2nd BioProScale Symposium

Inhomogeneities in large-scale bioprocesses

System biology and process dynamics

14 to 16 March 2012
Berlin – Germany

Language: English

Focus: Biosystems

Cellular stress responses / Metabolic adaptation /
Population dynamics / Analytical tools and modelling

Focus: Bioreactor environment

Large-scale bioprocess characterisation /
Bioprocess scale down: Systems, sensors, industrial relevance /
Scale up: Consistent bioprocess development considering
large-scale reality in industrial systems

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.ifgb.de/bioproscale

Institut für Biotechnologie
Technische Universität Berlin

Chair of Bioprocess Engineering

IfGB
Institute for Biotechnology and Fermentation in Berlin

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Introduction

Welcome address

Dear Colleagues, Ladies and Gentlemen
Dear Guests and Students

My co-workers and I would like to warmly welcome you to Berlin for the 2nd BioProScale Symposium. The aim of this symposium is to continue with the intensive discussions which started in the first symposium in 2009 especially to the cell physiology and systems biology in the large scale. Currently it is obvious that large-scale research is a very important basis for consistent bioprocess development. It is more and more accepted that a better understanding of the final industrial scale is an important issue, which includes technical challenges (sensors, modelling tools) and the cellular dynamic regulatory networks and population dynamics.

The 2nd BioProScale Symposium aims to discuss progresses and challenges in the different scientific disciplines. It summarises the current state of knowledge in relation to our understanding of processes in large scale bioreactors for different bioproducts. The symposium covers related applications starting from food production, white biotechnology and bio based energy/biorefinery processes up to the field of biopharmaceutical production.

We are delighted to have a wealth of promising lectures in our programme and we would like to extend our gratitude especially to the speakers who followed our invitation and give us the benefit of sharing and discussing their knowledge.

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

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

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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.

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Programm at a glance: Wednesday, 14 March 2012

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

13:15 **Opening Lecture:**
Lifelines of single cells and populations in large scale bioreactors – Complex dynamic interplay between extracellular environment and cell machinery (L01)
Matthias Reuss (University of Stuttgart, Germany)

Biosystems

Chair Henk Noorman (DSM), Stefan Junne (TU Berlin)

14:00 **Physiological heterogeneity and population dynamics in microbial cultures (L02)**
Chris Hewitt (Loughborough University, United Kingdom)

14:40 **Coffee break with poster session and exhibition**

15:10 **Key note lecture:**
Population composition and dynamics in biogas reactors (L03)
Edith Nettmann, Antje Rademacher, Kathrin Heeg, Michael Klocke (Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. (ATB), Germany)

15:40 **Reduced overflow of acetate in Escherichia coli by gene engineering and media design (L04)**
Kaarel Adamberg, Kaspar Valgepea, Ranno Nahku, Petri-Jaan Lahtvee, Liisa Arike, Sten Erm, Raivo Vilu
(Tallinn University of Technology and CCFFT, Estonia)

16:05 **Tuning protein production at level of translation (L05)**
Zoya Ignatova (University of Potsdam, Germany)

16:30 **Temperature and substrate-related stresses enhance recombinant protein production (L06)**
Mohammedhadi Jazini, Christoph Herwig
(Vienna University of Technology, Austria)

16:55 **Coffee break with poster session and exhibition**

17:10 **Synthesis of non-proteinogenic amino acid species in recombinant Escherichia coli fermentation – Looking for strain dependent differences (L07)**
Michael Biermann, Julia Linnemann, Bettina Bardl, Sebastian Vollstädt, Uwe Horn (Hans-Knöll Institut, Jena, Germany), Guido Seidel (Wacker Biotechnologie, Jena, Germany)

17:35 **Effects of oxygen transfer limitation and inhomogeneous supply in Corynebacterium glutamicum (L08)**
Friedrich Käss, Marco Oldiges
(Forschungszentrum Jülich, Germany)

18:00 **Effect of intensity and frequency of glucose pulse perturbations on transient E. coli behavior: A step toward the large scale bioreactor (L09)**
Nathalie Gorret, Sirichai Sunya, Jean-Louis Uribelarrea, Carole Molina-Jouve (Université de Toulouse, CNRS, France), Frank Delvigne (University of Liege, Belgium)

18:25 **The dynamics of the branched chain amino acid synthesis in Escherichia coli in a two-compartment reactor (L10)**
Stefan Junne, Eva Brand, Dennis Runge, Martin Baudis, Peter Neubauer (TU Berlin, Germany)

18:50 **Distinguished lecture: Engineering of the genetic code as a biosafety strategy in the industrially relevant bio-production? (L11)**
Nediljko Budisa (TU Berlin, Germany)

19:30 **Poster session & Exhibition**

21:30 **End**

Programm at a glance: Thursday, 15 March 2012

Biosystems

Chair Matthias Reuss (University of Stuttgart)
Peter Neubauer (TU Berlin)

9:00 **Key note lecture:**
Bioprocess monitoring by marker gene analysis (L12)
Thomas Schweder
(Ernst-Moritz-Arndt Universität Greifswald, Germany)

9:40 **Elaboration of a mini scale-down platform on the basis of the response of GFP microbial biosensors responsive to a substrate limitation: Detection of substrate heterogeneities at the single cell level and assessment of microbial viability (L13)**
Alison Brognaux, Philippe Thonart, Frank Delvigne (Université de Liège, Belgium), Peter Neubauer (TU Berlin), Jean-Claude Twizere

10:05 **Microthrix parvicella and Cloacamonas acidaminovorans: Indicator organisms for foam formation in large-scale biogas plants? (L14)**
Tobias Lienen (Helmholtz-Zentrum Potsdam Deutsches GeoForschungsZentrum GFZ, Germany)

10:30 **Evaluation of evolved xylose fermenting strains for bioethanol production: Comparison of single cells and mixed populations (L15)**
Elia Tomas-Pejo, Lisbeth Olsson
(Chalmers University of Technology, Sweden)

10:55 **Coffee break and poster session and exhibition**

11:25 **Systematic process development from microscale to pilot production at the example of a ribonuclease inhibitor in E. coli (L16)**
Juozas Siurkus (Thermo Fisher Scientific (formerly Fermentas), Lithuania), Peter Neubauer (TU Berlin)

11:50 **Engineering E. coli to increase plasmid DNA production in high cell-density cultivations in batch mode (L17)**
Alvaro R. Lara
(Universidad Autónoma Metropolitana-Cuajimalpa, México)

12:30 **Lunch break, poster session and exhibition**

Bioreactor environment

Chair Octavio Ramirez (Instituto de Biotecnologica, UNAM)
Thomas Schweder (Ernst-Moritz-Arndt Universität Greifswald)

14:00 **Key note lecture: Scale up of lactic acid production (L18)**
Joachim Venus (Leibniz-Institut für Agrartechnik, Potsdam)

14:40 **Generic methods for scale up of bioprocesses based on quality-by-design (QbD) principles (L19)**
Christoph Herwig (Vienna University of Technology, Austria)

15:15 **Shaken bioreactors with culture volumes of 0.1 ml to 200 liter: Effects of scale, vessel geometry and shaking parameters on kLa values, mixing times and hydrodynamics (L20)**
Wouter Duetz (Enzyscreen B.V., The Netherlands), Tibor Anderlei, Markus Kühner (Kühner AG, Switzerland)

15:50 **Physiological impacts of mixing at the docosahexaenoic acid production process with the heterotrophic marine microalgae Crypthecodinium cohnii (L21)**
Friederike Hillig, Stefan Jahns, Stefan Junne, Peter Neubauer (TU Berlin)

16:15 **Coffee break and poster session and exhibition**

16:30 **Design of a scalable single-use bioreactor (L22)**
Nico M. G. Oosterhuis, Anton Tromper (Cellution Biotech B.V., The Netherlands), Stefan Junne, Peter Neubauer (TU Berlin)

16:55 **Towards a complete single-use upstream process for an Escherichia coli high cell density fermentations (L23)**
Thomas Dreher, C. Zahnow, U. Husemann, G.Greller
(Sartorius-Stedim, Germany)

17:20 **Decreasing the uncertainty when increasing the scale (L24)**
Wouter van Winden, Rogier Meulenberg, Sybe Hartmans (DSM, NL)

17:50 **Insitu Biocell Vitality Analyzer:**
The on-line bridge to flow cytometry (L25)
Friedel H. Schwartz (Sequip S + E GmbH, Germany)

18:10 **EnBase: A solution for fed-batch conditions during process development (L26)**
Antje Neubauer (BioSilta Europe GmbH, Germany)

19:15 **Conference dinner**

Programm at a glance: Friday, 16 March 2012

Bioreactor environment

Chair Nico Oosterhuis (Cellution Biotech)
Kathrin Ralla (TU Berlin)

9:00 **Key note lecture: Consistent Bioprocess Development and Scaling: Study of Some Forgotten Variables (L27)**
Octavio T. Ramirez
(Universidad Nacional Autónoma de México, México)

9:40 **Scale-down of penicillin production in Penicillium chrysogenum (L28)**
Walter van Gulik
(Delft University of Technology, The Netherlands)

10:10 **Development of a scale-down model of hydrodynamic stress to study the performance of an industrial CHO cell line under simulated production scale bioreactor conditions (L29)**
Jochen Sieck (Novartis Pharma AG, Switzerland)

10:35 **Scale-down model of the inactivated Polio Vaccine production process: Unit operation cell and virus culture (L30)**
Yvonne E. Thomassen, Leo A. van der Pol, Wilfried A.M. Bakker
(RIVM, The Netherlands)

11:00 **Coffee break with poster session and exhibition**

11:30 **Are classical correlations suitable for high performance lab scale bioreactors? (L31)**
Sebastian Schaepe, Andreas Lübbert (Martin-Luther-Universität Halle-Wittenberg, Germany), Artur Kuprijanov, Rimvydas Simutis (Kaunas Technical University, Lithuania)

11:55 **Evaluation of process integration opportunities using process simulation and scheduling tools (L32)**
Jose Oscar Jimenez (Intelligen B.V., The Netherlands)

12:20 **Lunch break with poster session and exhibition**

13:30 **Bacteriophage contamination in bacterial fermentation as an important cause of fermentation failures (L33)**
Marcin Los, Piotr Golec, Joanna M. Los, Grzegorz Wegrzyn
(University of Gdansk, Poland)

13:55 **High throughput cultivation of recombinant Escherichia coli in microtiter plates (L34)**
Csilla Török (Austrian Centre of Industrial Biotechnology, Vienna), Monika Cserjan, Gerald Striedner (Austrian Centre of Industrial Biotechnology / University of Natural Resources and Applied Life Sciences, Vienna)

14:20 **Picoliter bioreactors for growth heterogeneity studies of industrial bacteria on single cell level (L35)**
Alexander Grünberger
(FZ Jülich, IBG-1: Biotechnologie, Germany)

14:45 **Concluding remarks**
Peter Neubauer (TU Berlin, Germany)

15:00 **End of symposium**

Wednesday, 14 March 2012

13:00 Welcome address

Peter Neubauer

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

13:15 Opening Lecture: Lifelines of single cells and populations in large scale bioreactors – Complex dynamic interplay between extracellular environment and cell machinery (L01)

Matthias Reuss

Centre Systems Biology, University Stuttgart – reuss@ibvt.uni-stuttgart.de

Abstract: The purpose of strategies for the integration of computational fluid dynamics (CFD) and quantitative physiology is the development of more reliable simulation tools to accelerate the process of scale-up. This lecture aims at introducing an Euler-Lagrange approach to characterize the behaviour of a heterogeneous cell population in a stirred-tank bioreactor with non-ideal mixing. It allows one to describe population behaviour as the outcome of the interaction between the intracellular state of its individual cell and the turbulent flow field in the reactor. The modelling approach and the numerical method employed are based on an Euler-Lagrange formulation of the system combined with a fractional-step method to allow for a stable, accurate, and numerically efficient solution of the underlying equations. The strategy permits one to account for the heterogeneity present in real reactors in both the abiotic and biotic phases. The example chosen to illustrate this approach deals with the impact of mixing on the sugar uptake (phophotransferase system, PTS) of E.coli cells growing in a fed batch culture with constant sugar feeding rate. The activity of the uptake system depends on the local concentration of glucose as well as the ratio of the intracellular concentrations of phosphoenolpyruvate and pyruvate, which in turn is a function of the history of the individual cell. The simulation results point to distinct differences in the viability of the cells at different scale of operation, a phenomenon which is compared with experimental result. An additional model for the dynamics of cAMP signalling leads to further inside into the expression of rpos.

References

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Biosystems

Chair Henk Noorman (DSM), Stefan Junne (TU Berlin)

14:00 Physiological heterogeneity and population dynamics in microbial cultures (L02)

Chris Hewitt, Loughborough University, United Kingdom – c.j.hewitt@lboro.ac.uk

Abstract: Microbiology is important to both human health and industry, therefore many methods have been developed to count as well as identify micro-organisms in the process environment. Accurate measurements relating to cell proliferation and viability are essential if informed decisions about a process are to be made, since process performance will depend largely upon cell number and individual cell physiological state. In this way, products can be harvested at optimum concentrations and inducible systems can be activated at the appropriate time so that high product yields are achieved. Such information is also important since a high number of dead or dormant cells present during any part of a process will have a detrimental effect on the synthesis of any desired products. The development of multi-parameter flow cytometric techniques in our laboratories has led to a functional classification of the physiological state of single celled micro-organisms, including both yeast and bacteria. This classification is based on the presence or absence of an intact fully polarised cytoplasmic membrane and the transport systems across it. Using these techniques it is possible to resolve a cells physiological state, beyond culturability to include metabolic activity enabling assessment of population heterogeneity. Importantly results are available in real-time, 1-2 minutes after a sample is taken enabling informed decisions to be taken about a process. These techniques have been extensively applied by us for monitoring the stress responses of micro-organisms in such diverse areas as brewing, bio-remediation, bio-transformation, food processing and pharmaceutical fermentation, some of which are discussed here.

14:40 Coffee break and exhibition

15:10 Key note lecture: Population composition and dynamics in biogas reactors (L03)

Edith Nettmann, Antje Rademacher, Kathrin Heeg, Michael Klocke

Leibniz-Institut für Agrartechnik Potsdam-Bornim e.V. (ATB), Germany – enettmann@atb-potsdam.de

Abstract: Despite the exploding number of biogas plants in Germany, the knowledge about the microbial community involved in this important bioprocess is still limited. As revealed by molecular analyses, the majority of participating microbes belongs to up to now uncharacterized species and seems to be not cultivable.
For the analysis of laboratory and full-scale biogas reactors, a number of culture-independent strategies for monitoring of process relevant micro-organisms were established. By molecular approaches based on the detection and analysis of species specific gene sequences, access to hardly or even non-cultivable micro-organisms becomes feasible. In detail, following approaches can be applied: (1) microscopic determination of cell numbers for major groups of micro-organisms w/o with application of fluorescent labeled oligonucleotide probes, (2) analysis of microbial community structure by cloning and sequencing of specific chromosomal regions (e.g. ribosomal RNA operon) up to sequencing of the total microbial DNA content of one single sample (metagenome), (3) following the dynamics within the microbial community structure by "whole"-community-fingerprint (e.g. DGGE, TRFLP), (4) development of species or group specific molecular markers for cell-independent quantification by quantitative ("realtime") PCR, and, finally, (5) monitoring of major enzymes fractions by mapping of the microbial proteome via 2D electrophoresis.
Generally, the microbial community structure differs depending on the apparent process conditions and the substrates used for biomethanization. Also, during ongoing fermentation, the microbial community underlies certain alterations. In mesophilic biogas reactors, the fermentative bacteria population consists predominantly of members of the classes Clostridia and Bacteroides. Also several members of the classes Spirochaetes, Flavobacteria as well as ε-Proteobacteria are present. The methanogenesis is performed by different members of the orders Methanomicrobiales, Methanobacteriales and Methanosarcinales. In most cases, hydrogenotrophic methanogens are detected in laboratory as well as in full-scale biogas reactors. Aceticlastic methanogenesis were only observed in case of a balanced acid turnover, which occurs mainly at lower organic loading rates. In thermophilic biogas reactors, the fermentative bacteria community consists nearly exclusively of Firmicutes, mostly Clostridia. Here, methanogenesis is conducted preferentially by hydrogenotrophic Methanothermobacter spp.
In conclusion, the structure of the microbial community is highly variable and dynamic. However, most of participating micro-organisms are still unknown meaning their role in anaerobic degradation remains unclear. Hence, the next tasks must be not only to develop fast tools for microbial monitoring, but also to explore the unknown "underwater portion of the iceberg".

15:40 Reduced overflow of acetate in Escherichia coli by gene engineering and media design (L04)

Kaarel Adamberg[2,3], Kaspar Valgepea[1,2], Ranno Nahku[1,2], Petri-Jaan Lahtvee[1,2], Liisa Arike[2,3], Sten Erm[1,2], Raivo Vilu[1,2]

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[3]Tallinn University of Technology, Department of Food Processing, Ehitajate tee 5, 19086 Tallinn, Estonia

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 increasedapparent 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.

Wednesday, 14 March 2012

Biosystems

16:05 Tuning protein production at level of translation (L05)

Zoya Ignatova

Department of Biochemistry & Biology, University of Potsdam, Germany – ignatova@uni-potsdam.de

Abstract: The ribosome is the central molecular machinery that translates the genetic information into a corresponding polypeptide by sequential addition of amino acids to the growing nascent chain in an mRNA template-directed manner through repetitive selection of aminoacylated-tRNAs. The tibosomes do not translate the mRNA with uniform speed and transiently attenuate the elongation at different positions along the coding mRNA sequence. Stable secondary structure of the mRNA and clustering of codons read by low-abundance tRNAs are among the factors that slow down the ribosomal movement during elongation. Intriguingly, these transient stops are not random and coordinate protein biogenesis with translation which enables the cell to efficiently use the translation resources for optimal production of a target protein. Alterations of the ribosomal pausing by stress or synonymous mutations influence severely the yield of a soluble protein. Many industrially relevant proteins cannot be expressed efficiently (in soluble form) in heterologous hosts which in part can be explained with differences between the translation profiles in the parental strain and in the heterologous host. Fine-tuning of the coding sequence to the tRNA pool of the host cell might be a successful strategy to improve the production of a target protein.

16:30 Temperature and substrate-related stresses enhance recombinant protein production (L06)

Mohammedhadi Jazini, Christoph Herwig

Vienna University of Technology, Institute of Chemical Engineering, Research Area of Biochemical Engineering, Austria – mjazini@mail.zserv.tuwien.ac.at

Abstract: Overall performance of biological system in small scale differs from large scale because microorganisms are exposed to fast changes in microenvironment in large scale bioreactors and they respond fast to the changes. To analyze the effects of dynamic variations in the environment, such variations are mimicked in small scale. In this work two different types of triangular oscillatory feeding profiles as the post induction feeding strategy as well as a temperature oscillation were applied in intracellular recombinant alkaline phosphatase production expressed in Escherichia coli. The results of the experiments were compared with those of the control run at which constant feeding rate and constant temperature was set. The results showed that oscillatory feeding at which cells were not starved led to higher yield of protein per substrate when compared to a constant feeding rate. Moreover, oscillatory temperature triggers more intracellular protein production. Hence, a one compartment scale down reactor can be used not only to understand the effects of inhomogeneities which may exist in large scale but also it could be intended to use to trigger and enhance product quantity.

16:55 Coffee break and exhibition

17:10 Synthesis of non-proteinogenic amino acid species in recombinant Escherichia coli fermentation – Looking for strain dependent differences (L07)

Michael Biermann[1], Julia Linnemann[1], Bettina Bardl[1], Sebastian Vollstädt[1], Guido Seidel[2], Uwe Horn[1]

[1] Hans-Knöll Institut, Jena, [2]Wacker Biotechnologie, Jena, Germany – michael.biermann@hki-jena.de

Abstract: Escherichia coli is one of the major microbial platforms for the recombinant production of proteins, which underlay an increasing demand in medical and industrial applications. Recent progress in protein analytics revealed the emerging problem of misincorporation of rare amino acids and the formation of protein byproducts during recombinant expression caused by overflow metabolism. Cultivation techniques based on carbon source limiting feeding strategies made it possible to reach high cell densities and lower inhibiting overflow compounds like secreted acetate, but minor investigations were done on amino acid overflow metabolism.
Here we adress this question by analysis of recombinant K12- and B-strain dependent differences of glucose metabolism related stress response and rare amino acid biosynthesis in Escherichia coli fermentations. By using different stress inducing fed-batch fermentation set-ups, precise U-HPLC measurement of involved metabolites and the expression analysis of the target protein we show that recombinant E. coli B-strain produce lower levels of non-proteinogenic amino acids under stress conditions and respond via the overflow synthesis of other branched chain amino acids in comparison to E. coli K12. Our data indicate that E. coli B is the favourable strain variant regarding the unwanted synthesis of rare amino acid species in fermentation processes.
These results enable a broad range of further studies on the molecular mechanism of overflow stress response. A closer look towards various connections of the central carbon and amino acid metabolism reveals possible strategies in the prevention of misincorporation of non-proteinogenic amino acids into recombinant proteins.

17:35 Effects of oxygen transfer limitation and inhomogeneous supply in Corynebacterium glutamicum (L08)

Friedrich Käss, Marco Oldiges

Forschungszentrum Jülich, Germany – f.kaess@fz-juelich.de

Abstract: The platform organism Corynebacterium glutamicum has a successful history of biotechnological usage and prevailing significance in Industrial Biotechnology. Impact of individual cultivation conditions on metabolism of new producer strains needs to be assessed before industrial scale-up in order to identify competitive candidates for emerging bulk and fine chemical markets. One of the most important challenges is the identification of ideal oxygen supply in research and production.
At the IBG-1, a tool for fast determination of oxygen transfer related effects on metabolism was developed and applied: A modified microtiter plate based cultivation system (BioLector, m2p-Labs Baesweiler) creates different oxygen supply conditions in 48-times parallel cultivation approach. The setup is based on a combination of varying filling volume and gas phase oxygen content, generating defined oxygen

transfer rates. Online data acquisition by optical methods combined with a liquid handling robotic system facilitates characterization and comparison of C. glutamicum strains with respect to growth and product secretion properties.
Systematic results and observed biological properties for C. glutamicum Wildtype and producer strains are discussed. Biomass and product yields for the complete scope of process-relevant oxygen transfer rates are presented. Findings were verified by scale-up to laboratory stirred-tank reactor. With growth and productivity as target for optimization, ideal oxygen transfer conditions can be identified from these results. Establishing this tool for future process-development with oxygen-dependent producer strains will lead to shortened development times and increased performance.

18:00 Effect of intensity and frequency of glucose pulse perturbations on transient E. coli behavior: A step toward the large scale bioreactor (L09)

Nathalie Gorret[1], Sirichai Sunya[1,2,3], Frank Delvigne[4], Jean-Louis Uribelarrea[1,2,3], Carole Molina-Jouve[1,2,3]

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[3] CNRS, Toulouse, France [4] University of Liège, Gembloux Agro-Bio Tech, Gembloux, Belgium

Abstract: Ineffective mixing entailing heterogeneity issue within industrial bioreactors has been reported to affect microbial physiology and consequently bioprocess performances. Alteration of these performances results from micro-organisms' ability to modulate their physiology at metabolic and/or transcriptional levels to survive in a given environment. Substrate gradients are part of the fluctuations which take place inside large scale bioreactor due to the fact that fed-batch mode using highly concentrated feed solution is commonly used in bioprocesses. Microorganisms circulating inside those bioreactors would encounter different concentrations of substrate; depending on the history of the cells, microorganisms may respond in a different manner. In order to get some quantitative data on the transient response of Escherichia coli to independent and repeated glucose perturbations of different intensities, a well-controlled experimental setting using rapid sampling device has been employed. Firstly, dynamic stimulus-responses of E. coli DPD2085, yciG::LuxCDABE reporter strain, to a glucose pulse of different intensities (0.08, 0.4 and 1 g L-1) were compared using glucose-limited chemostat cultures at dilution rates close to 0.15 h-1. After at least five residence times, the steady-state cultures were disturbed by a pulse of glucose, engendering conditions of glucose excess with concomitant oxygen limitation. In all conditions, substrate, metabolites and exhaust gases were quantified and specific uptake, production, and respiratory rates were determined using mass and redox balance equations. Furthermore, an in situ bioluminescence approach was used for real-time monitoring of the transcriptional induction of a characteristic stress response of E. coli containing yciG::luxCDABE reporter fusion. Despite many studies describing the dynamics of the transient response of E. coli to glucose perturbations, it is the first time that a direct comparison is reported, using the same experimental design (strain, medium, experimental set-up), to study the impact of the glucose pulse intensity on the dynamics of microbial behavior regarding growth, respiration, metabolite productions and on-line transcriptional induction of the stress response. Secondly, the dynamics of repeated glucose pulses (1, 2 and 4 successive pulses) of different intensities (0.25 and 0.5 g L-1) will be presented. Kinetic data will be also quantified both at the metabolic and transcriptional levels as previously described.

18:25 The dynamics of the branched chain amino acid synthesis in Escherichia coli in a two-compartment reactor (L10)

Stefan Junne, Eva Brand, Dennis Runge, Martin Baudis, Peter Neubauer

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Inhomogeneities regularly occur in industrial fermentation processes due to insufficient mixing [1,2]. The addition of feed in the top of a large scale fermenter leads to areas of high substrate concentration combined with low dissolved oxygen levels [3]. These conditions are leading to oscillations in the substrate uptake rate and in the following conversion to side products like the branched-chain amino acids (BCAA) in Escherichia coli. Further, due to these oscillations, the long-time synthesis profile is influenced compared to conditions, where no gradients are present in the liquid phase. One effect of this accumulation was found to be the synthesis of norvaline when cells were exposed to oscillating substrate and oxygen levels [4].
The presence of BCAA is likely influencing the composition of proteins. To study their synthesis, a scale down reactor (SDR) concept was established, in which inhomogeneities in industrial scale are simulated in lab scale. It consists of a stirred tank reactor (STR) connected to a plug flow reactor (PFR). The feed is introduced at the entrance of the PFR, representing the zone near the feeding spot in industrial bioreactors. Hence, while the STR represents a zone characterized by evenly distributed glucose and oxygen, the PFR characterizes a zone with a high glucose gradient. Since residence times in the PFR are of a minute, short term responses of Escherichia coli are observed in a rapid sampling unit, which allows the observation in a second time scale. By combining both strategies a picture of the dynamics is obtained covering the first few seconds after exposure to excess substrate until a minute. This allows for understanding the flux dynmaics when coupling concentration measurements of a high resolution to a dynamic flux analysis.
The concept presented should be understood as a bridge between systems biology for understanding the regulatory network of a cell and the industrial application for strain and process development.

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18:50 **Distinguished lecture: Engineering of the genetic code as a biosafety strategy in the industrially relevant bio-production? (L11)**

Nediljko Budisa (TU Berlin, Germany) – budisa@biocat.tu-berlin.de

Abstract: The expansion of the genetic code is gradually becoming a core discipline in Synthetic Biology. It offers the best possible platform for the transfer of numerous chemical reactions and processes from the chemical synthetic laboratory into the biochemistry of living cells. The incorporation of biologically occurring or chemically synthesized non-canonical amino acids in recombinant proteins and even proteomes via reprogrammed protein translation is in the heart of these efforts. Orthogonal pairs consisting of aminoacyl-tRNA synthetase and its cognate tRNA proved to be general tool for the assignment of certain codons of the genetic code with a maximum degree of chemical liberty.
Nature builds up proteins with the 20 canonical amino acids (cAAs) encoded by the 61 sense codons. However, these 20 side-chain functionalities are obviously not sufficient for proteins to cover all the chemical diversity necessary to maintain many vital biological functions in both unicellular and multicellular organisms. Evolution invented two strategies to increase the side-chain inventory: a small fraction of proteins are co-translationally equipped with special proteinogenic amino acids such as selenocysteine (Sec) and pyrrolysine (Pyl) by reassignment of termination codons. However, the major chemical classes of modifications that contribute to the protein structure/function diversity are post-translational modifications (PTMs). These reactions are selectively and timely coordinated chemistries performed by dedicated enzymes and enzymatic complexes, usually in specialized cell compartments.
Certainly, one of the main goals of Synthetic Biology is to generate new and emergent biological functions in streamlined cells which are equipped with "tailor-made biochemical production lines". However, it is extremely difficult to mimic nature's complex machineries such as the PTM-apparatus. Thus, we usually highjack and/or divert cellular systems such as protein translation to gain additional chemical diversity. To achieve this goal, we need to find a way for efficient cellular uptake, metabolic stability and translational activity (i.e. incorporation) of useful non-canonical amino acids (ncAAs) which are usually chemically synthesized. Furthermore, we need to (re)assign coding units (i.e. codons) in the genetic code to accommodate ncAAs into target proteins.
The rapid development of new orthogonal pairs and new aaRS specificities for various ncAAs will allow the assignment of novel functionalities of the genetic code with a maximum degree of chemical liberty. Orthogonal pairs should be designed to serve as generalist tools so that ncAAs-mediated protein engineering will not only be relevant for single recombinant proteins, but also feasible throughout the entire E. coli proteome. In the near future, I anticipate ground-breaking works on various systems with the codons in the genetic code emancipated and liberated from the current chemical function. In addition, the use of genome remodeling will enable stable and valuable ncAA additions to the entire proteome of a cell. As the whole research area moves towards maturity, more and more approaches will contribute to solve industrially relevant bio-production problems, including advanced peptide and protein production.

19:30 **Poster session & Exhibition**

Foyer of the Institute for Chemistry

21:30 End

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Biosystems

Chair Matthias Reuss (University of Stuttgart) / Peter Neubauer (TU Berlin)

9:00 **Keynote lecture: Bioprocess monitoring by marker gene analysis (L012)**

Thomas Schweder

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Abstract: The consideration of bacterial stress and starvation responses is of great importance for the successful establishment of an industrial large scale fermentation process. Suitable analysis techniques for stress and starvation specific genes are therefore particularly interesting for the monitoring and control of such processes. The combined methods of transcriptome analysis, high resolution two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry have been extensively applied for the physiological analyses of industrial microorganisms.
We used these techniques to get a detailed view on the physiology or the stress and starvation "stimulons" of the industrially relevant bacteria Bacillus subtilis and Bacillus licheniformis. By means of density gradient centrifugation and combined transcriptome and proteome analyses we were able to separate and characterize subpopulations from B. licheniformis fermentation cultures.
By these techniques we have filtered a set of indicator genes for critical situations during large-scale fermentation processes which could be used as biomakers in order to control the fitness and productivity of these industrial bacterial hosts. In this respect fast mRNA analytical techniques for an at-line monitoring of gene expression during bioprocesses are required. It will be demonstrated that electric DNA-chips loaded with mRNA specific DNA-probes represent a suitable technique for gene expression analyses during fermentation processes. The electric biochip combined with an automated sample preparation establishes a basis for continuous at-line monitoring of host cell physiology during industrial bioprocesses.

9:40 **Elaboration of a mini scale-down platform on the basis of the response of GFP microbial biosensors responsive to a substrate limitation: Detection of substrate heterogeneities at the single cell level and assessment of microbial viability (L013)**

Alison Brognaux[1], Peter Neubauer[2], Jean-Claude Twizere[3], Philippe Thonart[1], Frank Delvigne[1]

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[2]Technische Universität Berlin, Laboratory of bioprocess engineering, Department of biotechnology, Berlin, Germany
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Abstract: The basic principle adopted in our studies is to use substrate limitation responsive biosensors in order to detect spatial glucose heterogeneities inside industrial bioreactors (whole-cell biosensor). Indeed, such heterogeneities cause a lowering of the biomass yield and an increase of by-products concentration. In this work, we have used these biosensors for the elaboration of a mini-bioreactor platform that can be used as a scale-down tool. Three green fluorescent protein (GFP) transcriptional reporters have been chosen in Escherichia coli, i.e. uspA::gfp, csiE::gfp and yciG::gfp. Our previous studies have shown that these kinds of promoters are induced in response of substrate limitation and exhibit a strong fluorescence attenuation when cultivated in heterogeneous bioreactors. This sensitivity to substrate limitation has been confirmed in the case of the csiE and yciG biosensors. A mini scale-down platform has been proposed as a high throughput tool to investigate rapidly the usefulness of a given microbial biosensor. This platform is composed of shake flask able to operate in fed-batch mode either by using the slow release or the intermittent feeding principle. The first system is based on a commercial package (Enbase) based on the enzymatic release of glucose in the medium. The Enbase system allows the generation of a very smooth glucose profile without any perturbations. For comparison purpose, we have also used an intermittent feeding that induces strong fluctuation at the level of the glucose and the dissolved oxygen concentration. Local heterogeneities have thus been reproduced at the level of these mini-bioreactors and these one have caused a decrease of GFP expression, as in conventional scale-down reactor. The presence of GFP in supernatants has also been noticed and seems to be correlated with the substrate limitation signal for the three cultivation systems considered in this work (i.e., chemostat, conventional and mini-bioreactors) and with the membrane permeability.

10:05 Microthrix parvicella and Cloacamonas acidaminovorans: Indicator organisms for foam formation in large-scale biogas plants? (L14)

Tobias Lienen

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Abstract: Anaerobic co-fermentation of sewage sludge and waste with the objective to produce biogas is of growing interest to generate renewable energy and to reduce greenhouse gas emissions. An anaerobic digester is still operated as a so called „black box" and process failures such as foam, over-acidification or floating layers occur in various plants. Changes in the microbial community during process failures could already be observed in laboratory-scale fermenters. However, the alteration in the microbial biocenosis during process failures in largescale biogas plants is scarcely investigated.

In our studies the variances of the microbial community during a foam formation in a sewage sludge and grease fed biogas plant, consisting of four 8,000,000 litre biogas reactors, were analyzed. To compare the diversification in the microbial community, the partial 16S rDNA genes of the two microbial domains Bacteria and Archaea were analyzed by polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and microorganisms were identified by sequence alignment. A relative quantification of possible indicator organisms was carried out using real-time PCR. Activity measurements and analysis of spatial relationship are planned via fluorescence in situ hybridization (FISH).

The molecular fingerprinting revealed an altered microbial biocenosis during a foam formation event and over a one-year period in the foaming-prone reactor. Microthrix parvicella and Cloacamonas acidaminovorans seemed to be directly connected to the foam formation. Higher cell numbers of these two organisms were detected in the foam. Real-time PCR measurements verified higher DNA amounts of M. parvicella in the foaming reactor and foam. Additionally, higher cell numbers of M. parvicella could be detected in the winter months possibly caused due to temperature sensitivity.

M. parvicella and C. acidaminovorans could act as indicator organisms for a starting foam formation in large-scale biogas plants. Finding a threshold DNA concentration of M. parvicella or C. acidaminovorans could serve as earlywarning indicator to take countermeasures against a foam formation.

10:30 Evaluation of evolved xylose fermenting strains for bioethanol production: Comparison of single cells and mixed populations (L15)

Elia Tomas-Pejo, Lisbeth Olsson

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Abstract: Currently large-scale production of bioethanol is mainly based on sugar or starch richfeedstocks. These raw materials are also employed for animal feed and human use and seem not to be sufficient to the increasing demand. In this context, lignocellulosic raw materials are good alternatives because they do not compete with food crops and are widely distributed. However, its utilization for second generation ethanol production at large-scale still needs improvements. When using lignocellulosic biomass, not only sugars are contained in hydrolysates because toxic compounds derived from cellulose, hemicellulose and lignin degradation during pretreatment are also found in the media. Hence the importance of obtaining robust strains which ferment xylose to ethanol with high yields. In this study, different evolved xylose fermenting Saccharomyces cerevisiae strains were evaluated in ethanol production processes from lignocellulosic hydrolysates. The differences between using lifelines of single cells and mixed populations will also be compared in terms of ethanol production for large scale bioreactors.

10:55 Coffee break and exhibition

11:25 Systematic process development from microscale to pilot production at the example of a production of heterologeous ribonuclease inhibitor in E. coli (L16)

Juozas Šiurkus[1], Peter Neubauer[2]

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Abstract: Eukaryotic RNase ribonuclease/angiogenin inhibitors (RI) share a unique scaffold – they are composed of hydrophobic leucine-rich repeat motifs (LRR) and contain a high amount of reduced cystein residues. Members of this group are challenging to produce recombinantly in native state in prokaryotic hosts due to high aggregation level into insoluble protein fraction.

Here we describe the several RI fed-batch production process development and scale up strategies which resulted in a high production of the target as a cytoplasmic fusion protein with MBP [1], as periplasmic correctly processed authentic RI [2] and cytoplasmic authentic RI [2,3]. For scale-up of all processes we applied the fed-batch cultivation technology in the form of the EnBase® biocatalysis-based feeding system[4] allowing from the early screening steps to the final bioreactor process maintain highly similar growth mode. Thus, we were able to fast and systematically verify an original RI folding approaches based on utilization of

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reducing medium additives [2] and/or co-expression of molecular chaperones [3] at the fed-batch production mode already in the shaking flasks and utilized derived data for final production process tuning in stirred bioreactor. The actual study shows, how physiological knowledge combined with high throughput screening strategies can be successfully applied for a straight forward bioprocess development also for difficult-to-produce considered proteins.

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11:50 Engineering E. coli to increase plasmid DNA production in high cell-density cultivations in batch mode (L17)

Alvaro R. Lara

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Abstract: Recent advances in therapies using plasmid DNA (pDNA) imply that better strains for its production will be needed in a near future. pDNA is produced in high cell-density cultivations (HCDC) in fed-batch mode of Escherichia coli. The typical limitations in practical cultivations of E. coli, like aerobic acetate production and metabolic deviations due to the existence of substrate gradients remain as serious challenges for the fast and effective pDNA production. We have previously demonstrated that the substitution of the natural glucose transport system (PTS) by the over-expressed galactose permease in E. coli allows efficient growth while strongly decreases acetate production. The modified strain, named VH33, has been tested for pDNA production using high initial glucose concentrations in order to reach high cell densities in batch, un-fed mode. Notwithstanding the higher production of such strain, compared to its wild type (W3110), its production levels remain low if compared to commercial strains like DH5&61537;. In the present work, several genetic modifications were made to VH33 to further improve pDNA production.

Several genes were deleted from strain VH33: the recA (to reduce pDNA segregation and deregulate pDNA replication), deoR (to deregulate nucleotide synthesis) and nupG (to avoid nucleotide export) were inactivated independently and in combination.

The kinetic and stoichiometric effects of the mutations were evaluated in shake flasks during the production of a 6 kb plasmid bearing an antigen gene against mumps. The best producer strain was cultivated in a lab-scale bioreactor using 100 g/L of glucose to achieve HCDC in batch mode and compared with DH5&61537; under the same conditions. Plasmid supercoiling degree was also evaluated.

The different mutations had effects in the specific growth rate, glucose uptake rate and pDNA yields at different levels and will be shown during the presentation of the present work. Whereas the pDNA yield (YP/X) of VH33 was 1.16 mg/g, the triple mutant VH33  recA deoR nupG reached a yield of 4.22 mg/g, while the acetate production remained very low. When cultivated using a high glucose concentration, the engineered strain produced 182 mg/L of pDNA, 40 g/L of biomass and only 2 g/L of acetate. In contrast, DH5 produced only 70 mg/L pDNA and accumulated 9 g/L of acetate. Furthermore, the supercoiling degree of the pDNA produced by the triple mutant was higher than 80 %.

The pDNA concentration reached by the engineered strain is, as far as we know, the highest reported for batch cultivations. The quality of the produced pDNA (as indicated by the supercoiling degree) is good enough for therapeutic applications. The strain developed in the present work and its cultivation using elevated glucose concentrations represent an attractive technology for fast and efficient pDNA production and a valuable alternative to fed-batch cultivations of commercial strains.

12:30 Lunch break and exhibition

Bioreactor environment

Chair Octavio Ramirez (Instituto de Biotecnologica, UNAM)
Thomas Schweder (Ernst-Moritz-Arndt Universität Greifswald)

14:00 Key note lecture: Scale up of lactic acid production (L18)

Joachim Venus

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Abstract: Renewable feedstocks (e.g. crops, lignocellulosics, green biomass, residues) are being used as raw materials for the production of microbial lactic acid. Lactic acid, its salts and esters have a wide range of potential uses and are extensively used in diverse fields. The goal is to develop a fermentation process based on the substitution of expensive nutrient supple-ments by cheaper materials from biomass due to their main proportion of the whole proc-ess costs.

The scale-up to a technical scale of several processing steps have to be developed for transferable solutions of biotechnologies for renewables. For that purpose a multifunc-tional pilot plant was planned and built at the site of ATB to investigate different raw materials and products. First results of the lactic acid fermentation in a 450-L-bioreactor will be presented. One of the usual ways to keep the biomass inside of the system for increasing the overall productivity is the cell retention with hollow fibre membranes. In comparison to the process without cell recycle (e.g. chemostat) there is a triple up to four time's higher productivity of lactic acid.

Depending on the further processing of the lactic acid the separation of impurities after fermentation is a major process cost too. Therefore an optimization is necessary to find a balance between the substitution of expensive nutrients and the limitation of interfering or undesirable components of natural raw materials respectively. Exploitation of high qual-ity L(+)- and D(-) lactic acid for the production of biopolymers is one of the recent applica-tions. Conventional processes for down-streaming are based on precipitation steps that generate large amounts of chemical effluents. Consequently the environmental impact of traditional processes can be reduced by using alternative technologies, such as electrodi-alysis with monopolar and bipolar membranes.

14:40 Generic methods for scale up of bioprocesses based on quality-by-design (QbD) principles (L19)

Dr. Christoph Herwig

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Abstract: Following the PAT/QbD initiative, new strategies have to be developed in order to scale processes using scientifically based process understanding in a comprehensive but also effective way. QbD must also ensure that process understanding can be transferred from development to production. Therefore, data must be transformed to information and on to knowledge. Consequently, data need to be converted into specific parameters, most effectively using tools which operate in real time. The goal of the contribution is
i) to show which richness of specific parameters can be gathered from simple on-line analytics , which can be applied at the first scale up step, the quantitative screening.
ii) to suggest which key parameters can be efficiently derived from dynamic experiments and may serve as a basis for strain selection and design space determination, Those key parameters are independent of scale and of initial conditions, and therefore enable scale up
iii) to present an integrated approach how to transfer process development knowledge to (simpler equipped) production facilities
iv) to demonstrate how to compare strains by using a sequence of automated, quantitatively and in real time exploited, dynamic experiments even in early strain screening.
We demonstrate that scale up should focus on exploiting data to its root content and must be understood as an integrated approach combining automated experimental design, real time quantitative exploitation and the understanding of biological systems.

15:15 Shaken bioreactors with culture volumes of 0.1 ml to 200 liter: Effects of scale, vessel geometry and shaking parameters on kLa values, mixing times and hydrodynamics (L20)

Wouter Duetz[1], Tibor Anderlei[2], Markus Kuhner[2]

[1]Enzyscreen BV, Haarlem, The Netherlands – duetz@enzyscreen.com *[2]Kuhner AG, Basel, Switzerland*

Abstract: For a long time, orbital shaking as a method to mix and oxygenate microbial cultures has been mainly limited to Erlenmeyer cultures. In the past decade, however, shaking has been increasingly applied for both smaller cultures, (especially round and square well microtiter plates) and larger vessels (round vessels, up to 200 liters). The presentation will give an overview how culture volumes, vessel geometry and shaking parameters influence kLa values, mixing times and hydrodynamics in various types of bioreactors. These comparative data are useful when assessing to what degree experimental data (e.g. productivities of mutant libraries) generated in small shaken vessels may have relevance for the performance in large scale bioreactor systems.

15:50 Physiological impacts of mixing at the docosahexaenoic acid production process with the heterotrophic marine microalgae Crypthecodinium cohnii (L21)

Friederike Hillig, Stefan Jahns, Stefan Junne, Peter Neubauer

Technische Universität Berlin, Department of Biotechnology, Chair of Bioprocess Engineering, Berlin, Germany – f.hillig@tu-berlin.de

Abstract: Crypthecodinium cohnii is a heterotrophic marine microalgae which produces large amounts of docosahexaenoic acid (DHA), a polyunsaturated fatty acid (PUFA) [1]. This marine dinoflagellate can be an economically valuable and sustainable source for DHA production. While the growth (and synthesis of fatty acids) is characterized by a high oxygen demand [2], the algal cells are sensitive to high shear forces, which hamper the application of directly aerated and stirred systems [3]. In addition, the high chloride concentration of the media leads to the corrosion of steel. However, insufficient oxygen supply leads to an immediate reduction of viability and a further drastic increase in shear sensitivity. All these limitations result in the demand for a reactor design which is taylor-made for this process in order to compete with the traditional way of extracting DHA out of fish oil. Beside the physiological constraints which have to be fulfilled, the marine media demands for non-corrosive bioreactor systems.
This study describes a methodology to cultivate C. cohnii from µL to the L scale under fed-batch conditions in appropriate polymer-based cultivation systems under special considerations of the oxygen supply and shear forces. Different systems are compared and the impact of the fluid flow characteristics caused by different shaking and stirring methods is described. The impact of inhomogeneous oxygen concentrations on the physiology is demonstrated and the resulting restrictions for large scale applications are summarized.
A final proof of concept demonstrates which systems are suitable for the application at a process while the impact of inhomogeneities at some systems are not causing severe process disturbances in comparison to other non-favourable systems.

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16:15 Coffee break and exhibition

16:30 Design of a scalable single-use bioreactor (L22)

Nico M.G. Oosterhuis[1], Stefan Junne[2], Anton Tromper[1], Peter Neubauer[2]

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Abstract: In the biopharmaceutical industry is the application of single-use equipment nowadays common practice. Compared to the traditional glass or stainless steel bioreactors, single-use bioreactors offer clear advantages: a quicker turnaround time; minimal utilities required; greatly reduced potential of cross contamination; greater operational flex-

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ibility; reduced validation requirements. However, until today, single-use bioreactors have a restricted application to cell cultures, which are characterized by a low oxygen turnover, due to the low oxygen transfer achieved in single-use bioreactors.
The application of single-use has not only benefits forthese processes, but clearly also in microbial processes, which are demanding higher gas transfer rates. However, application in seed-trains and also in process development reduces risk for contamination. Another advantage of many single-use bioreactors is their design which has been obtained due to the most possible reduction of the shear stress applied to cells. Hence, the application of single-use bioreactors for cultivation of shear sensitive cells like heterotrophicmarine algae is beneficial.
The CELL-tainer® single-use bioreactor has been developed to create a superior oxygen mass-transfer compared to other single-use bioreactors.In the CELL-tainer® an oxygen mass transfer coefficient, kla, of 100-300hr^{-1} is easily achieved. This makes the system suitable for high-density mammalian cell cultures, but especially also for microbial cultures, which has been demonstrated at a 15L scale.
Due to the simple liquid flow pattern, generated by a 2-dimensional rocking movement of the pillow-shaped bag, the reactor is easily scalable. Mass transfer data of a scaled model will be presented.
Comparison of the CELL-tainer® performance with the standard stirred bioreactor and of cultivation results of different cell-lines like CHO-cells, PER.C6®-cells, but especially also in microbial cultures like E.coli, Corynebacteriumand Pichiashows the opportunity to carry out high-performance processes in single-useequipment.As the kla values in the CELL-tainer® can be controlled by changing the rocking speed, the equipment can be used for process development as well.
It can be concluded that the CELL-tainer® bioreactor opens a new area for bioprocesses optimization in a single-use system and using the advantages thereof.As a wide range of operational conditions and operational volumes is possible to achieve in one single piece of equipment, a real multi-purpose,scalable, single-use bioreactor is available now.

16:55 Towards a complete single-use upstream process for an Escherichia coli high cell density fermentations (L23)

Thomas Dreher, C. Zahnow, U. Husemann, G.Greller,

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Abstract: During the last decades single use systems have became widely accepted in the biopharmaceutical industry. This is motivated by many advantages they offer compared to conventional stainless steel bioreactors like lower contamination risk, less time for cleaning and sterilization, lower investment costs and a shorter time to market for pharmaceutical products. Different types of single use bioreactors are available on the market and commonly used for the cultivation of animal and plant cells. The main limitation for microbial use is the relative low mass transfer coefficient (k_La) of this systems compared to stainless steel bioreactors. In this study we present a complete single-use upstream process using Escherichia coli as a high oxygen demanding microbial model system. Two different types of single-use bioreactors a rocking motion type and a stirred bioreactor were considered. The finally applied process is based on the results of the individual kLa-mapping studies for each bioreactor type following the commonly used design space approach. For the seed culture a rocking motion type bioreactor with 5 L working volume was used. Although the mass transfer coefficient (k_La) of this system is lower compared to shaking flasks the oxygen transfer rate (OTR) is higher due to the possibility of using oxygen enriched air in the DO-feedback control loop. Based on an OTR_{max} model a batch cultivation in a chemical defined media was conducted resulting in a final optical density (OD_{600}) of 12 (dry cell weight = 4.6 g/L) under aerobic conditions. Only a small part (<10%) of that seed culture was used to inoculate a stirred single-use bioreactor which is a further development of a cell culture version (50 L working volume). A fed batch cultivation based on an OTR_{max} model was conducted. Using an exponential feeding strategy with a specific growth rate of 0.1 1/h a final optical density (OD_{600}) of 160 (dry cell weight = 60.8 g/L) could be reached. These first results show that by implementing the design space approach single-use bioreactors should be challenged already today for a useful integration in microbial seed train and process development approaches.

17:20 Decreasing the uncertainty when increasing the scale (L24)

Wouter van Winden, Rogier Meulenberg, Sybe Hartmans

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Abstract: Industrial fermentation research and development projects commonly start with laboratory scale strain testing and/or fermentation process development, later followed by transfer of selected strains and optimized fermentation protocols to production facilities. For some important fermentation products, business demands are shifting from maximized productivity to increasing speed of successful scaling up; time consuming iterations between the scales are no longer tolerated. One approach in preventing major mishaps in scaling up is proper testing of the sensitivity of key performance indicators of the fermentation process to the differences that are anticipated between the scales. Thus, good down scale models do not only have value in studying scale up effects, but also in business risk reduction.
This contribution presents some experience gained in industrial research and development practice on (absence of) scale up effects of various fermentation parameters on microbial enzyme production. The results lead to suggested improvements of the design of representative laboratory scale fermentation experiments.

17:50 Insitu Biocell Vitality Analyzer: The on-line bridge to flow cytometry (L25)

Friedel H. Schwartz *Sequip S + E GmbH, Germany – fhs@sequip.de*

Abstract: Batch costs of >500,000 € need safe measures! The quality of a product is influenced by the fermentation process. It is necessary that cells are fermented under optimal conditions, but often these conditions are evaluated by measures, which only give indirect results such as conductivity or optical density. The Insitu Biocell Vitality Analyzer from Sequip focuses the cells directly and thus allows a population analysis. The Insitu sensor offers the opportunity of precise real time in situ measurement of the cell vitality count, cell growth rate, morphology and optical density through the display of the fraction of single cell sizes directly in the fermenter and thus allows a direct control of cell properties. The Insitu Biocell Vitality Analyzer is a valuable sensor in view of the PAT (Process Analytical Technology) Initiative of the US-Food and Drug Administration (US FDA) through the direct, immediate observance of the process and the possibility to compare the analyses with available dates and specifications. For validation purposes it permits a specification-compliant product (Quality by Design).

18:10 EnBase: A solution for fed-batch conditions during process development (L26)

Antje Neubauer *BioSilta Europe GmbH, Germany – antje.neubauer@biosilta.com*

Abstract: Biosilta has developed an advanced technology for microbial cell cultivation, protein production and screening of biocatalysts. Moreover, the EnBase products for bacteria and yeasts are based on medium for high cell density cultivation with enzymatically controlled glucose release. This feature enables the medium to be used as tool for process optimization. Using pumps to change the feed rate externally is the way to control the concentration of the limited substrate glucose in fed-batch fermentations. No mechanical pumps are needed for investigation of the growth rate when the fed-batch medium is used in shake flasks or multi well plates, rather the added enzyme dose will be increased to raise the available glucose amount during the cultivation. Another application of such media is the screening process of production clones. Top performing clones selected under batch cultivation conditions have failed at the production scale while the possible best clones were not discovered. Using fed-batch like conditions in the screening phase can avoid such failure.

19:30 Conference dinner at the Aschingers Restaurant (End 22:30)

Friday, 16 March 2012

Bioreactor environment

Chair Nico Oosterhuis (Cellution Biotech), Kathrin Ralla (TU Berlin)

9:00 Keynote lecture: Consistent bioprocess development and scaling: Study of some forgotten variables (L27)

Octavio T. Ramirez, Universidad Nacional Autónoma de México, México – tonatiuh@ibt.unam.mx

Abstract: Deficient mixing in industrial-scale bioreactors is an important concern as it can result in a heterogeneous environment that may affect cell physiology and bioprocess performance. Adequate knowledge of the effects of environmental gradients on cells is required for consistent bioprocess development during translation of results obtained at the laboratory level to the full industrial scale. The effects of dissolved oxygen tension or substrate gradients has been widely determined for a number of microorganism using the scale-down methodology, however, the study of other relevant variables has been neglected. In this lecture, the use of one- and two-compartment scale-down systems will be presented for the study of the effect of dissolved carbon dioxide gradients and fluctuations in temperature and shear stress on the performance of bacterial and animal cell cultures. Results will be shown on the effects on traditional kinetic and stoichiometric parameters as well as on transcriptional levels of genes from relevant metabolic pathways and on product quality attributes, such as composition of inclusion bodies and glycosylation patterns of recombinant proteins. Finally, novel operation processes will be described, inspired on the results obtained from the scale-down studies.

9:40 Scale-down of penicillin production in Penicillium chrysogenum (L28)

Walter van Gulik, Delft University of Technology, The Netherlands – w.m.vangulik@tudelft.nl

Abstract: In large-scale production reactors the combination of high broth viscosity and large broth volume leads to insufficient liquid-phase mixing, resulting in gradients in, for example, the concentrations of substrate and oxygen. This often leads to differences in productivity of the full-scale process compared with laboratory scale. In this scale-down study of penicillin production, the influence of substrate gradients on process performance and cell physiology was investigated by imposing an intermittent feeding regime on a laboratory-scale culture of a high yielding strain of Penicillium chrysogenum. It was found that penicillin production was reduced by a factor of two in the intermittently fed cultures relative to constant feed cultivations fed with the same amount of glucose per hour, while the biomass yield was the same. Measurement of the levels of the intermediates of the penicillin biosynthesis pathway, along with the enzyme levels, suggested that the reduction of the flux through the penicillin pathway is mainly the result of a lower influx into the pathway, possibly due to inhibitory levels of adenosine monophosphate and pyrophosphate and lower activating levels of adenosine triphosphate during the zero-substrate phase of each cycle of intermittent feeding.

10:10 Development of a scale-down model of hydrodynamic stress to study the performance of an industrial CHO cell line under simulated production scale bioreactor conditions (L29)

Jochen Sieck, Novartis Pharma AG, Switzerland – jochen.sieck@novartis.com

Abstract: The objective of this study was to investigate the effect of hydrodynamic stress on the performance and robustness of an industrial CHO cell line in terms of growth, metabolism, production of monoclonal antibodies and their quality during fed-batch cultivation. Various levels of hydrodynamic stress were generated in 2L bioreactors mimicking those present in different locations of a large scale stirred tank bioreactor, i.e. impeller vicinity, liquid bulk, and low turbulence zone. It was observed that at elevated hydrodynamic stress, equivalent to the average energy dissipation rates, (e), equal to 0.4 W/kg, the specific monoclonal antibody productivity, qmAb, decreased by 25% compared to the standard cultivation conditions corresponding to (e) of 0.01 W/kg. Even stronger decrease of qmAb, in the order of 30%, was observed when (e) was periodically oscillating between 0.01 and 0.4 W/kg to simulate the repeated passage of cells through the highly turbulent impeller discharge zone of a production scale bioreactor. Despite this effect, no changes in metabolite consumption or byproduct formation were observed. Furthermore, product quality was independent of the applied (e). To achieve a molecular insight into the observed drop of cellular productivity, a transcriptome analysis using mRNA microarrays was performed. It was found that transcripts related to DNA damage and repair mechanisms were upregulated when high (e) was applied for cultivation. Based on these results, it can be concluded that the studied cultivation process may be less productive at full scale, due to the repeated exposure of cells to higher local values of (e). Keywords: Scale Down Model, local energy dissipation rate, CHO, production cell line, fedbatch cultivation, transcriptome analysis, mRNA microarrays

Friday, 16 March 2012

10:35 Scale-down model of the inactivated Polio Vaccine production process: Unit operation cell and virus culture (L30)

Yvonne E. Thomassen, Leo A. van der Pol, Wilfried A.M. Bakker

RIVM, Vaccinology, Process Development, Bilthoven, The Netherlands – yvonne.thomassen@rivm.nl

Abstract: Industrial scale inactivated polio vaccine (IPV) production dates back to the 1960s when at the „Rijks Institute voor de Volksgezondheid" (RIV) in Bilthoven a process was developed based on micro-carrier technology and primary monkey kidney cells [1]. Starting in the 1990s, the process was improved by scale-up, and in a later stage, the introduction of Vero cells as replacement of the primary cells [2]. The challenge of such long-running manufacturing processes is to keep the knowledge up to date and at the level required for future, or even inevitable, process changes.
To increase the knowledge on the IPV production process, data from over 50 production runs were analyzed using multivariate data analysis [3]. The explorative analysis performed on single unit operations indicated consistent manufacturing. Although variation in the dataset was large, this method allowed to detect outliers and to set specifications for important variables like cell densities and product yield. The information obtained from this analysis is being applied in process development studies for which a scale-down model of the production line was established.
Cell culture and subsequent virus production were scaled down from 750-L to 2.3-L bioreactor volume. Scale-down was done based on vessel aspect ratios and power input per unit volume. Growth curves and metabolite consumption and production rates were largely in agreement. In this contribution, results of the unit operation cell and virus culture will be presented.

[1] van Wezel, A. L. Nature 216, 64-65 (1967)
[2] van der Velden-de Groot, C. A. M. Cytotechnology 18, 51-56 (1995)
[3] Thomassen, Y. E., et al. Biotechnol.Bioeng. 107, 96-104 (2010)

11:00 Coffee break and exhibition

11:30 Are classical correlations suitable for high performance lab scale bioreactors? (L31)

Sebastian Schaepe[1], Artur Kuprijanov[2], Rimvydas Simutis[2], Andreas Lübbert[1]

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[2] Kaunas Technical University, Lithuania

Abstract: Laboratory-scale reactors are generally used for process development. This equipment is thus commercially available from many suppliers. For most experiments performed in the context of process design and optimization, reactors on the 10-L-scale are chosen. With E.coli cultures that are often used for recombinant protein formation, these reactors are usually operated at impeller speeds up to 1500 rpm to achieve high oxygen transfer rates. At these impeller speeds the motors usually draw electric power up to 1.5 kW. This leads to specific power consumptions of more than 100 kW/m^3. As the power input determines most transport processes within the cultures, much effort within the last 50 years was put into investigations on the relationships between the power input and the key transport variables. Unfortunately the engineering correlations published in literature depict two essential disadvantages: (i) They were measured in air-in-water dispersions and not during real cultivation processes and (ii) they were developed for power intervals (validity ranges) that are much lower than the intervals often applied during cultivations in laboratory scale reactors. In other words, for real cultures, data on the most important quantity kLa, are not yet available at the usually applied high power inputs. Recent developments of high performance high cell density cultivation techniques now allow for precise kLa measurements during the entire cultivation of E.coli cells for recombinant protein production processes. As the noise on the signals from these cultures are very low, detailed investigations of the oxygen transfer rates and oxygen mass transfer coefficients can be performed. The power drawn by the agitator system operated at a fixed impeller speed in a given culture does not only depend on the impeller choice but also on the baffles used to avoid swirling or vortexing. Although it is well known, that the mass transfer coefficient primarily depends on the power input, the problem how much power can be transferred into fluid motion with the various impeller/baffle combinations at a given aeration rate is still under debate. Some combinations are more others less prone to flooding. Thus, different baffle-impeller arrangements were investigated in high cell density cultures of E. coli BL21 with defined media. Detailed studies regarding the kLa and power input were performed to determine the relationships between kLa, power input by the impeller systems and the aeration rate. All investigations were performed during E.coli cultivations at high cell densities during recombinant protein production runs.
The results showed that the various impeller power numbers are varying substantially with changes in size and combination of various impellers and baffles. The data were compared with historical correlations. Finally an optimized bioreactor design is presented which is best suited for maintaining high oxygen transfer rates. This depicts an oxygen transfer performance which is more than an order of magnitude larger than for the reactors delivered by the manufacturers. The improvements make supplementation of the air with oxygen in high performance processes obsolete.

11:55 Evaluation of process integration opportunities using process simulation and scheduling tools (L32)

Jose Oscar Jimenez, Intelligen B.V., The Netherlands – jojimenez@intelligen.com

Abstract: The successful scale up and commercialization of biopharmaceuticals is a challenging task that requires collaboration of professionals from many disciplines. Process simulators and other computer aids can facilitate this task by assisting scientists and engineers to answer the following and other related questions: What is the impact of product titer increase on the capacity load of the downstream section, the overall throughput of a plant, and the cost of goods? What changes are required in an existing multi-product facility to accommodate the process of a new product? What is the range of variability that a process can accommodate if it operates under a tight cycle time? What is the impact of single-use equipment on the demand for utilities and the cost of goods? Our experience in addressing the above questions will be presented using industrial examples in which we evaluated alternative technologies for producing therapeutic monoclonal antibodies and vaccines.

12:20 Lunch break and exhibition

13:30 Bacteriophage contamination in bacterial fermentation as an important cause of fermentation failures (L33)

Marcin Los, Piotr Golec, Joanna M. Los, Grzegorz Wegrzyn

University of Gdansk, Poland – marcinlos@rocketmail.com

Abstract: All bacterial fermentations are prone to phage infection. The results of such infections may show some differences in symptoms and effect, but in most cases they result in process failure with subsequent contamination of facility. During the presentation some aspects of phage infections, based on industrial experience as well as on our research, will be discussed.

13:55 High throughput cultivation of recombinant Escherichia coli in microtiter plates (L34)

Csilla Török[1], Monika Cserjan[1,2], Gerald Striedner[1,2]

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[2] Department of Biotechnology, University of Natural Resources and Applied Life Sciences, Vienna, Austria

Abstract: The production of heterologous proteins in Escherichia coli has become a standard technique widely used in biotechnology and for therapeutic applications. The selection of a strain/vector combination for the production of a defined target protein, and the establishment of suitable cultivation/induction conditions are critical and time consuming steps. To accelerate the process optimization high throughput (HTP) applications with high information content are requested. The BioLector, a mini-bioreactor system (m2p-labs GmbH; Baesweiler; Germany) enables online monitoring of cell density via scattered light and measurement of pH and dissolved oxygen tension (DOT) via fluorescence intensities emitted by optodes implemented in shaken microtiter plates. During this work an enzyme based substrate delivery method – the EnBase® Flo cultivation medium (BioSilta Oy; Oulu; Finland) was applied for 1000 µl scale cultivations of E. coli in the BioLector, to establish a stable protocol for highly reproducible "fedbatch" like cultivations. Thereby the impact of the initial optical density of the inoculum, the inoculum preparation (glycerol stock, pre-culture in different media) and of varying enzyme concentrations on growth behavior was investigated. Based on these results a standard cultivation protocol was defined and a reproducibility study with 18 replicates was performed to ensure the well to well and plate to plate reproducibility Due to the good reproducibility of the chosen standard protocol characterization of different recombinant host strains or cultivation conditions is enabled.

14:20 Picoliter bioreactors for growth heterogeneity studies of industrial bacteria on single cell level (L35)

Alexander Grünberger

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Abstract: Since the last decade there is a rising interest in how and if cellular heterogeneities influence large scale-fermentation processes. Former approaches concentrate on the analysis of large cell populations, determining process parameters based on average cell behavior. Here a disposable microfluidic chip for single cell analysis of industrially relevant microorganisms, e.g., C. glutamicum and E. coli is presented. The device incorporates several hundred bioreactors of picoliter volume, enabling laminar flow, diffusion based transport and fast heat exchange. The bioreactor height of approx. 1 µm restricts bacteria growth to a cell monolayer, ideally suited for image based live cell microscopy. The system can be used to determine growth rates of isogenic microcolonies at various culture parameters. At the same time the growth and division heterogeneity of single cells can be investigated and compared. As a proof of principle the growth of C. glutamicum was studied intensively. This work lays the foundation for future system biological and metabolic investigations such as heterogeneity studies of bacterial populations and long-term growth and production perturbations studies on single cell level. The ability of investigating hundreds of microcolonies simultaneously makes the system ideal for statistical data collection and the investigation of rare events (occurring <1%) such as spontaneous morphological changes or cell deaths etc.

14:45 Concluding remarks

Peter Neubauer, TU Berlin, Germany

15:00 End of symposium

Poster abstracts

Modulation of the scale-down effect by the addition of oleic acid for the production of yeast in fed-batch bioreactor (P1)

Alison Brognaux (alison.brognaux@doct.ulg.ac.be)
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Oleic acid has been used in order to modulate the respiro-fermentative transition when yeast is exposed to glucose fluctuations in scale-down reactors (SDR). When oleate is added as a co-substrate in a scale-down bioreactor, a decrease of the ethanol production is noticed by comparison with a SDR operated with glucose only. Flow cytometry experiments show an increase of the peroxisome content of the cells cultivated in the presence of oleate, potentially explaining the modulation of the overflow metabolism by an increase of the total carbon flux capacity through the stimulation of the & 946;-oxidation pathway. The drawback associated with this methodology is the need for a conditioning phase decreasing the productivity of the process. However, this conditioning can be performed directly during the fed-batch process in order to optimize the productivity to 0.74 g cell dry weigth/L.h.

Investigations at full-scale biogas reactors: Characterization of mixing conditions and counter measures against process failures (P2)

Anne Kleyböcker (ankley@gfz-potsdam.de)
GFZ Potsdam, Potsdam,Germany

Characterization of mixing conditions
Three different full-scale biogas reactors were investigated in terms of their mixing conditions. Therefore three tracer studies were conducted. The tracer was uranine which is a fluorescent dye. It was introduced as a shock load to each reactor. The three reactors comprised a volume of two times 2300 m^3 and one time 8000 m^3. The tracer studies revealed stagnation zones and dynamic zones in the reactors. A correlation between short-circuits of undigested substrate from the inlet to the outlet and missing amounts in the methane yields (on average 15 - 20 %) could be observed.
Counter measures against process failures
After the introduction of p-cresols in a full-scale biogas reactor, the gas production rate decreased by more than 75 % and the organic acids increased by a factor of five resulting in an over-acidification. To recover the process of biogas formation, different counter measures were investigated (1) reducing the organic loading rate (2) introducing sodium hydroxide and (3) adding calcium oxide. After the reduction of the organic loading rate, the concentration of acetic acid decreased, while propionic acid further increased and the pH remained below 7.0. After the addition of sodium hydroxide, the pH increased above 7.0 for one week, but the concentration of propionic acid further increased still indicating an over-acidification. After the addition of calcium oxide, the acid concentration entirely decreased to its supposed level for a stable process of biogas formation, the pH increased above 7.0 and the gas production rate increased to its expected level.

Apparatus and model development for the investigation of scale-up dependent regulatory effects on cell metabolism (P3)

Jens Buchholz (buchholz@ibvt.uni-stuttgart.de), Bastian Blombach, Andreas Freund, Ralf Takors
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Large-scale industrial bioreactors at typical dimensions of up to 750 m^3 exhibit mixing times of up to several minutes. Thus a highly heterogeneous environment is the consequence in which the cells are exposed to rapidly changing process conditions. Various vertical and radial gradients are known and already examined in the literature: amongst others appear gradients of pressure, temperature, pH, dissolved gases and substrates. They obviously impair with successful bioprocess transfer strategies in terms of scale. The challenging requirement of maintaining comparable product amounts, yields and qualities is not easily done as detrimental physiological consequences as microbial stress exposure or metabolic shifts largely occur. In order to drive the analysis of multicellular responses forward, a novel scale-up simulator was developed. The latter facilitates the mimicking of large-scale mixing times and the application of desired reactor inhomogeneities in a cascade of multiple fully equipped stirred tank bioreactors. Desired process conditions can individually be applied to each fermenter of the cascade, reflecting the flow path of the cells through the various micro-environments at production-scale. Regulatory changes and responses of various biological platforms shall be followed on metabolome, transcriptome and enzymatic level in the future. The project is funded by the German Research Foundation (DFG) with the grant number: TA 241/5-1.

Anaerobic treatment of waste water derived from hydrothermal carbonization (P4)

Benjamin Wirth (bwirth@atb-potsdam.de)
Leibniz Institute for Agricultural Engineering Potsdam-Bornim, Germany

Hydrothermal carbonization (HTC) is an artificial coalification process, which produces a solid phase that is discussed as a potential soil amender and a carbon sink as well as a substitute for coal in power plants. Besides the solid phase, HTC also produces a liquid phase, which contains high amounts of soluble organic compounds and requires treatment. Current state-of-the-art are aerobic water treatment technologies. The aim of this experimental study was to investigate the feasibility of using HTC waste water for anaerobic digestion as a sole substrate. The experimental focus was placed on the process performance and influence of potential inhibitors contained in the HTC liquid phase.
The HTC liquor used in this study was obtained from an HTC plant processing corn silage. The liquor had a COD and TOC of 41 g L^{-1} and 15 g L^{-1} with a pH-value of 3.9. The experimental set-up consisted of two 3.8 L glass reactors – a completely stirred-tank reactor and an anaerobic filter. Both reactors were operated continuously for 13 weeks at 37 °C and a constant organic loading rate (OLR) of 1 gCOD L^{-1} d^{-1}. The overall methane rates of both reactors showed values of up to 0.65 LN $gTOC^{-1}$. Based on the methane production a COD removal rate of 50 to 80 % was calculated. TOC degradation was 10 to 20 percentage points lower. Relevant inhibition was not detected but the addition of trace elements seemed essential. The anaerobic filter showed less fluctuations during the whole experiment.
Further, a conducted economical feasibility analysis reveals increased economics for an industrial-scale HTC plant by integrating a digester operated at OLRs above 1.5 kgCOD m^{-3} d^{-1}. The produced methane allows replacing up to 94 % of the natural gas demand for steam production and can lead to an increase of the overall efficiency by 5 percentage points based on the higher heating value in case of municipal organic waste as feedstock.

Impact of different induction levels on inclusion body (IB) formation kinetics in recombinant E. coli fed batch cultivations (P5)

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Overexpression of heterologous proteins in Escherichia coli often results in accumulating of insoluble aggregates, called inclusion bodies (IBs). IB formation comprises complex multi-factorial metabolic pathways. Depending on the specific host/vector system, the intrinsic characteristics of the target protein, the physiology of the host cell and on process operation the distribution between soluble and aggregated recombinant protein can be influenced. Thereby the demands of the industry are to achieve profit-yielding and controllable high quality production processes by modulating IB formation.
The aim of this work was to investigate the impact of varying induction levels (IPTG) on yield of recombinant protein, distribution ratio of soluble and aggregated proteins, production rate, composition of IBs, cellular growth (BDM), gene dosage (PCN) and total cell number (TCN) during exponential carbon-limited fed-batch cultivations. In addition the metabolic load on the host cell was monitored by quantification of the global stress molecule ppGpp and transcription profiling. As model systems E. coli HMS174(DE3) and BL21(DE3) with plasmid or genome encoded T7 systems were used for the expression of GFP, hSOD and Npro fusion proteins.

The performed experiments showed significant differences in terms of growth behaviour and product formation kinetics. Under limiting induction increased solubility and even activity of the target protein inside the IBs was stimulated. Beyond that a cell differentiation process resulting in morphological different cells and in changes of IB formation kinetics from growth associated to fully growth decoupled recombinant protein production was observed.
In summary we have proven that the balance between heterologous and host protein production rate plays a fundamental role in IB formation and that modulation of induction level enables to exert influence on accumulation or prevention of IBs.

Assessment of rigid lab-scale disposable reactors (P6)

Gerco Van Eikenhorst (gerco.van.eikenhorst@rivm.nl), Yvonne E. Thomassen, Leo A. van der Pol, Wilfried A.M. Bakker
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In recent years more disposable stirred tank reactors, like the HyClone or Xcellerex single use bioreactor, have become available. Most of them are bag reactors and designed for production processes. Resulting form their design for production processes the minimal working volumes are 25-L. Recently, rigid lab-scale disposable bioreactors were introduced on the market, ranging from 2.5 to 14-L. An advantage of these reactors is that they can be connected to a normal bioreactor control system with none, or a few modifications. To implement these reactors, and compare them to conventional glass bioreactors, they should be characterized with respect to mass transfer coefficient kLA, mixing times and power consumption. Here these characteristics, relevant to animal cell cultures, are presented for the three mostly used types: the Mobius Cellready (Applikon/Millipore), CelliGen BLU single use (New Brunswick Scientific) and Univessel SU (Sartorius Stedim).
Power inputs varied from 0.5 to 380W/m^3, klA measured in water by means of headspace aeration ranged from 0.2 to 1.4 hr^{-1} and observed mixing times ranged from 9 to 50 seconds.
These results are in accordance with the characteristics of a conventional glass bioreactor.

Automated quantitative analysis of bubble size distribution in a gas/liquid system (P7)

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Technische Universität Berlin, Germany

The design of multi phase reactors usually requires expensive experimental investigations. Complete models for the particle size distribution as a function of power-input, material and process parameters are rare and relatively inaccurate. For an exact prediction of heat and mass transfer and reaction or growth rates during fermentation or in bubble columns, exact knowledge of the interfacial area is required. Therefore the quantification of bubble size distributions during such processes needs to be established.
For sophisticated quantitative results photo-optical probes have proved oneself as reliable measurement techniques. An in house developed endoscope technique presents distinguished in situ pictures.
The used endoscope probe allows real time recording of 2D images of the bubbles. A measurement for particles in the size range of 5 to 5000 µm is provided. In order to ensure robust and accurate bubble detection, every series of images is first pre-filtered to remove irrelevant and misleading image information. The subsequent bubble recognition consists of three steps: Pattern recognition by correlation of pre-filtered grey values with search samples, the pre-selection of plausible circle coordinates, and the classification of each of those circles by an exact edge examination. The software employs a normalized cross correlation procedure algorithm. Manual evaluation of the drops on the images was used to quantify the accuracy of the image algorithm software.
The deviations of the mean diameter between manual and automated analysis were below 10 %. However, the manual results show differences between single analyses of the mean Sauter diameter and expose the human bias. The automated image analysis gives uniform results, but can be improved in detecting non spherical bubbles.

Evaluation of bio-ethanol production from ligno-cellulosic biomass using process simulation tools (P8)

Jose Oscar Jimenez (jojimenez@intelligen.com)
Intelligen, Amsterdam, The Netherlands

Over the past three decades there has been intense investigation on the development of fuel producing processes that are based on the use of renewable agricultural materials as feedstock. This activity is driven primarily by the quest for fuel self-reliance and carbon oxides emission reductions. The main effort has been concentrated on bio-ethanol and bio-diesel which have been shown to give motor engine performance similar to that of conventional petroleum based fuels. In addition to product characteristics, however, process economics play an equally important role in any successful product commercialization. In this work, realistic process simulation models have been developed in order to analyze the economics of ethanol production from corn stover and other ligno-cellulosic sources of biomass. This presentation will illustrate how such models can guide research and development work and facilitate process optimization.

Biocatalytic whole-cell-screening in microwell plates: Determination of the best growth and screening-system for yeast reduction processes (P9)

Thomas Grimm[1,2] (thomas.grimm@berlin.de), R. Roller[1], A. Neubauer[3], P. Neubauer[1]
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In a microwell plate screening with aerobic conditions for cell growth a maximum volume of 2-4 mL is possible. To get enough cells for the following whole-cell-screening in 24 square deepwell plates a high cell density is necessary. In addition, a good metabolic status of the cells is required.
To meet these requirements in a screening for biocatalytic yeast reduction processes, a comparison of different batch and fed-batch media in microwell plates was carried out. Six yeasts where tested in 24 square deepwell plates and a volume and medium optimization was performed. Standard batch medium for yeast-cultivation where compared with three different cultivation media with the enzyme-based glucose-liberation system (EnBase® Flo Yeast Medium) from BioSilta, Finland.
It was shown that with a glucose feeding a significantly higher cell density compared to standard batch cultivation could be achieved. In addition the biocatalytic screening was directly performed in the 24 square deepwell plates. The reduction was done under aerobic as well as under anaerobic conditions after growth of the cells.
The results showed a significantly improved conversion and increased enantioselectivity by using the enzyme-based glucose-liberation system. There was also a different activity and enantioselectivity with aerobic and anaerobic reduction conditions. The entire resulting growth and screening system is an efficient process for a fast and representative whole-cell-screening in 24 square deepwell plates.

Model-based cultivation of Escherichia coli RB791 and BL21 strains: Process parameter comparison from laboratory bioreactor and shaking flask scale fermentations (P10)

Oskars Grigs (oskars.grigs@rtu.lv), Riga Technical University, Riga, Latvia

Model based bioreactor and flask scale cultivations of two different Escherichia coli species RB791 and BL21 were performed and results discussed. Aims of current research was to utilize mathematical modeling approach to calculate correct substrate feeding rates for desired cell specific growth rates (μ) and to evaluate and compare most of comonly used process parameters, such as yield coefficients (Y), specific substrate consumption rates (q), Monod saturation/inhibition constants (K) etc. for both strains and both scales. Due to this, under substrate limited conditions, were maintened biomass accumulation processes with specific growth rates of 0.15, 0.25 and 0.35 h^{-1} with value shift of ± 0.05 h^{-1}. Acetate measurements allowed to conclude, that in thous conditions were avoided undesirable accumulations of this by-product. In such a way smooth and controlled biomass growths according to the process model was achieved. Using well defined fed-batch media consisting from salts and glucose, were achieved E. coli biomasses up to 60 g/l.

Microelectronic biosensors for on-line monitoring of key molecules in bioprocesses (P11)

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The continuous monitoring of certain key molecules in bioreactors would enable an improved understanding of running processes and their effective and economic operation. Today, however, only a few parameters are recorded on-line on a regular basis. On the other hand, a small set of data like actual consumption of glucose or other metabolites would be required to follow the reactor processes and allow for a robust control. - A continuously operating sensor chip has been developed at IHP to monitor glucose in the interstitial fluid of diabetic patients [1], which might also apply to bioreactors. The microchip relies on a microelectromechanical system (MEMS) that operates by the principle of affinity viscosimetry, i.e. glucose concentrations cg are determined from viscosity variations of a sensoric fluid in which glucose and dextran compete for binding to the lectin concanavalin A. A cg-dependent viscosity is exhibited by the solution that depends on the degree of cross linking between dextran and ConA and that is determined from the velocity with which a MEMS actuator is deflected in the sensoric liquid. A temperature measurement is combined with the determination of cg in order to correct for possible temperature variations. The sensor chip has dimensions of $0.4 \times 1.3 \times 0.2$ mm, and a single measurement requires about 100-300 ms such that cg transients can be determined with a time resolution of 1 sec. The active volume of the measurement is on the order of nl allowing for a detection of only 1e10…1e13 analyte molecules. These characteristic numbers illustrate the potential that is offered by biosensors fabricated by semiconductor technology and which qualifies their usage in bioreactors and microfluidic devices.

[1] M Birkholz, K-E Ehwald, R Ehwald, M Kaynak, J Borngräber, J Drews, U Haak, J Klatt, E Matthus, G Schoof, K Schulz, B Tillack, W Winkler, D Wolansky, Mikroviskosimeter zur kontinuierlichen Glucosemessung bei Diabetes mellitus, Mikrosystemtechnik-Kongreß 2009, VDE-Verlag, Berlin (2009) pp. 124

Scale-down approach to enzyme production process with Pichia pastoris (P12)

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The methylotrophic yeast Pichia pastoris has become a popular host for the production of heterologous proteins. In this study we evaluated the performance of such process in production scale using a two-compartment scale-down simulator. P. pastoris strain (Mut+ phenotype) expressing Rhizopus oryzae lipase (ROL) was cultivated in an oxygen limited fed-batch process with a mixed feed of glucose and methanol. Results show that glucose gradient does not affect ROL productivity, and that P. pastoris is a robust host for the production of heterologous protein. It is also suggested that scale-down reactor is useful in reactor design and modeling reactors of different volumes. However, constraints on power consumption, oxygen transfer rate and heat transfer rate must be considered.

An overview: Microbioreactors and analytics for systems biology on single cell level (P13)

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Single cell analysis (SCA) solely enables overcoming fundamental problems of population level analysis and opens up possibilities to elucidate cellular mechanisms independent of cell-to-cell influences. The systems biological analysis on population level is often masked by unspecific average values [1]. Hence, SCA became one of the pioneer technologies of ´omics´ [2] and an integral part of biotechnology for the identification of new objectives for metabolic engineering and synthetic biology [3]. Lab-on-a-chip (LOC) systems are well suitable for SCA as they maintain a compatible size proportion to the dimension of single cells and facilitate precisely defined environmental conditions [4]. The most prominent analytical tools for SCA are capillary electrophoresis and flow cytometry, which enable a high throughput, but are limited to a snapshot analysis of a certain point in time.1 Alternatively, time-resolved analysis to receive data sets from a single cell for a certain period of time is conceivable. In order to achieve this, different contact and contactless trapping methods are feasible. Contact trapping techniques like microarrays or dam structures are easy to design, but might induce unspecific phenotypes by cell-surface contact. Contactless methods like hydrodynamic traps or nDEP (negative dielectrophoresis) field cages are generally more complicated, but allow the significant advantage of the feasibility to perform intra- and extracellular analysis. Hence, cellular functions can be mechanistically elucidated with the application of systems biological approaches by totalizing ´omics´ on single cell level.
Here we present an overview of methodological strategies for single cell analysis and the unique capability of LOC systems to analyse single cells in precisely defined microenvironmental conditions (Figure 1). Furthermore we demonstrate important corresponding analytical technologies to access information on all levels of ´omics´ research for the decryption of bio-mechanistic data at single cell resolution.

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Beyond the bulk: Analysis and cultivation of single yeast and bacteria in precisely controlled extracellular environments (P14)

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The acquisition of quantitative and time-resolved data from single cells is a central approach for the elucidation of cellular functions. In contrast to bulk measurements, single cell analysis grants undisturbed access to physiology and regulatory circuits beyond the population average [1]. A detailed knowledge about individual physiology and its response to perturbations is essential for the understanding and accurate prediction of how the dynamic system of a clonal microbial population behaves in inhomogeneous environments.
Here, we present growth kinetics of three different microorganisms under precisely defined (micro-) environmental conditions at single cell resolution in the Envirostat 2.0 system [2, 3]. In biotechnology, growth monitoring constitutes an important method for various assays for e.g. substance screening [4]. Therefore, growth is contemplated as a proxy for global cellular physiology because of its close connection to the functional state of the biochemical network. Two methylotrophic yeast strains, Hansenula polymorpha and Pichia pastoris, as well as the bacterium Corynebacterium glutamicum were chosen as model systems for eukaryotic and prokaryotic cells with industrial relevance. The microorganisms were contactless trapped by negative dielectrophoresis (nDEP) in a continuous medium flow to ensure unlimited availability of nutrients and oxygen and an immediate removal of secreted metabolites. Cultivation temperature was kept constant by a customized temperature control system, which also compensated the temperature shift caused by nDEP-induced Joule heating.
The obtained kinetic data from single cell cultivation experiments of all three microorganisms were compared to growth kinetics of populations grown from the respective strains singularized by the dilution-to-extinction method [5]. Populations originating from singularized cells were assumed to propagate under unlimited growth conditions at low cell concentrations occurring at the very early exponential growth phase. Interestingly, both methylotrophic yeast, Pichia pastoris and Hansenula polymorpha, showed a higher specific growth rate when cultivated in the Envirostat system of up to 35 % and 20 % compared to volumetric growth rates of populations grown from single cells, respectively. An even more significant increase of to 40 % could be observed in the specific volumetric growth rate of single Corynebacterium glutamicum. These findings question the common idea of unlimited growth prevailing during the exponential growth phase of microbial cultures with population-based cultivation technologies. This might have impact on all scientific areas focusing on the understanding and manipulation of cellular physiology from cancer research to metabolic engineering. Moreover, the results of this study nicely show the Envirostat 2.0 cultivation system to eliminate inhomogeneity occurring in bulk cultivation

approaches and for disclosing the impact of perturbations on cellular physiology on the single cell level.

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Contactless single cell analysis of industrial relevant bacteria in controlled microenvironments (P15)

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Time resolved analysis of single cells enables deciphering of cellular mechanisms, such as cause and effect in regulation cascades by systematic perturbation experiments [1]. The principal reasons for phenotypic diversity and heterogeneity in bioprocesses with isogenic populations are the varying microenvironments surrounding single cells [2]. Hence, microenvironments have to be controlled during single cell analysis (SCA) to allow systematic perturbation with the aim to identify biochemical targets for drug development or for metabolic engineering of hyper-producing microorganisms. First, total microenvironmental control considers chemical control, such as controlled nutrient supply and precisely defined chemical composition surrounding the isolated single cell. Second, it considers physical control, such as temperature control and defined mechanical impact, such as pressure or shear stress. Most lab-on-a-chip (LOC) devices are limited to a partial control of these major impacts and do not consider impacts of for example surfaces which can induce unknown changes in cell phenotypes. Up to now, LOC devices using more advantageous contactless single cell trapping by negative dielectrophoresis (nDEP) had the disadvantage of Joule heating and did not allow analyzing bacteria under continuous flow.
Here we present a next generation microfluidic platform which allows for the first time contactless manipulation and trapping of single bacteria in a stream of medium and overcomes all mentioned limitations (Figure 1 (A)). Computational modeling and cell experiments were used to develop a sophisticated microelectrode and microchannel design enabling: first, single bacterium isolation from a fermentation broth, second, a fluid removal of process medium residues and third, a precisely microenvironmental controlled nDEP trapping of a chosen bacterium in a microchamber for single cell analysis (Figure 1 (B)). In the Envirostat 2.0 the temperature and medium composition around an isolated bacterium can be set and alternated by the experimenter. We demonstrate single cell response of the lysine producer Corynebacterium glutamicum MH20-22 B on different phosphate concentration in the medium stream. Single cells can be analyzed by direct growth analysis, applying fluorescents reporter systems like GFP reporter coupled the product concentration and for the first time also by collection of extracellular molecules secreted from a living single bacterium for mass spectroscopy analysis. Subsequent sampling can be reached by an on-tube-seal microfluidic connection [3]. In contrast to other LOC devices for SCA, single cell trapping of our device is less depending on cell nature and allows microenvironmental controlled isolation and analysis of a broad range of cell types, which we demonstrate with 9 different cell strains. Additionally, next to environmental controlled single cell screening, selection, cultivation and analysis, the chip allows the separation and parallel analysis of daughter cells. Finally, the analyzed cell or daughter cells can be transferred in cultivation wells to retrieve and analyze the resulting population. Importantly, contactless single cell cultivation in a flow of growth medium enables total microenvironmental control by the experimenter and hence allows analyzing and identifying responses of this smallest catalytically active unit with decoupled perturbations like specific inhibitors or stress signals.

Online monitoring using EloTrace® measurements for optimization of bacterial fermentations (P16)

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Bacterial fermentation processes are applied in a wide range of industrial processes. A fast and reliable monitoring of the actual physiological fitness of the cultures is required to develop and maintain efficient fermentation processes. In the presented work bacterial fermentation processes are monitored by the new EloTrace® System which uses the electro-optical properties of rod shaped bacteria cells to characterize the morphological and physiological states of the population. The modified EloTrace® System is designed for the industrial application under GMP conditions, considering also requirements of hygienic design.
The achieved results of the online measurement allow a reproducible and reliable monitoring of the fermentation process of Lactobacillus sp. and Bacillus sp., two bacteria with many industrial applications. Furthermore, the additional insight in the physiological state of the cultures enables the optimization of the fermentation process influencing the yield as well as the survival rate during downstream processing. Exemplary results of on-line monitored batch fermentation of Lactobacillus plantarum will be presented and discussed.
The electro-optical analysis expands the possibilities of quality assurance (QA) in bacterial fermentation. The integration of this analytical technique should improve process quality and productivity for example in the production of bacterial cultures in agricultural field. Within the scope of a research project the system will be enhanced by cooperation between EloSystems GbR (Berlin), ABiTEP GmbH (Berlin), TU Berlin and HS Mannheim.
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Implementation of proton transfer reaction: Mass spectrometry (PTR-MS) as tool in bioprocess monitoring – measurement of volatile components in the bioreactor exhaust gas (P17)

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Common bioprocess conditions imply a gas-liquid-mixture with living cells as solid phase in a sterile environment which demands a great deal on sensor/analyzer technology and design. On-line access to physiology relevant process variables, the ultimate request of process engineers, is still very limited as complexity of biological systems additionally constrains direct measurements. Living cells can be described as a closed compartment in the bioprocess and information on physiology only can be gained by measuring the cell compartment and its components or by detection of molecules in the gas/liquid phase. Quantification of intracellular components definitely yielding the highest level of physiology related information is not possible via direct on-line measurement. Molecules in the gas/liquid phase either provided by medium and gas input or emitted by cells (exa-metabolome) represent a class of analytes better suited for on-line monitoring. Within these analytes a series of volatile organic compounds (VOCs) is expected which is easy accessible via analysis of the fermenter exhaust gas. The required high sensitivity for VOC measurements and a linearity range of multiple orders of magnitude are perfectly matched by the PTR-MS technology (http://www.ptrms.com). For implementation of PTR-MS technology into bio process monitoring recombinant protein production processes with E. coli were used as model system. In first experiments around 40 VOCs were detected and 15 to 20 of them showing significant changes during the course of the process. Due to a limited mass resolution explicit assignment of masses to substances was not always possible and additional measurements with a PTR-TOFMS were required. For some of the identified components a direct connection to host physiology is obvious but there are also unexpected molecules in the fermenter off-gas containing new maybe very valuable process information. The next steps will focus on detailed evaluation of the information content of PTR-MS data and how this information can be used in bioprocessing. Furthermore reproducibility of measurements and systems robustness will be tested.

Stock culture heterogeneity rather than new mutational variation complicates short-term cell physiology studies of Escherichia coli K-12 MG1655 in continuous culture (P18)

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Nutrient-limited continuous cultures in chemostats have been used to study microbial cell physiology for over 60 years. Genome instability and genetic heterogeneity are possible uncontrolled factors in continuous cultivation experiments. We investigated these issues by using high-throughput (HT) DNA sequencing to characterize samples from different phases of a glucose-limited accelerostat (A-stat) experiment with Escherichia coli K-12 MG1655 and a duration regularly used in cell physiology studies (20 generations of continuous cultivation). Seven consensus mutations from the reference sequence and five subpopulations characterized by different mutations were detected in the HT-sequenced samples. This genetic heterogeneity was confirmed to result from the stock culture by Sanger sequencing. All the subpopulations in which allele frequencies increased (betA, cspG/cspH, glyA) during the experiment were also present at the end of replicate A-stats, indicating that no new subpopulations emerged during our experiments. The fact that ~31% of the cells in our initial cultures obtained directly from a culture stock centre were mutants raises concerns that even if cultivations are started from single colonies, there is a significant chance of picking a mutant clone with an altered phenotype. Our results show that current HT DNA sequencing technology allows accurate subpopulation analysis and demonstrates that a glucose-limited E. coli K-12 MG1655 A-stat experiment with a duration of tens of generations is suitable for studying cell physiology and collecting quantitative data for metabolic modelling without interference from new mutations.

Multiposition sensor monitoring in industrial scale anaerobic bioprocesses for the detection of gradients in the bulk of the liquid phase (P19)

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Anaerobic digestion processes are usually operated with no or only restricted agitation. Mixing is often supported through the movement of gas bubbles when carbon dioxide and other gases are synthesized as by-products of the digestion. Hence, the hydrodynamics in industrial scale bioreactors are characterized by a laminar flow field. It can be assumed, that the mixing is not sufficient to prevent the occurrence of gradients in the core of the liquid phase, although the knowledge about their magnitude and importance on the process performance is still low.
The traditional sampling spots are mounted at the wall of the bioreactors. Samples taken there do not reflect the conditions in the core of the liquid phase. In order to elucidate the conditions in the core of the liquid phase, novel monitoring and sampling strategies have to be applied. Multiposition sensors that are miniaturized and equipped with wireless data transmission are introduced into yeast digestion tanks for beer production and into biogas reactors. The aim is to measure gradients directly in the bulk of the liquid phase. Therefore, also lances for location-independent sampling are currently developed. The combination of multiposition sensors, innovative sampling tools, metabolomics and the measurement of the cell physiology allows for an improved understanding of the impact of gradients on the cells' metabolism and activity. It further allows locating zones which are characterized conditions which can be regarded as critical for the process. In these zones, an on line observation is a suitable method to detect process disturbances in a very early stage and to stabilize digestion processes quickly by suitable control strategies. At biogas processes, a more efficient and reliable monitoring and sampling at critical zones will also allow an increased flexibility of the digestion process to variations in the composition of the substrate feed.

Electrooptical monitoring of polarizability in Escherichia coli cultivations for an enhanced process development (P20)

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The anisotropy polarizability (AP) can be monitored at line applying electrooptical methods [1] and automated sample preparation. In recent reports, we showed that the monitoring of the AP of rod-shaped bacteria in batch cultures supported a better insight into the cell's physiologic stages during the process [2, 3]. The application of the methodology to E. coli batch cultivations offered the possibility to identify phases characterized by different substrate availability. The growth phase could be divided in several parts with respect to the specific acetic acid synthesis rate.
Now the possibility for monitoring aerobic E. coli K12 fed-batch and continuous cultivations was investigated. The aim of the study was to observe whether conditions at different feed rates (and thus different substrate uptake rates) influence the AP. Then it might be a valuable parameter for the optimization of a fed-batch process.
Results indicate a strong relation between the AP and the biomass yield at chemostat cultivations. The obtained regression model was applied to fed-batch cultivations, which were conducted in a common glucose limitation mode and at excess glucose, and to auxostat cultivations, where the polarizability signal was kept stable by varying the dilution rate. A feed rate providing the highest biomass yield could be identified and controlled based on the AP signal. Since the AP seems to be related strongly to the overall metabolic activity, the impact of disturbances like substrate oscillations which are occurring at industrial scale cultivations should be measurable. Then, the signal can be used to adopt the feed rate so that the biomass and product yield will be maximized.

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