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Bioverfahrenstechnik

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**IfGB**  
Institut für  
Gärungsgewerbe  
und Biotechnologie  
zu Berlin

# 9<sup>th</sup> BioProScale Symposium

**Scaling Down and Up of Bioprocesses:  
Process Heterogeneities, Robustness, and Analytics**

APRIL 20-22, 2026

LANGENBECK-VIRCHOW-HAUS, BERLIN, GERMANY

Updated  
Online  
Edition

- **Scale Up and Industrial Scale Processes**
- **Bioprocess Scale Down Models for Heterogeneities**
- **Process-Driven Cell Performance**
- **Integrated Bioprocesses**
- **Process Analytical Technologies (PAT) and FAIR Data Management**



[biotechnologie.ifgb.de/bioproscale2026](https://biotechnologie.ifgb.de/bioproscale2026)



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Chair of Bioprocess Engineering, Prof. Dr. Peter Neubauer  
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## ■ Our venue

Exhibition, coffee & lunch breaks ..... Ground level  
Room "Bernhard von Langenbeck" (poster area) & Exhibition ..... 1<sup>st</sup> floor  
"Historical Lecture Hall" (presentations) ..... 2<sup>nd</sup> floor

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

Download this abstract book and the symposium programme at <https://biotechnologie.ifgb.de/bioproscale2026/docs>



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## ■ Welcome address

Dear colleagues,

it is my great pleasure to welcome you all to the 9<sup>th</sup> BioProScale Symposium in Berlin. I am grateful for the continued interest in the conference since its inauguration in 2009!

We are meeting at a time of profound transformation in biotechnology and biomanufacturing. Rapid advances in automation, data-driven modelling, and the integration of artificial intelligence are fundamentally reshaping how we design, analyze, and control bioprocesses. At the same time, cost-, sustainability, and productivity constraints are placing unprecedented demands on scalable, robust, and flexible production systems.

Emerging applications such as precision and gas fermentation, cellular agriculture, and the integration of alternative substrates are expanding the scope of industrial biotechnology. These developments highlight the growing importance of efficient and scalable bioprocesses for the sustainable production of food, materials, and chemicals.

At the same time, we are operating in a broader global environment characterized by increasing uncertainty and geopolitical tensions. These dynamics influence supply chains, energy availability, and international collaboration, and further emphasize the need for resilient and adaptable bioprocess technologies. Biotechnological production is closely connected to the actual global challenges, including rising energy demands, resource limitations, and evolving regulatory frameworks. At the same time, new opportunities are created through innovative process concepts, integrated bioprocessing, and continuous and automated manufacturing strategies.

In this context, the question of how to reliably translate processes across scales – from laboratory to industrial production – remains central. Process heterogeneities, complex cellular responses, and the interplay between transport phenomena and biological systems continue to challenge our ability to develop predictable and efficient processes. Addressing these challenges is essential not only for technological progress but also for enabling a sustainable, resource-efficient, and resilient bioeconomy.

The BioProScale Symposium provides a unique platform to discuss these topics at the interface of science and industry. This



year's program reflects key developments in scale-up and scale-down strategies, process modelling, and the analysis of heterogeneities, as well as advances in process analytical technologies, data integration, and FAIR data management. The increasing integration of mechanistic understanding with machine learning and hybrid modelling approaches highlights the growing convergence of engineering, biology, and data science in our field.

A key strength of the BioProScale community is its strong international network and the many personal interactions that foster exchange, collaboration, and innovation. We are grateful to all speakers, contributors, sponsors, and organizers who have made this event possible, and we especially thank all participants for sharing their expertise and perspectives.

I hope that the coming days will inspire fruitful discussions, stimulate new ideas, and initiate future collaborations. Let us use this opportunity to deepen our understanding and to jointly advance the development of robust, scalable, and sustainable bioprocesses.

With this, I wish you an inspiring and enjoyable symposium!

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

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MONDAY, 20 APRIL 2026

## WELCOME ADDRESS AND PLENARY TALK

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

## SESSION 1: MASS TRANSFER, SCALE-UP &amp; MODELLING (I)

Chair Robert Spann / Isabel Thiele

12:20 **Plenary Talk: The dilemma of scale-up/down and the promise of scaling out/modularized biomanufacturing (P01)**  
*Andreas Worberg, Center for Biosolutions DTU, Denmark*

13:05 **From pilot to production: development and application of a hybrid model across scales (L01)**  
*Marc Lemperle, Technical University of Denmark, Denmark*

13:30 **Lessons learned on dynamic simulation of heterogeneities using compartment models (L02)**  
*Hector Maldonado de Leon, TU Delft, The Netherlands*

13:55 **Industry promotion talks**

14:15 *Coffee break & exhibition*

## SESSION 2: MASS TRANSFER, SCALE-UP &amp; MODELLING (II)

Chair Ralf Takors / Rosa Haßfurth

14:45 **Challenges in oxygen mass transfer measurement for industrial-scale aerated stirred tank bioreactors (L03)**  
*Nicolas Nickel, Hamburg University of Technology, Germany*

15:10 **Using CFD-based compartment models to resolve spatial heterogeneities in industrial bioprocesses (L04)**  
*Johan Le Nepvou De Carfort, Technical University of Denmark, Denmark*

15:35 **Numerical scale-up and experimental validation of gas-liquid bioleaching reactors using LB-LES CFD modelling : from 2 L to 2000 L (L05)**  
*Saranath Sripriya, Bureau de Recherches Géologiques et Minières Orléans, France*

16:00 **Coupling genome-scale models with compartment bioreactor models to study intracellular responses under fluctuating conditions (L06)**  
*Titania Insani Sugiarto, RWTH Aachen, Germany*

16:25 **Scale-up challenges in *Pichia pastoris* fermentation processes (L07)**  
*Anamari Brdar, Sanofi Aventis Deutschland GmbH, Germany & Hannah Singer, Sanofi Aventis Deutschland GmbH, Germany*

16:50 *Coffee break & exhibition*

## SESSION 3: MASS TRANSFER, SCALE-UP &amp; MODELLING (III)

Chair Howard Ramirez Malule / Simon Täuber

17:20 **Multivariate population balance model resolution for bioreactor simulation (L08)**  
*Benjamin Casale, Toulouse Biotechnology Institute, France*

17:45 **Morphology-based scale-up of *Streptomyces* species cultivations using CFD simulations (L09)**  
*Philipp Eibl, SimVantage GmbH, Austria & Gesa Brauneck, RWTH Aachen, Germany*

18:10 **Balancing yield and sustainability: Modelling operational strategies for scaled cultivated meat production (L10)**  
*Ben Tumulero, TU Delft, The Netherlands*

## EVENING PROGRAM

18:35 **Melody of the genome 2.0 – The sound of DNA**  
*Langenbeck-Virchow-Haus, Foyer*  
*Artistic Contribution*

19:00 **Poster Session, Exhibition, Get-together for all participants**  
*Langenbeck-Virchow-Haus, Foyer*  
*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

## TUESDAY, 21 APRIL 2026

9:00 **Welcome note***Peter Neubauer, TU Berlin, Germany***SESSION 4: SCALING OF BIOPROCESSES**Chair *Annette Berg / Johannes Nicklisch*9:05 **Plenary Talk: Process modeling strategies for successful biopharmaceutical development (P02)***Thomas Wucherpfnig, Boehringer Ingelheim Pharma GmbH & Co. KG, Germany*9:50 **Hybrid modelling to assist scaling of mAb production processes (L11)***Sally Gras, University of Melbourne, Australia*10:15 **Model-based scaling of recombinant protein production in *Escherichia coli* fed-batch processes: Knowledge-based optimization of secretion performance and cell viability (L12)***Fabian Schröder-Kleeberg, TU Berlin, Germany*10:40 **Industry promotion talks**10:55 *Coffee break & exhibition***SESSION 5: BIOPROCESS INTEGRATION (I)**Chair *Alvaro Lara / Fatemeh Nejati*11:25 **FerroFlow: unlocking simulation software for end-to-end bioprocess design – A fermentation case-study (L13)***Pierre Guilloteau, Technical University of Denmark, Denmark*11:50 **Towards understanding the effect of upstream process parameters on the properties and downstream performance of inclusion bodies (L14)***Mohamed Elshazly, TU Wien, Austria*12:15 **Two-dimensional protein denaturation surfaces as a process development tool for solubilization and refolding of bacterial inclusion bodies (L15)***Robert Klausser, TU Wien, Austria & Martin Voigtmann, Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria*12:40 *Lunch break, poster session & exhibition***SESSION 6: BIOPROCESS SCALE-DOWN**Chair *Peter Neubauer / Tolve Kheirkhah*13:50 **Bioreactor simulation from the cellular perspective: application in scaling from cell cultures to syngas fermentation (P03)***Cees Haringa, TU Delft, The Netherlands*14:35 **BioProScale Lifetime Achievement Award***Awardee: Matthias Reuss, Prof. Emeritus of University of Stuttgart, Germany*15:00 **Predictive scale-down using AI-designed 3D-printed bioreactors (L16)***Lena Achleitner, print4biotech GmbH, Obersdorf, Germany*15:25 **Beyond mixing times: From industrial to lab-scale bioreactors using cell trajectories (L17)***Ryan Rautenbach, Technical University Hamburg, Germany*15:50 *Coffee break & exhibition*

**SESSION 7: PROCESS ANALYTICAL TECHNOLOGY (I)**

Chair Krist Germaey / Saskia Waldburger

16:20 **Unlocking the potential of inline process analytical technology (PAT) for advanced bioprocess development, scale-up, and control (L18)***Katharina Dahlmann, Hamilton Bonaduz AG, Switzerland*16:45 **Smart feeding: Raman-based glucose control for enhanced malic acid fermentation (L20)***Luca Antonia Grebe, RWTH Aachen, Germany*17:10 **Machine learning spatially offset time-gated Raman spectroscopy (TG-SORS) in-line sensors for endoglucanase production in *T. reesei* bioprocesses (L21)***Martin Kögler, VTT Finland, Finland*17:35 **From fed-batch to repetitive fed-batch: Soft-sensor-driven control of phosphate-regulated Fab production in *E. coli* (L22)***Rüdiger Lück, TU Wien, Austria*18:00 *End of presentation program***EVENING PROGRAM**19:30 **Conference Dinner***Conference Dinner: Brauhaus Georgbräu im Nikolaiviertel, Berlin (with voucher only)*22:30 *End of day 2**Presentation L19 was canceled at the short notice***WEDNESDAY, 22 APRIL 2026**9:00 **Welcome note***Peter Neubauer, TU Berlin, Germany***SESSION 8: BIOPROCESS INTEGRATION (II)**

Chair Jerome Morchain / Linda Cai

9:05 **Plenary Talk: Intensified fermentation with *E. coli* effects on the economic and ecological footprint (P04)***Oliver Spadiut, TU Wien Austria*9:50 **Hybrid modeling as a life cycle support solution of bioprocesses: from process development to manufacturing operations (L23)***Niels Krausch, DataHow AG, Zurich, Switzerland*10:15 **Development and application of an ANN-perception-based autonomous control system for an *Escherichia coli* cultivation process (L24)***Nikolai Mushnikov, T&J Bioengineering, Shanghai, China*10:40 *Coffee break & exhibition***SESSION 9: PROCESS ANALYTICAL TECHNOLOGY (II)**

Chair Katharina Dahlmann / Lara Santolin

11:10 **A novel view into aerated stirred tanks: Magnetic resonance imaging (L25)***Till Lenczyk, Technical University Hamburg, Germany*11:35 **Integration of online HPLC analytics into chemostat-based polyhydroxyalkanoate production from dark fermentation effluent (L26)***Saskia Waldburger, TU Berlin, Germany*12:00 **Integrating microfluidic analysis and MRI-based flow characterization for the scale-up of a novel bioelectrochemical reactor (L27)***Selma Iraqi Houssaini, Hamburg University of Technology, Germany & Daniel Bauer, Hamburg University of Technology, Germany*12:25 *Lunch break, poster session & exhibition*

## SESSION 10: INTENSIFIED AND CONTINUOUS PROCESSES

Chair Regine Eibl / Eike Janesch

14:00 **Plenary Talk: Process intensification in microbial and enzymatic conversions (P05)***Helen de Wever, VITO, Belgium*14:45 **Lipid production in *Schizochytrium limacinum* SR21 from biogenic residues (L28)***Simon Täuber, TU Berlin, Germany & Anne Kathrine Clausen, Aalborg University, Denmark*15:10 **A cell-machine interface for monitoring plasmid-loss events and population stability during continuous bioproduction of plasmid DNA (L29)***Hannah Sehr, University of Liège, Belgium*15:35 **Paving the way for continuous bioprocessing: Building robust *Pichia pastoris* strains for long-term cultivation (L30)***Arnau Gasset, Universitat Autònoma de Barcelona, Spain*16:00 **Award ceremony best talks and posters & Closing remarks***Peter Neubauer, TU Berlin, Germany & Stefan Junne, Aalborg University, Denmark*16:20 *End of symposium*

## About the organisers

Technische Universität Berlin, Institute of Biotechnology,  
Chair of Bioprocess Engineering

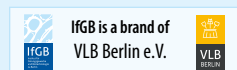
The research at the Chair of Bioprocess Engineering at the TU Berlin is directed to the development and application of new methods for faster bioprocess development, including genetic, cultivation, and analytical tools with a special focus on the industrial scale. It aims specifically in understanding 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](http://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.



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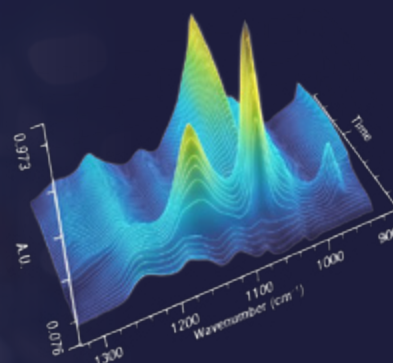
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## SCIENTIFIC POSTERS

- P1: Real-time CFD: Emulating 3D bioreactor gradients in milliseconds**  
Victor Puig I Laborda, Novo Nordisk Foundation Center for Biosustainability / TU Denmark, et al.
- P2: Machine learning-based 2D and hybrid 3D modeling for process intensification in iPSC expansion**  
Muriel M. Zumbihl, Zurich University of Applied Sciences, Switzerland, et al.
- P3: Machine-Learning framework for instantaneous prediction of the mixing time in a mechanical stirred bioreactor at both lab and industrial scales**  
Nathan Nourdin, Université de Lorraine, France, et al.
- P4: Physics-informed neural network surrogates for bioreactor hydrodynamics: Opportunities and current limitations**  
Veronika Trávníková, RWTH Aachen University, Germany, et al.
- P5: Physics-informed neural network framework for optimizing PHA bioreactor performance**  
Monesh Kumar Thirugnanasambandam, Universidade NOVA de Lisboa, Portugal, et al.
- P6: Deep hybrid modelling and control of microbiome evolution**  
José Pinto, Universidade NOVA de Lisboa, Portugal, et al.
- P7: A standardized template for modeling and simulation of upstream bioprocesses**  
Dingchang Xu, TU Berlin, Germany, et al.
- P8: withdrawn**
- P9: Development of a digital twin for a novel gas fermentation process**  
Maximilian Klein, Hamburg University of Technology, Germany, et al.
- P10: Dynamic 1D gas-liquid model to simulating heterogeneous bioreactor – in various cultivation modes**  
Julian Federico Sanchez Caldas, Université de Toulouse, France, et al.
- P11: Kinetic modelling of complex co-culture yoghurt fermentation**  
Susanne Musters, Delft University of Technology, The Netherlands, et al.
- P12: Dynamic feed strategies in *S. cerevisiae* fed-batch fermentation guided by model-based design of experiments: an in silico study**  
Ana H. V. Caetano, Technical University of Denmark, Denmark, et al.
- P13: Computational scale-up analysis of bioreactor design for cultivated meat production**  
Pieter Brorens, Delft University of Technology, The Netherlands, et al.
- P14: Agent-based simulation of N-glycosylation: Model parameter estimation using a genetic algorithm**  
Christian Jetschni, Peter Götz, Berliner Hochschule für Technik, Germany
- P15: Model-based strain selection using an industrial KPI**  
Okyanus Yazgin, TU Berlin, Germany, et al.
- P16: Towards a comprehensive decision support system for microbial strain discovery**  
Marco Anteghini, Lifeglimmer GmbH, Germany, et al.
- P17: withdrawn**
- P18: Engineering robust strains: Scale-down approaches for industrial optimization of *Pseudomonas putida***  
Gaffney Aoife, Stuttgart University, Germany, et al.
- P19: Scale-down characterization of an *E. coli* L-tyrosine producer in an STR-PFR simulator using transcriptomic iModulon analysis**  
Mykhaylo Semenov Petrov, Technical University of Denmark (DTU), Denmark, et al.
- P20: Investigation of the L-tyrosine crystallization kinetics under fermentation conditions**  
Deborah Pfaff, Technical University of Denmark, Denmark, et al.
- P21: Lifeline analysis of environmental perturbations in industrial bioreactors: A multi-organism, multi-reactor comparative study**  
Mario Balogh / Ralf Takors, University of Stuttgart, Germany
- P22: Heterogeneity amplification effect on cell environment feedback loop induced by metabolic burden**  
Tiphaine Gallet de Saint Aurin, University of Liège, Belgium, et al.
- P23: Reduced viscosity mutants of *Trichoderma reesei* with improved industrial fermentation characteristics**  
Fernando Silva, IFF, The Netherlands
- P24: From high-throughput microreactors to bench-scale: Modelling tunable recombinant protein production in a 2-feed *E. coli* process**  
Philipp Pably, Technical University of Denmark (DTU), Denmark, et al.
- P25: Co-cultivation dynamics of the filamentous microorganisms *Aspergillus niger* and *Streptomyces coelicolor* in a rocking motion bioreactor**  
Tolue Kheirkhah, TU Berlin, et al.
- P26: From shake flasks to bioreactor: assessing *Vibrio natriegens* for biotechnological plasmid DNA production**  
A. Rita Silva-Santos, Universidade de Lisboa, Portugal, et al.
- P27: Scale-down characterization of plasmid DNA production in *Escherichia coli* for digital twin development and process optimization**  
Sofia O. D. Duarte, Universidade de Lisboa, Portugal, et al.
- P28: Optimizing pDNA production in *Vibrio natriegens* via automated high-throughput process development**  
Rosa Hassfurth, TU Berlin, Germany, et al.
- P29: Enhancing efficiency in kombucha fermentation: A study on scaling up, fermentation time and sensory properties.**  
Alexander J. Gantenbein, TU Berlin, Germany, et al.
- P30: Foam as a source of bioprocess heterogeneity: A scale-down platform for automated foam decay analysis**  
Laura Barth, RWTH Aachen University, Germany, et al.
- P31: Control of lactic acid bacteria and yeast co-cultures in micro-aerated cultures**  
Anne Kathrine Clausen, Stefan Junne, Aalborg University, Denmark
- P32: Uncovering a population-level safety mechanism for bioprocesses handling inhibiting substrates**  
Maximilian Sehart, Frank Delvigne, University of Liège, Belgium
- P33: Scalability of in-situ gas supply via hydrophobic membranes to autotrophic biofilm systems**  
Andreas Netsch, Karlsruhe Institute of Technology, Germany, et al.
- P34: Benefits of off-gas analysis – Using OUR and CER as parameters for reliable “Batch-End-Detection”**  
Garry Schulze, BlueSens gas sensor GmbH, Germany, et al.

## SCIENTIFIC POSTERS

- P35: Local Oxygen limitation in multiphase bioreactors: A method for optical quantification**  
*Katharina Zörner, Hamburg University of Technology, Germany, et al.*
- P36: Scalable micro-sparger modifications to support improved oxygen supply within intensified upstream processing**  
*Karl Scheibenbogen, Sartorius Stedim Biotech, Germany, et al.*
- P37: Evaluation of a novel oxygen injection system on pilot scale for the production of single cell protein**  
*Koen J.A. Verhagen, dsm-firmenich, The Netherlands, et al.*
- P38: Precise measurements of high mass transfer coefficients in Single Use Bioreactors in microbial processes**  
*Mustafa Salli, Hamburg University of Technology, Germany, et al.*
- P39: Novel 96 well screening tool for time-resolved, independent and non-invasive determination of the oxygen transfer rate**  
*Juan C. Porras, Kuhner Shaker GmbH, Germany, et al.*
- P40: Three-angled X-ray tomography for investigating gas-phase dynamics in scalable bubble columns**  
*Rik Volger, Delft University of Technology, The Netherlands, et al.*
- P41: Multi-spectral fiber systems for life science applications**  
*Viacheslav Artyushenko, Ulm University, Germany, et al.*
- P42: Advances in fluid dynamic stirred tank characterization – opportunities of using MRI**  
*Noah von Schnitzler, Hamburg University of Technology, Germany, et al.*
- P43: Low-cost PAT and feedstocks for scalable biohydrogen production**  
*Andrés Barrera, Universidad del Valle, Colombia, et al.*
- P44: Smart multi-column chromatography for scaleable continuous bioprocessing**  
*Sebastian Thürmann, Tosoh Bioscience GmbH, Germany, et al.*
- P45: Spectroscopy-assisted Bayesian optimization for efficient refolding of inclusion body proteins**  
*Florian Gispeger, TU Wien, Austria, et al.*
- P46: Scale-up and optimization of recombinant protein production in E. coli in controlled conditions**  
*Vlad-Constantin Tofan, Cantacuzino National Military Medical Institute for Research and Development, Romania, et al.*
- P47: Driving performance and process reproducibility in recombinant protein manufacturing through optimal selection of yeast-derived bionutrients**  
*Burcin Boran, Ohly GmbH, Germany, et al.*
- P48: Scale-down bioreactor studies on heterologous protein production in stringent response modulated E. coli chassis.**  
*Sidharth Jaya Sankar, University of Stuttgart, Germany, et al.*
- P49: Biotechnological process development for amphiphilic elastin-like proteins**  
*Anzhelika Yashukova, Stefan M. D. Schiller, Goethe University Frankfurt, Germany*
- P50: High-yield production of Cupriavidus necator soluble NAD<sup>+</sup>-reducing hydrogenase in E. coli for sustainable biocatalysis**  
*Qin Fan, TU Berlin, Germany, et al.*
- P51: Scaling of a fed-batch process for efficient heterologous production of active recombinant [NiFe]-hydrogenase**  
*Francisco De La Fuente, TU Berlin, Germany, et al.*
- P52: Comparative evaluation of feeding strategies for glucose oxidase production in Pichia pastoris using a small-scale multi-bioreactor system**  
*Julia Schollmeyer, TU Berlin, Germany, et al.*
- P53: Enhanced production of phenazine-1-carboxylic acid using a genetically modified Pseudomonas chlororaphis strain**  
*Julie Mathiesen, Aalborg University, Denmark, et al.*
- P54: Carbon-to-phosphorus ratio optimization for PHA production in high cell density cultivation under phosphorus limitation condition**  
*Johannes T. Nicklisch, Berliner Hochschule für Technik, Germany, et al.*
- P55: Modulating PHA molecular weight in Cupriavidus necator through overexpression of native depolymerases**  
*Paul Cornehl, TU Berlin, Germany, et al.*
- P56: Why optimized bioprocesses fail at scale: an integrated framework for robust dextran EPS production**  
*Nicolás Armendáriz, National Centre for Food Technology and Safety (CNTA), Spain, et al.*
- P57: Biotechnological production of human milk oligosaccharides**  
*Anastasia Setiadi, Chr. Hansen HMO GmbH, Germany, et al.*
- P58: Life cycle assessment of acetate production using gas fermentation with different CO<sub>2</sub> sources**  
*Ramya Preethi Surendran, NORCE Norwegian Research Centre, Norway, et al.*
- P59: Upcycling waste apples into platform chemicals: pilot-scale production of lactic and succinic acids**  
*Laís Portugal Rios da Costa Pereira, Leibniz Institute for Agricultural Engineering and Bioeconomy, Germany, et al.*
- P60: Valorization of cassava wastewater via acidogenic fermentation: Process intensification and scale-relevant analytics for sustainable VFA production**  
*Howard Ramirez-Malule, Universidad del Valle, Colombia, et al.*
- P61: E-waste bioleaching in a bioreactor: Experimental and numerical studies of particles suspensions**  
*Marc-Antoine Besch, Université de Lorraine, France, et al.*
- P62: Sustainable bioprocess development for upcycling of industrial waste streams**  
*Tanja Caspary, TU Wien, Austria, et al.*
- P63: Microbial population shifts during compost-mediated degradation of anthropogenic polymers**  
*Marcin Damian Jasiak, Wrocław University of Environmental and Life Sciences, Poland, et al.*
- P64: Integrating microbial biodegradation of polymers into circular waste management frameworks**  
*Julia Alicja Dybka, Wrocław University of Environmental and Life Sciences, Poland, et al.*
- P65: SSF biodegradation of smoked cigarette butts and other hazardous cellulosic support materials**  
*Hassan Sreenath, H.K.S Consulting LLC, USA*
- P66: Managing deployable bioprocess soft sensors: A FAIR registry approach**  
*David Camilo Corrales, Université de Toulouse, France, et al.*
- P67: An ontology-based digital interlinked blueprint of the KIWI-biolab to support experimental reproducibility and traceability**  
*Ana S. Lodos, TU Berlin, Germany, et al.*
- P68: A Blockchain based digital thread for FAIR, trustworthy, and human-centric bioprocess development**  
*Jonas M. Karsten, TU Berlin, Germany, et al.*
- P69: From off-line to real-time: Probe-integrated electrochemical glucose sensing for bioprocess monitoring**  
*Aliyeh Hasanzadeh, Technical University of Denmark, et al.*

MONDAY, 20 APRIL 2026

## 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*



## SESSION 1: MASS TRANSFER, SCALE-UP & MODELLING (I)

Chair Robert Spann / Isabel Thiele

## PLENARY TALK

### 12:20 The dilemma of scale-up/down and the promise of scaling out/modularized biomanufacturing (PL01)

*Andreas Worberg*

*Center for Biosolutions DTU, Denmark  
Email: andwor@dtu.dk*

For decades, bioprocess development has followed a predictable path: optimize at laboratory scale, scale up through pilot facilities to production vessels, and hope the physics cooperate. Mass transfer, mixing dynamics, and heat dissipation behave fundamentally differently in 5L benchtop versus 15,000L production bioreactors, creating technical risk at each transition. Scale-down approaches offer elegant solutions for some questions but cannot fully capture large-scale phenomena.

We've been optimizing bigger vessels, higher titers, and maximum volumetric productivity. But what if we've been asking the wrong question at process and SynBio?

Modularized biomanufacturing inverts traditional thinking: replicating smaller production units rather than progressively larger vessels. Technical demonstrations now exist: containerized facilities achieving GMP certification in 18 months, pilot-scale systems producing chemicals from waste feedstocks, portable platforms generating biologics on-demand. Industry surveys show nearly half of Western European facilities pursuing modular approaches.

Yet peer-reviewed techno-economic analyses comparing scale-out versus scale-up remain conspicuously sparse. Perhaps because the answer is uncomfortably context-dependent. Perhaps because we're confronting a more fundamental problem: how many large-scale industrial biotechnology facilities have been built in the West recently? While Asia invests heavily in biomanufacturing infrastructure, Western development has stalled. Traditional scale-up demands massive capital that venture capital won't touch. VCs fund intellectual property, not stainless steel. "Tough tech" requiring patient capital struggles in ecosystems optimized for rapid exits.

Modular approaches offer different economics: lower upfront capital, faster deployment, incremental capacity expansion matching funding availability. Whether this addresses the financing gap remains unclear, but it reframes the question investors might answer differently.

This presentation challenges assumptions about optimization targets. When supply chain resilience matters more than marginal efficiency gains? When the ability to finance and build facilities matters more than theoretical operating costs? Perhaps the critical question isn't "scale-up or scale-out?" but "what are we actually trying to achieve?"



### 13:05 From pilot to production: development and application of a hybrid model across scales (L01)

*Marc Lemperle<sup>1</sup>, Pedram Ramin<sup>1</sup>, Julian Kager<sup>1</sup>, Benny Cassells<sup>2</sup>, Stuart Stocks<sup>2</sup>, Krist V. Gernaey<sup>1</sup>*

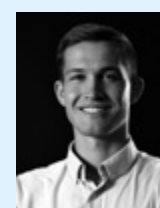
*<sup>1</sup>Process and Systems Engineering Center (PROSYS), department of Chemical and Biochemical Engineering, Technical University of Denmark, Building 228A, 28000 Kgs. Lyngby*

*<sup>2</sup>Novonosis, Fermentation Pilot Plant, Krogshøjvej 36, 2880 Bagsværd, Denmark  
Email: marlem@dtu.dk*

Scaling up aerobic filamentous fermentations remains a major challenge in the bioprocess industry due to the complex morphology-driven rheology and nonlinear dynamics of oxygen transfer [1,2]. In this study, we present a hybrid modeling framework that addresses these challenges by improving the prediction of oxygen transfer rate (OTR) across scales with the integration of mechanistic models and online data-driven viscosity estimations.

A cost- and time-efficient pilot-scale experimental procedure, the so-called 'Intra-batch experimental design' for the development of a mechanistic  $k_L$  model is presented. In parallel, the utilization of a machine learning model is demonstrated with a Light Gradient Boosted Machine (LGBM) model that is trained using historical process data and used to predict viscosity for different strains, scales and fermentation conditions. This includes an exploration of how machine learning methods can estimate process characteristics, such as viscosity, that are challenging to measure or model at production scale. In addition, the integration of external information, such as real-time weather forecasts, to enhance the accuracy of large-scale model deployment for digital twin applications will be described.

The framework has been developed with the goal of deploying the model within a digital twin system. Tech-transfer for large-scale implementation of the model will be demonstrated in the context of daily operations, including fermentation planning and process optimization aimed at minimizing the cost per unit of enzyme produced.



It will be shown that to fully realize smart manufacturing and ensure seamless model transfer between scales, major investments in online measurement infrastructure at industrial scale are crucial. Our findings support a shift towards greater reliance on high-frequency online process data to reduce dependency on inconvenient offline measurements. The study presents a practical roadmap for developing models for non-model organisms, implementing them at production scale, and understanding current limitations.

1. Garcia-Ochoa, F. and Gomez, E. (2004). Theoretical prediction of gas-liquid mass transfer coefficient, specific area and hold-up in sparged stirred tanks. *Chem. Eng. Sci.*, 59: 2489–2501.
2. Garcia-Ochoa, F. and Gomez, E. (2009). Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnol. Adv.*, 27: 153–176.

### 13:30 Lessons learned on dynamic simulation of heterogeneities using compartment models (L02)

**Hector Maldonado de Leon<sup>1</sup>, Adrie Straathof<sup>1</sup>, Cees Haringa<sup>1</sup>**

<sup>1</sup>*Bioprocess Engineering, Biotechnology Department, Delft University of Technology, Van der Maasweg 9, 2629 HZ, Delft, The Netherlands*  
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Predicting the occurrence of heterogeneities in bioreactors and their impact on cellular performance is crucial for scaling up fermentations, as heterogeneities can affect microorganisms and fermentation performance. Simulations based on Computational Fluid Dynamics (CFD) with integrated biokinetic models facilitate this analysis, but they are computationally intensive and expensive [1-3]. This hinders a rapid and reliable evaluation of process performance, particularly when dealing with dynamic operating conditions as encountered in fed-batch fermentations. Compartment models (CM) offer a computationally affordable yet effective alternative for capturing key hydrodynamic and mass transport features in stirred tank bioreactors [4-7]. By combining these models with machine-learning routines, it is possible to capture the effect of varying operating conditions, circumventing their inherent dependency on CFD-derived flow fields [8].



In this presentation, we share our experience in coupling compartment models and machine learning techniques to develop spatially resolved surrogate models for flexible exploration of bioreactor design and operational modes, with a strong focus on the impact of heterogeneities in industrial-scale bioreactors. Depending on the aim of the surrogate, either supervised and unsupervised learning techniques can be employed – for flow field calculations, thereby decoupling hydrodynamics from biokinetics [8], or to directly infer substrate gradients and metabolic regimes resulting from the interaction of these phenomena [9,10]. Furthermore, deep learning methods have enabled the incorporation of categorical variables, unveiling new opportunities for bioreactor design space exploration. The developed models show a reduction by up to three orders of magnitude in runtime when compared with a fully coupled CFD model, while providing results at a suitable resolution for industrial practitioners. Nonetheless, certain considerations must be addressed when deploying the surrogate: either enforcing linear constraints to ensure mass conservation or penalising spurious predictions outside the expected range.

1. Haringa, C., Deshmukh, A.T., Mudde, R. and Noorman, H. (2017). Euler-Lagrange analysis towards representative down-scaling of a 22m<sup>3</sup> aerobic *S. cerevisiae* fermentation. *Chem. Eng. Sci.* 170: 653–669.
2. Haringa, C., Tang, W., Wang, G., Deshmukh, A.T., van Winden, W., Chu, J., van Gulik, et al. (2018). Computational fluid dynamics simulation of an industrial *P. chrysogenum* fermentation with a coupled 9-pool metabolic model: Towards rational scale-down and design optimization. *Chem. Eng. Sci.* 175: 12–24.
3. Puijman, L., Almeida Benalcázar, E., Picioreanu, C., Noorman, H. J. and Haringa, C. (2023). Downscaling Industrial-Scale Syngas Fermentation to Simulate Frequent and Irregular Dissolved Gas Concentration Shocks. *Bioeng.* 10: 518

### 13:55 Industry promotion talks

#### 14:15 Coffee break & exhibition

## ■ ■ SESSION 2: MASS TRANSFER, SCALE-UP & MODELLING (II)

Chair Ralf Takors / Rosa Haßfurth

### 14:45 Challenges in oxygen mass transfer measurement for industrial-scale aerated stirred tank bioreactors (L03)

**Nicolas Nickel<sup>1</sup>, Noah von Schnitzler<sup>1</sup>, Jürgen Fitschen<sup>2</sup>, Thomas Wucherpennig<sup>2</sup>, Michael Schlüter<sup>1</sup>**

<sup>1</sup>*Institute of Multiphase Flows, Hamburg University of Technology, 21073, Hamburg, Germany*

<sup>2</sup>*Boehringer Ingelheim Pharma GmbH & Co. KG, 88400 Biberach, Germany*

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Oxygen supply is a key parameter in bio-pharmaceutical processes involving mammalian cell cultivation and is commonly characterized by the volumetric mass transfer coefficient  $k_La$  [1]. Although, methods for determining the oxygen mass transfer performance are well known for laboratory and pilot scale plants, reliable measurements in industrial size stirred tank (STR) reactors present significant challenges [2]. At large scales, factors such as hydrostatic pressure, fluid heterogeneities, and probe positioning can substantially influence oxygen measurements. Investigations conducted at the Institute of Multiphase Flows using an industrial-scale STR with a working volume of 12500 L demonstrate that both hydrostatic pressure and operating conditions have a pronounced effect on the measured oxygen saturation concentration. Accurate determination of the oxygen saturation concentration is, however, essential for reliable  $k_La$  calculation. Additionally, the hydrostatic pressure has an influence on the measured  $k_La$  value itself as the oxygen solubility is increased by rising hydrostatic pressure [1].



These findings highlight the limitations of conventional  $k_La$  determination methods when applied to large-scale bioreactors and emphasize the need for scale-aware measurement strategies. Improved understanding of pressure-related effects is crucial for reliable oxygen transfer characterization and for ensuring robust process design and scale-up in industrial mammalian cell cultivation.

This presentation will highlight the challenges for kLa measurements on industrial scale and will discuss how a reliable measurement procedure can be achieved.

1. Matsunaga, N., Kano, K., Maki, Y. and Dobashi, T. (2009). Culture scale-up studies as seen from the viewpoint of oxygen supply and dissolved carbon dioxide stripping. *J. Biosci. Bioeng.* 107: 419–424.
2. Garcia-Ochoa, F. and Gomez, E. (2008). Bioreactor scale-up and oxygen transfer rate in microbial processes: An overview. *Biotechnol. Adv.* 27: 153–176.

## 15:10 Using CFD-based compartment models to resolve spatial heterogeneities in industrial bio-processes (L04)

Johan Le Nepvou De Carfort<sup>1\*</sup>, Victor Puig I Laborda<sup>1,2</sup>, Lars K. Nielsen<sup>2</sup>, Krist V. Gernaey<sup>1</sup>, Ulrich Krühne<sup>1</sup>

<sup>1</sup>PROSYS, Department of Chemical and Biochemical Engineering, Technical University of Denmark

<sup>2</sup>Novo Nordisk Fondation Center for Biosustainability, Technical University of Denmark

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Industrial-scale bioprocesses often exhibit spatial heterogeneities in parameters such as pH and substrate concentration, that can alter cell performance and compromise the product quality. This presentation focuses on translating the complex behavior of large-scale reactors into reliable computational models. While computational fluid dynamics (CFD) can provide a detailed resolution of the flow field, turbulence levels, and mass transfer phenomena, CFD remains computationally too intensive to couple with chemical / biochemical reaction kinetics over realistic production times. CFD-based compartment models stand as a promising tool for converting short CFD snapshots into fast surrogate models that can be coupled with reaction kinetics and simulate extended time spans.



Compartment models provide an intuitive representation of industrial reactors as connected zones, allowing engineers to communicate where and why gradients arise and to test mitigation options in silico. In addition, the compartment structure offers a practical basis for scale-down design by translating large-scale heterogeneity into laboratory-relevant, quantitatively defined targets (e.g., zone volumes, exchange rates, and local hydrodynamic conditions), thereby enabling experiments that reproduce industrial micro-environments more faithfully than conventional well-mixed assumptions.

Overall, this presentation discusses how CFD-based compartment models can serve as computationally feasible, mechanistic models of industrial bioreactors, supporting robust scale-up/scale-down workflows, accelerated process design and optimization, and improved assurance of product quality in the presence of spatial gradients.

1. Le Nepvou De Carfort, J., Pinto, T. and Krühne, U. (2024). An Automatic Method for Generation of CFD-Based 3D Compartment Models: Towards Real-Time Mixing Simulations. *Bioeng.* 11: 169.
2. Le Nepvou De Carfort, J., Puig I. Laborda, V., Nielsen, L.K., Gernaey, K.V. and Krühne, U. (2026). Flow-informed clustering of bioreactor volumes to build CFD-based compartment models, *Chem. Eng. Sci.* 320: 122539.
3. Laborda, V.P.I., Puiman, L., Groves, T., Haringa, C. and Nielsen, L.K. (2024). Unsupervised Learning Bioreactor Regimes. *Comp. Chem. Eng.* 194: 108891.

## 15:35 Numerical scale-up and experimental validation of gas-liquid bioleaching reactors using LB-LES CFD modelling: from 2 L to 2000 L (L05)

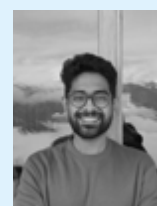
Saranath Sripriya<sup>1\*</sup>, Eric Olmos<sup>2</sup>, Celine Loubière<sup>2</sup>, Douglas Pino-Herrera<sup>1</sup>, Anne-Gwenaelle Guezennec<sup>1</sup>

<sup>1</sup>Bureau de Recherches Géologiques et Minières, BRGM, 45100 Orléans, France

<sup>2</sup>Université de Lorraine, CNRS, LRGP, F-54000, Nancy, France

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Metal recovery using bioleaching processes is strongly influenced by the reactor hydrodynamics and oxygen availability, directly affecting the microbial activity and leaching performance [1]. Therefore, the coupling of computational fluid dynamics (CFD) simulations incorporating gas-liquid mass transfer with kinetics provides a more detailed spatial and temporal insight into transport phenomena, that are difficult to access experimentally, making them powerful tools for reactor design and scale-up [2]. However, comparative studies of bioleaching reactors under similar hydrodynamic conditions during scale-up remain limited. In this work, we present a CFD-based model for bioleaching processes, validated through experiments at three scales -2 L, 20 L, and 2000 L. Additionally, oxygen consumption kinetics [3] were integrated to support further optimization and aid in reactor design.



The scale-up criteria are based on geometrical similarity and conservation of the dissipated power per unit volume to ensure comparable hydrodynamic conditions. Lattice-Boltzmann Large Eddy Simulations (LB-LES) are solved using M-Star software to predict reactor flow-fields with turbulence closure implemented via Lilly-Smagorinsky model. Oxygen is introduced through air sparging, and gas-liquid mass transfer is modelled on a bubble-by-bubble basis using advection-diffusion equations. Experimental measurements of the dissolved oxygen concentration and the volumetric mass transfer coefficient (kLa) are determined at each scale to validate the simulations.

The LB-LES approach demonstrates strong agreement with the experimental measurements of oxygen transfer across scales, highlighting the capability of the proposed model to efficiently map oxygen dynamics during scale-up. This work is aimed toward modelling and design of an industrial-scale pond bioleaching pilot [4] and enabling large-scale bioleaching operations.

1. Dew, D.W., Rorke, G.V. and Guezennec, A.-G. (2023). Engineering designs and challenges of stirred tank systems, in: *Biomining Technologies*. Springer International Publishing, pp. 41–65.
2. Chéron, J., Loubière, C., Delaunay, S., Guezennec, A.-G. and Olmos, E. (2020). CFD numerical simulation of particle suspension and hydromechanical stress in various designs of multi-stage bioleaching reactors. *Hydrometallurgy*, 197: 105490.
3. Guezennec, A.-G., Joulain, C., Jacob, J., Archane, A., Ibarra, D., De Buyer, R., Bodéan, F. and d'Hugues, P. (2017). Influence of dissolved oxygen on the bioleaching efficiency under oxygen enriched atmosphere. *Minerals Engineering*, 106: 64-70.
4. Guezennec, A.-G., et al. "Bioleaching method and facility." U.S. Patent Application No. 15/316,605.

**16:00 Coupling genome-scale models with compartment bioreactor models to study intracellular responses under fluctuating conditions (L06)*****Titania Sugiarto*<sup>1\*</sup>, *Samira van den Bogaard*<sup>1</sup>, *Tobias B. Alter*<sup>1</sup>**<sup>1</sup>*Institute of Applied Microbiology, Aachen Biology and Biotechnology, RWTH Aachen University, Aachen 52074, Germany*<sup>2</sup>*First affiliation according to the authors numbering**\*Email: titania.sugiarto@rwth-aachen.de*

The success of bioprocess development at lab scale is often accompanied by reduced yields at industrial scale. A major cause is nonideal mixing in large-scale bioreactors, which generates spatial gradients in substrate concentration, dissolved oxygen, and pH. Traditionally, this challenge has been addressed through digital twins and Computational Fluid Dynamics (CFD) simulations to improve reactor design and mixing performance. However, fully homogeneous conditions are practically unattainable due to physical and economic limits on power input.

Instead of aiming for perfect mixing, a more informative approach is to investigate how microbial cells respond to these spatial gradients. Understanding this interaction can reveal key process limitations and open new strategies for optimization. Many CFD-based studies treat the fluid as a continuous phase and model cells as discrete particles governed by simple biomass-based kinetics. While suitable for describing fluid–cell interactions, such models neglect intracellular metabolic complexity. Genome-scale metabolic models (GSMMs) provide detailed insight into cellular metabolism, but direct coupling with CFD is computationally prohibitive.

To overcome this limitation, we propose a reduced compartment modeling framework that translates complex hydrodynamics into a network of interconnected, well-mixed stirred tank reactors. Each compartment represents distinct environmental conditions while preserving essential hydrodynamic features. This structure enables coupling with GSMMs to analyze intracellular metabolic states under heterogeneous conditions, supporting improved strain design and bioreactor optimization.

1. Enfors, S.-O., Jahic, M., Rozkov, A., Xu, B., Hecker, M., Jürgen, B., Krüger, E., et al. (2001). Physiological responses to mixing in large scale bioreactors. *J. Biotechnol.* 85: 175–185.

2. Bafna-Rührer, J., Bhutada, Y.D., Orth, J.V., Øzmerih, S., Yang, L., Zielinski, D., Sudarsan, S. (2024). Repeated glucose oscillations in high cell-density cultures influence stress-related functions of *Escherichia coli*. *PNAS Nexus*. 3: pgae376.

**16:25 Scale-up Challenges in *Pichia pastoris* Fermentation Processes (L07)*****Anamari Brdar*<sup>1\*</sup> and *Hannah Singer*<sup>2\*</sup>**<sup>1</sup>*Sanofi, Global CMC Development Microbial Platform Clinical Trial Manufacturing*<sup>2</sup>*Sanofi, Global CMC Development Microbial Platform USP**\*Email: Anamari.Brdar@sanofi.com; Hannah.Singer@sanofi.com*

The successful scale-up of *Pichia pastoris* fermentation processes presents significant challenges to the biotechnology industry, as numerous subtle yet critical obstacles often emerge during process transfer from laboratory to production scale. These variables frequently manifest as scale-dependent phenomena during technology transfer from bench to production scale. In particular, large-scale harvest operations such as continuous centrifugation and subsequent filtration remain difficult to mimic accurately at laboratory scale. This study investigates a systematic approach to identifying and overcoming scale-up challenges during the transfer from 200 mL laboratory fermentations through 400 L pilot-scale operations to 2500 L production bioreactors. Through close interdisciplinary collaboration between development and operations teams, analytical tools were implemented that enabled precise identification of process vulnerabilities. Difficulties in separation of biomass from supernatant by continuous centrifugation that manifested only at production scale were successfully predicted at laboratory scale and subsequently resolved through targeted technical solutions addressing specific process parameters during the fermentation stage. The results demonstrate that solutions developed through applied testing during the development phase can be successfully translated into practice at pilot and production scale.

This study underscores the importance of developing laboratory-scale methods that accurately mimic large-scale harvest operations, enabling early mitigation of scale-up obstacles through systematic preliminary investigations and effective interdisciplinary communication. This approach yields considerable time and cost savings in bioprocess development.

**16:50 Coffee break & exhibition****SESSION 3: MASS TRANSFER, SCALE-UP & MODELLING (III)**

Chair Howard Ramirez Malule / Simon Täuber

**17:20 Multivariate population balance model resolution for bioreactor simulation (L08)*****Casale Benjamin*<sup>1\*</sup>, *Morchain Jerome*<sup>1</sup>, *Villedieu Philippe*<sup>2,3</sup>**<sup>1</sup>*TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France*<sup>2</sup>*ONERA/DMPE, Université de Toulouse, Toulouse, France*<sup>3</sup>*Institut de Mathématiques de Toulouse, Université de Toulouse, Toulouse,**\*Email: casale@insa-toulouse.fr*

Accurate modelling of macro-scale phenomena in industrial reactor systems requires describing the variability within cellular populations [1] as well as the two-way coupling between cells, liquid and gas leading to the formation of concentration gradients in large-scale stirred reactors [2]. A multivariate population balance model discriminates individuals according to their properties and, coupled with mass balances, it provides a framework for describing the dynamics of cell populations driven by internal noise and external forcing. We developed a particle-based Monte Carlo code to address these challenges in a general way. The proposed fixed modelling architecture allows the study of different biological processes within a unified simulation environment, using modifiable biological sub-models. It combines a CFD-based compartment model for scalars, a stochastic cell transport algorithm and particle-based methods for biological source term calculations.



The code captures local population heterogeneity in various operating conditions from laboratory-scale and scale-down experiments to industrial continuous fermenters. Mixing, gas–liquid mass transfer, population dynamics over time and space, uptake adaptation, metabolically governed cell division, and cell elongation processes are included. We validated the code through a comparison with available analytical solutions [3] considering length, age, instantaneous elongation rate, and metabolic reaction rates as cell properties. The redistribution of these properties at division (inheritance) produces, at the population scale, the observed population variability. With this tool, we now examine the impact of inheritance laws on population stability, adaptation to external fluctuations while cell size distribution is compared with experimental data [4]. Some examples, at different scales, will be presented.

1. Biswas, K. and Brenner, N. (2024). Universality of phenotypic distributions in bacteria. *Phys. Rev. Res.*, 6: L022043.
2. Brand, E., Junne, S., Anane, E., Cruz-Bournazou, M.N. and Neubauer, P. (2018). Importance of the cultivation history for the response of *Escherichia coli* to oscillations in scale-down experiments. *Bioprocess Biosys. Eng.*, 41: 1305–1313.
3. Quedeveille, V., Mochain, J., Villedieu, P. and Fox, R.O. (2019). A critical analysis of Powell's results on the interdivision time distribution. *Sci. Rep.*, 9: 8165.
4. Cullum, J. and Vicente, M. (1978). Cell growth and length distribution in *Escherichia coli*. *J. Bacteriol.*, 134: 330–337.

## 17:45 Morphology-based scale-up of *Streptomyces* species cultivations using CFD simulations (L09)

**Philipp Eibl<sup>1,\*</sup>, Gesa Brauneck<sup>2,\*</sup>, Christian Witz<sup>1</sup>, Marcel Mann<sup>2</sup>, Johannes Pastoors<sup>2</sup>, Dorothea M. Schütterle<sup>3,4</sup>, Miriam A. Rosenbaum<sup>3,4</sup>, Jorgen Magnus**

<sup>1</sup>SimVantage GmbH, Graz, Austria

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<sup>3</sup>Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena, Germany

<sup>4</sup>Faculty of Biological Sciences, Friedrich-Schiller-University Jena, Jena, Germany

\*Email: philipp.eibl@simvantage.com; gesa.brauneck@avt.rwth-aachen.de

Filamentous microorganisms, such as *Streptomyces* species, exhibit complex morphologies that influence product formation. Scale-up of these microorganisms remains challenging, as conventional approaches often fail to maintain reproducible morphology across scales. In this study, the morphology-based scale-up from shake flasks to stirred-tank reactors is investigated using energy dissipation rate (EDR) as scale-up parameter. This parameter quantifies hydromechanical stress, which affects the morphology of filamentous microorganisms, and was systematically analyzed using computational fluid dynamics (CFD) simulations.



Initial scale-up experiments focus on the average EDR, which prove insufficient to reproduce the morphology of *Streptomyces* species across scales. Instead, the maximum EDR is considered, but its determination is challenging and not well-established in literature.

Therefore, CFD simulations based on the lattice-Boltzmann method coupled with large eddy simulation are employed for investigation of the flow field statistics. The simulations enable a systematic evaluation of various EDR statistics for a wide range of operating conditions, including average, maximum, and percentile-based values.

A novel scale-up criterion based on the 95th percentile of the EDR distribution (95%) is proposed. To validate this approach, cultivations are performed according to this criterion, and the resulting morphologies of *Streptomyces* species are compared with that obtained using the conventional scale-up based on average EDR. These findings show that a similar 95% lead to a comparable morphology across scales, providing a strong basis for proposing 95% as a new scale-up parameter for filamentous microorganism cultivations [1].

1. Brauneck, G., Brass, A., Boreiko, A., Ueßeler, M., Mann, M., Pastoors, J., Steier, V. et al. (2025). Scale-up of *Streptomyces* species cultivations based on the morphological response to the energy dissipation rate. Submitted to *AIChE J.*

## 18:10 Balancing yield and sustainability: Modelling operational strategies for scaled cultivated meat production (L10)

**Ben Tumulero<sup>1,\*</sup>, Cees Haringa<sup>1</sup>, Marcel Ottens<sup>1</sup>**

<sup>1</sup>Delft University of Technology, Delft, the Netherlands

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As the world's food demand continues to expand while available resources remain limited, there is a pressing need to optimize global food production systems. Cultivated meat has emerged as a potential way to enable the growth of familiar meat products using fewer resources. Despite significant technological advances over the past decade, achieving large-scale animal cell cultivation to make these products widely available remains a major challenge [1].



To support the development of scalable production processes, various techno-economic models have been proposed to describe animal cell growth and predict industrial performance [2-4]. However, many existing models neglect key biological and operational dynamics such as the inhibitory effects of osmolality and oxygen, or overflow metabolism [5]. Moreover, differences in foundational assumptions make direct comparisons across operational modes difficult.

To address these gaps and enable more consistent benchmarking, this work compares animal cell proliferation using four operational modes (repeated fed-batch, chemostat, concentrated repeated fed-batch, and continuous perfusion) and several feed- or perfusion strategies in an ideally mixed 2000L stirred-tank reactor (STR), using minimal unstructured and unsegregated kinetic models based on literature parameters. Performance is evaluated through space-time yield, and nutrient -use and -efficiency metrics. A sensitivity analysis on kinetic input parameters such as the maximum growth rate and apparent stoichiometric yields further elucidates how different cell types might perform across these processes.

All simulation code is written in object-oriented Python, facilitating straightforward adaptation of the framework to accommodate for diverse cell lines, process conditions, and underlying models.

1. Post, M.J., Levenberg, S., Kaplan, D.L., Genovese, N., Fu, J., Bryant, C.J., Negowetti, N., et al. (2020). Scientific, sustainability and regulatory challenges of cultured meat. *Nat. Food.* 1: 403-415.
2. Humbird, D. (2020). Scale-Up Economics for Cultured Meat Techno Economic Analysis and Due Diligence. <https://doi.org/10.31224/osf.io/795su>

3. Risner, D., Li, F., Fell, J.S., Pace, S.A., Siegel, J.B., Tagkopoulos, I. and Spang, E.S. (2020). Preliminary Techno-Economic Assessment of Animal Cell-Based Meat. *Foods*. 10: 3.
4. Negulescu, P.G., Risner, D., Spang, E.S., Sumner, D., Block, D., Nandi, S. and McDonald, K.A. (2023). Techno-economic modeling and assessment of cultivated meat: Impact of production bioreactor scale. *Biotechnol. Bioeng.* 120: 1055-1067.
5. Frohlich, B., Harsini, F. and Swartz, E. (2025). Optimizing cultivated meat techno-economics: Cell growth modeling review and recommendations. <https://doi.org/10.62468/xcjx6040>

## 18:35 GET-TOGETHER & EXHIBITION & POSTER-SESSION FOR ALL PARTICIPANTS

- 18:35 **Melody of the genome 2.0 – The sound of DNA (Artistic Contribution), Langenbeck-Virchow-Haus, Foyer**  
*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**

### TUESDAY, 21 APRIL 2026

## ■ ■ WELCOME NOTE

- 9:00 **Welcome note 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*

## ■ ■ SESSION 4: SCALING OF BIOPROCESSES

- Chair **Annette Berg / Johannes Nicklisch**

## ■ ■ PLENARY TALK

- 9:05 **Process modeling strategies for successful biopharmaceutical development (PL02)**

*Thomas Wucherpennig*

*Global Development CMC Biologicals*

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Biopharmaceutical process development is undergoing a fundamental transition from empirically driven experimentation toward predictive, model-based decision-making. Increasing process complexity, accelerated timelines, and rising demands for robustness, sustainability, and facility fit require integrated modeling concepts that span scales, disciplines, and development phases. This talk outlines a holistic process modeling toolbox for upstream biopharmaceutical development that incorporates statistical, mechanistic, data-driven, and hybrid modeling approaches.

Classical Design of Experiments, multivariate data analysis, and selected data-driven models provide a quantitative backbone for describing process dynamics and variability across operating spaces. Hybrid models, combining first-principles knowledge with data-driven learning, offer a powerful means to capture both causality and correlation.

Detailed insight into scale-dependent phenomena is achieved through validated computational fluid dynamics (CFD) models that resolve hydrodynamics, mixing, mass transfer, and shear stress across equipment and scales. CFD is applied from microtiter plates and laboratory reactors to large-scale stirred tanks, perfusion systems, and downstream unit operations, enabling early identification of heterogeneities, short-circuit flows, and high-risk zones. This mechanistic understanding supports robust scale-up, technology transfer, and risk mitigation while reducing experimental effort.

The integration of process models with plant-level simulations enables quantitative assessment of facility fit, capacity utilization, debottlenecking strategies, and sustainability metrics such as resource consumption and carbon footprint. In combination, CFD-based equipment characterization and plant-wide modeling establish a digital representation of both process and facility, allowing scenario-based evaluation of process intensification strategies and informed decision-making early in development. Together, these approaches illustrate how integrated modeling can transform biopharmaceutical development into a predictive, scalable, and industrially resilient discipline.



## 9:50 Hybrid modelling to assist scaling of mAb production processes (L11)

Kenneth S.Y. Ng<sup>1,2,3</sup>, Kaleb F. Ferede<sup>1,2,3</sup>, Masih Karimi Alavijeh<sup>5</sup>, Yih Yean Lee<sup>1,4</sup>, Ellen Otte<sup>1,4</sup>, Sally L. Gras<sup>1,2,3\*</sup>

<sup>1</sup>The ARC Digital Bioprocess Development Hub, The University of Melbourne, Australia

<sup>2</sup>Department of Chemical Engineering, The University of Melbourne, Parkville, Victoria, 3010, Australia

<sup>3</sup>The Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Parkville, Victoria, 3010, Australia

<sup>4</sup>CSL Innovation, 655 Elizabeth Street, Melbourne, Victoria, 3000, Australia

<sup>5</sup>CSL Behring, 189 – 209 Camp Road, Broadmeadows, Victoria, 3047, Australia

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Hybrid modelling has the potential to assist the scale up or scale down of processes such as monoclonal antibody (mAb) production in mammalian cells. This approach couples digital algorithms and process data, together with mechanistic equations and understanding, to predict process performance across scales [1].

Digital, or data driven, model components can be used to represent some of the complexity of scaling, including bioreactor size, geometry, process conditions, hydrodynamics, mass transfer, as well as culture behaviour, whilst mechanistic equations can help to constrain possible solutions within physical limits, reducing the need for process data and improving extrapolation.

Here, a serial hybrid model was developed using a CatBoost algorithm and mechanistic component relating viable cell density (VCD) to growth rate. VCD was predicted up to 14 days ahead at 200 L scale for a new process, when trained using historical process data spanning bioreactor scales (5 L, 200 L and 2000 L) supplemented with 5 L data for the new process. The sensitivity of predictions to the 5 L and historical training datasets is also examined.

This approach builds on our prior study that predicted the P/V needed to achieve a target peak VCD using a Catboost algorithm in a purely digital approach [2], where additional small scale data was also found to assist predictions at larger scale for ANN and XGBoost models using a dataset of 55 bioreactors from 250 mL to 5000 L scale.

Together, these approaches illustrate the potential strengths and limitations of simple AI assisted scaling models.

1. Karimi Alavijeh, M., Baker, I., Lee, Y.Y. and Gras, S.L. (2022). Digitally enabled approaches for the scale up of mammalian cell bioreactors. *Dig. Chem. Eng.* 4: 100040.
2. Karimi Alavijeh, M., Lee, Y.Y. and Gras, S. (2024). A perspective-driven and technical evaluation of machine learning in bioreactor scale-up: A case-study for potential model developments. *Eng. Life Sci.* 24: e2400023.



## 10:15 Model-based scaling of recombinant protein production in Escherichia coli fed-batch processes: Knowledge-based optimization of secretion performance and cell viability (L12)

Fabian Schröder-Kleeberg<sup>1</sup>, Lucas Kaspersetz<sup>1</sup>, Sanchir Anar<sup>1</sup>, Markus Zoellkau<sup>2</sup>, Markus Glaser<sup>2</sup>, Markus Brunner<sup>3</sup>, Christian Bosch<sup>3</sup>, Mariano Nicolas Cruz Bournazou<sup>1</sup>, Peter Neubauer<sup>1\*</sup>

<sup>1</sup>Technische Universität Berlin, Institute of Biotechnology, Department of Bioprocess Engineering, 13355 Berlin, Germany

<sup>2</sup>Wacker Biotech GmbH, 07745 Jena, Germany

<sup>3</sup>Wacker Chemie AG, 81379 München, Germany

\*Email: peter.neubauer@tu-berlin.de

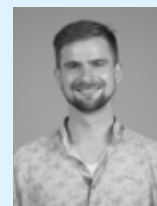
Scaling bioprocesses remains a fundamental challenge due to complex physiological responses and variable environmental conditions. This work presents an integrated, model-driven approach to optimize the production of Fab antibody fragments in the medium through periplasmic release by Escherichia coli across five distinct scales.

Initially, a mechanistic macro-kinetic model was extended with a two-fraction approach to accurately describe the influence of complex yeast extract additives [1]. This enabled precise predictions of growth dynamics and acetate metabolism for various media compositions and concentrations. Building on this, scalability from 15 mL mini-bioreactors to the 30 L pilot scale was investigated [2]. Model-based analysis identified bolus feeding in high-throughput systems as a critical parameter that enhances cell robustness and significantly lowers cell lysis rates compared to homogeneous reactor conditions.

Additionally, automated high-throughput optimization of 30 multifactorial conditions was conducted to examine the balance between product formation, release, and cell viability. The results revealed a fundamental trade-off: while high Fab titers correlated with low lysis but slow release, rapid release kinetics led to substantial cell lysis. Induction strength and temperature emerged as the primary control variables for managing this trade-off [3].

Overall, this study demonstrates that the combination of automated experimentation and mechanistic modeling significantly accelerates knowledge-based bioprocess development. This approach minimizes scaling uncertainties and provides a robust framework for the efficient development and optimization of secretion-based bioprocesses.

1. Schröder-Kleeberg, F., Zoellkau, M., Glaser, M., Bosch, C., Brunner, M., Cruz Bournazou, M.N. and Neubauer, P. (2025). Modelling of Escherichia coli Batch and Fed-Batch Processes in Semi-Defined Yeast Extract Media. *Bioeng.* 12: 1081.
2. Schröder-Kleeberg, F., Kaspersetz L., Zoellkau, M., Glaser, M., Bosch, C., Brunner, M., Cruz Bournazou, M.N. and Neubauer, P. (2026) Model-driven monitoring and parameter-based scale-down of an Escherichia coli fed-batch process for production of a Fab antibody fragment. Submitted to *Biotechnol. Bioeng.*
3. Schröder-Kleeberg, F., Kaspersetz, L., Anar, S., Zoellkau, M., Glaser, M., Bosch, C., Brunner, M., et al. (2026). Model-driven monitoring and parameter-based scale-down of an Escherichia coli fed-batch process for production of a Fab antibody fragment. Submitted to *Bioprocess Biosyst. Eng.*



10:40 Industry promotion talks

10:55 Coffee break &amp; exhibition

## SESSION 5: BIOPROCESS INTEGRATION (I)

Chair Alvaro Lara / Fatemeh Nejati

### 11:25 FerroFlow: unlocking simulation software for end-to-end bioprocess design - A fermentation case-study (L13)

*Pierre Guilloteau<sup>1,\*</sup>, Merlin Alvarado Morales<sup>1</sup>, Gürkan Sin<sup>1</sup>*

<sup>1</sup>Technical University of Denmark (DTU), Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Kgs Lyngby, Denmark

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Scaling-up is a critical part of process development for biomanufacturing, and laboratory success often fails to translate to industrial production. While the chemical industry relies on rigorous platforms to design entire plants, the bioprocess industry lacks this capability due to complex biological libraries.

This presentation introduces FerroFlow, a software-based solution that unlocks industry-standard simulators for end-to-end bioprocess design. By leveraging Aveva Process Simulation (APS), we provide the first platform capable of performing rigorous energy and mass balances for bioprocesses, based on APS strong thermodynamics. This integration allows for the assessment of different plant configurations, equipment sizing, utilities consumption, and techno-economic assessments with the same level of ambition as for modern chemical processes.

We present here a specific fermentation case study implemented in APS and validated with industrial full-scale data. Fermentation modeling itself is a well-established field, however industrial success requires more than standalone models.

Beyond biological reaction kinetics and transport phenomena, this contribution demonstrates the sizing of major equipment, specifically:

- Fermenter geometry: impeller type and sizing, liquid-gas mass transfer rate, broth viscosity correlations.
- Air Compressors, pumps, stirrers, and heat exchangers.
- Industrial Utilities: electricity, power requirements and cooling water demands.

FerroFlow distinguishes itself from known models by utilizing the native "equation-oriented" architecture, validated sizing heuristics and rigorous thermodynamic of APS, which enables full flowsheet integration of bioprocesses. While this case study focuses on fermentation, FerroFlow is expanding to include validated downstream unit operations to provide the exhaustive, standardized bio-library required to de-risk scale-up and significantly reduce operational expenses.



### 11:50 Towards understanding the effect of upstream process parameters on the properties and downstream performance of inclusion bodies (L14)

*Mohamed Elshazly<sup>1,2,\*</sup>, Eva Prada Brichtova<sup>1,2</sup>, Florian Gispe<sup>1,2</sup>, Robert Klausser<sup>1,2</sup>, Oliver Spadiut<sup>1,2</sup> and Julian Kopp<sup>1,2</sup>*

<sup>1</sup>Research Division Integrated Bioprocess Development, Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Gumpendorferstraße 1A, Vienna 1060, Austria

<sup>2</sup>Christian Doppler Laboratory for Inclusion Body Processing 4.0, Institute of Chemical, Environmental and Bioscience Engineering, Technische Universität Wien, Gumpendorferstraße 1A, Vienna 1060, Austria

\*Email: mohamed.elshazly@tuwien.ac.at

Ever since inclusion bodies (IBs) have been found to be valuable cultivation outcomes, their expression in *Escherichia coli* has become indispensable for recombinant protein production in the biopharmaceutical field [1].

Still, the current state-of-the-art IB processing remains highly empirical and inefficient. De novo process development still requires extensive experimental efforts to optimize conditions for specific proteins [2]. Numerous studies have already provided viable insights into the critical process parameters. Upstream process (USP) parameters such as the temperature, pH, carbon source, and feeding strategy have proven to affect IB titer and IB properties, such as the protein purity, size and preserved secondary structures [3–5].

Despite those efforts, to our knowledge, the current literature lacks a dedicated description of how USP strategies influence the consequent downstream processing (DSP) performance. This might provide insights into the complex relationship between intracellular aggregation and subsequent renaturation. To tackle this research challenge, the influence of USP variations on titer, properties like IB size and secondary structure composition, as well as refolding performance must be systematically assessed. In my talk, I will demonstrate how to alternate the USP using a design-of-experiment approach to capture its correlation with the selected responses, aiming to shed more light on the effects of the USP on the DSP for IB processing.

1. Ruiz, N. and Silhavy, T.J. (2022). How *Escherichia coli* Became the Flagship Bacterium of Molecular Biology. *J. Bacteriol.* 204: e0023022.

2. Metzger, K.F.J., Padutsch, W., Pekarsky, A., Kopp, J., Voloshin, A.M., Kühnel, H. and Maurer, M. (2020). IGF1 inclusion bodies: A QbD based process approach for efficient USP as well as early DSP unit operations. *J. Biotechnol.* 312: 23–34.

3. Slouka, C., Kopp, J., Hutwimmer, S., Strahammer, M., Strohmmer, D., Eitenberger, E., Schwaighofer, A., and Herwig, C. (2018). Custom made inclusion bodies: impact of classical process parameters and physiological parameters on inclusion body quality attributes. *Microb. Cell. Fact.* 17: 148.

4. Kopp, J., Slouka, C., Ulonska, S., Kager, J., Fricke, J., Spadiut, O., and Herwig, C. (2017). Impact of Glycerol as Carbon Source onto Specific Sugar and Inducer Uptake Rates and Inclusion Body Productivity in *E. coli* BL21(DE3). *Bioeng.* 5: 1.

5. Reichelt, W.N., Brillmann, M., Thurrold, P., Keil, P., Fricke, J., and Herwig, C. (2017). Physiological capacities decline during induced bioprocesses leading to substrate accumulation. *Biotechnol. J.* 12: 1600547.



**12:15 Two-dimensional protein denaturation surfaces as a process development tool for solubilization and refolding of bacterial inclusion bodies (L15)**

*Robert Klausser*<sup>1,2\*</sup>, *Martin Voigtmann*<sup>3</sup>, *Eva Prada Brichtova*<sup>1,2</sup>, *Florian Gisberg*<sup>1,2</sup>, *Robert Klausser*<sup>1,2</sup>, *Oliver Spadiut*<sup>1,2</sup> and *Julian Kopp*<sup>1,2</sup>

<sup>1</sup>Research Division Integrated Bioprocess Development, Institute of Chemical, Environmental and Bioscience, Technische Universität Wien, Vienna, Austria

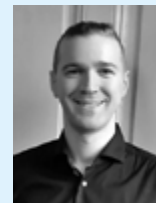
<sup>2</sup>Christian Doppler Laboratory IB Processing 4.0, Technische Universität Wien, Vienna, Austria

<sup>3</sup>Boehringer Ingelheim RCV GmbH & Co KG, Biopharma Austria, Development Operations Analytical Development, Vienna, Austria

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Solubilization and refolding remain the main bottlenecks in recombinant protein production from inclusion bodies in *Escherichia coli*. However, these unit operations are still mainly optimized empirically, which causes long development times and a lack of transferability to different products.

In this joint presentation, we show the potential of protein denaturation curves as a knowledge-based tool for the optimization of pH and denaturant concentration – two key parameters for solubilization and refolding processes. Two-dimensional denaturation surfaces were generated by measuring the average emission wavelength of intrinsic tryptophan fluorescence after incubation at different pH and denaturant concentrations. This knowledge was then used to efficiently define a rational design space for empirical process optimization, saving valuable development time and resources. Furthermore, we highlight how this approach could be leveraged in an industrial setting using automation tools. By exploring this approach rooted in the basics of protein science, we aim to contribute to more transferability and mechanistic understanding in the field of inclusion body refolding.

**12:40 Lunch break, poster session & exhibition****SESSION 6: BIOPROCESS SCALE-DOWN**

Chair Peter Neubauer / Tolué Kheirkhah

**PLENARY TALK****13:50 Bioreactor simulation from the cellular perspective application in scaling from cell cultures to syngas fermentation (PL03)**

*Cees Haringa*

*Department of Biotechnology, Delft University of Technology, Delft, The Netherlands*

*Email: C.Haringa@tudelft.nl*

For a successful transition towards a circular bioeconomy, bioprocesses need to compete at large scales with low margins: optimized processes which can be reliably scaled are key. Currently, much effort is dedicated to development of new bioprocesses in food and biobased chemicals, often with large synthetic biology efforts, only for processes to underperform or outright fail upon piloting or commercialization. Mass transfer limitation, gradient formation and metabolic shifts are often part of this failure: early development focuses on optimizing the biology, whilst at scale the interplay with physics is crucial. Computational tools, sometimes popularly referred to as 'digital twins', play a key role in predicting physics transitions upon scale-up, in characterizing expected industrial environments for scale-down, and ideally in predicting the biological response to such (variable) environments.



While several academic projects have shown the application of such tools, there are a number of factors that are restraining practical adaptation towards quantitative predictions and rational process optimization. These include: (a) lack of quantitative physical models for the specific conditions in bioreactors, (b) computational complexity of models leading to high computation times, (c) lack of kinetic models and data for cell behavior under dynamic conditions. In this talk, I will highlight the recent developments on these topics, as well as some possible futures.

**14:35 BioProScale Lifetime Achievement Award**

*Awardee: Matthias Reuss, Prof. Emeritus of University of Stuttgart, Germany*

**15:00 Predictive scale-down using AI-designed 3D-printed bioreactors (L16)**

*Peter Satzer*<sup>1\*</sup>, *Lena Achleiter*<sup>1</sup>, *Manfred Satzer*<sup>1</sup>

<sup>1</sup>print4biotech (p4b) GmbH, Gaertnerweg 4, 2120 Odersdorf

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Bioprocess scale-up remains a major challenge in translating laboratory fermentations to industrial production, primarily due to scale-dependent phenomena such as nutrient gradients and differences in oxygen transfer. Traditional scale-up strategies rely on incremental increases in vessel size, with deviations in yield, product quality, and process robustness often becoming apparent only after significant time and resource investment. Here, we introduce a novel technology that combines evolutionary artificial intelligence, computational fluid dynamics (CFD), and additive manufacturing to replicate large-scale mixing dynamics within laboratory-scale bioreactors.



Our workflow employs a generative evolutionary algorithm to design internal static structures that can be retrofitted into existing small-scale vessels, thereby altering their hydrodynamic behavior to mimic production-scale conditions. The algorithm iteratively modifies reactor wall geometries, automatically evaluates mixing performance using CFD, and converges on designs that reproduce target large-scale mixing profiles. This physics-based approach does not require extensive product- or host-specific training datasets, as it directly recreates the physical environment of large-scale bioreactors at bench scale.

We demonstrate that the resulting modified small-scale bioreactors achieve extended mixing times exceeding 150 s representative for large scale mammalian processes. Experimental validation confirmed the presence of large-scale effects, including delayed growth, reduced product titers, and increased by-product formation, despite comparable peak cell densities. This technology enables predictive scale-down experimentation without the need for costly pilot-scale studies, providing a flexible platform for early identification of scale-up risks and the development of robust upstream bioprocesses.

## 15:25 Beyond mixing times: From industrial to lab-scale bioreactors using cell trajectories (L17)

Ryan Rauterbach<sup>1,\*</sup>, Jonas Barczyk<sup>2</sup>, Marko Hoffmann<sup>1</sup>, Ralf Takors<sup>2</sup>, Michael Schlüter<sup>1</sup>

<sup>1</sup>Institute of Multiphase Flows, Hamburg University of Technology, Eißendorfer Str. 38, 21073 Hamburg, Germany

<sup>2</sup>University of Stuttgart, Institute of Biochemical Engineering, Allmandring 31, 70569 Stuttgart, Germany

\*Email: ryan.rautenbach@tuhh.de

Reliable scale-down bioreactors are essential for modern bioprocess development, most scale-down concepts, such as the Single Multi-Compartment Bioreactor (SMCB) are calibrated under single-phase conditions using global or local mixing times, neglecting the inherently multiphase nature of fermentation which reshapes flow structures, compartment boundaries, and the cell lifelines that define the cellular exposure to processes conditions.[1,2,3]

This study integrates experiments and numerical simulations to characterise a 15 m<sup>3</sup> mammalian cell culture stirred tank reactor (STR) as an industrial benchmark and to evaluate the scale-down accuracy of the SMCB. A validated numerical framework provides hydrodynamic metrics inaccessible experimentally, emphasizing a Lagrangian cell-centric perspective to quantify velocity distributions, circulation times, and residence times.[4]

Key findings show that at constant aeration, increased agitation does not enhance mixing intensity, while circulation and residence times become dominated by aeration rate rather than agitation. Comparative analysis in the SMCB reveals good qualitative agreement with the STR in single-phase flow but significant deviations under aeration. With the upper compartment behaving like a short bubble column, failing to reproduce the increasing circulation and residence times observed at scale. Mammalian cell fermentations in both the SMCB and a non-scaled reference reactor further confirm that ignoring multiphase effects leads to misleading performance, with initial heterogeneities diminishing over fermentation time as the effective bubble column grows.

This work establishes a validated experimental and numerical baseline and framework for multiphase lifeline analysis and demonstrates that robust scale-down design must integrate multiphase lifeline metrics, not solely single-phase mixing times.

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.

2. 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.* 121: 1244-1256.

3. Arulrajah, P., Lievonen, A.E., Subaşı, D., Pagal, S., Weuster-Botz, D. and Heins, A.L. (2025). Scale-down bioreactors—comparative analysis of configurations. *Bioprocess Biosys. Eng.* 48: 1619-1635.

4. Hofmann, S., Rautenbach, R., Buntkiel, L., Brouwers, I.S., Gaugler, L., Barczyk, J., Fitschen, J. et al. (2025). Lagrangian Sensor Particles for detecting hydrodynamic heterogeneities in industrial bioreactors: Experimental analysis and Lattice-Boltzmann simulations. *Chem. Eng. J. Adv.* 22: 100744.



## 15:50 Coffee break & exhibition

## SESSION 7: PROCESS ANALYTICAL TECHNOLOGY (I)

Chair Krist Germaey / Saskia Waldburger

## 16:20 Unlocking the potential of inline process analytical technology (PAT) for advanced bio-process development, scale-up, and control (L18)

Katharina Dahlmann<sup>1,\*</sup>,

<sup>1</sup>Hamilton Bonaduz AG

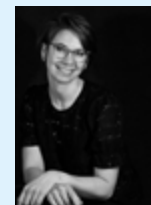
\*Email: kdahlmann@hamilton.ch

Inline PAT technologies provide a substantial advantage over classical offline analytics by enabling continuous, real time insight into cellular physiology. The integration of impedance spectroscopy with inline glucose, CO<sub>2</sub> and pH sensing captures metabolic transitions and physiological shifts with a temporal resolution unattainable by daily offline measurements. This high frequency data stream supports improved monitoring of preculture quality, early detection of process deviations, and robust batch to batch comparability while enabling cell centric approaches to process control.

Application data from multiple use cases demonstrate how multiparametric inline measurements generate a continuous, information rich dataset across small scale and scaled up bioprocesses. Comparisons with conventional offline analytics underline the superior metabolic sensitivity and process relevance of inline tools, offering new process perspectives that enhance development decisions. Of particular relevance is the ability to monitor inoculum performance and detect metabolic transitions in real time, providing a foundation for automated control strategies.

Inline PAT measurements revealed metabolic dynamics that remain hidden in offline sampling. Impedance spectroscopy resolved shifts in intracellular conductivity and cell size distribution associated with metabolic transitions, while inline glucose monitoring tracked rapid changes in nutrient uptake. Inline CO<sub>2</sub> and pH measurements enabled early identification of scale dependent effects, such as CO<sub>2</sub> accumulation and hydrostatic pressure influences. These combined insights allowed timely corrective actions and contributed to more stable and robust process operation.

Across multiple runs, the multiparametric PAT dataset generated distinct metabolic fingerprints that enhanced batch to batch comparability on the cellular performance level. Inline PAT technologies therefore establish a continuous, high resolution analytical foundation for advanced process control, reduce development timelines, and support future real time release strategies based on in process verification.



L19 was withdrawn at short notice

## 16:45 Smart feeding: Raman-based glucose control for enhanced malic acid fermentation (L20)

Luca Antonia Grebe<sup>1</sup>, Katharina Hürter<sup>1</sup>, Dominik Engel<sup>1</sup>, Mafalda Dos Santos<sup>2</sup>, Jörn Viell<sup>2</sup>, Jørgen Magnus<sup>1</sup>

<sup>1</sup>AVT-Biochemical Engineering, RWTH Aachen University, Germany

<sup>2</sup>AVT-Process Systems Engineering, RWTH Aachen University, Germany

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In fermentation processes, the final product titer serves as a key performance indicator that significantly influences downstream processing and overall economic viability. Maintaining optimal substrate availability throughout the process is essential for achieving high titers; however, traditional batch processes are often limited by substrate inhibition, overflow metabolism, or high osmolality. To address these challenges, substrate feeding is usually employed, yet without online monitoring of substrate concentrations, it suffers from imprecise control, fluctuating substrate levels, and potential carbon limitation.

This work introduces Raman spectroscopy, a non-destructive, molecular-specific technique, as a process analytical technology for closed-loop glucose feed control during malic acid production with *Ustilago trichophora*. Malic acid is a promising platform chemical with diverse applications in food, pharmaceuticals, and biodegradable plastics. Extended batch processes are commonly used for production processes with *Ustilago* spp., and maintain a constant substrate concentration well above the substrate affinity constant. However, the feed rate is severely affected by changes in consumption rates caused by biomass growth, cell aging, or product inhibition. Real-time monitoring of glucose and malic acid concentrations is achieved using a Partial Least Squares regression model, with a root-mean-square error of prediction of 2.7 g/L and 5.3 g/L, respectively. The implemented automated feedback system uses a PID controller that dynamically adjusts the feed rate based on real-time metabolic demands to maintain a constant glucose concentration during the production phase. Simultaneously, the product concentration is monitored, and the feed is stopped automatically to prevent substrate loss due to product inhibition.



## 17:10 Machine learning spatially offset time-gated Raman spectroscopy (TG-SORS) in-line sensors for endoglucanase production in *T. reesei* bioprocesses (L21)

Martin Kögler<sup>1\*</sup>, Hans Mattila<sup>2</sup>, Jari Havisto<sup>1</sup>, Aki Mäyrä<sup>1</sup>, Markku Alamäki<sup>1</sup>, Peter Blomberg<sup>2</sup> and Dorothee Barth<sup>2</sup>

<sup>1</sup>VTT Technical Research Centre of Finland, Oulu, Finland

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Inline monitoring of endoglucanase from *T. reesei* bioprocesses for sustainable applications in pulp & paper, food, brewing, biomaterials, and animal feed remains challenging. We addressed this using time-gated Raman spectroscopy (TG), which reveals more spectral features than standard methods by suppressing fluorescence, bioluminescence and ambient light backgrounds. Real-time measurement in bioreactors is often limited by probe sterilization requirements or port availability. Combining spatially offset Raman spectroscopy with time-gating (TG-SORS) allows non-invasive measurements directly through container walls without disrupting the process. TG-SORS uses an offset between laser excitation and collection to overcome signal disturbance from glass or plastic boundaries. We further enhanced this approach with optical multiplexing and advanced machine learning for efficient Raman data analysis. Ultimately, integrating TG-SORS, multiplexing, and machine learning enables more accurate and effective monitoring.



1. Mosca, S., Conti, C., Stone, N. and Matousek, P. (2021). Spatially offset Raman spectroscopy, *Nat. Rev. Methods Primers*. 1: 21.

2. Mojzita, D., Aro, N., Kontturi, J., Kögler, M., Nordlund, E., Hosia, W. and Casteleijn, M.G. (2025). Label-free analytical characterization of brazzein produced with the filamentous fungus *Trichoderma reesei*. *Front. Bioeng. Biotechnol.* 13: 1688495.

3. Lange, C., Borisyak, M., Kögler, M., Born, S., Ziehe, A., Neubauer, P. and Bournazou, M.N.C. (2025). Comparing machine learning methods on Raman spectra from eight different spectrometers. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* 334, 125861.

## 17:35 From fed-batch to repetitive fed-batch: Soft-sensor-driven control of phosphate-regulated Fab production in *E. coli* (L22)

Luca Antonia Grebe<sup>1</sup>, Katharina Hürter<sup>1</sup>, Paul Loebenstein<sup>1</sup>, Julian Kopp<sup>1</sup>, Oliver Spaduit<sup>1</sup>

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Advanced bioprocess monitoring and control are key enablers for Industry 4.0 in the biopharmaceutical sector. [1]. Soft-sensors have emerged as powerful process analytical technology (PAT) tools allowing the estimation of critical but hard-to-measure variables from secondary measurements [2]. In this study, a mechanistic model-based soft sensor was developed to enhance the production of the therapeutic recombinant fragment antigen-binding (Fab) ranibizumab using the microbial host *Escherichia coli* in different processing modes. The *E. coli* strain W3110 carried an pAT153 plasmid with an auto-inducible *phoA* promoter regulating the Fab transcription as a function of the extracellular inorganic phosphate (PO<sub>4</sub>) availability, whereby efficient monitoring and control strategies are limited by off-line PO<sub>4</sub> analytics.

The soft-sensor enabled the accurate prediction (NRMSE < 5%) of key process variables including biomass, substrate, PO<sub>4</sub>, Fab titer, CO<sub>2</sub> evolution and NH<sub>3</sub> demand. This capability facilitated optimized fed-batch operation with reduced substrate accumulation and maximized Fab titers at harvest. Furthermore, real-time integration via a Python-based REST-API enabled the transition from conventional fed-batch to intensified repetitive fed-batch (RFB) processing, which was demonstrated over three consecutive cycles in four independent cultivations.

The presented approach enabled precise monitoring of PO<sub>4</sub>-regulated Fab production in *E. coli* W3110. As promoter activation occurred at very low extracellular PO<sub>4</sub> concentrations (<1 mM), where conventional PAT tools such as Raman spectroscopy are not applicable, the mechanistic soft-sensor provided a suitable PAT tool by integrating first-principles modeling with secondary measurements.



1. Gargalo, C.L., Udugama, I., Pontius, K., Lopez, P.C., Nielsen, R.F., Hasanzadeh, A., Mansouri, S.S. 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.

18:00 End of presentation programme

## 19:30 SYMPOSIUM DINNER

Brauhaus GEORGBRAEU, Spreeufer 4, 10178 Berlin  
(with voucher only)

22:30 End of day 2

### WEDNESDAY, 23 APRIL 2026

09:00 Welcome and introduction

*Peter Neubauer, Technische Universität Berlin, Germany*

## ■ ■ SESSION 8: BIOPROCESS INTEGRATION

Chair Jerome Morchain / Linda Cai

## ■ ■ PLENARY TALK

09:05 Intensified fermentation with E. coli effects on the economic and ecological footprint (PL04)

*Oliver Spadiut<sup>1,2</sup>*

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<sup>2</sup>Christian Doppler Laboratory IB Processing 4.0, Technische Universität Wien, Vienna, Austria

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In my talk I will show and discuss different strategies for process intensification with recombinant E. coli and how this affects the economic as well as the ecological footprint. I will discuss aspects of strain engineering as well as bio-processing strategies and compare them to established state-of-the-art processes.



09:50 Hybrid modeling as a life cycle support solution of bioprocesses: from process development to manufacturing operations (L23)

*Niels Krausch<sup>1</sup>*

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Hybrid modeling is increasingly recognized as a transformative technology in biomanufacturing, offering a powerful framework to support bioprocesses across their entire life cycle. Achieving efficient and robust operations demands modeling technologies that capture and predict process dynamics with high fidelity, while remaining sufficiently interpretable for operators and regulators.

This work highlights hybrid modeling as a key life cycle support solution, bridging process development and manufacturing operations. On the development side, transfer learning and Pareto-based Bayesian optimization work closely together to systematically build process knowledge, enabling risk-aware experimental design and accelerated learning with reduced effort, time, and cost. On the manufacturing side, the knowledge captured during development is deployed to support operations, including offline simulation, online predictive monitoring, and human-in-the-loop or closed-loop control.

Through newly published works and industrial case studies, hybrid modeling is shown to reshape the role of models from a tool for understanding mature production processes to a continuous decision-support aid across the full product life cycle.



**10:15 Development and application of an ANN-perception-based autonomous control system for Escherichia coli cultivation process (L24)**

Mengxuan Zhou<sup>1</sup>, Beichen Zhao<sup>4</sup>, Zhiren Gan<sup>1</sup>, Jingyan Jiang<sup>2</sup>, Renquan Guo<sup>2</sup>, Nikolai Mushnikov<sup>2</sup>, Xueliang Li<sup>2</sup>, Jian Ding<sup>1\*</sup>, Zhenggang Xie<sup>2,3\*</sup>

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To address the challenges of overflow metabolism and the heavy reliance on manual intervention in high-density Escherichia coli fermentation, this study introduces an AI-driven, autonomous intelligent control system. Using superfolder green fluorescent protein (sfGFP) as a reporter, the research first optimized DO-stat feeding parameters and the induction process, achieving a 53% increase in cellular specific fluorescence intensity and significantly enhancing protein expression levels. Subsequently, an artificial neural network (ANN) model was developed and trained to achieve real-time recognition of dissolved oxygen (DO) baselines (R2 = 0.998). This model was integrated with feeding control logic to form the NeuroStat-Ctrl system, enabling fully autonomous control across the entire fermentation lifecycle. Utilizing this system, unattended E. coli fermentation was successfully achieved, yielding a 9.32% increase in fluorescent protein production. Experimental validation demonstrated that the system effectively mitigates feeding deviations inherent in traditional fixed-threshold strategies, prevents metabolic overflow, and enhances process stability and reproducibility. Furthermore, this system provides an efficient, standardized, and intelligent solution for high-throughput strain screening and process validation in parallel bioreactors.

**10:40 Coffee break & exhibition****SESSION 9: PROCESS ANALYTICAL TECHNOLOGY (II)**

Chair Katharina Dahlmann / Lara Santolin

**11:10 A novel view into aerated stirred tanks: Magnetic Resonance Imaging (L25)**

Till Lenczyk<sup>1\*</sup>, Noah von Schnitzler<sup>2</sup>, Stefan Benders<sup>1</sup>, Alexander Penn<sup>1</sup>

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The understanding of hydrodynamics and aeration processes in stirred tanks is crucial for reactor optimization and scale-up. Classic means of characterizing these phenomena rely on optical access, excluding system with high aeration rates, suspended particles or non-opaque fluids, or only provide local point-information using intrusive probes [1]. In contrast, Magnetic resonance imaging (MRI) is able to investigate such opaque systems and provide novel, non-invasive insights into small-scale bioreactor systems [2]. Because the signal in MRI originates from the spins of <sup>1</sup>H nuclei of fluids, it can provide contrast between these fluids and the MRI-silent gas phase. Moreover, the flow of liquids can be directly measured using phase contrast velocimetry, without the need for tracer particles. In this work, we extended MRI of commercially-relevant bioreactors to a larger scale of 250 ml, using a unique vertical MRI system which allows samples of diameters up to 400 mm and height of 3 m to be investigated. The contrast information allows the extraction of spatially resolved gas distribution, enabling the calculation of residence time and determination of stirrer efficiency. Using MR velocimetry, three-dimensional flow maps of whole vessels can be reconstructed, which reveal changing flow patterns with varying aeration rates and stirrer speeds. Both approaches will provide temporal averaged data with a spatial resolution up to 1 x 1 x 2 mm<sup>3</sup>. This data will improve our fundamental physical understanding of the hydrodynamics in reactor systems and provide datasets for the validation of numerical models as well as empirical models for stirred tank reactors.



1. Zlokranik, M. (2001). Stirring: Theory and Practice. Wiley-VCH Verlag GmbH, DOI: 10.1002/9783527612703.oth01

2. Legrand, C., Cheeks, M., Sellick, C. and Mantle, M. (2022). MRI hydrodynamic characterization of an ambr15® bioreactor. Biochem. Eng. J. 179: 108304.

**11:35 Integration of online HPLC analytics into chemostat-based polyhydroxyalkanoate production from dark fermentation effluent (L26)**

Saskia Waldburger<sup>1</sup>, Simon Täuber<sup>1</sup>, Friederike Gerstl<sup>1</sup>, Ulrike Krop<sup>2</sup>, Johannes Menke<sup>2</sup>, Peter Neubauer<sup>1</sup>, Stefan Junne<sup>3</sup>, Sebastian L. Riedel<sup>4\*</sup>

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<sup>2</sup>KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin, Germany

<sup>3</sup>Aalborg University Esbjerg, Department of Chemistry and Bioscience, Niels Bohrs Vej 8, 6700, Esbjerg, Denmark

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Measurement of short-chain carboxylic acids (SCCAs) and sugars commonly relies on labor-intensive offline analytics, which restrict temporal resolution and limit their use for advanced process control. In this study, an online HPLC system was developed and directly coupled to a pH-auxostat chemostat, enabling automated quantification of fructose and SCCAs at 30-min intervals without manual sample preparation. The analytical setup was applied to polyhydroxyalkanoate (PHA) production using the wildtype Cupriavidus necator H16 using SCCA-rich dark fermentation effluent as both carbon source and pH-controlling feed.

PHAs are intracellularly accumulated, biodegradable polyesters synthesized by numerous microorganisms as carbon and energy storage compounds. Owing to their thermoplastic properties and full biodegradability, PHAs represent a promising sustainable alternative to conventional fossil-based plastics. However, high production costs and the use of refined substrates remain major limitations, making the utilization of waste-derived feedstocks an important strategy for improving process economics and sustainability [1].



The comparatively low carbon concentration of the effluent necessitates high feed rates, which inherently impose dilution and volumetric constraints in continuous cultivation. These challenges were addressed by implementing a process intensification concept that enabled high medium throughput while maintaining elevated biomass over 20 g/L and PHA concentrations reaching >50 wt%. The integration of online HPLC provided continuous, time-resolved insight into substrate consumption and metabolite dynamics, supporting stable long-term operation and efficient effluent utilization. Overall, this work demonstrates that combining intensified chemostat operation with online HPLC analytics is a powerful approach for controlled PHA production from SCCA-rich waste streams.

1. Täuber, S., Riedel, S.L. and Junne, S (2025). Polyhydroxyalkanoate production from food residues. *Appl. Microbiol. Biotechnol.* 109: 171.

## 12:00 Integrating microfluidic analysis and MRI-based flow characterization for the scale-up of a novel bioelectrochemical reactor (L27)

*Selma Iraqi Houssaini*<sup>1\*</sup>, *Daniel Bauer*<sup>2\*</sup>, *Muhammad Adrian*<sup>3</sup>, *Till Lenczyk*<sup>3</sup>, *René Wurst*<sup>2</sup>, *Timo Merbach*<sup>1</sup>, *Felix Kexel*<sup>1</sup>, *Alexander Penn*<sup>3</sup>, *Johannes Gescher*<sup>2</sup>, *Michael Schlüter*<sup>1</sup>

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Bioelectrochemical systems (BES) represent a sustainable approach to produce hydrogen and platform chemicals. In BES, exoelectrogenic microorganisms drive oxidation and reduction reactions through extracellular electron transfer to or from the electrode. The reactor development of those systems is still at an early stage and a central challenge lies in balancing high mass transfer with low shear stress supporting stable biofilm growth [1].

This project introduces the Double Jet Loop Reactor (DJLR), a novel reactor concept featuring two separated circulation loops. The inner loop is characterized by high turbulence, enabling efficient gas dispersion and substrate mixing [2]. The outer loop provides a low-shear environment suitable for biofilm growth on additively manufactured lattice electrodes, allowing fine control of local flow conditions which enable a high productivity of the microorganisms [3].

To determine optimal operating conditions, a two-step characterization strategy is applied. A microfluidic platform with optical coherence tomography (OCT) enables non-invasive monitoring of the biofilm development, which can be correlated to the current density, produced by the microorganisms. Defined shear and flow conditions in the microfluidic system help to identify specific microbial requirements [4]. Magnetic resonance imaging (MRI) in TUHH's vertical large-bore scanner provides three-dimensional measurements of flow pattern and velocity fields for the whole reactor setup and especially in the optical inaccessible 3D-printed electrodes. This helps to evaluate how electrode geometry and process parameters can be adapted to meet biofilm needs [5].

This integrated approach forms a data-driven foundation for reactor scale-up and process optimization in bioelectrochemical applications.

1. Hackbarth, M., Gescher, J., Horn, H. and Reiner, J. E. (2023). A scalable, rotating disc bioelectrochemical reactor (RDBER) suitable for the cultivation of both cathodic and anodic biofilms. *Bioresour. Technol. Rep.* 21: 101357.
2. Maly, M., Schaper, S., Kuwertz, R., Hoffmann, M., Heck, J. and Schlüter, M. (2022). Scale-up strategies of jet loop reactors for the intensification of mass transfer limited reactions. *Processes.* 10: 1531.
3. Eckendörfer, L., Rudolf, D., Brix, A., Börnhorst, M. and Freund, H. (2024). Periodic open cellular structures in chemical engineering: Application in catalysis and separation processes. *Annu. Rev. Chem. Biomol. Eng.* 15: 163–186.
4. Klein, E., Wurst, R., Rehlund, D. and Gescher, J. (2024). Elucidating the development of cooperative anode-biofilm structures. *Biofilm.* 7: 100193.
5. Gladden, L. F. and Sederman, A. J. (2017). Magnetic resonance imaging and velocity mapping in chemical engineering applications. *Annu. Rev. Chem. Biomol. Eng.* 8: 227–247.

12:25 Lunch break, poster session & exhibition

## SESSION 10: INTENSIFIED AND CONTINUOUS PROCESSES

Chair Regine Eibl / Eike Janesch

## PLENARY TALK

### 14:00 Process intensification in microbial and enzymatic conversions (PL05)

*Heleen de Wever*,

*Flemish Institute for Technological Research, (VITO), Boeretang 200, 2400, Mol, Belgium*

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Process intensification is defined as 'any chemical engineering development that leads to a substantially smaller, cleaner, and more energy efficient technology'.

In this presentation, I will introduce several process intensification strategies applicable to fermentation and enzymatic processes. These approaches include integrating separation technologies with bioconversions to enable high cell density fermentations, in situ product recovery, and selective product separation.

Through discussion of generic concepts and case studies, I will demonstrate how these strategies can enhance productivity, yield and substrate utilization, remove inhibitory (by)products, avoid side reactions, shift reaction equilibria, and incorporate initial downstream processing steps. Particular attention will be given to evaluating these approaches under industrially relevant conditions, as well as their scalability.



14:45 Lipid production in *Schizochytrium limacinum* SR21 from biogenic residues (L28)

*Simon Täuber*<sup>1</sup>, *Anne Kathrine Clausen*<sup>2</sup>, *Peter Neubauer*<sup>1</sup>, *Stefan Junne*<sup>1,2,\*</sup>

<sup>1</sup>Chair of Bioprocess Engineering, Technische Universität Berlin, Germany

<sup>2</sup>Department of Chemistry and Bioscience, Aalborg University Esbjerg, Denmark

\*Email: sju@bio.aau.dk

Docosahexaenoic acid (DHA) is a polyunsaturated fatty acid (PUFA) that represents a major structural component of the human retina and brain grey matter, thus playing a key role in human health. At present, DHA is predominantly derived from fish meal and fish oil, which are becoming increasingly scarce and costly due to overfishing. Aquaculture further amplifies this pressure by relying heavily on fish-derived PUFAs. Therefore, microbial PUFA production represents a promising sustainable alternative, particularly when biogenic residues are used as substrates [1].

The marine protist *Schizochytrium limacinum* SR21 is well suited for the production of DHA and other PUFAs, as it can metabolize short-chain carboxylic acids (SCCA). This enables the use of complex acid mixtures such as effluents from dark fermentation and other waste streams, which contain SCCAs [2]. A production strategy was developed in which SCCA-rich effluent was applied both as a carbon source and pH controlling agent using a pH-auxostat feeding. Due to the comparatively low carbon concentration of the feed stream, high feed rates inherently lead to strong dilution and volumetric limitations. These were overcome by the choice of a suitable operation mode. Distinct SCCAs induced characteristic cell structures and morphologies. Image analysis was therefore applied to describe the individual cell status and compare it with data from other, rather conventional process conditions.

Acetate and butyrate-rich feeds promoted pronounced lipid body accumulation, while the many nutrients of dark fermentation effluent led to a different cell physiology status. Using this approach, biomass concentrations of 33.5 gL<sup>-1</sup> and DHA titres of 2.0 gL<sup>-1</sup> were achieved after 94 h, with an intracellular DHA content of 58 mgg<sup>-1</sup>. These results demonstrate that SCCA-rich residual streams are suitable substrates for PUFA production in *S. limacinum*, which is also underlined by techno-economic analyses.

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

2. Täuber, S., Janesch, E., Neubauer, P., Junne, S. (2025). An update on valorising dark fermentation effluent through microbial lipid synthesis. *J. Environ. Manag.* 395: 127912.



## 15:10 A cell-machine interface for monitoring plasmid-loss events and population stability during continuous bioproduction of plasmid DNA (L29)

*Hannah Sehrt*<sup>1,\*</sup>, *Juan Andres Martinez*<sup>1</sup>, *Romain Kinet*<sup>2</sup>, *Mathéo Delvenne*<sup>1</sup>, *Frank Delvigne*<sup>1</sup>

<sup>1</sup>Université de Liège - Gembloux Agro-Bio Tech, Belgium

<sup>2</sup>GSK groups of companies, Rixensart, Belgium

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The utilization of plasmid DNA (pDNA) in the medical field is on the rise, along with the production of pDNA in bacterial cells like *Escherichia coli* (*E. coli*). Most related processes currently rely on batch or fed-batch cultivations. While continuous processes offer the highest productivity, batch and fed-batch systems help reduce the occurrence of mutations and plasmid loss. We developed a cell-machine interface to monitor plasmid loss during continuous pDNA production. This interface incorporates a bioreactor with an integrated online flow cytometer enabling feedback control with single-cell resolution. Using a biosensor signal linked to plasmid copy number, real-time monitoring is possible throughout cultivation.

*E. coli* is well suited to be cultivated inside stirred-tank bioreactors, with high stirring speeds and oxygen transfer rates. However, with the cell-machine interface, it is possible to observe the influence of shear stress induced by stirring speed on the *E. coli* population containing a high-copy plasmid. By cultivating cells under high shear stress (1000 rpm), the cells lose fluorescence and plasmids over time. Lowering the shear stress (to 780 rpm) makes it possible to maintain fluorescence and plasmid content for 100 hours. This result was supported by microfluidic experiments under zero shear stress, where cells exhibited very high fluorescence levels, filamentation, and reduced growth rates due to the metabolic burden imposed by plasmid accumulation.

Taken altogether, these results indicate that shear stress impacts pDNA production in *E. coli*, and by reducing stirring speed, it is possible to improve population stability during continuous cultivation over 100 hours.

15:35 Paving the way for continuous bioprocessing: Building robust *Pichia pastoris* strains for long-term cultivation (L30)

*Guillermo Requena-Moreno*<sup>1,2</sup>, *Arnaud Gasset*<sup>1,2</sup>, *Jesper Wang Jensen*<sup>1</sup>, *Ulrich Krühne*<sup>1</sup>, *John M. Woodley*<sup>1</sup>

<sup>1</sup>Department of Chemical and Biochemical Engineering, Process and Systems Engineering Centre (PROSYS), Technical University of Denmark (DTU), Lyngby, Denmark

<sup>2</sup>The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark (DTU), Lyngby, Denmark  
Email: heljun@kt.dtu.dk

The yeast *Komagataella phaffii* (formerly *Pichia pastoris*) is a widely used cell factory for producing recombinant proteins in applications ranging from food and feed to biopharmaceuticals, biocatalysis, cosmetics, and biomaterials.

This organism is well known for its ability to reach very high cell densities, leading to high product titers. However, achieving comparable titers at lower cell densities would offer major advantages. It would simplify downstream processing, reduce oxygen transfer and cooling demands, improve product-to-carbon ratios, and lower CO<sub>2</sub> emissions. These benefits can be particularly well implemented in continuous cultivation, where stable performance at lower biomass concentrations in smaller tanks can maintain high productivity with reduced technical challenges.



Yet, long-term cultivation of *K. phaffii* remains difficult. Strain instability, flocculation, pseudo-hyphae formation, and declining productivity at late stages are significant barriers to continuous biomanufacturing.

To address these challenges, we explored the development of next-generation *K. phaffii* platform strains, vectors, and bioprocess strategies. Using a kinase and transcription factor knockout library, we identified mutant strains with enhanced productivity. We also investigated how flocculation, morphology, and physiology are influenced by carbon source, process duration, and cultivation conditions.

The resulting strains and cultivation strategies enable prolonged processes with high specific productivity and fewer technical hurdles. To demonstrate their performance, we applied these new tools to the production of technical enzymes, *Candida antarctica* lipase B (CalB) and VHH single-domain antibody fragments.

Together, these advances open new opportunities for robust, continuous, and high-titer recombinant protein production in *K. phaffii*.

## 16:00 Award ceremony best talks and posters by young scientists

*Peter Neubauer, TU Berlin, Germany & Stefan Junne, Aalborg University, Denmark*

## 16:20 End of symposium

**Publish your BioProScale contribution in**

### **Special Issue – Scaling Down and Up of Bioprocesses: Process Heterogeneities, Robustness, and Analytics**

We invite all participants to submit their research to this dedicated Special Issue focused on the key challenges discussed at our symposium.

We are looking for contributions on:

- Scale-up & scale-down approaches
- Process heterogeneities & reactor effects
- Modeling (mechanistic, hybrid, AI-driven)
- Digital twins & data-driven bioprocessing
- PAT, sensors & real-time analytics
- Integrated & continuous processes



## SCIENTIFIC POSTER ABSTRACTS

**P01: Real-time CFD: Emulating 3D bioreactor gradients in milliseconds**

*Victor Puig I Laborda<sup>1,2\*</sup>, Jorge Carrasco Muriel<sup>1</sup>, Andrea Castañeda Tena<sup>1</sup>, Cees Haringa<sup>3</sup>, Ignasi Puig de Dou<sup>4</sup>, Ulrich Krühne<sup>2</sup>, Johan le Nepvou de Cafort<sup>2</sup>, Krist Gaerney<sup>2</sup>, Lars Keld Nielsen<sup>1,5</sup>*

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Spatial heterogeneities (e.g., substrate starvation or pH excursions) remain a primary bottleneck in bioprocess scale-up. While Computational Fluid Dynamics (CFD) provides the necessary resolution to detect these gradients, its prohibitive computational cost prevents integration into real-time monitoring, spatially resolved Design of Experiments (DOE) of operating conditions or Digital Twin frameworks, which currently rely on OD models or simplified compartment approaches [1,2].

This work presents a Deep Learning framework that bridges this gap, predicting fully resolved 3D reaction-transport fields in milliseconds ( $>10^5 \times$  speed-up vs. CFD).

We position this as the second pillar of a dual-path data-drive strategy. Building on our previous work using unsupervised learning to compress hydrodynamic data and identify flow regimes without prior labeling [3-5], we here deploy a supervised learning to map global operating conditions directly to local scalar fields. Using a database of 750 simulations [6] (RANS-MRF) of an industry-standard bioreactor, we benchmarked architectures ranging from linear baselines to attention-based Neural Networks (NNs).

Our results demonstrate that successful emulation requires physics-informed data representations. By targeting biological saturation ratio ( $q_S/q_{S,max}$ ) rather than raw concentration, our surrogates stabilized learning across sharp gradients, achieving high generalization and accuracy ( $R^2 \approx 0.91$ ) on unseen test cases. We conclude that while unsupervised methods are essential for diagnosing flow structures, these supervised surrogates provide the speed required for predicting them in real-time, offering a foundation for spatially distributed Digital Twins.

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- Maldonado de León, H., Straathof, A. and Haringa, C. (2025). Dynamic compartment models: Towards a rapid modeling approach for fed-batch fermentations. *Chem. Eng. Sci.* 308: 121396.
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**P02: Machine learning-based 2D and hybrid 3D modeling for process intensification in iPSC expansion**

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Human induced pluripotent stem cells (iPSCs) are a promising allogenic cell therapy for a wide range of clinical indications. However, depending on the application, cell doses ranging from 105 to 1012 are required [1]. Although large-scale manufacturing of iPSCs is increasingly performed in three-dimensional (3D) suspension bioreactors, expansion is typically initiated in two-dimensional (2D) monolayer cultures to generate inoculum and define initial growth characteristics [2]. Quantitative and qualitative characterization of the iPSCs inoculum is therefore essential for enabling reliable seeding and successful initiation of the 3D bioreactor cultivation [3].

Therefore, there is a need for technologies that standardize the analysis of iPSC cultures and enable objective comparison across different cultivations. A freeware, high-throughput, non-invasive image analysis workflow was established for automated evaluation of phase-contrast microscopy images captured by EVOS from 2D iPSC cultures, enabling standardized morphology-based analysis similar to previously reported automated pipelines for iPSC colony quality and single-cell dynamics [4] using ilastik and Python.

Moreover, a 3D model was developed using historical real-time process data from 3D bioreactor cultivations within a discrete hybrid Gaussian Process modeling framework implemented in DataHowLab (DataHow, CH). This hybrid approach combines data-driven learning with mechanistic process knowledge to capture the dynamic behavior of iPSC cultures under varying operating conditions and to improve prediction of culture performance and product-relevant outputs [5]. Both models were successfully validated in the context of T-flask and benchtop bioreactor scale cultivations. By modelling real-time process data, the foundation for a digital twin is established, enabling the testing of real-time bioprocess optimization strategies.

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### P03: Machine-Learning framework for instantaneous prediction of the mixing time in a mechanical stirred bioreactor at both lab and industrial scales

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Over the last 20 years, the computational cost has been drastically reduced, leading to generalization of new tools such as Computational Fluid Dynamics (CFD). Some resolution times have reduced from days to a few hours [1]. With recent developments in artificial intelligence (AI), the next step is to reduce this computation time to enable quasi-instantaneous hydrodynamic solutions in bioreactors, thereby enhancing design optimisation capabilities and enabling implementation in digital twins for instantaneous prediction [2].

While some studies have used AI models to optimise the design of individual bioreactors, this was made only for a single configuration [3]. The aim of this work is to generalise optimisation across various set-ups and to create an AI that can rapidly predict key macroscopic quantities of mechanically agitated bioreactors.

The database was created using CFD with M-Star® software. This is based on a Lattice-Boltzmann approach for transport equations and a Large Eddy Simulation (LES) approach for turbulence. Replicas were performed due to the pseudo-stochastic results of LES [4]. The AI model used is a cascade of two Bayesian neural networks (BNNs) that provide the ( $\mu$ ;  $\sigma$ ) couple. This database was created for a single impeller and geometrical configurations [2.8 L; 2.800 L]. The first BNN predicts impeller characteristics based on geometric ratios (H/T; D/T) and impeller type. The second uses these outputs and the Reynolds number to estimate the mixing time ( $t_m$ ). For an unseen geometry at 100 RPM, the  $t_m$  agrees with the results of CFD. AI: 5.400±0.350 s; CFD: 6.065±0.177 s versus 8.34 s using the empirical method [5].

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### P04: Physics-informed neural network surrogates for bioreactor hydrodynamics: Opportunities and current limitations

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Hydrodynamics in stirred tank bioreactors determine mixing efficiency, mass transfer, and shear conditions, and thus strongly influence cell performance. Accurate yet computationally efficient models of the flow field are therefore essential for bioprocess development and scale-up. Physics-informed neural networks (PINNs) have attracted considerable attention

in recent years as a means to combine physical models and data within a unified deep learning framework [1]. However, their application to stirred tank hydrodynamics is only just emerging [2,3] and their practical value for bioprocess engineering remains to be systematically assessed.

In our work, we investigate the suitability of PINNs as surrogate models for the flow field in a stirred tank bioreactor. “Vanilla” PINNs are systematically compared with advanced variants employing adaptive loss balancing, adaptive sampling, and domain decomposition. We analyze how these strategies affect prediction accuracy, robustness, and training cost, and evaluate model configurations based on their capability to reproduce relevant hydrodynamic quantities.

A particular focus is placed on quantifying the amount of data required to obtain reliable surrogates [4]. We contrast PINNs with purely data-driven supervised neural networks trained on CFD data and discuss in which regimes physics-informed training is advantageous. The results provide an initial assessment of the opportunities and current limitations of PINN-based hydrodynamic surrogates and outline their potential role in future bioreactor design, scale-up, and digital twins.

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### P05: Physics-informed neural network surrogates for bioreactor hydrodynamics: Opportunities and current limitations

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Modelling and optimizing microbial bioprocesses are challenging due to nonlinear dynamics, limited experimental data, and low-throughput measurements [1]. To address these limitations, we developed a dual-ANN Physics-Informed Neural Networks (PINNs) framework for dynamic modelling of *Cupriavidus necator* batch cultivations using a lipid-rich extract from black soldier fly larvae (BSFL) as the primary carbon source for polyhydroxyalkanoates (PHAs) production. The dual-ANN structure combines two networks, one predicts the time evolution of state variables, and the other captures reaction kinetics, both constrained by ODE equations to preserve physical consistency [2, 3].

Hyperparameters were selected using random pointwise cross-validation with Optuna (Bayesian optimization), and the three best-performing models based on validation error were aggregated into an ensemble to enhance predictive accuracy [4,5]. This ensemble framework enabled detailed exploration of the design space and delivered reliable predictions of PHA accumulation and substrate consumption. Ensemble-based uncertainty quantification produced meaningful confidence intervals, identifying high yield, low-uncertainty operating regions and highlighting areas that require further experimental validation.

In silico validation showed that the ensemble closely matched observed PHA accumulation profiles under optimal condi-

tions, with narrow prediction intervals. Design space analysis revealed yields above 25 g L<sup>-1</sup> and optimal initial conditions of XA<sub>0</sub> = 2.84 g L<sup>-1</sup>, N<sub>0</sub> = 2.0 g L<sup>-1</sup>, and LA<sub>0</sub> = 29.2 g L<sup>-1</sup>. Overall, the dual-ANN PINN ensemble provides a rigorous, uncertainty-aware tool for model-guided optimization, supporting efficient experimental design and accelerating data-driven development of microbial bioprocesses.

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## P06: Deep hybrid modelling and control of microbiome evolution

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Hybrid modeling that combines first-principles knowledge with machine learning (ML) is emerging as a key methodology for Industry 4.0. By integrating prior knowledge, these approaches enhance predictive performance, improve transparency, and reduce data requirements for process development. However, a gap remains between advanced ML techniques and their application in bioprocess development [1].

In this work, we present an automatic control system that makes use of Physics Informed Neural Networks (PINNs) [2] to act as a digital twin of a natural microbiome in a Sequencing Batch Reactor (SBR) in order to maximize Polyhydroxyalkanoates (PHA) production through the control of 3 different feeds: acetate, ammonia, and culture medium.

The PINN model was trained using historical SBR data with deep learning optimization methods, including the Adaptive Moment Estimation (ADAM) algorithm. Once trained, the model was integrated into a Model Predictive Control (MPC) framework. The PINN predicts reactor dynamics based on process measurements and feed rates, enabling the MPC to optimize inputs for each feast–famine cycle by maximizing productivity, defined as biomass (VSS) multiplied by PHA concentration at the end of the feast phase.

After 14 cycles (three hydraulic retention times), PHA content increased from 5 to 226 mg/gVSS, while biomass decreased from 14 to 6.5 g/L VSS. A separate accumulation experiment confirmed the improvement, reaching 528 mg/gVSS at 10 g/L VSS.

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## P07: A standardized template for modeling and simulation of upstream bioprocesses

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Kinetic models are widely used to describe biological systems and are increasingly applied in bioprocess engineering. However, the lack of standardization, reproducibility, and interoperability remains a major challenge for kinetic models used in upstream bioprocess development. Existing modeling standards from systems biology are often not well suited to represent typical bioprocess concepts such as cultivation phases, feeding strategies, and process-related parameters.

To address this issue, a lightweight framework was developed to support the creation, documentation, and simulation of kinetic bioprocess models. The framework is implemented in Python and uses Jupyter Notebook as a structured format for model definition and execution. By deploying the framework with JupyterLite, the modeling environment can be accessed directly in the browser, allowing users to run and modify models without local software installation. In addition, the framework is designed to facilitate structured model storage and integration with the STAMM Model Registry [1], where models are stored, versioned, and described through structured metadata, to support standardized access, traceability, and reuse.

To evaluate the approach, established *E. coli* kinetic models were reconstructed using the proposed template and the simulation results were compared with the results reported in the original publication [2; 3]. The results showed close agreement with the published model behavior. The generated models follow a consistent structure and remain easy to interpret and reproduce.

The proposed framework represents an initial step toward more standardized and reusable kinetic models for upstream bioprocess applications and may support future model repositories for bioprocess modeling.

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## P08 withdrawn

## P09: Development of a digital twin for a novel gas fermentation process

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Gas fermentation with hydrogen-oxidizing bacteria enables the conversion of CO<sub>2</sub> into bioproducts without the use of plant-based resources and with a reduced carbon footprint.

This positions gas fermentation as a promising option for CO<sub>2</sub> utilization in a circular economy, contributing to climate action and reduced land use in line with the Sustainable Development Goals [1].

To translate such processes efficiently from laboratory and pilot scale to industrial application, early integration of a model-based process development allows for a more time and resource efficient scale-up and optimization as well as process control [2].

The objective of this work is to develop a digital twin for a gas fermentation process targeting the production of a sustainable palm oil substitute. This process model is intended to be used for the final process as a basis for process control and optimization, as well as to aid decision-making during scale-up by reducing experimental effort and associated costs.

As a first step, a mechanistic process model is developed to describe the core process dynamics, with a particular focus on gas transfer and microbial growth. The estimation of uncertain model variables or parameters is supported by data-driven models, which are combined with the mechanistic model in a hybrid structure to improve the predictive capability of the digital twin. The digital twin is designed to be coupled to the process control system, allowing continuous data integration and iterative model refinement as new experimental data become available.

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## P10: Dynamic 1D gas-liquid model to simulating heterogeneous bioreactor – in various cultivation modes

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The numerical simulation of heterogeneous gas-liquid bioreactors using CFD or CMA approaches remains challenging, especially when volume increase and long-term operation are at stake. In this context, we developed a dynamic two-phase 1D model to predict the evolution of gas volume fraction in both time and space, consistently coupled with biological reaction [1; 2]. The model also provides spatio-temporal profiles of gas and liquid phase making possible to analyse axial gradients and to identify locally limiting zones.

An illustrative example is the biomethanation process, since both substrates are supplied through the gas phase (H<sub>2</sub>, CO<sub>2</sub>) and their conversion into methane (CH<sub>4</sub>) is accompanied by a major change in gas volume fraction. Hence, gas-liquid hydrodynamics, interphase mass transfer, and bioreaction are strongly coupled [3]. Another application regards fed-batch processes, where a significant increase in liquid volume modifies gas-liquid mass transfer and can lead to under-aerated zones in the upper part of the reactor, i.e., creating a heterogeneous environment along the cell lifelines. The example considers the aerobic fed-batch cultivation with *S. cerevisiae*.

The proposed 1D framework allows to study the coupling between bioreaction, mass transfer and hydrodynamics and is designed for batch, fed-batch, semi-batch, and continuous cultivation modes, including variable feeding and level dynamics. The model offers a computationally efficient tool to study how operating strategies and scale-up impact both local and overall reactor performance. In practice, it enables rapid screening of feeding, aeration, and level-control strategies to identify limiting zones of concentrations along the reactor height.

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## P11: Kinetic modelling of complex co-culture yoghurt fermentation

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Yoghurt is one of the most widely consumed dairy products. Its production relies on the co-culture fermentation of bovine milk with *Streptococcus thermophilus* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB). Although their relationship is characterized as mutualistic [1], the underlying mechanisms are not quantitatively defined, forcing product development and process upscaling to depend on empirical, trial-and-error approaches. A deeper understanding of these mechanisms can accelerate innovation and improve process robustness.

To address this issue, we conducted kinetic experiments to investigate the complex metabolic relationship between ST and LB. These experiments were performed in both mono- and co-culture fermentations at varying temperatures. Samples were collected at high frequency (every 20 minutes for 7 hours), and the resulting dataset included measurements of relevant sugars (lactose, glucose, galactose), lactic acid, formic acid, free amino acids, dissolved oxygen, pH, and strain-specific cell counts. Based on this data we developed a mechanistic model that identifies the limiting nutrients and that quantifies their effect on growth and metabolite production.

The model provides critical insights into the dynamics of microbial mutualism and serves as a predictive tool for estimating fermentation endpoint, reducing reliance on offline analyses. The quantitative framework developed can be used to optimize existing processes and design novel yoghurt fermentation strategies. The novel insights of this study provide a solid foundation for exploring innovative fermentation conditions, which can be used to optimize existing yoghurt products or stimulate the development of new products

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## P12: Dynamic feed strategies in *Saccharomyces cerevisiae* fed-batch fermentation guided by model-based design of experiments: an in silico study

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This study presents an in silico implementation of Model-Based Design of Experiments (MBDoe) for optimizing dynamic glucose feed trajectories in *Saccharomyces cerevisiae* fed-batch fermentation. The goal is to demonstrate how MBDoe can improve parameter identifiability while reducing experimental effort [1]. A mechanistic model describing *S. cerevisiae* growth and ethanol formation [2], serves as the application case study for the biological system. The model includes ten kinetic parameters to calibrate and represents both oxidative and reductive metabolism and the metabolic shift associated with the Crabtree effect and serves as a benchmark under conditions of partial state observability and parameter correlation.

In this framework, the glucose feed rate profile acts as the manipulated variable. Each simulated run represents one full lab-scale fed-batch fermentation and generates synthetic off-gas data (oxygen, carbon dioxide, and ethanol), which are considered as the only available measurements. The synthetic datasets are produced using a process model previously calibrated with real lab fermentation data, ensuring realistic dynamics and noise.

Following each experiment, the parameters are re-estimated, and the local sensitivities are computed to construct the Fisher Information Matrix (FIM), which quantifies the information content of each experiment and is used to redesign the feed profile using selected optimality criteria. The resulting experiments are evaluated in terms of parameter identifiability, confidence intervals, and accuracy.

The in silico setup allows for the systematic investigation of how feed rate dynamics affect parameter estimation when only on-line data are available and establishes a foundation for real-time adaptive MBDoe applications in bioprocess automation.

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## P13: Computational scale-up analysis of bioreactor design for cultivated meat production

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Scaling up bioprocesses for cultivated meat remains challenging, driven by uncertain requirements for mass transfer, shear tolerance, and mixing performance [1]. We developed a two-stage evaluation approach for conceptual reactor designs. This uses a Monte Carlo-based timescale assessment for initial global screening of stirred tank, bubble column and airlift reactor (ALR) designs based on process timescales, followed by detailed compartmental modelling to quantify possible internal limitations and gradient formation. Designs were filtered against characteristic timescales for mixing, O<sub>2</sub>-transfer and CO<sub>2</sub>-accumulation limits while imposing a bubble shear constraint via the Hypothetical Killing Volume (HKV), translating cell

fragility into a maximum permissible gas flow [2]. The analysis identified mixing and O<sub>2</sub> transfer as dominant constraints and showed that geometric optimization and shear-protectants can expand the feasible design window.

Guided by these insights, we developed an 1D compartment model for ALRs up to 250 m<sup>3</sup>, [CH1.1] based on hydrodynamic behavior characterized for full-scale internal loop reactors [3]. The model resolves height-dependent gas hold-up, circulation velocities, and local mass-transfer coefficients, yielding liquid and gas phase O<sub>2</sub> and CO<sub>2</sub> profiles in riser and downcomer. The HKV criterion caps inlet gas flow to keep bubble-burst mortality below growth while a maximum gas-holdup limit preserves model validity at scale. The model quantifies starvation and toxicity thresholds that emerge along the reactor height and shows feasible biomass concentrations depend strongly on geometric parameters and cell characteristics. Together, the complementary frameworks provide a basis for ALR scale-up and guidance on cell engineering and operating windows for cultivated meat production.

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## P14: Agent-based simulation of N-glycosylation: Model parameter estimation using a genetic algorithm

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N-glycosylation in the Golgi apparatus is a crucial post-translational modification that influences protein folding, stability, and function, and plays a key role in cell signaling and disease development. Computational modeling of this process remains challenging due to the complexity of enzymatic networks, structural diversity of glycans, and the stochastic nature of intracellular transport and reactions [1]. In this work, we present a parameter estimation approach for a previously developed agent-based model of N-glycosylation [2] using a genetic algorithm (GA).

The Golgi apparatus with its cisternae is modeled as a sequence of reaction compartments characterized by distinct glycosyltransferase and glycosidase concentrations. The model integrates stochastic diffusion and biochemical reactions, allowing simulation of reproducible glycosylation patterns. To investigate matching of experimental glycan profiles, glycosylation data sets for normal and tumor tissue were chosen [3]. The GA optimizes enzymatic parameters by minimizing the difference between simulated and experimental N-glycan distributions. Optimization runs demonstrate that the GA efficiently explores the complex parameter space and identifies parameter sets that closely reproduce experimental data.

These results demonstrate the feasibility of using GA's for enzymatic parameter estimation in agent-based N-glycosylation models. The validated framework provides a powerful tool to study how altered reaction rates in the Golgi apparatus could affect glycosylation patterns in tumor tissue. Linking the model parameters to environmental conditions such as nutrient availability, pH, dissolved oxygen, or metabolic state could allow prediction of optimal processes, providing a foundation for consistent glycan quality, relevant for biopharmaceutical

manufacturing and quality assurance of glycosylated therapeutic proteins.

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### P15: Model-based strain selection using an industrial KPI

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Strain selection is one of the critical steps in bioprocess development [1,2]. However, it is often performed without considering large-scale process performance, relying instead on so-called “myopic” design of experiments and screening approaches focused on lab-scale metrics [3]. This frequently leads to inefficient experimentation and suboptimal performance at the intended industrial scale. To address this limitation, we propose a telescopic strain selection strategy that leverages large-scale key performance indicators (KPIs) to guide early-stage decisions under a limited experimental budget. The proposed framework employs a Thompson sampling-based ranking procedure, which evaluates candidate strains according to their probability of being the best-performing strain under large-scale process conditions. Specifically, parameter samples are drawn from the prior distribution of each strain. For every parameter sample, a large-scale process optimization is performed, and the resulting KPI is recorded. A strain is counted as “best” whenever it achieves the best KPI value among all candidates for a given sample. A fixed total number of bioreactors is subsequently allocated across strains proportional to their probabilities of being the best-performing one. For each strain, the KPI-based design of experiments framework described in [4] is then applied to select experimental conditions that most effectively reduce the uncertainty in the KPI. As a proof of concept, a sugarcane-to-ethanol biorefinery was considered as the large-scale process, with multiple candidate yeast strains and internal rate of return (IRR) as the KPI. Results based on in silico-generated experimental data indicate that the proposed telescopic strain selection strategy successfully identifies the best-performing strain using a minimal number of experiments.

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### P16: Towards a comprehensive decision support system for microbial strain discovery

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The rapid growth of omics data has created a critical bottleneck for microbial strain discovery in the bioindustry. Strain-relevant information is scattered across structured databases and vast unstructured scientific literature, making integration and intuitive exploration highly challenging. We present a web-based Decision Support System (DSS) designed to democratize access to microbial strain data by combining structured database querying, AI-driven literature mining, and built-in biosafety mechanisms.

The current system interfaces with BacDive (the leading prokaryotic strain database with over 16.5 million RDF triples) via its SPARQL endpoint [1]. A GPT-4-based natural language processing module translates domain-specific queries (e.g., “List all *Bacillus* strains isolated from soil in Germany”) into valid SPARQL, leveraging dynamic schema context and guided prompts for reliability. A fallback schema ensures full functionality even when the endpoint is temporarily inaccessible.

To further enrich the system's knowledge base, we are developing a literature semantification module that processes scientific publications into a structured semantic database, enabling retrieval-augmented generation (RAG) over curated microbial literature. This expands the DSS beyond structured databases into the unstructured knowledge landscape.

Critically, the system incorporates biosecurity and responsible-use measures, including query filtering and access controls, to prevent misuse for harmful applications that is an essential consideration when handling sensitive microbial and strain-level information.

Together, these components form a scalable, FAIR-aligned infrastructure that accelerates biotechnological research by making complex strain data accessible to a broad community of researchers and industrial practitioners [2].

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### P17 *withdrawn*

### P18: Engineering robust strains: Scale-down approaches for industrial optimization of *Pseudomonas putida*

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Biotechnological processes play a crucial role in advancing sustainable production within a circular, carbon-neutral economy. Achieving this objective requires enhancing current production strains for large-scale fermentations, where imperfect mixing introduces additional stress not typically encountered at laboratory scale. These stresses arise from starvation zones caused by fluctuations in factors such as dissolved oxygen, carbon, and pH, ultimately reducing overall productivity.

To investigate these effects, scale-down bioreactor systems have been developed to replicate industrial conditions in laboratory settings. One such system is the STR-PFR (Stirred Tank Reactor–Plug Flow Reactor), which creates gradients in nutrients and oxygen, simulating the cyclic exposure of cells to well-mixed and starvation zones. This configuration has facilitated studies using traditional microbial chassis such as *Escherichia coli* [1]. More recently, the Single Multi-Compartment Bioreactor (SMCB) has replicated industrial mixing times in lab-scale settings by using disks with varying exchange areas to prolong mixing within a single vessel [2]. Both these systems enable detailed proteomic and transcriptomic analyses, offering insights into cellular behaviour and metabolic modulation under conditions resembling industrial scale.

This project will use both the STR-PFR and SMCB systems to assess how *P. putida* responds to various limitation scenarios, including carbon, nitrogen, and oxygen. Multivariate analysis (MVA) will be used to identify patterns that guide the rational design of a genome-reduced strain optimized for industrial-scale applications. The resulting strains will be compared to wild-type and other genome-reduced variants to identify optimal candidates for robust, large-scale bioprocessing, using realistic production conditions by leveraging scale down systems.

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### P19: Scale-down characterization of an *E. coli* L-tyrosine producer in an STR–PFR simulator using transcriptomic iModulon analysis

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Industrial-scale aerobic fermentations exhibit spatial and temporal gradients in substrate and oxygen that are not captured in conventional lab-scale stirred-tank reactors (STRs), yet these heterogeneities can strongly affect pathway fluxes and product yields in aromatic amino acid production processes. To mimic substrate large-scale conditions, we used a scale-down stirred tank reactor coupled with a plug-flow reactor (STR-PFR) system to expose an *E. coli* L-tyrosine producer to glucose depletion cycles and compared its performance to a homogeneous STR baseline. Three independent STR-PFR fermentations and one STR reference were conducted with identical medium, feeding strategy and process analytics, including online gas analysis and offline quantification of biomass, glucose and L-tyrosine. In addition, samples for RNA-seq were taken along the STR-PFR trajectory and analyzed using an iModulon framework to resolve condition-specific transcriptional modules.

STR-PFR operation is expected to reveal differences in carbon partitioning, L-tyrosine yield on glucose and overflow metabolism compared to the homogeneous STR baseline, potentially indicating a sensitivity of the production pathway to environmental fluctuations. Ongoing iModulon activity analysis aims to uncover coordinated transcriptional responses across stress-response, central carbon metabolism and aromatic amino acid biosynthesis modules under cyclic conditions, which may highlight regulatory trade-offs between robustness and productivity. Together, scale-down STR-PFR experiments combined with

metabolomics and transcriptomic analysis are anticipated to provide a powerful approach to identify vulnerabilities of *E. coli* L-tyrosine producers to large-scale heterogeneities and to guide future strain and process engineering.

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### P20: Investigation of the L-tyrosine crystallization kinetics under fermentation conditions

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The aromatic amino acid L-tyrosine is a precursor to several commercially important compounds, including L-DOPA, melanin, and nutritional supplements such as flavonoids and stilbenoids [1]. During microbial production using a tyrosine-producing *Escherichia coli* strain, L-tyrosine begins to form crystals due to its low solubility in the fermentation solution conditions (pH = 6.5, T = 37 °C) [2]. This in situ crystallization offers benefits such as partial product purification but also presents challenges. The needle-shaped morphology of L-tyrosine impairs filtration due to poor flow properties, and its density, which is close to that of cells, complicates the separation by centrifugation [3]. Although the fermentation process has been described in the literature [4–5], the dynamics of tyrosine crystallization during fermentation have not yet been studied.

This study aims to characterize the crystallization kinetics of L-tyrosine to identify an operating design space that promotes the formation of larger, more easily separable crystals. To mimic the microbial production of tyrosine, solubilized L-tyrosine is fed into a reactor at controlled rates representing typical metabolic production patterns. Different feeding profiles are applied to investigate how different supersaturation trajectories affect nucleation and crystal growth. By systematically quantifying the impact of these profiles on crystal size and morphology, this work provides new insight into how production dynamics influence crystallization behavior. Ultimately, establishing this knowledge supports a more integrated understanding of microbial synthesis and crystallization, enabling improved process design for tyrosine-producing bioprocesses.

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## P21: Lifeline analysis of environmental perturbations in industrial bioreactors: A multi-organism, multi-reactor comparative study

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Large-scale industrial bioreactors exhibit spatial heterogeneity in dissolved oxygen and substrate concentration due to mixing limitations, exposing microorganisms to dynamic environmental fluctuations that impact metabolic performance and product yield. Understanding organism-specific responses is therefore critical for rational bioprocess scale-up and optimization.

We employ high-fidelity Large Eddy Simulations (LES) across different reactor types (stirred-tank and airlift) and scales (22–600 m<sup>3</sup>), coupled with offline Lagrangian particle tracking to generate individual cell "lifelines", the time-series data capturing the substrate and oxygen concentrations experienced by microorganisms as they circulate through the reactor. Statistical analysis reveals organism-specific fluctuation characteristics, including frequency distributions and residence times in distinct metabolic regimes.

A central question is whether perturbations, that can be characterized by their frequency, amplitude, and magnitude, are physiologically relevant or can be safely neglected in process and organism design. Comparative analysis across *Escherichia coli*, *Saccharomyces cerevisiae*, *Clostridium autoethanogenum*, and *Pseudomonas putida* shows that organism-specific sensing kinetics and metabolic switching timescales determine whether gradients manifest as metabolic stress or average into quasi-steady-state conditions, providing a rational basis for scale-down design and strain selection.

## P22: Heterogeneity amplification effect on cell environment feedback loop induced by metabolic burden

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Continuous production remains under-exploited in the biotechnological industry largely because population stability problems lead to both genotypic and phenotypic diversification, reducing yields, process control, and repeatability. Currently, *Escherichia coli* accounts for roughly 30% of approved therapeutic protein production in this sector [1]. Population instability partly arises from phenotypic heterogeneity generated by the metabolic cost of induction and heterologous protein expression. Often termed the switching cost, this phenomenon reflects a cell–environment interface in which subpopulations grow at different rates. Faster or non-induced cells leave residual nutrients that further amplify the emergence of phenotype-based subpopulations able to escape induction. Such phenotypic escape may therefore represent an important parameter for controlling stability in continuous fermentation systems.

To investigate interactions between nutrient availability, metabolic burden, and phenotype formation, experiments were performed with *E. coli* BL21(DE3) carrying GFP reporters under two metabolic burden conditions. In the first system, burden originated solely from expression of the T7-based production machinery. The second system combined T7-driven expression with heterologous pro-insulin inclusion body formation.

Additionally, glucose or arabinose was supplied as the primary carbon source. Microscopy imaging together with automated flow cytometry enabled single-cell monitoring of gene expression and quantitative assessment of population heterogeneity. Results show that when T7-related burden acts alone, population heterogeneity limits stable expression and interacts with carbon source to influence phenotypic escape. When inclusion bodies accumulate, metabolic burden further intensifies population dynamics and heterogeneity, highlighting key parameters governing instability in fermenters producing heterologous proteins and informing future modelling and machine-learning assisted bioprocess control.

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## P23: Reduced viscosity mutants of *Trichoderma reesei* with improved industrial fermentation characteristics

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Morphological mutants of *Trichoderma reesei* were isolated following chemical or insertional mutagenesis. The mutant strains were shown to have reduced viscosity under industrially relevant fermentation conditions and to have maintained high specific productivity of secreted protein. This allowed higher biomass concentration to be maintained during the production phase and, consequently, increased volumetric productivity of secreted protein. The causative mutations were traced to four individual genes (designated *sfb3*, *ssb7*, *seb1*, and *mpg1*). We showed that two of the morphological mutations could be combined in a single strain to further reduce viscosity and enable a 100% increase in volumetric productivity.

## P24: From high-throughput microreactors to bench-scale: Modelling tunable recombinant protein production in a 2-feed *E. coli* process

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*Escherichia coli* is a popular host organism for recombinant protein production, with a wide range of products spanning from biopharmaceuticals to industrial enzymes [1]. Commonly, the T7 promoter system is used to trigger production after an initial growth phase. In industrial applications isopropyl β-d-1-thiogalactopyranosid (IPTG) is used to deploy a strong transcription of the encoded protein [2]. The resulting metabolic stress can lead to enhanced inclusion body formation and cell death [3]. Lactose presents itself as a low-cost alternative that allows for a tuneable induction process in strains capable of a complete digestion of the disaccharide [4].

This contribution proposes a process that utilizes a separate glucose and lactose feed to improve the productivity of an *E. coli* strain expressing green fluorescent protein (GFP). The influence

of the two substrate uptake rates onto the product formation rates was investigated and described with a physiological bioprocess model. The model identification and parameterization were performed with data from a high-throughput miniature scale bioreactor system. This allowed to conduct a thorough design of experiment approach investigating a multitude of feed-rate combinations. For comparison, the same modelling procedure was applied for a small number of 1 L bench-scale fed-batch cultivations. The differences and implications of the two different experimental scales are discussed and the final models obtained from both approaches were evaluated for their performance. The results were further applied to determine optimal feed trajectories for specific process design objectives. The potential for integration into advanced model-based control strategies is outlined.

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## P25: Co-cultivation dynamics of the filamentous microorganisms *Aspergillus niger* and *Streptomyces coelicolor* in a rocking motion bioreactor

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*Aspergillus niger* and *Streptomyces coelicolor* are filamentous microorganisms widely used as microbial cell factories. Their co-cultivation offers a promising strategy to mimic natural microbial interactions and unlock novel metabolic potential. In this study, their co-culture was systematically investigated in a rocking motion bioreactor (RMB) under varying inoculation ratios and cultivation conditions in a low shear-force regime. As maintaining balanced and reproducible population dynamics is crucial in such systems, a population balance model incorporating pH-dependent growth and inhibition was developed to quantitatively describe these dynamics. Experimental characterization included growth, substrate consumption, citric acid production, pH, and oxygen consumption.

At an inoculation ratio of 1:1, *A. niger* outcompeted *S. coelicolor* by lowering the pH to 3.0, a condition unfavourable for *S. coelicolor*. Increasing the initial proportion of *S. coelicolor* (1:4 and 1:16) allowed it to grow in the early batch phase but ultimately still resulted in dominant growth of *A. niger*. When pH-control was applied at 6.5 (1:4 inoculation ratio), *S. coelicolor* grew effectively alongside *A. niger*, however, the overall biomass yield was reduced. Simulation results showed qualitative agreement with experimental observations. Overall, the findings highlight pH as a key factor shaping the population dynamics in this co-culture, either through active microbial responses or through the accumulation of metabolic byproducts that influence growth

and cell physiology of the competing species.

This study demonstrates the potential of combining experimental data with population balance modelling to improve the mechanistic understanding of microbial co-cultivation.

## P26: From shake flasks to bioreactor: assessing *Vibrio natriegens* for biotechnological plasmid DNA production

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The increasing demand for plasmid DNA (pDNA) in biopharmaceutical applications such as gene therapies, DNA vaccines, and mRNA vaccines has traditionally relied on *Escherichia coli* as a production host due to its high-density cultivation and robust pDNA yields. However, with growing interest in more efficient and sustainable alternatives, *Vibrio natriegens* has emerged as a promising alternative host. Discovered in 1958, this fast-growing, non-toxic Gram-negative bacterium features a doubling time of less than 10 minutes and remarkable metabolic flexibility [1]. While *V. natriegens* has been studied for cloning and protein expression, its potential for large-scale pDNA production remains underexplored [2], [3]. This study aimed to establish and evaluate *V. natriegens* as a novel host for pDNA production and to compare its performance with *E. coli* under controlled cultivation conditions. The results demonstrate that *V. natriegens* can produce high plasmid yields within significantly shorter cultivation times compared to *E. coli*, while maintaining plasmid quality and functionality. Furthermore, successful transfer of the cultivation strategy from shake flasks over high throughput mini bioreactors to a 1.5 L bench-top bioreactor confirmed the organism's suitability for controlled and scalable bioprocess development. These findings highlight *V. natriegens* as a promising alternative production host, supporting the advancement of faster and more sustainable biotechnological platforms for pDNA manufacturing.

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## P27: Scale-down characterization of plasmid DNA production in *Escherichia coli* for digital twin development and process optimization

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Plasmid DNA (pDNA) is a critical raw material for DNA vaccines and non-viral gene therapies, yet its industrial production remains constrained by limited process understanding and scalability. Digital twins have emerged as promising tools to support rational process design and scale-up; however, their development requires high-quality experimental datasets that capture the interaction between host physiology and cultivation conditions.

In this study, systematic shake-flask experiments were conducted as scale-down models to generate reproducible data for future digital twin development of batch pDNA production. Three *Escherichia coli* strains – DH5 $\alpha$ , MG1655 $\Delta$ endA $\Delta$ recA, and the metabolically engineered GALG20 ( $\Delta$ endA $\Delta$ recA  $\Delta$ pgi) – were cultivated in chemically defined media differing in carbon source (glucose or glycerol) and amino acid supplementation. Biomass formation, substrate consumption, by-product formation, pDNA yield, and plasmid isoform distribution were quantitatively assessed.

Medium composition was identified as the dominant factor affecting process performance. Glycerol-based media resulted in up to a fivefold increase in specific pDNA yield compared with glucose-based formulations, while amino acid supplementation enhanced the supercoiled fraction above 76%, a key quality attribute for pharmaceutical applications. Maximum specific and volumetric yields reached 11.37 mg gDCW<sup>-1</sup> (DH5 $\alpha$ ) and 11.44 mg L<sup>-1</sup> (MG1655 $\Delta$ endA $\Delta$ recA), respectively. In contrast, the pgi knockout strain did not outperform the reference strains, suggesting that nutrient limitations redirected carbon flux toward less productive pathways.

Overall, these results establish a robust experimental framework for scale-down characterization of pDNA production and provide quantitative benchmarks to support kinetic modeling and the development of predictive digital twins for industrial bioprocesses.

## 28: Optimizing pDNA production in *Vibrio natriegens* via automated high-throughput process development

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After the COVID-19 pandemic, which enabled the first widespread application of mRNA vaccines and accelerated the development of gene therapy approaches, the global demand for pDNA has increased steadily [1]. While *Escherichia coli* remains the standard host, alternative organisms with superior growth characteristics may offer significant advantages. In this context, *Vibrio natriegens*, exhibits the highest reported growth rate among non-pathogenic microorganisms and represents a promising candidate, as many established molecular biology protocols can be directly transferred to this novel organism [2].

In this study, a pDNA production process utilizing *V. natriegens* was established and successfully transferred to the high-throughput facility at TU Berlin, enabling systematic process development. Based on a Design of Experiments approach, key process conditions were investigated to identify factors that influence pDNA yield. The influence of varying feed rates, feedstock composition, and media additives was evaluated. In

particular, the impact of different fed-batch strategies and their contribution to pDNA production was assessed [3]. Available cultivation data was further used to estimate parameters of a growth model incorporating overflow metabolism. The results demonstrate the technical feasibility of pDNA production in *V. natriegens* and highlight the value of high-throughput approaches for rapid bioprocess optimization with comparable yield as traditional systems at shorter process time.

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## P29: Enhancing efficiency in kombucha fermentation: A study on scaling up, fermentation time and sensory properties

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Kombucha is a fermented beverage produced from sugared tea by a symbiotic culture of yeasts and acetic acid bacteria, traditionally cultivated under static conditions using an undefined microbial consortium. The fermentation process usually takes 7–14 days, depending on the microorganisms involved and the conditions of the process. Although kombucha fermentation has been extensively studied, fermentations under agitated conditions with defined microbial consortia and the role of oxygen consumption remain largely unexplored.

This study aimed to optimize the kombucha fermentation process to reduce overall fermentation time while maintaining high product quality. Optimization was achieved through improvements in the fermentation substrate, selection of high ethanol-performing yeast strains, and control of aeration to regulate microbial activity. Thus, a defined microbial consortium was employed, consisting of *Komagataeibacter hansenii* as the acetic acid bacterium and selected yeasts including *Dekkera bruxelensis*, *Zygosaccharomyces bailii*, and *Saccharomyces cerevisiae*. The optimized process was subsequently scaled up from laboratory to pilot scale.

Fermentation time was successfully reduced to two days, comprising one day under static conditions followed by one day under aerobic conditions, while maintaining a high-quality final product. Microbial community monitoring revealed that static conditions favoured yeast metabolic activity, whereas aerobic conditions enhanced bacterial activity. The optimized process was successfully scaled up to 5 L bioreactors.

Overall, this study provides new insights into kombucha process optimization using a defined microbial consortium, emphasizing the importance of oxygen management in achieving efficient and scalable fermentation.

### P30: Foam as a source of bioprocess heterogeneity: A scale-down platform for automated foam decay analysis

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Foam formation represents an often-underestimated source of heterogeneity in large-scale bioprocesses, particularly in biosurfactant production such as rhamnolipid fermentations. In industrial reactors, foam constitutes a distinct phase where cells experience physicochemical conditions differing from the liquid bulk, yet foam is rarely considered explicitly in scale-down models. [1]

The mechanisms governing foam stability in rhamnolipid fermentations remain insufficiently understood, particularly the relative contributions of rhamnolipids, cells, and by-products [2–4]. Existing foam analysis approaches are laborious, technically complex, and low-throughput [5–7], hindering systematic, statistically robust investigations linking foam behavior to process variables.

We present a scalable platform for reproducible, parallelized generation of foam-related heterogeneities as a scale-down model, enabling phase partitioning studies and automated quantification of foam decay kinetics. We combine a shaken mini-batch settling cell, originally developed for liquid–liquid phase separation [8], with a novel image-processing workflow providing robust, high-throughput analysis.

Using this platform, we investigate key factors influencing foam stability, including surfactant and cell concentrations, stabilizing by-products, e.g., biopolymers as polyhydroxyalkanoates [9–11], and pH effects [12, 13]. The workflow is validated across a broad range of operating conditions.

Beyond fundamental foam characterization, the platform informs foam-based in situ product recovery (ISPR) strategies, such as foam fractionation, by identifying operating windows that maximize product recovery while minimizing biomass co-removal [14–16]. Overall, this work demonstrates how foam can be explicitly integrated into bioprocess scale-down strategies as a relevant, quantifiable, and mechanistically accessible source of process heterogeneity.

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### P31: Control of lactic acid bacteria and yeast co-cultures in micro-aerated cultures

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Lactic acid bacteria and yeast co-cultures can contain multiple flavor and health beneficial compounds for probiotic food and beverage consumption. These co-cultures are not industrially applied, since the microbial complexity is difficult to monitor and control in real-time [1]. Growth control is hypothesized to be possible through oxygen availability as suitable dissolved oxygen concentration supports yeast growth, however put oxidative stress on many lactic acid bacteria, and low dissolved oxygen favors lactic acid bacteria but limits yeast growth. Therefore, controlled micro-aeration is coupled with automated fluid scanning and microscopy image-based cell counting and analysis to assess the individual growth applied to achieve a distinct distribution of *Saccharomyces cerevisiae* DBVPG10191, *Lactobacillus paracasei* DSM6433, and *Lactococcus lactis* DSM4366 in batch co-cultivation. Lactic acid bacteria respond to environmental change by an altered detachment behavior during binary fission. Therefore, a novel on-line measurement method with stop-flow was applied to monitor average cell length of the rod-shaped and chain forming lactic acid bacteria. Image analysis technologies in combination with average cell length determination were used to correlate high values of cell length with observable chain formation. The consideration of cell morphology on a single-cell level, automatic individual cell counting, and rapid average cell length determination offers a promising process analytical technology for the control of yeast and lactic acid bacteria cultures. In case of blurry media, the methods become applicable if automated dilution techniques are applied. In this way, also relevant application of the co-culture in the dairy industry becomes possible.

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### P32: Uncovering a population-level safety mechanism for bioprocesses handling inhibiting substrates

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Lactic acid Microbial plastic waste valorisation enables specific biodegradation and biotransformation under tailored conditions. Recent developments highlight new strains or combinations of strains to enhance process robustness and productivity. However, key challenges remain, including the pretreatment steps required for generating fermentable substrates, impurity removal, and feedstock homogenisation. Furthermore, most microbial consortia have thus far been investigated primarily in batch cultures, with limited integration into intensified continuous systems. The absence of advanced control strategies for such systems hampers their scalability and industrial implementation.

Emerging technologies such as the Segregostat, a cell-machine interface relying on automated flow cytometry, provide insights into phenotypic landscapes and their dynamics. Previous studies demonstrated that the induction of burdensome gene circuits broadens phenotypic heterogeneity, thereby compensating for fitness losses. Although this diversification leads to the accumulation of less productive cells, it simultaneously increases overall system robustness. [1]

In this study, we extend the investigation of phenotypic

diversification to the context of mixed carbon sources with inhibitory properties. The soil bacterium *Pseudomonas putida* KT2440, engineered with a benzoate-degradation biosensor, was repeatedly exposed to oscillations between glucose and benzoate under non-inhibitory, inhibitory, and toxic conditions. Online flow cytometry revealed that elevated benzoate concentrations delayed activation dynamics and increased phenotypic heterogeneity, likely reflecting the induction of tolerance networks. Moreover, increasing benzoate stress led to differential collapse rates among subpopulations, enabling the set-up of control strategies involving fitness differences between these subpopulations.

Based on these observations, we were able to design a robust continuous cultivation system for *P. putida* on glucose, with benzoate exposure up to 90 mM.

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### P33: Scalability of in-situ gas supply via hydrophobic membranes to autotrophic biofilm systems

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In biological gas fermentations, the delivery of poorly soluble gaseous substrates to the biocatalysts has frequently been identified as a bottleneck in their scalability. An enhancement of the gas transfer can be accomplished in membrane biofilm reactors (MBfR) by the counter-diffusional supply of substrate gases through the membrane and dissolved nutrients in the liquid phase. Here, the autotrophic microorganisms are fixed within the reactor by growing on the surfaces of submerged hydrophobic membranes, which additionally allows for continuous reactor operation.

Lab- and bench-scale experiments have shown promising results with hydrogen gas supplied via microporous polypropylene membranes. Such applications include the in-situ upgrading of biogas by hydrogenotrophic archaea. Biogas was mixed with hydrogen and provided continuously via the membranes in a cross-flow-mode, realizing a maximum specific methane production rate of up to 32 NL m<sup>-2</sup> d<sup>-1</sup> ( $k_{la,H_2} = 634 \text{ h}^{-1}$ ) with a methane purity in the exhaust gas higher than 97 %. The cross-flow membrane configuration of the gas and liquid phases allowed for short hydraulic retention times along with an efficient removal of products from the biofilm. In a similar reactor configuration with dead-end hydrogen supply through the membranes, autotrophic denitrification was investigated, demonstrating a maximum specific denitrification rate of up to 3.57 g N m<sup>-2</sup> d<sup>-1</sup> ( $k_{la,H_2} = 1187 \text{ h}^{-1}$ ).

However, the up-scaling MBfRs towards technical scale systems requires further optimization, with respect to process stability (e.g. pH control in denitrification), membrane selection (e.g. mitigation of wetting in biomethanation) and packing density, to further increase gas transfer coefficients ( $k_{la,H_2}$ ).

### P34: Benefits of off-gas analysis – Using OUR and CER as parameters for reliable “Batch-End-Detection”

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Fed-Batch processes are often the preferred mode of operation for microbial cultivation. Despite the chosen feeding strategy, it is important to find a reliable way to detect the end of the batch phase to start the feeding of substrate and avoid starvation. The most common way to detect substrate depletion is to observe events in the dissolved oxygen measurement.

But DO-values only provide slight insights into the reactions that take place during the many phases of cultivation. During a batch phase with different substrates (diauxic growth), the DO-value will show small events if the metabolism is changing from one substrate to the other, but will follow its course through the whole batch phase. Off-gas analysis of O<sub>2</sub> and CO<sub>2</sub> on the other hand can provide more information about the actual reaction that takes place.

For example, using a crab-tree positive yeast like *Saccharomyces cerevisiae* in fed-batch cultivation will lead to a separated batch phase in which ethanol is first produced due to metabolic overflow and later consumed after glucose is depleted. Observations of the off-gas parameters will show that the respiratory quotient (RQ) will change from >1, indicating ethanol production, to 0.66 indicating ethanol consumption. At the same time oxygen uptake rate (OUR) and carbon dioxide emission rate (CER) can be used to trigger the feed phase. In this case the drop of OUR in combination with a threshold of cumulative CER is used to identify the end of the batch phase after total depletion of substrate.

### P35: Local oxygen limitation in multiphase bioreactors: A method for optical quantification

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Aerobic bioprocesses in stirred-tank bioreactors are governed by complex flow, mixing, and mass transfer phenomena that are difficult to capture experimentally. While spatial heterogeneities have been extensively studied in other reactor systems, bioreactor characterisation still relies largely on point-wise measurements. As a result, local oxygen dynamics remain insufficiently resolved, complicating the identification of oxygen limitations and the development of representative scale-down models [1,2,3].

This work presents a method for visualising heterogeneities in oxygen availability, implemented in an aerated, transparent 3 L stirred-tank reactor. The oxidation of methylene blue by dissolved oxygen, which induces a colour shift to blue, is employed as a spatial and temporal indicator of local oxygen availability [4]. Global and local colourisation times are determined by greyscale analysis of the colourisation reaction in response to oxygen gassing and related to dissolved oxygen availability.

Experiments conducted at different agitation rates show that colourisation times are longest near the walls and bottom of the reactor and shortest in the centre, resulting in a cone-shaped gradient. At higher agitation rates, shorter colourisation times and a reduced gradient are observed. These results suggest that oxygen limitation is most likely to occur near the reactor walls and bottom, whereas higher agitation improves reoxygenation efficiency and homogeneity throughout the vessel.

The developed method proves effective for comparing different operating conditions and for identifying key areas of oxygen limitation. Due to its simplicity, spatial resolution, and compre-

hensive output, it is highly suitable to support the development of scale-transfer models and for validating numerical simulations.

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### P36: Scalable micro-sparger modifications to support improved oxygen supply within intensified upstream processing

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The drive to reduce manufacturing costs for biopharmaceutical drugs has led to the pursuit of process intensification, aiming for higher space-time yields. A key aspect of this is ensuring robust oxygen transfer rates in cultures across scales, particularly in single-use bioreactors with volumes up to 2000L. This study investigates how sparger orifice parameters affect O<sub>2</sub> mass transfer capabilities. Traditional scaling involved increasing the number of same-sized orifices [1], which resulted in high gas exit velocities (GEV) in dense cultures. Using transparent vessel replicas, bubble formation and spatial distribution were visually analysed [2]. The performance was evaluated through oxygen consumption in CHO N-1 runs using different micro-sparger variants under similar conditions.

The study confirmed a correlation between increased mean Sauter diameter and GEV, characterized by the Weber number (We). Maintaining  $We < 2$  is crucial to ensure formation of a stable single-bubble regime [3]. Contrary to expectations, reducing orifice diameter and increasing their number did not enhance mass transfer significantly with PBS buffer alone. The addition of Kolliphor, a cell protective agent, reduced surface tension, prevented bubble coalescence during formation, and enhanced oxygen transfer. A lower GEV proved more effective than smaller orifice sizes for enhanced transfer rates. Comparing oxygen consumption in perfusion processes with different sparger types showed a doubling in performance. These insights led to the development of an Intensified Sparger for Flexsafe STR<sup>®</sup> bags, featuring a lower but consistent GEV and scalable mass transfer performance from 50L to 2000L.

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### P37: Evaluation of a novel oxygen injection system on pilot scale for the production of single cell protein

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The Intensification of aerobic yeast fermentations is essential for enabling cost effective production of single cell protein (SCP) for sustainable aquafeed applications. In this pilot scale study (15 m<sup>3</sup>), the performance of a novel oxygen injection system was evaluated using *Cyberlindnera jadinii* grown on ethanol as the primary carbon source. After successful technology transfer of the baseline air-only process from lab scale, a series of oxygen enriched fermentation runs were conducted to quantify achievable oxygen transfer rates (OTR) and assess productivity impacts.

Across multiple test conditions, separate O<sub>2</sub> injection via the novel sparger delivered OTR values 2-2.5-fold higher compared to air-only sparging, which was below expectations from CFD modelling and comparable to oxygen enriched air without a dedicated sparger. Analysis of kLa revealed predominantly coalescing broth behaviour, limiting the benefit of small bubble O<sub>2</sub> injection. Two process-based strategies, reduced antifoam dosage and controlled ethanol accumulation, significantly improved gas holdup and mass transfer. Ethanol accumulation combined with oxygen injection increased OTR by fourfold compared to air-only sparging.

Three full fermentations operated under optimized conditions achieved stable performance with a threefold improvement of OTR compared to air-only sparging and a twofold improvement in biomass productivity compared to air-only operation. SCP product quality met project requirements, reaching crude protein levels  $\geq 70\%$  (dry matter).

Overall, while the evaluated sparger did not provide intrinsic mass transfer advantages, process driven adjustments enabled substantial performance gains. Future campaigns will focus on improved O<sub>2</sub> injection hardware, including supersonic nozzles, combined with optimized broth rheology to further intensify SCP fermentation.

### P38: Precise measurements of high mass transfer coefficients in Single Use Bioreactors in microbial processes

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The volumetric oxygen mass transfer coefficient kLa is a key parameter for the engineering characterization of bioreactors. In single-use bioreactors (SUBs), its determination is commonly performed according to established guidelines, such as the DECHEMA recommendations, which describe the saturation (gassing-out) method based on nitrogen stripping [1]. This approach is well suited for mammalian cell culture processes with moderate oxygen transfer requirements ( $kLa < 15\ 20\ h^{-1}$ ). In contrast, microbial processes impose higher oxygen demands, requiring bioreactors to deliver higher kLa values to sustain microbial metabolism and productivity, which challenge conventional measurement approaches [1-4].

In this study, the reliable measurement of high oxygen mass transfer coefficients kLa in a large-scale acrylic glass replica of a microbial SUB with a working volume of 500 L is presented. Compared to typical mammalian cell culture processes, a 32-fold increase in kLa values was achieved, with values reaching up to 1069 h<sup>-1</sup>. These mass transfer conditions were realized

by increasing the volumetric power input (P/V) up to 2.35 kWm<sup>-3</sup> through elevated impeller tip speeds and increased air gassing rates of up to 2 vvm. The measured mass transfer coefficients were evaluated against the widely used van't Riet correlation for different sparger designs.

Key experimental challenges associated with the gassing-out method required to achieve such high k<sub>L</sub>a values, like sufficient degassing, impeller-induced vortex formation leading to self-gassing, headspace effects, heterogeneities in the reactor and accumulation of gas bubbles at dissolved oxygen (DO) probes, were systematically investigated. The presented results provide practical guidance for accurate k<sub>L</sub>a measurements in microbial SUBS

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### P39: Novel 96 well screening tool for time-resolved, independent and non-invasive determination of the oxygen transfer rate

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Conventional micro- and mini-bioreactor systems with integrated online monitoring lack the technology to measure the essential process parameter, the Oxygen Transfer rate (OTR). This crucial parameter can be used to distinguish between typical biological phenomena such as substrate limitation, diauxic growth, or oxygen limitation.

At Enduro, OTR monitoring is essential to establish and transfer predictive small-scale screening when implementing titer-increasing genetic biosensor technology, EnduroSense®, into clients' industrial production strains of *E. coli*, *Bacillus subtilis* and yeasts.

The novel Kuhner microTOM is an online-monitoring tool for non-invasive, high throughput measurement of OTR in microtiter plates. The system operates as a multi-channel platform with 96 positions enabling independent analysis of all wells of standard 96 deep-well microtiter plates (DWP). The oxygen partial pressure is measured using optical sensor spots based on dynamic fluorescence quenching, allowing contactless detection of oxygen partial pressure without consumption of oxygen or interference with the biological system. A filling volume dependent Coefficient of Variation under 2% was reported for OTR determination, indicating good reproducibility of parallelized micro-scale respiration measurements and supporting the reliability of the device for comparative screening studies [1].

In this case study, microTOM was applied to map diauxic shifts and synchronize *E. coli* precultures into highly defined physiological states in 96-DWPs, to enable optimal outcomes when screening EnduroSense® strains for improved bioprocess performance, with further applications in cell culture and

microbiology, including strain screening [1,2,3,6], media screening and optimization [3], toxicity assessment [4], and bioprocess understanding [5,6].

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### P40: Three-angled X-ray tomography for investigating gas-phase dynamics in scalable bubble columns

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Bubble columns are cheap to construct and operate and thus might play a crucial role in scaling up bioprocesses for manufacture of products with small margins (e.g. bulk chemicals or food proteins). The dynamics and performance of bubble columns is dominated by the gas phase behaviour, which is strongly affected by fluid composition [1, 2]. Understanding the link between fluid composition and gas phase behaviour is therefore instrumental to efficient scale-up of bubble column systems. For this, accurate measurement of bubble sizes, velocities and local gas fraction are necessary. In clear fluids and at low gas fractions (<5%), high-speed camera measurements can provide these data. Unfortunately, this technique breaks down in opaque broths and at high gas fractions (>5%) [3], while high gas fractions are required for optimal gas-liquid mass transfer. One method to circumvent these limitations is optical fiber probes, another is to use higher energy radiation such as X-ray. In this work we explore the use of a unique X-ray setup with three sources and three detectors simultaneously recording a bubble column [4]. We compare the X-ray tomography results to simultaneously acquired fiber probe data, pressure probe data and literature. We find that the main sources of error are cross-scatter (i.e. X-rays from source 1 ending up on detector 2 & 3), and beam hardening (attenuation coefficient decreases over attenuation length) and propose corrections for these. These corrections make our X-ray tomography setup a plug-and-play environment to determine local gas fraction in scalable systems, at high gas fractions.

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## P41: Multi-spectral fiber systems for life science applications

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Review of the innovative fiber system applications used for biomedical applications in 0.3-16µm range, including multi-spectral diagnostics to define tumor margins, QCL-fiber coupled probes to detect osteoarthritis in 5-15µm range, and unique systems with combi-fiber probes which enable to fuse data for 2-3 spectroscopy methods for more precise molecular analysis of media in-line.

Composition of tissue and bioliquids can be analyzed by several spectroscopic methods: Mid IR-absorption, reflection and transmission, fluorescence, and Raman scattering. Tiny fiber-optic probes enable flexible, sterilizable, and compact solutions for media analysis using several spectroscopic modalities. The innovative results to be presented on use of multispectral systems with combi-fiber probes for biomedical applications [1]. The synergy of data fusion from 2 complimentary methods helps to differentiate malignant and normal tissues for cancer diagnostics ex-vivo [2].

Broad spectra applications for various fiber coupled spectrometers and sensors open many promising applications in spectral diagnostics of tissues, bioliquids, bioreaction monitoring in-line, spectral analysis in multi-organ biochips, etc.

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## P42: Advances in Fluid Dynamic Stirred Tank Characterization – Opportunities of using MRI

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Stirred tank reactors (STRs) are still the most widely used reactor types for chemical and biochemical reactions. Stirred tanks exist for their various use cases in sizes ranging from the Milliliter

scale in biotechnology to the production scale of bulk chemicals in the order of several hundred cubic meters [1]. Depending on the use case, stirred tanks are tasked with very different challenges such as the mixing of one or multiple phases, dispersion of a gaseous phase, suspending solid particles in liquids or simple storage. In the past, the characterization of stirred tanks could only be based on local measurement data from probes, or in case of transparent reactors, on optical investigations, whereby dense dispersed phases quickly led to limitations [2]. Magnetic resonance imaging (MRI) offers a method to obtain tomographic data with sub millimeter spatial resolution of flow velocities, temperature, and concentration in various media [3].

This study analyzes the fluid mechanics of multiple stirred tank reactors, a Sartorius Ambr®250 single-use bioreactor for bioprocess development, with a working volume of 250 mL and both Rushton and segment impellers. The measurements are done on a unique specialized MRI system, which enables measurements of STRs in operando through its vertical orientation. In the Sartorius Ambr®250, the focus of this work lies in the fluid dynamical characterization, namely the analysis of velocity spectra, gas dispersion and gas holdup in respect to stirrer geometry, agitation rate and aeration rate.

Changes in gas distribution and gas-holdup can be observed in respect to stirrer geometry and agitation rate.

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## P43: Low-cost PAT and feedstocks for scalable biohydrogen production

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Dark fermentation of agro-industrial residues is a promising pathway for sustainable biohydrogen production; however, large-scale implementation is often constrained by high substrate costs and the limited availability of affordable online monitoring tools. This study addresses these challenges by integrating low-cost carbon sources with a low-cost hydrogen sensor implemented as a process analytical technology (PAT) for real-time monitoring. Sugarcane vinasse and sugarcane straw - abundant byproducts of the sugar and ethanol industries - were evaluated as carbon sources for biohydrogen production through co-digestion in batch dark fermentation reactors [1]. These residues provide readily fermentable substrates while offering a sustainable pathway for diversifying the sugarcane agro-industrial sector and strengthening its circular economy. Hydrogen production dynamics were continuously monitored using an MQ-8-based sensor system, and sensor performance was validated through statistical indicators including coefficient of variation, RMSE, and mean bias error. The sensor exhibited stable and repeatable performance, enabling real-time identification of hydrogen production phases as well as substrate inhibition effects associated with high vinasse fractions. The results confirm that low-cost feedstocks can sustain effective biohydrogen generation, while affordable PAT tools deliver the process insight required for monitoring, control, and optimization. By integrating economically accessible substrates with cost-efficient analytical technologies, this work supports a scalable, resource-efficient, and industrially relevant approach to biohydrogen production.

The proposed strategy reduces key economic barriers to scale-up and demonstrates strong potential for decentralized and industrial bioprocess applications within a circular bioeconomy framework.

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#### P44: Smart multi-column chromatography for scalable continuous bioprocessing

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This work presents the integration of multi-column chromatography (MCC) using the Octave BIO system into a continuous biomanufacturing platform. The complete, bacteria-based continuous manufacturing system was developed through a six-partner collaboration within the EConti project. We will highlight the software interface, command architecture, and the MCC's responsiveness to titer fluctuations. Furthermore, we will explore the broader potential of this approach for seamless integration into end-to-end processes as well as scale-up opportunities - simplifying the adoption of MCC in continuous downstream processing.

1. www.econti.eu

#### P45: Spectroscopy-assisted Bayesian optimization for efficient refolding of inclusion body proteins

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The production of recombinant proteins in *Escherichia coli* often yields insoluble inclusion bodies, which require denaturation and refolding to obtain the native product. The refolding step represents a major bottleneck. Conventional development and optimization typically rely on sequential Design of Experiments with high-performance liquid chromatography readouts. This approach is slow, labor-intensive, and requires a chromatographic method as well as purified protein standards. At the beginning of process development, these prerequisites may not be met, especially for proteins that can only be expressed as inclusion bodies.

We introduce a more efficient, data-driven workflow that pairs Bayesian optimization with an in-line readout from intrinsic tryptophan fluorescence. Using a disulfide-bonded single-chain variable fragment, we explored a five-dimensional design space of refolding buffer composition (dithiothreitol, oxidized glutathione, dilution factor, pH, and final urea concentration) guided by two spectroscopy-derived objectives. We showed that the spectral shift correlates with chromatographic yields, supporting its use as a fit-for-purpose sensor for process development.

With 25 experiments, Bayesian optimization identified conditions that delivered a refolded protein concentration of  $1.29 \pm 0.06 \text{ g L}^{-1}$  at  $58.7 \pm 1.3\%$  refolding yield with a dilution factor of 3.14, whereas a three-stage Design of Experiments with more than 60 experiments concluded at  $0.37 \pm 0.02 \text{ g L}^{-1}$  and  $61.4 \pm 3.1\%$  with a dilution factor of 11.39. Thus, the presented workflow achieved roughly 3.5-fold higher product concentration at comparable yield while operating at substantially higher protein concentrations. Therefore, spectroscopy-assisted Bayesian optimization is a sample-efficient tool for refolding optimization, especially in early development stages.

#### P46: Scale-up and optimization of recombinant protein production in E. coli in controlled conditions

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Recombinant protein synthesis in *E. coli* is the foundation for many biomedical applications both in diagnostics and in research. Widely recognized and standardized workflows allowing for small scale protein production often need fine tuning in order to generate higher yields and properly folded protein. Moreover, scaling up introduces additional challenges, but allows precise control of several parameters. Here we aimed at studying recombinant protein production in BL21(DE3) strain using, as a model protein, the ectodomain of influenza virus A hemagglutinin (HA-ECTO). Bacteria carrying a pET-24a(+) with HA-ECTO DNA sequence were cultivated in a vertical stirred tank reactor with 1L working volume. Protein expression was tested in three different culture media (Luria-Bertani (LB), ZYM-5052 and EnPresso®), while also evaluating the need to maintain antibiotic selection pressure. Samples were collected regularly to monitor dynamics of protein expression by SDS-PAGE, to assess plasmid copy number using qPCR and to paint the transcriptomic landscape via RNA sequencing.

Results showed that lab-scale expression kinetics are applicable to a 1L bioreactor setting when cultivating in LB, and the absence of antibiotic selection did not affect plasmid copy number per cell nor the amount of expressed recombinant protein. Autoinduction media and the enzyme based substrate delivery system, EnPresso®, yielded higher biomass amounts, yet longer cultivation intervals resulted in highly degraded protein.

Precise parameter control in a bioreactor setting and the possibility of on-line monitoring allowed better characterization of a process that is, ultimately, scarcely ever reviewed on lab-scale, thus, opening the possibility for targeted optimization of a protein yield and quality.

#### P47: Driving performance and process reproducibility in recombinant protein manufacturing through optimal selection of yeast-derived bionutrients

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In industrial biomanufacturing, media composition is a key lever for improving recombinant protein expression, productivity, batch consistency, and overall process economics. Among media ingredients, yeast-derived bionutrients (YDBs), including yeast extracts and peptones, are particularly important because they

provide amino acids, peptides, vitamins, trace elements, and other growth-promoting factors that support microbial growth and protein biosynthesis.

The performance of YDBs depends on multiple factors, including the host organism, expression system, fermentation conditions, downstream processing requirements, and the physicochemical properties of the target protein. As a result, selecting the most suitable YDB(s) is critical for maximizing yield and ensuring a robust and reproducible process. At the same time, the compositional complexity and natural variability of YDBs make batch-to-batch consistency essential for reliable bioprocess performance.

This work presents case studies showing that key process performance indicators in recombinant protein production are strongly influenced by the choice of Ohly's X-SEED® YDBs. High-throughput small-scale fermentation screening enabled rapid comparison of YDBs, followed by validation in controlled bench-scale fermentations under process-relevant conditions. The data underline the importance of targeted nutrient selection to unlock the full potential of microbial expression systems and demonstrate consistency and functional reproducibility across batches.

By prioritizing consistent, high-performing nutrient sources, manufacturers can improve process reliability, support scale-up, and enhance the economic efficiency of recombinant protein manufacturing.

#### P48: Scale-down bioreactor studies on heterologous protein production in stringent response modulated E. coli chassis

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*Escherichia coli* is a widely adopted host for large-scale recombinant protein production due to its genetic tractability and fast growth. However, industrial bioreactors introduce significant challenges including generating spatial and temporal heterogeneities, resulting in oscillating gradients of nutrients and dissolved oxygen. These fluctuating conditions trigger stress responses in *E. coli*, activating protective mechanisms that increase maintenance energy requirements, reduce growth rates, impair protein yields, and lower volumetric productivity. Consequently, final product titers decline, adversely affecting the economic viability of large-scale manufacturing. To overcome these limitations, engineered robust chassis strains are being developed. The *E. coli* SR strain has been specifically modified to suppress the global stringent response by maintaining ppGpp (guanosine tetraphosphate/pentaphosphate) at basal levels. This genetic modification has the potential to tolerate to industrially relevant stresses, making the strain a promising candidate chassis for reliable large-scale bio manufacturing. Scale-down bioreactors, combining a stirred-tank compartment with a plug-flow section, effectively mimic the heterogeneities and stress profiles encountered at production scale. These systems enable monitoring of metabolic, transcriptomic, and production responses under controlled stress conditions. Transcriptomic profiling confirms that SR strain adopts a stress-adaptation mode, whereas wild-type cells activate the stringent response under nutrient limitation. Current efforts apply the Design-Build-Test-Learn cycle to perform genome reduction and further refine the stringent-response-modulated platform, targeting superior robustness and productivity. Studies will evaluate recombinant production of two high-value model proteins: streptavidin and human carbonic anhydrase II, comparing its performance against standard *E. coli* producer strains to quantify advantages under simulated large-scale conditions.

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#### P49: Biotechnological process development for amphiphilic elastin-like proteins

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Precision biomacromolecular systems based on elastin-derived motifs enable the design of amphiphilic, intrinsically disordered protein architectures with defined molecular composition and programmable physicochemical behavior [1]. Amphiphilic elastin-like proteins (aELPs) consist of repetitive elastin-based sequences that exhibit a reversible lower critical solution temperature (LCST) phase transition, resulting in stimulus-responsive aggregation and resolubilization [2].

To enable their broader technological application, a biotechnological production and work-up scheme is currently being established. Recombinant expression in *Escherichia coli* is optimized in shake flask cultivations and is being transferred to controlled stirred-tank systems to facilitate scalable production and improved process control. Cultivation parameters and induction strategies are systematically evaluated with respect to protein yield and reproducibility.

Downstream processing focuses on exploiting the intrinsic transition behavior of aELPs as a material-inherent purification handle. Temperature- and salt-induced phase separation allows selective enrichment of the target protein and separation from host cell proteins [3]. This transition-based strategy is developed as an alternative to IMAC-dependent workflows, enabling tag-free constructs and aiming at a cost-efficient, rapid, and simplified purification process while avoiding the use of affinity tags and critical additives such as imidazole. The reversibility of the transition is assessed with regard to product integrity and process robustness.

The presented approach integrates upstream expression and transition-driven purification as a foundation for efficient and scalable production of programmable elastin-like protein materials.

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## P50: High-yield production of Cupriavidus necator soluble NAD<sup>+</sup>-reducing hydrogenase in E. coli for sustainable biocatalysis

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Academic and industrial fields are increasingly turning to biocatalysis to make chemical manufacturing more sustainable. Among [NiFe]-hydrogenases, the soluble NAD<sup>+</sup>-reducing hydrogenase from *Cupriavidus necator* (CnSH) is a premier candidate for H<sub>2</sub>-driven enzymatic cofactor recycling systems. By using H<sub>2</sub> as a direct, atom-economical electron donor, CnSH enables the byproduct-free regeneration of NADH/NADPH and inexpensive synthetic cofactor analogs driving diverse in vitro biotransformations [1-3]. However, the structural complexity of its hexameric assembly (HoxFUYH<sub>12</sub>), exceptionally low native yields (<1 mg/L), and long cultivation times remain significant bottlenecks for its industrial integration [4].

Building on our established bioprocess for the regulatory hydrogenase (CnRH) model [5; 6], we here present a robust bioprocess for the high-yield heterologous production of active CnSH. By implementing a dual-expression platform that coordinates structural subunits with the Hyp maturation machinery and nickel permease (HoxN), we optimized cultivation parameters specifically for scalable performance.

Using a fed-batch-like strategy with EnPresso media, we achieved an active soluble SH titer of 40 mg/L, corresponding to approx. 40-fold increase in yield and a significantly shorter process time compared to the native host [4]. The purified enzyme demonstrated a specific activity of ~20 U/mg, with correct cofactor insertion confirmed by IR spectroscopy. This study establishes a reliable scalable production strategy, providing the essential foundation for scaling up H<sub>2</sub>-driven, nicotinamide-free recycling systems and sustainable chemical manufacturing.

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## P51: Scaling of a fed-batch process for efficient heterologous production of active recombinant [NiFe]-hydrogenase

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Hydrogenases are key enzymes for utilizing molecular hydrogen as an alternative energy source and hold significant promise as biocatalysts due to their unique redox properties. However, their large-scale application is constrained by low expression in native hosts, the high cost of homologous production systems, and challenges arising from their complex structure, maturation requirements, and sensitivity to gases such as O<sub>2</sub> and CO. For the development of a bioprocess for heterologous hydrogenase production in *E. coli*, we selected the oxygen-tolerant regulatory hydrogenase (RH) from *Cupriavidus necator* H16 as a model enzyme. Previous work using the fed-batch-like EnPresso B medium enabled active RH production in shake flasks [1 2]. This strategy was subsequently transferred to a glucose-limited fed-batch process in a 2 L stirred-tank bioreactor, which yielded considerably higher overall RH titers, but at the expense of lower specific yields and decreased RH solubility [3].

In the present study, we aimed at improvement of the fed-batch process with the primary focus on increasing RH activity and solubility. To this end, we used the 2mag mini-bioreactor system as a scale-down platform to systematically screen critical cultivation parameters (e.g. inducer concentration, metal ion supplementation). We were able to enhance hydrogenase production, achieving higher soluble RH yields and increased enzymatic activity. Finally, the optimized process was scaled up again, confirming the findings from the scale-down experiments and demonstrating the reproducibility and robustness of the developed production strategy.

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## P52: Comparative evaluation of feeding strategies for glucose oxidase production in *Pichia pastoris* using a small-scale multibioreactor system

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The methylotrophic yeast *Pichia pastoris* is a well-established host for recombinant protein production, combining high cell density cultivation with the ability to perform eukaryotic post-translational modifications. However, efficient process control remains crucial to maximize product yield while minimizing metabolic burden and oxygen limitations. In this study, three fed-batch feeding strategies were systematically compared for the production of glucose oxidase from *Aspergillus niger* (AnGOx) using a small-scale multi-bioreactor system ( $\leq 150$  mL) with automated liquid handling. Cultivations were performed using *P. pastoris* SMD1168H carrying the plasmid pPICZnA-AnGOx. All processes included an initial glycerol batch phase followed by a glycerol fed-batch and methanol pulse induction. Subsequently, one of three feeding strategies was applied: (I) methanol-limited fed-batch (MLFB) [1], (II) temperature-controlled methanol fed-batch with a linear temperature decrease from 30°C to 16°C (TCFB) [1], and (III) methanol-limited fed-batch with minimal glycerol co-feeding (MGFB) [2]. Key process parameters, including dissolved oxygen, pH, and off-gas  $O_2/CO_2$  concentrations, were monitored online. Biomass and metabolite concentrations were assessed at-line, and enzyme activity was quantified throughout the process. All strategies enabled high cell density cultivations, reaching up to 100 g L<sup>-1</sup> cell dry weight. Glucose oxidase activity exceeded 100 U mL<sup>-1</sup> under optimized conditions. The comparative analysis highlights the impact of feeding strategy on process performance and provides insights for improving recombinant protein production in *P. pastoris*.

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## P53: Enhanced production of phenazine-1-carboxylic acid using a genetically modified *Pseudomonas chlororaphis* strain

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Phenazine-1-carboxylic acid (PCA) is a promising nitrogen-containing, heterocyclic compound with antibiotic, antifungal, and antitumor properties [1]. It is used as a natural biocontrol agent against fungal plant infections [2]. PCA can be produced as an extracellular, secondary metabolite by bacteria like *Pseudomonas*, *Burkholderia*, or *Streptomyces* [1]. This study focused on enhancing PCA production for a genetically modified strain of *P. chlororaphis* subsp. *aurantiaca* (DSM19603) through the development of an improved media composition.

After a series of statistical experiments, the following media recipe (per litre) was established: 37g glycerol, 20g tryptone,

1g MgSO<sub>4</sub> × 7H<sub>2</sub>O, 0.5g K<sub>2</sub>HPO<sub>4</sub>, 1.25g KH<sub>2</sub>PO<sub>4</sub>, 3g NaCl, and 0.0275g CaCl<sub>2</sub>. When using the determined media composition, and the genetically modified strain (denoted as DSM 19603ΔphzOΔrpeAΔrsmEΔlonΔpykFΔlon3) The production increased from 273 mg/L to about 1683 mg/L.

A bioreactor fed-batch strategy was implemented using a modified version of the designed media composition. The fed-batch strategy consisted of a 22-hour batch phase, followed by a 30-hour constant feeding fed-batch with the feed composed of 300 g/L glycerol and 400 g/L tryptone, and a feeding rate of 3.3 mL/h. The bioreactors were operated with dissolved oxygen control at 20% saturation and varying stirrer speed, and with pH control at 7. Using this strategy, it was possible to achieve an average maximum concentration of 2.83 g/L of PCA after 46 hours of cultivation.

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## P54: Carbon-to-phosphorus ratio optimization for poly(hydroxybutyrate co-hydroxyhexanoate) production in high cell density cultivation under phosphorus limitation condition

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The class of polyhydroxyalkanoates (PHAs) comprises a group of naturally occurring biopolymers that are characterised by excellent biodegradability [1]. The biodegradability of these biopolymers renders them a sustainable alternative to conventional plastics. However, economic feasibility and scalability remain major obstacles to their commercial success [2]. In this study, optimization of the carbon to phosphorus (C/P) ratio was carried out for the production of the PHA copolymer poly(hydroxybutyrate co hydroxyhexanoate) [P(HB co HHx)] under phosphorus limiting conditions using the recombinant strain *Cupriavidus necator* Re2058/pCB113 and canola oil as feedstock. Optimization was performed in cultures with low cell densities by systematically varying the C/P ratio in order to increase the yield of P(HB co HHx) and to estimate the optimal range of the C/P ratio. Cultivations were performed in 1 L laboratory scale parallel bioreactor systems, allowing for efficient screening of multiple C/P ratios simultaneously under controlled conditions. After defining the optimal range of the C/P ratio, experiments were conducted using high cell density fed batch cultures at C/P ratios of 275 and 225. With cell densities >300 g L<sup>-1</sup> containing >80 wt% of PHA (~23 mol% HHx) and productivities of up to 4.8 g L<sup>-1</sup>h<sup>-1</sup>, we demonstrate the efficient use of canola oil as carbon source for P(HB co HHx) production under phosphorus limitation. This study highlights the importance of the C/P ratio, which influences the yield, substrate utilisation efficiency and ultimately the cost of production.

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### P55: Modulating PHA molecular weight in *Cupriavidus necator* through overexpression of native depolymerases

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Polyhydroxyalkanoates (PHAs) are biodegradable, bio-based polyesters produced by many microorganisms as intracellular storage compounds for carbon and energy. Because of their biocompatibility, adjustable material properties, and complete biodegradability, PHAs are considered promising alternatives to petrochemical plastics for applications ranging from packaging to medical devices. The physicochemical properties of PHAs, including mechanical strength [1], melting temperature [2], and degradation behaviour [3], are influenced by their molecular weight.

The Gram-negative bacterium *Cupriavidus necator* is the established model organism for PHA production, owing to its high polymer accumulation capacity and well-characterized metabolic network. While high molecular weight (HMW) PHAs are typically targeted for structural materials, low molecular weight (LMW) PHAs and oligomers have gained increasing attention due to their enhanced processability, faster biodegradation, and suitability for specialized applications such as drug delivery systems or biodegradable additives for the production of coatings and films [2; 4].

Here we employ a strain engineering strategy to modulate the molecular weight of the PHA copolymer Poly(hydroxybutyrate-co-hydroxyhexanoate) P(HB-co-HHx) in *Cupriavidus necator*. By inducing the overexpression of different native PHA depolymerases, which catalyze the intracellular degradation of stored PHA, the average polymer chain length can be reduced. This enables the production of tailored LMW PHAs and offers potential for control over polymer properties directly during biosynthesis.

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### P56: Why optimized bioprocesses fail at scale: an integrated framework for robust dextran EPS production

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Microbial bioprocess scale-up frequently leads to performance losses due to nonlinear sensitivities not fully captured at laboratory scale. This challenge is particularly relevant for exopolysaccharide (EPS) production by lactic acid bacteria, where viscosity depends not only on total polymer concentration but also on molecular attributes such as chain-length distribution and structural organization [1,2]. This work presents an integrated framework to reduce scale-up uncertainty in the production of highly viscous dextran-type EPS by *Leuconostoc citreum* CNTA 860, combining multivariable experimental design, mechanistic modelling and hybrid predictive tools.

At 500 mL scale, a fractional Design of Experiments identified initial sucrose concentration as the primary determinant of EPS synthesis and broth viscosity, with agitation speed, C/N ratio and inoculum concentration also contributing significantly. Under optimized conditions, EPS concentration reached 70 g/L and viscosity 8200 cP after 24 h, exceeding previously reported values for *L. citreum* fermentations (e.g., 63 g/L under optimized conditions [3]).

A mechanistic kinetic model describing substrate consumption, biomass growth, EPS formation and by-product accumulation improved process interpretability under optimal conditions. Scale transfer to 2 L and 30 L bioreactors, following scale up criteria, resulted in partial performance retention at 2 L but significant deviations at 30 L, indicating scale-dependent sensitivities. Physics-informed neural networks (PINNs) were explored to enhance predictive capacity and accelerate robust scale transfer. This case study demonstrates that defining a robust laboratory-scale operating window is necessary but not sufficient to ensure industrial scalability, highlighting the need for integrated experimental-mechanistic-hybrid strategies to anticipate scale-related performance losses.

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### P57: Biotechnological production of human milk oligosaccharides

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Human milk oligosaccharides (HMOs), indigestible by humans, constitute the third most abundant solid component in human breast milk. They are essential for infant nutrition, playing a crucial role in early development and providing numerous health

benefits such as: 1) serving as prebiotics that promote beneficial gut bacteria, 2) enhancing immune system function, 3) supporting brain development, and 4) reducing inflammation. Over 150 structurally distinct HMOs have been identified and categorized into three main branches: neutral fucosylated, neutral non-fucosylated, and acidic (sialylated) sugars [1].

Biotechnological production of HMOs using *Escherichia coli* offers a sustainable and scalable alternative to methods such as chemical synthesis. The development of *E. coli* production strains involves extensive metabolic engineering to reconstruct HMO biosynthetic pathways. Key steps include the heterologous expression of glycosyltransferases, optimization of nucleotide sugar biosynthesis, and elimination of competing pathways.

Fermentative production in large-scale bioreactors is central to achieving industrially relevant yields. Key aspects of fermentation optimization include designing fed-batch processes to keep cell growth low while maintaining high metabolic activity, controlling pH and oxygen levels to support consistent enzyme function, and implementing feeding strategies to prevent by-product accumulation that can inhibit growth or HMO production. Precise monitoring and control of fermentation parameters are essential to ensure reproducibility and scalability [2]. Advances in strain engineering and fermentation processes have enabled cost-effective, high-yield production of diverse HMOs, paving the way for their widespread use in infant formula and other functional foods.

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### P58: Life cycle assessment of acetate production using gas fermentation with different CO<sub>2</sub> sources

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Gas fermentation is a key enabler of circular economy with potential to support decarbonization by converting gaseous feedstocks such as CO<sub>2</sub> and CH<sub>4</sub> into value-added biochemicals [1; 2]. Acetogenic microorganisms, including *Moorella thermoacetica*, a thermophilic strain, can utilize CO<sub>2</sub> as a carbon source to produce acetic acid [3]. This study aims to establish a circular value chain by utilizing CO<sub>2</sub> from different sources for the production of acetic acid via gas fermentation. An early-stage Life Cycle Assessment (LCA) was conducted to evaluate the environmental impacts of acetic acid production and to assess future large-scale scenarios. The analysis included CO<sub>2</sub> sources such as direct air capture (DAC), point-source emissions, and biogas-derived CO<sub>2</sub>. Baseline experimental data were obtained from a 150 L gas fermenter to evaluate process performance run at the open-access research pilot at NORCE ([www.nbioc.no](http://www.nbioc.no)). Process design and scale-up assumptions were based on a techno-economic assessment (TEA) shows that challenges of scaling up gas fermentations [4; 5]. The developed scenarios were compared with conventional fossil-based acetic acid production to assess environmental competitiveness and support decision-making prior to commercialization. The results indicate that hydrogen production via electrolysis is a major contributor to overall environmental impacts. Furthermore, the choice of CO<sub>2</sub> source significantly affects process sustainability. Integration of biogas-derived CO<sub>2</sub> within the gas fermentation process shows improved environmental performance compared to conventional production routes. Overall, the findings support

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### P59: Upcycling waste apples into platform chemicals: pilot-scale production of lactic and succinic acids

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The conversion of renewable biological resources into value-added products is essential for the transition to a sustainable bioeconomy. In this context, the valorization of agri-food waste represents a viable strategy for waste reduction and sustainable resource management [1]. Among agri-food wastes, surplus and discarded apples are a promising feedstock due to their availability and biochemical composition [1]. In this regard, the European Union is among the world's leading apple producers, with an estimated 11.5 million tons harvested in 2023 [2], indicating a substantial potential for valorization. This study explores the use of waste apples for the production of two industrially relevant platform chemicals: lactic acid (LA) and succinic acid (SA), which serve as monomers for the bioplastics polylactic acid (PLA) and polybutylene succinate (PBS), environmentally friendly alternatives to fossil-based materials. A screening identified *Heyndrickxia coagulans* A203 and *Actinobacillus succinogenes* DSM 22257 as the most suitable strains for LA and SA production from apple biomass. A simplified procedure, omitting costly centrifugation and sterilization steps between enzymatic liquefaction and fermentation, was successfully scaled up to 30 L. This process resulted in 73.8 g/L of LA with a yield of 0.91 g/g, and 36.8 g/L of SA with a yield of 0.69 g/g. These results demonstrate the potential of waste apples as a low-cost, effective feedstock for the biotechnological production of high-value platform chemicals, contributing to a circular and sustainable bioeconomy.

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## P60: Valorization of Cassava wastewater via acidogenic fermentation: Process intensification and scale-relevant analytics for sustainable VFA production

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Acidogenic fermentation of agro-industrial wastewater represents a scalable strategy for sustainable production of volatile fatty acids (VFA), contributing to circular bioeconomy frameworks in industrial bioprocessing. In this study, cassava sour starch extraction wastewater - an abundant low-cost substrate - was examined under controlled batch conditions to investigate critical operational factors influencing VFA yields and composition. Key process drivers, including fermentation time, pH, temperature, and substrate-to-microorganism (S/M) ratio, were systematically evaluated. Maximum VFA concentrations reached 3444 mg HAC/L, with yields up to 0.58 gCODVFA/gCOD, indicating strong substrate conversion performance dominated by acetic acid, followed by propionic and butyric acids. While S/M ratio showed negligible effects within the tested range, temperature and pH were identified as significant modulators of VFA production kinetics and product profile.

Complementary kinetic modeling captured soluble organic matter consumption and dynamic VFA generation, providing reliable fit parameters for process design and control. Integrated data from analytical monitoring and kinetic frameworks yield actionable insights for process intensification and scale-relevant decision-making, enabling translation from lab-scale optimization toward robust industrial applications. These findings underscore the potential of process analytical technologies and systematic process understanding to enhance industrially relevant bioprocess efficiency aligned with circular economy and sustainability objectives.

## P61: E-waste bioleaching in a bioreactor: Experimental and numerical studies of particles suspensions

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Printed Circuit Boards (PCBs) contains metals like copper. Bioleaching, with acidophiles microorganisms like *L. ferrophilum*, is a promising environmentally friendly process [1]. Before bioleaching, waste PCBs are grinded into particles of dozens  $\mu\text{m}$  to a few mm to improve reactive surface, metals accessibility and shorten process duration. Adequate agitation is needed to keep particles suspended; but higher agitation raises power consumption and can cause microbial damages. PCBs particles are heterogeneous in size, shape, and composition (mean density of 2.446), making a sufficient suspension hard to manage.

PCBs were mechanically suspended in a 2 L bioreactor containing culture medium and aerated with gas bubbles. The minimal agitation rate for suspension (Njs) was determined using the Zwietering criterion [2] to assess the effects of solid loading (40, 100 and 200 g-L<sup>-1</sup>), particle granulometry (2 mm to 63  $\mu\text{m}$ ) and gas flow rate (15, 30, 60 and 100 l-h<sup>-1</sup>). A least 122 measurements were performed. This dynamic and heterogeneous system was studied by coupling CFD (Lattice-Boltzmann) methods for fluid flow [3] and Discrete Element Method for particles modelling. Three representative classes of particles,

namely metals (7549 kg-m<sup>-3</sup>), plastic (2715 kg-m<sup>-3</sup>) and glass (1029 kg-m<sup>-3</sup>) were simulated.

Njs values were dispersed, ranging from 300 to 1500 RPM. Njs was most sensitive to solid concentration, then gas flow rate, and mean particle diameter, with power-law exponents of 0.226, 0.155, and 0.114, respectively. The higher Njs may increase energy consumption and cell damage. Simulations showed a heterogeneous particle distribution within the bioreactor. Most particles at the tank bottom were metals (60.5%), with little plastic (1.2%).

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## P62: Sustainable bioprocess development for upcycling of industrial waste streams

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The increasing demand for industrial products leads to the generation of large volumes of technical waste streams posing environmental and economic challenges. These waste streams often contain organic and inorganic compounds that remain underutilized. Upcycling such waste offers a promising approach by converting it into valuable products or reintegrating recovered compounds into circular bioprocesses.

The aim of this project is the development of sustainable bioprocesses for the upcycling of technical waste streams. Examples of such streams include agro-industrial residues, municipal wastewater and side streams from the food and beverage industry [1,2,3]. To achieve upcycling, various analytical tools, such as high-performance liquid chromatography (HPLC), high-performance ion chromatography (HPIC) and inductively coupled plasma optical emission spectroscopy (ICP-OES), are used to characterize the waste streams and identify potential substrates for bioprocessing. Furthermore, both established and novel biotechnological hosts will be evaluated for their ability to metabolize these substrates, with a focus on yeast, algae and moss [1,2,4]. Combinations of these organisms and selected waste substrates will be tested in bioreactors to assess efficiency and scalability.

This project is part of the Cluster of Excellence for Circular Bioengineering, funded by the Austrian Science Fund (FWF, project number: 10.55776/COE17). It contributes to a holistic approach towards the development of a circular bioeconomy. Other research areas within the cluster include the use of natural feedstocks for producing biodegradable materials, the creation of enzyme libraries to enable sustainable synthesis of chemicals and pharmaceuticals, and the integration of these principles into a broader socio-environmental framework.

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### P63: Microbial population shifts during compost-mediated degradation of anthropogenic polymers

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Microbial communities in composting systems play a pivotal role in the degradation of anthropogenic polymers, yet the temporal dynamics and functional implications of these populations remain incompletely resolved. In this study, we investigate population shifts during compost-mediated degradation of conventional and biodegradable polymers, linking community succession to polymer disintegration and mineralization. Laboratory-scale composting assays were established using representative polymers (e.g., PLA, PCL, PET, PE), and degradation was monitored via mass loss, molecular weight reduction, and CO<sub>2</sub> evolution, coupled with 16S rRNA sequencing. Our aim was to identify nodes in a network of key degraders. Collectively, our results demonstrate that polymer chemistry and composting stages jointly govern microbial succession, interaction patterns, and functional potential during polymer turnover, providing an ecological framework for designing composting strategies and microbial consortia to enhance the End-of-life treatment of anthropogenic polymers.

### P64: Integrating microbial biodegradation of polymers into circular waste management frameworks

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Anthropogenic pollution from polymer waste is a critical environmental threat, emphasizing the need to explore the long-term effects of plastics on compost biomes. Developing alternative waste utilization methods within circular economy frameworks requires understanding biodegradation mechanisms and influencing factors. This study evaluated the potential of native compost microbiota to degrade six distinct polymers (PET, PETG, PLA, PHB, PCL, and PE), encompassing both conventional and biodegradable materials. To assess the dynamics of biodegradation and microbial community succession, an experimental composting setup was conducted over six weeks in biological triplicates. The study investigated the impact of various polymer concentrations (1%, 5%, 10%) and two incubation temperatures (37 °C and 60 °C). The degree of degradation was

analyzed based on the polymer form, physicochemical properties, temperature, and process duration. Results demonstrated substrate-specific microbial community shifts, as the presence of these polymers selectively influenced the development of specific microbial groups, highlighting a significant impact on the compost microbiome structure. By optimizing composting conditions, this approach offers a promising integrated bioprocess strategy for mitigating plastic waste and enhancing the circularity of polymer life cycles.

### P65: SSF biodegradation of smoked cigarette butts and other hazardous cellulosic support materials

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This preliminary work tested environmentally friendly way to biodegrade exceptionally hazardous industrial materials comprising cellulosic support material\* such as smoked cigarette butts for producing lactic acid or ethanol using simultaneous saccharification and fermentation (SSF). Commercial cellulosic preparation with *Lactobacillus plantarum* 14431 in SSF of smoked cigarette butt materials gave ~25 % weight loss (g/ 100 g substrate) and ~28 g lactic acid/ 100 g substrate in first 24 h. The SSF of smoked cigarette butts along with *Kluyveromyces marxianus* IMB-3 and commercial cellulase preparation gave ~3.8 g ethanol/ 100 g substrate.

The SSF biodegradation of other hazardous cellulosic materials such as photographic black and white prints as well as color prints with commercial cellulase preparation with *Lactobacillus plantarum* 14431 gave 65% weight loss (g/ 100 g substrate) producing 60 g lactic acid/ 100 g substrate in 48 h. The SSF of *Kluyveromyces marxianus* IMB-3 of photographic prints gave 13 g ethanol/ 100 g substrate.

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## P66: Managing deployable bioprocess soft sensors: A FAIR registry approach

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The increasing use of machine learning in bioprocess digitalisation requires not only accurate predictive models but also infrastructures that support their deployment, traceability, and long-term maintenance. In particular, soft sensors must remain accessible, interpretable, and reusable as processes, data streams, and operational conditions evolve.

This work presents a FAIR-oriented model registry designed to manage deployable machine-learning-based soft sensors for bioprocess applications. The registry, developed within STAMM (Soft sensor monitoring and maintenance framework for Machine learning Models) [1], enables storage, versioning, validation tracking, and lifecycle management of models implemented in Python and R. Structured metadata capture training context, validation conditions, input-output specifications, and operational constraints, supporting reproducibility, traceability, and long-term maintainability. The model registry offers both an interactive interface for model exploration and a REST API for integration into deployment, orchestration, and monitoring pipelines, enabling the reliable operation of soft sensors in real-time industrial environments. In our implementation, curated models from the model registry can be published in metadata catalogues such as the IBISBA Knowledge Hub (IBISBAhub; <https://hub.ibisba.eu>), a tailored instance of FAIRDOME-SEEK [2-4] for industrial biotechnology. This allows models to be versioned, attributed to authors, and linked to projects, experiments, and documentation, extending their discoverability, accessibility, and reuse beyond the local operational environment. By leveraging standardized metadata schemas, the registry ensures that soft sensor models remain traceable, interoperable, and reusable, supporting FAIR principles and long-term sustainability.

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## P67: An ontology-based digital interlinked blueprint of the KIWI-biolab to support experimental reproducibility and traceability

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Bioprocess engineering laboratories are becoming increasingly automated and consist of heterogeneous, tightly interconnected systems [1]. Despite this complexity, laboratory infrastructure is often documented in fragmented or unstructured ways. By this, essential contextual information required to reproduce the experiment, such as device configurations and software dependencies, is missing. This lack of structured documentation significantly hinders experimental reproducibility [2]. Addressing these issues aligns with the goals of the FAIR principles, which promote research infrastructures and data that are findable, accessible, interoperable, and reusable [3].

This work presents the development of a structured and machine-readable representation of the KIWI-biolab at TU Berlin, a highly automated laboratory for bioprocess development [4]. The project establishes an interactive blueprint of laboratory infrastructure that links hardware, software, and experimental workflows in a hierarchical, semantically structured model.

Devices are represented within their integration context and described through standardized specification profiles that contain operating parameters, communication protocols, calibration procedures, constraints, and software dependencies. Experimental workflows are formally represented and linked to the underlying infrastructure, enabling traceability from experimental execution to specific device configurations.

To provide semantic interoperability and enable machine-actionable knowledge, the device infrastructure is modeled based on the SOSA/SSN ontology [5], implemented in Protégé. This approach formalizes sensors, actuators, observations, and system relationships within the laboratory environment.

The resulting model transforms the laboratory from a collection of instruments into a structured digital infrastructure representation. This enables transparent documentation, improved traceability of experimental configurations, and a foundation for future integration with knowledge graphs, digital twins, and automated laboratory management systems.

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## P68: A blockchain based digital thread for FAIR, trustworthy, and human-centric bioprocess development

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Bioprocess development is characterized by complex workflows, heterogeneous datasets, and fragmented knowledge distributed across experimental records, computational models, and expert experience. These limitations hinder reproducibility, transparency, and systematic knowledge reuse across development projects. The concept of the Cognitive Digital Thread (CDTh) addresses these challenges by establishing a digital infrastructure that links experimental data, workflows, models, and decision rationale across the entire bioprocess lifecycle [1,2]. By integrating semantic technologies such as knowledge graphs with artificial intelligence methods, the CDTh enables machine-actionable representation of contextual knowledge, facilitating traceability, explainable decision support, and improved collaboration in bioprocess engineering [3].

Within this context, Bioprocessing 5.0 represents a shift toward a human-centric and knowledge-driven paradigm [4]. Instead of focusing solely on automation and efficiency, this approach emphasizes the systematic digitalization of FAIR data, reproducible experimental workflows, interoperable models, and collaborative knowledge generation. The CDTh functions as the central backbone of this paradigm by connecting data, models, and expertise into a coherent knowledge system that supports faster and more reliable bioprocess development [5].

To operationalize this vision, a five-layer architecture is proposed. The architecture includes: (1) a local integration layer enabling standardized device connectivity and data acquisition; (2) an AI-based automation layer supporting reproducible workflows and automated metadata capture; (3) a knowledge digitalization layer using knowledge graphs to represent relationships between data, models, and decisions; (4) a secure knowledge exchange layer ensuring governance, provenance, and trust through blockchain technologies; and (5) a collaborative bioprocess development layer enabling human-AI interaction and distributed decision making.

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## P69: From off-line to real-time: Probe-integrated electrochemical glucose sensing for bioprocess monitoring

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Real-time process analytical technology (PAT) is essential for translating lab-optimized bioprocesses into robust large-scale operations, yet key variables are often tracked via off-line sampling [1]. To overcome this issue, we are developing a miniaturized multi-parameter electrochemical sensor platform for continuous monitoring of critical metabolites in bioreactors across scales, from laboratory to industrial manufacturing.

Here, we present an enzyme-free electrochemical glucose monitoring sensor that is robust for continuous measurements under bioreactor conditions, enabled by our specially designed sensor chips and base material. Our unique sensor architecture and chemical composition are tailored for operation in complex media, addressing selectivity and signal stability under varying process conditions. We designed a compact probe and housing concept that ensures reproducibility in stirred tanks while protecting electrical contacts from liquid ingress during real-time mixing. The probe geometry and placement are optimized to reduce bubble-related artifacts and to ensure consistent mass transport at the sensing surface. We also combine our sensor sets (probe and sensor chips) with our in-house pocket potentiostat, making our sensors independent and well-controlled. The sensor performance evaluation was conducted under bioreactor-relevant conditions (agitation, temperature, and process-like media), including repeatability, drift behavior, and benchmarking against off-line reference analytics. The resulting platform supports real-time PAT workflows and provides a practical route toward scalable, data-rich bioprocess monitoring for more than 24 hours.

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# 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 discussion about the challenges of scaling-up biotechnological processes from laboratory to industrial scale.

- **1<sup>st</sup> BioProScale Symposium**  
**"Inhomogeneities in large-scale bioreactors: Description – scaling – control"**  
24 to 27 November 2009
- **2<sup>nd</sup> BioProScale Symposium**  
**"Inhomogeneities in large-scale bioprocesses: System biology and process dynamics"**  
14 to 16 March 2012
- **3<sup>rd</sup> BioProScale Symposium**  
**"Inhomogeneities in large-scale bioprocesses: System biology and process dynamics"**  
2 to 4 April 2014
- **4<sup>th</sup> BioProScale Symposium**  
**"Bioprocess intensification through Process Analytical Technology: (PAT) and Quality by Design (QbD)"**  
6 to 8 April 2016
- **5<sup>th</sup> BioProScale Symposium**  
**"Innovative scale up and scale down for bioprocess intensification"**  
20 to 22 March 2018
- **6<sup>th</sup> BioProScale Symposium Online**  
**"Scale-up and scale-down for accelerated bioprocess development and optimisation"**  
29 to 31 March 2021
- **7<sup>th</sup> BioProScale Symposium**  
**"Scaling Up and Down of Bioprocesses: Technological Innovation and Cell Physiology Insights"**  
28 to 31 March 2022
- **8<sup>th</sup> BioProScale Symposium**  
**"Scaling Down and Up of Bioprocesses: Strategies, Tools and Process Performance"**  
9 to 11 April 2024

The abstract books of all symposia are available at [biotechnologie.ifgb.de/bioproscale/history](https://biotechnologie.ifgb.de/bioproscale/history)

