BioProScale Symposium

Inhomogeneities in large-scale bioreactors

Description – scaling – control

24 to 27 November 2009
Berlin – Germany

Language: English

- **Large-scale bioreactors**
- **Cell physiology in large-scale bioprocesses**
- **Large-scale modeling and control**

Organisers

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

Conference days

November 24 – 27, 2009

Location

TU Berlin, TIB Gebaeudekomplex Humboldthain, Building 13-B, Room 13A
Gustav-Meyer-Allee 25, 13355 Berlin, Germany

Target groups

Producers of biogas and enzymes, antibiotic production, bioprocess development, pharmaceutical bioprocesses, brewery processes – experts from research & development and industrial practice

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

www.ifgb.de/bioproscale

IfGB

Institute for Biotechnology and Fermentation in Berlin

TU berlin

Chair of Bioprocess Engineering

In cooperation with

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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 first BioProScale Symposium. The aim of this symposium is to bring stakeholders which work with different large-scale bioprocesses (e.g. biogas, brewing, enzymes and metabolite production, pharma) together to discuss their experiences and research needs.

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 Peter Neubauer
Technische Universität Berlin
Chair of Bioprocess Engineering

■ 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 of microorganisms in inhomogenous bioreactor systems,

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

(iii) recombinant proteins – optimisation of systems and procedures for process development with complex proteins.

www.bioprocess.tu-berlin.de

IfGB – Institut für Gärungsgewerbe und Biotechnologie zu Berlin

Founded in 1874, under the umbrella of the Institute of Fermentation and Biotechnology in Berlin (IfGB) fermentation oriented research and education has been conducted for more then 140 years – always in close cooperation with the Technische Universität Berlin (resp. its predesessor institutions). Since 2003 the Versuchs- und Lehranstalt für Brauerei in Berlin (VLB) e.V. is the soule holder of IfGB.
Since 2003, under the brand name IfGB services and training for the spirits industry and distillers have been offered. Starting in 2009 our service and training programmes will be expanded into the field of biotechnology – again in close cooperation with the Institute of Biotechnology of TU Berlin.

www.ifgb.de

■ Exhibitors

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BioSilta

BlueSens gas sensor GmbH

Chemineer
Reliability & Technology in Mixing

BIO PROSCALE network

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Programme at a glance

Tuesday, 24 November 2009

16:00 **Welcome address** and chair
Prof. Peter Neubauer (TU Berlin)

17:00 **Opening Lecture: Large-scale industrial bioprocesses – state of the art and needs**
Henk J. Noorman (DSM, The Netherlands)

18:00 **Welcome Reception**
Get-together with drinks and light food in the foyer of the congress hall

Wednesday, 25 November 2009

Large-scale bioprocesses

Chair Peter Neubauer (TU Berlin) /
Henk Noorman (DSM)

9:00 **Key Note Lecture: Bench-scale studies for understanding large-scale stirred bioreactors**
Alvin W. Nienow (University of Birmingham, UK)

10:00 Coffee break

10:30 **Analysis of particle strain in stirred bioreactors by drop breakage investigations**
M. Kraume, S. Maaß (TU Berlin, Germany)

11:00 **Mixing, shear & mass transfer in production scale cell culture reactors**
Marco Jenzsch (Roche Diagnostics, Germany)

11:30 **The BIOSTAT Cultibag RM: A single-use bioreactor also for microbial fermentations**
Julia Glazyrina (TU Berlin, Germany) /
Gerhard Greller (Sartorius Stedim Biotech, Germany)

12:00 Lunch break & poster session

14:00 **How to integrate bioreactor inhomogeneities into QbD and PAT design concepts for biopharmaceutical production processes**
Carl-Fredrik Mandenius (University of Linköping, Sweden)

14:30 **Scale-up of continuous mode lactic acid fermentation**
Joachim Venus (Leibniz-Institute for Agricultural Engineering, Germany)

15:00 **Large-scale fermentation processes in the brewery**
Roland Pahl (VLB Berlin Germany)

15:30 Coffee break

16:00 **PAT – new possibilities by universal sampling devices**
Bernd-Ulrich Wilhelm (BBI-biotech GmbH, Germany)

16:30 **Transcriptomic and proteomic approaches for understanding and optimisation of bioprocesses**
Thomas Schweder (Ernst-Moritz-Arndt Universität Greifswald, Germany)

17:00 **Cell physiology and heterogeneity in the production of antibody fragments**
Florian David, C. Korneli, E. Franco-Lara (TU Braunschweig)

Thursday, 26 November 2009

Studying cellular responses in relation to large-scale bioprocesses

Chair Thomas Schweder (Universität Greifswald) /
Sven-Olof Enfors (Royal Institute of Technology)

9:00 **Keynote Lecture: Cell physiology in large scale bioprocesses**
Sven-Olof Enfors (Royal Institute of Technology KTH, Sweden)

10:00 Coffee break

10:30 **Bioprocess scale up – tracking the informations relevant for up-scaling by GFP reporter strains**
Frank Delvigne (University of Liège / Gembloux Agro-Bio Tech, Belgium)

11:00 **New insights in biological dynamics and relaxation time to well-characterized environmental stress using innovative microbial and process engineering tools**
Natalie Gorret (Laboratoire d'Ingénierie des Systèmes Biologiques et Procédés, France)

11:30 **Bioreactor scale-down studies of temperature-inducible recombinant protein production in E.coli**
Luis Caspeta and Octavio T. Ramirez (Universidad Nacional Autónoma de México)

12:00 Lunch break & poster session

14:00 **Fast sampling methods for quantitative metabolomics of industrial micro-organisms: How to get the right numbers?**
Walter M. van Gulik (Delft University of Technology, The Netherlands)

14:30 **Metabolic flux analysis of pulse experiments: The impact of product concentration gradients on the acetone-butanol-ethanol fermentation**
Stefan Junne (Technische Universität Berlin, Germany)

15:00 **Redesigning microbial processes to circumvent heterogeneities issues in large scale bioreactors: Examples in Saccharomyces cerevisiae**
Stephane E. Guillouet (Toulouse, France)

15:30 Coffee break

16:00 **Formate – an indicator of anaerobic zones in large scale bioreactors?**
Peter Neubauer (Technische Universität Berlin, Germany)

16:30 **Overcoming environmental heterogeneities in E. coli cultivations through metabolic engineering**
Alvaro R. Lara (Universidad Autónoma Metropolitana-Cuajimalpa, Mexico)

17:00 **Transcriptome and proteome data analysis infers gene regulatory networks in Escherichia coli**
Reinhard Guthke (Hans-Knöll-Institute, Germany)

17:30 **Downstream processing for biotransformation products – two case studies employing membrane fractionation**
Günther Laufenberg (TU Berlin / Bayer BioScience, Germany)

18:30 **Dinner** in the Peter-Behrens-Hall at the congress venue

Friday, 27 November 2009

Large-scale modeling and control

Chair Alvin Nienow (University of Birmingham, UK) /
Matthias Reuss (University Stuttgart)

9:00 **Key Note Lecture: Modeling the heterogeneity of microbial metabolism in large scale bioreactors**
Matthias Reuss (University of Stuttgart, Germany)

10:00 **Design space roadmap: From development to production**
Lennart Eriksson (Umetrics AB, Sweden)

10:30 Coffee break

11:00 **Exactly reproducible process operation in microbial and animal cell cultures**
A. Lübbert (Martin-Luther-Universität Halle-Wittenberg, Germany)

11:30 **EnBase® – a scalable fed-batch technology**
Antje Neubauer (BioSilta Oy, Finland)

12:00 **Bioprocessing of stem cells for regenerative medicine purposes – scale-up or scale-out**
Chris Hewitt (Loughborough University, United Kingdom)

12:30 **Closing remarks and farewell**
Peter Neubauer (TU Berlin)

12:45 Lunch

14:00 End of symposium

Programme

Tuesday, 24 November 2009

16:00 **Welcome address and chair**

Peter Neubauer
(Chair of Bioprocess Engineering, Technische Universität Berlin, Germany)

17:00 **Opening Lecture:**
Large-scale industrial bioprocesses – state of the art and needs

Henk J. Noorman (DSM, Den Haag, The Netherlands)

Abstract: Design, operation and control of industrial fermentation-based processes has matured over the last 140 years, i.e. since the first industrial wave in the 1870's. Current standards have been set by a delicate mix of practical experience, engineering rules, and applied science. Continuous process improvements over many decades have shown to be feasible, via cycles of debottlenecking rate-limiting hardware in the manufacturing plant, implementing new microbial strains (software), and adjustment of fermentation conditions to harvest the full potential of the new strains. Low cost and quick solutions generally prevail, but progress and improvements have a solid foundation in a proper use of process technology principles. A leading principle is to start development work from the perspective of the large-scale and then step down the scale, not vice versa. In this way, a solid industrial practice has been established for vitamins (B2), antibiotics (penicillin, cephalosporin), polymer building blocks (1,3-propanediol, lactic acid), food/feed ingredients (citric acid, lysine, enzymes), biofuels (ethanol), etc. The scope is expected to increase further, following global bio-based trends to replace petro-chemical feedstocks. Needs and challenges for future bio-based processing in large-scale reactors will be summarized.

18:00 **Welcome Reception**

Get-together with drinks and light food in the foyer of the congress hall

21:00 End of the conference day

Wednesday, 25 November 2009

Large-scale bioprocesses

Chair *Peter Neubauer* (TU Berlin) / *Henk Noorman* (DSM)

9:00 **Key Note Lecture:**
Bench-scale studies for understanding large-scale stirred bioreactors

Alvin W. Nienow (Centre for Bioprocess Engineering, Dept. of Chemical Engineering, University of Birmingham, UK)

Abstract: The scale-up of stirred aerobic bioreactors requires information on physical (transport phenomena) and biological aspects. The physical aspects are very generic and essentially apply to any reactor or bioreactor involving the need to disperse gas (air) in order to achieve mass transfer. These topics will initially be briefly reviewed including the development of improved impellers and the concept of high and low 'shear' types compared to the traditional Rushton turbine. The biological aspects are very specific to the particular organism being used in the bioreactor. Recently, many studies have suggested better ways of using microreactors and shake flasks to obtain such data and the appropriateness of work at this scale will be discussed. Some aspects cannot be investigated at less than the stirred bench scale (mechanical stresses) and some require a combination of two bench scale bioreactors in a loop to mimic the loss of homogeneity found at large scale. The former studies should cover mechanical stresses due to turbulence, from bursting bubbles, high air velocities from spargers or from collisions between microcarriers. The latter studies are associated with the loss of environmental homogeneity imposing stresses due to temporal and spatial deviations in nutrients, pH, dO_2, pCO_2, temperature and cell concentration. The organisms covered in this talk will include animal cells (free suspension and attached), yeast, bacteria and mycelia and in relation to cell lysis, plasmids. There are many myths that they have significantly impacted on the design and operation of large-scale bioreactors. Given the present content of courses in biochemical engineering, these myths are likely to continue.

10:00 Coffee break

10:30 **Analysis of particle strain in stirred bioreactors by drop breakage investigations**

M. Kraume, *S. Maaß* (Field of Process Technology, Technische Universität Berlin, Germany)

Abstract: High energy dissipation rates are necessary for fast and efficient turbulent mixing in a stirred vessel. In many biochemical, pharmaceutical and food industries processes is also a need for gentle mixing. The key factor for optimizing such processes is to fulfil both needs. Therefore the prediction of particle strain is of major importance for these industries.
It is broadly known that different stirrer types create divers particle strains with the same energy input (P/V). Primarily the differences between axial and radial stirrers are obvious and well described in literature. The reason for these differences has not been analysed yet.
In this study liquid/liquid-systems are used as model systems for bioreactors. For systematic analysis and quantitative understanding of particle strain and single drop breakage a special experimental set-up for the investigation of such events was developed and operated [Maaß et al. 2009b]. Furthermore comparative experiments in a stirred tank have been carried out to gain additional results not only from single particles but also from drop swarms [Maaß et al. 2009a]. This paper presents the results of breakage probabilities, breakage time and location of single drops depending on power input, particle size and particle viscosity. These experimental results will be compared to models from literature. Additionally experimental and simulation results of drop swarm experiments in a stirred vessel are matched.
Those experiments allow valuable insights into the mechanisms of particle breakage and particle strain and helps to predict those phenomena. This knowledge can be used for an improved design or scale-up of bioreactors.

- Maaß, S., Metz, F., Rehm, T. and Kraume, M., 2009a. Prediction of drop sizes for PVC-production in slim reactors part I: Single stage stirrers. submitted to AiCHE J.
- Maaß, S., Wollny, S., Sperling, R. and Kraume, M., 2009b. Numerical and experimental analysis of particle strain and breakage in turbulent dispersions. Chem. Eng. Res. Des., 87(4): 565–572.

11:00 **Mixing, shear & mass transfer in production scale cell culture reactors**

Marco Jenzsch (Roche Diagnostics GmbH, Penzberg, Germany)

Abstract: In modern biotechnology the production of recombinant proteins by animal cell culture in reactors up to 25m³ is state of the art technology. One major task from target identification to market launch is the acceleration of timelines during development and scale up for clinical phases and production. In general, comparable process performance and product quality in all scales from bench top to full commercial has to be achieved. To identify potential issues and to minimize risk during scale up detailed knowledge of physical unit operations in bioreactors is required. In particular this includes mode of operation control and scale dependent differences in mass transfer, mixing and hydrodynamic shear force. Based on this knowledge a suitable scale up strategy with respect to cell physiology and identified key process parameters can be derived.
In this presentation methods for the characterization of bioreactors will be discussed and data describing mixing, mass transfer and acting shear force for different scales from bench top to full commercial will be compared. Based on these methods several scale up activities were performed. The comparability of these processes in the different scales with respect to cell culture performance, especially product quality, will be presented.

11:30 **The BIOSTAT Cultibag RM:**
A single-use bioreactor also for microbial fermentations

Julia Glazyrina (TU Berlin, Germany), *Gerhard Greller* (Sartorius Stedim Biotech, Göttingen, Germany)

Abstract: The BIOSTAT Cultibag RM a single-use bioreactor system will be presented. The Wave induced motion imparts mixing and promotes oxygen transfer in the disposable, flexible, sterile gamma-irradiated bag. The combined advantages of a fully closed, disposable system with process monitoring capabilities (pH and dissolved oxygen), makes it highly attractive over traditional systems for animal cell culture such as shake flasks and stirred tanks. Cultivations of mammalian, insect and plant cells in single-use bioreactors have been reported in details elsewhere. However, the BIOSTAT Cultibag RM has not been well evaluated for aerobic microbial growth so far.
Our results show that the BIOSTAT Cultibag RM allows a glucose-limited growth of E. coli BL21(DE3) to high cell densities of OD600nm = 60 (corresponding 18 g/L cell dry weight). The yeast Pichia pastoris GS115/HSA could be grown in a two stage process to a final cell density of 50 g/L dry cell weight with a rHSA concentration of 0.7 g/L.
The BIOSTAT Cultibag RM is robust and simple-to-apply single-use bioreactor which can be used for microbial seed expansion steps. The control tower solves the limitations of shaken cultures like uncontrolled growth, oxygen limitation, and pH drops. Additionally high cell density cultivations for protein expression and DNA production can be performed.

12:00 Lunch break & poster session

14:00 How to integrate bioreactor inhomogeneities into QbD and PAT design concepts for biopharmaceutical production processes

Carl-Fredrik Mandenius (Division of Biotechnology, University of Linköping, Sweden)

Abstract: The Quality-by-Design (QbD) and Process Analytical Technology (PAT) concepts have interesting connections to bioreactors and their characteristics. The design and control space approach of QbD is directly dependent on the variability of the bioreactor. Several of the recommended PAT methodologies are essential tools for characterizing, understanding and interpreting the dynamics of the bioreactor during operation.

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The talk will overview the relevant current QbD and PAT methodologies and exemplify how the bioreactor design and operation on large scale could integrate QbD and PAT in accordance to the regulators' guidelines and to the benefit for biopharmaceutical production.
In particular, the use of new process analytical techniques, the application of novel design science concepts and data optimization methods such as design-of-experiments will be highlighted with examples from production of biopharmaceuticals and other biologics.

- Mandenius C-F (2006) The role of Process Analytical Technology in biotechnology, European Pharmaceutical Review 11 (3), 69-76
- Mandenius C-F, Derelöv M, Detterfelt J, Björkman M (2007) PAT and design science, European Pharmaceutical Review, 12 (3), 74-80
- Mandenius C-F and Brundin A (2008) Bioprocess optimization using design-of-experiments methodology, Biotechnol Prog 24, 1191-1203
- Mandenius C-F, Graumann K, Schultz TW, Premsteller A, Olsson I-M, Periot E, Clemens C, Welin M. 2009. Quality-by-Design (QbD) for biotechnology-related phamaceuticals. Biotechnol. J. 4, 11-20
- Mandenius CF, Björkman M. 2009. Process analytical technology (PAT) and Quality-by-Design (QbD) aspects on stem cell manufacture. European Pharmaceutical Review 14(1), 32-37

14:30 Scale-up of continuous mode lactic acid fermentation

Joachim Venus (Dept. Bioengineering, Leibniz-Institute for Agricultural Engineering, Potsdam, Germany)

Abstract: Renewable resources (e.g. starch containing crops, lignocellulosic feedstocks, green biomass) 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 supplements by cheaper materials from renewable resources due to their main proportion of the whole process costs. Besides the basic research projects respecting the screening and characterization of microorganisms, phenotypic optimization, down-stream processing of fermentation products, application and refining of lactic acid, economic assessment of bioconversion processes the scale-up to a technical scale of several processing steps have to be developed for transferable solutions of bioconversion technologies of agricultural or renewable materials respectively. For that purpose a multifunctional pilot plant was planned and built at the site of ATB to investigate different raw materials and products. First results of the utilization of biomass after enzymatic pre-treatment for the continuous 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 mode) there is a triple up to four times higher productivity of lactic acid.

15:00 Large-scale fermentation processes in the brewery

Roland Pahl (Research Institute for Engineering and Packaging, VLB Berlin Germany)

Abstract: Brewery is often said to be one the oldest biotechnology-industries of the world. For the process of fermentation during beer production many different types of tanks have been used through the ages. Nowadays, although there are still open fermenting tubs in use, the so called CCV (cylindro-conical-vessel) dominates the brewing equipment.
These tanks are built up to enormous sizes, often reaching 1000 m³ or more. Cleanability of tanks that big is an important topic. Additionally it is clear that within such gigantic tanks inhomogeneities are a threat to any standardized production process. Possible effects in that direction are e.g. varying CO_2-concentrations which may lead to differences in the biological status of the fermenting yeast. Also it is possible that according to specific densities in different areas of a fermenting vessel, varying concentrations of substances occur. Moreover changing concentrations of fermentation by-products or pH-influencing compounds might be detected. All of these factors lead to a high necessary effort in process control and a necessary good understanding of possible counter-measures. Temperature, pressure and off-gas composition are regularly checked by many breweries inline during fermentation. Other important parameters such as pH, extract-decline, yeast concentration in the medium and others are normally controlled off-line. The measures to keep the vessel contents as homogenous as possible reach from circulation systems to use of cooling jackets for mixing purposes.

15:30 Coffee break

16:00 PAT – new possibilities by universal sampling devices

Bernd-Ulrich Wilhelm (BBI-biotech GmbH, Wildau, Germany)

Abstract: Bioprocess control is lacking reliable data from the process. Beside online analysis – which is limited on pH, temperature, DO – most of the interesting parameters are only available as offline-data.The PAT initiative of the FDA is targeting this problem. Bringing offline data closer to the process is becoming a major issue for making model-based quality and process control available for the daily work in the biotech industry and research.
The new bbi-biotech sampling device is combining any bioreactor system to any kind of analytics. Offline analytical systems as flow cytometers, microscopes, enzyme analyzers and others will become atline analytics. Sampling is performed fast, automated, dead-volume free and sterile. Data are available immediately after analysis. Therefore, the results can be integrated into process control.
Since the system only needs the volume which is really necessary for the analysis, it can already be used in early phases of the development of new processes in small bench top parallel reactor systems.

16:30 Transcriptomic and proteomic approaches for understanding and optimisation of bioprocesses

Thomas Schweder (Institute of Pharmacy, Ernst-Moritz-Arndt Universität Greifswald, Germany)

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 Escherichia coli, Bacillus subtilis and Bacillus licheniformis. We have filtered a set of marker genes, which could be used as indicators for process-relevant stress and starvation situations during large-scale fermentation processes.
Such process-critical genes can be used as biomarkers 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.

17:00 Cell physiology and heterogeneity in the production of antibody fragments

Florian David, *C. Korneli*, *E. Franco-Lara* (Institute of Biochemical Engineering, Technische Universität Braunschweig, Germany)

Abstract: Recombinant antibodies and antibody fragments are indispensable tools for research, diagnostics and therapy. These proteins are traditionally produced either in mammalian cells or in recombinant microbial strains like E. coli. In mammalian systems the titres are usually very high, but at the cost of very long process times. In microbial systems the production itself is very efficient, but it is associated with high downstream processing costs since the intracellular product must be gained through cell disruption, protein extraction, separation and purification. A promising alternative is the use of the gram positive Bacillus megaterium, which is an expression host with high secretion capacities. Due to the lack of the outer membrane the produced antibody fragments can directly be harvested from the medium supernatant.
In this work the cell heterogeneity of B. megaterium regarding the state of production and membrane potential from inoculum in shake flasks through lab-scale up to pilot-scale (GMP process design) cultures were investigated. As model recombinant product the antibody fragment anti-lysozyme (D1.3 scFv) was chosen. The quantity and functionality of the single chain fragment were determined by antigen binding ELISA. Several methods and stains were evaluated and established to determine cell heterogeneity during production and secretion of D1.3 scFv. Culture heterogeneity was detected in cell morphology, membrane properties and productivity by means of flow cytometry.
To characterize the production capabilities of B. megaterium, two different approaches were followed. The first approach focussed on the characterization of the membrane properties, since the membrane influences not only the product secretion, but plays a very important role in the cell physiology. By measuring the membrane potential metabolic active cells could be distinguished from non-active cells giving a hint on the effectiveness of a bioprocess regarding its productivity. The membrane potential as a component of the proton motive force is involved in generation of ATP, sugar transport, chemotaxis and survival at low pH. In a second approach, the state of antibody fragments production itself was determined via immunofluorescence. A first antibody directed against the His-tag linked to the intracellularly produced antibody fragment and a second antibody fluorescently labelled was used for specific detection of producing cells, i.e. for those cells secreting functional antibodies allowing a very easy assay to differentiate between producing and non-producing populations.

17:30 End of conference day

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Studying cellular responses in relation to large-scale bioprocesses

Chair *Thomas Schweder* (Universität Greifswald) /
Sven-Olof Enfors (Royal Institute of Technology KTH, Stockholm)

9:00 Keynote Lecture: Cell physiology in large scale bioprocesses

Sven-Olof Enfors (School of Biotechnology, Royal Institute of Technology KTH, Stockholm, Sweden)

Abstract: This review deals with physiological responses of E. coli and S. cerevisiae in aerobic large-scale fed-batch processes. This environment is characterised by measurable concentration gradients of limiting substrate (glucose) in the reactor, gradients both in time (seconds) and place in the reactor (distance from substrate feed point). Measurements in a 22 m³ E. coli fed-batch process with an average glucose concentration of 77 mg/L revealed that the concentrations in the same point oscillated between about 30 and 350 mg/L. These gradients are likely to generate gradients also in dissolved oxygen when

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the cell concentration is high enough. By using a specially designed scale-down reactor (SDR) with controlled repeated exposure to fluctuating glucose concentration, physiological responses could be demonstrated in the gradients. Comparison of large scale (a 170 m³ baker's yeast process and 6-22 m³ E. coli processes) with corresponding homogeneous lab scale processes and processes in the SDR indicated that the observed lower yield of biomass per sugar in large scale processes were due to rapid shifts in the metabolism between production and re-assimilation of overflow metabolites and/or anaerobic metabolites. Also corresponding shifts between synthesis and degradation of stress induced genes may contribute to the observed scale-up effect on the biomass yield. Other observed physiological responses (of E. coli) to the heterogeneous environment in the large scale reactors and the SDR are: increased viability, reduced segregation to non-dividing cells, improved proteolysis pattern of human growth hormone, and reduced yield of a recombinant protein, probably due to reduced product expression rather than to increased proteolysis.

10:00 Coffee break

10:30 Bioprocess scale up – tracking the informations relevant for up-scaling by GFP reporter strains

Frank Delvigne (University of Liège / Gembloux Agro-Bio Tech, Belgium)

Abstract: Three GFP based reporter systems associated respectively with prpoS, pcisE anb puspA promoters have been investigated in several bioreactor configurations. These stress promoters are known to respond, among other, to glucose limitation and their potential use as tool for estimating the bioreactor mixing efficiency has been evaluated. A first set of experiments, involving lab-scale fed-batch bioreactor running under different degree of glucose limitation, has been performed in order to estimate the responsiveness of the different GFP clones to standard culture conditions. Flow cytometry analysis have led to the observation of an interesting bimodal expression profile in the case of prpoS::GFP construct. The lab-scale experiments have been compared with cultures performed in the same conditions in a two-compartment scale-down bioreactor. A drastic drop of GFP expression level has been noticed for the rpoS clone and has been in a first time attributed to the passage of the cells in the tubular part of the scale-down reactor in which exposure to local glucose excess repress the expression of rpoS. The scale-down sensitiveness of the puspA and pcsiE clones is lower.
In a second set of experiments, a mini-bioreactor platform, based on shaken flasks, has been elaborated to estimate the sensitivity of the GFP clones to different fed-batch regimes. For all the fed-batch conditions, a very low GFP expression level has been observed for the three reporter systems, suggesting that cell density plays a role in the expression of these particular promoters associated with the carbon starvation regulon.
On the basis of the set of results obtained throughout this work (i.e., 15 experiments performed in stirred bioreactor and 9 experiments performed in mini-bioreactor and 24 experiments performed in micro-bioreactor), the potentialities of the three stress promoter in the context of the bioreactor scaling-up procedures have been highlighted.

11:00 New insights in biological dynamics and relaxation time to well-characterized environmental stress using innovative microbial and process engineering tools

Sunya S.[1], *Bideau C.*[1], *Uribelarrea J. L.*[1], *Delvigne F.*[2], *Molina-Jouve C.*[1], *Natalie Gorret*[1]

[1] Laboratoire d'Ingénierie des Systèmes Biologiques et Procédés , CNRS UMR 5504 – INRA UMR 792, INSA Toulouse Cedex, France

[2] Centre Wallon de Biologie Industrielle, Unité de Bioindustries, Faculté des Sciences Agronomiques de Gembloux, Belgium

Abstract: Improving the performances of large scale bioprocesses demands to better understand dynamic interactions between microorganism and physical phenomena (transport, mixing) inside bioreactors. We are especially interested in:
(i) analysing and modelling the impact of local gradients (liquid/gas, temperature, pH) on microorganism behaviour
(ii) identifying limiting biological and physical mechanisms by determining the respective relaxation times.
An experimental scale-down approach was performed using two complementary equipments:
- a fully equipped 1l chemostat bioreactor
- a homemade fully automated platform composed of a two stage bioreactors (1l and 100 ml bioreactors) connected to a fast autosampler. The small bioreactor was designed to provide a perfect mixing between a physiological steady-state microbial culture and a micro-environment. This platform allows a dynamic analysis of biological and physical phenomenon at different scales (macroscopic, microscopic and molecular) within a very short period of time (few seconds).
Experiments were conducted with reporter bioluminescent strains of E. coli in which the luxCDABE operon was fused to promoters responding to different selected environmental stresses (dissolved oxygen, pH, T). On-line measurement of luminescence allows real-time recording of the expression of genes involved in stress responses. Kinetic analyses of biomass, extracellular metabolites, inlet/exhaust gas, are carried out in order to determine mass balances and biological kinetic parameters. We will present and discuss our approach and the results concerning continuous culture of E. coli DPD2417 (nirB::luxCDABE fusion) to monitor the microbial responses to oxygen limitation.

11:30 Bioreactor scale-down studies of temperature-inducible recombinant protein production in E.coli

Luis Caspeta and *Octavio T. Ramirez* (Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México)

Abstract: A major focus of our research group during the past 14 years has been the study of spatial gradients in fundamental culture parameters, such as dissolved oxygen, dissolved carbon dioxide, pH and concentration of substrates that can commonly occur in large-scale bioreactors. Such a problem is due to a deficient mixing, that results from inherent limitations in the traditional scaling-up methods as well as practical constraints of the design and operation of large-scale bioreactors [1].
The approach taken by our group has been to simulate in scale-down systems various environmental gradients relevant to a variety of industrially important organisms, including bacteria, insect, and mammalian cells [2]. In this presentation, we will discuss a scale-down study that simulates the typical heating rates that can be achieved in large-scale bioreactors during expression of recombinant proteins by an E. coli thermo-inducible expression system [3].
At the laboratory scale, sudden step increases from 30 to 42 °C can be readily accomplished for attaining full induction conditions. However, for large scale-cultures only slow ramp-type increases in temperature are possible due to heat transfer limitations, where the heating rate decreases as the scale increases. In this presentation we will show how heating rate affects the transcriptional and metabolic responses of recombinant an E.coli strain during temperature-induced synthesis of pre-proinsulin in fed-batch high cell density cultures. Heating rates between 6 and 0.4 °C/min were tested in a scale-down approach to mimic fermentor scales between 0.1 and 100 m³. As the heating rate increased, the yield and maximum recombinant protein concentration decreased, whereas a larger fraction of carbon skeletons was lost as acetate, lactate, and formate. Such results, in addition to the transcriptional profiles from six relevant heat shock genes (as measured by RT-PCR), three genes from the transcriptional and translational machinery, five stress control genes, and the gene coding for the heterologous protein, revealed that cells subjected to slow temperature increases can adapt to stress. The results that will be discussed indicate that slow heating rates, such as those likely to occur in conventional large-scale fermentors, favor heterologous protein synthesis by the thermo-inducible expression system used in this report. Furthermore, based on the knowledge of the effect of heating rate on bacterial physiology and product formation, a novel strategy for temperature induction of recombinant protein will be presented. Altogether, the present study should be useful for the rational design of scale-down and scale-up strategies and optimum recombinant protein induction schemes.

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12:00 Lunch break & poster session

14:00 Fast sampling methods for quantitative metabolomics of industrial micro-organisms: How to get the right numbers?

Walter M. van Gulik (Department of Biotechnology, Delft University of Technology, The Netherlands)

Abstract: Rational identification of gene targets for the metabolic engineering of industrial micro-organisms requires quantitative information on the in vivo kinetics of metabolism. Obtaining reliable and accurate experimental data is therefore crucial. If detailed and extensive mathematical models of biological systems are based on erroneous data, the insights and predictions obtained from those models are of little use. It is therefore of prime importance that the experimental procedures to generate these data are thoroughly checked for their reliability and reproducibility and that they are improved if they do not meet the required criteria. This presentation focuses on the development and application of an experimental platform for quantitative microbial metabolomics, whereby it is shown how the applied experimental and analytical procedures, e.g. the cultivation of the micro-organisms, rapid sampling, fast quenching, metabolite extraction and analytical procedures can be evaluated and optimized. Subsequently, the optimized procedures were applied in research aimed at unraveling the metabolic regulation of central metabolism in different industrial micro organisms, i.e. Saccharomyces cerevisiae, Penicilium chrysogenum and Escherichia coli, thereby showing that often the experimental procedures have to be adapted for a particular micro organism.

14:30 Metabolic flux analysis of pulse experiments: The impact of product concentration gradients on the acetone-butanol-ethanol fermentation

Stefan Junne, *Peter Neubauer*, *Peter Götz* (Chair of Bioprocess Engineering, TU Berlin, Germany)

Abstract: The production of biobutanol is gaining increased interest due to strengthened efforts in developing economically viable processes for fuel production based on renewable feed stocks [1]. Biobutanol is produced by the anaerobic bacterium Clostridium acetobutylicum in the acetone-butanol-ethanol (ABE) fermentation. The metabolism of C. acetobutylicum can be divided into two stages: During the exponential growth phase, it produces mainly CO_2, H_2, acetate, and butyrate. Then the metabolism switches to solvent production. The acid producing phase is commonly called acidogenesis, the solvent producing phase solventogenesis [2]. The mechanisms which are causing the switch from acidogenesis to solventogenesis are still unclear. However, it can be assumed, that high intracellular concentrations of acids and their precursors play a key role in regulation [3, 4]. Hence, concentration

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gradients within a fermentation containing C. acetobutylicum, as e.g. in digestion processes for biogas production, are crucial for process stability. In order to elucidate the regulatory interactions caused by extracellular metabolite concentration changes, pulse experiments in the batch fermentation mode are investigated, in which each main acid and solvent metabolite is added. Based on concentration measurements, fluxes are estimated with a non-stationary metabolic flux analysis. Results can provide a picture of the impacts of the pulses on the in vivo reaction rates. The data can be further used to create a kinetic model of the central carbon metabolism, which is able to reflect the impacts of acid and solvent synthesis, when the environment of the cells is changing.

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15:00 Redesigning microbial processes to circumvent heterogeneities issues in large-scale bioreactors: Examples in Saccharomyces cerevisiae

J.R. Mouret, *D. Feria-Gervasio*, *A. Ochoa-Estopier*, *J. Lesage*, *N. Gorret* and *Stephane E. Guillouet* (UMR5504, UMR792 Ingénierie des Systèmes Biologiques et des Procédés, CNRS, INRA, INSA, Toulouse Cedex, France)

Abstract: The yeast Saccharomyces cerevisiae belongs to such yeast species which switch from a pure oxidative metabolism to a respiro-fermentative metabolism even under fully aerobic conditions as soon as the glucose exceeds a concentration of about 0.5-1 g l-1. This specific characteristic of S. cerevisiae has been supportive for all fermentative processes such as alcoholic beverage or ethanol production but represents a disadvantage for production of yeast biomass (baker's yeast production) as well as biomass-related yeast products (such as heterologous proteins). Therefore, yeast biomass production bioprocesses have been usually carried out under aerobic fed-batch mode where the glucose feeding is finely controlled to keep a very low residual concentration (< 1 g l -1) and to maintain the specific growth rate below a critical value, called critical dilution rate (Dc). Such strenuous monitoring conditions become challenging when the production is carried out in large scale bioreactors where microorganisms, due to mixing issues, are submitted to fluctuations in concentrations of e.g. substrate and oxygen with an intensity and a frequency depending on the operating scale.
A potential alternative to controlled feeding of sugar based feedstock would be the use of additives able to delay the metabolic switch and/or the use of cheap non-fermentable (oxidative) carbon sources. We will present here an example of each strategy applied to the production of S.c. under pure oxidative metabolism.

15:30 Coffee break

16:00 Formate – an indicator of anaerobic zones in large-scale bioreactors?

Peter Neubauer (Chair of Bioprocess Engineering, Technische Universität Berlin, Germany)

Abstract: Formate has been repeatedly assigned as an indicator of anaerobic zones in Escherichia coli large-scale bioprocesses. Here we show that its accumulation is mainly due to the lack of important trace elements [1] and that the process robustness can be increased by proper medium design. A good function of the fermentative metabolism is also important for poorly controlled laboratory scale cultures with low oxygen transfer rates.

[1] Soini J, Ukkonen K, Neubauer P. 2008. High cell density media for E. coli are generally designed for aerobic cultivations – consequences for large-scale bioprocesses and shake flask cultures. Microb Cell Fact 7:26

16:30 Overcoming environmental heterogeneities in E. coli cultivations through metabolic engineering

Alvaro R. Lara (Dept. de Procesos y Tecnología, Universidad Autónoma Metropolitana-Cuajimalpa, Mexico)

Abstract: Environmental gradients in large-scale bioreactors are known to cause deleterious effects on bacterial cultures. Up to now, most of the experimental work has dealt with the simulation of environmental gradients at laboratory scale and the evaluation of their effect on cultures. Our research group not only has applied scale-down models to study the physiological effects of gradients at molecular level, but also has developed metabolic engineering concepts to obtain bacterial strains with an improved performance under environmental heterogeneities. The effects of dissolved oxygen (DO) gradients in the physiology of Escherichia coli have been studied in a two compartment bioreactor scale-down system consisting of two interconnected stirred tank reactors. The transcriptional response of E. coli to DO gradients was studied, and the results applied to obtain strains with a partially blocked mixed-acid fermentation metabolism that were able to grow faster, while accumulating less organic acids and producing more recombinant protein under DO fluctuations. We have also evaluated the effect of glucose gradients using a stirred tank bioreactor coupled to a mini-plug flow (PFR) reactor. This has allowed us to evaluate the fast dynamic response of the fermentation metabolism of E. coli to

glucose gradients under fully aerobic conditions, taking 11 samples in the glucose-rich region in a time frame of 92 s. In a separate experiment, glucose gradients were combined with anaerobiosis in the PFR, and the effects on fermentation metabolism was analyzed. In both cases, a strong increase on specific glucose uptake rate was measured, which resulted in a fast accumulation of organic acids. We restricted the glucose uptake rate at molecular level by replacing the natural glucose transport system (PTS) by the galactose permease (GalP), which resulted in a glucose uptake rate 60% lower than the wild-type strain. This allowed us to cultivate such E. coli strain in batch mode using elevated glucose concentrations (up to 100 g/L), while attaining high cell-densities (52 g/L) and high recombinant protein titers (8 g/L), while very low acetate accumulation. The use of such culture system avoids the concomitant presence of substrate gradients in large-scale fed-batch cultures.
This approach is now being evaluated for the production of gene therapy vectors. Altogether, our results show the potential of combining in-depth scale-down studies with metabolic engineering approaches to overcome large-scale limitations.

17:00 **Transcriptome and proteome data analysis infers gene regulatory networks in Escherichia coli**

Reinhard Guthke (Systems Biology / Bioinformatics, Hans-Knöll-Institute, Jena, Germany)

Abstract: Different network inference algorithms were applied to generate hypotheses on main interactions of genes and proteins in Escherichia coli, in particular during the recombinant synthesis of the recombinant proteins. Starting from genome-wide analysis on transcriptome and proteome level, we focused a detailed analysis on few selected genes and proteins. For detailed analysis by dynamic modeling, we selected representative genes that are coding for transcrption factors and/or are differentially expressed and belong to clusters with different kinetic behavior or are coding for transcription factors.

17:30 **Downstream processing for biotransformation products – two case studies employing membrane fractionation**

Günther Laufenberg (Chair of Food Process Engineering, TU Berlin and Bayer BioScience, Germany)

18:30 Dinner in the Peter-Behrens-Hall at the congress venue

Programme

Friday, 27 November 2009

Large-scale modeling and control

Chair *Alvin Nienow* (University of Birmingham, UK) / *Matthias Reuss* (University Stuttgart)

9:00 **Key Note Lecture:**
Modeling the heterogeneity of microbial metabolism in large scale bioreactors

Matthias Reuss (Institute of Biochemical Engineering, University of Stuttgart, Germany)

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. Two examples are used to illustrate the application of the approach. The first example deals with the impact of mixing on the synchronisation of glycolytic oscillations in a population of yeast cells. The second examples deals with 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 phosphoenolpyruivate 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 further discussed with the aid of an additional model for the dynamics of cAMP signalling.

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10:00 **Design space roadmap- From development to production**

Lennart Eriksson (Umetrics AB, Uppsala, Sweden)

Abstract: Throughout the development process the combination of Design of Experiments (DoE) and Multivariate data analysis (MVDA) ensures maximum value from collected data and provides proof of process understanding. In production MVDA allows real-time monitoring to ensure operation within the Design space. This talk will focus on the relationship between DoE and MVDA for QbD within biopharmaceutical development and production. Umetrics will also demonstrate how MVDA allows comparison of batches over time, between scales and sites.

10:30 Coffee break

11:00 **Exactly reproducible process operation in microbial and animal cell cultures**

Aehle, M., *Schaepe, S.*, *Kuprijanov, A.*, *Simutis, R.* , *Lübbert, A.* (Center for Bioprocess Engineering, Martin-Luther-Universität Halle-Wittenberg, Germany)

Abstract: Only a few initiatives of FDA/EMEA led to a more intense discussion in production companies and universities than PAT. We show, how reproducible we currently can produce recombinant proteins using the standard industrial host systems E. coli and CHO.
It is mainly the online measurement information from the processes that is the bottle neck in reaching high batch-to-batch reproducibility. With proper measurement data, we are able to counteract random fluctuations in the key process variables. Central measurements in industrial production processes using microorganisms are the offgas volume fractions, base consumptions and culture weights. In most production processes, these data are sufficient for reliable estimates of biomass and product concentrations. However, there are prerequisites often neglected in the past as they were considered of second order importance: These are pH and pO_2 controls in the culture media. With sufficiently accurate data, even simple control procedures can keep the fermentation processes exactly on the desired paths.
Not only microorganisms can be cultivated in a highly reproducible way. Animal cell cultures can also kept exactly on given paths with simple control approaches, even on predefined profiles of the specific cell growth rate μ. This is shown at the example of CHO cells producing EPO.

11:30 **EnBase® – a scalable fed-batch technology**

Antje Neubauer (BioSilta Oy, Oulu, Finland)

Abstract: EnBase® is an advanced microbial cultivation technology for high cell density growth [1] in different formats from micro-well plates up to shake flasks and bioreactors. The most exploited technique to reach high cell densities in E. coli cultivations are fed-batch fermentations, where the specific growth rate is controlled by the substrate-limited feeding. The feeding process in bioreactors is operated by pumps. EnBase® technology enables the glucose supply in closed systems by a biocatalytic reaction using a polymeric substrate. The enzyme has the function of a pump in the bioreactor. Process factors which have impact on the product yield, such as strains, vectors, media, additives or

induction modus, should be screened under fed-batch conditions. Investigations for bioreactor process development like impact of glucose gradient, dissolved oxygen concentration, induction and growth rate on product formation were successfully performed in shake flask fed-batch cultivations by using EnBase®.
Current studies in a 10L and 100L bioreactor showed that EnBase® Flo allows glucose-limited high cell density cultivation at big scale. After the batch phase of about 10h the concentration of glucose and acetate decreased and remained nearly zero during the whole cultivation period.
A new application area is the WAVE single use bioreactor. The growth conditions were optimized, so that optical densities (OD600) of 30 to 40 after 48 hours were obtained, with a very similar growth behavior to small scale cultures. Also the expression of recombinant protein was proven to be possible and yield high amounts of soluble protein.

1. Panula-Perala J, Siurkus J, Vasala A, Wilmanowski R, Casteleijn MG, Neubauer P. Enzyme controlled glucose auto-delivery for high cell density cultivations in microplates and shake flasks. Microb Cell Fact 2008, 7: 31

12:00 Bioprocessing of stem cells for regenerative medicine purposes – scale-up or scale-out

Chris Hewitt (Department of Chemical Engineering, Loughborough University, United Kingdom)

Abstract: Continued improvement in the world's health depends upon the development of affordable and effective therapeutic treatments. Since typical pharmaceutical screens of a single compound using 384 well-plates would require ~6x106 hESC-derived cells/plate, our current differentiation efficiencies calculate that 120x106 hESCs or iPSCs are needed. Similarly, the 5x109 cardiomyocytes required to repair an infarcted heart in a single patient would require 100x109 starting pluripotent cells. Manually a single operator can culture ~10 x T175 flasks (300x106) of pluripotent cells per week, well short of the manufacturing scale required to produce a feasible product and representing the single most limiting bottleneck in developing products from these cell populations. The key to the successful larger-scale cultivation and harvest of stem cells is the development of well-defined, controlled conditions with known starting points that ensure desirable specific outcomes. Preliminary data using an automated hESC culture platform has reproducibly produced 3.1x109 cells over a 1-2 week period, dependent on pluripotent cell line, a sufficient scale for cell banking and pharmaceutical screens. However, for larger-scale applications, the surface area limitations of T flasks could be addressed by the use of microcarrier beads maintained within STRs that could enable the production of >500x109 cells.

12:30 Closing remarks and farewell

Peter Neubauer (TU Berlin)

12:45 Lunch

14:00 End of symposium

Poster abstracts

Poster 1: New development in online-fermentation-control using MIR-Sensors

Bader, J.[1], *Castritius, S.*[2], *Harms, D.*[2], *Kron, A.*[3], *Schäfer, T.*[3], *Rädle, M.*[3], *Stahl, U.*[1]

1) Chair of Microbiology and Genetics, TU Berlin, Germany
2) Central Laboratory, Versuchs- und Lehranstalt für Brauerei in Berlin (VLB), Germany
3) Institut für Prozesstechnik und innovative Energiesysteme, HS Mannheim, Mannheim, Germany

A fiber-optic based Middle-Infrared (MIR)-Spectrometer will be developed, which enables the quantitative determination of substrates and products in bioreactors. In comparison with existing similar systems, the main advantages of this development are the online-detection and definite cost reduction as well as an increase in process stability accompanied with low maintenance effort. These achievements are based upon the application-oriented-development of the MIR-Technology by the use of sapphire elements in the optical components. The excellent chemical and thermal resistance of sapphire enables a sterilisation of the in situ sensor. Different sugars, alcohols and acids can be detected simultaneously with the MIR-Sensor. At this point of the development, the lower detection limits of the substances of interest are between 20 to 50mg/L (depending on the substance itself). Improvements in the electronic components of the evaluation unit and the reduction of the background noise as well as constructional advancement of the sensor head are expected to decrease the detection limit of the MIR-Sensor. Various sensor heads will also be developed to increase the field of application. According to these achievements, the sensor system is highly suited for controlling/ monitoring fermentation processes. A medium-sized company will launch the sensor system after the development.

Poster 2: Culture heterogeneity of Bacillus megaterium is a function of the fed-batch control strategy

Claudia Korneli (Institute of Biochemical Engineering, TU Braunschweig, Germany)

Bacillus megaterium is a relatively large bacterium with a length of 2x5 µm. The ability for stable plasmid replication as well as its enormous secretion capacity turns it to be an attractive host for industrial production of recombinant proteins like antibodies, for which an increasing demand for diagnostic and therapeutic applications exists. A main problem by scaling up a suitable process strategy to industrial scale is the appearance of inhomogeneous areas in the bioreactor during oxygen and substrate supply exposing the microorganisms to unnecessary stress conditions. An optimal adjusted cultivation strategy therefore plays a crucial role trying to avoid stress, which may cause a loss in product and biomass yield. For a B. megaterium strain different fed-batch control strategies were developed. As target proteins a recombinant antibody fragment as well as the model protein GFP (green fluorescent protein) was produced. Alterations in the cell population were examined by means of flow cytometry. The existence of heterogeneities during the course of cultivation, e.g. in membrane potential and GFP-content, was observed. In the contribution three

fed-batch control strategies (constant, exponential and DO regulated feeding profiles) are compared to each other and the correlated results from flow cytometric analyses are discussed. Since cell heterogeneity arises principally as result of bioreactor inhomogeneities, e.g. in concentration gradients, actual development and results of a scale-down experimental set-up are described as well.

Poster 3: Reliability of optical dissolved CO_2 measurements in fermentation medium during the production of thermostable α-amylase and protease by B. caldolyticus

Skelats, L.[1], *Wewetzer, S.*[1], *Bader, J.*[2], *Karschöldgen, A.*[3], *Popovic, M.K.*[1]
[1)] Beuth University of Applied Science Berlin, Germany
[2)] Chair of Microbiology and Genetics, TU Berlin , Germany
[3)] Kreienbaum Wissenschaftliche Messsysteme, Langenfeld, Germany

In many cases yields of fermentative products in large scale bioreactors are lower than in small scale fermenters. Reasons for this are inhomogenities of temperature, pH, dissolved oxygen and carbon dioxide concentrations and substrate in the liquid phase (Xu et al., 1999). This may result in mixed-acid fermentation, overflow metabolism or other unfavorable changes in metabolic activity. These inhomogenities also appear in the gas phase and can hardly be detected by simple exhaust gas analysis because only the overall concentration is measured (Popovic et al., 1980). Whereas small amounts of dissolved CO_2 have advantageous effects on the production of amylase and proteases (Popovic et al., 2009), increased CO_2 concentrations result in a decreased pH value in the cells followed by altered metabolic activity. Only in situ measurement of the carbon dioxide concentration offers the possibility to detect dissolved CO_2 inhomogenities in the bioreactors. Reliability of the used optical CO_2-probe was tested during the fermentation of the thermophilic microorganism Bacillus caldolyticus at a temperature of 70°C over a period of 10 hours in 5 L fermenters. The given results of the probe were compared with the exhaust gas analysis. The applied optical dissolved CO_2-probe showed comparable and reliable results during the whole fermentation indicating the possibility of using this measuring method even under the extreme fermentation temperature.

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Poster 4: Sprout culture of different plants

Yaroslav Shevchenko (TU Berlin, Germany)

Establishment of sprout culture of three different plants is presented (Lippia, Stevia rebaudiana, Red Mint). Sprout culture cultivation is considered to be a preliminary stage for possible cultivation in a bioreactor (large scale production). The conditions of sprout culture cultivation impose specific conditions on the plants. The plant metabolic system tends to respond through different adjustments reactions. It is reflected specifically in biosynthesis of the secondary metabolites like natural sweeteners (Lippia and S.rebaudiana) or essential oil production in the Red Mint culture. One of the remarkable qualities of the sprout culture cultivation is a rapid biomass gain and possibility to influence biosynthetic reactions through application of stressors and precursors.

Poster 5: CFD-based compartment model for the description of the heterogeneities experienced by microorganisms in bioreactors

Angélique Delafosse (Laboratory of Chemical Engineering, Liège University, Belgium)

Understanding and modelling the complex interactions between biological reactions and hydrodynamics is a key problem when dealing with bioprocesses. Indeed, the scale-up of a bioprocess from lab-scale to industrial scale often results in a decrease of the productivity compared to the lab-scale one. This problem can be partly attributed to the decreasing mixing efficiency with the increasing bioreactor size. It is thus fundamental to be able to accurately predict the hydrodynamics of bioreactors of different size and its interaction with the biological reaction.
An important challenge is the characterization of the concentration heterogeneities observed by microorganisms in bioreactors and their influence on the biological performance. For this purpose, we propose to develop a modelling strategy based on an Euler-Lagrange approach in order to predict (1) the scalar mixing process and (2) the displacement of microorganisms and their history in terms of heterogeneities encountered during their displacement. The mixing modelling is achieved using a compartment model defined from CFD data. In order to take into account the displacement of the microorganisms, a stochastic model will be implemented in the compartment model. This model will be a useful tool to study the influence of scale-up on biological performance and to propose scale-down experiments in order to characterize the response of microorganisms to perturbations of their environment composition.

Poster 6: Advanced process control stragies in animal cellculture

Mathias Aehle (Centre for Bioprocess Engineering, Institute of Biotechnology, Martin-Luther University Halle-Wittenberg, Halle/Saale, Germany)

Control strategies are presented that allow keeping animal cell cultures on their optimal growth rate profiles, thus combating the missing batch to batch reproducibility of most industrial cell protein production processes. As the quantities that one would like to control such as the specific growth rate or the biomass concentration are not measurable on-line in a production environment. Indirect measurement techniques must form the basis of control. The solutions presented consider the fact that the cell cultures show up a considerable response time upon changes in the adjustable variables such as feed and aeration rates. Two different closed-loop control solutions are illustrated at fed batch cultures of CHO cells where the control variable is the total mass of oxygen consumed by the cells. This quantity can be measured on-line. It is shown that the process can be kept on its predefined path even if it starts with considerably different cell densities.

Poster 7: Rapid process development method for high performance recombinant protein production processes in Escherichia coli

Sebastian Schaepe (Centre for Bioprocess Engineering, Institute of Biotechnology, Martin-Luther University Halle-Wittenberg, Halle/Saale, Germany)

For the development of a high performance production process it is necessary to determine the cells maximal respiratory capacity. Here we present a feedback Feed-pulse approach which allows tracking the cells performance based on changes in the dissolved oxygen level of the fermentation broth. Most organisms are able to take up more substrate than oxygen. Overflow metabolites are the consequence of this effect. These metabolites can negatively influence the rates of product formation and growth, so the formation has to be strictly prevented. For accurate detection of sub- and supra critical states a cross-correlation algorithm is used. The results of the Feed-pulse experiments are critical specific substrate uptake rate profiles. Under these process conditions overflow metabolites are not formed. If the critical substrate uptake rate is determined, the focus is put on a highly reproducible fermentation procedure. Therefore we use an improved algorithm for control of the total produced carbon dioxide profile. The combination of both control strategies allows a reproducible protein production under high performance conditions.

Poster 8: Electrooptical monitoring of polarizability and cell size in Escherichia coli fed-batch and continuous cultivations

S. Junne[1], *H. Stosch*[1], *I. Zavodni*[1], *A. Angersbach*[2], *P. Goetz*[3], *P. Neubauer*[1]
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[2)] Biotronix GmbH, Hennigsdorf, Germany
[3)] Beuth University of Applied Sciences, Berlin, Germany

Exposing rod-shaped microorganisms to an alternating electrical field, the average polarizability and the average size of cells can be

monitored with electrooptical measurements (EOM) [1]. In previous studies, it could be demonstrated that the polarizability and cell length measured at-line with EOM could provide valuable information about the morphological state and the dimension of main product fluxes in an anaerobic fermentation process [2].
We investigated the possibility of EOM for the online-monitoring of metabolic activity in Escherichia coli K12 aerobic fed-batch, chemostat and auxostat cultivations. The response of the signals gained by EOM was investigated from low dilution rates (D < 0.1 h-1) under strong starvation conditions up to dilution rates near the wash-out point. This response was compared to the development of substrate and oxygen consumption, product synthesis and biomass growth. Results indicate a strong relation between the polarizability and the biomass yield. The combination of the two parameters polarizability and cell size makes it possible to distinguish between strong starvation, optimal process conditions and metabolic overflow.
Applying a regression model, it was possible to predict the biomass yield based on the polarizability and cell length at all experiments. It is assumed that both parameters together can be used as a suitable tool for monitoring effects of inhomogeneities in aerobic E. coli cultivations. Since the parameters are related to the overall metabolic activity of the cell, disturbances during cultivation can be detected immediately.

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Poster 9: SENBIT® – wireless process control of shaken cultures

Stefan Junne[1], *Eva Materne*[1], *Julia Glazyrina*[1], *Christian Hälsig*[2], *Peter Neubauer*[1]
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[2] teleBITcom GmbH, Teltow, Germany

For the cordless and location-independent process control, a modular radio system for electrodes was developed in cooperation with teleBITcom GmbH (www.telebitcom.de). The system can be used in laboratory scale as well as in production scale. The difficulties arising when sensors are applied in shake-flasks or in the bulk of liquid cultures in bioreactors can be avoided when using SENBIT®. The emitter is devolving the data via the free- available ISM-band to a personal computer. The receiver is allocating the signals to the appropriate sensor, so that data from up to 32 sensors can be measured and analyzed in parallel.
So far, the system was applied for measuring the pH and the dissolved oxygen concentration in shaken cultures. The system was further successfully applied for the determination of the oxygen mass transfer coefficient in shake flasks of different geometries using the sulfite method.
With the application of multiposition sensors, monitoring of gradients in large-scale bioprocesses will be enabled, when the sensors move freely inside the reactor. While combining this system with various sensors, new possibilities of location-independent process control arise.

Poster 10: Flounder – a novel technique for recovery of biosurfactants

Julia Glazyrina[1], *Stefan Junne*[1], *Klaus Lunkenheimer*[2], *Peter Neubauer*[1] and *Peter Götz*[3]
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A new method based on the enrichment of surface active compounds at an air/liquid-interface in a custom-built glass body called 'flounder' is presented. Biosurfactants are removed from complex mixtures and separated by compressing and harvesting the liquid surface layer. This method was applied to Bacillus subtilis cultures, in which the lipopeptide antibiotic fengycin as well as the polyketide antibiotic bacillaene were produced. The automated harvesting and collection of the biosurfactant fengycin was repeated several hundred times. The fengycin concentration in the fractions was found to be four times higher than in the culture centrifugate. 50 % (w/w) of the overall fengycin were recovered after 300 cycles, 95 % (w/w) after 800 harvesting cycles. A separation of fengycin from the less surface-active bacillaene could be achieved due to stronger surface activity of fengycin. The ratio of partition coefficients of fengycin and bacillaene was nine times higher compared to foam fractionation. A step-wise increase of the equilibrium surface tension in the centrifugate from 29 to 33 mN/m indicated a fractionated separation of different surface active substances. The utilization of cell containing culture broth instead of centrifugate had only slight effects on separation efficiency. These results demonstrate the possibility to separate biosurfactants directly from cultivation without the use of additional recovery methods.

Poster 11: Bioprocess scale-up from deep well plates to 100 L fermentation with Liquid-EnBase™

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EnBase™ is a unique microbial cultivation platform for high cell density growth of microorganisms (Panula-Perälä et al., Microb. Cell Fact. 2008, 7:31). It is based on the principle of the glucose-limited fed-batch technology but applies an enzyme controlled internal delivery system for the controlled feed of glucose from a glucose-containing polymer which allows easy scaling to any cultivation volume, from micro liter cultures to the liter scale, with the benefit that no external feeding is needed. The previous EnBase system was characterized by a 3-phase system, a storage gel containing the polymer, a gel for controlling the release to the medium, and the overlaying liquid medium. Although this set up is advantageous by providing the possibility to supply high amounts of substrate, its preparation is laborious and its application is limited to small volumes. Here we demonstrate a new liquid form of EnBase (EnBase-Flo) which consists of a simple one-phase system only. Controlling the growth with Liquid-EnBase is easily applied in any scale. Cell densities above 8 g/l are obtained and the new liquid system has the same beneficial characteristics as the gel-based EnBase for recombinant proteins, a higher amount of soluble protein is produced and aggregation is low. Whilst the ultimate cell densities have been shown in the gel based format compared the liquid format is much more convenient, and cultures can be directly centrifuged to obtain the cell pellet for extraction of the target product.
EnBase-Flo is especially advantageous for bioprocess scale up, which we demonstrate here by performing a scale up from micro liter cultures to the 100 L scale. Preliminary investigations with WAVE show that the same beneficial results will also be achieved in disposable Bio-Reactor technologies.

Poster 12: Application of a short intracellular pH method to flow cytometry for determining Saccharomyces cerevisiae vitality

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The measurement of yeast's intracellular pH (ICP) is a proven method for determining yeast vitality. Vitality describes the condition or health of viable cells as opposed to viability which defines living versus dead cell. In contrast to fluorescence photometric measurements, which show only average ICP values of a population, flow cytometry allows the presentation of an ICP distribution. By examining six repeated propagations with three separate growth phases (lag, exponential, stationary), the ICP method previously established for photometry was transferred successfully to flow cytometry by using the pH-dependent fluorescent probe 5,6-carboxyfluorescein (CF). The correlation between the two methods was good ($r^2 = 0.898$, $n = 18$). With both methods it is possible to track the course of growth phases. While photometry didn't yield significant differences between exponentially and stationary phases ($p = 0.433$), ICP via flow cytometry did ($p = 0.012$). Yeast in an exponential phase has a

unimodal ICP distribution, reflective of a homogeneous population; however, yeast in a stationary phase displays a broader ICP distribution and subpopulations could be defined using the flow cytometry method. In conclusion, flow cytometry yielded specific evidence of the heterogeneity in vitality of a yeast population as measured via ICP. In contrast to photometry, flow cytometry increases information about the yeast population's vitality via a short measurement, which is suitable for routine analysis.

Poster 13: Type of Agitators

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Mainly three types of unit operations are involved in bioreactorMIXING-used to mix the three phases,microorganisms,media and oxygen. MASS TRANSFER-oxygen being transfered from blk gas to microbes. HEAT TRANSFER-heat is dissipated from the the bioreactor or is provided to bioreactor.
Agitator is used to provide mixing and several types of agitator are being used in bioreactor and their effect in large scale bioreactos will be summarised in this paper.

Poster 14: Simulating fluctuations in environmental parameters using small scale continuous cultivations

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In large scale bioreactors microorganisms experience variations in environmental parameters e.g. temperature, pH as well as in nutrient and oxygen availability. These variations cause evolvement of a microorganism leading to a decrease in production yield of a desired metabolite or recombinant protein. Cost-efficient research simulating environmental variations in small scale bioreactors may provide insights into the impact caused by environmental variations. The method enabling the latter is dilution rate stat (D-stat). D-stat is a continuous cultivation method (chemostat) where one or more environmental parameters are changed smoothly and when desired periodically. D-stat experiments with Lactococcus lactis IL1403 were carried out at dilution rate 0.2 h-1 while changing pH with the following profile: 6.3 → 5.4 → 6.3 during 30 generations. We observed asymmetrical behavior (hysteresis) in culture characteristics comparing leaving and returning to the initial environmental conditions. Hysteresis was observed in the yield of biomass (Yxs) lactate (Ylact) as well as on gene expression level. Mentioned controlled variations in pH resulted in 12 % smaller Yxs and 5 % larger Ylact and different regulation in arginine pathway genes at the end of the experiment. No significant change in Yxs and Ylact was observed during three parallel chemostat experiments (70 generations). Similar hysteretic phenomena were also seen in Lactococcus lactis IL1403 and Escherichia coli MG1655 D-stat cultivations with changing temperature. In conclusion, D-stat could be used for simulating environmental fluctuations in large scale bioreactors as well as for testing the stability of production strains.