Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik

BioProScale e.V.

Bio-PAT

IfGB
Institut für Gärungsgewerbe und Biotechnologie zu Berlin

6th BioProScale Symposium

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

Scale-up and scale-down for accelerated bioprocess development and optimisation

+ Industrial-scale bioprocessing
+ Scaling up and down of bioprocesses
+ Bioprocesses for a circular economy
+ Process Analytical Technologies (PAT)
+ Microfluid Systems
+ Modelling Bioprocesses

ONLINE EVENT

Live Programme: 29 to 31 March 2021

Access to the Convention Site:
Friday, March 26, 2021, 10:00 (CET) until Tuesday, April 6, 2021, 22:00 (CEST)

VLB-BERLIN.6CONNEX.EU/EVENT/VIRTUAL-CONFERENCE/LOGIN

www.bioproscale-conference.org

VENUE: VLB VIRTUAL CAMPUS (ONLINE EVENT)

LANGUAGE: ENGLISH

Exhibitors / Sponsors / Media Partner

Bio-PAT

BECKMAN COULTER Life Sciences | mp labs
Now part of Beckman Coulter Life Sciences

BlueSens
understanding bioprocesses

aquilabiolabs

Roche

Cell tainer
BIOSOLUTIONS GmbH

HAMILTON
THE MEASURE OF EXCELLENCE®

ENPRESSO
Advanced Ready-to-use Culture Media

microorganisms
bioengineering

iST Innovative Sensor Technology
physical. chemical. biological.

ino Biotech AG
Discover molecular interactions

q&more
Excellence in Lab and Process

Welcome address

Dear Colleagues, Ladies and Gentlemen

It's my previledge and pleasure to welcome you to our 6th BioProScale Symposium. Although I would have loved to welcome you to Berlin for this, unfortunately, like so many other events, we are forced to organize this conference as a digital event. Since the meeting, which normally took place every 2 years, unfortunately had to be cancelled last year, we think it is time to bring our community together once again and discuss what interesting developments and new findings there are in the field of industrial biotechnology and especially in the area of bioprocess development and scaling. A lot has happened since our last meeting in 2018, for example in the area of bioprocess scaling methodology, implementation of new sensors, and automation of laboratory operations for faster bioprocess development. In particular, there are also increased efforts for a circular bioeconomy in relation to the concept of biorefineries and a diversification of approaches to the production of valuable materials based on residues. During the same time, consumers became more sensitive for probiotic food and the replacement of animal-based food production, e.g. through cellular agriculture. And last but not least, in the context of the current pandemic, the pharmaceutical industry demonstrated that modern technologies can be used to develop new vaccines and therapeutics more quickly within the last year, but nevertheless, many challenges remain for large scale production.

The current trends show the great importance of efficient strategies for the development of bioprocesses – not least with a currently very high level of public awareness by the Covid-19 pandemic. This process of implementing methods, e.g. from the fields of modelling and even artificial intelligence, is not trivial and needs intensive interdisciplinary discussions. That is my understanding why this conference is so important at this time. I believe that not only the pandemic, but also the social discussions that preceded it in the context of the worldwide movement for a climate-neutral, sustainable economy have brought the field of biotechnology and especially the branch of sustainable processes more into the public interest and represent an opportunity towards a biotechnology-based economy.

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

We are also very pleased that our symposium is attractive to a number of companies in its digital format and would like to thank our exhibitors and sponsors in particular.

Last but not least, I personally would like to express my sincere thanks to all the people and entities who helped to organize this event. My sincere thanks go to the VLB Berlin for their close and very professional cooperation in realizing the very interactive on-line platform for the conference. I hope that this will also excite all of you and give you the opportunity not only to follow even more lectures and posters than at real events, but also to activate and make new contacts via our online platform. I would also like to thank my colleagues and the Scientific Advisory Board who have always actively supported the idea of the conference and its implementation and have unselfishly taken on many detailed tasks.

With this in mind, I wish us all an interesting and exciting symposium. Enjoy the talks and fill the open meeting platform with live by fruitful discussions!

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

Scientific advisory board

Mario Birkholz (IHP Frankfurt/Oder, Germany)
Anika Bockisch (Bio-PAT e.V., Germany)
Nicolas Cruz-Bournazou (DataHow, Switzerland)
Frank Delvigne (University of Liege, Belgium)
Regine Eibl-Schindler (ZHAW, Switzerland)
Jörn Emmerich (SOPAT, Germany)
Krist V. Gernaey (DTU, Denmark)
Matthias Gimpel (TU Berlin, Germany)
Thomas Grimm (ANiMOX, Germany)
Peter Götz (Beuth University of Applied Sciences, Germany)
Sebastian Hans (TU Berlin, Germany)
Christoph Herwig (TU Wien, Austria)
Stefan Junne (TU Berlin, Germany)
Ulrich Krühne (DTU, Denmark)
Anja Lemoine (TU Berlin, Germany)
Jerôme Morchain (INSA, France)
Peter Neubauer (TU Berlin, Germany)
Henk Noorman (DSM, The Netherlands)
Marco Oldiges (Research Center Jülich, Germany)
Nico Oosterhuis (Celltainer Biotech BV, The Netherlands)
Saija Rasi (LUKE Research, Finland)
Sebastian L. Riedel (TU Berlin, Germany)
Rigoberto Rios Estepa (Universidad de Antioquia, Colombia)
Stuart Stocks (LEO Pharma, Denmark)
Ralf Takors (University of Stuttgart, Germany)
Joachim Venus (ATB, Germany)

About the organisers

Co-organizer: BioProScale e.V.

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.

www.bioprocess.tu-berlin.de

IfGB – Institut für Gärungsgewerbe und Biotechnologie zu Berlin

Founded in 1874, under the umbrella of the Institute of Fermentation and Biotechnology in Berlin (IfGB) fermentation oriented research and education has been conducted in Berlin for more than 140 years – always in close cooperation with the Technische Universität Berlin (resp. its predesessor institutions). Since 2003 the Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V. is the soule holder of the IfGB. Under the brand name "IfGB", services and training for the spirits industry and distillers have been offered and expanded also into the field of applied biotechnology.

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

www.ifgb.de

MONDAY, 29 MARCH 2021

WELCOME ADDRESS AND PLENARY TALK

10:00	**Welcome address and introduction** *Peter Neubauer, TU Berlin, Germany*
10:20	**Plenary talk: Zero concepts in bioprocessing – towards the ultimate performance (PL01)** *Henk Noorman, DSM, The Netherlands*
11:05	*Break & exhibition*

Best Presentation Award sponsored by:

microorganisms
bioengineering

SESSION 1A
INDUSTRIAL-SCALE BIOPROCESSING AND SCALE DOWN

Chair	Stefan Junne, TU Berlin, Germany
11:35	**Keynote talk: Digital twins for improved bioprocess operation? (L01)** *Krist V. Gernaey, Technical University of Denmark, Denmark*
12:05	**Towards increased stability in large-scale bioreactors by bacterial co-cultures (L02)** *Pauli Losoi, Tampere University, Finland*
12:25	**Dynamic genome scale modeling of** Streptomyces clavuligerus **metabolism for studying ist metabolic performance under different shear stress conditions (L03)** *David Gómez-Rios, Universidad de Antioquia, Colombia*
12:45	*Break, poster session & exhibition*

SESSION 1B
PROCESS ANALYTICAL TECHNOLOGIES (PAT)

Chair	Theresa Menzel, TU Berlin, Germany
11:35	**Keynote talk: Interfacing single cell technologies for stabilizing microbial cell population incontinuos cultivation (L04)** *Frank Delvigne, Université de Liège, Belgium*
12:05	**Continuous** E. coli **bioprocessing: Monitoring of sub-populations and how to deal with them (L05)** *Julian Kopp, TU Wien, Austria*
12:25	**What is beyond the average - a step toward quantifying the specific reactivity of single cells? (L06)** *Martin Schirmer, University Leipzig, Germany*
12:45	*Break, poster session & exhibition*

SESSION 2A
INDUSTRIAL SCALE BIOPROCESSING AND SCALE DOWN

Chair	Fabian Schröder, TU Berlin, Germany
14:30	**Keynote talk: New experimental methods for detecting heterogeneous flow structures in bioreactors pave the way for a more reliable scale-down and scale-up (L07)** *Michael Schlüter, TU Hamburg, Germany*
15:00	**CFD-based dynamic compartment modelling for the time-series prediction of gradients in industrial-scale aerobic fed-batch fermentation processes (L08)** *Gisela Nadal Rey, Technical University of Denmark, Denmark*
15:20	**Heat balance and CFD coupling strategy for the scale-up of an innovative bioleaching process (L09)** *Céline Loubiere, Bureau de Recherches Géologiques et Minières, Orléans, France*
15:40	**CFD and mass transfer in industrial gas-lift reactors for syngas fermentation (L10)** *Lars Puiman, Delft University of Technology, The Netherlands*
16:00	*Break, exhibition & virtual Get-together*
17:00	**Poster discussion 1: Poster 1–4** *Moderation: Tolue Kheirkhah, TU Berlin, Germany*
18:00	**Poster discussion 3: Poster 9–12** *Moderation: Niels Krausch, TU Berlin, Germany*
19:00	End of day 1

SESSION 2B
PROCESS ANALYTICAL TECHNOLOGIES (PAT)

Chair	Marion Longis, TU Berlin, Germany
14:30	**Keynote talk: PAT for the assessment of population heterogeneity in scale up and down (L11)** *Stefan Junne, TU Berlin, Germany*
15:00	**In-line application of Photon Density Wave spectroscopy as a PAT sensor in high-cell-density bioprocesses: Monitoring of** E. coli **growth and PHA formation in R. eutropha (L12)** *Björn Gutschmann, TU Berlin, Germany* *Thomas Schiewe, Universität Potsdam / innoFSPEC, Germany*
15:20	**Design and development of electrochemical sensors for bioprocess monitoring (L13)** *Aliyeh Hasanzadeh, Technical University of Denmark, Denmark*
15:40	**Control and optimization of polyhydroxyalkanoates production at pilot plant scale in real-time (L14)** *Silvia Ochoa, Universidad de Antioquia, Colombia*
16:00	*Break, exhibition & virtual Get-together*
17:00	**Poster discussion 2: Poster 5–8** *Moderation: Fatemeh Nejati, TU Berlin, Germany*
18:00	**Poster discussion 4: Poster 13–16** *Moderation: Lara Santolin, TU Berlin, Germany*
19:00	End of day 1

All times Central European Summer Time (UTC+2)

TUESDAY, 30 MARCH 2021

OPENING AND PLENARY TALK

10:00	**Opening** *Peter Neubauer, TU Berlin, Germany*
10:05	**Plenary talk: Systematic bioprocess devlopment in advanced microtiter plate and shake flask culture systems with online monitoring and feeding options Industrial scale bioprocessing and scale down (PL02)** *Jochen Büchs, RWTH Aachen, Germany*
10:50	*Break & exhibition*

Best Presentation Award sponsored by:

microorganisms
bioengineering

SESSION 3A
INDUSTRIAL-SCALE BIOPROCESSING AND SCALE DOWN

Chair: Joana Carvalho Pereira, TU Berlin, Germany

11:20	**Keynote talk: Exploiting Scale-down Tests for Engineering a Robust *E. coli* Host as a Platform for Industrial Production Processes (L15)** *Ralf Takors, University of Stuttgart, Germany*
11:50	**Investigation of scale dependent factors in industrial human milk oligosaccharide production (L16)** *Greta Gecse, Technical University of Denmark*
12:10	**Secretory protein producing *Bacillus subtilis*: Withstanding process inhomogeneities expected in a large-scale stirred tank bioreactor (L17)** *Marco Oldiges, Forschungszentrum Jülich, Germany*
12:30	*Break, poster session & exhibition*

SESSION 3B
INDUSTRIAL-SCALE BIOPROCESSING AND SCALE DOWN

Chair: Sebastian L. Riedel, TU Berlin, Germany

11:20	**Keynote talk: Feedstock potential and valorisation of organic side-streams for value-added products (L18)** *Saija Rasi, Natural Resources Institue Finland, Finland*
11:50	**Scale up from the cellar - the LX-Process as pretreatment for microbial conversion (L19)** *Friedrich Streffer, LXP Group GmbH, Germany*
12:10	**Upscaling butanol production using mixed microbial cultures (L20)** *Tiago Pinto, Technical University of Denmark, Denmark*
12:30	*Break, poster session & exhibition*

SESSION 4A
BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair: Matthias Gimpel, TU Berlin, Germany

14:30	**Keynote talk: Potential of genome and proteome-reduced strains for protein and plasmid DNA production (L21)** *Alvaro R. Lara, Universidad Autonoma Metropolitana-Cuajimalpa, Mexico*
15:00	**Scale-down of high cell density Fab production in *E. coli* (L22)** *Florian Mayer, University of Natural Resources and Life Sciences, Vienna, Austria*
15:20	**N-1 perfusion-based IgG productions in stirred single-use bioreactors (L23)** *Jan Müller, Zurich University of Applied Sciences, Switzerland*
15:40	**Evaluation of the clavulanic acid production integrating process simulation and systems biology (L24)** *Rigoberto Rios Estepa, Universidad de Antioquia, Colombia*
16:30	*Break, exhibition & virtual Get-together*
17:00	**Poster discussion 5: Poster 17–20** *Moderation: Eike Janesch, TU Berlin, Germany*
18:00	**Poster discussion 7: Poster 25–28** *Moderation: Lucas Kaspersetz, TU Berlin, Germany*
19:00	End fo day 2

SESSION 4B
MICROFLUID SYSTEMS

Chair: Mario Birkholz, IHP-Leibniz

14:30	**Keynote talk: High-throughput single-cell-resolution microfluidics to accelerate microbial bioproduction bioprocess development (L25)** *Arum Han, Texas A&M University, USA*
15:00	**Seperation of microalgae and polystyrene particles by dielectrophoresis (L26)** *Danai Malti, TU Berlin, Germany*
15:20	**Dynamic microfluidic single-cell cultivation: Growth of Corynebacterium glutamicum at fluctuating environmental conditions (L27)** *Sarah Täuber, Bielefeld University, Germany*
15:40	**Reproducing dynamic environment in microfluidic single-cell cultivation based on computational lifeline analysis (L28)** *Phuong Ho, Forschungszentrum Jülich, Germany*
16:30	*Break, exhibition & virtual Get-together*
17:00	**Poster discussion 6: Poster 21–24** *Moderation: Marie-Therese Schermeyer, TU Berlin, Germany*
18:00	**Poster discussion 8: Poster 28–32** *Moderation: Annina Kemmer, TU Berlin, Germany*
19:00	End fo day 2

All times Central European Summer Time (UTC+2)

WEDNESDAY, 31 MARCH 2021

OPENING AND PLENARY TALK

10:00 **Opening**
Peter Neubauer, TU Berlin, Germany

10:05 **Plenary Talk: Digitalization platform and supervisory control for continuous integrated manufacture of monoclonal antibodies (PL03)**
Massimo Morbidelli, Politecnico di Milano, Italy

10:50 *Break & exhibition*

Best Presentation Award sponsored by:

microorganisms
bioengineering

SESSION 5A HIGH-THROUGHPUT BIOPROCESSING AND AUTOMATION

Chair Sebastian Hans, TU Berlin, Germany

11:20 **Keynote talk: Robot and machine learning assisted protein engineering on the high-throughput screening platform LARA (L29)**
Mark Dörr, University Greifswald, Germany

11:50 **Towards accelerated bioprocess development: Using cell-free protein synthesis to screen for promising biocatalysts (L30)**
Katrin Rosenthal, TU Dortmund, Germany

12:10 **Fed-batch like microtiter cultivations as high-throughput screening tool for *E. coli* production process development (L31)**
Mathias Fink, University of Natural Resources and Life Sciences, Vienna, Austria

12:35 *Break, poster session & exhibition*

SESSION 5B MODELING BIOPROCESSES

Chair Nicolas Cruz-Bournazou, TU Berlin, Germany

11:20 **Keynote talk: Improvements for scalability of Lagrangian-Eulerian approaches for tracking lifelines of single cells in large bioreactors (L32)**
Matthias Reuss, University of Stuttgart, Germany

11:50 **Black box modelling approaches to judge a yeast extracts influence on microbial growth and production (L33)**
Stefanie Kaul, Hamburg University of Applied Sciences, Germany

12:10 **On the modelling of microbial population dynamics using partial differential equations (L34)**
Jerome Morchain, Toulouse Biotechnology Institute, France

12.35 *Break, poster session & exhibition*

SESSION 6A HIGH-THROUGHPUT BIOPROCESSING AND AUTOMATION

Chair Stefan Born, TU Berlin, Germany

14:30 **Keynote talk: Smart digital solutions for USP and DSP to master bioengineering challenges towards industry 4.0 in biopharma (L35)**
Michael Sokolov, DataHow / ETH Zürich, Switzerland

15:00 **Keynote talk: The CompuGene automated platform for the construction and characterization of genetic parts and microbial cell factories (L36)**
Johannes Kabisch, TU Darmstadt, Germany

15:30 **Modelling approaches with a fully-automated microbial fermentation platform (L37)**
Vignesh Rajamanickam, Boehringer Ingelheim, Vienna, Austria

15:50 **Towards an autonomous model based high throughput bioprocess development and clone discrimination (L38)**
Sebastian Hans, TU Berlin, Germany

16:10 *Break & exhibition*

SESSION 6B BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair Björn Gutschmann, TU Berlin, Germany

14:30 **Keynote talk: Process development of polyhydroxyalkanoate (PHA) bioplastics production from lipid based waste and raw materials (L39)**
Sebastian L. Riedel, TU Berlin, Germany

15:00 **Keynote Talk: Usage of mealworms to recover and purify polyhydroxyalkanoate granules from *Cupriavidus necator* cells (L40)**
Kumar Sudesh, Universiti Sains Malaysia, Malaysia

15:30 **Extraction of chitin from American lobster (*Homarus americanus*) shells and fabrication of membranes for potential biomedical use (L41)**
Christopher Brigham, Wentworth Institute of Technology, USA

15:50 **Low quality by-products for high quality products – Processing strategy and application development for the circular economy (L42)**
Thomas Grimm, Animox GmbH, Germany

16:10 *Break & exhibition*

PLENARY TALK AND CLOSING

16:30 **Plenary talk: Integrated and networked systems and processes – A perspective for digital transformation in (bio) process engineering (PL04)**
Michael Maiwald, BAM, Germany

17:15 **Closing remarks / Best Poster & Presentation Award**
Peter Neubauer, TU Berlin, Germany

17:30 *End of Congress*

All times Central European Summer Time (UTC+2)

SCIENTIFIC POSTERS

Best Poster Award sponsored by: eppendorf

P01 **Design and upscaling of *Pseudomonas putida* fermentations for robust biomanufacturing**
Jesper W. Jensen et al., Technical University of Denmark

P02 **Insights into the role of sigma factor orf21 in *clavulanic acid* production by Streptomyces clavuligerus ATCC27064**
Patiño Cervantes L.F. et al., Universidad de Antioquia,

P03 **Genome mining of Streptomyces strains and its potential to identify new compounds with antimicrobial activity**
Carlos Caicedo-Montoya et al., Universidad de Antioquia

P04 **Cell-free biosynthesis of the nonribosomal peptide antibiotic valinomycin**
Jian Li, ShanghaiTech University

P05 **Development of a *P. pastoris* strain for the recombinant production of peptide-based antibiotics in eukaryotic cells**
Lisa Michel et al., Hamburg University of Applied Sciences

P06 **Experimental studies on the isolation of photobiont and mycobiont partners of lichens for controlled cocultivation and production of secondary substances**
Zakieh Zakeri et al., TU Berlin

P07 **Comparison of different analytical methods for the assessment of viability during the production and storage of yeast preparations**
Martin Senz et al., VLB Berlin

P08 **Applying dielectrophoresis to improve a microring resonator biosensor platform**
Anders Henriksson et al., TU Berlin

P09 **Effect of oscillatory cultivation conditions on the macromorphology in *Yarrowia lipolytica* cultivations**
Jasmina Cziommer et al., TU Berlin

P10 **Control of macromorphology and the implications on product formation in *Aspergillus niger***
Tolue Kheirkhah et al., TU Berlin

P11 **Cultured meat production in a 2D rocking bioreactor**
Tobias Höing, MosaMeat

P12 **From micro to macro: a study on the volumetric power input in microtiter plates and its use as a strategy for scale-up in downstream processing**
Ignacio Montes-Serrano et al., Austrian Centre of Biotechnology

P13 **Towards smart factories: Data-driven modeling approaches in bioprocessing**
Jonathan Sturm, Westfälische Hochschule Recklinghausen

P14 **A novel gradient-based monitored dark fermentation of biogenic feedstocks for material use in plug-flow reactors**
Marion Longis et al., TU Berlin

P15 **BioProdPacific: A platform to accelerate the design of integrated and sustainable bioprocesses in Colombia**
Erika Y. Ortiz et al., Universidad Icesi

P16 **A small-scale hydrocyclone system as a biotechnological application for continuous cell separation**
Huschyar Al-Kaidy et al., Beuth University of Applied Sciences Berlin

P17 **Optimization of H2-sensing regulatory hydrogenase production from *Ralstonia eutropha* in *Escherichia coli***
Qin Fan et al., TU Berlin

P18 **Evaluation of microbial hydrolysis for anaerobic digestion in a plug-flow reactor**
Theresa Menzel et al., TU Berlin

P19 **Exploring the potential of biofilms for fermentation-based biomanufacturing**
Pascal S. Leonov et al., Technical University of Denmark

P20 **Production of enzyme laccase at pilot scale by using loofah-immobilized biomass of *Ganoderma chocoense***
Natalia Andrea Llanos et al., Universidad Icesi

P21 **Integration of a robotic small-scale bioreactor system as a prerequisite for a selflearning and autonomous cultivation platform**
Lucas Kaspersetz et al., TU Berlin

P22 **Importance of oxygen signal shape matching for robust parameter estimation in bioprocess development**
Judit Aizpuru et al., TU Berlin

P23 **Determination of plasmid mutation rates in *Escherichia coli* using an automated high-throughput Quasi-Turbidostat**
Matthias Gimpel et al., TU Berlin

P24 **Feasibility analysis of a non-stirred miniature bioreactor**
Jonathan Poit et al., TU Berlin

P25 **Developing a unified IT Platform covering the whole development cycle – A Case study for Enzyme Production**
Simon Seidel et al., TU Berlin

P26 **Reproducing dynamic environment in microfluidic single-cell cultivation based on computational lifeline analysis**
Phuong Ho et al., Forschungszentrum Jülich

P27 **Rapid and cost-effective fabrication of microchromatography integrated with microelectrode impedance sensor for determination and characterization of column efficiency and effluent**
Amin Javidanbardan, Universidade de Lisboa

P28 **Advanced robotic workflows for integrating mass spectrometry based multi-component analysis into metabolic phenotyping**
Alexander Reiter et al., Forschungszentrum Jülich

P29 **From screening to production: a holistic approach of high-throughput model-based screening for recombinant protein production**
Niels Krausch et al., TU Berlin

P30 **An automated method that enables high-throughput screening of knockout libraries with focus on industrial important metabolic cell properties**
Fabian Schröder et al., TU Berlin

P31 **The batch brewing process represented by a mechanistic model**
Maximilian Schmacht, VLB Berlin

P32 **Unraveling the microbial dark matter using picolitre gel droplets**
Bianka Kästner et al., Beuth University of Applied Sciences-Berlin

MONDAY, 29 MARCH 2021

Welcome Address & Plenary Talk

10:00 Welcome address and introduction

Peter Neubauer

Technische Universität Berlin, Institute for Biotechnology,

Chair of Bioprocess Engineering, Berlin, Germany, peter.neubauer@tu-berlin.de

10:20 Plenary talk: Zero concepts in bioprocessing: towards the ultimate performance (PL01)

Henk Noorman

DSM Biotechnology Center, Delft, The Netherlands, Henk.Noorman@DSM.COM

The transition to a bio-economy will be much facilitated when 'ideal' bioprocesses will become the standard in industrial bioprocessing. For this, we advise that conceptual bioprocess design and development should fulfil as much as possible eight requirements, all addressing a zero element:

- Zero carbon spills: develop feedstock-conversion-product combinations that minimize the formation of CO2 and other byproducts. Examples include cell retention for zero cell growth, full product recovery, valorization of all byproducts incl. CO2, and radical metabolic re-engineering.
- Zero energy spills: develop approaches that eliminate energy-intensive aseptic methods and product recovery methods using extremophiles or xenobiotic nutrients for nonsterile fermentation, and aim at water-insoluble products (gaseous, oily, or solid).
- Zero water spills: maximize water management, using water-free feedstocks and microbial hosts that tolerate high substrate and product concentrations.
- Zero pH swings: minimize pH-controlling agents using acidophilic/alkaliphilic production hosts for production of acids/bases.
- Zero O2 fermentation: use anaerobic conversions, for the highest conversion efficiency from feedstock to product.
- Zero N2 fermentation: if O2 is unavoidable, consider using pure O2 rather than air to enable intensification, while keeping dissolved O2 at nontoxic concentrations.
- Zero process variability: minimize variability in processes across the duration of a fermentation and between fermentations, maintaining optimal process conditions over a long production period using robust, continuous processes at steady state.
- Zero development time: minimize R&D costs; the organism-process combination should be developed quickly, at low costs, and scaled-up without losses or failures.

Examples will be provided where already good progress is being made, both at the drawing table and in industrial practice. There are still grand challenges on the way forward, however, once ultimate performance will be accomplished then fossil-based production processes will be outcompeted.

11:05 Break & exhibition

Session 1A: INDUSTRIAL-SCALE BIOPROCESSING AND SCALE DOWN

Chair Stefan Junne, TU Berlin

11:35 Keynote talk: Digital twins for improved bioprocess operation? (L01)

Krist V. Gernaey and Isuru A. Udugama

Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, Building 229, 2800 Kgs. Lyngby, Denmark
Email: kvg@kt.dtu.dk

The "Digital twin" concept has been coined by NASA in 2011, to describe a digital model that simulates the behavior of an aircraft. For the process industries, the digital twin concept has been widely adopted in the chemical industry, where for example the Tennessee Eastman Process benchmark was introduced in the 90's as a benchmark for chemical processes [1]. Digital twins are also very popular in the study of biological wastewater treatment processes, where the Benchmark Simulation Model No. 2 (BSM2) was developed as a state-of-the-art comprehensive plant-wide simulation of a wastewater treatment plant that has later been adapted and validated on specific full-scale cases [2]. For fermentation and bio-based processes, mathematical models often only contain the fermentation unit operation. This is of practical use for the development of process monitoring and control strategies for the fermentation process, but it does not consider the connection with upstream and downstream processes, which are also an integral part of a bio-based production process. Starting with a brief overview of digital twins used in the process industries, this presentation will focus on the development of digital twins for bio-based production processes. The main requirements for such a digital twin will be highlighted, as well as the need for detailed process data to allow for model validation. Furthermore, the benefits to be expected from availability of a plant-wide digital twin of a bio-based production process are discussed and put in perspective in terms of current process operation practice.

1. Downs, J.J. and Vogel, E.F. (1993) A plant-wide industrial process control problem. Comput. Chem. Eng., 17: 245-255.
2. Kazadi Mbamba, C., Lindblom, E., Flores-Alsina, X., Tait, S., Anderson, S., Saagi, R., Batstone, D.J., Gernaey, K.V. and Jeppsson, U. (2019) Plant-wide model-based analysis of iron dosage strategies for chemical phosphorus removal in wastewater treatment systems. Water Res., 155: 12-25.

12:05 Towards increased stability in large-scale bioreactors by bacterial co-cultures (L02)

Pauli Losoi, Suvi Santala, Jukka Konttinen, Ville Santala

Faculty of Engineering and Natural Sciences, Tampere University, Hervanta campus, Korkeakoulunkatu 8, Tampere, 33720, Finland
Email: pauli.losoi@tuni.fi

Scale-up of microbial fermentations from laboratory to production-scale is hampered by mass transfer limitations, reactor heterogeneity, and their biochemical consequences such as harmful and potentially inhibitory side-product formation. Two approaches are readily available: either mass transfer limitations and reactor heterogeneity need to be diminished or the tolerance of the production host against perturbations needs to be increased. A form of the latter is to utilize a co-culture of strains, which can for example recover some of the potentially inhibitory side-products back into the desired products. We have explored the potential of co-cultures in a large-scale reactor by simulating biomass productivity with a previously published compartmental model of a 30m3 reactor and a population balance framework coupled to a metabolic model. The performance at large scale was compared with small scale by simulating also an ideal, homogeneous reactor. Based on the simulations a synergistic or mutualistic co-culture may outperform respective monocultures in terms of productivity, titer and yield in both small and large scale. However, the stability of a co-culture is a prerequisite to realize potential benefits. We have therefore constructed a co-culture using mutually dependent strains, of which neither can outgrow the other. Our modeling and experiments show that such an obligatorily mutualistic co-culture is stable even in longer term cultivations.

1. Losoi P., Santala V. and Santala S. (2019) Enhanced population control in a synthetic bacterial consortium by interconnected carbon cross-feeding. ACS Synth. Biol. 8: 2642–2650.
2. Santala S., Karp M. and Santala V. (2014) Rationally engineered synthetic coculture for improved biomass and product formation. PLoS ONE. 9: e113786.

12:25 Dynamic Genome-scale modeling of Streptomyces clavuligerus metabolism for studying its metabolic performance under different shear stress conditions (L03)

David Gómez-Ríos[1], Howard Ramírez-Malule[2], Peter Neubauer[3], Stefan Junne[3], Silvia Ochoa[1], Rigoberto Ríos-Estepa[4]

[1]Grupo de Investigación en Simulación, Diseño, Control y Optimización de Procesos (SIDCOP), Departamento de Ingeniería Química, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín 050010, Colombia.
[2]Escuela de Ingeniería Química, Universidad del Valle, A.A. 25360, Cali 76001, Colombia.
[3]Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Ackerstr. 76, ACK 24, D-13355 Berlin, Germany.
[4]Escuela de Biociencias, Universidad Nacional de Colombia sede Medellín, Calle 59 A 63-20, Medellín 050010, Colombia.

Streptomyces clavuligerus (S. clavuligerus) is a Gram-positive filamentous bacterium producer of ß-lactamase inhibitor clavulanic acid (CA), which is widely used in the treatment of resistant infections. Recent studies have shown S. clavuligerus responds differently to cultivation conditions, affecting the synthesis of CA production [1]. Cultivations under high and low shear stress conditions in stirred tank and 2-D rocking motion bioreactors have shown that shear stress conditions may change the oxygen uptake and the carbon flux distribution along the metabolic network as well as its morphological phenotype . Antibiotics biosynthesis and secretion in the Streptomyces genus is closely related with nutritional and environmental perturbations. The use of stoichiometric representations of the metabolism has been widely used for in silico studies of carbon flux distribution in organisms and optimization of culture and genetic conditions aimed to increase the production rate of specialized metabolites [2]. Nevertheless, the pseudo-steady state assumption behind the stoichiometric models does not capture the dynamic nature of the bioprocesses carried out under batch or fed-batch operations. In this work, the integration of a dynamic flux balance analysis approach (DFBA) with a validated genome scale metabolic network (GSMN) of S. clavuligerus (iDG1237) [3] was used for the description of intracellular metabolic fluxes of S. clavuligerus, cultivated under high and low shear conditions in fed-batch operation mode. The application of the DFBA framework provided insights about the role of central and amino acids metabolism in CA biosynthesis, the changes arisen in the carbon flux distribution under the different shear stress conditions and its relationship with CA biosynthesis.

DFBA simulations suggested that in cultivations with high growth rates at high shear stress, the substrate starvation causes a sudden drop in the reaction fluxes of glycolysis, TCA and urea cycles; which led to a decrease in the flux toward the CA pathway, at batch stage. However, the feeding condition (from 37 h onwards), restored the metabolic activity boosting the urea cycle in arginine direction, causing a favorable peak in the fluxes of the central metabolism and CA biosynthesis. In contrast, at low shear stress, DFBA showed no drop in the metabolic fluxes since lower growth rates and substrate uptakes were attained leading to a more balanced metabolism. Although lower CA synthesis rates were observed under this condition, the performance of the metabolic fluxes indicated that low shear stress conditions in 2-D rocking motion reactors avoid sudden changes in the intracellular metabolism, which is desired in cultivation of sensitive cells.

1. Gómez-Ríos, D., Junne, S., Neubauer, P., Ochoa, S., Ríos-Estepa, R. and Ramírez-Malule, H. (2019) Characterization of the metabolic response of Streptomyces clavuligerus to shear stress in stirred tanks and single-use 2D rocking motion bioreactors for clavulanic acid production. Antibiotics. 8: 168.
2. Orth, J.D., Thiele, I. and Palsson, B.O. (2010) What is flux balance analysis? Nat. Biotechnol. 28: 245–248.
3. Gómez-Ríos, D., López-Agudelo, V.A., Ramírez-Malule, H., Neubauer, P., Junne, S., Ochoa, S. and Ríos-Estepa, R. (2020) A genome-scale insight into the effect of shear stress during the fed-batch production of clavulanic acid by Streptomyces clavuligerus. Microorganisms. 8: 1–19.

12:45 Break, poster session & exhibition

Session 1B: PROCESS ANALYTICAL TECHNOLOGIES (PAT)

Chair Theresa Menzel, TU Berlin, Germany

11:35 Keynote Talk: Interfacing single cell technologies for stabilizing microbial cell population in continuous cultivation (L04)

Frank Delvigne

University de Liège, Belgium, Email: F.Delvigne@uliege.be

Predicting the fate of a microbial population (i.e., growth, gene expression...) remains a challenge, especially when this population is exposed to very dynamic environmental conditions, such as those encountered during continuous cultivation. Indeed, microbial populations exhibit inherent diversification process leading to heterogenous population of cells. Here, we describe an approach for controlling such diversification process in the context of continuous culture of E. coli when cells are facing a choice between the utilization of glucose or arabinose. We observed that, under typical chemostat conditions when glucose and arabinose are fed simultaneously, a bet-hedging strategy is favored with the simultaneous occurrence of subpopulations of cells able specialized either in the utilization of arabinose or glucose. However, when arabinose and glucose are pulsed at a given frequency, bet-hedging breaks down and a responsive switching mechanism is favored. This frequency has been automatically adjusted based on a technology we developed for this purpose i.e., the segregostat. Besides the fundamental aspects developed in this, there are a lot potential applications useful for microbiologists and biotechnologists, e.g., such as running more efficiently continuous microbial cultivations. Additionally, these results suggest that constraining individual cells into a given phenotypic trajectory is maybe not the best strategy for directing cell population. Instead, allowing individual cells switching around a predefined threshold seems to be a robust strategy leading to oscillating, but predictable, cell population behavior.

12:05 Continuous E. coli bioprocessing: Monitoring of subpopulations and how to deal with them (L05)

Julian Kopp

Institute of Chemical, Environmental and Biological, EngineeringTU Wien
Email: julian.kopp@tuwien.ac.at

Recombinant protein production with E. coli is commonly carried out in fed-batch cultivations as high product titers can be achieved in short cultivation times. To further boost the space-time yield, industry leans towards long-term cultivations, trying to achieve stable productivity at constant CQAs (=critical quality attributes).

The main challenge of long term microbial chemostat cultivations, however, seems to be a fluctuating productivity. Even though the exact cause of the fluctuating productivity remains in the dark, population heterogeneities are believed to initiate process deviations in industrial biotechnology. Trying to generate a PAT-tool to shed more light on this phenomenon, led us to the employment of an online flow cytometry device. Analysing the production of the model protein GFP (=green fluorescent protein), provided the possibility to monitor population heterogeneities within microbial chemostat cultivations.

Results indicated that we have to deal with a highly heterogeneous system throughout chemostat processing. Trying to avoid the occurrence of non-productive subpopulations, we implemented a continuous cascaded system consisting of two sequentially operated continuous reactors. This allows spatial separation of biomass growth and recombinant protein production. By optimizing this continuous cascaded cultivation mode, we were able to achieve stable productivity for 250 hours of cultivation time.

12:25 What is beyond the average – a step toward quantifying the specific reactivity of single cells? (L06)

Martin Schirmer[1], Andreas Schmid[1] Konstantin Wink[2] Detlev Belder[2] and Christian Dusny[1]

[1]Department Solar Materials, Helmholtz Centre for Environmental Research GmbH – UFZ, Leipzig, Germany
[2]Institute of Analytical Chemistry, University Leipzig, Germany
Email: Martin.schirmer@ufz.de

It has been shown that cellular heterogeneity can significantly impact the efficiency/yield of industrial bioprocesses, implying that the idea of cell population homogeneity is outdated [1] To make biotechnological processes more efficient, it is necessary to study and understand the image of the "single cell" in such processes to estimate its influence on population heterogeneity.

One of the major challenges of single-cell analysis is the very small amount of catalytic products and tiny sample volumes. As a consequence, the focus of single-cell research is on analytical methods with low detection limits, such as fluorescence microscopy. However, product quantification based on fluorescence is limited in the range of compounds that can be detected.

We here present a microfluidic approach to study single-cell reactivities. We demonstrate how droplet microfluidics can be used to incubate thousands of single-cell batch reactors in parallel and analyze the batch supernatants one by one via mass spectrometry coupled to microfluidics to obtain an overall picture of population heterogeneity in terms of single-cell reactivity [2].

1. Xiao, Y., Bowen, C.H., Liu, D. and Zhang, F. (2016) Exploiting nongenetic cell-to-cell variation for enhanced biosynthesis. Nat Chem Biol. 12: 339–344.
2. Schirmer, M., Wink, K., Ohla, S., Belder, D., Schmid, A. and Dusny, C. (2020) Conversion efficiencies of a few living microbial cells detected at high throughput by droplet-based ESI-MS. Anal Chem. 92:10700-10708.

12:45 Break, poster session & exhibition

Session 2A: INDUSTRIAL SCALE BIOPROCESSING AND SCALE DOWN

Chair Fabian Schröder, TU Berlin, Germany

14:30 Keynote talk: New experimental methods for detecting heterogeneous flow structures in bioreactors pave the way for a more reliable scale-down and scale-up (L07)

Michael Schlüter, Jürgen Fitschen, Sebastian Hofmann, Marko Hoffmann, Alexandra von Kameke

Institute of Multiphase Flows, Hamburg University of Technology, Eißendorfer Str. 38, 21073 Hamburg
Email: michael.schlueter@tuhh.de

Aerated stirred tank reactors are frequently used for mixing as well as heat and mass transfer processes in chemical and biochemical process engineering. Especially in cell culture processes, the optimal cell growth and product quality depends not only on the level of substrate, pH, oxygen and carbon dioxide concentration but as well on its homogeneous distribution. Furthermore, the influence of shear stress is still controversially discussed. But not only the spatial distribution of such parameters within a reactor is of importance but additionally the residence time of cells within different regions and their trajectory throughout such compartments. This has been already addressed with Computational Fluid Dynamics by Lapin et al. [1] and Kuschel et al. [2] with the concept of lifelines. A closer view on such lifelines reveals, that the spatial and temporal distribution of mixing structures in bioreactors is a crucial parameter for a reliable scale-up and scale-down because the large-scale flow structures are depending strongly on the geometry and overall dimension of the reactor such as stirrers, baffles, etc. and aeration [3]. To characterize and model such three-dimensional plus temporal (4D) distributions of flow structures, new experimental methods are needed, that allow more precise numerical simulation which right now still fails to predict bubble size distribution, rheology and surface tension.

Such new methods have been developed during the last years and its application to bioreactors will be presented in this keynote lecture. With a novel experimental evaluation method [4], it is possible to determine the local mixing time distribution in transparent bioreactors from laboratory scale up to a 12000L production scale. With the 4D Particle Tracking Velocimetry using the "Shake-the-box" algorithm, cell trajectories can be measured to track the lifeline of certain cells through the different compartments. With sophisticated mathematical methods like Lagrangian analysis, much deeper insights into the "life" of a cell within an aerated bioreactor becomes possible [5]. These new methods are promising enabling technologies that should pave the way to a much more reliable scale-up and scale-down in future process engineering.

1. Lapin, A., Klann, M. and Reuss, M. (2010) Multi-scale spatio-temporal modeling: lifelines of microorganisms in bioreactors and tracking molecules in cells. Adv Biochem Eng Biotechnol. 121: 23-43.
2. Kuschel, M. and Takors, R. (2020) Simulated oxygen and glucose gradients as a prerequisite for predicting industrial scale performance a priori. In: Biotechnol. Bioeng., Biotechnol. Bioeng.
3. Rosseburg, A., Fitschen, J., Wutz, J., Wucherpfennig, T. and Schlüter, M. (2018). Hydrodynamic inhomogeneities in large scale stirred tanks – Influence on mixing time. Chemical Engineering Science. 188, 208-220.
4. Fitschen, J., Hofmann, S., von Kameke, A., Hoffmann, M., Wutz, J., Wucherpfennig, T. and Schlüter, M. (2021) Novel evaluation method to determine the local mixing time distribution in stirred tank reactors. Journal Chemical Engineering Science X, Manuscript Number: CESX-D-20-00005R1 (under review).
5. Llamas, C.G., Spille, C., Kastens, S., Paz, D.G., Schlüter, M. and von Kameke, A. (2020). Potential of Lagrangian analysis methods in the study of chemical reactors. Chemie Ingenieur Technik. 95: 540-553.
6. Padberg-Gehle, K. and Schneide, C. (2017) Network-based study of Lagrangian transport and mixing. Nonlin. Processes Geophys., 24: 661–671.

15:00 CFD-based dynamic compartment modelling for the time-series prediction of gradients in industrial-scale aerobic fed-batch fermentation processes (L08)

Gisela Nadal-Rey[1,2], Dale D. McClure[3], John M. Kavanagh[3], Benny Cassells[2], Sjef Cornelissen[2,4], David F. Fletcher[3], Krist V. Gernaey[1]

[1]Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, Building 228A, 2800 Kgs. Lyngby, Denmark
[2]Novozymes A/S, Krogshoejvej 36, 2880 Bagsvaerd, Denmark
[3]The University of Sydney, School of Chemical and Biomolecular Engineering, Building J01, Camperdown, NSW, Australia 2006
[4]Current address: Centrient Pharmaceuticals, Alexander Fleminglaan 1, 2613 AX Delft, The Netherlands
Email: gsnr@novozymes.com

In industrial-scale fermentation processes, the potential formation of gradients can reduce process performance and productivity due to suboptimal cell performance [1,2] Modelling of gradients is a valuable approach for understanding their causes and consequences, as it provides a detailed description of the local fermentation environment. To this end, computational fluid dynamics (CFD) [3,4] and/or compartment models (CMs) [5] are typically combined with microbial kinetic models. Nevertheless, both CFD and CM approaches have the limitation that volume addition cannot be considered and, consequently, only batch or snapshots of fed-batch fermentation processes can be investigated.

In this contribution, a novel strategy to develop and solve dynamic compartment models that describe the fluid dynamics of industrial bioreactors and consider volume addition and local differences in oxygen transfer is presented. This methodology is applied to an industrial-scale (40 to 90 m3) aerobic fed-batch fermentation process of Saccharomyces cerevisiae operated with a stirred tank with different feeding positions. For the first time, this modelling approach allows the fast spatio-temporal characterization of all process variables (e.g., glucose and DO concentrations) and of the metabolic regimes that the cells undergo (e.g., glucose starvation and oxygen limitation) over the course of the entire fermentation process. The proposed strategy is flexible in the sense that it can be immediately generalized to any type of bioreactor, microorganism and fermentation process.

1. Nadal-Rey, G., McClure, D.D., Kavanagh, J.M., Cornelissen, S., Fletcher, D.F. and Gernaey, K. V. (2021) Understanding gradients in industrial bioreactors. Biotechnol. Adv. 46: 107660.
2. Bylund, F., Collet, E., Enfors, S.O. and Larsson, G. (1998) Substrate gradient formation in the large-scale bioreactor lowers cell yield and increases by-product formation. Bioprocess Eng. 18: 171-180.
3. Haringa, C., Tang, W., Wang, G., 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.
4. Spann, R., Glibstrup, J., Pellicer Alborch, K., et al. (2018) CFD predicted pH gradients in lactic acid bacteria cultivations. Biotechnol Bioeng. 116: 769–780.
5. Pigou, M. and Morchain, J. (2015) Investigating the interactions between physical and biological heterogeneities in bioreactors using compartment, population balance and metabolic models. Chem. Eng. Sci. 126: 267–282.

15:20 Heat balance and CFD coupling strategy for the scale-up of an innovative bioleaching process (L09)

Céline Loubiere[1], Eric Olmos[2], Yannick Menard[1], Anne-Gwenaëlle Guezennec[1]

[1]Bureau de Recherches Géologiques et Minières, BRGM, 45100 Orléans, France
[2]Laboratoire Réactions et Génie des Procédés, Université de Lorraine, CNRS, LRGP, 54000 Nancy, France
Email: c.loubiere@brgm.fr

The global metal demand is steadily growing for both industrial and domestic applications [1]. Combined to high-grade deposits exhaustion, it motivates the exploitation of low-grade ores. Within this context, bioleaching represents an alternative when traditional processes are not economically viable.

An innovative bioleaching concept was recently developed, using floating agitators to inject gases and to mix solids in cheap ponds instead of costly tanks [2,3]. This system was validated at pilot scale (2 m3 reactor with mini-floating agitator 1:4-scale at a culture temperature of 40°C using a moderate thermophilic consortium) [4].

A scale-up strategy has been set up to propose a process design and operating conditions for a demonstration pilot in Sotkamo (Finland), where the temperature regulation is achieved without any heat exchanger. To this end, a dynamic heat balance model was developed and validated on current continuous stirred tank plants. In the case of bioleaching application, the temperature requires to be regulated due to very exothermic biological reactions ($\Delta rH0$ = -12.9 MJ kg-1 for pyrite oxidation). It was demonstrated that for treatment of low sulphides grades ores or tailings, a process without heat exchanger was theoretically possible by controlling the fresh pulp inlet conditions (flow rate, temperature) and the aeration (flow rate, O2 partial pressure). In parallel, Computational Fluid Dynamics was used to characterize the hydrodynamics and to define the volume of influence around one agitator with periodic conditions (single-phase, multiphase conditions).

The coupling of these two models would allow then to propose a design for a demonstration pilot and opportunities for scenarios comparison and optimization.

1. Johnson, B. (2018) The evolution, current status, and tuture prospects of using biotechnologies in the mineral extraction and metal recovery sectors. Minerals, 8: 343.
2. Guezennec, A.-G., et al.: Bioleaching method and facility, 2017. Patent US20170175223A1.
3. Guezennec, A.-G., Delclaud, F., Savreux, F., Jacob, J. and d'Hugues, P. (2014) The use of bioleaching methods for the recovery of metals contained in sulfidic mining wastes. Hydrometallurgy 2014, Victoria, Canada. 7 p. (hal-00988746).
4. Guezennec, A.-G., Joulian, C., Archane, A., Ibarra, D., de Buyer R., Bodénan, F. and d'Hugues, P. Influence of dissolved oxygen on the bioleaching efficiency under oxygen enriched atmosphere. Minerals Engineering. 106: 64-70.

15:40 CFD and mass transfer in industrial gas-lift reactors for syngas fermentation (L10)

Lars Puiman[1], Henk J. Noorman[1,2], Cristian Picioreanu[1]

[1]Department of Biotechnology, Delft University of Technology, van der Maasweg 9, 2629 HZ, Delft, The Netherlands.
[2]DSM Biotechnology Centre, Alexander Fleminglaan 1, 2613 AX, Delft, the Netherlands.
Email: l.puiman@tudelft.nl

The microbial fermentation of synthesis gas (CO, CO2, H2 mixture) is a promising process for the production of valuable chemicals. Its success has been demonstrated by the LanzaTech company for the production of ethanol from steel mill off-gasses [1]. A main limitation in syngas fermentation is the low solubility of the syngas constituents, leading to low mass transfer rates in lab [2]. However, in industrial reactors the transport characteristics can be completely different from those determined in the lab scale reactors. Because external-loop gas-lift reactors is being used by LanzaTech, we took this reactor as a reference case for evaluating mass transfer characteristics in industrial syngas fermentation.

A three-dimensional computational model has been developed in Ansys FLUENT to determine the gas and liquid flow patterns in the gas-lift reactor, using an Euler-Euler two-phase approach. Gas-liquid mass transfer, microbial CO uptake and the production of CO2 were implemented and related with the maximum uptake rate. Model results were compared with the reported and expected performance of the LanzaTech process. It was found that increasing the biomass concentration would only poorly influence the CO uptake rate, as this decreases the dissolved CO concentration and thus counteracting the rate intensification given by more biomass. However, decreasing the bubble size leads to a significant improvement in the CO conversion within the reactor.

Further steps to be taken to increase the microbial CO uptake rate would be by increasing the gas and liquid residence time in the reactor. Such methods will increase the conversion and the uptake rate of waste gasses and could potentially advance the industrial production of valuable and sustainable chemicals.

1. Teixeira, L.V., Moutinho, L.F. and Romão-Dumaresq, A.S. (2018) Gas fermentation of C1 feedstocks: commercialization status and future prospects. Biofuels, Bioproducts and Biorefining, 12: 1103–1117.
2. Yasin, M., Jang, N., Lee, M., Kang, H., Aslam, M., Bazmi, A.A., and Chang, I.S. (2019). Bioreactors, gas delivery systems and supporting technologies for microbial synthesis gas conversion process. Bioresource Technology Reports. 7: 100207.

16:00 Break, exhibition & virtual Get-together

17:00 Poster discussions P1-4, P5-8

18:00 Poster discussions 9-12, P13-16

Session 2B: PROCESS ANALYTICAL TECHNOLOGIES (PAT)

Chair Marion Longis, TU Berlin, Germany

14:30 Keynote talk: PAT for the assessment of population heterogeneity in scale up and down (L11)

Stefan Junne

Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
Email: stefan.junne@tu-berlin.de

Monitoring tools for the liquid phase of bioprocesses are often restricted to a very few parameters. This might be insufficient, if data from the single-cell level is required. While several off line tools have been commercialized, not many were adjusted and applied in line in bioprocess development and control.

Nevertheless, there has been great progress in recent years with regard to single cell based methods for monitoring across scales. This has also shown the relevance for scale up and scale down to consider population heterogeneity. Often, already a relation exists between morphology and the viability and vitality of an organism so that a lot of relevant data can be already gained by photo-optical monitoring [1]. The impact of stress due to gradient formation, as it appears in large scale production, on the macromorphological level is seen in many microbial processes [2].

Intracellular product accumulation can be assessed with a similar approach, such as lipid production in algae and yeast. Single-cell monitoring in co-cultures is evident to enable a balancing of the population. Together with fluorescence-assisted methods, quite some tools are nowadays available that allow for the consideration of population distributions with a certain degree of automation to achieve statistical evidence.

The talk aims to provide examples from several areas of microbial bioprocesses, where population heterogeneity related to scaling effects is investigated with suitable monitoring tools in the process, either with or without additional sample preparation [2]. The resulting data can be used to understand the effect of scaling on the process performance and to validate population balance models that aim to consider these effects on the population for process simulation.

1. Marbà-Ardébol, A.M., Emmerich, J., Muthig, M., Neubauer, P. and Junne, S., (2018) Real-time monitoring of the budding index in Saccharomyces cerevisiae batch cultivations with in situ microscopy. Microb. cell fact., 17: 73.
2. Lemoine, A., Delvigne, F., Bockisch, A., Neubauer, P. and Junne, S. (2017) Tools for the determination of population heterogeneity caused by inhomogeneous cultivation conditions. J. Biotechnol. 2017, 251: 84–93.

15:00 In-line application of photon density wave spectroscopy as a PAT sensor in high cell-density bioprocesses: Monitoring of E. coli growth and PHA formation in R. eutropha (L12)

Björn Gutschmann[1], Thomas Schiewe[1,2,3], Marvin Münzberg[2], Peter Neubauer[1], Roland Hass[3], Sebastian L. Riedel[1]

[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]innoFSPEC, University of Potsdam, Am Mühlenberg 3, 14476 Potsdam, Germany
[3]PDW Analytics GmbH, Geiselbergstr. 4, 14476 Potsdam, Germany
Email: riedel@tu-berlin.de

A cost-efficient production process often involves high-cell-density cultivations, which represent challenging surroundings for optical process analytical technologies, due to signal saturation effects and probe fouling. An accurate process monitoring, and control strategy is a key concern for industrial bioprocesses. Addressing this issue, the integration of photon density wave (PDW) spectroscopy into highly turbid multiphase systems enables a novel approach for optical process monitoring. It allows for independent quantification of absorption and scattering properties by measuring the optical coefficients μa and μs' while being suitable for highest particle concentrations (i.e. >40 vol%) in stirred or flowing systems [1, 2].

This contribution shows the application of a fully autoclavable in-line PDW spectroscopy probe during high-cell-density fed-batch cultivations. Results from Escherichia coli cultivations at 3.7-L scale show excellent correlations between the reduced scattering coefficient μs' and biomass concentrations up to 76 g L-1. The results will be discussed in comparison to multiple established analytical methods.

Additionally, for the first time polyhydroxyalkanoate (PHA) biopolymers formation and growth during cultivations of Ralstonia eutropha, was monitored in-line, simultaneously by separation of absorption and scattering properties using the PDW technology. Results of high-cell-density fed-batch cultivations at 6.7-L scale with rapeseed oil [3] and the adaption to waste animal fats will be presented.

1. Bressel, L., Hass, R. and Reich, O. (2013) Particle sizing in highly turbid dispersions by Photon Density Wave spectroscopy. JQRST. 126: 122-129.
2. Hass, R., Munzke, D., Ruiz, S.V., Tippmann, J. and Reich, O. (2015) Optical monitoring of chemical processes in turbid biogenic liquid dispersions by Photon Density Wave spectroscopy. Anal Bioanal Chem. 407: 2791-802.
3. Gutschmann, B., Schiewe, T., Weiske, M.T., Neubauer, P., Hass, R. and Riedel, S.L. (2019) In-Line monitoring of polyhydroxyalkanoate (PHA) production during high-cell-density plant oil cultivations using Photon Density Wave Spectroscopy. Bioengineering. 6: 85.

15:20 Design and development of electrochemical sensors for bioprocess monitoring (L13)

Aliyeh Hasanzadeh[1], Helena Junicke[1], Daria Semenova[1], Maria Dimaki[2], Mogens Kilstrup[2], Krist V. Gernaey[1]

[1]Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, Lyngby, Denmark
[2] Department of Biotechnology and Biomedicine, Technical University of Denmark, Lyngby, Denmark
Email: alhas@kt.dtu.dk

The Process Analytical Technology (PAT) guidance advocates that industrial bioprocesses should be monitored and controlled to enable consistent product quality by validated processes. High quality products and efficient processes must be obtained on the basis of a deep understanding and knowledge of the whole process, which is significantly enhanced by availability of a suitable sensor system. At many production sites nowadays, simple real-time monitoring systems are already implemented [1]. These systems rely on measurements providing a limited number of variables such as pH, temperature, dissolved oxygen concentration, and carbon dioxide evolution rate (CER). Contrary to that, information concerning biologically important variables such as the concentration of different nutrients, me-

tabolites, and biomass is mainly available via laborious at-line or off-line analyses [2]. In this context, the development of novel (bio) sensors is pursued to provide a more detailed insight into bioprocesses. The combination of different sensors with knowledge-based process models opens up towards holistic and intelligent bioprocess monitoring and process control [3].

This work focuses on the development and automation of electrochemical sensors for on-line monitoring of compounds like ammonium, lactate, and glucose. A robust and reproducible approach for sensor fabrication was developed. The sensor requirements for industrial diagnostic systems will be discussed. In the novel concept, microfabricated electrochemical sensors allow for an improved spatial surveillance of production units with sensors placed at several critical locations, and even with the option to incorporate the electrochemical sensors into free-floating sensor particles for data collection in bioreactors.

1. Vojinović, V., Cabral, J.M.S. and Fonseca, L.P. (2006) Real-time bioprocess monitoring: Part I: In situ sensors. Sens Actuators B Chem. 114: 1083-1091.
2. Lopez, P.C., Feldman, H., Mauricio-Iglesias, M., Junicke, H., Kjøbsted Huusom, J. and Gernaey, K.V. (2019) Benchmarking real-time monitoring strategies for ethanol production from lignocellulosic biomass. Biomass Bioenergy. 127: 105296.
3. Semenova, D., Fernandes, A.C., Bolivar, J.M., et al. (2020) Model-based analysis of biocatalytic processes and performance of micro-bioreactors with integrated optical sensors. New Biotechnol. 56: 27-37.

15:40 Control and optimization of polyhydroxyalkanoates production at pilot plant scale in real-time (L14)

Silvia Ochoa[1], Cesar Garcia[1], Wilman Alcaraz[2]
[1]SIDCOP Reseearch group, University of Antioquia, Colombia
[2]Biotransformación Research group, University of Antioquia, Colombia
Email: silvia.ochoa@udea.edu.co

Real-time optimization is implemented in this work at pilot plant scale for the fed-batch production of Polyhydroxyalkanoates from a vinasses-molasses mixture. Soft-sensor predictions for substrate concentration are used at the core of the real-time implementation, which use information about the on-line measurement of CO2 at the exhaust gas. Such information is then used by a model-based online estimator of the polymer concentration. Two optimization based-control strategies were developed and implemented online in order to calculate the optimal feed flow rate, required for optimizing the process operation. Results have shown that considering different objectives at different process stages on the optimization-based control strategy leads to an improved process operation, performing much better than a simple feedback control strategy. Finally, the implemented on-line optimization-based strategies, which updated the feeding policy during process operation, have shown to be a suitable alternative for being implemented when pursuing optimal operation in bioprocesses application.

16:00 Break, exhibition & virtual Get-together

17:00 Poster discussions P1-4, P5-8

18:00 Poster discussions P9-12, P13-16

TUESDAY, 30 MARCH 2021

Opening

10:00 Welcome address and introduction

Peter Neubauer, TU Berlin, Germany

Plenary Talk

10:05 Plenary talk: Systematic bioprocess development in advanced microtiter plate and shake flask culture systems with online monitoring and feeding optionsperformance (PL02)

Jochen Büchs
AVT - Chair of Biochemical Engineering, RWTH Aachen University, Germany
Email: Jochen.Buechs@avt.rwth-aachen.de

The development of bioprocesses usually starts on agar plates and continues in microtiter plates and in shake flasks. These last two types of bioreactors are used for primary and secondary screening and for medium (ingredients) optimization. Final strain selection and evaluation and process optimization require more elaborated experiments, for which small numbers of bench scale stirred tank bioreactors are usually applied. This traditional bioprocess development scheme has several crucial disadvantages. 1) No information about the kinetic properties of the cultured microorganisms is collected during work in micro titre plates and shake flasks, as options for online monitoring are scarce. Very frequently, only endpoint determinations of product concentration are performed. Based on this scant information, strains are selected and transferred to the next steps of bioprocess development. 2) Micro titre plates and shake flasks are operated in batch mode, whereas production processes are most often conducted in fed-batch mode. This results in completely different physiological conditions for the cultured microbes. Strains selected in batch screening programs might not be the most suited for fed-batch production. 3) In micro titre plates and shake flasks the pH has to be controlled by pH buffers. This results in elevated levels of osmotic stress. In stirred tank bioreactors the pH-value is usually controlled by titration, which results, again, in a different culture environment.

In this talk, alternative concepts for improved bioprocess development will be presented, which minimize the disadvantages of the

traditional methodology. The new concept is based on different dedicated small scale culture technologies with e.g. 48, 96 or 384 parallel bioreactors. These offer a lot of online monitoring options, avoid to a large extent the necessity of sampling and offline analysis. Process development is accelerated, as a maximum of stoichiometric and kinetic information about the cultured microbial system is already collected in early steps of process development at much higher throughput than according to the traditional procedure. In addition, also in micro titre plates and shake flasks fed-batch operating mode as well as pH control by supplementation of pH controlling agents is enabled. It has been proven that bioprocesses can readily be transferred from these new small scale culture techniques to bench scale and larger stirred tank reactors, as long as the fundamental engineering parameters are kept constant during scale-up.

10:50 Break & exhibition

Session 3A: INDUSTRIAL-SCALE BIOPROCESSING AND SCALE DOWN

Chair Joana Carvalho Pereira, TU Berlin, Germany

11:20 Keynote talk: Exploiting Scale-down Tests for Engineering a Robust E. coli Host as a Platform for Industrial Production Processes (L15)

Martin Ziegler, Julia Zieringer, Ralf Takors

Institute of Biochemical Engineering, University of Stuttgart, 70569 Stuttgart, Germany
takors@ibvt.uni-stuttgart.de

Successful metabolic engineering aims to create microbial hosts that produce the targeted compound under industrial production conditions. Often enough, large scale bioreactors suffer from non sufficient mixing creating micro-environmental heterogeneities. Fluctuating cells experience the resulting gradients (e.g. of substrates) as repeated stimulations, triggering metabolic and transcriptional responses. As a consequence, the key performance criteria TRY (titer, rates, yields) may deteriorate.

In order to investigate the large-scale performance of novel producers, scale-down experiments should be applied to unravel cellular responses. Thereof, metabolic engineering measures could be deduced for optimizing cells ensuring equal phenotype in large scale as observed in small bioreactors.

A series of scale-down experiments mimicking large scale conditions was performed with E. coli [1]. Thereof, metabolic and transcriptional dynamics were identified allowing the conclusion that the repeated on/off switching of stringent response caused additional ATP needs. Accordingly, E. coli SR, a stringent response knockdown strain, was engineered. Exposure to said large-scale conditions revealed a strongly reduced transcriptional response compared to the wildtype [2]. Besides, further exploitation of the transcriptional dynamics [1] lead to the genome-reduced strain E. coli RM214. Taking the plasmid encoded eGFP production as a proxy for metabolic production capacities, E. coli RM214 revealed superiority by showing 45% higher eGFP production rates than E. coli wildtype when being exposed to large-scale conditions [2].

1.Löffler, M., Simen, J.D., Jäger, G., Schäferhoff, K., Freund, A. and Takors, R. (2016) Engineering E. coli for large-scale production - Strategies considering ATP expenses and transcriptional responses. Metabolic engineering, 38: 73–85.
2. Ziegler, M., Zieringer, J. and Takors R. (2020). Transcriptional pofiling of the stringent response mutant strain E. coli SR reveals enhanced robustness to large-scale conditions. Microbial biotechnology. DOI: 10.1111/1751-7915.13738.

11:50 Investigation of scale dependent factors in industrial human milk oligosaccharide production (L16)

Greta Gecse[1,2], Peter Becker[1], Ted Johanson[1]

[1]DSM, Kogle alle 4, Hørsholm, DK-2970
[2]DTU Bioengineering, Kgs. Lyngby, DK-2800
Email: greta.gecse@dsm.com

Human milk oligosaccharides (HMO's) constitute an important, highly abundant part of mother's milk bringing many health benefits to the neonate. Industrial scale manufacturing of HMO's is currently being performed in large fermentation vessels of 200-400 m3 [1,2]. Perfect mixing on such enormous scales is not feasible leading to gradients of e.g. carbon source, oxygen and pH. Microbial cells traversing such concentration gradients can become negatively affected influencing the stability and productivity of the overall fermentation [3]. As large-scale fermentations are extremely expensive, scale-down reactors are invaluable tools for strain and process development to study cell physiology and behavior under such conditions.

This study aims to characterize and optimize an Escherichia coli strain for the industrial scale production of HMO's. The project involves scale-down fermenter approaches combined with physiological characterization and transcriptomics analysis on selected conditions. The project aims to identify targets for cell factory engineering to make the strains more robust and have increased yields and productivity in industrial settings.

Here, results are presented from the development of scale-down models with a 2'-FL producing E. coli strain including a direct comparison with an industrial scale fermentation in multi-hundred-ton scale. Several scale-down approaches such as plug-flow compartment, pulse feeding, and CO_2-enriched setup were used to study the impact of oxygen- and glucose gradients and inhibition of high dissolved CO_2, respectively. The results suggested that in particular increased dCO_2 concentrations can potentially impair yield and productivity.

1. Ammann, R. (2017) Achieving the impossible: Jennewein Biotechnologie is dedicated to the production of human milk oligosaccharides. Eur. Dairy Mag. 29: 30–31.
2. Bych, K., Mikš, M.H., Johanson, T., Hederos, M.J., Vigsnæs, L.K. and Becker, P. (2019) Production of HMOs using microbial hosts—from cell engineering to large scale production. Current opinion in biotechnology. 56: 130-137.
3. Enfors, S.O., Jahic, M., Rozkov, A., et al. (2001) Physiological responses to mixing in large scale bioreactors. J. Biotechnol. 85: 175–185.

12:10 Secretory protein producing Bacillus subtilis: Withstanding process inhomogeneities expected in a large-scale stirred tank bioreactor (L17)

Marco Oldiges[1], A. Hütterott[1], Valentin Steier[1], Matthias Moch[1], Maria del Carmen Álvarez[2], Wolfgang Wiechert[1]

[1]Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich GmbH, Jülich, Germany
[2]Competence Center for Fermentation, Puratos Group, Rue Bourie 12, Andenne, Belgium
Email: a.huetterott@fz-juelich.de

Transfer of biotechnological production processes from laboratory to industrial scale is often accompanied by performance loss. This is typically caused by mixing deficiencies and resulting gradient formation in large bioreactor vessels. Although this issue is well known, its extent as well as effect on the metabolism of the production host is often poorly understood. As there is currently no efficient and consistent method for a priori predictions concerning cellular responses, scale-up still mainly relies on physical criteria and experience. Enhanced insight would thus offer great potential to reduce time-to-market and increase competitiveness and sustainability of new bio-products.

Scale-down systems, such as compartmented stirred tank bioreactors, can be used to mimic inhomogeneities experienced in industrial production reactors already at laboratory scale. While commonly one reactor represents the homogeneous bulk zone, coupled reactors reflect cultivation conditions in badly mixed disturbance zones of the large-scale system to be mimicked. This setup allows the study of short-term, space-resolved metabolic adaptations of the production host.

A two-compartment stirred tank bioreactor setup, implemented in a parallel cultivation platform, was used to analyze the robustness of a B. subtilis enzyme producer. A low oxygen, high substrate feed zone was partitioned off as a disturbance compartment. Cells were cultivated in a rich complex medium and spent an average of three minutes in the small stirred tank. Strikingly, fluctuations in oxygen and substrate availability were found to not reduce growth and enzyme production. In fact, cell densities and enzyme activities were elevated by an average of 26 % and 68 % in the scale-down setup compared with a one-compartment bioreactor reference process, respectively. This metabolic response stands in stark contrast to previous scale-down investigations presented in literature and illustrates that dynamic process conditions might show beneficial effects for a biotechnological process. In addition, scale-down approaches can reveal previously unknown potential of production systems.

12:30 Break, poster session & exhibition

Session 3B: INDUSTRIAL-SCALE BIOPROCESSING AND SCALE DOWN

Chair Sebastian L. Riedel, TU Berlin, Germany

11:20 Keynote talk: Feedstock potential and valorisation of organic side-streams for value-added products (L18)

Saija Rasi

Natural Resources Institute Finland, Email: saija.rasi@luke.fi

In Finland, the potential amount of different organic waste and side-streams and agricultural biomass (as energy crops and crop residues) is approximately 54 million t/a. From this the amount of municipal biowaste is about 300 000 t/a [1]. This accounts for about 24 TWh (all) and 460 GWh (biowaste) is used for biogas production. Other option would be the volatile fatty acid (VFA) production though anaerobic digestion (AD) process. These renewable intermediates are needed to replace the fossil resources in extensive industrial production of chemicals and fuels. Compared to methane formed during AD of biodegradable biomasses, VFAs have significantly higher added value and more versatile utilization possibilities. In the optional AD-process, 10% of the wet weight and 41% of the organic matter were converted to VFA. The remaining solid fraction after separation of digestate could be used as raw material for biogas processing since approximately 40% of the biodegradable organic matter of the original substrate is still left in the solid residues. A shift from the present fossil economy to a truly biobased economy increases the need of all renewable biomass. Along with the increasing demand, the use of biomass has to be prioritized for food, feed, biomaterials, biochemicals and biofuels. Because the need of biomasses for the production of both energy and chemicals is increasing, the resource-effectiveness of the VFA production is increasingly important to ensure the availability and sustainability of raw materials.

1. Marttinen, S., Luostarinen, S., Winquist, E. andTimonen, K. (2015) Rural biogas - feasibility and role in the Finnish energy system. Research report no 1.1.3-4. BEST project final report. www.bestfinalreport.fi

11:50 Scale up from the cellar – the LX-Process as pretreatment for microbial conversion (L19)

Friedrich Streffer

LXP Group GmpH, Email: friedrich.streffer@lxp-group.com

The LX-Process is a low temperature, low pressure pretreatment process for lignocellulosic material like straw, leaves, wood and also the fibrous residues form a biogas plant, providing a fermentable carbohydrate stream as well as a high purity lignin.

Recently, the LX-Process has been scaled form a cellar in Berlin to the 500t p.a. scale at a biogas plant in Aholfing (Bavaria). The talk will show the hurdles as well the major stepping stones in the journey. A full over view of the LX-Process will be given as well as examples of the integration into anaerobic biogas digestion, bacterial lactic acid fermentation, as well as ethanol fermentation with high solids saccharification and conversion by Ethanol Red™ of Lesaffre, showing the competitiveness of the process with current cellulosic ethanol processes with the C6-sugar content. An estimated 4t of straw per 1t of ethanol are needed when utilizing C5 and C6 sugars from the hydrolysate.

12:10 Upscaling butanol production using mixed microbial cultures (L20)

Tiago Pinto[1], Xavier Flores-Alsina[1], Krist V. Gernaey[1], Helena Junicke

[1]Process and Systems Engineering Center (PROSYS), Department of Chemical and Biochemical Engineering, Technical University of Denmark, Søltofts Plads, Building 228A, 2800 Kgs. Lyngby, Denmark
Email: tiapi@kt.dtu.dk

The transportation sector contributes significantly to global carbon dioxide emissions and accounts for 65% of the world's consumption of liquid fossil fuels [1] stressing the need for alternatives to energy sources like gasoline and diesel that allow for a more sustainable economy. Due to their similarities, butanol can already today be used as direct replacement for gasoline but its production still relies on expensive pure substrates (e.g. glucose), which carries some

challenges such as competition with food production, and low adaptability to variations in substrate composition. Due to their microbial diversity, mixed microbial cultures (MMC) can overcome such limitations and potentially become the predominant bio-based production platform for butanol. Previous studies have shown that butanol formation by anaerobic mixed cultures was feasible using only butyrate and hydrogen (H2) [2] Both substrates are common intermediates during the decomposition of organic residues, as is the case for industrial waste streams, offering a cheap feedstock alternative that avoids competition with food production.

In this work, we investigate the feasibility of butanol formation by anaerobic MMCs. In order to suppress competing side reactions such as anaerobic butyrate conversion and methanogenesis, elevated H2 partial pressures (2 bar) and acidic conditions (pH 5.5) were applied. Anaerobic butyrate conversion was selectively inhibited under these conditions and the butanol concentrations obtained were about three times larger than previously reported by Steinbusch et al. [2], hinting at future applications of MMC for butanol production from waste streams.

1. International Energy Agency: Key world energy statistics, 2017.
2. Steinbusch, K.J., Hamelers, H.V., Buisman, C.J. (2008) Alcohol production through volatile fatty acids reduction with hydrogen as electron donor by mixed cultures. Water Research, 42: 4059–4066.

12:30 Break, poster session & exhibitionn

Session 4A: BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair Matthias Gimpel, TU Berlin, Germany

14:30 Keynote talk: Potential of genome and proteome-reduced strains for protein and plasmid DNA production (L21)

Alvaro R. Lara

Universidad Autónoma Metropolitana-Cuajimalpa
Email: alara@correo.cua.uam.mx

The design of optimal cell factories requires engineering resource allocation for maximizing product synthesis. Genome or proteome minimization could be useful strategies for better the development of better cell factories. Genome minimization eliminates sections of the genome considered non-essential for growth under certain conditions. In turn, proteome minimization focuses on reducing or eliminating the expression or proteins that are non-essential under certain conditions and therefore result in a waste of cellular resources. While both approaches focus on aspects like growth rate and biomass formation, little attention has been paid to the performance of minimized cells as factories. In this contribution, the expression of recombinant protein in genome-reduced strains will be compared to that of its wild type. Furthermore, the production of plasmid DNA (intended for DNA vaccines) using the classical pUC and a novel minimized R1 replicons was also evaluated in proteome-reduced strains. Results of batch cultures in mineral and complex media, as well as small scale fed-batch cultures will be shown. The advantages and potential disadvantages of genome or protein minimization for recombinant protein or plasmid DNA vaccines will be discussed.

15:00 Scale-down of high cell density Fab production in E. coli (L22)

Florian Mayer[1], Monika Cserjan-Puschmann[1], Christian Sam[2], Gerald Striedner[1]

[1]Christian Doppler Laboratory for production of next-level biopharmaceuticals in E. coli, University of Natural Resources and Life Sciences Vienna
[2]Boehringer Ingelheim RCV GmbH & Co. KG

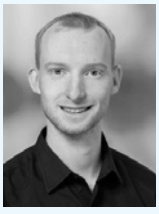
In industry, large cultivation vessels of up to several hundred m3 are used which result in long mixing times. As a consequence, cells are exposed to inhomogeneities, which can have negative effects on process performance, such as lower biomass yields [1]. In this work, genome-integrated E. coli HMS174(DE3) host strains expressing a Fab antibody fragment in the periplasm were cultivated to high cell densities of more than 70 g/L in a 20 L stirred tank bioreactor (STR). To investigate scale effects, the STR was connected to a custom-build plug-flow bioreactor (PFR) and then operated either as two- or one-compartment system. The residence time of the cells in the PFR compartment was adjusted according to the mixing time difference between the 20 L and an industrial scale bioreactor. To generate gradients in 2-stage mode, the feed was directly added at the PFR entrance. In general, we observed a decreased glucose yield coefficient (-12%) in scale down experiments. However, during product formation, cells exposed to gradients showed higher robustness and reduced lysis. In contrast to the reference experiments, cells were able to maintain growth and thus higher specific intracellular product concentrations were obtained. Based on these findings, process development can follow a more rational approach and allows for design of fully scale-able processes.

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

15:20 N-1 perfusion-based IgG productions in stirred single-use bioreactors (L23)

Jan Müller, Dieter Eibl, Regine Eibl

Zurich University of Applied Sciences, Wädenswil,
Email: jan.mueller@zhaw.ch



Monoclonal antibodies (mAb) are an important group of biopharmaceuticals being mainly used for detection and therapy of cancer and autoimmune diseases [1]. Already more than 20 years ago, mAb were produced with mammalian cells in perfusion mode. Meanwhile, increasing product titres in fed-batch processes led to a predominance of this cultivation mode, but in current researches, the focus of process intensification is on perfusion-based processes again [2]. In particular, N-1 perfusions executed in wave-mixed bioreactors and following high-seed fed-batch productions as well as continuous productions are of increasing interest [3, 4].

In this work, the successful establishment of an N-1 perfusion and a high-seed fed-batch process, inoculated with the cells grown in perfusion mode, is shown for benchtop and pilot scale. Two different mAb-producing CHO cell lines were cultivated. The N-1 perfusion (maximum perfusion rate of 2.5 d-1) was conducted in wave-mixed bags with 1 L working volume. Within 11 days, viable cell densities (VCD) exceeded 2° 108 cells mL-1. On days 5 and 9, the cells were used for inoculation of 250 mL stirred single-use bioreactors with different cell densities (up to 10° 106 cells mL-1). Independently of the inoculum cell density, comparable maximal VCD (25–30 x 106 cells mL-1) and mAb titres (≈ 400 mg L-1) were obtained. However, the process time of the IgG production process could be reduced in high-seed mode by up to 50 %. The process was successfully transferred to pilot scale.

1. Walsh, G. (2018) Biopharmaceutical benchmarks 2018, Nature Biotechnology. 36: 1136–1145.
2. Pollock, J., Ho, S.V. and Farid, S.S. (2013) Fed-batch and perfusion culture processes: Economic, environmental, and operational feasibility under uncertainty. Biotechnology and Bioengineering. 110: 206–219.
3. Woodgate, J.M. (2018) Perfusion N-1 culture-opportunities for process intensification. in Biopharmaceutical Processing: Development, Design, and Implementation of Manufacturing Processes. 755-768.
4. Bielser, J.-M., Wolf, M., Souquet, J. Broly, H. and Morbidelli, M. (2018) Perfusion mammalian cell culture for recombinant protein manufacturing – A critical review. Biotechnology Advances. 36: 1328–1340.

15:40 Evaluation of the clavulanic acid production integrating process simulation and systems biology (L24)

Sofía Toro-Vásquez, David Gómez-Ríos, Rigoberto Rios Estepa

Grupo de Investigación en Simulación, Diseño, Control y Optimización de Procesos (SIDCOP), Departamento de Ingeniería Química, Universidad de Antioquia UdeA, Calle 70 No. 52-21, Medellín 050010, Colombia.
Email: sofia.toro@udea.edu.co

Clavulanic acid (CA) is a specialized metabolite produced by Streptomyces clavuligerus in submerged cultivations under nutrients limitation. This compound is widely used in the pharmaceutical industry owing its ability to bond the serine residues in the active site of the ß-lactamases enzymes, creating a stable and non-reacting compound. Thus, the CA is commercialized in combination with other ß-lactam antibiotics as part of the treatment strategy against antibiotic resistance infectious diseases. Unfortunately, the CA production is expensive, mainly due to the low yield of the specialized metabolite, its degradation in aqueous solution and the costly separation and purification strategies [1]. Currently, the studies are focused on improving the productivity and profitability of the process, either optimizing culture media, strain genetics and downstream processing. In the literature, few works have evaluated the industrial application of the experimental studies. In this work, an approach of bioprocess simulation and systems biology was implemented in the evaluation of the process based in the current state-of-the-art in the regional context.

The integration of in silico metabolic studies with simulation were focused in the product yield and plant profitability in Colombia. Considering the reported effect of some amino acids on CA production Flux Balance and Dynamic Flux Balance Analyses were performed using a validated genome scale model (iDG1237) of S. clavuligerus metabolism [2]. The in silico results suggested that Glutamic acid, Arginine, Ornithine, Threonine and Lysine affects positively the biomass and CA concentration in the cultivation under batch operation [3]. A conceptual process was stablished considering the medium composition explored in silico and the unit operations required for downstream processing based on experimental data. The simulation results for the whole production process allowed to identify the critical points affecting the direct cost of fabrication and hence, hampering the plant profitability. The most remarkable points were the CA concentration obtained during the batch cultivation and the required organic to aqueous solvent ratio during liquid extraction for CA recovery from fermentation broths. Those results suggest that CA downstream processing is still an open matter in the CA production and further studies are required for obtaining higher yields during the separation and purification stages.

1. López-Agudelo, V.A., Gómez-Ríos, D. and Ramirez-Malule, H. (2021) Clavulanic acid production by Streptomyces clavuligerus: Insights from systems biology, strain engineering, and downstream processing. Antibiotics. 10: 84. 2. Gómez-Ríos, D., López-Agudelo, V.A., Ramírez-Malule, H., Neubauer, P., Junne, S., Ochoa, S. and Ríos-Estepa, R. (2020) A genome-scale insight into the effect of shear stress during the fed-batch production of clavulanic acid by Streptomyces clavuligerus. Microorganisms. 8: 1–19.
3. Ser, H.-L., Law, J.W.-F., Chaiyakunapruk, N., Jacob, S.A., Palanisamy, U.D., Chan, K.-G., Goh, B.-H. and Lee, L.-H. (2016) Fermentation conditions that affect clavulanic acid production in Streptomyces clavuligerus: A systematic review. Front. Microbiol. 7: 522.

16:30 Break, exhibition & virtual Get-together

17:00 Poster discussions P17-20, P21-24

18:00 Poster discussions P25-P28, P29-32

■ ■ Session 4B: MICROFLUID SYSTEMS (Bernhard von Langenbeck Hall, 1st floor)

Chair Mario Birkholz, IHP-Leibniz

14:30 Key note talk: High-throughput single-cell-resolution microfluidics to accelerate microbial bioproduction bioprocess development (L25)

Arum Han

Dept. Electrical and Computer Engineering & Dept. Biomedical Engineering, Texas A&M University, TX, USA
Email: arum.han@ece.tamu.edu

High-throughput microfluidic systems have the capability to conduct cellular assays and analysis with single-cell resolution at extremely high throughput. Lab-on-a-chip systems, which can integrate multiple functionalities onto a single chip, enable conducting a series of biological assays fully automatic at high throughput. Taken together, these technologies can transform and greatly accelerate the pace of discovery and engineering of cellular systems. Microorganisms are diverse with extremely broad ranges of characteristics, and have the potential to be used as producers of various high-value products through metabolic and genetic engineering. Here I will present several examples of high-throughput microfluidic lab-on-a-chip systems that we have developed for rapid discovery in microbiological systems. The first example is towards developing better algal biofuel,

where I will introduce several microfluidic platforms, including a photobioreactor microarray system that can screen how different light exposure conditions influence growth and oil production, droplet microfluidics technology for ultra-high-throughput screening of engineered algal strains to identify strains with higher growth and oil production capabilities, and dielectrophoresis microsystem-based algal cell separation based on their lipid content. In the second example, I will introduce the development of droplet microfluidics-based microsystems that we are using to identify new antibiotics from environmental microorganisms.

15:00 Separation of microalgae and polystyrene particles by dielectrophoresis (L26)

Danai E. Malti[1], Arohi Barai[2], Maria E. P. Emmerich[1], Lea I. M. Hintze[1], Peter Neubauer[1], Mario Birkholz[2]

[1]Chair of Bioprocess Engineering, Institute of Biotechnology, Technische Universität Berlin, Ackerstr. 76, ACK24, 13355 Berlin, Germany
[2]IHP – Leibniz-Institut für innovative Mikroelektronik, Im Technologiepark 25, 15236 Frankfurt (Oder), Germany
Email: d.malti@tu-berlin.de

Microalgae are investigated due to their high production rates of useful biochemical compounds like polyunsaturated fatty acids (PUFA). The present work demonstrates separations of cells from PUFA-producing microalgae species Crypthecodinium cohnii which is developed in comparison to well defined polystyrene-carboxylate microbeads of different sizes (2, 4.5, and 10µm in diameter). The particle separation is implemented with the use of dielectrophoresis (DEP) which is the locomotion of dielectrically polarized particles in a non-uniform electric field and allows a separation due to different polarizabilities as caused by varying PUFA content [1]. DEP fields are generated in a microfluidic channel of 40µm height by integrated top-bottom electrode structures. This electrode design allows the particle suspension to flow in between, however, with the appliance of an AC electric field the particles are being deflected along the electrode's edges and thus changing their direction of flow.

This study uses polystyrene-carboxylate microbeads of different sizes diluted in water of 13.6 µS/cm conductivity, in order to identify the size separation efficiency of DEP for various frequencies and amplitudes of AC voltages applied. Effective separation of 10µm polystyrene particles from 4.5µm ones is observed at 5 Vpp for a frequency range between 100 kHz up to 10 MHz. The DEP separation of C. cohnii cells, which are 8-20µm in size and have a spherical shape, is studied in M1 cell culture medium with a conductivity of 35.7 µS/cm [2,3]. The microalgae cells were tested in the same microfluidic channel for multiple experimental parameters and the separation performance was identified by quantitative analysis of the microscopic videos.

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

1. Pethig, R. (2017) Review-Where is dielectrophoresis (DEP) going?, Journal of The Electrochemical Society. 164: B3049-B3055.
2. Abt, V., Gringel, F., Han, A., Neubauer, P. and Birkholz, M. (2020) Separation, characterization, and handling of microalgae by dielectrophoresis. Microorganisms. 8: 540.
3. Mendes, A., Reis, A., Vasconcelos, R., Guerra, P. and Lopes da Silva, T. (2009) Crypthecodinium cohnii with emphasis on DHA production: a review. J Appl Phycol. 21: 199–214.

15:20 Dynamic microfluidic single-cell cultivation: Growth of Corynebacterium glutamicum at fluctuating environmental conditions (L27)

Sarah Täuber[1], Luisa Blöbaum[1], and Alexander Grünberger[1]

[1]Multiscale Bioengineering, Technical Faculty, Bielefeld University, Bielefeld, Germany
Email: sarah.taeuber@uni-bielefeld.de

In large-scale bioreactors, different gradients occur, which lead to a fluctuating supply of oxygen, nutrients and other process parameters that strongly influence the growth and production behaviour of the microbial strains and endanger the success of the scale-up [1]. Scale-down experiments are an established tool to investigate the growth and production related effects of industrial-scale gradients on microorganisms using lab-scale fermenters [2]. However, traditional scale-down approaches are bulk measurements and cannot provide the direct answer to how cells are affected by gradients on a single-cell level [3]. Novel analytical methods need therefore to be developed [4].

In this contribution, we introduce a microfluidic single-cell workflow for the cultivation of microbial cells under dynamic environmental conditions [5]. This system allows oscillation between different environmental input parameters e.g., between pH values or carbon sources. We give an overview into the technology and show how oscillating environmental conditions (here C source and pH value) affect the cellular physiology. In a first study, we cultivated C. glutamicum under oscillating medium conditions (medium rich and buffer) with different oscillation frequencies ranging from hours to seconds intervals [5]. A significant difference within the overall growth behaviour was observed at different oscillations frequencies. Oscillations time between 5 and 15 minutes significantly affect the overall growth rate. At higher oscillations frequencies growth was not impaired significantly. In a second study, we investigated the growth behaviour of C. glutamicum under specific pH oscillations that varied in their pH amplitude and frequency [6]. pH oscillations between discrete pH units decreased the overall growth rate. The decrease was dependent on the stress pH values (e.g., pH=5) and the ratio of pH stress phases to regeneration phase at pH=7. Latest results, hypothesis regarding the observed growth pattern and potential application will be shown.

Our results show that the concept of dynamic microfluidic single-cell cultivation has a high potential to investigate cellular physiology at dynamic environmental conditions. This paves the way for an improved understanding of how environmental conditions shape metabolic heterogeneity and thus the cellular response within growth and production upon nutrient and pH gradients within large-scale bioprocesses, often referred as lifeline. In future it is essential to validate the technology with conventional scale-down approaches to further optimize the technology towards novel single-cell scale-down reactors.

1. Takors, R. (2012) Scale-up of microbial processes: impacts, tools and open questions. Journal of biotechnology, 160: 3–9.
2. Delvigne, F. and Noorman, H. (2017) Scale-up/Scale-down of microbial bioprocesses: a modern light on an old issue. Microbial biotechnology, 10: 685–687.
3. Lemoine, A., Maya Martinez-Iturralde, N., Spann, R., Neubauer, P. and Junne, S. (2015) Response of Corynebacterium glutamicum exposed to oscillating cultivation conditions in a two- and a novel three-compartment scale-down bioreactor. Biotechnology and bioengineering, 112: 1220–1231.
4. Grünberger, A., Wiechert, W. and Kohlheyer D. (2014) Single-cell microfluidics: opportunity for bioprocess development. Current opinion in biotechnology, 29: 15-23.

15:40 Reproducing dynamic environment in microfluidic single-cell cultivation based on computational lifeline analysis (L28)

Phuong Ho[1], Sarah Täuber[2], Alexander Grünberger[2], Eric von Lieres[1]

[1]Forschungszentrum Jülich, IBG-1: Biotechnology, Wilhelm-Johnen-Str. 1, 52428 Jülich, Germany
[2]Universität Bielefeld, Multiscale Bioengineering, Universitätsstraße 25, 33615 Bielefeld
Email: p.ho@fz-juelich.de; sarah.taeuber@uni-bielefeld.de

The biotechnological production of valuable substances is typically complicated by the loss of microbial performance upon scale-up [1-3]. This challenge is mainly caused by discrepancies between homogeneous environmental conditions at laboratory scale, where organisms are optimized, and inhomogeneous conditions in large-scale bioreactors, where the production takes place. To improve strain selection and process development, it is thus of major interest to characterize these fluctuating conditions at large scales and investigate their impact on microbial cells.

In this contribution, we will demonstrate the high potential of dynamic microfluidic single-cell cultivation combined with computational fluid dynamics (CFD) simulation of large-scale bioreactors. CFD simulations of a 300 L bioreactor were applied to characterize environmental conditions in large-scale bioreactors. So-called lifelines were determined by simulating multiphase turbulent flow and mass transport combined with particle tracing. Glucose availability experienced by the microorganism Corynebacterium glutamicum was traced. Resulting lifelines were discretized into low, medium and high glucose availability regimes. Discretized lifelines were used as feeding profiles of a dynamic microfluidic single-cell cultivation (dMSCC) system to investigate how the fluctuating glucose concentration affects cellular physiology and colony growth rate.

The presented approach paves the way for an improved understanding of how the cellular lifelines of large-scale bioreactors influence the cellular response within growth and production. It also provides insights into how to understand the conditions in large-scale bioreactors from the view of a microorganism and the dependence of cell wellbeing on the observed conditions.

1. Oosterhuis N.M.G. and Kossen N.W.F. (1984) Dissolved-oxygen concentration profiles in a production-scale bioreactor. Biotechnol. Bioeng. 26: 546-550.
2. Larsson G. and Enfors S.O. (1985) Influence of oxygen starvation on the respiratory capacity of Penicillium chrysogenum. Appl. Microbiol. Biotechnol. 21: 228-233.
3. Enfors S.O., Jahic M., Rozkov A., et al. (2001) Physiological responses to mixing in large scale bioreactors. Journal Biotechnol. 85: 175-185.

16:30 Break, exhibition & virtual Get-together

17:00 Poster discussions P17-20, P21-24

18:00 Poster discussions P25-P28, P29-32

WEDNESDAY, 31 MARCH 2021

Opening

10:00 Welcome address and introduction

Peter Neubauer, TU Berlin, Germany

Plenary Talk

10:05 Plenary talk: Digitalization platform and supervisory control for continuous integrated manufacture of monoclonal antibodies (PL03)

Massimo Morbidelli

Department of Chemistry, Materials and Chemical Engineering Politecnico di Milano, Milano, Italy
Email: massimo.morbidelli@polimi.it

In the last years, there has been an increased interest in continuous and integrated manufacturing of biopharmaceuticals, due to increasing cost and time pressure in industry as well as diversifying pipelines asking for more flexible production concepts. However, we are still far from an efficient implementation of modern process analyzers, a centralized data mining combined with online use of advanced analysis algorithms and the integration of process knowledge into a supervisory control frame.

In this contribution, we present an automated end-to-end integrated platform for the production of a monoclonal antibody. The process consists of a perfusion bioreactor, a continuous protein A capture step, which is followed by low-pH virus inactivation, and frontal and flow-through chromatographic steps for final polishing. Automated at-line HPLC systems equipped with protein A and size exclusion columns give insight into critical process parameters without the necessity of manual sampling. Additionally, the potential of Raman spectroscopy was investigated in USP and a flow cell was developed to enable important real-time information from downstream units. The presented results will show the need for an efficient process data collection and hierarchical control system to handle process perturbations and drifts, facilitating robust product yield and quality. This concept provides a very important basis to intensify the main advantages of continuous integrated manufacturing in agreement with the trends of industry 4.0.

10:50 Break & exhibition

Session 5A: HIGH-THROUGHPUT BIOPROCESSING AND AUTOMATION

Chair Sebastian Hans, TU Berlin, Germany

11:20 Keynote talk: Robot and machine learning assisted protein engineering on the high-throughput screening platform LARA (L29)

Mark Doerr[1], Uwe Bornscheuer[1], Stefan Born[2]

[1]Inst. f. Biochemistry, University Greifswald
[2]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
Email: mark.doerr@uni-greifswald.de

In this talk, we summarise recent developments on our robotic high-throughput protein/enzyme screening platform LARA (lara.uni-greifswald.de) [1]. Examples of recent screening campaigns, like, e.g., transaminases [2], carboxylic acid reductases (CAR) [3], and oxidases, will be shown under the aspect of automated robotics. Special focus will be further laid on advanced, machine learning based, experimental planning and automated data evaluation using the open source software suite LARA (gitlab.com/larasuite). Open source and open lab-automation protocols are a central part of LARA, therefore novel lab automation approaches (SiLA 2) and semantic long-term data storage (AnIML / SciData / RDF) will also be presented.

1. Dörr, M., Fibinger, M.P.C., Last, D., et al. (2016) Fully automatized high-throughput enzyme library screening using a robotic platform. Biotechnol. Bioeng., 113: 1421–1432.
2. Calvelage, S., Dörr, M., Höhne, M. and Bornscheuer, U.T. (2017) A systematic analysis of the substrate scope of (S)- and (R)-selective amine transaminases. Advanced Synthesis & Catalysis, 359: 4235–4243.
3. Schwendenwein, D., Ressmann, A.K., Dörr, M., Höhne, M., Bornscheuer, U.T., Mihovilovic, M.D., Rudroff, F. and Winkler, M. (2019) Random mutagenesis-driven improvement of carboxylate reductase activity using an amino benzamidoxime-mediated high-throughput assay. Advanced Synthesis & Catalysis, 361: 2544–2549.

11:50 Towards accelerated bioprocess development: Using cell-free protein synthesis to screen for promising biocatalysts (L30)

Katrin Rosenthal

Chair for Bioprocess Engineering, TU Dortmund University, Emil-Figge-Str. 66, D-44227 Dortmund
Email: katrin.rosenthal@tu-dortmund.de

Cell-free protein synthesis (CFPS) is a simple and sensitive method to study various proteins in parallel and to rapidly investigate the influence of genetic modifications on the biocatalytic performance of enzymes with regard to activity, stability, substrate and product specificity [1].

In this study, we demonstrated the potential of CFPS for the screening of enzymes from various classes and origins. We synthesized different hydroxylases and azoreductases with CFPS to evaluate the optimal expression temperature and the biocatalytic activity of various homologues. We also used CFPS for a pre-screening to study the expressibility of several genes coding for cGAS (cyclic GMP-AMP synthase) in bacterial systems. The nucleotidyltransferase cGAS naturally occurs in metazoans and catalyzes the formation of cyclic dinucleotides, which can act as second messengers and activates the type I interferon signaling pathway [2]. Synthetic template genes from yet uncharacterized cGAS homologues were used and all tested variants were successfully expressed. They were subsequently characterized using an in vivo expression system to supply higher amounts of the enzymes. Most of the variants catalyzed the synthesis of cyclic dinucleotides, however, exhibited different specific activities.

In conclusion, we demonstrated the potential of CFPS as a tool for rapid synthesis and screening of biocatalysts, which will definitely accelerate biocatalyst development for biotechnological processes.

1. Rolf, J., Rosenthal, K. and Lütz, S. (2019) Application of cell-free protein synthesis for faster biocatalyst development. Catalysts. 9: 190.
2. Ablasser, A., Goldeck, M., Cavlar, T., Deimling, T., Witte, G., Röhl, I., Hopfner, K., Ludwig, J. and Hornung, V. (2013) cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. Nature. 498: 380–384.

12:10 Fed-batch like microtiter cultivations as high-throughput screening tool for E. coli production process development (L31)

Mathias Fink[1], Monika Cserjan[1], Daniela Reinisch[2], Gerald Striedner[1]

[1]Christian Doppler Laboratory for production of next-level biopharmaceuticals in E. coli, University of Natural Resources and Life Sciences Vienna;
[2]Boehringer Ingelheim RCV GmbH & Co KG, Dr. Boehringer-Gasse 5-11, 1121 Vienna Austria
Email: mathias.fink@boku.ac.at

The development of bioprocesses is a cost and time-consuming procedure, which makes it necessary to have strong and feasible tools that can accelerate this step and save costs. These facts bring the microbioreactors into action as they allow high-throughput screenings (HTP) providing controlled environmental conditions and reliable data in the course of the early stage process development 1[1]. Additionally, it is considered that during these experiments fed batch mode should be operated to gain higher reliability and greater utility for the process development [2, 3]. The present work focused on the evaluation of a microbioreactor system as HTP screening platform for recombinant E. coli cultivation/production processes. Lab-scale bioreactor fed-batch cultivations were used as reference and benchmark. We employed a BioLector® system using a fed-batch like process, enabling a carbon limited growth during production phase by enzymatic glucose release from a polysaccharide. As model organisms we used genome integrated E. coli strains HMS174(DE3) and BL21(DE3), producing four different Fabs translocated to the periplasm either by co-(dsbA) or post-translational (ompA) leader sequences. Lab-scale bioreactor fed-batch cultivations were performed in the DASGIP® parallel bioreactor system. Our results showed that trends in growth behavior and Fab expression level rankings observed in the microbioreactor can directly be transferred to bench-top bioreactor cultivations. Concluding, we found that the microbioreactor system is a time-saving, reliable tool perfectly suited for clone- and condition screening in the course of production process development for E. coli expression systems.

1. Lladó Maldonado, S., Panjan, P., Sun, S., Rasch, D., Sesay, A.M., Mayr, T. and Krull, R. (2019) A fully online sensor-equipped, disposable multiphase microbioreactor as a screening platform for biotechnological applications. Biotechnology and Bioengineering. 116: 65-75.
2. Toeroek, C., Cserjan-Puschmann, M., Bayer, K. and Striedner, G. (2005) Fed-batch like cultivation in a micro-bioreactor: screening conditions relevant for Escherichia coli based production processes. SpringerPlus 4: 490.
3. Keil, T., Dittrich, B., Lattermann, C., Habicher, T. and Büchs, J. (2019) Polymer-based controlled-release fed-batch microtiter plate - Diminishing the gap between early process development and production conditions. Journal of Biological Engineering. 13: 18

12:35 Break, poster session & exhibition

Session 5B: MODELING

Chair Nicolas Cruz-Bournazou, TU Berlin, Germany

11:20 Keynote talk: Improvements for scalability of Lagrangian-Eulerian approaches for tracking lifelines of single cells in large bioreactors (L32)

Matthias Reuss and Alexey Lapin

Stuttgart Research Center Systems Biology, University Stuttgart, Nobelstr. 15, 70569 Stuttgart
Email: reuss@ibvt.uni-stuttgart.de

Detailed mathematical models capturing the variation in both the extracellular environment and the metabolism of a segregated biophase promise to aid significantly in describing the behavior of cell populations in bioreactors. This requires the combination of discrete and continuum modeling manifested in the Lagrange – Euler approach. For the first time, these interactions between the intracellular state of the individual cells of the population and the turbulent flow field in the bioreactor was tackled by Lapin et al. [1-3]. With special focus on using these models for the model- based design of scale down experiments this approach has been further developed and significantly updated by Haringa et al. [4].

When applying these modeling approaches for simulation of bioreactors at very large scales we are faced with serious problems of performing the simulations with comparable resolution regarding the size of grids and number of cells per volume. The detailed microscopic modeling will therefore require tracking and evolving an extremely high-dimensional configuration space at high computational cost. Similar problems arise when using larger dynamic models at the subcellular level.

The lecture aims at introducing new hardware and software developments applicable to multiscale modelling based on the strategy of structural consistency between the multiscale structure of the model and the architecture of the computer hardware. This should help to efficiently solve the problems of interactions between the response of subcellular networks to local variations of extracellular concentrations. The presentation summarizes efforts made to create multiscale simulators based on hybrid structures of parallelized graphic and central processors. This approach enables micro-macro (multiscale) simulations of higher complexity and resolution than would be otherwise feasible.

1. Lapin, A., Müller, D., Reuss ,M. (2004) Dynamic behaviour of microbial populations in stirred bioreactors simulated with Euler-Lagrange methods: traveling along the lifelines of single cells. Ind.Eng,Chem.Res. 43:4647-4656.
2. Lapin, A., Schmid, J., Reuss, M. (2006) Modeling the dynamics of E.coli populations in the three dimensional turbulent flow field of a stirred tank bioreactor – A structured segregated approach. Chem.Eng,Sci., 61: 4783-4797.
3. Lapin, A., Klann, M., Reuss, M. (2010) Multi-scale spatio-temporal modeling: lifelines of microorganisms in bioreactors and tracking molecules in cells, in Biosystems Engineering II , Springer, pp.23-43.
4. Haringa, C., Tang, W., Deshmukh, A.T., Xia, J., Reuss, M., Jeijnen, J.J., Mudde R.F., Noorman H.J. (2016) Euler-Lagrange computational fluid dynamics for (bio)reactor scale down: An analysis of organism lifelines. Eng. Life. Sci., 16: 652-663.

11:50 Black box modelling approaches to judge a yeast extracts influence on microbial growth and production (L33)

Stefanie Kaul, Nafiseh Esfandiari, Maria Scheunemann, Gesine Cornelissen

Hamburg University of Applied Sciences, Hamburg, Germany
Email: stefanie.kaul@haw-hamburg.de

The use of yeast extract is diverse: Animal food, food additives, as a complex media component. Because of their content profile, they are useful as a media component, as they generally contain everything common used cell strains need, but they also have a clear disadvantage: Because of the way they are produced, they generally show a high product-to-product and batch-to-batch variance. The top-level project of this work deals with the challenge of this variance and the complexity of the yeast extract twofold: On the one hand, the specific components of the yeast extract which influence the growth of the model organism, B. licheniformis, are determined and on the other hand a black box model is to be developed which classifies the yeast ex-tract without knowing it's specific make-up.

In this presentation, the focus is on the second part of this work. The model organisms, B. licheniformis DSM 13 and P. pastoris BSGYBG11 GFP, were grown on 23 different yeast extracts with non-limiting nitrogen, carbon and phosphate content. The yeast extracts furthermore were subjected to different analytical methods – UV/VIS spectroscopy, various HPLC-methods (IC, IEXC, RP), Raman and NIR spectroscopy, fluorescence measurements. Raman and NIR spectroscopy were also used in development of an on-line cell density calculation, which indicates that those could be used also as an off-line tool to indicate a yeast extracts performance. The resulting chromatograms and spectra of the yeast extracts were used untreated and treated via various pretreatment algorithms like SNV, mean centering, peak analysis, etc. Both the untreated and treated analytical data in combination with the fermentation results, both from shaking flask and bioreactor cultivations, – end cell density, growth rates, and yield were then used to build the black box models.

Ten to 14 of the 23 yeast extracts were then used as training sets, the rest for testing. The classification and modelling algorithms employed were simple data clustering, support vector machines, one- and two-layered feed forward neural networks, principle component regression, etc. where each showed different advantages and disadvantages as well as different resulting errors.

The goal at the end is to develop an easy to use toolbox, which other labs can use to just read in the necessary analytical data to give them an idea about their purchased yeast extracts performance.

12:10 On the modelling of microbial population dynamics using partial differential equations (L34)

Jérôme Morchain[1], Vincent Quedeville[2], Rodney O. Fox[3], Philippe Villedieu[4]

[1]Toulouse Biotechnology Institute, CNRS, INRA, INSA Toulouse, France
[2]FERMAT, Université de Toulouse, CNRS, INPT, INSA, UPS, Toulouse, France
[3]Department of Chemical and Biological Engineering, Iowa State University, 618 Bissell Road, Ames, Iowa 50011-1098, USA
[4]ONERA/DMPE, Institut de Mathématiques de Toulouse, Université de Toulouse, F-31062 Toulouse, France
Email: jerome.morchain@insa-toulouse.fr

The use of Population Balance Equations in the field of microbiology emerged in the 60's [1,2]. New questions arise from the use of this mathematical formalism because we miss the fundamental laws at the cell scale. Indeed, many biological laws are actually correlations dealing with population averaged properties. They describe a macroscopic consequence of unexplained interactions between individual microbial cells. The direct use of such laws in PBE is questioning [3]. Obviously, multiple variables are necessary if one intends to model cell elongation, division, nutrient uptake and metabolic behavior. The redistribution of properties at division is critical. The formulation of closure laws becomes a very challenging task and raises new questions. The definition of growth itself deserves some attention: should we distinguish between growth in mass and growth in number, why? Does a cell divide because of its age, its length, its DNA content or any combination of these? What is the relationship between the interdivision time distribution, the doubling time and the dilution rate? These questions will be addressed and illustrated with experimental data [4], mathematical developments [5] and numerical results [6]. In particular, the simulation of the dynamic response to a glucose pulse in a chemostat will be investigated and discussed in terms of uptake rate, metabolic response and growth rate. This example illustrates the choices we made in terms of internal variables and closure laws. Moreover, we will provide some insights into some numerical methods available for solving multivariate population balance models.

1. Eakman, J.M., Fredrickson, A.G. and Tsuchiya, H.M. (1966). Statistics and Dynamics of microbial cell populations. Chem Eng Prog Symp Ser 69: 37–49.
2. Fredrickson, A.G. and Tsuchiya, H.M. (1963) Continuous Propagation of Microorganisms. AIChE J. 9: 459–468.
3. Morchain, J., Pigou, M. and Lebaz, N. (2017) A population balance model for bioreactors combining interdivision time distributions and micromixing concepts. Biochem. Eng. J. 126: 135–145.
4. van Heerden, J., Kempe, H., Doerr, A., Maarleveld, T., Nordholt, N. and Bruggeman, F.J. (2017), Statistics and simulation of growth of single bacterial cells: Illustrations with B. subtilis and E. coli. Sci. Rep. 7: 1–11.
5. Quedeville, V., Morchain, J., Villedieu, P. and Fox, R.O. (2019) A critical analysis of Powell's results on the interdivision time distribution. Sci. Rep. 9: 8165.
6. Quedeville, V., ouazaite, H., Polizzi, B., Fox, R.O., Villedieu, P., Fede, P., Létise, F. and Morchain, J. (2018) A two-dimensional population balance model for cell growth including multiple uptake systems. Chem. Eng. Res. Des. 132: 966–981.

12:35 Break, poster session & exhibition

Session 6A: HIGH-THROUGHPUT BIOPROCESSING AND AUTOMATION

Chair Stefan Born, TU Berlin, Germany

14:30 Keynote talk: Smart digital solutions for USP and DSP to master bioengineering challenges towards industry 4.0 in biopharma (L35)

Michael Sokolov[1,2], Fabian Feidl[2], Nicolas Cruz[2], Harini Narayanan[1], Martin Luna[1], Alessandro Butté[2], Massimo Morbidelli[1,2]

[1]Institute of Chemical and Bioengineering, Department of Chemistry and Applied Biosciences, ETH Zurich, Vladimir-Prelog-Weg 1, Switzerland
[2]DataHow AG, Vladimir-Prelog-Weg 1, Zurich, Switzerland
Email: m.sokolov@datahow.ch

Following the advanced examples of successful process digitalization and automation in the 'older industries' such as automotive and finance, several trends could be observed in the biopharmaceutical industry. Over the past decade, one witnessed a shift from yield maximization to quality optimization, the utilization of miniaturized and parallelized high throughput techniques, continuous bioprocessing and continuous data acquisition as well as the utilization of data- and knowledge-driven tools for process analysis, forecasting, monitoring, control and digital twin creation. In order to eventually fulfill the standards and goals of the industry 4.0 era, the methodologies and technologies associated to previous trends must be further developed and extensively utilized in the biopharmaceutical process industry.

Throughout the past years, we elaborated several digital solutions for the analysis, modeling and interpretation of cell culture processes based on advanced engineering statistics, hybrid modeling approaches and advanced process sensors. Furthermore, we successfully integrated them into the USP and DSP development workflow in several collaboration projects with the biopharmaceutical industry as well as into our end-to-end integrated continuous bioprocessing demonstration laboratory at ETH Zurich. Based on the achieved results of these projects, the presentation will address central challenges in digitalization and big data analytics in biopharma and will demonstrate the potential to provide systematically value through integration of smart digital technologies as digital twins into the work stream of USP and DSP development.

15:00 Keynote talk: The CompuGene automated platform for the construction and characterization of genetic parts and microbial cell factories (L36)

Johannes Kabisch[1,2], Thomas Zoll[1], Aron Eiermann[1], Silke Hackenschmidt[1], Niels Schlichting[1]

[1]Technische Universität Darmstadt, Computer-aided Synthetic Biology, Germany
[2]Center for Synthetic Biology at TU Darmstadt
Email: johannes@kabisch-lab.de

The design-build-test-learn cycle driving many experiments in Synthetic Biology strongly relies on automation. Robotics can provide the throughput to build many variants of genetic constructs and test these in a reproducible manner to provide sufficient data for learning what to improve in the next design round. The CompuGene robotics platform at the Kabisch-lab was designed to perform two workflows: (1) DNA assemblies for rapid prototyping of different genetic switches and circuits and (2) testing as well as characterizing bacterial and yeast cell factories implementing these genetic designs. We will present the tools developed to realize these workflows. This includes our results for optimizing the ligase cycling reaction (LCR) for DNA assemblies as well as software tools to expedit the design process.

The characterization is done both through simple growth and expression experiments and moreover a first implementation of an active learning approach during which the platform autonomously explores the solution space to find optimal inducer conditions. Additionally we will present our approach of an open colony picking solution that can be transferred to most liquid handling robots without hardware upgrades.

1. Schlichting, N., Reinhardt, F., Jager, S., Schmidt, M. and Kabisch, J. (2019) Optimization of the experimental parameters of the ligase cycling reaction. Synthetic Biology. 4: ysz020.
2. Bruder, S., Melcher, F.A., Zoll, T., Hackenschmidt, S. and Kabisch, J. (2019) Evaluation of a Yarrowia lipolytica strain collection for its lipid and carotenoid production capabilities. European Journal of Lipid Science and Technology, 122: 1900172.

15:30 Modelling approaches with a fully-automated microbial fermentation platform (L37)

Joseph Newton[1], Annina Sawatzki[1], Vignesh Rajamanickam[1,2], Jeannine Gesson[1], Stefan Haider[1], Sandra Abad[1], Daniela Reinisch[1]

[1]Boehringer Ingelheim RCV GmbH & Co KG, Vienna, Austria
[2]Institute of Chemical Engineering, Technische Universität Wien, Vienna, Austria
Email: joseph.newton@boehringer-ingelheim.com

The fully-automated fermentation platform at Boehringer Ingelheim in Vienna has been developed to the point that it can routinely carry out up to 100 fed-batch fermentation runs per week. DoE studies have demonstrated scalability to 5L bench-scale fermenters and the platform is now being routinely implemented in development projects, where applications include screening (e.g. clones, process parameters), optimization and process characterization.

The large amount of data generated each week on the fermentation platform opens up a wide range of opportunities for mechanistic or statistical modelling applications, enabling smart process design for integrated process optimization. A recent case study applied machine learning models to rapidly optimize a fermentation process in combination with the automation platform. The model recommended optimum process conditions that achieved a 15% increase in titer in comparison to results obtained with a traditional DoE approach.

Many scale-down fermentation platforms use intermittent feeding of the carbon source, which may lead to an impact on growth and product formation due to stress on the cells. In a recent project, we implemented and characterized an enzymatic feed for glucose release and mathematically described the release from dextrin by an enzyme (glucoamylase), using the mathematical model to predict and plan the glucose release during a fermentation (based on temperature, pH, product inhibition, enzyme inactivation).

The major obstacle for widespread industrial use of model-based technologies is a lack of standardized methodologies for modelling approaches. In an ongoing project, we are developing adaptive mechanistic modelling workflows for model development, calibration and validation that are transferable across different platforms, from small-scale high-throughput platforms to large-scale fermentation. The developed computational workflows combine methods for recursive state and parameter estimation, as well as search techniques for best-fitting subsets and have been verified by proof of superior efficiency compared to the state-of-the-art process development workflows in academic and industrial research.

In summary, approaches using a combination of mechanistic and statistical models together with automation platforms strive towards a next-generation approach for in silico process development, ultimately leading to deeper process understanding and better manufacturing processes.

15:50 Towards an autonomous model based high throughput bioprocess development and clone discrimination (L38)

Sebastian Hans

Technische Universität Berlin, Chair of Bioprocess Engineering
Email: sebastian.hans@tu-berlin.de

The number of potential drugs or products in the biochemical and pharmaceutical sector is ever increasing. At the same time, the bioprocess industry is going through a period of digitalisation and automation with major upheavals. Many companies are already well advanced in automation in particular. Liquid Handling Stations and mini bioreactor systems are commercially available from different manufacturers in a broad range of designs and can be found in many laboratories. These are mostly used for large screenings or static bioprocess development. However, these experiments differ only slightly from classical screening. The advancing digitalisation further ensures that measured values and experimental data are available online and enables the application of new methods of control engineering or machine learning.
All this together led to the development of the developed a high-throughput bioprocess development facility, supported by online data evaluation and a model-based framework. With this facility, a large number of strains can be cultivated in parallel using Mini-Bioreactors. Here we present our latest results towards an autonomous bioprocess development platform, where online glucose consumption, the end of the acetate shoulder and the feed rate were determined for 8 strains in parallel during the running

cultivation. Our results showed that the feed start calculated and executed in this way deviated on average only 7 min from the optimal time. The executed feed was calculated for each strain and allows an extremely robust process with uncharacterized strains and allows screening under production relevant process conditions.

16:10 Break & exhibition

Session 6B: BIOPROCESSES FOR A CIRCULAR ECONOMY

Chair Björn Gutschmann, TU Berlin, Germany

14:30 Key note talk: Process development of polyhydroxyalkanoate (PHA) bioplastics production from lipid based waste and raw materials (L39)

Sebastian L. Riedel[1], Björn Gutschmann[1], Lara Santolin[1], Peter Neubauer[1], Stefan Junne[1]*
[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
Email: riedel@tu-berlin.de

The worldwide plastic pollution and the continuing climate change are among the biggest challenges of today's society. Substitution of conventional plastics with polyhydroxyalkanoate (PHA) bioplastics addresses both issues, since PHAs are fully biodegradable in nature and PHAs can be produced with a lower CO2–footprint than fossil-based plastics. However, the worldwide PHA production is still negligible, due to high production costs, partly caused by the substrate supply. To address this issue, biogenic feedstocks, which show little competition to other industries are utilized in this study to enable a low cost PHA production. Additionally, the process is tended to work substrate flexible to avoid dependencies on a single feedstock [1–3].

Process development from mL- to 100 L-scale with various waste animal fats for the production of polyhydroxybutyrate and the copolymer poly(hydroxybutyrate-co-hydroxyhexanoate) with Ralstonia eutropha is shown. Difficulties through inhomogeneous distribution of the fat in the aqueous media, especially caused through the high melting temperatures (up to 60°C), had to be overcome to reach reproducible results. A cultivation method for 24-microwell plates was developed to perform parallel cultivations at small scale. A feeding strategy, which leads to a productivity of >1 g L-1 h 1 and 71 g L-1 PHA in bioreactors and a novel method for in-line PHA monitoring by Photon Density Wave spectroscopy [4] was developed. To decrease set-up times, a repeated fed-batch strategy was performed, where the batch phase could be shortened to 1/3, with no sterilization between these cultivations. The presented results will allow to lower the PHA production costs further in the future.

1. Brigham, C.J. and Riedel, S.L. (2018) The potential of polyhydroxyalkanoate production from food wastes. Appl. Food Biotechnol. 6: 7–18.
2. Riedel, S,L, and Brigham, C,J. (2019) Polymers and adsorbents from agricultural waste. In: Byprod. from Agric. Fish. 22: 523–544.
3. Riedel, S.L., Jahns, S., Koenig, S., Bock, M.C.E., Brigham, C.J., Baader, J. and Stahl, U. (2015) Polyhydroxyalkanoates production with Ralstonia eutropha from low quality waste animal fats. Biotechnol. 214: 119–127.
4. Gutschmann, B., Schiewe, T., Weiske, M.T., Neubauer, P., Hass, R. and Riedel, S.L. (2019) In-Line monitoring of polyhydroxyalkanoate (PHA) production during high-cell-density plant oil cultivations using Photon Density Wave Spectroscopy. Bioengineering. 6: 85.

15:00 Usage of mealworms to recover and purify polyhydroxyalkanoate granules from Cupriavidus necator cells (L40)

Kumar Sudesh
School of Biological Sciences, Universiti Sains Malaysia
Email: ksudesh@usm.my

Polyhydroxyalkanoates (PHAs) are microbial storage polyesters that have thermoplastic and biodegradable properties. Therefore, there is much interest in using PHAs as substitute for some non-biodegradable petrochemical plastics, especially single-use plastics. However, the production of PHA is costly in part because of the tedious downstream processes where the microbial cells need to be lysed before the PHA granules can be liberated for further purifications. Cupriavidus necator (formerly known as Alcaligenes eutrophus H16) was initially studied for its potential as a single-cell protein. [1,2] It is currently not only a model bacterial strain used for studying PHA biosynthesis but also a robust and versatile industrial strain that can be grown to high cell density using a diverse range of carbon sources such as sugars, fatty acids, lipids, CO2 and methanol. The PHA granules accumulated in the cell cytoplasm of C. necator can be recovered by feeding the dried cells to mealworms.[3,4] It was previously found that other animals such as rats can also eat the C. necator cells and release the PHA granules in their feces.[1,2,5] The mealworms consume about 10-15 wt% daily of their body weight dried cells of C. necator.[3-5] The PHA granules in the cell cytoplasm are not digested but released in the feces which can be further purified by washing with water. Currently, this method allows the recovery of kilogram quantities of PHA in the laboratory at low cost. The scaling-up study is currently ongoing to address the various challenges of this biological recovery process. [6,7]

1. Kunasundari, B., Murugaiyah, V., Kaur, G., Maurer, F.H.J. and Sudesh, K. (2013) Revisiting the single cell protein application of Cupriavidus necator H16 and recovering bioplastic granules simultaneously. PLoS One. 8: e78528.
2. Kunasundari, B., Rodriquez Arza, C., Maurer, F.H.J., Murugaiyah, V., Kaur, G. and Sudesh, K. (2017) Biological recovery and properties of poly(3-hydroxybutyrate) from Cupriavidus necator H16. Sep. Purif. Technol. 172: 1-6.
3. Zainab-L., I. and Sudesh, K. (2019) High cell density culture of Curiavidus necator H16 and improved biological recovey of polyhydroxyalkanoates using mealworms. J. Biotechnol. 305: 35-42.
4. Murugan, P., Han, L., Gan, C.-Y., Maurer, F.H.J. and Sudesh, K. (2016) A new biological recovery approach for PHA using mealworm, Tenebrio molitor. J. Biotechnol. 239: 98-105.
5. Ong, S.Y., Zainab-L, I., Pyary, S. and Sudesh, K. (2018) A novel biological recovery approach for PHA employing selective digestion of bacterial biomass in animals. Appl. Microbiol. Biotechnol. 102: 2117-2127.
6. Ong, S.Y., Kho, H.-P., Riedel, S-L., Kim, S.-W., Gan, C.-Y., Taylor, T.D. and Sudesh, K. (2018) An integrative study on biologically recovered polyhydroxyalkanoates (PHAs) and simultaneous assessment of gut microbiome in yellow mealworm. J. Biotechnol. 265: 31-39.
7. Chee, J.Y., Lakshmanan, M., Jeepery, I.F., Mohamad Hairudin, N.H. and Sudesh, K. (2019) The potential application of Cupriavidus necator as polyhydroxyalkanoates producer and single cell protein: A review on scientific, cultural and religious perspectives. Appl. Food Biotechnol. 6: 19-34.

15:30 Extraction of chitin from American lobster (Homarus americanus) shells and fabrication of membranes for potential biomedical use (L41)

Christopher Brigham

Department of Interdisciplinary Engineering, Wentworth Institute of Technology
Email: brighamc2@wit.edu

As a component of crustacean shells and fungal cell walls, chitin is the second most abundant polysaccharide in nature. Chitin is a structural polysaccharide that exhibits favorable biological, chemical and mechanical properties, indicating its promise as a bio-based polymer for a variety of structural applications. We developed a bio-based extraction method to isolate chitin from ground Homarus americanus shells, which involves coculturing with Serratia marcescens (a protease producing bacterium) and Lactobacillus plantarum (a lactic acid producing bacterium). This coculture method was shown to be comparably effective to traditional chitin extraction methods using harsh acids and bases. Chitin purified in this way was cast into thin membranes. Since chitin is insoluble in water as well as organic solvents, we used the ionic liquid 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]) as the solvent. Chitin membranes fabricated by solvent casting using [C2mim][OAc] were shown to be durable, flexible and porous. These membranes were shown to be suitable as physical substrates for growth of mammalian cells and for release of bioactive compounds. Blending of chitin with hydroxyapatite or polylactide (PLA) altered the material properties and supported growth of osteocytes. We have demonstrated the potential of chitin a promising bio-based polymer with applications in the medical and life sciences industries. Our work on small-scale, bio-based processing of crustacean shells for chitin raw material suggests that environmentally friendly processing of this polysaccharide material is possible, thus mitigating the impact of seafood processing waste and potentially providing a new value stream.

15:50 Low quality by-products for high quality products - Processing strategy and application development for the circular economy (L42)

Thomas Grimm

ANiMOX GmbH, Max-Planck-Str. 3, D-12489 Berlin, Germany
Email: t.grimm@animox.de

In recent years, by-products have been increasingly reused in material cycles. Particularly in food and feed processes, more high-quality materials are being used. The use of these proteins, fats and carbohydrates results in the loss of biogenic raw materials for technical applications. Due to the increasing number of people, decreasing areas for agriculture and climate change, the use of low-quality biogenic residues is necessary for the bioeconomy. For a more effective use of these sources, processes in which these biogenic residues are produced must be re-evaluated and redesigned so that the by-products are also separated and processed as effectively as possible.

ANiMOX works on projects for the treatment of biogenic residues of plant and animal origin. Using the example of a plant process based on rapeseed material and animal raw materials, options for biogenic raw material extraction and application development are presented. The focus is on the utilization of materials that are difficult to recycle and extract in bioprocesses.

1. Winter, B., Hphling, A. and Venus, J. (2015) Tierische Proteinhydrolysate als N-Quelle für die Milchsäurefermentation BIOspektrum. 21: 228-229.
2. EP 1835816 B1. ANiMOX GmbH. Pr.: DE 102004063258 23.12.04. – Method for producing protein hydrolysates
3. Fetzer, A., Herfellner, T., Stäbler, A., Menner, M. and Eisner, P. (2018) Influence of process conditions during aqueous protein extraction upon yield from pre-pressed and cold-pressed rapeseed press cake. Industrial Crops and Products. 112: 236-246.

16:10 Break and exhibition

16:30 Plenary Talk and Closing: Integrated and Networked Systems and Processes – A Perspective for Digital Transformation in (Bio) Process Engineering (PL04)

Michael Maiwald

Bundesanstalt für Materialforschung und -prüfung (BAM), Berlin
Email: Michael.maiwald@bam.de

The competitiveness of the process industry is based on ensuring the required product quality while making optimum use of equipment, raw materials and energy. Chemical companies have to find new paths to survive successfully in a changing environment, while also finding more flexible ways of product and process development to bring their products to market more quickly – especially high-quality high-end products like fine chemicals or pharmaceuticals. The potential of digital technologies belongs to these.

One way is knowledge-based production, taking into account all essential equipment, process and regulatory data of plants and laboratories. Today, the potential of this data is often not yet consistently used for a comprehensive understanding of production. Another approach uses flexible and modular chemical plants, which can produce different high-quality products using multi-purpose equipment with short downtimes between campaigns and reduce the time to market of new products. Digital transformation is enabling completely new production concepts that are being used increasingly. Intensified continuous production plants also allow for difficult to produce compounds.

This contribution aims to encourage a more holistic approach to the digitalization in (bio) process engineering by introduction of integrated and networked systems and processes, which have the potential to speed up the high-quality production of specialty chemicals and pharmaceuticals.

17:15 Closing remarks / Best Poster & Presentation Award

Peter Neubauer, TU Berlin, Germany

17:30 End of congress

POSTER ABSTRACTS

P01: Design and upscaling of Pseudomonas putida fermentations for robust biomanufacturing

Jesper W. Jensen[1], John M.Woodley[1], Pablo I. Nikel[2] and Helena Junicke[1]

[1]Department of Chemical and Biochemical Engineering, Technical University of Denmark
[2]The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark
Email: jewaj@dtu.dk

The bacterium Pseudomonas putida is becoming a cell factory of choice due to its versatile metabolism and high stress tolerance, the latter being of prime importance when considering production at large scale and bioconversion of harsh chemicals. These phenotypic features are interconnected through a cyclic glucose catabolism that allows the bacterium to adapt to environmental stresses by altering the production of co-factors [1]. However, glucose metabolism is linked to a high oxygen requirement as P. putida is an obligate aerobe. Oxygen availability has for a long time been known to be an issue in large scale bioreactors due to insufficient mixing and the resulting oxygen gradients [2]. We postulate that the presence of oxygen gradients and long mixing times in large scale bioreactors could affect the metabolism of P. putida and thereby the applicability and performance of this cell factory under industrially-relevant conditions. We therefore aim to investigate the influence of oxygen availability on the metabolism of P. putida and develop a suitable bioreactor control strategy. This strategy will be based on oxygen availability in the bioreactor as well as on the parameters used to control this. An appropriate control strategy will aid to the development of scale-down models to investigate the phenomena that influence P. putida metabolism in large-scale bioreactors.

1. Nikel, P.I., Chavarría, M., Fuhrer, T., Sauer, U. and de Lorenzo, V. (2015) Pseudomonas putida KT2440 strain metabolizes glucose through a cycle formed by enzymes of the Entner-Doudoroff, Embden-Meyerhof-Parnas, and the pentose phosphate pathways. Journal of Biological Chemistry. 290: 25920-25932.
2. Wehrs, M., Tanjore, D., Eng, T., Lievense, J. Pray, T.R. and Mukhopadhyay, A. (2019) Engineering robust production microbes for large-scale cultivation. Trends in Microbiology. 27: 524-537.

P02: Insights into the role of sigma factor orf21 in clavulanic acid production by Streptomyces clavuligerus ATCC27064

Patiño_Cervantes L.F., Aguirre V., Pinilla L., Rios-Estepa R.

Chemical Engineering and Bioengineering, Universidad de Antioquia, Colombia

Sigma factors play an essential role in cells adaptation to environments [1]. Cellular balance in prokaryotes is achieved through signal transduction mechanisms that connect the extracellular medium with the cytoplasm. Extracytoplasmic function factors (ECFs) are the core components of one of the major signal transduction mechanisms in bacteria in terms of abundance and importance of the stress responses they mediate [2]. The Streptomyces genome encodes various ECF sigma factors, which reflects the complexity of their metabolic processes [3]. In the clavulanic acid (CA) producer organism, Streptomyces clavuligerus ATCC 27064, the orf21 gene located downstream of CA gene cluster encodes a sigma ECF factor. The role of orf21 on CA biosynthesis has been previously studied [4;5]; nevertheless, its performance as regulator of CA production is not fully understood.

In this contribution, orf21 gene was overexpressed in the wild-type strain of S. clavuligerus (S. clavuligerus/ pIORF21) and its effect on CA production was evaluated in GSPG and ISP media. The findings obtained from this contribution suggest that the role of orf21 on CA biosynthesis is strongly determined by the environmental conditions of the fermentation. In GSPG, a defined medium, S. clavuligerus/ pIORF21 increased CA production 2.6-fold. Likewise, orf21 overexpression in GSPG stimulated the expression of the late CA gene, gcas and the regulatory gene, adpA, as displayed by real-time PCR. In contrast, in ISP, a medium based on isolated soy protein, S. clavuligerus/ pIORF21 decreased CA production by 1.8-fold compared to the control strain. Thus, orf21 overexpression in ISP modulate CA biosynthesis in a negative manner, repressing (indirectly) the expression of the regulatory gene, claR. The regulatory network that controls morphological differentiation in S. clavuligerus is related to CA production [6;5]. In this work, we corroborated that orf21 overexpression in solid medium favors aerial mycelium formation in S. clavuligerus/pIORF21. Furthermore, our results suggest that orf21 overexpression also plays a role in the carbon metabolism of S. clavuligerus, affecting the glycerol uptake rate profile.

1. de Bruijn, F.J. (2016) Stress and environmental regulation of gene expression and adaptation in bacteria, John Wiley & Sons, Inc., Editor: F.J. de Bruijn vol. I.
2. Casas-Pastor, D., Müller, R.R. Jaenicke, et al. (2021) Expansion and re-classification of the extracytoplasmic function (ECF) sigma factor family. Nucleic Acids Research. 49: 986-1005.
3. Helmann, J.D. (2002) The Extracytoplasmic Function (ECF) Sigma Factors. Adv. Microb. Physiol. 46: 47-110.
4. Yeon J.Y., Eun S.K., Kim, D.W., Jensen S.E. and Lee, K.J. (2009) A gene located downstream of the clavulanic acid gene cluster in Streptomyces clavuligerus ATCC 27064 encodes a putative response regulator that affects clavulanic acid production. J. Ind. Microbiol. Biotechnol. 36: 301-311.
5. Jnawali, H.N., Liou, K. and Sohng, J.K. (2011) Role of σ-factor (orf21) in clavulanic acid production in Streptomyces clavuligerus NRRL3585. Microbiol. Res. 166: 369–379.
6. Yepes-Garcia, J., Caicedo-Montoya, C., Pinilla, L., Toro, L.F. and Rios-Estepa, R. (2020) Morphological differentiation of Streptomyces clavuligerus exposed to diverse environmental conditions. Processes. 8: 1038.

P03: Genome mining of Streptomyces strains and its potential to identify new compounds with antimicrobial activity

Carlos Caicedo-Montoya, Monserrat Manzo-Ruiz and Rigoberto Ríos-Estepa

Departamento de Ingeniería Química, Universidad de Antioquia, Colombia
Email: candres.caicedo@udea.edu.co

Streptomyces is the most prolific genus of the Actinobacteria phylum, discovered so far, for the production of bioactive secondary metabolites with high societal impact, including immunomodulators, immunosuppressant, chemotherapeutics and mostly antibiotics. With the arrival of next-generation sequencing technologies a vast quantity of genomes of Streptomyces strains have been produced. This richness of data can lead to the discovery of new compounds of diverse chemical structures and bioactivities that might have novel biotechnological and pharmaceutical applications.

In this work we focus on the in-silico detection of compounds with antimicrobial activity based on the mining of 121 complete published genomes for determining biosynthetic gene clusters (BCG) in this genus. Genomes were analyzed using ARTS 2.0 to prioritize BGC more likely to produce an active metabolite based on the presence of self-resistance enzymes co-localized within BGC, as well as the presence of duplicated core genes with evidence of horizontal gene transfer (HGT) which could serve for uncovering those BCG with potential antibiotic activity.

The analysis revealed that Streptomyces strains harbor 41 different types of BGC out of 52 types defined by antiSMASH 5.0, between 10 to 26 types of BGC per genome, and 23 to 83 BGC per genome, with Nonribosomal peptide synthetases (NRPS), terpenes and siderophores being the most frequently found in the set of genomes analyzed.

To prioritize the search for antibiotics, ARTS use BGC prediction from antiSMASH and display the presence of self-resistance enzymes co-localized with BGC. In all 121 genomes analyzed, 593 self-resistance genes were identified, distributed in 480 clusters. On average we identified 5 self-resistance genes per genome, with the notable example of S. alfalfae ACCC40021 that carry 12 of these genes. Additionally, core genes along with self-resistance genes were found in only 242 clusters. The number decreased to 33 clusters in 31 genomes if only duplicated core genes with evidence of HGT along with self-resistance genes are considered; most of these clusters were NRPS. Thus, these could be considered bona fide clusters for the production of antibiotics which can be further experimentally tested to combat emerging new strains with resistance to known antibiotics.

P04: Cell-free bosynthesis of the nonribosomal peptide antibiotic valinomycin

Jian Li

School of Physical Science and Technology, ShanghaiTech University, Shanghai 201210, China
Email: lijian@shanghaitech.edu.cn

The foundational principle of cell-free synthetic biology is that precise, complex biomolecular systems can be constructed without the use of living, intact cells. Because of the absence of cell walls, these open systems allow for easy manipulation, monitoring, sampling, and optimization. Therefore, in vitro cell-free systems have many advantages over in vivo cell systems including high product yields, fast reaction rates, tolerance of toxic precursors and/or products, and highly controlled reaction conditions. Recently, cell-free synthetic systems are becoming important as platforms for enabling biosynthetic routes to proteins, novel biopolymers, and small molecule chemicals. In this context, we take advantage of cell-free biology to design and construct efficient platforms for the rapid and cost-effective next-generation biomanufacturing. In this poster, I will show the cell-free biosynthesis of nonribosomal peptide natural products at the example of valinomycin, which demonstrates that in vitro, cell-free, platforms are robust to produce "difficult-to-express" proteins like the very large nonribosomal peptide enzymes, and more importantly, complex natural products that could be biosynthesized via the in situ cell-free expressed enzymes. We expect that cell-free biosynthetic system will provide a new avenue to express, discover, and characterize natural product gene clusters of interest in vitro.

P05: Development of a P. pastoris strain for the recombinant production of peptide-based antibiotics in eukaryotic cells

Lisa Michel, Alexander Thoma, Jörg Andrä, Stephan Noll, Gesine Cornelissen

Hamburg University of Applied Sciences, Hamburg, Germany
Email: lisa.michel@haw-hamburg.de

Soon increasing bacterial resistances to conventional antibiotics will cause a lack of potent pharmaceuticals against nowadays harmless infections. Peptide-based antibiotics also known as antimicrobial peptides (AMPs) are a promising alternative to common antibiotics.

For recombinant AMP production, a P. pastoris strain, expressing an inactive fusion construct, is designed. The construct comprises of a modified sequence of the membrane active region of porcine NK-lysin merged with a green fluorescent protein (GFP). A TEV-protease recognition site allows cleavage of the fusion construct. Extracellular secretion is attained using the -mating factor secretion signal sequence. GFP fusion is likely to improve cellular production. Moreover, it enables on-line process analytics (PAT) via in-line fluorescence detection.

The fusion construct coding sequence is cloned under the transcriptional control of alcohol oxidase 1 promotor using Golden Gate Cloning. Then, the linearized fusion construct vector is homologously integrated into the genome of P. pastoris.

As proof of principle, the clones are applied in small-scale expression experiments. The secreted fusion construct is concentrated from the supernatant either by size or its affinity tag. Then, the pooled construct is cleaved by TEV-protease and the fission product is examined by Tricine-SDS-PAGE.

A fully automated 3-stage upstream process is carried out as 3 l high cell density cultivation (up to 75 g l-1). During batch phase P. pastoris is grown on glycerol followed by a controlled exponential fed-batch phase. An on-line methanol measurement control circuit controls gene expression and maintains constant methanol concentrations and thus constant production rates.

The correct integration of the coding sequence is demonstrated by agarose gel electrophoresis and Sanger sequencing. In contrast to the wild type strain, the recombinant strain shows a fluorescent signal after methanol induction. A SDS-PAGE of the supernatant shows a product band of the expected size. The antimicrobial activity of chemically synthesized peptides was already proven using agar diffusion tests. If the AMP is active after the cleavage has to be shown next.

P06: Experimental studies on the isolation of photobiont and mycobiont partners of lichens for controlled co-cultivation and production of secondary substances

Zakieh Zakeri[1], Fabia Jäger[1], Marcel Dostert1, Stefan Junne[1], Volker Otte[2], Peter Neubauer[1]

[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany.
[2]Senckenberg Museum of Natural History, Am Museum 1, 02826 Görlitz, Germany
Email: zakeri@tu-berlin.de

Lichens represent natural symbionts of algae and fungi. It is estimated that 6% of Earth's land surface is covered by lichens. Although there are around 25,000 identified species of lichens worldwide and about 600 secondary metabolites. Nevertheless, they are currently not biotechnologically exploited. This is partly caused by the difficulties in cultivating lichens in vitro. It is necessary to develop a suitable methodology to control growth of a lichen so that its secondary metabolites are synthesized in considerable amounts for screening and subsequent purification for further exploitation of potential medical and industrial application.

Previous studies have shown that cultivating lichen from thalli (the entire body) is very difficult due to contamination by parasites and a lack of growth control. To overcome these problems, we have tested various methods to optimize the isolation of symbiotic partner (fungi and algae). The isolation processes took 2-4 weeks to reach the axenic culture of algae and fungi. Taxonomic confirmation of isolated symbionts was verified by ITS gene sequencing. The axenic cultures of the fungi and algae were cultivated in various media and under different conditions in agar plates and shake flasks, in order to optimize the cultivation method.

After two months of cultivation of the axenic culture and co-cultivation of the symbiotic partner in solid and liquid media, the production of secondary substances was analyzed by HPLC. The presence of lichen substances such as usnic acid, lecanoic acid, fumarprotosetraric acid was detected in the cultures.

P07: Comparison of different analytical methods for the assessment of viability during the production and storage of yeast preparations

Sarah Köhler, Maximilian Schmacht, Stefanie Malchow, Martin Senz

Research and Teaching Institute for Brewing in Berlin, Research Institute for Biotechnology and Water, Department Bioprocess Engineering and Applied Microbiology, Berlin, Germany
Email: m.senz@vlb-berlin.org

Dried yeast preparations are used in different kinds of industrial applications because of advantages in practice, logistics and storage. Thereby the different steps in the preparation process can negatively affect yeast viability. Thus, measuring viability is crucial for proper sample characterization and a key quality attribute for producer and user. The aim of this study was the assessment and comparison of viability based on different analytical methods during the production and storage of yeast Saccharomyces cerevisiae. Yeast cells were dried via vacuum drying at room temperature and freeze drying, the latter with and without a protection matrix, and stored at 4 °C and 26 °C for 3 months. Viability was detected via classical plate count in combination with impedance measurement (Multisizer™ 3, Beckman Coulter GmbH), and different staining based methods, i. e. flow cytometry (CyFlow® Cube 8, Sysmex Inc.; cFDA, PI), NucleoCounter® YC-100™ (ChemoMetec A/S ©; PI) and Oculyze (Oculyze GmbH; methylene violet). Freeze dried yeast with protection matrix showed highest viabilities even after 3 months of storage at 4 °C and 26 °C in comparison to pure yeast as well as vacuum dried pure yeast. The different analytical methods for determination of viability of yeast preparations showed significant differences over the whole process of production and storage. Results obtained by NucleoCounter and Oculyze revealed continuously much higher values than plate count method and flow cytometry for the respective samples. The results show the importance of choosing the adequate method for viability determination. Both the expected measuring range and the corresponding sample amount must be taken into account in order to ensure satisfactory measuring reliability with the lowest possible measuring outlay.

P08: Applying Dielectrophoresis to Improve a Microring Resonator Biosensor Platform

Anders Henriksson[1], Matthias Jäger[2,3], Laura Kasper[2], Peter Neubauer[1], Mario Birkholz[4]

[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]Department of High Frequency Technology/Photonics, Technische Universität Berlin, Einsteinufer 25, 10587 Berlin, Germany
[3]Tethir Limited, The Core, Bath Lane, Newcastle Helix, Newcastle Upon Tyne, NE45TF, United Kingdom
[4]IHP - Leibniz Institute for Innovative Microelectronics, Im Technologiepark 25, 15236 Frankfurt/Oder, Germany
Email: henriksson@tu-berlin.de

Silicon-based photonic microring resonators have many advantages as biosensor platforms. These include high sensitivity, real time response, small footprint and a simple integration into conventional semiconductor processes, allowing a straight forward miniaturization of the system. As biosensors are approaching the nanoscale, analyte mass transfer and bonding kinetics have been ascribed as crucial factors that limit their performance. In the current work we have designed a platform that applies dielectrophoretic forces to increase the mass transfer of a microring resonator-based biosensor. Dielectrophoresis, the migration of polarized dielectric particles in a non-uniform AC field has previously been successfully applied to achieve a 1000-fold improved detection efficiency in nanopore sensing1 and may potentially significantly also increase the sensitivity in microring resonator biosensors. The chip design with two different electrode configurations, packaging strategies and electric field gradient simulations are presented. FEM simulations calculated for both electrode configurations resulted in a E2 of 1016-1020 V2m-3 around the sensor areas2. This is comparable to E2 previously reported for interaction with common analytes such as proteins and antibodies. The sensor chip was subsequently realized using the SGH25_PIC technology from IHP. The position of the resonance peak of the realized ring resonator was confirmed to be linearly correlated to the refractive index of the environment, confirming its function as a sensor with a sensitivity of 4.9 ±0.03 nm/RIU and a Q factor of approximately 1-1.5x105. Finally, as a proof of principle, the microalgae C. cohni was successfully aligned onto the sensor surface by applying an AC field of 10 MHz and 10 Vpp.

1. Freedman, K.J., Otto, L.M., Ivanov, A.P., Barik, A., Oh, S.-H. and Edel, J.B. (2016) Nanopore sensing at ultra-low concentrations using single-molecule dielectrophoretic trapping. Nature communication. 7: 10217.
2. Henriksson, A., Kasper, L., Jäger, M., Neubauer, P. and Birkholz, M. (2020) An approach to ring resonator biosensing assisted by dielectrophoresis: Design, simulation and fabrication. Micromachines. 11: 954.

P09: Effect of oscillatory cultivation conditions on the macromorphology in Yarrowia lipolytica cultivations

Jasmina Cziommer[1], Simon Briel[1], Nico Feuser[1], Eva Moldenhauer[2], Johannes Kabisch[2], Peter Neubauer[1], Stefan Junne[1]

[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany[2]Department of Biology, Computer-aided Synthetic Biology, Technische Universität Darmstadt, Schnittspahnstr. 12, 64287 Darmstadt, Germany
Email: j.cziommer@tu-berlin.de

Oleaginous microorganisms (yeasts, moulds and algae) are interesting hosts for the exploitation of high-value lipids, which mainly comprise nutritionally important polyunsaturated fatty acids (PUFAs) and lipids suited for the production of biofuels. Monitoring tools for the liquid phase of bioprocesses are often restricted to a very few parameters. This might be insufficient, if data from the single-cell level is required. Often, already a relation exists between morphology and the viability and vitality of an organism so that a lot of relevant data can be gained, e.g. by photo-optical monitoring [1]. In this case, three-Dimensional digital holographic microscopy (DHM) [2] was used to monitor intracellular lipid accumulation in oleaginous yeast Yarrowia lipolytica fed-batch bioreactor cultivations under homogeneous and under oscillatory cultivation conditions. Several parameters, which are gained by measurements with DHM were evaluated for sensitivity to monitor the increasing lipid content in cells of wild type and genetically modified strains. The optical volume, a ratio of the cell volume and its refractive index as measured by DHM, was identified as most sensitive parameter, which showed a direct correlation to the lipid content as determined volumetrically by gas chromatography. Flow cytometry with stained cells revealed a very similar development of the population inhomogeneity concerning the intracellular lipid accumulation. It is shown that the increasing inhomogeneity, e.g. induced by an oscillatory growth environment, is captured simultaneously by both methods throughout a serious of repeated substrate pulses.

The measurement of the optical volume by DHM provide quantitative information about the lipid content of individual cells and the impact of a fluctuating oxygen concentration. It allows the rapid evaluation of scale-down methodologies on the basis of subpopulation similarity.

1. Lemoine, A., Delvigne, F., Bockisch, A., Neubauer, P. and Junne, S., (2017) Tools for the determination of population heterogeneity caused by inhomogeneous cultivation conditions. J. Biotechnol. 251: 84–93.
2. Marbà-Ardébol, A.-M., Emmerich, J., Neubauer, P. and Junne, S. (2016) Single-cell-based monitoring of fatty acid accumulation in Crypthecodinium cohnii with three-dimensional holographic and in situ microscopy. Proc. Biochem. 52: 223–232.

P10: Control of macromorphology and the implications on product formation in Aspergillus niger

Tolue Kheirkhah[1], Tutku Kurt[2] Peter Neubauer[1], Stefan Junne[1]

[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]Celltainer Biotech BV, Bothoekweg 9, 7115 AK Brinkheurne, Winterswijk, The Netherlands
Email: tolue.kheirkhah@tu-berlin.de

The product portfolio of filamentous fungi is diverse and includes proteins, enzymes, secondary metabolites, and organic acids. However, the development of the macroscopic morphology of fungi is still largely an unpredictable multifactorial process that influences productivity.

An oscillatory shear-force environment as it appears in large-scale bioreactors has an impact on the macromorphology of filamentous fungi. Usually, this situation can't be mimicked in small scale stirred tank bioreactors as a low shear-force environment is coupled to low stirring rates, and thus a low gas-mass transfer. Any effect that would be observable would be probably related, at least partly, to severe oxygen limitation in any aerobic process.

In order to achieve a similar macromorphology in the lab scale than that one obtained in large scale stirred tank bioreactor cultivations with shear force gradients in the liquid phase, cultivation conditions in the two-dimensional rocking motion bioreactor CELL-tainer are tailor-made. By a certain set up of rocking motion and additives, the impact on macromorphology as induced by an oscillating shear-force environment on substrate uptake, product secretion and side metabolite accumulation is studies. It is shown that macromorphology, pellet density and process performance are related. It is further possible to use the data for a prediction of the process performance, if the macromorphology that is obtained under certain shear-force conditions is known.

The methodology developed will then be used to investigate optimization options for the industrial scale at the laboratory scale in a knowledge-driven manner.

P11: Cultured meat production in a 2D rocking bioreactor

Tobias Höing

MosaMeat, Email: tobias.hoeing@mosameat.com

To revolutionize the food industry by bringing cultured meat to the market, a scalable process for the proliferation of primary bovine stem cells is required. Overcoming difficulties of an adherent growing cell culture on an industrial scale is a key factor for success. In this study, a rocking bioreactor (CELL-tainer) has been investigated for its potential to sustain such a process.

The bovine muscle and fat precursor cells are adherent cells, growing on microcarriers and as such, they are sensitive to shear stress. It was hypothesized that by the high mixing efficiency and mass transfer provided by the 2-dimensional rocking motion of the CELL-tainer, the culture could be successfully scaled up to 3L. It was found that cell densities at least as high as in a conventionally stirred bioreactor could be reached in the CELL-tainer. An intermittent rocking strategy on the day of inoculation was necessary and the cells attached and grew on the microcarriers with a doubling time of down to 27 hours. The same method was used when the microcarrier density was subsequently increased throughout the culture to expand the cells further. The cells were able to populate and grow on the freshly added microcarriers. The bead to bead transfer efficiency was evaluated as % of carriers with at least one cell on them every day and compared to the current standard (stirred tank reactor). To supply the cells with sufficient nutrients and to decrease any metabolites built up in the culture, medium exchanges at specific timepoints were performed through an integrated sieve.

P12: From micro to macro: a study on the volumetric power input in microtiter plates and its use as a strategy for scale-up in downstream processing

Ignacio Montes-Serrano, Peter Satzer, Alois Jungbauer, Astrid Dürauer

Austrian Centre of Biotechnology (acib), Muthgasse 18, 1190, Vienna
Email: ignacioserrano@acib.at

Scale-up strategies for downstream processing have been developed over the last decades in order to accelerate process development time and enhance efficiency of production processes. Microtiter plates, which allow for the development of different downstream processes under different conditions, are widely used in laboratories. Volumetric power input (VPI) determines the hydrodynamic stress and the fluid dynamics of the system and has already been proved as an adequate scale-up option. In microscale, it can be obtained via measuring the temperature increase produced by the energy dissipation which can be done with high sensitivity and precision. For this study, three types of microtiter plates (96-, 24- and 6-well) have been studied in different shaking conditions. In parallel, the hydrodynamics of the different wells have been studied by Comutational Fluid Dynamics (CFD) to find a correlation between the motion and the VPI. So far, results have proven that, under the same conditions such as 600rpm under a 3mm orbital diameter, the 96-well displays a higher temperature increase and thus a higher amount of VPI compared to 24- and 6 well, 31, 15 and 9 W/m3 respectively. similar to the displayed effect on stirred tanks for larger volumes under the same stirring frequency. The CFD simulations also allow for experiment with different conditions, demonstrating a power trend of the volumetric power input as the shaking frequency increases. The established mathematical correlation follows a power trend that can be utilized as a proper scale-up tool for large scale mixing in batch or continuous mode.

P13: Towards smart factories: Data-driven modeling approaches in bioprocessing

Jonathan Sturm[1], Sylvia Denter[2], Michael Weniger[2], Frank Eiden[1]

[1]Westfälische Hochschule Recklinghausen, AG BioProzessTechnik
[2]ARTES Biotechnology GmbH, 40764 Langenfeld, Germany
Email: Jonathan.Sturm@w-hs.de

Data-driven modeling methods represent a great potential for the optimization of biotechnological processes. They are focussed on the development of so-called soft sensors, which are used to allow automation of processes and aim to establish innovative process control strategies. Therefore, the results shown are related to PAT aswell as the digital transformation of the biotechnology sector. Thus, they are of great interest for science and industry alike. [1]

For this purpose, fed-batch cultivations of the yeast Hansenula polymorpha were used as a dataset for developing models of the dependent variables product and biomass concentration. The techniques used for these approaches included artificial neural networks (ANNs), multiple linear regression (MLR), or support vector machines (SVM). The different modeling approaches were compared and validated. Afterwards, the optimal model for creating a soft sensor was selected for each target. [2]

The best estimation of dry cell weight was based on a gaussian kernel SVM and showed a RMSE of 2,7% of maximum biomass. The model for estimating product concentration was based on an ANN. Here, the deviation from the measured values was 8,4 %. The error size of both presented soft sensors is comparable to literature values [3]. Finally, the soft sensors were implemented into the software ProcessShield, for ease of use.

Overall, the possibilities of data-driven modeling for automation and optimization of biotechnological processes could be successfully presented based on these examples. Further studies on the basis of the presented results could drive the evolution towards fully autonomous, self-learning processes in bioprocessing forward. [4]

1. Rathore, A.S., Bhambure, A. and Ghare, V. (2010) Process analytical technology (PAT) for biopharmaceutical products. Analytical and bioanalytical chemistry. 398: 137-154.
2. Guerra, A.C. and Glassey, J. (2018) Machine learning in biopharmaceutical manufacturing. European Pharmaceutical Review. 23: 62-65.
3. Desai, K., Badhe, Y., Tambe, S.S. and Kulkarni, B.D. (2006) Soft-sensor development for fed-batch bioreactors using support vector regression. Biochemical Engineering Journal. 27:225-239.
4. Narayanan, H., Luna,M.F., von Stosch, M., Cruz Bournazou, M.N., Polotti, G., Morbidelli, M., Butté, A. and Sokolov, M. (2020) Bioprocessing in the digital age: The role of process models. Biotechnology Journal. 15: 1900172.

P14: A novel gradient-based monitored dark fermentation of biogenic feedstocks for material use in plug-flow reactors

Marion Longis, Joana PC Pereira, Peter Neubauer, Stefan Junne

Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
Email: marion.longis@tu-berlin.de

Anaerobic digestion (AD) of biogenic feedstocks is mainly applied for energetic uses as the main end-product is methane. In dark fermentation (DF), the methanogesis prevention results in H2 production and short-chain carboxylic acids (SCCAs) accumulation in the liquid phase for material uses. SCCAs offer a great potential as substrate in many sub-sequent bioprocesses, like polyunsaturated fatty acid production in microalgae and yeast or bacterial bioplastic production. Most of the AD or DF are performed in stirred tank reactors or plug-flow reactor (PFRs) in semi-continuous processes. While in STRs the reactions are homogeneously distributed in the reactor, in PFRs, the reactions are distributed regarding the hydraulic retention time (HRT) along the reactor resulting in a lengthwise AD step segregation namely hydrolysis, acidogenesis and methanogenesis. The AD of biogenic feedstocks in PFR is well-investigated but the monitoring is restricted to influent-effluent analysis and gas monitoring. In this work, the gradient formation along the reactor have been investigated by on-line monitoring of pH, oxidoreduction potential (ORP) and conductivity (ARC sensor series, Hamilton Bonaduz AG), in the liquid phase, in three points evenly distributed along a 12L tailor-made PVC and Teflon PFR. The total gas volume and gas composition in CO2, H2 and CH4 using a mass-flow controller BlueVcount coupled to BCP-sensors (BlueSens GmbH, DE) are recorded. Additionally, the liquid phase has been analyzed by evaluating the SCCAs accumulation, sCOD, FOS/TAC and electropolarizability (FDAP) as metabolic activity control. The combination of the on-line recorded parameters and off-line analysis have shown that the artificial acidification of the AD process led to an increased FOS/TAC values, ORP and SCCAs accumulation at the inlet and center ports of the reactor and a decrease in the conductivity resulting in an efficient AD to DF transition. Also, correlation analysis showed that the gradients formation, with the pH, ORP and conductivity variations along the reactor, appears in parallel to the SCCAs accumulation in the liquid phase and an enhanced H2 production. Regression analysis showed that the SCCAs accumulation is correlated to FDAP spectra. When no stirring is applied, the microbial accessibility to the substrate and, as a result, the hydrolysis, acidogenesis and acetogenesis occur mainly in the inlet zone of the PFR. Gradient formation was affected by the different feedstock loadings and process states, which provided sufficient data to analyze the relevance of gradient formation for each individual case and the potential of their on-line measurement for the achievement of higher process robustness at flexible feedstock load.

P15: BioProdPacific": a platform to accelerate the design of integrated and sustainable bioprocesses in Colombia

Erika Y. Ortiz, Carlos A. Alvarez, Andres A. Ceballos, Andrés Vargas, Nelson H. Caicedo.

Biochemical Engineering Department, Engineering, Universidad Icesi, Colombia
Email: nhcaicedo@icesi.edu.co

Five years ago, Universidad ICESI made a great deal on the Colombian future through the opening of its Biochemical Engineering undergraduate program, having, among other purposes, to train professionals to meet the needs of biotechnology-based industries located in the region. For this, the department's staff conceived and designed a pilot plant called "';BioProdPacific,"; which, after being built in early 2019, has been consolidated as a useful tool for both teaching and developing (research and innovation) new bioprocesses and biorefinery schemes using residual biomasses from local industries.

So far, 8 different theses have been developed at the pilot plant and a biorefinery scheme for Chontaduro peel is being tested. Chontaduro (Bactris gasipaes) peel is the primary residue originated from this fruit consumption and due to the significant amount represents both an environmental problem which can be switched to an economic growth opportunity. Our proposed integrated biorefinery scheme is able to use all the main components of Chontaduro peel (i.e., starch, cellulose, hemicellulose, lipids, phenols and carotenoids) to produce a broad range of bioproducts including succinic acid, polyphenols, biosurfactants, animal feed, antioxidants theses and CO2 capture. Our biorefinery scheme uses fermentation, biocatalyst and solvent extraction using green chemistry and circular economy principles to give value to agricultural waste and mitigate its carbon footprint. Additionally, the pilot plant's facilities (up-stream, transformation, and down-stream areas) support several courses of undergraduate and graduate programs to develop several competencies for the student under the alignment of outcomes of ABET for biochemical engineering.

P16: A small-scale hydrocyclone system as a biotechnological application for continuous cell separation

Huschyar Al-Kaidy[1], Jan Millauer[2], Bernd-Ulrich Wilhelm[2], Johannes Bader[1]

[1]Beuth University of Applied Sciences Berlin, Life Sciences and Technology, Berlin, Germany
2bbi-biotech GmbH, Berlin/Germany
Email: hal-kaidy@beuth-hochschule.de

Hydrocyclones are widely used in all areas of particle separation [1]. However, commercially available systems have not been used for cell harvesting since cells are sensitive to shear forces. In order to use hydrocyclones for cell culture separation, a two-stage, fully automated continuous separation process is being developed in the present project. In the first step of the separation, the cells will be separated from the liquid phase in a hydrocyclone and will then be returned to the fermenter. Afterwards, the solution is led through another hydrocyclone

where cell fragments and released DNA will be separated. This can be achieved by an automatized precipitation process, which will be developed in this project. Finally, the clear supernatant will be further processed by a continuous chromatography system.

In different prototypes, developed using CAD and 3-D printing, the cell separation of different cells is model-tested with yeasts and animal cells and characterized with respect to the cell separation behavior in the hydrocyclone. Flow rates are correlated with separation rates, the process stability will be determined with respect to different process parameters.

At a yeast concentration of 25 g L-1 a separation of ca. 56 % is possible. This concentration is about 100 times higher than the cell concentration used in the literature for separation experiments [2].

The prototypes showed that at flow rates up to 1500 ml min-1, the viability of the CHO cells after separation is about 85 percent. A hydrocyclone with four entrances enables a ca. 23 % higher viability of the CHO cells compared to a hydrocyclone with only two entrances.

In order to optimize the separation process, there is an ongoing research to find the optimal design of the hydrocyclone, the best precipitation medium and even more suitable process parameters.

P17: Optimization of H2-sensing regulatory hydrogenase production from Ralstonia eutropha in Escherichia coli

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

[1] Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]Technische Universität Berlin, Institut für Chemie, Straße des 17. Juni 135, 10623 Berlin, Germany
Email: qin.fan@campus.tu-berlin.de

Concerns about exhaustion of fossil fuels and global warming have led to increasing attention to clean and renewable energy. Here, biohydrogen is a very attractive alternative. The most efficient biohydrogen producers are hydrogenases. These enzymes display fascinating redox-chemical properties with tremendous promise as a biocatalyst for hydrogen fuel. However, due to the complex structure and maturation process of hydrogenases, their heterologous production has been a challenging task and their sensitivity to O2, CO, etc has seriously limited the potential applications [1].

The ß-proteobacterium Ralstonia eutropha H16 hosts four different O2-tolerant [NiFe]-hydrogenases (MBH, SH, AH and RH) [2]. The R. eutropha RH was selected as model for development of a heterologous [NiFe]-hydrogenase production system in Escherichia coli.

We investigated relevant cultivation parameters and obtained an initial production yield of 14 mg/L of purified RH in the initial batch E. coli shake-flask cultures. A 18-fold improvement of the production yield was achieved in EnPresso-based fed-batch-like shake-flask cultures. The RH productivity was further enhanced by a simple and highly efficient IPTG/lactose-autoinduction with shorter cultivation time, corresponding to several 1000-fold increase in the amount of RH purified from the homologous host R. eutropha. Moreover, different spectroscopic methods were used to analyze the catalytic activity in vitro and cofactor content of E. coli produced RH with or without co-expressing the corresponding R. eutropha maturation genes. Our results lay a good basis for the future production of functional hydrogenases for basic as well as applied science.

1. Fan, Q., Neubauer, P., Lenz, O. and Gimpel, M. (2020) Heterologous hydrogenase overproduction systems for biotechnology—An overview. Int. J. Mol. Sci. 21: 5890.
2. Lenz, O., Lauterbach, L., Frielingsdorf, S. and Friedrich, B. (2015) Oxygen-tolerant hydrogenases and their biotechnological potential. In Biohydrogen; DE GRUYTER: Berlin, München, Boston, pp 61–96.

P18: Evaluation of microbial hydrolysis for anaerobic digestion in a plug-flow reactor

Theresa Menzel, Peter Neubauer, Stefan Junne

Technische Universität Berlin, Institute of Biotechnology, Chair of Bioprocess Engineering, Straße des 17. Juni 135, 10623 Berlin, Germany
Email: t.menzel@tu-berlin.de, stefan.junne@tu-berlin.de

Valorization of residual biomass and waste streams into valuable products are the main aim in biorefinery processes like anaerobic digestion or bioplastic production. In most processes however, substrate hydrolysis is a limiting factor, requiring extensive pretreatment for good performance [1]. In this study, the microbial hydrolysis of maize silage in a plug-flow-reactor was evaluated to gain insight into important process parameters and enable the introduction of a microbial hydrolysis stage as a green method for yield optimization. As hydrolysis measurement is not a standard method, we evaluated the potential of several parameters for quick estimation of hydrolytic activity by on- or at-line parameters to enable process control.

Hydrolysis was examined in a plug-flow reactor in fed-batch mode for 60 days. After inoculation and a two week adaptation phase, anisotropic polarizability and CO2 % production increased. Organic loading above 8 kgTVSm-3d-1, pH < 3.7 and acidification > 27 % lead to process failure after 33 days with a quick drop of gas production and anisotropic polarizability while lactic acid accumulated up to a concentration of 10 gL-1. Correlation analysis suggest the evolution of an adapted microbial consortium in this second phase, able to withstand harsher pH-conditions. Main parameters with high influence on gas productivity and anisotropic polarizability found in this study are pH and total acid accumulation, while no significant correlation to hydrolytic activity was found.

1. Menzel, T., Neubauer, P. and Junne, S. (2020) Role of Microbial Hydrolysis in Anaerobic Digestion. Energies. 13: 55.

P19: Exploring the potential of biofilms for fermentation-based biomanufacturing

Pascal S. Leonov[1,2], Krist V. Gernaey[2], Claus Sternberg[1]

[1]Department of Biotechnology and Biomedicine, Technical University of Denmark, Kgs. Lyngby, DK
[2]Department of Chemical and Biochemical Engineering, Technical University of Denmark, Kgs. Lyngby, DK

Harnessing the potential of biocatalytic conversion of renewable biomass into value-added products is still hampered by unfavorable process economics. This partly arises from limitations of traditional suspended culture systems regarding (i) productivities and product yields, (ii) sensitivity towards operation under hazardous environments, and (iii) limitations in converting complex and crude substrates.

We are focusing on harnessing the benefits of biofilm-systems for continuous fermentations to improve process metrics of bulk chemical production. Furthermore, we want to utilize the enhanced tolerance of biofilm-encased cells for improving the utilization of cheap waste as feedstock for reducing process costs. Thereby, we are aiming to foster the sustainable production of bulk chemicals for substituting their petrochemical-derived counterparts.

P20: Production of enzyme laccase at pilot scale by using loofah-immobilized biomass of Ganoderma chocoense

Natalia Andrea Llanos[1,2], Luisa Fernanda Navarrete[1,2], Juan Camilo Gutierrez[2], Andrés Felipe Vargas[2], Aida M. Vasco-Palacios[3], Nelson H. Caicedo[2].

[1]Department of Biology, Natural Sciences,Universidad Icesi, Colombia
[2]Department of Biochemical Engineering, Engineering, Universidad Icesi, Colombia
[3]Biomicro-School of Microbiology, Universidad de Antioquia, Colombia.
Email: nataliallanos26@hotmail.com

Through a protocol of bioprospecting, a native white-rot fungus strain isolated from humid low mountain forest, La Carolina, Cali, Colombia, and identified as Ganoderma chocoense, showed a high potential to synthesize lignocellulolytic enzymes such as laccase and to biotransforms some synthetic colorants. Willing to increase the development of bioprocesses in Valle del Cauca region and the sustainable use of biodiversity as well, a process concept by using cells of this fungus immobilized on pieces of loofah sponge was evaluated at a pilot-scale for the production of the enzyme laccase. During the design process, it was demonstrated that sugar cane vinasse, a massive by-product of the sugar mills in the region, can be used as an inductor of this enzyme during the fungus cultivation.

The experimental set-up was performed in the "BioProdPacific" pilot-plant at Universidad Icesi, where the fermentation was carried out in a stirred tank (40 liters) and also came up with the enzyme separation process to purify it partially, as a product 0.5 L of a laccase solution with an enzymatic activity of 366.1 UI/L was obtained. The complete layout of the process was schematized and modeled by using SuperPro Designer software, and a preliminary techno-economic feasible study was run. Even though a financial analysis showed a promissory scenario by considering the partial purification (ultrafiltration, precipitation with ammonium sulfate, centrifugation and dialysis), the results showed an essential loss of activity with a low purification factor. Additionally, through electrophoresis, the molecular weight of two laccase isoenzymes present in the partially purified enzyme was determined (50-75 kDa). This demonstrates that G. chocoense is a potential biotransformation agent applicable to future industrial processes in the region, both to produce the enzyme laccase and to develop bioremediation process.

P21: Integration of a robotic small-scale bioreactor system as a prerequisite for a selflearning and autonomous cultivation platform

Lucas Kaspersetz, Marie-Therese Schermeyer, Sebastian Hans; Peter Neubauer, Mariano Nicolas, Cruz Bournazou

[1] Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
Email: L.Kaspersetz@tu-berlin.de

The process industries are moving towards digitalization and automation, known as industry 4.0, where smart factories allow different unit operations to interacting with each other. In contrast to that, the biotechnological/bio-pharmaceutical sector lacks this transition and does not even fulfill the automation standards of industry 3.0, while facing the necessity of faster development cycles [1]. As a prerequisite for this transition, cultivation platforms featuring parallelization, miniaturization and automation have become inevitable, accounting for high-throughput bioprocess development (HTBD) [2].

In order to enhance automated high-throughput microbial process development and optimization under consistent scalable conditions, we present the integration of a commercially available small-scale bioreactor system (BioXplorer 100, HEL), a liquid handling system (LHS) & a microplate photometer into our HTBD-robotic facility [3]. The system consists of eight individually controllable vessels (20 - 150 mL), each equipped with three pumps (acid, base, feed) and a mass flow controller (up to 200 mL/min). This allows for flexible cultivation modes (batch, high-cell-density-fed-batch, continuous cultivations) and online, pH, DO, turbidity measurements. Moreover, the LHS guarantees an automated sampling procedure, followed by photometric measurements of enzymatic-based assays for substrate, metabolite or product quantification. The integration and data management follows the F.A.I.R data principle [4], storing the corresponding online, at-line & offline measurements of each bioreactor, as well as the executed liquid handling steps, in a centralized database by using standardized communication protocols (SiLA 2). Additionally, communication between the devices enables trigger-based events forcertain tasks e.g. inducer addition. The usability & benefits are assessed during an initial cultivation with two different expression systems under four different conditions. As a first step, this will enable further automated data processing, data analysis, implementation of adaptive modeling frameworks and deployment by dynamical machine learning models. Finally, leading towards autonomously performed experiments.

1. Narayanan, H., Luna,M.F., von Stosch, M., Cruz Bournazou, M.N., Polotti, G., Morbidelli, M., Butté, A. and Sokolov, M. (2020) Bioprocessing in the digital age: The role of process models. Biotechnology Journal. 15: 1900172.
2. Hemmerich, J., Noack, S., Wiechert, W. and Oldiges, M. (2018) Microbioreactor systems for accelerated bioprocess development. Biotechnology Journal. 13: 1700141.
3. Haby, B., Hans, S., Anane, E., Sawatzki, A., Krausch, N., Neubauer, P. and Cruz Bournazou, M.N. (2019) Integrated robotic mini bioreactor platform for automated, parallel microbial cultivation with online data handling and process control. SLAS Technology. 24: 569–82.
4. Wilkinson, M.D., Dumontier, M., Aalbersberg, I, et al. (2016) The FAIR guiding principles for scientific data management and stewardship. Scientific Data. 3: 160018.

P22: Importance of oxygen signal shape matching for robust parameter estimation in bioprocess development

Judit Aizpuru[1], Annina Kemmer[1], Niels Krausch[1], Peter Neubauer[1], Mariano Nicolas Cruz Bournazou[1], Tilman Barz[2]

[1] Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]Austrian Institute of Technology, Center for Energy, Giefingasse 4, 1210 Vienna, Austria
Email: judit.aizpuru@tu-berlin.de

The characterization of macro kinetic growth models is one of the main tasks in high throughput bioprocess development, where accurate parameter estimations can enable a better understanding of the system and improve the performance of model predictive control actions, among others [1]. The experiments performed for this characterization typically gather different measurements at varying sampling rate, e.g. at-line measurements for biomass and metabolites, versus online monitoring for gases and pH. The usual procedure to fit these data to the systems model, is to minimize the sum of the squared residuals known as Least Square Estimation (LSE), where the measurement errors are assumed to be normally distributed [2]. However, oxygen signals manifest very rapid dynamics, and the optical sensors typically used for its measurement have shown a delayed response. Even if this latter drawback can be solved by calibrating the sensor with a first order step response [3], the variance of the response time remains still high in a system of parallel bioreactors, leading to a poor measurement accuracy and therefore a wrong estimation of the model parameters using LSE. The hypothesis of this work is that using a different estimator which considers the signals shape and not

just amplitude, as some estimator derived from Dynamic Time Warping, the overall quality of the fitting problem improves. The comparison study and validation are made via an in-silico data generator with assumed error models and known parameter values.

1. Hans, S., Haby, B., Krausch, N., Barz, T., Neubauer, P. and Cruz-Bournazou, M.N. (2020) Automated conditional screening of multiple Escherichia coli strains in parallel adaptive fed-batch cultivations. Bioengineering. 7: 145.
2. López, D.C., Barz, T., Körkel, S. and Wozny, G. (2015) Nonlinear ill-posed problem analysis in model-based parameter estimation and experimental design. Computers & Chemical Engineering. 77: 24–42.
3. Anane, E., López, D.C., Neubauer, P. and Cruz-Bournazou, N.M. (2017) Modelling overflow metabolism in Escherichia coli by acetate cycling. Biochemical Engineering Journal. 125: 23–30.

P23: Determination of plasmid mutation rates in Escherichia coli using an automated high-throughput Quasi-Turbidostat

Isabel Schumann, Fabian Schröder, Sebastian Hans, Peter Neubauer, Matthias Gimpel

Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
Email: matthias.gimpel@tu-berlin.de

The use of plasmids as genetic carriers to produce recombinant proteins, pharmaceuticals or chemicals is an established method and will continue to be used in the future. The functionality of the plasmid contributes significantly to the quality and quantity of the products. Mutations that occur during cultivation usually have a negative influence on the plasmid and consequently on the cell. Possible consequences are thus a reduction in product quantity or also contamination through increased by-product formation. While mutation processes of chromosomal DNA have already been described in the literature [1, 2], the mutation rate of plasmids has been neglected until now.

The aim of this work is to determine the plasmid mutation rate and to establish existing relationships between mutation rate, replication mechanism and specific copy number of the plasmid. To test this, different reporter systems were constructed, which differ in the characteristic properties of plasmids. An automated Quasi-Turbidostat [3] was used to perform the cultivation, as this allows parallel, individual, and stress-free cultivation of multiple constructs up to a certain generation.

The results show a clear influence of the copy number on the mutation rate. A low specific copy number favours a high mutation rate. Differences can also be seen in the replication mechanism. The rolling circle mechanism seems to be less susceptible to mutations compared to Θ-type replication. Furthermore, it was shown that a mutation of the plasmids affects the entire cell as soon as a small proportion of the plasmids per cell have this mutation.

It is shown that the plasmid mutation rate is a non-negligible factor. With a growing knowledge of the plasmid mutation rate and the avoidance of the use of susceptible plasmids, bioprocesses can be further optimised and a better quality of the product can be ensured.

1. Matic, I., Radman, M., Taddei, F., Picard, B., Doit, C., Bingen, E., Denamur, E., and Elion, J. (1997) Highly variable mutation rates in commensal and pathogenic Escherichia coli. Science. 277: 1833-1834.
2. Williams, A. B. (2014) Spontaneous mutation rates come into focus in Escherichia coli. DNA Repair. 24: 73–79.
3. Hans, S., Gimpel, M., Glauche, F., Neubauer, P. and Cruz-Bournazou, M.N. (2018) Automated cell treatment for competence and transformation of Escherichia coli in a high-throughput quasi-turbidostat using microtiter plates. Microorganisms. 6: 60.

P24: Feasibility analysis of a non-stirred miniature bioreactor

Jonathan Poit[1], Frederik Steiner[1], Jonas Mader[1], Nicolas Feuser[2], Katharina Paulick[1], Sebastian Hans[1] and Peter Neubauer[1]

[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]Lonza AG, Lonzastrasse 2, 3930 Visp VS, Switzerland
Email: frederik.steiner@tu-berlin.de

High-throughput minibioreactor systems are used to accelerate the upscaling process in bioprocess development. To transfer knowledge from the lab-scale to the pilot- and industrial scale, reliable scale-up parameters are required. The volumetric oxygen- and energy inputs constitute critical parameters. Modern multi-bioreactor systems rely on impellers, magnetic stirrers or agitation to supply oxygen to the culture medium. Disadvantages of these systems include temperature shifts caused by changes of stirrer speed, shear force gradients and high energy inputs. To overcome these drawbacks, the aim of this work was to assess the miniaturization capabilities of a non-stirred 3D-printed bioreactor system and its use in 12 mL scale. The CAD software SolidWorks was used to generate CAD-files, 3D-printing was conducted with the Formlabs printer Form 2. To assess the oxygen input, kLa-values were determined by the gassing-out method with nitrogen. Temperature shifts were measured as an indication of energy input. The 3D-printed system was compared to a commercially available stirred system. This study revealed lower oxygen input and lower temperature shifts for the designed system (kLa: 109 - 352 h-1, temperature shifts: 0.38 - 0.66 K) compared to the stirred system (kLa: 333 - 443 h-1, temperature shifts: 0.96 - 4.04 K). The results indicate the potential of the non-stirred system in multi-bioreactor-systems.

P25: Developing a unified IT Platform covering the whole development cycle - A Case study for Enzyme Production

Simon Seidel[1], Sebastian Hans[1], Julia Katharina Schollmeyer[2], Katharina Paulick[1], Anke Kurreck[2], Stefan Krauss[3], Mariano Nicolas Cruz Bournazou[1,4], Peter Neubauer[1]

[1] Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]BioNukleo GmbH, Ackerstraße 76, D-13355 Berlin, Germany
[3]Siemens AG, Digital Industries, Process Automation, Technology & Concepts, DI PA PHA OPS TC, Siemensallee 84, 76187 Karlsruhe, Germany
[4]DataHow AG, c/o ETH Zurich, HCI, F137, Vladimir-Prelog-Weg 1, 8093 Zurich, Switzerland
Email: simon.seidel@tu-berlin.de

Through a protocol of bioprospecting, a native white-rot fungus strain isolated from humid low mountain forest, La Carolina, Cali, Colombia, and identified as Ganoderma chocoense, showed a high potential to synthesize lignocellulolytic enzymes such as laccase and to biotransforms some synthetic colorants. Willing to increase the development of bioprocesses in Valle del Cauca region and the sustainable use of biodiversity as well, a process concept by using cells of this fungus immobilized on pieces of loofah sponge was evaluated at a pilot-scale for the production of the enzyme laccase. During the design process, it was demonstrated that sugar cane vinasse, a massive by-product of the sugar mills in the region, can be used as an inductor of this enzyme during the fungus cultivation.

The experimental set-up was performed in the "BioProdPacific" pilot-plant at Universidad Icesi, where the fermentation was carried out in a stirred tank (40 liters) and also came up with the enzyme separation process to purify it partially, as a product 0.5 L of a laccase solution with an enzymatic activity of 366.1 UI/L was obtained. The complete layout of the process was schematized and modeled by using SuperPro Designer software, and a preliminary techno-economic feasible study was run. Even

though a financial analysis showed a promissory scenario by considering the partial purification (ultrafiltration, precipitation with ammonium sulfate, centrifugation and dialysis), the results showed an essential loss of activity with a low purification factor. Additionally, through electrophoresis, the molecular weight of two laccase isoenzymes present in the partially purified enzyme was determined (50-75 kDa). This demonstrates that G. chocoense is a potential biotransformation agent applicable to future industrial processes in the region, both to produce the enzyme laccase and to develop bioremediation process.

P26: Reproducing dynamic environment in microfluidic single-cell cultivation based on computational lifeline analysis

Phuong Ho[1], Sarah Täuber[2], Alexander Grünberger[2], Eric von Lieres[1]

[1]Forschungszentrum Jülich, IBG-1: Biotechnology, Wilhelm-Johnen-Str. 1, 52428 Jülich, Germany
[2]Universität Bielefeld, Multiscale Bioengineering, Universitätsstraße 25, 33615 Bielefeld
Email: p.ho@fz-juelich.de; sarah.taeuber@uni-bielefeld.de

The biotechnological production of valuable substances is typically complicated by the loss of microbial performance upon scale-up [1-3]. This challenge is mainly caused by discrepancies between homogeneous environmental conditions at laboratory scale, where organisms are optimized, and inhomogeneous conditions in large-scale bioreactors, where the production takes place. To improve strain selection and process development, it is thus of major interest to characterize these fluctuating conditions at large scales and investigate their impact on microbial cells.

In this contribution, we will demonstrate the high potential of dynamic microfluidic single-cell cultivation combined with computational fluid dynamics (CFD) simulation of large-scale bioreactors. CFD simulations of a 300 L bioreactor were applied to characterize environmental conditions in large-scale bioreactors. So-called lifelines were determined by simulating multiphase turbulent flow and mass transport combined with particle tracing. Glucose availability experienced by the microorganism Corynebacterium glutamicum was traced. Resulting lifelines were discretized into low, medium and high glucose availability regimes. Discretized lifelines were used as feeding profiles of a dynamic microfluidic single-cell cultivation (dMSCC) system to investigate how the fluctuating glucose concentration affects cellular physiology and colony growth rate.

The presented approach paves the way for an improved understanding of how the cellular lifelines of large-scale bioreactors influence the cellular response within growth and production. It also provides insights into how to understand the conditions in large-scale bioreactors from the view of a microorganism and the dependence of cell wellbeing on the observed conditions.

1. Oosterhuis N.M.G. and Kossen N.W.F. (1984) Dissolved-oxygen concentration profiles in a production-scale bioreactor. Biotechnol. Bioeng. 26: 546-550.
2. Larsson G. and Enfors S.O. (1985) Influence of oxygen starvation on the respiratory capacity of Penicillium chrysogenum. Appl. Microbiol. Biotechnol. 21: 228-233.
3. Enfors S.O., Jahic M., Rozkov A., et al. (2001) Physiological responses to mixing in large scale bioreactors. Journal Biotechnol. 85: 175-185.

P27: Rapid and cost-effective fabrication of microchromatography integrated with microelectrode impedance sensor for determination and characterization of column efficiency and effluent

Amin Javidanbardan[1,2], Virginia Chu[3], João P. Conde[2,3], Ana M. Azevedo[1,2]

[1]IBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal
[2]Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal
[3]Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC MN) and IN – Institute of Nanoscience and Nanotechnology

Chromatography is considered an indispensable operation unit for purifying therapeutic biomolecules in biopharmaceutical processes [1]. Generally, the common lab-scale and pilot-scale strategies for defining suitable resin and chromatographic operations are tedious and costly [2]. Hence, more economical and rapid bioprocess development and scale-up strategies are in demand in the industry. For this purpose, many research works have been focused on strategies to further scale-down and parallelize experimentations by implementing good representative models such as microfluidic structures [3,4].

Our research work defined a fast and straightforward method to fabricate microchromatographic columns- with a circular cross-section- using micromilling technology outside the cleanroom environment. PMMA polymer was selected as a substrate for micromilling and then used as a first master mold for PDMS patterning. The surface roughness of the PMMA master mold was minimized by optimizing influential parameters in the micromilling process using Design of Experiment (DOE), Response Surface Methodology (RSM). Via exploiting the intrinsic flexibility of micromilling fabrication, our microstructure was designed in a way to easily integrate the specifically developed interdigitated planar microelectrodes in the microchromatography outlet channel. The presence of impedance spectroscopy allows us to determine the conductivity of the effluent during the process, which could also be used to assess resin packing quality and hydrodynamic dispersion factor. Besides, it could provide more information about the effluent's other compositions by measuring impedance in the other electrical frequency ranges.

1. Hahn R. (2012) Methods for characterization of biochromatography media. J Sep Sci. 35: 3001–3032.
2. Stamatis, C., Goldrick, S., Gruber, D., Turner, R., Titchener-Hooker, N.J. and Farid, S.S. (2019) High throughput process development workflow with advanced decision-support for antibody purification. J Chromatogr A. 1596: 104–116.
3. Pinto, I.F., Soares, R.R.G., Aires-Barros, M.R., Chu, V., Conde, J.P. and Azevedo, A.M. (2019) Optimizing the performance of chromatographic separations using microfluidics: Multiplexed and quantitative screening of ligands and target molecules. Biotechnol J. 14: 1–11.
4. Keller, W.R., Evans, S.T., Ferreira, G., Robbins, D. and Cramer, S.M. (2017) Understanding operational system differences for transfer of miniaturized chromatography column data using simulations. J Chromatogr A. 1515: 154–163.

P28: Advanced robotic workflows for integrating mass spectrometry based multi-component analysis into metabolic phenotyping

Alexander Reiter, L. Herbst, Wolfgang Wiechert, Marco Oldiges

Institute of Bio- and Geosciences, IBG-1: Biotechnology, Forschungszentrum Jülich, Jülich, Germany
Email: a.reiter@fz-juelich.de
Email: frederik.steiner@tu-berlin.de

In recent years, highly parallelized and automated cultivation systems were established as valuable tools for bioprocess

development and microbial strain characterization. Integrating these systems into robotic platforms enabled automated sampling and sample processing procedures. This allowed substrate and product concentrations to be monitored at-line using spectroscopic assays [1]. While easy to automate, these assays are generally specific for a single target product only, which limits information gain and raises the need for additional laborious and time-intensive off-line analytics like UPLC or LC-MS/MS.

In this study, we present robotic workflows for microbial producer strain phenotyping by multicomponent analysis of small molecules via ultra-fast dilute-and-shoot flow-injection-analysis tandem mass spectrometry (DS-FIA-MS/MS). Automated modules for microtiter scale inoculation, pre-cultivation, sacrifice and repeated low-volume sampling were developed and applied to microtiter batch cultivations of Corynebacterium glutamicum strains. Time-resolved product and by-product concentrations were determined by automated sample preparation and subsequent amino acid analysis by DS-FIA-MS/MS with an analysis time of 1 min / sample.

By implementing an integrated pre-culture, reproducibility of growth behavior was improved and volumetric productivity increased distinctly. Applying these workflows to an L-Histidine producing library of 96 strains highlighted the potentials for screening of strain performance as the most promising producer was identified within three automated experiments. The workflows are very generic and can be easily adapted to other strains and products to facilitate and accelerate bioprocess development as they decrease time and effort, while increasing detailed insight into the metabolic phenotype.

1. Hemmerich, J., Tenhaef, N., Steffens, C., et al. (2019) Less sacrifice, more insight: Repeated low-volume sampling of microbioreactor cultivations enables accelerated deep phenotyping of microbial strain libraries. Biotechnol J. 14: e1800428.

P29: From screening to production: a holistic approach of high-throughput model-based screening for recombinant protein production

Niels Krausch[1], Sebastian Hans[1], Felix Fiedler[2], Sergio Lucia[2], Peter Neubauer[1], Mariano N. Cruz Bournazou[3]

[1] Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[2]Technische Universität Berlin, Internet of Things for Smart Buildings, Einstein Center Digital Future, Einsteinufer 17, 10587 Berlin, Germany
[3]DataHow AG, Vladimir-Prelog-Weg 1, 8093 Zurich, Switzerland
Email: n.cruz@datahow.ch

Efficient and robust screening of production strains in early bioprocess development is usually hampered by the limited cultivation resources and identification of dynamical cell parameters for the complete design space. Even though High-Throughput (HT) liquid handling stations enable a large number of strains to be tested, these experiments provide no insight into the dynamical phenotype of the strains. This is especially critical in scale-up, since cultivations in industrial bioreactors expose the microbial cell factories to significant stresses due to substrate, oxygen, and pH gradients among others. In an effort to address this challenge and reduce the risk of failure during scale-up, new HT scale down systems based on model-based operation strategies have been developed and extended to conditional screening experiments.

In this work we further extend the existing platform to enable a feedback control of the 24-parallel mini-bioreactor setting, using a recursive moving horizon parameter estimation combined with a model-predictive control approach to calculate an optimal feeding regime, which exposes the cells to stress conditions similar to those present in large-scale bioreactors. We present a case study showing the advantages of the framework by screening a set of E. coli strains for obtaining highest biomass at the end of the process. The results show that the prediction and selection of the most suitable strain for industrial production is significantly improved.

P30: Feasibility analysis of a non-stirred miniature bioreactor

Fabian Schröder, Sebastian Hans, Matthias Gimpel, Peter Neubauer

[1] Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
Email: fabian.schroeder@tu-berlin.de

Industry is always looking to optimise its processes. One major area is the optimisation of the production strain itself. The modification of promoters, regulation by sRNA or targeted metabolic engineering are just a few of the diverse and creative solutions that bring about an increase in the organism's performance. Metabolic engineering includes the hypothesis that a genetically determined deactivation of unnecessary regulatory cascades or other synthesis pathways can bring about such a performance-enhancing effect. On the one hand, increasingly efficient genome editing strategies lead to large mutant libraries such as the Keio Collection, but on the other hand, there is a lack of methods to screen these large libraries for targeted purposes just as rapidly.

This study focuses on filling this gap. Using the Quasi-Turbidostat (Hans et al., 2018), the plasmid-based reporter system pAG032 (Garwin et al., 2019) is transformed into a large selection of strains from the Keio Collection. This reporter system allows the observation of industrially important cell properties such as productivity, ribosomal capacity and metabolic stress responses. Subsequently, enzyme-based fed-batch screening is performed in the same 96-well plate scale.

The results show a significant and partly surprising influence on the target parameters. While hardly any differences in growth behaviour can be detected, reductions of up to -88% or increases of 65% can be observed for the synthesis of recombinant proteins. In combination with the associated other two cell parameter, minimum requirements were set, from which 7 potential genes emerged whose deletion causes an increase in performance.

It has been shown how important it is to reduce production strains not only to the growth potential of a microorganism alone. Additionally, it confirms the assumptions that a reduction of unused regulatory cascades leads to an optimisation of performance and that gene functions can be better understood using specific reporter systems. The final step for the project would be to screen the favourites in stress-like conditions of a production scale.

P31: The batch brewing process represented by a mechanistic model

Maximilian Schmacht[1,2], Caroline Hamelmann[2], Sebastian Hans[2], M. Nicolas Cruz Bournazou[2,3,4], Peter Neubauer[2], Martin Senz[1]

[1]Research and Teaching Institute for Brewing in Berlin (VLB), Department Bioprocess Engineering and Applied Microbiology, Berlin, Germany
[2]Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[3]Department of Chemistry and Applied Biosciences, ETH Zurich - Institute of Chemical and Bioengineering, Zurich, Switzerland
[4]DataHow AG, Zurich, Switzerland
Email: m.schmacht@vlb-berlin.org

On the way to a more economic process management, different possibilities such as saving energy or manpower by faster and more efficient fermentations are followed. A profound and proper understanding of the fermentation itself is crucial for a

valuable exploitation of the process. Mathematical modeling serves as a powerful tool to deepen and structure the process knowledge and can beneficially be used for process development.

A mechanistic mathematical model for batch brewing fermentations is presented considering the stepwise utilization of the three main sugars of brewer's wort, i. e. glucose, maltose and maltotriose for the formation of biomass, ethanol and CO2. Thereby the aerobic conditions in the beginning as well as the anaerobic conditions in the progress of the fermentation are taken into account. The model was fitted to experimental data from 5 L-benchtop bioreactors as well as µL-scale experimental data from the miniaturized bioprocessing platform BioLector® Pro serving as a screening platform. Having this model as a base, further research on the development of a fed-batch brewing fermentation under high gravity conditions and model extensions can be conducted.

P32: Unraveling the microbial dark matter using picolitre gel droplets

Bianka Kästner[1,2,3], Sundar Hengoju[3], Ashkan Samimi[3], Johannes Bader[1], Peter Neubauer[2], Miriam Agler-Rosenbaum[3]

[1]Beuth University of Applied Sciences, Life Sciences and Technology Department of Biotechnology – Berlin
[2] Technische Universität Berlin, Chair of Bioprocess Engineering, Ackerstraße 76, 13355 Berlin, Germany
[3]Leibniz Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Bio Pilot Plant – Jena
Email: miriam.rosenbaum@leibniz-hki.de

The urge to explore the microbial dark matter is more pressing than ever. A deeper understanding of the microcosmos around us may offer new possibilities to cope with urgent problems, like the acidification of soil [1], rising antimicrobial resistance [2], or the transfer from fossil resources to renewables [3, 4]. Metagenomic analyses of environmental samples have revealed a large unknown world of microorganisms with an impressive biosynthetic potential [5].

Although the understanding of the microbial diversity is improving with these investigations, it still remains a challenge to bring the so far uncultured into stable lab culture. In recent years, microfluidic technologies have been introduced for massively parallel cultivation and possesses huge potential for domestication so far uncultured microbes. Individual cells can easily be singularised, compartmentalised and cultivated from mixed cultures using droplet microfluidics. Cell encapsulation has been applied in various studies improving of the culturability of bacteria from environmental samples [6-8].

We hypothesise that bacterial growth can be further enhanced in gelled microdroplets by a barrier that protects against inhibitory factors and cell stress. At the same time, the membrane pores still allow the exchange of small molecules, thereby enabling cell-to-cell interactions. In this proof-of-principle development, environmental cells are incubated at single cell level inside of small gel vessel. These gel microdroplets are produced using microfluidic chips that disperse either ultra-low melting agarose or alginate in an inert oil phase at droplet frequencies up to 800 Hz. Formed droplets are gelled by ionic crosslinking and serve as miniaturised incubation systems with volumes of 100 to 250 pL. Due to the entrapment in a membrane, subsequent analysis can be carried out using fluorescence-activated cell sorting (FACS) that facilitates sorting mechanisms by frequencies higher than 10,000 drops per second. This allows for rapid analysis of cell growth including separation of cell-containing droplets for further analysis and incubation. Thus, slow- and fast-growing microorganisms can be separated from each other and the enrichment of slow growers is enabled. The presentation will focus on the initial steps of cell encapsulation, gel droplet generation, and sorting.

1. Sullivan, T. S., Barth, V. and Lewis, R.W. (2017) Soil acidity impacts beneficial soil microorganisms WSU Extension. FS247E: 1-6.
2. Willyard, C. (2017) The drug-resistant bacteria that pose the greatest health threats. Nature, 543: 15.
3. Horn, S.J., Vaaje-Kolstad, G., Westereng, B. and Eijsink, V.G. (2012) Novel enzymes fort he degradation of cellulose. Biotechnol Biofuels. 5: 45.
4. Sharma, S., Basu, S., Shetti, N.P. and Aminabhavi, T.M. (2020) Waste-to-energy nexus for cicular econom and environmental protection: Recent trends in hydrogen energy. Sci Total Environ. 713: 136633.
5. Wohlleben, W., Mast, Y., Stegmann, E. and Ziemert, N. (2016) Antibiotic drug discovery. Microb Biotechnol. 9: 541-548.
6. Alkayyali, T., Pope, E., Wheatley, S.K., Cartmell, C., Haltli, B., Kerr, R.G. and Ahmadi, A. (2020) Development of a microbe domestication pod (MD Pod) for in situ cultivation of micro-encapsulated marine bacteria. Biotechn Bioeng. 118: 1166-1176.
7. Dichosa, A.E., Daughton, A.R., Reitenga, K.G., Fitzsimons, M.S. and Han, C.S. (2014). Nat Protoc. 9: 608-621.
8. Mahler, L., Niehs, S., Martin, K., Weber, T., Scherlach, K., Agler-Rosenbaum, M., Hertweck, C. and Roth, M. (2019) Higly parallelized microfluidic droplet cultivation and prioritization on antibiotic producers from complex natural microbial communities. BioRxiv. doi: 10.1101/2019.12.18.877530.

EXHIBITORS / SPONSORS / MEDIA PARTNER

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

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

BioProScale e.V. (VR 38529 B)