Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik

BioProScale e.V.

Bio-PAT

IfGB
Institut für Gärungsgewerbe und Biotechnologie zu Berlin

7th BioProScale Symposium

Scaling Up and Down of Bioprocesses: Technological Innovation and Cell Physiology Insights

28 TO 31 MARCH 2022
LANGENBECK-VIRCHOW-HAUS, BERLIN, GERMANY

In-person event with online access to the presentation sessions

Part 1: Industrial Scale – Scaling up and down – PAT
(28-29 March 2022, Mon/Tue)

Part 2: High Throughput Bioprocess Development – Advances in Software, Hardware, and Integration
(30-31 March 2022, Wed/Thu)

www.bioproscale-conference.org

VENUE: LANGENBECK-VIRCHOW-HAUS, BERLIN, GERMANY LANGUAGE: ENGLISH

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Our venue

Exhibition, coffee & lunch breaks ..... Ground level

Room "Bernhard von Langenbeck":
Exhibition, poster areas, coffee & lunch breaks ..... 1st floor

"Historical Lecture Hall", poster areas ..... 2nd floor

Online access: vlb-berlin.6connex.eu/event/virtual-conference/login

Welcome address

Dear Colleagues, Ladies and Gentlemen,

I want to welcome you all to our 7th BioProScale Symposium in Berlin. I am very glad that we have the possibility to perform the symposium as in-person event in Berlin again after the last year´s online conference and the cancellation of the conference that was planned for March 2020.

Our symposium is taking place in a very turbulent time, in which not only the impact of climate change is becoming apparent, but the pandemic and its consequences are still present, and the Russian invasion into the Ukraine is causing a human tragedy. It threatens the entire social, economic and political system, and by this also the scientific progress and the global cooperation on which our modern science is built on.

All these developments create challenges for biotechnology, ranging from the demand for new process development and production concepts for the pharmaceutical industry, to a sustainable, from fossil resources -independent production of materials and energy and to new concepts for a healthy nutrition of a steadily growing world population. The implementation of processes, however, in economically and ecologically viable solutions is extremely demanding and requires completely new ways of thinking in combination with the development of new technologies in the frame of bioprocess engineering.

We are very pleased that we have once again been able to attract leading scientists from both academia and industry to our symposium and would like to express our special thanks to the speakers who have accepted our invitation to share and discuss their expertise with us.

We are also very pleased that our symposium is also attractive to a number of companies which take the symposium as a chance to present their new products and developments. We would like to thank in particular our exhibitors and sponsors.

Last but not least, I personally would like to express my sincere thanks to all the people and organizations who helped to plan and conduct this event. My sincere thanks go to the VLB Berlin for their close and very professional cooperation in realizing the in-person event, but also for the provision of the interactive conference platform. In this hybrid concept, interested scientists that are not able to join physically in Berlin, have the opportunity to follow the presentations from all over the world.

I hope that the four days excite all of us and give us the opportunity not only to follow the new scientific developments by the talks and posters, but also to make new or re-activate former contacts. I would also like to thank my colleagues and the members of the scientific advisory board who have always actively supported the idea of the conference and its implementation and have unselfishly taken on many detailed tasks.

With this I wish us all an exciting symposium, enjoy the talks and discussions!

Professor Dr. Peter Neubauer
Technische Universität Berlin – Chair of Bioprocess Engineering
peter.neubauer@tu-berlin.de

Scientific advisory board

Anika Bockisch (Bio-PAT e.V., Germany)
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Joachim Venus (ATB, Germany)

About the organisers

Technische Universität Berlin: Institute 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 and sustainability.

www.tu-berlin.de/ bioprocess

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 sole holder of the IfGB. Under the brand name "IfGB", services and training for the spirits industry and distillers have been offered and expanded also into the field of applied biotechnology.

IfGB is a brand of VLB Berlin e.V.

www.ifgb.de

Co-organizer:
BioProScale e.V., Berlin

Co-organizer:
Cooperation Network Bio-PAT e.V., Berlin

MONDAY, 28 MARCH 2022

INDUSTRIAL SCALE – SCALING UP AND DOWN – PAT (PART 1)

WELCOME & PLENARY TALK

12:00 **Welcome address and introduction**
Peter Neubauer, TU Berlin, Germany

12:20 **Plenary Talk: Large scale production of mRNA vaccines (PL01)**
Steffen Panzner, BioNTech Delivery Technologies, Germany[1]

SESSION 1: SCALE UP AND SCALE DOWN OF BIOPROCESSES

Chair Cees Haringa / Simon Täuber

13:05 **Dedicated single-use bioreactor designs, making cellular agriculture feasible (L01)**
Nico Oosterhuis, The Netherlands

13:30 **Assessment of Lagrangian sensor particle designs in a transparent 15,000 L acrylic glass bioreactor (L02)**
Sebastian Hofmann, Hamburg University of Technology, Germany

13:55 **CFD supported scale-up in biopharma – an overview with focus on hydrodynamic stress (L03)**
Maike Kuschel, Boehringer Ingelheim Pharma GmbH & Co.KG, Germany

14:20 *Coffe break & exhibition*

SESSION 2: BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair Krist V. Gernaey / Sebastian Riedel

14:50 **High yield bioconversion of animal by-product streams to polyhydroxyalkanoates (L04)**
Björn Gutschmann, TU Berlin, Germany

15:15 **Continuous bio-succinic acid production from alternative feedstock (L05)**
Pascal Leonov, Technical University of Denmark, Denmark

15:40 **Combining in-line FTIR spectroscopy with MIMO control and feedback linearization for a continuous *C. glutamicum* bioprocess fed with lignocellulosic waste streams (L06)**
Daniel Waldschitz, TU Wien, Austria

16:05 **Improving biochemical hydrolysis by bioaugmentation in plug-flow based reactors (L07)**
Theresa Menzel, TU Berlin, Germany

EXHIBITOR SHORT PRESENTATIONS

16:30 **Exhibitor short presentations**
Thomas Skov, SANI Membranes A/S, Denmark / Richard Salliss, Keit Spectrometers Ltd, United Kingdom / Julius Muno, aquila biolabs GmbH, Germany / Anika Bockisch, Bio-PAT e.V., Germany

16:50 *Coffee break & exhibition*

SESSION 3: BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair Howard Ramirez-Malule / Tolue Kheirkhah

17:20 **Production of methane and carboxylic acids from organic residues (L08)**
Ilmari Laaksonen, Natural Resources Institute Finland, Finland

17:40 **Production of biotechnological fish feed by microalgae and oleaginous yeast (L09)**
Stefan Junne, TU Berlin, Germany

PLENARY TALK

18:00 **Plenary Talk: Fungal biotechnology as innovation driver for a circular economy (PL02)**
Vera Meyer, TU Berlin, Germany

EVENING PROGRAMME

18:45 **Poster session, exhibition, get-together**
Langenbeck-Virchow-Haus

21:00 End of day 1

All times Central European Time (CET/MEZ)

TUESDAY, 29 MARCH 2023

INDUSTRIAL SCALE – SCALING UP AND DOWN – PAT (PART 2)

WELCOME & PLENARY TALK

9:00 **Welcome & introduction**
Peter Neubauer, TU Berlin, Germany

9:05 **Plenary Talk: An industrial perspective on the gas-liquid flow of stirred multistage bioreactors: challenge and a novel scaling approach (PL03)**
Sören Bernauer, BASF Ludwigshafen, Germany

SESSION 4: SCALE UP AND SCALE DOWN OF BIOPROCESSES

Chair Ralf Takors / Stefan Junne

9:50 **CFD-kinetic modelling for the scale-up of *P. putida* fed-batch fermentations (L10)**
Maryam Jamshidzadeh, Technical University of Denmark, Denmark

10:15 **Impact of local solid concentration on anaerobic digester hydrodynamics at industrial scale: a CFD study (L11)**
Liliane Megue Kamkeng, Université de Lorraine/Air Liquide, France

10:40 **Scale-down of high cell density Fab production in *E. coli* (L12)**
Florian Mayer, University of Natural Resources and Life Sciences, Vienna, Austria

11:05 *Coffee break & exhibition*

SESSION 5: SCALE UP AND SCALE DOWN OF BIOPROCESSES

Chair Stuart Stocks / Eike Janesch

11:35 **Development of a single multi-compartment bioreactor (SMCB) for CHO scale-down studies in heterogeneous cultivation environments (L13)**
Lena Gaugler, University of Stuttgart, Germany

12:00 **Suitability of various scale-down bioreactor designs at the example of the oleaginous yeast *Yarrowia lipolytica* (L14)**
Jasmina Cziommer, TU Berlin, Germany

12:25 **Syngas fermentation: a scale-down approach to simulate large-scale gradients at lab-scale (L15)**
Lars Puiman, TU Delft, The Netherlands

12:50 **Optimization and scale-up of itaconic acid production on complex substrates (L16)**
Paul-Joachim Niehoff, RWTH Aachen, Germany

13:15 *Lunch break & exhibition*

SESSION 6: PROCESS ANALYTICAL TECHNOLOGIES

Chair Holger Müller / Annina Kemmer

14:45 **Enabling continuous co-culture bioprocesses (C3BIO) based on oscillating environmental conditions promoting genetic and/or metabolic requirements of individual species (L17)**
Vincent Vandenbroucke, Université de Liège, Belgium

15:10 **Dead or alive: a novel PAT tool for the extremophile *Sulfolobus acidocaldarius* (L18)**
Kerstin Rastädter, TU Wien, Austria

15:35 **Population heterogeneity in *E. coli* chemostat cultivation: An investigation of alternating gene expression levels between observed phenotypes (L19)**
Julian Kopp, TU Wien, Austria

16:00 **Heavyweight data: Microscale material balancing in microfluidics (L20)**
Katharina Smaluch, UFZ Leipzig, Germany

16:25 *Coffee break & exhibition*

All times Central European Time (CET/MEZ)

SESSION 7: PROCESS ANALYTICAL TECHNOLOGIES

Chair: Anika Bockisch / Carmen Walczak***

16:55 **On-line monitoring of key metabolites in *E. coli* fermentations by near-infrared (NIR) spectroscopy (L21)**
Jakob Forsberg, University of Copenhagen, Denmark

17:20 **Real-time inline monitoring of *Trichoderma reesei* cultivation in industrial environment and prediction of protein folding by time-gated Raman spectroscopy (L22)**
Martin Kögler, VTT Technical Research Centre of Finland, Finland

PLENARY TALK

17:45 **Plenary Talk: Engineering, processing and application of recombinant spider silk proteins – from lab to market (PL04)**
Thomas Scheibel, University of Bayreuth, Germany

EVENING PROGRAMME

19:30 **Conference Dinner**
Lindenbräu im Sony-Center am Potsdamer Platz, Bellevuestr. 3-5, 10785 Berlin, www.bier-genuss.berlin
Access mit Dinner Voucher only!

23:00 *End of day 2*

WEDNESDAY, 30 MARCH 2022

HIGH THROUGHPUT BIOPROCESS DEVELOPMENT – ADVANCES IN SOFTWARE, HARDWARE, AND INTEGRATION (PART 1)

WELCOME & PLENARY TALK

9:00 **Welcome & introduction**
Peter Neubauer, TU Berlin, Germany

9:05 **Plenary Talk: Smart tools for high cell density perfusion process producing monoclonal antibodies (PL05)**
Veronique Chotteau, Royal Institute of Technology, Sweden

SESSION 8: BIOPROCESS DEVELOPMENT

Chair: Robert Spann*** / Sarah Westarp

9:50 **Application of a microfluidic single-cell cultivation platform for mammalian suspension cell lines in bioprocess research and development (L23)**
Julian Schmitz, Bielefeld University, Germany

10:15 **Raining fresh red blood cells: scale-up of ex vivo erythroblast expansion for transfusion purposes (L24)**
Sebastian Aljoscha Wahl, Friedrich-Alexander Universität Erlangen-Nürnberg, Germany

EXHIBITOR SHORT PRESENTATIONS

10:40 **Exhibitor short presentations**
Nico Oosterhuis, Celltainer Biotech BV, Netherlands / Mirko Fraulob, INFORS HT, Germany / Simon Lucht, I&L Biosystems GmbH, Germany / Anja Dürasch, KNAUER Wissenschaftliche Geräte GmbH, Germany / Jochen Uhlenküken, Hamilton Bonaduz AG, Germany

11:00 *Coffee break & exhibition*

SESSION 9: BIOPROCESS DEVELOPMENT

Chair: Marco Oldiges / Niels Krausch

11:30 **Development of a bioprocess for heterologous hydrogenase production in *E. coli* (L25)**
Matthias Gimpel, TU Berlin, Germany

All times Central European Time (CET/MEZ)

*** *last-minute change*

11:55	**Development of a scalable production for recombinant horseradish peroxidase (L26)** *Julian Ebner, TU Wien, Austria*
12:20	**Innovative bioprocess strategies combining physiological control and strain engineering of *Pichia pastoris* to improve recombinant protein production (L27)** *Xavier, Garcia-Ortega, Universitat de Vic, Universitat Central de Catalunya, Spain*
12:45	**Insights into the physiology of *Streptomyces clavuligerus* by constraint-based models for the enhancement of clavulanic acid (L28)** *David Gómez-Rios, Howard Ramirez-Malule, Universidad del Valle, Colombia*
13:10	*Lunch break & exhibition*

SESSION 10: HIGH-THROUGHPUT BIOPROCESSING AND AUTOMATION

Chair: Klaus Pellicer Alborch / Katja Winkler

14:40	**Analytical imaging: non-invasive, high-throughput, multiplexed, and automatable (L29)** *Marieke Klijn, TU Delft, The Netherlands*
15:05	**Development of a filamentous defined co-culture process with high-throughput online-monitoring (L30)** *Maurice Finger, RWTH Aachen, Germany*
15:30	**Online 2D-fluorescence monitoring in microtiter plates using a fully tunable monochromator-based spectroscopical setup (L31)** *Christoph Berg, RWTH Aachen, Germany*
15:55	**Mulit-vendor test automation to support process automation (L32)** *Jan Dahinden, Sebastian Gross, wega Informatik, Germany / Switzerland*
16:20	*Coffee break & exhibition*

SESSION 11: HIGH-THROUGHPUT BIOPROCESSING AND AUTOMATION

Chair: Mario Birkholz / Lucas Kaspersetz

16:50	**Acetoin detection in alcoholic beverages and fermentation broth with a capacitive enzyme biosensor (L33)** *Melanie Welden, FH Aachen, Germany*
17:15	**Development of a multi-parameter biosensor for bioprocess monitoring based on a flow system (L34)** *Aliyeh Hasanzadeh, Technical University of Denmark, Denmark*

PLENARY TALK

17:40	**Plenary Talk: Bioprocess modelling: Have we moved forward sufficiently (PL06)** *Jarka Glassey, Newcastle University, Great Britain*

EVENING PROGRAMME

18:25	**Poster session, exhibition & get-together**
21:00	*End of day 3*

Best Presentation Award sponsored by:

microorganisms

bioengineering

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THURSDAY, 31 MARCH 2022

HIGH THROUGHPUT BIOPROCESS DEVELOPMENT – ADVANCES IN SOFTWARE, HARDWARE, AND INTEGRATION (PART 2)

WELCOME & PLENARY TALK

9:00 **Welcome & introduction**
Peter Neubauer, TU Berlin, Germany

9:05 **Plenary Talk: The automation of science (PL07)**
Ross King, Chalmers University of Technology, Sweden

SESSION 12: HIGH-THROUGHPUT BIOPROCESSING AND AUTOMATION

Chair Nico Oosterhuis / Marie-Therese Schermeyer

9:50 **Fast production of complex proteins (L35)**
Vicky Goralczyk, FyoniBio, Germany

10:15 **Eliminating gas-liquid mass transfer limitations in shake flasks by a new reactor design based on perforated ring walls (L36)**
Sven Hansen, Evonik Operations GmbH, Germany

10:40 *Coffee break & exhibition*

SESSION 13: MODELING BIOPROCESSES

Chair Nikolas Cruz Bournazou*** / Judit Aizpuru

11:10 **Accelerated microbial phenotyping: How process modelling and a decision policy can enhance high-throughput screening of PETase-secreting *Corynebacterium glutamicum* variants (L37)**
Laura Marie Helleckes, Forschungszentrum Jülich, Germany

11:35 **Hybrid modelling coupled with Raman spectroscopy for enhanced bioprocess understanding via particle filtering (L38)**
Joao Alcantara, Politecnico di Milano, Italy

12:00 **Model-based optimal control of parallel mini-bioreactors (L39)**
Niels Krausch, TU Berlin, Germany

12:25 **Machine learning meets scientific understanding: New approaches for holistic process models (L40)**
Jens Smiatek, Boehringer Ingelheim Pharma GmbH & Ko.KG, Germany

12:50 *Lunch break & exhibition*

SESSION 14: MODELING BIOPROCESSES

Chair Sören Bernauer / Stefan Born

14:20 **Stochastic parcel tracking in an Euler-Lagrange compartment model for fast simulation of fermentation processes (L41)**
Cees Haringa, TU Delft, The Netherlands

14:45 **CFD based particle-tracking tools for quantifying large-scale bioreactor performance (L42)**
Dale McClure, Brunel University London, Great Britain

PLENARY TALK

15:10 **Plenary Talk: Towards automating active learning in collaborative bioprocess development (PL08)**
Ernesto Martinez, National Research Council of Argentina & KIWI Biolab, Germany

15:55 **Closing Remarks**
Peter Neubauer, TU Berlin, Germany

16:10 *End of conference*

All times Central European Time (CET/MEZ)

*** *last-minute change*

SCIENTIFIC POSTERS *(Abstracts see p. 34 ff)*

P01: Phosphate assimilation in co-culture of *Acinetobacter tjernbergiae* and *Pseudomonas stutzeri*
Simon Täuber et al., TU Berlin, Germany

P02: Utilizing straw-derived hemicellulosic hydrolysates for feed manufacturing with *Chlorella vulgaris* – a new waste to value approach
Ricarda Kriechbaum et al., TU Vienna, Austria

P03: PHA bioplastic with tunable monomer content by flexible substrate mixtures
Lara Santolin, Isabel Thiele et al., TU Berlin, Germany

P04: Novel methanol-free expression system PDH: a potential alternative to classical *P. pastoris* promoters for recombinant protein production
Núria Bernat-Camps et al., Universitat Barcelona, Spain

P05: Influence of oxygen levels on a genome reduced *Pseudomonas putida* strain
Jesper W. Jensen et al., Technical University of Denmark

P06: *withdrawn*

P07: Parallel scale-down tool to accelerate fermenterphile selection
Jonas Bafna-Rührer et al., Technical University of Denmark

P08: Controlling *Aspergillus niger* morphology in a rocking motion bioreactor
Tolue Kheirkhah et al., TU Berlin, Germany

P09: Progress in characterisation of liquid flow in oscillatory rocked disposable bioreactors: experimental procedures and empirical models for mixing time evaluation
Mateusz Bartczak et al., Warsaw University of Technology, Poland

P10: Dynamically adjusting extracellular environmental conditions leads to robust oscillations in gene expression: toward a generalizable cell population control strategy
Lucas Henrion et al. University of Liège, Gembloux, Belgium

P11: Small scale mechanical cell disruption: A workflow to screen for ideal disruption conditions for recombinantly produced proteins in *E. coli*
Stefan Kittler et al., TU Vienna, Austria

P12: Bioprocess development for the heterologous production of a hyperthermostable 5'-methylthioadenosine phosphorylase in *E. coli*
Julia Schollmeyer et al., TU Berlin, Germany

P13: Thermostable adenosine 5'-monophosphate phosphorylase from *Thermococcus kodakarensis* forms catalytically active inclusion bodies
Sarah Kamel et al., TU Berlin, Germany

P14: XenoGlue – Scale up of a recombinant mussel protein analog as photoactivatable bioglue
Christian Schipp et al., TU Berlin, Germany

P15: Model-based rational design for aerobic industrial fermentation: *P. chrysogenum* and *S. cerevisiae* as model organisms
Wenjun Tang et al., Delft University of Technology, The Netherlands

P16: Across scales: An integrated robotic cultivation platform for accelerated bioprocess development
Lucas Kaspersetz et al., TU Berlin, Germany

P17: Analyzing growth kinetics of cyanobacterial photobiocatalysts in microfluidic droplets
Paul Böhme et al., Helmholtz-Zentrum für Umweltforschung, Germany

P18: Beyond the average – quantifying the specific reactivity of single cells
Martin Schirmer et al., Helmholtz Centre for Environmental Research

P19: Electroporation of PUFA-producing Dinoflagellate
Stephan Hartmann et al., TU Berlin, Germany

P20: A semi-automated luciferase-based substrate screening assay for nucleoside kinases
Katja F. Winkler et al., TU Berlin, Germany

P21: Automated cell line characterization in shake flasks for multiple organisms
Rüdiger W. Maschke et al., Zurich University of Applied Sciences, Switzerland

P22: Focal molography a new real-time PAT solution for bioprocess
Volker Gatterdam, lino Biotech AG, Germany

P23: PAT for the automation of a recombinant antimicrobial peptide production process
Lisa Michel et al., University of Applied Sciences Hamburg

P24: Kuhner TOM for off-gas analysis in shake flasks
Juan Camillo Porras Correa, Kuhner Shaker GmbH, Germany

P25: Application of a novel high resolution volumetric gas measurement system for the determination of the biochemical methane potential
Marius Conrady et al., Humboldt Universität Berlin, Germany

P26: Flexibilization of two-phase digestion through monitoring of dissolved hydrogen
Eike Janesch et al., TU Berlin, Germany

P27: Raman spectroscopy as an analytic tool in upstream bio-processing
Christoph Lange et al., TU Berlin, Germany

P28: Monitoring of fermentation processes by gas chromatography-ion mobility spectrometry (GC-IMS) and machine learning
Joscha Christmann et al., Mannheim University of Applied Sciences, Germany

P29: Xcom, a multi-objective function for the metabolic modeling of microbial consortia
Xavier Marbehan et al., Laboratoire Réactions et Génie des Procédés, Vandoeuvre Cedex, France

P30: Model based real-time estimation of maximum substrate uptake capacity in microbial fermentation
Don Fabian Müller et al., TU Wien, Austria

P31: Modeling enzymatic glucose release to facilitate continuous feeding in miniaturized fermentations
Annina Kemmer et al., TU Berlin, Germany

P32: Modeling *Saccharomyces cerevisiae* central carbon metabolism at steady state and under glucose perturbations, *David Lao-Martil et al., Eindhoven University of Technology, The Netherlands*

P33: Reconstruction of a genome-scale model of *Cupriavidus* necator for PHA production
Martha Ascencio-Galvan et al., Universidad Valle, Colombia

P34: Computer simulation of the glycosylation of proteins in the Golgi apparatus
Christian Jetschni, Peter Götz, Berliner Hochschule für Technik, Germany

P35: *withdrawn*

P36: Creating educational software inspired by digital twins
Carina L. Gargalo et al., Technical University of Denmark

MONDAY, 28 MARCH 2022

Welcome Address

12:00 Welcome address and introduction

Peter Neubauer
Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Ackerstraße 76, D-13355 Berlin, Germany, peter.neubauer@tu-berlin.de

Plenary Talk

12:20 Large scale production of mRNA vaccines (PL01)

Steffen Panzner
BioNTech Delivery Technologies, Germany
Email: steffen.panzner@biontech.de

This talk will first provide an overview on the research and development of current delivery systems for mRNA, starting from the initial finding of cationic carriers via seminal findings on transient PEGylation through to the mode of action of modern LNP.

In a second section the lecture provides a description of the manufacturing process of Comirnaty®, the BioNTech/Pfizer vaccine to prevent Covid-19 infections. The focus will be on quality concerns during upscale of the original method and will identify developmental and control aspect critical for the vaccine's quality

Session 1: Scale up and scale down of bioprocesses

Chair Cees Haringa / Simon Täuber

13:05 Dedicated single-use bioreactor designs, making cellular agriculture feasible (L01)

Tutku Kurt[1] and Nico M.G. Oosterhuis[2]
[1]Celltainer Biosolutions GmbH, Berlin, Germany
[2]Celltainer Biotech BV, Winterswijk, The Netherlands
Email: n.oosterhuis@celltainer.com

Cellular agriculture is a relatively new and popular field of bioprocessing, although fermentation has been known for food for thousands of years. The cultivation of mammalian, plant and insect cells has been known and applied for decades already, but their use up to now has been limited to the production of mostly biopharmaceuticals. Since the successful introduction of the "cultured meat burger" in 2013 by the company Mosa Meat BV, many new companies have started and are currently developing the production of cultured meat in an economically feasible way.

Application of suspension plant cells to produce cocoa products is becoming increasingly important and technically feasible as well, while also microbial or fungi-based fermentation processes are also being developed to produce several proteins for food application, like milk protein replacers, single-cell proteins for feed and food, and others.

Bioreactor designs, and especially single-use bioreactors designed for biopharmaceutical production, can serve as a basis for a dedicated bioreactor design for such type of processes. These types of reactors may not fully address the capacity needs of cellular agricultural products, although single-use bioreactors, due to their flexibility, can contribute to enable cellular agricultural production by adopting a scale-out approach as well as making the seeding steps of these processes more flexible and more reliable in terms of sterility.

The development of a bioreactor system dedicated for the production of cultured meat requires more than merely adopting the stirred tank reactors commonly used for mammalian cells. More sophisticated reactor designs are likely to be required to achieve optimal conditions and minimal cost of goods. A new concept will be presented that opens new avenues for cellular agricultural applications.

13:30 Towards increased stability in large-scale bioreactors by bacterial co-cultures (L02)

Sebastian Hofmann[1], Hugo Vernier-Lambert[4], S. Meriguet[4], Paramveer GopalSingh[1], Jürgen Fitschen[1], Peter Neubauer[2], Eric von Lieres[3], Michael Ferguson[4], Michael Schlüter[1]
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[2]Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Ackerstraße 76, D-13355 Berlin, Germany
[3]Department of 'Modeling and Simulation', Jülich Research Centre, Jülich, Germany
[4]Gymetrics France SAS, Sainte-Hélène-du-Lac, France
Email: sebastian.hofmann@tuhh.de

Due to occurring compartments in stirred tank reactors (STR) cells are exposed to heterogeneities during fermentation processes [1–3]. Even modern local probes, which are usually located near the reactor wall, are not able to depict this characteristic spatiotemporally varying cell stress alongside the cell's trajectory. This stress depends strongly on multiple variables like the reactor dimension, impeller speed, gassing and subsequently emerging concentration gradients (dissolved oxygen, pH, etc.) that lack a detailed quantification particularly in industrial-scale STRs. In this case, the state-of-the-art way to represent both experimentally-based lifelines [4] of a cell and their temporally resolved conditions are Lagrangian sensor particles, which may portray a characteristic footprint of

aforementioned variables, strongly depending on their design. In the past years, many research groups devoted their focus on developing such free-floating sensor particles to obtain insight into a bioreactor without necessarily having optical access [5–7]. However, prior utilizing them in real-case scenarios, their hardware must be subjected to various tests. Therefore, the Institute of Multiphase Flows (IMS) offers a transparent 15,000 L acrylic glass bioreactor as a platform to investigate different sensor particle designs, evaluate measured results and enhance understanding of gained sensor data. In a close collaboration with the ERA CoBioTech funded team around Gymetrics, their sensor particles have been tested and compared to another design. First results show that the shell material, shape and density have a big impact on (a) the durability of the hardware and (b) the overall axial distribution, which eventually indicates to the residence time distribution of a finite-sized, inertial particle and occurring compartments inside a production-scale bioreactor.

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13:55 CFD supported scale-up in biopharma – an overview with focus on hydrodynamic stress (L03)

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Global sales for biopharmaceutical products are continually on the rise. Accordingly, the demand for optimized production processes is substantial. Main prerequisite for this optimization is detailed process understanding of the single steps involved in the manufacturing of biopharmaceuticals. In the recent years computational fluid dynamics (CFD) has emerged as an important modelling tool to support the characterization of upstream and downstream equipment with focus on stirred tanks and vessels. Boehringer Ingelheim (BI) is currently applying CFD over multiple steps of the biopharmaceutical production chain from early process development over bioreactor cultivation to formulation fill & finish. Thereby, dedicated simulation approaches are used for the determination of power numbers, flow fields, energy dissipation, shear rates and mixing times to support the scale-up from lab to commercial scale, helping to mitigate risks during process transfer and supporting trouble shooting activities. Additionally, the design of scale-down models is facilitated by CFD, leading to a reduction of experimental work, consequently saving time, capacities, and personnel.

This talk will give examples of said CFD approaches at BI but also provide insights into how experimental methods can be used to validate CFD results. Special focus will be drawn to the characterization of upstream equipment by hydrodynamic stress and the effect of shear stress on the cell culture performance. Within ambr250® bioreactor system stress threshold values have been determined, below which no significant influence on cell behavior is observed [1]. Such calibrated systems can serve as scale-down model and are of particular interest for the development of high cell density perfusion processes.

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14:20 Coffee break & exhibition

Session 2: Bioprocesses for a circular economy

Chair Krist V. Gernaey / Sebastian Riedel

14:50 High yield bioconversion of animal by-product streams to polyhydroxyalkanoates (L04)

Björn Gutschmann, Peter Neubauer, Sebastian L. Riedel

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To date, polyhydroxyalkanoate (PHAs), easily biodegradable plastic alternatives, have held only a small share of the bioplastic market, mainly due to high production costs. Oleaginous animal by-product streams (ABS) are locally available in large quantities and depending on quality they face little competition from other industries. To accelerate industrial PHA production, the development of efficient bioprocesses based on ABS is a promising approach to reduce the overall production costs. However, the solid nature of ABS requires a tailor-made process development [1,2].

This contribution will focus on the production of PHA with Ralstonia eutropha from different oleaginous ABS under nitrogen limitation. Multiple ABS were screened and porcine waste animal fat (WAF) and a porcine waste fat-protein emulsion (FPE) were identified as promising substrates for further bioprocess development [3]. FPE is a mixture of WAF and protein hydrolysate, which was used as sole carbon and nitrogen source. Tailored feeding systems were developed for WAF and FPE, respectively, which enabled high-cell-density fed-batch cultivations. A thermostat connected to double jacket tubing system was employed to thermally liquefy low-quality WAF, which is solid at room temperature, and constantly pump it to the bioreactor. Up to 120 g/L CDW containing 54 wt% PHA with an overall process space time yield (STY) of 0.9 g/L/h were produced using this feedstock. During WAF cultivations, growth and PHA formation was monitored in real-time by in-line photon density wave spectroscopy. FPE could not be thermally liquefied, but an automated pneumatic feeding system was developed to enable fed-batch cultivations. Up

to 65 g/L CDW containing 80 wt% PHA with a STY of 0.6 g/L/h without using any other carbon or nitrogen sources were achieved with FPE in a pulse-based fed-batch cultivation. So far, the WAF process was transferred to 150-L pilot scale, which is promising for a further planned scale-up to industrial-scale

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15:15 Continuous bio-succinic acid production from alternative feedstock (L05)

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Bio-succinic acid is one of the twelve most promising future bio-based building blocks [1]. However, it still requires a reduction of the overall process costs to make it economically viable [2]. Techno-economic and lifecycle assessments have identified glycerol as one of the most promising feedstocks for bio-succinic acid production [3]. However, very few studies have investigated the microbial conversion of glycerol into succinic acid and they mostly reached comparably low titers and volumetric productivities [4-7].

During my project, I am investigating the performance of continuous succinic acid production from glycerol by the bacterial strain Actinobacillus succinogenes. My goal is to characterize the growth and succinic acid production kinetics for eventually performing a model-based process optimization to maximize the product yield, titer, and volumetric productivity.

Growth on glycerol by Actinobacillus succinogenes was initially improved during an adaptive evolution experiment during which the lag-phase was reduced from over a week to less than a day.

For characterizing the growth kinetics and product inhibition, a glycerol-pulsed chemostat experiment was performed with increasing succinic acid concentrations in the feed. The system could cope with succinic acid titers of up to 40 g l-1 at a volumetric productivity of 2.4 g (l·h)-1 but at the expense of the product yield which decreased to below 0.3 gSA/gGly.

This data will be used for a kinetic characterization to eventually perform a model-based process optimization to find the optimum process conditions for maximizing product yield, titer, and volumetric productivity.

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15:40 Combining in-line FTIR spectroscopy with MIMO control and feedback linearization for a continuous C.glutamicum bioprocess fed with lignocellulosic waste streams (L06)

Daniel Waldschitz, Yannick Bus, Johanna Bartlechner, Eva Karner, Peter Sinner, Bence Kozma, Christoph Herwig

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Spent sulfite liquor (SSL) from the pulp mill process is a lignocellulosic waste stream for valorisation with fermentation due to its sugar content such as glucose, mannose and xylose. However, the exact sugar composition depends on the wood raw material used and thus variable. *Corynebacterium glutamicum* is the industrial workhorse for the production of amino acids. Its high tolerance for organic acids such as acetate as well as its ability to be genetically engineered to utilize different C5 and C6 sugars make it an ideal host for SSL-based fermentations. Continuous bioprocesses would be optimal to valorize SSL that is produced in the industry continuously, in high quantities however, varying sugar concentrations present a challenge, because each sugar results in different growth rates and yields. Our goal was to develop a continuous bioprocess solution, evolving from our previous works [1,2] and which fits into the circular economy concept, by employing Fourier transform infrared spectroscopy (FTIR) and a multivariate model, to determine the concentration of the fermented sugars. FTIR spectra are acquired in-line and used as an input for multiple-input multiple-output control (MIMO) with feedback linearization. The multivariate model was able to accurately predict the sugar concentrations within the SSL matrix, even different C6 sugars like glucose and mannose simultaneously. This strategy based upon the combination of the FTIR and MIMO control demonstrated that the steady-state can be maintained even for variable sugar content and concentration in the feed over time that makes it a potential solution for SSL valorisation.

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2. Sinner, P., Stiegler, M., Herwig, C. and Kager, J., Noninvasive online monitoring of *Corynebacterium glutamicum* fed-batch bioprocesses subject to spent sulfite liquor raw material uncertainty. (2021) Bioresour. Technol., 321: 124395.

16:05 Improving biochemical hydrolysis by bioaugmentation in plug-flow based reactors (L07)

Theresa Menzel, Peter Neubauer, Stefan Junne

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The process of anaerobic digestion is an important and widely applied bioprocess to make use of biogenic resources. In Germany, residual biomass comprises of straw and other cellulolytic resources, which pose a challenge for regular anaerobic digestion due to their lignocellulosic structure. The application of hydrolysis reactors might improve digestion and increase the feedstock flexibility of those processes. The addition of specialized microbes – so called bioaugmentation – is one method to further improve the hydrolytic process.

Due to gradient formation in a plug-flow based hydrolysis reactor, a hydrolytic phase is formed at the entrance of the reactor and acidification occurs in the end part, thus providing different microenvironments for specialized microorganisms. Partial recirculation of the liquid phase increases the hydraulic retention times of hydrolytic organisms, including those, that were added through bioaugmentation.

Here, we added individually two species of hydrolytic bacteria to a semi-continuous operated plug – flow reactor, which was operated with maize silage, and in a later stage, with 30% (w/w) of straw. For process monitoring, a gradient-based measurement method was applied, monitoring the pH-value, conductivity and redox-potential on three spots along the reactor.

For each bioaugmentation phase, a significant increase in conductivity and hydrolysis efficiency was shown compared to the non-augmented process. This led to higher acid yields and better overall digestion.

The addition of hydrolytic organisms for process improvement with lignocellulosic substrates seems quite promising. Further work needs to clarify the amount and frequency of bacterial addition that is required to maintain the improved process performance.

16:30 Exhibitor Short Presentations

Thomas Skov, SANI Membranes A/S, Denmark / Juan C. Porras Correa, Kuhner Shaker GmbH, Germany / Richard Salliss, Keit Spectrometers Ltd, UK / Julius Muno, aquila biolabs GmbH, Germany / Holger Müller, BlueSens gas sensor GmbH / Anika Bockisch, Bio-PAT e.V., Germany

16:50 Coffee break & exhibition

Session 3: Bioprocesses for a circular economy

Chair Howard Ramirez-Malule / Tolue Kheirkhah

17:20 Production of methane and carboxylic acids from organic residues (L08)

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The increasing demand on renewable and sustainable products is challenging us to improve our treatment of organic residues. More attention is needed for efficient use of raw materials as resources are scarce [1]. The aim of this study was to evaluate the possibilities of anaerobic digestion and plug flow process as flexible conversion of agricultural feedstocks as organic acids, methane and nutrients. A laboratory scale plug flow-based digester was used to treat manure and grass silage. Organic loading rate (ORL) of 2 gVS/Ld was used with 20-day hydraulic retention time (HRT) until the process was slowly overloaded to optimize the conditions for organic acid production. ORL was increase gradually with grass silage from 5 and 8 to 10 gVS/Ld, HRT decreasing from 15, 10 and 5, respectively. Acid phase was reached with ORL 10 gVS/Ld and this ORL and HRT was kept for 4 HRT's. After 4th HRT, pH started to decrease and ORL was decreased to 8 gVS/Ld. pH, redox potential, conductivity as well as gas production (amount and quality) were monitored continuously. Acid production as well as nutrient balance in residue was analyzed frequently. Acid production raised up to 9.4 g/L during the experiment. As a result, continuous processing was successful in both phases, methane phase with manure as well as acid phase with grass silage, although acid phase was unstable and needed pH adjusting. Next steps are the purification and concentration of acetic liquids to be used in hydrometallurgical process for rare earth metal recovery.

1. Bartek, L., Strid, I., Henryson, K., Junne, S., Rasi, S. and Eriksson, M., Life cycle assessment of fish oil substitute produced by microalgae using food waste. (2021) Sustain. Prod. Consum. 27: 2002-2021.

17:40 Production of biotechnological fish feed by microalgae and oleaginous yeast (L09)

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Fish feed contains long-chain fatty acids and proteinogenic sources from fish meal and oil which cannot be fully replaced by plant sources. Especially docosahexaenoic acid (DHA), which is a sixfold unsaturated fatty acid, is obtained from fish oil. In recent years, however, also the microbiological production of it was established in industrial scale.

In a current project, this and other fish feed components like middle-chain fatty acids, proteins and vitamins were produced in heterotrophically and phototrophically grown microalgae as well as in the oleaginous yeast *Yarrowia lipolytica* up to a m³ scale. A comparison between the process performance in the lab and large scale is conducted and implications on the product synthesis are analysed. Beside the technical view, also economic values are compared with the so far dominating use of fish meal and oil. Potentials for process optimization and improved monitoring are presented.

The utilization of alternative feedstock such as short-chain carboxylic acids in the production phase, e.g. derived from digestion processes of biogenic residues and the efforts required for process coupling are discussed.

Finally, the achievable fish quality and the remaining challenges for the creation of a qualitatively competitive fish feed based on biotechnologically derived compounds are summarized.

■ ■ Plenary Talk

18:00 Fungal biotechnology as innovation driver for a circular economy (PL02)

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The opportunities that research in fungal biotechnology offers us today are breathtaking. We could, in the not-so-distant future, live in houses built with the power of fungi and sit on furniture grown with the help of fungi. We could wrap ourselves in fungal clothing, as textiles as well as leather are made from them. These new fungal-based products will significantly change the way we live and work and could prove disruptive for some industries.

In my lecture I will illuminate the roots of fungal biotechnology, will highlight the current-state-of-the-art and will discuss the paradigm shifts that the fungal biotech community is aiming for to drive a circular economy and to contribute to a sustainable future.

18:45 Get-together & exhibition & Poster-Session

21:00 End of day 1

TUESDAY, 29 MARCH 2022

Opening

09:00 Welcome and introduction

Peter Neubauer, TU Berlin, Germany

Plenary Talk

09:05 An industrial perspective on the gas-liquid flow of stirred multistage bioreactors: challenge and a novel scaling approach (PL03)

Sören Bernauer

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In high-performance industrial fermentation processes, stirring and aeration may account for a significant proportion of the production costs. In this study a 160 m3 bioreactor equipped with four Rushton impellers was investigated regarding its potential for saving of energy costs. Therefore, a scaling concept was applied, scaling the flow regime into pilot-scale [1]. The individual flow regime, power input and gas hold-up of each impeller compartment were measured, and the bubble flow was analyzed by a novel two-phase CFD model. The results reveal that due to pre-dispersion of air by the bottom impeller, a more homogenous distribution of bubbles is found in the upper compartments with fewer bubbles in the vicinity of the impellers. This leads to a higher power draw and a decreased efficiency of the upper Rushton impellers [2]. Based on these findings, the large-scale reactor was adjusted by applying pitched blade impellers on the upper levels, retaining a second Rushton impeller for gas dispersion. For equal operating conditions, the proposed configuration yielded similar gas hold-ups and better mixing times (-35 %) compared to the Rushton-only configuration. Hence, applying a radial impeller on an upper level for improving gas dispersion maintains the benefits of axial impellers in terms of reducing energy demand (up to -50 %) [3]. We conclude that this effect is significant only at large-scale when bubbles substantially expand due to the release of the hydrostatic pressure and have time to coalesce. The work thus extends current knowledge on mixing and aeration of large-scale reactors using classical impeller types.

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2. Bernauer, S., Schopf, M., Eibl, P., Witz, C., Khinast, J. and Hardiman, T., Characterization of the gas dispersion behavior of multiple impeller stages by flow regime analysis and CFD simulations. (2021) Biotechnol. Bioeng. 118: 3058-3068.
3. Bernauer, S., Eibl, P., Witz, C., Khinast, J. and Hardiman, T., Analyzing the effect of using axial impellers in large scale bioreactors. (2022) Authorea. doi: 10.22541/au.164591554.42826271/v1.

Session 4: Scale up and scale down of bioprocesses

Chair Ralf Takors / Stefan Junne

09:50 CFD-kinetic Modelling for the Scale-up of *P. putida* Fed-batch Fermentations (L10)

Maryam Jamshidzadeh[1], Jesper Wang Jensen[1], Ulrich Krühne[1], John M. Woodley[1], Pablo Ivan Nikel[2], Helena Junicke[1]

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Bioprocess industry has always struggled with the generation of oxygen and substrate gradients in large-scale bioreactors due to the non-ideal mixing. In microbial processes, scale-down simulators are a practical tool for assessing the microorganism response to the heterogeneous condition of large-scale systems. Scale-down simulators use local gradients of various compartments formed in the large-scale system and recreate the condition on a smaller scale. However, experimental monitoring of local concentrations is a demanding task. It is not possible to record experimentally the conditions each cell experiences during its residence time in a fermenter. For this reason, Computational Fluid Dynamics modelling and in particular, coupling the Eulerian model with Lagrangian particle tracking can be of assistance [1]. CFD modelling of large-scale bioreactors enables the prediction of biomass trajectories in a detailed manner in the form of the microorganism's lifelines and it provides a platform for developing scale-down simulators [2].

In this study, an Euler-Lagrange CFD model was developed and was coupled to a kinetic model of Pseudomonas putida in fed-batch mode [3]. The numerical model comprises an Eulerian multiphase system representing the aeration and the liquid phase in combination with a Lagrangian phase representing the biomass. The simulations were performed at three different stages of the fermentation to account for the increase in the broth's height with continuous feeding and its effect on the mixing and gradient formation. The effect of operating conditions, such as number of impellers and their speed, was also studied on the generation of gradients inside the system..

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2. Demling, P., Ankenbauer, A., Klein, B., Noack, S., Tiso, T., Takors, R., and Blank, L.M. Pseudomonas putida KT2440 endures temporary oxygen limitations. (2021) Biotechnol. Bioeng. 118: 4735-4750.
3. Davis, R., Duane, G., Kenny, S.T., Cerrone, F., Guzik, M.W., Babu, R.P., Casey, E., and O'Connor, K.E. High cell density cultivation of Pseudomonas putida KT2440 using glucose without the need for oxygen enriched air supply. (2015) Biotechnol. Bioeng. 112: 725-733.

10:15 Impact of local solid concentration on anaerobic digester hydrodynamics at industrial scale: a CFD study (L11)

Liliane Megue Kamkeng[1,2], Philippe Marchal[1], Benjamin Le Creurer[2], Eric Olmos[1]

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To limit the environmental impact of energy production, the use of anaerobic digestion to produce biogas from waste finds renewed interest. Consequently, the hydrodynamics within anaerobic digesters has been widely studied to describe the transport phenomena occurring in these systems. However, models used [1-2] generally consider non-newtonian fluids that are homogeneous in terms of solid concentration (and thus rheology) throughout the digester, which is probably not representative of real process conditions. Industrial digesters are indeed generally fed with a heterogeneous substrate (liquid phase + solid suspension) whose viscosity is higher than the one of the digestate (fluid contained in the digester). Moreover, locally, too low liquid velocities may cause sedimentation problems. To improve the robustness of existing numerical models, the impact of solid concentration on mixture rheology was coupled with Computational Fluid Dynamics (CFD) to model the hydrodynamics in a continuous stirred industrial digester (2000 m^3). First, a detailed rheological study highlighted a non-newtonian behavior of the fluids and a specific mass-weighted law was established to model the local viscosity variations due to solid concentration gradients. Using a laminar sliding mesh CFD approach and a passive scalar transport equation, the mixing of substrate and digestate was simulated. Furthermore, sedimentation phenomena were considered by transporting a passive scalar in the liquid phase of the digestate. The simulations were validated using the total power required by the agitators and its dissipation by the viscosity forces (CFD) and the electrical power measured on the production site.

1. Yu, L., Ma, J. and Chen, S., Numerical simulation of mechanical mixing in high solid anaerobic digester. (2011) Bioresour. Technol. 102: 1012–1018.
2. Hu, Y., Zheng, X., Zhang, S., Ye, W., Wu, J., Poncin, S. and Li, H.Z., Investigation of hydrodynamics in high solid anaerobic digestion by particle image velocimetry and computational fluid dynamics: Role of mixing on flow field and dead zone reduction. (2021) Bioresour. Technol. 319: 124130.

10:40 Scale-down of high cell density Fab production in *E. coli* (L12)

Florian Mayer[1], Monika Cserjan[1], Christian Sam[2], Miroslav Soos[3], Gerald Striedner[1]

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In industry large cultivation vessels of up to several hundred m^3 are used which result in long mixing times. As a consequence, cells are exposed to inhomogeneities, which can have negative effects on process performance, such as lower biomass yields [1]. In this work genome integrated *E. coli* HMS174(DE3) and BL21(DE3) host strains expressing a Fab in the periplasm, were cultivated to high cell densities of more than 70 g/L in a 20 L stirred tank bioreactor (STR). To investigate scale effects, the STR was connected to a custom-build plug-flow bioreactor (PFR) and then operated either as two- or one-compartment system. The residence time of the cells in the PFR compartment was adjusted according to the mixing time difference between the 20 L and an industrial scale bioreactor. To generate gradients in 2-stage mode the feed was directly added at the PFR entrance. In addition, the miss incorporation of Norleucine into the Fab and the influence of scale-effects on several downstream relevant parameters were investigated. In general, we observed a decreased glucose yield coefficient (-10%) in scale-down experiments. We showed that the incorporation of Norleucine in the Fab is highly strain dependent and also that downstream relevant parameters, just as viscosity and settling velocity, are influenced by scale-effects. Based on these findings, process development can follow a more rational and integrated approach, which allows for design of fully scale-able processes.

1. Neubauer, P. and Junne, S., Scale-up and scale-down methodologies for bioreactor. (2016) In Bioreactors: Design, Operation and Novel Applications, C.-F. Mandenius, Editor. 2016, Wiley-VCH Verlag GmbH & Co.KGaA: Weinheim, Germany. 323 - 354.

11:05 Coffee break & exhibition

■ ■ Session 5: Scale up and scale down of bioprocesses

Chair Stuart Stocks / Eike Janesch

11:35 Development of a single multi-compartment bioreactor (SMCB) for CHO scale-down studies in heterogeneous cultivation environments (L13)

Lena Gaugler[1], Yannic Mast[1], Jürgen Fitschen[2], Sebastian Hofmann[2], Michael Schlüter[2], Ralf Takors[1]

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With the biopharmaceutical industry facing high research and development costs at decreasing approval rates, efficient and reliable scale-up/ scale-down strategies are essential [1]. However, at production scale with bioreactors larger than 10 kL mammalian cells are likely to experience heterogeneities of for example shear stress, pH, dissolved oxygen and carbon dioxide [2]. Commonly applied scale-down approaches either fail to address potential process impairments due to mixing heterogeneities or impose biasing shear stress by pumping cells through multiple reactors. Here we present a single multi-compartment bioreactor (SMCB) that allows the recreation of large-scale mixing times in laboratory scale while avoiding biasing shear stress by pumping and additional contamination risks. Originally described by Schilling et al. [3] this scale-down concept uses horizontally installed stainless steel discs to impair the vertical mixing behavior and to generate different compartments within a single bioreactor. Applying optical mixing time deter-

minations and CFD, a targeted manipulation of mixing times could be achieved. The exchange area provided by the disc was identified as an essential design parameter. Further considering the power input and vertical fluid velocities different ways to install large-scale mixing times in the SMCB could be identified. In fed-batch cultivations with CHO DP-12 the applicability of the developed scale-down concept was evaluated for a generic scale-down scenario. This way, the SMCB could aid in unravelling metabolic mechanisms induced by large-scale cultivation environments and increasing the reliability of scale-up/ scale-down approaches.

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12:00 Suitability of various scale-down bioreactor designs at the example of the oleaginous yeast *Yarrowia lipolytica* (L14)

Jasmina Cziommer, Peter Neubauer, Stefan Junne

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Scale-down has been well established for mimicking large-scale conditions in the lab scale. While scale-down systems that exert either a one-compartment or a compartmentation of two or more stirred tank- or plug flow reactors are widely applied, it is still not investigated thoroughly how comparable the various designs are among each other. Oleaginous microorganisms such as *Yarrowia lipolytica* are interesting hosts for the exploitation of high-value lipids. In this work, the process performance of Y. lipolytica cultures in one- and more-compartment scale-down reactors are compared. The emphasis lies on the investigation of the cellular stress response to fluctuating dissolved oxygen supply with respect to growth, fatty acid formation, amino acid depletion and main carbon metabolites, as well as macromorphology and viability down to the single-cell level.

The comparison of the accumulation profile of fatty acids and amino acids provides insides in metabolic proceedings of Y. lipolytica during oscillating conditions – and shows, how profound the impact of the different conditions evoked by the scale-down design is.

Furthermore, the relation of the population's appearance to process performance parameters was investigated. It is demonstrated how process analytical technologies, such as in situ light microscopy, facilitate the capture of macromorphologic heterogeneity within a culture and the monitoring of intracellular lipid droplet formation on a single-cell basis. The results were related to the prevalent stress conditions and also compared to at-line flow cytometry measurements.

The workflow that was finally obtained provides a suitable tool for comparing scale-down reactor designs and evaluate their suitability to reproduce the large scale in case of oleaginous yeasts.

12:25 Syngas fermentation: a scale-down approach to simulate large-scale gradients at lab-scale (L15)

Lars Puiman[1], Cees Haringa[1], Henk Noorman[1,2], Cristian Picioreanu[3]

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Syngas (CO, H_2) fermentation is a promising process for the conversion of waste gases [1]. At this moment, a highlighted research topic is the expansion of the product spectrum obtained by syngas fermentation, from acetate and ethanol towards higher value-chemicals, such as PHB, butanol and isoprene [2]. For successful scale-up of such processes, scale-down studies should be used: The industrial heterogeneity as experienced by micro-organisms in large-scale bioreactors has to be represented at a smaller scale [3]. Here, we present an approach for down-scaling an industrial syngas-to-ethanol fermentation process.
By employing Euler-Euler simulations, we determined the gradients in dissolved CO and H_2 concentrations in a large-scale external-loop bioreactor, using black-box kinetics. Lagrangian particle tracking was employed to obtain microbial lifelines in the reactor, which were used to determine the design characteristics of a scale-down simulator. It is observed that microbes observe very short, semi-periodic, peaks in CO/H_2 concentrations, whilst they stay predominantly in lower concentration zones.
We propose several methods to represent these large-scale concentration fluctuations in lab-scale bioreactors, with different trade-offs between complexity and representability. A single-vessel approach with changing the stirrer speed is proposed as the most simple version of a scale-down simulator. Meanwhile, a more challenging yet more accurate approach would be a stirred tank, with an hollow fibre membrane gas-liquid contactor to simulate the concentration peaks, with variable residence times in the STR. Lastly, microfluidic devices to represent these fluctuations could be developed, although the gas-liquid mass transfer might be challenging in the these set-ups.

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12:50 Optimization and Scale-Up of Itaconic Acid Production on Complex Substrates (L16)

Paul-Joachim Niehoff[1], Johannes Brockkötter[2], Philipp Ernst[3], Robert Kiefel[2], Katharina Saur[2], Nick Wierckx[3], Andreas Jupke[2], Jochen Buechs[1]

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Substrate costs still have the largest impact on the economy of biologically produced bulk chemicals. To reduce these costs, cheap and abundantly available substrates have to be used. Especially, side streams from agriculture and the food industry are excellent substrate sources for biotechnological processes, due to their high carbon load and regional availability. In this study, thick juice from local sugar industry was used to produce itaconic acid.
In a first step, critical key performance indicators like titers and yields needed to be quantified. Using an optimized Ustilago cynodontis strain1 and an extended batch mode in a 2 L fermenter, itaconic acid titers of up to ~100 g/L with a yield comparable to glucose fermentations [1] (0.46 g/g) were reached. A pH shift from 6.5 to 3.6 and a reduction of the initial nitrogen amount were introduced. This led to a process with reduced overall costs, due to a significant reduction in added acid and saline waste costs, while still reaching the same yield. Feasibility of large-scale production was proven by batch cultivation at 150 L scale. In conclusion, a production process on a complex carbon source with high titers and yields could be established and scaled up to 150 L. In the future, the feeding protocol from the 2 L reactor will be transferred to the scaled up process.

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13:15 Lunch break & exhibition

Session 6: Process analytical technologies

Chair Holger Müller / Annina Kemmer

14:45 Enabling continuous co-culture bioprocesses (C3BIO) based on oscillating environmental conditions promoting genetic and/or metabolic requirements of individual species (L17)

Vincent Vandenbroucke, Lucas Henrion, Fabian Moreno-Avitia, Frank Delvigne, Juan Andres Matinez,

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Continuous Co-Culture Bioprocesses (C3BIO) is one the key enabling technologies for the next generation of bioprocesses, due to increased metabolic capabilities, efficiency and modularity. However, the effective implementation of co-cultures in bioreactors is impeded by growth imbalances, leading to an unstable population. Not only does each organism have a different growth rate, but in addition each organism can have a phenotypically heterogeneous population with distributed growth rates. We have addressed the latter problem based on a previously developed technique, i.e., the segregostat, that can force a single-strain culture to adopt a homogeneous oscillatory behaviour in terms of growth and gene expression by periodically changing the environmental conditions at a rate close to the phenotypic diversification rate of the targeted population.

We want to transpose those results to co-cultures by providing oscillatory behaviours to each organism, and use these oscillations to stabilise the population. To this end, the key aspect is to synchronise the growth for each strain, and generate a phase shift between the growth phase of the different strains. We considered two approaches for providing this phase shift. In a first case study, we used the natural difference in metabolic capabilities of wild-type *E. coli* and S. cerevisiae to stabilise their co-culture using oscillating feeding profiles. In a second case study, we will engineer each strain of a co-culture with specific gene circuits, the toggle-switch and the repressilator, providing phase-shifted oscillatory behaiours. These behaviours will be coupled to growth by the production of essential genes.

Altogether, this approach and our current results point towards a viable strategy for the effective implementation of continuous co-culture bioprocesses.

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15:10 Dead or alive: a novel PAT tool for the extremophile *Sulfolobus acidocaldarius* (L18)

Kerstin Rastädter

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Viability of *Sulfolobus acidocaldarius*, an extremophilic Archaeon thriving at 75 °C and pH 3.0, is still determined via laborious, time- and material-intensive plating assays. Sulfolobus harbor great potential as still untapped production hosts and biocatalysts in biotechnological applications, nevertheless no industrial processes have been developed yet. During development and scaling of industrial bioprocesses it is crucial to monitor the impact of process parameters on the cultivated organism. Flow cytometry (FCM) combines the ability to analyze single-cell properties in a cell

population with viability assessment via the use of fluorescent-active dyes. In this study, commercially available fluorescent-active dyes applicable in S. acidocaldarius were identified. The dyes, Fluorescein diacetate and Concanvalin A conjugated with rhodamin, are suitable for viability determination via FCM. For showing the applicability of the developed at-line PAT tool for bioprocess monitoring a chemostat cultivation at 75°C and pH 3.0 was conducted. Over 800 hours, this novel PAT tool successfully allowed monitoring of viability. In my talk, I will introduce our exciting PAT tool paving the way for an untapped Archaeon into industrial application.

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15:35 Population heterogeneity in *E. coli* chemostat cultivation: An investigation of alternating gene expression levels between observed phenotypes (L19)

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State-of-the-art recombinant protein production with *E. coli* is commonly carried out in fed-batch cultivations. Still, both industry and research, struggle in achieving long-term cultivations with stable productivity. Continuous cultivations boost space-time yields of established fed-batch cultivations. The main challenge of microbial continuous cultivations, however, is the fluctuating, instable productivity. Subpopulation development (i.e. occurrence of non-producing populations) is believed to cause these process deviations in long-term cultivations.
We monitored subpopulation evolvement throughout *E. coli* chemostat cultivations using an online flow cytometry device. Trying to understand the evolvement of population heterogeneity, we performed transcriptomic analysis. As single cell RNA sequencing approaches for bacterial cultivations are problematic due to fast cell growth, we separated subpopulations due their time-dependent occurrence during chemostat cultivation. In my talk, insights on alternating gene expression patterns of different subpopulations in *E. coli* chemostat cultivations are presented, aiming to shed more light in the dark of occurring population heterogeneities.

16:00 Heavyweight data: Microscale material balancing in microfluidics (L20)

Katharina Smaluch[1], Bastian Wollenhaupt[2], Heiko Steinhoff[3], Dietrich Kolhheyer[2], Alexander Grünberger[3], Christian Dusny[1]

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Modern microbial biotechnology can profit from novel physiological insights obtained with microfluidic cultivation and single-cell analysis to make biotechnological processes more efficient. However, obtaining quantitative data on single-cell kinetics, growth stoichiometry, cell-cell and cell-reactor interactions is difficult to access due to a lack of corresponding conceptual and analytical principles [1-2].

We implemented biochemical engineering principles based on the kinetics and stoichiometry of growth in microscopic spaces to develop a combination of non-invasive optical cell mass determination with sub-pg sensitivity, microfluidic perfusion cultivations for establishing physiological steady-states, and picoliter batch reactors. This enabled the quantification of physiological parameters relevant for material balancing in microfluidic reaction environments.

At the core of this study, we used quantitative phase imaging as a novel tool that gave access to individual microbial cells' mass and dry matter density data. Specific growth rates based on these mass parameters bring the basic concepts of quantitative physiology to the single-cell level, whereas also being adaptable to other cell types relevant for bioprocesses.

We here present an advanced single-cell approach which delivers novel insights regarding biomass concentration based on single-cell dry weight and mass density, specific kinetics of substrate uptake, biomass synthesis, as well as substrate affinities and growth stoichiometry at single-cell resolution.

Our unique framework gives access to microscale material balancing beyond the averaged values obtained from populations as a basis for integrating heterogeneous kinetic and stoichiometric single-cell data into generalized bioprocess models and descriptions [3]

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16:25 Coffee break & exhibition

Session 7: Process analytical technologies

Chair Anika Bockisch / Carmen Walczak***

16:55 On-line monitoring of key metabolites in *E. coli* fermentations by near-infrared (NIR) spectroscopy (L21)

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The transition from recipe based to automated manufacturing of recombinant proteins requires real-time monitoring of critical quality attributes (CQAs) during the fermentation process. Rapid, non-destructive spectroscopic techniques such as near-infrared (NIR) spectroscopy enable real-time monitoring of key chemical compounds and thus facilitate improved process control and optimization. This study investigates the use of on-line NIR spectroscopy to monitor the concentration of key metabolites such as glucose and acetate during a fed-batch fermentation using *E. coli* as host organism. Glucose is crucial for cell viability, whereas elevated levels of acetate caused by glucose overflow inhibits cell growth and product formation. The experiments were carried out in a 5 L bioreactor mounted with a closed bypass loop made of silicone hose. A peristaltic pump was applied to ensure constant circulation in the loop, and a flow cell enabled real-time spectral data acquisition by NIR spectroscopy. The concentration of key metabolites in at-line samples were determined by quantitative NMR and used as reference values for multivariate modelling by Partial Least Squares (PLS) regression. The concentration of acetate was successfully monitored with an uncertainty of approximately 2 mM, thus demonstrating that NIR spectroscopy can be used to accurately monitor key metabolites in complex fermentation media. In the future, model-predictive control of glucose feeding based on real-time monitoring of acetate might be a promising strategy to avoid formation of inhibitory acetate.

17:20 Real-time inline monitoring of Trichoderma reesei cultivation in industrial environment and prediction of protein folding by time-gated Raman spectroscopy (L22)

Martin Kögler[1], Alexey Popov[1], Caj Södergård[2], Timo Laakko[2], Juha Tähtiharju[2], Jaana Uusitalo[2], Mauri Aikio[1], Marco G. Casteleijn[2]

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Real-time inline monitoring of protein folding for design and development of biological drugs, foods, and biomaterials remains a challenge. To tackle this challenge, we used time-gated Raman spectroscopy (TG-Raman). It is capable to reveal more spectral features than commonly used continuous-wave Raman spectroscopy due to the ability for fluorescence background suppression. TG-Raman was utilized for monitoring the development of substrates and protein secondary structures throughout a 60-hour-long T. reesei cultivation. Directly harvested protein secretion pathways for protein production were further predicted by comparing the obtained TG-Raman spectra of 21 different commercial proteins during step-wise heating from the room temperature up to 65 °C. The protein folding can be monitored via decoding of their changing Raman spectra..

Finally, the influence of different parameters such as, frequency, amplitude and flow velocity on the separation efficiency was examined.

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*** last-minute change

Plenary Talk

17:45 Engineering, processing and application of recombinant spider silk proteins - from lab to market (PL04)

Thomas Scheibel

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Proteins reflect one fascinating class of natural polymers with huge potential for technical as well as biomedical applications. One well-known example is spider silk, a protein fiber with excellent mechanical properties such as strength and toughness [1]. During 400 million years of evolution spiders became outstanding silk producers. Most spider silks are used for building the web, which reflects an optimized trap for flying prey. We have developed biotechnological methods using bacteria as production hosts which produce structural proteins mimicking the natural ones [2]. Besides the recombinant protein fabrication, we analyzed the natural assembly processes [3,4] and we have developed spinning techniques to produce protein threads closely resembling natural silk fibers [5,6]. Importantly, we can employ the bio-inspired proteins also in other application forms such as hydrogels, particles, non-woven mats, foams or films [7], and we have been able to use spider silk proteins as novel bioinks for biofabrication [8,9]. Our bio-inspired approach serves as a basis for new materials in a variety of medical, biological, or technical applications. Our technology has been further the basis for the biotech spin-off company AMSilk GmbH founded in 2008 focusing on scale-up and product development. First products containing spider silk-based proteins are already established

19:30 Conference Dinner

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23:00 End of Day 2

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WEDNESDAY, 30 MARCH 2021

Opening

09:00 Welcome and introduction
Peter Neubauer, TU Berlin, Germany

Plenary Talk

09:05 Smart tools for high cell density perfusion process producing monoclonal antibodies (PL05)

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AdBIOPRO, Competence Centre for Advanced BioProduction by Continuous Processing, KTH
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The biopharmaceutical field is strongly evolving towards its intensification, where high cell density perfusion processing has globally received a very high interest in industry.

We review here approaches and tools created for high cell density perfusion processes (HCDP), e.g. at steady-state, using Chinese Hamster Ovary cells or HEK293 cells for the production of biologics within AdBIOPRO, Competence Centre for Advanced BioProduction by Continuous Processing. A goal has been to stably maintain healthy cells at a density of 100 x 1E6 cells/mL with sustained productivity and quality of the product of interest, e.g. monoclonal antibody (mAb), and a medium renewal rate of 1 to 1.5 reactor volume/day.

Our development work of HCDP has relied on our system of 200 mL stirred tank bioreactor in perfusion mode, recently complemented with a micro-bioreactor of 2 mL working volume, Erbi Breez, providing very good prediction of stirred tank bioreactor performances. Issued from our development work, a 30 L pilot-scale process was operated for two weeks at 100 x 1E6 cells/mL density, while fully integrated to a 4-steps continuous purification process (ICB), showing a yield of 90%.

We have developed a new targeted feeding strategy, named 'TAFE', for sugar feeding in HCDP to control the level of the toxic by-product lactate. This approach was also successfully applied to feed combinations of carbon sources, such as glucose, mannose and galactose. Although these sugars impact differently the glycosylation, this feature has not been used since the cells have a much higher glucose uptake. The TAFE strategy was extended to the simultaneously feeding of different sugar combinations in HCDP with successful uptake of non-glucose sugar, generating various glycan profiles, and combined to a new mathematical model of the mAb glycosylation, Glycan Residue Balance Analysis, providing a new approach to tune the glycosylation. Another achievement derived from the TAFE concept to balance the amino acids of the perfusion medium, has been to develop a HCDP and enhanced fed-batch for a difficult-to-express therapeutic enzyme based on an established fed-batch process. The novel HCDP process at 100 x 106 cells/mL resulted in stable and more mature galactosylation, increased sialylation, and comparable product specific activity in comparison with the fed-batch.

Process analytical technology (PAT) for process monitoring and control are instrumental for perfusion processes. Within the EU project iConsensus several tools have been created to monitor the culture such as quantification of mAb or host cell protein by microfluidics-based affinity detection, amino acids and metabolites detection by chip capillary electrophoresis, optical sensors or Raman spectroscopy probe. For HCDP, we have developed an approach providing a dynamic culture environment favorable for the Raman model calibration to quantify the amino acids, and then indirectly the antibody N-glycosylation.

Session 8: Bioprocess development

Chair Robert Spann*** / Sarah Westarp

09:50 Application of a microfluidic single-cell cultivation platform for mammalian suspension cell lines in bioprocess research and development (L23)

Julian Schmitz[1,2] Thomas Noll[2,3], Alexander Grünberger[1,2]
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Chinese hamster ovary (CHO) cells are a workhorse for mammalian biopharmaceutical production of e.g., monoclonal antibodies, growth factors or pharmaceutical enzymes. In this context, it is assumed that cell-to-cell heterogeneity in monoclonal populations plays an important role in unexpected process outcomes and unsteady bioprocess robustness, which makes the analysis of these phenomena desirable. Yet bioprocess related single-cell investigations of industrially relevant CHO cell lines are limited and not systematically performed, due to the unavailability of suitable tools [1].

In the last years we developed a PDMS-glass based microfluidic single-cell cultivation (MSCC) device that allows reliable long-term cultivation and analysis of CHO cells under highly controllable environmental conditions. The versatility of this MSCC platform was demonstrated on selected examples. First, we analyzed cellular heterogeneity in the growth behavior of CHO suspension cells [2]. Afterwards, systematic scale comparison between shake flask, bioreactor, and MSCC verified the setup's suitability as miniaturization tool for bioprocess related research questions, by revealing matching growth behavior as well as morphology across all scales [3]. Recently, first cell-to-cell heterogeneity studies of eGFP production and stress response have been initiated.

*** last-minute change

Our results demonstrate that MSCC holds a huge potential within the field of bioprocess research and development. Latest results and insights will be shown. Additionally, technical limitations, necessary improvements, and future application fields will be discussed.

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10:15 Raining fresh red blood cells: scale-up of ex vivo erythroblast expansion for transfusion purposes (L24)

Joan Gallego Murillo[1,2], Christina Bernal[3], Tom van Arragon[3], Luuk van der Wielen[1,2], Emile van den Akker[2], Marieke von Lindern[2], Sebastian A. Wahl[1,2,4]

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Transfusion of donor-derived red blood cells (RBCs) is the most common form of cell therapy. Nevertheless, it faces challenges such as emerging blood-borne diseases, and supply limitations; for instance, in low-income countries, or for chronically transfused patients requiring special blood groups. Ex-vivo (in vitro) production of cultured RBCs (cRBCs) is one solution [1]. Erythroid precursors can be cultured from hematopoietic progenitors, and differentiated into transfusion-ready cRBCs. The large number of cRBCs required for a single transfusion unit (2×1012 RBCs) requires major innovations in the culture process [2].

Expansion and differentiation was successfully scaled from static culture conditions (culture dishes of 10 – 12 mL) to 0.5 L stirred bioreactors. Next step is to further scale-up the erythroblast expansion, the stage in which most of the cell proliferation takes place. We have performed expansion cultures in single-use ST 3.0 L stirred bioreactors, following a repeated batch cultivation strategy, and using tip speed as scale-up parameter. Furthermore, perfusion enabled to significantly increase cell density in bioreactors compared to culture dishes.

Erythroblast growth rate, viability, and differentiation dynamics were maintained during 9 days of culture when scaling up the cultivations from the 0.5 L to the 3.0 L bioreactors. Concentration of lactate in the supernatant of bioreactor cultures was lower than in culture dishes, probably reflecting to a better control in oxygen availability in the bioreactors. This study demonstrates the first steps towards the scaling up of erythroblast expansion in a GMP-compatible bioreactor setup.

1. Pellegrin, S., Severn, C.E. and Toye, A.M., Towards manufactured red blood cells for the treatment of inherited anemia. (2021) Haematologica. 106: 2304-2311.
2. Zeuner, A., Martelli, F., Vaglio, S., Federicic, G., Whitsett, C. and Migliaccio, A.R., Concise Review: Stem cell-derived erythrocytes as upcoming players in blood transfusion. (2021) Stem Cells. 30: 1587-1596.

10:40 Exhibitor short presentations

Ricardo Egea, Bionet & Celltainer Biotech BV, Spain & Netherlands / Mirko Fraulob, INFORS HT, Germany / Simon Lucht, I&L Biosystems GmbH, Germany / Anja Dürasch, KNAUER Wissenschaftliche Geräte GmbH, Germany / Jochen Uhlenküken, Hamilton Bonaduz AG, Germany

11:00 Coffee break & exhibition

Session 9: Bioprocess development

Chair Marco Oldiges / Niels Krausch

11:30 Development of a bioprocess for heterologous hydrogenase production in *E. coli* (L25)

Qin Fan[1], Giorgio Caserta[2], Christian Lorent[2], Peter Neubauer[1], Oliver Lenz[2], Matthias Gimpel[1]

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Concerns about exhaustion of fossil fuels and global warming have led to increasing attention to clean and renewable energy. Here, biohydrogen is a very attractive alternative. The most efficient biohydrogen producers are hydrogenases. These enzymes display fascinating redox-chemical properties with tremendous promise as a biocatalyst for hydrogen fuel. However, due to the complex structure and maturation process of hydrogenases, their heterologous production has been a challenging task and their sensitivity to O_2, CO, etc has seriously limited the potential applications [1].
The β-proteobacterium Ralstonia eutropha H16 hosts four different O_2-tolerant [NiFe]-hydrogenases (MBH, SH, AH and RH) [2]. The R. eutropha RH was selected as model for development of a heterologous [NiFe]-hydrogenase production system in Escherichia coli.
Initial trials with fed-batch-like EnPressoB medium demonstrated the possibility of heterologous hydrogenase production [3]. The yields achieved were several 100-fold higher compared to production in the native organism R. eutropha. Since spectroscopic studies showed the absence of the NiFe cofactor, we further improved the production process by varying different parameters (e.g. strain, production temperature, co-expression of maturation genes etc.). Hereby, we succeeded in producing a RH with an activity similar to that of native RH, but with a significantly higher yield and shorter process time [4]. Finally, the process was transferred to a glucose fed-batch in a 3.7 L stirred tank bioreactor. Thus our results lay a good basis for the future production of functional hydrogenases for basic as well as applied science.

1. Fan, Q., Neubauer, P., Lenz, O. and Gimpel, M. Heterologous hydrogenase overproduction systems for biotechnology - An overview. (2020) Int. J. Mol. Sci. 21: 5890.
2. Lenz, O., Lauterbach, L., Frielingsdorf, S. and Friedrich, B. Oxygen-tolerant hydrogenases and their biotechnological potential. (2015) In Biohydrogen; DE GRUYTER: Berlin, München, Boston, pp 61–96.
3. Fan, Q., Caserta, G., Lorent, C., Lenz, O., Neubauer, P. and Gimpel, M. Optimization of culture conditions for oxygen-tolerant regulatory [NiFe]-hydrogenase production from Ralstonia eutropha H16 in Escherichia coli. (2021) Microorganisms, 9: 1195
4. Fan, Q., Caserta, G., Lorent, C., Lenz, O., Neubauer, P. and Gimpel, M. Production of functional regulatory [NiFe]-hydrogenase from Ralstonia eutropha in Escherichia coli by metabolic engineering and co-expression of maturation genes. (2022) Submitted to Front. Microbiol.

11:55 Development of a scalable production for recombinant horseradish peroxidase (L26)

Julian Ebner, Diana Humer, Julian Kopp, Oliver Spadiut

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The enzyme Horseradish Peroxidase (HRP) is used in a broad variety of biotechnological fields. A prominent application of HRP is its utilization as a reporter enzyme for enzymatic assays and clinical diagnostics. Up to date, HRP is still isolated from its natural source, the horseradish root. However, this production process exhibits several disadvantages such as low yields, varying isoforms and alternating glycosylation patterns. Therefore, a scalable and industrially feasible production process providing a steady supply of the required HRP isoform is highly desirable. We recently developed a process for the production of non-glycosylated, recombinant HRP from *E. coli* inclusion bodies. Initial refolding conditions were established using a Design of Experiments approach in small scale. Targeted refolding conditions were transferred and optimized in a lab-scale bioreactor, allowing a scalable production process. Using this integrated development approach, a highly pure enzyme (>99%) with a competitive final yield of 960 mg/L fermentation broth was achieved. Based on the established refolding protocol, selected process development steps were transferred to a microbioreactor setup in a scale-down approach aiming to further improve the developed process. Microbioreactor scale down models showed significant differences for redox conditions correlating to the dissolved oxygen input monitored in the microbioreactor setup.

In this talk, I will present the general feasibility and applicability of microbioreactors for refolding processes. As these systems allow for in-line measurements and realize a controlled environment at small scales the implementation of microbioreactors has the potential to revolutionize future screening of refolding conditions.

1. Ebner J., Humer, D. and Spadiut, O., Scalable high-performance production of recombinant horseradish peroxidase from *E. coli* inclusion bodies. (2020) Int. J. Mol. Sci., 21: 4625.
2. Ebner J., Humer, D. and Spadiut, O., Von der Wurzel ins Labor: Meerrettichperoxidase produziert in *E. coli*. (2021) Biospektrum 27: 773–775.
3. Ebner J., Humer, D., Klausser, R., Rubus, V., Pell, R., Spadiut, O. and Kopp, J., At-line reversed phase liquid chromatography for in-process monitoring of inclusion body solubilization. (2021) Bioeng. 8: 78.

12:20 Innovative bioprocess strategies combining physiological control and strain engineering of *Pichia pastoris* to improve recombinant protein production (L27)

Arnau Gasset[1], Xavier Garcia-Ortega[1,2], Javier Garrigós-Martínez[1], Francisco Valero[1], Jose Luis Montesinos-Seguí[1]

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The combination of strain and bioprocess engineering strategies should be combined to obtain the highest levels of recombinant protein production (RPP) while assuring product quality and process reproducibility.

In this work, a successful combination of both was achieved on the yeast *Pichia pastoris*. Firstly, the performance of two C. rugosa lipase 1 producer clones with different gene dosage under the regulation of the constitutive PGAP were compared in chemostat cultures with different oxygen-limiting conditions. Secondly, hypoxic conditions in carbon-limited fed-batch cultures were applied by means of a physiological control based on the respiratory quotient (RQ). Stirring rate was selected to maintain RQ between 1.4 and 1.6, since it was found to be the most favorable in chemostat.

As the major outcome, between 2-fold and 4-fold higher specific production rate (qP) values were observed when comparing multicopy clone (MCC) and single-copy clone (SCC), both in chemostat and fed-batch. Additionally, when applying oxygen limitation, between 1.5-fold and 3-fold higher qP values were obtained compared with normoxic conditions. Thus, notable increases of up to 9-fold in the production rates were reached.

Furthermore, transcriptional analysis of certain key genes related to RPP and central carbon metabolism were performed. Results seem to indicate the presence of a limitation in post-transcriptional protein processing steps and a possible transcription attenuation of the target gene in the strains with high gene dosage.

The combined approach, represents a relevant novelty involving physiological control in Pichia cell factory too boost the RPP bioprocess efficiency towards the promotion of bioeconomy.

1. Gasset, A., Garcia-Ortega, X., Garrigós-Martínez, J., Valero, F. and Montesinos-Seguí, J.L., Innovative bioprocess strategies combining physiological control and strain engineering of *Pichia pastoris* to improve recombinant protein production. (2022) Front. Bioeng. Biotechnol. 10:818434.

12:45 Insights into the physiology of *Streptomyces clavuligerus* by constraint-based models for the enhancement of CA production (L28)

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Streptomyces clavuligerus is a filamentous Gram-positive bacterial producer of the β-lactamase inhibitor clavulanic acid (CA), which is used together with wide spectrum antibiotics to deal with resistant infections. Nevertheless, CA yield in all scales is highly limited by several metabolic restrictions [1]. Flux balance analysis is a mathematical framework that helps to understand the physiology of the organisms and currently it has become one of the most used approaches for systems biology given their flexibility to include omics data of different levels [2]. However, one of the drawbacks of constraint-based genome-scale models is the pseudo-state condition that underlies the mathematical development of the models. The extension of the Flux Balance Analysis to dynamic conditions allows to model the cells physiology in non-steady state process such as batch and fed-batch cultivations. In this study, the enhancement of CA production in cultivations of wild-type S. clavuligerus through the application of constraint-based modeling is discussed.

The use of genome-scale models of the organism in steady and non-steady conditions provided insights about the physiology of the strain. This information was used to guide the application of environmental perturbations based on the analysis of the metabolic fluxes. Particularly, the dynamic experiments in silico allowed to identify scenarios of increased CA production by maintaining the conversion rates within the central and secondary metabolism. The experimental validation of the in silico scenario showed that rational operation based on constraint-based modeling allowed to maintain the carbon flux in the secondary metabolism, thus increasing CA production (up to 0.38 mmol gDCW-1).

1. López-Agudelo, V.A., Gómez-Ríos, D. and Ramirez-Malule, H., Clavulanic acid production by *Streptomyces clavuligerus*: Insights from systems biology, strain engineering, and downstream processing. (2021) Antibiotics. 10: 84.
2. Ramirez-Malule, H., López-Agudelo, V.A., Gómez-Ríos, D., Ochoa, S., Ríos-Estepa, R., Junne, S. and Neubauer, P., TCA cycle and its relationship with clavulanic acid production: A further interpretation by using a reduced genome-scale metabolic model of *Streptomyces clavuligerus*. (2021) Bioeng. 8: 103.

13:10 Lunch break & exhibition

Session 10: High-throughput bioprocessing and automation

Chair Klaus Pellicer Alborch / Katja Winkler

14:40 Analytical imaging: non-invasive, high-throughput, multiplexed, and automatable (L29)

Marieke Klijn

Delft University of Technology
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Non-invasive and high-throughput analytical technology is highly desired during characterization and development of protein-based biopharmaceutical products, as it allows for larger and faster experimental screenings, which in turn leads to a deeper product understanding. Imaging corresponds well with these requirements and recent technological advancements resulted in applications across the electromagnetic spectrum, broadening the information content of imaging. Therefore, it is not surprising to see an increasing number of imaging-based methodologies applied for characterization and development of protein-based biopharmaceutical formulations. Exemplary setups range from thermal stability screening to subvisible particle detection and residual moisture analysis. As imaging technology continues to develop, it will play a pivotal role as non-invasive, high-throughput, multiplexed, and automatable analytical technique. This presentation will outline the current role and highlight the future role of analytical imaging across the electromagnetic spectrum for (protein-based) biopharmaceutical characterization and development studies. This includes a comprehensive overview of analytical imaging in the field of biopharmaceutical formulation characterization and development studies. The presentation will cover ultraviolet (UV), visible (Vis), and infrared (IR) imaging techniques applied for the determination of several (bio)physical properties that are commonly used for the assessment of protein-based biopharmaceutical formulations. In addition, personal work on high-throughput imaging setups and automated image analysis strategies for (long-term) stability screening of protein formulations will be discussed and placed within a broader landscape of imaging initiatives by other research groups in the field.

15:05 Development of a filamentous defined co-culture process with high-throughput online-monitoring (L30)

Maurice Finger[1], Ana María Palacio-Barrera[2], Ivan Schlembach[2], Miriam Agler-Rosenbaum[2], Jochen Büchs[1]

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Co-culture processes offer great potential for process intensification as well as the discovery of novel compounds. Despite these opportunities, co-cultures are scarcely seen in industrial production. The high complexity and therefore, resulting lack of process understanding impedes their use for possible enhanced production processes. In this work, we showcase how a co-cultivation process for the cellulolytic filamentous fungus Trichoderma reesei RUT-C30 and filamentous soil bacterium Streptomyces coelicolor A3(2) can be established by utilization of high-throughput online-monitoring. Thereby, enabling the biosynthesis of natural products from renewable cellulosic material. Online-monitoring of oxygen transfer rates was used to evaluate cellulose consumption while fluorescence was used for monitoring the growth trajectories of the involved strains as well as product formation for pigmented antibiotics. This was achieved applying an in-house developed 48-well microtiter plate-based system. A suitable process window for pigment formation was identified by varying the inoculation ratios for both co-culture partners. Depending on the initial co-culture composition, the glucose release profile as estimated from cellulose hydrolysis varied. Low glucose release rates and availability of glucose resulted in a pigment formation by S. coelicolor. To investigate to what extent the pigment formation was related to the interaction of the co-culture partners or to slow glucose release, S. coelicolor was grown in axenic cultures. Different glucose release rates were tested by the addition of cellulase cocktails in varying concentrations. Similar to co-cultivations, low glucose release rates correlated with enhanced pigment production. We successfully demonstrate that online-monitoring is a valuable tool to gain in-depth process understanding, which assists in developing novel and beneficial co-cultivation processes.

15:30 Online 2D-fluorescence monitoring in microtiter plates using a fully tunable monochromator-based spectroscopical setup (L31)

Christoph Berg and Jochen Büchs

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Bioprocess online monitoring using fluorescence measurements has been established for 30 years in large-scale and for more than 15 years in small-scale cultivations. However, while often filter- and photodiode-based setups have been used in the past, setups for continuous emission spectra recording have been introduced only recently [1,2]. By including CCD-based detectors, a large amount of spectral data becomes easily accessible while still allowing for experiments at elevated throughput. In common data-processing workflows, the recorded spectra are finally used for generating multivariate models, thereby allowing a detailed analysis of microbial growth performance as well as the extraction of critical process parameters such as pH or carbon consumption rates [2,3].

Yet, for the currently existing microtiter plate-based setup, the scattered light represents the most dominant signal in the spectra, and fluorescence intensities are only measured with limited resolution. Therefore, a revised spectroscopical setup was developed, including a tunable grating for the emission monochromator. By enabling a precise selection of the emission spectra range, significantly higher sensitivities for biogenic fluorescence can be realized. Based on data from Escherichia coli cultivations, the advantages of the new spectroscopic system and its potential are presented using unsupervised (Principal Component Analysis (PCA)) and supervised (Partial Least-Square Regression (PLS-R)) multivariate data analysis methods. In conclusion, a fast and in-depth evaluation of the metabolic activity of Escherichia coli cultures can be conducted without the need for synthetic fluorophores or reporter proteins.

1. Graf, A., Claßen, J., Solle, D., Hitzmann, B., Rebner, K. and Hoehse, M., A novel LED-based 2D-fluorescence spectroscopy system for in-line monitoring of Chinese hamster ovary cell cultivations – Part I. (2019) Eng. Life Sci. 19, 352–362.
2. Ladner, T., Beckers, M., Hitzmann, B. and Büchs, J., Parallel online multi-wavelength (2D) fluorescence spectroscopy in each well of a continuously shaken microtiter plate. (2016) Biotechnol. J. 11: 1605–1616.
3. Berg, C., Ihling, N. and Büchs, J., Combination of multivariate data analysis and 2D fluorescence spectroscopy in high-throughput cultivation experiments. (2020) Chemie Ing. Tech. 92: 1206.

15:55 Multi-vendor test automation to support process automation (L32)

Jan Dahinden[1], Sebastian Gross[2], Sabrina Leuenberger[1]

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The laboratory of the future consists of an increasing number of connected devices and software applications that must interoperate with each other (e.g. LIMS, eLN, workflow schedular, lab instruments, mobile robots). While the software and interfaces are individually checked (tested) by the vendors, it is up to the user, or a third party integrator, to ensure the proper operation of the devices and software applications among each other. Updates to one or more systems can quickly disrupt automated processes. However, no updates mean an increasing security risk in the connected lab of the future. To ensure the robustness especially of automated processes, they must be re-tested after each update to check the interoperation of each entity involved, ideally all the way up to the user level, i.e., at the user interface level (GUI). This complex testing procedure is hardly feasible in the required cycles and time and must be automated to enable secure and robust automated processes.

Therefore, automated testing on GUI and application interface (API) level has a high potential to increase process security, robustness and flexibility and is therefore a key for a high throughput R&D and low idle times in production.

Here we present the possibilities of automated testing. As an example, we use a state-of-the art bioprocess automation with multiple instrument vendors, in combination with third party and homemade software applications [1] and transfer it further in a regulated environment.

1. Kaspersetz, L., Waldburger, S., Schermeyer, M.-T., Riedel, S.L., Groß, S., Neubauer, P. and Cruz-Bournazou, M.N., Automated bioprocess feedback operation in a high throughput facility via the integration of a mobile robotic lab assistant. (2022) bioRxiv 2022.01.13.476044.

16:20 Coffee break & exhibition

Session 11: High-throughput bioprocessing and automation

Chair Mario Birkholz / Lucas Kaspersetz

16:50 Acetoin detection in alcoholic beverages and fermentation broth with a capacitive enzyme biosensor (L33)

Melanie Welden[1,2], Johannes Bongaerts[1], Torsten Wagner[1,3], Michael Keusgen[2], Petra Siegert[1], Michael J. Schöning[1,2]

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In food industry, quality control is a major factor including maintaining the specific taste. To ensure a consistent taste, flavorings are frequently applied. These include acetoin, which has a buttery-like aroma and can be found in e.g., cheese, yogurt or alcoholic beverages. The acetoin concentration in wine and beer changes during different fermentation stages and can serve as an indicator for the maturation degree of the products. Since flavor maturation is the time-limiting factor during the beer-brewing process, regular control of the acetoin level can avoid unnecessary long maturation time. Apart from its role in alcoholic beverages, the detection of acetoin has a crucial impact in biotechnological applications: Its concentration provides information about the metabolic activity of bacteria during fermentation processes [1].

Off-line determination of acetoin by gas chromatography (GC) allows accurate quantification of the acetoin concentration. However, this technique is time-consuming, requires sample preparation, equipment and trained personnel, which can result in high costs. A biosensor could avoid these drawbacks by providing on-site measurements with a fast response time. For this reason, our group developed a field-effect biosensor based on an enzyme-modified electrolyte-insulator-semiconductor (EIS) sensor [1,2].

In the present study, the storage stability of the acetoin biosensor was investigated and acetoin was detected in real samples of beer, red wine and fermentation broths. The functionality of the acetoin biosensor was characterized by measurements in the capacitance-voltage and constant-capacitance mode. Experiments were performed in buffer solution, beer-, red wine-, and fermentation-broth samples spiked with different acetoin concentrations. Finally, the naturally formed acetoin during fermentation in in-house fermenters producing subtilisin proteases with Bacillus subtilis DB104 was successfully detected with the developed capacitive field-effect biosensor.

1. Jablonski, M., Münstermann, F., Nork, J., Molinnus, D., Muschallik, L., Bongaerts, J., Wagner, T., et al., Capacitive field-effect biosensor applied for the detection of acetoin in alcoholic beverages and fermentation broths. (2021) Phys. Status Solidi A 218: 2000765.
2. Molinnus, D., Muschallik, L., Osorio Gonzalez, L., Bongaerts, J., Wagner, T., Selmer, T., Siegert, P., et al., Development and characterization of a field-effect biosensor for the detection of acetoin, (2018) Biosens. Bioelectron. 115: 1-6.

17:15 Development of a multi-parameter biosensor for bioprocess monitoring based on a flow system (L34)

Aliyeh Hasanzadeh[1], Babak Rezaei[2], Pedram Ramin[1], Mogens Kilstrup[3], Krist V. Gernaey[1]

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Bioprocesses require strict control of metabolites and nutrients to achieve optimal cell growth and maximize product yields. However, when comparing full-scale operation to lab-scale, the number of on-line sensors for monitoring of full-scale bioreactors is usually limited, consisting of traditional sensors (e.g. temperature, pH, and dissolved oxygen) [1]. Especially in a production environment, the detailed monitoring of cell performance is usually achieved by taking regular samples from the fermentation medium during the process, followed by off-line measurement of substrate, metabolite, and product levels in the samples. Thus, real-time monitoring of such critical process parameters is often lacking, and therefore, controller set points are often based upon experience rather than on-line data (e.g. golden batch approach). Some of these challenges can be alleviated by using biosensors for on-line monitoring which is also aligned with Process analytical technologies (PAT) [2].

In this work, we report on the design and development of a multi-parametric analytical flow system integrating three electrochemical sensors. The developed flow-system allows for simultaneous determination of ammonium, lactate, and glucose remotely in real-time. Amperometric biosensors for the determination of glucose and lactate are based on a thin film of platinum nanoparticles and immobilized glucose oxidase and L-lactate oxidase. A metal-organic framework (MOF) based amperometric sensor was developed for ammonium measurement. A fast detection was recorded for the sensors over wide concentration ranges for the mentioned analytes. The as-prepared sensors indicated highly reproducible responses and stable sensitivity. The successful application of the sensors set-up is demonstrated in different fermentation processes.

1. Vojinović, V., Cabral, J.M.S. and Fonseca, L.P., Real-time bioprocess monitoring: Part I: In situ sensors. (2006) Sens Actuators B Chem. 114: 1083-1091.
2. Gargalo, C.L., Udugama, I., Pontius, K., Lopez, P.C., Nielsen, R.F., Hasanzadeh, A., Mansouri, S. et al., Towards smart biomanufacturing: a perspective on recent developments in industrial measurement and monitoring technologies for bio-based production processes. (2020) J. Ind. Microbio.l Biotechnol. 47: 947-964.

Plenary Talk

17:40 Bioprocess modelling: Have we moved forward sufficiently? (PL06)

Jarka Glassey

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The benefits of advanced modelling and control procedures are significant in biopharmaceutical industry, given the high value of the products and the quality demands on the Advanced Therapy Medicinal Products (ATMPs). Whilst the use of advanced modelling and control procedures is becoming more universally accepted in this sector partly due to the widespread awareness of Quality by Design (QbD) and Process Analytical Technologies (PAT), significant challenges remain using these methods in the information limited development environment. This is even more pronounced in areas such as ATMP manufacturing, in cell culture processing for production of a range of products, e.g. monoclonal antibodies, where knowledge of critical quality attributes is limited. This presentation will overview the advances to date in this area and demonstrate examples of model-based bioprocess development and advanced process monitoring to assist those who are seeking to make fundamental changes to the drug manufacturing philosophy. The emphasis is on the whole process optimization based on a continuous processing strategy and the development of effective frameworks for advanced modelling and control schemes for such processing in monoclonal antibody production form the key technical focus with emphasis on overcoming the current constraints of use arising through process complexity, data limitation and uncertainty.

18:25 Poster session, exhibition & get-together

21:00 End of day 3

Best Presentation Award sponsored by:

microorganisms
bioengineering

Best Poster Award sponsored by:

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THURSDAY, 31 MARCH 2021

Opening

09:00 Welcome and introduction

Peter Neubauer, TU Berlin, Germany

Plenary Talk

09:05 The automation of science (PL07)

Ross King
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A Robot Scientist is a physically implemented robotic system that applies techniques from artificial intelligence to execute cycles of automated scientific experimentation. A Robot Scientist can automatically execute cycles of hypothesis formation, selection of efficient experiments to discriminate between hypotheses, execution of experiments using laboratory automation equipment, and analysis of results. The motivation for developing Robot Scientists is to both to better understand the scientific method, and to make scientific research more efficient. The Robot Scientist 'Adam' was the first machine to discover scientific knowledge autonomously. The Robot Scientist 'Eve' was originally developed to automate early-stage drug development, with specific application to neglected tropical disease such as malaria, it is currently working on anti-corona drugs. I am now developing Genesis, a next-generation Robot Scientist designed to work on yeast systems biology. Genesis will soon be able to run 10,000 cycles of hypothesis-led experiment in parallel per day. Advances in AI and lab automation will drive the development of ever-smarter Robot Scientists. Therefore, I am co-organising the 'Nobel Turing Challenge' to develop: AI systems capable of making Nobel- quality scientific discoveries highly autonomously at a level comparable, and possibly superior, to the best human scientists by 2050.

Session 12: High-throughput bioprocessing and automation

Chair Nico Oosterhuis / Marie-Therese Schermeyer

09:50 Fast production of complex proteins (L35)

Vicky Goralczyk
Director Cell Line and Bioprocess Development FyoniBio (Service Brand of Glycotope), Germany
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Early development of new drugs, therapeutics or other biomolecules often heavily relies on fast availability of newly designed proteins for early screening purposes. For complex proteins sufficient amounts of said protein can only be obtained after months as unfit production systems prolong timelines and increase costs. GEX® is a human cell host which is proven to increase productivity for difficult to express proteins and expresses fully human processed proteins. We present data for a low-cost fast track process to supply early needs and compare that to traditional CHO based approaches. Also, we show timelines and yields for a reduced up-stream program allowing early production of GMP material, e.g. for tox studies.

10:15 Eliminating gas-liquid mass transfer limitations in shake flasks by a new reactor design based on perforated ring walls (L36)

Sven Hansen, Andreas Gumprecht, Linda Micheel, Hans-Georg Hennemann, Franziska Enzmann, Wilfried Blümke
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For nearly a century shake flasks have widely been applied for biotechnological screening and process development in lab scale [1,2]. However, up to today, a direct scale-down of production fermenter conditions often remains challenging due to the different engineering characteristics of the reactor types. The most prominent difference certainly is the usually significantly lower maximum oxygen transfer capacity of unbaffled shaken bioreactors compared to bubble aerated fermenters [2,3]. The implementation of baffles can overcome this problem but introduces other disadvantages like low reproducibility, instable hydrodynamics, and intense foam formation [2]. In both cases, severe problems in cultivation procedures may occur and even remain unseen, especially in the absence of online monitoring systems.

A newly invented shake flask design eliminates the problem of oxygen transfer limitations without the introduction of baffles. For that purpose, perforated concentric ring walls are implemented into the design of the shaken bioreactor. The increased film area allows for oxygen transfer rates that are comparable to many stirred tank bioreactors. Thus, this technology allows new possibilities for the operation of shaken bioreactors in scale-down and screening approaches.

The talk introduces the design principle of the new shaken bioreactor type, shows fermentation examples, and implies future potentials

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10:40 Coffee break & exhibition

Session 13: Modeling bioprocesses

Chair Nicolas Cruz Bournazou*** / Judit Aizpuru

11:10 Accelerated microbial phenotyping: How process modelling and a decision policy can enhance high-throughput screening of PETase-secreting *Corynebacterium glutamicum* variants (L37)

Laura Marie Helleckes, Tim Griesbach, Carolin Müller, Michael Osthege, Wolfgang Wiechert, Marco Oldiges

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In the European pursuit of a sustainable bioeconomy, the development of efficient biotechnological production processes is a key factor. Modern methods in high-throughput strain generation require equivalently fast screening and data analysis, which is often performed in small-scale reaction systems such as microtiter plates (MTP). However, even in these parallelised systems such as microbioreactors [1], the questions remain which strains and conditions to choose in each run and how many replicates to perform before advancing to larger scales for process validation. In this context, a suitable metric for candidate rankings as well as an efficient decision policy are key to success.

In this work, we aim at a holistic modelling approach to screen two libraries of *Corynebacterium glutamicum*, secreting heterologous polyethylene terephthalate hydrolase (PETase) with different Sec secretion signal peptides of Bacillus subtilis. Combined with the decision policy of Thompson sampling, our Bayesian hierarchical process model allows to describe reaction kinetics, to capture biological and technical batch effects and to provide useful experimental suggestions for consecutive screening rounds.

For the two different PETase variants examined, the Leaf-Branch Compost Cutinase [2] and the Polyester Hydrolase [3], we found that the rankings of best signal peptides differ significantly. Due to the efficient screening policy, only three batch runs were needed to make a statistically sound distinction between a set of 24 signal peptide variants. Additionally, the process model pointed to a column-specific bias in the assay, which could not only be accounted for in the ranking, but also led to experimental improvements for consecutive experiments.

Overall, the process model assists a deeper understanding of the underlying biological and technical processes. With the successful application of the screening policy, this work paves the way for more efficient, high-throughput screening in early-stage bioprocess development. In the future, this will especially play a role for large libraries with hundreds of variants, where manual selection and data analysis are not feasible.

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11:35 Hybrid modelling coupled with Raman spectroscopy for enhanced bioprocess understanding via particle filtering (L38)

João Medeiros Garcia Alcântara[1,2], Claus Wirnsperger[2], Hugo Santos[2], Fabian Feidl[2], Michael Sokolov[2], Mattia Sponchioni[1], Davide Moscatelli[1], Massimo Morbidelli[1,2], Alessandro Butté[2]

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In the last years, increased process complexity and the more and more stringent regulations in bioprocessing has driven the necessity for enhanced process understanding and control. In this context, new process analyzers such as online Raman Spectroscopy, can heighten process knowledge and allow for the implementation of suitable control strategies ensuring that the critical quality attributes (CQAs) of the product remain within the guidelines. This approach is supported and encouraged by the regulatory authorities. Evidence of this is the long-established PAT initiative and the upcoming ICH Q14. On the other hand, advanced modelling methodologies such as hybrid modelling can draw both on mechanistic knowledge and data-driven evidences, creating a powerful control and optimization tool.

This work reports the integration of Raman spectroscopy for the online monitoring of a fed-batch mammalian cell bioreactor producing therapeutic proteins. Based on the data from the Raman spectrometer, a soft-sensor was developed and demonstrated able to reliably predict the cell density and multiple metabolites. Secondly, founded on mechanistic knowledge, a hybrid model for the process was created. These two models were then combined using a particle filter. Specifically, the predictions coming from the hybrid model are filtered and improved based on the collected online Raman spectra.

This approach has showed heightened predictive power, leading to applications in real-time monitoring, control and possibly automated decision-making in pharmaceutical bioprocessing

*** *last-minute change*

12:00 Model-based optimal control of parallel mini-bioreactors (L39)

Niels Krausch[1], Jong Woo Kim[1], Sergio Lucia[2], Peter Neubauer[1], Mariano N. Cruz Bournazou[1,3]

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Bioprocess development is characterized by long development times. The screening of a multitude of potential strains and process conditions is a key element of this procedure. Even though High-Throughput (HT) liquid handling stations enable a large number of strains to be tested, these experiments provide no insight into the dynamical phenotype of the strains without using model-based approaches. However, this information is highly important for further scale-up [1]. To assure optimal growth and process control without knowledge about the growth behavior of the possible strains to be tested, we propose and implement a combination of model-predictive control (MPC) with adaptive moving horizon estimation (MHE) to estimate the parameters of an underlying macro-kinetic growth model, so this information can be used already while the cultivation is running. MPC is widely used in chemical process control, but so far very less prominent in the bioprocess industry, because of the difficulties originating from the different dynamics among the state variables in bioprocess (fast for dissolved oxygen, slow for growth) and higher nonlinearity of such systems [2]. The proposed framework allows for online parameter estimation and subsequent optimization of the remaining feeding profile of the running process to reach a predefined goal, fulfilling all experimental constraints, e.g. starvation and oxygen depletion [3]. As a proof of concept, we test our developed method in 24 *E. coli* cultivations in parallel mini bioreactors on our high-throughput facility with automated sample analysis. We demonstrate that the proposed framework is capable of maintaining the cultivation within the defined experimental design space even for highly complex dynamics as e.g. dissolved oxygen in bolus feeding processes and outperforms classical feeding approaches.

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12:25 Machine learning meets scientific understanding: New approaches for holistic process models (L40)

Jens Smiatek

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Computational modeling and statistical analysis are now standard approaches to support process development and experimental analysis in the biopharmaceutical industry [1]. In addition to these now almost traditional methods, other non-parametric machine learning approaches are also gaining increasing attention. In this talk we will highlight certain applications of machine learning methods for the holistic modelling of bioprocesses in combination with the detailed analysis of the outcomes.

As a first application, we will shed light on the role of recurrent neural networks (RNNs) in predicting upstream cultivation processes [2]. We will discuss the basics as well as the corresponding implications for interpreting the results. Closely related to RNNs are artificial neural networks (ANNs) that can be used to predict unknown thermodynamic properties of drug formulations [3]. However, the validation of such models is associated with some requirements [4]. In the last part of our talk, we will outline how individual models for different process steps can be combined within the framework of hierarchical Bayesian functions in order to enable a holistic modeling of the entire bioprocess.

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12:50 Lunch break & exhibition

Session 14: Modeling bioprocesses

Chair Sören Bernauer / Stefan Born

14:20 Stochastic parcel tracking in an Euler-Lagrange compartment model for fast simulation of fermentation processes (L41)

Cees Haringa[1], Wenjun Tang[1,2], Henk Noorman[1,2]

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Recently, computational fluid dynamics with integrated reaction dynamics (CFD-CRD) has gained increasing attention as a tool to assess the impact of spatial heterogeneity on fermentation processes [1,2]. However, the excessive computational expenses required for such simulations prohibit routine usage. Compartment Modeling (CM) are a well-established alternative; often, CFD is used to calibrate inter-compartment flows, allowing for a coarser but substantially faster estimation of spatial heterogeneity in bioreactors. In order to estimate the effect on overall process performance, simulations also need to include the impact of said heterogeneity on the response of the micro-organism, which is not the case for the black-box kinetic models typically incorporated in CM. Of the two leading strategies for incorporating cellular response, population balance modeling is established in the framework of CM [3], but to date, agent-based (parcel-tracking) is not. In this presentation, we build upon the stochastic parcel tracking methodology of Delafosse et al. [4] and incorporate a multi-pool agent based reaction model in the CM framework. The penicillin production case study, studied extensively with full agent-based CFD, is used as a case study. Both the extra-cellular concentration gradient and the intra-cellular pool response agree with the full CFD simulation, provided the CM simulation is configured to properly reproduce the mixing time of the full CFD simulation. Whereas the full CFD simulation, even with considerable simplifications, required approximately 2 weeks to compute 80 h flow-time, the coarsest CM requires a few minutes. This hence alleviates one of the major bottlenecks towards routine analysis of spatial heterogeneity in bioprocesses.

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14:45 CFD based particle-tracking tools for quantifying large-scale bioreactor performance (L42)

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The performance of large-scale bioreactors depends on the complex interaction between the hydrodynamics and the physiology of the microorganism being grown. Use of Computational Fluid Dynamics (CFD) models combined with Lagrangian particle-tracking approaches offers a sophisticated way to understand the impact of bioreactor design on a population of microorganisms [1]. The aim of this work is to demonstrate how particle tracking models can be used to understand process performance and improve reactor designs.

In this work we use CFD based particle-tracking methods to quantify the performance of two different processes; a traditional large-scale biotechnological process (the heterotrophic production of baker's yeast) as well as an emerging photoautotrophic process using the microalga Phaeodactylum tricornutum for the production of eicosapentaenoic acid. The effect of the reactor design and operating conditions on the performance of the process was quantified. For example, it was found that light-dark cycle frequency could be increased by a factor of 2-3 by modifying the photobioreactor design and operating conditions, leading to a predicted 10-30% increase in the specific growth rate. Comparison was also made between results determined using the particle-tracking approach and those calculated using other methods (e.g. those based on average concentrations). The advantages and disadvantages of each method will be examined, along with suggestions for future work in this area

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Plenary Talk

15:10 Towards automating active learning in collaborative bioprocess development (PL08)

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The challenges of automating complex cognitive tasks in the framework of Industry 4.0/5.0 give rise to a key question: what is needed for the next industrial revolution? More specifically, what means for collaborative bioprocess development the promise of autonomously gathering highly informative data sets using high-throughput robotic facilities and networked experimentation using ubiquitous digitization. In the talk it is argued that the next revolution is about automating learning, planning and model building alongside with creating cognitive interfaces for more skilled humans to inter-act with semi-autonomous robotic systems. The main enablers for automating active learning of all data needed throughout the development lifecycle are then highlighted. The pillars for next revolution are put forward as cognitive digital threads, using FAIR principles for data, workflows, experimental protocols and computational pipelines, and more importantly, active inference. Active inference is a powerful new theory in neuro-science that characterizes brain function prediction capability using mathematical for-malisms and first principles that provide an entirely new approach to automate active learning in robotic platforms. Active inference is most appealing for high-throughput bioprocess development because it unifies state-estimation, control and bioreactor model learning as inference processes that are solved by optimizing a single objective functional: the free energy as it is used in Variational Bayesian Inference. Some pre-liminary results obtained for two case studies are presented.

15:55 Closing remarks

Peter Neubauer, TU Berlin, Germany

16:10 End of conference

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POSTER ABSTRACTS

P01: Phosphate assimilation in co-culture of *Acinetobacter tjernbergiae* and *Pseudomonas stutzeri*

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Phosphorus is a key element in all biological processes and is indispensable as a fertiliser. Current commercial phosphate mining is largely harmful to the environment and causes major social problems. Furthermore, phosphate is a finite resource. Current recycling methods recover phosphate through chemical enrichment but are energetically costly and may not achieve the level of sustainability that natural phosphate-binding processes by microbes can. Phosphate-accumulating organisms (PAOs) represent an attractive solution. PAOs can store phosphate as polyphosphates intracellularly. The cell acts as an adsorptive or desorptive material for phosphate depending on the ratio of external substrate and oxygen availability, making a PAO cell an interesting alternative to take up or release dissolved phosphate in a controlled manner in a continuous process.

In order to take use of the potential to assimilate phosphate microbially, a biological recycling method is being developed in the form of a plug flow reactor with a carrier material. Phosphate is continuously taken up from the wastewater by a co-culture of two sewage sludge bacteria, Acinetobacter tjernbergiae and Pseudomonas stutzeri. First fed-batch experiments show that a phosphate reduction of 90 % in the medium is possible with subsequent enrichment, depending on the process conditions. The phosphate is stored in the biofilm, which is then detached for further processing. The stability and vitality of the co-culture are monitored employing electrooptical polarizability measurements, among other. Future work will include the monitoring of polyhydroxyalkanoates as value-adding side products of the bioprocess.

P02: Utilizing straw-derived hemicellulosic hydrolysates for feed manufacturing with *Chlorella vulgaris* – a new waste to value approach

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As the human population is rising, the need for novel food approaches and a reduction of greenhouse gas emission is steadily increasing in importance. An approach to tackle both concerns would be microalgal cultivation. Microalgae can be cultivated on non-arable land and require less space than the production of currently established protein sources, such as meat and soy.

Feed manufacturing via Chlorella vulgaris results in products being high in unsaturated fatty acids. Moreover, C. vulgaris protein content includes all essential amino acids needed for a balanced human and animal nutrition and simultaneously fixates atmospheric CO_2. However, the cultivation of microalgae in a sustainable and economically viable manner still phases major challenges.

To reduce production costs and increase obtained biomass concentrations, hydrolysates from abundantly available lignocellulosic waste streams, could be used as substrates for heterotrophic and mixotrophic cultivations of C. vulgaris.

In this presentation, I discuss the impact of different oligomeric and monomeric sugar concentrations of wheat straw hydrolysates on the biomass composition of C. vulgaris.

A successful implementation of wheat straw hydrolysates as a substrate for microalgal cultivation could be demonstrated. Comparing mixotrophic to standard photoautotrophic cultivation showed a 1.5 fold increase in biomass production whilst repurposing those waste streams.

Presented results thus demonstrate an alternative utilization for lignocellulosic hydrolysates, generate novel foods, while fixating CO_2.

P03: PHA bioplastic with tunable monomer content by flexible substrate mixtures

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Fossil-based plastics are a major pollutant of our environment. While around 400 million tons of plastics are produced annually worldwide, the global demand is estimated to double within the next 20 years [1]. Polyhydroxyalkanoates (PHAs) are bacterially synthesized polyesters that serve as a green alternative to conventional plastics due to their comparable properties but full biodegradability in natural environments [2]. Poly(hydroxybutyrate-co-hydroxyhexanoate) [P(HB-co-HHx)] is a PHA copolymer of great interest as the HHx molar fraction may be used to modulate the thermal processability, toughness and degradation rate of the final polymer [3]. We developed a simple feeding strategy that enabled to precisely control the HHx content in engineered Ralstonia eutropha cultivations from the mL-scale in deep-well-plates to 1-L high-cell-density fed-batch bioreactor cultivations. Adjusting the ratio of fructose to canola oil as substrates, permitted to tune the HHx molar fraction in a range from 2–17 mol%. The developed strategy allows the production of tailor-made PHAs with defined properties for various applications.

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P04: Novel methanol-free expression system PDH: a potential alternative to classical *P. pastoris* promoters for recombinant protein production

Núria Bernat-Camps[1,2], Miguel Angel Nieto-Taype[1], Jasmin E. Fischer[2], Katharina Ebner[2], Francisco Valero[1,2], Anton Glieder[2], Xavier Garcia-Ortega[1,3]

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In the widely used yeast cell factory Pichia pastoris (Komagataella phaffii), the recombinant protein production (RPP) has often been driven by the methanol-induced AOX1 promoter (PAOX1) and the constitutive GAP promoter (PGAP). In the

last years, strong efforts have been focused on finding novel expression systems, since they are crucial for the bioprocess performance.

In this work, a study of a methanol-free expression system based on a heat-shock gene promoter (PDH) was conducted using glycerol as sole carbon source. Using this promoter, the recombinant expression is strongly induced in carbon-starving conditions. The classical PGAP was used as reference expression system, taking for both strains the lipase B from C. antarctica (CalB) as model protein. PDH expressing CalB exceeded PGAP in shake-flask cultivations when using a slow-release feeding technology, confirming that PDH was induced in conditions of no growth.

This increase was confirmed in fed-batch cultivations. Three optimization rounds were carried out for PDH under different feeding strategies. In all of them the PDH outperformed the CalB titer achieved by PGAP. The single biomass production and induction phase at a very low constant feeding rate was the best approach, reaching 2.8-fold higher specific productivity than PGAP fed-batch at low μ (0.025 h-1). Unless fed-batch at high μ (0.15 h-1) with PGAP strain presented 36.2 % higher specific productivity, the best PDH fed-batch strategy had 2.6-fold more titer. To summarize, PDH is an inducible promoter that exhibited a higher efficiency, consuming less substrate and oxygen than the benchmark. Thus, this novel system emerges as a potential alternative for P. pastoris RPP bioprocess.

P05: Influence of oxygen levels on a genome reduced *Pseudomonas putida* strain

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The bacterium Pseudomonas putida is becoming a cell factory of choice due to its versatile metabolism and high stress tolerance, the latter being of prime importance when considering production at large scale and bioconversion of potentially toxic chemicals. These phenotypic features have led to the development of P. putida as a platform organism including the development of genome reduced chassis strains like the SEM10 [1]. However, thorough characterization of the genome-reduced strain under industrially relevant conditions is still lacking.
In order to compare the wild type KT2440 with the genome reduced SEM10 we cultivated both strains under varied oxygen levels. The oxygen levels were varied by changing the partial pressure of oxygen in the cultivation gas, thereby reducing the influence of shear. SEM10 showed better growth properties compared to the wild type during cultivation with the pO2 equal to ambient air. The wild type KT2440 strain accumulated 3.84±0.06 g/L CDW compared to 4.42±0.23 g/L CDW by SEM10 under conditions of ambient air. When cultivated under a partial pressure of oxygen equal to 25% of ambient air, SEM10 accumulated 4.56±0.23 g/L CDW compared to 3.64±0.28 g/K CDW by KT2440 within the same time span. The ability of SEM10 to accumulate more biomass during conditions of both ambient and low partial pressure of oxygen suggests that the genome reduced strain is able to retain its superior growth properties even at poor oxygen availability.

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P06: withdrawn

P07: Parallel scale-down tool to accelerate fermenterphile selection

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Successful scale-up of fermentation processes relies on the selection of fermenterphiles, i.e. robust production strains that maintain optimal performance under industrial conditions [1]. Screening and selection of strains with fermenterphile phenotype require suitable scale-down simulators that mimic relevant industrial conditions [2]. Commonly used scale-down simulators often have a large footprint and low throughput [3], hence they are used at later stages of bioprocess development, whereas including high-throughput scale-down systems at the early stages of bioprocess development would improve the chances of finding fermenterphiles [4]. In this study, we present a parallel scale-down tool based on the Sartorius Ambr® 250 high-throughput system to accelerate fermenterphile research in the early stage of bioprocess development. For the scale-down cultivation, we designed an intermittent feeding regime scale-down strategy to simulate the impact of substrate gradients in large bioreactors on Escherichia coli. In our design, intermittent feeding occurs in cycles of 1-2 minutes to impose repeated glucose starvation, however, the intermittent feeding regime can be adjusted freely to simulate a wide range of substrate gradients and liquid mixing times expected in industrial-scale bioreactors. With the liquid handling solutions of Ambr® 250, metabolome and transcriptome analysis can be performed to elucidate the response of the strains to industrially relevant conditions. We postulate that the presented scale-down tool can be used to efficiently characterize the performance of microbial production strains in industrial fermentation processes and accelerate fermenterphile selection. This would allow the development of fermenterphile strains to be implemented at an early stage of bioprocess development.

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P08: Controlling *Aspergillus niger* morphology in a rocking motion bioreactor

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The filamentous fungus Aspergillus niger has been widely utilized in the production of organic acids, proteins, and secondary metabolites. Its productivity, however, is highly affected by the unpredictable morphological form that it develops in a bioprocess. In particular, shear force regimes as they appear in stirred tank bioreactors have a huge impact on the macromorphology and finally the product selectivity and yield. The mechanical stress imposed by the stirrers lead to either fragmented hyphae or pellets of varying density. The zones of different shear forces in large scale reactors also lead to different macromorphologies than in lab scale stirred tank reactors (STR).

In this study, the development of the macromorphology and its implication for the process performance is investigated both in lab-scale STR and a 2-dimensionally rocking motion bioreactor (RMB). Moreover, the possibility of controlling the pellet size under low shear stress regimes independently from oxygen limitation in RMB is studied. To do so, talcum microparticles are added at different concentrations for the purpose of getting a distinct macromorphology.

The high shear regimes in STR, no matter whether an intermittent or continuous stirring was applied, led to the formation of a mixture of dispersed morphology and clumps of varying sizes. Oxygen limitation, however, was unavoidable in STR when intermittent stirring was applied. On the contrary, it was possible to achieve different macromorphologies in dependence on the talcum concentration without the appearance of oxygen limitation in the RMB.

At 1.00 % and 0.25 % (w/v) talcum, dispersed hyphae and loose clumps were formed, whereas at 0.1 % and 0.05 % (w/v) talcum homogenous pellets were dominating. Further analysis of the quantitative data confirmed the reduction of 150-200 µm in average pellet diameter at 0.1 % in comparison with 0.05 % (w/v) talcum. The pellet size distribution was similar to what was observed in large scale cultivations.

This enables the investigation of the dependence of the process performance and certain macromorphologies independently from oxygen limitation.

P09: Progress in characterisation of liquid flow in oscillatory rocked disposable bioreactors: experimental procedures and empirical models for mixing time evaluation

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Oscillatory rocked disposable bioreactors are prevalent in the bioprocess and biopharmaceutical industries, with their recent applications in modern areas such as human mesenchymal stem cells cultures [1] for cell therapy and scaling-up of viral vaccines manufacturing [2]. Despite this, a factor that hampers the application of rocked bioreactors is the small number of useful mathematical models for predicting important parameters describing the conditions inside the disposable culture bags.

The aim of the research was to examine the characteristics of mixing time inside a Cellbag 2 L disposable container in the ReadyToProcess WAVE 25 (Cytiva, US) bioreactor system. The experiments were designed according to the DoE methodology. The included operational parameters were: the wave-mixing angle α and frequency ω, the volume of liquid VL and the acceleration parameter acc. The measurements were performed using a sensor method based on recording changes in pH values after tracer addition.

Acquired experimental data were analysed and used to derive model equations for estimating mixing time values in the studied disposable bioreactor system. Provided correlations allow for calculations of mixing time for the entire available range of operational parameters for mixing time values estimation in analogous oscillatory rocked disposable bioreactors. The equations show a satisfactory level of accuracy, with the predictions falling within ± 30 % of experimental values [3]. These findings will facilitate the successful utilisation of disposable bioreactor systems supported with wave-assisted agitation in the future.

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P10: Dynamically adjusting extracellular environmental conditions leads to robust oscillations in gene expression: toward a generalizable cell population control strategy

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Cellular systems have evolved to adapt to fluctuating environmental conditions based on phenotypic diversification. Such adaptation relies on a complex set of molecular sensors embedded in various gene circuits architectures. Consequently, for optimizing cell culture performances, environmental conditions (i.e., process parameters and medium composition) are carefully selected for promoting the occurrence of productive phenotypes. However, such physiological traits are neither stable over time nor homogeneously expressed across the cell population, leading to heterogeneous and unstable cell populations.

Biological noise is the main source for population diversification but, among all the components of this very complex mechanism, we determined that the feedback effect excerted by cell population on the extracellular environment is key. Indeed, a population of cells alters their environmental conditions by consuming substrate and excreting metabolites. Our work focuses on elaborating a general methodology for stabilizing and homogenizing the occurrence of target phenotypes in continuous bioreactors.

The approach relies on a previously developed experimental platform, called the segregostat, and involving the use of automated flow cytometry (FC). On-line FC allowed for the automated determination of cell switching rate the culture according to the use of specific fluorescent reporters. Based on this approach, we dertermined that, when the rate of environmental fluctuations are set to the rate of phenotypic switching, gene expression in cells become synchronized, leading to a robust and oscillating gene expression profile during the cultivation. This observation point out the fact that gene circuits exhibit a "critical" stimulation frequency, as previously determined based on simulations.

This method has been applied successfully on three model systems i.e., Escherichia coli, Bacillus subtilis and S. cerevisiae, leading for each case to the effective control of gene expression among cell population. Several gene circuits involved in key cellular processes (adaptation to the utilization of alternative carbon sources, general stress response, sporulation) were targeted, suggesting that the approach could be generalized to many other gene circuits.

From an applied perspective, our data could be exploited for optimizing productivity during continuous bioreactor operations or for designing more robust scaling-up strategies.

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P11: Small scale mechanical cell disruption: A workflow to screen for ideal disruption conditions for recombinantly produced proteins in *E. coli*

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The majority of processes using Escherichia coli require cell disruption to release the intracellularly produced target protein. Several mechanical and non-mechanical methods are commonly applied. The most frequently employed method in industry, high pressure homogenization, requires large sample volumes (>20 mL), which is problematic for small scale screening. Hence, for screening experiments enzymatic methods (e.g. Lysozyme) are often used. These methods lack in reproducibility, scalability and might influence purity patterns of samples due to the additional enzyme addition.

Thus, the need for a small scale mechanical disruption method is given. Even tough an ultrasonic homogenization device is already reported in literature, application of standardized protocols might lead to fluctuating recoveries for various proteins. Depending on the expression state (inclusion body/soluble), target protein characteristics and localization (cytoplasm/periplasm) different conditions for ultrasonic cell disruption are required. To test this hypothesis, we investigated the factors power input, sonication time and cycles of sonication for three different target proteins. Disruption efficiency was determined in comparison to high pressure homogenization and enzymatic cell lysis by applying a variety of analytical methods.

Based on this study, we show that (i) the ultrasonic lance is suited for mechanical cell disruption in small scale screening and (ii) a workflow has been developed to screen for suitable cell disruption conditions, measuring DNA content (absorbance at 260 nm) and protein concentration using an established RP-HPLC method.

P12: Bioprocess development for the heterologous production of a hyperthermostable 5'-methylthioadenosine phosphorylase in Escherichia coli

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Nucleoside phosphorylases are important biocatalysts for the chemo-enzymatic synthesis of nucleoside analogues which are, among others, used for the treatment of viral infections or cancer. S-methyl-5'-thioadenosine phosphorylases (MTAP) are a rarely studied group of nucleoside phosphorylases. The thermostable MTAP of Aeropyrum pernix (ApMTAP) was described to accept a wide range of modified nucleosides as substrates. Therefore, it is an interesting biocatalyst for the synthesis of nucleoside analogues in industrial applications. However, so far, no information is available on the efficient expression of thermostable nucleoside phosphorylases in Escherichia coli high cell density cultivations. In this study, we report the heterologous expression of the thermostable ApMTAP through high cell density cultivation from 24-well plate scale to stirred-tank bench-top bioreactor cultivations. Glucose was used as a carbon source in mineral salts medium (MSM) and the addition of media additives like, peptone and yeast extract were investigated. However, no significant improvement in ApMTAP expression was observed with any additive. Comparing different growth rates in cultivations in bench-top bioreactors revealed that growth at maximum growth rates until induction resulted in higher yields of ApMTAP. This study paves the way towards the application of thermostable nucleoside phosphorylases in industrial applications due to their improved heterologous expression in E. coli.

P13: Thermostable adenosine 5'-monophosphate phosphorylase from *Thermococcus kodakarensis* forms catalytically active inclusion bodies

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Catalytically active inclusion bodies (CatIBs) produced in Escherichia coli are an interesting but currently underexplored strategy for enzyme immobilization. They can be purified easily and used directly as stable and reusable heterogenous catalysts. However, very few examples of CatIBs that are naturally formed during heterologous expression have been reported so far. Previous studies have revealed that the adenosine 5'-monophosphate phosphorylase from Thermococcus kodakarensis (TkAMPpase) forms large soluble multimers with high thermal stability. Herein, we show that TkAMPpase additionally forms inclusion bodies during heterogeneous expression in E. coli which are catalytically active against all tested natural 5'-mononucleotides. Our results show that the recombinant thermostable TkAMPpase is one of rare examples of naturally formed CatIBs. Moreover, heat treatment of the soluble protein from crude extract induces aggregation of active protein. Inclusion bodies were found to be similarly active with 2–6 folds higher specific activity compared to these heat-induced aggregates. Surprisingly, differences in the substrate preference were observed between both insoluble preparation.

P14: XenoGlue – Scale up of a recombinant mussel protein analog as photoactivatable bioglue

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In nature, mussels secret adhesive proteins (Mfp-5) and efficiently adhere to stone and other inorganic surfaces and even to man-made products such as metal and plastic materials. The secret of these bio-glues is the presence of catechol groups in the side chain of the non-proteinogenic amino acid L-DOPA. A photoactivatable non-canonical amino acid (ncAA) analog was synthesized [1] and integrated in the protein chain by stop codon suppression [2].

The main challenge is the protein production yield due to low solubility or toxicity of the compounds [3].

In a host strain with 6 nitro-reductases which were knocked out, the mussel protein construct was equipped with a six times his-tag (6-HIS). Various regimes were applied in shake flask and bioreactor experiments to identify favorable amino acid supply. A downstream processing (DSP) protocol that combined inclusion body purification and immobilized metal affinity chromatography (IMAC), with a subsequent dialysis and lyophilization, was accomplished. Educt and product analytics of free ncAAs and ncAA of proteins was examined as well as the resulting adhesive power of the product.

The cultivation under AA supplementation enables an enhanced protein yield. The combined inclusion body purification and IMAC offers an easy and scalable downstream process. The maximal yield in fed-batch bioreactor cultivations was 352 mg*L of the unlabeled Mfp-5 analogue and 354 mg*L of five-fold meta-oNB-DOPA-labeled Mfp-5 analogue. An adhesion strength of 0.093 MPa [N/mm²] was achieved.

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P15: Model-based rational design for aerobic industrial fermentation: *P. chrysogenum* and *S. cerevisiae* as model organisms

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Bioprocesses use the power and versatility of nature via microorganisms that make bio-products from renewable feedstocks. Microorganisms can very well be engineered as efficient cell factories. However, the gap between the cell environment at lab and production scales is causing gross resource and asset utilization inefficiencies and is a barrier to fast and successful scale-up [1,2]. This research study target on a robust and model-based simulation platform to ramp up the scale-up techniques driven by profound biological and physical understanding. More specifically, our work particularly focuses on systematic ways of constructing computational reaction dynamics (CRD) models, using industry favored Penicillium chrysogenum [3.4] and Saccharomyces cerevisiae [5] as model systems. Beginning with the end in mind, we use pool-based kinetic models to find a good balance between model capability and complexity. Those models were designed to reproduce cellular response at different time scales and trained/validated on collected bioinformatic data under steady states, long-term adaptation, and short-term pulse conditions [2,3]. Development and application of these computational approaches to design better scale-down simulators, enable faster scale-up, and improve the energy and resource efficiency of fermentations, will accelerate bringing biotech-innovations to the markets.

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P16: Across scales: An integrated robotic cultivation platform for accelerated bioprocess development

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The degree of automation in modern high-throughput (HT) laboratories has reached unprecedented levels. Liquid Handling Stations (LHS) with embedded micro- or mini- bioreactor systems can perform a large number of sophisticated cultivations with a very high degree of automation. Still, important steps of the developmental process require human intervention leading to bottlenecks in automated bioprocess development. In this work, we address two important limitations namely, (1) integration of automated advanced analytics and (2) automated operation of experiments at different cultivation scales (from µl up to 150 mL) in an automated workflow. To solve these limitations, we extended the capabilities of a high-throughput bioprocess facility [1] from 200 µL to 150 mL with the integration of a small scale cultivation system automatically operated in a LHS. Our approach demonstrates the automated sample transfer via a mobile robotic lab assistant allowing for flexible workflows throughout the facility. Hence, spatial separation of laboratory devices or unit operations is no longer a restriction for automation, enabling integrated development of process steps of different scale and sequence. In combination with a central data infrastructure, the basis for a data-driven and fully automated platform is given. The design and conceptualization of the facility, is assessed in a case-study for the production of elastin like proteins (ELPs) [2]. A clone library of ELP producing E. coli strains was screened in 24 parallel mini-bioreactors (2mag, 10 mL) and their kinetic parameters were estimated by

fitting a macro-kinetic growth models [3]. The findings were evaluated under industrial relevant high cell density cultivations in 8 small scale reactors (HEL bioreactor, 150 mL). Thus, allowing for automated screening and process optimization in different cultivation scales under process relevant conditions in order to accelerate bioprocess development.

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P17: Analyzing growth kinetics of cyanobacterial photo-biocatalysts in microfluidic droplets

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The interest in cyanobacteria as photoautotrophic whole-cell biocatalysts for sustainable biotechnological applications increases since they allow to tap the potential of oxygenic photosynthesis. Nevertheless, the reported productivities for phototrophic microorganisms are low compared to their heterotrophic counterparts and make light-driven cyanobacterial biocatalysts unattractive for economically viable applications. As production and growth kinetics are tightly linked in cyanobacteria, growth optimization is an important objective function for improving the productivity of photobiotechnology. However, linking reaction conditions and growth is an intricate task, as the growth architecture of a population might be masked behind averaged values from bulk experiments. Latest findings imply a pronounced heterogeneity in single-cell growth kinetics and indicate that this heterogeneity is an overlooked productivity-determining trait in cyanobacterial populations. Our work, therefore, aims to gain insights into the condition-dependent growth behavior of cyanobacteria with single-cell resolution. We applied microfluidic droplets
as parallelized microscale photobioreactors and monitored the droplet-specific growth kinetics via automated image analysis to unravel the links between growth heterogeneity and cultivation conditions. Microfluidic cultivations enable defined cultivation conditions, e.g. light input without shading effects, at high throughput. The results of our experiments give strong hints that light is a major factor that determines the extent of growth heterogeneity. The low cell densities inside the droplets allow the cultivation under only limited light conditions, thereby making light-dependent changes in physiology experimentally accessible. The obtained results might become an important basis for tuning cyanobacteria and their reaction conditions towards competitive productivities of photobiotechnological processes.

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P18: Beyond the average – quantifying the specific reactivity of single cells

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It has been shown that cellular heterogeneity can have a significant impact on the efficiency/yield of industrial bioprocesses [1]. To increase the efficiency of biotechnological processes, it is therefore also necessary to understand the behavior of individual cells in such processes and to highlight their influence on the population.

Current single-cell analysis focuses on fluorescence spectroscopic methods, which are limited to fluorophores. One reason for the limited analytical methods available to date is the low quantity of catalytic products and the tiny sample volume of individual cells.

In our work, we seek to establish a microfluidic, label-free approach to determine single cell efficiencies. Here, thousands of single cell batch reactors are incubated in parallel using droplet microfluidics and subsequently analyzed by mass spectrometry to present an overall picture of population heterogeneity with respect to single cell reactivity [2].

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P19: Electroporation of PUFA-producing Dinoflagellate

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Crypthecodinium cohnii, which belong to the dinoflagellate subphylum, are primary producers of polyunsaturated fatty acids (PUFA), especially the ω3-fatty acid docosahexaenoic acid (DHA) used as a food supplement. For rapid characterization of bioprocessed C. cohnii cultures, the dielectrophoresis (DEP) method is currently being investigated, which uses electrical fields to separate cells with different lipid contents. However, the electric field strengths must not be chosen too high, since above a threshold value electroporation of cells begins and their integrity is destroyed. The threshold value is usually given as the product of field strength and exposure time $(Et)_{th}$ and has already been determined for various microorganisms. We show here the results of our studies on electroporation of C. cohnii. The heterotrophic protists have a complex cell envelope that includes theca plates composed of cellulose. The effect of electroporation was tested both in conventional electroporation cuvettes with 4 mm plate spacing and in a microfluidic lab-on-chip system with integrated metal electrodes with spacing of 20 µm. For this case, which has hardly been investigated microbiologically so far, a method for quantitative determination of the threshold $(Et)_{th}$ is developed in order to work only with completely intact cells in the aforementioned DEP characterization. For detection, propidium iodide was used as a reporter molecule that fluoresces only after intercalation into a DNA double strand in the detection window around 617 nm. In electroporation cuvettes, onset of fluorescence is observed at

about 1,200 V, resulting in a threshold value $(Et)_{th}$ of 1,5kV·s·m^{-1} for C. cohnii. Interestingly, however, even up to 2,5 kV·s·m^{-1} only a subset of all cells actually fluoresces, which is observed in a similar form in the microfluidic device.

P20: A semi-automated luciferase-based substrate screening assay for nucleoside kinases

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Nucleoside analogues are applied as pharmaceuticals for the therapy of cancer or viral infections. Since they are phosphorylated in vivo by nucleoside kinases (NKs), the characterization of the NKs is essential. In addition, NKs can be used for the in vitro synthesis of nucleoside analogues. Thus, a fast and reliable activity assay for NKs is of great importance.

In this study, we used a well-known luciferase-based assay to establish a semi-automated detection of NK activity. The developed assay showed good linearity ($r^2 > 0.98$) in the range of 0–500 µM ATP and comparable results to HPLC analysis. Besides ATP, alternative phosphate donors such as dATP and CTP were accepted by the luciferase. The results of the substrate screening of four NKs using in total 20 natural as well as base- and sugar-modified substrates were in good accordance with the literature. Furthermore, hitherto undescribed substrates were found for three of the kinases.

Our results demonstrate that the developed semi-automated high-throughput assay is applicable to validate the substrate scope of NKs in a simple and parallelizable method.

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P21: Automated cell line characterization in shake flasks for multiple organisms

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Shaken Erlenmeyer flasks are a commonly used cultivation system in biotechnology and are regularly employed in early-stage process development (e.g., inoculum production, media development, and strain characterization). However, they are mostly used as black box systems without the sensoring capabilities a stirred tank bioreactor can provide. Several measurement systems have been developed to overcome this issue, but there is still a lack of comparability or a uniform, automated approach to using online data for process characterization in shake flasks.

To overcome this, we compared online backscattered light, dissolved oxygen, and pH data for plant, animal, E. coli, and S. cerevisiae cultivations using the PreSens SFR vario. With these data, key performance indicators (KPIs), such as the specific growth rate and the cell-specific oxygen demand, were evaluated automatically.

For algorithm validation, manually calculated KPIs based on offline biomass data, online data and algorithm-based automatically calculated KPIs using online data were compared. The developed algorithm is based on Python and searches for the exponential phase in the corresponding online signal. The exponential fit set by the algorithm and the observed signal were compared and the fit optimized so that the root-mean-square error was as low as possible. With this technique, an accurate estimation of the growth rate and further calculation of the cell-specific oxygen demand can be performed using either the oxygen uptake rate or a biomass estimation based on the backscattered light signal. This enables the comparison and evaluation of different media, strains, and process conditions in a standardized cost-effective and automated manner, reducing human effort and errors.

P22: Focal molography a new real-time PAT solution for bioprocess

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Focal Molography is a new nanotechnology-based bioanalytic technology [1] that allows the detection and characterization of various biological partners in extreme crude samples [2]. Based in this technology platform lino Biotech develops a new purification free process analytical technology (PAT) solution for hazzle-free and direct product characterization in fermentations media without unpleasantly long analytics time.

The aim of this real-time PAT solution is to measure critical quality attributes (CQAs) of a process directly on the biological product level without making guesses on the product quality from more basic process parameters like pH, gas or nutrients concentration. The technology is directly addressing the modern challenges in bioprocess and cell and gene therapy like identity, purity, safety and potency of the final product. Lino Biotech is developing new solution for direct determination of host cell protein contamination, titer determination and other end product quality control attributes. The goal is to give leading players in the field access to this new PAT solution via the product and services of lino Biotech and establish it as a new gold standard in bioprocess quality control.

At the center of focal Molography is a patented sensor chip architecture which can be decorated with any type of binder molecule recognizing for example AAVs, host cell DNA contamination, bacterial contaminations, or membrane receptors of CAR-T cells. Unspecific protein binding to the sensor surface or signal fluctuations due to temperature changes or medium inconsistencies as observed with other technologies is nearly not observed because of the underlying physical sensing principle.

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P23: PAT for the automation of a recombinant antimicrobial peptide production process

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Antibiotic resistances are rising to dangerously high levels worldwide. The recombinant production of antimicrobial peptides targets this problem. Process Analytical Technology enables the establishment of a fully automated P. pastoris process.

The three-stage process starts with an unlimited batch phase on glycerol. Due to the pO_2/agitation control mode, substrate depletion causes a significant alteration of the dissolved oxygen level and stirrer speed. Monitoring these two parameters the transition to the fed-batch phase is initiated. The pO_2/agitation control requires parameter adjustment in each process phase.

The initial feeding rate is calculated based on the reservoir substrate concentration (300 g l^{-1}), the actual cell density (turbidity probe), the set growth rate (0.1 h^{-1}), the yield coefficient (0.80 g g^{-1}) and the cell maintenance rate on the substrate (0.04 h^{-1}). During the fed-batch phase, the feeding rate is increasing exponentially according to the set growth rate.

After the cells are grown to cell densities above 50 g l^{-1} the production phase starts. The methanol concentration is controlled to 1.5 g l^{-1} using the inline measurement system Alcosens® from Biotechnology Kempe GmbH. Due to the fusion of the antimicrobial peptide with the green fluorescent protein, the product formation is monitored using the BioView® sensor from DELTA Lights and Optics.

To gain further process information's the oxygen consumption rate and the carbon dioxide production rate as well as the respiration quotient are calculated. Therefore, the oxygen and carbon dioxide mole fraction in the off-gas is measured using the BlueInOne Cell from BlueSens gas sensor GmbH.

P24: Kuhner TOM for off-gas analysis in shake flasks

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Shake flasks are a widespread reactor type for microbial and cell cultivations. They are the link between early process development and strain selection in microplates and large-scale fermentations in stirred tank or shaken bioreactor systems. During process development and scale-up, a good process understanding is essential. To facilitate and speed up the process, online measurement techniques for shake flasks accompany manual sampling for maximum information output per cultivation. Off-gas analysis gives oxygen transfer rate (OTR), carbon dioxide transfer rate (CTR) and respiratory quotient (RQ) as quantitative measures of the physiological state of the culture. Growth rate, substrate consumption and limitation, product formation and inhibition, balancing of carbon dioxide, oxygen limitation and kLa can be derived from off-gas analysis data. Online monitoring of shaken cultures also helps scheduling of manual sampling to most significant points. In production, off-gas analysis in shake flasks can be used for preculture observation and substrate quality validation.

We developed a shake flask off-gas analysis system for non-invasive online determination of OTR, CTR and RQ. TOM (Transfer rate Online Measurement) is built modular for off-gas analysis in 4, 8, 12 or 16 individual shake flasks. For gas phase analysis, sensitive oxygen and carbon dioxide sensors were applied. The new Kuhner TOM can be applied to various shake flask sizes and types (baffled, plastic, glass) enabling the user to get information about their existing cultivation procedures.

A new versatile online measurement tool for shake flasks is presented that enhances process development and facilitates scale-up.

P25: Application of a novel high resolution volumetric gas measurement system for the determination of the biochemical methane potential

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Aiming at an optimal utilization of biomethane as a robust source of renewable energy, accurate methods to determine the biochemical methane potential (BMP) of various organic (waste) materials are mandatory. Despite numerous available methods based on chemical analysis [1] or volumetric, manometric and gravimetric detection [2], there is high demand for research and development [1,3,4]. The most important aspects are standardization and automation of BMP tests for time saving and error reduction [3,4]. Moreover, standardization is also crucial to improve the data needed for simple and fast forecast of BMP by kinetic modeling [4].

Objective of this work was the evaluation of a novel method for determination of the BMP. The used experimental setup comprises completely stirred tank reactors that were operated in batch mode combined with a novel automated volumetric gas measuring system. The novel measuring system is highly scalable with a volumetric resolution of 1 mL and a flow rate range from 0 to 80 mL min-1 at a headspace overpressure of 3 hPa, and can be read by commercial software.

The novel measuring system was evaluated and compared to an established system according to guideline VDI 4630 [5]. Monodigestion of the common biogas substrate maize silage under mesophilic conditions resulted in biogas yields of 814 ± 16 L $kgVS^{-1}$ and 779 ± 35 L $kgVS^{-1}$ determined with the novel measuring system and the established VDI 4630 system, respectively. The corresponding methane concentrations, at both systems as determined by standard methods, showed slightly higher deviation with 53.7 vol% and 57.8 vol%. Therefore, further development should include automatic methane quantification. Overall, the novel measuring system provided results, which are in good agreement with those of the established system, but was more precise and easier to handle.

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P26: Flexibilization of two-phase digestion through monitoring of dissolved hydrogen

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Energy from biomass has the potential to partially compensate for the temporarily fluctuating energy generation from wind and solar power. For this purpose, it is necessary that the processes of anaerobic digestion can be monitored and controlled properly to enable flexible methane production – at the same time a combination of material and energy use to increase value generation would be a benefit. To increase flexibility in substrate utilization and product formation, a lab-scale two-stage bioreactor separated into a hydrolytic/acidic part and methanogenic part is used. The concept includes a thin sludge recirculation. In this way, the steps of the anaerobic digestion can be controlled separately. In order to achieve this, monitoring of the liquid phase with the help of reliable on-line and in-situ measurement of dissolved gases in the liquid phase, in particular hydrogen, is desirable. Therefore, a novel measuring system is developed, tested and adapted to biogas media, which includes a membrane-free extraction unit for the dissolved gases in the liquid phase of biogas plants, in particular hydrogen, and a measurement with a highly selective and sensitive metal-oxide (MOX) gas sensor.

Experiments with maize silage and straw manure (50/50 m/m) show a good functionality of the reactor system made of 3D-printed parts. At values of the chemical oxygen demand of 15-20 g/L, short-chain carboxylic acids with a concentration of up to 10 g/L (mostly butyric and acetic acid) are produced in the first stage. The effluent of this first stage is converted into biogas at a rate of 500 mL/d with a methane share of up to 65 % in the second stage. Experiments with and without recirculation show, that methane yields can be increased by more than 20 %. Monitoring of dissolved hydrogen was successfully tested and shows a good detection response, which makes the technology suitable for its consideration in process control within the applied two-stage digestion process.

P27: Raman spectroscopy as an analytic tool in upstream bio-processing

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In upstream bio-processing, the aim is to optimize cultivations concerning cell density, product concentration, or other parameters. This optimization involves taking actions, i.e., feeding various amounts of glucose to the cells. Choosing the optimal sequence of treatments depends on their respective state. The cells' state is partially characterised by the concentrations of glucose, acetate, and other process parameters. Inferring these process parameters from Raman spectra happens within seconds, as opposed to at-line methods, which take more than 10 minutes. But which Raman spectrometer is most suited to the different process parameters?

Estimating process parameters from Raman spectra presents a supervised learning problem that we address via machine learning. Imitating an experimental setup, we created one set of samples with known glucose, acetate, and magnesium concentrations. Using six Raman spectrometers from various manufacturers we gathered spectra from each sample. Using a well-established combination of a Savitzky-Golay filter and a partial least squares model, we compared how well the models predict the concentrations from the respective spectra. We found that all spectrometers allow for reasonable glucose predictions. However, for acetate only the spectra from Anton Paar and Metrohm are useful. For magnesium all spectra deliver reasonable results except those from Tec5 and Tornado.

Some spectrometers are more sensitive to specific molecular vibrations that correlate to the respective substrate concentrations. Consequently, one needs to choose a spectrometer according to one's use-case. Though, more sophisticated modelling approaches might improve the process parameter predictions from certain spectrometers.

P28: Monitoring of fermentation processes by gas chromatography-ion mobility spectrometry (GC-IMS) and machine learning

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In recent years, highly parallelized and automated cultivation systems were established as valuable tools for bioprocess development and microbial strain characterization. Integrating these systems into robotic platforms enabled automated sampling and sample processing procedures. This allowed substrate and product concentrations to be monitored at-line using spectroscopic assays [1]. While easy to automate, these assays are generally specifiGC-IMS is a powerful technique for the fingerprint analysis of complex samples in conjunction with multivariate analysis. Due to the high sensitivity of IMS, headspace sampling is commonly used. The analysis of the exhaust air of fermentations allows for the generation of profiles of volatile, extracellular metabolites without disturbing the process or risking contaminations. The obtained GC-IMS data can be correlated to variables that cannot be measured directly in real-time, e.g., the formation of a product or the presence of contaminations.

This poster presents an offline proof-of-concept-study which demonstrates that microorganisms can be categorized simply by headspace analysis as a first step towards detecting contaminations. For this experiment E. coli, S. cerevisiae, L. brevis and P. fluorescens were cultivated on an incubation shaker with an hourly sampling by headspace GC-IMS. Classification was carried out by a newly developed, open-source, Python package for chemometric analysis of such data called gc-ims-tools. Additionally visualized feature selection with variable importance in projection (VIP) scores and PLS coefficients reveals discriminating metabolites.

P29: Xcom, a multi-objective function for the metabolic modeling of microbial consortia

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Bacterial consortia are interesting in bioprocessing because they may offer advantages such as robustness and the use a wide range of substrates. Moreover some communities have demonstrated .new capacities through metabolites exchanges between their members which can connect metabolic pathways from each member of the consortium [1]. While solving metabolic models for pure microbial cultures can be achieved by using Flux balance analysis, this method is not the most suitable for microbial consortia as it does not allow to achieve simultaneous multiple objectives [2]. Therefore, specific tools for solving consortia metabolic models have to be developed. In this context, a new metabolic modeling tool named XCOM is proposed. It has been developed under Matlab environment to be combined with CobraToolbox. A previous tool for metabolic modeling of microbial consortia is already present in CobraToolbox [3], but it permits the optimization of only two objectives such as, for example, biomass synthesis and one metabolite production. XCOM offers to go further by optimizing up to five synthesis reactions simultaneously. As a consequence, microbial consortia containing more than two microorganisms can be modeled. Moreover, compared to Opticom [2], the weighting of each objective is possible with this new function. XCOM was validated by simulating the metabolic behavior of a previously published microbial consortium. This will highlight the inputs and the advantages of XCOM.

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P30: Model based real-time estimation of maximum substrate uptake capacity in microbial fermentation

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The monitoring of internal physiological cell parameters poses a big challenge in biotechnological processes. Especially the specific maximum uptake rate of substrate (qSmax) is of high interest since it provides information about the cells metabolic capacity. It can also be utilized to prevent possible substrate accumulation in the fermentation media and control the substrate feed rate accordingly. It has been already shown in literature, that qSmax is not a static value. It is often times declining throughout the course of a cultivation [1]. However, the state-of-the-art approach of qSmax identification by pulsing substrate during the induction phase and determine the subsequent decrease rate of substrate concentration intervenes deeply in the cell metabolism and negatively acts on process performance. Also, the identified qSmax can hardly be utilized for real-time control since glucose concentration is usually measured offline in low frequency. That is why model based real time estimation of qSmax provides a promising alternative to substrate pulsing. We present a model based qSmax estimation technique built upon a Bayesian filtering approach incorporating compliance with elemental balances. Measurements driving the estimator include oxygen uptake rate (OUR) and carbon evolution rate (CER) as well as the substrate feed rate (FR). Identifiability of the nonlinear differential equation model was structurally analyzed based on Lie Derivatives [2]. The approach was verified in simulation and successfully applied to real fermentation datasets. The method could now be used to facilitate optimization of input feed trajectories in order to control substrate uptake to the maximum possible uptake rate.

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P31: Modeling enzymatic glucose release to facilitate continuous feeding in miniaturized fermentations

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Stirred miniaturized parallel bioreactors combine the advantages of microtiter plates and benchtopscale bioreactors. Nutrient-limited fed-batch conditions can be applied, however differences in the culture handling still exist if the feed is pipetted into the mini-bioreactors by the needles of a liquid handler. Compared to bench-top scale fermentations with pumped feeding, this results in short periods of substrate excess followed by starvation, which might have an influence on growth and product formation [1]. Striving for maximal throughput and minimal volumes, simple and robust continuous feeding strategies offer important advantages. One way of implementing continuous glucose feed in small systems is enzymatic cleavage of glucose from polysaccharides [2]. Here, the release rate can be influenced easily by enzyme additions. Nevertheless, a proper control of the feeding rate might become cumbersome. The enzymatic activity is influenced by the experimental conditions, such as the polysaccharide concentration, pH, and temperature, resulting in a complex, non-linear process.
A mathematical model able to predict the release rate considering all above mentioned factors is hence necessary to assure an accurate control of the feeding strategy. We present a model that extends the Michaelis Menten kinetics by considering different substrate fractions [3], and has been fitted using 48 high throughput glucose release experiments under different conditions. As a proof of concept, the model is used to plan enzyme additions during Escherichia coli fermentations to mimic exponential feeding. This approach enables continuous fed-batch conditions in miniaturized systems, increases the comparability to the larger scale and thus reduces bioprocess development times.

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P32: Modeling *Saccharomyces cerevisiae* central carbon metabolism at steady state and under glucose perturbations

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How cells deal with perturbations in industrial bioreactors is still a recurring question. Kinetic metabolic models of glycolysis have been proposed to understand how Saccharomyces cerevisiae navigates through nutrient perturbations. Yet, these representations lacked important variables that constraint metabolism in industrial fermentations, hindering their industrial implementation [1]. To generate mechanistic models that do not only suit the lab, but industry as well, we have developed a model of yeast glycolysis connected to central carbon metabolism. By considering the effect of precursors of anabolic reactions, mitochondria and the trehalose cycle, the model simulates a growing cell within dilution rates from 0.02 to 0.38 h^{-1} and during a glucose perturbation of 20 g L^{-1}. Gas exchange and ATP synthesis and maintenance are described during respiratory and respirofermentative metabolism. To overcome parameter estimation challenges presented by the model scale and dataset complexity, a model decomposition approach was developed. Our results suggest that missing regulation is required to explain glucose perturbation response and is responsible for a transient glycolytic imbalance [2]. Finally, the model developed here can be used as a chassis for representations of central metabolism in other organisms and to examine metabolic landscapes not limited to steady states or a single glucose perturbation. For instance, it can be used to explore how a cell adapts to prolonged perturbations in a fermenter or integrated into computational fluid dynamics (CFD)-generated lifelines to consider cellular physiology information in an area so far restricted to black box models [3].

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P33: Reconstruction of a genome-scale model of *Cupriavidus necator* for PHA production

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Cupriavidus necator is a Gram-negative bacterium characterized by its ability to accumulate up to 80% of its biomass in the form of Polyhydroxyalkanoates (PHA) under nitrogen limiting conditions [1]. Constraint-based genome-scale modeling is a valuable method for evaluating and exploring the carbon flux distribution along multiple metabolic pathways. It provides insights into nutrient uptake, growth rate, and production of metabolites under specific conditions [2]. This work presents the most recent and manually curated genome-scale model (GEM) of C. necator (iREH22A) for PHA production. The automated tool CarveMe was used to generate the first draft reconstruction of C. necator H16 based on the most recently reported genome assembly (RefSeq: GCF_004798725.1). The first draft contained 2,839 reactions, 1,835 metabolites, and 2,092 genes. After curation, 156 reactions associated with the production of PHA were included. Mass-balancing was applied to 1.07% of the reactions, and 7.40% of them required charge-balancing. Additionally, the tool ModelPolisher was used to annotate metabolites, reactions, and subsystems extensively. The Model and Constraint Consistency Checker highlighted only 1.34% dead-end metabolites and 1.3% zero flux reactions. 30 thermodynamically infeasible cycles were identified and curated. Finally, our iREH22A GEM reached a MEtabolic MOdel TEsting (MEMOTE) score of 84%, indicating iREH22A accomplishes the metabolic modeling community standards in terms of annotation, consistency, and completeness. It is encoded in SBML Level 3 Version 1 with extension packages for flux balance constraints and groups. The obtained GEM of C. necator could be used as an analytical tool for exploring metabolic scenarios in silico under different environmental/genetic conditions, focused on reaching a cost-effective PHA production.

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P34: Computer simulation of the glycosylation of proteins in the Golgi apparatus

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The process of protein glycosylation occurs in the cytosol, the endoplasmic reticulum (ER) and the Golgi apparatus. It is crucial for a broad spectrum of biological processes, e.g. protein folding, quality control and biological recognition events. Although glycosylation is one of the major post-translational modifications of proteins, it has not yet been possible to uncover the full extent of the biological role of this modification.

Computational modeling offers hereby a fascinating opportunity to provide analysis and control components to understand the subjacent mechanisms of glycosylation using a mathematical model. This is challenging through the sheer quantity and diversity of involved enzymes and their distribution within the cellular compartments. To cope with these problems an agent-based approach was chosen with defined movement patterns and reaction rules between the involved proteins and enzymes acting as agents.

The path of the protein from the ER through the Golgi apparatus is simulated and a prognosis for the final glycan protein and additional information about the system is given. A complete program in Java has been developed, that successfully characterizes the glycosylation processes and visualizes their results in the form of a mass spectrometer file. The program provides a suitable tool to characterize glycoproteins during synthesis with the opportunity to control and ensure the quality and consistency of these glycoproteins.

P35: withdrawn

P36: Creating educational software inspired by digital twins

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Digitalization and digital twinning are expected to be the future of the bioprocess industries. A digital twin is a virtual world based upon a software model that communicates with the real world (lab, pilot plant, or industrial facilities). There are several layers and building blocks in the digital twin architecture, where data repositories (databases) and simulation play a key role. Engineering education must keep up with the industry's requirements. To achieve this, in this work, we present the first stages of a DT-based education prototype/platform based on the BioVL tool previously developed by the authors [1]. For example, this platform incorporates two database layers represented by two chatbots, the "good" and the "evil" twin. This aims at aiding the learning process by being interactive and engaging and emphasizing the development of critical thinking and decision-making capabilities.

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Notes:

BioProScale Symposium History

As a joint project of Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, and the Institute für Gärungsgewerbe und Biotechnologie zu Berlin (IfGB) the first BioProScale Symposium took place in November 2009 in Berlin. In the following years, the event has been well established in industry and science as a platform for the dicussion about the challenges of scaling-up biotechnological processes from laboratory to industrial scale.

- **1st BioProScale Symposium**
  "Inhomogeneities in large-scale bioreactors: Description – scaling – control"
  24 to 27 November 2009
- **2nd BioProScale Symposium**
  "Inhomogeneities in large-scale bioprocesses: System biology and process dynamics"
  14 to 16 March 2012
- **3rd BioProScale Symposium**
  "Inhomogeneities in large-scale bioprocesses: System biology and process dynamics"
  2 to 4 April 2014
- **4th BioProScale Symposium**
  "Bioprocess intensification through Process Analytical Technology: (PAT) and Quality by Design (QbD)"
  6 to 8 April 2016
- **5th BioProScale Symposium**
  "Innovative scale up and scale down for bioprocess intensification"
  20 to 22 March 2018
- **6th BioProScale Symposium Online**
  "Scale-up and scale-down for accelerated bioprocess development and optimisation"
  29 to 31 March 2021

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