3rd BioProScale Symposium

# Inhomogeneities in large-scale bioprocesses

## System biology and process dynamics

2 to 4 April 2014
Berlin – Germany

Three-day symposium about development and application of bioprocesses in industrial scale

- **Quality by design in bioprocess development**
  High throughput applications, in-line process analytics, data handling and analysis, multivatiate data processing, scale down methods, evolutionary strategies for strains and processes
- **Scale down approaches and process analytical technologies for advanced process design**
  Comprehensive process monitoring, non-invasive sensors for all process scales, multiparameter sensors, addressing inhomogeneities by sensor applications and multiposition sampling, modelling and control approaches, influence of early process steps on later down-stream operations
- **Novel reactors, automation and control concepts**
  Process design including feed-back and feed-forward strategies, process control by data based and mechanistic models, model-based experimental design strategies

**Organisers** Technische Universität Berlin – Chair of Bioprocess Engineering & Institute for Biotechnology and Fermentation in Berlin (IfGB)

**Location** Technische Universität Berlin, Institute for Chemistry, Lecture hall C130
Strasse des 17. Juni 115, 10623 Berlin (Charlottenburg), Germany

**Institute for Biotechnology and Fermentation in Berlin (IfGB)**
**Seestrasse 13, 13353 Berlin, Germany**

www.bioproscale-conference.org

Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik
Chair of Bioprocess Engineering

IfGB
Institute for Biotechnology and Fermentation in Berlin

In cooperation with

BIO PROSCALE network

unicat

## Welcome address

Dear Colleagues, Ladies and Gentlemen
Dear Guests and Students

My co-workers and I would like to warmly welcome you to Berlin to the 3rd BioProScale Symposium. The aim of this symposium is to continue the intensive discussions which started during the previous symposia in 2009 and 2012. This year we extend the focus of large-scale considerations to the whole process development line and will also take into consideration how to implement more consistency into the development of new processes. We are especially excited about the fascinating opportunities which become possible through automation technologies and new analytical principles and devices. The systematic implementation of Quality by design (QbD) and Process Analytical and Control Technologies increase the efficiency of bioprocess development and the robustness of the processes themselves.

An important aspect of this Symposium is the participation of scientists and engineers from different biotechnological disciplines which are all connected by the similarity of the principles which are relevant for the industrial scale. Consequently, also the requirement to start bioprocess development from the perspective of the large scale process is the same for all areas of applied biosciences and a basic need to save time and costs, and decrease the investment risks.

We are very delighted to have a wealth of promising lectures in our programme and would like to extend our gratitude especially to the speakers who followed our invitation and will share and discuss their expertise with us , as well as to our exhibitors and sponsors, who provided a substantial basis for a pleasant atmosphere.

I wish you all a very interesting symposium and a great stay in Berlin!

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

## Scientific advisory board

Dr. Henk Noorman (DSM, The Netherlands)
Prof. Dr. Matthias Reuss (Universität Stuttgart, Germany)
Prof. Dr. Nico Oosterhuis (Cellution Biotech, The Netherlands)
Dr. Claus Lattemann (Sanofi, Frankfurt, Germany)
Dr. Frank Delvigne (University of Liege, Belgium)
Prof. Dr. Krist V. Gernaey (DTU Chemical Engineering, Lyngby, Denmark)
Dr. Stefan June (TU Berlin, Germany)
Prof. Dr. Peter Neubauer (TU Berlin, Germany)

## About the organisers

### Technische Universität Berlin: Department of Biotechnology – Chair of Bioprocess Engineering

The Department of Biotechnology has six chairs: Bioprocess Engineering, Medical Biotechnology, Bioanalysis, Microbiology and Genetics, Brewing Technology and Applied Biochemistry.

The Chair of Bioprocess Engineering, which was newly established in 2008, has three focus areas:

(i) Large-scale bioprocessing including cell physiology and flux analysis of microbial processes in inhomogenous bioreactor systems, scale-down simulators, mobile sensors and sampling devices for measurements in the bulk liquid of large-scale bioreactors

(ii) Biocatalysis – development of new biocatalysts and biocatalytic products on the basis of structure based evolution and metabolic engineering

(iii) High throughput bioprocessing, robots in bioprocess development, especially for recombinant proteins

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 for more then 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 IfGB.

Since 2003, under the brand name IfGB services and training for the spirits industry and distillers have been offered. Starting in 2009 our service and training programmes will be expanded into the field of biotechnology – again in close cooperation with the Institute of Biotechnology of TU Berlin.

www.ifgb.de

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

**www.bioproscale-conference.org**

## Exhibitors & Sponsors

PreSens PRECISION SENSING
HAMILTON
infoteam software AG
sequip
Cell tainer OPTIMAL MASS TRANSFER
BELACH BIOTEKNIK
EloSystems Products for Biotechnology
SANOFI
sartorius

***Compliance note:*** *For the placement of the company logo on our website and in the conference documentation we received from Sanofi-Aventis a financial support of 1000 €.*

## Programm at a glance, Wednesday, 2 April 2014

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

**13:30** **Plenary Lecture: Exploring the heterologous genomic space for building, stepwise, complex, microbial strains for large-scale bioprocessing (L01)**
Eleftherios Terry Papoutsakis (Delaware Biotechnology Institute and Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, USA)

### Quality by design in bioprocess development

Chair Andreas Knepper (TU Berlin, Dep. of Bioprocess Engineering)

**14:15** **Key note lecture: Approaching the microbial growth space for systematic bioprocess development (L02)**
Raivo Vilu
(Technical University of Tallinn and CCFFT, Tallinn, Estonia)

14:45 Coffee break with poster session and exhibition

**15:20** **Reviving QbD – A mechanistic approach (L03)**
Patrick Sagmeister (TU Wien, Austria)

**15:50** **Key note lecture: Plasmid DNA production by E. coli: Cell engineering, cultivation techniques and process monitoring (L04)**
Alvaro R. Lara (Universidad Autónoma Metropolitana-Cuajimalpa, UAM, Mexico)

**16:20** **From shake flasks to bioreactor in the production of a recombinant glycoprotein in a filamentous bacterial culture (L05)**
Mauricio A. Trujillo-Roldán
(Instituto de Investigaciones Biomédicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico)

**16:40** **Sponsor talk:**
**Navigator software for process development and optimisation**
Tomi Aho (Bioptima Oy, Tampere, Finland)

16:45 Coffee break with poster session and exhibition

Chair Nicolas Cruz-Bournazou
(TU Berlin, Dep. of Bioprocess Engineering)

**17:15** **Optimisation of a single-use bioreactor for the expansion of human mesenchymal stem cells at bench-top scale by means of CFD: A QbD approach (L06)**
Stephan Kaiser (Zürcher Hochschule für Angewandte Wissenschaften, Switzerland)

**17:35** **Consistent high throughput bioprocess development under process relevant conditions (L07)**
Florian Glauche (Dep. of Bioprocess Engineering, TU Berlin, Germany)

**18:05** **Mini Pilot Plant (MPP) for fast and reliable upstream development (L08)**
Simon Unthan (Forschungszentrum Jülich, Jülich, Germany)

**18:25** **Low-cost devices and technologies for bioprocess, monitoring, control, modeling and optimisation (L09)**
Gueguim Kana Evariste Bosco
(University of Kwazulu Natal, Pietermaritzburg, South Africa)

**18:45** **Single-cell cultivation in picoliter scale: Opportunity for bioprocess development (L10)**
Alexander Grünberger (Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany)

**19:05** **From extracellular environment to functional phenotype: Quantitative comparison of single cell physiology in static and steady environments (L11)**
Christian Dusny (Laboratory of Chemical Biotechnology,TU Dortmund University, Dortmund, Germany)

**19:30** **Poster session, Exhibition & Welcome Reception**

21:30 End

# Programme

## Programm at a glance, Thursday, 3 April 2014

### Scale down approaches and process analytical technologies for advanced process design

Chair Stefan Junne (TU Berlin, Dep. of Bioprocess Engineering)

**9:00** **Key note lecture: Investigating the impact of different $CO_2$/$HCO_3$-levels on metabolism and regulation – Example: Corynebacterium glutamicum (L12)**
Ralf Takors (University of Stuttgart, Stuttgart, Germany)

**9:30** **Response of Corynebacterium glutamicum to oscillations in a three-compartment scale-down reactor concept (L13)**
Anja Lemoine
(Department of Bioprocess Engineering, TU Berlin, Germany)

**9:50** **Scale-down meets Omics: Challenging metabolism of Corynebacterium glutamicum by oxygen inhomogeneity (L14)**
Marco Oldiges (Forschungszentrum Jülich / Institute of Bio- and Geosciences- IBG-1: Biotechnology, Jülich, Germany)

**10:20** **Simulating large scale conditions in a scale-down bioreactor: Impacts on the cell physiology of Escherichia coli (L15)**
Christian Reitz
(Department of Bioprocess Engineering, TU Berlin, Germany)

**10:40** **Sponsor talk: Capacitance based measurement of online viable cell density and monitoring of changes in physiological states**
Jens Rupprecht (Hamilton-Messtechnik, Bonadouz, Switzerland)

**10:45** **Sponsor talk: EloTrace – Analysis of the cell polarizability as process analytical tool**
Stefan Junne (TU Berlin, Dep. of Bioprocess Engineering)

10:55 Coffee break and poster session and exhibition

Chair Julia Glazyrina (TU Berlin, Dep. of Bioprocess Engineering)

**11:25** **Towards a single empirical correlation to predict kLa across scales and processes (L16)**
Daniela Quintanilla-Hernandez (Technical University of Denmark, Lyngby, Denmark)

**11:45** **Redesigning yeast processes to circumvent heterogeneities issues in large scale bioreactors (L17)**
Stephane Guillouet (INSA-LISBP, Toulouse, France)

**12:05** **Key note lecture: Microbial heterogeneity affects bioprocess robustness: future of dynamic single-cell analysis for large-scale bioprocess control (L18)**
Frank Delvigne (University of Liege, Belgium)

12:35 Lunch break, poster session and exhibition

Chair Mario Birkholz (TU Berlin, Dep. of Bioprocess Engineering)

**14:00** **Monitoring functions in managed microbial systems by cytometric bar coding (L19)**
Susann Müller (UFZ – Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany)

**14:30** **Use of on-line flow cytometry for the characterisation of microbial stress dynamics during the bioprocess (L20)**
Alison Brognaux (Gembloux Agro Bio-Tech, Gembloux, Belgium)

**14:50** **Mobile multi-sensor systems for the 3-D characterisation of industrial scale processes and the investigation of gradients (L21)**
Anika Bockisch (Department of Bioprocess Engineering, TU Berlin, Germany)

**15:10** **Application of wireless sensors for the detection of inhomogeneities in stirred tanks (L22)**
Joachim Venus (Leibniz-Institute for Agricultural Engineering, Potsdam-Bornim, Dept. Bioengineering, Potsdam, Germany)

**15:40** **Sponsor talk: In situ analysis of cell count and size distribution**
Friedel H. Schwartz (Sequip S+E GmbH, Düsseldorf, Germany)

**15:50** **Sponsor talk: GRETA – High throughput multifermenter system**
Gyorgy Rajkai (Belach Bioteknik AB, Skogås, Sweden)

15:55 Coffee break and poster session and exhibition

Chair Peter Neubauer (TU Berlin, Dep. of Bioprocess Engineering)

**16:25** **Key note lecture: Engineering biology: Enabling a knowledge-based bioeconomy (L23)**
Jürgen Eck (BRAIN AG, Zwingenberg, Germany)

**16:55** **Key note lecture: Fermentation scale-down – A view from industrial practice (L24)**
Henk Noorman
(DSM Biotechnology Center, Delft, The Netherlands)

**17:25** **Distinguished lecture: Concepts for scale down studies based on CFD agent based modelling approaches (L25)**
Matthias Reuss (University of Stuttgart, Germany)

**19:30** **Conference dinner**
Restaurant Nolle, S-Bahnbogen 203, Georgenstraße, 10117 Berlin (Bahnhof Friedrichstrassse)

## Programm at a glance, Friday, 4 April 2014

### Novel reactors, automation and control concepts

Chair Mirja Krause (TU Berlin, Dep. of Bioprocess Engineering)

**9:00** **Plenary lecture: Bioprocess development for the production of gaseous biofuels from agroindustrial wastes (L26)**
Gerasimos Lyberatos (School of Chemical Engineering, National Technical University of Athens, Athens, Greece)

**9:45** **Single-use bioreactors for microbial application: Feasibility and recent advances (L27)**
Nico Oosterhuis
(CELLution Biotech B.V., Nieuwendijk, The Netherlands)

**10:05** **Design space definition for a stirred single-use bioreactor family (L28)**
Thomas Dreher
(Sartorius Stedim Biotech GmbH, Göttingen, Germany)

**10:25** **Solutions for the application of disposable technologies in a bio-production plant (L29)**
Benjamin Minow
(Rentschler Biotechnologie GmbH, Laupheim, Germany)

10:45 Coffee break with poster session and exhibition

Chair Erich Kielhorn (TU Berlin, Dep. of Bioprocess Engineering)

**11:15** **Key note lecture: From High throughput process development to PAT applications in downstream processing (L30)**
Jürgen Hubbuch (KIT, Karlsruhe, Germany)

**11:45** **Hybrid cybernetic modeling of Escherichia coli metabolic adaptations to carbon source availability: A step forward in the modeling of microorganism behavior within industrial bioreactors (L31)**
Nathalie Gorret (INRA, Toulouse, France)

**12:05** **infoteam iLAB: A software platform for optimised bioprocess development (L32)**
Ingrid Schmid (infoteam Software AG, Bubenreuth, Germany)

**12:25** **Key note lecture: Modelling across bioreactor scales: methods, challenges and limitations (L33)**
Krist V. Gernaey (DTU Chemical Engineering, Lyngby, Denmark)

**12:55** **Concluding remarks**
Peter Neubauer (TU Berlin, Germany)

13:00 End of symposium

## Wednesday, 2 April 2014

### Opening session

**13:00 Welcome address and introduction**

*Peter Neubauer*

*Technische Universität Berlin, Institute for Biotechnology, Chair of Bioprocess Engineering*
*peter.neubauer@tu-berlin.de*

**13:30 Plenary Lecture:**
**Exploring the heterologous genomic space for building, stepwise, complex, microbial strains for large-scale bioprocessing (L01)**

*Eleftherios Terry Papoutsakis, Stefan M. Gaida, Sergios A. Nicolaou, Nicholas R. Sandoval, Kyle A. Zingaro, Yongbo Yuan*

*Delaware Biotechnology Institute and Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, USA.*
*papoutsakis@dbi.udel.edu*

Abstract: The repertoire of metabolites that can be produced by bio-based processes as commodity or specialty chemicals is ever expanding, and includes organic acids, alkanes, long-chain alcohols and aldehydes. Several molecules are also considered suitable as biofuels, from the established ethanol to higher alcohols, hydrocarbon-like molecules, and precursors such as butyric acid. Most industrially relevant chemicals are toxic to microbes, an issue that limits the development of economically attractive fermentation and bioremediation processes. How does one generate then microbial strains that are considerably more resistant to the intended metabolite under real bioprocessing conditions? We will argue that the heterologous genomic space and the metagenomic space remain largely unexplored for this and other metabolic-engineering applications. A key limitation in using heterologous genomic or metagenomic libraries in functional genomics and genome engineering is the low-level expression of heterologous genes in screening hosts, such as Escherichia coli. We will discuss a novel strategy (based on flow-cytometry screening of GFP-trap libraries) to overcome this limitation and demonstrate the application of this strategy in building complex tolerance capabilities. Using E. coli, we developed first separate and then a composite phenotype of improved growth and survival under ethanol stress by utilizing the Lactobacillus plantarum genomic space. We will show that this strain can significantly improve ethanol productivity in a practically significant Melle-Boinot-like fermentation process.

## Wednesday, 2 April 2014

## Quality by design in bioprocess development

Chair Andreas Knepper (TU Berlin, Chair of Bioprocess Engineering)

### 14:15 Key note lecture: Approaching the microbial growth space for systematic bioprocess development (L02)

*Raivo Vilu*

*Technical University of Tallinn and CCFFT, Tallinn, Estonia – raivo@kbfi.ee*

**Abstract:** Recent developments of systems biology and synthetic biology have created new challenges - needs to develop and introduce high throughput omics-methods together with the high throughput data handling and modelling methods, which allow to carry out comprehensive quantitative diagnostics of physiology of bacterial cells not only in one point of possible growth conditions but in the region of growth space interesting for the researchers and/or process developers. An approach including changestat cultivation methods (A-stat, D-stat etc.), absolute quantitative omics-methods (proteomics, metabolomics etc.) and modelling methods (FBA, SCM etc.) will be described and its use in studies of evolution of genetic variability during the continuous cultivation of E. coli, acetate overflow metabolism in E. coli and growth rate dependent metabolism in L. lactis will be illustrated. It was shown using the A-stat cultivation together with the next generation sequencing that genetic variability of bacteria in the culture collections and during the continuous cultivations should be taken into account if fully reliable quantitative data need to be generated. The results of the studies of levels of growth rate dependent regulation mechanisms showed that in the both bacteria investigated the post-translational regulation is playing a more important role in comparison with transcriptional and translational controls. It was possible to formulate and prove a novel regulation mechanism of acetate overflow in E. coli using the systems biology platform developed and studying different knock-out mutants of the bacteria. Future challenges of growth space studies will be discussed.

14:45 Coffee break and exhibition

### 15:20 Reviving QbD – A mechanistic approach (L03)

*Patrick Sagmeister, Christoph Herwig*

*Vienna University of Technology, Institute of Chemical Engineering, Research Area Biochemical Engineering, Vienna, Austria, patrick.sagmeister@tuwien.ac.at*

**Abstract:** Following the pharmaceutical quality by design (QbD) initiative, the development and demonstration of process understanding emerged as a primary demand from the regulatory authorities. This should enable the pharmaceutical industry to actively handle wanted or unwanted process variability and hence to guarantee a quality product "by design". In the last decade, statistical methods (statistical experimental design, data-driven models, statistical process control) emerged as the primary tools to put QbD into practice. Although statistical methods are suitable to capture the relationships between process parameters and quality attributes, they allow little insight and understanding in the underlying technical or biological mechanistic. Furthermore, scale-effects cannot be ruled out and the transferability of knowledge to other processes is difficult.
Here, we sketch the reviving of QbD using mechanistic methods. We show how mechanistic methods (kinetic models, first principle relationships) can be used for the bioprocess lifecycle and interlinked with statistical methods. This includes i) mechanistic models assisted efficient and precise experimentation to extract the same knowledge from less experiments, ii) sound demonstration of scalable process understanding by revealing true underlying relationships iii) clear identification of true process optima and iv) consistent use of models from the use in the screening phase to model-based control strategies within manufacturing.

### 15:50 Key note lecture: Plasmid DNA production by Escherichia coli – Cell engineering, cultivation techniques and process monitoring (L04)

*Alvaro R. Lara*

*Universidad Autónoma Metropolitana-Cuajimalpa (UAM), México – alara@correo.cua.uam.mx*

**Abstract:** Escherichia coli cultivations are accompanied by acetate excretion (overflow metabolism), which has harmful effect on the growth and wastes carbon to undesired products. Growth substrates (glucose, amino acids) and specific growth rate (μ) are important parameters influencing cell physiology and acetate metabolism. In this study systems biology approach was used – advanced continuous cultivation methods (A-stat and D-stat) with transcriptome, proteome and metabolic flux analysis were used to monitor regulation patterns of mRNA vs protein and protein vs flux at different specific growth rates. It was shown that acetate overflow was started at $\mu = 0.27 \pm 0.02\ h^{-1}$ in parallel with excretion of pyrimidine pathway intermediates carbamoylphosphate, dihydroorotate and orotate. Almost 11 % carbon was wasted at specific growth rate 0.5 $h^{-1}$. Overflow of acetate was caused by the carbon catabolite repression of acetyl-CoA synthethase (Acs) and disruption of the PTA-ACS node indicating imbalance between carbon consumption and biosynthetic pathways. It was observed that on the level of protein abundance in reproduction pathways (synthesis of biopolymer building blocks) covers large part of proteome synthesis (25 % of ATP cost of total proteome) and is higher than that for energy generation (10%). On the contrary, carbon overflow was observed in parallel with the reduction of ATP spilling (36 %), TCA cycle and glycolysis fluxes indicating more effective energy metabolism. It can be explained by increased apparent catalytic activities of enzymes (almost 3.5 times at specific growth rate 0.1 $h^{-1}$ vs 0.5 $h^{-1}$, especially ATP generating enzymes) and by the fact that less proteins was needed to be synthesized for biomass production. To improve E. coli growth several mutants were designed which had Acs repression or activation mutations. Repression released mutants had increased biomass yield and postponed acetate production. It was also shown that balanced growth media (addition of amino bases or amino acids) can effectively reduce carbon wasting to unwanted byproducts indicating big potential to design proper media for bioprocesses.

## 16:20 From shake flasks to bioreactor in the production of a recombinant glycoprotein in a filamentous bacterial culture (L05)

*Mauricio A. Trujillo-Roldán*

*Instituto de Investigaciones Biomédicas, Universidad Nacional Autonoma de Mexico, Mexico City, Mexico – maurotru@gmail.com*

**Abstract:** Culture conditions in shake flasks affect filamentous Streptomyces lividans morphology, as well the productivity and O-mannosylation of recombinant Ala-Pro-rich O-glycoprotein (known as the 45/47 kDa or APA antigen) from Mycobacterium tuberculosis. Three different shake flask geometries were used to provide different shear and oxygenation conditions; and the impact of those conditions on the morphology of S. lividans and the production of rAPA was characterized and evaluated. Small and dispersed mycelial aggregates obtained in baffled and stainless steel coiled flasks improve the production and increase the degree of O-mannosylation of the recombinant protein, in comparison with large aggregates obtained in conventional shake flasks. In order to scale-up from shake flasks to bioreactor, the effect of agitation on morphology of Streptomyces strains were used to obtain volumetric power input (P/V) values that can be used to obtain a morphology of S. lividans in bioreactor similar to the morphology reported in coiled/baffled shake flasks. Morphology of S. lividans was successfully scaled-up, obtaining similar mycelia sizes in both scales (baffled and coiled shake flasks and bioreactor). More interestingly, the quality of the recombinant glycoprotein measured as the amount of mannoses attached to the C-terminal of APA was also scaled-up; with up to five mannoses residues in cultures carried out in shake flasks; and six in bioreactor. However, final biomass concentration was not similar, indicating that although the process can be scaled-up using the power input, others factors like oxygen transfer rate, tip speed or energy dissipation/circulation function can be an influence on bacterial metabolism. Recent studies on power input and oxygen transfer rates in shake flasks allow us to conclude that P/V taken as a global parameter, is not a definitive parameter that can determine filamentous bacteria growth and morphology, not even recombinant glycoprotein production. But it can be proposed that that oxygen transfer in the center of the pellets and hydromechanical stress might be the more relevant parameters than P/V.

## 16:40 Sponsor Talk: Navigator software for process development and optimisation

*Tomi Aho, Bioptima Oy, Tampere, Finland*

16:45 Coffee break, poster session and exhibition

Chair Nicolas Cruz-Bournazou (TU Berlin, Chair of Bioprocess Engineering)

## 17:15 Optimisation of a single-use bioreactor for the expansion of human mesenchymal stem cells at bench-top scale by means of CFD: a QbD approach (L06)

*Stephan C. Kaiser[1], Valentin Jossen[1], Carmen Schirmaier[1], Ann Siehoff[2], Silke Brill[2], Gerhard Greller[3], Alexander Tappe[3], Dieter Eibl[1], Regine Eibl[1]*

*[1]Zurich University of Applied Sciences, School of Life Sciences and Facility Management, Institute of Biotechnology, Grüental, 8820 Wädenswil, Switzerland, stephan.kaiser@zhaw.ch. [2]Lonza Cologne GmbH, Nattermannallee 1, 50829 Köln, Germany, [3]Sartorius Stedim Biotech, Landstrasse 94-108, Göttingen, Germany.*

**Abstract:** The in-vitro expansion of human mesenchymal stem cells (hMSCs) is of growing interest, because of their limited availability and the increasing demands for medical applications, where the primary cells have shown a great potential for treatment of a wide range of diseases. Currently, the cells are expanded in 2-D cultures because of their adherence. However, those systems are mostly non-instrumented and limited in scale because of the lack of mixing. This results in concentration gradients and, therefore, supply of high cell amounts with consistently high quality is difficult or impossible to ensure. A promising alternative may be provided by suspension cultures of hMSCs cultivated on microcarriers (MCs) in stirred SU bioreactors, which offer the benefit of higher process safety due to the lower contamination risk. However, these bioreactors were originally designed for the cultivation of animal cell cultures that are characterized by higher robustness than the primary hMSCs. Thus, they are equipped with impellers, which are not optimized for the cultivation of hMSCs at typical operation conditions.
This study presents an optimization approach of a commercially available, stirred SU bioreactor by means of Computational fluid dynamics (CFD). Based on Design of Experiments (DoE), different geometries of the impellers were investigated in terms of fluid flow, power input and shear stress. In subsequent suspension investigations, the required impeller speeds for MC suspension were identified, which provides sufficient mixing while preventing excessive shear stresses. Consequently, a design space for the successful in-vitro expansion of hMSCs was defined.

## 17:35 Consistent high throughput bioprocess development under process relevant conditions (L07)

*F. Glauche[1], A. Knepper[1], L. Theuer[1], M. Heiser[1], F. Wollny[1], S. Bigesse[1,2], A. Neubauer[2], S. Arain[3], G. John[3], J. Aschoff[4], B. Stehlik[4], I. Schmidt[4], N. Violet[5], R. King[5], D. Gölling[6], A. Raab[6], G. Kiesewetter[1,6], R. Nolte[1,6], P. Neubauer[1]*

*[1]Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany, florian.glauche@tu-berlin.de, [2]BioSilta Europe GmbH, Berlin, Germany, www.biosilta.com, [3]PreSens PreSens Precision Sensing GmbH, Regensburg, Germany, www.presens.de, [4]Infoteam Software AG, Bubenreuth, Germany, www.infoteam.de, [5]Technische Universität Berlin, Chair of Measurement and Control, Berlin. Germany, www.mrt.tu-berlin.de, [6]Organobalance GmbH, Berlin, Germany, www.organobalance.de*

**Abstract:** In the field of biotechnology, the development of cost-effective production processes is a time- and labor-intensive task due to the vast design space to be screened. Quite often, process parameters result from trial and error based experimentation. Through the eyes of an engineer, most screening systems lack from relevant information and the consistency in scaling-up is limited. Although there is a general trend towards miniaturized and parallelized development of bioprocesses, the degree of automation is mostly limited to single steps. With a combination of high throughput cultivation systems, sensor technology, data handling, mathematical modeling and experimental design strategies, the AUTOBIO consortium is working on platform technologies in order to significantly shorten process development time and costs. The Neubauer lab has set-up two robot stations, which allow automated microbial cultivations in multiwell plate formats and in a 48-stirred tank bioreactor system.

## 18:05 Mini Pilot Plant (MPP) for fast and reliable Upstream Development (L08)

*Simon Unthan, A. Radek, M. Oldiges, W. Wiechert, S. Noack*

*Forschungszentrum Jülich, Jülich, Germany, s.unthan@fz-juelich.de*

**Abstract:** For industrial biotechnology, it is highly important to select the most promising production hosts and medium compositions in early stages of upstream development. However, the opposing demands of detailed process insight during high throughput are generally not fulfilled by classical cultivation systems. Today, this gap is therefore often bridged by use of high quality MTP-cultivation devices, enabling the non-invasive monitoring of pH, pO2 and biomass while providing sufficient oxygen transfer. However, these devices still depend on manual work to inoculate, sample or induce cultivations, which limits the overall speed and throughput of upstream development.
To address this issue, we constructed a mini pilot plant (MPP) by embedding a BioLector in a robotic environment to automate complete workflows for upstream development. The connected liquid handling platform enables triggered addition of substances (i.e. inductors, precursors or fed substances) to individual cultivation experiments. Moreover, different growth media are prepared and inoculated in a sterile environment established by laminar flow. To gain a deeper process understanding, cultivation samples are harvested and centrifuged automatically to provide supernatants for subsequent quantitative analysis with various fully automated assays in MTP scale.
With this MPP at hand we evaluated 22 novel L-Lysine producing Corynebacterium glutamicum strains in different media for growth and glucose uptake rate as well as L-Lysine yield and productivity within two weeks. Concentrations of glucose and L-Lysine in cultivation samples were automatically measured on the MPP by an enzymatic or biochemical assay, respectively. As a result the novel strain C. glutamicum D67 was identified who showed above 20 % increased L-Lysine titers compared to the reference producer DM1933. The increased L-Lysine production by D67 was subsequently also observed in lab scale (1 L), basically confirming our results obtained on the MPP. This exemplary finding shows the potential of our MPP approach and more generally speaking, how future upstream development will benefit from robotic automation.

## 18:25 Low-cost devices and technologies for bioprocess, monitoring, control, modeling and optimisation (L09)

*Gueguim Kana Evariste Bosco*

*University of Kwazulu Natal, Pietermaritzburg, South Africa, kanag@ukzn.ac.za*

**Abstract:** Bioreactors are central to bioprocess development and production. At the present state, their high cost impedes parallel multivariate experimentations to generate high throughput data for process development. In this work, the potentiality of using a low-cost open-source microcontroller such as the Arduino board for bioprocess monitoring and control is presented. Then, we reviewed more than thirty low-cost actuators and sensors, which can interface the bioreaction and the controller. These sensors generate a stream of data on bioprocess behavior, required for modeling and optimisation.

## 18:45 Single-cell cultivation in picoliter scale: opportunity for bioprocess development (L10)

*Alexander Grünberger, Christopher Probst, Simon Unthan, Nurije Mustafi, Julia Frunzke, Stephan Noack, Wolfgang Wiechert and Dietrich Kohlheyer*

*Forschungszentrum Jülich GmbH, IBG-1: Biotechnology, Jülich, Germany, a.gruenberger@fz-juelich.de*

**Abstract:** Novel analytical techniques have provided more detailed information on cellular heterogeneity in biotechnological processes in the last years. Nevertheless, the complex interplay between environmental changes and cellular response is far from being completely understood [1]. To tackle some of these challenges, microfluidic single-cell analysis offers promising technical concepts to perform in depth cell-to-cell heterogeneity investigations.
In this contribution, we will present a systematic and comprehensive study utilizing microfluidic bioreactors to analyze bacterial processes on single-cell level [2]. In comparison to droplet based single-cell cultivation and simple agar pad cultivation methods, the presented device are operated under well-defined and constant environmental conditions. Corynebacterium glutamicum, which is an industrial workhorse for amino acid production, was analyzed at single-cell level to gain novel insides into industrial bioprocesses. The growth rate of C. glutamicum wildtype under various environmental conditions was investigated systematically. Surprisingly, single-cell cultivations in a continuously perfused microfluidic bioreactor system revealed elevated growth rates μ=0.62±0.02 h-1) in contrast to large-scale batch cultivations (μ=0.42±0.02 h-1) [3]. Based on these findings, potential effectors were investigated. It was found that protocatechuate - an iron chelator -, significantly influences growth rates of C. glutamicum [4].
Furthermore, the L-valine producer C. glutamicum aceE was investigated by means of our recently developed genetically encoded fluorescence reporter systems for the intracellular detection of amino-acids at single-cell resolution [5]. Interestingly, the strain exhibited strong population heterogeneity with respect to growth as well as productivity with individual cells expressing different fluorescence levels. Latest results will be discussed in the presentation.
Our results demonstrate that microfluidic picoliter bioreactors are powerful tools to gain single-cell knowledge of biotechnological processes essential for upcoming process optimization and research. Nevertheless, single-cell bioreactors are still in an early phase of development, making a critical discussion of advantages, disadvantages, existing challenges and necessary improvements necessary.

**References:** [1] Grünberger et al. Curr Opin Biotechnol, 2014, in revision. [2] Grünberger et al. Lab Chip, 2012. 12(11): p. 2060-2068. [3] Grünberger et al. Biotechnol Bioeng, 2013. 110(1): p. 220-228. [4] Unthan et al. Biotechnol Bioeng, 2014: 111:359-371. [5] Mustafi et al. PlosOne, 2014. 14(4): 9:e85731.

## 19:05 From extracellular environment to functional phenotype: Quantitative comparison of single cell physiology in static and steady environments (L11)

*Christian Dusny[1], Alexander Grünberger[2], Oliver Frick[1], Dietrich Kohlheyer[2], Wolfgang Wiechert[2], and Andreas Schmid[1]*

*[1]Laboratory of Chemical Biotechnology, TU Dortmund University, Germany – christian.dusny@bci.tu-dortmund.de*
*[2]Institute of Bio- and Geosciences, IBG-1, Forschungszentrum Jülich GmbH, Germany*

**Abstract:** Nature has equipped microorganisms with an extensive repertoire of sophisticated cellular mechanisms to adapt to physicochemical changes in the extracellular environment. These mechanisms include stochastic alterations of regulatory circuits as well as specific adaptations to external stimuli, which strongly vary from cell to cell.1 Consequently, cells of a microbial population exhibit significant physiological diversity despite of clonality, which can be accessed exclusively by single cell analysis.
In order to unambiguously assign the origin of cell-to-cell differences to either environmental factors or intrinsic stochasticity, the control of the extracellular (micro-) environment is a fundamental requirement. To date, only microfluidic cultivation systems offer the possibility to precisely control the extracellular environment and eliminate the unavoidable environmental inhomogeneities occurring during bulk cultivations.2 We here present a systematic comparison of inherently different microcultivation technologies in order to quantify the response of cellular physiology to the extracellular environment at a single cell level. We compared microfluidic contactless trapping with the negative dielectrophoresis (nDEP/ Envirostat) and contact-based hydrodynamic single-cell cultivation systems (MGC), both allowing for constant environmental conditions by continuous cell perfusion, with solid agarose-based cultivation pads on the basis of physiological and morphological data.3, 4 In order to allow a quantitative comparison of the three systems, unified analytical methods for the precise quantification of specific growth rates, cell morphology and division characteristics of single microorganisms were developed. Corynebacterium glutamicum ATCC13032 served as a model organism during this study and was investigated under otherwise identical cultivation conditions. Independent of the employed cultivation system, exceptionally high specific growth rates of 0.6 h-1 could be observed on both single cell and colony level. These findings lead to the conclusion that an optimal nutrient and oxygen supply was present in all employed systems and maximal growth was rather limited by the physiological capacity of the cells than by the environment. It can also be deduced that all cells exhibit a highly similar metabolic capacity. In contrast to growth, the static environment of agarose pads manifested itself in considerable differences in the snapping division angle and cell length distribution before and after division, while cells cultivated with nDEP and MGC showed highly similar length and division angle distributions.
To the best of our knowledge, this study represents the first systematic analysis of physiological responses to steady and static extracellular environments at single cell resolution. Moreover, the results nicely show the potential of observing physiological phenomena at a single cell level unbiased by population activity and uncontrollable environmental influences.

**References:** 1. Fritzsch et al. Annu Rev Chem Biomol Eng (2012). 2. Dusny et al. Appl. Environ. Microbiol. 78, 7132-7136 (2012). 3. Grünberger et al. Biotechnol. Bioeng. 110, 220-228 (2013). 4. Young et al. Nat Protoc 7, 80-88 (2012).

## 19:30 Poster session & Exhibition & Welcome Reception

*Foyer of the Institute for Chemistry*

21:30 End

Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik

Chair of Bioprocess Engineering

## Thursday, 3 April 2014

## Scale down approaches and process analytical technologies for advanced process design

Chair Stefan Junne (TU Berlin, Chair of Bioprocess Engineering)

### 9:00 Key note lecture: Investigating the impact of different $CO_2$/$HCO_3$-levels on metabolism and regulation – Example: Corynebacterium glutamicum (L12)

*J. Buchholz, B. Blombach, Ralf Takors*

*Institute of Bioprocess Engineering (IBVT), University of Stuttgart, Germany – takors@ibvt.uni-stuttgart.de*

**Abstract:** It is a well-known fact that large scale bioreactors show mixing heterogeneities mirrored by gradients of substrates, dissolved gases or pH. While impacts of varying substrate or dissolved oxygen levels were investigated manifold, our contribution focuses on the distinct interaction of changing $CO_2$/$HCO_3$- levels on cellular performance – a topic which has been discussed rarely for microbial cells. Studying the industrial workhorse Corynebacterium glutamicum a series of lab-scale fermentations was performed analyzing the metabolic and transcriptional response on different $CO_2$/$HCO_3$- stimuli. Besides a novel scale-down device was developed enabling the in–depth analysis of intracellular dynamics caused by extracellular heterogeneities. After characterization of this device sub-cellular dynamics were elucidated as a function of external $CO_2$/$HCO_3$- heterogeneities mirroring typical scenarios of large scale conditions.

### 9:30 Response of Corynebacterium glutamicum to oscillations in a three-compartment scale-down reactor concept (L13)

*Anja Lemoine, Nina Maya, Robert Spann, Stefan Junne, Peter Neubauer*

*Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany – anja.lemoine@tu-berlin.de*

**Abstract:** Corynebacterium glutamicum is widely applied for the industrial production of the amino acid lysine. These large-scale nutrient limited fed-batch processes are characterized by gradients of substrate and oxygen. Scale-down experiments are used for the systematic investigation of the impact of heterogeneous conditions on the physiologic and morphologic state of the cells. Therefore, two scale-down concepts are compared in this study: (i) a two-compartment reactor comprising of a stirred tank reactor and a plug flow reactor, in which the feed is supplied to the culture (high substrate availability and oxygen limitation), and (ii) a three compartment reactor, where the two compartment reactor is extended by a second plug flow module (low substrate availability and oxygen limitation). These zones are occurring in industrial-scale lysine production.
Results show a reduced lysine production and a concomitant accumulation of the free amino acids alanine, aspartate, glutamine and glutamate under heterogeneous conditions. The intracellular lactate concentration showed an increase only in the three compartment reactor, underlining the necessity to broaden the concept of a two-compartment scale-down reactor to a reactor concept reflecting multiple zones.

### 9:50 Scale-down meets Omics: Challenging metabolism of Corynebacterium glutamicum by oxygen inhomogeneity (L14)

*Friedrich Käß[1], Ioanna Hariskos[1], Andrea Michel[1], Robert Spann[1,2], Peter Neubauer[2], Stefan Junne[2], Wolfgang Wiechert[1], Marco Oldiges[1]*

*[1]Forschungszentrum Jülich; Institute of Bio- and Geosciences IBG-1: Biotechnologie, Jülich, Germany – m.oldiges@fz-juelich.de,*
*[2]Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany*

**Abstract:** C. glutamicum has proven its potential as platform organism for Industrial Biotechnology with annual production of millions of tons of amino acids, organic acids and derivatives at bioreactor scales of several hundred cubic meters. In this work, a two-compartment reactor system (aerobic STR / non aerated PFR) have been used to generate defined oscillations of oxygen and substrate supply during cultivation of C. glutamicum wt and lysine producing strain C. glutamicum DM1933. With residence times in non-aerated PFR from 40 seconds up to minutes, robustness and performance of microbial metabolism is challenged in batch as well as fed-batch experiments. Glucose consumption, respiratory activity and side product concentrations show fundamental differences when comparing homogeneous and oscillating conditions. Most strikingly, omics-data from the metabolome, proteome and transcriptome show only marginal effects with respect to oscillation. This indicates that the regulon of C. glutamicum is very robust against oscillatory conditions, which constitutes an important mechanism for robustness in industrial processes.

### 10:20 Simulating large scale conditions in a scale-down bioreactor: Impacts on the cell physiology of Escherichia coli (L15)

*Christian Reitz, Eva Brand, Ping Lu, Robert Spann, Sergej Trippel, Stefan Junne, Peter Neubauer*

*Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany – christian.reitz@tu-berlin.de*

**Abstract:** Escherichia coli is an important production strain for recombinant protein production in large-scale fed-batch bioprocesses. Due to limited mixing capacities, gradients exist concerning the nutrient and oxygen availability when a certain scale and cell density is reached. In order to investigate the impact of these heterogeneous conditions

on the physiologic state of the cells, experiments in a scale-down two-compartment reactor are performed. It consists of a stirred tank reactor connected to a plug-flow reactor, which represents conditions at the feed zone by a combination of oxygen limitation and a high concentration of substrate.
Metabolome analysis at cultivations of E. coli K12 W3110 show an accumulation of metabolites deriving from pyruvate (acetate, formate and lactate) as well as an increased production of (non-canonical) branched-chain amino acids. As revealed by experiments with the rapid sampling unit BioScope, the short time response to a glucose pulse is dependent whether cells had been exposed to oscillating conditions before. As non-canonical amino acids are introduced into recombinant proteins, the incorporation into recombinant proteins under oscillating conditions has to be investigated.

## 10:40 Sponsor talk: Capacitance based measurement of online viable cell density and monitoring of changes in physiological states

*Dr. Jens Rupprecht, Hamilton-Messtechnik, Bonadouz, Switzerland – j.rupprecht@hamilton-messtechnik.de*

**Abstract:** With respect to the FDA's Process Analytical Technology (PAT) framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance, further analytical tools are requested to be implemented for the supervision and controlling of fermentation processes. Goal is an improvement of product quality and reduction of production costs and time. This can be achieved by an increase in process automation, a more efficient energy and material use and continuous process supervision and quality assurance. However todays established pool of reliable and autoclavable in-line tools is restricted to pH, dissolved oxygen, conductivity, redox potential, temperature and cell mass as optical density.
In particular optical density provides only information via the turbidity of a culture without being able to distinct between living and dead cells, gas bubbles and cells as well as micro-carrier and cells also being sensitive to external light sources: Resulting in a restricted quantity and quality of information on the fermentation batch.
In the recent years capacitance based measurements have been employed in an increasing amount of fermentations covering a growing range of single cell organisms. Beside its ability to measure the viable cell volume in a bioreactor, it was shown that this technique is able to provide useful information on fermentation events like nutrient depletion, virus infection or optimal induction time. Supported by other analytical tools like gas analysis (e.g. respiration or photosynthetic oxygen evolution) and off-line investigations of nutrient availability, precise time points of those events can be determined and used to supervise and control fermentation runs.
In January 2014 Hamilton incorporated the BioTech division of Fogale NanoTech. With this acquisition Hamilton is now the only company offering the main parameters of in-line process analytics under one roof, promising comprehensive and intensive support and service.

## 10:45 Sponsor talk: EloTrace – Analysis of the cell polarizability as process analytical tool

*Alexander Angersbach[1], Viktor Bunin[1], Anja Lemoine[2], Peter Neubauer[2], Stefan Junne[2]*

[1]EloSystems GbR, Berlin, Germany, angersbach@biotronix.de.
[2]Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany.

**Abstract:** The cell polarizability can be monitored at line applying electrooptical methods and automated sample preparation with EloSystems' monitoring device EloTrace. In several experiments, we showed that the monitoring of the anisotropy of polarizability of rod-shaped bacteria in batch cultures supported a better insight into the cell's physiologic stages during the process. The application of the methodology to Escherichia coli batch cultivations offered the possibility to divide the growth phase in several parts with respect to the specific acetic acid synthesis rate. Furthermore, a correlation between the anisotropy and biomass yield in E. coli K12 fed-batch and chemostat cultures is found. Currently, the system is applied for the quantification of impacts of oscillating nutrient and oxygen supply in C. glutamicum cultures in scale-down experiments. Since the polarizability seems to be related strongly to the overall metabolic activity of the cell, the impact of stress during cultivation can be monitored for the purpose of process optimization and control.

10:55 Coffee break, poster session and exhibition

Chair Julia Glazyrina (TU Berlin, Chair of Bioprocess Engineering)

## 11:25 Towards a single empirical correlation to predict kLa across scales and processes (L16)

*Daniela Quintanilla-Hernandez[1], Krist V. Gernaey[1], Mads O. Albæk[2], Stuart M. Stocks[2]*

*[1]Department of Chemical and Biochemical Engineering, Technical University of Denmark, Lyngby, Denmark – danaquh@kt.dtu.dk*
*[2]Novozymes A/S, Fermentation Pilot Plant, Bagsværd, Denmark*

**Abstract:** Mathematical models are increasingly used in fermentation. Nevertheless, one of the major limitations of these models is that the parameters they include are process specific, e.g. the volumetric mass transfer coefficient (kLa). Oxygen transfer was studied in order to establish a single equation to predict kLa, and data from a range of processes – pilot and production scale – were extracted. On-line viscosity was measured for all processes (56 batches). Off-line rheological measurements were performed for the pilot scale processes (26 batches). The apparent viscosity was evaluated with 5 different calculations of the average shear rate. The experimental kLa value was determined with the direct method; however, eight variations of its calculation were evaluated. Several simple correlations were fitted to the measured kLa data. The standard empirical equation was found to be best for predicting kLa in all processes at pilot scale using off-line viscosity measurements, and using the equation from Henzler and Kauling (1985) to evaluate the shear rate. In addition, a parameter set of the standard empirical equation was found that can predict oxygen transfer in Bacillus processes at all scales using on-line viscosity measurements. A single correlation for all processes and all scales could not be established.

## Thursday, 3 April 2014

### 11:45 Redesigning yeast processes to circumvent heterogeneities issues in large scale bioreactors (L17)

*Stephane Guillouet*

*INSA-LISBP, Toulouse, France – stephane.guillouet@insa-toulouse.fr*

**Abstract:** The yeast Saccharomyces cerevisiae belongs to such yeast species which switch from a pure oxidative metabolism to a respiro-fermentative metabolism even under fully aerobic conditions as soon as the glucose exceeds a concentration of about 0.5 gl-1. This specific characteristic of S. cerevisiae has been supportive for all fermentative processes such as alcoholic beverage or ethanol production but represents a disadvantage for production of yeast biomass (baker's yeast production) as well as biomass-related yeast products (such as heterologous proteins). Therefore, yeast biomass production bioprocesses have been usually carried out under aerobic fed-batch mode where the glucose feeding is finely controlled to keep a very low residual concentration (<0.5 gl-1) and to maintain the specific growth rate below a critical value, called critical dilution rate (Dc). Such strenuous monitoring conditions become challenging when the production is carried out in large scale bioreactors where microorganisms, due to mixing issues, are submitted to fluctuations in concentrations of e.g. substrate and oxygen with an intensity and a frequency depending on the operating scale.
An alternative to controlled feeding of sugar based feedstock would be the use of additives able to delay the metabolic switch. We will present here an example of strategy applied to the production of S. c. under pure oxidative metabolism. We demonstrated that addition of oleic acid allowed delaying and modulating the transition from respiratory to fermentative metabolism in S. cerevisiae. The response of yeast to oleic acic was studied through systemic analysis coupling metabolics and transcriptomics. We further investigated the potential of oleic acid to circumvent heterogeneities issues in large scale bioreactors by mimicking these effects in scale-down bioreactor coupled with cell population analysis and computer simulation.

### 12:05 Key note lecture: Microbial heterogeneity affects bioprocess robustness: Future of dynamic single-cell analysis for large-scale bioprocess control (L18)

*Frank Delvigne*

*University of Liege, Belgium – f.delvigne@ulg.ac.be*

**Abstract:** Microbial phenotypic heterogeneity strongly impacts bioprocesses, as demonstrated for several cell factories. Phenotypic heterogeneity in bioprocessing conditions is often investigated by using fluorescent transcriptional reporters in combination with single cell detection technique, such as flow cytometry. However, there are now some evidences that gene induction is not the only mechanism subjected to stochasticity. Indeed, variability at the level of metabolic network can also lead to different corresponding phenotype, such as the specialization of a fraction of the cell population to the uptake of a specific substrate or a specific by-product. These considerations are of primary importance for the understanding of phenotypic heterogeneity since classical detection techniques are not directed towards metabolic processes. Based on these theoretical considerations, a scientific strategy involving on-line flow cytometry coupled to different bioreactor configurations will be presented and the importance for the selection of an appropriate fluorescent reporter system will be demonstrated. E. coli will be used as a representative cell factory exhibiting significant phenotypic heterogeneity with severe consequences on bioprocesses.

12:35 Lunch break, poster session and exhibition

Chair Mario Birkholz (TU Berlin, Chair of Bioprocess Engineering)

### 14:00 Monitoring functions in managed microbial systems by cytometric bar coding (L19)

*Susann Müller[1], Christin Koch[2], Ingo Fetzer[1], Susanne Günther[1], Hauke Harms[1]*

*[1]UFZ – Helmholtz Centre for Environmental Research, Department of Environmental Microbiology, Leipzig, Germany – susann.mueller@ufz.de*
*[2]UFZ - Helmholtz Centre for Environmental Research, Department of Bioenergy, Leipzig, Germany*

**Abstract:** The still poorly explored world of microbial functioning is about to be uncovered by a combined appliance of old and new technologies. Especially bacteria are still in the dark both in view of their phylogenetic affiliation as well as their metabolic capabilities and functions.
However, with the advent of sophisticated flow cytometric and cell sorting technologies in microbiological labs there is now the possibility to gain this knowledge without cumbersome cultivation approaches.
A single-cell based approach called cytometric bar coding (CyBar) for fast identification of reactive sub-communities was used. Functionality of specific sub-communities was uncovered by processing CyBar data with abiotic reactor parameters using Spearman's correlation coefficient. The dynamics of cellular DNA-contents and cell sizes as growth indicators were determined to detect the most active individuals/sub-communities and to determine their phylogenetic affiliation upon cell sorting and correlate their abundance to certain abiotic factors. New biostatistic tools are developed to evaluate and interpret the resulting huge data sets. The procedure is easy to perform, quick and has the capacity to design and control biotechnological processes. The workflow is shown to be suited for complex microbial community analysis on every desired time and investigation level with higher automatization potential as described elsewhere. Applications are shown for wastewater treatment plants and biogas reactors.

**References:** Koch et al. 2013. Nature Protocols 8/1, 190-202. Koch et al. 2013. Environm Sci Technol 47, 1753-1760. Günther et al. 2012. Environm Sci Technol 46(1), 84-92

## 14:30 Use of on-line flow cytometry for the characterization of microbial stress dynamics during the bioprocess (L20)

*Alison Brognaux*

*Gembloux Agro Bio-Tech, Gembloux, Belgium – alison.brognaux@doct.ulg.ac.be*

**Abstract:** Microbial cell population heterogeneity is now recognized as a major source of issues for the development and optimization of bioprocesses. Flow cytometry is a very powerful tool for the follow up of physiological properties of microbial cells in process-related conditions at the single cell level, and can be used to study the dynamics of segregation directly in bioreactors. In this context, specific interfaces have been developed in order to connect flow cytometer (FC) directly on bioreactor for automated analyses. In this work, we propose a simplified version of such interface and demonstrated its usefulness for multiplexed experiments.
This automated FC system has been tested for the follow up of the dynamics of an E. coli pfis::gfpAAV fluorescent bioreporter and its PI uptake, correlated with membrane permeability. This bioreporter is composed of a fis promoter, a growth dependent promoter-indicator of the nutrient status of cells, fused to a gene expressing an unstable variant of GFP. The results obtained showed that the dynamics of the GFP synthesis is complex and can be attributed to a complex set of biological parameters. Segregation in the membrane permeability has been noticed.
This work demonstrates that a simplified version of on-line FC can be used at the process level for the investigation of the dynamics of complex physiological mechanisms.

## 14:50 Mobile multi-sensor systems for the 3-D characterisation of industrial scale processes and the investigation of gradients (L21)

*Anika Bockisch, S. Junne, P. Neubauer*

*Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany, anika.bockisch@tu-berlin.de*

**Abstract:** In large scale fermentation processes, there is a limitation in the power of mixing. Consequently, the occurrence of gradients is likely. However, the available sensor- and sampling-technology is not sufficient to quantify these gradients and characterize large scale processes. In order to increase the knowledge about gradients and their impact on the process performance, multi-position sensors have been developed for in situ monitoring at different zones in the liquid core of industrial bioreactors. These analyses are combined with off line studies on the cell's metabolism, morphology, and physiology.
It has been shown that the on line monitoring is achievable for the pH-value, redox potential, DO-concentration, conductivity, pressure, and temperature. The data from large scale is suitable for the design of experiments in a two-compartment scale-down reactor, mimicking the conditions of the large scale. Results of S. cerevisiae cultivation experiments indicate the impact of oscillating conditions on the metabolic activity, cell size, and respiratory activity. The combination of multi-position sensors and physiologic studies allows the identification of critical reactor zones. On line monitoring of these zones will allow a fast detection of disturbances, while targets for process optimization are identified.

## 15:10 Application of wireless sensors for the detection of inhomogeneities in stirred tanks (L22)

*Joachim Venus[1], Thormann, S.[1], Hälsig, C.[2]*

*[1]Leibniz-Institute for Agricultural Engineering Potsdam-Bornim, Dept. Bioengineering, Potsdam, Germany – jvenus@atb-potsdam.de*
*[2]teleBITcom GmbH, Teltow, Germany*

**Abstract:** Renewable feedstocks can be utilized directly, e.g. as energy carriers, as packaging materials, as fibres, for the production of colouring agents or as lubricants. However, they can also be converted biotechnologically by enzymes and microorganisms, giving us access to a multitude of new, biocompatible products and possible uses. Often the economy of bioprocesses is still the problem because in the case of huge product quantities the price is affected mainly by raw material costs and the overall yield, respectively.
For the performance improvement of the entire fermentation, which is still often carried out in big stirred tanks, a new approach has been investigated based on wireless sensors. The aim of the study was to identify any zones of inhomogeneities for the bulk liquid in order to optimize the process conditions. Besides the sensors, a specific experimental set-up (Fig. 1) has been developed to avoid disturbances of the hydrodynamic behaviour of the system.
For the specific case of lactic acid fermentation, first results will be presented up to 1 $m^3$ bioreactor volume. Thanks to the analytical devices for parameters like pH value and conductivity a better understanding of the turbulences has been achieved. The arrangement of several sensors allows an individual positioning for further measurements concerning the influences of process-determining parameters.

## 15:40 Sponsor Talk: In situ analysis of cell count and size distribution

*Friedel H. Schwartz (Sequip S+E GmbH, Düsseldorf, Germany)*

## 15:50 Sponsor talk: GRETA – High throughput multifermenter system

*Gyorgy Rajkai (Belach Bioteknik AB, Skogås, Sweden)*

15:55 Coffee break, poster session and exhibition

Chair Peter Neubauer (TU Berlin, Chair of Bioprocess Engineering)

## 16:25 Key note lecture: Engineering biology: Enabling a knowledge-based bioeconomy (L23)

*Juergen Eck*

*BRAIN AG, Zwingenberg, Germany – je@brain-biotech.de*

**Abstract:** Heavily engineered whole-cell biocatalysts and highly effective enzymes promise improvement for existing processes or could enable novel product ideas, paving the way to a knowledge-based Bioeconomy [1]. Both, the replacement of traditional processes for the production of industrial chemicals by multi-step biosynthetic processes and the establishment of novel products and corresponding sustainable processes are based on fairly recent technological innovations in fields such as microbial genomics, metagenomics, producer strain engineering and coordinated process development [2;3;4]. The dramatic increase of information about the true biosynthetic potential of microorganisms and their enzymes makes it possible to discover and optimize applicable enzymes according the process requirements and to design biological routes and processes for the synthesis of industrial chemicals.

**References:** [1] European Commission. 2012. Innovating for Sustainable Growth: A Bioeconomy for Europe. http://ec.europa.eu/research/bioeconomy/pdf/201202_innovating_sustainable_growth_en.pdf. [2] Lorenz & Eck. 2005. Nat Rev Microbiol 3: 510-516. [3] Gabor et al. 2012. J Mol Biol 418: 16-20. [4] Mampel et al. 2013. Trends Biotechnol 31:52-60

## 16:55 Key note lecture: Fermentation scale-down – A view from industrial practice (L24)

*Henk Noorman*

*DSM Biotechnology Center, Delft, The Netherlands – Henk.noorman@dsm.com*

**Abstract:** Innovations in (molecular) biotechnology and chemistry for the conversion of biomass into bio-based products, now are rapidly advancing to bioprocess scale-up and implementation in industry. There, the manufacturing value chain connects biomass as novel feedstock, biomass pretreatment and hydrolysis, (bio)conversion of sugars into monomeric intermediates, and novel purification schemes to meet final product specifications. All need to be proven economic and robust in industry. In particular, the shift from sugars to biomass as feedstock presents the bioprocess technology field with a formidable additional challenge, and new solutions are required. Process modeling, high-throughput screening, scale-down simulation, process intensification and pilot/demo-scale campaigns are important for de-risking and faster implementation. On top of this, sustainability metrics such as less greenhouse gas emissions, energy input and waste need to be clearly linked to the process mass and energy balances.
A leading principle is to take the industrial fermentation as reference point, and from there scale-down to the labs where the optimization research is being done. For a proper scale-down, experimental data from the industrial reactor provide guidance to design and operation of scale-down simulators, but also computations to get sufficient high-resolution information. It is important to use as thoroughly as possible the scarce data from industrial fermentations to validate computational results.
In recent years, good progress in this area has been demonstrated as shown in this presentation via examples from DSM, applied to viable business cases for e.g. antibiotics, vitamins, lignocellulosic bio-ethanol, and bio-plastics.

## 17:25 Distinguished lecture: New concepts for designing scale down operations based on CFD coupled agent-based modelling and simulation (L25)

*Matthias Reuss, Alexei Lapin*

*Stuttgart Research Centre Systems Biology and Institute of Biochemical Engineering, University Stuttgart, Nobelstr. 15, 70569 Stuttgart – reuss@ibvt.uni-stuttgart.de*

**Abstract:** The lecture aims at discussing a new strategy for a model based design of scale-down experiments to optimally mimic the lifelines of individual cells in large bioreactors. The design concept is based on the agent-based segregated modelling approach, in which the liquid is treated as a continuum (Euler) and the dispersed biophase is tracked with the aid of a Lagrangian representation (Lapin et al., 2004, 2006, 2010). Application of this modelling strategy is exemplified with the sugar uptake rate of E. coli (Lapin et al., 2006). An important outcome of the simulation is a quantitative portray of the adventures of individual cells in terms of variation of sugar uptake rate during the spatio-temporal tracking of the agents. The new approach suggested in the lecture further analyses this information with the aid of a Fourier-transformation. Applying the same approach to the scale down experiment – well mixed vessel coupled with a plug flow in the recycling flow – allows an adaptation of the parameters of the scale-down operation to optimally portray the situation in the large scale reactor.

**References:** Lapin et al. 2004. Ind. Eng, Chem. Res. 43, 4647-4656. Lapin et al. 2006. Chem. Eng. Sci. 61, 4783-4797. Lapin et al. 2010. Adv. Biochem. Eng./Biotechnol 121, 23-43

19:30 **Conference dinner**

*Restaurant Nolle, S-Bahnbogen 203, Georgenstraße, 10117 Berlin*

*(directly located at Bahnhof/Station Friedrichstrassse, individual journey)*

## Friday, 4 April 2014

## Novel reactors, automation and control concepts

Chair Mirja Krause (TU Berlin, Chair of Bioprocess Engineering)

### 9:00 Plenary lecture: Periodic bioprocesses and their use for nutrient removal and for the production of gaseous biofuels from agroindustrial wastes (L26)

*Gerasimos Lyberatos*

*School of Chemical Engineering, National Technical University of Athens, Athens, Greece – lyberatos@chemeng.ntua.gr*

**Abstract:** Periodic bioreactor operation provides several possibilities that are not possible with either batch or continuous stirred tank bioreactors operated at steady-state. These include flexibility of operation, enhanced bioreactor performance, prevalence of selected microbial populations and indefinite retention of recombinant strains. Apart from periodic operation of CSTR-type reactors, sequencing batch reactors are periodically operated reactors by nature. It is demonstrated, as an example that partial nitrification/denitrification may be effectively carried out through appropriate operation of an SBR. Another bioreactor type that is inherently periodic by nature is the Periodic Anaerobic Baffled Reactor. It is a high rate anaerobic reactor that is maintained in a periodic state at all times, despite receiving constant feed, with distinctive advantages. The optimization of the process operation is greatly facilitated by appropriate dynamic modeling. Examples of the use of this reactor for the production of gaseous biofuels, such as hydrogen and methane, from agroindustrial wastes are presented.

### 9:45 Single-use bioreactors for microbial application: feasibility and recent advances (L27)

*Nico M.G. Oosterhuis[1], S. Junne[2], P. Neubauer[2]*

*[1] CELLution Biotech BV, Assen, The Netherlands – nico.oosterhuis@cellutionbiotech.com*
*[2]Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany*

**Abstract:** Nowadays single-use bioreactors are fully accepted in the biopharmaceutical industry. Reactors up to 2000L working volume are commonly used. However, these bioreactors are limited in terms of mass-transfer and mixing capabilities and therefore only suited for application in mammalian cell culture. Single-use processing offers the same advantages for microbial processes as for mammalian processes, so there is a need for single-use bioreactors applicable for microbial processes as well. The CELL-tainer® technology, based on a 2-dimensional rocking motion is available now with a working volume from 0.15 – 25L in one and the same bag as well as from 10 – 150L in one bag size. KLa values of 300 h-1 and above have been reported for both sizes of reactors. Culture data of E. coli and a Rhodutorula yeast show that the CELL-tainer® single-use bioreactor is comparable to stirred fermenters and thus suitable for microbial cultivations. Application in both the seed train and as final production system for small batches in GMP is possible now.

### 10:05 Design space definition for a stirred single-use bioreactor family (L28)

*Thomas Dreher, Ute Husemann, Christian Zahnow, Gerhard Greller*

*Sartorius Stedim Biotech GmbH, Göttingen, Germany – thomas.dreher@sartorius-stedim.com*

**Abstract:** Single-use bioreactors continue to gain large interest in the biopharmaceutical industry. They are excessively used for mammalian and insect cell cultivations, e.g. production of monoclonal antibodies and vaccines. This is motivated by several advantages of these bioreactors like reduced risk of cross contaminations or short lead times. Single-use bioreactors differ in terms of shape, agitation principle and gassing strategy. Hence, a direct process transfer or scale-up can be a challenge. Conventional stirred stainless steel or glass bioreactors are therefore still considered as the gold standard due to their well-defined and characterized properties.
Based on this knowledge a stirred single-use bioreactor family was developed with geometrical ratios similar to conventional reusable systems. To follow a Quality by Design (QbD) approach the single-use bioreactor family evaluated here was characterized by using process engineering methods. For definition of a design space the power input per volume (P/VL), mixing time and kLa-values were determined for the different scales. Based on the results conclusions about the suitability and fields of application for the single-use bioreactors are possible.

### 10:25 Solutions for the application of disposable technologies in a bioproduction plant (L29)

*Benjamin Minow*

*Rentschler Biotechnologie GmbH, Laupheim, Germany – benjamin.minow@rentschler.de*

**Abstract:** In biopharmaceutical industry, characterized of cost pressure and increased competition, disposable technology is a welcome measure to reduce timelines and cost for the development and production of drugs. Rentschler is one of the pioneers in terms of employment of disposable equipment and as a CMO the need to ensure a certain degree of equipment diversity that can be offered is obvious. This in turn complicates the incorporation of new Disposable Technologies into already existing facilities whether stainless steel or "plastic". In order to reach a satisfying degree of harmonization tailor made solutions for certain Disposable equipment was developed. This presentation aims for reviewing the latest improvements and the impact on process performance.

10:45 Coffee break, poster session and exhibition

## Friday, 4 April 2014

Chair Erich Kielhorn (TU Berlin, Chair of Bioprocess Engineering)

### 11:15 Key note lecture: High throughput process development for the purification of biopharmaceuticals (L30)

*Jürgen Hubbuch*

*Institute of Process Engineering in Life Science, Section IV: Biomolecular Separation Engineering, Karlsruhe Institute of Technology (KIT), Germany, juergen.hubbuch@kit.edu*

**Abstract:** Modern architecture of biopharmaceutical development and production has seen a rapid change over the past decade and is currently composed by initiatives such as Quality by Design, Process Analytical Technology and early product assessment to mitigate risks during development and manufacture. In the downstream area of biopharmaceutical production, the demand for fast process development with limited material has become everyday life. To meet this demand, high throughput process development (HTPD) strategies for application in downstream process development have been developed. In order to gain maximal benefit from this experimental approach, the analytical methods applied to evaluate the experiments performed require automated performance and a throughput matching the experimental speed. In addition, even though HTS applies low volume set-ups, experimental space should be minimized in terms of feedstock volume needed while at the same time maximising the level of information gained. While the methodology is currently based on statistical data evaluation on the long run a fundamental mechanistic understanding is required. The presentation will give a short overview on and case studies from the application of the HTPD methodology and examples showing its linkage within the current development framework of the biopharmaceutical industry.

### 11:45 Hybrid cybernetic modeling of Escherichia coli metabolic adaptations to carbon source availability: A step forward in the modeling of microorganism behavior within industrial bioreactor (L31)

*Nathalie Gorret*

*INRA, Toulouse, France – ngorret@insa-toulouse.fr*

**Abstract:** Knowledge of the microbial kinetics in complex dynamic environment is required in order to understand and describe the behavior of cells cultivated in large-scale bioreactor where concentration gradients take place [1,4]. Growth of microorganisms on substrate mixtures display diverse growth responses characterized by simultaneous or sequential/preferential uptake of carbon sources. With the objective to simulate the metabolic behavior of microorganisms facing local concentration gradients, a hybrid metabolic and hydrodynamic model has to be intended; as a consequence the degree of complexity of both building blocks has to be considered. In this idea, the aim of this work is to compare different dynamical metabolic frameworks and identify the optimal model complexity i.e. the trade-off between complexity (number of parameters, variables) and realism (prediction range and accuracy) in order to model systematically E. coli metabolic adaptations to carbon source availability. Three different metabolic modeling approaches were compared: a published Kinetic model with enzymatic and transcriptional regulation [2], which theoretically presents a higher degree of complexity, a Hybrid Cybernetic Model and a Lumped Hybrid Cybernetic model with lower degree of complexity. Hybrid Cybernetic modeling (HCM) was previously used to predict successfully diauxic growth patterns and/or simultaneous consumption of substrates [3]. The hybrid cybernetic framework focuses on a dynamic description of metabolism based on a set of sub-networks of the global metabolic pathways called elementary modes. The regulatory processes are interpreted as optimal allocation of resources required for enzyme synthesis among different modes in order to maximize a global objective function such as carbon uptake [5]. In this work, a Hybrid Cybernetic (HCM) and a Lumped Hybrid Cybernetic (L-HCM) models were developed and optimized independently using a global estimation algorithm to find specific parameters based on experimental data. Numerical simulations using the 3 formalisms have been carried out from various set of experimental data in order to evaluate the predictability and genericity of the models. Both developed models can predict the behavior of E. coli in batch environments with very good coefficient of determination R2 ranging from 0.87 to 0.98. This study shows that HCM and especially L-HCM have a good trade-off between number of parameters and accuracy contrary to the large scale metabolic model. L-HCM shows very good accuracy to simulate biomass, glucose and acetate concentrations; consequently it could be an efficient tool to simulate dynamical metabolic behavior of E. coli facing fluctuating environment when integrated with a hydrodynamic model.

**References:** [1] Enfors et al. 2001. J Biotechnol 85, 175–185. [2] Kotte et al. 2010. Molec Syst Biol 6. [3] Kim et al. 2008. Biotechnol Prog 24, 993–1006. [4] Lara et al. 2006. Molec Biotechnol 34, 355-381. [5] Patnaik. 2000. Biotechnol Adv 18, 267–288

### 12:05 Infoteam iLAB: A Software Platform for Optimized Bioprocess Development (L32)

*Ingrid Schmid*

*Infoteam Software AG, Bubenreuth, Germany, Ingrid.Schmid@infoteam.de*

**Abstract:** Biotechnology being one of the key technologies of the 21st century is the basis for the production of many pharmaceuticals, industrial biocatalysts and fine chemicals. It is expected that in 2030 one third of the world wide industrial production will originate from biotechnological processes. The biggest challenge to achieve the demanding aims is the reduction of the development time and costs for new bioprocesses.
infoteam is developing a new lab automation process data management and process control software platform called iLAB. iLAB is developed as part of the research project AutoBIO, sponsored by the German federal Ministry of Education and Research.

The basic idea of AUTOBIO is to apply automation technologies from the high-throughput application domain on the development of bioprocesses. Bioprocess development is miniaturized and parallelized. Methods and technologies usually only established for big scale fermentation are adapted to the small screening and lab scale. Therefore, the quality of the process itself and of data generated during the development is enhanced, product yields are increased and development times are reduced significantly.
iLAB is a flexible and device-independent software platform that provides an easy-to-use interface for the monitoring and visualizing of biotechnological processes. Based on existing laboratory automation standards, the core of iLAB is a well-structured data base.
The integration of DoE and dynamic process modeling tools are possible. The feedback of resulting actuating variables realizes a well-documented online process optimization in a closed loop.
iLAB is developed according to the IEC 62304 software development life cycle process and the EU-GMP Guideline and includes key features like tracking and tracing, audit trail, user and role management and electronic signatures.
In this presentation we provide an overview on the basic features of iLAB and its open architecture that is designed to be flexibly adapted to specific application requirements.

## 12:25 Key note lecture: Modelling across bioreactor scales: methods, challenges and limitations (L33)

*Krist V. Gernaey*

*Department of Chemical and Biochemical Engineering, Building 229, Technical University of Denmark (DTU), DK-2800 Kgs. Lyngby, Denmark. kvg@kt.dtu.dk*

Abstract: Scale-up and scale-down of bioreactors are very important in industrial biotechnology, especially with the currently available knowledge on the occurrence of gradients in industrial-scale bioreactors. Moreover, it becomes increasingly appealing to model such industrial scale systems, considering that it is challenging and expensive to acquire experimental data of good quality that can be used for characterizing gradients occurring inside a large industrial scale bioreactor. But which model building methods are available? And how can one ensure that the parameters in such a model are properly estimated? And what are the limitations of different types of models? This paper will provide examples of models that have been published in the literature for use across bioreactor scales, including computational fluid dynamics (CFD) and population balance models. Furthermore, the importance of good modeling practice will be highlighted as well.

## 12:55 Concluding remarks

*Peter Neubauer*

*Technische Universität Berlin, Institute for Biotechnology, Chair of Bioprocess Engineering – peter.neubauer@tu-berlin.de*

13:00 End of symposium

Institut für Biotechnologie
Technische Universität Berlin
Bioverfahrenstechnik

Chair of Bioprocess Engineering

Poster abstracts

## Determination of cultivation conditions for automated bioprocess development (P01)

*M. Heiser[1] , A. Knepper[1], F. Glauche[1], L. Theuer[1] , F. Wollny[1], S. Bigesse[1,2], A. Neubauer[2], P. Neubauer[1]*
*[1] Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany, www.bioprocess.tu-berlin.de*
*[2] BioSilta Europe GmbH, Berlin, Germany, www.biosilta.com*

With „Quality by Design" (QbD) as the leitmotif, consistent bioprocess development can start at micro liter scale. Setting up a process at that stage must therefore take the same considerations into account, as one would apply to large-scale bioprocesses. Here, the implementation of auto-induction, automated process monitoring and scale-up on a multiwell plate and mini bioreactor platform are shown. Important features that were included are monitoring of key parameters (optical density, pH, evaporation, glucose- and acetate level in the medium). They were applied to micro litre scale cultivations in shaken multiwell plates and scaled up to a millilitre scale stirred tank mini bioreactor system.

## Sensor equipment for quantification of spatial heterogeneity in large bioreactors (P02)

*Anders Nørregaard[1], Luca R. Formenti[1], Stuart M. Stocks[2], Brian Madsen[3], John M. Woodley[1], Krist V. Gernaey[1] [1] Department of Chemical and Biochemical Engineering, Technical University of Denmark, Building 229, DK-2800 Kgs. Lyngby, Denmark*
*[2] Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsværd, Denmark*
*[3] Novo Nordisk A/S, Hallas Allé, DK-4400 Kalundborg, Denmark*

Suspension cultivation in large stirred tank reactors suffers from imperfect mixing and pressure gradients due to the large size of the liquid column in the bioreactors. This leads to gradients of substrate concentrations and in turn cell population heterogeneity. The processes in large scale cannot be directly compared to laboratory scale experiments due to these reasons, and thus, in order to understand the large scale processes, experimental data has to be collected at large scale.
The cost of acquiring data at large scale is high. The bioreactors are usually run with a limited array of sensors and in order to apply more sensor equipment the bioreactor has to be modified which is both costly and results in production downtime. The presence of three phases (gas, liquid, and solid), and the opaque nature of the fermentation broth together with the necessity of heat sterilization further increases the requirements to the sensor equipment. In order to address these issues this study aims to make an investigation into freely floating, battery driven sensor particles that can follow the liquid movement in the reactor and make measurements while being distributed in the whole volume of the bioreactor. The method leaves a minimal footprint and can be applied to running production to gather large scale fermentation data, without the need of dedicated experimental cultivations.
Ultimately, data describing the spatial heterogeneity can be used to enhance existing process models and to create better scale-down strategies for lab-scale experiments. Accurate process models and lab-scale experiments could in turn lead to a more scientific approach to scaling of biotechnological processes.

## Investigation of the scale-up related $CO_2$/$HCO_3^-$-stimulus in Corynebacterium glutamicum (P03)

*Jens Buchholz, Bastian Blombach, Tobias Busche, Jörn Kalinowski, Ralf Takors, Institute of Biochemical Engineering, University of Stuttgart, Stuttgart, Germany, buchholz@ibvt.uni-stuttgart.de*

C. glutamicum is a facultative anaerobic, Gram-positive organism that grows on a variety of sugars and organic acids and is the workhorse for the large scale production of a number of amino acids, such as L-glutamate and L-lysine. In large scale production processes typical bioreactor volumes reach up to 750 $m^3$ to keep production costs low, resulting in mixing times up to 240 s [1, 2]. Consequently, cells are exposed to gradients of temperature, substrate concentrations, pH-values or partial pressures of the various diluted gases as e.g. $O_2$ and $CO_2$ [3]. The latter is additionally enforced due to the hydrostatic pressure, caused by the reactor height and the existence of poorly ventilated regions due to larger volumes [4]. Here, we investigated the scale-up related $CO_2$/$HCO_3^-$ stimulus on the metabolism of C. glutamicum. Batch fermentations with glucose as sole carbon and energy source with elevated (quasi-stationary) $pCO_2$ levels of up to (300–400) mbar in the liquid phase showed no difference in the growth rate but slightly higher biomass yield compared to standard conditions indicating that C. glutamicum can tolerate considerable high $CO_2$/$HCO_3^-$ concentrations. In contrast, experiments with significantly reduced $CO_2$ concentrations obtained by stripping conditions (3 vvm) with air led to three phasic growth, which can be complemented by increasing the proportion of $CO_2$ in the inlet gas flow. Transcriptome profiles obtained by microarray analyses in combination with the determination of enzyme activities led to a comprehensive picture of the adaptation to $CO_2$/$HCO_3^-$ limiting conditions, indicating an indirect activation of decarboxylation reactions by triggering the thiamin biosynthesis. Under high $CO_2$/$HCO_3^-$ conditions, microarray experiments, revealed a complex transcriptional response with most genes assigned to the complete dtxR/ripA regulon controlling the iron homeostasis in C. glutamicum.

**References:**

[1] R. Kelle. L-Lysine production, in: Eggeling & Bott (Eds.) Handbook of Corynebacterium glutamicum, CRC Press, Boca Raton, 2005
[2] Junker. J. Biosci. Bioeng., 97 (2004) 347-364. [3] Lara et al. Mol. Biotechnol., 34 (2006) 355-381. [4] Baez et al. Ramírez. Biotechnol. J., 6 (2011) 959-967

## Development and application of a bioreactor scale-down simulator for fermentations of Corynebacterium glutamicum under dynamic $CO_2$/$HCO_3^-$-gradients (P04)

*Jens Buchholz, Michaela Graf, Bastian Blombach, Andreas Freund, Tobias Busche, Jörn Kalinowski, Ralf Takors*
*Institute of Biochemical Engineering, University of Stuttgart, Stuttgart, Germany, buchholz@ibvt.uni-stuttgart.de*

The use of scale-down systems to imitate industrial-relevant reactor inhomogeneities and to determine concluding metabolic and physiological impacts has been recently reviewed [1, 2]. In principal, most concepts focus on (i) the simulation of the reduced mixing quality occurring at production scale in combination with (ii) oscillating nutrient or gas supply during fermentation processes. Even though, the general application of C. glutamicum as platform organism in scale-up/down studies was recently intensified [3, 4], the metabolic and transcriptional response to large scale-relevant dissolved $CO_2$ levels has been rarely investigated [5, 6].

In dependence on our results obtained under (quasi-)stationary $CO_2/HCO_3^-$ levels [6], we present the development and application of a cascade bioreactor system (CBS) comprising of three stirred tank reactors (STRs) put in series: main reactor (MR), cascade reactor 1 (CR1) and CR2. In total circulation, the application of flow rates between F = (0.5–4) L min–1 facilitated the imitation of residence times in the range of about τ= (0.6–4) min (CR1; CR2). After successful process technical characterization of the system, batch fermentations using C. glutamicum wild-type (WT) were performed. Industrial relevant $CO_2$ levels of about $pCO_2$ = 150 mbar (CR1) and 300 mbar (CR2) were installed by variation of the gas inlet composition and/or gas flow rates. As a result, a similar growth phenotype as obtained in reference batch fermentations performed in the MR under standard $CO_2/HCO_3^-$ levels was observed, in summary leading to growth rates of $\mu = (0.42\pm0.03)\ h^{-1}$, glucose consumption rates of qS = $(0.87\pm0.06)\ g\ g^{-1}\ h^{-1}$, and biomass yields of YXS = $(0.48\pm0.01)\ g\ g^{-1}$. Additionally, the transcriptional response observed by DNA microarrays was recorded. Samples were therefore withdrawn simultaneously from the CBS (MR as reference) revealing a fast transcriptional response, despite the short residence times in the individual CRs of <2 min, as a consequence of the applied $CO_2/HCO_3^-$ variations. Moreover, it was observed that the absolute number of differently expressed genes increased in analogy to the extent of the $CO_2/HCO_3^-$ gradient with CR2 vs. MR >> CR1 vs. MR.

**References:**

[1] Neubauer & Junne. 21 (2010) Curr Opinion Biotechnol 21: 114-121
[2] Takors. J. Biotechnol., 160 (2012) 3-9
[3] Käß et al. Biopr Biosyst Eng. (2013) 1-12
[4] Käß et al. Microb Cell Fact, 13 (2014) 6
[5] Bäumchen et al. J. Biotechnol., 128 (2007) 868-874. [6] Blombach et al. J. Biotechnol., (2013)

## Scale-down model of manufacturing bacterial cultivation: A case study (P05)

*V. Mangiafridda[1], C. Boucher[1], M. Allagadda[1], S.K. Guddeti[2], G. De Conciliis[2], W. Vassillo[2], A. D'Avino[2]*
*[1]Novartis Vaccines & Diagnostics, Technical Development Bacterial Drug Substance Development, 53100 Siena, Via Fiorentina 1, Italy, valentina.mangiafridda@novartis.com. [2]Novartis Vaccines & Diagnostics, Manufacturing Science & Technology Primary, 53100 Siena, Via Fiorentina 1, Italy*

A down-scale model of a manufacturing process is required in order to support manufacturing troubleshooting and improvements and to execute process characterization activities. In this poster, the activities performed to establish a lab scale model of a 50x scale manufacturing process are described, with particular focus on the down-scaling strategy applied to the fermentation step. In order to reproduce the fermenter manufacturing mixing and mass transfer properties, several adjustments of the lab scale bioreactor configuration were required, including optimization of the impeller position, dissolved oxygen cascade control parameters and working volume , through a KLa study. Finally, two feasibility runs were carried out to demonstrate the suitability of the down-scaled process. Growth profiles, productivity of fermentation batches, and other product quality indicators demonstrated the comparability between the lab-scale and large-scale material.

## Integration of rapid sampling experiments into a scale-down two-compartment bioreactor study of Escherichia coli

*Sergej Trippel, Eva Brand, Robert Spann, Dennis Runge, Ping Lu, Basant El Kady, Stefan Junne, Peter Neubauer*
*Chair of Bioprocess Engineering, Department of Biotechnology, Technische Universität Berlin, Ackerstr. 71, ACK 24, D-13355 Berlin, Germany*

Since the residence time of sample ports of the PFR part in scale down reactors is in the range of >10 seconds, short term responses are observed by combining the scale-down reactor and the rapid sampling unit Bioscope. The response to a pulse of glucose under aerobic and anaerobic conditions is investigated. The response of cells, which had been grown under oscillatory conditions, is compared to the response of cells, which had been grown under heterogeneous conditions. It is observed that the preferred route towards the synthesis of branched-chain amino acids after a pulse depends on the history of growth conditions. The presented methodology should be understood as a bridge between systems biology for the investigation of the regulatory network under industrially relevant conditions.

## Process development for Rhodotorula glutinis: From shake flasks to pilot scale (P06)

*Eric Lorenz[1], Runge D.[1], Schmidt L.[2], Stahl U.[1], Bader J.[3]*
*[1] University for Technology Berlin, Germany, eric.lorenz@campus.tu-berlin.de*
*[2] University of Applied Sciences Jena, Germany, [3] Beuth University of Applied Sciences Berlin, Germany*

Regarding further increase of human population, conscious use of natural resources becomes more and more important. In the next years, one of the main problems is the sufficient supply of protein-rich food. Fish is an efficient way to meet this demand. Another advantage of fish is its high content of vitamins, minerals and polyunsaturated fatty acids (PUFAs), (FAO report 2012). During the last three decades, consumption of fish has more than doubled. In 2010, 148 mmt of fish as a nutrition source were harvested. However, yield of fish from industrial fishing is not sufficient to cover the worldwide demand. Nowadays, 45% of seafood is already produced by aqua culturing.
Fishmeal (FM) and fish oil (FO) are essential ingredients of fish feed to ensure suitable fish quality and quantity. Due to overfishing, the quantity of FM and FO decreased, simultaneously leading to higher prices.
The objective of the cooperative project FENA, supported by BLE, was to find a strategy for the substitution of fishmeal and fish oil by algae and yeast. Therefore, we successfully developed a high-cell-density process for oleaginous yeast Rhodotorula glutinis. This strain was selected after a screening of 32 oleaginous yeast strains. Crucial selection criteria were protein and lipid content, as well as amino and fatty acid profiles. For initial shaking flask experiments and the subsequent process optimization, design of experiments (DoE) was conducted. Hence, various factors such as the C/N-ratio, temperature, and nitrogen and carbon sources were examined. The obtained results were transferred to a 5 L bioreactor cultivation resulting in excellent growth performance (80 g $L^{-1}$) and lipid accumulation (30 %). Finally, this valid optimized process was successfully scaled-up to 150 L pilot scale bioreactor. Achieved biomass will be used to supplement fish feed in animal trials.
In conclusion, the production process enables a partial substitution of fishmeal and fish oil for sustainable and high-value fish nutrition.

## Modeling regulation of carbon and nitrogen metabolism in large-scale processes with Escherichia coli (P07)

*Michael Löffler, Joana Simen, Alexander Broicher, Andreas Freund, Ralf Takors*
*Institute of Biochemical Engineering, University of Stuttgart, Stuttgart, Germany, michael.loeffler@ibvt.uni-stuttgart.de*

Development and optimization of production strains and processes is commonly performed in lab-scale fermenters (1–100 L). Consequently, bioprocesses need to be scaled-up to industrial scale following the constraint that performance data of the lab are equally achieved in large production reactors. However, cells in production scale fermenters experience substrate and oxygen gradients while travelling through the different zones of the reactor causing a large number of underperformances. It is the goal of the project to address the urgent scale-up problem by profound systems biology studies using E. coli as a relevant model strain.
Multi-scale systems biology analysis will be performed focusing on the interplay of substrate-gradient based stimuli and the dynamic metabolic and transcriptional response in cells. Metabolome and transcriptome data will be measured in scaled-down experiments (Plug-Flow-Reactor, Cascade-Bioreactor) simulating the gradients of the substantial substrates carbon (C-, glucose) and nitrogen (N-, ammonia). Quantitative transcript data, based on next generation sequencing will be gathered using diverse mutant and reporter strains of E. coli for setting-up data-driven metabolic and regulatory models.

## Kinetic modelling of the central carbon metabolism of Escherichia coli considering flux estimations (P08)

*Sebastian Hans, Anja Lemoine, Nicolas Cruz, Peter Neubauer, Stefan Junne*
*Chair of Bioprocess Engineering, Department of Biotechnology, Technische Universität Berlin, Ackerstraße 76 ACK24, D-13355 Berlin, Germany, sebastian.hans@campus.tu-berlin.de*

Kinetic models are increasingly used for model based process-development and –control. The quality based process design can be enhanced by statistically relevant parameters and measurements. However, there is a lack of suitable models, which are able to describe dynamical fed-batch processes. In the presented work, a model of the central carbon metabolism of Escherichia coli K12 W3110 is developed, which represents a fusion of a model of the TCA cycle [1] with models describing the amino acid syntheses and acetate formation. A dynamic metabolic flux analysis based on a mass balance approach [2] was applied for the kinetic parameter estimation, when reaction rates where adjusted to the fluxes. This allows improved parameter estimation, when not all intermediates can be measured accurately. Using this combinatorial method, the kinetic model could be adjusted successfully in the TCA pathway.

**References:**
[1] Singh & Ghosh. Theor. Biol. Med Model, 2006. 3-27
[2] Desai et al. Biotechnology.1999. 71, 191-205

## Screening of Klyveromyces marxianus strains isolated from traditional dairy products for ethanol production from lactose (P09)

*Fatemeh Nejati[1], Ralf Greiner[2]*
*[1] Islamic Azad University-Shahrekord Branch, Shahrekord, Iran*
*[2] Department of Food Technology and Bioprocess Engineering, Max Rubner Institut, Federal Research Institute of Nutrition and Food, Karlsruhe, Germany*

In this study 11 yeast strains previously isolated from dairy products and identified as Klyveromyces marxianus were evaluated for ethanol tolerance and ethanol production. The results showed that ethanol concentration from 6% can have a reduction on growth of the yeasts. In a medium containing 25 g/l lactose, ethanol concentrations were determined to be 0.61 to 1.70 g/l after 24 hrs of fermentation. Although, ethanol production was not high, it seems that changing and modifying some parameters in fermentation such as carbon source and nutrient ingredient amount, temperature, incubation time and inoculation size would results in higher yields of ethanol.

## Docosahexaenoic acid production with marine organism Crypthecodinium cohnii in the wave-mixed single-use bioreactor CELL-tainer (P10)

*Friederike Hillig, Nadine Porscha, Stefan Junne, Peter Neubauer*
*Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany, f.hillig@tu-berlin.de*

Cultivation of marine organisms exhibits the big challenge to avoid corrosion in conventional stainless steel bioreactors due to the high chloride ion concentration of marine media. The application of single-use bioreactors (SUB) offers a possibility to circumvent this problem. However, most of the organisms have also a high oxygen demand and, in the case of Crypthecodinium cohnii, they are sensitive to shear forces. Therefore, the choice of the appropriate bioreactor system is crucial. The CELL-tainer (CELLution Biotech BV, Assen, Netherlands) provides high oxygen transfer rates and was therefore applied for the cultivation of C. cohnii. A comparison of this SUB with the conventional stirred tank reactor was performed and the growth and production performance in both devices is presented. Additionally, special attention was addressed to the cell physiology determined by the application of flow cytometry. Results prove the feasibility of the concept as an alternative to stainless steel reactors in early stages of marine process development.

## Effect of phosphate concentration and oxygen oscillation conditions on clavulanic acid production in Streptomyces clavuligerus (P11)

*Howard Ramirez[1,2], Juan Arzate[2], Nicolas Cruz-Bournazou[2], Stefan Junne[2], Peter Neubauer[2], Rigoberto Rios[1]*
*[1]Bioprossess Research Group, University of Antioquia, Colombia*
*[2]Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany, howardrm13@gmail.com*

Clavulanic acid (CA) is produced by fermentative processes with Streptomyces clavuligerus (Sc). Despite its weak antibacterial activity, it is a powerful inhibitor of β-lactamase enzymes with some important applications already available in the market. Large-scale production of antibiotics is performed at growth-limiting concentrations of inorganic phosphate. This plays an important role in CA synthesis, because it is related with the availability of glyceraldehyde-3-phosphate, which is the first precursor for the clavulanic acid production. In this work, two cultivations were carried out using either 0.58 g/L $K_2HPO_4$ or 0.8 g/L $K_2HPO_4$ in 250 mL UltraYield© shake flask. The cultures were exposed to oxygen oscillation conditions at 30 h and 48 h separately by the repeated interruption of shaking and the accumulation of CA was investigated. A process model of the batch fermentation was developed, applied in a Matlab® environment and compared with experimental data. Results indicate a good simulation performance at the biomass and substrate concentration.

## Mobile multi-sensor systems for the 3-D characterization of industrial scale processes and the investigation of gradients (P12)

*Anika Bockisch, Erich Kielhorn, Stefan Junne, Peter Neubauer*
*Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany, anika.bockisch@tu-berlin.de*

Anaerobic processes are often characterized by laminar flow. Hence, gradients in the liquid phase occur. However, the knowledge of their impact on process performance is poor. The traditional sampling spots are usually mounted at the wall of the bioreactors. Samples or values, which have been taken without the consideration of gradients, might therefore not reflect the conditions of the core of the liquid phase. Therefore, mobile sampling systems and miniaturized multiposition sensors have been developed and applied in brewing tanks and biogas reactors. It has been shown that the on line monitoring of gradients is achievable with microsensors for the pH-value, the redox potential, $O_2$- and $CO_2$-concentration, and temperature, respectively.
The coupling of sensors with a process-state dependent sampling frequency at the spot where process disturbances can be detected very early offers the possibility to save time and costs for off line analytics. The combination of this data and studies on the cell's metabolism and physiology allows an improved understanding of gradients and their impact on a process, and a further improved design of scale-down experiments close to the conditions in the industrial scale.

## Multiparameter sensor system for process monitoring in biogas plants (P13)

*Sandra Päßler, Ute Enseleit, Winfried Vonau*
*Kurt-Schwabe-Institut für Mess- und Sensortechnik e.V. Meinsberg, Waldheim, Germany, paessler@ksi-meinsberg.de*

The poster presents electrochemical sensors which can be used to investigate biotechnological processes e.g. in biogas plants. The monitoring is focused on insufficiently mixed areas and inhomogeneities in the biogas media. The sensors have to be adapted to the media composition and the measurement requirements. Consequently, two different systems were constructed. For the measurement in a hydrolysis basin a lance with three sensor heads in different depths was developed. Each sensor head contains electrodes to measure pH, redox potential and temperature. The second system is a multi-parameter probe with miniaturised sensors for the same parameters which is used in the fermenter of a biogas plant.

## Monitoring of Bacillus sporulation via electo-optical measuring method (EloTrace®) (P14)

*Dounia, Sandra[1], Schütze, O.[2], Mucha, O.[2], Angersbach, A.[3], Bunin. V.[3], Stahl, U.[1], Bader, J.[4]*
*[1] Research and Teaching Institute for Brewing in Berlin (VLB), Research Institute for Microbiology, Berlin, Germany, s.dounia@vlb-berlin.org*
*[2] ABiTEP GmbH, Berlin, Germany*
*[3] EloSystems GbR, Berlin, Germany*
*[4] Beuth University of Applied Sciences, Life Sciences and Technology, Department of Biotechnology, Berlin, Germany*

New tools for monitoring of fermentation processes are required to achieve additional process control and to meet the increasing demand on reproducibility and quality control in biotechnological production.
In this work the electro-optical module EloTrace was used for online measurement and characterization of changes in cell physiology and morphology during aerobic growth and sporulation of Bacillus amyloliquefaciens.
Some representatives of the species Bacillus amyloliquefaciens belong to the group of plant-growth-promoting rhizobacteria (PGPR) and were successfully used as biofertilizers in agriculture others were used for the production of enzymes and antibiotics. During fermentation of Bacilli high aeration rates are required to achieve optimal growth and high product formation. These demands on the fermentation conditions result in finely dispersed gas bubbles and foam formation which cause severe problems during automatic sampling and online measurement.
Key point of the presented work was to develop an automatic sampling tool able to handle samples including foam and gas bubbles. The successful development of the sampling unit by the collaboration of EloSystems GbR (Berlin), ABiTEP GmbH (Berlin), Technical University of Berlin and HS Mannheim enabled online measurement of cell length, optical density and anisotropy of polarizability (AP), a parameter for the physiological state of cells, with EloTrace even in strongly aerated fermentation processes. Specific curves of the AP-level of the cells reflect alterations of intracellular substances and modifications of membrane surface. Gained AP-signals could be correlated with onset of sporulation, which is based on conversion of cellular structures.
This work was supported by AiF e.V. (Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto von Guericke" e.V.).

## Sensor equipment for quantification of spatial heterogeneity in large bioreactors (P15)

*Anders Nørregaard[1] (andno@kt.dtu.dk), Luca R. Formenti1, Stuart M. Stocks[2], Brian Madsen[3], John M. Woodley[1] and Krist V. Gernaey[1]*
*[1] Department of Chemical and Biochemical Engineering, Technical University of Denmark, Building 229, DK-2800 Kgs. Lyngby, Denmark*
*[2]Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsværd, Denmark; 3Novo Nordisk A/S, Hallas Allé, DK-4400 Kalundborg, Denmark.*

Suspension cultivation in large stirred tank reactors suffers from imperfect mixing and pressure gradients due to the large size of the liquid column in the bioreactors. This leads to gradients of substrate concentrations and in turn cell population heterogeneity. The processes in large scale cannot be directly compared to laboratory scale experiments due to these reasons, and thus, in order to understand the large scale processes experimental data has to be collected at large scale.
The cost of acquiring data at large scale is high. The bioreactors are usually run with a limited array of sensors and in order to apply more sensor equipment the bioreactor has to be modified which is both costly and results in production downtime. The presence of three phases (gas, liquid, and solid), and the opaque nature of the fermentation broth together with the necessity of heat sterilization further increases the requirements to the sensor equipment.
In order to optimize the experimental setup, different types of sensor equipment are currently investigated with regard to describing the flow patterns and shear stress, mass transfer of oxygen and substrate gradients in the fermentation broth. Ultimately, data describing the spatial heterogeneity can be used to enhance existing process models and to create better scale-down strategies for lab-scale experiments. Accurate process models and lab-scale experiments could in turn lead to a more scientific approach to scaling of biotechnological processes.

## Continuous production of biobutanol: Design and evaluation of bioreactor configurations (P16)

*Peter Götz[1], Katja Karstens[1], Horst Niebelschütz[2], Richard Görlitz[2], Giuseppe Olivieri[3], Francesca Raganati[3], Antonio Marzocchella[3]*
*[1] Beuth University of Applied Sciences, Berlin, Germany, goetz@beuth-hochschule.de*
*[2] ARGUS Umweltbiotechnologie GmbH, Berlin, Germany*
*[3] Università degli Studi di Napoli - Federico II, Napoli, Italy.*

Fermentative solvent production by the Acetone-Butanol-Ethanol (ABE) fermentation had its peak in the first half of the last century. Initial process development started from batch operation, but due to the advantages of continuous production, there have been several attempts to implement this operating mode. The biphasic nature of the ABE fermentation, caused by a metabolic switch from acid to solvent production, degeneration of production strains by plasmid loss and the occurrence of contaminations (bacteriophages) are some of the main challenges which are encountered in development of a continuous ABE fermentation.
A review of continuous reactor configurations, including single stage CSTR, series of CSTRs and (fixed-bed) flow reactors will be given. Based on research of metabolism of C. acetobutylicum, a laboratory setup for investigating new reactor configurations will be presented. Problems addressed by using this configuration for research are regeneration of degenerated cells, induction of solvent production and suppression of contaminations. Special attention will be given to scalability of the process.
The presented work is related to the authors´ research in the Era-Net EuroTransBio 7 Project OPTISOLV: German partners are supported by the BMBF, the Italian partner is supported by MSE.

## Influence of biotransformation media and C-Source on biotransformation with fed-batch-cultivated yeasts (P17)

*Thomas Grimm, Technische Universität Berlin, Chair of Bioprocess Engineering, Berlin, Germany, thomas.grimm@berlin.de*

In 24 square deepwell plates yeast were cultivated with fed-batch media to get a high cell density for a following whole-cell screening. With the best volume for optimal aerobic growth in 24 square deepwell plates six promising yeast strains were grown on EnPresso medium (Biosilta).
After cultivation the whole-cell biotransformation for the enantioselective reduction of 2-butanone to R/S-2-butanol was investigated. The influence of biotransformation conditions on the conversion and enantioselectivity of the six cultured yeasts were examined under anaerobic conditions after 24h. Our results shows, that with fed-batch like EnPresso cultivation a higher biomass per volume and a higher enzyme activity per biomass with higher selectivity could be reached.
The results of the influence of the biotransformation media and the C-source during the biotransformation where shown.

## Production and partial characterization of exopolysaccharide from Rhodococcus erythropolis growing brewer´s spend grain from pilot plant (P18)

*Enriqueta Martinez-Rojas*
*Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V., Berlin, Germany, e.martinez@vlb-berlin.org*

Biopolymers like the Exopolyssacharide (EPS) have been extensively studied but only a few of them have been industrially developed over the last decades. Exopolyssacharide are highly heterogeneous polymers containing a number of distinct monosaccharides and noncarbohydrate substituents that are species specific. Due to the broad metabolic diversity and array of unique enzymatic capabilities, Rhodococcus erytbropolis has been used to produce EPS in submerged fermentation with Brewer's spent grain from pilot plant as alternative carbon source. The results obtained suggest an opportunity for exploring this alternative strategy to produce EPS and other high valuable chemicals using economically feasible substrates.

## Two compartment reactor concepts for bioprocess scale-down studies (P19)

*Michael Limberg, Viola Pooth, Wolfgang Wiechert, Marco Oldiges*
*Forschungszentrum Jülich; Institute of Bio- and Geosciences IBG-1: Biotechnologie, Jülich, Germany, m.limberg@fz-juelich.de*

The relevance of biotechnological production increases with the demand for sustainable bulk products. But performance losses during the scale-up from laboratory into production scale and the associated increase of production costs, imperils the competitiveness of biotechnological production. One reason for the losses is based on the decreasing mixing quality and the resulting gradient formation at the industrial scale. To consider the influence of inhomogeneous cultivation conditions during bioprocess development they have to be transferred down to laboratory scale. This is the so called scale-down reactor concept. As there are no commercial scale- down systems available, the most widely used setups are custom-made devices with their individual scope of application. Predominantly the setups are based on the two compartment reactor concept [1]. The cell suspension oscillates between an ideal and a disturbed compartment with different residence times.
In this study a two compartment scale-down device composed of two connected stirred tank reactors (STR) is set up and characterized, for flow and residence time distribution. The reactor compounds consist of standard laboratory scale parts in a commercial cultivation platform. As reference setup an established and well characterized two compartment reactor which consists of an aerobic STR connected to an anaerobic plug flow reactor (PFR) is used. The PFR contains 18 % of total working volume of 5 L and is equipped with three sampling ports in defined intervals [2]. Across the PFR a space / time resolution of the limiting conditions could be achieved, but the occurrence of secondary effects, like pH oscillations, makes it very difficult to allow a separate discussion of the critical parameters. Both scale-down setups were used for comparative cultivations with C. glutamicum production strains under oscillating oxygen supply conditions.
The STR-STR two compartment scale-down reactor, which was build up in this work, is based on a DASGIP parallel fermentation platform and enables thereby a high level of flexibility. By combining different vessel sizes, fluid levels and sensor equipment, the volume ratio between the two compartments as well as the online analytics could be adapted to a broad scope of applications. Additionally the possibility of monitoring and controlling the conditions in each compartment enables the observation of separated or combined effects of oscillating pH, temperature, pO2 and substrate concentration. This study aims at comparative analysis of the concepts of a defined space / time resolution (STR-PFR) with the concept of an average residence time resolution (STR-STR) for an industrially relevant microbial system.

**References:**

[1] Neubauer & Junne. Current Opinion in Biotechnology 2010. 21: 114-121
[2] Käß et al. Bioprocess Biosyst Eng., 2013

## Microfluidic chip development for sampling of metabolites secreted by a single cell (P20)

*Katrin Rosenthal, C. Dusny, V. Oehling, F. Fritzsch, O. Frick, A. Schmid*
*TU Dortmund University, Laboratory of Chemical Biotechnology, Germany, katrin.rosenthal@bci.tu-dortmund.de*

Classic biotechnology offers several analytical methods, based on population level with a plenty of available application ranges. However, exact investigation of cell-to-cell differences and individual effects on cell physiology, as well as metabolic and production performance may only be obtained by single cell analysis (SCA).1, 2
Specific single cell research questions necessitate specific and new lab-on-a-chip systems. In order to establish a system suitable for the precise definition of physical and chemical parameters, the microfluidic chip Envirostat has been developed previously. In the Envirostat, cell trapping is applied by negative dielectrophoresis (nDEP) allowing the contactless cultivation of microorganisms.3 This method provides the advantage of avoiding unspecific effects on cell phenotype resulting from cell-surface-interactions. Additionally, a continuous microfluidic medium flow enables the application of specific chemical perturbations to the target cell. Proof of the Envirostat concept was demonstrated by cultivating various microorganisms and by growth studies of single bacteria and yeast cells.3, 4 The manufacturing process of the Envirostat chip has been optimized particularly with regard to the establishment of analytical methods for the evaluation of cellular productivity at single cell level. The previously used indirect glue bonding process was replaced by a direct fusion bonding process, which enhances the microfluidic stability of the chip and thereby the reproducibility of single cell experiments. Two glass substrates are covalently bound, leading to a homogeneous connection exhibiting high bond strength and chemical resistance. Besides their biocompatibility, glass made chips are compatible to further analytics of excreted cell metabolites. The electric and microfluidic design was changed to improve the microfluidic stability and the reproducibility of single cell isolation. Unreliable electrical isolation sections were replaced by validated electrode geometries, which simplify cell separation, as well as the removal of interfering cells or medium from the inoculum.
We present the most recent development of the microfluidic chip Envirostat, which was optimized to enable analysis of excreted metabolites of a single cell.

**References:**

1. Fritzsch et al. Annu Rev Chem Biomol Eng 3, 129-155 (2012)
2. Delvigne & Goffin. Biotechnol J 9, 61-72 (2014).
3. Fritzsch et al. Lab on a chip 13, 397-408 (2013)
4. Dusny et al. Appl Environ Microbiol (2012).

## From shake flasks to pilot-scale production and application of the plant-growth-promoting bacterium Azospirillum brasilense for a liquid inoculant formulation (P21)

*Norma A. Valdez-Cruz, Cesar Gonzalez-Monterrubio, Carlos Martinez-Salinas, Ramses I. García-Cabrera, Ramses A. Gamboa-Suasnavart, Luz D. Marín-Palacio, Jesus Villegas, Abel Blancas-Cabrera and Mauricio A. Trujillo-Roldán*
*Unidad de Bioprocesos, Departamento de Biología Molecular y Biotecnología, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México. Biofabrica Siglo XXI S.A. de C.V. México, adrivaldez1@gmail.com*

Azospirillum brasilense is the best well known plant growth promoting rhizobacteria for its capacity to fix nitrogen, and also for the ability to produce key signals and components of plant growth promotion. This bacteria are widely used in several plantations, mainly wheat, sorghum, barley, rice, and maize. However, there is no report in the literature disclosing a liquid product produced in pilot scale bioreactors, and able to be stored at room temperature for more than two years. The aim of this work was scaled-up a process for the production of a liquid inoculant formulation based on A. brasilense, from shake flask to 1,000 L pilot-scale bioreactor using the oxygen mass transfer coefficient (KLa) as criteria. Furthermore, we aimed to determine the shelf life of the liquid formulation stored at room temperature, and the increase in maize crops yields in greenhouses. A concentration ranging from 3.5 to 7.5 x 108 CFU/mL was obtained in shake flasks and bioreactors, and after two years stored at room temperature, the liquid formulation showed a one order of magnitude decrease. Applications of the cultured bacteria in maize yields resulted in increases of up to 95 % in corncobs, and 70 % in aboveground biomass.

## Microbial analysis of a biofiltration system – Do heterotrophic bacteria contribute to metal removal? (P22)

*Lorika S. Beukes, Stefan Schmidt, Discipline of Microbiology, School of Life Sciences, University of KwaZulu-Natal, Private Bag X01, Pietermaritzburg 3209, South Africa, lorikabeukes@live.co.za*

The objective of this study was to verify the presence of iron and manganese oxidizing bacteria in a biofiltration system employed to purify borehole water and to assess their potential contribution to metal removal. In addition to the quantification of these target microorganisms in biofilter samples, the contribution of individual isolates to the removal of Fe (II) and Mn (II) from borehole water and the presence of metabolically active biofilms on the filter matrix were verified. Presumptive Fe (II) and Mn (II) oxidizing bacteria were quantified using MSVP medium plus either added iron or manganese. Selected isolates were characterized by light and electron microscopy, physiological tests, MALDI-TOF MS and 16S rRNA gene sequence analysis. Colorimetric assays using ferrozine and leucoberbelin blue as well as XRD analysis were employed to confirm Fe (II) and Mn (II) oxidation using selected bacterial isolates. The presence of metabolically active biofilms was verified by CLSM analysis of biofilter matrix samples. A non-motile, Gram and oxidase-negative plump rod-shaped isolate able to form biofilms was identified as a member of the genus Acinetobacter which was confirmed by MALDI-TOF MS and 16S rRNA gene sequence analysis. The ability of this isolate to oxidize both Fe (II) and Mn (II) was confirmed by XRD analysis of crystals formed in aerobic batch cultures containing MSVP plus added iron or manganese. In the presence of active cells of Acinetobacter sp. strain LB1, the rate of Mn (II) oxidation was significantly higher at neutral pH than that determined for abiotic controls. However, at neutral pH the rate of Fe (II) oxidation in the presence of active cells of Acinetobacter sp. LB1 was in the same range as that determined using abiotic controls. CLSM analysis of biofilter matrix samples confirmed the presence of metabolically active biofilms via CTC staining.
Our data indicate that heterotrophic bacteria such as members of the genus Acinetobacter present in the system water or within metabolically active biofilms on the biofilter matrix can contribute to the oxidative removal of Mn (II) at neutral pH. However, the removal of iron by microbial activity appears to be indistinguishable from abiotic iron oxidation at neutral pH.

## Pharmaceutical protein synthesis and screening in vitro with a cell free platform (P23)

*Marco G. Casteleijn, Centre for Drug Research, University of Helsinki, Helsinki, Finland, marco.casteleijn@helsinki.fi*

Therefore, the cell-free systems bypass cell walls, organelle structures and avoid the issues of cellular genetic regulation. The absence of the cellular barriers allows easy incorporation of substrates, efficient product removal, rapid system monitoring and convenient sampling. This should promote production of synthetic proteins with novel properties as well as the production of sustainable fuels, chemicals and new classes of designer (nano)materials with tuneable properties.

Here we describe the experimental outline to develop a protein expression and screening platform geared to translate biologically active proteins towards pharmaceutical drug candidates. Our aim is to product proteins as native as possible and to add functionality to the proteins post-transitionally by means of conjugation techniques. The focus is on development of innovative tools for biobased drug discovery and drug delivery.

## Wealth from waste? A case study using some selected citrus peels (orange, lime and lemon) (P24)

*Esonu Marvellous, Department of Animal Science and Technology, Federal University of Technology, Owerri, P.M.B.1526, Imo State, Nigeria, ugwum1@yahoo.com*

A study on the extraction of essential oils from Lemon peels, Lime and Orange using N-Hexane was carried out. The yield of oil from Lemon, Lime and Orange was 3.6%, 6.87% and 6.13% respectively. The effects of Temperature, Time and particle sizes were also studied. The result obtained show that the yield of oil increased in small particle size and longer contact time. The yield at a temperature of 25 °C in 24hrs with 0.5 mm particle size for orange and lemon was 0.846 and 0.69 % respectively, at 40 °C for 1.0mm particle size and 30 minutes was 0.615 and 0.31% respectively and that of 60 °C for 1.5 mm particle size for 1 hour was 0.39 and 0.015 % respectively. Further experiments were done considering two different solvent. The yield was 0.2 and 0.7 % respectively for lemon and orange using Methanol and 0.9 and 1.5 % respectively for N-Hexane. The oil was characterized and Specific gravity was found to be 0.9016, 0.9020, 0.9039 respectively for lemon, lime and orange, Free fatty acid value was 2.62, 2.06, 1.78 respectively, Peroxide value in mill equivalent per kilogram was 9.26, 9.46, 9.54 respectively, Saponification value was 173.48, 179.10, 174.33 respectively, Unsaponification was 18.3 %, 16.5 %, 17 % respectively and Acid value was 5.213, 4.11, 3.55 respectively.

## Effect of overexpression of inhibin α fragment on subsequent maturation of preantral follicles (P25)

*Ahmed Allam, KSU, South Africa, aallam@ksu.edu.sa*

The present study aimed to detect the effect of inhibin α on the growth and apoptosis of the bovine preantral follicles and on the expression of genes of follicle stimulating hormone receptor (FSHR), luteinizing hormone receptor (LHR), insulin-like growth factor receptor (IGF-1R) and Activin genes as well. To enhance expression of the inhibin α fragment, different types of preantral follicles were transfected with pEGISI (Fig. 1) then in vitro cultured for 25 days (D). The inhibin DNA vaccine was constructed with inhibin α fragment inserted into the C terminus of HBsAgS. Transfection increased the growth and development of all types of preantral follicles till D10 but occurred to followed by sharp decreasing at D15. The incidence of apoptosis was higher ($P < 0.01$) in transfected preantral follicles than in controls at D15 and increased further (P, 0.01) by D25. The levels of gene expression in transfected follicles were increased by the first 10 days of culture then dramatically decreased in the remaining times of culture period to reach to the minimum level of expression. In conclusion, overexpression of inhibin α fragment induce preantral follicle growth, just till D10 of in vitro culture but occurred significant decreasing in the growth due to apoptosis and decreased gene expression.

## Expression efficacy of dual gene mediated by the internal ribosome entry site element of Encephalomyocarditis virus (P26)

*Chao-Lin Liu, Ming Chi University of Technology- New Taipei City, f2402002@ms16.hinet.net*

Internal ribosome entry sites (IRESs) allow the translation of a transcript independent of its cap structure. However, it is unclear whether the efficiency of IRES-mediated translation is comparable to that of cap-dependent translation. Here, we generated various bicistronic constructs with quantifiable reporter genes, luciferases from fire fly (Fluc) as well as jellyfish (Rluc) and enhanced green fluorescence protein (EGFP), as upstream and downstream cistrons of the encephalomyocarditis virus (EMCV) IRES to precisely evaluate the efficacy of IRES-mediated translation in mammalian cells. There was no significant difference in protein production when the reporter gene was cloned as an upstream cistron. However, lower levels of protein production were obtained when the reporter gene was located downstream of the IRES. Moreover, in the presence of an upstream cistron, a markedly reduced level of protein production was observed. In conclusion, our findings demonstrate what EMCV IRES-mediated translation is relatively less efficient than cap-dependent translation and provide valuable information regarding the utilization of IRES to facilitate the expression of more than one protein from a transcript.

## Novel environmental friendly deacetylation method for chitin (P27)

*Min-Lang Tsai, Department of Food Science, National Taiwan Ocean University- Keelung, Taiwan, tml@mail.ntou.edu.tw*

Commercial preparation of chitosan applies with hot-alkaline method. It is not only to produce lots waste water, but also to inefficiently consume energy. This study utilizes explosive puffing to treat chitin performing large scale of deacetylation. This Green Processing with advantages of energy-save, environment-friendly and convenience-simple is expected to make chitin-chitosan products great reaction activity due to their porous structures. The color of puffed chitin will be browner due to increasing moisture contents, puffed pressure, puffed times, and pH. The degree of deacetylation (DD) of explosive puffed chitin was from 32.2 % increased to 41.4 %. The DD of puffed chitin was increased with puffed pressure increasing. X-ray diffraction patterns of chitin and puffed chitins exhibited two characteristic crystalline peaks, close to 10° and 20°, and exhibited the small crystalline peaks at 27°. The characteristic crystalline peaks of puffed chitins were decreased with moisture contents, puffed pressure and puffed times increasing. However, the small crystalline peaks at 27° were increased as puffed pressure increasing. The structures of break and wrinkles of puffed chitins were increased due to moisture contents and puffed pressure increased. The puffing treatment could reduce particle sizes of puffed chitins.