - the biorefineries. Currently biorefineries produce chemical base materials on an industrial scale from readily available sugar -or starch-containing plant components. However, sugar and/or starch, the substances that are also food, account only for about 1% of the available plant biomass. By far the greater part of the plant biomass is made up of inaccessible lignocellulose, the main component of plant residues. Significant cost determining factors of a biorefinery are commodity prices, costs and expenses of the fermentation / chemical process and for product workup in the downstream process. Only effective solutions in all three steps will secure the economic energy and raw material production at today's conditions.

LXP is a tech company, active in the field of industrial biotechnology, developing, marketing and licensing of technical solutions for the economic and ecological processing of plant residues on the basis of closed carbon and mineral cycles to maximize the ecological and economic efficiency of biotechnological processes. The core technology is based on a patent protected pretreatment process called LX-Process. The process provides alternative/2G-carbohydrates/-sugars and is easy to integrate into biotechnological processes. It allows the conversion of virtually all carbohydrates of lignocellulosic non-food materials to chemicals or biofuels. Additionally, sulphur free lignin can be provided.

17:45 From waste to value: Green chemical production in mixed microbial cultures (L49)

Helena Junicke[1], *Xavier Flores-Alsina*[1], *Krist V. Gernaey*[1]
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Today's chemical production and energy supply strongly depend on fossil feedstocks. This is not only linked to problematic greenhouse gas emissions. The limitation of fossil resources also means there is an imminent end to traditional hydrocarbon economy and industries will need to deploy production concepts on the basis of renewable commodities [1]. Mixed culture biotechnology holds promise to become a corner stone in next generation chemical production platforms [2]. Unlike microbial pure cultures requiring sterile substrates, mixed cultures can produce valuable chemicals from low-value feedstocks and even waste streams. However, controlling the product spectrum in mixed microbial bioconversions remains a key challenge and progress in this direction is hampered since generalized process models are not yet encompassing the formation of higher value products [3].

Here we present general extensions to state-of-the-art process models that allow an improved implementation of resource and energy recovery in the frame of mixed microbial conversions. Taking the example of butanol, an energy-rich biofuel with properties similar to gasoline, we demonstrate how continuous production strategies can be established. These strategies rest on ecological selection principles aimed to direct the microbial population structure towards a desired product space [4]. We anticipate our model framework to be the starting point for intensified research at the intersection of environmental and industrial biotechnology with a mission to leverage the full potential of circular economy.

[1] Mansouri, S. S. et al.(2017), Current opinion in chemical engineering, 18, 1-9 [2] Kleerebezem, R. et al. (2007), Current opinion in biotechnology, 18(3), 207-212 [3] Batstone et al. (2002), Water science and technology, 45(10), 65-73[4.] Junicke, H. et al.(2016), Applied microbiology and biotechnology, 100(2), 915-925

18:05 Keynote talk: Feedstock tolerance of continuous mode fermentation using agri-food residues (L50)

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Enormous amounts of agricultural residues and food waste are produced globally every year and mostly burned or disposed. Alternatively, their use as feedstocks in biotechnological processes provides an innovative way to convert 'no-value' material into 'value-added' products. This lecture introduces to opportunities of using those materials as feedstocks in fermentative lactic acid production.

Originally, lactic acid was produced from starch-based substrates like glucose. Increasingly, however, non-food biorenewables such as lignocellulosic feedstocks, agri-food residues and by-products are also being used as raw materials for the production of microbial lactic acid. The goal is to develop a fermentation process based on the substitution of expensive nutrient supplements by cheaper materials due to their main proportion of the whole process costs.

Examples of lactic acid fermentation using different types of organic residues as substrates are shown. Furthermore, pre-treatment methods and future perspectives in view of a so called feedstock-tolerant process are presented. As an option for local/regional availability of lower quantities of different feedstocks the question raises to what extent a continuous mode fermentation process would be able to tolerate changing input materials. First results will be illustrated for experimental set-up in lab-scale.

Exploitation of high quality lactic acid for the production of biodegradable polymers is one of the recent applications. Conventional processes for down-streaming are based on precipitation steps that generate large amounts of chemical effluents. Consequently the environmental impact and the operating costs of traditional processes can be reduced by using alternative technologies, such as electrodialysis with monopolar and bipolar membranes.

[1] Pleissner, D. et al. (2014), ACS Symp. Series, Vol. 1186 "Green Technologies for the Environment", 13, 247–263

19:30 Conference dinner at the Brauhaus Lemke am Alexanderplatz

(individual arrival)

■ ■ Session 6B: Microsensors for Bioprocesses (Historical lecture hall, 2nd floor)

Chair Mario Birkholz / Anders Henriksen

16:55 Keynote talk: Standards and guidelines for microfluidic interconnections, building blocks and verification testing (L51)

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Microfluidic devices are increasingly used for measuring and processing. They offer several benefits over conventionally sized systems by using less volume of samples, chemicals and reagents reducing the global fees of applications. Many operations can be executed at the same time thanks to their compact size, shortening the time of experiment. They also offer an excellent data quality and substantial parameter control which allows process automation while at the same time improving the yield. Integrating microfluidic components from different suppliers into systems often needs ad hoc solutions leading to reliability problems. Cleary industry agreements about interconnections and component formats would simplify matters for designers and producers of microfluidic devices. That goes even more for industry wide supported quality standards. Such agreements will make working with microfluidic devices easier and more reliable; i.e. will lead to plug & play microfluidics. A particular problem in the microfluidic industry is the wide variation in technologies and materials used. Therefore, to make the standards widely applicable, they should be formulated in such a way that they are independent of technology and application. A multinational group of microfluidic companies and institutes agreed on such specifications.

The proposed standards and guidelines are published or will be published as White Papers to ensure wide dissemination. Several of the standards developed so far are currently being transferred to the International Standard Organization (ISO) to become official standards.

17:25 "Pipe based bioreactors" as tool for miniaturization and numbering up of bioprocesses (L52)

Stefan Wiedemeier

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Droplet based microfluidics possesses a promising application potential in the field of drug screening for personalized medicine or for pharmaceutical research, to mention only a few. Precondition for all applications is a stable and reproducible generation, manipulation, and cultivation of droplets. As droplet based microfluidics bases on the application of at least two immiscible fluids, a technical system for droplet manipulation has to be adapted to the properties of the used fluids. iba has developed a technological platform called "pipe based bioreactors" that meets these requirements and thus allows for the cultivation of microorganisms, cells and even 3D cell structures [1, 2]. Up to 500 droplets, acting as bioreactors, could be serially arranged in a microchannel were the droplets are separated by a water-immiscible fluid like oil. The platform is composed of functional chip-based modules e.g. for droplet generation, addition of fluids like drugs, and for droplet detection [3, 4]. The special chip manufacturing technology allows for manufacturing of chips for the generation of droplet volumes down to 10 nL. The chip manufacturing technology as well as representative applications and the application potential of the "pipe based bioreactors" platform will be presented.

[1] Lemke, K. et al., (2015), J Biotechnol, 205: 59-69.[2] Spitkovsky, D. et al., (2016), Cell Physiol Biochem, 38: 1883-1896. [3] Wiedemeier, S. et al., (2017), Eng Life Sci, 17: 1271–1280.[4] Wiedemeier, S. et al., (2017), Microfluidics and Nanofluidics, 21: 167 ff.

17:45 Droplet-based microfluidics and mass spectrometry: Detection of metabolites in nano-/picoliter reactors (L53)

Konstantin Wink, Martin Schirmer, Julia Beulig, Detlev Belder

Institute for Analytical Chemistry, University of Leipzig, Germany, Email: konstantin.wink@uni-leipzig.de Germany

Droplet-based microfluidics involve the manipulation of volumes down to nanoliter or picoliter in "Lab-On-A-Chip" systems. Current monitoring of bioprocesses and their analytes in droplet-based microfluidics is mainly achieved by fluorescence-based readouts. Further information however can be generated also with mass spectrometry which offers a tool for unlabelled detection and identification of analytes.

The combination of droplet-based microfluidics with mass spectrometry allows mass spectrometric detection of droplet contents. While droplet-microfluidics provide the generation and controlled manipulation over minuscule volumes, mass spectrometry offers a tool with high sensitivity and specificity, broad molecular coverage and the potential for relative quantitation and structural determination.

By merging these two methods we demonstrate possible applications of droplet-mass spectrometry in microfluidic systems and possible applications for bioanalytical investigations.

18:05 Keynote talk: Minimalistic impedance instrumentation for process monitoring (L54)

Uwe Pliquett, Danny Echtermeyer, Chris Gansauge, Yahor Zaikou

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Impedance sensors are increasingly important for noninvasive, fast and label free detection [1]. Although electrical impedance as sole characteristic is not sufficient selective for many applications, it becomes a strong tool in combination with different transducer principles [2]. A general bottleneck in early days was the demanding instrumentation such as gain-phase- or network analyzers. During the last two decades several low cost devices, often based on a single chip [3], were developed. In order to reduce hardware requirements but also measurement time, such devices are often very specific but still too expensive for single use applications.

Impedance spectroscopy based on processing of the step response of a material is compatible with ultra-low power operation but also with minimalistic hardware requirements. The most important limitation is the huge data volume if the step response is equidistantly sampled. Besides this, uncertainties due to noise are hardly suppressible. A way out is adaptive sampling – fast where the step response changes fast and with larger distance of sampling points where slow changes occur. In order to fulfill the sampling constraints, partial integration of the signal is used before sampling. This reduces greatly the noise but keeps the bandwidth of the signal. Here we present a single chip solution based on microcontrollers with incorporated analog frontend. This allows a high bandwidth of spectroscopic measurements (e.g. 10 Hz – 5 MHz) with more than two orders of dynamic range of the magnitude. Applications are cell detection, biomass monitoring, or impedometric biosensors. Grant from Thüringer Aufbaubank, FKZ FGR0040

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19:30 Conference dinner at the Brauhaus Lemke am Alexanderplatz

(individual arrival)

THURSDAY, 22 MARCH 2018

9:00 Plenary Talk: Industria scale process optimization for manufacturing of scFv-class antibody with FOLDTEC® (L55)

Guido Seidel

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Wacker Biotech will present a case study for development and optimization of a scalable process employing microbial system to produce the scFv-class antibody for commercial application. Using its E. coli-based FOLDTEC® technology, WACKER completely overhauled a process employed by customers to facilitate production on an industrial scale. WACKER's proprietary refolding technology was able to produce the desired product in enhanced yields and streamline the purification process with achieving higher purity than was hitherto possible.

Session 7A: Process Analytical Technologies (Historical lecture hall, 2nd floor)

Chair Alain Sourabié / Anika Bockisch

9:55 Key note talk: Controlling microbial population at a single cell resolution: From cybergenetics to bioprocess engineering (56)

Frank Delvigne[1], Hosni Sassi[1], Samuel Telek[1], Ruddy Wattiez[2], Guillermo Gosset[3], Alexander Grünberger[4,5]

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Phenotypic heterogeneity is a key mechanism providing microbial population with increased fitness in front of nutrient limitation [1][2] and nutrient shift. However, little is known about the dynamics of these processes. We'll take the adaptation of E. coli to glucose limitation at the single cell level as a case study, and show that this mechanism exhibits a strong stochastic component leading to bistable behavior. An important question about phenotypic heterogeneity is its impact on the global fitness of the whole population and whether it depends on timescale and subpopulations size. However, this important question cannot be experimentally addressed since, in classical cultivation devices (i.e. batch, fed-batch or chemostat), subpopulations size change continuously with time. In the context of this study, we have developed a special continuous cultivation device allowing the stabilization of subpopulations size, i.e. the segregostat. Based on the experimental data acquired with this device, transcriptional

10:25 Budding index, a real time parameter for monitoring (L57)

Anna Maria Marbà-Ardébol[1]; Jörn Emmerich[2], Peter Neubauer[1], Stefan Junne

[1]Chair of Bioprocess Engineering, Institute of Biotechnology, TU Berlin, Germany [2]SOPAT GmbH, Berlin a.marbaardebol@tu-berlin.de

The maturation state of yeast cells becomes trackable in real-time through the measurements of a photo-optical in situ microscopy device, which was adapted and applied to monitor morphological features of single cells directly in the cell suspension during cultivation. The real-time measurement is conducted by coupling of the photo-optical probe to an automated image analysis, which is performed based on a Neural Network approach.

Several parameters, which are gained from the captures of the microscope, can be related to process relevant features like growth and cell activity. Moreover, the single-cell distribution of these parameters, provided not only information about the cell activity, but also about the population heterogeneity. Based on the relation of budding and non-budding cells (budding index, BI), a distinction was feasible between growth stages. The BI is valuable for large-scale industries like beer, wine, protein, probiotics or biofuel production, since it can assess the quality, quantity or the cultivation times. This knowledge can be used in the industry to compare the dynamics from golden-batches with actual cultivations and help to predict deviations.

As the imaging as such is fast, the application of ISM for process development and control purposes to achieve or maintain population homogeneity across scales becomes feasible. This approach will allow the assessment of the population status and of the cultivation conditions in situ. The direct monitoring of the microorganism can provide a better understanding of the overall process.

[1] Marbà-Ardébol, A.-M. et al., (2017), Process Biochem, 52: 223-232.[2] Marbà Ardébol, A. M. et al., (2017), 13. Dresdner Sensorymposium, P2: 222 - 225

10:45 In-line determination of spectral light distribution and biomass production in photobioreactors by fibre-optical methods: Spatially distributed light sensing and photon density wave spectroscopyg (L58)

Stephanie Schönfelder[1], Marvin Münzberg[1], Christopher McHardy[2], Roland Hass[1]

[1]innoFSPEC Potsdam, Institute of Chemistry, Physical Chemistry, University of Potsdam, Germany
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Light is essential for photosynthesis and, therefore, it also is one of the most critical factors limiting biomass production during algal cultivation in photobioreactors (PBR). A detailed knowledge of the light distribution within a PBR is crucial for optimisation of both reactor design and culturing conditions. Here, fibre-optical setups for the in-line assessment of light distribution are presented, which provide high spectral, spatial, and temporal resolution.

Fibre-optical spatially distributed light sensing was implemented in bubble columns and in an industrial Mesh-Ultra-Thin-Layer (MUTL)-PBR, thus showing its applicability at small and large scale, respectively. Data obtained in the bubble columns offered an in-line optical density measurement and also served as a basis for simulations of light-intensity profiles by a Lattice-Boltzmann approach. Results from the MUTL-PBR provided valuable information for optimising the light distribution within the reactor.

The second fibre-optical technology used in the MUTL-PBR is Photon Density Wave (PDW) spectroscopy. It enables the simultaneous and independent assessment of the absolute absorption and scattering properties of highly turbid liquid material. Thus, based on the scattering properties of the algae suspension, biomass development could be assessed in-line, with no calibration or dilution necessary. The reduced scattering coefficient of the algal culture, as obtained by PDW spectroscopy, exhibited a good correlation with its dry mass content, which reached values of approx. 35 g L-1. The presented technologies are powerful innovative fibre-optical tools complementing each other. They open up new vistas for the control and optimization of algal biomass production in both small and large scale.

Session 7B: Automated Bioprocess Development (Bernhard von Langenbeck Hall, 1st floor)

Chair Krist Gerney / Nico Cruz

9:55 Multi-feed simultaneous saccharification and fermentation: model-based development of high gravity lignocellulose-based bioprocesses (L59)

David Nickel and Carl Johan Franzén – franzen@chalmers.se

Chalmers University of Technology, Dept Biology and Biological Engineering, Div. Industrial Biotechnology, 412 96 Gothenburg, Sweden

Second generation bioethanol production can be viewed as a model biorefinery concept for biotechnological conversion of recalcitrant lignocellulosic raw materials to chemicals and other products. High Gravity operation, i.e. fermentation at high concentrations of water insoluble solids, pushes the process towards higher product concentrations and productivities, and improved energy and water economy. However, lignocellulosic processes are highly prone to batch-to batch variability in e.g. raw materials and enzyme activities. This variability can be propagated throughout system scales during process development and optimization, influencing the outputs of bioreaction models, techno-economic analyses and life cycle assessments. We have developed the variance-stabilizing Multi-Feed SSCF process: a systematic, model-driven design of fed-batch simultaneous saccharification and co-fermentation of lignocellulosic materials in standard stirred tank reactors. The design includes feeding of the solid fraction of steam-pretreated material, enzymes, and robust cell factories propagated on the liquid fraction of the substrate. It has been applied to lignocellulosic ethanol production using S. cerevisiae, and to lactic acid production from wheat straw by the thermophilic, cellulolytic strain Bacillus coagulans MA-13. We used uncertainty analysis to quantify the effects of model input variations on outputs in the multi-feed simultaneous saccharification and co-fermentation of wheat straw. We show how uncertainty analysis can be used to guide process development by comparing different modes of operation, defining possible process ranges and developing experimental designs at laboratory scale. The method can identify economically feasible process ranges with low environmental impact while increasing the robustness of bioprocesses with high variation in raw material inputs.

[1] Wang et al. (2014) Bioresource Technology, 172:303-311

[2] Wang et al. (2016) Biotechnology for Biofuels, 9:88.

[3] Aulitto et al. (2017) Biotechnology for Biofuels, 10:210

[4] Westman et al. (2017) Biotechnology for Biofuels, 10:213

10:25 Entering the next dimension: microfluidic single-cell analysis of bacterial interaction (L60)

Alina Burmeister[1,2], Wolfgang Wiechert[1], Dietrich Kohlheyer[1], Alexander Grünberger[2]

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Robustness and division of labour are key characteristics of microbial communities. The interactions in these fascinating complex systems bear a nearly unlimited diversity of biological processes that are by far not understood [1]. To date, microbial communities play an important role in waste water treatment and biogas production, but other biotechnological processes could benefit from the diversity of

metabolic interaction in microbial populations as well [2]. To understand these microbial communities in detail, novel analytical methods need to be developed. ere, we present a microfluidic cultivation setup with single-cell resolution for the analysis of a twostrain co-culture. The two strains are cultivated in picolitre bioreactors with two compartments. Cells are spatially separated by a sieve structure in nanometer scale that enables exchange of metabolites by diffusion. Similar to single-cell cultivation of isogenic cell colonies, cell growth is restricted to

monolayers with full spatio-temporal resolution in image-based live-cell microscopy [3]. The microfluidic setup enables precise environmental control and can be used to determine growth rates, interactions and cell-to-cell heterogeneity of two interacting microbial strains. As a proof of principle the behavior of Corynebacterium glutamicum ΔLysA, a lysine auxotrophic strain, and its interaction

with a lysine producing C. glutamicum strain was investigated. Our results show that the concept of single-cell co-cultivation has the potential to investigate bacterial

interactions of multiple strains and species in more detail. This lays the foundation for an improved understanding of natural and

synthetic co-cultures and could lead to novel strategies of designing and establishing novel microbial bioprocesses.

[1.] Hays, S. G., et al.(2015), Current Opinion in Biotechnology, 36: 40-49. [2.] Jagmann, N., et al. (2014), Journal of Biotechnology, 184:209-218. [3.] Grünberger, A., et al.(2012), Lab on a Chip, 12(11):2060-2068

10:25 Using integrated process modelling towards understanding process variation across unit operations (L61)

Christopher Taylor, Exputec

Christopher.taylor@exputec.com

A holistic approach to bioprocess industrialization requires a technique that will optimize inference from small number of small scale and large scale runs while maintaining the stringent requirements of regulatory guidelines. An integrated process modelling approach (IPM) is presented that will use early DoE data and risk assessment information to build an iterative and concatenated modelling of process variation over the various unit operations. The primary performance response is determined (e.g. impurity clearance, yield, clearance) which can be tracked through all unit operations. Attention is focused on the behaviour of the parameter-response relationship across unit operations rather than within unit operations. That is, the variation from previous unit operations is not overlooked, but rather included in the consideration for planning studies in subsequent steps.

Variation within and across unit operations is processed via application of Monte Carlo (MC) simulation that enables estimation of 'variation space' (i.e. Design Space). Using the range of existing variation, the process is modelled and the broad process capability is established. In early development, this information can be used either to assist in scale-up qualification or, if large scale runs are present, can be verified against existing results. That is, IPMs may be trained with development data and then further refined with qualification and large scale runs. Upon further model training, routine parameter settings may be established. The model will be used continuously throughout the process lifecycle, increasing in sensitivity and being used to identify outliers and trends.

Literature [1.] Zahel, Thomas et al.(2017), Bioengineering, 4(4), 86

Session 8A: Process analytical technology (Historical lecture hall, 2nd floor)

Chair Peter Götz / Anna Maria Marbà-Ardébol

11:55 Laser-based in situ back reflection analysis of particle sizes during feedstock pre-treatment by multi capture signal interpretation (L62)

Stefan Junne[1], Hermann B. Schwartz[2], Chiara Barilaro[1], M. Quietsch[1], Peter Neubauer[1]

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Laser-light back reflection is applied for the determination of the particle and cell-size size distribution is adapted for the optimization of feedstock pre-treatment and measurement of the fluid phases of mono-cultivation and complex anaerobic digestion bioprocesses. Due to the different patterns of back reflection by multi capture signal interpretation at the edges and cores of particles in a laser beam, sizes of them can be determined in the culture broth.

The method allows to determine size distributions over a typical range of cell and particle densities, which are usually achieved in bioprocesses. The resolution of the technology reaches up to < 0.5 µm, which makes it also applicable for bacterial cultivations. The results show that the method is robust and allows the rapid identification of suitable operation conditions substrate pre-treatment, e.g. milling or ultrasound exposure, w/o the requirement of time-consuming monitoring of the process performance, e.g. carboxylic acid production or methane formation.

The detection of agglomerates due to unfavorable stress conditions is feasible as well. Population heterogeneities can be determined if there exists a relation between form and function, down to the typical bacterial cell size. Besides, the monitoring of co-cultivation processes becomes possible, if the co-cultures differ in morphologic features. The applicability of the method and the potential to improve monitoring of bioprocesses is demonstrated, and challenges at high dry biomass concentrations and complex media matrices are discussed.

The laser-based back reflection allows to tailor feedstock and cell suspension treatment based on the particle size distribution, thus reducing the power consumption and increasing the efficiency of a feedstock pre-treatment process.

Acknowledgements: The authors kindly acknowledge funding of the German Federal Ministry of Economics and Energy within the framework programme "Biomass Energy Use", project "Lasersize", grant no. 03KB120

12:20 Comparison of vibrational spectroscopic techniques for PAT in industrial biotechnology (L63)

Edo Becker

The complex nature of biochemical processes makes real time analysis of reaction progression very difficult, with development teams having to resort to off-line extractive techniques, such as HPLC. This can provide highly sensitive analysis of the process, but is a slow and complicated method.

Vibrational spectroscopy if a common method of studying chemical processes, and is slowly transitioning to the biochemical space. Raman and NIR are the most common techniques for aqueous environments, but suffer from fluorescence and low sensitivity respectively. FTIR spectroscopy is not commonly used because of a strong absorbance for water, and an inherent lack of stability in the industrial environment. It is therefore important to establish what are true strengths and weaknesses, and what is myth and conjecture.

Here we present the results of a comparison of process NIR, Raman, conventional FTIR and a novel solid-state FTIR instrument to monitor a S. cerevisiae fermentation of sucrose in real-time. We observe the concentration of sucrose, glucose and fructose sugars, as well as ethanol (the key product) and organic acids. The sugars range in concentration from 0 – 30 %wt whilst the acids are in the ppm range.

We also present how important the use of chemometrics is to spectroscopic PAT in biotechnology, demonstrating how it can differentiate and quantify up to seven different sugars from aqueous solutions. We also show how solid state FTIR improves model robustness and enables calibration transfer from instrument to instrument – a must for industrial scale up.

12:40 In situ Raman spectroscopy as a promising PAT tool for monitoring of macro and micro-heterogeneity of monoclonal antibody glycosylation produced by CHO cells in bioreactors (L64)

Meng-Yao Li[1], Bruno Ebel[1], Cédric Paris[2], Fabien Chauchard[3], Emmanuel Guedon[1], Annie Marc[1]

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[2] Structural and Metabolomics Analyses Platform, SF4242, EFABA, Lorraine University, Vandœuvre-lès-Nancy, France
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Therapeutic monoclonal antibodies (mAbs) are mostly produced by Chinese hamster ovary (CHO) cells due to their capacity of performing human-like glycosylation profile. Indeed, glycosylation is a critical quality attribute that plays an important role in the safety and efficacy of the therapeutic mAbs. However, the glycosylation profile of the mAbs can vary greatly during the cell culture (upstream) process, and is therefore an essential parameter to be monitored and controlled. Current glycosylation characterization techniques involve time and labor consuming analyses, which are often carried out at the end of the culture when a majority of the product is obtained. To ensure the quality of the product, the glycosylation status of the mAbs needs to be monitored in real time by using a Process Analytical Technology (PAT) approach. In our study, both macro and micro-heterogeneity of mAb glycosylation were accurately predicted in real time during cell cultures, by combining in situ Raman spectroscopy analyses with chemometric techniques. Concentrations of total mAbs, non-glycosylated mAbs, galactosylatied and fucosylated mAbs, as well as the high mannose form of the mAbs were predicted simultaneously by online Raman spectra using chemometric models developed in this study. For the first time, we showed the potential of in situ Raman spectroscopy as a PAT tool for monitoring both macro and micro-heterogeneity of the mAb glycosylation. This real time information is essential for process understanding and control, and could be rapidly integrated in mAb quality control strategies in process scale up operations until the final production scale.

13:00 SERS and Time-Gated (TG-SERS) Raman spectroscopy as advanced process analytical tools for monitoring of lactic acid bacteria fermentations in complex media (L65)

Martin Kögler [1,3,] Klaus Pellicer Alborch[1], Andrea Pau[2], Tapani Viitala[3], Alex Bunker[3], Michael Maiwa[2], Stefan Junne[1], Peter Neubauer[1]

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Raman spectroscopy is of increasing importance for the concentration measurements in cell-free supernatant and even cell-suspension samples. It is increasingly regarded as a powerful process analytical technology (PAT) tool. Until now, Raman-technology has not yet shown its full potential in bioprocess on-line monitoring. Nevertheless, process Raman-spectrometers are in general suitable for a fast measurement in rather pure environments, but are getting applicable to follow rapid changes of metabolite concentrations. In comparison to IR-spectroscopic approaches, Raman bands of most analytes exhibit no interference with vibrations from water molecules [1]. Spectra derived from conventional continuous-wave (CW) Raman-spectrometers are often obscured by background-fluorescence. Thus, Raman spectroscopy needs i) an enhancement of analytes' bands to increase the limit of detection (LOD), and ii) a reliable method to distinguish the Raman signal from background-fluorescence. SERS (surface-enhanced Raman spectroscopy) is able to significantly enhance the Raman signal while time-gated (TG) Raman-spectroscopy is a technique that is able to measure Raman signal before the fluorescence arrives at the detector [2-6]. Here we demonstrate a combination of SERS- and TG-Raman spectroscopy on cell-free supernatant samples of a lactic acid bacteria fermentation with complex media. As reference method for estimating amino acids and other metabolites, HPLC was used to evaluate the Raman-based detection. Quantitative evaluation of Raman data was performed by multivariate data analysis using PCA/

PLSR. For the first time, we can show that both qualitative and quantitative measurements are conducted successfully with both, SERS and time-gated Raman-methods in industrially relevant media, so that fast and reliable in-situ measurements become feasible.

[1] Smith, E. & Dent, G., (2005), Modern Raman Spectroscopy: A Practical Approach, West Sussex: John Wiley and Sons. [2] Fleischmann, M. et al., (1974), Chem Phys Lett, 26: 163–166. [3] Kneipp K. et al., (1997), Phys Rev, 78: 1667–1670. [4] Li, Z. & Xu, H. (2016), Adv Phys X, 1: 492–521. [5] Schlücker S., (2014), Angew Chem - Int Ed, 53: 4756–4795. [6] Rojalin, T. et al., (2016), Anal Bioanal Chem, 408: 761–774

13:20 Lunch break, poster session and exhibition

Session 8B: Automated bioprocess development (Bernhard von Langenbeck Hall, 1st floor)

Chair Carl Johan Franzén / Florian Glauche

11:50 Keynote talk: Beyond miniaturization and parallelization: standard and tailor-made automated workflows for smart microbial bioprocessing (L 66)

Marco Oldiges

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The biotechnological production of fine and bulk chemicals as well as heterologous protein production using microbial systems is an 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 engineering microbial strains in a fast way, easily providing strain libraries harbouring large biological variance.

However, the capability for detailed phenotyping of such libraries at well-defined bioprocess level is orders of magnitude slower and represents a substantial bottleneck in strain engineering. Besides, the best performing strain need to be identified under bioreactor process conditions instead of artificial screening conditions as well as bioprocess parameters need to be optimized.

Increased experimental cultivation throughput in microbial phenotyping at well-defined bioprocess conditions can be provided by miniaturization and automation [1]. However, to fully release the potential of the approach, standard and tailor-made workflows need to be put in place, comprising the full experimental pipeline from upstream processing, cultivation, process analytics, data management and design-of-experiment. This is illustrated using case studies with different microbial systems [2-4], demonstrating how developments in miniaturized cultivation combined with smart lab automation and data processing are amalgamated in workflows for more efficient microbial phenotyping and bioprocess development.

Literature[1] Hemmerich, J. et al., (2018), Biotechnol J, DOI:10.1002/biot.201700141 (in press) [2] Morschett, H. et al., (2017) Biotechnol Biofuels, 10, DOI:10.1186/s13068-017-0711-6 [3] Hemmerich, J. et al., (2016), Microb Cell Fact, 15: 208.[4] Freier, L. et al., (2016), Eng Life Sci, 16: 538-549.

12:20 Model based design of experiments for high throughput bioprocess development (L 67)

M. Nicolas Cruz Bournazou, Florian Glauche, Sebastian Hans, Emmanuel Anane, Benjamin Haby, Robert Giessmann, Peter Neubauer

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Current advances in laboratory automation enable highly complex parallel experiments in Liquid Handling Stations (LHS), these facilities can be combined with central and cloud data management systems, computer aided modeling, simulation, and optimization tools, and distributed control systems. The lab of the future at the TU Berlin has developed a holistic framework that covers all levels from experimental facility over closed loop controls and data handling up to the online optimization of the experimental strategy. We present some advances in the use of model based tools to design and operate High Throughput experiments efficiently while complying with all relevant constraints to significantly accelerate bioprocess development from the conditional screening phase to scale up. We prove the potential of this framework in High Throughput Bioprocess Development with two case studies. First, we achieve scalable, accurate, and reproducible scale-down experiments in parallel mini-bioreactors to investigate the misincorporation of non-canonical amino acids in recombinant proteins due to reactor heterogeneities. Pulse based feeding profiles are designed and performed to reproduce the substrate and oxygen fluctuations that dominate in large scale reactors. With this approach experimental results as well as product quality have an improved transferability to real production compared to typical screening batch experiments. Secondly, we combine an online parameter estimation approach with an adaptive input design strategy to enable the accurate phenotyping of eight different strains including substrate uptake, growth, acetate production and consumption capacities, and product to increase the success rate of the screening phase while reducing the experimental time and efforts.

12:40 Mastering the digital transformation challenge in biopharmaceutical processing (L68)

Michael Sokolov[1,2], Alessandro Butté[1,2], Fabian Feidl[1,2], Massimo Morbidelli[1,2]

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Following the advanced examples of successful process digitalization and automation in the 'older industries' such as automotive and finance, several trends could be observed in the biopharmaceutical industry. Over the past decade, one witnessed a shift from yield maximization to quality optimization, the utilization of miniaturized and parallelized high throughput techniques, continuous bioprocessing and continuous data acquisition as well as the utilization of data- and knowledge-driven tools for process analysis, forecasting, monitoring and control. These trends face substantial challenges, as for instance, therapeutic proteins feature several dozen critical quality attributes (CQAs) including their glycosylation and charge variant profiles as well as their aggregated and low molecular weight forms, all which are highly important for the efficacy and safety of the drug. In order to eventually fulfill the standards and goals of the industry 4.0 era, the methodologies and technologies associated to previous trends must be further developed and extensively utilized in the biopharmaceutical process industry. Throughout the past years, we elaborated several digital solutions based on advanced engineering statistics, machine learning and deterministic approaches for the analysis, modeling and interpretation of bioprocesses. Furthermore, we integrated them into the process development workflow in several collaboration projects with the biopharmaceutical industry. This presentation will outline key technology and business drivers to master the digital transformation challenge in bioprocessing. It will show the possibilities to accelerate development as well as to reduce risks and resources in scale-up and production based on several industrial case studies.

13:00 CHO cell-free protein synthesis for mammalian protein production and future bioprocess development (L 69)

Nils Janzen

Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria

Nowadays, bioprocesses based on mammalian cell culture plays an important role for drug development and production in the pharmaceutical industry. The continuous development and improvement of mammalian cell culture processes and generation and engineering of host cells led to increased productivity and applicability of this bioprocesses especially addressing complex mammalian proteins. A broad range of mammalian cell hosts are available, but Chinese Hamster Ovary (CHO) cells are of particularly importance for industrial bioprocesses due to their simple and stable cultivation, established protocols for gene modifications and gene amplifications and their safety approval for nearly 4 decades. The mammalian cell cultivation has major drawbacks concerning the time and effort for process development. To circumvent these issues, novel CHO cell-free protein synthesis systems are developed, which are not based on complete cells, but on cell lysates including a completely active protein translation machinery. These systems not only contain the molecular components for protein translation, but also microsomal structures derived from the endoplasmic reticulum to perform posttranslational modifications. The set-up of CHO cell-free protein synthesis enables a fast production of proteins within hours or a few days depending on the applied reaction mode. The CHO cell-free system was already applied for DNA template evaluation and for the production of diverse protein types including antibody formats and difficult-to-express membrane proteins leading to yields up to 1 g/l. The results underline the potential as a future down scaled platform for the efficient pre-evaluation of DNA-templates for mammalian bioprocesses and the high-throughput production of pharmaceutical relevant "difficult-to-express" proteins.

[1] Thoring, L. et al., (2017), Sci Rep, 7: 11710. [2] Stech, M. et al., (2017), Sci Rep, 7: 12030. [3] Jérôme, V. et al., (2017), Eng Life Sci, 17: 1097-1107. [4] Thoring, L. et al., (2016), PLoS one 11: e0163670.

13:20 Lunch break, poster session and exhibition

Session 9A: Bioprocess development (Historical lecture hall, 2nd floor)

Chair Frank Delvigne / Matthias Gimpel

14:20 Taking the microbial perspective: Computational fluid dynamics for bioprocesses design, operation and optimization (L70)

Cees Haringa[1], Neil Tang[1], Guan Wang[2], Robert F. Mudde[1], Henk J. Noorman[1]

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Euler-Lagrange CFD simulations allow to assess industrial fermentations from the perspective of microbes, gathering timeseries -lifelines- of perceived variations in the extra-cellular environment (e.g. substrate or oxygen concentration) [1;2]. These lifelines can be analyzed towards the design of experimental scale-down simulators, with fluctuations representative of industrial-scale processes [2;3]. Dynamic metabolic models based on perturbation and downscaling experiments [4] can be coupled to CFD simulations to resolve Computational Reaction Dynamics (CFD-CRD), and predict the intra-cellular response to extra-cellular heterogeneity [1;4]. The combination of CFD-CRD simulations and scale-down experiments is a promising tool to evaluate bioreactor performance, and design and optimize bioprocess such as to minimize the impact of extra-cellular heterogeneity [4].

The potential of CFD-CRD simulations is outlined based on baker's yeast and penicillin fermentation case-studies. Both the analysis of lifelines towards scale-down simulator design, and the use of reaction models to predict yield losses and study the emergence of population heterogeneity are discussed. It is furthermore demonstrated how simulations can be used to test the impact of reactor design alterations on process performance. To conclude, a longer-term perspective is provided. If challenges in computational expense can be overcome, real-time simulations have a potential application in quantifying the impact of operational disturbances [5]. Furthermore, CFD-lifelines may be used to control a new generation of microfluidic scale-down simulators [6].

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14:50 CFD-based strategy to optimize the impeller design for mesenchymal stem cells cultures in bioreactors (L71)

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In mixed bioreactors, adherent-dependent mesenchymal stem cells (MSC) are cultivated on 100-200 µm diameter solid particles, called microcarriers. When mixing this solid-liquid suspension, a compromise has to be found allowing sufficient mixing to suspend the microcarriers without damaging the cells1. Thus, optimization strategies have to be established to define the best choice of impeller design and agitation rate.

Coupled with multi-objective optimization, Computational Fluid Dynamics (CFD) has been already validated in literature as a tool to design impellers maximizing the solid distribution throughout the vessel2 or maximizing the effective gas holdup with power consumption minimization3. Based on these results, CFD-based method to screen and to provide impeller designs suitable for MSC cultures is proposed in the present study.

40 Ear-Elephant impeller designs were considered in Design of Experiment, varying the D/T ratio, defining the impeller diameter on the vessel diameter, the C/T ratio defining the clearance off the base on the vessel diameter and blades slope angle. Simulations successfully predicted the just-suspended state agitation rate according to a method based on the particles velocity. To characterize the hydromechanical stress, mixing representative parameters were calculated, including the power per unit of volume applied on particles and a fluid energy dissipation circulation function. On the basis of 114 simulations, response surfaces were built and optimizations were performed by minimizing the previous parameters. A set of impeller designs was finally selected and printed in 3D to validate experimentally the numerical results.

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14:50 CHO cell-free protein synthesis for mammalian protein production and future bioprocess development (L72)

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Nowadays, bioprocesses based on mammalian cell culture plays an important role for drug development and production in the pharmaceutical industry. The continuous development and improvement of mammalian cell culture processes and generation and engineering of host cells led to increased productivity and applicability of this bioprocesses especially addressing complex mammalian proteins. A broad range of mammalian cell hosts are available, but Chinese Hamster Ovary (CHO) cells are of particularly importance for industrial bioprocesses due to their simple and stable cultivation, established protocols for gene modifications and gene amplifications and their safety approval for nearly 4 decades. The mammalian cell cultivation has major drawbacks concerning the time and

effort for process development. To circumvent these issues, novel CHO cell-free protein synthesis systems are developed, which are not based on complete cells, but on cell lysates including a completely active protein translation machinery. These systems not only contain the molecular components for protein translation, but also microsomal structures derived from the endoplasmic reticulum to perform posttranslational modifications. The set-up of CHO cell-free protein synthesis enables a fast production of proteins within hours or a few days depending on the applied reaction mode. The CHO cell-free system was already applied for DNA template evaluation and for the production of diverse protein types including antibody formats and difficult-to-express membrane proteins leading to yields up to 1 g/l. The results underline the potential as a future down scaled platform for the efficient pre-evaluation of DNA-templates for mammalian bioprocesses and the high-throughput production of pharmaceutical relevant "difficult-to-express" proteins.

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15:30 Reactors for electrobiotechnology: Upgrading bioreactors for bioelectrosynthesis (L73)

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Electrobiotechnology is the recent offspring of microbial electrochemical technologies (METs) [1]. This technology platform allows the conversion of electric energy into chemical energy stored in biomass and platform and fine chemicals with its reactors being termed bioelectrochemical systems (BES).

The BES-based bio-productions so far reported in literature [2] are relatively small in scale, and the diverging requirements of the electrochemical and the biological processes, make the engineering of these BES a special challenge. The current plethora of approaches designs and operating conditions make standardization and normalization and systematic engineering almost impossible. This lack of standardization and scalability is the driving force for our work. We will show an evolving series of prototypes for integration of electrochemistry with existent bioreactor systems using an upgrade kit. These allow the utilization of existing infrastructure of conventional bioreactors for running bioelectrosynthesis, for electricity driven microbial synthesis. Using the model organism Shewanella oneidensis sp. [3], we show that these systems can be used for engineering and scale up.

The different prototypes of our BES upgrade set [4] were studied regarding fluid dynamics using finite element modelling. Alterations done to the standard bioreactors, like not including baffles, or the introduction of extra volume bodies (electrodes) and a centralized protrusion (counter-electrode chamber) and custom agitators/stirrers in the reactor vessel were evaluated. Using classical methodologies and modelling including a rotating stirrer enabled the acquisition of important physical information (e.g. kLa, mixing time, Reynolds number, velocity fields, etc.) In summary we show that conventional bioreactors can be seized for bioelectrosynthesis utilizing a tailor-made upgrade kit.

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15:50 Keynote talk: Opportunities and challenges of model-based control of pellet growth in industrial-scale penicillin fermentation (L74)

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Industrial-scale fermentation strategies aim at robust and optimal conditions for achieving economic operation. Today, biotechnology industry widely applies filamentous fungi for producing various products. Growth of the fungal hyphae might be useful in the dispersed or pelleted form. The present contribution focuses on the advanced monitoring and control of growing Penicillium chrysogenum cell pellets for a robust large-scale production of penicillin. Unsolved questions for targeting optimal conditions with respect to mixing and reactor characterization will be addressed.

An understanding of the underlying complex processes of pellet growth is available since decades. Implementation of a soft sensor for the real-time estimation and control of pellet growth in industrial-scale operation will be demonstrated. This includes highlighting (i) the development of a simple mechanistic process model applying a genome-scale metabolic model, (ii) the integration of process information, and (iii) a concept of two particle filters and their communication.

Advanced monitoring and control of pellet growth enables a robust and economic operation based on the deeper process understanding and process data. However, optimizing operation requires more than simplified growth models. The comprehensive analysis of transport processes of oxygen and the carbon source within the reactor and within pellets as well as particle strain associated with mixing remain a challenge.

16:20 Closing remarks

Peter Neubauer

16:30 End of symposium

Poster abstracts

P01: Experiences with an automated small scale fermentation system regarding scalability of results in process development

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DoE experiments have become a standard tool during upstream process development. Screening experiments with full factorial designs are mostly set up in high throughput robotic systems which are highly automated but limited e.g. in pH control. As pH is seen as one of the most critical parameters for cell growth and product quality the best results from such screening experiments are confirmed in parallelized small scale fermentation systems with reduced DoE designs. In Pharma Technical Development the use of a parallelized small scale fermentation system for cultivation of mammalian cell lines was established in order to increase the throughput of fermentations under controlled conditions. The parallelized and highly automated 250 mL fermentation system fills the gap between the robotic screening system and the 2 L scale which is mainly used for process design studies.

Results from fermentations in the 250 mL system will be compared with cultivations in 2 L and 1000L scale for different projects. Despite cell growth and metabolic data also product quality data will be shown. Initial differences between data from the 250 mL and 2 L scale were eliminated by implementing a consistent pH measurement device and method as well as using the information generated by offgas analysis.

P02: Scale-Up of Escherichia coli Fermentation from small scale to pilot scale using Eppendorf fermentation systems

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The scale-up of fermentation processes is critical to the success of industrial fermentation. Eppendorf bioprocess systems are available with autoclavable, single-use and sterilize-in-place vessels and together cover a wide range of working volumes from less than 1 L to as large as 1,200 L. We used E. coli fermentation to demonstrate the scale-up capabilities of Eppendorf fermentation systems from small scale to bench scale and pilot scale.

To determine suitable parameters and setpoints for the operation of each fermentor, we considered critical scalability-related engineering parameters. The parameters described include proportional vessel/impeller geometry, oxygen transfer rate (OTR), impeller power numbers (Np) and impeller power consumption per volume (P/V).

We carried out E. coli fermentation runs at three different scales (1 L, 10 L, and 100 L) following a constant P/V strategy, and represented the E. coli biomass growth trends by plotting optical density curves over time. The fermentation runs at each of the three scales produced very similar biomass yields over time, indicating excellent scalability within the Eppendorf fermenter product family.

P03: Development and scale-up of aqueous two-phase extraction as clarification and capture operation in the manufacturing process of biologics

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Continuous processes are known in downstream processing, but their implementation is rarely transferred on a production scale. Upstream cultivation technology on the other hand has already created and partially implemented a number of solutions, such as perfusion technology [1, 2]. Clarification processes and the associated devices that are already established in other industries, such as flocculation, precipitation or flotation, are increasingly being considered as alternatives in the production of monoclonal antibodies (mAb) despite individual restrictions such as process robustness, process costs, toxicity of flocculants or precipitants and easy scale-up. A promising concept is the application of the aqueous two-phase extraction (ATPE) as a combined harvesting and catching step, especially since this approach covers all the above-mentioned issues [3, 4]. Nowadays aqueous two-phase extraction is regarded as a practicable unit operation, e.g. for the recovery of monoclonal antibodies or recombinant proteins. However, most of the work published so far investigates the applicability of ATPE in antibody processes on a laboratory scale and mostly only as a capture step. In contrast, this presentation shows approaches for the integration of ATPE as a combined harvesting and capture step in a downstream process. In addition, a model is used that allows early prediction of settler dimensions with high prediction accuracy. Up-scaled processing in a pilot scale settler (DN150) of 160 L cultivation broth in a time frame of 3 h was achieved. Finally, a reliable process development concept is presented, which covers the necessary steps from the definition of the separation task to the final stages of integration and scale-up.

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P04: Characterization and development of milli-devices for membrane-supported ATPE in the manufacturing of biologics

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While devices based on micro effects are increasingly accepted in reaction engineering, there is still no breakthrough for separation applications like liquid-liquid extraction (LLE). Additionally, the limited throughput potential, mostly caused by complex fluid dynamics and difficult phase separation, is problematic [1].

The implementation of milli-scale devices in engineering processes is a new and steadily growing field. In contrast to conventional industrial techniques, a milli process is characterized by improved mass and heat transfer and small equipment size. The majority of current applications are established in the field of reaction technology. However, the transfer of knowledge from these scientific focuses to separation technology is performed hesitantly. For the transfer of milli technology to separation processes like liquid/liquid extraction (LLE), a combination of efficient mass transfer and fast phase separation is necessary [2, 3].

In this work, a membrane-supported LLE-device in milli-scale is introduced. This concept offers several advantages like small void volume and less required effort for piping and utility space. It is applicable for batch and continuous operation and suitable for single-use and disposable implementation. For different aqueous two-phase systems and throughput, the influence on phase separation and extraction efficiency is determined. Finally, integration into production scale is discussed for different fields of application like the manufacturing of biologicals, like monoclonal antibodies.

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P05: Scale-up and optimization of the continuous Acetone-Butanol-Ethanol (ABE) bioprocess

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Butanol is a promising candidate for a fuel from renewable resources with physicochemical properties superior to Ethanol or Biodiesel. In order to optimize the continuous acetone-butanol-ethanol (ABE) fermentation with Clostridium acetobutylicum on a process engineering level, a cascade of six continuous stirred tank reactors (CCSTR) was designed. Main optimization parameters in the cascade are the dilution rate and the controlled pH-value in the first reactor. We currently achieve a final butanol concentration of 8,2 g/L and a butanol productivity of 0,75 g/Lh in steady state of the CCSTR. Operational stability makes the CCSTR a flexible evaluation system for solvent producing species. Other wild types of solvent producing bacteria and a genetically modified strain of C. acetobutylicum with enhanced butanol tolerance are currently under investigation.

Following the results from the lab scale, a scale-up of the continuous ABE fermentation to pilot plant scale is being prepared. The pilot plant will utilize 1 m3 whey per week and will include substrate preparation, fermentation, product recovery and waste water treatment units. The fermentation unit combines one stirred tank reactor with four packed bed reactors into a cascade. The butanol recovery will occur inline in adsorption/desorption columns. Besides butanol, other product streams like whey proteins, produced gases, biomass, acetone, ethanol, acetic acid and butyric acid will be valorized. The fermentation process will be optimized towards a higher solvent productivity. The pilot plant will allow to assess the quality of the products, an economic analysis of the continuous, whey-based ABE process will follow.

P06: Large scale demonstration for the bio-based bulk chemical itaconic acid aiming at cost reduction and improved sustainability

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Itaconic acid (IA) is an important dicarboxylic acid used as a platform chemical; for example, it serves as a co-monomer for the synthesis of emulsion polymers, as a substitute for petrochemically produced acrylic acid or for the production of paints and varnishes and biodegradable polymers in the packaging industry. Itaconic acid can be produced biochemically by fermentation of sugar substrates with e.g. filamentous fungi of species Aspergillus [1]. Within the European project "Bio-QED" (2014-2017) Fraunhofer (IGB and CBP) dealt with the selection of suitable raw materials for the second generation as well as the production of sugars from these raw materials and the associated toxicity test for microbiological utilization. The focus was on developing and scale-up of the fermentation process for the production of IA with regard to optimal growth morphology, oxygen supply, feeding and inoculation strategy. Finally the process could be successfully demonstrated in pilot scale (10-m³) at Fraunhofer CBP. The downstream process for obtaining purified products – developed by TNO – was demonstrated using a 2-step continuous crystallization (equipment provided by SoliQz). A purity of 91-95 % was achieved which could be increased to 99 % by performing a 3rd crystallization at TNO.

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P07: HD cell banking for intensified seed trains

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In recent years perfusion technologies experienced a comeback in biopharmaceutical processes, especially in the context of intensified seed trains. An intensified seed train promises simultaneous cost and time savings as well as safety benefits. Traditionally, the biopharmaceutical industry is slow to adopt new technologies and only few processes have been reported to make use of an intensified seed train [1,2]. Here, we present an intensified seed train concept for an industrial bioprocess aiming at the production of a monoclonal antibody in CHO cell cultures.

In a first step, high density (HD) cell banking protocols for CHO DG44 and CHO K1 cell lines were optimized for cell concentrations of up to 150x106 mL-1. HD cell banks were then used to inoculate a ReadyToProcess WAVE25 bioreactor (GE healthcare) operated in perfusion mode. Applying a cell-specific perfusion rate CHO cells were grown to cell concentrations of up to 145x106 mL-1, sufficient to directly inoculate a 250 L seed bioreactor. In comparison to the conventional seed train, time to inoculate the production bioreactor was reduced by ~50 %, obsoleting a 50 L seed stage. In addition, fewer manual handling steps were required, minimizing the contamination risk and personnel cost. Ultimately, we demonstrated that seed train intensification is a simple but powerful route to boost bioprocesses efficiency without affecting bioprocess performance.

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P08: Establishment of a process strategy dedicated to hMSC cultures derived from umbilical cords on microcarriers in a hPL supplemented culture medium

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Mesenchymal stem cells isolated from the Wharton's jelly of umbilical cords (WJ-MSC), are promising due to their abundance and their high proliferative and immunomodulatory properties [1]. However, they are still widely cultivated in 2D static mode, limiting the available surface to adhere and grow. Dynamic cultures of these MSCs on microcarriers suspended by mechani-

cal agitation remain poorly reported in the literature. Moreover, replacement of the serum in the culture medium is required to assure a clinically acceptable quality. The human platelet lysate (hPL) has been validated as a substitute and is already used in clinical trials [2].

WJ-MSC cultures on microcarriers led to several issues in a culture medium supplemented with hPL. After seeding, a jelly matrix precipitate was formed, entrapping microcarriers, and led to high variability of cell adhesion and expansion. Microcarriers and agitation mode selections were thus crucial.

To overcome these issues and find suitable operating conditions, a methodology has been established, based on a cell recovery strategy from static cultures, avoiding clusters or aggregates formation, and mechanical fibrinogen depletion from the culture medium [3]. Different cultures were then performed to compare cell adhesion, expansion and detachment performances. To do that, a counting method has been developed using Matlab, allowing the direct measurement of the number of cells per microcarrier in situ, without detachment step. A process strategy has finally been validated after microcarrier selection, coated or not with hPL, and considering the agitation impact between orbital (Erlenmeyer) and mechanical (Wheaton) mixing.

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P09: Improve-Stem (Interreg Project developing new bioMaterials for PROliferation and in vitro expansion of STEM cells)

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The potential applications of stem cells in medicine are very promising but but usual cell cultures methods (T-Flasks) do not provide sufficient amounts for clinicals studies nor clinical application. Upscaling and automation of the process will be required in the near future to ensure reproductibility and quality of the cell acquisition process. The most promising expansion technique to date is based on the use of microcarriers, on which the cells multiply, suspended in a stirred tank bioreactor. However, this technique poses problems in the specific case of stem cells, which need to be recovered without being altered.

Improve is a project that involves 7 teams in 4 countries with key competencies in material sciences, bioprocess engineering and cell biology. It aims to develop an integrated set of tools necessary for the multiplication of stem cells. The achievement of this objective is based on three aspects:

- the development of microcarriers whose surface properties will be optimized to allow the adhesion of stem cells and an easy detachment after the expansion phase;
- the design of an adapted bioreactor with controlled operating conditions adjusted to ensure a suitable environment for the stem cells and to guarantee their quality;
- the development of standardized characterisation protocols to ensure purity, homogeneity and quality of the cells, through all steps of the process (from the stem cell collection stage to the detachment and harvesting phase).

The project is co-funded by FEDER within the framework of INTERREG VA Greater Region initiative.

P10: Model-assisted strategies for design, optimization and control of bioprocesses

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Biopharmaceuticals produced with Chinese hamster ovary (CHO) cells are the key drivers for the medication of former untreatable diseases. The development of their production processes is still challenging because of regulatory requirements and variabilities concerning cell line changes or changes in cultivation conditions. Therefore, a high number of process parameters needs to be optimized. New design and process control strategies mainly based on mathematical process models are en route from academia to industry.

The key component is a mathematical model, which aims to describe the real phenomena as simple as possible and as accurate as necessary. This enables the development of model-assisted strategies for design, optimization, monitoring and control.

In this contribution, model-assisted concepts for design of experiments, seed train design as well as optimal control are presented. In brief, the model-assisted design of experiments method can be used to reduce the number of experiments during DoE and the time needed for the development of more knowledge-based cell culture processes1. Seed train design and optimization can be applied to the analysis and optimization of existing seed trains as well as design of new seed train protocols for novel cell lines/clones and design of seed train scales for new facilities or seed train transfers to a different facility2. The model predictive "open-loop-feedback-optimal" (OLFO) – strategy allows high-performance processes for the transferability of different cell lines and cultivation systems3.

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P11: Optimization of computer fluid dynamics modeling using experimentally obtained characteristics of stirred-tank fermenters

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Scale-up of bioprocess cultures from bench scale to pilot or production scale requires identifying key engineering characteristics to produce predictable and proportional product yields at each scale. The power number is an important scale-up characteristic of stirred-tank bioreactors as it allows the calculation of impeller power at each scale, thus, enabling constant power-based scale-up. As computer fluid dynamics (CFD) simulation is becoming an economically necessary part of the industrial bioreactor and fermenter design, it is important that CFD results closely match experimentally measured characteristics such as the power number. The default parameters of commercially available CFD software, such as ANSYS® Fluent®, are set to give good results in the most general range of fluid flow conditions; however, they may not be optimal for the specific operating conditions used in bioprocess applications. In this

study, we show how we tailored the general default parameters of a CFD model to allow CFD results to better match a specific flow condition used in bioprocess, the flow inside of a stirred-tank fermentor. The characteristic we used as our benchmark is the fermenter's experimentally measured power numbers over a practical range of impeller tip speeds suitable for bacterial fermentation. The results are CFD settings which produced power numbers that agree with our experimental result, and a validated CFD model system that will better predict the performance of new fermenters as we scale up our designs.

P12: Modelling oxygen transfer in minibioreactors with induced draft aeration

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One of the limiting factors in bioreactor cultivation is the mass transfer characteristics of the bioreactor, particularly the volumetric mass transfer coefficient (KLa) for transfer of oxygen from the gas phase into the broth. In effect, the KLa determines the maximum achievable biomass concentration under aerobic conditions in batch cultivations. It is therefore desirable to understand the dependence of the KLa on operating conditions of the bioreactor, namely, the aeration and agitation rates. A major difference between lab scale bioreactors and the minibioreactors used in high throughput systems is the configuration of the mixing and aeration systems. For instance, the 2mag bioREACTOR® system uses distributed airflow through the head plate coupled with a hollow shaft that induces broth aeration by the differential pressure along the shaft. Thus, the aeration and agitation rates are coupled, such that the aeration is controlled in tandem with the agitation. This coupling of operating conditions and other differences means conventional oxygen transfer equations for larger bioreactors may not be applicable to minibioreactors. Therefore, it is important to establish suitable mathematical models to predict the mass transfer characteristics of minibioreactors.

In this work, we model the relationship between the Kla and operating conditions for minibioreactors employing the induced draft sparging mechanism and radial flow impellers. The models are validated with experimental data obtained from gassing in—gassing out experiments at varying aeration and agitation rates. The established correlations are then used to determine the theoretically maximum amount of biomass that can be supported by each of the 48 parallel minibioreactors under fully aerobic conditions. Such validated models can be used to expand the usability of minibioreactor systems for application to oxygen-intensive cultures in future fed-batch experiments.

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P13: Model-based automated optimization of a microbial production process using parallel miniaturized stirred tanks reactors

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Microbial production processes are often designed based on personal experience and trial-and-error experiments in sequential benchtop fermentations. Today, parallel cultivation systems, laboratory automation solutions and computational methods for experimental planning and evaluation are available and can be used to accelerate bioprocess development.

Here we present a data-driven approach using principles adapted from statistical experimental planning and machine learning.

As an example, a Saccharomyces cerevisiae AH22 culture producing a secretory enzyme is optimized toward the best space time yield. The parameters medium pH, substrate and feeding strategy are investigated. In the parallel automated minibioreactor system, the product formation rate should be maximized in a very limited amount of individual experimental runs.

To achieve this, we integrate computer aided experimental design tools with automated cultivation facilities in a closed loop setting to enable data collection, storage and processing coupled with a "real-time" coordination of the next steps in the experimental run. The data-driven methods are synergically combined with available knowledge and trends providing a robust mechanistic guidance to the statistical tools allowing efficient experiments in very early development stages

The workflow developed in this project serves as a blueprint for highly effective, model-supported and automated development of microbial cultivation protocols.

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P14: Generating a laboratory for the automated screening and production of antimicrobial agents

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The joint research project "AdvancedBioPro" intends to establish an automated laboratory for biotechnological process development. State-of-the-art robot-based technology for high throughput screening will be combined with already established methods. The laboratory will eventually provide a complete bioprocess chain at Beuth University of Applied Sciences: from initiation and development, over optimization, to upscaling for industrial application.

In the era of rising antibiotic resistances, innovative approaches for the development of new antibiotics are needed [1]. Therefore, the automated laboratory will be applied for the discovery and production of new antimicrobials.

At the beginning, a robot-based high throughput screening was developed to isolate bacteria from environmental samples. The isolated bacteria were tested for their antimicrobial activity against human pathogenic bacteria and identified by MALDI Biotyper System. A promising candidate, an Escherichia coli (E. coli) strain exhibiting antimicrobial activity against enteropathogenic E. coli (EPEC), was chosen for further analysis. To purify the antimicrobial substance, a strategy for downstream processing, combining different precipitation and chromatography steps, was established. Additionally, the purified substance was tested for cytotoxicity using state-of-the-art cell imaging technology. Further steps include the identification of the substance using LC-MALDI-TOF MS-based analysis, fermentation development and large-scale production and purification of the product. Every step of the process chain will be scaled up and optimized.

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P15: Expression and purification of an extremophilic superoxide dismutase from Deinococcus radiodurans

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Microorganisms from extreme environments, e.g. locations with high temperatures or high osmotic pressure, express enzymes called extremozymes due to their robustness to harsh conditions. The SUPPLE project (Sustainable plant-based production of extremozymes) focuses on the development of novel applications for extremozymes in industrial processes. Together with partners from Italy and Germany, the production of antioxidative and de-toxifying enzymes from extremophiles is investigated.

A superoxide dismutase (SOD) from Deinococcus radiodurans (Juan, Keeney et al. 1991) was expressed in E. coli in shaking flask cultures and 1 L reactors using IPTG induction. We show an easy purification strategy employing high-pressure homogenization, Ion exchange chromatography and hydrophobic interaction chromatography techniques leading to a highly pure SOD, according to SDS-PAGE analysis. This allows for direct purification of the SOD from the lysate without intermediate buffer exchanges or concentration steps and an easy scale up of the individual unit operations. Employing complex and defined media we were able to obtain up to 50 mg/l of purified SOD showing a specific activity of up to 3000 U/mg protein on pyrogallol as test substrate.

These results indicate a promising approach for scaling up the production of the extremozyme for potential commercial application. To improve expression yields in future experiments, we aim to establish a scaled up fed-batch process supported by a mathematical model (Calleja, Kavanagh et al., 2016) combined with scaled up purification systems based on the results presented here. Furthermore, the results obtained will be used as reference for the development of the plant cell-based expression system.

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P16: Engineering of Penicillium chrysogenum for rapid prototyping of biosynthetic gene clusters

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Penicillium chrysogenum is an industrially relevant cell factory producing ß-lactam antibiotics. Several decades of strain improvement resulted in strains optimized for high β-lactam yield [1], mainly by accumulation of point mutations [2]. The improved fermentation characteristics of these strains were successfully employed for the production of the polyketide drug Pravastatin [3] and cephalosporins [4].

However, for expression of novel heterologous biosynthetic gene clusters (BGCs), deletion of remaining and active BGCs would be beneficial. Employing a penicillin BGC null strain, we stepwise deleted highly expressed BGCs encoding for chrysogine [5], roquefortine [6] and fungisporin [7], utilizing Cas9 (RNPs) [8]. The obtained strain was characterized in a glucose-limited chemostat for changes in amino acid fluxes, mRNA transcripts and off-target events of Cas9. Our results suggest that the flux towards valine remains and reduced intracellular levels of methionine lead to a stronger induction of autophagy under starvation conditions as encountered in glucose-limited chemostats.

Subsequently, we investigated the potential of P. chrysogenum for direct in vivo assembly of BGCs to shorten the design-build-test cycle of novel pathways. Using short overlaps in all genetic parts, we (re-)introduced the penicillin (21.8 kbp) and decumbenone (38.3 kbp) BGC via direct transformation of PCR products and obtained reasonably high success rates of in-vivo recombination. We further refactored the native Penicillin cluster with various promoter variants and a previously characterized polycistronic expression design [9]. Our approach is suitable for parallelization and library-style-assemblies and increases the speed of probing the potential of so far uncharacterized BGCs from fungal hosts.

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P17: Evaluation of the microbial L-malic acid production by Aspergillus oryzae

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L-Malic acid is a C4-dicarboxylic acid and a potential key building block for a bio based economy. Currently, malic acid is synthesized petrochemically. It is mainly used in the food and beverages industry, in metal cleaning and pharmaceuticals. Due to its bifunctionality, malic acid can also serve as polymerization starter unit. A sustainable alternative to petroleum based synthesis is the microbial production of malic acid from renewable resources. As CO2 fixation is involved in biosynthesis, high yields are possible and at the same time greenhouse gases can be reduced. The mould Aspergillus oryzae is known for its high production capacity for malic acid and has received GRAS status, making it a promising candidate for industrial scale fermentations.

The microbial L-malic acid production is optimized in laboratory scale experiments to enhance the economic efficiency and to identify obstacle for the transformation into the industrial scale. Different aspects including media composition, cultivation strategy and pH regulation are studied, aiming for improved process characteristics (e.g. product titre, yield and productivity), minimized fermentation costs, and a better process understanding.

P18: Development of cultivation strategies for Aspergillus utilizing micro cultivation

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Limited throughput in bioprocess development still hampers the full potential of industrial biotechnology. Miniaturization and automation are viable tools to accelerate bioprocess development. However, the application of such technologies focuses on bacterial systems. Filamentous microorganisms offer unique advantages, going along with multiple obstacles due to their complex morphology.

During the cultivation, the microorganisms can undergo drastic morphological changes, creating challenging cultivation conditions. Depending on the process and product, one specific state of morphology might lead to an increased productivity or yield. Different approaches to control morphology have been investigated, such as micro particle enhanced cultivation. However, the addition of solid micro particles impedes the scattered light measurement used by most micro cultivation systems and alternatives are needed.

Aspergillus giganteus was used to develop a fast and reproducible workflow allowing microscale cultivation with higher throughput. The effect of micro plate geometry, inoculum, power input, temperature and medium additives on homogeneity of culture morphology and reproducibility were analyzed via online biomass measurement, microscopic imaging and cell dry weight. Furthermore, a set of medium additives was tested regarding their impact on growth and morphology to develop an improved medium allowing for online biomass determination. The optimized conditions were used for lab scale bioreactor as well as micro cultivation in order to verify the reproducibility and scalability. Proteomic analysis revealed that antifungal protein (AFP) is the most abundant one, being an attractive candidate for the application in the medical field. It could be shown that AFP production was increased for the morphology optimized conditions.

P19: Impact of glycerol as carbon source onto specific sugar and inducer uptake rates and inclusion body productivity in E. coli BL21(DE3)

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The Gram-negative bacterium E. coli is the host of choice for a multitude of used recombinant proteins. Generally, cultivation is easy, media are cheap and a high product titer can be obtained. However, harsh induction procedures using isopropyl β-D-1 thiogalactopyranoside as inducer are often referred to cause stress reactions, leading to a phenomenon known as "metabolic" or "product burden". Therefore, approaches tend to use "soft" induction with lactose and reduce the stress level of the production host. The usage of glucose as energy source in combination with lactose as induction reagent causes catabolite repression effects on lactose uptake kinetics and as a consequence reduced product titer. Glycerol as an alternative carbon source is already known to have positive impact on product formation and has been referred to show no signs of repression when cultivated with lactose concomitantly. In recent research activities, the impact of different products on the lactose uptake using glucose as carbon source was highlighted, and a mechanistic model for glucose-lactose induction systems showed correlations between specific substrate uptake rate for glucose or glycerol (qs,C) and the maximum specific lactose uptake rate (q,s lac, max). In this study, we investigated the mechanistic of glycerol uptake when using the inducer lactose. We were able to show that a product-producing strain has significant higher inducer uptake rates when being compared to a non-producer strain. Additionally, it was shown that glycerol has beneficial effects on viability of cells and on productivity of the recombinant protein compared to glucose.

P20: Wide range of bacterial strains employed for 1,3-PDO microbial synthesis, during batch/fed-batch fermentations

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A petroleum free glycol and a major intermediate for the production of polytrimethylene terephthalate (PTT), 1,3-propanediol (1,3-PDO) could be obtained in controlled conditions by several microbial cells. Species like Clostridium, Citrobacter, Lactobacillus, Klebsiella, Escherichia coli are good examples known as efficient bio-converters of media containing glycerol, resulting in important yields and productivity for 1,3-propanediol [1,2]. Some strains isolated from soil are competent to use both crude and pure glycerol as carbon source up to 200 g/L of media component [3], synthesizing up to 130 g/L of the final product [4]. Some of them present fast growth speed and high productivity (> 2.0 g/l/h) when glycerol is the main component in the culture broth [3]. The cell growth, the substrate digestion and the final product synthesis during batch / fed-batch fermentations might be predicted by using Monod's equation and Contois-type modelling [2]. Further studies aiming the improvement of 1,3-PDO production, implies the analysis of different growth condition, media components and genetically engineered strains, when aerobic/anaerobic batch and fed-batch fermentations are proposed.

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P21: Foam adsorption as a new generation unit operation for recovery of amphiphilic compounds

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During fermentations foam might occur, especially during the production of amphiphilic compounds such as biosurfactants. An innovative method for process intensification is employed to solve this problem, as well to intensify the production process. The highly concentrated product is being adsorbed from the foam phase during the fermentation, while the rest of the lean stream containing fermentation broth and cells is recycled back into the fermenter which is operated as a closed loop. Two adsorption columns operated automatically were used for continuous product adsorption and elution. The selection of an appropriate adsorbent for this purpose was the central point of this technology development. Based on hydrophobic-hydrophobic biosurfactant specific interaction ex situ separation was performed.

This integration of the upstream and downstream process resulted in stable batch and fed-batch fermentations. An implementation of this technology in the fermentative production of surfactants resulted in reduced number of unit operations with very high biosurfactant recoveries and purities. More important, at the end of the fermentation, 95% of the product was adsorbed in the integrated adsorption column. The foam adsorption method can be applied in all kind of processes where an amphiphilic product is enriched in the fermentation foam.

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P22: BioKat – Biocatalysts in bioreactors

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Main aim of this research project is the characterization of microbial metabolic activities in semi-continuously fed biogas reactors, based on predominantly occurring microbial proteins and enzymes. The results of this study will be used for the development of strategies to support the microbial hydrolysis of renewable raw materials by multi-criteria optimization. In particular, the targeted addition of supplementary enzymes of fungal origin complementary to the endogenous hydrolytic potential present in the biogas fermenters will be analyzed. Different supplementary enzymes will be used in this study, including a novel enzyme preparation generated from remained growth substrates after edible white-rot fungi cultivation. Characterization of the novel enzyme preparation will be conducted in terms of composition, selected enzyme activities as well as its potential to improve the degradation of biomass in the semi-continuously fed biogas processes and batch fermentation trials.

P23: Efficient gas fermentation for the sustainable Single Cell Protein production from N_2, CO_2 and H_2 by single- and two-step anaerobic bioprocesses.

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Unsustainable use of water and land, environmental degradation and food insecurity clearly demand for alternatives to agriculture as only food supply. We are developing efficient, fail-safe, truly sustainable and economically reasonable bioprocesses for the production of edible microbial biomass starting from N_2, CO_2 and H_2. So far experiments focused on the versatile microbe Rhodobacter whose biomass contains 60 % protein with excellent amino acid profile and other valuable products such as Cofactor Q10 and pigments. Rhodobacter can grow anaerobically on H2 + CO_2 or non-fermentable substrates with infrared photosynthesis, both factors contributing to greatly reduced contamination risks. Microaerobic H_2 oxidation also permits light-independent growth. Gas fermentation was tested in CSTR reactors with gas flowthrough, on agar plates, in a closed horizontal fermenter with „rocking wave", and in closed, gas cycling silicon hoses as „intermittent flow". Biofilms may facilitate direct gas mass transfer and cell harvesting by dry cell scraping. Foams are another option to be explored. Growth coupled with a sterile gas loop to dark fermentation of starch powder was attempted. Gas analysis currently being established will confirm H_2 and CO_2 in off-gas from anaerobic digestion of biowaste as well as its concomitant consumption by Rhodobacter. Homoacetogenic bacteria fix CO_2 with half the required ATP and [H], widen substrate range to CO from biowaste gasification and secrete 90 % of fixed C as acetate, which can be consumed in a cocultivation with Rhodobacter or established heterotrophic bioprocesses. Cocultivation avoids inherent problems of acetate such as expensive extraction, product inhibition and toxicity.

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P24: Biosorbents from biowastes versus algae for heavy metals removal from industrial effluents

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Biosorption is a promising and versatile technique to remove dissolved species of heavy metals from industrial effluents. Different researchers have already accomplished a large number of laboratory investigations on biosorption aimed at the pollution removal from aqueous solutions with different kinds of biomass. The most investigated biosorbents are different micro- and macro algae, as well as various biowastes. The algae show relatively high metal-sequestering capacity, although there are very serious disadvantages of the algae biosorption process. In case of micro algae this is the necessity of effective and economically feasible separation of pregnant biosorbent from the solution, or of the algae immobilization. In case of the latter that is the disadvantageous composition of harvested and processed macro algae. Nevertheless, the economically feasible conversion of the biowaste into biosorbent results in the value-added product, as well as meets the endeavors of circular economy.

There are countless research reports on the biosorption process using waste-based sorbents, although the mechanism of their biosorption is not fully revealed yet. The first objective of the present study was to investigate and compare the adsorption behavior of biowastes (e.g. natural and chemically modified sunflower seed hulls and soybean hulls) and that of algae (e.g. Lyngbya taylorii micro algae and Undaria pinnatifida makro algea) in mono-cationic systems containing lead and cadmium. Another objective was to reveal the mechanism of the Pb- and Cd-cations on biowastes above. Several examinations like BET specific surface, FT-IR analysis and ξ-potential measurements were performed to understand the mechanism of metal uptake process.

P25: Continuous enrichment strategies for biobutanol production

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New national and international policies are pushing for a more sustainable production landscape. Once seen as waste, many resources are now the focus of valorization and recovery [1]. Mixed culture biotechnology is held as promising solution for producing valuable biofuels and chemicals from such low-value substrates and waste streams [2]. In this context, butanol is a biofuel of particular attention. With an energy density that is higher than ethanol and properties similar to gasoline, it is a qualified substitute for fossil transport fuels [3]. Biobutanol is currently produced by Clostridia pure cultures with strict glucose requirement [4]. However, reports from Junicke et al. [5] and Steinbusch et al. [6] lead to suggest an alternative production pathway using butyrate and hydrogen as the sole substrates and with an improved environmental balance.

This work presents a novel strategy for butanol production based on microbial mixed cultures. Starting from non-defined methanogenic communities fed on butyrate and hydrogen, butanol-producing microorganisms are enriched through directed ecological selection in a continuously stirred tank reactor. Defined operating conditions are key to obtaining a stable microbial population of desired function and suitable design criteria are discussed. This strategy is entirely different from the pure culture approach as it engineers the environment

rather than the organism. Key advantages include the absence of contamination risks and the ability to use low-value feedstock for the recovery of valuable chemicals such as butanol. The presented work thus contributes to a wider adoption of sustainable production concepts in a circular economy.

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P26: Kinetics of cell disruption during pulsed electric field processing of microalgae Chlorella vulgaris

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Pulsed Electric Fields (PEF) is an emerging technology for the disruption of microbes, making intracellular metabolites accessible for extraction. Due to its low energy requirements, the PEF technology was recently applied for cell disruption of different microalgae species. However, most studies remain descriptive and lack of a mechanistic background.

The present work investigates the kinetics of PEF-mediated pore formation in the cell membrane of Chlorella vulgaris. Cell suspensions were treated at varying temperature and electric field strength and the pore formation was subsequently estimated by means of fluorescent dye staining and flow cytometry. With the experimental data a mechanistic model for pore formation was calibrated, allowing the prediction of pore formation on basis of a profound thermodynamic background. The calibrated model can be used in future research to design, optimize or scale up PEF processing of Chlorella vulgaris.

P27: Effects of the aerobic capacity and growth phase in the adaptation of Saccharomyces cerevisiae to biomass hydrolysate: A single cell analysis approach

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The inhibitors generated during the pretreatment of lignocellulocic biomass reduce the performance of yeast to produce 2G bioethanol. In this context, several studies reported that adaptation of the cell culture during the propagation step results in significant improvements in the ethanol production [1].

In this study, multi-parametric flow cytometry (MPFC) was used to study the changes in the physiology of Saccharomyces cerevisiae during fermentation containing different combinations of common inhibitors found in biomass hydrolysate (vanillin, furfural and acetic acid). Measurements of the cytosolic ROS concentration, membrane potential and membrane permeability were used to assess the physiological state of the cells during the adaptation process. The results showed that the presence of inhibitors results in long lag-phases characterized by high concentration of ROS compounds and low membrane potentials (corresponding to detoxification processes). Only cases with combinations of inhibitors resulted in premature loss of cell viability. MPFC is then applied to determine the effects of the aerobic capacity and of growth phase on the capacity of S. cerevisiae to adapt to hydrolysate. Seed cultures are propagated in high and low aerobic conditions and inoculated, at middle exponential and stationary phases, in a synthetic media simulating hydrolysate. The physiology of yeast is periodically followed with MPFC for a period of 3 – 6 days.

MPFC was first used to understand the adaptation of yeast to biomass hydrolysate, and then applied to assess the effect of aerobic capacity and growth phase on the capacity of adaptation of yeast.

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P28: Simulation assisted development of microfluidic system with integrated electrodes for dielectrophoresis based analysis and sorting of micro algae C. cohnii

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Micro algae are a key element in UN's Sustainable Development Goal number 7. Whereas 3rd generation biofuels are produced by conventional micro algae, 4th generation is looking at specially designed algae for a sustainable biofuel production. Beside biofuels multiple other applications such as nutrition are currently investigated for different types of micro algae. A promising target is the dinoflagellate Crypthecodinium cohnii for production of Docosahexaenoic acid (DHA) as a supplement for fish oil. To improve algae research and process development, methods for population analysis are needed. Dielectrophoresis (DEP) is such a method and currently rarely used with micro algae. DEP enables label free and vital cell analysis and separation by size and polarizability. Among others, C.cohnii is suited for such analysis due to its change of volume and polarizability during DHA production.

We present the development process towards a microfluidic system with integrated electrodes for DEP application. Different manufacturing techniques including 3D-printing and lithography are compared. Design of the microfluidic platform was derived from literature and validated by finite element simulations. We aim to manufacture a cell sorting device based on DEP to investigate the heterogeneous DHA production phase and realize extraction of target cells

P29: On the use of a bienzymatic biosensor for electrochemical detection of propionate in fermentation samples

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Volatile fatty acids (VFAs) are important intermediates in anaerobic digestion that greatly affect the performance and stability of the biogas process. Accumulation of VFAs, often provoked by organic overload and resulting in acidification, reflects a deterioration of the fermentation process and is therefore a reliable indicator for process disturbances [1]. Especially, propionic acid is considered one of the most important single parameters for

the current state of the system, due to its low degradation rate and high inhibitory effect on methanogenic bacteria [2].

Hence, a bienzymatic biosensor for electrochemical quantification of the fatty acid is presented. Propionate CoA-transferase (PCT) from Clostridium propionicum and short-chain acyl-CoA oxidase ($SCAO_x$) from Arabidopsis thaliana were recombinantly produced in Escherichia coli cells, purified by affinity chromatography and immobilized on a platinum working electrode by crosslinking with glutaraldehyde. The reaction principle is based on the enzymatic conversion of propionate into propionyl-CoA, catalyzed by PCT. Subsequently, the activated fatty acid is oxidized by SCAOx, resulting in acrylyl-CoA and hydrogen peroxide (H_2O_2). At an applied potential of +0.6 V vs. Ag/AgCl, the amperometric detection is realized by anodic oxidation of hydrogen peroxide.

The proposed system enables an immediate and accurate determination of this critical process parameter. Moreover, the broad substrate spectrum of $SCAO_x$ allows further extension of the system by substitution of PCT with other enzymes providing activated short chain fatty acids. Observation of different key factors allows an improved understanding of the complex dynamics inside a biogas reactor.

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P30: Printed biosensor for L-lactate monitoring of cell-cultivations

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Optimal process control of mammalian cell cultures in bioreactors requires monitoring of the important process parameters. Some of them, like temperature, dissolved oxygen and pH are being measured continuously and standardly in most cultivation systems on the market today. Other important parameters like glucose and lactose concentrations have not yet found a simple and stable enough measurement method and are in many places still determined once or twice a day by hand. Herein we report the development of a bio-sensing platform for at-line monitoring of lactate. The proposed platform exploits the latest advances in printed electronics to achieve low cost, fast, sensitive and accurate single-measurement biosensors.

In the reported work the development of an amperometry based reagentless biosensor, suitable for the at-line monitoring of lactate, is presented. A robust and reproducible approach for biosensor fabrication was developed and will be shown together with results on the stability of the biosensor under different storage conditions. Furthermore, results from monitoring a cell culture running in a lab scale stirred bioreactor will be compared with those of a commercially available spectrophotometric lactate assay.

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P31: Comparison of impedance and permittivity based dielectric sensors for cell density measurements

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For cell density measurements of cell cultures, two different biosensor concepts were integrated and tested in specially designed microtiter plates. The first sensor operates with interdigitated electrodes (IDEs) in the MHz frequency range, allowing impedance spectroscopy measurements in this well-investigated frequency range. In contrast to that, the second sensor makes use of frequencies around 32 GHz and enables permittivity measurements. It is based on high-frequency hetero-bipolar SiGe:C transistors and contains integrated readout circuits determining the oscillation frequency and power. Thus, the results are provided at DC outputs of the chip, which allows a convenient setup with DC inputs and outputs. Both sensors were tested with different concentrations of NaCl and S. cerevisiae cell suspensions to find an optimal micro-sensor for online cell density determinations in microwell plates with sub-mL sample volumes. The measurements indicate that the 32 GHz sensor could not yet provide permittivity ranges suitable for cell density determinations. On the other hand, the IDE structured sensor was able to distinguish different concentrations of saline solutions as well as S. cerevisiae and E. coli.

P32: Qualitative analysis of organic compounds in bioprocess off-gas

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Bioprocesses are an important tool for the production of desired products such as active biopharmaceuticals. Especially for manufacturing of substances for clinical applications a high product quality needs to be achieved. Therefore control and regulation over certain parameters during the process is required. Within this work a method for off-line measurements of volatile organic carbons (VOCs) in the off-gas stream was established as a reference method for online proton transfer reaction – mass spec. (PTR-MS) measurements. Extraction of VOCs from the off-gas stream was performed using Solid Phase Micro Extraction (SPME)-fibers. Desorption and analysis from the fibers was accomplished using a gas-chromatograph coupled to a mass-spectrometer (GC-MS). Aim of the work was to identify the emitted VOC profile of a model microorganism during the progress of the fermentation as well as the identification of potential key analytes for the process. To accomplish those goals retention times of pure analytical standard substances were determined. This retention times in combination with comparison of mass spectra with a database was sufficient for proper identification. Using PTR-MS and GC-MS a broad spectrum of substances could be determined and identified during fermentation off-gas measurements. SPME fibers coupled with GC-MS analysis prove to be a potent tool for analysis and identification of VOCs in the fermentation off-gas stream and represent a non-invasive way to gain more knowledge over the process itself.

P33: Improving product quality through Raman based bioprocess control

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The quality of therapeutic CHO cell culture proteins are a result of the entire production process. Producing therapeutic proteins with consistent characteristics is a challenging task which requires a high level of process understanding and maintaining process conditions within an optimal batch trajectory.

The aims were to enable – via in situ Raman spectroscopy - simultaneous monitoring of multiple product quality relevant parameter and allowing for in-process corrections.

Process development, pilot, and manufacturing scale CHO cell culture batches were utilized to demonstrate the feasibility of monitoring and effect of controlling multiple critical process parameters in real-time using Raman spectroscopy. Case studies in the presentation illustrate the impact of Raman based bioprocess control to the cells, the cell culture environment, the yield, and finally to the quality of therapeutic proteins [1]. Results of the case studies provide valuable insights into effective control strategies and explicate the successful transfer of analytical Raman methods from process development to GMP manufacturing.

Precise navigation and smart adaptation of critical upstream process conditions based on Raman spectroscopy reduced the complexity of producing high quality products.

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P34: Online Raman spectroscopy for the quantitative evaluation of a Ralstonia cultivation

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Spectroscopic methods possess important advantages for the observation of bioprocesses: they are robust, gain many data within a short time scale, and allow for the measurement of multiple relevant information at the same time [1]. Next to Near-infrared spectroscopy, Raman spectroscopy is increasingly applied as a powerful online tool to monitor bioprocesses [2]. However, Raman spectra may be obscured by straylight, cosmic rays and fluorescence. To achieve the best performance, optimal conditions for spectral quality and stability should be pursued. This comprises not only the choice of highly-sensitive equipment, but also the way of implementation into the process. Here we compared the potential of introducing a sterilized fiber optical Raman probe, and a flow-through cell into the bypass of a stirred tank reactor to monitor an autotrophic cultivation of Ralstonia eutropha where gasses are the energy and carbon sources. Raman spectra were continuously recorded over seven days and 22 samples were taken for the reference analytics of biomass, optical density, and protein. In addition, data for of phosphate and ammonium were available. The quantitative evaluation of Raman spectra by Partial Least Squares Regression (PLSR) yielded comparable results for both approaches, however, there was a general trend of lower prediction errors with the immersion probe. The better performance of the non-contact optic may be either related to a more representative interaction of the measurement optics with the probe or simply to the absence of probe-fouling, which was observed with the immersion optic in the interstice between window and metal frame.

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[2] Berry, B., et al., (2015), Biotechnol. Prog., 31,2, 566-577

P35: Spectroscopic analytical technology for mammalian bioprocesses

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According to the PAT initiative of the FDA, industrial bioprocesses should be monitored and controlled to enable product quality by valid processes. High quality products and efficient processes must be obtained by a deep understanding and knowledge of the whole process, enhanced by a suitable sensor system. For bioprocesses the biological component as well as the chemical environment of the cells should be monitored extensive. For mammalian cell cultivations substrate, metabolite and product concentrations are particularly of interest, as well as the amount and viability of the cells. By spectroscopic sensors a couple of critical process variables are accessible simultaneously, in-line and non invasive.

Different sensor applications such as UV-VIS, NIR, and fluorescence spectroscopy are discussed to establish an effective process monitoring. The possibilities and limitations of various spectroscopic methods for on line prediction of variables are presented for CHO cell cultivation in a 15 liter stirred tank bioreactor. The essential data analysis for spectral data offer many possibilities of qualitative and quantitative process monitoring. On the other hand, extensive data analysis marks the limitations of this technique. The combination of different sensors including knowledge based process models opens the horizon for a holistic and intelligent bioprocess monitoring and to build up a process control system.

P36: Photon Density Wave spectroscopy as a new approach for in-line monitoring of biomass formation during fermentation processes

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The ability to monitor the formation of biomass and hence the determination of the specific growth rate during fermentations are of high interest for the development of process control strategies. This is of high importance for industrial applications, as factors like maximal biomass production, the physiological state of cells, product formation rate and product quality are directly related to the specific growth rate [1].

A fully autoclavable and chemically sterilizable, calibration-free process analytical technology suitable to highest concentrations (i.e. > 40 vol%) in stirred or flowing systems is found in Photon Density Wave (PDW) spectroscopy [2]. It allows for the independent quantification of the absorption and scattering properties of disperse materials, i.e. the absorption and the reduced scattering coefficients, respectively. The absorption coefficient exhibits information about concentration changes of various media components during the investigated process. The reduced scattering coefficient however is related to the size and concentration of the dispersed cells.

In this contribution, PDW spectroscopy will be introduced as a new in-line analytical tool for bioprocess monitoring and control. Information obtained from changing scattering and absorption properties during fermentations are shown to be directly related to biomass formation and the adjustment of feeding strategies. As practical examples, results of fed-batch high-density fermentations of yeast cultures will be presented, analyzing process parameters of importance by PDW spectroscopy and in comparison to already established optical process analytical

technologies. Furthermore the potential for the control of the specific growth rate by an adaptive carbon source feed strategy based on in-line PDW spectroscopy will be discussed.

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P37: Application of flow following sensor particle: Flow characteristics in stirred vessels

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In this study, a novel process analytical technology (PAT) tool for online monitoring of industrial bioreactors is presented. The technology consists of a flow following sensor device which is robust, steam sterilizable and capable of measuring critical culture parameters, as well as linking the measured data to the depth at which they are measured. It is therefore possible to analyze flow characteristics and concentration gradients when scaling to industrial bioreactors.

The sensor particles have been tested in a pilot scale reactor with 630 L of water. From pressure measurements of the particles, axial distribution, velocities and circulation times have been determined at four agitation intensity levels. The results have been compared with CFD simulations of the reactor. In addition, the circulation times have been correlated with mixing times determined from traditional tracer experiments in the reactor.

It was demonstrated that circulation times determined by the particles are proportional to the mixing time determined in tracer experiments and by CFD simulations. In addition, the axial velocities determined by the sensor particles are comparable to the velocities obtained from CFD simulations.

Using free-floating sensors rather than fixed sensors can provide unexplored data which has not previously been available. This can serve as an important tool in validation, control and regulation of industrial processes, as well as a tool for process optimization where it should for example be possible to reduce development cycle time during process scale-up.

P38: Adaptive control of microbial fermentation processes using modular soft-sensor based platform

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Cultivating microbial or CHO cells in an optimal way is a challenging task especially when scale is changing. Many parameters, like pH, temperature or substrate feeding have to be controlled precisely. Soft-sensors can help in achieving this, by processing sensor data in real time to automate process control, which is an important component of the process analytical technology (PAT) and is essential for effective quality control. The ProcessShield platform, which is presented here, combines different soft-sensor modules designed for intelligent, adaptive process control. Several extendable modular tools grant deeper insights into the process at hand. For example, the Biomass-Observer module estimates the current cell density, based on a logistic model, as an addition to hardware cell density sensors or to reduce the frequency of offline probing. Another module called Flux-Calculator computes intracellular flux rates of different metabolites to detect bottlenecks in production pathways or enable novel process control strategies. Other modules are built for dynamic process control, like the Feed-Distributor tool, which adapts the feeding strategy in response to the current substrate consumption and improves product formation, and the Process-Protector module, which improves process stability by scanning for faulty sensor data and monitoring the other modules. The Brain-Pool module uses artificial neural networks to improve process control. The model automatically creates clusters of similar data (e.g. log-phase, under-fed log-phase) based on legacy data sets, to categorize online data and show the reason for standard process deviations. With neural networks, predictions about the expected batch behaviour and thus predictive control could be feasible

P39: Monitoring of a yeast insulin production process using image analysis and IR spectroscopy for integral process understanding and control

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Fermentation processes are often the most complex step within bio-manufacturing. Nevertheless, the detailed understanding of the fermentation process is often lacking and manual control decisions are frequently based on experience instead of process data. We want to access industrially relevant fermentation processes with advanced but still rather uncommonly used cutting-edge technologies thus developing new monitoring methods while improving the integral understanding and success of the process. Based on an insulin production process by yeast, we investigate and develop monitoring strategies based on microscopy image analysis and IR spectroscopy. Using the former, the lately developed oCelloScope instrument1 enables rapid imaging and image analysis of a growing yeast culture facilitating the monitoring of growth and morphology dynamics. Ideally, a snap shot of the culture can represent a set of process performance parameters within a minute (cell concentration, physiological state). As image analysis, IR spectroscopy can access multiple types of process information at a time: A single spectrum can be used to detect various nutrients in the fermentation broth. Focus here is on glucose, phosphate, ammonium, ethanol and acetate quantification representing typical process performance data on yeast processes. As in the liquid phase, molecules get excited in the gas phase when exposed to the IR spectrum thus enabling quantitative analysis. Based on a tunable laser spectroscopy detection principle, the BioInsight gas analyzer is a high-precision, flexible, cost-effective and easy-to-use multi-gas analyzer. It features real-time monitoring on a high dynamic range, making it possible to measure a variety of gasses relevant for the process.

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P40: Usage of various process analytical technologies (PAT) for evaluation of Lactobacillus acidophilus fermentation

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For the production of probiotic and starter cultures, the viability and vitality of the cells is of major interest, since the declared amount of living microorganisms at the end of shelf-life is a main quality criterion. Thus, it is of fundamental importance to produce cultures which are as robust as possible to withstand

the conditions during the different processing steps and storage until the time of usage or consumption. The culture media and the process conditions during the fermentation can significantly impact the stability properties of produced cultures and preparations. One aspect of relevance is the media alteration during heat sterilization that can influence the growth performance, the cell morphology as well as the robustness of the cultures. Therefore, monitoring of Lactobacillus fermentations by adequate PAT and the correlation of relevant monitored parameters among each other are of high interest for an optimal fermentation progress leading to constant high quality products.

In this contribution, different methods for monitoring growth kinetics, cell morphology and changing physiological characteristics caused by cultivation in different media are shown for the fermentation process of L. acidophilus DSM 9126. Besides the control and measurement of online parameters like DO, pH and redox potential, culture performance and cell-size distribution were characterized by online automated electrooptical measurements (EloTrace®, Biotronix GmbH), and several offline methods, e.g. impedance measurement in combination with digital pulse processing (Multisizer™ 3, Beckman Coulter GmbH), flowcytometry (CyFlow® Cube 8, Sysmex Inc.), microscopic cell-size mapping (Olympus CX41; cellSens Entry 1.14; Olympus Deutschland GmbH), cell dry weight and microbial metabolites.

It was shown that a continuous measurement of different parameters led to a better understanding of the fermentation progress, especially for the here shown process where the cell morphology is of relevance. The influence of the differently treated media on the monitored parameters are discussed in respect to optimal fermentation strategies for the production of robust cultures.

P41: Online monitoring of the cell specific oxygen uptake rate in CHO-cell cultivation using a softsensor in different scales of STR

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The cell specific oxygen uptake rate qOUR is a key parameter to evaluate the productivity of biotechnology production systems. Knowledge off the qOUR implements the opportunity to judge the profitability to continue the cultivation and can easily be detected for microbial cell cultivations. In mammalian cell cultivation it is difficult to detect the very small difference of oxygen consumption, only expensive devices like mass spectrometer can detect the concentration difference.

The qOUR depends on the oxygen uptake rate OUR and the current cell concentration. The OUR is related to the oxygen transfer coefficient kLa. The kLa itself depends on process variables like stirrer speed, aeration rate, liquid volume, oxygen rate at the gas inlet and antifoam concentration. For each physical process condition the kLa can be estimated by design of experiments DoE and used to calculate the OUR and the qOUR during cultivation. Another possibility to determine the qOUR is the dynamic method, evaluating the decreasing pO_2 signal during stopped aeration.

With both approaches a softsensor was established, being able to follow the course of the qOUR during mammalian cell cultivation in stirred-tank reactors of different scales (2 L and 15 L). Now the qOUR can be monitored without expensive measuring devices, online and in a simple manner, being always able to evaluate the process condition and cell behaviour.

P42: Mobile multiparameter sensor technology for gradient characterization in the liquid phase of large scale bioreactors

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Since the power input in large-scale bioreactors is limited for various reasons, gradients occur, e.g. for the pH-value, dissolved oxygen (DO), dissolved carbon dioxide (DCO_2) or temperature.

Since the available sensor technology, which is usually located at an arbitrarily chosen spot is not designed for the consideration of such heterogeneities, the knowledge about gradients and their magnitude is rather low.

Therefore, mobile, multi-parameter sensor tools were developed for in-situ and on-line measurements of various process parameters in order to characterize gradient formation in anaerobic fermentation and identify suitable positions to install fixed monitoring devices and sampling ports. Sensors for the pH-, DO-, DCO_2-value, redox potential, conductivity, temperature, and pressure were integrated into two sensor units. One unit was comprising 6 newly developed miniaturized sensors, the other 3 commercially available sensors. These monitoring tools were applied in various fermenters of different geometries and scales, like they are applied in the brewing industry and for biogas production. The miniaturized unit was integrated into lance and rope-based systems for in-situ monitoring in radial and axial direction at various fermenters. Recently, also the unit with larger sensors was applied at different positions in a research biogas plant, using different ports installed on the fermenter roof and positioning at different heights.

The long-term application of miniaturized sensors with a small membrane diameter and electrolyte volume is challenging in complex brewing media due to the risk of clogging or toxification of the electrolyte by chemical compounds, e.g. DCO_2. This affects the measurement stability and sensor drift. With larger sensors, however, the pH-value, redox potential, conductivity, temperature, and DO-value were measured up to 3 months without significant losses in the measurement stability and with a negligible increase in the sensor drift. In the biogas process, measurement stability and low drift were achieved also with the miniaturized system.

The mobile multi-parameter measurement devices allow for a fast detection of gradients in large-scale processes. The correlation of the sensor data from multi-position measurements with the data from metabolite and physiological analysis enables a description of the impact of gradients on the bioprocess performance. Scale-down experiments in the lab scale can be designed so that they mimic the industrial case properly.

P43: A software platform for quality by control realization of mammalian cell culture bioprocesses

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Mammalian cell culture bioprocesses are nowadays the system of choice for the production of complex therapeutic proteins such as monoclonal antibodies in biopharmaceutical industry. In order to improve production processes, reducing over-processing and enhancing consistency of the final product, the FDA defined in 2004 a regulatory framework for process analytical technology (PAT) implementation. The concept aims for better process understanding by measuring and monitoring (in-line and on-line) the critical process parameters (CPPs) which affect the critical quality attributes (CQAs). Implementation of PAT into the bio-production process enables moving from the quality by testing to a more flexible quality by design approach. Hereby, extensive process monitoring by advanced sensor systems in combination with mathematical modeling techniques allows real-time product quality monitoring, control and release. In this project we are establishing a software platform that enables integrated process development and advanced process control. A design of experiment set-up was applied to CHO bioprocesses at different scales (300 mL – 100 L) in order to generate soft-sensors for the estimation of process-related data which are generally not directly measurable such as OUR and biomass. In addition, hybrid models were built to better understand the overall process and optimize the outcome. We furthermore want to apply the software platform for model based and predictive control of the bioprocess.

P44: LED-Based two-dimensional fluorescence spectroscopy for bioprocess monitoring

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Fluorescence Spectroscopy is already well established in laboratory analytics. At the same time, its monitoring capabilities of different bioprocesses have been thoroughly researched over the last decades. These studies have shown that through detection of naturally occurring fluorophores inside and outside of cells, the metabolic state of the cells can be monitored. Through application of multivariate methods other important process parameters like cell count and viability can be quantified as well [1].

The aim of this study is to develop a new fluorescence spectrometer, which is specifically designed for industrial applications of on-line monitoring of mammalian cell cultures. The device consists of three high-power LEDs with different peak-wavelengths for excitation of the different fluorophores, a specially designed probe that connects to the bioreactor, as well as a back-thinned CCD-spectrometer for detection of the Rayleigh backscattering and the fluorescence signal.

First laboratory tests showed the sensors high sensitivity and large dynamic range. Further tests conducted in CHO cultivations proved the sensor's capability of process monitoring. Multivariate models that correlate to the cells' metabolic state were established, as well as quantitative models for viability, total- and viable cell count.

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P45: Morphology monitoring of bacteria for better process understanding and control

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Food bioprocesses such as probiotic manufacturing only use conventional pH and OD for monitoring, even though they represent the system's physical, not biological state. Cell morphology is the combined result of genotype and environmental factors, and therefore a better indicator of biology (Barry & Williams, 2011). Bioprocess monitoring in pharmaceutical industry has evolved into employing a wide range of at-line, in-line and on-line sensors for better process understanding and control (Zhao, et al., 2015).

To evaluate the potential of rapid, robust monitoring strategy that measures morphology of cells, we performed oCelloScopeTM (BioSense Solutions ApS, Denmark) analysis of Lactobacillus acidophilus samples collected at regular intervals during fermentation. The analysis provides parameters like area, skeleton length and circularity that characterize morphological state of cells (ApS, 2017). Emphasis is the development of a morphological investigation method and its potential industrial applicability in a low-value, high-volume biotech industry such as food ingredients. oCelloScope results of different pH fermentations showed no significant change in morphology, proving that the industrial strain used is robust and resistant to pH gradients prevailing in large scale bioreactors. Unlike traditional time-consuming methods (i.e. plating), oCelloScope provides results in few minutes. 96-well plate used makes multiple recordings easier, giving better statistics and less potential errors in at-line applications. Furthermore, it can detect contamination, which is not readily possible in other monitoring strategies. The combination of image acquiring and analysis provides operators with a tool to 'look into the reactor' and make informed feedback control decisions.

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[2] Barry, D. J. & Williams, G. A., (2011). Journal of Microscopy, Volume 244, pp. 1-20

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P46: Increase the profit of a bacteria-based PDO production facility – The process simulation cup 2018

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In 2015, Chemstations Europe initiated the first edition of the Process Simulation Cup (PSC), a contest for students interested in process engineering. This contest was related to a dynamic distillation problem. Two further contests have been realized, one addressing energy optimization and the other controller optimization for biogas plants. Details of these contests and benefits for students and teachers are presented in [1].

The PSC2018 problem is related to a world scale bio process, the bacteria-based production of 1,3-propanediol (PDO) from cane juice, see e.g. [2]. Participants must increase the profit of the production facility by adjusting several design variables. The process is modelled in CHEMCAD as a dynamic fed-batch process coupled to a continuous downstream process. The latter is modelled based on information gathered from the patent [3].

This case study shows how a flowsheet simulator is used to model and to optimize coupled batch and continuous process parts as they commonly occur in bio-refineries. A workflow for

converting macro-kinetic fermentation models into first-principle flowsheet simulator models is presented. The implementation of typical downstream process related unit operations like cross-flow filtrations is discussed.

Having a rigorous process model in a flowsheet simulator allows a detailed calculation of the operational expenditures (OPEX) and thus enables the application of process improvement tools like sensitivity studies and optimization algorithms. But a multivariate optimization with a complex process model and several process constraints is still a challenging task and this task is to be tackled by the participants of the PSC2018.

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P47: Microcarrier culture scale-up via bead-to-bead transfer

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Intravacc

Culturing anchorage dependent cells, such as Vero cells and stem cells, in bioreactors require microcarriers as a surface. Scale-up of such cultures is a time intensive process and often results in cell damage. Scale-up requires the spend culture medium to be removed, a washing step and enzymatic detachment of the cells from the microcarriers. After detachment, the cells need to be separated from the microcarriers before they can be transferred to the new larger bioreactor containing fresh medium and empty microcarriers. We present a method devoid of these steps where scale-up is achieved by the transfer of cells from full microcarriers to new empty microcarriers. An intermittent stirring profile causes the microcarriers to settle and allows contact between the occupied and empty microcarriers. During this contact dividing cells can transfer and attach to the empty microcarrier and subsequently proliferate. This transfer procedure was shown to be scalable up to 100L.

P48: Custom made inclusion bodies: Triggering QAs using classical process parameters like pH and T

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E. coli is the host of choice for about 30% of the worldwide production of recombinant pharmaceuticals. In a multitude of processes, recombinant proteins are expressed as inclusion bodies (IB) in E. coli – an (in)active aggregate of misfolded produced protein. Respective IB properties can be modified by the cultivation conditions in the upstream process and these have severe impact on the performance of the subsequent process chain, like the refolding unit operation. However, only a very few studies deal with the general quality attributes of IBs.

In this study, we deal with the interaction of different IB QAs like size, titer and purity. Temperature and pH are altered using a design of experiment (DoE) approach in a CCF design. QAs are monitored as a function of induction time and analyzed for all given timepoints. It is shown, that different QAs already show a strong time dependence within a single cultivation run and are highly correlated to each other. Furthermore, DoE evaluation give the possibility to determine QAs as a function of pH, temperature and induction time and receive custom made IBs with optimized performance for further process steps.

P49: Risk of product heterogeneities caused by population heterogeneity

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Novel analytical techniques will lead to major improvements in the field of biotechnological processes. In this work, an unsupervised image analysis technique was established and used to determine multidimensional cell properties in various cell cultures [1]. As model processes, lipid production in different algal-biotechnological model cultivations (Lab to pilot-scale) were investigated. Single-cell analysis was based on epifluorescence microscopy with spectral information. Object recognition and single-cell characterization in the microscopic data was done via algorithms programmed in MATLAB®. Use of spectroscopic information from fluorescence microscopes enables quantitative determination of cell size and quantification of different cellular ingredients such as pigments and lipids in parallel. Reliability of the technique was proven via different reference analyses. On the single-cellular level, heterogeneity of the culture in multiple dimensions and correlations between different cellular properties were quantified over time. The algal cells show an unexpected heterogeneity in all single-cell parameters and exhibit very dynamic correlations between cell size, chlorophyll content, and lipid content. Furthermore, the cells differentiated into two distinct sub-populations with clearly different TAG productivities. Understanding and optimization of these single-cell dynamics will contribute to gain higher lipid yields in future and opens new perspectives in biotechnology and systems biology. Based on the described cell-to-cell heterogeneity the question raises how the product quality and quantity is possibly negatively influenced.

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