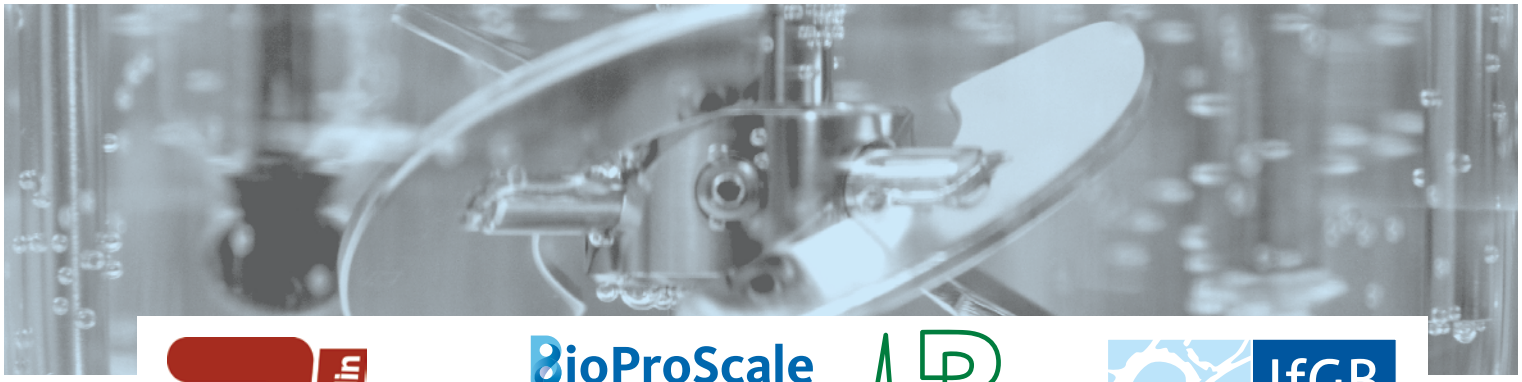

BioProScale
DEVELOP · IMPROVE · SUSTAIN

Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik

Bio-PAT

IfGB
Institut für Gärungsgewerbe und Biotechnologie zu Berlin

8th BioProScale Symposium

Scaling Down and Up of Bioprocesses: Strategies, Tools and Process Performance

APRIL 9-11, 2024
LANGENBECK-VIRCHOW-HAUS, BERLIN, GERMANY

Online Edition, 10/Apr/2024

- Industrial scale process performance and optimization
- Scale down and scale up of bioprocesses
- Process-driven cell performance
- Integrated bioprocesses
- Process analytical technologies (PAT)

biotechnologie.ifgb.de/bioproscale2024

VENUE: LANGENBECK-VIRCHOW-HAUS, BERLIN, GERMANY

LANGUAGE: ENGLISH

EXHIBITORS / SPONSORS

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Institut für Gärungsgewerbe und Biotechnologie zu Berlin (IfGB)
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Seestraße 13, 13353 Berlin, Germany
Geschäftsführer: Dr.-Ing. Josef Fontaine, Gerhard Andreas Schreiber
Ust.-IdNr.: DE 136 621 351, Steuernummer: 640/50721

Technische Universität Berlin – Institute of Biotechnology
Chair of Bioprocess Engineering, Prof. Dr. Peter Neubauer
Ackerstrasse 76, 13355 Berlin, Germany

BioProScale e.V. (VR 38529 B), c/o TU Berlin, Ackerstr. 76, ACK 24, 13355 Berlin
Bio-PAT e.V. (VR 35530 B), c/o TU Berlin, Ackerstr. 76, ACK 24, 13355 Berlin

Table of content

Our venue

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

Room "Bernhard von Langenbeck" (poster area) ..... 1st floor

"Historical Lecture Hall" (presentations) ..... 2nd floor

Historical site: No coffee/drinks/food inside the lecture hall, please!

Welcome address

Dear colleagues,

I welcome you to our 8th BioProScale Symposium in Berlin. Our symposium is taking place in a turbulent time when many production networks and processes that have been evolved since decades are being called into question. In my opinion, it is now up to each individual to give their best personal contribution to society with the aim of preserving our social standards while fulfilling our ethical principles. In this context, I see our contribution with the BioProScale Symposium to the further development of a sustainable, resource-conserving, fossil-independent bioeconomy and efficient biopharmaceutical processes. Strong international networking and the many associated personal contacts are an important element in taking responsibility in our fields of activity.

Biotechnological production is currently facing major challenges in many regions of the world: high energy costs, an ever-growing awareness of limited resources in the shadow of the climate crisis and the associated high demands on new processes, and finally new regulations and standards for production have caused some initially prosperous ideas to disappear. However, with the increased awareness of healthy nutrition and new therapies for an ever-growing world population and a circular economy in general, also new opportunities for bioprocesses arise. This leads to an even increasing demand for new production concepts for biotechnological production. However, the implementation of processes in economically and ecologically viable solutions is extremely difficult and requires completely new ways of thinking in combination with the development of new technologies in the field of bioprocess engineering. Nowadays, topics around scale up and scale down of bioprocesses are even more important as they were when the 1st BioProScale Symposium took place in 2009.

We are very pleased that the BioProScale Symposium has developed into an attractive, internationally visible event that builds on a stable scientific community and is attractive to many scientists from academia and industry for already 15 years now!

Our special thanks go to the speakers who accepted our invitation to share and discuss their expertise with us. We are especially grateful to the companies that support this event as sponsors and exhibitors as we depend on their support. We can only guarantee reasonable prices for all participants through their contribution! I personally would like to thank all the people and organizations that helped to plan and to realize this event, especially the VLB Berlin, the Bio-PAT e.V. and the BioProScale e.V. for their close and always very professional cooperation in organizing this event. 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.

I hope that the three days will inspire us all and give us the opportunity not only to follow the new scientific and technological developments in talks and posters, but also to make new and reactivate personal contacts and perhaps initiate future collaborations – always with the demand of improving comprehensive understanding and fostering developments towards more sustainable and efficient industrial bioprocesses in mutual cooperation projects.

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

Mario Birkholz (TU Berlin, Germany)
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Michael Schlüter (Hamburg University of Technology, Germany)
Ralf Takors (University of Stuttgart, Germany)
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, the Institute for Fermentation and Biotechnology in Berlin (IfGB) has been conducting fermentation-oriented research and education in Berlin for more than 140 years – always in close cooperation with the Technische Universität Berlin (or its predecessors). Since 2003, the Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V. has been the sole owner of the IfGB. Under the brand name "IfGB", services and training for the spirits industry and distillers were offered and extended to 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

TUESDAY, 9 APRIL 2024

WELCOME ADDRESS AND PLENARY TALK

12:00 **Welcome address & introduction**
Peter Neubauer, Technische Universität Berlin, Germany

12:20 **Plenary Talk: Proper scale down – a prerequisite for engineering robust microbial chassis and for model-based prediction of industrial scale performance (PL01)**
Ralf Takors, University of Stuttgart, Germany

SESSION 1: INDUSTRIAL SCALE PROCESS PERFORMANCE AND OPTIMIZATION

Chair Michael Schlüter / Lucas Kaspersetz

13:05 **Model applications in scale-up of mammalian cell culture (CHO) processes (L01)**
Emmanuel Anane, Fujifilm Diosynth Biotechnologies, Denmark

13:30 **Valorising syngas in a coupled fermentation via acetate: Techno-economic analysis for SCP production and pilot-scale implementation (L02)**
Elodie Vlaeminck, Ghent University, Belgium

13:55 **Industry talks**

14:15 *Coffee break & exhibition*

14:45 **Optimizing bioreactors for microbial gas fermentation: An approach for sustainable valorization of industrial off-gas (L03)**
Carolin Bokelmann, University of Stuttgart, Germany

15:10 **Analytical modeling of large-scale bioreactors with diffusion equations (L04)**
Pauli Losoi, Tampere University, Finland
– withdrawn –

15:35 **Resolved particle Lattice-Boltzmann Large Eddy Simulation in a 15,000 L bioreactor to mimic Lagrangian sensor particles (L05)**
Ryan Rautenbach, Hamburg University of Technology, Germany

SESSION 2: PROCESS-DRIVEN CELL PERFORMANCE

Chair Robert Spann / Isabel Thiele

16:00 **Ecological forces dictate microbial community assembly processes in bioreactor systems (L06)**
Susann Müller, Helmholtz Centre for Environmental Research, Leipzig, Germany

16:25 **Morphology control for supporting scalability of *Aspergillus niger* cultures (L07)**
Tolue Kheirkhah, Technische Universität Berlin, Germany

16:50 *Coffee break & exhibition*

17:20 **Transport-controlled growth decoupling for self-induced protein expression with a glycerol-repressible genetic circuit (L08)**
Alvaro Lara, Aarhus University, Denmark

17:45 **Phenotypical heterogeneity in bioprocesses: Better think positive!? (L09)**
Anna-Lena Heins, Hamburg University of Technology, Germany & Alexander Grünberger, Karlsruhe Institute of Technology, Germany

18:10 **Plenary Talk: Managing cell population entropy: Navigating from ecosystems to bioprocesses (PL02)**
Frank Delvigne, University of Liège, Belgium

EVENING PROGRAM

18:55 **Poster Session, Exhibition, Get-together for all participants**
Langenbeck-Virchow-Haus
We encourage all poster authors to be at their posters at least from 19:30-20:15 (even poster numbers) and 20:15-21:00 (odd poster numbers).

21:00 End of day 1

WEDNESDAY, 10 APRIL 2024

9:00	**Welcome & introduction** *Peter Neubauer, Technische Universität Berlin, Germany*
9:05	**Plenary Talk: The role of bioeconomy on the path to net-zero (PL03)** *Daniela Thrän, Helmholtz Centre for Environmental Research, Leipzig, Germany*

SESSION 3: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair: Alvaro Lara / Tolue Kheirkhah

9:50	**Integrating hybrid modelling and transfer learning for novel bioprocess predictive modelling (L10)** *Harry Kay, University of Manchester, United Kingdom*
10:15	**Small scale models for process intensification: How to migrate a months-long perfusion process to intensified fed-batch (L11)** *Lena Tholen, FyoniBio GmbH, Berlin, Germany*
10:40	**Industry talks**
10:55	*Coffee break & exhibition*

SESSION 4: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair: Ralf Takors / Annina Kemmer

11:25	**Scale-up of stem cell cultures from shake flask to bioreactor: A CFD-based comparison of hydrodynamic stress (L12)** *Ramon van Valderen, Delft University of Technology, The Netherlands*
11:50	**Fully automated growth media optimization employing a new Machine Learning algorithm (L13)** *Frédéric Lapierre, Munich University of Applied Sciences, Germany*
12:15	**Robustness of microbial functions in dynamic environments: A microfluidic approach (L14)** *Luisa Blöbaum, Bielefeld University, Germany*
12:40	*Lunch break, poster session & exhibition*
14:00	**Plenary Talk: Towards closed-loop bioprocess development: Robotic workflows for automated Design-Build-Test-Learn cycle (PL04)** *Marco Oldiges, Forschungszentrum Jülich, Germany*

SESSION 5: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair: Klaus Pellicer / Niels Krausch

14:45	**Human induced pluripotent stem cell scale-up and expansion under consideration of bioengineering aspects (L15)** *Misha Teale, Zurich University of Applied Sciences, Switzerland*
15:10	**Workflow automation for reproducible high-throughput cultivations (L16)** *Lucas Kaspersetz, Technische Universität Berlin, Germany*
15:35	*Coffee break & exhibition*

SESSION 6: PROCESS ANALYTICAL TECHNOLOGIES (PAT)

Chair: Regine Eibl / Simon Täuber

16:05	**Innovative sensor solutions for bioprocess monitoring: Design and implementation (L17)** *Aliyeh Hasanzadeh, Technical University of Denmark, Copenhagen, Denmark*
16:30	**Gasphase-based bioprocess monitoring by untargeted volatilomics with gas chromatography – ion mobility spectrometry (GC-IMS) (L18)** *Joscha Christmann, Mannheim University of Applied Science, Germany*
16:55	**Lensfree microscopy for real-time bioprocess monitoring in PAT: Holographic reconstruction and fluorescence integration (L19)** *Phil Thiel, Leibniz University Hannover, Germany*
17:20	**Online monitoring of protein refolding in inclusion body processing using intrinsic fluorescence (L20)** *Eva Prada, TU Wien, Austria*

17:45	**Multi parameter sensors and dissolved oxygen (DO) sensor pills for shake flasks: Removing black boxes for improved bioprocess development (L21)** *Christina Dickmeis, Scientific Bioprocessing Inc., Baesweiler, Germany*
18:00	**Off-gas analysis in shake flasks (L22)** *Andreas Schulte, Kuhner Shaker GmbH, Herzogenrath, Germany*
18:15	*End of presentation program*

EVENING PROGRAM

19:30	**Symposium Dinner** *ERDINGER am Gendarmenmarkt, Jägerstraße 56, 10117 Berlin, erdingerberlin.de* *(by invitation or separate booking only)*
22:30	*End of day 2*

THURSDAY, 11 APRIL 2024

9:00	**Welcome & introduction** *Peter Neubauer, Technische Universität Berlin, Germany*
9:05	**Plenary Talk: How fundamental research on multiphase flows can support a reliable scale up (PL05)** *Michael Schlüter, Hamburg University of Technology, Germany*

SESSION 7: INTEGRATED BIOPROCESSES

Chair	Marco Oldiges / Saskia Waldburger
9:50	**Towards a circular bioeconomy: Polyhydroxyalkanoate bioplastic production (L23)** *Sebastian Riedel, Berliner Hochschule für Technik, Germany*
10:15	**Multiscale modelling of polyhydroxyalkanoate biopolymer production (L24)** *Stefanie Duvigneau, MPI for Dynamics of Complex Technical Systems, Magdeburg, Germany*
10:40	*Coffee break & exhibition*

SESSION 8: INTEGRATED BIOPROCESSES

Chair	Stefan Junne / Lara Santolin
11:10	**Production of PUFAs from dark fermentation effluent with *Schizochytrium limacinum* SR21 (L25)** *Simon Täuber, Technische Universität Berlin, Germany*
11:35	**Bioprocess optimization for lactic and succinic acid production from a pulp and paper industry side stream (L26)** *Agata Olszewska-Widdrat, Leibniz Institute for Agricultural Engineering and Bioeconomy, Potsdam, Germany*
12:00	**Is it possible to produce cultured meat at a farm? Scale-up strategy and realization aspects (L27)** *Nico Oosterhuis, Respect Farms BV, Den Haag, The Netherlands*
12:25	*Lunch break, poster session & exhibition*
14:00	**Plenary Talk: Single-use bioreactors: Applications and scaling-up (PL06)** *Regine Eibl-Schindler, Zurich University of Applied Sciences, Switzerland*

SESSION 9: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair Cees Haringa / Sarah Westarp

14:45 **Scalable microbioreactor system enabling efficient *Pichia pastoris* clone screening for production of biopharmaceuticals (L28)**
Eva Maria Palmqvist, Sanofi-Aventis GmbH, Frankfurt, Germany

15:10 **Automating the DBTL-cycle for *E. coli*: Integration of modular cloning, CRISPR-Cas9 and proteomics for advanced strain engineering (L29)**
Tim Stoltmann, Forschungszentrum Jülich, Germany

15:35 **Model-based scaling strategies of *Pseudomonas putida* fed-batch fermentations (L30)** *– withdrawn –*
Helena Junicke, Technical University of Denmark, Copenhagen, Denmark

16:00 **Closing remarks and awards for the three best posters and talks by young scientists**
Peter Neubauer, Technische Universität Berlin

16:20 *End of symposium*

Best Poster & Presentation Award supported by: SCI where science meets business

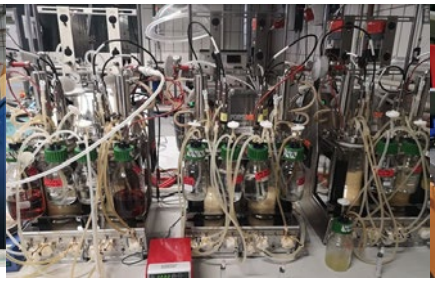
AFTER SYMPOSIUM EXCURSION

AFTER SYMPOSIUM EXCURSION

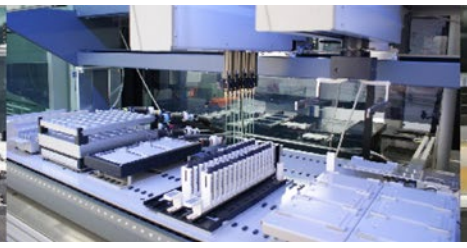
17:30 **Visit to the laboratory of the Chair of Bioprocess Engineering, TU Berlin, Ackerstraße 76, 13355 Berlin**

For those interested to visit the Pilot Plant and High Throughput Bioprocess Development (KIWI-biolab) at TU Berlin, we will organize a small tour following the closure of the symposium. The tour includes:

***Pilot Plant:** It consists of stainless steel bioreactors from 1L to 100L and a 200 L CELL-tainer single use system for pilot-scale production, a Flonamics automatic sampling system and cutting-edge process analytical tools (PAT, e.g. in-situ / in-line technologies for particle / cell analysis, namely Photon Density Wave spectroscopy and SOPAT microscopy). Also new approaches for process monitoring systems are developed.*


*At **KIWI-biolab** we are pioneering the development of innovative bioprocesses through automation, AI integration and advanced analytics. During your visit, we will share insights, discuss potential collaborations, and explore how our expertise can complement each other. If you have any questions or would like to explore something specific during your visit, please feel free to contact mariano.n.cruzbournazou@tu-berlin.de.*


***Location:** Ackerstraße 76, 13355 Berlin (20 min by tram, 8 min by car):*

The number of participants is limited to 40. Please register before (no extra charge) at

18:30 *End*

SCIENTIFIC POSTERS

P01: Towards a biological-driven bioprocess development
Nadja Alina Henke, Karlsruhe Institute of Technology, Germany

P02: Creating a closed process semi-automated workflow for human MSC Expansion, harvest, and final fill
Julien Muzard, Entegris

P03: Mimicking large-scale mixing times in a laboratory scale single multi compartment bioreactor
Jonas Barczyk, Stuttgart University, Germany

P04: CFD-guided scale-down for end-in-mind bioreactor development: from 2000 L to 2 L
Miki Segami, Delft University of Technology, Biotechnology

P05: CFD simulation of pH gradients and their effect on ester hydrolysis by *Candida antarctica* lipase B
Caroline Hamelmann, Technical University of Denmark - DTU, Denmark

P06: Challenges of bioprocess scale-down on an automated platform
Linda Lantian Cai, TU Berlin, Germany

P07: Microfluidic single-cell cultivation: A game-changer in predicting bioprocess scalability?
Julian Schmitz, Bielefeld University, Germany

P08: Bridging the protein gap using single cell protein
Koen Verhagen, dsm-firmenich, Science, Research & Innovation

P09: Robustness characterization of AMP producing *C. glutamicum* strains on single-cell level
Yannick Scholz, Karlsruhe Institute of Technology, Germany

P10: Analysis and control of expression heterogeneity of microbial gene circuits on a single-cell level
Boris Yermakov, Karlsruhe Institute of Technology, Germany

P11: Scaling-up of microbial biomass production, with immunomodulating potential
Mihaela Palela, The National Institute of Medical-Military Research and Development, Bucharest, Romania

P12: Design and optimization of animal component-free media for plasmid DNA production in E. coli
Kyle Probst, Kerry, Beloit, WI, USA

P13: From microtiter plate to fermenter: scale-up of a *Vibrio natriegens* fed-batch process
Clara Lüchtrath, RWTH Aachen University, Germany

P14: Process characterization across scales of an industrial Aspergillus oryzae aerobic fed-batch fermentation Process
Mariana Albino, Technical University of Denmark – DTU, Denmark

P15: Intensified proliferation of BY-2 plant cells in structurally modified culture bags for wave-mixed single-use bioreactor
Mateusz Bartczak, Warsaw University of Technology, Poland

P16: Development of an automated online flow cytometry method to quantify cell density and fingerprint bacterial communities
Juan Lopez Galvez, Helmholtz-Zentrum für Umweltforschung, Angewandte Mikrobielle Ökologie

P17: To feed or not to feed? The challenges of glycerol fed-batch for Pichia pastoris expression in shake flasks
Christina Dickmeis, Scientific Bioprocessing Inc., Baesweiler, Germany

P18: Effect of cell culture production methods on the survival of probiotic yeast *Saccharomyces cerevisiae var. boulardii* in gut-like conditions
Dorotea Rzechonek, Chalmers University of Technology, Sweden

P19: Elementary Flux Mode Analysis predicts co-culture stability in continuous bioprocesses
Juan Andres Martinez Alvarez, Université de Liège, Belgium

P20: Benefits of Off-gas Analysis – Improved volume calculation for fermentations by monitoring the absolute humidity
Nils Arto, BlueSens gas sensor GmbH, Development

P21: Oxygen transfer in non-Newtonian liquids
Emilie Overgaard Willer, Technical University of Denmark – DTU, Denmark

P22: Membrane-free dissolved hydrogen monitoring in hydrolytic and methanogenic bioprocesses
Eike Janesch, TU Berlin, Germany

P23: Investigating signal attenuation in raman spectra of bacterial fermentations
Christoph Lange, TU Berlin, Germany

P24: Mathematical modelling of the oxygen transfer rate (OTR) as a first step towards the development of a digital twin
Marc Lemperle, Technical University of Denmark – DTU, Denmark

P25: Digital twin modeling of a pilot-plant disk centrifuge in GFPUV production downstream
Alina Anamaria Malanca, Technical University of Denmark – DTU, Denmark

P26: Robust tube-based MPC for controlling bioprocesses under uncertainty
Niels Krausch, TU Berlin, Germany

P27: Potential of predictive model-based dissolved oxygen control for intermittent fed-batch processes
Philipp Pably, Technical University of Denmark – DTU, Denmark

P28: Machine learning based compartment models for dynamic simulation of heterogeneous fed-batch processes
Hector Maldonado, Delft University of Technology, The Netherlands

P29: Accelerating Bioprocess Optimization and Scale-Up for a CHO Cell Culture Process Using Digital Models
Jannik Richter, Leibniz University Hannover, Germany

P30: Enzyme-Mediated Exponential Glucose Release: A Model-Based Strategy for Continuous Defined Fed-Batch in Small-Scale Cultivations
Annina Kemmer, TU Berlin, Germany

P31: Real-time Analysis of Multicomponent Bioprocesses Using Raman Spectroscopy and RAMANMETRIX(™)
Jörg Weber, Biophotonics Diagnostics GmbH

P32: An open access platform for bioreactor
Xiyan Li, Technical University of Denmark – DTU, Denmark

P33: Data Management in Automated transdisciplinary laboratories
Simon Seidel, TU Berlin, Germany

P34: Silicon-based photonic biosensors for label-free detection of microorganisms
Philipp Schrenk, TU Berlin, Germany

SCIENTIFIC POSTERS

P35: Mechanistic soft-sensor design for protein refolding processes based on intrinsic fluorescence measurements
Florian Gisperg, TU Wien, Austria

P36: Developing a low cost, highly parallel, scalable, bacterial protein production workflow based on single-use bubble column reactors
Nathan Wright, University of Oxford, United Kingdom

P37: Secretory production of bifunctional proteins with *Corynebacterium glutamicum*
Vera Waffenschmidt, Forschungszentrum Jülich, Germany

P38: Accelerated secretion efficiency screening for the production of microplastic-binding peptides in *C. glutamicum*
Rebecca Hamel, Forschungszentrum Jülich, Germany

P39: Development of a non-canonical amino acid-labeled [NiFe]-hydrogenase production system in Escherichia coli
Qin Fan, TU Berlin, Germany

P40: Heterologous production of an active hydrogenase using lactose-based autoinduction
De La Fuente Kratzborn Francisco, TU Berlin, Germany

P41: Bioprocess development to produce a hyperthermostable S-methyl-5'-thioadenosine phosphorylase in Escherichia coli
Julia Schollmeyer, TU Berlin, Germany

P42: Characterization and optimization of peroxidase production in *Komagataella phaffii* with accelerated bioprocess development through automation and miniaturization
Christian Wagner, Forschungszentrum Jülich, Germany

P43: Automated strain library screening and bioprocess optimization of heterologous production of sakacin P in *Corynebacterium glutamicum*
Lisa Prigolovkin, Forschungszentrum Jülich, Germany

P44: Advanced workflows for the systematic identification of metabolic optimization targets in DBTL-cycles: A demonstrator for producing aromatic compounds in *C. glutamicum*
Niels Hollmann, Forschungszentrum Jülich, Germany

P45: Trans-cinnamic acid production by whole-cell biotransformation of recombinant Pseudomonas putida KT2440
Sompot Antimanon, Technical University of Denmark – DTU, Denmark

P46: Biosynthesis of phenazine-1-carboxylic acid in *Pseudomonas chlororaphis* DSM19603 through media factor optimization and genetic engineering
Anne Clausen, Aalborg University Esbjerg, Denmark

P47: Lactic acid production from tropical agro-food waste. An overview to opportunities in cuba
Anabel V Sánchez-Díaz, Universidad Tecnológica de La Habana José A. Echeverría, Cuba

P48: Purine nucleoside antibiotics: recent synthetic advances harnessing chemistry and biology
Jonas Motter, TU Berlin, Germany

P49: Biocatalytic nucleobase diversification of 4'-thianucleosides and de novo RNA synthesis detection with 5-ethynyl-4'-thiouridine in proliferating HeLa cells
Sarah von Westarp, TU Berlin, Germany

P50: Design of a production process for resistance structures and metabolites of Metarhizium robertsii MT008 for the control of Anastrepha obliqua through submerged fermentation on a laboratory scale using agroindustrial waste
Ginna Quiroga, Agrosavia, Bioproducts

P51: Opportunities of waste bioprocessing towards a circular approach in Cuba
Ileana Pereda-Reyes, Universidad Tecnológica de La Habana José A. Echeverría, Cuba

P52: Substituting raw materials: Animal by-product streams for polyhydroxyalkanoate production
Saskia Waldburger, TU Berlin, Germany

P53: Polyhydroxyalkanoate production by *Cupriavidus necator* using apple juice residues
Lena Kranert, Otto-von-Guericke- Universität Magdeburg, Germany

P54: Microbially produced monomers for biopolymers: Bioprocess development for 2-oxoglutarate production with *Corynebacterium glutamicum*
Lars Halle, Forschungszentrum Jülich, Germany

P55 A comparative study of Python and Julia programming for downstream process simulation
Fiammetta Caccavale, Technical University of Denmark, Lyngby

P56 Optimisation of the oxygen regime for the accelerated production of kombucha with defined co-cultures
Marie Ludszuweit, VLB Berlin

P57 Cost-to-go model predictive control for enhanced optimization of bioprocesses
Don Fabian Müller, Competence Center CHASE GmbH, Linz, Austria

TUESDAY, 9 APRIL 2024

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 Proper scale down: a prerequisite for engineering robust microbial chassis and for model-based prediction of industrial scale performance (PL01)

Ralf Takors
University of Stuttgart, Germany
Email: takors@ibvt.uni-stuttgart.de

The successful scale-up of lab performance to industrial application is essential for making novel bioprocesses happen. Often, key performance indicators such as titer, rates, and yields (TRY values) deteriorate while transferring a process from lab to production. However, thorough analysis of so-called lifelines, i.e. the recorded experiences of floating microbes inside a bioreactor, reveal that even complicated scenarios may be well mimicked by 2-3 bioreactor compartments in lab scale [1]. The single multi-compartment bioreactor 'SMCB' of IBVT reflects this mindset [2]

Even further, strain engineering benefits from proper scale-down tests. The engineered chassis *E. coli* RM214 is characterised by a 'smartly reduced genome' lacking genes that showed apparently non-necessary on/off-switching under large scale conditions [3]. Said strain turned out to be superior to the wild-type e.g. for producing heterologous proteins.

Model-based scaling up heavily depends on the predictive power of the applied models. This hold particularly true for bubble populations that are crucial for modelling gas/liquid mass transfer, gas hold-up, and turbulence inside bioreactors. Here, an experimental setting for measuring bubble dynamics is presented that successfully lead to the identification of a novel bubble breakage model [4]. The latter was successfully applied for correctly predicting bubble breakage not only in water but also in fermentation solutions. Theron, the model was used to analyse the performance of a 600 m³ industrial bubble column that served for the microbial production of L-Phenylalanine with *E. coli*. Real large-scale date is compared with simulated conditions showing good agreement. The results provide the basis for further process optimisation.

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2. Gaugler, L., Mast, Y., Fitschen, J., Hofmann, S., Schlüter, M. and Takors, R. (2022), Scaling-down biopharmaceutical production processes via a single multi-compartment bioreactor (SMCB), Eng. Life Sci., 23: e2100161.
3. Ziegler, M., Zieringer, J., Döring, C.L., Paul, L., Schaal, C. and Takors, R. (2021), Engineering of a robust *Escherichia coli* chassis and exploitation for large-scale production processes, Metab. Eng., 67: 75-87.
4. Mast, Y. and Takors, R. (2023), Novel experimental data-driven bubble breakage model for universal application in Euler-Lagrange multiphase frameworks, Chem. Eng. Sci., 284: 119509.

SESSION 1: INDUSTRIAL SCALE PROCESS PERFORMANCE AND OPTIMIZATION

Chair Michael Schlüter / Lucas Kaspersetz

13:05 Model applications in scale-up of mammalian cell culture (CHO) processes (L01)

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Approximately 70 % of the world's biopharmaceutical drugs are produced using CHO cells in suspension culture. The short turnaround times required to develop these processes means that most process development facilities have adopted single-use bioreactors (SUBs) up to 2000L, during the early phases of a drug candidate. However, when product demand exceeds certain thresholds, it is no longer economically viable to use SUBs, and hence, large-scale manufacturing in 10,000-25,000 L stainless-steel bioreactors becomes relevant.

To scale-up from one geometrically dissimilar bioreactor to another, we present a scale-up strategy where the historical data of the process is used to derive scale-independent mass transfer, hydrodynamic and cellular metrics that are unique to the cell line and to the process. We perform an assessment of these metrics, comparing the mass transfer and hydrodynamic requirements of the process to equipment capabilities, site experience and industry standards. This is followed by advanced simulation of the process at scale, with the objective of matching donor site process performance within the simulations. The simulations are performed with an integrated framework of mechanistic models on a backdrop of CFD validation. Concurrently, the simulations are used to define a scale-down version of the process, which is validated in 5 L bioreactors. The 5 L results and the simulations of the 20,000L process allow a final, holistic strategy to be put in place on how to operate the 20,000L bioreactor. The integrated model ensures that the scale-up of a new process is based on a full assessment of mass transfer, cellular kinetics and hydrodynamic considerations, as opposed to matching a single criterion like P/V or KLa.

As a large-scale CDMO, FDB receives processes that are fundamentally variable, with different requirements for each process, such as meeting specific pH/pCO_2, osmolality and metabolite profiles. The model-based scale-up strategy has proven to be versatile, cost effective and a fast way to de-risk the scale-up of CHO cell culture processes, irrespective of the donor site bioreactor type and process requirements.

13:30 Valorising syngas in a coupled fermentation via acetate: techno-economic analysis for SCP production and pilot-scale implementation (L02)

Elodie Vlaeminck [1,2], Koen Quataert[2], Evelien Uitterhaegen[2], Karel De Winter[2], Tom Delmulle[1], Wim K. Soetaert[1,2]
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Third-generation biorefineries using CO2 as their feedstock have garnered considerable interest for the carbon-neutral biological production of fuels and chemicals [1]. To make this technology economically competitive, energy-efficient fixation of the gaseous carbon is crucial. In this respect, syngas fermentation with acetogenic bacteria appears to be a propitious route, albeit with a limited product spectrum of mainly small organic acids and alcohols. To enhance the product portfolio, we focus on a coupled fermentation approach with acetate, the natural product of the acetogens, serving as the intermediate.

More specifically, we have been investigating the techno-economic feasibility of this intricate process for the production of single-cell protein (SCP) from steel-mill off gas. Experimental data from lab and pilot scale fermentations were used to build a model and conduct economic analyses. Significant cost reductions could be achieved through optimization of the gas-to-acetate fermentation process, for which a target concentration (45 g/L) and productivity (4 g/L/h) were identified, laying the foundation for further development of the acetate platform [2].

Another key aspect in this research field is the utilization of real industrial syngas as fermentation feedstock since the varying gas composition and presence of contaminants influence the acetate production. To assess the performance directly at the emission source, a mobile pilot plant for gas fermentation (the Bio Base Mobile Pilot Plant) was utilized to convert biomass-derived syngas into acetate [3].

Conclusively, our work sheds light on practical strategies to advance towards the sustainable and economically viable implementation of third-generation biorefineries.

1. Liu, Z., Wang, K., Chen, Y., Tan, T. and Nielsen, J. (2020), Third-generation biorefineries as the means to produce fuels and chemicals from CO2, Nat. Catal. 3: 274-288.
2. Vlaeminck, E., Uitterhaegen, E., Quataert, K., Delmulle, T., Kontovas, S.-S., et al. (2023), Single-cell protein production from industrial off-gas through acetate: Techno-economic analysis for a coupled fermentation approach, Fermentation, 9: 771.
3. BioSFerA project (EU Horizon 2020 No 884208), url: https://biosfera-project.eu/scaling-up-the-integrated-syngas-fermentation-pilot-process.

13:55 Industry talks

14:15 Coffee break & exhibition

14:45 Optimizing bioreactors for microbial gas fermentation: An approach for sustainable valorization of industrial off-gas (L03)

Carolin Bokelmann, Ralf Takors
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Addressing the European Commission's ambitious target to achieve carbon neutrality by 2050, which even considers reduction of net greenhouse gas emission by at least 55% until 2030, requires innovative approaches in industrial and municipal waste gas use. These include the microbial production of drop-in building blocks for the chemical industry utilizing off-gases. Thereby, less greenhouse gases are released while avoiding further oil consumption. Here, we showcase ethanol production.

Such microbial conversions with acetogens require optimal performing, low-cost, large-scale fermentations operating with the least technical effort to meet economic demands. Gas lift reactors and bubble columns are the bioreactors of choice to fulfill the technical expectations.

In our study, we investigate and compare the hydrodynamics, mass transfer, and mixing characteristics of different pilot- to large-scale reactor setups for gas fermentation. The reactor types investigated include a bubble column reactor, an annulus-rising and a center-rising internal loop airlift reactor, and an external loop airlift reactor. We use computational fluid dynamics tools for this analysis. Our objective is to identify the most suitable reactor for ethanol production with Clostridium autoethanogenum. Further optimization of the different setups using different internals to improve the bubble residence time and thereby the overall performance is conducted.

Next, the best-performing reactor type is used to further investigate large-scale limitations. The results form the basis for the scale-down of the system to a lab-scale single multi-compartment bioreactor [1] and large-scale optimization.

1. Gaugler, L., Mast, Y., Fitschen, J., Hofmann, S., Schlüter, M. and Takors, R. (2022), Scaling-down biopharmaceutical production processes via a single multi-compartment bioreactor (SMCB), Eng. Life Sci., 23: e2100161.

15:10 Analytical modeling of large-scale bioreactors with diffusion equations (L04)

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– withdrawn –

Modeling is a widely employed tool in the study of bioreactor scale-up, where mixing, reaction, mass transfer, and biological phenomena interact. Heterogeneous large-scale reactors have usually been modeled with numerical models, but an analytical predictive model has been lacking. To fill the gap, we studied one-dimensional axial diffusion equations as a generalized model of high aspect ratio bioreactors, and developed a predictive formula for its main parameter, the axial dispersion coefficient, in mechanically agitated reactors. Over 800 previously published experimental mixing times were collected and used to validate the model without optimizing the dispersion coefficient. Mixing in typical multi-impeller configurations was well predicted when flooding due to strong aeration was avoided. Condensation of the whole flow field into a single spatially homogeneous dispersion coefficient did not work equally well in all considered configurations, though. We then used steady-state diffusion equations with first- and zeroth-order kinetics to develop analytical approximations to the axial profiles of substrate, oxygen, temperature, carbon dioxide, and pH during substrate-limited fed batches. The profiles of substrate were compared to previously published experimental and numerical data, and good similarity was found especially with the numerical references. In conclusion, analytical solutions to axial diffusion equations could be used for preliminary quick estimations of how mixing and reaction affect the relevant variables in large-scale fed-batch fermentations. In future, the model could be used to initialize more involved numerical simulations or in the design of scale-down setups mimicking the environment found in large-scale reactors.

1. Losoi, P., Konttinen, J. and Santala, V. (2023), Modeling large-scale bioreactors with diffusion equations. Part I: Predicting axial dispersion coefficient and mixing times, Biotechnol. Bioeng., 121: 1060-1075.
2. Losoi, P., Konttinen, J. and Santala, V. (2023), Modeling large-scale bioreactors with diffusion equations. Part II: Characterizing substrate, oxygen, temperature, carbon dioxide, and pH pro-files, Biotechnol. Bioeng, 121: 1102-1117.

15:35 Resolved particle Lattice-Boltzmann Large Eddy Simulation in a 15,000 L bioreactor to mimic Lagrangian sensor particles (L05)

Ryan Rautenbach[1], Sebastian Hofmann[1], Lukas Buntkiel[2], Jonas Barczyk[3], Sebastian Reinecke[2], Marko Hoffmann[1], Ralf Takors[3], Uwe Hampel[2,4], Michael Schlüter[1]

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This study researches the application of experimentally available Lagrangian Sensor Particles (LSP) [1] in bio-pharmaceutical production, focusing on their role in characterizing mixing processes by mimicking the lifelines [2] of cells during steady state mixing conditions through numerical simulations. Lattice Boltzmann Large Eddy Simulations are utilized to simulate the lifelines [2] of LSPs in a 15,000L Stirred Tank Reactor (STR) across various Reynolds numbers (Re = $2.94 \cdot 10^5$ to Re = $4.64 \cdot 10^5$), aiming to contribute to the development of a scale-down industrial STR model similar to models proposed by Haringa et al. [3]. The simulations are driven by the Lattice Boltzmann solver based software provided by M-Star Simulations, LLC. And supported by high-performance computing resources. The 60 simulated LSPs are treated as resolved particles, each 40mm in diameter to closely mimic their physical behaviour in the STR. The reactor's setup mirrors an industrial STR, equipped with a Rushton and Pitched blade impeller and three wall-mounted baffles, which has been experimentally studied in a similar manner using the LSPs [4]. Findings reveal three distinct axially distributed compartments within the STR, significantly impacting the dispersion and movement of LSPs. Results provide valuable insights into specific compartments, highlighting the importance of the analysis and quantification of mixing conditions. Offering a detailed understanding of mixing heterogeneities and compartmentalization, crucial to the advancement of scaled down biopharmaceutical manufacturing models [3;5]. The study identifies potential nutrient and media depletion zones during steady state cultivation, suggesting avenues for enhancing process efficiency and product quality in bio-pharmaceutical production.

1. Buntkiel, L., Reinecke, S.F. and Hampel, U. (2023), Acceleration measurement for flow tracking in bioreactors with sensor particles, Tech. Mess., 90: 43-48.
2. Blöbaum, L., Haringa, C. and Grünberger, A. (2023), Microbial lifelines in bioprocesses: From concept to application, Biotechnol. Adv., 62: 108071.
3. Haringa, C., Deshmukh, A.T., Mudde, R.F. and Noorman, H.J. (2017), Euler-Lagrange analysis towards representative down-scaling of a 22 m3 aerobic S. cerevisiae fermentation. Chem. Eng. Sci., 170: 653–669.
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5. Gaugler, L., Hofmann, S., Schlüter, M. and Takors, R. (2024), Mimicking CHO large-scale effects in the single multicompartment bioreactor: A new approach to access scale-up behavior. Biotechnol Bioeng., Epub ahead of print, doi: 10.1002/bit.28647.

SESSION 2: PROCESS-DRIVEN CELL PERFORMANCE

Chair Robert Spann / Isabel Thiele

16:00 Ecological forces dictate microbial community assembly processes in bioreactor systems (L06)

Susann Müller[1], Shuang Li[1], Volker Grimm[2]

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Microbial communities are indispensable for future biotechnology to produce valuable platform chemicals and reduce the exploitation of fossil resources. Yet, the stability of microbial communities in classical continuous reactor setups is best brief or non-existent. The population ecology of microbial communities is still poorly understood and their notorious instability makes them impossible to control. Much of the instability is caused by the stochastic assembly of microorganisms, especially in highly diverse microbiomes where structural and hence functional changes occur rapidly due to the short generation time of their members. Usually, to maintain organismic proportions in communities, their niches are deterministically reinforced, but stochasticity strongly counteracts this.

Looped mass transfer was found to be a means of stabilizing microbial communities over long periods of time via increasing the mass transfer rate RC. Mass transfer i) reduced local and temporal variations, and the stochastic behavior was reduced. All microbiomes showed high constancy and increasing resistance as well as unaffected functions at high mass transfer rates. Mass transfer ii) synchronized structures of the microbiomes by the mechanism of homogeneous dispersal, resulting in the lowest inter-community ß-diversity at the highest mass transfer. Persistence of particular SCs was highest at high mass transfer. High turnover of community structures was observed only when no mass transfer occurred. An increase in mass transfer iii) increased cell numbers, thereby decreasing netgrowth rates μ^'. Subcommunities that showed no growth (μ' SC_x=0) in one locality were rescued by growth at another locality and by their redistribution via the loop design.

The rescue effect, known from metacommunity theory, was the main stabilizing mechanism leading to synchrony and survival of subcommunities, despite differences in cell physiological properties, including growth rates. This study fills a long-standing gap and enables continuous and proportionally equal growth of community members using an unprecedented operational design that addresses an acute need in healthcare and biotechnology industries.

1. Li, S., Abdulkadir, N., Schattenberg, F., Rocha, U.N., Grimm, V., Müller, S. and Liu, Z. (2022), Stabilising microbial communities by looped mass transfer, Proc. Natl. Acad. Sci. U S A, 119: e2117814119.
2. Li, S. and Müller, S. (2023), Ecological forces dictate microbial community assembly processes in bioreactor systems, Curr. Opin. Biotechnol., 81: 102917.

16:25 Morphology control for supporting scalability of *Aspergillus niger* cultures (L07)

Tolue Kheirkhah[1], Karin Engelbert[1], Henri Müller[2], Charlotte Deffur[2], Fangxing Zhang[2], Heiko Briesen[2], Vera Meyer[1], Peter Neubauer[1], Stefan Junne[1,3]

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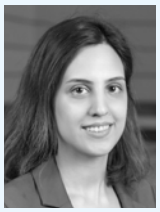
Scale-dependent unpredictable morphology is known to adversely affect the productivity in *Aspergillus niger* cultivations. While high shear forces are one of the primary factors contributing to this challenge, a rocking motion bioreactor (RMB) was applied with a substantially lower shear force regime similar to shake flasks. Sufficient kLa-values are achievable in such a bioreactor with 2-dimensional rocking so that oxygen limitation was avoided. Addition of talcum micro particles have proven to be effective in controlling the morphology in earlier studies. Therefore, in this study, different concentrations of talcum were added to the RMB to investigate the possibility of directing the development of a certain morphology. Results showed that, while at higher talcum concentrations (10 and 2.5 g L^{-1}) A.niger grew into dispersed forms or clumps, further adjustment of talcum at a lower concentration of 1.0 g L^{-1} allowed distinct pellet formation with controlled sizes [1].

Given the crucial role of seed cultures, we then investigated the morphological development under various shear stress regimes in comparison to the initial morphology. To achieve defined seed culture morphologies, A. niger was cultivated under 48 distinct cultivation conditions with various spore and talcum concentrations. Two identical seed cultures (with 1 and 10 g L-1 talcum) were selected and simultaneously inoculated in the RMB and in a stirred tank reactor (STR). The 20-hour batch cultivation included the analysis of the overall growth behavior and pellet micro- and macromorphology with automated 2-dimensional image and 3-dimensional structural analysis based on synchrotron radiation μ-computed tomography. In the STR, stirrer induced shear stress led to immediate breakage of the pellets, restricting growth beyond a specific diameter (500-600 μm). In contrast, all pellets in the RMB exhibited a continuous increase in the pellet diameter (800 μm).



This research pioneers the estimation of hyphal growth rates and pellet breakage as functions of shear forces, enhancing our understanding of cell-bioreactor interactions. Such insights could also be useful for optimizing the process performance in the seed train and achieving a customized morphology as inoculate for a larger scale or scale-down studies.

1. Kheirkhah, T., Neubauer, P. and Junne, S. (2023), Controlling *Aspergillus niger* morphology in a low shear-force environment in a rocking-motion bioreactor, Biochem. Eng. J., 195: 108905.

16:50 Coffee break & exhibition

17:20 Transport-controlled growth decoupling for self-induced protein expression with a glycerol-repressible genetic circuit (L08)

Alvaro R. Lara[1], Flavio Kunert[2], Vincent Vandenbroucke[3], Hilal Taymaz-Nikerel[4], Luz María Martínez[5], Juan-Carlos Sigala[6], Frank Delvigne[3], Guillermo Gosset[5], Jochen Büchs[2]

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[2]RWTH Aachen University, Germany
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Decoupling cell formation from recombinant protein synthesis is a potent strategy to intensify bioprocesses. *Escherichia coli* strains with mutations in the glucose uptake components lack catabolite repression, display low growth rate, no overflow metabolism and high recombinant protein yields. Fast growth rates were promoted by the simultaneous consumption of glucose and glycerol, and this was followed by a phase of slow growth, when only glucose remained in the medium. A glycerol-repressible genetic circuit was designed to autonomously induce recombinant protein expres-

sion. The engineered strain bearing the genetic circuit was cultured in 3.9 g L^{-1} glycerol + 18 g L^{-1} glucose in microbioreactors with online oxygen transfer rate monitoring. The growth was fast during the simultaneous consumption of both carbon sources, while expression of the recombinant protein was low. When glycerol was depleted, the growth rate decreased, and the specific fluorescence reached values 17 % higher than those obtained with a strong constitutive promoter. Despite the relatively high amount of carbon sources used, no oxygen limitation was observed. The proposed approach eliminates the need for the substrate feeding or inducers addition and is set as a simple batch culture while mimicking fed-batch performance.

17:45 Phenotypical heterogeneity in bioprocesses: better think positive!? (L09)

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Phenotypic cell-to-cell heterogeneity (Pc²h) in industrially relevant biotechnological production processes has lately been the subject of much research, however the real impact of this phenomenon on bioprocess performance remains poorly understood. Traditionally, the presence of Pc²h has been viewed as a drawback for bioprocesses leading to lowered productivities. However, recent research proposes several bioprocess beneficial effects of Pc²h such as enhanced robustness of cellular populations in response to changing environmental conditions. Intrigued by these findings, we give an overview into Pc²h and discuss advantages and disadvantages on bioprocess performance, especially in the context of recent studies. The studies show, that the current negative opinion about Pc²h needs to be reconsidered as it holds immense potential to revolutionize bioprocesses. Based on this, we identify research directions and necessary steps to further demystify the role of Pc²h on bioprocesses. Deepened insights will enable the development of bioprocess specific management strategies for Pc²h which includes either the reduction of Pc²h with a negative impact on bioprocesses, or exploit positive features of Pc²h to optimize future bioprocesses.

■ ■ PLENARY TALK

18:10 Managing cell population entropy: navigating from ecosystems to bioprocesses (PL02)

Frank Delvigne

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Isogenic cell populations can cope with stress conditions by switching to alternative phenotypes. Even if it can lead to increased fitness in a natural context, this feature is typically unwanted for a range of applications (e.g., bioproduction, synthetic biology, and biomedicine) where it tends to make cellular response unpredictable [1;2]. However, little is known about the diversification profiles that can be adopted by a cell population. We then characterized the diversification dynamics for various systems (bacteria and yeast) and for different phenotypes (utilization of alternative carbon sources, general stress response and more complex development patterns), based on a metric named "information entropy". This information entropy is a robust proxy reflecting the degree of cell-to-cell difference within a population [3]. Our results suggest that cell population entropy and the fitness cost associated with cell switching are tightly coupled [4]. We further demonstrated that the activation of burdensome gene circuits is systematically compensated by an increase of population entropy, ensuring the stability of the cell population in continuous cultivation. More importantly, cell population entropy can be manipulated based on the use of a cell-machine interface [5;6]. This feature will be demonstrated for the control of a T7-based expression vector in *E. coli*, the mitigation of the general stress response for various microbial cell factories, as well as for the control of yeast-bacteria co-cultures [7].

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6. Delvigne, F. and Martinez, J.A. (2023), Advances in automated and reactive flow cytometry for synthetic biotechnology, Curr. Opin. Biotechnol., 83: 102974.
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18:45 GET-TOGETHER & EXHIBITION & POSTER-SESSION FOR ALL PARTICIPANTS

We encourage all poster authors to be at their posters at least from 19:30-20:15 (even poster numbers) and 20:15-21:00 (odd poster numbers).

21:00 End of day 1

WEDNESDAY, 10 APRIL 2024

OPENING

09:00 Welcome and introduction

Peter Neubauer, Technische Universität Berlin, Germany

PLENARY TALK

09:05 The role of bioeconomy on the path to net-zero (PL03)

Daniela Thrän[1, 2], Alberto Bezama[1], Romy Brödner[2], Kathleen Meisel[2], Walther Zeug[1]

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The transformation of the resource base is key to realise the net zero climate emission targets till 2045. Next to the provision of renewable energy from wind and solar biomass and biobased products or of increasing importance as renewable carbon source. However, the production of biomass depends on land and a healthy environment. Circular bioeconomy combine both, sustainable supply chains and its efficient application, to make the best use out of the limited biomass. Behind this background this presentation provides an introduction into circular bioeconomy concepts in Germany and the European Union and strategies for ist implementation. Also, key performance indicators for sustainability are presented and discussed, using some examples from the biofuels and biomaterial sector.

SESSION 3: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair Alvaro Lara / Tolue Kheirkhah

09:50 Integrating hybrid modelling and transfer learning for novel bioprocess predictive modelling (L10)

Harry Kay, Sam Kay, Alexander W. Rogers, Dongda Zhang

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Hybrid modelling is a novel technique incorporating both mechanistic and data-driven information into a single model, providing effective solutions to the modelling of complex (bio)chemical systems where underlying mechanisms are not entirely understood. Even though within the literature, it has been shown more data efficient than traditional data-driven models, time consuming experiments must still be performed when constructing such models to simulate a novel system. To accelerate this process, transfer learning can be implemented to retain information (transfer knowledge) from a well understood source domain to the novel transfer domain. In this study, we develop integrate hybrid models with transfer learning methodologies to extract knowledge from a well know strain Desmodesmus sp., of which abundant data and high-fidelity models exist to describe the system. This knowledge is then used to accelerate the development (using limited process data) of a hybrid model for a recently isolated microalgal strain, *Chlorella sorokiniana*. In addition, the mode of operation was changed from fed-batch to a batch operating mode. To test the predictive capabilities of the constructed models, state predictions of biomass growth, nitrate consumption and lutein production were made under differing operating conditions, and bootstrapping used to estimate model uncertainty. It was shown, through experimental validation, that hybrid models can accurately describe complex (bio)chemical systems and, when used in conjunction with transfer learning, can model novel systems under extremely limited data conditions with representative uncertainty bounds.

10:15 Small scale models for process intensification: How to migrating a months-long perfusion process to intensified fed-batch (L11)

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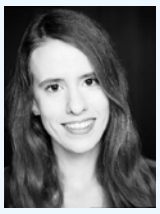

The demand for process intensification in the biopharmaceutical industry has increased steadily over the past decade, driven by the need to maximise efficiency and reduce production costs. Suitable strategies for intensification of existing bioprocesses are therefore urgently required. The data presented will show a case study where an existing perfusion process for a difficult-to-express human therapeutic protein expressed in the human cell host GEX® was converted to an intensified fed-batch process as demand increased due to the protein's potential for new applications. The conversion strategy was based on well established small-scale perfusion as well as intensified fed-batch models to enable efficient high throughput process optimisation. In a first step, protein-producing cell clones selected for their ability to produce well in a perfusion process were cultured in different media in a small-scale mock perfusion approach to select for the medium that promotes growth in n-1 perfusion. Cells from high cell density cultivation, either from said small scale mock perfusion or from 1L perfusion runs, were inoculated at high cell densities in different media and cultivated in an intensified fed-batch mode. Finally, after 20 days of n-1 perfusion, the 29-day perfusion process was successfully migrated to a 13-day fed-batch for expansion with comparable product quality. While the titer in the intensified fed-batch was increased 70-fold and the total harvest volume to be purified was reduced by almost a factor of 5, the total protein yield for the intensified fed-batch process was only about 60% at the same material and GMP facility production costs as the master perfusion process.

10:40 Industry talks

10:55 Coffee break & exhibition

SESSION 4: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair Ralf Takors / Annina Kemmer

11:25 Scale-up of stem cell cultures from shake flask to bioreactor: a CFD-based comparison of hydrodynamic stress (L12)

Ramon van Valderen[1], Tom van Arragon[2], Emile van den Akker[3], Marcel Ottens[1], Marieke Klijn[1], Cees Haringa[1]

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[2]Getinge-Applikon, Delft, The Netherlands
[3]Sanquin Research, Department of Hematopoiesis, Amsterdam, The Netherlands
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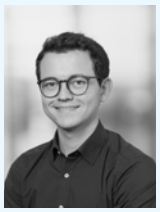

Ex-vivo cultivation of inducible pluripotent stem cells (iPSCs) for the production of Red Blood cells (RBCs) is a promising therapeutic alternative to donor-based blood cell transfusions. During early-stage development of such biopharmaceutical processes, the shake flask is often used due to its low cost and ease to operate. However, scaling up from shake flask to stirred tank bioreactor is challenging, as mass transfer, mixing and hydrodynamic conditions can become limiting factors or difficult to translate [1]. Especially for human stem cell cultures, as their sensitivity to hydrodynamical stress is well-known and must be accounted for during scale-up [2].

In this work, computational fluid dynamic simulations were performed to compare the hydrodynamics of a 125 mL shake flask and 250 mL MiniBio® bioreactor (Getinge) for various operating conditions, using highly resolved large-eddy simulations (LES). Specifically, the heterogeneities in fluid strain (1/s) and energy dissipation rate (W/kg), originating from either shaking or agitation, were compared as these parameters serve as proxy for hydrodynamic stress. Furthermore, Lagrangian particle tracking was used to simulate how single cells move through the time-evolving flow field, known as lifelines, to characterize the spatial-temporal fluctuations in fluid strain and energy dissipation rate from the cellular perspective.

This work shows how highly resolved CFD can be used to compare the hydrodynamic stress of two fundamentally different cultivation systems and how the operating conditions (RPM) of the shake flask can be translated to the bioreactor, which contributes to faster process development time when scaling up.

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2. Chalmers, J.J. (2015), Mixing, aeration and cell damage, 30+ years later: What we learned, how it affected the cell culture industry and what we would like to know more about. Curr. Opin. Chem. Eng., 10: 94–102.

11:50 Fully automated growth media optimization employing a new machine learning algorithm (L13)

Frédéric M. Lapierre[1,3], Dennis Raith[2,3], Mariela Castillo[2,3], Jonas Bermeitinger[3], Robert Huber[1]

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[2]University of Freiburg
[3]LABMaiTE GmbH
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Bioprocessing plays a crucial role in producing specialized products like antibiotics and enzymes. Optimizing culture conditions is essential for economic success. A key challenge is developing a fermentation medium. Traditionally, Design of Experiments (DoE) methods have been the standard for growth media development. Although they reduce the number of necessary cultivation experiments, these methods have limitations, particularly with nonlinear systems. Moreover, two-stage DoE methods may overlook significant parameters in an initial screening that become crucial in later optimization stages. Machine Learning (ML) algorithms, such as Bayesian Optimization, emerge as a promising, more goal oriented and efficient alternative.

We introduce an innovative, fully automated microbioreactor system that autonomously prepares and tests nutrient media from various stock solutions. This system combines a commercial microbioreactor with real-time monitoring of vital bioprocess parameters, and an open-source liquid handling system for medium preparation, plate sterilisation, and inoculation.

Initially, randomly generated media compositions are tested. After this cycle, the system automatically cleans and sterilizes the plate and prepares it for the next cycle. An ML algorithm suggests new media formulations to explore in this following cycle based on the previous results. This iterative process continues until there are no more improvements found or the optimization budget is exceeded, e.g. due to time or cost constraints.

Initial data sets are promising. The ML-optimized growth medium shows a 34% higher maximum backscatter value compared to the DoE-optimized medium. It is planned to diversify and test the ML algorithm with more strains to prove broad applicability.

12:15 Robustness of microbial functions in dynamic environments: a microfluidic approach (L14)

Luisa Blöbaum, Luca Torello Pianale[2], Lisbeth Olsson[2], Alexander Grünberger[3]*

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[2]Industrial Biotechnology Division, Department of Life Sciences, Chalmers University of Technology, Gothenburg, Sweden
[3]Microsystems in Bioprocess Engineering, Institute of Process Engineering in Life Sciences, Karlsruhe Institute of Technology, Karlsruhe, Germany. Email: luisa.bloebaum@uni-bielefeld.de

Microbes are challenged with rapidly changing environments in large-scale bioprocesses, due to gradients of substrates and products [1]. The microbe's exposure and response to non-optimal production conditions affects yield, titers, and rates [2]. These effects are challenging to predict and address during development in lab-scale fermenters. Subsequent scale-up can lead to non-profitable bioprocesses [3]. Tools to mimic large-scale conditions early in strain development are therefore desirable.

This contribution presents an approach to assess robustness (function stability) and performance of cellular functions of microbial cell factories in changing environments. As proof-of-principle, the lab strain *Saccharomyces cerevisiae* CEN.PK113-7D was cultivated in glucose feast-starvation cycles using dynamic microfluidic single-cell cultivation [4]. Growth and other cellular functions (e.g. intracellular ATP-level) were monitored using live-cell imaging and fluorescent biosensors. The data were analysed with a recent robustness quantification method [5,6].

Under feast-starvation cycles, growth behaviour and intracellular ATP-level depended on the frequency of environmental change. A decrease in specific growth rate, but an increase in intracellular ATP levels with slower cycles were observed. In a next step, the comparison using robustness quantification to two industrial yeast strains (PE2 and EthanolRed) was performed. Recent results will be shown.

The workflow enabled the investigation of function stability and performance of microbial functions in dynamic environments. Benefits of this approach include fast adaption of the set-up to other organisms, cultivation conditions and cycles. It therefore promises to be a useful tool to include large-scale bioprocess conditions in early strain selection and development.

1. Lara, A.R., Galindo, E., Ramírez, O.T. and Palomares, L.A. (2006), Living with heterogeneities in bioreactors: Understanding the effects of environmental gradients on cells. Mol. Biotechnol., 34: 355–382.
2. Enfors, S.O., Jahic, M., Rozkov, A., Xu, B., Hecker, M., et al. (2001), Physiological responses to mixing in large scale bioreactors. J. Biotechnol. 85: 175–185.
3. Crater, J.S. and Lievense, J.C. (2018) Scale-up of industrial microbial processes. FEMS Microbiol. Lett., 365: fny138.
4. Blöbaum, L., Torello Pianale, L. et al, Quantifying microbial robustness in dynamic environments using microfluidic single-cell cultivation. In review at Microbial Cell Factories, DOI: 10.21203/rs.3.rs-3644873/v1
5. Trivellin, C., Olsson, L. and Rugbjerg, P. (2022), Quantification of microbial robustness in yeast. ACS Synth. Biol. 11: 1686–1691.
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12:40 Lunch break, poster session & exhibition

PLENARY TALK

14:00 Towards closed-loop bioprocess development: Robotic workflows for automated Design-Build-Test-Learn cycles (PL04)

Marco Oldiges

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In times of global efforts to change from fossil to renewable resources, microbial biotechnology is a key technology to convert renewable substrates into sustainable value products. Modern approaches of microbial strain and bioprocess engineering facilitate faster development cycles. Recent approaches making use of miniaturization and parallelization of microbial cultivation experiments were successful enablers to allow for improved throughput. However, addition of key enabling technologies from lab automation and digitalization provide additional capabilities paving the way from lab automation to partly autonomous experimental workflows. Such approaches can be connected in Biofoundry concepts enabling rapid cell factory design for production of chemicals or proteins with higher throughput in small volumes. This covers the design of new strain variants or process conditions (´Design´), molecular biology to generate new strain variants (´Build´), characterization of their performance (´Test´) and gain of knowledge to understand relevant factors of impact (´Learn´) to close the DBTL cycle.

The concept of the DBTL cycle will be demonstrated using case studies on catalytically active inclusion bodies (CatIB). CatIBs can be induced from soluble cytoplasmic enzymes by adding a linker structure and an aggregation inducing protein tag. Since structure-function relationship of CatIB formation is in its infancy, a library of CatIB variants using different linker and aggregation inducing tag combinations need to be constructed and characterized, to find inclusion body variants with remaining catalytic activity. Starting with a small library for lysine decarboxlase CatIBs, further studies focused on CatIBs of glucose dehydrogenase, a well known enzyme in biocatalysis used for NAD(P)H cofactor regeneration.

SESSION 5: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair Klaus Pellicer / Niels Krausch

14:45 Human induced pluripotent stem cell scale-up and expansion under consideration of bioengineering aspects (L15)

Misha Teale[1], Marcos Sousa[2], Stefan Seidel[1], Elin Vesper[2], Samuel Schneider[1], Martin Poggel[2], Dieter Eibl[1], Regine Eibl-Schindler[1]

[1]Zurich University of Applied Sciences, Wädenswil, Switzerland
[2]Bayer AG, Leverkusen, Germany
Email: misha.teale@zhaw.ch and marcos.sousa1@bayer.com

The recent clinical and commercial success of cell therapeutics underscores its substantial developmental potential within the healthcare sector. Despite these achievements, challenges persist, particularly in improving patient accessibility and product affordability. In this context, therapeutic approaches based on allogeneic human induced pluripotent stem cells (hiPSCs) may prove pivotal, as they allow for both an economy-of-scale approach and the production of a wide range of specialized cell types. Our study therefore focused on the bioengineering aspects which must be addressed when transitioning the serum-free expansion of hiPSCs from mL- to L-scale while ensuring both product quantity and quality, especially under stirred conditions. Furthermore, we applied and improved upon proven bio-

logical and technical approaches and toolboxes used to support the developmental process. Using such a framework allowed for the efficient production of functional pluripotent cells, improving on similar reported perfusion processes with hiPSCs [1–3]. The model expansion processes and toolboxes discussed lay the foundation for subsequent upstream processing steps in the manufacturing of hiPSC-based cell therapeutics, namely directed differentiation.

1. Pandey, P.R., Tomney, A., Woon, M.T., Uth, N., Shafighi, F., et al. (2020), End-to-end platform for human pluripotent stem cell manufacturing, Int. J. Mol. Sci., 21: 89.
2. Laco, F., Lam, A.T.-L., Woo, T.-L., Tong, G., Ho, V., et al. (2020), Selection of human induced pluripotent stem cells lines optimization of cardiomyocytes differentiation in an integrated suspension microcarrier bioreactor, Stem. Cell. Res. Ther., 11: 118.
3. Manstein, F., Ullmann, K., Kropp, C., Halloin, C., Triebert, W., et al. (2021), High density bioprocessing of human pluripotent stem cells by metabolic control and in silico modeling, Stem. Cells. Transl. Med., 10: 1063–1080.

15:10 Workflow automation for reproducible high-throughput cultivations (L16)

Lucas Kaspersetz[1], Federico M. Mione[2], Martin F. Luna[2], Annina Kemmer[1], Ernesto C. Martinez[2], Peter Neubauer[1], M. Nicolas Cruz Bournazou[1]

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[2]INGAR, CONICET - UTN, Santa Fe, Argentina
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In complex high-throughput experiments, such as parallel fed-batch cultivations [1], guaranteeing reproducibility of computational workflows is of paramount importance to generate FAIR data. Particularly, if model-based decisions under uncertainty are taken during the scale-up of a process. A Workflow Management System (WMS) not only contributes to the automation of computational tasks, but also accompanies the generated data with metadata of the executed workflows. This is necessary for others to understand the context of the experiment and allows users to understand how data was obtained, manipulated, and analysed [2]. Thus, the transparency throughout the bioprocess development chain is facilitated and increased. In this work, we present the implementation of a WMS based on Directed Acyclic Graphs, offering a modular design for plug-and-play integration of different computational tools in high-throughput experimentation. We demonstrate the proposed approach using a case-study, where we have successfully online re-designed the feeding rate of parallel *E. coli* fed-batch cultivation, based on Variational Bayesian Analysis [3, 4]. The feeding profiles were re-computed with the objective of maximizing the biomass concentration at the end of each parallel experiment while enforcing a DOT constraint. This approach provides a solid foundation for increasing trust and FAIRness in generated data, while reducing integration effort for computational tools in robotic experimental facilities.

1. Haby, B., Hans, S., Anane, E., Sawatzki, A., Krausch, N., et al. (2019), Integrated robotic mini bioreactor platform for automated, parallel microbial cultivation with online data handling and process control. SLAS Technol. Transl. Life Sci. Innov. 24: 569–582.
2. Mitchell, S.N., Lahiff, A., Cummings, N., Hollocombe, J., Boskamp, B., et al. (2022), FAIR data pipeline: provenance-driven data management for traceable scientific workflows. Philos. Trans. R. Soc. Math. Phys. Eng. Sci. 380: 20210300.
3. Daunizeau, J., Adam, V. and Rigoux, L. (2014), VBA: A probabilistic treatment of nonlinear models for neurobiological and behavioural data. PLoS Comput. Biol. 10: e1003441.
4. Luna, M.F., Cruz-Bournazou, N.M. and Martínez, E.C. (2022), Online Bayesian re-design of parallel experiments based on asynchronous posterior sampling, in: Montastruc, L., Negny, S. (Eds.), Computer Aided Chemical Engineering, Elsevier, 51: 1111–1116.

15:35 Coffee break & exhibition

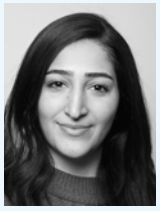
SESSION 6: PROCESS ANALYTICAL TECHNOLOGIES (PAT)

Chair Regine Eibl-Schindler / Simon Täuber

16:05 Innovative sensor solutions for bioprocess monitoring: design and implementation (L17)

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

[1]Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, Lyngby, Denmark
[2]National Centre for Nano Fabrication and Characterization, DTU Nanolab, Technical University of Denmark, Lyngby, Denmark
Email: alhas@kt.dtu.dk



Achieving optimal cell growth and product yields in bioprocesses necessitates precise control of metabolites and nutrients. Current challenges in full-scale bioreactor operation involve limited on-line sensors, primarily encompassing traditional parameters such as temperature, pH, and dissolved oxygen. Detailed monitoring of cell performance often relies on periodic off-line sampling, resulting in a lack of real-time data and reliance on experiential-based control strategies [1].

To bridge this gap, we present a novel multi-parametric approach integrating three advanced electrochemical sensors for real-time determination of ammonium, lactate, and glucose, crucial parameters in bioprocessing. Notably, these sensors demonstrate fast detection across wide concentration ranges for the analytes, offering highly reproducible responses and stable sensitivity.

This study showcases the successful application of the sensor system in diverse fermentation processes, emphasizing its adaptability and robust performance. The integration of these electrochemical sensors aligns with the principles of Process Analytical Technologies (PAT), addressing the need for real-time monitoring in bioprocessing.

1. Gargalo, C.L., Udugama, I., Pontius, K., Lopez, P.C., Nielsen, R.F., et al. (2020), Towards smart biomanufacturing: a perspective on recent developments in industrial measurement and monitoring technologies for bio-based production processes, J. Ind. Microbiol. Biotechnol., 47: 947-964.

16:30 Gasphase-based bioprocess monitoring by untargeted volatilomics with gas chromatography – ion mobility spectrometry (GC-IMS) (L18)

Joscha Christmann[1,2], Sascha Rohn[2], Philipp Weller[1]

[1]Mannheim University of Applied Sciences
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Gas chromatography hyphenated to ion mobility spectrometry (GC-IMS) is a powerful, two-dimensional separation and detection technique for volatile organic compounds (VOC) [1]. Its high sensitivity, paired with a robust setup, GC-IMS is an ideal tool for on-site bioprocess monitoring. Fermentation exhaust gas is easily available without interference of the process and features a plethora of characteristic VOCs and is currently not utilized in comprehensive process monitoring. The aim of this study was the development of a non-targeted screening approach of volatile metabolites and medium compounds based on GC-IMS and the combination of the obtained "VOC-fingerprints" with chemometric data analysis to predict parameters that are difficult to measure directly, such as the presence of contaminations.

For non-targeted analysis, the go-to strategy is the use of the obtained data as spectral fingerprints and subsequently, multivariate calibration and machine learning. Leading machine and deep learning libraries are implemented in Python, however, the missing link were GC-IMS specific routines to bridge the gap between analytical data and the existing data science ecosystem, such as file readers and preprocessing methods. This talk presents an exemplary workflow and results for the profiling of biomass and the detection of contaminations in exemplary fermentation processes by GC-IMS data and the necessary instrument and software development behind the results.

Innovative aspects:

- Fast and sensitive headspace GC-IMS setup for online process monitoring.
- Free and open-source Python package for chemometric GC-IMS data analysis [2].
- Non-target screening of volatile metabolites applied bioprocess monitoring.

1. Capitain, C. and Weller, P. (2021), Non-targeted screening approaches for profiling of volatile organic compounds based on gas chromatography-ion mobility spectroscopy (GC-IMS) and machine learning, Molecules, 26: 18.
2. Christmann, J., Rohn, S., and Weller, P. (2022) gc-ims-tools – A new Python package for chemometric analysis of GC–IMS data, Food. Chem., 394: 133476.

16:55 Lensfree microscopy for real-time bioprocess monitoring in PAT: Holographic reconstruction and fluorescence integration (L19)

Phil Thiel[1], Lukas Rüpke[1], Jannik Richter[1], Sascha Beutel[1], Mathias Belz[2], Dörte Solle[1]

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To fulfill the strict regulatory and quality requirements of the U.S. Food and Drug Administration (FDA), the Process Analytical Technologies (PAT) should be employed during pharmaceutical manufacturing processes. Sensor development, particularly for online bioprocess monitoring, is one of the main challenges of PAT tools. To detect various biological components of a bioprocess, a new lensfree microscopy based online sensor has been developed. The lensfree microscope has a compact and inexpensive design, which has now been adapted for online monitoring. To enable high-resolution imaging, the holographic images obtained were reconstructed using numerical algorithms. In initial experiments, this technique was employed for online monitoring of the concentration of Chinese Hamster Ovary (CHO) suspension cells. Through the integration of simultaneous fluorescence measurements, more detail about cell metabolism can be obtained, improving the microscope's ability to differentiate between living and non-living cells. This integrated approach holds the promise of advancing bioprocess monitoring by providing insights into cell concentration, cell viability, and the overall state of the process.
In conclusion, the combination of holographic reconstruction, fluorescence integration, and online measurements makes the lensfree microscope a powerful tool for efficient real-time bioprocess monitoring in the PAT field.

17:20 Online monitoring of protein refolding in inclusion body processing using intrinsic fluorescence (L20)

Chika Linda Igwe[1,2], Don Fabian Müller[1], Florian Gisperg[1], Jan Niklas Pauk[1,2], Matthias Kierein[1], Mohamed Elshazly[1], Robert Klausser[1], Julian Kopp[1], Oliver Spadiut[1], Eva Prada Brichtova[1]

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Inclusion bodies (IBs) are protein aggregates formed as a result of overexpression of recombinant protein in *E. coli*. The formation of IBs is a valuable strategy of recombinant protein production despite the need of additional processing steps, i.e., isolation, solubilization and refolding. Industrial process development of protein refolding is a labour-intensive task based largely on empirical approaches rather than knowledge-driven strategies. A prerequisite for knowledge-driven process development is a reliable monitoring strategy1.

We implemented intrinsic tryptophan and tyrosine fluorescence as a tool for real-time and in situ monitoring of protein refolding for the first time. In contrast to commonly established process analytical technology (PAT), this technique showed high sensitivity with reproducible measurements for protein concentrations down to 0.01 g L-1.

The change of protein conformation during refolding is reflected as a shift in the position of maximum of tryptophan and tyrosine fluorescence spectrum as well as change in the signal intensity. The shift in the peak position, expressed as average emission wavelength of a spectrum, was correlated to the amount of folding intermediates whereas the intensity integral to the extent of aggregation. These correlations were implemented as an observation function into a mechanistic model.

The versatility and transferability of the technique was demonstrated on the refolding of different proteins with varying structural complexity. The technique was also successfully applied to detect the effect of additives and process mode on the refolding process efficiency. Thus, our methodology poses a generic and reliable PAT tool enabling real-time process monitoring of protein refolding.

1. Buscajoni, L., Martinetz, M.C., Berkemeyer, M. and Brocard, C. (2022), Refolding in the modern biopharmaceutical industry, Biotechnol. Adv., 61: 108050.

17:45 Multi parameter sensors and dissolved oxygen (DO) sensor pills for shake flasks: removing black boxes for improved bioprocess development (L21)

Christina Dickmeis

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Shake flasks are one of the most frequently used bioprocessing vessels. Especially in strain and bioprocess development for screening applications they offer a cost-effective way to achieve necessary throughputs. However, shake flasks differ significantly from benchtop bioreactors and other more advanced fermentation systems in their design, operation, and size and present black boxes due to a lack of compatible sensor technology. Therefore, careless screening design can easily lead to non-productive conditions which can subsequently lead to incorrect screening conclusions, resulting in expensive consequences for the overall process.

In this presentation, we first describe the development and functionality of a novel multiparameter sensor (MPS) and dissolved oxygen (DO) sensor pills, designed to remove the black boxes in shake flask processes.

Secondly, we will show how we achieved improved protein production in Komagataella phaffii (Pichia pastoris) using data collected with the new sensors. Using the MPS and DO pills, we were able to translate different phases of bioreactor runs to shake flasks (e.g., batch, fed-batch, adaptation, and induction phase) and gain important knowledge about the protein production in our strains. Through real-time measurements of DO, backscatter, and fluorescence in our shake flasks, we observed that longer phases of oxygen limitation led to a larger drop in pH, causing reduced protein production in the cultures. We achieved better control and reduced oxygen limitation in the flasks by triggering methanol feeding into the cultures based on real-time DO measurements, allowing for a higher protein yield and better comparability to bioreactor runs.

18:00 Off-gas analysis in shake flasks (L22)

Andreas Schulte, Tibor Anderlei

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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. These measures come with various information that speed up media-and process development and scale-up. Growth rate, substrate consumption and limitation, product formation and inhibition, balancing of carbon dioxide, oxygen limitation, kLa and out of phase operating conditions 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.

Therefore, 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.

18:15 End of presentation programme

19:30 SYMPOSIUM DINNER

ERDINGER am Gendarmenmarkt, Jägerstraße 56, 10117 Berlin, erdingerberlin.de
(by invitation or separate booking only)

22:30 End of day 2

THURSDAY, 11 APRIL 2024

OPENING

09:00 Welcome and introduction
Peter Neubauer, Technische Universität Berlin, Germany

PLENARY TALK

09:05 How fundamental research on multiphase flows can support a reliable scale up (PL05)

Michael Schlüter
Institute of Multiphase Flows, Hamburg University of Technology, Eißendorfer Straße 38, 21073 Hamburg, Germany
Email: michael.schlueter@tuhh.de

Despite the long tradition of successful industrial scale bioprocessing, there is still a lack in models for a reliable scale down, scale up and process transfer. One reason is the challenging scalability of fluid dynamic heterogeneities in two-phase flows as well as the uncertainty in predicting mass transfer performance in bubbly flows under industrial conditions. This plenary lecture will provide insights into fundamental research on fluid dynamics and mass transfer in typical bioreactors from laboratory to production scale. Challenges for scale down, scale up and process transfer will be addressed and discussed. With exemplary results from experiments and numerical simulations the frontiers of a reliable scaling from the perspective of fluid dynamics and mass transfer will be highlighted and discussed.

SESSION 7: INTEGRATED BIOPROCESSES

Chair Marco Oldiges / Saskia Waldburger

09:50 Towards a circular bioeconomy: polyhydroxyalkanoate bioplastic production (L23)

Sebastian L. Riedel[1,2]
[1]*Berliner Hochschule für Technik, Department VIII – Mechanical Engineering, Event Technology and Process Engineering, Environmental and Bioprocess Engineering Laboratory, Seestraße 64, 13347 Berlin, Germany*
[2]*Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany*
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Globally, the production of the fully bio-based and biodegradable bioplastic polyhydroxyalkanoate (PHA) is still insignificant, mainly due to the cost of carbon substrates and polymer recovery. In addition, the specific monomer composition determines the area of application prior to polymer compounding and processing, which increases the demand for individual material samples for specific product development. The flexible use of regionally available carbon raw materials achieves independence from the overall rising market prices of an individual raw material due to seasonal availability or increasing competition with other industries [1]. As part of the bioprocess development, plant raw [2] and waste streams as well as animal by-product streams [3-5] were considered for cost-efficient PHA production from mL- to 750 L-scale using different feeding strategies and partial implementation of novel inline PAT tools for online determination of biomass and PHA accumulation. High-cell-densities of up to 150 gL-1 biomass and up to 2 g(Lh)-1 PHA were achieved, and a total PHA production of > 100 kg was obtained. The monomer content of the PHA copolymer poly(hydroxybutyrate-co-hydroxyhexanoate) [P(HB-co-HHx)] was adjusted between 0 and >30 mol% HHx by adjusting the feeding. Downstream processing with non-halogenated solvents [6] was further optimized, including mechanical and enzymatic treatments. Together with cooperation partners, efforts are being made to optimize the recovery of the intracellular PHA granules from the surrounding biomass and to produce tailor-made biopolymers for various applications such as injection molding, textile fibers, packaging materials, coatings, and material for medical applications. The latest findings will be discussed.

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3. Riedel, S.L., Donicz, E.N., Ferré-Aparicio, P., Santolin, L., Marbà-Ardébol, A.M., et al. (2023), Workflow for shake flask and plate cultivations with fats for polyhydroxyalkanoate bioproduction, App. Microbiol. Biotechnol., 107: 4493-4505.
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5. Gutschmann, B., Maldonado Simões, M., Schiewe, T., Schröter, E.S., Neubauer, P. et al. (2023), Continuous feeding strategy for polyhydroxyalkanoate production from solid waste animal fat at laboratory-and pilot-cale, Microb. Biotechnol., 16: 295-306.

10:15 Multiscale modelling of polyhydroxyalkanoate biopolymer production (L24)

Stefanie Duvigneau[1], *Robert Dürr*[2], *Achim Kienle*[1,3]

[1]*Max Planck Institute for Dynamics of Complex Technical Systems, Magdeburg, Germany*
[2]*Magdeburg-Stendal University of Applied Sciences, Germany*
[3]*Otto von Guericke University, Magdeburg, Germany*
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The term "multiscale" has a variety of different meanings in biotechnology. It usually refers to different production scales, such as laboratory, pilot and industrial scales. In the area of mathematical modelling, the term "multiscale" model defines a combination of different abstraction levels in one model construct with the aim of obtaining a more detailed model description and thus providing a broader information spectrum.

This contribution aims to present a multiscale approach to describe the polyhydroxyalkanoate (PHA) production process in *Cupriavidus necator* [1]. With this approach, consumption and production dynamics from a metabolic model are linked to the current chain length distribution dynamics in the reactor. A key advantage of this model compared to other process models is that both product quantity and quality can be estimated. More precisely, with the multiscale model developed for this example case, it becomes possible to simulate PHA yield and composition as well as polymer chain length distribution at different time points.

In the talk, we will present the simulation results using our multiscale approach for poly(3-hydroxybutyrate) production using fructose and acetate [2] and the production of the copolymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) in a fed-batch process using propionic acid as feed [3]. Those simulation results are compared with experimental data from lab-scale bioreactor experiments.

1. Dürr, R., Duvigneau, S. and Kienle, A. (2021), Microbial production of polyhydroxyalkanoates – modeling of chain length distribution, Comput. Aided Chem. Eng., 50: 1975–1981.
2. Duvigneau, S., Dürr, R., Wulkow, M. and Kienle, A. (2022), Multiscale modelling of the microbial production of polyhydroxyalkanoates using two carbon sources, Comput. Chem. Eng., 160: 107740.
3. Duvigneau, S., Wilisch-Neumann, A., Dürr, R. and Kienle, A. (2023), Modelling and experimental validation of Poly(3-Hydroxybutyrate-co-3-Hydroxyvalerate) chain length distribution, Comput. Aided Chem. Eng., 52: 2223-2228.

10:40 Coffee break & exhibition

SESSION 8: INTEGRATED BIOPROCESSES

Chair Stefan Junne / Lara Santolin

11:10 Production of PUFAs from dark fermentation effluent with *Schizochytrium limacinum* SR21 (L25)

Simon Täuber[1], *Peter Neubauer*[1], *Stefan Junne*[2]

[1]*Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Berlin, German*
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Docosahexaenoic acid (DHA), a polyunsaturated fatty acid (PUFA), is the predominant structural fatty acid in the human retina and brain's grey matter. It plays a crucial, health-promoting role in the body. Due to overfishing, DHA from fish meal and fish oil represents an unsustainable and increasingly expensive source. Additionally, DHA and other PUFAs from fish are also used in fish feed, making sustainable fish production from aquaculture currently unsustainable in comparison to PUFAs from microbes, in particular if biogenic residues are used as substrate [1].
The marine protist *Schizochytrium limacinum* SR21 offers a viable alternative for sustainable DHA production, as it can grow on various carbon sources, including short-chain carboxylic acids (SCCAs) [2]. While acetic acid and butyric acid are preferred carbon sources, with which intracellular concentrations of 47 mgg-1 and 60 mgg-1 DHA are obtained after 78 h, *S. limacinum* can also grow on otherwise rarely applicable acids like propionic and lactic acid, with which a DHA concentration of 31 mgg-1 and 46 mgg-1 is reached. These features allow the application of acid mixture feed, like effluents from microbial hydrolysis in dark fermentation operation mode (DF), rich in SCCAs and other nutrients.
A two-phase fed-batch process in pH-auxostat mode was developed: In the initial growth phase, glucose served as the primary carbon source. In the subsequent production phase, DF effluent was employed both as a secondary carbon source and a pH regulator for PUFA production at the highest feasible feeding rate. Given the limited carbon content compared to pure SCCA feed, an increased amount is required. To prevent a concomitant dilution of the cell suspension, a hollow fibre membrane for cell retention was successfully applied. It was shown that this process design enabled the complete consumption of the SCCAs while yielding specific concentrations of PUFAs and a productivity comparable to pure feed of SCCAs among the tested scenarios.
Coupling DF effluent utilization to a subsequent cultivation of *S. limacinum* for PUFA accumulation, was proven to be successful. While microbial hydrolysis in DF mode offers the possibility to apply a wide range of residual feedstock, substrate costs are considerably low in comparison to many other feedstocks currently applied for the microbial production of PUFAs. Future work will consider how to deal with the typical alternations in the DF effluent's composition, e.g. with a suitable monitoring concept.

1. Bartek, L., Strid, I., Henryson, K., Junne, S., Rasi, S. and Eriksson, M. (2021), Life cycle assessment of fish oil substitute produced by microalgae using food waste, Sust. Prod. Consumpt., 27: 2002-2021.
2. Junne, S., Cziommer, J., Täuber, S. and Neubauer, P. (2022) Chapter 8: From cellulose to lipids. In: Biorefinery - From Biomass to Chemicals and Fuels: Towards Circular Economy, M. Aresta, A. Dibenedetto, F. Dumeignil (eds.), Berlin, Boston; De Gruyter, pp. 229-264.

11:35 Bioprocess optimization for lactic and succinic acid production from a pulp and paper industry side stream (L26)

Agata Olszewska-Widdrat, Joachim Venus

Leibniz Institute for Agricultural Engineering and Bioeconomy (ATB).
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The effective and cheap production of platform chemicals is a crucial step towards the transition to a bio-based economy. In this work, biotechnological methods using sustainable, cheap, and readily available raw materials bring bio-economy and industrial microbiology together: Microbial production of two platform chemicals is demonstrated lactic (LA) and succinic acid (SA) from a non-expensive side stream of pulp and paper industry (fibre sludge) proposing a sustainable way to valorise it towards economically important monomers for bioplastics formation. This work showed a promising new route for their microbial production, which can pave the way for new market expectations within the circular economy principles. Fibre sludge was enzymatically hydrolysed for 72 h to generate a glucose rich hydrolysate (100 $g{\cdot}L^{-1}$ glucose content) to serve as fermentation medium for *Bacillus coagulans* A 541, A162 strains and Actinobacillus succinogenis B1, as well as *Basfia succiniciproducens* B2. All microorganisms were investigated in batch fermentations, showing the ability to produce either lactic or succinic acid, respectively. The highest yield and productivities for lactic production were 0.99 $g{\cdot}g^{-1}$ and 3.75 $g{\cdot}L^{-1}{\cdot}h^{-1}$ whereas the succinic acid production stabilized at 0.77 g·g−1 and 1.16 $g{\cdot}L^{-1}{\cdot}h^{-1}$. This work shows a promising route for side streams utilization in terms of pulp and paper industry.

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12:00 Is it possible to produce cultured meat at a farm? Scale-up strategy and realization aspects (L27)

Nico Oosterhuis, Ira van Eelen, Charilaos Korkontzelos

Respect Farms BV, The Netherlands
Email: nico@respectfarms.com

Production of cultured meat is a relatively new and fast-growing field of bioprocessing. Since the successful introduction of the "cultured meat burger" in 2013 by Mosa Meat BV, many new companies started and are currently developing the production of cultured meat in an economically feasible way.

Most new companies focusing on large-scale production, aiming to produce thousands of tonnes of cultured meat per year, which requires the application of very large-scale bioreactors (> 100 m3) at high investment levels. Apart of the financial risks of such investments, also scale-up of mammalian cell cultures to volumes > 20 m3 never has been realized, which introduces a big technical risk as well.

The concept of Respect Farms is after proving feasibility of production at a more limited scale, to offer present farmers a reasonable alternative in case they may lose their traditional business.

For such a concept we need a process design which is simple to operate and guarantees a high level of sterility. Single-use bioreactors designed for biopharmaceutical production, can serve as a basis for such a dedicated process design for a farm-scale operation. These single-use bioreactors available today, may not fully address the capacity needs of cultured meat production, but due to their flexibility, a scale-out approach is possible, and application of such reactor types makes the seeding steps of these processes more flexible and more reliable in terms of sterility which is an important aspect for successful operation at a farm.

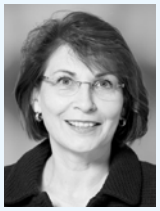
12:25 Lunch break, poster session & exhibition

■ ■ PLENARY TALK

14:00 Single-use bioreactors: Applications and scale-up (PL06)

Regine Eibl-Schindler, Stefan Seidel, Jan Müller, Dieter Eibl

1Zurich University of Applied Sciences, Department N, ICBT.
Email: eibs@zhaw.ch



The WAVE Bioreactor 20 entered the market in 1998. It was the first bag-based, wave-mixed single-use bioreactor. This bioreactor had a maximum working volume of 10 liters and was originally developed to replace the spinner flasks used in inoculum production in production processes with human and animal cells. The success of the Wave Bioreactor 20 resulted in its scale-up and culminated in further single-use bioreactor types. Today, the main single-use bioreactors used are wave-mixed, stirred and orbitally shaken systems, which are available from various manufacturers from milliliter to cubic meter scale. Existing single-use bioreactor platforms make it easier for users to scale-up processes within a bioreactor series with comparable geometry.

Currently, single-use bioreactors are primarily used in R&D and the production of pre- and clinical samples in mammalian cell culture processes. However, their use in the production of commercial biotherapeutics, both in inoculum production and product expression, has been increasing rapidly in recent years. This can be explained, among other things, by the trend towards intensified and continuous production processes, in which 1 m^3 or 2 m^3 production bioreactors are often sufficient. In addition, single-use bioreactors are recommended for the production of cell and gene therapeutics, which are considered to have enormous growth potential within the pharmaceutical sector in the future.

Based on an overview of frequently used single-use bioreactors, examples of process developments realized with them from our own research will be presented. We will show that single-use bioreactors are successfully applied beyond the cultivation of animal and human cells. The process scale-up was also carried out for bioreactors that are not geometrically similar.

SESSION 9: SCALE DOWN AND SCALE UP OF BIOPROCESSES

Chair Cees Haringa / Sarah Westarp

14:45 Scalable microbioreactor system enabling efficient *Pichia pastoris* clone screening for production of biopharmaceuticals (L28)

Eva Palmqvist

R&D CMC, Microbial Platform, Upstream Processing, Sanofi Frankfurt.
Email: EvaMariaAkke.Palmqvist@sanofi.com

Pichia pastoris (syn. Komagataella phaffii) is a widely used expression host for production of recombinant proteins, often using the methanol-inducible expression system based on the strong PAOX1 promoter. For large scale production, the use of methanol requires special consideration to address health, safety and environmental issues, thereby increasing the complexity of the process.

Therefore, Sanofi's microbial expression platform is exploring the use of a de-repressible promoter, originated from *Hansenula polymorpha*, that delivers high titers of recombinant protein without the drawbacks associated with the use of methanol.

In order to improve and expand the clone screening and bioprocess development platform for early-stage projects using the methanol-free promoter technology, we established a scalable microbioreactor system enabling online monitoring, mimicking production scale conditions using the BioLector Pro technology (Beckmann Coulter).

15:10 Automating the DBTL-cycle for *E. coli*: Integration of modular cloning, CRISPR-Cas9 and proteomics for advanced strain engineering (L29)

Tim Stoltmann[1], Julia Tenhaef[1], Till Redeker[1], Luisa Wachtendonk[1], Susana Matamouros[1], Jan Marienhagen[1,2], Stephan Noack[1]

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Email: tim.stoltmann@fz-juelich.de

The current process for developing microbial strains is both time-consuming and costly, mainly due to the many manual steps involved. Through miniaturization, parallelization, automation and digitalization, there is great potential for a significant acceleration of strain engineering. Here, we present automated workflows tailored to *E. coli* based on the seamless integration of modular cloning techniques (MoClo), genome editing tools (CRISPR-Cas9) and quantitative omics methods (proteomics).

MoClo enables the rapid screening of libraries with various DNA building blocks such as promoters or ribosomal binding sites. The plasmid-based workflow includes the entire Golden Gate reaction up to the transformation and plating of 96 assemblies on one plate. The additional integration of an automated CRISPR-Cas9 module enables the precise and targeted modification of the *E. coli* genome, which is crucial for the further metabolic engineering of producer strains. The latter covers the entire process from the generation of single guide RNA plasmids to the actual knock-out and curing step. Finally, to accelerate the subsequent phenotypic characterization of the genetically modified strains, an automated high-throughput module for untargeted proteomics was set up. The entire workflow, spanning from cultivation through bead mill processing to the Bradford assay, can be represented on the platform.

In summary, the automated MoClo, CRISPR-Cas9 and proteomics workflows provide a comprehensive toolset for the rapid engineering of *E. coli* strains. Each module can be operated with minimal human interaction and high robustness, also facilitating the exploration of the genetic diversity of this important model organism.

1. Iverson, S.V., Haddock, T.L., Beal, J. and Densmore, D.M. (2016), CIDAR MoClo: improved MoClo assembly standard and new *E. coli* part library enable rapid combinatorial design for synthetic and traditional biology, ACS Synth. Biol., 5: 99-103.

2. Jiang, Y., Chen, B., Duan, C., Sun, B., Yang, J. and Yang, S. (2015), Multigene editing in the *Escherichia coli* genome via the CRISPR-Cas9 system, Appl. Environ. Microbiol., 81: 2506-2514.

15:35 Model-based scaling strategies of *Pseudomonas putida* fed-batch fermentations (L30)

Maryam Jamshidzadeh[1], Antonia Ursula Griesz[1], Jesper Wang Jensen[1], Ulrich Krühne[1], John M. Woodley[1], Krist V. Gernaey[1], Pablo Ivan Nikel[2], Helena Junicke[1]

[1]Department of Chemical and Biochemical Engineering, Process and Systems Engineering Centre (PROSYS), Technical University of Denmark (DTU), Lyngby, Denmark
[2]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark (DTU), Lyngby, Denmark
Email: heljun@kt.dtu.dk

– withdrawn –

Fed-batch fermentation processes are marked by challenging variations of the reactor liquid volume, mixing time, feed rate, mass transfer, and shear rate. Computational fluid dynamics (CFD) is a valuable tool to increase our understanding of the local distribution of key process parameters, to identify gradient formation and to propose the most suitable scale-up strategy with minimized scaling losses. Using a CFD approach, the scale-up of Pseudomonas putida fed-batch fermentations was assessed, starting from a 2 L lab-scale bioreactor to a 200 L pilot-scale reactor. The multiphase Euler-Euler CFD model was coupled to a kinetic model of *P. putida*. The model was validated against experimental data of the volumetric mass transfer coefficient and mixing times. By applying response surface methodology, a performance map of the pilot bioreactor was generated at different aeration rates, agitation rates, and reactor filling volumes. Different scale-up strategies were evaluated, including constant aeration rate, constant tip speed, constant power to unit of liquid volume, and constant volumetric mass transfer coefficient. Eventually, optimum operating conditions were identified at different stages of the fed-batch process, and the process performance was evaluated. The resulting performance map opens up the opportunity to evaluate the potential and severity of oxygen and substrate gradient formation at different reactor liquid volumes of the pilot bioreactor.

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2. Davis, R., Duane, G., Kenny, S.T., Cerrone, F., Guzik, M.W., et al. (2015), High cell density cultivation of *Pseudomonas putida* KT2440 using glucose without the need for oxygen enriched air supply, Biotechnol. Bioeng., 112: 725-733.

3. Lara, A.R., Galindo, E., Ramírez, O.T. and Palomares L.A. (2006), Living with heterogeneities in bioreactors, Mol. Biotechnol. 34: 355–381.

16:00 Closing remarks and awards for the three best posters and talks by young scientists
Peter Neubauer, TU Berlin, Germany

16:20 End of symposium

Best Poster & Presentation Award supported by: SCI where science meets business

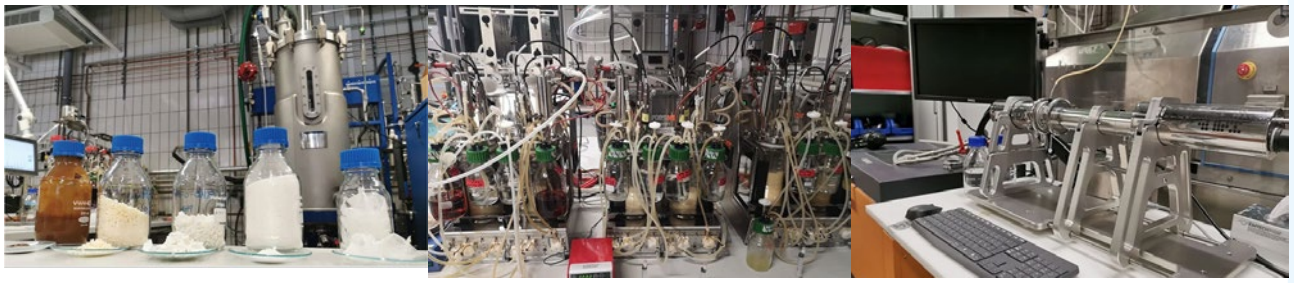
AFTER SYMPOSIUM EXCURSION

AFTER SYMPOSIUM EXCURSION

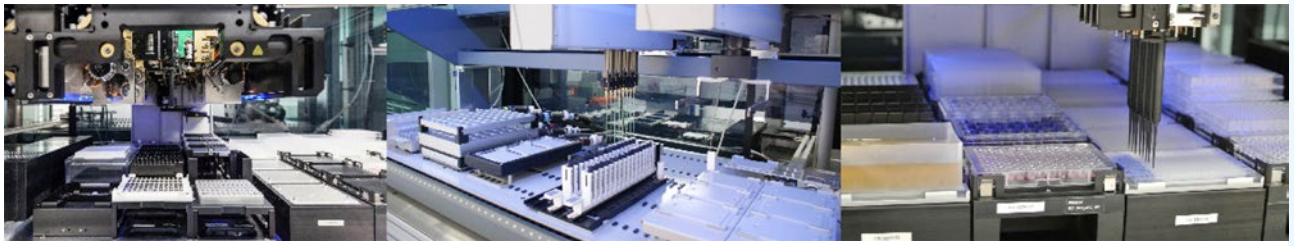
17:30 **Visit to the laboratory of the Chair of Bioprocess Engineering, TU Berlin, Ackerstraße 76, 13355 Berlin**

For those interested to visit the Pilot Plant and High Throughput Bioprocess Development (KIWI-biolab) at TU Berlin, we will organize a small tour following the closure of the symposium. The tour includes:

***Pilot Plant:** It consists of stainless steel bioreactors from 1L to 100L and a 200 L CELL-tainer single use system for pilot-scale production, a Flonamics automatic sampling system and cutting-edge process analytical tools (PAT, e.g. in-situ / in-line technologies for particle / cell analysis, namely Photon Density Wave spectroscopy and SOPAT microscopy). Also new approaches for process monitoring systems are developed.*



*At **KIWI-biolab** we are pioneering the development of innovative bioprocesses through automation, AI integration and advanced analytics. During your visit, we will share insights, discuss potential collaborations, and explore how our expertise can complement each other. If you have any questions or would like to explore something specific during your visit, please feel free to contact mariano.n.cruzbournazou@tu-berlin.de.*



***Location:** Ackerstraße 76, 13355 Berlin (20 min by tram, 8 min by car):*

The number of participants is limited to 40. Please register before (no extra charge) at

18:30 *End*

SCIENTIFIC POSTER ABSTRACTS

P01: Towards a biological-driven bioprocess development

*Jonas Lange[1], Vasundhara Karthikeyan1, Alexander Grünberger[2], Nadja Alina Henke[1]**

[1]CZS Junior research group BIOSCALE, Institute for Process Engineering in Life Science, Karlsruher Institute of Technology (KIT), Karlsruhe, Germany
[2] Microsystem in Bioprocess Engineering, Institute for Process Engineering in Life Science, Karlsruher Institute of Technology (KIT), Karlsruhe, Germany
Email: nadja.henke@kit.edu

Bioprocess engineering is challenged by the forthcoming transition into a circular economy. The number and complexity of newly designed microbial bioprocesses will steadily increase upon the upcoming decades in order to realize sustainable and carbon-optimized processes. Nowadays, microbial bioprocesses represent a minority of industrial processes whereas a limited number of microorganisms are used. It could be expected that both the quantity of processes as well as the quantity of cell factories will grow in the future. Thus, bioprocess engineering and scale-up has to be performed more rapidly and with high precision to guarantee new production processes with high quality. In contrast to state of the art technical-driven bioprocess engineering concepts that consider the biology of the bioprocess in terms of key performance indicators (e.g. growth, biomass/product titer, volumetric productivity), a holistic and better understanding of the biological mechanisms within a bioprocess should be considered [1] in order to build new and powerful concepts for the prediction of the optimal bioprocess parameter space.

In the here presented work, I would like to discuss the idea of a biological-driven concept, that considers holistic and biological transcriptomic data sets from microbial bioprocesses. Next generation sequencing approaches such as RNA-seq [2] are powerful tools that have the potential to understand bioprocesses with a better spatial temporal resolution. The aim of the study is a transcriptional profiling that allows a mapping of the biology of a bioprocess towards the technical parameter space and thus has the potential to allow for the prediction of optimal strain and bioprocess engineering strategies.

1. Masson, H.O., Karottki, K.J.C., Tat, J., Hefzi, H. and Lewis, N.E. (2023), From observational to actionable: rethinking omics in biologics production, Trends Biotechnol., 41: 1127-1138.
2. Wang, Z., Gerstein, M. and Snyder, M. (2009), RNA-Seq: a revolutionary tool for transcriptomics, Nat. Rev. Genet., 10: 57-63.

P02: Creating a closed process semi-automated workflow for human MSC expansion, harvest, and final fill

Julien Muzard[1], Donnie Beers[1], Joy Chen[1], Joseph Hauptman[1], Ross Acucena[1]

[1]Entegris Inc, Billerica, MA, United States
Email: julien.muzard@entegris.com

Costly all-in-one cell processing systems are commonly used in the development and production of cell and gene therapies. Large scale production of these therapies requires bioreactors to generate enough biological material, with reproducible and aseptic workflows, often warranting precious and costly cleanroom space. While commercially available bioreactor platforms allow for large scale production of cell therapies, they are often limited in scaling processes, are inherently inefficient, require skilled personnel, and lack flexibility. Employing a decentralized manufacturing strategy, cost-effective production of advanced therapeutics using modular closed-system production workflows may be achieved at a fraction of the cost of all-in-one systems. This presentation describes the results of expanding bone-marrow derived mesenchymal stem cell (bMSC) on Corning CellBIND microcarrier beads in a modular closed-system. The workflow was assembled using specialized single-use off-the-shelf bioprocessing consumables from Entegris, Chemglass rocking bioreactor modules, and the BioLife Signata CT-5 liquid-handling system. The modular workflow allowed for closed-system processing over multiple steps, including bioreactor MSC expansion, aseptic culture monitoring, cell-microcarrier detachment, and final filling. The entire workflow was arranged and performed on a single table in a standard BSL2 laboratory. Samples were collected aseptically daily for seven days, and over this period three population doublings were recorded. The study results indicate that this modular system is suitable for both suspension and adherent cell growth, capable of scale up and scale out from lab to commercial levels.

P03: Mimicking large-scale mixing times in a laboratory-scale single multi compartment bioreactor system

Jonas Barczyk[1], Carolin Bokelmann1, Lena Gaugler[1], Ryan Rautenbach[2], Sebastian Hofmann[2], Michael Schlüter[2], Ralf Takors[1]

[1] University of Stuttgart, Institute of Biochemical Engineering, Allmandring 31, 70569 Stuttgart, Germany;
[2] Hamburg University of Technology (TUHH), Institute of Multiphase Flows, Eißendorfer Str. 38, 21073 Hamburg, Germany
Email: jonas.barczyk@ibvt.uni-stuttgart.de

In industrial-scale cell culture processes, the formation of heterogeneous zones due to slow mixing is an important topic for bioprocess development [1,2]. However, doing research on cell culture performance in large-scale is difficult, due to the cost of operating a reactor with large volume.

This study addresses the issue of investigating heterogeneities in large-scale bioprocesses which influence cell culture performances and product quality. In recent publications a single multi-compartment bioreactor system (SMCB) was developed to mimic slow mixing times and therefore potential oxygen, pH and substrate heterogeneous zones in laboratory scale stirred tank bioreactors of approximately 3 litres working volume [3]. For this system, discs with different exchange areas are implemented inside the reactor to alter the mixing properties. The SMCB scale-down model could already link different power inputs to changes in product quality based on glycan analysis of the produced antibodies [4].

As a next step, a scale-down reactor model containing three compartments is designed and tested with an optical mixing time determination method [5]. The mimicked compartments and mixing times are based on recent measurements with Lagrangian sensor particles of a 12 500 litre reactor [6]. Additionally, there are first attempts to model the SMCB system with a computational fluid dynamics (CFD) model. With this CFD guided approach, the aim is to predict the placement of discs with different exchange areas to mimic a wide range of industrial scale bioreactors with different size and stirrer setup in a small-scale laboratory environment.

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2. Zakrzewski, R., Lee, K. and Lye, G.J. (2022) Development of a miniature bioreactor model to study the impact of pH and DOT fluctuations on CHO cell culture performance as a tool to understanding heterogeneity effects at large-scale, Biotechnol. Prog. 38: e3264.
3. Gaugler, L., Mast, Y., Fitschen, J., Hofmann, S., Schlüter, M. and Takors, R. (2022), Scaling-down biopharmaceutical production processes

via a single multi-compartment bioreactor (SMCB), Eng. Life Sci., 23: e2100161.

4. Gaugler, L., Hofmann, S., Schlüter, M. and Takors, R. (2024), Mimicking CHO large-scale effects in the single multicompartment bioreactor: A new approach to access scale-up behavior. Biotechnol Bioeng., Epub ahead of print, doi: 10.1002/bit.28647.
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6. Fitschen, J., Hofmann, S., Kursula, L., Haase, I., Wucherpfennig, T. and Schlüter, M. (2023), Advances in characterization of industrial bioreactors for cell culture process, Biopharm. Manuf., 11: 67–111.

P04: CFD-guided scale-down for end-in-mind bioreactor development: from 2000 L to 2 L

Miki Segami[1], Marieke E. Klijn[1], Tim Overkleeft[2], Marcel Ottens[1], Cees Haringa[1]

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The development of upstream manufacturing processes for novel biotherapeutics progresses gradually from the laboratory to larger scales. However, often lab-scale bioreactor processes do not perform as expected when translated to manufacturing scale. Different dimensions and mixing/sparging capacities have a significant impact on hydrodynamics and mass transfer, which consequently affect mammalian cells. Computational Fluid Dynamic (CFD) modelling allows the study of hydrodynamics and mass transfer inside bioreactors, posing a promising method to understand environmental conditions in large scale bioreactors and replicate these in lab-scale bioreactors. The use of Reynolds-Averaged Navier Stokes (RANS) models in the Finite Volume (FV) framework is well established for this purpose, but computational expense and challenges in modelling gas-liquid flows prohibit routine application. As an alternative, Large Eddy Simulations (LES) in the Lattice-Boltzmann framework are gaining traction, but are less mature in their application. In this work, both approaches are compared in regard to simulating lab-scale bioreactors for mammalian cell applications characterized in literature. The best approach is selected based on accuracy of velocity fields, turbulent kinetic energy, mixing time and gas hold-up. Subsequently, the selected method is used to simulate a 2000 L bioreactor to study the limiting environmental conditions (mixing, oxygen transfer, or shear stress) at industry-relevant scale to determine appropriate process parameters for a lab-based scale-down model. This model will serve as a proof-of-concept for routine application of CFD models for scale-down and end-in-mind bioreactor development for biotherapeutics manufacturing.

P05: CFD simulation of pH gradients and their effect on ester hydrolysis by *Candida antarctica* lipase B

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Computational fluid dynamics (CFD) simulations are an important tool which, when utilized correctly, contribute significantly towards understanding problems that are being observed in the biotech industry. However, the complexity of bioprocesses is challenging for such deterministic modelling tools [1].

In this work, CFD is used to understand the impact of gradients in pilot scale bioreactors on biocatalysis (example case: 200L, 150rpm, mixing time ~37s). The potential of biocatalysis has been widely recognised and used in the small-molecule pharma industry but has not yet been widely applied to lower-priced chemicals. To improve its feasibility in this industry, understanding the effect of gradients on biocatalysis is of significance [2]. Though the relevance of gradients in fermentation has been well established, this topic is yet unexplored for biocatalysis [2]. To explore this phenomenon for biocatalysis ester hydrolysis by Candida antarctica lipase B forming an acid and an alcohol has been identified as a model reaction. To neutralize the acid production, a base is added to the reactor, which will result in pH gradients, with a high pH around the base dosing point. To implement this process in a simulation, experiments were executed to measure the reaction rate (~ 0.001 s^(-1),pH 7) at different pH values to mathematically implement the reaction rate as a function of pH and enzyme concentration. The resulting simulation has been used to investigate different scenarios, like position and number of base dosing points, impeller speed and concentration of dosed base. In the future the simulation will be validated experimentally.

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P06: Challenges of bioprocess scale-down on an automated platform

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Biopharmaceuticals are indispensable for global healthcare with many running production processes. New research insights enable the optimization of established industrial processes. During the optimization process, scale-down platforms increase the experimental throughput. Large data sets can be generated which serve as a basis for further model-based process optimization.

We present the systematic scale-down of an established industrial antigen-producing fed-batch process in *Escherichia coli* into a small-scale screening platform using 24 parallelized mini-bioreactors in the mL-scale [1]. Process conditions and the computational backbone for automated process control were adapted.

Since the transition between scales impacts the growth, optimizing the oxygen supply and nutrient availability is important in the process of down-scaling [2]. This includes the medium adaptation, as the amino acids uptake from yeast extract promotes bacterial growth and influences biomass production, metabolic pathways, and overall product formation. To ensure robustness in the high-throughput platform, feeding profiles alongside medium composition and aeration must be considered. Furthermore, operating fed-batch cultivations in robotic platforms poses additional challenges. Feed solutions are administered through pulse-based needles on liquid handling stations, rather than pumps. Additionally, high sample volumes must be handled and limited analysis options are present.

The generated data for initial modeling approaches with a macro-kinetic model [3] will be used to extend the model to encompass various carbon sources, facilitating the comparison of their respective influences on microbial growth. As a result, automation and modeling thus accelerate the development of bioprocesses, enabling in silico screenings for the expression of an antigen.

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P07: Microfluidic single-cell cultivation: A game-changer in predicting bioprocess scalability?

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Identifying the most promising producer strain and scaling up of a novel bioprocess comes with different issues: Due to environmental gradients and varying physical properties [1], unrepresentative behaviour of cells in larger stages compared to lab-scale can be the result [2,3]. Consequently, scale-up becomes time-consuming, expensive, and there is no guarantee for a sufficient bioprocess [4].

Hence, there is a need for new approaches to predict scalability of bioprocesses already during the lab-scale development stage. In this context, especially the robustness of the novel bioprocess concerning key performance indicators should be focus of investigations. To get a representative result, analyses should optimally be performed under relevant bioprocess conditions equalling those occurring in production-scale vessels. At the end, a prognosis of a bioprocess' scale-up potential should be the result of such an investigation.

We believe that a combination of purposeful CFD simulation and dynamic microfluidic single-cell cultivation (dMSCC) represents an answer to this challenge. By simulating production-scale bioreactors in terms of respectively occurring gradients and lifelines of cells within the bioreactor [5], the relevant conditions for subsequent scalability analysis are determined. Following, dMSCC can be used to emulate these conditions on-chip. With its special properties dMSCC allows for abrupt changes in cultivation conditions and the analysis of key performance indicators on the single-cell level [6]. Therefore, cellular behaviour can be examined under production-scale equivalent environments [7]. In the future, applying the proposed approach might lead to a workflow that allows reliable predictions of bioprocess scalability already during bioprocess development.

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P08: Bridging the protein gap using single cell protein

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At dsm-firmenich, we aim to develop alternative protein sources, for use as ingredients in the aquaculture industry and in pet feed, which are more sustainable compared to commonly used protein sources. Current sources of protein in animal feed, like fish meal and soy protein hydrolysate, are associated with sustainability issues, such as land use change, loss of biodiversity, carbon footprint and impact on life in oceans. Protein produced using microorganisms, also known as single cell protein (SCP), however, has a far more sustainable footprint in comparison.

To additionally limit the carbon footprint, we aim to produce this SCP in a two-stage process, in which already commercialized processes to convert CO_2/H2 to alcohols using renewable energy, are combined with aerobic fermentation. A screening protocol, executed in microtiter plates, shake flasks and bioreactors, was devised to select for yeast strains producing SCP with various criteria: 1) high protein content, 2) essential amino acid composition and 3) beneficial effects on health.

Protein content in yeast cells is highly dependent on the growth rate at which the yeast is cultivated. As such, a fermentation process was designed around the selected yeast maintain high growth rates throughout the fermentation and produce biomass with high protein content. Additionally, process optimization was performed towards improving the overall process productivity.

An initial SCP prototype was produced at pilot scale and extensively evaluated in aquaculture fish trials. These fish trials showed comparable yields for growth on SCP compared to the conventional protein sources.

P09: Robustness characterization of AMP producing *C. glutamicum* strains on single-cell level

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Preservation of food by fermentation is based on the exclusion of pathogens and food-spoiling organisms by microorganisms associated with fermentation [1]. One important mechanism of exclusion is the production of antimicrobial peptides (AMPs) by the bacteria during the fermentation process [2]. Here, AMP producing strains are exposed to fast changing conditions, e.g., pH value and substrate concentration. This can potentially impact the performance of the cultivated microorganisms, leading, for instance to a reduction in productivity or a decrease in growth rates [3]. These aspects contribute to poor predictability and reproducibility during scale-up of bioprocesses. Consequently, it is imperative to select robust microorganisms that exhibit reduced susceptibility to environmental heterogeneity [4]. For this, microfluidic cultivation systems could lead to a fast determination of robust AMP producing strains under oscillating environmental medium conditions. One example is dynamic microfluidic single-cell cultivation (dMSCC), whereby single cells can be cultivated dynamically under varying environmental conditions with a temporal resolution from seconds to minutes [5]. This project aims to use dMSCC systems to determine growth robustness of AMP producing strains to bioprocess relevant environmental conditions. Focus will be given to understand how oscillation in cultivation conditions impact

the dynamics of microbial growth. For this, selected oscillating profiles of different carbon concentrations, pH values and AMP levels will be investigated. With this approach, the probability of success in developing robust bioprocesses could potentially be increased through the selection of robust production strains using microfluidic cultivation systems. Latest progress and results will be shown and discussed.

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P10: Analysis and control of expression heterogeneity of microbial gene circuits on a single-cell level

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In the realm of bioprocess engineering, cellular heterogeneity, characterized by the presence of distinct subpopulations, poses a significant challenge, as it can adversely affect key performance indicators such as growth rate, product yield, and overall process robustness, thereby impeding bioprocess development and scale-up. Effectively addressing the challenge posed by cellular heterogeneity necessitates the utilization of novel approaches that enable its analysis [1;2]. Among such approaches are dynamic microfluidic single-cell cultivation (dMSCC) systems. In combination with modern live-cell-imaging technologies, the application of dMSCC allows for the precise control of environmental conditions with unparalleled spatio-temporal resolution, enabling the analysis of cellular heterogeneity on a single-cell level [3;4]. In this contribution, we aim to present a concept for controlling expression heterogeneity at the single-cell level through the application of dMSCC systems, with the following objectives: (i) identifying environmental parameters influencing expression heterogeneity, (ii) developing a novel dMSCC system designed to enable precise oscillation of multiple environmental parameters on a timescale ranging from seconds to hours, (iii) determining optimal parameter combinations for effective control of expression heterogeneity, and (iv) devising multi-parameter oscillation patterns for gene expression synchronization and control. By investigating the impact of various environmental conditions on expression heterogeneity, we aim to deepen our understanding of the relationship between the environment and cellular heterogeneity, with the goal of enabling robust control through specific environmental perturbations. The insights gained will lay the foundation for an improved understanding of heterogeneity dynamics and the development of novel bioprocess control strategies.

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P11: Scaling-up of microbial biomass production, with immunomodulating potential

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The bacteria in the group of Enterococci, Streptococci and Staphylococci offer good prospects in order to obtain biomass with immunomodulating effect. The feasibility of scaling up the fermentation process for the industrial application needs to be tested at laboratory and pilot scale. This study aims the scaling up of the biomass production, from the working volume of 1 L up to 10 L, in 3.6 L and 13 L STR bioreactors (InforsHT). Therefore, the growth dynamics, as well as the reproducibility grade of the batch mode fermentation processes in glucose - peptone like industrial medium of the following strains such as Enterococcus faecalis, Streptococcus pyogenes and Staphylococcus aureus have been tested. Therefore, the critical scaling up point has been identified monitoring parameters such as turbidity, specific growth rate, pH and the biomass productivity. The growth profiles showed good reproducibility of the growth dynamics, good growth and scalable potential from 1 L to 10 L culture, in aerobic conditions for all three strains. The growth profiles revealed the critical scaling up points after 3, 4 and 8 hours of cultivation for E. faecalis, S. pyogenes and S. aureus, respectively, in both working volumes of 1L and 10L, with a slightly higher specific growth rate (μmax) for lactic acid bacteria E. faecalis and S. pyogenes after scaling up the cultivation process to 10 L. The online data were processed with the eve® soft (InforsHT) and Sigma Plot 14 programmes. The reproducibility grade has been statisticaly calculated using the Tukey test.

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P12: Design and optimization of animal component-free media for plasmid DNA production in *E. coli*

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In this study, we demonstrate the use of screening and optimization to design ACF media that supports similar or greater plasmid DNA production compared to animal containing media. Laboratory scale batch E. coli fermentations performed under unoptimized processing conditions were able to achieve plasmid volumetric yields >50 mg/L using ACF media. While plasmid yields greater than 1 g/L have been reported for larger scale optimized fed-batch fermentation processes, the yields obtained in this work are comparable to reports using batch fermentation at <100-L scale. Insights from this work will be used to develop novel ACF media for upstream plasmid DNA manufacturing and further process development.

P13: From microtiter plate to fermenter: scale-up of a *Vibrio natriegens* fed-batch process

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Vibrio natriegens is a promising new host for biotechnological processes due to its high growth rates and its nutritional flexibility. Rapid growth is beneficial to accelerate fermentation processes and thereby improving the efficiency of industrial bioprocesses. One challenge when working with V. natriegens is its strong overflow metabolism and mixed acid fermentation [1]. To avoid such phenomena, comparable industrial processes are often carried out in fed-batch mode [2]. To date, only a few fed-batch processes for V. natriegens have been reported in the literature. Hence, a comprehensive fed-batch process development should be carried out.

First, a fed-batch process in microtiter plates with online monitoring of the oxygen demand was conducted. This was followed by an oxygen demand-based scale-up to membrane-based fed-batch shake flasks. Finally, a scale-up into a lab-scale fermenter was performed. Oxygen consumption, pH value, optical density, glucose consumption, and acetate production were monitored during cultivation to gain a better understanding of the process and to compare between different scales. In all tested scales, overflow metabolism and mixed acid fermentation were prevented during the feed phase.

In summary, scale-up from microtiter plates to liter scale works successfully for V. natriegens. Additionally, overflow metabolism and mixed acid fermentation can be prevented by performing fed-batch cultivation instead of batch.

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P14: Process characterization across scales of an industrial *Aspergillus oryzae* aerobic fed-batch fermentation process

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One of the biggest challenges of biomanufacturing is understanding the sources of batch-to-batch variation at industrial scale. This is important when developing process models that can be used to improve fermentation processes. This contribution focuses on an *Aspergillus oryzae* fermentation process, which exhibits significant variation from batch-to-batch at both production and pilot scales. The objective is to comprehend the influence of process variables and parameters on the performance of the fermentation process. A critical aspect of filamentous fungi fermentation processes is understanding the relationship between biomass concentration and the broth's rheology, since an increase in broth viscosity leads to a decrease in mass transfer which can affect process performance. The study first examined the levels of biomass concentration and rheology in the industrial-scale process. Then, online process data was analysed to pinpoint potential causes of batch-to-batch variation in production. The process was also scaled-down to pilot scale to conduct an in-depth investigation of key process parameters, feeding settings and agitation power, and their effect on the process' KPIs (product titre and yield coefficients). The results were compared to the large-scale process, where it could be observed that the product yield on substrate was in general higher than at the pilot-scale. Finally, a multi-omics (metabolomics, proteomics and transcriptomics) analysis was conducted for fermentation processes at different agitation and aeration conditions, to evaluate and better understand their impact on the cells' metabolic behaviour. In conclusion, the data gathered and analysed lays a solid foundation for the development and testing of a model.

P15: Intensified proliferation of BY-2 plant cells in structurally modified culture bags for wave-mixed single-use bioreactor

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Wave-mixed single-use bioreactors are widely used in specialized bioprocesses involving high cell-density cultures of shear-sensitive biomass of human, animal, plant, and microorganism origin [1,2]. Results of Nicotiana tabacum Bright Yellow No. 2 (BY-2) cells batch cultures in the ReadyToProcess WAVE™ 25 system revealed limitations that hampered the growth of the dispersed callus cells. As the cell density increased during the culture performed at the wave agitation parameters suggested by the bioreactor manufacturer [3], the dissolved oxygen was depleted by the cells on day 6 of 10 of the culture. Increasing the wave agitation rate did not improve the culture performance – the cells started disintegrating due to excessive shear stress. The aim of the work was to intensify the N. tabacum BY-2 culture by placing passive 3D structural elements on the inner bottom side of the culture bag, which could mitigate the negative effect of increasing culture broth viscosity by positively increasing the mass transfer capabilities of the system.

Initial screening of the modifications' geometry was done using CFD simulations, followed by comparing experimentally acquired t95 and kLa values in original and modified culture bags. Several modifications were selected based on their impact on the maximum shear stress values, mixing efficiency, and mass transfer in the modified culture bag. Bioprocess verification of the structural modifications was done through cultures of tobacco BY-2 cells and evaluated by comparison of obtained fresh biomass values and specified protein bioproduct efficiency.

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P16: Development of an automated online flow cytometry method to quantify cell density and fingerprint bacterial communities

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Cell density is an important factor in all microbiome research, where interactions are of interest. It is also the most important parameter for the operation and control of most biotechnological processes. In the past, cell density determination was often performed offline and manually. While there are now some online methods for rapid and automated cell density determination, they are unable to distinguish between the different cell types in bacterial communities. To address this gap, an online automated flow cytometry procedure is proposed for real-time high-resolution analysis of bacterial communities. On the one hand, it allows for the online automated calculation of cell concentrations and, on the other, for the differentiation between different cell subsets of a bacterial community. To achieve this, the OC-300 automation device (onCyt Microbiology, Zürich, Switzerland) was coupled with the flow cytometer CytoFLEX (Beckman Coulter, Brea, USA). The OC-300 performs the automatic sampling, dilution, fixation and 40 ,6-diamidino-2-phenylindole (DAPI) staining of a bacterial sample before sending it to the CytoFLEX for measurement. It is demonstrated that this method can reproducibly measure both cell density and fingerprint-like patterns of bacterial communities, generating suitable data for powerful automated data analysis and interpretation pipelines. In particular, the automated, high-resolution partitioning of clustered data into cell subsets opens up the possibility of correlation analysis to identify the operational or abiotic/biotic causes of community disturbances or state changes, which can influence the interaction potential of organisms in microbiomes or even affect the performance of individual organisms.

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P17: To Feed or Not To Feed? The challenges of glycerol fed-batch for *Pichia pastoris* expression in shake flasks

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Shake flasks are frequently used for screening steps in process development because they offer a cost-effective way to achieve the necessary throughputs. However, shake flasks differ significantly from industrial production bioreactors in their design, operation, and size. We developed a novel multiparameter sensor (MPS) and dissolved oxygen (DO) sensor pills to unlock the black boxes in shake flask processes. We used protein production in Komagataella phaffii (Pichia pastoris) and collected data with the new sensors to better compare shake flask cultivations with bio reactor runs. Using our cell growth quantifier (CGQ) and liquid injection system (LIS), we were able to translate different phases of bioreactor runs to shake flasks (e.g., batch, fed-batch, adaptation, and induction phase) and gain important knowledge about the protein production in our strains. The glycerol-fed batch phase posed some challenges. Protein production was completely abolished in shake flasks experiments with an "uncontrolled" glycerol addition after the batch phase. In contrast, a glycerol fed-batch in bio reactor runs increases the biomass and improves the product yield. Using the MPS and DO sensor pills, we were able to gain further insights into the process and yielded a 20-fold product increase in shake flask with a successful glycerol fed-batch process.

P18: Effect of cell culture production methods on the survival of probiotic yeast *Saccharomyces cerevisiae var. boulardii* in gut-like conditions

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Saccharomyces cerevisiae var. boulardii is a probiotic yeast used in the treatment of diarrhea. The quality of produced cells is a key to functionality. S. boulardii exercise its activity in the colon, but before it reaches this location, it is exposed to harsh conditions. The challenges include digestive enzymes, low pH of the stomach, sudden pH raise upon entering duodenum and high concentration of bile salts in the duodenum and small intestine. Methods to ensure high survival include encapsulation techniques or addition of prebiotics. In our studies we focused on adaptation of yeast to ensure the required cell functionality.

We have developed a model system that mimics the conditions in the digestive track, which allows us to study growth variation between different strains of S. boulardii with the respect to challenges. The type of medium used to proliferate the cells proved to be crucial for their subsequent survival in a low pH or in presence of bile salts. Our further goal is to investigate how the quality of cells changes with the upscaling of their production.

P19: Elementary Flux Mode Analysis predicts co-culture stability in continuous bioprocesses

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Cell phenotype and metabolism adaptation are common in natural and human-controlled biological processes. Whereas in nature, it usually translates into extended strain fitness, within industrial bioprocessing, it usually results in a loss in process productivity. This problem is more relevant for new-generation bioproducts which require more controlled and specialized bioprocesses. This is the case of the production of specialized compounds by microbial consortia, where the challenge is to constrain populations with specific metabolic functionalities into a designed threshold of productivity. Assessing metabolic distributions is critical for accurately controlling the functionality of these complex and specialized continuous co-culture bioprocesses. In this work, we use Elementary Flux Mode Analysis as a metabolic state profiling tool to characterize the metabolic distributions in bacteria-yeast co-culture couples and establish diverse strategies for their control. The consumption and production yield analysis ,composed from the microorganisms central metabolism network, resulted in a mixed cybernetic model, which described the population and metabolic flux distributions phenomena during co-culture. Yield analysis showed that the higher the degree of separation between substrate consumption capabilities, the more extensive the stability range of the microorganism pair. While the cybernetic model also suggested that dynamic interplay between two different substrates helps alleviate competition by enabling each strain to dominate in different substrate niches, even when both strains are capable of their utilization. An online control of alternate substrate pulsing strategy was then used in a proof-of-concept bioprocess to optimize the production of p-coumaric acid in a continuous co-culture.

P20: Benefits of off-gas analysis – Improved volume calculation for fermentations by monitoring the absolute humidity

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Using sensors for off-gas analysis in fermentation processes is still considered as an add-on instead of an obligatory tool. But beside the obvious benefit of knowing the concentrations of CO_2 and O_2 in the exhaust gas, off-gas analytic can be used as an input for various soft-sensor applications to calculate a variety of physical parameters, such as the evaporation of media. In most fermentations the current process volume is calculated without consideration of the evaporation caused by aeration with dry air. This leads to discrepancies up to 10 %, even if exhaust gas condensers were used to minimize the evaporation. Off-gas sensors can give many information about the gas beside its composition such as pressure, temperature and moisture content. These parameters were usually used to normalize the determined concentrations, but can also help to calculate process related parameters. We created a soft-sensor based on these additional values measured by a BlueVary O_2/CO_2 analyzer to calculate the evaporation caused by aeration. The determined loss of water content can further be used to calculate the corrected fermentation volume. With this we were able to minimize the effect of vaporized medium on the total volume and to optimize volume dependent calculations during the process. Examples like this show how exhaust-gas analytic can help to improve fermentation processes without additional equipment.

P21: Oxygen transfer in non-Newtonian liquids

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For many bioprocesses, gas-liquid mass transfer plays a significant role in reaching optimal process conditions. Therefore, this is a well-studied area, but there is still a lot to learn, especially considering process performance and scaling. Some of the main issues for oxygen mass transfer are the low solubility of oxygen and liquid rheology [1]. Many bioprocesses, like fungi cultivation and biopolymer production, have been shown to start Newtonian and change into non-Newtonian behaviour a long with process evolving. [2]

To focus on scaling, it is important to be able to predict the volumetric mass transfer coefficient (KLa) in case of ensuring a sufficient oxygen supply. Therefore, multiple empirical correlations have been proposed over time, also considering the viscosity. However, many bioprocesses have been shown to have dynamic rheology, making it more difficult to make a correlation for the changing KLa.

For this study, experimental work has been carried out in a 200L working volume bioreactor equipped with baffles, a ring sparger, and three 6-blade Rushton turbines. One of the baffles was equipped with four oxygen probes to ensure homogeneity of the media. The dynamic gassing out method was used to determine KLa. To mimic an increasing non-Newtonian liquid, four concentrations of carboxymethyl cellulose (CMC) were used, creating varying viscosities.

The results showed that the KLa was drastically affected with increasing viscosity, creating oxygen limitations. Furthermore, it was shown that the rheology had an impact on the effect to which the KLa was affected and thereby on the prediction.

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P22: Membrane-free dissolved hydrogen monitoring in hydrolytic and methanogenic bioprocesses

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Flexible feedstock utilization for energy and material use is of great importance to gain locally sufficient substrate for bioprocesses. One way to increase feedstock flexibility is to use microbial hydrolysis to break down complex carbohydrates into short-chain carboxylic acids. These can be used as substrate in subsequent processes. Another byproduct of such processes is hydrogen. It can also be used as substrate, however, monitoring is important due to a low solubility in aqueous media and to avoid excess or starvation when added from outside. Therefore, a novel measurement apparatus for dissolved hydrogen in anaerobic digestion systems has been developed. This system comprises a membrane-free extraction chamber and a metal oxide sensor for hydrogen detection. This was applied in a two-stage digestion process with a microbial hydrolysis of maize silage and bedding straw and a subsequent methanogenesis [1].

In the hydrolytic stage, up to 10 g L-1 of short-chain carboxylic acids accumulated. These were, together with hydrogen, converted so that 60% of methane were achieved in the off-gas.

During the operation of the sensor (for three months in its final version), it responded reliably to dynamic changes in both process stages. Depending on the process conditions, a partial pressure between <10 and >4000 Pa was detected, corresponding to dH2 concentrations of <0.074 to >30 µmol L-1. The sensor itself exhibited a detection limit of 50 ppb (0.37 * 10-4 µmol L-1), a response time (t90) of < 5 s and a selectivity of > 0.8 [2]. The sensor thus allows for better controllability of hydrogenotrophic bioprocesses.

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P23: Investigating signal attenuation in Raman spectra of bacterial fermentations

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In biotechnology, Raman spectroscopy has emerged as a powerful tool for studying fermentations due to its non-destructive nature and ability to provide molecular fingerprinting information. While Raman spectroscopy is used more frequently in cell cultures with lower cell densities, for bacterial fermentation, the adaption rate is low. One of the key challenges in the analysis of Raman spectra obtained from bacterial cultures is high signal attenuation. In this study, we investigate the phenomenon of signal attenuation and its causes in Raman spectra acquired during fermentation processes from two model bacterial species, *Escherichia coli* and *Ralstonia eutropha*. We employed Raman spectroscopy to monitor the spectral changes during the fermentation of both bacterial strains. Our analysis focused on identifying and characterizing the factors contributing to signal attenuation. By systematically varying parameters like antifoam concentration and cell density, we assessed their impact on signal attenuation and spectral quality. Our results reveal a decrease of the signal strength due to increasing cell density and an increased amount of antifoam. Notably, at high cell densities and antifoam concentrations, we observe an extinction of the signal. Finally, we discuss practical strategies for optimizing experimental conditions to improve spectral quality and data interpretation in the presence of high cell densities.

P24: Mathematical modelling of the oxygen transfer rate (OTR) as a first step towards the development of a digital twin

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The oxygen transfer rate (OTR) is often a limiting factor when targeting maximum yield in a fermentation process. Understanding the OTR is therefore critical for improved bioreactor performance, as dissolved oxygen often becomes the limiting factor in aerobic fermentations due to its inherent low solubility in liquids such as in fermentation broths [1]. With the long-term aim of establishing a digital twin framework, the initial phase of development involves mathematical modelling of the OTR in a pilot-scale bioreactor, hosting the filamentous fungus *Aspergillus oryzae* using an elaborate experimental design.

The experimental design is specifically tailored to the interplay of the factors influencing the OTR e.g., airflow, back-pressure and agitation speed. Through a set of 4 fermentation, a full-factorial experimental design with three factors (aeration, agitation, and pressure) and two levels (high and low) is designed. Concluding the 2^3 factorial design, 8 different unique patterns of factors and two centre points were investigated in four different fermentation processes.

Since viscosity plays a crucial role in determining the mass transfer properties in the chosen fungal process, understanding its effects is essential for modelling the OTR [2].

Cell dry weight and off-line viscosity measurements were taken from each of the above-mentioned industrial based fermentation processes throughout the fermentation. The subsequent analysis aims to decipher the relationships between the OTR and the agitation, aeration, head pressure and viscosity, thus providing the basis for an accurate and reliable mathematical model of OTR [2].

This physical OTR model presents the first step towards developing a digital twin, aiding with operational decisions for fermentation processes.

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P25: Digital twin modeling of a pilot-plant disk centrifuge in GFPUV production downstream

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Downstream processing in bio-based manufacturing often constitutes a significant portion of production costs, especially when high purity requirements are involved [1]. Digitalization of these processes aims to provide adequate tools for process optimization by integrating physical (sensors, control elements, etc.) and digital elements (soft sensors, digital twins, etc) [2]. Despite significant improvements in the digital infrastructures, the full potential in value creation remains unrealised. This is especially true in the bioprocess industry due to the complexity of fermentation broth and its effect in the downstream [3].

Pilot-scale university-based bioprocesses provide an excellent opportunity for testing potential value creation through digitalization before rolling it out in production. Stevnsborg et al. developed a digital-twin for a pilot-scale fermentation process for producing recombinant green fluorescent protein (GFPUV) with *E. coli BL21* (DE3). The physical plant is located at the North Carolina State University's (NC State) Golden LEAF Biomanufacturing Training and Education Center (BTEC) [4].

This study adopts the same approach and leverages the same tech stack in an effort to digitize a disk centrifuge, used for cell separation after fermentation. Consequently, we develop a model to predict separation efficiency by providing inputs such as feed flowrate, backpressure, bowl rotation speed, solid discharge time, and the geometric configuration of the unit. The

main goal is to provide a tool for monitoring and optimizing the processes by providing insights to the input-output effects. The model is purely mechanistic and utilizes real plant data and advanced analytics such as uncertainty and sensitivity analyses.

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P26: Robust tube-based MPC for controlling bioprocesses under uncertainty

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Recent developments in biotechnology have led to the need for rapid and robust bioprocess development, especially in the early stages of a project. However, these processes are often characterized by nonlinear uncertain dynamics, presenting challenges for model predictive control (MPC) algorithms which rely on a model to optimize predicted performance. Various approaches can be used to address these challenges, using robust and stochastic MPC. However, these approaches can be over-conservative and computationally intensive if the system is nonlinear [1]. Recent research has shown that coupling tube-based MPC with difference of convex functions (DC) programming techniques enables robust online process control with reduced computation [2]. The approach is based on systematic DC decompositions of the dynamics and successive linearizations around feasible trajectories. The convexity property allows linearization errors to be tightly bounded and treated as bounded disturbances in a robust tube-based MPC framework. However, it can be a difficult task to find the DC composition analytically. To overcome this problem, we use a neural network with a convex structure to learn the dynamics in DC form. We apply this technique to the problem of maximizing the product formation rate of a cultivation with uncertain substrate concentration in the feed, obtaining bounds on the uncertainty in future model states in terms of simplexes. The results show that this is a promising approach for computationally tractable data-driven robust MPC of bioprocesses.

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P27: Potential of predictive model-based dissolved oxygen control for intermittent fed-batch processes

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Ensuring sufficient dissolved oxygen (DO) levels during a cultivation is one of the most essential tasks in aerobic bioprocesses [1]. Therefore, control strategies are employed that act on the stirring speed, aeration rate and oxygen partial pressure in the reactor, often by utilizing PID algorithms of varying complexity [2]. While these controllers perform well in batch or continuously fed systems, their purely reactive nature flashes its limitations when challenged with abrupt changes in nutrient addition. This can occur during the transition between phases or with intermittent feeding profiles in high-throughput small scale multi-reactor systems, where a robot adds the substrate in intervals through bolus shots. The resulting sudden drops in the DO signal can lead to the system being oxygen limited due to the response time of the control loop and consequently lead to the organism changing its metabolism and physiological state [3].

This work investigates the opportunities that model-based predictive DO control algorithms offer to avoid limitations in oxygen supply during the process. The combination of elemental balances, simple growth kinetics and the Van't Riet equation present enough tools to create a process model for the oxygen demand. Provided with the scheduled feeding profile it can predict the resulting metabolic activity and needed changes in stirring speed and aeration within a selected bioprocess. The impact and potential benefits over basic PID control are shown through in-silico simulations for a microbial intermittent fed-batch process.

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P28: Machine learning based compartment models for dynamic simulation of heterogeneous fed-batch processes

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The occurrence of heterogeneous conditions and their impact on cellular metabolism in large-scale bioreactors due to mixing limitations forms a major scale-up risk. Computational Fluid Dynamics (CFD) simulations are a powerful tool to assess their impact [1-3]. However, its inherent computational demand means CFD is limited to evaluating limited process snapshots. This hampers a fast and reliable evaluation of process performance, especially when considering full fed-batch operation with dynamic operating conditions. As an alternative, compartment models (CM) have shown the capability to capture hydrodynamic features present in stirred tank bioreactors at an affordable computational cost [4-6], but incorporating changes in hydrodynamics remain challenging due to their reliance on CFD-derived flow fields [5-7].

In this work, we propose the use of a machine learning (ML)

approach to compute the flow fields in a stirred tank bioreactor, reducing the need for repeated CFD calculations upon changes in operating conditions, such as the stirring speed, or the continuously increasing volume such in fed-batch fermentations. The obtained models allow us to predict the mixing phenomena and substrate gradients with a reduction by two orders of magnitude of runtime when compared with a fully-coupled CFD model.

This work forms a step towards a flexible, rapid simulation framework to optimize the design and dynamic operation of bioreactors, including the impact of heterogeneous environments in industrial-scale bioreactors.

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P29: Accelerating bioprocess optimization and scale-up for a CHO cell culture process using digital models

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Optimization and digitalization of bioprocesses play a central role in current research in biotechnology and bioprocess engineering. In this scientific environment the terms artificial intelligence (AI), automation, digital twin/shadow or digital model are ubiquitous and become more and more important. The increase of efficiency, productivity and yield, as well as the reduction of time, costs and materials are the main benefits of these digitalization efforts. The development and application of different types of digital models - whether it is mechanistic, data-driven or hybrid - is usually the first step in the digitization of the bioprocess, and these models alone offer numerous possibilities for in-silico simulation of various process conditions/modes and also to estimate the scale-up effects in advance. [1;2]

In this contribution, an industrial CHO cell cultivation process for recombinant monoclonal antibody (mAb) production is employed and will be described and optimized by using digital models. To this end, the first step is to identify the optimal bioprocess parameters for the models using historical and highly diverse cultivation data sets from small-scale bioreactors (ambr15® system). With this process knowledge, the models can be applied to simulate and predict the best cultivation conditions to produce higher titers and also to transfer the process in larger scales or in perfusion mode. Real validation experiments then provide information on the accuracy of the in-silico predictions and allow further optimization cycles of the models.

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P30: Enzyme-mediated exponential glucose release: A model-based strategy for continuous defined fed-batch in small-scale cultivations

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Maximum cell growth under substrate-unlimited conditions, as in the batch phase, results in a number of negative effects, including engineering limitations and increased biomass by-product formation. Thus, fed-batch is the preferred cultivation mode in bioprocesses [1]. Consequently, screening for the best strains and optimal growth conditions in miniaturized cultivations should also be performed by applying defined substrate-limited feeding. However, small-scale bioreactors usually lack the miniaturized pumps necessary for fed-batch mode. An alternative are enzyme-mediated glucose release systems from starch-derived polymers, facilitating continuous glucose supply. Nevertheless, while the glucose release, and thus the feed rate, is controlled by the enzyme concentration, it also strongly depends on the type of starch derivative, and the culture conditions. So far it was not possible to implement controlled feeding strategies (e.g., exponential feeding).

In this context, we propose a model-based approach to achieve precise control over enzyme-mediated glucose release in cultivations. An existing mechanistic model [2] was integrated into a computational framework [3] to calculate setpoints for enzyme additions.

We demonstrate the ability of the tool to maintain different pre-defined exponential growth rates during Escherichia coli cultivations in parallel mini-bioreactors integrated into a robotic facility. Although in this case study, the intermittent additions of enzyme and dextrin were performed by a liquid handler, the approach is adaptable to manual applications. Thus, we present a straightforward and robust approach for implementing defined continuous fed-batch processes in small-scale systems, where continuous feeding was only possible with low accuracy or high technical efforts until now.

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P31: Real-time analysis of multicomponent bioprocesses using Raman spectroscopy and RAMANMETRIX™

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In the realm of bioprocess analysis, particularly in pharmaceutical and food production, accuracy is crucial. These complex processes depend on living organisms, making it essential to have rapid, high-performance analytical tools for immediate control and response to any irregularities. Our study emphasizes the effectiveness of combining RAMANMETRIX™, an AI-driven tool, with Raman spectroscopy for real-time monitoring of intricate multicomponent bioprocesses.

Our experiment involved growing a genetically modified E. coli strain in a fermenter. We periodically collected samples for analysis, employing a Wasatch Photonics Raman spectroscope for spectral data collection. These samples were also analyzed using HPLC. RAMANMETRIX™ excels in data importation, calibration, preprocessing, and model creation. This methodology facilitates swift assessment of new samples, bypassing the delay of laboratory analysis. The developed model enables immediate online tracking of key metabolites, such as carbon sources and organic acids, allowing for timely process management.

Furthermore, acknowledging the need for rapid analysis, we have advanced our system to support online capabilities in line with Process Analytical Technology (PAT). By integrating a probe in a viewport, we achieved continuous spectrum recording, enabling live sample analysis without interrupting the bioprocess. This online technique, complemented by traditional HPLC assessments, forms the basis of our reference framework.

To summarize, the integration of AI-powered software with Raman spectroscopy presents a novel method for the real-time analysis of complex bioprocesses. This approach not only promotes efficient production in the pharmaceutical and food industries but also highlights the importance of quick, non-invasive monitoring and control of these processes.

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P32: An open-source online platform for bio-processing

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A sub-project was developed within the WP17 of Inno4vac. The purpose is integrating the compartment models and the kinetic models implemented in an open access software platform. This presentation shows the work together with two master students on it. The platform takes the results from CFD modelling and does the compartmentation through a linked computer and gives the results. After that the kinetic was linked to another platform--CADET, to close the mass balance. The presentation here will mainly show the design of the platform.

P33: Data management in automated transdisciplinary laboratories

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In the field of automated laboratories, the integration of high-throughput liquid handling operations is crucial for advancing research in genomics, drug discovery, and bioinformatics. This poster introduces a platform that integrates both physical and virtual devices to improve the efficiency and adaptability of liquid handling experiments. Central to this system is an orchestrator, a controller that oversees the coordination of various laboratory equipment and the precise management of extensive data sets and sample data. The orchestrator ensures procedural consistency, prevents impractical operations, and facilitates seamless hardware and software interactions. A case study on enzyme characterization demonstrates the system's capability to handle complex, dynamic workflows, via the Cylc version 8 scheduler. The poster concludes that the innovative setup improves scalability, modular design, reproducibility and efficiency in automated experiments.

P34: Silicon-based photonic biosensors for label-free detection of microorganisms

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Silicon-based photonic biosensors represent a promising approach for the detection of suspended biomolecules and microorganisms. The measuring principle of photonic biosensors relies on the evanescent field next to a waveguide and variations of the refractive index n associated with the sample in its vicinity. Utilizing micro-ring resonator waveguides on photonic-electronic integrated microchips as produced with IHP's EPIC technology allowed the combination with dielectrophoretic electrodes which should cause significantly shorter detection times due to overcoming the diffusion required in other biosensors. In contrast to conventional methods, photonic-electronic biosensors are cost-efficient, have a small footprint of a few mm2, and notably, possess the capability to deliver real-time results. In this study, we employed a biofunctionalization with a polydopamine (PDA) layer followed by specific antibodies. Biofunctionalized photonic sensor chips showed the attachment of Legionella cells as successfully demonstrated by shifts of resonance minima in the spectra of micro-ring resonators using wavelengths in the range from 1551 to 1555 nm. According to our recent results, we expect to enable the detection of Legionella pneumophila cells at concentrations below the legal limit value of 100 CFU/100 mL. If appropriately adapted antibodies are used, the detection of other microorganisms is of course also possible with very low detection thresholds.

P35: Mechanistic soft-sensor design for protein refolding processes based on intrinsic fluorescence measurements

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In protein refolding processes the scarce availability of on-line measurements hampers effective process monitoring. In this work we developed a mechanistic soft-sensor for protein refolding based on online intrinsic fluorescence measurements of tryptophan and tyrosine. In validation experiments using two model proteins, lactate dehydrogenase (LDH) and galactose oxidase, the soft-sensor showed accurate estimates for the prediction of the total sum of folding products (NRMSE < 6.1%) by calculating the changing rate of the average emission wavelength. For refolding of the enzyme LDH it was possible to obtain separate predictions of native protein and insoluble aggregates. The soft-sensor design was further extended by a model-based observer approach using particle filtering to incorporate kinetic formulations as well as physical constraints.

The novel approach enabled the analysis of kinetic mechanisms during rapid reaction dynamics and can therefore be seen as an enabler to achieve a better understanding of kinetic refolding mechanisms.

P36: Developing a low cost, highly parallel, scalable, bacterial protein production workflow based on single-use bubble column reactors

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The Centre of Medicines Discovery (CMD) was created with the ambition of developing medicines that are developed through to clinic. The Protein crystallography small research facility (PX-SRF) through its co-evolution with the XChem facility, contributes to this ambition by supporting structure-based drug discovery (SBDD), ranging from protein engineering, crystal optimisation, fragment screening, fragment hit progression, and structure analysis.

A major blocker in SBDD is producing the right protein for the assays and crystallization. One strategy the CMD employs to address this is by exploring a diversity in constructs to probe crystallisation space (10 to >100 variants). However, this increases the experimental burden. Since labs operational exigency is typically more valuable that yield efficiency, and since there is not regulatory burden (e.g. FDA), the motivation is production of enough, consistent protein for downstream assays, whilst avoiding time-consuming process steps (e.g. washing-up and centrifugation).

Presented is a single-use bubble column reactor array that allows for 1 L E.coli fermentations of recombinant proteins in parallel. Each reactor is constructed of a very low aspect-ratio polythene tube, sealed at the bottom, with a removable closure at the top. Mixing and aeration is supplied via a sparger, and heat by immersion in a water bath. This simple, compact system is intended to have an exceptionally low barrier to entry, whilst being highly scalable. This approach facilitates mixed operation of either single target Scale-Out, or highly parallel construct screening.

P37: Secretory production of bifunctional proteins with *Corynebacterium glutamicum*

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Bifunctional proteins (BiFuProts) are a promising tool for a more sustainable industry. Their bipartite structure consists of an adhesion-promoting peptide and a functional domain. This enables specific targeting and functionalisation of surfaces. Exemplary future applications are novel biosensors or sustainable plastic packaging.

While conventional intracellular production of BiFuProts is possible, it requires costly downstream processing. The aim of this work was therefore to establish the secretory production of BiFuProts with *Corynebacterium glutamicum*.

Unfortunately, it is not yet possible to predict under what conditions secretion efficiency will be optimal, requiring the screening of large secretion strain libraries under different process conditions. To accelerate this process, central experimental steps were automated. This allowed the rapid construction of strain libraries expressing BiFuProts fused to 24 different Sec-type signal peptides (SP). With the aid of lab automation, it is also possible to cultivate, sample, and assay these strains in a shorter time.

Because BiFuProts are usually not enzymatically active, the activity-independent split-GFP assay was established as a fast and easy quantification method to evaluate secretion performance. Its reliability was validated using conventional protein quantification methods such as Bradford assay and SDS-PAGE.

By using these previously established methods, the influence of different factors on secretion efficiency was evaluated. It became apparent that BiFuProt secretion is strongly influenced by the choice of SP. Using the most efficient SP, further bioprocess parameters were optimised. The production process was transferred from microscale cultivation to lab-scale bioreactors, enabling the secretory production of sufficient quantities of BiFuProts for application tests.

P38: Accelerated secretion efficiency screening for the production of microplastic-binding peptides in *C. glutamicum*

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Micro- and nanoplastic particles (MP/NP) in food and beverages have been associated with health risks. Therefore, an approach is needed by which MP/NP can be detected in and removed from foodstuffs. Adhesion promoting peptides (APs) present an elegant solution by binding specifically to different types of polar and nonpolar MP/NP. They can be used to label MP/NP with fluorescent dyes for specific detection. Protein secretion

in *C. glutamicum* can be utilised in heterologous production by expressing a fusion protein consisting of a secretion signal peptide (SP) and the target protein.

A secretion strain library expressing 24 SPs from Bacillus subtilis was generated for the selected AP using automated molecular biology workflows. *C. glutamicum* was cultivated in a microbioreactor with a backscatter-triggered inoculation protocol and secreted APs were harvested by an automated liquid handling platform. For the quantification of secretion efficiency, an automated split GFP assay was optimised on the same platform. The SP YwaD displayed the highest secretion efficiency for the selected AP. In stirred tank reactors, a suitable glucose feeding strategy was identified.

Automated molecular biology workflows were successfully established for the construction of secretion strain libraries. A library with 24 different SPs was generated for a selected AP. In an automated screening experiment, a secretion efficiency ranking was obtained for the different SPs. The best performing SP YwaD was investigated in bioprocess development experiments.

With the optimised workflows, strain construction, secretion efficiency screenings and bioprocess optimization experiments can be conducted in high throughput for selected APs.

P39: Development of a non-canonical amino acid-labeled [NiFe]-hydrogenase production system in *Escherichia coli*

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[NiFe]-hydrogenases not only utilize complex nickel and iron cofactors to split H2, but also transfer the resulting electrons to an electron-acceptor via iron-sulfur clusters1,2. This distinctive structure and function has attracted interest in developing H2-driven chemo-bio catalysts endowed with valuable functionalities. Labeling of [NiFe]-hydrogenases with non-canonical amino acids (ncAAs) enables coupling of the hydrogenase with chemical catalysts via click chemistry to drive interesting enzymatic activities such as CO2 reduction by utilizing electrons released from H2-splitting.

Recently, a bioprocess for heterologous production of the O2-tolerant regulatory [NiFe]-hydrogenase (RH) from Cupriavidus necator H16 in Escherichia coli has been successfully developed, exhibiting RH activity similar to the native form but with significantly higher yield and shorter process time3,4. Based on this model enzyme, our study focused on site-specific incorporation of the ncAA S-allyl-cysteine (Sac), abundant in garlic extract with clickable properties, into RH variants by amber stop codon suppression in *E. coli*.

To do this, an orthogonal translation system, comprising the engineered psychrophilic pyrrolysyl-tRNA synthetase and tRNA pair was introduced into *E. coli* by co-expressing RH and maturation enzymes. The incorporation efficiency and Sac-labeled protein yield were optimized by screening conditional parameters. The successful incorporation of Sac at the target mutation site within the RH was verified by LC-MS. In this way, the active production yield of Sac-labeled RH reached up to 85% of the corresponding wildtype protein3.

The results showcase the potential for in vivo high-yield production of ncAA-labeled hydrogenases, opening avenues for the development of chemo-bio catalysts applicable in bioenergy and biotechnology.

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P40: Heterologous production of an active hydrogenase using lactose-based autoinduction

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Hydrogenases play a crucial role in the utilization of molecular hydrogen (H2) as an alternative energy source, making them promising catalysts for several industrial and scientific applications. Unfortunately, the industrial-scale production of hydrogenase remains a challenge due to limited yields in the native hosts and the high cost associated with homologous expression systems. Additionally, the heterologous production of hydrogenases still represents a challenge, due to the complex maturation process, structure, and optimum production conditions [1].

In previous studies, more than 100-fold higher yields compared to production in the native host R. eutropha were achieved using the fed-batch like EnPresso B medium [2]. Additionally, a lactose-based autoinduction allowed to obtain even higher protein yields in comparison to classic IPTG induction, making it a promising alternative to increment protein expression [3]. However, no activity was detected for strain BQF8RH [3].

Here, we used the improved strain BQF8RH18, which produced an active RH with activities comparable to RH purified from R. eutropha [3], and applied lactose-based auto induction, using EnPresso B medium. We also monitored important process parameters (DOT and pH) online using the PreSens system. Even though the RH yields could not reach the yields obtained with BQF8RH [2], they are far higher than the yields obtained with strain BQF8RH18 so far [3]. Moreover, the RH showed enzymatic activity demonstrating the suitability of auto induction for heterologous production of an active hydrogenase. This serves as a basis for the improvement of future autoinduction based processes.

Future work will be aimed at the scale-up of the process, as this would be required for the biotechnological application of these enzymes.

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P41: Bioprocess development to produce a hyperthermostable S-methyl-5'-thioadenosine phosphorylase in *Escherichia coli*

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Nucleoside phosphorylases catalyze the reversible phosphorolysis of pyrimidine and purine nucleosides in the presence of phosphate. They are important biocatalysts for the chemoenzymatic synthesis of nucleosides and their analogs which are, among others, used for the treatment of cancer and viral infections. The S-methyl-5'-thioadenosine phosphorylases (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 analogs for therapeutic applications. However, so far, no information is available on the efficient expression of thermostable nucleoside phosphorylases in *Escherichia coli* high cell density cultivations. Here, 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. The optimal conditions for expression were determined in 24-well plates with a fed-batch medium and HCD cultivations with *Escherichia coli* BL21-Gold cells were performed in stirred-tank bioreactors using a glucose-limited fed-batch strategy. A comparison of different growth rates revealed that growth at the maximum growth rate up to induction resulted in the highest ApMTAP yields. Volumetric yields increased by a factor of 136 compared to the originally applied shake flask cultivations with Terrific Broth medium. After purification of ApMTAP activity assays revealed specific activities ranging from 0.21 ± 0.11 (low growth rate) to 3.99 ± 1.02 U mg-1 (growth at maximum growth rate). The present study opens the way for the use of thermostable nucleoside phosphorylases in industrial applications based on improved heterologous expression in *Escherichia coli*.

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P42: Characterization and optimization of peroxidase production in *Komagataella phaffii* with accelerated bioprocess development through automation and miniaturization

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The polymerization of unsaturated polyester resins to manufacture compound materials is currently accelerated with cobalt siccatives. Because of the carcinogenic nature and limited availability of cobalt, a bio-based alternative would be beneficial. Therefore, a promising approach is the utilization of peroxidases, that can substitute cobalt in this application. A suitable host for the production of these peroxidases is Komagataella phaffii. This methylotrophic yeast can be cultivated to high cell densities with strongly inducible protein secretion for easier downstream processing.

Because there is no a priori prediction possible for optimal process conditions, bioprocess development and optimization need to be performed. To enable sufficient exploration of process parameters, tools for cultivation and sample analysis need to be developed in high throughput.

High throughput sample processing in a reproducible and time-efficient manner can be realized with lab automation platforms. This technology enables versatile sample analysis and enhances operator walk-away time. Additionally, microcultivation systems such as the BioLector allow for up to 32 parallel

pH-controlled fed-batch cultivations with online data monitoring of biomass, oxygen and pH value. With this technology, process parameters can be analyzed in a thorough manner.

An enzymatic activity assay and protein quantification assay were automated to streamline sample analysis. Furthermore, cultivation conditions for peroxidase production through glycerol derepression were transferred from lab- to microscale to develop a high throughput screening workflow for the most suitable cultivation parameters. These results form a foundation for the implementation of an automated, miniaturized workflow for bioprocess development of peroxidase production processes in K. phaffii.

P43: Automated strain library screening and bioprocess optimization of heterologous production of sakacin °P in *Corynebacterium glutamicum*

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Bacteriocins, antimicrobial peptides produced by bacteria, exhibit potential in inhibiting pathogens relevant to the food and healthcare industry. Despite many bacteria producing bacteriocins, only a few are well characterized and commercially available. To overcome this limitation, automation and microcultivation are used for accelerated strain construction and bioprocess development.

The class °IIa bacteriocin, sakacin°P, naturally produced by Latilactobacillus°sakei, targets the mannose phosphotransferase system of susceptible bacteria inducing pore formation and cell death. *Corynebacterium glutamicum* is not susceptible and a suitable host for the heterologous production of sakacin P. As heterologous production may be limited by secretion, different Sec secretion signal peptides are tested for optimal production.

Automated tools expedite strain construction of heterologous bacteriocin producers harbouring 23 different secretion signal peptides. A systematic automated workflow encompasses cultivation, harvesting, sample processing and quantification of antimicrobial activity. The antimicrobial activity is determined by pHluorin2 assay reporting membrane damage [1].

By using the automated workflows, LipB was identified as best performing secretion signal for heterologous production of sakacin°P in *C. °glutamicum*. The analysis of sakacin°P production in BioLector°Pro and 1-liter bioreactors revealed the highest antimicrobial activities at the end of the exponential growth phase.

The demonstrated methods enable a widely autonomous strain

construction and screening workflow for the heterologous production of bacteriocins in *C. °glutamicum*. In the future, further strain libraries may be rapidly evolved and evaluated in a fast and robust manner to set the foundation for larger-scale production processes of bacteriocins.

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P44: Advanced workflows for the systematic identification of metabolic optimization targets in DBTL-cycles: A demonstrator for producing aromatic compounds in *C. glutamicum*

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The establishment of rational Design - Build - Test – Learn (DBTL) cycles based on miniaturization, parallelization, automation and digitalization enables a reduction in development times and an increase in reproducibility and effectiveness of microbial strain construction.

Here, we present an advanced strain design and analysis workflow to systematically improve the understanding of microbial production pathways and to enable faster identification of metabolic optimization targets. As first demonstrator, we focus on aromatic compound production in *Corynebacterium glutamicum* with the specific goal to enhance the carbon flux towards L-tyrosine through the shikimate pathway.

The workflow harnesses the power of in-silico metabolic modelling with parallelized strain phenotyping. A standardized library of small- to genome-scale stoichiometric network models of *C. glutamicum* in combination with thermodynamic data and available kinetic information was established. For the simulation of intracellular metabolic states, using parsimonious flux balance analysis, a python-based workflow was set up. Quantitative and fast characterization of growth and production phenotypes is realized by employing robotic-assisted micro-cultivation experiments with (un)targeted metabolomics via LC/ GC-ToF-MS. Data processing and modelling is performed using recently developed python tools [1-4]. Resulting specific rate estimates are applied to further constrain reaction fluxes for in-silico strain design. The wider range of potential metabolic engineering targets for enhancing L-tyrosine production can then be realized using MoClo-based workflows on our AutoBioTech platform [5].

Ultimately, a standardized and validated modelling toolbox enables the design and analysis of a wide range of production strains and thus represents an essential building block for the operation of biofoundries.

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P45: Trans-cinnamic acid production by whole-cell biotransformation of recombinant *Pseudomonas putida* KT2440

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Trans-cinnamic acid is a phenylpropanoid that can be used in a broad spectrum of applications including antioxidant, anti-inflammatory, anticancer and antidiabetic properties in the pharmaceutical industry, as well as use in the food and cosmetic industry [1,2]. Currently, chemical synthesis or extraction methods from plants are used to produce trans-cinnamic acid. However, these processes require expensive conditions, and are not suitable for large scale production [3]. Hence, microbial production processes converting L-phenylalanine to trans-cinnamic acid catalysed by the enzyme, phenylalanine ammonia-lyase (PAL), has become of interest. In this study, trans-cinnamic acid production using a whole-cell biotransformation of recombinant *Pseudomonas putida* KT2440, an organic-solvent tolerant host, was examined with four different PAL genes under a rhamnose-inducible (PrhaB) promoter. The highest trans-cinnamic acid production was obtained from the strain expressing RmXAL (*Rhodotorula mucilaginosa*) with a concentration and conversion rate of 2.62 g/L and 0.29 g/L.h, respectively. Interestingly, the biotransformation rate was almost doubled when operating at 37oC compared to 30oC. Furthermore, a fed-batch fermentation was conducted in a stirred-tank bioreactor to achieve high cell concentration. In this way, the production of trans-cinnamic acid in terms of product concentration and conversion rate reached 12.74 g/L and 1.82 g/L.h, respectively. Future work will include cell recycle to further increase the volumetric productivity.

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P46: Biosynthesis of phenazine-1-carboxylic acid in *Pseudomonas chlororaphis* DSM19603 through media factor optimization and genetic engineering

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The aim of this project is to produce phenazine-1-carboxylic acid (PCA) from *Pseudomonas chlororaphis* DSM19603. PCA is a member of the group of phenazines, which are produced by plant-beneficial *Pseudomonas spp.* and used for protection against plant pathogens1. Hence, one important application can be the use of PCA as a biological pesticide2. This chosen strain can only produce 26 mg/L of PCA in standard media and there is a complex regulation of phenazine biosynthesis which hinders high production of PCA. As a first step, optimization of media composition was conducted in the wild type. Statistic-based experimental designs were used, including Full Factorial experimental design for determining the suitable carbon and nitrogen source, Plackett-Burman experiment for factor screening, and Full Factorial experimental design again for finding the most significant factor together with determining its optimal content. Due to the desire of exclusively producing PCA, the gene coding for the enzyme (phzO) that converts PCA to 2-hydroxyphenazine-1-carboxylic acid was knocked out. From this genetically modified strain, a 1 L batch cultivation with the optimized media resulted in 554 mg/L of PCA. Furthermore, five negative regulators of the phenazine production (lon, rsmE, psrA, rpeA, and parS) were knocked out together with a pyruvate kinase (pykF) for enhancing the production of a phenazine biosynthesis precursor. Subsequently, it is intended to further enhance the PCA accumulation through the deletion of the regulators.

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P47: Lactic acid production from tropical agro-food wasre. An overview to opportunities in Cuba

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A large number of chemical compounds that are used to obtain products of primary need and high demand are extracted from petroleum and its derivatives. The alternative of substituting these chemicals from bio-based raw materials is becoming increasingly clear and viable [1]. Lactic acid is one of these chemical compounds, it can be a source of products from various industrial sectors such as pharmaceuticals, textiles, plastics and others [2]. There are several organic residues from tropical fruits present in Cuba, as mango, guava, banana, pineapple, orange and sugar cane, which may serve as sources of lactic acid by fermentation using different types of microorganisms [3-5]. Production of biogas from these residues is also feasible [6]. To achieve adequate lactic acid production yields, recent findings cover the stimulation of the fermentation process through the use of metallic compounds, obtaining yield rates five times higher [7;8]. Moreover, Cuban agro-industry bears a vast potential of lignocellulosic raw materials. In summary, thousands of tons are generated as waste. Therefore, it is necessary to expand research actions to propose technological solutions that promote the circularity of the processes. On the other hand, it is essential to foster the standards that promote the use of bio-based materials, such as biodegradable plastic [10]. In this sense, and as a part of the BMBF-funded project Biotechnological concepts for regionally implementable material cycles: Coupling of acid fermentation and material utilization in pure cultures (BioKreiS), the present work provides an approach to utilize the potential of acid lactic production in Cuba assuming the experiences on bioprocess stimulation [9; 10] and the waste potential previously referred.

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P48: Purine nucleoside antibiotics: recent synthetic advances harnessing chemistry and biology

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Nucleoside analogues represent one of the most important classes of small molecule pharmaceuticals and their therapeutic development is successfully established within oncology [1;2] and for the treatment of viral infections [3;4]. However, there are currently no nucleoside analogues in clinical use for the management of bacterial infections. Despite this, a significant number of clinically recognised nucleoside analogues are known to possess antibiotic activity, thereby establishing a potential source for new therapeutic discovery in this area [5;6]. Furthermore, given the rise in antibiotic resistance, the discovery of new clinical candidates remains an urgent global priority and natural product-derived nucleoside analogues may also present a rich source of discovery space for new modalities. This poster presents a current perspective surrounding the construction of natural purine nucleoside antibiotics [7]. By amalgamating recent efforts from synthetic chemistry with advances in biosynthetic understanding and the use of recombinant enzymes, prospects towards different structural classes of purines are detailed.

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P49: Biocatalytic nucleobase diversification of 4′-thianucleosides and de novo RNA synthesis detection with 5-ethynyl-4′-thiouridine in proliferating HeLa cells

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Nucleoside and nucleotide analogues are valuable tools for the treatment of viral infections and cancer [1]. Possible structural modifications to deliver new nucleoside analogue classes include replacement of the canonical ribose oxygen with sulfur [2]. The biological activity of such analogues has been shown in some cases [3;4], but wider exploration of this class of analogue is hampered by the lack of a straightforward and universal nucleobase diversification strategy. Herein we present a biocatalytic platform to enable nucleobase diversification from 4'-thiouridine in a one-pot process employing thermophilic nucleoside phosphorylases. We show that this approach makes both, pyrimidine and purine 4'-thianucleosides accessible, thereby paving a way for the widespread synthetic and biological exploration of this analogue class. We chose 5-iodo-4'-thiouridine as example for a multimilligram scale synthesis and from here completed the chemical synthesis of a novel nucleoside analogue probe, 5-ethynyl-4'-thiouridine. We demonstrate the biological activity of this probe by monitoring de novo RNA synthesis in proliferating HeLa cells, validating its capability as a new metabolic labelling tool.

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P50: Design of a production process for resistance structures and metabolites of Metarhizium robertsii MT008 for the control of Anastrepha obliqua through submerged fermentation on a laboratory scale using agroindustrial waste

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Fruit flies are limiting pests in fruit production in Colombia with a quarantine nature due to the risk of introduction to other countries. An alternative for controlling fruit flies is entomopathogenic fungi and their active compounds. Thus, in previous projects, the extract from Metarhizium robertsii Mt008 fermentation was selected for its promising results in controlling the pest in mango. For the development of a dual biopesticide (fungus structures and its extract), culture medium optimization was developed by assessing a carbon source (liquid agroindustrial waste) and a nitrogen source (complex organic compound). A central rotating composite was designed with 11 experiments Erlenmeyer at a stirring speed of 300 rpm and 28 °C. The response variables were resistance structures (mycelium pellets, submerged conidia, and blastospores), destruxins concentration (dtx A and dtx B), and insecticidal activity (biomass and dtx) before 18 days-fermentation. The results showed that a C source concentration of 6 g/L and N of 10 g/L would optimize the production of structures, destruxins, and insecticidal activity on Anastrepha obliqua. Subsequently, a central rotating composite design was developed to define the fermentation time and inoculum concentration (48h seed culture), using the same medium, agitation, and temperature conditions. It was found that a fermentation time of 9 days and a seed culture percentage of 7.5% would achieve the maximization of structures with a 62% mortality on preimaginals of A. obliqua, although destruxin concentration was not the highest. However, the extract achieved 100% mortality on adult A. obliqua.

P51: Opportunities of waste bioprocessing towards a circular approach in Cuba

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The path towards sustainability implies triggering a system change to seek for new raw materials ob-tainable from waste management. In this sense, waste bioprocessing is called to be the core of integrated analysis in the material life cycle in which biomass is involved. Chemicals, energy and other by-products from renewable sources foster new strategies for the rethinking of established process-es, resulting in higher added value. Numerous studies and research projects dealing with waste man-agement for such purpose was conducted in Cuba in strong collaboration with research institutes in Germany and others countries [1-3]. The biomass potential in Cuba is remarkably huge, but its utiliza-tion does not meet the actual social and industrial necessities. Therefore, the current situation characterized by anon-utilized resources is far from satisfactory, which has a strong impact on the Cuban economy. According to recent statistics [4], in the last decade an average of about 5 Mt of Municipal Solid

Waste (MSW) are disposed annually in dumps across the country in the last decade. The Sugar Cane industry is depressed due to the lack of fertilizer, among other reasons. Nevertheless, still 225 Mt of organic solid waste were generated in the last harvesting season, without closing the material cycle. The present work is aimed to present the opportunities of waste bioprocessing in Cuba to-wards a circular approach, combining the use of biomass waste not only for the generation of energy but also for the production of chemicals, such as lactic acid, as part of the BMBF-funded project ¨Biotechnological concepts for regionally implementable material cycles: Coupling of acid fermenta-tion and material utilization in pure cultures¨ (BioKreiS).

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P52: Substituting raw materials: Animal by-product streams for polyhydroxyalkanoate production

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With the still-increasing demand for plastic materials, the modern society calls for environmentally friendly alternatives to fossil-based plastics which are not only bio-based but also biodegradable. Polyhydroxyalkanoates (PHAs) are polymers produced by various microorganisms as an energy reservoir. The properties of PHA can vary depending on their structure showing similarities to a lot of fossil-based polymers.
To further reduce the environmental and ecological impact of plastic production, animal by-products can be used as a cheap alternative to raw materials [1]. Pre-treating animal materials is a crucial step to achieve high substrate yields as well as pure substrate phases. The animal materials are pre-treated, hydrolysed and subjected to phase-separation for the production of a fat, fat-protein and protein phase [2]. Not only the carbon source but also the nitrogen source in Ralstonia eutropha cultivations can be substituted with animal by-product streams of different qualities. In this work, fat and protein fractions of porcine by-product streams hydrolysed at temperatures ranging from 130 – 160°C were evaluated in R. eutropha laboratory-scale bioreactor cultivations under nitrogen-limited conditions. Up to 59 g L-1 cells containing 80 wt% P(HB-co-18 mol% HHx) could be produced using different animal fat-phases, yielding similar results to raw substrates like fructose or canola oil. Protein phases were evaluated regarding their molecular weight and their influence on growth and PHA production as the sole nitrogen source. Their use can reduce the lag-phase by boosting cell growth at the beginning of the cultivation.

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P53: Polyhydroxyalkanoate production by *Cupriavidus necator* using apple juice residues

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The production of biodegradable and biobased polymers is one way to overcome the present plastic pollution. One promising group of such biopolymers are polyhydroxyalkanoates, which can be produced by various microorganisms. The production process is, however, far more expensive than the production of conventional petroleum-based plastic. A way to reduce production costs is by using alternative, cheap feedstocks derived from waste streams, e.g. from agriculture or food production. One of these potential feedstocks is trub, which is derived from fruit juice production.
In our contribution, we explore the process of using apple trub as a basis to produce polyhydroxyalkanoates. For this, we investigate different pretreatment strategies such as autoclaving, boiling, and filtration and conduct experiments in shake flasks and lab-scale bioreactors to observe polyhydroxyalkanoate production in Cupriavidus necator using apple trub. We compare our results based on apple trub as feedstock with polyhydroxyalkanoate production using fructose as a carbon source.

P54: Microbially produced monomers for biopolymers: Bioprocess development for 2-oxoglutarate production with *Corynebacterium glutamicum*

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Efficient bioprocesses open the door for the transition from a fossil-based economy to a sustainable circular economy. However, the development of new bioprocesses is very time-consuming and labor-intensive due to manual execution. To save time, costs and resources, miniaturization, parallelization and automation are state of the art to modern bioprocess development [1].
In our study, molasses, a sucrose-containing 2nd generation feedstock from the sugar industry, is used as raw material to synthesize 2-oxoglutarate as a higher-value product for biopolymers. *Corynebacterium glutamicum* is used as a suitable platform organism for the production of biobased AKG, the precursor of the eponymous amino acid L-glutamate. Most importantly, *C. glutamicum* is naturally able to metabolize a broad range of carbon sources, including monomers such as acetate, ethanol and fructose contained in widely available industrial side streams [2, 3, 4]. To our knowledge, there is no data available for the efficient production of AKG from molasses. Therefore, a suitable bioprocess was developed from scratch and rapid metabolic engineering was supported by automated high-throughput experiments in combination with data-driven modeling and analysis.

In silico strain design followed by targeted metabolic engineering of *C. glutamicum* and automated phenotyping in microliter scale resulted in a first strain for AKG production from defined sucrose-media with a batch-titer of 22 mM and a yield of 0.16 g g-1. Further screening on molasses-media revealed another strain variant, which was able to produce 80 mM AKG at a yield of 0.6 g g-1. Interestingly, this strain showed no grow and production from sucrose-based media. A first scale up to a 1 L fed-batch process on molasses resulted in a AKG titer of 350 mM. For product purification an organic extraction approach was carried out and a concentrated AKG solution with a purity of > 91% was obtained.

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P55: A comparative study of Python and Julia programming for downstream process simulation

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Recent years have seen the Chemical Engineering field shifting from MATLAB to Python for demanding simulation and data science applications. Python is considered a well-grounded and valid choice to be adopted in the field thanks to its widespread usage, straightforward syntax, readability, available libraries, broad documentation, support, and suitability for machine learning applications. Moreover, contrary to tools like MATLAB, it is open-source. It follows that Python has been attracting the interest of both students and professionals in the community [1].
However, a new programming language, Julia, is being established in the field and has the potential to improve simulation speed and accuracy further. This work aims to explore the differences between the two languages and to propose a detailed overview of the best-suited applications for each language. Additionally, a GitHub repository (open-source) containing all the work will be introduced. The repository includes tutorials on how to get started with Julia and examples of comparable code in both Python and Julia. In this work, the simulation of a chromatography separation is used as an example of downstream processing.
Overall, our aim is to provide a solid comparison of how various processes can be implemented in the two discussed languages and compare and discuss the speed and efficiency of their implementations.
The integration of Process Analytical Technology (PAT) with advanced modelling and simulation is greatly enhanced by the performance capabilities of modern programming languages like Julia and Python, underscoring the critical role of language selection in optimizing analytical processes. This study will hopefully aid researchers and practitioners in making an informed decision about which language might fit a specific project best.

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P56: Optimisation of the oxygen regime for the accelerated production of kombucha with defined co-cultures

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The traditional production of kombucha, a sweetened fermented tea beverage, is characterized by an undefined, long process with a tendency towards self-regulation. From a microbiological perspective, the beverage matrix and process conditions are not optimal for the growth and metabolism of the existing bacteria and yeasts. This influences the characteristic taste and aroma of the final product. Therefore, efforts to accelerate the fermentation process must be carried out in a manner that ensures the maintenance of product quality.
This study investigates methods for kombucha production under defined conditions in bioreactors with active aeration, taking into account the metabolic interaction of the defined co-culture of acetic acid bacteria and yeast as well as the product sensory. Various process engineering options for accelerated fermentation are presented along with other factors relevant to product quality and the subsequent production process are discussed.

P57: Cost-to-go model predictive control for enhanced optimization of bioprocesses

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The development of monitoring & control applications for bioprocesses is usually a labour and time intensive task, since it requires accurate models for the complex biological systems. Specifically in bioprocessing, challenges are posed by measure-

ment uncertainties, limited model transferability and significant plant-model mismatches.
In this study we investigated the possibility to predict and control the recombinant protein production of Escherichia coli using a mechanistic production model and a novel controller framework, the cost-to-go MPC. The model was developed based on bioreactor cultivations of E. coli producing the model protein lactate dehydrogenase (LDH), specifically aiming to distinguish soluble protein formation from insoluble inclusion body (IB) formation. After analysis, selection and parameter identification, the model was used as a basis to develop a cost-to-go model predictive controller, a novel concept in the field of bioprocessing. First, the optimal control input trajectory is obtained prior to the start of the process (offline) by means of a dynamic programming optimization. Subsequently, the obtained cost-to-go matrix is used in combination with an MPC controller during the process. This structure enabled the MPC controller to optimize the control input beyond the prediction horizon globally until the end of the process, which led to improved product yields in simulation studies compared to a standard MPC approach. Since optimal trajectories are defined for each feasible point in time and in the state space, the chances to find the global optimum are increased for highly nonlinear systems. However, the method also requires high demands of computational power and the time for optimization scales exponentially with the dimensions/states considered in the process model. Also, the tuning procedure to obtain good values for the meta parameters of the cost-to-go MPC is intricate and not straightforward. Further development and better process models are necessary in order to increase the applicability for this concept in a real production environment.
However, there might be more interesting applications of the concept with slightly varied focus. For example, the optimization problem could be reformulated in such a way, that it optimizes for the best possible time point to stop the process and harvest the cells instead of optimizing for the optimal control input. When disturbances come into play, the controller could adapt the optimal harvest time according to the model. Another interesting application could be the incorporation of the whole oxygen transfer and uptake dynamics, which includes additional complexities and non-linearities into the model. In this setting, the cost-to-go controller might show even better performance increases than obtained in this work, which might account for the additional overhead of the method.

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
- **7th BioProScale Symposium**
  "Scaling Up and Down of Bioprocesses: Technological Innovation and Cell Physiology Insights"
  28 to 31 March 2022

The abstract books of all symposia are available at biotechnologie.ifgb.de/bioproscale/history