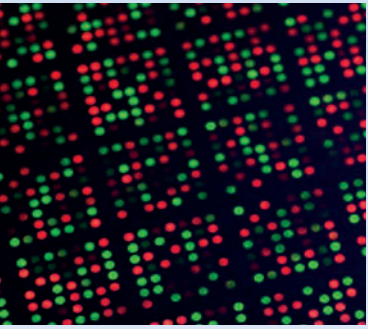
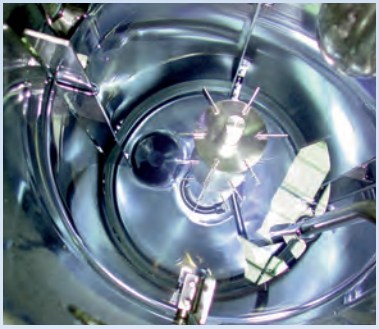
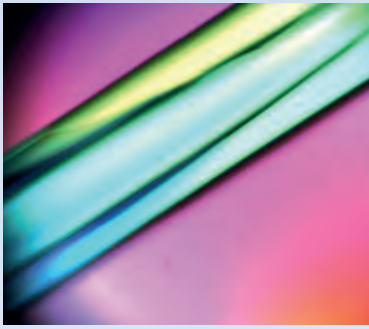
Industrial Biotechnology Forum 2016

March 14/15, 2016 · Technische Universität München (TUM) · Garching

PROGRAM

Content

Welcome to the IBF 2016!

Industrial Biotechnology ("White Biotechnology") is the technology that uses enzymes or cells for a large-scale production of substances like fine chemicals, food and feed ingredients, agrochemical and pharmaceutical products, industrial additives, and – with increasing importance – bulk chemicals and fuels. Compared to chemical conversions, successful biotechnological processes require less heat and pressure, and result in economic and environmental benefits. In particular the bioconversion of renewable raw materials is a promising approach towards lowering greenhouse gas emissions and preserving resources.

Scientific challenges in the field of Industrial Biotechnology are tailoring of enzymes and multi-enzyme systems for industrial applications (enzyme engineering) as well as redirection of metabolic fluxes or establishment of new artificial pathways in microorganisms (metabolic engineering) to develop new catalysts for biotechnological processes. Bioprocess engineering provides the tools for a promising process design and makes the newly designed biocatalysts work on an industrial scale. Bioseparation engineering is essential to overcome one of the main limitations in Industrial Biotechnology – a cost-efficient purification of bio-products from diluted aqueous solutions.

The Industrial Biotechnology Forum (IBF) 2016 brings together recent developments from all these fields – enzyme engineering, metabolic engineering, bioprocess engineering, and bioseparation engineering. As launching event of a biennial conference, the IBF 2016 intends to initiate an interdisciplinary exchange and productive discussion between the different scientific areas forming the core of Industrial Biotechnology science.

In the name of the organizers, the Industrielle Biotechnologie Bayern Netzwerk GmbH and the TUM-Research Center for Industrial Biotechnology we would like to thank all speakers and poster presenters for contributing and sharing their newest scientific results. We hope for many intensive and fruitful discussions and we look forward to many new insights into the emerging field of Industrial Biotechnology science. Enjoy the lectures and the poster session with beer and Brez'n, the poster awards ceremony, the conference dinner and the guided tours to the TUM-Pilot Plant for Industrial Biotechnology (Garching) and the TUM Algae Pilot Plant (Ottobrunn).

Prof. Dr.-Ing. Dirk Weuster-Botz
TUM-Research Center for Industrial Biotechnology

Prof. Dr. Haralabos Zorbas
Industrielle Biotechnologie Bayern Netzwerk GmbH

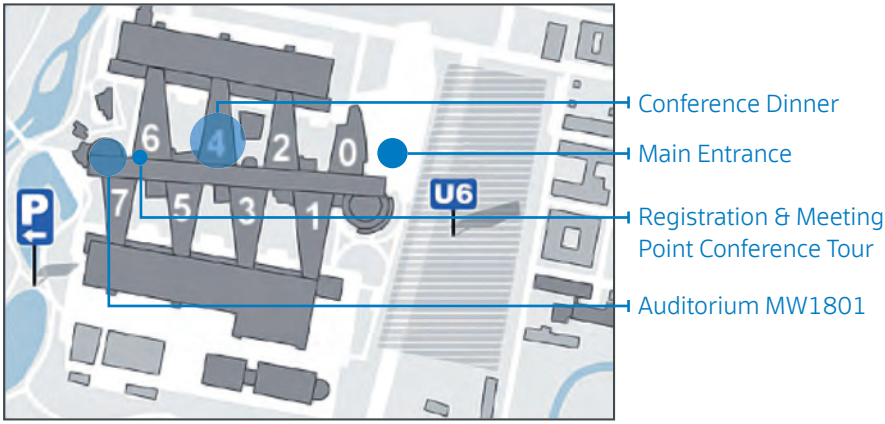
Where, when & what?

Industrial Biotechnology Forum 2016
March 14-15 (Monday-Tuesday)

Conference Venue

TUM-Department of Mechanical Engineering
Boltzmannstraße 15 · 85748 Garching b. München

Underground station: "Garching-Forschungszentrum" (U6)



All presentations will take place in lecture hall MW 1801 in the rear part of the department, 1st floor. The registration desk is located in front of the lecture hall (ground floor) and will be open from Monday 12:00 am. Industry presentations and exposition of the joint project „Advanced Biomass Value" will be open from Monday 10:00 am. Posters can be placed from Monday 10:00 am.

Conference Dinner

Monday 14th March from 7:30 pm at the TUM-Department of Mechanical Engineering, court 4

Conference Tour

Tuesday 15th March from 2 to approx. 4:30 pm. Meeting point is at the registration desk. The participants will be split into two groups; group 1 will start with the TUM-Pilot Plant for White Biotechnology (Garching), group 2 will start with the TUM-Algae Pilot Plant (Ottobrunn). All further information is available at the registration desk.

Program

Monday, March 14, 2016

10:00	Industry presentations and exposition of the joint project „*Advanced Biomass Value*“ funded by the Federal Ministry of Education and Research	
12:00	Registration with snacks and beverages	
12.45	**Opening and welcome address**	
	Speech of Prof. Dr. Dr. h.c. mult. Wolfgang A. Herrmann, President of TUM	
	Speech of Dr. Ronald Mertz, Head of Section “Innovation, Research, Technology”, Bavarian Ministry of Economic Affairs	
13:30	**Session 1: Enzyme Engineering**	
	Chairs: Prof. Dr. Volker Sieber, Prof. Dr. Thomas Brück	
13:35	Keynote: **Dr. Miguel Alcalde**, Institute of Catalysis, CSIC Madrid, *“Engineering the Ligninolytic Enzyme Armoury by Directed Evolution”*	p.8
14:20	**Prof. Dr. Ulrich Schwaneberg**, Institute of Biotechnology, RWTH Aachen, *“Directed Enzyme Evolution: Concepts and Lessons“*	p.9
14:50	**Dr. Wolfgang Schwarz**, Department of Microbiology, Technical University of Munich, *“Enzymatic Biomass Hydrolysis: Effective Enzyme Complexes for Non-Starch Plant Materials”*	p.35
15:10	**Dr. Martin Hofrichter**, International Institute Zittau, Technical University of Dresden, *“Fungal Peroxidases: A New Generation of Oxygen-transferring Biocatalysts”*	p.30
15:30	**Coffee break**	

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16:00	**Session 2: Metabolic Engineering**	
	Chairs: Prof. Dr. Wolfgang Liebl, Prof. Dr.-Ing. Andreas Kremling	
16:05	Keynote: **Prof. Dr. Uwe Sauer**, Institute for Molecular Systems Biology, ETH Zürich, *"From Tinkering to Engineering"*	p.10
16:50	**Prof. Dr. Volker F. Wendisch**, Genetics of Prokaryotes, Bielefeld University, *"Metabolic Engineering of Microbial Cell Factories: Flexible Feedstock Concept and New Bioproducts using Corynebacterium glutamicum"*	p.11
17:20	**Dr. Alberto Marin-Sanguino**, Systems Biotechnology, Technical University of Munich, *"Design Principles as a Guide for Metabolic Engineering: Application to Halophiles"*	p.49
17:40	**Dr. Mislav Oreb**, Institute of Molecular Biosciences, Goethe University of Frankfurt, *"Engineering Subcellular Compartmentalization in Yeast"*	p.51
18:00	**Poster session** with Beer and Brez'n	
19:30	**Conference Dinner – Piano: Sarah Mettenleiter**	

Tuesday, March 15, 2016

08:30	**Session 3: Bioprocess Engineering**	
	Chair: Prof. Dr.-Ing. Dirk Weuster-Botz	
08:35	Keynote: **Prof. Dr. John Woodley**, Department of Chemical and Biochemical Engineering, DTU Denmark, *"Integrated Bioprocess and Biocatalyst Engineering"*	p.12
09:20	**Prof. Dr. Wolfgang Wiechert**, Institute of Bio- and Geosciences, Research Center Jülich, *"Bioprocesses from a Single Cell Perspective"*	p.13
09:50	**Dr. Kathrin Castiglione**, Institute of Biochemical Engineering, Technical University of Munich, *"One-step Expression and Enzyme Immobilization in Bacterial Cellular Envelopes"*	p.61
10:10	**Dr. Doris Hafenbradl**, Electrochaea, Planegg (Germany), *"Power-to-Gas: From Lab to a 1 MW Scale Plant"*	p.67
10:30	**Coffee break**	
11:00	**Session 4: Bioseparation Engineering**	
	Chair: Prof. Dr. Sonja Berensmeier	
11:05	Keynote: **Prof.dr.ir. Luuk van der Wielen**, Department of Biotechnology, TU Delft, *"Integral biorefining: downstream processing move up"*	p.14
11:50	**Prof. Dr. Jürgen Hubbuch**, Biomolecular Separation Engineering, KIT Karlsruhe, *"Bioseparation engineering in the biopharmaceutical industry – screening, analytics and modelling"*	p.15
12:20	**Dr. Paula Fraga Garcia**, Bioseparation Engineering, Technical University of Munich, *"Magnetic Nanoparticles as New Carriers in Downstream Processing"*	p.92
12:40	**Dr. Wouter Van Hecke**, VITO, Mol (Belgium), *"Process Intensification – A Variety of Solutions Rendering Bioprocesses more Viable"*	p.98
13:00	**Poster awards**	
13:15	**Lunch**	
14:00	**Tours** – Guided tour for registered participants to the TUM-Pilot Plant for Industrial Biotechnology (Garching) and the TUM Algae Pilot Plant (Ottobrunn)	
16:00	Return to conference venue, arrival in Garching approx. at 16:30	

Dr. Miguel Alcalde

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Research Focus:

Our research primarily focusses on the engineering of enzymes by directed evolution and hybrid/semi-rational design for a wide range of biotechnological purposes; the development of high-throughput biomolecular screening tools and genetic methods for library construction and exploration, as well as synthetic biology studies for environmental and energy applications. Within this research field, our main line focuses on the laboratory evolution of the ligninolytic enzymatic consortium secreted by white-rot fungi. Typically composed of different oxidoreductases (mostly laccases, peroxidases, peroxygenases and H_2O_2-supplying oxidases), this lignin-degrading army represents the front line of green chemistry, with potential applications in the production of second generation biofuels, pulp biobleaching, the design of nanobiodevices (biosensors and biofuel cells), organic synthesis and bioremediation, among others. In the last decade we have used laboratory evolution to tailor specific properties of laccases, peroxidases, peroxygenases and H_2O_2 enzymes (e.g., enhanced secretion levels, kinetics and total turnover numbers for the production of chemicals, improved stability over a range of pHs and temperatures, and adaptation to the presence of non-natural environments ranging from human blood to organic co-solvents).

Scientific Career:

Dr. Miguel Alcalde studied Biology at the Universidad Complutense de Madrid, Spain (1988-1993) and in 1994, he obtained an M.Sc in Plant Physiology. In 1999, he completed his doctoral studies in Biochemistry and Molecular Biology under the supervision of Prof. A. Ballesteros and Dr. F.J. Plou at the Institute of Catalysis (CSIC, Madrid). From 2001 to 2003, Dr. Alcalde took up a postdoctoral fellowship in Biochemistry in Prof. F.H. Arnold's group at the Division of Chemistry and Chemical Engineering at the California Institute of Technology, CALTECH. He was later appointed as a Staff Scientist of the CSIC in 2007 and promoted to Researcher in 2014. Dr. Alcalde has participated in several consecutive European Projects and research contracts with industries with several patents and over 70-SCI papers in the field (for further information please visit www.miguelalcaldelab.eu).

Prof. Dr. Ulrich Schwaneberg

RWTH Aachen University
Institute of Biotechnology
and DWI-Leibniz Institute for Interactive Materials
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Research Focus:
The Schwaneberg group is a world leader in protein engineering by directed evolution and rational design. Projects range from fundamental science to understand structure-function relationships to methods development for directed evolution and optimization of biocatalysts for sustainable production from renewable resources. In addition, within the DWI-Leibniz Institute for Interactive Materials triggerable proteins (FhuA, anchor peptides) are designed. Within the research field of protein engineering and biocatalysis 24 patents (mainly with industrial partners (BASF, DSM, Evonik, Roche, Henkel, BRAIN) have been filed, three engineered enzymes are currently in industrial use and more than 150 publications have been published.

The group is located at the RWTH Aachen University and the DWI-Leibniz Institute for Interaktive Materials and divided in five subgroups (Diversity Generation, High-Throughput Screening, Computational Biology, Biohybrid Systems, Biomedical Systems) with >50 post-docs, PhD fellows and staff. Recent highlights comprise the KnowVolution protein engineering strategy, the OmniChange mutagenesis method for simultaneous site saturation mutagenesis (generation of 3.2 million enzyme variants in one afternoon), and the FUR-Shell screening technology based on flow cytometry and fluorescent hydrogels.

Scientific Career:
Ulrich Schwaneberg studied chemistry at the University of Stuttgart and received his diploma in 1996. His doctoral studies were also carried at the University of Stuttgart in the Institute of Technical Biochemistry under the supervision of Prof. R. D. Schmid. After his graduation in 07/1999, he moved as post-doctoral fellow to the lab of Prof. Frances H. Arnold at Caltech (USA) for two years. In January 2002, he was appointed as Assistant Professor at the Jacobs University Bremen and was promoted in 2006 to Associate Professor. In January 2009, he moved to the RWTH Aachen University as Head of the Institute of Biotechnology and since 2010 co-appointed in the Scientific Board of Director at the DWI Leibniz Institute for Interactive Materials. Furthermore he has been appointed in the board of directors in the Bioeconomy Science Center and servers as speaker of HICAST (Henkel Innovation Campus for Advanced and Sustainable Technologies) at RWTH Aachen University.

Prof. Dr. Uwe Sauer

ETH Zürich
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Research Focus:

The mission of our systems biology research is to identify and quantify the key regulation mechanisms that control cellular metabolism in microbes.

The speciality of our interdisciplinary team of biologists, engineers, and computer scientists are high-throughput experimental technologies for quantitative metabolomics, 13C-based intracellular flux analysis, and dynamic GFP analysis in mini-scale cultivations. Different types of mathematical models are then used to quantitatively relate metabolomics, expression and proteomics data to the functional network output in terms of fluxes.

Scientific Career:

- President ETH Research Commission, ETH Zurich
- Professor Systems Biology, ETH Zurich, 06-present
- Group leader Metabolic Engineering, ETH Zurich, 96-05
- Postdoc Biotechnology, ETH Zurich, 93-95
- Ph.D. Microbiology, University of Göttingen, 89-93

Prof. Dr. Volker F. Wendisch

Bielefeld University
Genetics of Prokaryotes
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web.biologie.uni-bielefeld.de/genetik

Research Focus:

Prof. Wendisch's research interests concern genome-based metabolic engineering of industrially relevant microorganisms, systems and synthetic microbiology. In particular, the Wendisch Lab focuses on

- Characterization of global gene regulation and metabolic pathways
- Systems Biology on Escherichia coli and Corynebacterium glutamicum as model organisms
- White Biotechnology with emphasis on rational strain development for the production of industrially relevant chemicals

Scientific Career:

Prof. Dr. Volker F. Wendisch is Chair of Genetics of Prokaryotes at the Faculty of Biology at Bielefeld University. Since 2010 he is member of the board of the university's Center for Biotechnology CeBiTec and speaker of its Institute for Genome Research and Systems Biology. Volker F. Wendisch received his diploma in biology from Cologne University. After having completed his PhD at the Institute of Biotechnology 1 of the Research Center Jülich in 1997, Volker F. Wendisch worked as postdoctoral researcher at the University of California, Berkeley, CA, USA. In 2004 he received the venia legendi in microbiology from Heinrich-Heine-University Düsseldorf. From 2006-2009 he was Professor for Metabolic Engineering at the Westfalian Wilhems University Münster.

Prof. Dr. John Woodley

Technical University of Denmark
Department of Chemical and
Biochemical Engineering
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www.kt.dtu.dk

Research Focus:

John M. Woodley leads a research group focused on Bioreaction Engineening, with emphasis in four main areas as follows:

1. Multi-step biocatalysis, including systems biocatalysis and flow chemistry
2. Downstream processing from biocatalytic reactions and fermentations, including in-situ product recovery (ISPR)
3. Modelling tools for the assessment of bioprocesses, including property prediction, thermodynamics, kinetics, process simulation and economic evaluation
4. Bio-oxidations, including oxygen supply methods to biocatalytic reactions and fermentations

The work is largely experimental, supported by engineering calculations. Reactions currently under investigation include those catalyzed by alcohol oxidases, carbohydrate oxidases, cytochrome P450s, Baeyer-Villiger monooxygenases and transaminases. The work in collaborative with a range of industrial and academic partners from around Europe. Asia and USA.

Scientific Career:

John M. Woodley (originally from the UK) is currently a Professor of Chemical Engineering at the Department of Chemical and Biochemical Engineering at the Technical University of Denmark (DTU, Lyngby, Denmark), a position he took up in 2007 after 20 years at University College London (UCL, London, UK). He has published around 160 ISI journal papers, 60 conference proceedings, 20 book chapters and 400 conference abstracts. He has industrial experience from ICI (UK) where he held one of the first two Academic Research Fellowships (1989-1994). He sits on numerous scientific advisory and editorial boards. In 2010 he was a joint recipient of the Rita and John Cornforth Award of the RSC (UK). In 2014 he was a Gambrinus Forum lecturer (TU Dortmund, Germany). He is a Fellow of the Institution of Chemical Engineers (UK) and a Fellow of the Royal Academy of Engineering (UK).

Prof. Dr. Wolfgang Wiechert

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IBG-1: Biotechnology
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www.fz-juelich.de/ibg/ibg-1

Research Focus:

- Industrial Biotechnology: Production of bulk chemicals, fine chemicals, pharmaceuticals and proteins by using microorganisms and biocatalysts
- Systems Metabolic Engineering: Development of industrial production strains and bioprocesses based on Systems Biology methods
- Synthetic Biology: Using and functionalizing biological components to gain biological insight and to develop new processes
- Modeling and Simulation of biochemical networks: model based experimentation, data analysis and visualization in System Biology
- Single cell analysis and Optogenetics: Using tailor-made microfluidic devices for analyzing single microbial cells with integrated genetically encoded biosensors
- Raw data evaluation for Omics Technologies: Generating meaningful information for metabolic flux analysis, quantitative metabolomics and proteomics, single cell analysis
- Automated Experimentation: Accelerating bioprocess development by intelligent lab robotics and integrated experimental design

Scientific career:

2011 – now: Professor for Computational Systems Biology, RWTH Aachen University
2009: Director at IBG-1, Forschungszentrum Jülich and Professor for Systems Biology, University of Düsseldorf
2006: Guest professor, ETH Zürich
2002-09: C4 professor for Simulation and Computer Science in Mechanical Engineering, University of Siegen
1996-02: C3 professor for Simulation, University of Siegen
1995: Habilitation in Theoretical Biology, University of Bonn
1990-96: PostDoc at the Institute of Biotechnology, Forschungszentrum Jülich
1985-90: PhD in Theoretical Biology and Biotechnology, University of Bonn
1979-85: Diploma in Mathematics and Computer Science, University of Bonn

Prof.dr.ik. Luuk A.M. van der Wielen

Delft University of Technology
Bioprocess Engineering (BPE)
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www.tnw.tudelft.nl/en/about-faculty/departments/biotechnology/research-groups/bioprocess-engineering

Research Focus:

- thermodynamics for biotechnological processes
- process chromatography on several scales, crystallization and precipitation processes
- multifunctional bioreactors
- in-situ-product recovery as well as miniaturized ('on-chip') and computational, high-throughput technology for rapid process development
- process integration for bioenergy, biofuels and biochemicals manufacturing

Scientific Career:

Prof.dr.ir. Luuk A.M. van der Wielen (Amsterdam, 16-06-1964) holds a M.Sc. degree in Chemical Engineering from Twente University (Netherlands), and a PhD degree (with honours) from Delft University of Technology (TUD). Currently, he is Distinguished Professor for Biobased Economy of TUD and Full Professor at its Dept of Biotechnology at (www.bt.tudelft.nl), where he heads the Bioprocess Engineering Section effectively since 1998.

Since 2004, Luuk van der Wielen is director of BE-BASIC (www.be-basic.org), the globally operating private-public research organisation for Biobased Sustainable Industrial Chemistry & Energy, which is based in The Netherlands with hubs in South East Asia and Brazil, and a cumulative budget exceeding 250 M €. BE-BASIC executes a R&D, training and innovation program in the field of industrial and environmental biotechnology, via a consortium of 50 academia and industries. He initiated the multipurpose pilot facility (www.bpf.eu, ~ M € 80). In 2012, he coordinated the Netherlands' Bioenergy and Biochemicals Innovation plan under the new Dutch Topsector Policy (budget exceeding 1 billion euro), and was appointed in the 1st Board of Directors of Foundation TKIBBE. In 2007, he joined (part-time) Royal Dutch Shell as Principal Scientist Biotechnology. He was Visiting Professor at the Univ. San Carlos, the Philippines until 2008; and 2009-'13 at Univ. of Technology Malaysia. The last Google Scholar count shows over 250 publications and patents as of feb 2014 (H-index 30).

Prof. Dr. Jürgen Hubbuch

Karlsruhe Institute of Technology (KIT)
Biomolecular Separation Engineering
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Research Focus:

The chair of Biomolecular Separation Engineering focuses on all aspects of modern downstream processing: protein purification, formulation as well as analytics in the biopharmaceutical and biotech industry. The area of research ranges from assessing structural parameters of proteins on a molecular level, transport and surface interaction phenomena of proteins, purification and characterization of bio-nanoparticles and cells to industrial process development based on high throughput process development methodologies and mathematical models.

Scientific Career:

By training a process engineer Jürgen Hubbuch holds a Diploma / MSc in Chemical Engineering from University of Karlsruhe, Germany and an M.Phil. in Process Engineering from Herriot-Watt University Edinburgh, Great Britain. During his PhD Jürgen Hubbuch worked on integrated capture operations targeting crude feedstocks in the field of downstream processing at the Danish Technical University, Denmark. Following this his focus was shifted to molecular mechanisms occurring during chromatography during his PostDoc work at the Institute for Enzyme Technology, Research Center Jülich, Germany, followed by work on high throughput process development as a group leader at the same institution. Since 2008 Prof. Hubbuch holds a chair for Biomolecular Separation Engineering at the Karlsruhe Institute of Technology, Germany.

Sarah Mettenleiter, Piano

Vocals & Piano
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www.sarahmettenleiter.de

Already in early years, Sarah Mettenleiter came in contact with music. At the age of six she started playing the piano, later saxophone, clarinet and vocals followed. After her high school graduation, she spent two years at the New Jazzschool Munich with focus on rock/pop/jazz and graduated in 2010. Her major subject was vocals which she learnt from Max Neissendorfer and Barbara Mayr. During this time, she also collected first experiences with own bands and with the Federal Youth Jazz Orchestra of Bavaria. From 2010 to 2014, Sarah Mettenleiter studied jazz singing at the University of Music and Performing Arts Munich with supervisors Sanni Orasmaa and Philipp Weiss. After her Bacherlor's degree, she continued her Master studies with major subject jazz singing. Besides her artistic activities, she works as teacher for vocals and piano at the New Jazzschool Munich.

TUM Research Center for Industrial Biotechnology

The TUM Research Center for Industrial Biotechnology is an interdisciplinary association of 9 TUM professors and their research groups of 3 faculties of the Technical University of Munich (Department of Mechanical Engineering, Department of Chemistry and TUM School of Life Sciences Weihenstephan) with the objectives

Prof. Dirk Weuster-Botz, Director TUM Research Center for Industrial Biotechnology (Photo: TUM)

- to establish Industrial Biotechnology („White Biotechnology“) as a strongly interdisciplinary research and educational program at TUM,
- to constitute interdisciplinary research networks across multiple faculties regarding prospective topics in Industrial Biotechnology,
- to promote transfer of technologies by an early integration of companies, in particular small and middle-sized companies, in research projects and
- to organize the scientific education within the Master‘s Program Industrial Biotechnology of the Munich School of Engineering (MSE).

The main technical facility of this interfacultative research center is the TUM Pilot Plant for Industrial Biotechnology located in Garching, which

- enables the production of sufficiently large amounts of new biocatalysts, functional proteins, and biomaterials for TUM scientists,
- offers the possibility to evaluate novel biotechnological production processes on a technically relevant scale,
- promotes transfer of technologies via the demonstration of technical processes, and
- contributes to a practically oriented education of graduate students in the field of Industrial Biotechnology.

Researchers in the TUM Pilot Plant for Industrial Biotechnology (Photo: TUM)

The TUM Pilot Plant for Industrial Biotechnology enables to perform bio-processes up to the m3-scale and to obtain highly purified biotechnological products up to the kg-scale. The pilot plant facilities can be used by scientists of TUM and by qualified scientists of other collaborating research institutions or private enterprises.

The industrial demand for outstanding individuals who can work across a number of disciplines and have a broad and applicable expert knowledge of biosciences and process engineering is continually rising. TUM is meeting this increased demand by offering a Master's program in Industrial Biotechnology (IBT) at the Munich School of Engineering (MSE). In the first two semesters, graduates with very good bachelor's degrees in biosciences or engineering are taught the complementary interdisciplinary fundamentals, based on a specially tailored curriculum. The program continues with scientific training in the following four key areas: enzyme engineering, metabolic engineering, bioprocess engineering and bioseparation engineering. The intensive study program is complemented by a mentoring program. The design of the IBT study program is unique in Germany and one of the first of its kind in Europe. Successful completion of the Industrial Biotechnology master's program offers most successful graduates a seamless transition to a doctorate, especially in the research groups associated in the TUM Research Center for Industrial Biotechnology.

Contact and information:

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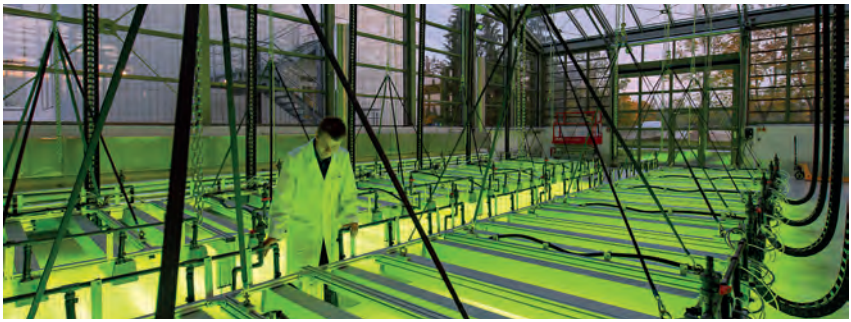
Algae Pilot Plant at the Ludwig Bölkow Campus (Ottobrunn)

The Technical University of Munich (TUM) has built a worldwide one-of-a-kind technical facility for microalgae cultivation at the Ludwig Bölkow Campus in Ottobrunn to the south of Munich in cooperation with Airbus Group.

Scientists estimate that there are some 150,000 different types of algae. Around 5,000 of them have been fundamentally characterized, yet only around ten species have been implemented for commercial exploitation. With their new algae facility, researchers at TUM aim to change this. They hope to develop new and efficient bioprocesses for the production of biokerosene and valuable chemical products there.

The 1,500 square meter building houses three areas for algae cultivation, as well as laboratory and office space. What sets the Ottobrunn algae center apart is the



Dipl.-Ing. Andreas Apel (Institute of Biochemical Engineering, TUM) launching the LED lighting in the Algae Tec facility (Photo: TUM)

fact that lighting and climate conditions for practically any location on Earth can be simulated. Airbus Group and the Bavarian Ministry of Education, Cultural Affairs, Science and the Arts are sharing the costs of just over ten million euros for the facility and the equipment. The AlgaeTec facility was inaugurated in October 2015 in the presence of the Bavarian Minister of Education, Cultural Affairs, Science and the Arts, Dr. Ludwig Spaenle, Airbus Group's Chief Technical Officer, Dr. Jean Botti, and TUM president, Professor Wolfgang A. Herrmann.

The building's façade is comprised of special highly transparent glass that also lets ultraviolet light pass through. Elaborate climate technology ensures that both tropical and very dry climate conditions can be created. Two different climate zones can be simulated at the same time in the two outer halls. Additional LED lighting allows the simulation of light and climate conditions of practically any location on Earth. These highly efficient LEDs provide light in the 300 to 800 nanometer wavelength range and an intensity distribution that closely mimics that of sunlight. Since the different LED types can be individually controlled and adjusted, researchers can also configure specific spectra that differ from that of the sun.

Cultivation is not limited to just one type of photo-bioreactor. Various open and closed systems will be developed by TUM-scientists and can be operated in parallel in the halls, under the same or different climate conditions. Thanks to the facility's elaborate building automation systems, the algae center's operations are highly energy efficient.

Contact and information:

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Prof. Dr. Wolfgang Liebl
Microbiology,
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Prof. Dr. Volker Sieber
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Prof. Dr. Arne Skerra
Biological Chemistry,
TUM School of Life Sciences Weihenstephan (Freising)

Prof. Dr.-Ing. Dirk Weuster-Botz
Biochemical Engineering, Department of Mechanical Engineering,
TUM (Garching)

Media Partners

Session 1: Enzyme Engineering

1. Chain length and isomer determinants in actinobacterial olefin biosynthesis

Angel Angelov, Maximilian Surger, Maria Übelacker, Wolfgang Liebl*
Department of Microbiology, Technische Universität München

Abstract: Aliphatic hydrocarbons are the predominant components of petroleum-based fuels. Certain bacteria produce such compounds naturally and are therefore regarded as sources of enzymes and pathways for the biotechnological production of aliphatic hydrocarbons. One of the routes by which bacteria synthesize hydrocarbons involves the head-to-head Claisen condensation between two fatty acyl-CoA molecules. This type of alkene biosynthesis is well documented in many representatives of the Actinobacteria phylum, which produce primarily anteiso- and iso-branched, long-chain (C25 to C29) alkenes. The first reaction of this pathway is catalyzed by enzymes of the thiolase superfamily and it is currently debated if and to what extent these proteins (termed OleA) determine the nature of the condensation products formed by an organism.

In order to better understand the role of the OleA enzymes in determining the type of olefins formed, we initiated a detailed study of the oleA genes, alkene profiles and free fatty acids composition of a collection of 23 isolates belonging to the *Micrococcus*, *Kocuria* and *Kytococcus* genera. GC-MS analysis of hexane extracts showed that all tested strains were capable of producing olefins and displayed a substantial variation in the olefin amounts, the distribution of chain lengths and of isomers. We cloned selected oleA genes or ole gene clusters from our strain collection in an engineered *Micrococcus luteus* strain lacking its native ole genes. This allowed us to probe the role of different OleA proteins in shaping the product profile under the same conditions (growth conditions, fatty acid precursor pools etc.). The results from these experiments showed strong selectivity differences between OleA proteins from different sources. Several oleA genes conferring characteristic olefin profiles were selected for cloning in *E. coli* in order to obtain recombinant proteins for further structural studies which will address the relations between Ole protein structure and substrate specificity.

2. Enzymatic functionalization of algae-derived fatty acids

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Abstract: Material utilization of renewable resources represents the most promising way to hamper "food vs fuel" discussions. The need of alternative and sustainable processes is obvious in nearly all industrial sectors. Especially the conventional petroleum-based industry wants to overcome the demand for a sustainable future and therefore they are in the need of "green" solutions for bulk chemicals as well as niche products. With a percentage of 1 % of mineral oil products, the lubricant industry represents a highly attractive sector to replace petrol based products by renewable alternatives.

Fresh and salt water microalgae, which can be cultivated on wasteland areas without competing with food agriculture, represent a perfect alternative resource. Additionally, algae can convert CO_2 towards biomass production much more efficiently than terrestrial plants. Specified, strain dependent, cultivation conditions of microalgae can be used to target lipid production. These lipids represent the base as renewable resources for further functionalized algae based lubricants.

In the frame of the "Advanced Biomass Value" (ABV) project we deal with an innovative process for the enzymatic functionalization of algal lipids. Saturated long-chained fatty acids, e.g. obtained by lipase catalyzed hydrolysis of triglycerides, are oxidative decarboxylized to yield the corresponding chain-shortened terminal alkenes. This represents a simple way to produce lubricants or fuel additives directly from biomass derived C sources. The cytochrome P450 peroxygenase $OleT_{JE}$ from *Jeotgalicoccus sp.* ATCC 8456 is perfectly equipped to realize this process by a one-step reaction, requiring just H_2O_2 as cofactor. To overcome the limited stability of this enzyme in the presence of the peroxide, several methods for the *in situ* production of H_2O_2 in combination with the decarboxylation reaction were investigated. A FMN-dependent light-driven H_2O_2 generation method resulted in 90 % conversion of stearic acid for both cell-free lysate and purified enzyme. The formation of olefins and β-hydroxy fatty acids was monitored by GC with FID and MS detection.

Whilst saturated fatty acids are suitable substrates for decarboxylation, their unsaturated counterparts can be used for epoxidation approaches of fatty acids. Via a lipase-mediated chemo-enzymatic "self-epoxidation" reaction around 90 % conversion of oleic acid was achieved.

3. Dihydroxy-acid dehydratase from *Sulfobus solfataricus*: A key enzyme for the conversion of carbohydrates into chemicals

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Abstract: Dihydroxyacid dehydratases (DHADs) are excellent biocatalysts for the defunctionalization of biomass. Here we report on the recombinant production of DHAD from *Sulfolobus solfataricus* (SsDHAD) in *E. coli* and its characterization with special focus on activity towards non-natural substrates, thermo-stability, thermo-inactivation kinetics and activation capabilities and its application within multi-step cascades for chemicals production.

Using a simple heat treatment of cell lysate as major purification step we achieved a specific activity of 4.4 units per gram cell mass towards the substrate D-gluconate. The optimal temperature and pH value for this reaction are 77 °C and pH 6.2. The inhibitory concentration (IC50, 50 % residual activity) of different alcohols was determined to be 15 % (v/v) for ethanol, 4.5 % (v/v) for butanol and 4 % (v/v) for isobutanol. In addition to D-gluconate and the natural substrate 2,3-dihydroxyisovalerate (DHIV), SsDHAD is able to convert the C3-sugar-acid D-glycerate to pyruvate with a specific activity of 10.7 ± 0.4 mU/mg, a reaction, which does not occur in natural metabolic pathways.

The specific activity of the enzyme can be increased 3-fold by incubation with 2-mercaptoethanol. The activation has no impact on temperature dependence, but modulates the thermo-inactivation tolerance at 50 °C. The total turnover numbers for all of the three reactions was found to be $35.5 \times 10^3 \pm 1.0 \times 10^3$ for the conversion of Dgluconate to 2-keto-3-deoxygluconate (KDG), $28.2 \times 10^3 \pm 0.8 \times 10^3$ for DHIV to 2-ketovalerate (KIV) and $943 \pm 0.28 \times 10^2$ for D-glycerate to pyruvate. With activated SsDHAD these values could be further increased 5- and 4-fold for the D-gluconate and D-glycerate conversion, respectively.

4. Biocatalytic oxygenation catalyzed by human FMO5

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Abstract: The insertion of oxygen atom(s) from molecular dioxygen into an organic molecule facilitates the metabolism of xenobiotic compounds and allows oxyfunctionalization. To this end, Nature has developed an arsenal of cofactor-dependent enzymes.

Human flavin-containing monooxygenases (hFMOs) are nicotinamide-dependent proteins which oxygenate organic molecules via a 4a-hydroperoxyflavin species. Among the five human isoforms, FMO5 stands out: while it shows the most abundant expression in the liver, its functional activity has not yet been clearly established and classical FMO substrates are either poorly converted or non-reactive [1]. Isoenzyme hFMO5 was heterologously expressed in *E. coli*, extracted by surfactants from the microsomal fraction and purified to homogeneity. Thermo-FAD and gel-filtration analysis allowed us to find the requirements offering the best stability to the hFMO5-detergent complex and, accordingly, to study its activity towards NADPH and oxygen via analysis of the spectral behaviour of the FAD cofactor.

A comprehensive substrate profiling revealed that hFMO5 acted as a Baeyer-Villiger monooxygenase (BVMO) [2] with a rather large substrate spectrum. The regio- and stereoselectivity pattern of hFMO5 on aldehydes and aliphatic and cyclic ketones was fully characterized and compared to that of classical BVMO enzymes [3].

Acknowledgements:

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5. *E. coli* based production of cyclooctatin via consecutive functionalization with a new redox cascade

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Abstract: De novo production of multi-hydroxylated diterpenoids is challenging due to the lack of efficient redox systems. Our engineered *Escherichia coli* strain provides a 43-fold higher yield of anti-inflammatory cyclooctatin, a tri-hydroxylated fusicoccane, than the native producer *Streptomyces melanosporofaciens*. Consecutive, two-step hydroxylation of the parent olefinic macrocycle was feasible with absolute stereospecificity using two sequence divergent P450 type hydroxylases in conjunction with a newly identified, generic redox system from *Streptomyces afghaniensis*. Performance comparison of this redox system with characterized equivalents of *Pseudomonas putida*, showed that the *Streptomyces* system provided an improved product titer. We demonstrate that redox system engineering can boost and harmonize the catalytic efficiency of class I hydroxylase enzyme cascades.

Talk

6. Fungal peroxygenases: A new generation of oxygen-transferring biocatalysts

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Abstract: The enzymatic oxyfunctionalization of organic molecules under moderate conditions has attracted keen interest from the chemical community. Unspecific peroxygenases (EC 1.11.2.1) secreted by fungi represent an intriguing enzyme type that selectively transfers peroxide-borne oxygen with high efficiency to diverse substrates including unactivated hydrocarbons. An enzyme of this type with aryl alcohol-oxidizing activity had first been found in the mushroom *Agrocybe aegerita* (Black poplar mushroom) in 1995, still without knowing what kind of biocatalyst it was. Ten years later, the enzyme was recognized as a heme-thiolate protein that acts as a true mono-peroxygenase. In the following years, similar enzymes were found in other mushroom genera such as *Coprinellus*, *Coprinopsis*, *Marasmius* and *Auricularia*. Over 2,500 of putative peroxygenase sequences, which form at least two distinct clusters, can nowadays be found in genetic data bases indicating the widespread occurrence of such enzymes in the whole fungal kingdom including true fungi and fungus-like protists. In February 2011, this enzyme type was included as unspecific peroxygenase (UPO) into the EC system where it is the first enzyme of a second peroxidase subclass. On the phylogenetic level, UPOs represent together with chloroperoxidase (CPO; 1.11.1.10) a separate (super)family of heme proteins. The catalytic cycle of UPOs combines that of heme peroxidases with the “peroxide shunt” of P450s. The crystal structures of the major UPO from *A. aegerita* has been solved and reveals a funnel-shaped, hydrophobic heme-access channels rich in phenylalanine residues. There are indications that differences in the molecular architecture of UPOs may affect the catalytic activity, in particular regarding the size of the oxidized substrates and the peroxide binding mode. Reactive UPO intermediates (compounds I and II) have been characterized by stopped-flow techniques and proved to be the oxygen key species in hydroxylation reactions. Heterologous expression studies are being currently carried out in cooperation with academic and industrial partners and have already been successful in expressing recombinant UPO in *Saccharomyces* and *Aspergillus*. If the reasonable heterologous (over)expression of peroxygenases will succeed, a powerful biocatalytic tool will be available for organic synthesis and other applications.

7. Enzymatic recycling of synthetic nicotinamide cofactors

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Abstract: The cell-free biocatalytic production of fine chemicals by oxidoreductases has continuously grown over the past years [1]. Since especially dehydrogenases depend on the stoichiometric use of nicotinamide pyridine cofactors, an integrated efficient recycling system is crucial to allow process operation under economic conditions. Lately, the variety of cofactors for biocatalysis was broadened by the utilization of totally synthetic biomimetics. They are cheap alternatives for the nicotinamide pyridine cofactors and can be prepared in a few steps. The stereospecific hydroxylation by cytochrome P450 BM-3 R966D/W1046S from *Bacillus megaterium* or different reactions of Old Yellow Enzymes could be performed under the consumption of biomimetics [2]. However, the regeneration is limited to chemical or electrochemical methods using mediators like rhodium (II) complexes, since there is a lack of an enzymatic recycling system [3]. We show a detailed investigation in the stability of biomimetics and its enzymatic regeneration. We investigated commercially and non-commercially available recycling systems and found a dehydrogenase which performed the substrate oxidation under the consumption of the biomimetic. Subsequently, determination and evaluation of the kinetics constants for the natural and biomimetic cofactor was performed.

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8. High throughput platform for generating *Pichia pastoris* expression libraries

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Abstract: Heterologous protein expression and secretion requires suitable expression hosts. *Pichia pastoris* is well established as it combines the benefits of high cell density cultivation, efficient secretory capabilities, can be easily genetically modified and is able to perform post-translational modifications.

Despite the large number of proteins that have been expressed using this host, finding the optimal expression conditions can be time-consuming. This process is influenced by the differing structural features of proteins and modifications that need to be made, but their impact is not well understood.

Here we present standardized elements for the rapid assembly of *Pichia pastoris* expression cassettes. By exploiting existing hierarchical MoClo techniques and expanding them with *Pichia* specific regulatory elements, it is possible to efficiently generate large diverse libraries.

This synthetic biology toolkit will assist development of efficient cell factories to meet ever increasing demands in biotechnology.

9. Plant glucosyltransferases for the biotechnological production of small molecule glucosides

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Abstract: The number of publicly available plant genomes is increasing continuously. Currently, more than 50 genomes have been sequenced which provide a rich source of information on novel biocatalysts such as glucosyltransferases (GT). GTs are able to glucosylate/esterify a large number of molecules, including secondary metabolites like phenols, terpenoids and alkaloids and thus, they are offering numerous possibilities for biotechnological application. Polyphenol glucosides for example are known for their health-promoting and anti-oxidative properties, while others contribute to flavor and colour of fruits. The production of glucosides is therefore a promising market niche regarding food, cosmetics and pharmaceutical industry. We are collecting and building a plant GT library and focus on enzymes catalyzing the glucosylation of small molecules. The candidates are tested as whole cell biocatalysts on lab scale. Here we report the characterization of selected GTs from various plant systems involved in the formation of terpenoid and phenolic glucosides and the optimization of the glucoside/ester production.

10. Magnetic low-cost enzyme carriers for Industrial Biotechnology

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Abstract: The constant increase of sustainable products on the global markets demands new biotechnological strategies, such as recycling and recovering biocatalysts. Iron oxide nanoparticles (IONs) might be the most promising magnetic nanostructures for a plethora of applications in health, life and environment science. IONs as carrier material in enzyme catalysis facilitate excellent recovery properties from high viscous liqueurs by high-gradient magnetic separation. Since most carriers utilized industrially need expensive coatings, the application of bare nanoparticles seems economical worthwhile.

We present simple and fast electrostatic assemblies of cellulase (CEL) and low-cost IONs at different buffer conditions. Three different ION species were synthesized by co-precipitation methods and were thoroughly characterized. The iron oxides were synthesized at comparable conditions and completely without stabilizing agents. XRD identifies the crystal phases of IONs and indicates the size of nanoparticles which is validated by TEM. Here average sizes range between 10-30 nm for each ION facilitating the comparison. Mössbauer, infrared and Raman spectroscopy identify the chemical composition of the synthesized materials while SQUID measurements reveal their magnetic properties and BET adsorption isotherms the specific surfaces.

The adsorption behavior of the CEL on IONs follows electrostatic interactions which are revealed for each surface by potentiometric titrations and adsorption isotherms for different pH values and buffer systems. The driving force for the interaction between the biomolecules and the nanoparticles is the molecular adhesion that leads to adsorption at the bio-nano interface. For successful further advance, a fundamental understanding of these surface interactions is essential and main focus of our work.

We want to emphasize the complex adsorption processes of biomolecules on nanoscale surfaces. While the formation of a so called corona around nanoparticles in contact with proteins is widely discussed, the influence of pH and ionic strength as well as composition of the buffer is an interesting topic. Here the nanoparticle surface plays a significant role for adsorption processes and even protein-protein interactions in the adsorption. We investigated different protein-particle systems where the amount of adsorbed enzyme can be controlled. CEL activity can be directly related to the binding conditions on the ION surfaces.

We demonstrate high enzymatic activity, excellent colloidal stability and stable enzyme bonding. The high CEL loading could be sustained without leaching of biocatalyst and high recovery yields over ten magnetic recycling steps, preserving high enzymatic activities and live-cycle stabilities.

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Talk

11. Enzymatic biomass hydrolysis: Effective enzyme complexes for non-starch plant materials

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Abstract: The growing demand for a sustainable alternative to crude oil not competing with food supply has resulted in the obvious but so far elusive need for utilization of the abundantly available plant biomass. The hydrolysis of plant biomass delivers sugar, which then can serve as a basis for the industrial production of bio-based platform chemicals and fuels. However, plant biomass consists of a naturally resilient matrix of cellulose, hemicellulose and lignin. To date, no economically competitive technology to hydrolyze the cellulose has been developed. Filling this gap, our product FasCiPlex is a specially designed, engineered enzyme complex efficiently converting cellulose to sugar.

Currently available cellulose degrading enzymes (cellulases) are mainly derived from fungi, which have been intensively investigated and developed for already over 70 years. In contrast, our product FasCiPlex is based on the most effective cellulase in nature, the cellulase complex of the anaerobic bacterium *Clostridium thermocellum*, called cellulosome. The cellulosome connects all the different enzymes required for cellulose degradation in a protein complex, thereby enhancing enzymatic synergy. Tremendous research effort has already been spent on the characterization of the >70 single cellulosomal enzymes which are available to us as a toolbox for the *in vitro* assembly to functional synthetic complexes. Based on our proprietary HTS screening system the optimal mixture of enzymes is evaluated for each individual substrate. The prototype FasCiPlex already outperforms marketed fungal cellulases. Our future research and development will concentrate on FasCiPlex's adaption to different biomass substrates.

12. New halophilic enzymes out of the Deep Red Sea Brine Pools

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Abstract: The success of biotechnological processes is based on the availability of efficient and highly specific biocatalysts, which can satisfy industrial demands. Extreme and remote environments like the deep brine pools of the Red Sea which reveal high salt concentrations of up to 4.3 M and elevated temperatures of up to 68°C are highly interesting for the investigation of novel halophilic and thermophile enzymes. *Haloferax volcanii* was chosen as a suitable expression system for halophilic enzymes because expression of first enzymes out of the Deep Red Sea Brine Pools were not successful with *E. coli*. A batch process was developed for the cultivation of *Haloferax volcanii* H1895 with knockouts of components of the flagella assembly system in a controlled stirred-tank bioreactor (T = 45°C, pH 7.3, DO > 50%). The complex medium Hv-YPC was supplemented to achieve higher cell densities. Hence, two halophilic alcohol dehydrogenases and two dihydrodipicolinat reductases obtained from Discovery Deep and Atlantis II Deep were expressed under the control of the p.tna promotor in *Haloferax volcanii*. The yields of the two expressed alcohol dehydrogenases are 16.5 mg/gCDW and 3.9 mg/gCDW at a maximum cell dry weight of 6.5 g/L and 5.8 g/L, respectively. Activity of the alcohol dehydrogenases was determined and reached in a maximum specific activity of 2.5 U/mg at 75°C, 3 M KCl, pH 10 and 0.1 mM $MnCl_2$ using cinnamylalcohol as substrate. Expression of dihydrodipicolinat reductases yielded in 17.3 and 8.9 g/L at a maximum cell density of 8.5 and 6.8 g/L, respectively. They showed a maximum specific activity of 7.1 U/mg with citraconic anhydride as substrate at 3 – 4 M KCl, 40°C and pH 7.5. Compared to the so far applied uncontrolled shake flask cultivations dry cell mass concentrations were improved by a factor of 10 and cell-specific enzyme activities showed an up to 28-fold increased yield of heterologous proteins. Thus, new halophilic enzymes can easily be made available with scalable batch processes with *Haloferax volcanii*. The new enzymes pose an opportunity to perform biocatalytic reactions at elevated temperatures at almost saturated salt solutions.

Session 2: Metabolic Engineering

13. Construction of plasmid-free bacterial strains for the synthesis of human milk oligosaccharides (HMO)

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Abstract: Topic: Beneficial effects of human milk oligosaccharides (HMOs) on infants' well-being have raised attention for HMOs as potential nutritional additives for infant formula [1]. However, chemical or *in vitro* enzymatic syntheses are laborious or costly. Objective of this work was the construction of plasmid-free *Escherichia coli* strains capable of synthesizing HMOs using recombinant glycosyltransferases in combination with intracellularly generated nucleotide-activated sugars (e.g. GDP-L-fucose) to allow further research on these compounds.

Methods: Strain construction was based on the *E. coli* K-12 strains (JM109 or LJ110) using a site-specific λ-red recombineering technique for chromosomal integration of heterologous genes in combination with a screening on differential agar plates [2]. Strain evaluations and HMO syntheses were conducted in shake-flask cultivations and fed-batch fermentations, allowing quantification and improvement of intracellular precursor molecule levels via HPLC.

Results and Conclusions: With up to 6 consecutive integrations in one strain, plasmid-free *E. coli* strains were constructed for the synthesis of different HMOs. During strain improvement, different copy-numbers of genes allowed leveling of gene expression in order to raise HMO productivity. Furthermore, chromosomal stability allowed syntheses in fed-batch fermentations without the need for antibiotics as selection markers, resulting in product titers of up to 20 g/L (2´-fucosyllactose) [3,4]. Utilizing chromosomal integration, we could also demonstrate the combination of specific glycosyltransferases (LgtA, WbgO) together with enhanced intracellular synthesis of UDP-nucleotide-activated sugars for the efficient preparative synthesis of oligosaccharide core-structures such as lacto-N-tetraose (LNT) [4,5]. Subsequent combination of these syntheses of core-structures with chromosomally integrated fucosyltransferases and enhanced intracellular supply of GDP-L-fucose resulted in fucosylated HMOs up to penta- and hexasaccharides such as LNF I and LNDFH II [6]. Thus, chromosomal integration turned out to be a powerful tool for synthetic

microbiology, allowing multiple rapid and site-specific insertions and construction of genetically stable strains. Using this method, we could construct strains for gram-scale syntheses of complex HMOs.

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14. Usage of acetic acid bacteria as engineered oxidative catalysts

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Abstract: Acetic acid bacteria (AAB) such as *Gluconobacter oxydans* are diverse, nonpathogenic organisms that contain several membrane-bound dehydrogenases (mDH), which allow them to oxidize a multitude of sugars, sugar derivatives and other polyols in a stereo- and regio-selective manner. Electrons from the oxidations are channeled into the electron transport chain of the AAB and are finally transferred to oxygen. Various AAB-catalysed oxidation reactions are not easily achievable by organic chemistry, which is why the organisms have been employed in many biotechnological processes. Processes using AAB can be hampered by overlapping substrate spectra of their mDHs or because specific strains are not cultivable in the laboratory or have growth characteristics not usable for fermentations. We have developed methods to engineer *G. oxydans* strains that were already used in biotechnology, in a way that the native mDHs are removed from the strain in order to circumvent competing reactions by several mDHs and unwanted reaction cascades. Then, mDHs solely with the envisaged activities could be functionally expressed. Usage of *G. oxydans* as expression host is very favorable for biotechnology because the organism tolerates high substrate and product concentrations and features the correct cell physiology for the functional expression of homologous and heterologous mDHs. This could be a very flexible general approach for the construction of strains for new specific oxidative fermentations, because it also allows expression of mDHs not only from a multitude of cultured AAB genera and species but also from uncultured AAB with variations in substrate specificity, opening oxidative fermentations for many new biotechnological applications.

Shuttle vectors for *G. oxydans* and *E. coli* with several promotors allowing the problem-prone expression of the membrane-bound enzymes were constructed and their strength was determined by a *lacZ* reporter system and activity measurements of the mDHs. The weak activity of those promotors in *E. coli* is advantageous for the cloning of those membrane located enzymes. Many mDHs where expressed using those vectors and characterized by a specifically developed high-throughput activity assay in microtiter plates, including mDHs from various AAB and a mother of vinegar metagenome, featuring new substrate specificities.

15. Dynamic flux balance modeling to increase the production of high-value compounds in green microalgae

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Abstract: Photosynthetic organisms can be used for renewable and sustainable production of fuels and high-value compounds from natural resources. Costs for design and operation of large-scale algae cultivation systems can be reduced if data from laboratory scale cultivations are combined with detailed mathematical models to evaluate and optimize the process.

In this work we present a modeling formulation for accumulation of high-value storage molecules in microalgae that provides quantitative predictions under various light and nutrient conditions. The modeling approach is based on dynamic flux balance analysis (DFBA) and includes regulatory models to predict the accumulation of pigment molecules. The accuracy of the model predictions is validated through independent experimental data followed by a subsequent model-based fed-batch optimization. In our experimentally validated fed-batch optimization study we increase biomass and β-carotene density by factors of about 2.5 and 2.1, respectively. Our study shows that a model-based approach can be used to develop and significantly improve biotechnological processes for biofuels and pigments.

Keywords: β-carotene; Dynamic flux balance analysis; process design

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16. Parameter estimation for genome-scale models of cellular processes

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Abstract: Holistic mechanistic understanding of cellular processes requires the integration of gene regulation, signal transduction and metabolism. This is mostly approached using ordinary differential equation (ODE) models. The resulting genome-scale ODE models possess thousands of state variables and parameters. To achieve reliability of model predictions, the parameters of these ODE models have to be estimated from omics data. This task is computationally challenging and appropriate methods for genome-scale models are so far missing.

Most parameter estimation algorithms employ gradient information for efficient optimization. In this project, we propose a method exploiting adjoint sensitivity equations for efficient gradient evaluation. We demonstrate that this method is for large-scale models several orders of magnitude faster than state-of-the-art methods, i.e. finite differences and forward sensitivity equations. Using this boosted computational efficiency, we inferred the parameters of a large-scale model for a variety of cellular processes, demonstrating the feasibility of quantitative large-scale modeling. Accordingly, the proposed method and the provided implementation facilitate the development of more quantitative models for health care and biotechnology.

17. Evolution of *E. coli* for diterpene synthesis

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Abstract: The demand of diterpenoids increased over the last decades due to their diverse applications as important pharmaceuticals, food additives, cosmetics and fragrances. Their natural occurrence is mostly limited and chemical synthesis are polluting and difficult because of their high structural complexity. Using engineered *E.coli* systems is a sustainable approach for future supply.

Herein we report a metabolic engineering approach to optimize the production of hydroxylated diterpenoids harboring a Taxadiene skeleton. Essential genes were isolated from natural sources and randomly combined in artificial Operons. Different Promotor Systems and varying ribosomal binding site strengths give us the opportunity to up- and down regulate specific enzymes to gain a balanced flux. For a sustainable process the usage of lignocellulosic hydrolysates is favorable. While xylose is a major component of lignocellulosic feedstocks, it cannot be metabolized efficiently by *E. coli* in the presence of glucose and other hexoses. One prerequisite for targeted pentose utilization is its conversion to glyceraldehyde-3-phosphate and pyruvate, which are subsequently condensed to form geranyl-geranyl diphosphate (GGPP), the universal metabolic precursor of all diterpenoids. We have addressed the enhancement of xylose uptake and improved carbon flux in the pentose phosphate cycle in order to increase the intracellular concentration of diterpenoid precursors without affecting glucose metabolism. To achieve xylose uptake an engineered Xylose/H+-Symporter is expressed that resolves limited xylose uptake in the presence of glucose. Subsequent metabolomic analyses guide genomic optimization strategies to enable efficient xylose and glucose uptake and metabolism. In further steps xylose usage is balanced to the need of the cell by expression of xylose isomerase and xylulose kinase. Each enzyme will be specifically controlled by selection of specific promoter systems to avoid metabolic stress.

18. An integrated systems biology platform for the sustainable production of structurally minimized bioactive taxoids

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Abstract: The project "SysBioTerp" (An integrated systems biology platform for the sustainable production of taxoid based bioactives) focusses onto the development and optimization of a microbial production platform for structurally minimized derivatives of the anticancer drug Taxol.

Initially Taxol was extracted from the bark of the pacific yew (*Taxus brevifolia*). Today Taxol is a product of an 11 step semi-synthetic process starting from deacetylbaccatin III extracted from the needles of the european yew (*Taxus baccata*). As this production system is still depending on a natural product a scale–up to cope with the rising demand for the anticancer drug Taxol, cannot be realized under economical and ecologically sound conditions. Therefore, the SysBioTerp consortium aims to develop and optimize a fermentative production system based on an engineered microbe to obtain structurally minimized taxoids that maintain and expand the bioactivity of Taxol. To reach this goal SysBioTerp unites the scientific expertise of 7 academic research entities and a SME partner.

In one domain of SysBio Terp project we focus on the microbial production system. To optimize the production platform a plasmid-based production strain was characterized. The fermentation process was optimized for high product titers and gained data about growth rate and uptake of nutrients was used for metabolic flux analysis. Based on these results a refined in-silico metabolic flux model will be constructed to predict further genetic improvements of the production host.

In cell culture produced Taxadiene, its derivates and further metabolites were characterized by GC/MS-, LC/MS and NMR-Analysis to get insight into the diversity of accumulated compounds. Quantification of Taxadiene by GC/MS was used for optimization of growth, harvest and extraction conditions. In parallel an approach for genomic integration of the enzymes needed for Taxadien production was made.

The other domain addresses the component engineering. A compilation of putative hydroxylases was screened for activity to succeed necessary stereoselective hydroxylation on distinct positions on the one hand, and to obtain new components

on the other. Additionally acetylation and phenylation will be introduced, to enable the biosynthesis of an array of novel non-natural taxoid structures, which have to be characterized by MS and NMR methods, as it is already done for the standard substances Taxol and 10-DAB III. The novel taxoid structures will be characterized further towards anti-tumor, antibiotic and neuro-protective activities. Therefore several novel testing systems will be developed to supplement the available DIN testing methods.

19. Modeling gene regulation and signaling in systems biology

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Abstract: Cellular systems biology aims at a holistic description of metabolic, signaling, and gene regulatory networks, including their interactions. Nowadays, advanced mathematical models of metabolism are frequently applied to real biological systems. In contrast, fundamental research is still required to accurately describe signaling as well as gene regulation. As a consequence, their inclusion into a metabolic model is intricate, although this would greatly improve the reliability of the model. The difficulty in describing regulation and signaling is mainly due to small molecule copy numbers of involved components, leading to significant random fluctuations (noise). As a consequence, the use of stochastic models might be necessary. Such models are usually harder to analyze than deterministic ones. Moreover, they exist in various levels of complexity, and it is not a trivial task to determine a priori whether a simplification would significantly distort the outcome of the model or not.

Our first goal was to extend the theoretical foundations of stochastic modeling. We have studied general connections between deterministic and stochastic models, and we have identified and analyzed factors which lead to severe discrepancies. They include features of biochemical reactions like large stoichiometric coefficients, or nonlinearity. This study facilitates the critical evaluation of the reliability of a deterministic model based on the specific biological system.

We have then used this theoretical framework for systematically studying factors within gene regulatory circuits which have an impact on noise. In that context, the effect of protein bursts in gene expression, of network topologies, and of reaction kinetics have been considered. We have investigated in which ways they can modulate noise strength and how population heterogeneities can emerge. Such identifications and characterizations of design principles in signaling and regulation are expected to facilitate their integration into systems biological models.

20. Optimizing xylose metabolism for heterologous terpene production in *E. coli* systems

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Abstract: Biotechnological production of highly valuable diterpenoids, such as bioactive taxoids, using engineered *E.coli* systems is a sustainable approach for future pharmaceutical supply. While xylose is a major component of lignocellulosic feedstocks, it cannot be metabolized efficiently by *E. coli* in the presence of glucose and other hexoses. To enable an economically sensible utilization of lignocellulosic hydrolysates for heterologous taxoid production, the xylose fraction needs to be converted efficiently by engineered *E.coli* strains. In our work we have addressed the enhancement of xylose uptake and improved carbon flux in the pentose phosphate cycle. One prerequisite for successful heterologous terpene production in bacteria is the transformation of sugar into the metabolic intermediates glyceraldehyde-3-phosphate and pyruvate. Targeted upregulation of the intracellular concentration of diterpenoid precursors such as isopentenyldiphosphate (IPP) and dimethylallyldiphosphate (DMAPP) should thereby not affect glucose metabolism. To achieve xylose uptake an engineered Xylose/H+-Symporter is expressed that resolves limited xylose uptake in the presence of glucose. Subsequent metabolomic analyses guide genomic optimization strategies to enable efficient xylose and glucose uptake and metabolism. Therefore the influence of xylose regulator XylR is tested to optimize xylose utilization. In further steps xylose usage is balanced to the need of the cell by expression of xylose isomerase and xylulose kinase. Each enzyme will be specifically controlled by selection of specific promoter systems to avoid metabolic stress.

21. Maximizing succinate production in *Escherichia coli*

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Abstract: Succinic acid is an intermediate of the TCA cycle and therefore part of the central carbon metabolism of cells. In wild-type *Escherichia coli* it is secreted in small amounts as part of the mixed acid fermentation under anaerobic condition, with acetic acid, lactic acid, formic acid and ethanol as by-products. Maximizing succinate production (while minimizing by-products) for its use as a base chemical has been the aim of extensive research.

Important achievements have been reported in the microbiological succinate production. The identification of genetic targets has relied almost exclusively on the expertise and/or experience of research staff rather than on model-based predictions. However, model-driven strain design can be a fast and efficient tool for rational strain design. Here, we describe the application of computational tools for optimizing succinate production in *E. coli*.

Flux Balance Analysis (FBA) based on a stoichiometric network reconstruction can be used to identify an optimal mass balanced flux distribution through the underlying network corresponding to the optimization goal. Allowing uptake and fixation of additional CO_2 FBA gives a maximum theoretical yield of 1.69 mol succinate per mol glucose under anaerobic condition, which can be increased to 1.71 mol/mol when a heterologous pyruvate-kinase reaction is added to the network. For optimal succinate production also the production and consumption of the redox equivalent $NADH/H^+$ must be finely balanced. This we want to reach by a very specific activity level of the respiratory chain through micro-aerobic condition and/or quinon manipulation. Based on FBA and Flux Variation Analysis (FVA) beneficial and non-beneficial reactions can be identified and Gene knock-outs for achieving the optimization target can be predicted. Additionally, we could identify the CO_2 fixing enzyme phosphoenolpyruvate carboxylase (PPC) as a key enzyme in the succinate biosynthesis by using an *in silico* sensitivity analysis of the exchange reactions of an *E. coli* core model. The overexpression of this enzyme led to an improvement in the rate of succinate production, when compared to a reference strain. Finally, the analysis of experimental data sets for many mutant strains (Δ*ldhA;* Δ*ldhA* Δ*pflB;* Δ*ldhA* Δ*pflB and ppc over-expression*) under different cultivation conditions have allowed to gain new insights into limiting factors of succinate production in *E. coli.*

As maximum growth rate and maximum production rate can rarely be achieved at the same time, we also want to design a synthetic toggle-switch that allows to switch our strain from growth to production. This allows for a fast and highly productive process. After successful strain design dynamic process optimization will be used to calculate optimal switching-time, switching-intensity and process time.

22. Microbial olefin production

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Abstract: Cost efficient production of chemicals from biomass is often hampered by cumbersome product purification from aqueous solution. One solution to alleviate this problem is the production of gaseous compounds right in the fermentation. We are aiming to provide ethylene via fermentation, firstly investigating the ethylene building capacity of various microorganisms.

Plants use the Yang cycle to form ethylene starting from L-methionine and ending up with the direct precursor of ethylene: 1-aminocyclopropane-1-carboxylic acid, which is converted to ethylene by the ACC oxidase. In bacteria ethylene is formed by two distinct pathways depending on the organism: from α-ketoglutaric acid and L-methionine.

In the project farm-stead biorefinery we screened for microorganisms capable of ethylene production from various substrates. In our environmental screening we isolated and identified microorganisms producing ethylene from L-methionine. To further investigate the pathway we applied a variety of substrates such as α-ketoglutaric acid, ATP and L-methionine in different combinations and concentrations to elucidate the trigger for ethylene production.

In the later process organisms will grow on silage in order to produce ethylene from renewable resources.

23. Design principles as a guide for metabolic engineering: Application to halophiles

Talk

Alberto Marin-Sanguino*, Christiana Sehr, Viktoria Kindzierski, Andreas Kremling
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Abstract: Microbes are extremely sophisticated factories that convert the most mundane substrates into all kinds of products. Some of these products have been produced by microbes for half a century and the processes and microbial strains used to obtain them have been highly optimized. This has led to the establishment of a handful of microbes as industrial workhorses. But in order to take the next step towards a real bioeconomy, a greater diversity of industrial microorganisms is needed, and that is not going to be achieved by traditional methods. The long term goal of our project is to better understand industrially relevant halophilic microorganisms towards model driven strain development. Our current focus is ectoine production by the halophilic bacteria *Halomonas elongata* due to the interest of ectoine as a novel product for medicine and cosmetics but also because of the potential of this microbe for further biotechnological applications.

A relevant question for any attempt to understand unusual microbes is: how much can be generalized from the microbes we know well? This is a difficult question since variety and exceptions are the very essence of Biology. Although the specifics are very seldom generalizable, there are certain patterns that get observed repeatedly in very different settings. These patterns of organization are called design principles. We can think of a "design principle" as a reusable solution to a commonly occurring problem in an evolutionary context. This definition, borrowed from software engineering, can be very useful for engineering biological systems. Understanding how evolution has molded the characteristics of certain metabolic pathways will not only improve our understanding of them but will also enable their rational modification to achieve goals other than those favored by evolution. We will present some examples on how different modeling techniques, oriented to the detection of design principles, have been valuable in our research on the industrial performance of *Halomonas elongata*.

The underlying philosophy of this project is that as technological progress facilitates the collection of data through high throughput techniques, the challenges will shift from collecting data to integrating information. Assembling data from many different sources into a meaningful whole as well as solving the contradictions between different datasets requires both a robust modelling platform and additional (more focused) experimental data. For that reason, the methodological focus of our group is on interaction between computational and experimental approaches in order to achieve that integration. On the modeling side, highly standardized formalisms: stoichiometric, thermodynamic and power-law kinetics. These methods enable a smoother transition from big genome scale networks to dynamic models of the subnetwork that is relevant for a particular bioprocess. On the experimental side, the project combines molecular biology, classical biochemistry and cultivation in a biorreactor as mean to collect information, test models/hypothesis and suggest means of improvement. This two sides of the project should converge into a knowledge base for *Halomonas elongata* that will stand as a proof of concept for the efficient incorporation of novel organisms into biotechnology.

24. Sustainable production of novel bio-insecticides

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Abstract: Intensive crop cultivation requires the increasing application of petroleum based, non-specific insecticides associated with a negative impact on the environment and human health. Biological insecticides represent an ecologically friendly alternative, as these natural products are rapidly biodegradable and can be tailored to act only on specific pests. Since most bio-based insecticides are of plant origin, their production is competing with agricultural food production. The sustainable, biomass-based production of insecticides has not been addressed. We have tackled this issue by primarily converting agricultural residues, such as wheat bran to an efficient fermentation base medium. We have developed a proprietary enzyme system for the quantitative conversion of milling residues to a high sugar containing fermentation medium. We have engineered an *E. coli* system to fermentatively produce an insecticidal diterpenoid. Using iterative process engineering in conjunction with continuous strain improvements we aim to design a process, which provides for economical, bio-based production of this novel bioactive. We are developing a turnkey- ready demonstration process, which will allow potential downstream partners already active in the agrochemical business to commercialize the generated technology platform.

Talk

25. Engineering subcellular compartmentalization in yeast

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Abstract: Metabolic engineering is a powerful tool to increase the synthesis of interesting compounds naturally produced by yeast or even to establish the synthesis of new valuable chemicals. However, competing metabolic pathways, formation of toxic or inhibitory intermediates, loss of intermediates across the plasma membrane or inefficient kinetics of the pathway enzymes often limit the yield of desired products.

Naturally, such limitations can be circumvented by the formation of enzyme complexes (e.g. fatty acid synthase complexes) or by subcellular compartmentalization (e.g. glycosomes of trypanosomes). To improve the efficiency of engineered pathways, it could therefore be advantageous to construct synthetic enzyme complexes or membrane-surrounded artificial compartments. Different strategies to achieve this goal in *Saccharomyces cerevisiae* will be presented.

In xylose fermentation with recombinant yeast cells, xylose is taken up into the cells by sugar transporters and converted by xylose isomerase into xylulose which is further fermented to ethanol. In competition with xylose isomerase endogenous aldose reductases, however, convert xylose into xylitol which accumulates in the cells and inhibits xylose isomerase. By connecting xylose isomerase directly to xylose transporters via protein-protein interaction domains and heterospecific coiled-coil zipper motifs we could significantly improve ethanol production and reduce formation of the side product xylitol.

Pyruvate is a central metabolite in yeast carbon metabolism. Under fermentative conditions it is mainly converted to ethanol. However, other products derived from pyruvate are also interesting for biotechnological applications. We tested whether the formation of artificial complexes between pyruvate kinase and different downstream enzymes, mediated by coiled-coil zippers, can be employed to redirect the flux of pyruvate towards the products of interest.

Moreover, we have developed synthetic membrane surrounded organelles in yeast into which we can selectively target specific enzymes or whole metabolic pathways. Formation of these ER-derived metabolosomes is initiated by heterologous protein body-inducing peptides. Transporters for substrates, cofactors and products are integrated into the membrane of the organelles. A proof of concept will be presented.

26. Multiparameter quantification in high-throughput – Automated qNMR in aqueous samples

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Abstract: In the development and optimization of bioprocesses, microbial strains or media large numbers of samples may apply. Thus, an efficient and low-cost analysis is essential. It is also important to ensure a reliable quality control in the production of bio-based fine chemicals or drugs following the successful development of appropriate systems.

numares AG uses an innovative, highly standardized and automated approach based on nuclear magnetic resonance (NMR) spectroscopy to support research institutes, development departments and the processing industries in the analysis and optimization of fermentation systems, process flows or in quality control. numares systems, both in human diagnostics and in the bioprocess optimization, are in routine use and allow the analysis of over 100 samples per day and device.

Using the numares-platform all organic ingredients present in the sample are measured simultaneously in a single experiment, in an identical matrix and over a large dynamic concentration range of 6 orders of magnitude. In aqueous fermentation samples for example this means a range between 1 and 100,000 ppm. There are almost no restrictions regarding solvents. Expensive deuterated solvents are contained only in additives and are not required in large quantities. The measurement of a 1D 1H spectrum is performed at 400 or 600 MHz, depending on the complexity of the matrix, and takes 15 minutes at the maximum. The resulting spectra contain the qualitative and quantitative information of all substances that are above the detection limit of about 1 ppm. Subsequently, data are processed and analyzed in a fully automated manner by tailor-made numares software. Signal overlaps are processed and deconvoluted. After a single signal assignment using reference spectra or the numares database, a fully automated identification and quantification against internal standards is possible. The results are reported in customized form. For processing aqueous fermentation samples in the daily routine, a modular system is available, using the 96-tube format and analyzing target metabolites from various classes. Depending on the module and matrix up to 70 metabolites from different substance classes are analyzed. Integrated control routines guarantee a very high reproducibility and reliability, combined with low hands-on time. In summary, a 96-tube rack can be completely processed per day - the results are available the next day.

27. Chemical evolution of a bacterial proteome

Christian Schipp*, Stefan Oehm, Michael Georg Hösl, Lauri Peil, Juri Rappsilber, Nediljko Budisa

Department of Chemistry, Technical University of Berlin

Abstract: The introduction of alternative biochemical building blocks to the standardized chemistry of a living cell remains an important milestone in synthetic biology. Changing the fundamental components of live such as amino acids or nucleic acids has mostly detrimental effects on biochemical processes. Therefore, the system must adapt towards the new substrate in an evolving manner rewiring its genetic basis to overcome the structurally unfavorable replacement.

During a long-term evolution experiment (LTEE), a tryptophan auxotroph *E. coli* strain was forced by a serial-transfer regime in a tryptophan (Trp) limiting environment to adapt towards the non-canonical analog L-ß-(thieno[3,2-b]pyrrolyl)-alanine ([3,2]Tpa). Genomic and proteomic analysis revealed deep changes on genetic and translational level. Some mutations obviously led to the shutdown of *E. coli*'s stringent response, which is a well-known artifact of LTEE. Nevertheless, the replacement of Trp --> [3,2] Tpa in more than 20,000 UGG codons yielded robust growing descendants in minimal glucose medium only containing the surrogate [3,2]Tpa as a tryptophan source.

This study illustrates an approach for the construction of synthetic cells with alternative biochemical building blocks exploiting the flexibility of the genetic code. Furthermore, the experiment will deepen the understanding of the evolvability of living cells in respect to non-canonical substrates.

28. Engineering of *E. Coli* for enantiomeric pure (R)-α-ionone production

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Abstract: Ionones are major fragrance compounds in plants and highly valuable for fragrance industry. In nature they are derived from carotenoids and therefore belong to the largest class of plant secondary metabolites, the terpenes. The group of ionones comprises the single compounds α-, β- and γ-ionone with α-and γ-ionone occurring in (R)- and (S)-enantiomeres – all of them varying in scent.

In fact, in natural sources ionones are always found as mixtures of different composition. Providing pure compounds for fragrance industry is intricate and cost intensive, especially regarding the industrial relevant (R)-α-ionone which only occurs in low amounts. By engineering an optimized carotenoid pathway including a modified plant derived lycopene epsilon cyclase, we effectively synthesized highly pure ∈-carotene in *E. coli* and further converted it to enantiomeric pure (R)-α-ionone by a carotenoid cleaving dioxygenase. Since high product yields are most important for cost-effective fermentative systems, it is crucial to further enhance Terpene production in *E. coli*. By implementation of the mevalonate (MVA) pathway we were able to increase carotenoid production. Furthermore, we could successfully replace individual bacterial MVA enzymes by their plant-derived counterparts which showed equal to higher activity in kinetic studies. Obtained data show that plant derived enzymes can be successfully applied in bacterial production systems and in the assembly of synthetic metabolic pathways leading to high value compounds with enantiomeric purity. Therefore their use in microbial fermentative systems for production of high value terpenoid compound is notably and should bear higher consideration.

29. Bridging the gap between constrained based and dynamical modeling

Christiana Sehr*, Andreas Kremling, Alberto Marin-Sanguino
Specialty Division for Systems Biotechnology, Technische Universität München

Abstract: Halophiles, like *Halomonas elongata*, show great potential with respect to biotechnological applications, especially concerning Ectoine production. The aim of our project is to use mathematical modeling in order to gain a better understanding of the metabolism of *H. elongata* and to figure out ways to optimize the product yield.

The metabolism of the organism still presents some unknowns, which need to be examined closely. Such an analysis requires the quantification of the pathway fluxes as well as the metabolite concentrations. It is further characterized by the sequential application of constraint based techniques, which reduces the dimensionality of the parameter space, moreover, it enables an optimal use of available information through its focus on evolutionary sound strategies. In this work, we apply a framework, which includes different kinds of modeling techniques.

First, we apply the law of mass conservation via FBA and thereby concentrate especially on the anaplerotic reactions, which have been shown to play an important role concerning Ectoine production. Second, we further analyze the resulting flux distributions with respect to thermodynamic feasibility in order to arrive at a deeper knowledge of the genome-scale modeling of metabolite concentrations. Third, we use the gained insights to build a dynamical model, which will then be analyzed via the Biochemical Systems Theory (BST) to account for regulatory aspects.

30. Genome editing strategies to realize tailor made exopolysaccharides

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Abstract: With ongoing re-orientation of the chemical industry towards renewable resources, microbiologically synthesized exopolysaccharides (EPS) gain more and more importance. Besides sustainable production and biodegradability, bacterial polymers have another promising feature. Their chemical structures can specifically be modified by engineering the genome of the production strain. Targeted alterations in the molecular structure of EPS can deliver new physicochemical traits and open up yet unknown application areas.

We are working on effective strategies to engineer the producers' genome in order to design tailor-made EPS. These strategies include genome sequencing of environmental isolates, identification of specific gene clusters and functional assignment of genes involved in EPS biosynthesis. Current approaches for targeted alterations of the EPS biosynthesis include knock-out, knock-down and overexpressing variants of genes encoding specific enzymes. Especially the levels of nucleotide sugar precursors are promising targets to influence the monomer compositions of EPS and to investigate substrate specificity of glycosyltransferases. EPS variants produced via genetic engineering are evaluated with sophisticated analytic tools (HPLC-UV-ESI-MS/MS, SEC-MALLS). Furthermore rheological and other physicochemical traits as temperature and pH stability are assessed to unravel structure-function relationship of EPS.

Altogether our genetic engineering strategy gives detailed insights into the different EPS biosynthesis pathways and therefore paves the way to realize tailor-made EPS fulfilling the needs for specific applications.

31. Phosphoenolpyruvate as effector for metabolic analysis of *Escherichia coli* producing L-phenylalanine

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Abstract: Phosphoenolpyruvate (PEP) is an important factor for the microbial production of aromatic amino acids as it is a precursor in two enzymatic steps of the biosynthetic pathway. Therefore, it is of interest to use PEP as a perturbation substrate for metabolic analyses of such processes. Here the obstacle is that *Escherichia coli* cells hardly metabolize PEP that is provided as carbon source by nature whereas high uptake-rates are necessary to establish clear perturbations in metabolism.

A process for the production of the aromatic amino acid L-phenylalanine on 15 L scale with *E. coli* is established at the Institute of Biochemical Engineering. The process strategy was transferred to a production strain that additionally carried a gene coding a transporter for PEP. This transporter had no influence on process performance. As a consequence, a metabolic analysis during the production process with PEP as effector could be executed. For this, the established method of parallel short-time (20 min) perturbation experiments was used. Glycerol (86 g L^{-1}) and glucose (54 g L^{-1}) were used as fed-batch carbon sources in two reactors, respectively. The reactors with the same carbon sources varied in the feeding rate (25 mL h^{-1} and 50 mL h^{-1}). PEP was added as a secondary batch substrate with a target concentration of 1 g L^{-1} in all reactors after ~9 min, so there was sufficient time to establish steady-state fluxes before and after addition of PEP. Off-gas analysis and frequent sampling for HPLC analysis enabled a precise determination of uptake- and production rates.

Before the addition of PEP, the available carbon sources were metabolized completely and L-phenylalanine and CO_2 were formed. L-phenylalanine production rates in the four reactors varied from 0.08 to 0.16 mmol g_{CDW}^{-1} h^{-1}, whereas the feeding rates of both carbon sources only had a small influence on product formation. After adding PEP, it was taken up by the cells immediately. Uptake rates of 0.9 mmol g_{CDW}^{-1} h^{-1} occurred in the reactors with high feeding rates for glycerol and glucose. Even higher values for uptake of PEP (1.5 mmol g_{CDW}^{-1} h^{-1}) were reached with the small feeding rates. These are the highest PEP uptake rates reported so far. The addition of PEP influenced all extracellular rates except the uptake rates of glucose and glycerol. The respiration rates (oxygen uptake and carbon dioxide production) increased in all reactors, especially with low feeding-rate of glucose (OUR +39 %, CPR +63 %). But the most pronounced difference in all setups was observed in L-phenylalanine production rate. These rates increased between +64 % (low glycerol feeding) and +200 % (high feeding of glycerol or glucose). So a clear effect of additional PEP on several measurable parameters was achieved. It indicates a possible PEP-limitation during L-phenylalanine production.

Thus, the application of a modified strain with a transporter that allows metabolic analyses with PEP as an effector was demonstrated successfully. This approach enables perturbations of metabolism via metabolites that are of special interest for a specific problem but cannot be incorporated sufficiently without an additional transporter.

32. Systems biotechnological approaches to overcome metabolic burden

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Abstract: In biotechnological applications, it is often necessary to genetically modify a bacterial host system, e.g. *Escherichia coli*, by introducing a multiplicity of heterologous genes into its genome. The induced additional load can lead to metabolic stress, as bacterial cells have a limited transcriptional, translational and energetic capacity. This phenomenon is referred to as Metabolic Burden. One concrete example of a biotechnological application in which the metabolic burden plays an important role is the production of terpenoids in *E. coli*.

Terpenoids are functionalized terpenes, which are naturally synthetized compounds formed by the ligation of isoprene molecules. They have a wide range of applications in the food, cosmetic, biotechnology and pharmaceutical industry. Lycopene, taxoids and artemisinin are some prominent examples of terpenoid molecules with remarkable applications. The microbiological terpenoid production is a relatively new field of research and consequently, much work has yet to be done to improve current experimental product yields and productivities. Here, we report some achievements on the strain and process optimization of the *E. coli* based terpenoid production.

We recently developed a protocol for Metabolic Engineering which guides the development and improvement of both the production strain and its subsequent usage in a bioreactor [1]. The protocol provides clear advices on the right order of application of many standard computational methods nowadays widely used for rational Metabolic Engineering. By applying the tools described in the protocol, we could identify optimal carbon source, minimize carbon leakage via pathway modifications as well as identify optimal feeding strategies, *in silico*. Further improvements of current host strains requires a deep understanding of the metabolic stress induced by the expression of large enzyme cascades typically required for the synthesis of complex terpenoids. Therefore, our project also addresses the metrological data acquisition, quantification and evaluation of the cellular response of *E. coli* during production of recombinant enzymes. To this end, we introduced a reporter plasmid expressing the fluorescent protein mCherry under the control of a housekeeping promoter. Additionally, we introduced a second plasmid that provides a controllable load. The used pSEVA system supplies resistance cassettes and origins of replication in a highly modular way. This system enables us to systematically analyse genes regarding their replicative, transcriptional, and translational burden. A mathematical model which will not only consider metabolic fluxes but also transcriptional and translational processes is being developed and validated based on the experimental data. By using this model, we will be able to calculate and predict energy and resource requirements for a heterologous burden and consequently, estimate if and to what extent inserted genes can be expressed. The developed model should also be useful at proposing genetic modifications as well as process parameters for resource rearrangement and energy delivery to accomplish the burden. Ultimately and to confirm the forecasting power of this model, the concept will be transferred to the taxoid synthesis pathway in *E. coli*.

[1] Springer Protocols Handbooks, Chapter: "Computer-guided Metabolic Engineering", http://dx.doi.org/10.1007/8623_2015_118

33. Designed enzyme cascades and inexpensive high-level production of synthetic proteins

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Abstract: Synthetic biology offers powerful methods for DNA assembly and genome manipulation. We have successfully used them for biotransformations and metabolic engineering for the production of synthetic proteins with non-canonical amino acids.

The co-expression of multiple enzymes to set up an artificial reaction cascade in *E. coli* has become an effective approach to generate highly efficient designer cell catalysts. We used a combination of Gibson cloning and overlap extension PCR for the assembly of a multi-enzyme cascade in *E. coli*. Combinatorial assembly of the three enzymes L-amino acid deaminase, 2-hydroxyisocaproate dehydrogenase, and formate dehydrogenase on a single expression plasmid as well as the variation of promoters allowed an optimal balance of the individual catalytic steps. The designer cell catalyst quantitatively transformed L-amino acids to the corresponding optically pure (R)- and (S)-α-hydroxy acids at up to 200 mM substrate concentration.

The residue-specific incorporation of non-canonical amino acids into target proteins using amino acid auxotrophic *E. coli* strains has become a routine method for academic applications. Its prowess for the engineering of proteins with special traits was demonstrated at many examples. However, the transfer of the technology to the industrial context raises major concerns about yield and the costs for the non-canonical amino acids.

To reassess these concerns, we used genome manipulation to metabolically engineer a Met auxotrophic strain for the biosynthesis of the Met analog norleucine. Under optimized bioprocess conditions, this strain biosynthesized 4 g/L of norleucine and facilitated its translational incorporation into two target enzymes. We were able to produce up to 1 g/L of labeled enzymes with excellent incorporation efficiencies. This indicates that the high-level production of residue-specifically labeled proteins is feasible in inexpensive medium.

Session 3: Bioprocess Engineering

34. Towards economic microalgae mass production: Realistic reproduction of environmental conditions

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Abstract: Microalgae could become an important renewable source for chemicals, food, and energy if process costs can be reduced. Despite considerable global research efforts on economic microalgae mass production in the past decades, today there are only few industrial-scale processes for high-value products such as nutraceuticals or fine chemicals, while commercial viability of processes for low-value commodity products such as biofuels remains to be demonstrated.

A major reason for this is the difficult scale-up of photobioreactors from laboratory to industrial scale. Since photobioreactors are optimized for large light-receiving surface areas, they are completely different from well-characterised classic bioreactors such as the stirred tank. Therefore, reliable scale-up methodologies for photobioreactors have yet to be developed.

Since late 2015, the Technical Center for Algae in Munich provides a novel and globally unique solution to this problem: With an area of more than 1,000 m^2, this specialized microalgae research facility allows pilot-scale open photobioreactor experiments under controlled climate conditions that closely mimic real outdoor environmental conditions of any desired location in the world. Faithful reproduction of the most important environmental conditions in microalgae cultivation—sunlight, temperature, and humidity-speeds up research as experiments can be conducted year-round with no weather- or season-related downtimes. The custom-built sunlight reproduction technology relies on both real sunlight and artificial sunlight from high-power LED arrays with 8 different wavelengths, providing an irradiance of more than 2,000 µmol photons m^{-2} s^{-1} at a sun-like spectral energy distribution for unprecedented realism in indoor microalgae cultivation.

This presentation will cover relevant factors for realistic indoor microalgae cultivation as well as the experience gained using the climate reproduction system in the Technical Center for Algae. The focus will be on the accuracy of climate reproduction, discussing the congruence of target and actual values, system reaction speeds, and effects on several microalgae experiments conducted in open thin-layer cascade photobioreactors.

35. One-step expression and enzyme immobilization in bacterial cellular envelopes

Talk

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Abstract: In biocatalysis, enzymes or whole cells (containing the desired enzyme or enzyme system) are routinely used as catalysts for chemical reactions. Both approaches have their specific advantages and challenges. Whole cells are disadvantageous if substrate(s) or product(s) are toxic to the cells or undesired byproducts are formed due to the cellular metabolism. In addition, severe mass transfer limitations can be caused by the cell membrane. The use of isolated enzymes, in comparison, is more expensive due to the required downstream processing. Immobilization of enzymes after purification increases preparation costs for biocatalysts significantly, but allows for the efficient reuse of the enzymes.

The aim of this study was the development of a new technology to overcome typical limitations of using whole cells or isolated enzymes in biocatalytic processes. The combination of enzyme production and immobilization in one process step holds great potential to reduce the costs for a biocatalytic preparation considerably. For this purpose, the βgalactosidase from *Escherichia coli* K12 was genetically fused to a C-terminal membrane anchor originating from cytochrome b_5 from rabbit liver. Thus, the enzyme was *in-situ* immobilized to the cytosolic membrane of *Escherichia coli* during protein production in a batch cultivation using a stirred-tank reactor. The subsequent expression of a lytic phage protein (gene E from PhiX174) caused the formation of a pore in the cellular membrane, which resulted in release of the cytosol. Lysis yields > 99 % were obtained. Crossflow microfiltration was used to remove host cell proteins and non-immobilized enzymes and to concentrate the cellular envelopes, which were subsequently characterized.

Up to 27,200±10,460 enzyme molecules per cellular envelope were immobilized, according to sandwich enzyme-linked immunosorbent assay (ELISA) quantification of the β-galactosidase (753±190 U/$g_{dry\ weight}$). The specific activity of the immobilized β-galactosidase was 57 % higher than the activity of the soluble enzyme without membrane anchor. The cellular envelopes with immobilized enzymes were applied in biotransformations and compared to whole-cell biocatalysts. The activity of cellular envelopes was three-fold higher than the activity of whole cells prior to lysis at equivalent particle concentrations. This result demonstrates that the mass transfer limitation of the substrate *ortho*-nitrophenyl-β-galactoside (oNPG) was significantly reduced in cellular envelopes due to the pore formation in comparison to whole-cells.

In conclusion, a new one-step expression and immobilization technique for the generation of biocatalytic preparations was established. The technique could be a useful tool especially for enzyme systems which are not suitable for whole-cell biocatalysts due to severe mass transfer limitations or undesired side reactions mediated by cytosolic enzymes. [1]

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36. Valorization of CO_2-rich off gases to polymers

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Abstract: Carbon dioxide or CO_2 is considered to be the major cause of climate change by its accumulation in the atmosphere and its greenhouse properties. Nowadays it is recognized that rather than just storing it, emitted CO_2 can be a valuable source of carbon for the production of commercially valuable products. This Carbon Capture and Utilization (CCU) approach provides much needed additional capacity in the move towards a low carbon economy. Clearly, CO_2 is the ultimate sustainable resource, available everywhere, in unlimited quantities, and forever.

Poly(3-hydroxybutyrate) (PHB) is a biodegradable and bio-based plastic, synthesized by a variety of organisms as an intracellular storage material from renewable resources. Hydrogen oxidizing bacteria such as *Cupriavidus necator* have the ability to store PHB using CO_2 as a carbon source. This CCU approach can answer multiple needs: the production of biopolymers without the use of petroleum derived or agricultural feedstock, the replacement of petroleum derived plastics with biodegradable renewable products, further improvement of the sustainability profile of PHB production and the capture of CO_2 to prevent greenhouse gas accumulation.

This contribution will focus on the development and optimization of a new and sustainable fermentation process for pure culture PHB production using CO_2-rich off-gases as feedstock. First, the technical feasibility of sustainable PHB production was investigated on synthetic gas mixture (CO_2, H_2, O_2). Then, a CO_2-rich off-gas stream was sampled and transported to VITO to compare performances. The experimental work was complemented with and supported by model-based approaches.

Further developments will focus on the bioconversion of CO_2 from industrial point sources into lactic acid (the monomer of PLA, polylactic acid) and polyhydroxyalkanoate (PHA) copolymers for their application in 3D printing and aquaculture.

Acknowledgments:

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37. Syngas fermentation with *Clostridium carboxidivorans* for alcohol production

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Abstract: Limited nature of fossil fuels results in search of alternatives based on renewable sources. Microbial conversion of suitable substrates could be a promising way. Synthesis gas (syngas), a mixture of gases with mainly carbon monoxide, carbon dioxide and hydrogen can be used as carbon and energy source for this approach. Syngas can be obtained by industrial waste gas or by gasification of biomass from agricultural or municipal waste. So, it does not compete with the food or fuel discussion. Acetogens are able to use these different gas components via the ancient acetyl-CoA pathway with acetic acid as the main metabolic product. But also ethanol is one of the products from a variety of these acetogens. Among these, *Clostridium carboxidivorans* is of special interest, because it is capable of producing even longer organic carbon compounds as butyric acid and butanol beside acetic acid and ethanol.

For these reasons *C. carboxidivorans* is investigated by reaction engineering means. Cultivations with *C. carboxidivorans* are carried out in a 1 L stirred tank bioreactor (Bioengineering, KLF2000) with continuous gas supply and batch mode for the liquid phase. Metabolic product quantification is followed with HPLC. Exhaust gas analysis (µGC 490, Agilent Technologies) is applied for quantification of gas uptake rates. The often mentioned observation for clostridia to be shear rate sensitive was not confirmed for *C. carboxidivorans*. Therefore high power input (15.1 W L^{-1}) for improved mass transfer from gas to liquid phase can be applied. The effects of extracellular pH were studied in detail to clarify the necessity of an acetogenic phase for acid production followed by a solventogenic phase for the conversion of the acids to alcohols with *C. carboxidivorans*.

Cultivation with an initial pH of 6.0 and no further pH-control showed growth associated acetic acid production accompanied by pH decrease (aceotigenic phase). Having passed through a pH minimum of 4.4 the pH rises again (solventogenic phase) and conversion to ethanol and formation of butanol is promoted, whereas growth stagnates. Maximum product concentrations were 19.0 mM acetic acid, 44.1 mM ethanol, 1.6 mM butyric acid and 6.1 mM butanol. Cultivation with constant pH of 6.0 during growth phase (not controlled after end of cell growth) resulted in higher concentration of acetic acid (36.1 mM), but alcohol concentrations were lower (33.3 mM ethanol, 2.0 mM butanol). Therefore an additional batch process with continuous syngas supply was performed with pH-control in the growth phase at pH 6.0 followed by a pH shift to pH 5.0 after end of growth phase and a subsequent biphasic pH control in the range between 5.0 and 5.5. This resulted in significantly higher maximum concentration of ethanol (57.8 mM) with complete conversion of acetate.

38. An at-line PAT tool determining spore quality in filamentous bioprocesses

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Abstract: Spore inoculum quality in filamentous bioprocesses is a critical parameter associated with viable spore concentration and spore germination [1]. It influences pellet morphology and, consequently, process performance [2]. An essential step before inoculation is the determination of the viable spore concentration, in order to apply quality control and decrease batch-to-batch variability. The state-of-the-art method to investigate this variable is tedious, associated with significant inherent bias, and not applicable in real time. Therefore, it is not usable as a process analytical technology (PAT). PAT aims to design, analyse and control pharmaceutical production in order to ensure product quality [3].

Monitoring of spore germination is not only necessary to investigate further spore quality attributes but can also be used as validation of the beforehand mentioned method. Quality attributes connected to spore germination are not only the amount of germinating spores but also how long spores need for germination and whether all spores germinate at the same time or not. Those elements were so far monitored using image analysis [4], which is hampered by complex medium background often observed in filamentous bioprocesses [5].

The method presented here is based on the combination of viability staining and large-particle flow cytometry which enables measurements in real-time and hence aims to be applicable as PAT tool. It is compatible with the complex medium background, and allows the quantification of metabolically active spores. Furthermore, spore swelling as a previous step of spore germination and germination itself may be monitored. A distinction of germinated spores from not germinated spores was based on a logistic regression using multiparameteric data from forward scatter and green fluorescence channel.

In an industrial bioprocess with filamentous fungi, a good correlation to CFU counts was found. The morphological parameters as spore swelling and spore germination as well as metabolic activity were followed over the initial process phase with close temporal resolution. The validation of the method to measure spore germination showed an error of spore classification of less than 5%.

Differences in spore germination for various spore inocula ages and spore inoculum concentrations were monitored. Not all spores showing metabolic activity germinated. The amount of spores germinated was found to be dependent on the quality of the spore inoculum.

This combination of viability staining and flow cytometry is a promising tool to not only determine spore inoculum quality before batch inoculation but also to at-line monitor spore swelling and spore germination in order to react in real-time on deviations and hence decrease batch-to-batch variability. Therefore the method could be applicable for the implementation as PAT tool in filamentous bioprocesses [6].

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39. Transcriptional profiling of biotransformation process towards α,ω-dicarboxylic acids in small scale bioreactors

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Abstract: Long chain α,ω-dicarboxylic acids (DCA) are widely used as precursors for various polyamides, polyesters, fragrances, macrolide antibiotics and adhesives in the chemical industry. They have a high potential to serve as biobased building blocks, since they can be produced from renewable resources. Some well-known microorganisms, such as *Candida tropicalis*, are able to convert n-alkanes and fatty acids into their corresponding diacid molecules. Due to the complex reaction mechanism of conversion process, whole cell biotransformation represents the preferred method for the DCA production. Within our scale down approach, we designed a production process by following optimizations to achieve comparable and reproducible process design. A pH-shift, from 5.8 to 8.0 was applied to increase solubility of the product and to enhance the transport of DCA efficiency out of the cells. Additionally, this approach prevents product inhibition, which was confirmed by transcriptional analysis. Since most fatty acids have toxic or inhibitory effects on the cells, optimal substrate feed was investigated to obtain the most efficient conversion rate. Moreover, substrate accumulations might inhibit expression of P450 enzymes, involved in first and rate limiting conversion step. Optimized process parameters for production of 1,12-dodecanedioic acid resulted in volumetric productivity of 0.4 g/l/h. Furthermore, using this reproducible small scale production system screening of different strains or substrates can be applied.

40. Reaction engineering analysis of different acetogenic bacteria for the production of chemicals from carbon dioxide

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Abstract: One of the future challenges is the development of sustainable processes for the production of chemicals and fuels from non-food feedstocks to diminish the dependency on fossil carbon reserves. A promising approach may be the conversion of synthesis gas (CO_2, H_2, CO) with acetogenic bacteria to produce C2- and C4-chemicals, which is why interest in these microbial processes has increased significantly in the last few years. For the purposeful selection of acetogenic bacteria for industrial applications, it would be useful to characterize and compare the process performances of as many autotrophic strains as possible under identical, defined and technically relevant process conditions.

To facilitate the comparative characterization of growth and product formation of acetogenic bacteria a new general medium for gas fermentation was designed to avoid the distorting influence of varying medium compositions on the process performances. Furthermore, standardized process conditions were specified for the operation of fully controlled batch operated stirred-tank bioreactors with continuous gas supply on an L-scale without gas-liquid mass transfer limitations.

Acetobacterium fimetarium, *Acetobacterium wieringae*, *Blautia hydrogenotrophica*, *Clostridium magnum*, *Eubacterium aggregans*, *Sporomusa acidovorans*, *Sporomusa ovata* and *Terrisporobacter mayombei* were selected for comparative reaction engineering analysis of their gasfermentation capabilities because they depict a representative cross section of the diversity of acetogenic bacteria based on the phylogenetic tree. All of them have not been studied in stirred-tank bioreactors so far.

The comparison of the autotrophic batch process performances of these bacteria with continuous supply of CO_2 and H_2 as gas phase revealed enormous differences: growth rates, product formation rates as well as maximum biomass and product concentrations (formate, acetate, ethanol and butyrate) differ in parts by a factor of up to 20.

41. Power-to-gas: From the lab to a 1 MW scale plant

Talk

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Abstract: The increasing deployment of renewable energy is creating challenges for managing the electricity system. In today's grid, electricity demand and supply need to be balanced at all times. With a growing share of wind and solar energy, which generate electricity intermittently and generally independent of demand, the electricity grid will experience more frequent and prolonged periods of supply/demand imbalances. Such imbalances will lead to more volatile power prices and, in severe cases where grid capacity is constrained, to the curtailment of wind and solar energy production.

Energy storage is being proposed as a powerful solution to address these problems.

In power-to-gas (P2G) energy storage, electrical energy is converted to chemical energy in the form of methane. If wind, solar, or other sources of renewable energy is used, a low-carbon gas can be produced that is fully compatible with the currently existing gas delivery and utilization infrastructure.

The first step in the P2G production chain consists of the production of hydrogen and oxygen through electrolytic water splitting. 70-80% of the electric energy required for electrolysis is conserved in the hydrogen (H_2) molecule. In the second step, hydrogen is combined with carbon dioxide (CO_2) and catalytically reacted to methane (CH_4), the principal component of natural gas. If the composition of the product gas meets the gas quality requirements of the natural gas network, it can be injected into a natural gas pipeline at the point of production.

Electrochaea is using a selectively evolved strain of methanogenic archaea for the methanation reaction. The Electrochaea strain is a single-celled, autotrophic organism and feeding exclusively on hydrogen and carbon dioxide, while producing almost exclusively methane.

The strain exhibits many characteristics that make it particularly suitable for industrial environments:

- Extremely fast reaction rates
- Very high tolerance to contaminants
- High substrate and product gas selectivity
- Mild operating temperatures (60-65°C)
- High longevity (self-reproducing, self-maintaining)
- Fast ramping rates (quick response to fluctuations in H_2 supply)
- Very high carbon conversion efficiency (98.6% of carbon is converted to CH_4)

The high contamination tolerance of the microbes allows the process to use a wide range of carbon dioxide sources, including untreated biogas.

Electrochaea believes that the technical characteristics of its microbes will translate to lower capital and operating costs as well as higher operating flexibility compared with the Sabatier process.

The BioCat Project marks the latest step in Electrochaea's scale-up and de-risking pathway. Building on a pre-commercial demonstration project executed in 2013 in Denmark, BioCat is anticipated to lift the company's technology to market readiness. The Project is partially funded by ForskEL, a technology development support program administered by the Danish transmission grid operator Energinet.dk.

The BioCat Project is located at the wastewater treatment plant Avedøre south of Copenhagen, Denmark, which is operated by BIOFOS.

Once operational, the BioCat facility will be one of the world's largest P2G plant operating with biological methanation.

42. Anodic respiration of *Pseudomonas putida* KT2440 in a stirred-tank bioreactor

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Abstract: Aerobic processes have the disadvantage of low product but high CO_2 and biomass yields whereas in anaerobic processes unwanted by-products are formed to balance the redox metabolism. In this study an anodic electro-fermentative process was developed for production of *para*-hydroxybenzoic acid (*p*HBA) by the obligate aerobic microorganism *Pseudomonas putida* KT2440 Δ*pobA*/pSEVA234-*ubiC*. To realise a three dimensional process the external electron transfer was mediated by the artificial mediator potassium ferricyanide.

For the first time growth and *p*HBA formation could be achieved for *P.putida* under strictly anaerobic conditions in a bio-electrochemical system. The analysis of this new anodic electro-fermentative process was conducted in a fully controlled stirred-tank bioreactor that was electrically insulated. Effects of working electrode (WE) surfaces areas (graphite rod, felt, brush), mass transfer and mediator concentrations were investigated.

It was found that WE surface areas do not limit external electron transfer in mediated systems. None of the electrodes tested showed complete reduction of 3 mM $K_3Fe(CN)_6$ at a stirring speed of 400 rpm (volumetric power input = 0.61 W/L) . The highest current density (referred to projected surface area) was measured with the rod electrode (0.45 mA cm^{-2}) for which no visible biofilm was observed.

To reduce mass transfer limitations stirrer speed and mediator concentration were increased to 700 rpm (2.93 W/L) and 47 mM $K_3Fe(CN)_6$ resulting in maximal current densities of 8.46 mA cm^{-2} for the rod electrode. Furthermore it could be shown that the metabolism and therefore the *p*HBA production strongly depends on the bulk redox potential (EB) which is determined by the ratio of oxidized to reduced mediator concentration. The optimal EB for the anodic *p*HBA production was identified to be 225 mV (vs. Ag/AgCl). For improved process conditions (700 rpm, EB = 225 mV, pH = 7, EWE = 0.5 V, 30 °C, graphite rod WE) and bulk redox potential control a maximal pHBA yield of 9.91 $mmolC_{pHBA}\ molC^{-1}_{citrate}$ could be reached which exceeds aerobic *p*HBA yields (5.87 $mmolC_{pHBA}\ molC^{-1}_{citrate}$) by 68 %. As an intermediate malate was formed for which an almost stoichiometric conversion was observed. Compared to the maximal theoretical malate yield determined by elementary flux mode analysis a conversion efficiency of 94.7 % could be achieved. The current densities of 12.5 mA cm^{-2} achieved for the optimized electro fermentation are to our knowledge the highest ever reported for a bio-electrochemical processes.

43. Biosensoring growth dependent terpene-production-systems in *E. coli*

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Abstract: Intensive research together with continuing development and refinement of the production of terpenes in *E. coli* has led to significant progress within the last years. Concerning the metabolism many influences have been demonstrated. But most of the results solely concern on the maximal reached terpene concentration itself and neglect loss in growth rate and standard deviations. By the introduction of a biosynthetic route for the production of Lycopene using constitutive promotors, a growth depending terpene production system was established. The calculation of the specific Lycopene production rate gave a reproducible parameter. In contrast to other analysis methods this quantification also takes growth rate and cell dry weight into account which results in a more accurate estimation of modifications and optimizations within the process. The improvements achieved can be easily transferred for the production of other terpenes due to their same precursors.

44. Phage-based ssDNA production for scaffolded DNA-origami

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Abstract: User defined three dimensional nanostructures like for example nanotubes, nanosponges or hollow DNA boxes with a switchable lid can be fabricated with single-stranded DNA molecules (ssDNA) in a self-assembly reaction under controlled conditions. In this "bottom up" method ssDNA as a scaffold interacts with short oligonucleotide sequences called staples and build three dimensional nanostructures with precision in subnanometer range (Linko und Dietz 2013). Scaffolded DNA origami enables the fabrication of a variety of complex nanostructures that promise utility in diverse fields of application, ranging from biosensing over advanced therapeutics to metamaterials. The broad applicability of DNA origami as a material beyond the level of proof-of-concept studies critically depends, among other factors, on the availability of large amounts of pure single-stranded scaffold DNA. The most promising method for production of scaffold ssDNA is the fermentation of filamentous, non-lytic M13 bacteriophages containing genomic ssDNA using *Escherichia coli* as host organism.

Based on preliminary studies for the production of ssDNA on a shake flasks scale, reaction engineering studies were performed in fully controlled batch and fed-batch operated stirred-tank bioreactors on a L-scale. A high-cell-density fermentation of *Escherichia coli* was established with bacteriophage M13mp18 infection leading to a 50-fold increase in maximal phage titer compared to literature (Hofschneider 1963; Grieco et al. 2012; Kick et al. 2015). After liquid-solid separation of host organism, the bacteriophages are precipitated using polyethylene glycol. The protein coat is chemically lysed while the ssDNA is purified by ethanol precipitation. We obtained up to 410 mg of high-quality single-stranded DNA per one liter reaction volume, thus upgrading DNA origami-based nanotechnology from the milligram to the gram scale.

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45. Nano-scale enzyme membrane reactors: Surface functionalization using non-antibacterial peptide anchors

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Abstract: Hollow vesicles formed from block copolymers, so-called polymersomes, have been extensively studied in the last decade for their various applications in drug delivery, in diagnostics and as nano-scale enzyme membrane reactors. For each application, the immobilization of proteins on the polymersomes' surface is desired as it can aid in cell targeting, lead to functional biosensors or add an additional reaction space for multistep syntheses. In almost all surface functionalization strategies to date, a chemical pre-conjugation of the polymer with a reactive group or ligand and the functionalization of the protein are required, which leads to extensive chemical modification. To avoid this chemical pre-conjugation, we investigated the functionalization of unmodified, preformed poly(2-methyloxazoline)-poly(dimethylsiloxane)-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA) polymersomes through the spontaneous insertion of hydrophobic peptide anchors into the membrane. As a model protein, enhanced green fluorescent protein (eGFP) was genetically fused to the peptide anchors and displayed on the polymersomes' surface. Because the polymersomes are to be used as drug carriers, in diagnostics or as bioreactors, uncontrolled diffusion across the polymer membrane may be detrimental, making antibacterial peptides not applicable for surface functionalization due to their potential to disrupt membrane integrity and cause leakage of entrapped molecules. Thus, non-antibacterial peptide anchors were chosen for surface functionalization of polymersomes. Three of four investigated peptide anchors, the transmembrane domains of cytochrome b5 (Cytb5'), of the lysis protein L (L') and of the syntaxin VAM3 (Vam3p') could be recombinantly expressed as soluble fusion proteins. The peptide insertion into the membrane was spontaneous at high salt concentrations and was linearly dependent on the protein concentration with 20.7 ± 0.7, 4.8 ± 0.2 and 3.7 ± 0.4 molecules being immobilized per polymersome per µM applied protein for Cytb5', L' and Vam3p', respectively. Up to 2320 ± 280 eGFP molecules could be immobilized on a single polymersome, which is in congruence with the calculated maximum loading capacity of 2250 molecules per polymersome at the highest-density hexagonal packing. This represents a 100-fold higher degree of surface functionalization compared to chemical functionalizations found in literature. The peptide insertion was stable without disrupting membrane integrity, as shown in calcein leakage experiments, or polymersome morphology, as shown by dynamic light scattering, and the functionalized polymersomes remained stable for at least 6 weeks. Furthermore, both peptide anchors inserted into the polymeric membrane within 4-48 h at a broad temperature range of 4-42°C and neutral pH, allowing for extremely mild insertion conditions compared to chemical functionalization. The transferability of the surface functionalization of polymersomes as nano-scale enzyme membrane reactors to an industrially relevant enzyme has been demonstrated with the enzyme CMP-sialic acid synthetase.

46. Rapid and in-depth strain characterization for filamentous microorganisms

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Abstract: Bacteria of the genus *Streptomyces* are interesting candidates for heterologous protein expression and production of pharmacologically active compounds. While genetic engineering tools are in place, only very few approaches focused on sophisticated strain characterization methods for time efficient and reliable identification of phenotypic differences. Due to their complex filamentous growth behavior, *Streptomyces sp.* show larger variability between independent biological experiments, than bisecting bacteria. Therefore, a larger number of parallel cultivations are needed to verify differences with sufficient statistical confidence demanding for higher cultivation throughput.

By bringing together ideas from automated and parallelized small scale cultivations with special aspects of filamentous organisms, we achieve to accelerate the strain characterization process for this important class of producer organisms.

A microtiter-plate based cultivation system is applied in combination with a liquid-handling robot to gain a comprehensive set of biological information, such as biomass formation, substrate/product kinetics and morphology in short time, compared to traditional lab-scale cultivation approaches. To prove the usability of this small scale workflow we conducted comprehensive comparison to our established 1L lab-scale cultivation system. The results show no significant differences with respect to growth rate, substrate uptake, by-product spectrum and morphology. This striking discovery is not obvious, especially due to the different energy input (shaking at small scale vs. stirring at lab-scale). To further underpin these studies, comparative omics analysis were applied at both scales.

When applying our novel workflow to characterize a set of 48 genome-reduced *S.lividans* mutants, only a small number of strains showed significant growth deficiencies or morphological effects and could be selected for more detailed analysis. The novel small scale cultivation workflow provides very efficient screening of *Streptomyces* strain libraries, enabling acceleration of strain phenotyping and bioprocess development with filamentous organisms.

47. Model-supported development of a lighting profile for cultivation of *Scenedesmus obtusiusculus* in a flat-plate photobioreactor

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Abstract: Due to the soaring consumption of finite fossil resources an inevitable search for alternative renewable raw materials is necessary. The production of microalgae is one of the major promising bio-economic approaches as these microorganisms use sunlight and carbon dioxide for their growth and do not compete with conventional crop cultivation. As part of bio refinery concepts algae biomass can be exploited completely concerning material and energetic utilization. For successful application, appropriate photobioreactors are required just as exceedingly robust microalgae strains that are able to grow fast and produce desired products efficiently. Therefore, a comprehensive knowledge is necessary about growth influencing parameters. Since cell size and pigmentation, as well as reactor geometry have great impact on absorption and scattering behavior, phototrophic growth and light penetration within a photobioreactor needs to be characterized in more detail.

In this study, the light dependent specific growth kinetic of the novel green algae isolate *Scenedemus obtusiusculus* was considered. *S. obtusiusculus* was cultivated in batch processes with BG11 medium at different constant photon flux densities using a pH-controlled flat-plate photobioreactor Labfors 5 LUX (Infors HT) with warm white LED as lighting source and a thickness of 2 cm. Best performance was achieved by illuminating *S. obtusiusculus* with 1400 µmol photons m^{-2} s^{-1} on the surface of the flat-plate photobioreactor. Under this condition the highest biomass concentration (5.27 ± 0.33 g L^{-1} within 3.5 d) and the highest maximum specific growth rate (0.22 h^{-1}) were observed in the experimental batch process. For characterization of light attenuation within the flat-plate photobioreactor two different models, Lambert-Beer's Law and the Two-Flux-Model from Schuster, were explored. Experimental data were used to estimate a mean integral average photon flux density that is supposed to be available within the entire flat-plate photobioreactor. Based on this set of data, growth parameters for different growth models that consider light inhibition were successfully identified for *S. obtusiusculus*. A lighting profile was derived from the identified parameters and process performance was compared to cultivation at constant photon flux density. Thereby, the experimental verification of both light attenuation models was possible.

48. Substrate consumption and lipid production of microalgal sugars by *Trichosporon oleaginosus*

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Abstract: In recent years the interest in producing microbial oils with oleaginous yeasts using regenerative feedstocks or waste resources has steadily grown. One approach is the utilization of waste microalgae biomass as media for the lipid production of oleaginous yeasts. Microalgae mass production does not compete with conventional agriculture and microalgae can use waste CO_2 for biomass generation. To enhance the economic viability, residual microalgae biomass, which remains after extraction of valuable products and contains mainly carbohydrates (and proteins) from the cell wall, can be hydrolyzed and used as media for the cultivation of oleaginous yeasts. The produced microbial oil can then be used for biofuel production. However, the ability of known oleaginous yeasts to metabolize microalgae sugars is poorly characterized and needs further investigation.

Therefore, the growth and lipid production of the well-known oleaginous yeast *Trichosporon oleaginosus* DSM 11815 using microalgal carbohydrates has been examined in this research study. *T. oleaginosus* was cultivated in defined media with six different carbohydrates, which are mainly present in microalgae, and growth and metabolization in terms of optical density and monosaccharide concentration measurement was recorded. The six monosaccharides D-(+)-glucose, D-(+)-mannose, D-(+)-galactose, L-rhamnose, D-(+)-xylose and L-(+)-arabinose were tested as single and mixed substrates in controlled batch processes in 48 parallel stirred-tank bioreactors on a mL-scale. Growth and metabolization was characterized by the determination of specific substrate uptake rates and biomass yields. Lipid production studies were performed with single and mixed monosaccharide solutions on a mL-scale in controlled fed-batch experiments.

The highest specific substrate uptake rates and biomass yields were observed with the substrates D-(+)-glucose, D-(+)-mannose and D-(+)-xylose. D-(+)-galactose and L-rhamnose were also fully metabolized, but the yeast cells showed slower uptake rates and longer lag phases. L-(+)-arabinose could not be consumed by *T. oleaginosus* DSM 11815. Furthermore, *T. oleaginous* metabolizes the monosaccharides in carbohydrate mixes simultaneously. The use of single carbohydrate solutions and artificial microalgae hydrolysate (60 % v/v D-(+)-glucose, 20 % v/v D-(+)-mannose and 20 % v/v D-(+)-galactose) as feed medium resulted in high lipid contents of up to 60 % (w/w). *T. oleaginosus* showed good growth on real microalgae hydrolysate, but no lipid accumulation was observed.

49. Revealing the diversity of algal carbohydrates: Fingerprinting of microalgal crude biomass and sugar distribution in *Chlorella vulgaris* by biomass fractionation

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Abstract: The quantitative and qualitative analysis of carbohydrates from microalgae is highly significant for optimal utilization of biomass in a biorefinery concept. A previously established high throughput method for the identification of complex carbohydrates was adapted to microalgae. It is based on the selective derivatization of monosaccharides by 1-phenyl-3-methyl-5-pyrazolone, UHPLC separation and MS analysis. The crude biomass of six representative microalgae was analyzed and the monosaccharide composition after trifluoroacetic acid digestion is reported. In addition to the usually found neutral sugars, uronic acids, and amino sugars, with this method also methylated and possibly phosphorylated or sulfated monosaccharides were identified. The monosaccharide recovery of the method was very good in five of the algal species. Further, a fractionation process with an overall recovery of 82 % was designed for the analysis of the carbohydrate distribution in the well-known microalga *Chlorella vulgaris*. The relative amounts of starch, soluble sugars, carbohydrates associated to glycoproteins and glycolipids, as well as the structural polysaccharides were determined using this procedure. Due to its advantages, this analytical method can help in strain screening and designing of proper strategies for maximal biomass development as well as utilization in the emerging field of algal bio refinery.

50. Application of an optimized radiation profile for enhanced microalgae productivity

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Abstract: Due to scarcity of fossil fuels, growth of the transportation sector and increase of greenhouse gas emissions an alternative source for liquid fuels is urgently required. Microalgae are one promising alternative for the manufacturing of bio-based regenerative liquid fuels. Beside cost-efficient open photobioreactors, novel high-lipid microalgae are required, which are able to thrive at elevated pH and salinity as well as fluctuating irradiance.

Light is the most important substrate for photoautotrophic growth of microalgae, therefore a deeper understanding of the light-dependent growth kinetics is essential. The crucial point in microalgae cultivation in photobioreactors is the attenuation of incoming photon flux density caused by mutual shading and absorption effects. Hence, variously illuminated zones arise in the bioreactor and determine the availability of photoactive radiation and thereby the growth rate and productivity. By establishing an optimized radiation profile instead of constant irradiation, extending the exponential growth phase and thus increasing the productivity shall be achieved.

Flat-plate photobioreactors on a L-scale with a maximum attainable photon flux density (PFD) of 3000 µmol m^{-2} s^{-1} were used to investigate the growth kinetics of the high-lipid microalgae *Nannochloropsis salina*. The dependency of the specific growth rate on the radiation was investigated by carrying out diverse cultivations with varying incoming PFD. Assuming that light availability is the only factor influencing the phototrophic growth and that light is homogenously distributed in the photobioreactor, the calculation of the mean integral photon flux density (I*) during exponential growth is possible. The mean integral PFD as a system-independent variable was correlated with the specific growth rate for every cultivation resulting in a three-zone graphical representation: The first zone refers to photolimitation where the growth rate increases linearly with I*, the second zone corresponds to photosaturation where the growth rate does not increase despite increasing I* and the third zone – photoinhibition – where the growth rate decreases with increasing mean integral PFD. By describing the curve with a model-based inhibition function, kinetic parameters like maximum growth rate, saturation and inhibition constant could be determined. Furthermore, the optimal mean integral PFD was calculated and used to draft the optimized radiation profile for *Nannochloropsis salina*: During exponential growth the specific growth rate was adjusted by increasing incoming PFD with increasing biomass concentration aiming the optimal I* to be constant. At the end of the exponential growth phase, the culture was diluted with nitrogen-free medium to induce lipid accumulation as a result of environmental stress. Incoming PFD was repeatedly increased with increasing cell density.

Implementing the optimized radiation profile resulted in significantly increased biomass and lipid productivity compared to cultivating *Nannochloropsis salina* under constant irradiation with the same value as I* in the radiation profile: Biomass productivity was increased by almost 100 % while lipid productivity was increased by 33 %. Additionally, the exponential growth phase could be extended by implementing the radiation profile. Beyond that, the profile is transferable to other reaction systems because of the system-independency of the mean integral photon flux density.

51. Cascades in catalysis

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Abstract: In the course of the developing bio-economy an increasing interest of the chemical industry in novel processes based on renewable raw materials, which guarantee an optimal supply of high value chemicals, is observed. Carbohydrates derived from a wide range of renewable resources are perfectly suited to act as cheap and reliable starting material. Sugar based substrates can be converted by classical fermentation processes, biotransformation approaches or by enzymatic as well as chemical reactions. Industrial production processes are mainly based on strict separation of the different biological and chemical conversion steps. The combination of chemo-enzymatic cascade reactions is rarely realized in existing processes due to limitations such as incompatibility or instability of the (bio)catalysts used. The combination of synthetic biotechnology and classical chemical catalysis is able to realize novel multi step process routes. Enzyme engineering towards optimal process conditions and parallel utilization of chemical catalysts enables completely new and synthetic cascades for chemical building blocks, fine chemicals as well as active pharmaceutical ingredients.

This general strategy of CASCAT is presented based on one of our patent-registered processes via a synthetic cascade for the production of the non-natural chemical building block 1,4-butane diol from glucose. This process is realized by a shortened enzymatic route, which is enabled by the use of promiscuous enzyme variants to reduce the number of biocatalysts used.

52. Nano-scale enzyme membrane reactors: Efficient high-quality production and characterization

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Abstract: In natural cells compartmentalization and selective mass transport allow different chemical reactions to take place at the same time. Nevertheless, undesired side reactions catalyzed by endogenous enzymes are a frequently encountered drawback in whole-cell biocatalysis. Instead of whole cells, vesicles at the nano-scale made from self-assembling, amphiphilic polymers can be used as artificial reaction compartments. Side reactions are avoided, since the polymer vesicles contain only those components necessary to synthesize a desired product.

So far, these polymer vesicles have been produced at inchoate laboratory conditions only. Most often, the polymer solution is added dropwise to the aqueous phase using a pipette and stirred for up to 72 h afterwards. This hardly reproducible procedure leads to vesicle dispersions of rather broad particle size distributions (PSD), where both undesired micelles and vesicles coexist. In order to narrow the PSD, several subsequent extrusion-steps through polycarbonate membranes with defined pore size are necessary. As micelles cannot be removed by extrusion, an additional size exclusion chromatography is needed. It was therefore the major goal of this study to develop an efficient, reproducible and scalable production process where vesicles of monomodal, narrow PSD can be achieved in only one production step. The amphiphilic triblock copolymer poly(2-methyloxazoline)-poly(dimethylsiloxane)-poly(2-methyloxazoline) (PMOXA-PDMS-PMOXA) was dissolved in ethanol and either piped continuously or dosed intermittently by a liquid handler into the stirred buffer solution. To enable spontaneous self-assembly of the polymer, the dispersion was agitated at completely different flow conditions. For this purpose, four diverse stirrer types were used in baffled and unbaffled miniaturized stirred tank reactors at varying agitation speed.

Dynamic light scattering measurements showed that using a S-shaped stirrer at 4000 rpm in unbaffled milliliter reactors (vol. power input ~12 W L^{-1}) leads to vesicles of about 200 nm in mean diameter. A monomodal PSD with low polydispersity index (PDI<0.2) could be achieved within only one production step in less than 1 h.

53. Exploitation and utilisation of carbohydrate derivatives recovered from lipid extracted algae

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Abstract: The marine microalga *Dunaliella salina* is an industrially cultivated micro-organism, which produces natural ß-carotene. The colorant is used in the food industry, nutritional supplement or in the pharmaceuticals. The lipophilic ß-carotene is removed by hexane extraction in the production process, and lipid extracted algae (LEA) remnant remains as surplus. The valorization of the LEA fraction in the algal industry is crucial for the improvement of the overall process economy. The present contribution considers alternative approaches where the low valued LEA remnant could be exploited and valorized.

In the chemical approach, the potential of the remnant biomass was investigated by treating it thermally under ethanol-water solvent mixture to support decompositions and liquefaction. The *Dunaliella* algae grow in highly saline conditions and lack the typical rigid, recalcitrant cell wall, which makes the species highly suitable for such a mild liquefaction process. After the hydrothermal treatment with various ethanol-water solvent ratios, the yields of the soluble fraction were determined and the remaining solid was separated from the solubles. The influence of ethanol was found to be crucial to the yield of the soluble fraction and its chemical characteristic under the liquefaction conditions. The soluble fraction was characterized and product chemical compounds were identified. The fraction contained e.g. free fatty acids, aldehydes and other functionalized hydrocarbons which have high potential to be used as chemical feedstock or fuel compounds. An extraction process was designed applying modern computational chemistry methods for the solubility estimation for 5-hydroxy methyl furfural, which was identified as one of the main valuable compound in the soluble fraction. The quantum chemically based screening model was found to be highly suitable for predicting the chemical solubilities of the biomass hydrocarbon derivatives in various solvent mixtures, for which reliable solubility data is scarcely available.

The other promising option for the valorization of the remnant fraction is the recycle of the available hydrocarbons for the use as carbon source in further cultivations. Therefore, the remnant biomass was decomposed by water as sole reaction solvent during mild liquefaction. The generated aqueous phase yielded in a glucose concentration of up to 80% of the appropriate biomass. Three biotechnologically highly relevant microorganisms were cultivated to investigate the usability of the liquefaction derived glucose as carbon source for biomass generation, namely the green microalga *Chlorella vulgaris*, the bacterium *Escherichia coli* and the yeast *Saccharomyces cerevisiae*. In all microorganisms presently studied, a satisfying utilization of the alternative glucose source and a comparable growth to the standard glucose were visible. Consequently, the aqueous phase represents promising carbon source for biotechnological application.

54. Development of a biotransformation process for terpene-based biopolymers

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Abstract: Biopolymers are a promising alternative to common plastics which are mostly produced on the basis of crude oil. To overcome the use of this limited and environmentally harmful raw materials *Pseudomonas putida* KT2240 was genetically modified to produce new monomers for biopolymers. Therefore parts of the P450cam operon, which are necessary for selective hydroxylation of different terpenes, were cloned in the vector pBBR122 and the resulting plasmid was transformed in *P. putida*. First experiments showed that borneols like (+)/(-)-borneol and (+)/(-)-isoborneol were successfully hydroxylated by the evolved strain. For an upscale it was focused on the biotransformation of (-)-borneol to the corresponding hydroxylated 5-hydroxyborneol and a batch process with cell recycling was developed, which allowed the semi continuous production of 5-hydroxyborneol. After purification by recrystallization 5-hydroxyborneol was used for polymerization with succinic acid dimethyl ester resulting in a polymer which is structural similar to the commercial available polybutylene terephthalate. First polymerization experiments showed that the new biopolymer has different properties like transparency and color in comparison to the commercial one, but further investigations have to be made to get more details about the new material.

55. Protein production with *Komagataella pastoris* from milliliter to pilot scale

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Abstract: The methylotrophic yeast *Komagataella pastoris* (formerly known as *Pichia pastoris*) is widely used for efficient production of heterologous proteins. The major advantages are the simplicity of molecular genetic manipulation, the possibility of high level intra- and extracellular protein expression as well as the ability of performing higher eukaryotic protein modifications.

High-cell-density cultivations (HCDC) with methylotrophic yeasts need continuous methanol feeding at controlled process conditions. However, in conventional small-scale screening applications *K. pastoris* is commonly cultivated in non-controlled batch or intermittent fed-batch processes at low cell densities. As a consequence, the received small-scale process performance data (e.g. protein expression yields) are not representative for high-performance processes on a laboratory or pilot scale.

Hence, continuous feeding of methanol in pH-controlled 48-fold parallel stirred-tank bioreactors on a milliliter scale was ensured by supplying air with varying methanol concentrations in the gas phase. The gas-liquid methanol transfer rates were characterized as a function of the operation conditions. Methanol transfer rates of up to 5.3 g/(L h) were possible without oxygen limitation at the chosen process conditions. HCDC processes with 50-60 g/L cell dry weight within 49-69 h were established on the milliliter scale with extracellular production of *Candida antarctica* lipase B2 (CaL-B2) variant as an example (1,03 g/L CaL-B2 with an activity of 16,604 U/L). The scalability was demonstrated by transferring the *Komagataella pastoris* HCDC processes to the liter and cubic meter scale with the maximum oxygen transfer rate as the scale-up criterion. Biomass concentrations and lipase activities were the same at all scales within the estimation error.

Hence, the continuous feeding of methanol into pH-controlled stirred-tank bioreactors via the gas phase facilitates the 48-fold parallel fed-batch cultivation of methylotrophic yeasts at high cell densities with high-level expression of heterologous proteins on a milliliter scale. Due to the direct scalability of fermentations in stirred-tank bioreactors between the milliliter, liter, and cubic meter scales, the experimental effort and costs to determine the optimal process conditions can be reduced significantly. Furthermore, high-level expression strains can be screened at production process conditions. Thus, high-producing mutants can be selected at technical process conditions for the first time. As the pH in the bioreactors can be controlled individually and the feeding of substrates like glycerol by the liquid handling system as well as the methanol transfer rate through the gas phase can be varied for each vessel, the milliliter scale bioreactors can be used to optimize different process parameters like the pH or feeding strategies in parallel.

56. Processing of grass silage in a biorefinery concept

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Abstract: In order to maintain the competitive position of the European economic system in global affairs, broadening the feedstock mix for chemical production is an important goal with respect to shortage of fossil-derived resources. Concepts of industrial biorefineries are described to enhance the inclusion of renewable resources creating a novel, local and bio-based industry. Performance of multiple product systems force up the competitiveness of the biorefinery plants by integrated processing of applied natural raw materials and provide a line-up of products, as well as many applications. Modular plant concepts can account for a rapid and easy adaptation to alternating market and policy situations.

Biomass from permanent pastures shows excellent potential as feedstock for biorefinery plants due to abundant availability at moderate cost and high yields in temperate regions. Furthermore, its utilization prevents the fallow of arable land and avoids direct competitiveness with food production. Conservation by auto-fermentative ensiling enables all-season availability of the biomass by production of value-added compounds, such as lactic acid.

The proposed green biorefinery process schedules the direct conversion of the liquid fraction to bio-polymers after mechanical separation. Regarding the complete hydrolysis of the fiber enriched lignocellulosic solid fraction different methods were examined to establish a mild procedure renouncing the addition of further chemicals. Besides a mechanical supported high total solid load enzymatic hydrolysis, different hydrothermal methods were tested to disintegrate the feedstock and to increase the availability of polysaccharides to release monosaccharides by enzymatic activity. The hydrolysate offering high saccharide concentrations is meant to feed a biotechnological conversion step towards gaseous hydrocarbons, which can be applied for downstream production of biofuels.

57. Nano-scale enzyme membrane reactors: High-yield refolding of membrane transport proteins

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Abstract: Synthetic polymer-based vesicles represent a new type of nano-scale membrane reactor that can be loaded with enzymes and functionalized with additional proteins that span the membrane. By using block copolymers instead of lipids, thicker membranes which are more stable can be generated. The resulting drop in permeability can be advantageous if substrates and products are channeled through transport proteins incorporated into the membrane, thus increasing the selectivity of the biocatalyst.

Outer membrane proteins of gram-negative bacteria represent interesting candidates for the incorporation into polymer membranes because they are extremely sturdy proteins and have various substrate specificities. The small monomeric ß-barrel outer membrane proteins AlkL (*Pseudomonas oleovorans*) OmpW (*Escherichia coli*), TodX (*Pseudomonas putida* F1) and OprG (*Pseudomonas aeruginosa*) are possible FadL-type transporters for hydrophobic substances. Although structurally well characterized, their transport characteristics are far less well known. Unfortunately, reconstitution experiments for functional characterization and especially the later use in biocatalysts typically require high amounts of concentrated protein.

As with most membrane proteins, native expression of these four proteins suffers from very low yields and tedious downstream processing. Leader peptide removal directs the protein into inclusion bodies, which can be purified with yields of 0.1-0.2 g pure unfolded protein per litre culture. The challenge then was to establish proper refolding conditions to revert the proteins into their functional form.

Small scale refolding experiments (1.5 mL) were performed using the rapid dilution method in order to investigate classical refolding parameters, such as the influence of the nature and concentration of selected detergents and supplementary folding additives as well as the initial protein concentration. The optimization was performed by varying one parameter at a time, thus narrowing down the search space. While AlkL and OprG folded properly over a wide range of conditions, TodX and to a lesser degree also OmpW required a more specific folding environment. For all four proteins optimal conditions were established, which yielded high refolding efficiencies (47-96%) at room temperature. AlkL: 2 % (w/v) N,N-Dimethyl-n-dodecylamine N-oxide, 1 M urea, pH 8; OmpW: 4 % (w/v) N lauroylsarcosine, 1 M urea, pH 9; TodX: 2 % (w/v) N lauroylsarcosine, 25 mM glycine, pH 8; OprG: 2 % (w/v) N-lauroylsarcosine, 100 mM glutamate, pH 8. The employment of folding additives, which is rather more common when refolding soluble proteins, was effective in improving the refolding efficiency especially in the intermediate protein concentration range, allowing the proteins to be refolded at concentrations as high as 0.5-1 mg/mL.

58. Computational fluid dynamics assisted development of an open photobioreactor system

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Abstract: Due to diminishing reserves of crude oil, new greenhouse gas regulations, and at the same time a growing aerospace sector, alternatives to fossil fuels have to be considered. A promising approach is the production of biofuels from CO_2 by cheap mass cultivation of microalgae for lipid production in open photobioreactors.

Since the construction of a novel thin-layer cascade photobioreactor system is a time-consuming and cost-intensive process, computation fluid dynamic (CFD) simulations were used to optimise the reactor geometry and simplify the design process. Therefore the reactor was separated in several, independent units.

One of these units is the broth inlet pipe and distributor, which has several functions: Through a vertical influent pipe carbon dioxide and additives for algal growth are added to the algal broth entering the inlet chamber of the thin-layer cascade. A baffle directly above the inlet serves to slow the high volume flow (200 l/min on a pilot-scale) and contributes to the mixing. Finally the algal suspension leaves the inlet chamber by an overflow weir, which should ensure the equal distribution of the suspension over the complete width (1 m on a pilot-scale). Consequently, to investigate the performance of the inlet with respect to these functions a three dimensional CFD simulation was set up. The resulting simulation allowed predictions on the flow behaviour in the inlet, including the water surface level at the weir, the formation of dead zones and the mixing behaviour, which are all vital for above mentioned functions.

These results were then used to vary geometric parameters of the inlet in order to optimise the geometry. It was found that an increased weir height over the bottom of the inlet chamber leads to a more even surface, and thus a better distribution of the suspension over the reactor width. Further modifications of the inlet's ground surface helped to avoid the formation of dead zones in the corners.

In the finally constructed inlet the thin-layer photobioreactor behaviour was observed to be in agreement with the predictions of the simulation: The water surface at the outlet showed an even, equally distributed surface over the entire width. Moreover, the simulative predicted streamlines of the fluid flow resemble the experimentally found results.

Conclusively, simulations with varying geometric parameters were performed, hence an optimal configuration could be found: With a weir height of 14 cm an even surface could be achieved, having a level distribution of algal broth over the entire width of the reactor. By further modification of the inlet chamber's ground, the dead volume in the reactor could be minimized. In a following physical experiment, these results could also be confirmed.

59. An experimental and modelling strategy for optimising production of AHL from *Exiguobacterium Sp.*

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Abstract: AHL (N-Acyl Homoserine Lactones) are a class of signalling molecules believed to be responsible for bacterial communication by the process of Quorum Sensing. Some applications of AHL have been explored by researchers, these include its use as immunosuppressant, as inhibitor of cancer cell growth, for treatment of congestive heart disease and insulitis, for increasing plant yield and as an additive in animal feed. The present study involved cultivating a bacteria belonging to the *Exiguobacterium Sp.* which has shown high production of AHL and is currently not used commercially. Optimal conditions are required for an economically feasible process. In this study an approach combining experiments and modelling is attempted.

Initial work involved media screening where six different media were screened. Among them Zobell Marine media showed the highest production of AHL and was used for further studies. Reactor runs were carried out in a 2.5L bioreactor under batch mode where the temperature, pH and aeration rate were maintained at 30°C, 7.0 and 2 L/min respectively. The agitation rate was varied from 300 to 750 rpm. Variations in carbon and nitrogen ratio were also carried out. Maximum AHL concentration of 89µg/l was observed at the 9th hour of fermentation. Biomass weight, dissolved oxygen and carbohydrate concentration were measured at hourly intervals throughout the culture duration and CHN elemental analysis of a few of these samples was also carried out.

Simulation was combined with the experiments in order to understand the reaction kinetics and identify the important parameters that control the process. Therefore, a model was developed for the production of AHL with the help of the acquired data and process conditions. One interesting phenomena that has been observed by us and also reported in the literature is that the AHL concentration spikes after a certain time. Being able to predict and then extract the product at the optimal time would strongly increase the economic feasibility of the process. A set of ordinary differential equations that describes the dynamic behaviour of biomass, dissolved oxygen, carbohydrates and AHL over the culture duration was developed. We are currently in the process of estimating values of parameters for these developed equations. With the help of this model it may be possible to determine whether to run it in fed batch mode as well as estimate the best reaction time. Finally, these predictions will be experimentally validated.

Keywords: N-Acyl Homoserine Lactones, Exiguobacterium Sp., Marine Medium, Bioreactor, Mathematical modelling.

60. Exploiting alcohol dehydrogenases in redox neutral processes: Disproportionation of α-substituted aldehydes

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Abstract: Redox biocatalysis offers attractive tools for the asymmetric synthesis of enantiopure molecules [1] by combining high chemo- and stereo-selectivity with environmentally friendly process conditions. The alcohol dehydrogenase (ADH)-catalyzed asymmetric Cannizzaro[2]-type reaction (Figure 1) allows the overall redox-neutral disproportionation of alpha-substituted aldehydes using catalytic amounts of nicotinamide cofactor, leading to the formation of the corresponding alcohol and carboxylic acid in up to >99% ee.[3] This parallel dynamic asymmetric transformation relies on the spontaneous racemization of the substrate, while formation of both products in high enantiopurity requires exquisite enzyme stereoselectivity in both redox half-reactions, not necessarily stereoconvergent.

Both cofactor and enzyme concentrations affect activity and stereochemical outcome, while electronic properties of the substrate substituents are expected to influence racemization rate and reactivities. In order to efficiently exploit the enzymatic system for the production of a broad range of enantiopure prim-alcohols and α-substituted carboxylic acids, critical parameters were investigated and experimental design (facilitated via Design-Expert® software) led to surprising findings.

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61. Resource efficient bio-production of atactic polyhydroxyalkanoates

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Abstract: The aim of this project is to develop a sustainable and a biotechnological process for the production of bio-derived and bio-degradable plastic, poly-hydroxybutyrate (PHB). Current microbial production of PHB generates an isotactic form of the polymer, which affects its properties adversely; high crystallinity, brittle structure, and undesirable thermoplastic properties. While production of the atactic form of PHB is chemically possible (re-catalysis), this process is very costly.

Large quantities of bran can be obtained as a cheap by-product and can be hydrolyzed enzymatically to a high quality fermentation medium. Consequently, cost-effective and resource-efficient monomer production, in *E. coli*, is possible with the use of pentose-containing bran hydrolysate. Upon conversion of pentose sugars to 3HB monomers, a novel synthesis strategy is implemented. Accordingly, atactic PHB of improved physicochemical properties is achieved via coupling of biocatalytic lactonization and lipase-mediated ring-opening polymerization.

This strategy establishes a platform for the fermentative preparation of improved PHB, which is an alternative to petrochemical polymers production. This developed process allows for energy and mass-efficient production of this high quality biopolymer. The potential of a continuous process and the improved product properties lead to an economically and ecologically optimized value chain.

Session 4: Bioseparation Engineering

62. Electrochemically modulated liquid chromatography on carbon nanotubes for preparative biomolecule separation

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Abstract: The separation principle in the Electrochemically modulated liquid chromatography (EMLC) is to control the adsorption behavior of ions and charged molecules on the solid/liquid interface through application of an electrical voltage on the stationary phase. In contrast to electrophoretic principles, the molecule migration is not led along the electrical field between two external electrodes but towards the stationary phase in the column, which itself serves as the working electrode. The proof-of-concept of EMLC for biomolecule separation has already been demonstrated in the literature. However, in order to be competitive with conventional chromatographic methods (e.g. ion-exchange chromatography with high salt concentrations in the elution buffer), the sorption capacity of the conductive matrices has to be strongly increased and a deeper theoretical understanding of the adsorption mechanism is necessary.

Optimal resin materials for a EMLC separation need large surface area, enough conductivity as well as electrochemical stability and must be packable into a column. Multiwalled carbon nanotubes, with a specific surface area of approx. 220 m^2/g, fulfill all these requirements and are therefore one of the central resins in our research. We have developed a new chromatography column (1) to ensure the adequate packing of the nanomaterials investigated and (2) to guarantee a homogeneous potential distribution on the stationary phase. The potential is applied using a three electrode system. Compared to earlier designs, in this new concept an increased surface of the counter electrode is given to enhance the adsorption capacity and to decrease the current density. Our experiments show the ability to influence the retention behavior of small and large biomolecules on the resin surface using an electrical potential. For example, about 20 µmol maleic acid per gram stationary phase are bound for a potential of 800 mV; this means that almost 73% of the applied electric charge is used in the retention of short carboxylic acids. Salt concentration, pH and the charge of the buffer ions also influence strongly the dynamic binding capacity and make optimizing working conditions possible. This research evidences that EMLC could become a promising alternative to conventional chromatographic processes.

63. Modelling-based analysis of the hydrodynamic behavior of preparative chromatography columns

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Abstract: Preparative packed-bed chromatography using polymer-based, compressible, porous resins is an essential bioseparation method especially for macromolecular bioproducts. Due to a downstream processing bottleneck because of limited purification capacities, the chromatography equipment is often operated at its hydrodynamic limit leading to a complex, hysteretic, thus, history-dependent packed bed behavior. The theoretical understanding of the causes is still limited. Therefore, a rigorous modeling approach of the chromatography column on the particle scale has been made which takes into account the interparticle micromechanics and fluid-particle interactions for the first time. A three-dimensional deterministic model was created by applying Computational Fluid Dynamics (CFD) coupled with the Discrete Element Method (DEM). The column packing behavior during either flow or mechanical compression was investigated *in-silico* as well as in laboratory experiments using a novel micro chromatography column. The simulation satisfactorily reproduced the measured behavior regarding packing compression as well as pressure-flow dependency. Pronounced axial compression-relaxation profiles were identified that differed for both compression strategies. During compression, void spaces were clearly visible in the packed bed being surrounded by a force chain network. Simulation results indicate that this packing anisotropy as well as the packing dynamics are governed by particle-wall and interparticle friction effects. Therefore, it was concluded that compaction of the chromatographic bed is rather due to particle rearrangement than particle deformation.

64. High capacity affinity resins for antibody purification

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Abstract: Protein A chromatography is one of the most extensively used technique in the purification of therapeutic antibodies. The high costs in the manufacturing of these affinity resins are mainly caused by the need of substantial amounts of purified affinity ligands in order to achieve the desired material performance. Additionally, low capacities of the resins are still limiting the productivity of the process.

Due to these reasons, the first objective was the development of an extracellular Protein A production process in *Escherichia coli*. The optimized process yielded a final product concentration of 4.5 g Protein A L^{-1} in the culture supernatant after 48 h of total process time. Furthermore the highly active secreted Protein A ligand was successfully recovered from the clarified cell culture supernatant using microfiltration, and a final purity of more than 98 % and a yield of 90 % was achieved by one single ion-exchange chromatography step.

The second part of the project mainly focused on improving the binding activity of novel affinity ligands. Protein A related affinity ligands currently consist of four to five domains, which can independently interact with one molecule of IgG. This optimization was achieved by the polymerization of one single IgG-binding domain (B-domain) of Protein A into ligands with up to nine distinct binding domains (B2-B9). Solution binding experiments revealed a clear linear dependency of the molecular stoichiometry in relation to the amount of IgG binding domains per ligand. The highest molecular stoichiometry of 6.5 molecules of bound antibody per ligand molecule was determined for the B9 ligand.

Furthermore the impact of the optimized ligand preparations on the performance as immobilized affinity ligand was evaluated. Purified ligands were covalently immobilized on Profinity Epoxide™ and the performance of the prototype resins was studied regarding its static and dynamic binding capacity. These results confirmed a correlation between the solution binding and the saturation capacity. Using a ligand comprised of eight IgG-binding domains (B8) yielded a chromatographic material with a binding capacity of 80 mg_{hIgG} mL^{-1}. Using a modified B8 affinity ligand with a reactive amino acid tag, this overall high capacity was furthermore slightly improved up to 88 mg_{hIgG} mL^{-1} and a dynamic binding capacity of 68 mg_{hIgG} mL^{-1} at a residence time of 4.8 min could be achieved. Compared to commercially available materials the method of ligand polymerization allowed the production of high capacity materials which could not be achieved using Protein A. In order to exclude a negative impact of the prolonged ligands on the diffusional properties of the novel materials, diffusion of hIgG into the pores was studied. Despite a detectable steric exclusion effect of materials coated with extended ligands, no negative effect could be observed when studying breakthrough curves. In addition, reusability of the resins is improved.

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Talk

65. Magnetic nanoparticles as new carriers in downstream processing

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Abstract: To ensure the continuing development of biotechnology and to establish it more solidly at the scientific and industrial levels, it is necessary to enhance fundamental research as well as technical approaches. Particularly in downstream processing, advanced separation and purification processes are in great demand. Although methodologies to ensure the high purity of the target product usually exist, it is still a huge challenge to achieve acceptable productivity levels with efficient costs; to maintain a balance between the final concentration degree, purity, yield, time, energy consumption, and waste remains a difficult task to deal with.

High-gradient magnetic separation (HGMS), which can separate nonporous magnetic nanoparticles with high specific areas without a mass transfer limitation, seems to be one new alternative. Compared to classical chromatography, HGMS works directly with cell homogenate or fermentation broth without any previous solid-liquid separation steps. Accordingly, this method also often leads to a significant reduction in time and costs. High purity and yield are guaranteed with a suitable carrier system. We present here an example of bioseparation using iron oxide nanoparticles with a new pentadentate chelate ligand. The high capacity of this carrier for recombinant histidine-tagged Green Fluorescent Protein (His-GFP) is not influenced by the other components of *E. coli* cell lysate; the values reach about 250 mg protein/g nanoparticle with the highest selectivity for the His-tagged protein. Recycling studies on a laboratory scale showed that after 9 cycles 90% of the initial capacity was still reached for His-GFP. Upon analyzing the aggregation in the system, we concluded that the formation of nanocrystal clusters fundamentally impacts the number of available interaction sites for large biomolecules.

Processing with an automated HGMS with a rotor/stator prototype and one liter process chamber enabled us in one batch to isolate 12 g His-GFP per hour using 100 g of carrier. The eluate purity levels of 96% and the 93% yield demonstrate the high efficiency of the process.

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66. Purification of recombinant proteins by large-scale crystallization

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Abstract: Large-scale crystallization may constitute an attractive economical alternative to common protein purification processes like preparative chromatography if this method can be carried out reproducibly and up-scalable with high yield and fast kinetics from impure solutions. This presentation will show that this aim was achievable in case of purification of a therapeutic monoclonal antibody from pre-treated harvest [1]. Pre-treatment consisted of a simple pH-shift followed by ultrafiltration/diafiltration (UF/DF). Crystallization could replace two out of three chromatography steps including protein A chromatography. Scale-up of the crystallization process from the mL-scale to the L-scale in geometrically similar stirred tanks was achieved successfully by keeping the maximum local energy dissipation coefficient constant [2]. In another case, it will be shown that a recombinant diagnostic protein, enhanced green fluorescent protein (eGFP), was purified by an attractive novel combination of three-phase partitioning, UF/DF, and crystallization [3]. Chromatography steps were not required anymore.

However, information regarding proper crystallization conditions of proteins from impure solutions is scarce in literature. Therefore, new results on the quantitative crystallization of exemplary proteins from solutions containing microbial host cell proteins (HCP) will be presented. The crystallization of two enzymes, lysozyme and a recombinant fungal lipase, from solutions being spiked with up to 15 % HCP in stirred tanks was investigated with regard to reproducibility and scalability. Furthermore, stirred-tank crystallization of eGFP from pre-treated clarified homogenized fermentation broths containing different levels of residual HCP was investigated. Fast, high yield, reproducible, and scalable crystallization was achieved in all three cases [4]. Robust crystal morphologies with narrow crystal size distributions could be obtained. HCP contained in the solvent channels of the protein crystals were removed by diffusive washing. Crystallization may lead to significant reductions of costs and process time compared to conventional preparative chromatography and become part of a platform process in near future.

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67. Semi-continuous microbial taxoid production with integrated product removal

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Abstract: Taxanes, like Paclitaxel and its diterpenic derivatives, are natural cytostatic compounds with diverse medical applications, especially in anti-tumor treatment. High technical and monetary efforts are necessary for their production in semisynthetic or plant-based processes. Therefore,

synthesis of taxanes and taxoids in heterologous but well-known organisms like *Escherichia coli* recently become a topic of interest. However, major challenges are low production titers due to multiple enzymatic steps involved as well as potential product toxicity. To address these obstacles, a semi-continuous fermentation process for taxane production in *E. coli* combined with an integrated product removal as well as subsequent downstream processing was established.

Early taxane or diterpene precursors taxa-4,11-diene and taxadiene-5a-ol were produced in *E. coli* in a stirred-tank fed-batch cultivation process. Cell densities up to 40 g L^{-1} allowed increased diterpene titers in a 3-day fermentation process with minimal medium. Further product yield enhancement has been carried out in terms of optimizing production temperature and inducer concentration. For further processing hollow-fiber crossflow filtration was implemented for cell recirculation and semi-continuous fermentation. Additionally two different capture steps will be demonstrated for the isolation of the taxoids from the cell-free medium. Both direct adsorption on octadecyl silica beads and membrane extraction with hollow-fiber membrane contactors show high potential.

The final purification of these taxanes was accomplished with chromatography. In order to create a broad purification platform for further taxoid compounds, different chromatographic techniques like size exclusion and countercurrent chromatography have been successfully investigated with Paclitaxel and its precursor 10-Deacetylbaccatin III as model substances.

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68. Purification of mucins from porcine stomach

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Abstract: Porcine gastric mucin shows promising properties in respect to lubrication and hydrogel formation and has gained much attention regarding the coating of biomaterials and antiviral supplements. Mucins are high molecular weight glycoproteins and are predominantly found in the gastrointestinal tract and bronchia of animals and humans and serve as a selective barrier for molecules and protection of underlying tissue from pathogens. Because the naturally formation of a protein hydrogel is lost for commercially porcine mucins at acidic pH we established a purification process for mucin from pig stomach (mainly Muc5AC) that maintains the desired properties. We focused on volume reduction for increased concentration in the early purification stage, functionality of the protein in terms of gel formation and the increase of protein yield and productivity. Different cross-flow module systems for pre-concentration of the mucus from pig stomach were evaluated using mass transfer relations. The mucus was successfully conducted with 100 kDa MWCO membranes. Subsequent size exclusion chromatography of the retentate resulted in a total recovery of the gel forming Muc5AC. Further diafiltration to remove salts was essential for preserving the functionality in terms of gel formation. The scale-up by factor 10 was successfully implemented for each process unit and led to an increase of the overall productivity. The functionality of the purified mucin was confirmed with rheological measurements at pH 2.

69. Investigation of bio-nano interactions with magnetic nanoparticles

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Abstract: Downstream processing of therapeutic proteins accounts for more than 80% of their total production costs; making the development of cheap adsorbing materials topic of great interest. In this context, particularly magnetic nanoparticles become more and more important in several fields such as biotechnology, medicine and catalysis. The driving force for the interaction between the biomolecules and the nanoparticles is the molecular adhesion that leads to adsorption at the bio-nano interface. For successful further advance, a fundamental understanding of these surface interactions is essential and main focus of our work.

Magnetite and other magnetic nanoparticles can be synthesized from metal chlorides cost-effectively by wet chemical co-precipitation processes. We have investigated different parameters which influence their synthesis, such as temperature, stirring rate and ion stoichiometry and achieve high saturation magnetization values and uniform size distributions. Thereby, our focus lies on the specific and reproducible design of surface properties such as surface charge and zeta potential. Based on the characterization of the surfaces, biophysical models are being developed in order to model the binding of amino acids, the building blocks of proteins and peptides to the surface. The free energy of binding of these molecules to the surface is determined as a function of the distance. Effective models permit the simulation of the binding behavior of larger peptides and therefore enable the prediction of potential binding peptide tags.

The binding of amino acids and short peptides is investigated and evaluated on the different iron oxide surfaces using spectroscopic approaches. Raman, attenuated total reflection infrared and X-ray photoelectron spectroscopy reveal novel insights into the binding mechanisms of small biomolecules. The adsorption is strongly dependent on electrostatic interactions and occurs mainly through the carboxyl group although influences of the side chains can be observed. The pH, ionic strength as well as buffer composition remain main parameters for controlling the adsorption process. They affect the charge of biomolecules as well as the charge distribution on the nanoparticle surface in a decisive way and therefore play a significant role for biomolecule adsorption on inorganic surfaces and biomolecule-biomolecule interactions.

With respect to the technical application in the field of selective tagged-protein purification or immobilization, an expression system in *E. coli* is being established which allows the peptides to be expressed as a fusion protein with GFP that can

be easily detected. With the addition of selective cutting sites, it is possible to systematically characterize the peptides as tag and their influence on the binding behavior of the fusion proteins.

Finally, this systematic approach for small biomolecules serves to enhance the knowledge on protein adsorption and desorption processes and their directed evolution for other carrier materials.

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Talk

70. Process intensification – A variety of solutions rendering bioprocesses more viable

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Abstract: The global trend towards sustainability and resource efficiency urges us to transform the concept of chemical plants and strive for compact, safe, energy-efficient, and environment-friendly sustainable processes. These developments share a common focus on "process intensification" — an approach that has truly emerged the last decade. Process intensification can come from reducing the size of individual pieces of equipment, from reducing the number of unit operations or apparatuses involved. Our concept of process intensification entails combining reaction and separation in such a way that the overall result is more sustainable, delivers better product quality, reduces the equipment size, and/or lowers solvent use.

Several bio-based processes are plagued by limited product titers and volumetric productivities due to product inhibition. Other processes suffer from side reactions decreasing the yield of the process. This leads to substantial downstream processing costs, high waste water volumes, high fermentor costs and an increased substrate cost in the case of a decreased yield. Therefore, it can be advantageous to invest in a recovery technology that allows the selective separation of the product during fermentation or biocatalysis: 1. To enrich the product leading to a decrease in downstream processing costs; 2. To improve the volumetric productivity by alleviation of product inhibition; 3. To reduce the process flows (decrease amount of wastewater per unit of product); 4. To improve the yield by removing the target product from the fermentation broth and rendering it unavailable for side reactions. These are the rationale behind *in-situ* product recovery (ISPR) technologies and intensification of the processes in general. This is the key technology platform we developed and applied during the last years to intensify bioprocesses. Specific cases will be presented and the benefits for the selected processes explained: processes with integrated separation technology for whole cell fermentations (butanol, succinic acid, short to medium chain fatty acids, itaconic acid) and for enzymatic conversions (chiral amines).