Zürcher Hochschule
für Angewandte Wissenschaften

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Life Sciences und
Facility Management

BioTech 2016

Biopharmaceutical Manufacturing and Single-Use Technologies

Campus Grüental, Wädenswil, Switzerland

5th and 6th September 2016

Post-conference workshop:
7th September 2016

Mini-symposium:
7th & 8th September 2016

www.biotech2016.ch

Zürich University of Applied Sciences and Arts

Welcome to the conference

Biopharmaceutical manufacturing and single-use technologies

Single-use devices in USP, DSP and Fill&Finish have undoubtedly made their impact on biopharmaceutical productions. They have not only contributed to savings in time and money when chosen and handled correctly, but have also given rise to new technologies and, during the last few years, become accepted in applications outside mammalian cell cultivation. As examples, large volume cell banking procedures have been developed and continuous processes are becoming more and more popular. Furthermore, there is growing demand for the application of single-use technologies in production processes relying on microorganisms, insect cells, and human primary cells.

All of these topics will be covered at BioTech 2016, to which I would like to welcome you warmly on behalf of the Institute of Chemistry and Biotechnology. As our guests, partners, developers and users of single-use technology, it is my pleasure to invite you to take an active part in the oral and poster presentations, as well as in an exchange of know-how and experience in the workshops and conference breaks.

May I also encourage you to attend the events in the social programme in order to enhance your network and foster personal contacts, to share your ideas for new training opportunities in the field of single-use technologies in our post-conference workshop, and to learn about the latest plant cell developments in the mini-symposium which will take place after BioTech 2016.

Finally, I would like to wish you a pleasant and memorable stay in Wädenswil.

Christian Hinderling

Director of the Institute of Chemistry and Biotechnology

ZHAW, Wädenswil

Life Sciences and Facility Management

ICBT Institute of Chemistry and Biotechnology
ZHAW Zurich University of Applied Sciences
Campus Grüental, 8820 Wädenswil, Switzerland

Single-use systems and single-use technology in biopharmaceutical manufacturing

Summary

Single-use systems (SUS) whose product-contacting parts consist of FDA-approved plastics are increasingly used in biopharmaceutical production. They are predominant in upstream processing (see Figure 1), but also exist for downstream processing and Fill & Finish. Their usage has not only contributed to savings in time and money when chosen and handled correctly, but SUS have also entailed new technologies (e.g. large volume cell banking procedures, one step cell expansions, high seed fed batch productions, see Kaiser et al., 2015). Furthermore, they have become accepted in applications exceeding mammalian cell cultivation in recent years. For example, there is growing demand for applying single-use technologies (SUT) in production with microorganisms, insect cells and human primary cells recognizable.

Figure 1: SUS often used in upstream processing (with kind permission from Sartorius Stedim Biotech)

Services and research for appliers

- Selection and bioengineering characterization of SUS: Fluid flows, mixing times, k_La values, shear stresses, identification of critical bag films by using a cell culture test (DECHEMA recommendation)
- Development and scale-up of upstream processes up to pilot scale: cell productions, productions of antibodies and vaccines
- Customised training courses: Bioengineering characterization of single-use bioreactors, cell cultivations in orbitally shaken, wave-mixed and stirred single-use bioreactors, scale-up of bioprocesses up to 50 L working volume

Selected Publications

- Single-use bioreactors for animal and human cells. S.C. Kaiser et al., In: Animal cell culture: Cell Engineering 9, Springer, 2015
- Recommendation for leachables studies. R. Eibl et al., DECHEMA, 2014
- Single-use technology in biopharmaceutical manufacture. R. & D. Eibl, John Wiley & Sons, 2011
- Single-use bioreactors I and II. R. & D. Eibl, Springer, 2009 and 2014

Vision

Our vision is to support suppliers in developing novel processes and to optimize existing SUS for the upstream processing and first stages in downstream processing (cell and product separations). Usage of both classical and also modern engineering tools (e.g. Computational Fluid Dynamics and Particle Image Velocimetry) allows reliable equipment qualifications while reducing the number of experiments. In addition, optimized equipment prototypes are designable and verifiable within a very short period of time. Furthermore, we can help users to choose the optimum SUS for their applications, to use them without any trouble and to develop and scale-up upstream production processes. We have long-term experience in process developments based on mammals, insects, plants and cells, but have also the knowledge to expand human mesenchymal stem cells.

Services and research for suppliers

- Bioengineering characterizations and optimizations of SUS: Mixers and bioreactors (DECHEMA Guideline), peripheral elements (filters and pumps, see Figure 2)
- Development of novel SUS: Mixers, bioreactors
- Biological tests with model cell lines (writing of application notes included): Chinese hamster ovary (CHO) cells, *Spodoptera frugiperda* cells (subclone 9, *Sf*-9) in combination with a model baculovirus expression vector system, Different plant suspension cell lines, *E. coli* cell line, immortalized adipose tissue-derived human mesenchymal stem cells

Figure 2: Magnetically levitated, centrifugal pump PuraLev (with kind permission from Levitronix). This single-use pump for shear sensitive liquids was optimized within a government-funded research project. Research partners were the company Levitronix and our group.

Collaboration Opportunities

We have carried out, and are carrying out, projects in cooperation with companies which are suppliers (e.g. Adolf Kühner, Finesse, GE Healthcare, Levitronix, Presens, Sartorius Stedim Biotech) and appliers (e.g., Cytos Biotechnology, Laves Arzneimittel, Lonza, Hoffmann-La Roche) of both SUS and SUT. In addition, we are closely linked with companies as well as research groups which are members of biotechnet Switzerland and the DECHEMA expert group “Single-Use Technology”.

Contact

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WiFi

Network: event-ZHAW
Password: 19ZHAW91event

Partners

DECHEMA
Gesellschaft für Chemische Technik und Biotechnologie e.V.

biotechnet switzerland

NETWORK inartis SWISSLIFESCIENCESCOMMUNITY

In cooperation with the CTI

KTT-Support
National thematic networks

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Swiss Confederation

Commission for Technology and Innovation CTI

SWISS BIOTECH™
National Thematic Network
Thematic Platform
Single-Use-Technology

Scope of the conference

Single-use devices and corresponding technologies have become well-accepted in biopharmaceutical manufacturing over the past 15 years. This mainly concerns processes with animal cell lines, which are aimed at products at small- and medium volume, for example monoclonal antibodies, hormones, enzymes or vaccines. Meanwhile, single-use devices are obtainable for all process steps of upstream- and downstream processing, formulation and filling and finally, the first complete single-use production facilities are in operation (WuXi PharmaTech in Shanghai) or under construction (Alvotech in Reykjavik or Polpharma Biologics in Gdansk). In addition, there is an obvious trend to replace re-usable equipment at existing production facilities by its single-use counterparts.

This development can be ascribed to the undoubted advantages of single-use systems as well as the concerted effort made by their appliers and manufacturers to overcome the existing limitations of these systems. For example, sensor technology was enhanced, automation solutions were improved, uncritical bag films and new single-use devices allowing applications beyond animal cell culture technique were developed.

Our conference will update the participants on the latest knowledge and developments in the field of biopharmaceutical manufacturing and single-use technology. It provides a perfect opportunity for networking and discussions with manufacturers, suppliers, users, regulatory affair specialists and representatives of organizations supporting the implementation of single-use devices worldwide. The conference program includes speeches and poster presentations, followed by a number of parallel workshops, run by leading companies in the sector. The main topics of the conference are:

- Challenges in mab and and vaccine productions
- Biosimilars and microbial products
- Implementation of single-use technology in the development and manufacturing of cell therapeutics
- Production facilities of the future
- Training for specialists in flexible biopharmaceutical manufacturing which is based on single-use systems and technologies

Target participants include:

- Those using or planning to use single-use devices in R&D and manufacturing, process engineers from the pharmaceutical industry and related sectors, specialists in apparatus engineering and facility design, and students in the fields of biotechnology, chemistry, pharmacy and medicine.
- Quality assurance and regulatory affair specialists interested in an update and the chance to exchange knowledge on the topics of biopharmaceutical manufacturing and single-use technology.

By taking part in this event on modern biopharmaceutical manufacturing, participants will gain a better understanding of how to beneficially choose and implement single-use systems and single-use process platforms, how to trouble-free design and operate production facilities and how to meet regulatory requirements.

Committee

Scientific Committee

Regine Eibl, Zurich University of Applied Sciences, CH

Detlef Eisenkrätzer, Roche Diagnostics GmbH, DE

Dethardt Müller, ViraTherapeutics GmbH, AT

Thorsten Peuker, Sartorius Stedim Biotech, DE

Ralf Pörtner, Technical University Hamburg, DE

Andreas Schneider, Roche Diagnostics International Ltd., CH

Markus Tanner, Werthenstein BioPharma GmbH, CH

Christian van den Bos, Mares Ltd., DE

Organising Committee

Dieter Eibl, Zurich University of Applied Sciences, CH

Dieter Beckmann, Institute of Bioporcessing and Analytical Measurement Techniques e.V., DE

Karin Tiemann, DECHEMA, DE

Domenico Alexakis, Swiss Biotech Association and NTN Biotech, CH

Daniel Gygax, University of Applied Sciences and Arts Northwestern Switzerland and Biotechnet, CH

Valentin Rüttimann, GEMÜ GmbH and ALUMNI ZHAW Life Sciences, CH

Philipp Kutter, Mayor of Wädenswil, CH

Conference programme

Monday, 5th September 2016, Morning

Biopharmaceutical manufacturing and single-use technologies: Update about latest achievements

09.00 – 09.30	Registration and coffee	(Building GA)
Session Chair	**Cathy Kroll**, Swiss Biotech Association, CH	(Room GA 203)
09.30 – 09.50	Welcome message **Jean-Marc Piveteau**, ZHAW, CH	
09.50 – 10.20	Current trends in single-use technologies: How future SUS developments will affect therapeutics and biosimilars productions over the next 5 years **Eric Langer**, BioPlan Associates, Inc., US	
10.20 – 10.50	Making drugs more affordable: Smart design & implementation of fully single-use bioprocess platforms & facilities for mAb's, ADC's & vaccines: client case studies **Miriam Monge**, Sartorius Stedim Biotech, FR	
10.50 – 11.20	Modular facility & flexible manufacturing platform solutions for modern single-use based biomanufacturing **Robert Morenweiser**, GE Healthcare Life Sciences, DE	
11.20 – 13.00	Lunch, exhibition and poster session	(Building GC and Building GA)

Monday, 5th September 2016, Noon

Parallel session 1	**Novel developments in applications with mammalian, insect and microbial cells**	
Session Chair	**Ralf Pörtner**, Technical University Hamburg, DE	(Room GA 203)
13.00 – 13.25	Reinventing commercial biomanufacturing **Sarfaraz K. Niazi**, Therapeutic Proteins International, LLC, US	
13.25 – 13.50	Breakthrough from the developer's perspective **Klaus Kaiser**, Bayer Pharma AG, DE	
13.50 – 14.15	Bioreactor options for continuous cell culture-derived viral vaccine production: Less defective interfering particles through process intensification **Yvonne Genzel**, Max Planck Institute Magdeburg, DE	
14.15 – 14.40	Increasing efficiency of insect cell-based vaccine production processes **Ina Dittler**, ZHAW, CH	
14.40 – 15.05	The influence of bisphenol A (BPA) on mammalian cell cultivation **Iris Pavenstädt**, Thermo Fisher Scientific, DE	

Parallel session 2	**Implementation of single-use technologies in the development and production of cell and gene therapeutics**	
Session Chair	**Regine Eibl,** ZHAW, CH	(Room GB 217)
13.00 – 13.25	Applying concepts of mAbs and vaccines development and manufacturing to cellular immune therapy **Alain Pralong**, Cell Medica Ltd., UK	
13.25 – 13.50	Commercial manufacture of cell therapeutics: Status and path forward **Christian van den Bos**, Mares Ltd., DE	
13.50 – 14.15	GMP production of immune cells as ATMPs with novel technology **Christian Leschke**, Zellwerk GmbH, DE	
14.15 – 14.40	Single-use technologies in hMSC and iPSC productions: Status quo and future needs **Erika M. McAfee**, Lonza Inc., US	
14.40 – 15.05	Scaling microcarrier-based expansion processes for production of high quality cells **Mark S. Szczypka**, PALL Life Sciences, US	
15.05 – 15.35	Coffee break and exhibition	(Building GA)

Monday, 5th September 2016, Afternoon

Parallel session 1	**Novel developments in applications with mammalian, insect and microbial cells**
Session Chair	**Rainer Glöckler**, Swissfillon AG, CH (Room GA 203)
15.35 – 16.00	Single-use technology for microbial processes: Potentials and challenges **Peter Neubauer**, Technical University Berlin, DE
16.00 – 16.25	Finding the missing link in complete single-use fermentation line: Evaluation of single-use large scale systems for aerobic bacteria **Nicolas Chaudet**, Bioprocess R&D Sanofi Pasteur, FR
16.25 – 16.50	A simple model-based strategy for microbial process design in single-use bioreactors **Patrick Sagmeister**, Exputec GmbH, AT
16.50 – 17.15	Single-use fermentation: Understanding process economy and process performance **Angus Thompson**, Fujifilm Diosynth, UK and **Sebastian Rothe**, GE Healthcare Life Sciences , CH
17.15 – 17.40	Heat characteristics of stirred single-use bioreactors **Matthias Müller**, Anhalt University of Applied Sciences, DE

Parallel session 2	**Implementation of single-use technologies in the development and production of cell and gene therapeutics**
Session Chair	**Daniel Gygax,** NTN Swiss Biotech, CH (Room GB 217)
15.35 – 16.00	Novartis cell & gene therapy **Christian Leist**, Novartis Cell & Gene Therapy, CH
16.00 – 16.25	Manufacturing of natural killer cell products for clinical applications **Volker Huppert**, Miltenyi Biotec GmbH, DE
16.25 – 16.50	Building the T cell factory of the future **Clive Glover**, GE Healthcare Life Sciences, UK
16.50 – 17.15	How to use Computational Fluid Dynamics in the development of cell therapeutics **Valentin Jossen**, ZHAW, CH
17.15 – 17.40	Evaluation of Eppendorf BioBLU single-use bioreactors for cell and stem cell culture and scale-up **Sebastian Kleebank**, Eppendorf, DE

17.40 – 18.45	Exhibition and poster session	(Building GA)
19.30 – open end	Conference dinner (boat trip on Lake Zurich)	

Tuesday, 6th September 2016, Morning

08.30 – 09.00	Registration and coffee	(Building GA)
Session Chair	**Thorsten Peuker**, Sartorius Stedim Biotech, DE	(Room GA 203)
09.00 – 09.30	Processes and factory of the future: Single-use and flexible for faster, cheaper and safer manufacturing **Sue Walker**, Merck BioDevelopment, FR	
09.30 – 10.00	Continuous GMP biomanufacturing **Dirk Tillich**, Finesse Solutions AG, CH	
10.00 – 10.30	Coffee break and exhibition	(Building GA)

Workshops and networking part 1 (Building GA and GB)

10.30 – 12.30

W1: Setting single-use technology standards for biopharmaceutical production processes (Room GB 217)

DECHEMA`s expert group single-use technology

- ⇨ Methods for the characterization of single-use bioreactors
- ⇨ How to early identify critical bag films for cell cultures
- ⇨ Important points to consider when implementing single-use bioreactors: A risk analysis recommendation
- ⇨ Are single-use bioreactors for microbial products required?

Sartorius Stedim Biotech

- ⇨ Container closure integrity (CCI) for single-use systems (SUS): Regulatory trends, challenges and test methods

W2: Facility of the future (Room GA 207)

GE Healthcare Life Sciences

- ⇨ Facility of the future – points to consider

Sartorius Stedim Biotech

- ⇨ Connect upstream = Sartorius Stedim Biotech

W3: Single-use technologies for the development and production of cell and gene therapeutics (Room GA 215)

Merck KGaA

- ⇨ Single-use technologies enable closed and scalable cell therapy manufacturing

Micro-Sphere SA and Cell Culture Technologies

- ⇨ Biodegradable/implantable microcarriers for the chemically defined cultivation of human adipocyte-derived stem cells

Thermo Fisher Scientific

- ⇨ The new Thermo Scientific™ Nunc™ High Density Cell Factory™ system to scale-up cells for a working or master cell bank

W4: Continuous bioprocessing with single-use systems (Room GA 217)

Filtrox AG

⇨ High performance single-use continuous microfiltration system for high cell density clarification

GE Healthcare Life Sciences

⇨ Integrated continuous cell culture utilising single-use technologies

Levitronix GmbH

⇨ The centrifugal revolution: Low-shear, pulsation free, precise control fluid management for different applications – Live demo and hands-on workshop

PALL Life Sciences

⇨ Enhanced single-use clarification of CHO feedstocks using cadence acoustic separation technology

Repligen

⇨ Process intensification and continuous bioprocessing: Tools and solutions

W5: Recent developments in the field of USP, DSP and PAT (Room GA 219)

Adolf Kühner AG

⇨ The orbitally shaken single-use bioreactor SB10-X

Infors AG

⇨ eve® – answering the question "Why bioprocess software needed to change"!

Purolite Life Sciences

⇨ The future of Protein A

smartINST

⇨ Connected *in situ* measurement system

Thermo Fisher Scientific

⇨ Point of use integrity testing; the power of knowledge

12.30 – 13.30	Lunch, exhibition and poster session	(Building GC and Building GA)

Tuesday, 6th September 2016, Afternoon

Workshops and networking part 2 (Building GA and GB)

13.30 – 15.30

W1: Setting single-use technology standards for biopharmaceutical production processes (Room GB 217)

DECHEMA`s expert group single-use technology

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- ⇨ Important points to consider when implementing single-use bioreactors: A risk analysis recommendation
- ⇨ Are single-use bioreactors for microbial products required?

Sartorius Stedim Biotech

- ⇨ Container closure integrity (CCI) for single-use systems (SUS): Regulatory trends, challenges and test methods

W2: Facility of the future (Room GA 207)

GE Healthcare Life Sciences

- ⇨ Facility of the future – points to consider

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W3: Single-use technologies for the development and production of cell and gene therapeutics (Room GA 215)

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	Repligen ⇨ Process intensification and continuous bioprocessing: Tools and solutions **W5: Recent developments in the field of USP, DSP and PAT** (Room GA 219) **Adolf Kühner AG** ⇨ The orbitally shaken single-use bioreactor SB10-X **Infors AG** ⇨ eve® – answering the question "Why bioprocess software needed to change"! **Purolite Life Sciences** ⇨ The future of Protein A **smartINST** ⇨ Connected *in situ* measurement system **Thermo Fisher Scientific** ⇨ Point of use integrity testing; the power of knowledge	
15.30 – 16.00	Coffee break and exhibition	(Building GA)

Podium discussion and networking

Session Chair	**James Dean Vogel,** The BioProcess Institute, US	(Room GA 203)
16.00 – 16.15	Standardization and single-use technology **James Dean Vogel**, The BioProcess Institute, US	
16.15 – 17.00	**Podium discussion about standardization:** **John Boehm**, Chair, BPSA, US **Jeffrey Carter**, Director of Filtration R&D, GE Healthcare Life Sciences, US **Christel Fenge**, Vice President, Sartorius Stedim Biotech, DE **Miriam Monge**, Committee member, ISPE, FR **Wolfgang Paul**, Senior Scientist, Roche Diagnostics GmbH, DE **Markus Tanner,** Director Biotechnology, Werthenstein BioPharma GmbH, CH	
17.00 – 17.15	Poster awards and closing remarks **Christian Hinderling**, ZHAW, CH	
18.00 – open end	Barbecue with draft beer made by ZHAW (50.- CHF, interested participants are asked to send a registration mail to katharina.blaschczok@zhaw.ch)	(Building GC)

Wednesday, 7th September 2016

DECHEMA working group meeting (By invitation only)

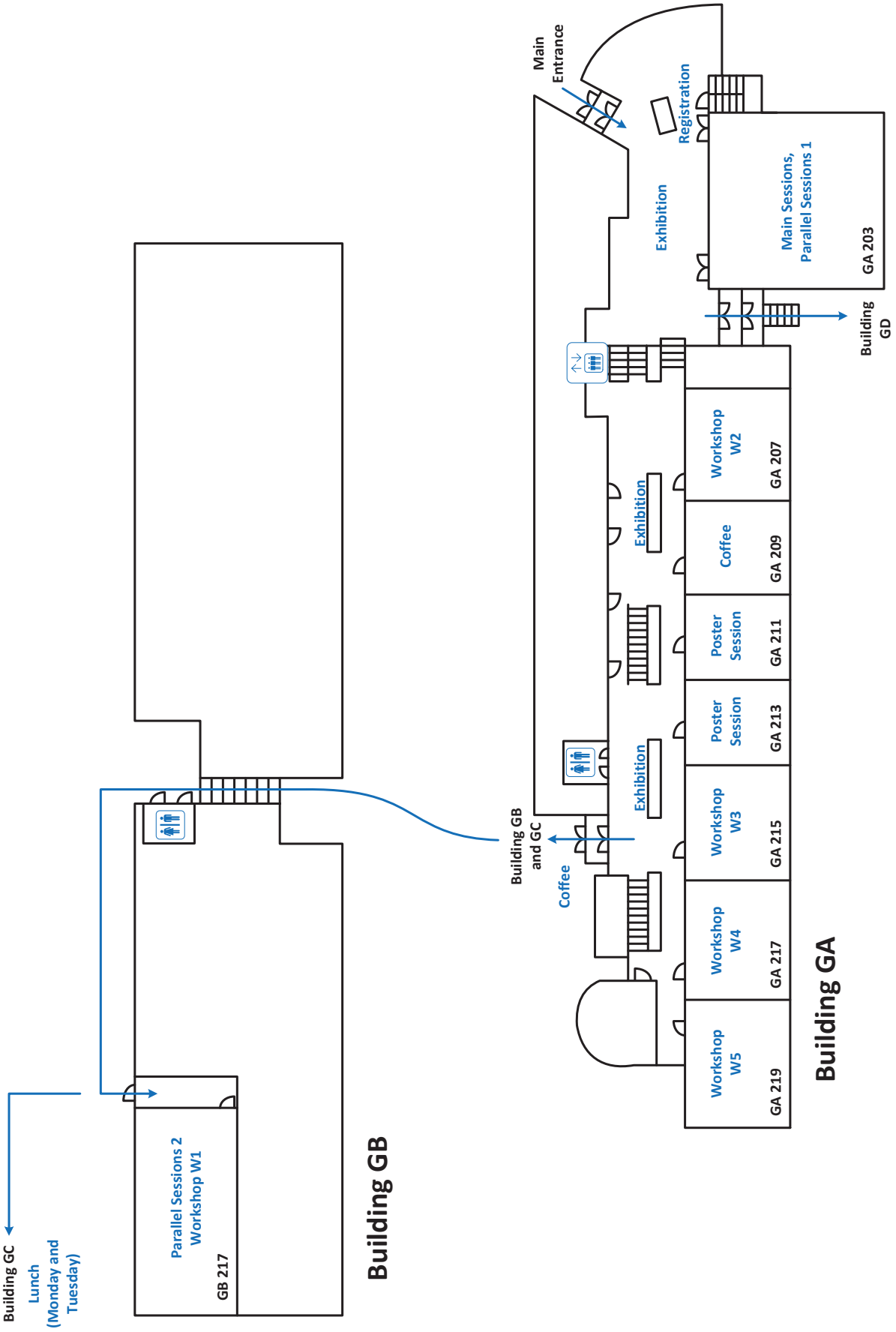
09.00 – 10.00	**DECHEMA working group "Single-use technologies in biopharmaceutical production"** Meeting	(Building GD 203)
10.00 – 10.30	Coffee break	

Post workshop 2016 (Interested participants are asked to send a registration mail to katharina.blaschczok@zhaw.ch)

The purpose of the workshop is to discuss the existing training opportunities in the field of flexible biopharmaceutical manufacturing with single-use systems and technologies. In addition, we would like to investigate requirements from both supplier and user perspectives. The goal is that the discussions from this meeting will be developed into a DECHEMA recommendation or position paper to be published in Engineering in Life Sciences.

Session Chair	**Karin Tiemann,** DECHEMA, DE	(Building GD 203)
10.30 – 10.35	Welcome and introduction **Karin Tiemann**, DECHEMA, DE	
10:35 – 11:55	**Keynotes and discussion**	
10.35 – 10.55	What do suppliers need? **Thorsten Peuker**, Sartorius Stedim Biotech, DE	
10:55 – 11:15	User requirements **Stefan Robert Schmidt**, Rentschler Biotechnologie GmbH, DE	
11:15 – 11:35	Situation in the USA **James Dean Vogel,** The BioProcess Institute, US, and **Eric Langer,** BioPlan Associates, Inc., US	
11:35 – 11:55	DECHEMA group and the ZHAW: Ongoing and planned activities **Regine Eibl,** ZHAW, CH	
11:55 – 12:00	**Summary and outlook**	
12:00	Lunch and informal discussions	(Building GD Mensa)

Floor plan BioTech 2016



Zurich University of Applied Sciences

Life Sciences and Facility Management

Location Wädenswil
Campus Grüental and Campus Reidbach

Arrival via Public Transport
From Wädenswil train station

Campus Grüental
Stop «Campus Grüental»
bus line 123, 126, 150, 160

Conference dinner
(boat trip on Lake Zurich)

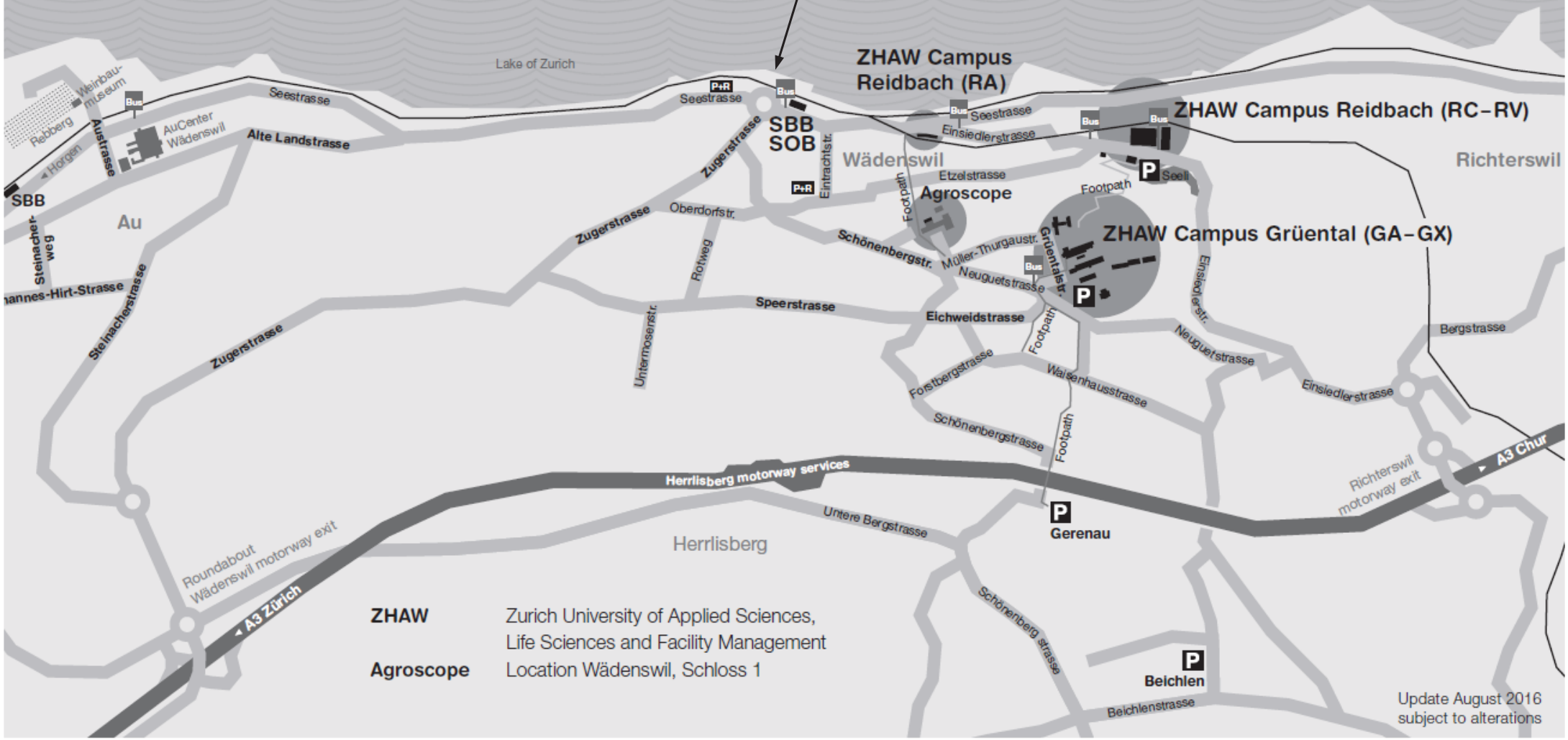
SÜRICHSEE SCHIFFFAHRT & GASTRO

Arrival via Private Transport
Campus Grüental: Exit Wädenswil
(Satnav: Grüentalstrasse 14)

Campus Reidbach (Building RC–RV):
Exit Richterswil (Satnav: Einsiedlerstrasse 31)

Campus Reidbach (Building RA):
Exit Wädenswil (Satnav: Seestrasse 55)

Note: Parking space at Campus Grüental and Campus Reidbach (RC–RV) is limited (central parking meter)! NO parking at the Campus Reidbach (RA) on Seestrasse – Public parking at Wädenswil train station.



ZHAW Zurich University of Applied Sciences,
Life Sciences and Facility Management

Agroscope Location Wädenswil, Schloss 1

Update August 2016
subject to alterations

Abstracts

Monday, 5th September 2016, Morning

Chair **Cathy Kroll**, Swiss Biotech Association, CH (Room GA 203)

09:30 **Welcome message**

Jean-Marc Piveteau[1]

[1]*ZHAW, CH*

09:50 **Current trends in single-use technologies: How future SUS developments will affect therapeutics and biosimilars productions over the next 5 years**

Eric Langer[1]

[1]*BioPlan Associates, Inc., US*

The world market for biopharmaceuticals is now over $200 billion; growing at a healthy ~15% rate. New products and new markets, particularly internationally, continue to support growth. This increasing production is being supported by single-use manufacturing as the platform creates new opportunities for future production. This presentation includes data and analysis from BioPlan's 13th Annual Report and Survey of Biopharmaceutical Manufacturing. Single-use bioprocessing equipment now thoroughly, ≥85%, dominates the smaller pre-commercial bioprocessing market. The market for single-use equipment will grow rapidly, doubling in 5 years as new commercial-scale facilities are projecting they will split certain unit operations about 50:50 between stainless steel and single-use in 5 years. Further, biosimilars capacity demands are projected to increase 6-fold over the next 5 years. This demand for capacity is likely to be met with a mix of production platforms, and single-use technologies are already playing a major role. Trends driving the shift toward single-use include: Demand for greater cost-containment and controls; more productivity and efficiency, manufacture in developing regions, worldwide standardization of biomanufacturing; the use of contract manufacturing organizations; the dominance of single-use bioprocessing equipment for clinical trials scale manufacturing, especially for smaller and under-funded companies to reduce facilities construction costs; the need for greater manufacturing flexibility, rapid deployment and shorter time to run; and demand for modular technologies that provide greater processes interconnectivity. For all bioprocessing, new product areas in demand include 6 of the top 7 area involving single-use devices; topping the list are downstream continuous bioprocessing technologies. These SUS 'enabling technologies' will permit better, faster, more consistent bioprocessing at ever-larger scales. In fact, single-use batch-fed bioreactors are most likely to be recommended by 71.5% of the industry for new clinical-scale products. The most rapid adoption of SUS devices today include the most complex devices, such as mixing systems, membrane adsorbers and bioreactors. Yet some hurdles remain, including problems with breakage, and with leachables/extractables data, where 76% and 73%, respectively, of the industry may still be holding back adoption until these issues are resolved. This presentation discusses and quantifies the industry's views on a broad range of topics, from the need for greater standardization, demand for single-use sensors and analytical devices, the need for better recycling programs and many others; we cover the industry's level of satisfaction with current suppliers, and we cover the most important attributes vendors must address today.

Notes

10:20 **Making drugs more affordable: Smart design & implementation of fully single-use bioprocess platforms & facilities for mAb's, ADC's & vaccines: client case studies**

Miriam Monge[1]

[1]*Sartorius Stedim Biotech, FR*

The biotechnology industry faces considerable challenges such as the need to increase supply to meet demand, greater complexity, the need for greater productivity at a lower cost and increased regulatory oversight. Flexible manufacturing operations will help companies overcome these challenges allowing them to operate smaller and smarter facilities all around the globe. Flexible facilities can shorten time to market and minimize both capital outlays and cost of goods.

Drug manufacturers can build facilities that utilize single-use technology in 12 months compared to the 24 months required for a stainless steel facility. Plant utilization is higher because of reduced downtime between batches, which operators need for cleaning, and sterilizing multi-use equipment. Lower utility requirements enable a 20 to 40% reduction in facility footprint. CAPEX savings are in the region of 25 to 40%. Flexible facilities are greener; generating around half the CO_2 emissions of stainless steel plants while also being safer for the patient because of the reduced risk of product cross contamination.

The Sartorius Integrated Solutions team is designing entire processes based on Single Use and/or hybrid design philosophies. We design and implement rapid and cost-effective biomanufacturing solutions from early phase development through scale-up to commercial manufacturing. Our customers benefit from the most comprehensive bioprocess technology portfolio coupled with our expertise in Single Use bioprocess engineering ensuring a robust manufacturing process**.**

The operation of flexible facilities requires great assurance of supply from the manufacturers of single-use technologies. Sartorius uses its material science, QbD, film extrusion, bag making and product design expertise to ensure it is always able to deliver consumable to customers. The company's assurance of supply policy is built on partnerships, transparency, supply contracts and quality agreements with our suppliers of raw materials, films and components. It relies on the complete control of our supply chain & manufacturing process from the resins to the final SU Pre Designed Solution

The industry can benefit from the standardized single-use bioprocess platforms. Sartorius is developing these for molecule classes such as monoclonal antibodies, ADCs and vaccines. Based upon the company's extensive experience with projects for these molecules our process development consultants can recommend how Sartorius can implement these platforms to meet the specific needs of customers without compromising on the speed or cost benefits associated with this approach.

This presentation will explain how flexible facilities are revolutionizing bioprocessing and illustrate the concepts with case study examples.

Notes

10:50 **Modular facility & flexible manufacturing platform solutions for modern single-use based biomanufacturing**

Robert Morenweiser[1]

[1]*GE Healthcare Life Sciences, DE*

The manufacture of biopharmaceuticals drugs is conducted under current good manufacturing practice (cGMP) standards and includes multiple unit operation steps for upstream production and downstream purification. Until recent years, manufacturing facilities were based on relatively inflexible, hard-piped equipment including large stainless steel bioreactors and tanks to hold product intermediates and buffers. In the meantime, there is an increasing trend towards the use of single-use technologies across the manufacturing process. Technology improvements do now more and more allow to using an end-to-end single-use manufacturing platform. The presentation provides a perspective on the current state-of-the-art in single-use technologies and indicates trends that will improve biomanufacturing performance as well as economics and increase the market penetration of disposable manufacturing in the future.

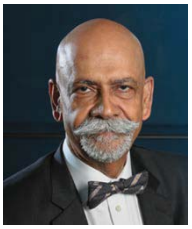
11:20 Lunch, exhibition and poster session (Building GC and Building GA)

Notes

Monday, 5th September 2016, Noon

Parallel session 1	Novel developments in applications with mammalian, insect and microbial cells	
Chair	**Ralf Pörtner**, Technical University Hamburg, DE	(Room GA 203)

13:00 **Reinventing commercial biomanufacturing**



Sarfaraz K. Niazi[1]

[1] *Therapeutic Proteins International, LLC, US*

The origins of the fermentation processes can be traced back thousands of years. Fast-forward to the present, and science has advanced to the point of enabling highly targeted and complex biologics. As the industry shifts to develop more complex and costly biologics, how will the commercial biomanufacturing process contribute to faster and more cost effective biologics to address patient needs? Further, how can companies successfully overcome legal, regulatory and financial challenges associated with biomanufacturing?

This presentation will review the evolution of biologics, how biomanufacturing has changed over the years, the future direction of biomanufacturing and what may be possible in the years to come.

Notes

13:25 **Breakthrough from the developer's perspective**

Klaus Kaiser[1]

[1]*Bayer Pharma AG, DE*

Antibodies are still dominating the field of biopharmaceutical drugs and many of them fill the pipelines and are waiting to be produced for added patient benefit. When antibodies are combined with a small molecule, for example a very toxic one, they can be transformed into precise weapons killing cancer cells very specifically. Our lead candidate Anetumab Ravtansine is such an antibody drug conjugate (ADC) targeting the cell surface glycoprotein Mesothelin which is overexpressed in various very aggressive cancers. Strong efficacy signals in ongoing clinical trials, e.g. ovarian and mesothelioma cancer, point to an accelerated development scenario. How can a CMC function prepare for such a potential breakthrough?

The development program depends on the complexity of the product, availability of platform technologies, relevant prior knowledge, and timing of designation. Risk assessments and comparability protocols are key considerations. It is a challenging task to ensure launch readiness at manufacturing scale against the background of shortened timelines. The clinical manufacturing process and corresponding facility already need to meet similar quality/GMP expectations as the later commercial manufacturing.

Usually ADCs are produced in dedicated facilities. The use of disposable systems and glove box technology enables us to perform clinical manufacturing in multi-purpose facilities where antibodies as well as ADCs can be produced in the same cleanroom. Cross-contamination is prevented by using dedicated equipment and single-use technologies in a closed system for conjugation and purification. This could support the use of initial product supply from a clinical process and site.

Notes

13:50 **Bioreactor options for continuous cell culture-derived viral vaccine production: Less defective interfering particles through process intensification**

Yvonne Genzel[1], Felipe Tapia[1], Daniel Vazquez[1], Ilona Behrendt[1], Ingo Jordan[2], Tim Bürgin[3], Dave J. Gangemi[4], Udo Reichl[1]

[1]Max Planck Institute Magdeburg, DE
[2]ProBioGen AG, DE
[3]Adolf Kühner AG, CH
[4]Viracell Advanced Products LLC, US

Defective interfering particles (DIP) may interfere with cell culture-based viral vaccine production processes and reduce yields. High concentrations of viruses within the bioreactor increase the opportunity for co-infections of defective and infectious particles, which then may result in increased production of defective virions. Different process strategies that avoid accumulation of high virus concentrations in bioreactors will be presented for MDCK.SUS2 and AGE1.CR.pIX cells infected either with influenza A or Modified Vaccinia Ankara (MVA) virus.

High cell density cultivation (HCD) within the extracapillary space (ECS) of a hollow fiber bioreactor allowed multiple harvests of virus by removal of the cell culture medium of the ECS and addition of fresh medium. This positive effect of multiple harvests was then first implemented in small-scale experiments (shaker flasks) and subsequently in lab-scale HCD perfusion cultivation using either alternating tangential flow filtration (ATF) or tangential flow filtration (TFF). Both, conventional stirred tank or orbital shaken bioreactors were used. Cell expansion exceeding 60 x 10^6 cells/mL was followed by either perfusion, fed-batch or multiple medium exchange/harvests during the virus propagation phase, resulting in up to 10-fold increase in virus titer compared to typical processes operated at 4 x 10^6 cells/mL. Further substrate feeding optimization resulted also in improvements in cell-specific virus yields and volumetric productivity.

Furthermore, a two-stage stirred tank bioreactor set-up (1 L scale; scale-down model in shaker flasks) was evaluated. Here, influenza A DIP accumulated and prevented production at a steady state as virus titers oscillated by more than 4 log scales. For the large DNA virus MVA virus however, observation of stable titers suggests an absence of the so-called „von-Magnus" effect.

The „von-Magnus" effect for influenza virus may be overcome with a plug-flow tubular bioreactor system that will be presented here. Accumulation of virions within the bioreactor is avoided by moving the virus-infected cells through a tube in a plug-flow mode, with a residence time sufficient to allow virus infection and release within the tube. This way, constant infection of new cells without chance of co-infection with already released new viruses and thereby control of the number of virus passages is accomplished.

In summary, all evaluated strategies present new options to improve significantly the productivity in cell culture-based viral vaccine manufacturing.

Notes

14:15 **Increasing efficiency of insect cell-based vaccine production processes**

Ina Dittler[1], Regine Eibl[1]
[1]ZHAW, CH

The baculovirus-expression vector system (BEVS) has been proven as a suitable expression system for insect cell-based vaccine manufacturing because of its high gene expression, scalability and safety compared to traditional egg-based vaccine production. However, extended use of baculovirus-derived vaccines for human and veterinary medicine, especially for pandemic scenarios, is constrained by high production costs as well as time-intensive development and manufacturing processes. New strategies for efficient baculovirus-based production technologies therefore need to be investigated. The titerless infected-cells preservation and scale-up (TIPS) method provides a suitable alternative to the classical 2-phase production process due to its shorter production time, stable storage condition of the recombinant baculovirus (r*Autographa californica* multicapsid nucleopolyhedrovirus rAcMNPV) enclosed in insect cells (*Spodoptera frugiperda Sf*9), and the titerless infection of up to 100 L cell culture broth in one scale-up step.

The aim of the investigation was to scale-up the TIPS method for orbitally shaken SU bioreactors for the first time. After establishment of the TIPS method at mL-scale, infection studies were conducted in 3 L shake flasks and in the orbitally shaken SU bioreactor SB10-X (Kühner AG) using the classical 2-phase production process and the TIPS method. To do this, a k_La value of 14 h^{-1} was used as a scale-up criterion. The maximal activities of the recombinant model protein secreted alkaline phosphatase (rSEAP) expressed were 144 – 158 U mL^{-1} for the TIPS method on day 4 post infection (pi) and 112 – 128 U mL^{-1} for the classical 2-phase production process on day 6 – 7 pi. The shortened production time and, thus, the increased efficiency of the BEVS platform is of special interest for vaccine developers and manufacturers.

Notes

14:40 **The influence of bisphenol A (BPA) on mammalian cell cultivation**†

Iris Pavenstädt[1]

[1] *Thermo Fisher Scientific, DE*

BPA plays a substantial role in industry, as it is used for polycarbonate (PC) plastics and epoxy resins which are required for various plastic consumer products. Due to the large application spectrum, BPA is one of the chemicals that are produced with the highest amount worldwide. However, BPA is known to be an endocrine disruptor, and its influence on humans, animals, and various cell lines was addressed in diverse studies. As the burden of BPA can be increased by using disposable plastic articles and single-use technologies for cultivation, it is essential to examine the consequences of BPA presence on mammalian cells, as they are a contributing factor in the production of complex pharmaceutical therapeutics.

We selected three industrially relevant cell lines (CHO, hybridoma and CAP) producing either a monoclonal antibody or a Fab fragment and analysed systemic effects of BPA by comparing cell culture performance in BPA-free poly-ethylene terephthalate glycol (PETG) and in PC shaking flasks. We focused on the influence of BPA on cellular growth, viability, and several metabolic parameters. In addition, we determined the product concentration and aggregation behaviour of the recombinant proteins expressed by these cell lines and the BPA concentration within the medium caused by leaching. Moreover, we performed EC_{50} studies to determine the toxic concentration of BPA.

Our results indicated that leached BPA had no effect on specific growth rates and viability and toxicity appeared at about 10^4 times higher concentrations; however, it influenced the specific productivity rate and metabolic activity parameters of our CHO cell line. Consequently, one can neglect BPA from leaching in the culture as long as the selected cell line is BPA tolerant. For sensitive cell lines BPA can be a hurdle for pharmaceutical production influencing the specific productivity of recombinant proteins. In this case, one has to critically control putative BPA sources as we observed it to be volatile under incubation conditions.

† published in Appl Microbiol Biotechnol. 2016 Jan;100(1):113-24

Notes

Parallel session 2	**Implementation of single-use technologies in the development and production of cell and gene therapeutics**
Chair	**Regine Eibl,** ZHAW, CH (Room GB 217)

13:00 **Applying concepts of mAbs and vaccines development and manufacturing to cellular immune therapy**

Alain Pralong[1]

[1]*Cell Medica Ltd., UK*

The number of clinical trials conducted in cancer has significantly increased over the last years reaching more than 5400 studies currently on-going at all stages of product development. This increase has primarily been driven by cellular immune therapy made possible by significant progress in understanding cancer biology, which in turn offered a variety of novel therapeutic approaches.

Autologous cellular immune therapies are today focusing on using the patient's own immune system ready and capable to combat its cancer. Either immune cells are specifically selected, activated and expanded before administration back into the donor patient as it is the case for Cell Medica's CMD-003 or the patient's immune cells are transformed with a chimeric antigen receptor (CAR) to provide the patient cancer antigen recognition capabilities after administration not present before the treatment as it is the case for leads investigated by Novartis, Kite, Juno and others.

Spectacular clinical results have generated a rush into cellular immune therapy and have massively increased the value of companies involved given the economical promise associated with successful completion of clinical trials and forecasted demands.

Materializing these promises will though not only depend on successful clinical results but also on the capability to build a robust and sustainable industry enabling full GMP compliance, supply continuity, and last but not least also accessibility.

In this presentation, current issues in mAb and Vaccine development and manufacturing will be discussed and concepts presented developed and proven to resolve these in order to provide potential solutions for facing the challenges cellular immune therapy will encounter going forward.

Notes

13:25 **Commercial manufacture of cell therapeutics: Status and path forward**

Christian van den Bos[1]

[1]*Mares Ltd., DE*

Therapeutic cells are currently largely manufactured in static systems such as cell factories. It is widely acknowledged that closed processes are advantageous, yet, converting processes based on such technology into fraught with difficulty.

Consequently, production modalities for therapeutic cells have begun to emulate lessons learned from bioproduction and initial promising work has been done on producing quantities of therapeutic cells in bioreactor systems - systems compatible with down stream processing systems such as counterflow centrifugation, all connected through established tubing/aseptic connection systems.

Another concern in producing therapeutic cells is the use of complex animal products such as bovine serum - for reasons of safety as well as economy. We believe that using bioreactor technology in conjunction with micro-carriers is likely to generate processes devoid of such animal products thus providing for processes which satisfy quantitative and qualitative requirements.

Beyond these immediate concerns we view bioreactors and associated systems as tools to approach physiological conditions during culture expansion of therapeutic cells; we propose this to be the central tool to maximise production efficiency, both in quantity as well as in quality, i.e. maximized therapeutic potential.

Notes

13:50 **GMP production of immune cells as ATMPs with novel technology**

Christian Leschke[1], Shreemanta K. Parida[2], Evguenyi Sinelnikow[1], Hans Hoffmeister[1]

[1]Zellwerk GmbH, DE
[2]Justus-Liebig University, DE

Immunotherapy of cancer adjudged as "Breakthrough of the year 2013" by the journal "Science" has been a paradigm shift and a "game changer" in the conquest of cancer by targeting immune-system rather than the tumour. These principles have been extended with some clinical success and promise in combating graft vs. host disease in transplant patients as well as in preventing infections in them. Immune cell therapy has opened up a plethora of options to address unmet clinical needs.

However, there have been major constraints in achieving the optimal numbers and the desired phenotypes of immune cells avoiding inactivation of in vivo applied cell preparations by conventional expansion culture methods. The increasing use of cell therapy towards myriads of clinical conditions has led to production processes in accordance with Good Manufacturing Practice (GMP) for ATMP. In cellular therapy, safety remains of paramount importance and refers to donor validation, choice of starting material, processes, and controls used, not only at the batch release level but also during the ex vivo expansion processes. Moreover, expansion of immune cells for therapy should be adapted to closed systems that are easy to use. Implementing dynamic controls during the manufacturing of clinical-grade cells for therapy is essential to ensure microbiological safety and to avoid potential adverse effects linked to genomic instability driving transformation and senescence or decrease of cell functions.

At Zellwerk, we have been engaged over many years to meet these growing requirements. The need of clinical grade immune cells in high numbers has been successfully conquered by innovative solutions. At Zellwerk, standard operation procedures have been worked out for mass expansion of purified NK cells, T Cells, MSCs etc., starting with common samples of peripheral blood, bone marrow or other tissues. The perfusion is consistently performed in controlled, reproducible, robust and efficient processes, offering reliable, validated ex vivo expansion of immune cells. Specific activation, triggering or differentiation during processing can easy be realised. Several types of single use bioreactors have been developed for mass production of immune cells. All types of our proprietary bioreactors can be operated inside the unique and indispensable Z®RP cell cultivation platform. Key elements of the platform are the Z®RP GMP Breeder, combining features of a laminar flow work bench and a cell culture incubator, and the Z®RP Control Unit for automatic regulation of essential parameters of perfusion cell culture processes, e.g. pH, pO_2, medium temperature, medium perfusion, feeding rates. For regulation as well as for monitoring of the cultivation process, a proprietary software is provided in the system to collect, document and evaluate the data as mandated in the GMP production. The single use bioreactors are certified as medical products class 2a.

Z®RP cell cultivation system, a proprietary platform is well suited for individual immune cell manufacturing near to bedside as well as for industrial production of ATMPs. Tools and technologies have evolved offering in the form of a GMP compliant, certified closed system. Platform and bioreactors provide a closed system suited for clinical use as well as for research on standardized immune cell preparations, characteristics, manipulations etc. There is opportunity to push the limits to achieve the best outcomes for patients by validating tools and technologies as well as performing robustly designed clinical trials as way forward.

Notes

14:15 **Single-use technologies in hMSC and iPSC productions: Status quo and future needs**

Erika M. McAfee[1]

[1]*Lonza Inc., US*

Many in biopharmaceutical manufacturing have begun to incorporate the use of single-use technologies in various aspects of their processes. From buffer and media preparation, through cell expansion, harvest, and fill/finish, the use of single-use technologies has proved advantageous in regards to process robustness and cost-effectiveness. Key drivers for this shift in industry from the use of stainless steel, glass, and other reusable technologies to single-use disposables include increased production output of high quality final product with a decrease in risks and associated costs.

With the recent breakthroughs in the cell therapy field in the last few years, autologous and allogeneic stem cell procedures, used to treat a variety of clinical indications, are quickly moving from the research lab to clinical trials and soon into commercial manufacturing. With this, innovative single-use technologies have made it possible to produce smaller amounts of high-quality drug product for use in pre-clinical and clinical trials than previously capable in reusable systems. In addition, single-use technologies for large-scale production reduce costs associated with GMP suite space, utilities, cleaning, validation testing, and labor requirements for the commercial manufacture of a product, as well as decrease risks of cross-contamination/sterility breaches, and increase the flexibility in GMP manufacturing suites by using equipment which is mobile as opposed to large, fixed, complicated manufacturing equipment with high initial capital investments.

This presentation will highlight the advantages of adopting novel, scalable, and closed single-use systems for upstream expansion and downstream harvesting of human mesenchymal stem cells (hMSCs) and induced pluripotent stem cells from the research to production scale. We will also identify common bottlenecks and challenges when using these systems in cell therapy processes and discuss strategies for reducing cost of goods without compromising final product quality.

Notes

14:40 **Scaling microcarrier-based expansion processes for production of high quality cells**

Dave Splan[1], Grishma Patel[1], Mark S. Szczypka[1]

[1]PALL Life Sciences, US

Leading edge cell therapy and regenerative medicine products require simple, robust and reproducible methods to generate large numbers of high quality cells to be commercially viable. Traditional planar cell culture technologies for expansion of adherent cells are labor intensive, require multiple open steps that introduce risk, and lack the environmental control of bioreactor systems. Microcarrier-based processes offer an attractive and tractable platform for cell expansion by providing a large surface area for growth that is amenable to implementation in a closed bioreactor system. Microcarriers have also been successfully used for decades in bioreactors at hundred to thousand liter scales for growth of adherent cell types. Pall develops scalable microcarrier-based processes for expansion of high quality primary cells and stem cells isolated from adult tissue sources. Data describing processes developed with adult stem cells and application of Pall's single use technologies to reproducibly generate high quality cells suitable for therapeutic purposes will be presented.

15:05 Coffee break and exhibition (Building GA)

Notes

Monday, 5th September 2016, Afternoon

Parallel session 1	Novel developments in applications with mammalian, insect and microbial cells	
Chair	**Rainer Glöckler**, Swissfillon AG, CH	(Room GA 203)

15:35 **Single-use technology for microbial processes: Potentials and challenges**

Peter Neubauer[1], *Anna Maria Marbà Ardébol*[1], *Stefan Junne*[1]

[1] *Technical University Berlin, DE*

Single-use bioreactors have succeeded to replace stainless steel bioreactors in the cultivation of higher cells. It is important to recognise that the wide range of various design approaches provides a benefit for tailored applications to fulfil the demands considering shear forces, power input, gas mass transfer and mixing time. This portfolio of existing reactor types is especially useful in the area of microbial cultivations where the breadth of the biological systems and their specific requirements may strongly benefit from the existing developments, but also may challenge new ideas. The presentation shows for some microbial processes that single-use systems offer a significant advantage and discusses remaining challenges for the use of single use bioreactors from the microliter to the cubic meter scale.

Notes

16:00 **Finding the missing link in complete single-use fermentation line: Evaluation of single-use large scale systems for aerobic bacteria**

Nicolas Chaudet[1], Grégoire Exbrayat[1], Marjorie Monnet[1], Jean Marc Guillaume[1]

[1]Bioprocess R&D Sanofi Pasteur, FR

The application of single use technologies for the bioproduction represents a major technology innovation advance. Single use bioreactors for cell culture have been largely implemented for manufacturing, providing significant flexibility, costs reduction and fast implementations. In contrast, few technologies are available for aerobic bacterial fermentation mainly due to limitations for oxygen and heat transfer. Like clinical products derived from bacterial expression system still represent a significant number of bio therapeutics/vaccines, some investigations have been performed in Sanofi Pasteur to evaluate new single use fermenters suitable for aerobic processes and matching to clinical manufacturing scale. 2 systems have been evaluated with an yeast and an E.coli strain to produce a recombinant protein versus a 100L stainless steel fermenter: the 200L celltainer system (Cellution) and the HyPerforma SUF (ThermoFisher).

Notes

16:25 **A simple model-based strategy for microbial process design in single-use bioreactors**

Patrick Sagmeister[1], Valentin Steinwandter[1], Thomas Zahel[1], Sophia Ulonska[2], Christoph Herwig[2]

[1]Exputec GmbH, AT
[2]Vienna University of Technology, AT

Background

Microbial fermentation is still the primary platform for the production of biopharmaceutical products. In recent years, single use technologies emerged as a valuable alternative to stainless-steel bioreactors even for microbial bioprocessing. Single-use technologies show clear advantages such as no cleaning validation and reduced initial investment costs. However, process design is highly challenging due to technical limitations as regards heat- and oxygen transfer and reduced instrumentation. This urges for novel bioprocess design strategies and platforms that can enable the fast- and efficient design of high performing microbial bioprocesses in single-use bioreactors.

A novel strategy for microbial bioprocess design in single-use bioreactors is presented. The simple two-step strategy is based on microbial feeding and process parameter optimization carried out in lab-scale (parallel) bioreactors (step 1) and model-based transfer and in-silico optimization of the developed processes for the targeted single use scale (step 2).

The two-step strategy for single-use bioprocess design is demonstrated on an industrial case study. The impact of the specific substrate, inducer uptake rate and cultivation temperature on product formation kinetics was investigated in a parallel bioreactor system. The data was used for the development of a mathematical model capable of predicting product, by-product and biomass formation as a function of the investigated process parameters (temperature, specific substrate, inducer uptake rate). This step was semi-automated for *E. coli* systems following a hybrid modelling approach. In the second step, based on data on maximum heat- and oxygen transfer rates measured in coalescing (real) media, in-silico time-space yield optimizations for three single use scales (50, 200 and 1000 liter) were performed The model predicted 5 key bioprocess design parameters: 1) optimal initial substrate concentration, 2) optimal batch temperature, 3) optimal induction phase temperature, 4) optimal non-induced fed-batch feeding profile and 5) optimal induction phase feeding profile. The in-silico optimizations resulted in strongly different optimal bioprocess designs depending on the scale of the single use bioreactor.

Conclusions

A simple but powerful model-based strategy for microbial process design in single use bioreactors was developed. Due to a strong scale-dependency of maximum heat- and oxygen transfer rates, single-use bioreactor scale demands a tailored process design for each scale. These optima can be identified using the presented strategy. The strategy is applicable for the development of single-use processes involving microbial systems as well as feasibility- and proof of concept studies based on historical data.

Notes

16:50 **Single-use fermentation: Understanding process economy and process performance**

Angus Thompson[1], Sebastian Rothe[2]

[1]Fujifilm Diosynth, UK
[2]GE Healthcare Life Sciences, CH


Biomanufacturing is now very much a global activity yet quality and affordability remains high on everyone's agenda. Consequently, the demands from consumers, regulators and manufacturers to drive cost and time 'out' and quality, productivity and flexibility 'in' to products life cycle continues to increase. To meet such demands, innovations are required throughout a products life cycle whether that be in discovery, development or manufacturing.

Within this presentation we plan to discuss one such innovation which is single use fermentation and the role that it can play in development and manufacturing to help manufacturers meet those demands. Using a combination of economic modelling (presented by GE Healthcare) and three industrial case studies (presented by Fujifilm Diosynth Biotechnologies), we plan to show how single use bioreactors up to the 500L scale are being used as a real alternative to conventional stainless steel bioreactors for both *E.coli* and *P. pastoris* type fermentations. We will show how single use fermentation achieves equivalent performance and reliability of conventional yet by retaining all benefits and flexibility of a single use format. We will also discuss when and where single use fermentation may make sense but also where it may not.

Notes

17:15 **Heat characteristics of stirred single-use bioreactors**

Matthias Müller[1], Wolfram Meusel[1], Ute Husemann[2], Gerhard Greller[2]

[1]Anhalt University of Applied Sciences, DE
[2]Sartorius Stedim Biotech GmbH, DE

Single-use equipment is mainly used in the biopharmaceutical industry where it is used for media preparation, storage, filtration and performing whole fermentations. The devices come presterilized thus they are ready to use for the dedicated application. Many of those applications need to take place under defined thermal conditions. For example during a fermentation it is needed to discard heat produced by the metabolic activity of the cells from the bioreactor. This topic becomes even more relevant when designing microbial processes. Even if current single-use bioreactors are mainly used for mammalian cell culture, microbial fermentations of *E. coli* and yeast are desired. Since such cells are growing several magnitudes faster, e.g. compared to CHO-cells, they produce a proportional greater amount of heat. Another frequently task single-use equipment needs to perform are fast heating and cooling cycles. This affects bioreactors as well as media and buffer storage tanks. Both situations face the drawback that additional cooling surfaces can hardly be introduced that are known from stainless steel tanks, e.g. cooling coils. Single-use systems often only transfer heat through the wall to a liquid cooled jacket. It is well known that the heat transfer to volume ratio worsens with increasing scale. One main difference between stainless steel vessels and bag-based single-use bioreactors is the film-layer, which acts as an additional barrier for the heat transfer. Although the film layer only has a thickness of about 100 to 400 µm the heat conductivity is with 0.33 W/(m K) low. Additionally, film-crinkles and a thin air layer between the film and the steel wall might further increase the resistance. The influence of the film layer is not yet characterized well giving the motivation to receive a deeper knowledge of the heat transfer capabilities of such reactor concepts.

In this work methods are described of how to characterize stirred single-use bioreactors by means of heat transfer. The general approach includes transient, as well as steady-state experiments according to the two main applications that occur during standard operation. Therefore both possible tasks of bioreactors are covered. The steady-state experiments are split in two sections. First, where the heat source is introduced via electrical heaters of defined power and second, where an exothermic chemical reaction is used. The chemical method has the advantage that it is more flexible by means of accessible geometry and scales. Both techniques are adequate to simulate the metabolic heat usually produced by the cells during a real cultivation. Transient experiments are performed simply by cooling the vessel content through the jacket. A heat balance is then set up where all major heat flows are present. By changing process conditions like stirring speed and jacket flow the effects of these changes are determined. The objective here is to estimate the heat transfer coefficient which is a suitable global parameter objectively describing the process of heat transfer. Finally results are shown, where the described methods are applied and compared at different scales, namely 5 L (glass vessel) and stirred single-use bioreactors of 50 L and 200 L. The data is then generalized using model equations and first attempts of scale-up considerations are given. Also the results are discussed in context of established correlations from VDI-Wärmeatlas to show the performance of single-use reactors compared to their counterparts produced from steel.

Notes

Parallel session 2	**Implementation of single-use technologies in the development and production of cell and gene therapeutics**	
Chair	**Daniel Gygax,** NTN Swiss Biotecg, CH	(Room GB 217)

15:35 **Novartis cell & gene therapy**

Christian Leist[1]

[1]*Novartis Cell & Gene Therapy, CH*

Novartis is fortifying the traditional oncology pipeline by implementing new technologies getting involved into personalized medicines. Making use of global logistics and distribution channels within Novartis represents a competitive advantage.

Cell and Gene Therapy represent a perfect approach for personalized medicines according to the definition of Cell & Gene Therapy (CGT) by FDA:
Gene therapy products are all products that mediate their effects

- by transcription and/or translation of transferred genetic material

and/or

- by integrating into the host genome and that are administered as nucleic acids, viruses, or genetically engineered microorganisms.

The products may be used to modify cells *in vivo* or transferred to cells *ex-vivo* prior to administration to the recipient.

For thes ex-vivo or in-vivo gene modifications currently replication incompetent viral vectors are applied. Common challenges for therapy development and application of viral vectors are:

- Developing tissue specific tropism of viral vectors
- Immune responses to viral capsids and transgene
- Transduction efficiency: intracellular transport to the nucleus, DNA conversion/translation
- Efficient and cell specific transgene expression
- Safety: Mutagenesis, activation of oncogenes, multiple injections (if required)
- Delayed adverse events as a consequence of persistent biological activity of the genetic material
- Long term observation of patients

The CGT pipeline of Novartis contains both, cell and gene therapy projects as well as a combination thereof. This combination, ex-vivo gene modified autologous Chimeric Antigen Receptor transduced T cells which are reinfused is the example of a cell based personalized cancer which will be presented here.

Notes

16:00 **Manufacturing of natural killer cell products for clinical applications**

Volker Huppert[1], Markus Granzin[1], Martha Elia Luevano Salinas[1], Regina Alex[1], Gerd Steffens[1], Mario Assenmacher[1]

[1]Miltenyi Biotec GmbH, DE

Although significant advances have been made for therapy of severe diseases, there are still indications with a medical need for improved treatment, including B cell acute leukemias ("B-ALL") and acute myeloid leukemia ("AML"). Cell based therapies now offer new approaches for treatment, utilizing natural or modified functions of stem cells or immune cells. Dendritic cells, antigen specific T cells and Natural Killer cells ("NK cells") have been evaluated in clinical trials and especially T cells modified with chimeric T cells ("CAR-T cells") have raised interest due to their reported efficacy in treatment of B-ALL. NK cells have demonstrated some clinical effect, especially in patients diagnosed with AML (Miller 2005, Rubnitz 2010, Leung 2013, Koehl 2015, Handgretinger 2016).

Manufacturing of a cell therapy product, i.e. a drug that contains living cells, typically requires special media, growth factors, cell modulation reagents, cell purification reagents, equipment and disposables that avoid contamination of the product and analytical protocols for in-process analysis and quality control of the final cell product. Cell Therapy products can be manufactured from cells donated by the patient ("autologous") or a healthy donor ("allogeneic"), and in the later case specifically for a single patient or generically for many patients ("off-the-shelf"). Those different settings contain individual arguments for preferred centralized or de-centralized manufacturing, depending on equipment required, transport distances acceptable (e.g. due to shelf life) and infrastructure.

NK cell products can be manufactured as an off-the-shelf product in an industrial scale or as an individual product for a specific patient. Patient specific NK cell products can be derived by isolation from a healthy donor apheresis harvest (McKenna 2012) or by cultivation. Both isolation and cultivation require disposables and devices providing appropriate scale and conditions (e.g. a closed system to minimize the risk of bacterial or fungal infection). Especially combinations of isolation and cultivation benefit from "all-in-one" devices and those devices also allow to perform viral transduction and/or final formulation steps (Apel 2013, Buckler 2016). The CliniMACS Prodigy System as an "all-in-one" system has been used to manufacture Dendritic cell, T cell, CAR-T cell (Mock 2016), hematopoietic progenitor cell and NK cell products (Granzin 2015).

Protocols for cultivation of NK cells have been improved and processed NK cells have been analyzed for their function (Granzin 2016), allowing further improvements for patient specific manufacturing in "all-in-one" devices or industrial manufacturing of "off-the-shelf" products. Universal manufacturing devices and related disposables in single-use technology can be used for manufacturing of a variety of cell therapy products both for patient-specific and centralized large scale manufacturing and will help to make cell based therapies more broadly available.

Notes

16:25 **Building the T cell factory of the future**

Clive Glover[1]
[1]*GE Healthcare Life Sciences, UK*

Autologous engineered T cell therapies are currently undergoing clinical trial to treat a variety of cancers. As these therapies are showing very strong clinical efficacy, there is a need to design new equipment and factories in order to ensure that the therapies are able to provided to the market in a safe and scalalbe way. This talk will describe the requirements to get a factory capable of delivering these exciting therapies.

Notes

16:50 **How to use Computational Fluid Dynamics in the development of cell therapeutics**

Valentin Jossen[1]

[1]*ZHAW, CH*

Computational Fluid Dynamics (CFD) is an established method in fluid mechanics that allows fluidic problems to be solved through numerical methods. In recent years, CFD has established itself as a modern tool in biochemical engineering, where it is mainly used to characterise and optimize devices (e.g. bioreactors, pumps, etc.). By using CFD, main bioengineering parameters (e.g. specific power input, mixing time, shear gradients) can be predicted independently on time and location. This allows defining process related parameters *in silico*, which reduces the number of experiments and costs. This is particularly important for the development of cell therapeutics, where the starting cell material is restricted and the batch costs are high. Recent economic reports have prognosticated a significant increase in cell therapeutics over the next few years, especially for human mesenchymal stem cells (hMSCs). This situation is also recognizable in the high number of clinical trials (269 trails, August 2016; clinicaltrails.gov) which are currently running with hMSCs for the treatment of e.g. myocardial infarct, Crohn's disease and graft versus host disease. However, large amounts of hMSCs are required for one single therapeutic dose (35-350 million cells per dose), which explains the demand for efficient and scalable *in vitro* expansion procedures.

Following a brief introduction of CFD, we aim to highlight the capabilities of CFD for the development of bioprocesses and scale-up procedures. For this purpose, we will show how CFD data can be used to support the scale-up of a microcarrier-based hMSC expansion process in stirred and wave-mixed single-use bioreactors. In this context, the advantageous combination of bioengineering and cell biological expertise will be presented.

Notes

17:15 **Evaluation of Eppendorf BioBLU single-use bioreactors for cell and stem cell culture and scale-up**

Sebastian Kleebank[1], Bin Li[2], Muriel Art[3], Kevin Han[2], Amanda Suttle[2], Francoise Delongueville[3], Stacey Willard[2], Ma Sha[2]

[1]Eppendorf AG Bioprocess Center, DE
[2]Eppendorf, US
[3]Eppendorf Application Center, BE

Single-use bioreactors gain increasing importance for cultivation of mammalian cells in biopharmaceutical research and manufacturing. Different technologies are in place which differ amongst others in the bioreactor material and in the way the culture is mixed. A rigid-wall stirred-tank vessel design is favorable, as it reduces the risk of issues caused by leachables and extractables and is especially suitable for the implementation of process scale-up. Different biopharmaceutical applications create different demands on the bioreactor systems, depending for example on cell type, working volume, and culture mode. In proof of concept studies we investigated the suitability of the Eppendorf BioBLU Single-Use Vessel portfolio for cell culture applications relevant in the biopharmaceutical industry: We exemplarily present results on the cultivation of stem cells, bioreactor scalability, and adaption for perfusion processes.
Cultivation of stem cells in highly controlled processes is crucial for the manufacturing of cell therapeutics. We successfully cultivated hMSCs on microcarriers using two rigid-wall stirred tank single-use vessels with maximum working volumes of 250 mL and 3.75 L, respectively. The cells retained their stem cell properties and their differentiation potential. Stem cell cultivation in a 3.75 L working volume facilitated the production of 1.6 x 10^9 cells per batch which is in the range estimated to be required per treatment dose in stem cell therapy.
In bioprocess development process scale-up is crucial. We investigated the scale-up capabilities of BioBLU Single-Use Vessels covering working volumes of 100 mL to 40 L. For different size vessels we compared critical parameters like k_La values, vessel geometries, mixing times, and fluid flow patterns. Besides volume scale-up, product amounts can also be raised by increasing the cell density in the culture. We present CHO process data at a 3 L scale in fed-batch and perfusion mode, respectively, demonstrating the usability of the stirred-tank single-use vessels for high-yield perfusion applications.
Taken together the presented examples demonstrate the suitability of the BioBLU Single-Use Vessel portfolio for diverse cell culture applications.

17:40 Exhibition and poster session (Building GA)

19:30 Conference dinner (boat trip on Lake Zurich)

Notes

Tuesday, 6th September 2016, Morning

08:30	Registration and coffee	(Building GA)
Chair	**Thorsten Peuker**, Sartorius Stedim Biotech, DE	(Room GA 203)

09:00 **Processes and factory of the future: Single-use and flexible for faster, cheaper and safer manufacturing**

Sue Walker[1]

[1]*Merck BioDevelopment, FR*

Despite the introduction of new technologies, the majority of Biotech processes and facilities still contain a number of stainless steel and multi-use equipment. We have made the decision to move away from this traditional setup and implement full Single-Use processes at Laboratory and manufacturing scale. This change from Multi-use to Single-use was developed in parallel with the facility revamping and a global strategic development of flexible facilities concepts.

We have developed an integrated USP & DSP that offers significant practical and economic advantages without sacrificing performance and robustness. This new holistic process can be run in either batch or continuous mode of operation. This single use leverages existing technologies with the potential to "change the game". A fed-batch process was performed in different types of disposable bioreactors in parallel with the revamping and installation of our hybrid facility. The process performances compared single use, glass and stainless steel bioreactors of different sizes including 3L, 50L, 200L, 1,250L, and 2,000L. Our study demonstrated the benefits of using disposable equipment in several key areas with a particular focus on Upstream activities. The comparison of stainless steel, glass and disposable equipment showed how comparable they are regarding titers, molecule quality…and how different they are regarding organization and financial aspects. The flexible factory concept includes a number of topics, such as single-use equipment implementation, facility design, modular vs. classical engineering, and enhanced flexibility. The concept allows for reduced capital expenditures while increasing facility modularity and adaptability to meet local market demand. Proper implementation of single-use technologies allows drug manufacturers to get the best possible outcome from those technologies: easy and fast modification of the set up for a variety of processes, increased capacity with rapid changeovers between batches, minimization of steam in place (SIP) and CIP steps with associated time and costs savings, ease of use, minimized contamination risk, and the ability to run some process steps closed and continuously with potentially lower cleanroom classifications. The integration of a new train at 2,000L scale in a flexible factory is presented as well as future development in flexible factories modular-based.

Notes

09:30 **Continuous GMP biomanufacturing**

Dirk Tillich[1]

[1] *Finesse Solutions AG, CH*

The current biopharma development and manufacturing models create challenges for the enterprise to meet their goals for high success rates, lower cost and faster time to market. With the paradigm shifting from titer to process optimization and analytics, the biopharma business model can be improved using continuous processing to manage complexity. Smart technology exists today which can increase flexibility, decrease cost, and improve production. Three prevalent perfusion platforms being utilized today will be introduced, and use cases on how best to match upstream and downstream capacity will be presented.

10:00 Coffee break and exhibition (Building GA)

Notes

Workshops and networking part 1 (Building GA and GB)

10:30 **W1: Setting single-use technology standards for biopharmaceutical production processes** (Room GB 217)

DECHEMA`s expert group single-use technology: Methods for the characterization of single-use bioreactors

Jörg Kauling[1], Stephan C. Kaiser[2]

[1]Bayer AG, DE
[2]Finesse Solutions, Inc., US

Even though a growing number of single-use bioreactors (SUB) has been introduced to the market over the last decade, there is a lack of engineering data available in the literature. Furthermore, the huge variety of SUB designs causes a lack of compatibility and comparability of the different systems amongst each other, as well as compared to their conventional counterparts. Finally, various methods to determine main engineering characteristics have been described in the past. This can make it difficult for users to compare the outcomes of different studies, which is increasingly perceived as a disadvantage by potential operators and customers who desire objective criteria for comparison.

The DECHEMA Upstream Processing (USP) expert group on "Single-use technology in biopharmaceutical manufacturing" developed standardized test protocols to determine the oxygen transfer rate (represented by the mass transfer coefficient, kLa), the mixing time (θ) and the power input (P) in single-use bioreactors. These protocols are based on published research studies and experiences within the companies and university institutes involved in the characterization of bioreactors. Even though the procedures were mainly developed for mechanically driven SUB, which include wave-mixed, orbitally shaken and stirred systems ranging from laboratory to production scale, they are also applicable for re-usable systems.

Based on a short outline about theoretical aspects of the methods applied, the workshop demonstrates the procedures to determine the kLa value and the mixing time in two stirred, single-use bioreactors at benchtop scale. The kLa value is determined using the dynamic gassing-out method, where special attention will be given to the response time of the sensors used. The mixing time is determined using the decolorization method, which is executed by the visual observation of the decolorization reaction. Potential mistakes during the experiments and the data evaluation of both methods are discussed.

Notes

DECHEMA`s expert group single-use technology: How to early identify critical bag films for cell cultures

Elke Jurkiewicz[1]

[1]*Sartorius Stedim Biotech, DE*

Over the last decades an increasing number of single-use products have been introduced on the biopharmaceutical market. However, besides many advantages it was reported that a variety of compounds, some of them with bioactive properties, migrate from disposable laboratory plastic ware into pharmaceutical formulations or process fluids. Since all these plastic materials consist of polymers which contain additives such as antioxidants, plasticizers, lubricants and stabilizers, biocompatibility assessment is absolutely necessary to avoid materials releasing potential harmful leachables into the process stream.

This workshop will focus on leachables and the biological tests used to evaluate their impact on mammalian cells. Advantages and drawbacks of existing tests will be discussed, giving guidance for future test protocols. Furthermore, case studies will be presented both from biopharmaceutical and other applications like food- and beverage systems and diagnostically equipment.

Notes

DECHEMA`s expert group single-use technology: Important points to consider when implementing single-use bioreactors: A risk analysis recommendation

Tobias Merseburger[1], Ina Pahl[2], Daniel Müller[3], Markus Tanner[4]
[1]ZHAW, CH
[2]Sartorius Stedim Biotech, DE
[3]Regierungspräsidium Tübingen, DE
[4]Werthenstein BioPharma GmbH, CH

Equipment in Pharma Industry made from Stainless steel is widely accepted and does not raise any concerns in terms of leachables or extractables. This is because extensive dates on physical and chemical properties are available. In addition the steel industry is continuous updating information on new developments.

Plastics and their manufacturing processes are rather complex. Often trade secrets does not permit to publish the details of the entire manufacturing process and used components. Even if the base for the plastic from two different suppliers is the same, that does not mean the final film is the same or comparable. Additives, slipping agents and different procedures for gamma irradiation are directing the properties of the final consumption material. Depending on the application, there could be a potential impact to cell growth, quality of the drug substance or drug product.

In order to assess the risks when using single use components, a solid risk analyses helps to make sure single used components or assemblies does not create growth inhibition or create a risk for a patient.

Notes

DECHEMA`s expert group single-use technology: Are single-use bioreactors for microbial products required?

Rainer Glöckler[1], *Katharina Blaschczok*[2], *Marco Leupold*[3], *Dieter Eibl*[2]

[1]*Swissfillon AG, CH*

[2]*ZHAW, CH*

[3]*Sartorius Stedim Biotech GmbH, Göttingen, DE*

Till now, if you have heard about single-use bioreactors mostly it was in combination with mammalian cell technology only! Is there really no market for microbial-based systems? Or do we not know much about how to use in combination with microbial systems? What about secondary metabolites? What about anaerobic systems or highly toxic products produced in small quantities by microbial systems? Is there really no need for microbial-based single-use bioreactors (SUBs)?

The DECHEMA Upstream Processing (USP) expert group on "Single-use Microbial Technology" developed a standardized test procedure to determine growth performance of a standard *E. coli* strain growing on glucose up to an OD_{600nm} of around 80 in a pure Batch fermentation to compare different single-use bioreactors based on growth performance. In addition, an Excel sheet was established to standardize calculation of critical growth parameters as k_La, OUR, CPR…! Even though the growth promotion test was mainly developed for mechanically driven SUBs, which include wave-mixed, orbitally shaken and stirred systems ranging from laboratory to production scale, they are also applicable for re-usable systems.

The workshop will start with a short summary of results obtained based on the k_La characterization of available "microbial-based single-use bioreactor systems" using the gassing out method of the DECHEMA Upstream Processing expert group on "Single-use technology in biopharmaceutical manufacturing". Furthermore, a method to characterize SUBs based on a standard microbial growth promotion test using an easy available *E. coli* strain will be discussed. Finally, first results will be presented using a single-use system compared to stainless steel and glass systems at scale.

Notes

Container closure integrity (CCI) for single-use systems (SUS): Regulatory trends, challenges and test methods

Carole Langlois[1]

[1]*Sartorius Stedim Biotech, FR*

Key Words: Container Closure Integrity, Helium Integrity Test, Point of Use Leak Test

Single-use fluid management solutions are being applied in critical downstream and final filling applications including applications requiring container closure integrity testing. Single-use bags are increasingly used for the storage, mixing, shipping, freezing of drug substances and drug products. Since single use bags are migrating towards more critical process steps and applications, there is a growing regulatory scrutiny and industry requirement for testing the containers in order to guarantee their integrity and their sterility before their use in commercial production processes.

This presentation will consider the regulatory trends for Container Closure Integrity of single-use systems and the challenges for vendors and users in the implementation of single-use systems in critical applications subject to container closure integrity testing.

The presentation will show a case study of the development of two different non-destructive test methods, one being a Helium test method at supplier, the second one being a point-of-use pressure decay leak test method.

The sensitivity, reproducibility and accuracy of each method are explored as well as the relevance to the required level of closure integrity.

All through the presentation, the emphasis will be given on container closure integrity as a concern throughout the product life cycle from its development, validation and manufacturing phase at supplier to its intended final use.

An analysis of the defect type expected at each step of the life cycle of a flexible bulk container is presented as well as a proposed implementation strategy for these test methods to manage the risks of defects.

Notes

10:30 **W2: Facility of the future** (Room GA 207)

Facility of the future – points to consider

Günter Jagschies[1]

[1]*GE Healthcare Life Sciences, SE*

Concepts for the facility of the future are driven by increased bioreactor productivity, responsiveness upon changes in production schedules and quantities or product portfolio to be manufactured, and in general the desire to reduce cost for manufacturing. We will lead you through a hierarchy of economic optimization opportunities and a process, equipment, and facility design approach that would allow to achieve the desired features of a future facility.

Notes

Connect upstream = Sartorius Stedim Biotech

Miriam Monge[1]

[1]*Sartorius Stedim Biotech, FR*

Sartorius Stedim biotech has developed the first fully integrated upstream platform. It connects a top performing expression system with outstanding equipment and process control for the rapid development and scale-up of robust, high titer commercial manufacturing processes. The 4 themes Speed to clinic + increased titers + Quality by Design + Robust production will be presented & discussed during this workshop.

Notes

10:30 **W3: Single-use technologies for the development and production of cell and gene therapeutics** (Room GA 215)

Single-use technologies enable closed and scalable cell therapy manufacturing

Antoine Heron[1]

[1]*Merck KGaA, DE*

Single-use technologies enable closed and scalable cell therapy manufacturing.

- Transitioning to a commercialization platform (from cell expansion to harvest and volume reduction)
- Challenges and opportunities across cell types (adherent/ suspension)
- Strategy for a safe and sustainable manufacturing (scale, raw materials, services).

1. Merck activities in field of Cell Therapies
   ➔ focus on hMSCs from cell expansion to harvest and volume reduction
2. Data generated by Merck for hMSCs / T-Cells
   ➔ The process is the product:
   - Importance to consider the process from phase I
   - Autologous / Allogeneic
3. Share experiences / challenges and drivers:
   ➔ ask question to audience: what are the 4 "pillars" and what is your understanding of these challenges?
   - COGs
   - Scalability
   - Sustainability
   - Quality

Conclusion

Notes

Biodegradable/implantable microcarriers for the chemically defined cultivation of human adipocyte-derived stem cells

Michele Müller[1], Ferruccio Messi[2]

[1]Micro-Sphere SA, CH
[2]Cell Culture Technologies, CH

Human adipose tissue-derived stem cells (hASCs) are mostly generated through conventional static adherent culture vessels under chemically undefined culture conditions. At present, protocols for hASCs expansion do not generate adequate high cell densities and quality for cell therapeuty use. Here we discuss on biodegradable/implantable microcarriers to be used with chemically defined media for the in vitro cultivation of hASCs.

Notes

The new Thermo Scientific™ Nunc™ High Density Cell Factory™ system to scale-up cells for a working or master cell bank

Iris Pavenstädt[1]

[1]*Thermo Fisher Scientific, DE*

In the Workshop we will present a customer application by using the new Thermo Scientific™ Nunc™ High Density Cell Factory™ system to scale up cells for a working or master cell bank. As CMO several companies outsource projects to receive high cell densities for antibody production, HTS and Stem Cells application. In this workshop we will present the whole workflow how to generate high cell densities e.g. 65 x 10 to 8 cells per harvest and freeze them in controlled conditions.

Notes

10:30 **W4: Continuous bioprocessing with single-use systems** (Room GA 217)

High performance single-use continuous microfiltration system for high cell density clarification

Ralf Daumke[1]

[1]*Filtrox AG, CH*

Midstream, defining the process step between upstream and downstream, is one of the most important steps in biotech processes. These work shop shows an efficient and economical way to clarify fermentation broths continuously and single-use with FILTRODISC™ BIO SD.

The goal of the selected method is to remove the cells and cell debris as well as to reach the maximum product yield in compliance with the existing regulatory environment.

Standard technologies (centrifugation, separators, membrane and depth filtration) can no longer handle the high particle loads (>10^8 cells/ml) in an economical way.

FILTRODISC™ BIO SD is the state of the art single-use system for cell removal from lab to production scale.

Notes

Integrated continuous cell culture utilising single use technologies

Sebastian Rothe[1]

[1]*GE Healthcare Life Sciences, CH*

In this workshop we will demonstrate how to set up and run a bioreactor with integrated perfusion capabilities for working volumes up to 25 L. Such set ups can be used for R&D, small scale GMP productions or for intensified seed train or cell banking.

Notes

The centrifugal revolution: Low-shear, pulsation free, precise control fluid management for different applications – Live demo and hands-on workshop

Adrian Ljutic[1]

[1]*Levitronix GmbH, CH*

In this workshop, the magnetically levitated pump and Ultrasonic flow measurement principles will be introduced, demonstrating their unique advantages for continuous bioprocessing. You will learn how to set up a very precise flow control system including non-invasive single-use flowmeters and reliable, non-pulsating, low shear force single-use (SU) pumps.

After this workshop you will have new possibilities to dynamically control and gently regulate the flow or pressure within your single-use disposable applications such as TFF and perfusion, depth filtration, final fill, chromatography, etc.

You will have a first look at our latest plug-and-play lab platform with the new fist-sized SU-Pump + Console able to manage flow rates from few ml/min up to GMP scale and the first high precision Single-Use Ultrasonic Inline Flowmeters on the market.

Company Introduction: Levitronix is the worldwide leader in magnetically levitated bearingless motor technology and offers single-use and multi-use pumps with a low shear design for sensitive fluids like cells and proteins for the Life Science market. The product range enables full manufacturing scalability from ml/min up to flow rates of 200 l/min. Levitronix also provides cost-effective clamp-on sensors and high-accuracy single-use inline flowmeters, both based on non-invasive Ultrasonic Technology.

Notes

Enhanced single-use clarification of CHO feedstocks using cadence acoustic separation technology

Beatrice Werlen[1]

[1]*PALL Life Sciences, CH*

Cadence Acoustic Separation is a gentle cell separation technology that enables clarification of CHO cell containing feedstocks. The technology is compatible with single-use operations and dramatically reduces the quantity of depth filtration required and the footprint of clarification operations. A demonstration will illustrate the mechanism of separation of this technology and the disposable flow path utilized.

Notes

Process intensification and continuous bioprocessing: Tools and solutions

Wolfgang Hohenauer[1]

[1]*Repligen, AT*

In our workshop we will highlight tools and solutions to achieve next level of productivity in Bioprocessing.

We will explain, show and discuss with you:

XCell ATF Systems, easy scalable cell retention systems for cell culture application. Now also available as pre sterilized SU version: Principles of operation and performance.

OPUS prepacked chromatography columns, including the ATOLL products, for resin screening, process development and GMP processes: Aspects to look into, when working with prepacked columns and how these columns can support continuous chromatography.

Repligen is a bioprocessing-focused life sciences company bringing over 30 years of expertise and innovation to our customers. We are inspiring advances in bioprocessing through the development and commercialization of high-value products and flexible solutions that address critical steps in the production of biologic drugs, principally monoclonal antibodies

Notes

10:30 **W5: Recent developments in the field of USP, DSP and PAT** (Room GA 219)

The orbitally shaken single-use bioreactor SB10-X

Tim Bürgin[1]

[1]*Adolf Kühner AG, CH*

The OrbShake SB10-X single-use bioreactor provides a new possibility for single-use cell-culture applications in the lab scale. The bioreactor has a volumetric working range of 3 – 12 L. It consists of a simple cylindrically formed 3D-bag. The implemented optical chemosensors for pH and DO allow the online measurement and control of pH and DO. Furthermore the control of medium temperature, shaking frequency and gas flow rate and mixture is possible.

The compact and functional designed control unit provides an easy handling and monitoring of the bioprocess. The control unit has an integrated and adjustable 12" touch screen with USB and Ethernet connection. The Kuhner Insight application software is user friendly and 21 CFR part 11 compliant. Three peristaltic pumps allow aseptic fluid management and the integrated thermal mass flow controllers for the gases enable a highly accurate control of the gas flow and mixture.

The SB10-X closes the scale-up gap between common disposable shake flasks and the Kuhner Shaker's OrbShake bioreactor family. Hence with the orbitally shaken technology a scale-up with disposable vessels starting in the µL scale (microtiter plates) all the way up to 2500 L (SB2500-X) is possible. This enables future facilities the possibility of a consequent, more flexible and efficient scale-up.

The workshop allows an insight of the bioreactor system SB10-X. It provides the possibility to get in touch with the bioreactor and to experience the possibilities in the shaken world.

Notes

eve® – answering the question "Why bioprocess software needed to change"!

Béla Brühlmann[1], Eric Abellan[1]

[1]Infors AG, CH

The traditional approach of separate Supervisory Control and Data Acquisition (SCADA) and database has been the de-facto-standard for bioprocess control. SCADA solutions, manly based on Windows® with user interfaces designed to either mimic standard Office applications or pictorial analogues of physical instrumentation. New devices, user interfaces and whole process-oriented ways of thinking are leading to a different solution for the twenty-first century.

The bioprocess platform software eve® takes this new path to a richer, more diverse blend of information, usability and integration of all bioprocess equipment, not only the bioreactor. Some key elements which illustrate this concept of "change for the better":

- A web-browser interface, familiar to anyone with any type of computer with a browser, allowing multiple, secure log-in of users independent from location and system.
- A NoSQL database, which allows much more than simple logged data to be stored and accessed. All the information about your complete bioprocess is in one place and easily accessible for analysis via the browser interface.
- Workflows which make defining the steps of a process match the ways in which biotechnologists think, so creating key stages and transitions is simple and intuitive.
- Close integration of the modern tools for bioprocess control, such as Design of Experiment (DoE) options and Process Analytical Technology (PAT) process – fully integrated not added on!

Come to this workshop and design your complex batch strategy within minutes.

Notes

The future of Protein A

Hans J. Johansson[1], Chris Major[1], Michael Kaleja[1]

[1]Purolite Life Sciences, UK

Protein A is still the work horse of almost all MAb processes and will continue to be the dominating capture resin for a long time. In this presentation Purolite will address different areas of Protein A affinity chromatography where we see substantial room for improvement:

1. Competition is the mother of invention. There are very few vendors with the capability to produce high performance agarose-based Protein A resins. To address this and facilitate second sourcing programmes Purolite is building a full scale manufacturing plant for production of a new, alkaline stable, Protein A resin.

2. Cost. One common concern is the high price of Protein A resins, especially in clinical manufacturing. For this purpose we have designed a cost effective resin based on a recombinant version of the native Protein A.

3. High eluting Protein A. Typically a MAb process involves elution at pH 3-3.5 followed by virus inactivation at a pH below 3.8. However, there are antibodies and antibody conjugates that are sensitive to acid conditions. For this purpose a new Protein A resin with a genetically engineered ligand allowing elution at a substantially higher pH has been developed.

4. Continuous manufacturing. One concern in multicolumn chromatography is the reproducibility of column packing. The use of a proprietary jetting technology that will generate agarose beads with a very narrow particle distribution will contribute and facilitate packing of columns with less column to column variation and improved pressure flow properties

Notes

Connected *in situ* measurement system

Yoann Gasteuil[1], Céline Vinson[1]

[1]smartINST, FR

The flow in a mixing tank like a bioreactor depends on several parameters as the reactor geometry, the fluid properties, the liquid volume, the impeller geometry and the rotation speed.

Numerical simulations help to optimize the processes, but they usually provide only a crude approximation of the real dynamics.

The smartINST system allows true measurement of the way each control parameter impacts the flow and therefore the mixing and internal reactor process. The system consists of the smartCENTER, which is the data logger, a data analyzer, and communication center with smartCAPS. The smartCAPS are embedded sensors, which freely move around the core of the fluid in order to monitor simultaneously, and in real-time, the evolution of products and processes. A smartCAPS in the reactor characterizes the flow and provides new possibilities for process optimization, scale-up and scale-down studies and reactor optimization.

The smartCAPS characterize the flow thanks to new data like agitation rate and hydrodynamic signature.

The agitation rate represents the energy supplied in the fluid. This rate is obtained in real time thanks to accelerometers embedded in the smartCAPS.

The hydrodynamic signature characterizes flow, providing detailed understanding of it, arising from the agitation rate statistics connected to control parameter settings.

It forms a measurable quantitative description of the flow, allowing:

- Flow comparison
- Detection of zones of very strong agitation, which can damage cells
- Detection of dead areas

Moreover, the wireless measurement devices, smartCAPS, measure also at the most relevant place: at the core of the fluid where the reaction is taking place. It can measure pH, turbidity, conductivity and temperature, within the process. This unique feature makes the system extremely reactive and reliable. Having the ability of in-line measurements of these parameters makes the instrument very suited for advanced process design using PAT and QbD approach. Such, it can also contribute to better controlled processes, being the basis of reproducible product quality.

Notes

Point of use integrity testing; the power of knowledge

Johannes Kirchmair[1], Rainer Marzahl[1], Camille Desrousseaux[1]

[1]Thermo Fisher Scientific, DE

The Power of Knowledge

As the value of product increases with each step downstream, sterility and leak detection for bioprocess containers (BPCs) becomes even more critical. The confidence of a final integrity test prior to use, at the point-of-use, is what the Thermo Scientific™ inSITE™ Integrity Testing System provides to customers. Our BPCs undergo rigorous quality checks and integrity testing before leaving the facilities, but true confidence can only be generated at point of use.

Quality Assurance

The advantages of the inSITE Integrity Testing System extend the level of Quality Assurance that Single Use Manufacturers employ to their end users. Although manufacturers maintain stringent standards for all of its products, such as raw material inspections, in-process pressure decay testing, sealing validations, packaging and shipping procedures and more, there is a potential for damage after the product leaves the manufacturers facility, specifically during bag handling and placement. The inSITE Integrity Testing System offers a level of confidence to biopharmaceutical manufacturers at the critical stage of fluid filling, ensuring that their product will enter an integral bag.

Pressure Decay Testing

Pressure decay refers to the change of pressure (P) inside a pressurized containment during a leak test. The test is an inflation test in which a bag is pressurized to a preset level. After the bag system has stabilized, the decay in pressure over time is evaluated to determine if a leak is present. The pressure decay method of testing was chosen due to its sensitive results and practicality at the point-of-use. In comparison to alternative methods of leak detection, pressure decay yields quantitative information and measurable data points that can be recorded and upon which decisions can be made.

12:30	Lunch, exhibition and poster session	(Building GC and Building GA)

Notes

Tuesday, 6th September 2016, Afternoon

Workshops and networking part 2 (Building GA and GB)

For workshop abstracts see "workshops and networking part 1"

13:30

W1: Setting single-use technology standards for biopharmaceutical production processes (Room GB 217)

DECHEMA`s expert group single-use technology / Sartorius Stedim Biotech

W2: Facility of the future (Room GA 207)

GE Healthcare Life Sciences / Sartorius Stedim Biotech

W3: Single-use technologies for the development and production of cell and gene therapeutics (Room GA 215)

Merck KGaA / Micro-Sphere SA and Cell Culture Technologies / Thermo Fisher Scientific

W4: Continuous bioprocessing with single-use systems (Room GA 217)

Filtrox AG / GE Healthcare Life Sciences / Levitronix GmbH / PALL Life Sciences / Repligen

W5: Recent developments in the field of USP, DSP and PAT (Room GA 219)

Adolf Kühner AG / Infors AG / Purolite Life Sciences / smartINST / Thermo Fisher Scientific

15:30 Coffee break and exhibition (Building GA)

Podium discussion and networking

Chair **James Dean Vogel,** The BioProcess Institute, US (Room GA 203)

16:00 **Standardization and single-use technology**

James Dean Vogel[1]
[1]*The BioProcess Institute, US*

16:15 **Podium discussion about standardization:**

John Boehm, Chair, BPSA, US

Jeffrey Carter, Director of Filtration R&D, GE Healthcare Life Sciences, US

Christel Fenge, Vice President, Sartorius Stedim Biotech, DE

Miriam Monge, Committee member, ISPE, FR

Wolfgang Paul, Senior Scientist, Roche Diagnostics GmbH, DE

Markus Tanner, Director Biotechnology, Werthenstein BioPharma GmbH, CH

17:00 **Poster awards and closing remarks**

Christian Hinderling[1]
[1]*ZHAW, CH*

18:00 – open end Barbecue with draft beer made by ZHAW (50.- CHF, interested participants are asked to send a registration mail to katharina.blaschczok@zhaw.ch) (Building GC)

Wednesday, 7th September 2016

DECHEMA working group meeting (By invitation only)

09:00	**DECHEMA working group "Single-use technologies in biopharmaceutical production"** Meeting	(Building GD 203)
10:00	Coffee break	

Post workshop 2016

The purpose of the workshop is to discuss the existing training opportunities in the field of flexible biopharmaceutical manufacturing with single-use systems and technologies. In addition, we would like to investigate requirements from both supplier and user perspectives. The goal is that the discussions from this meeting will be developed into a DECHEMA recommendation or position paper to be published in Engineering in Life Sciences.

Chair	**Karin Tiemann,** DECHEMA, DE	(Building GD 203)
10:30	**Welcome and introduction** *Karin Tiemann*[1] [1]*DECHEMA, DE*	
10:35	**What do suppliers need?** *Thorsten Peuker*[1] [1]*Sartorius Stedim Biotech, DE*	
10:55	**User requirements** *Stefan Robert Schmidt*[1] [1]*Rentschler Biotechnologie GmbH, DE*	
11:15	**Situation in the USA** *James Dean Vogel*[1]*, Eric Langer*[2] [1]*The BioProcess Institute, US* [2]*BioPlan Associates, Inc., US*	
11:35	**DECHEMA group and the ZHAW: Ongoing and planned activities** *Regine Eibl*[1] [1]*ZHAW, CH*	
11:55	**Summary and outlook**	
12:00	Lunch and informal discussions	(Building GD Mensa)

List of posters

Abstracts of posters

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Poster No. 1

Baculovirus based r-protein production in orbitally shaken flasks

Britta Badertscher[1], Ina Dittler[1], Katharina Blaschczok[1], Dieter Eibl[1], Regine Eibl[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

Insect cell culture coupled with the expression capacity of the baculovirus expression vector system (BEVS) is a well-established system for the production of biopharmaceuticals or biopesticides. The titerless infected-cells preservation and scale-up (TIPS) method promises to reduce the time and cost of the traditional insect cell BEVS production. In the TIPS approach, baculovirus-infected insect cells (BIICs) are generated, stored in liquid nitrogen, and used to infect insect cultures. The aim of this project is to establish the TIPS method using *Spodoptera frugiperda Sf*-9 cells infected with *Autographa californica* multicapsid nucleopolyhedrovirus (AcMNPV) for recombinant secreted alkaline phosphatase (rSEAP) expression in orbitally shaken flasks.

Expression studies were carried out with *Sf*-9 suspension cells growing in Sf-900 III SFM. A second virus generation of baculovirus AcMNPV encoding for the model protein rSEAP was used in the TIPS expression. In the first step of the TIPS infection, BIICs were generated by infecting shake flasks with a Multiplicity of Infection (MOI) of 3 pfu $cell^{-1}$, and harvesting and freezing of the infected cells after 7, 12, 24, 48 or 72 hours of infection (hpi) in order to establish a Master Cell Bank. Thawed BIICs were then used to infect *Sf*-9 cultures in 125 mL shake flasks by adding them with an estimated MOI (eMOI) of either 0.1 or 0.05 pfu $cell^{-1}$. In a second step the 48 hpi BIICs were used for a statistical evaluation of optimal infection parameters in 125 mL shake flasks modelled by MODDE 10.1.

All established BIICs resulted in a successful cell infection and revealed an infection profile dependent on their point of harvest. The highest SEAP activities of 136.9 ± 12.3 and 90.3 ± 17.3 U mL^{-1} were reached with the 24 and 48 hpi BIICs respectively. A statistical evaluation with the 48 hpi BIICs considering cell counts of infection (CCIs) between 0.5 and 2 x 10^6 cells mL^{-1} and eMOIs between 0.01 and 0.2 pfu $cell^{-1}$ revealed that high CCIs (2 x 10^6 cells per mL^{-1}) combined with low eMOIs (0.01 pfu $cell^{-1}$) result in the highest SEAP activities of > 150 U mL^{-1}. The results indicate that virus stability is improved and time and cost are reduced when using the TIPS method.

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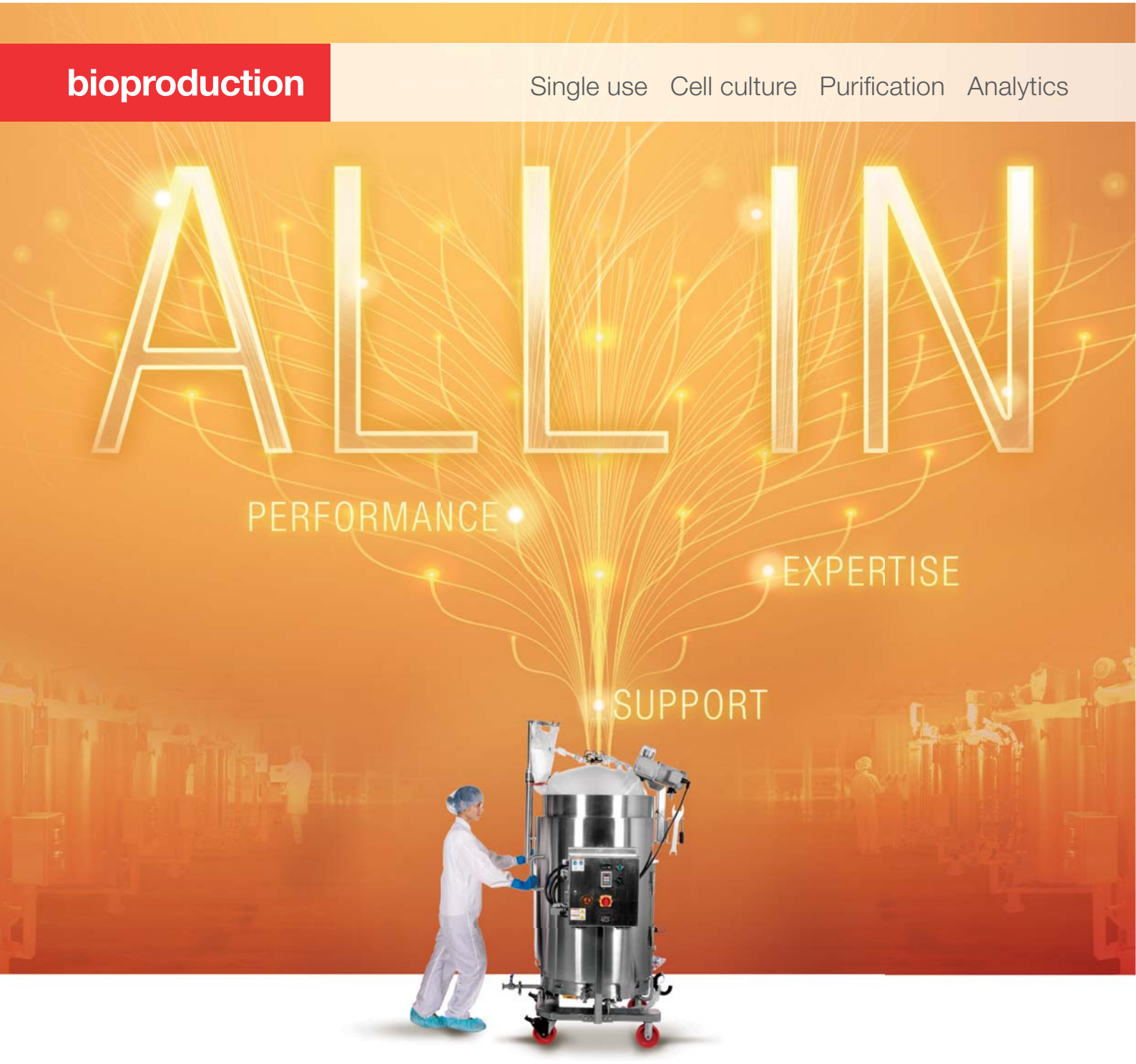

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Poster No. 2

Rational bioprocess design: a liaison between data evaluation and process simulation

Matthias Barmettler[1], Dominik Mächler[1], Iwo Zamora[1], Béla Brühlmann[1,2], Thomas Heimen[1,3], Straumann Marcel[1], Verena Looser[1], Karin Kovar[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

[2] present address: Infors AG, Bottmingen, CH

[3] present address: Lonza AG, Visp, CH

The development of bioprocesses typically requires many cultivation experiments. Consequently, translation of a product idea into a manufacturing process needs developmental times of 5-10 years, the long duration of which represents a major hurdle for the future success of biotechnologies.

The large amount and specificity of data generated while monitoring biotechnological cultivations is a challenge to process evaluation as proprietary software solutions and expert knowhow are required to extract the relevant information. Instead of manual steps, automated (optionally parallelised), standardised and, thus, efficient procedures are sought after to reduce the effort of evaluation and the number of experiments, so as to shorten developmental times and improve the chances of successful optimisation of a bioprocess.

This long-term conceptual research, which comprised several industrial and students' project over the last ten years, is aimed at developing and describing a generalised strategy to design bioprocesses for Pichia pastoris (Looser et al., 2015), a highly successful production system for manufacturing of biopharmaceuticals as well as industrial enzymes. As a basis for rational process development, the relationship between specific product formation rate (qp) and specific growth rate (μ) has to be described for each combination of host strain, recombinant protein and specific genetic construction. Experimentally determined product formation kinetics, i.e. qp=f(μ) are implemented in a generic process model, which is then used to perform simulation experiments aimed at identifying the optimum process strategy. This optimum strategy corresponds to the optimum μ, controlled at a certain set-point or a set-point-profile, and the optimum biomass concentration, through which, volumetric productivity (rp) reaches its maximum. Finally, an optimum feeding strategy to maintain the required optimum μ under bioreactor conditions is derived from the simulation.

Determination of the qp=f(μ)-relationship is a highly demanding experimental step in bioprocess development. Fundamental steps to accomplish a paradigm shift away from classical trial and error-based developmental strategies to more rationally designed and optimised bioprocesses (i.e. in alliance with the Quality-By-Design, QbD, initiative) are therefore: (a) efficient assessment of product formation kinetics in early stages of bioprocess development, and (b) knowledge (sensitivity analysis) about how different types of kinetic relationships affect the optimum process strategy.

Looser V., Bruhlmann B., Bumbak F., Stenger C., Costa M., Camattari A., Fotiadis D., Kovar K. (2015) Cultivation strategies to enhance productivity of Pichia pastoris: A review. Biotechnol. Adv. 33(6), 1177-1193.

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Poster No. 3

Scaling-up a model protein production process in stirred-tank single-use bioreactors from mL to L scale

Katharina Blaschczok[1], Sebastian Kleebank[2], Regine Eibl[1], Dieter Eibl[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

[2] Eppendorf AG Bioprocess Center, Jülich, DE

Single-use technology has become the state-of-the-art solution for rapid bioprocess development in the biopharmaceutical industry. Within the broad variety of bioprocess containers available on the market, stirred-tank bioreactors are particularly relevant as they still represent the gold standard for large scale manufacturing. For screening and optimization studies, small volume bioreactors with parallel operation are highly desirable, while good scalability to higher working volumes is a must.

In this study, we present data from a CHO cell-based model protein expression process using single-use stirred-tank bioreactors from Eppendorf. In a first step, 4-vessel parallel cultivations were performed in the BioBLU® 0.3c Single-Use Vessels (250 mL working volume) using the Eppendorf DASbox Mini Bioreactor System. Subsequently, the process was scaled up to the BioBLU 5c (3.75 L working volume), which was operated with an Eppendorf New Brunswick CelliGen® BLU Controller. This scale-up was based on the mixing time determined in previous bioengineering studies. Both bioreactor systems were controlled simultaneously via PC-based DASGIP Control software (now DASware® control 5).

Within the DASbox Mini Bioreactor System, we achieved excellent reproducibility with standard deviations < 5 % for the peak viable cell density (6.98 ± 0.16 x 10^6 cells/mL) and the maximum activity (33.7 ± 1.5 U/mL) of the model protein SEAP (secreted alkaline phosphatase). During parallel cultivations in the mL (BioBLU 0.3c with DASbox) and L scale (BioBLU 5c with CelliGen BLU) bioreactors, we were able to generate comparable results in regard to growth, metabolism and SEAP expression with standard deviations for critical process values < 10 %.

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Poster No. 4

Development and characterisation of the orbitally shaken pilot scale single-use bioreactor SB10-X and growth characterisations of *Sf*9 cells

Tim Bürgin[1], *Ina Dittler*[2], *Katharina Blaschczok*[2], *Simon Knobel*[1], *Andreas Richter*[1], *Tibor Anderlei*[1], *Dieter Eibl*[2], *Regine Eibl*[2]

[1] *Adolf Kühner AG, Birsfelden, CH*

[2] *Zurich University of Applied Sciences, Wädenswil, CH*

In the pharmaceutical market there is an increasing demand for single-use bioreactors to culture shear sensitive cells. A new orbitally shaken pilot scale single-use bioreactor SB10-X (Kuhner Shaker) was developed and characterised to demonstrate the suitability for shear sensitive cells. The determined k_La values were the crucial factor used to perform a scale-up process starting with a 250 mL Erlenmeyer flask, moving up to a 3 L Erlenmeyer flask and on to the SB10-X bioreactor (wv: 3 - 12 L). During this process growth characterisation tests with *Sf*9 cells were performed and the results from the different sized orbitally shaken vessels were compared.

Poster No. 5

Development and optimization of production of supercoiled plasmid DNA utilized in cancer immunotherapy in *Escherichia coli*

Daria Byrikhina[1], *Andrey Gosudarev*[1], *Fatima Izhaeva*[1], *Valentina Gusarova*[1], *Yury Novikov*[2], *Dmitriy Gusarov*[3]

[1] *Gamaleya Research Center of Epydemiology and Microbiology, Moscow, RU*

[2] *Information-Technology Center "Sartorius" – LLC "Sartoros", Moscow, RU*

[3] *Research-Educational Internet Portal «ExpertBIOTECH», http://expert-biotech.com*

Cancer remains one of the leading causes of morbidity and mortality worldwide. Immunotherapy is seen as the most promising approaches in cancer therapy including adoptive cell therapy which is one of the main treatment modalities within cancer immunotherapy. It is based on genetically modified lymphocytes possess chimeric T cell receptors (chimeric antigen receptors (CARs)) specific to tumor antigens. Large number of tumor-associated antigens which can be used separately or together to induce immune response against cancer has been identified to date.

Carcinoembryonic antigen (CEA)-secreting tumors are widespread among oncological diseases. High-level CEA-expression is observed in almost all cases of gastro-intestinal cancer, in 70% of non-small cell lung cancer and 50% of breast cancer and in other types of cancer.

Recent topic describes development and optimization of robust and cost and time effective production process of supercoiled plasmid DNA which is capable of *in vitro* transfection of patients with CEA-secreting tumors lymphocytes and may have therapeutic effect on oncological pathology. In this work we have demonstrated the feasibility and advantages of using a Wave-mixed bag bioreactor in fed-batch mode for *E. coli* strain cultivation. The downstream stage in production process consisted of: cell lysis, cell debris separation, three-step chromatographic purification procedure, buffer exchange and sterile filtration steps. All purification steps were elevated after investigation of influence of parameters and conditions on process and product yield and quality. On top of that, the composition of the formulation buffer was optimized and mixture stability during storage was controlled.

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Poster No. 6

A novel approach to increase efficiency of insect cell baculovirus expression vector system-based production processes

Ina Dittler[1], *Britta Badertscher*[1], *Katharina Blaschczok*[1], *Tim Bürgin*[2], *Tibor Anderlei*[2], *Peter Neubauer*[3], *Dieter Eibl*[1], *Regine Eibl*[1]

[1] *Zurich University of Applied Sciences, Wädenswil, CH*
[2] *Adolf Kühner AG, Birsfelden, CH*
[3] *TU Berlin, Department of Biotechnology, Berlin, DE*

Insect cell baculovirus expression vector systems provide a suitable production system for biopharmaceutical applications such as the production of recombinant protein complexes and virus-like particle vaccines. As the importance of rapid vaccine development and production processes increases, new strategies for efficient baculovirus-based production technologies need to be investigated. Among these is the implementation of single-use (SU) bioreactors, which have been proven as cost-effective (48 % less capital cost), scalable, ready-to-use systems. A further strategy for increasing efficiency is the use of the titerless infected-cells preservation and scale-up (TIPS) method as an alternative to the classical 2-phase production process (Phase 1: growth phase (1-2 days), Phase 2: production phase (3-5 days)). This method advantageously dispenses with the growth phase of the production process, eliminates virus titer determination, and enables stable storage conditions for the baculoviruses.

In this study, the TIPS method was successfully scaled up for orbitally shaken SU bioreactors for the first time. Firstly, the TIPS method was established with *Spodoptera frugiperda Sf*9 insect cells infected with the recombinant baculovirus *Autographa californica* multicapside nucleopolyhedrovirus (rAcMNPV) to express recombinant secreted alkaline phosphatase (rSEAP) in 125 mL shake flasks. After process establishment, the TIPS method and the 2-phase production process were conducted in 3 L shake flasks and in the orbitally shaken SU bioreactor SB10-X (Kuhner AG) at similar process engineering parameters. The maximum rSEAP activities of 144 – 158 U mL^{-1} and 112 – 128 U mL^{-1} were obtained for the TIPS method on day 4 post infection (pi) and for the 2-phase production process on days 6 – 7 pi respectively.

Poster No. 7

The development of a production scale technology of recombinant follicle stimulating hormone bioprocess in CHO cell line

Andrey Gosudarev[1], Daria Byrikhina[1], Fatima Izhaeva[1], Valentina Gusarova[1], Dmitriy Gusarov[2], Yury Novikov[3]

[1] Gamaleya Research Center of Epydemiology and Microbiology, Moscow, RU

[2] Research-Educational Internet Portal «ExpertBIOTECH», http://expert-biotech.com

[3] Information-Technology Center "Sartorius" – LLC "Sartoros", Moscow, RU

The problem of infertility is one of the most pressing health issues in Russia and in the World. It is estimated that every sixth couple in our country is barren. Finding a solution to this problem is in both the clinical issues (creation of new drugs, new treatment regimens), and in the field of embryology (new media and methods of embryo culture, new tools and equipment).

One of the promising drugs for the treatment of infertility is based on the preparations of follicle stimulating hormone (FSH) which are used to treat infertility in women since the mid-twentieth century.

The research discusses the development of a production technique for recombinant follicle-stimulating hormone based on the biosynthesis in the culture of Chinese hamster ovary (CHO) cells. As part of the creation of technological platform receiving hormone the bioprocess in wave-induced motion bioreactor has been optimized. A number of parameters that influence on the bioprocess were studied, as follows: optimal cell density, concentration of metabolites, temperature shift and its duration.

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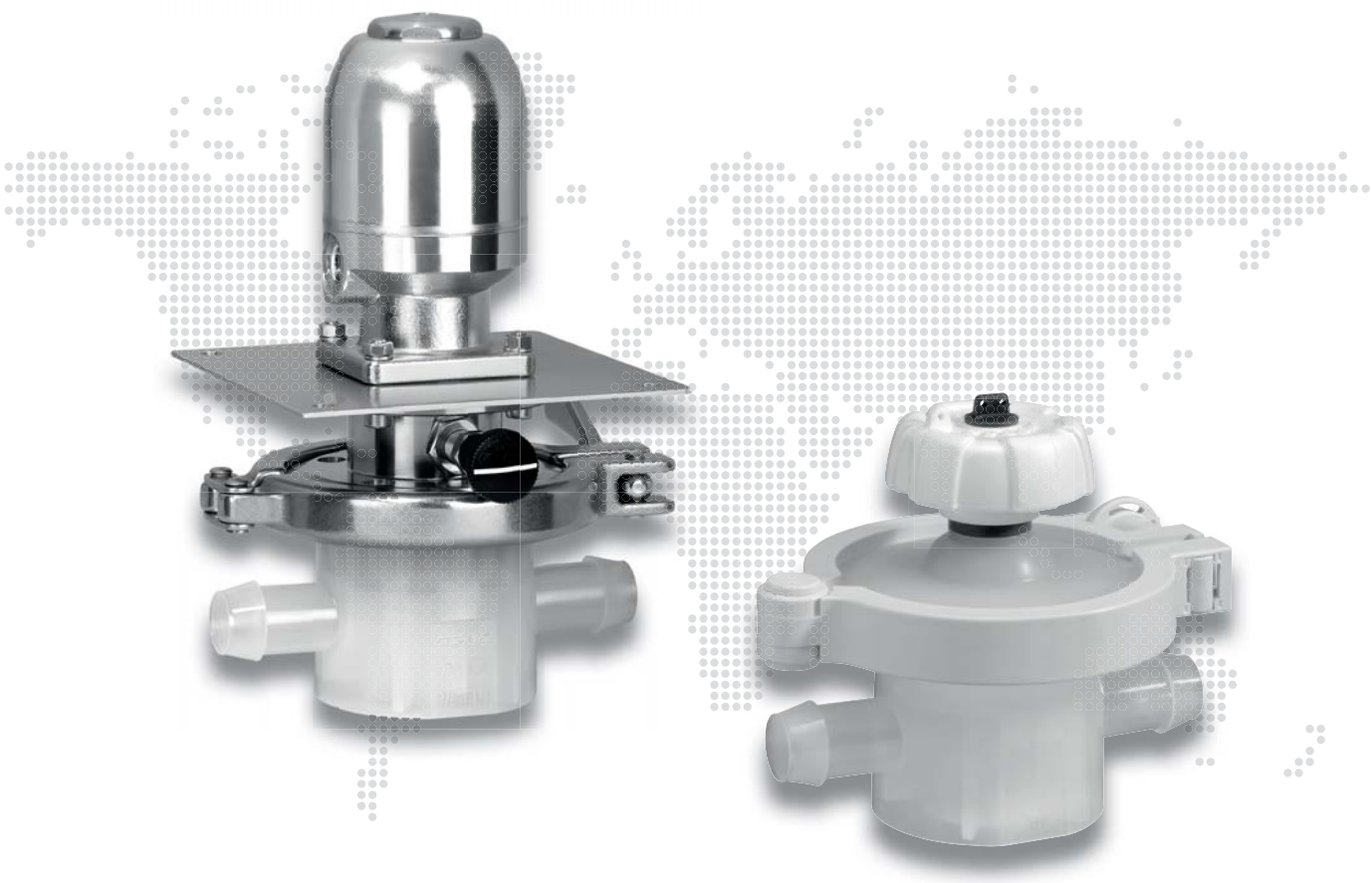

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Poster No. 8

3D printing technology for medical-pharmaceutical applications

Alexander Hämmerli[1], *Simona Iliev*[1], *Luginbühl Vera*[1], *Andrea Baier*[1]

[1] *Zurich University of Applied Sciences, Wädenswil, CH*

3D Printing (3DP) reveals opportunities for new applications within personified medicine and pharmaceutical technology. The two most auspicious 3D printing technologies are Fused Deposition modelling (FDM) and 3D Inkjet powder printing (3DPP). 3D printed implants based on polymer and polymer-coated bioceramics have become increasingly important. Patient-specific 3D printed implants can be produced with full functionalities and implemented active pharmaceutical ingredients for osteo-inductive bone regeneration. For 3D printed personalized bone grafts, magnetic resonance imaging data are the best available sources.

FDM 3D printing is an additive manufacturing method. It is based on melting thermoplastics and defined positioning in X, Y and Z directions. Precursor hot melt extruded polymer cable, entitled a filament, is the feed material, where the polymers used must meet biocompatability or biodegradability requirements. The latter has the potential to avoid a second surgical procedure for removal of a medical device.

As a further application of FDM 3D printing, thermostable active pharmaceutical ingredients can be incorporated into filaments. The result is a locally supported active substance e.g. for bone regeneration, and simultaneous reduction in systemic administrations. Heat stability during hot melt extrusion and the 3D printing process may be a limitation.

3D Inkjet powder printing is a low temperature additive manufacturing method. Pre-treated osteo-conductive bioceramic granules become etched with the elected liquid binder system. Layer by layer deposition of granules builds up the 3D structure. In-process insertions of small molecules, as well as biopharmaceuticals are advantageous. Regulation of the quantity and composition of the binder-/active substance- system enables the production of highly specific personalized implants comprising a variety of biomolecules.

Poster No. 9

Single-use membrane adsorbers in the recombinant human growth hormone production

Fatima Izhaeva[1,3], Daria Byrikhina[1], Andrey Gosudarev[1], Valentina Gusarova[1], Dmitriy Gusarov[2], Kirill Krivenko[3], Yury Novikov[4]

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Somatotropin, also known as growth hormone, is a protein hormone produced in the anterior pituitary glande. Somatotropin is involved in several metabolism processes, and it is extremely important for the growth of bones and muscles. Deficiency of growth hormone results in growth failure and short stature in children, and in adults it increases fat, increases risk of heart disease and weak heart, muscles and bones.

Today the recombinant somatropin is used for the treatment of growth failure and short stature, Shereshevsky-Turner syndrome, Prader-Willi syndrome, Noonan syndrome. Somatropin is also used to prevent severe weight loss in people with AIDS, or to treat short bowel syndrome, and in complex therapy for the treatment of chronic kidney failure.

This research is refer to the production of recombinant somatropin in the *E.coli.* Such process include biosynthesis of somatropin in bacterial cells as inclusion bodies (IBs) and further purification of protein in several steps. As a part of purification strategy the procedure of somatropin capture and polishing with applying the single-use membrane adsorbers was considered. The membrane adsorbers are a kind of ion-exchange chromatography and they are becoming more popular in the downstream processes. In this work the separation ability of membranes from different manufacturers with different ligands was compared.

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Poster No. 10

Insect cell-based rSEAP production in a wave-mixed single-use bioreactor with 3D-motion

Sandra Jäggi[1], Ina Dittler[1], Renate Lombriser[1], Katharina Blaschczok[1], Regine Eibl[1] and Dieter Eibl[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

In recent years the cultivation of insect cells in combination with the baculovirus expression vector system has become increasingly important for the production of biopharmaceuticals and biopesticides. For this purpose, scalable stirred and, in particular, wave-mixed bioreactors with 1D-motion have been advantageously applied. PALL recently introduced the XRS 20, the first waved-mixed bioreactor with 3D-motion. It has already been successfully used for the cultivation of mammalian suspension cells from 2 to 25 L working volume.

In this study, cell growth and protein production experiments were conducted with *Spodoptera frugiperda* (*Sf*-9) suspension cells, a recombinant model baculovirus (*Autographa californica* multicapsid nucleopolyhedrovirus), *Sf*-900 III SFM containing Pluronic F-68 and L-Glutamine, and the XRS 20 (maximum 20 L working volume) for the first time. Cell maintenance, seed inoculum production, virus generation and amplification were executed as described by Imseng et al. 2014 [1]. The XRS 20-based cell growth studies running over 11 days at a rocking angle of 15 ° in X direction and 5 ° in Y direction, and a rocking rate ranging from 18 to 28 cpm, were performed in feeding mode and at an initial cell density of 1×10^6 cells mL^{-1}. Similarly, in the subsequently carried out productions of the recombinant model protein, the secreted alkaline phosphatase (rSEAP), the XRS 20 was operated at 27 °C and the minimum DO was set to 50 %. The model protein secretion was induced by adding the working virus of the generation V_2 at a MOI of 0.01 pfu $cell^{-1}$ and a CCI of 2×10^6 cells mL^{-1}. Samples were taken daily in order to determine cell growth (Cedex HiRes), substrate and metabolite concentrations (BioProfile 100 Plus), and product formation (monitoring of rSEAP activity as reported in [1]).

All the experiments resulted in expected cell densities and rSEAP activities. The cell productions delivered peak cell densities of $13.33 \pm 0.49 \times 10^6$ cells mL^{-1} on day 7. The maximum rSEAP activity of 96.49 U mL^{-1} was obtained on day 9 post infection. Future studies will focus on rapidly realizable increases in process efficiency for the XRS 20 and insect cell-based production processes.

[1] Imseng, N., Steiger, N., Frasson, D., Sievers, M., et al., Single-use wave-mixed versus stirred bioreactors for insect-cell/BEVS-based protein expression at benchtop scale. Eng. Life Sci. 2014, 14, 264-271.

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Poster No. 11

Purpose-designed resins in prepacked, disposable columns substantially lower MAb processing costs

Hans J. Johansson[1], *Hans Berg*[1], *Patrick Gilbert*[1], *Mark Hicks*[1], *Caroline Tinsley*[1], *Charlotte Vassay-Jones*[1]

[1] *Purolite Life Sciences, Llantrisant, UK*

The cost of Protein A resins is very high, commonly 5-10 times higher compared to standard chromatography resins. One way to address this issue is to use a less expensive Protein A resin during early clinical trials, where the risk of failure is higher and fewer cycles are run, and subsequently switch to a resin designed for manufacturing if the product makes it to PIII and beyond. The higher cost for a manufacturing resin is amortized over a large number of purification cycles. To avoid any increasing regulatory burden offsetting the potential savings, it is important that the two types of resin perform in a very similar way with respect to purification performance.

Two different Protein A resins have been used in the study. Both resins are based on the same chemistry and the same alkaline-stable Protein A ligand. This should make the transition from one resin to the other very straight forward. For the particular resin designs used in the study, the only performance differences are dynamic binding capacity at higher residence times and long term CIP stability. Characterization and performance data of the Protein A resins will be presented, including comparability data with respect to host cell proteins, DNA, leaked Protein A and aggregate content.

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Poster No. 12

Expansion of human adipose tissue-derived stromal/stem cells in a wave-mixed single-use bioreactor: a proof-of-concept

Valentin Jossen[1], Cedric Schirmer[1], Regine Eibl[1], Matthias Kraume[2], Ralf Pörtner[3], Dieter Eibl[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

[2] Berlin University of Technology, Berlin, DE

[3] Hamburg University of Technology, Hamburg, DE

Cell-based therapies have become increasingly important in the field of regenerative medicine, as global revenues of approximately 1 billion US$ indicate. A special focus in the clinical field is placed on human mesenchymal stem cells (hMSCs), especially on those derived from the adipose tissue. Due to their clinical efficacy and safety, they can be used for allogeneic cell therapies (e.g. acute myocardial infarct, orthopaedic surgery). It therefore comes as no surprise that a large number of Phase 1, 2 and 3 clinical trials are currently running with them. However, the required number of therapeutically active human adipose tissue-derived stromal/stem cells (hASCs) for allogeneic applications is in the range of one trillion cells per year. Thus, alternatives to the 2-dimensional planar cultivation systems, typically applied to propagate hASCs, and suitable scale-up strategies are urgently required. Recent studies have demonstrated the applicability of stirred and wave-mixed single-use bioreactors as a promising alternative to the planar systems. In these dynamically mixed systems, the required growth surface for the adherent growing cells is provided by microcarriers (MCs), which are suspended in the bioreactor. Besides the high volume to growth surface ratio, these systems allow measurement and control of important culture parameters (e.g. DO, pH) online. Although these systems are often used in combination with MCs for the production of vaccines, working with hASCs is more complex due to their high shear sensitivity. This raises the question of how such an MC-based expansion process of hASCs can be performed in wave-mixed single-use bioreactors.

A proof-of-concept study is presented for hASCs in a wave-mixed single-use bioreactor (2L; benchtop scale). The optimum microcarrier-medium combination was determined in extensive screening studies at mL-scale, based on design of experiment. In addition, experimental investigations and Computational Fluid Dynamics simulations were performed for the BIOSTAT CultiBag RM 2L in order to find suitable conditions for cell expansion. The proof-of-concept cultivation resulted in peak cell densities of $1.9 \cdot 10^5$ hASC mL^{-1} ($2.85 \cdot 10^8$ hASCs) in the BIOSTAT CultiBag RM 2L without any loss of stem cell properties. The established processes can serve as a basis for allogeneic therapies with hASCs.

Poster No. 13

Impact of shear stress on cell growth and microcarrier-cell-agglomerate formation in microcarrier-based cultivations of adipose tissue-derived stromal/stem cells

Valentin Jossen[1], Regine Eibl[1], Matthias Kraume[2], Ralf Pörtner[3], Dieter Eibl[1]

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[2] Berlin University of Technology, Berlin, DE

[3] Hamburg University of Technology, Hamburg, DE

Cell-based therapies have become increasingly important in the field of regenerative medicine, as global revenues of approximately 1 billion US$ indicate. A special focus in the clinical field is placed on human mesenchymal stem cells (hMSCs), especially on those isolated from the stromal vascular fraction of the adipose tissue. Due to their good clinical efficacy and tolerability, they can be used for allogeneic cell therapies (e.g. Crohn's disease, graft versus host disease, acute myocardial infarct). However, the required number of therapeutically active human adipose tissue-derived stromal/stem cells (hASCs) for allogeneic applications is in the range of trillion cells per year. Thus, alternatives to the 2-dimensional planar cultivation systems, typically applied to propagate hASCs are urgently required. Different studies have recently demonstrated the applicability of stirred single-use bioreactors as a promising alternative to the planar cultivation systems. In these dynamically mixed culture systems, the required growth surface for the adherent growing cells is provided by microcarriers (MCs), which are suspended in the bioreactor. Although adherent mammalian cells are well investigated in combination with MCs for the production of vaccines, working with hASCs is more complex due to their higher shear sensitivity. This raises the question of how the induced fluid shear stress affects the cell expansion, the cell quality and the formation of MC-cell agglomerates during the cultivation in dynamic bioreactors.

For this purpose, MC-based cell expansions were performed in single-use spinner flasks at different impeller speeds below (25, 43 rpm) and above (90 and 120 rpm) the specific suspension criteria N_{S1u} (49 rpm) and N_{S1} (63 rpm) for polystyrene-based MCs. Cell numbers, metabolites and the formation of MC-cell agglomerates were measured during the cell expansion process. Furthermore, the expression of standard surface markers (CD14, CD20, CD34, CD45, CD73, CD90, CD105) for hASCs were analysed after harvesting of the cells. Beside the biological investigations, Computational Fluid Dynamics simulations were performed in order to predict the velocity gradients and the hydrodynamic forces. The results indicated that too low (25 rpm) and too high impeller speeds (120 rpm) result in statistically significant lower cell densities (0.81 and 0.25 x 10^6 cells/mL) compared to those at the suspension criteria (1.25 and 1.11 x 10^6 cells/mL). These lower cell densities can be ascribed to mass transport limitations at low impeller speeds and to too high hydrodynamic strains at high impeller speeds. The effect of the shear stress on the MC-cell agglomerate formation at high impeller speeds was not significant, when compared the Sauter mean diameters to those resulting at the suspension criteria. In contrast, impeller speeds below the suspension criteria resulted in a strong MC-agglomerate formation, which may support mass transfer limitations.

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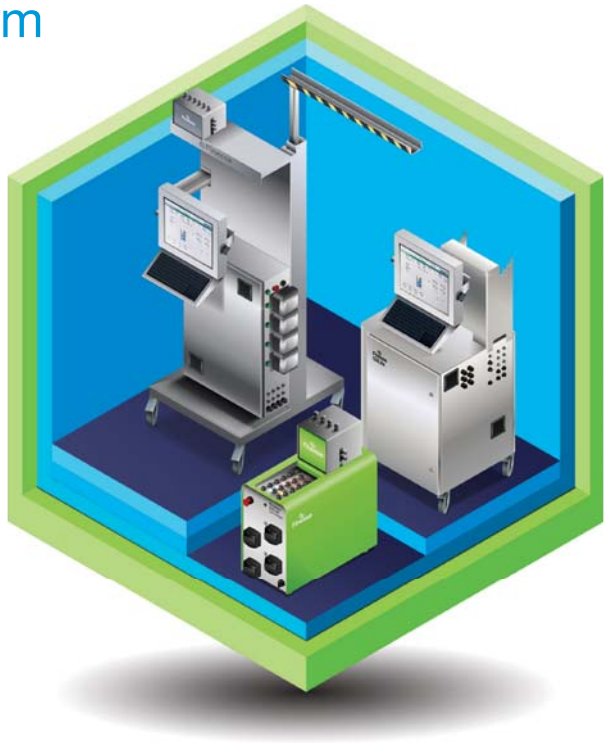

Poster No. 14

Comparison of the SmartGlass™ and SmartVessel™ 3L bioreactors – engineering data and cell cultivation results

Stephan C. Kaiser[1], Philipp Meier[2], Valentin Jossen[2], Dieter Eibl[2]

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[2] Zurich University of Applied Sciences, Wädenswil, CH

In this study, the engineering data about power inputs, mixing times and oxygen transfer rates in the single-use Finesse SmartVessel™ 3L bioreactor are compared with its reusable counterpart, the SmartGlass™ 3L bioreactor. With identical main geometrical parameters of the tank and impellers, the plastic bioreactor mimics its glass counterpart as close as possible. Nevertheless, small modifications are required due to manufacturing of the plastic bioreactor. Furthermore, the SmartVessel™ bioreactor is equipped with wall-mounted single-use sensors, whereas conventional probes are used in the SmartGlass™ bioreactors.

Using standard experimental methods, specific power inputs (≤ 76 $W \cdot m^{-3}$), mixing times (≥ 5 s) and k_La values (≤ 8 h^{-1}) were determined for cell culture typical operational conditions in both bioreactors. Good agreements were found between both bioreactors, irrespective of the selected agitation and aeration parameters, which is supported by flow field analyses realized using Computational Fluid Dynamics (CFD) models.

Data from material tests, as recommended by the DECHEMA working group, indicated no negative effects of the plastic materials on cell growth and metabolism of two industrially relevant insect and mammalian cell lines. This was also confirmed by first cultivations of a transfected Chinese hamster ovary cell line (CHO XM111-10) expanded in chemically defined minimal medium, where peak viable cell densities of up to $6.4 \cdot 10^6$ $cells \cdot mL^{-1}$ were achieved in a fed-batch process.

Poster No. 15

A new biotechnological system for mass production of rosmarinic acid based on cell cultures of *Satureja khuzistanica*

Abbas Khojasteh[1,2], Javier Palazon[1], Regine Eibl[2], Rosa M. Cusido[1]

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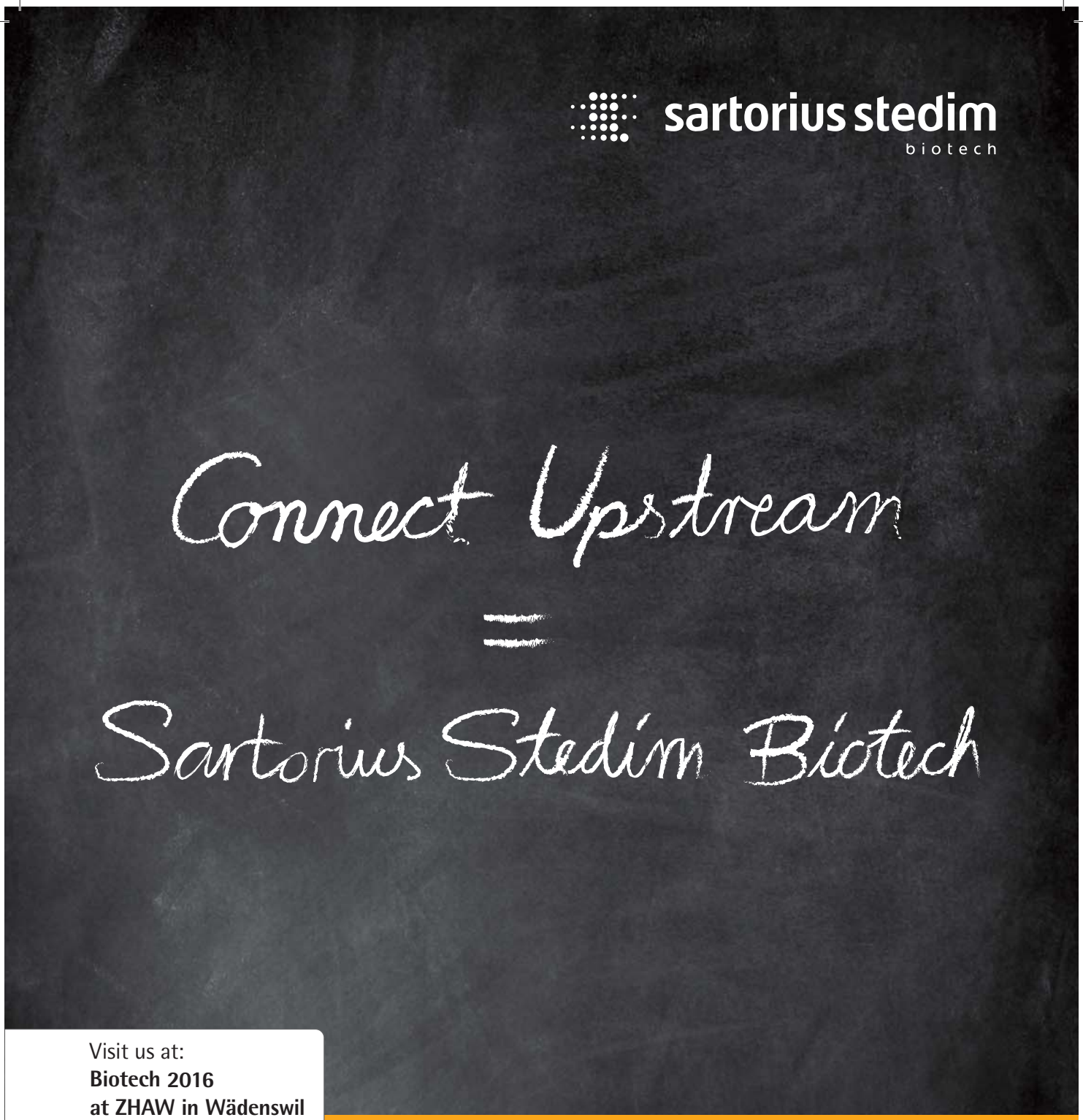
Rosmarinic acid (RA), an ester of caffeic acid and 3,4-dihydroxyphenyl lactic acid, is widely distributed in the plant kingdom, including *Satureja khuzistanica*, a native Iranian plant species. Interest in RA as a pharmaceutical or dietary supplement is growing with increasing awareness of its potential benefits for human health. Among its most promising biological activities are cognitive-enhancing and cardioprotective effects, cancer chemoprevention properties and a potential use in the treatment of Alzheimer's disease. The current possibilities that plant biotechnology offers for the production of plant secondary metabolites and the important bioacative properties of RA have prompted many researchers to establish RA-producing biotechnological platforms, such as shoots, cell suspensions and hairy root cultures of numerous plant species. In order to meet the growing demand for RA and to preserve its natural sources, the aim of this work was to develop a new biotechnological platform for RA production based on elicited cell suspension cultures of *S. khuzistanica* scaled up to a 2 L single-use wave-mixed bioreactor.

To achieve this goal, we established a cell line of the RA-producing plant species *S. khuzistanica*, which was cultured in B5 medium supplemented with 30 g L^{-1} sucrose, 20 mg L^{-1}, L-glutamine, 200 mg L^{-1} casein hydrolysate, 5 mg L^{-1} BA and 1 mg L^{-1} IBA, in 125 mL shake flasks, with a working volume of 25 mL and an inoculum pcv of 10%. The culture conditions were: 25ºC, 110 rpm, and darkness.

The results obtained in shake flasks show that *S. khuzistanica* cell suspensions synthesized high amounts of RA, which accumulated mainly inside the cells. In order to improve the production of this biotechnological system, the effect of two elicitors, methyl jasmonate (MeJA) at a concentration of 100 µM and cyclodextrin (CD) at a concentration of 40 mM, were tested, individually or in combination. MeJA increased RA productivity more than 3-fold, reaching an RA production of 3.9 g L^{-1}, without significantly affecting the system's biomass productivity. CD did not have a clear effect on RA production, and under the combined treatment of MeJA + CD only a small amount of RA was released to the culture medium. When the process was scaled up to a wave-mixed BioSTAT CultiBag RM of 2 L (WV of 1 L) working in batch mode, a maximum RA production of 3.6 g L^{-1} and a biomass productivity (CDW) of 22.4 g d^{-1} was achieved, demonstrating the suitability of *S. khuzistanica* cell suspensions for the biotechnological production of this bioactive plant secondary metabolite.

In conclusion, we can infer that plant cell cultures of *S. khuzistanica* represent an excellent biotechnological platform for RA production, having achieved a content of up to 3.9 mg L^{-1}, one of the highest production levels of this bioactive compound reported to date. Scaling up the process to a wave-mixed BioSTAT® CultiBag RM improved both the biomass and RA production of the system, thus confirming the suitability of the wave-mixed bioreactor for the culture of *S. khuzistanica* cells.



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Poster No. 16

Evaluation of a stirred small scale single-use bioreactor for microbial applications

Marco Leupold[1], *Thomas Dreher*[1], *Ute Husemann*[1], *Mwai Ngibuini*[2], *Gerhard Greller*[1]

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KEYWORDS: Single-use stirred tank bioreactor, process engineering, *Escherichia coli*, high cell density cultivation

For a successful process transfer, as well as for bioprocess development and optimization a suitable small scale approach is essential to reduce time and costs. To further increase the efficiency of these transfers single-use technology is very attractive. Unfortunately, small scale single-use systems often cannot mimic the conditions of established bioreactors, in terms of oxygen transfer and heat removal, especially, for microbial applications. Consequently, having such a model in small scale is of special interest.

Based on mentioned limitations, a small scale single-use bioreactor, the ambr® 250 modular (250 mL maximal working volume), was developed and evaluated. The design criteria and geometrical ratios are within the same range of established bioreactors. By its compact design the bioreactor, where all needed parts and reservoirs are already implemented, is easy to set up. Due to integrated pumps, processes with high automation efforts can be realized. A further feature is the integrated exhaust gas analyzer, fulfilling all requirements for PAT. For a further characterization the specific power input, the mixing time, the heat transfer and the volumetric mass transfer coefficient (k_La) were determined. Afterwards, an industrial relevant high cell density *Escherichia coli* fed batch cultivation was performed.

The process engineering characterization revealed that the system has a performance comparable to established multi-use bioreactors. For example a k_La of 400 h^{-1} was achieved (OTR_{max} = 410 mmol/(L·h)), determined by gassing out method. During the following *E. coli* cultivation a maximal OD_{600} of 360 (dry cell weight = 133 g/L) was reached and an expression of a target protein was demonstrated. The results of the exhaust gas analyzer showed that a maximal oxygen uptake rate of 600 mmol/(L·h) was obtained, indicating an even higher k_La than those determined during process engineering characterization. Overall, the ambr® 250 modular is a useful tool for the transfer, development and optimization of industrial bioprocesses. Furthermore, process development cycles can be shortened and the costs are reduced including a high flexibility due to the advantages of the single-use technology.

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References

AstraZeneca, Bayer, B. Braun, Boehringer Ingelheim, Dr. Franz Köhler Chemie, EVER Neuro Pharma, Glycotope, LTS Lohmann, Merck KGaA, NCPC, Pfizer, PHARMAQ, Polpharma Biologics, Rentschler Biotechnologie, Sandoz, Sanofi, TEVA (Merckle Biotec), WALA

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Poster No. 17

miwelt: Discover the hidden world of microbes

Verena Looser[1], Iwo Zamora[1], Marcel Straumann[1], Julia Dürr[2], Marcel Raabe[3], Caroline Ulli[1], Karin Kovar[1]

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[2] Freelance Children's Books Illustrator, Berlin, DE
[3] Independent Journalist, Leipzig, DE

The miwelt-project (funded by the Agora instrument of the Swiss National Science Foundation) uses an innovative didactic approach, a combination of arts and science, to familiarise children from 7 to 11 years of age with fundamental concepts in microbial physiology and biotechnology. The partners in miwelt open a dialogue that encourages scientists to explain their research, and how it becomes the scientific foundation for applications in industrial manufacturing, while using simple terms and language combined with illustrations. Although primarily child-centred, whose curiosity should be stimulated, this approach is also an asset to adults who lack a strong scientific background.

The materials developed within the scope of the miwelt project are well-documented in a series of booklets combining elements of a comic and a non-fictional book. The narrative scaffolding around the main characters, Alina and Conrad, incorporates 'phenomenological' observations as seen through the eyes of these children, along with the relevant technical subject matter. In addition, scientific and ethical questions are raised. Some of the subject matter appears casual (non-didactic) and is absorbed into the larger context.

The first booklets of miwelt comprise the following topics:

Issue 1, entitled "Visible invisible - Alina and Conrad discover the small-side of the universe", using cubes of yeast from the supermarket as an example, or rather the invisible single-celled organism Saccharomyces cerevisiae, the relationship between substrate consumption, growth and division is explained.

Issue 2, entitled "Crowding in Cytoplasm - Alina and Conrad see green", using Chlorella vulgaris microalgae isolated from nature as an example, the focus is on the cells' interior, and the topic of 'food' for microorganisms is examined in terms of energy and storage materials.

Issue 3, entitled "Faeces and Codes - Alina and Conrad decipher the blueprint for life", using examples from research and industry and discussing the genetic modification of the intestinal bacterium Escherichia coli, it shows how observed natural principles have become the basic methods of modern biotechnology.

Issue 1, which will be published in autumn 2016, can be ordered at: www.miwelt.net

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Poster No. 18

Scale-up principles for single-use bioreactors

Rüdiger W. Maschke[1], *Eric Abellan*[2], *Sören Werner*[1], *Mischa Stalder*[1], *Dieter Eibl*[1]

[1] *Zurich University of Applied Sciences, Wädenswil, CH*

[2] *Infors AG, Bottmingen, CH*

The scale-up of bioprocesses is one of the main challenges for an economical feasible production of biopharmaceuticals. Brilliant laboratory experiments may wither away in the spotlight of cost analysis if a proper scale-up strategy is missing. Hence, the quest for universally applicable scale-up parameters and strategies may be as old as commercial biotechnology itself. However, the philosopher's stone is yet to be found.

Nevertheless, notable progress was made in recent decades, especially for the scale-up of geometrical similar reactors (in particular stirred tank bioreactors). A widespread method is the application of similar process parameters, especially the volumetric power inputs (P/V), volumetric oxygen transfer rates (often represented by the corresponding k_La-value), geometric ratios, or stirrer tip speeds. More advanced approaches include the usage of computational fluid dynamics, multivariate analysis, or artificial neural networks.

Plenty of literature sources are available for all methods mentioned above, encompassing fundamental books like "Scale-up" by Marko Zlokarnik [1] to promising research papers like "An Approach for Scale-Up of Geometrically Dissimilar Orbitally Shaken Single-Use Bioreactors" by Werner *et al* [2]. However, the transfer of these principles succeeds only in rare cases.

With the increasing usage and acceptance of single-use bioreactors, the demand for reliable scale-up strategies has grown, too. In this poster presentation, several characterization principles for single-use and classical bioreactor systems are introduced and evaluated. Different scale-up approaches, based on process parameters and statistical correlations will be compared and exemplary scale-up approaches will be presented.

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Poster No. 19

Cultivation of CHO cells in Thomson Optimum Growth™ shake flasks and scale-up

Jolanda Meister[1], Rüdiger W. Maschke[1], Nicole Stichling[2], Sören Werner[1], Dieter Eibl[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

[2] Infors AG, Bottmingen, CH

In biotechnology, the usage of shaking flasks in upstream processing is widely common due to the easy handling. Frequent applications are process screening and optimization. Thereby, the focus lies mostly on a homogeneous and fast distribution of substrates and gases whilst power consumption and shearing force are meant to be kept low, which ideally results in high biomass concentrations and product titers.

For characterization purposes, the mixing time and the oxygen mass transfer coefficient (k_La) were measured in 5 L and 500 mL Thomson Optimal Growth™ shaking flasks, using the de-colorization or the dynamic gassing-out method, respectively. Those geometrical optimized bioreactors are promising higher space-time yields compared to the predominant Erlenmeyer-shake flask design.

According to the results of the procedural experiments, CHO (Chinese hamster ovary) cells were cultivated at selected, auspicious parameter combinations. The effectiveness of the predetermined parameters was evaluated and a scale-up method elaborated. The results can be summarized as follows:

The key parameter for all experimental setups is the shaking rate. In contrast, the filling volume showed to have a more ambiguous role. Modeling results in the 500 mL flasks showed no significant influence of the filling volume to the maximal cell density, in contrast to the 5L flask (both shaken at 50 mm throw). The highest viable cell density (up to $4.8 \cdot 10^6$ cells mL^{-1}) was reached using the 500 mL flask with high shaking rates at 50 mm shaking diameter. Thereby, a μ_{max} of 0.038 h^{-1} was achieved that correlates with a t_d of less than 19 h. All in all, the highest μ_{max} of 0.055 h^{-1} was reached during the scale-up process, whereby higher viable cell densities were reached compared to the batch cultivations using the same parameter settings.

In addition to the experiments performed to date, simulations with computational fluid dynamics and experimental determination of specific power consumption rates are already in progress, increasing the range of applicability and the validity of the proposed model correlations.

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Poster No. 20

Improving mammalian cell culture process development by combining model-based simulations with DoE tools

Johannes Möller[1], Regine Eibl[2], Dieter Eibl[2], Ralf Pörtner[1]

[1] Hamburg University of Technology, Hamburg, DE

[2] Zurich University of Applied Sciences, Wädenswil, CH

Bioprocesses involving mammalian cells are most frequently based on optimized feeding profiles within a fed-batch. Statistical Design of Experiment (DoE) tools are widely used for this kind of process optimization because of their simple structure and easy handling. Limitations occur with respect to the high number and duration of experiments during process development. Even if high-throughput systems, preferably based on single-use-technology, can perform these numbers of experiments in parallel, the heuristic restriction of boundaries results in stepwise iterations with multiple runs. This is time-consuming, cost-intensive and further complicates the path from process development to process establishment.

The use of DoE tools in combination with an appropriate growth model might be a valuable method for evaluating fed-batch strategies and decreasing the experimental space required for statistical optimization methods *in silico* before experiments are carried out. To prove this concept, a model was used to describe the dynamics of cell metabolism of CHO-XM-111 cells in a two-step growth process with medium exchange followed by a fed-batch. Model parameters were fitted to averaged data from three parallel shake flask cultivations, and model predictions were used to decrease the experimental space and the number of cultivation parameters *in silico*. Therefore, the concentration of L-glutamine (feed), constant feed rate, point in time for the medium exchange, and the start of feeding were identified as critical process parameters. At first, simulations were applied to minimize experimental space. Therefore, the point in time for medium exchange and the starting point for feeding were determined from a batch simulation at a minimal L-glutamine concentration of 0.1 mmol l^{-1}. Secondly, to increase the total cell number in a fed-batch, the L-glutamine concentration and constant feed rate were optimized by simulated DoE. In total, two simulated and iterative response surface plots were evaluated. Instead of performing multiple experiments to generate data for response surface plots, data gained from simulation was used. Finally, one appropriate combination of cultivation parameters was tested and compared with the initial model.

The method shown is suitable for the generation of deeper understanding of processes, e.g. the linkage of different process parameters to quality attributes. Furthermore, cultivation strategies for mammalian cell lines can be compared and evaluated. This results in a significant reduction in the number of experiments required during process development establishment. The strategy is especially intended for use in multi-single-use-devices to speed up process development.

Poster No. 21

Oxygen transport phenomena in single-use shaking flasks under reduced oxygen conditions

Johannes Möller[1], Niklas Widderich[1], Tibor Anderlei[2], Andreas Richter[2], Stefan Rüegg[2], Ralf Pörtner[1]

[1] Hamburg University of Technology, Hamburg, DE

[2] Adolf Kühner AG, Birsfelden, CH

Process development for mammalian cell lines is most often performed in disposable shaking flask systems. Advantages are the simple handling, the low investment costs and the possibility to perform many experiments in parallel single-use systems. Nevertheless, due to the complex physio- and biochemical parameters influencing the growth and viability of mammalian cells, the development of robust bioprocesses in shaking flasks can be challenging. Beside the pH, temperature, osmolality, concentration of substrates and metabolites, the concentration of dissolved oxygen can be critical. In contrast to fermentations in controlled bioreactors, most common shakers do not control the concentration of oxygen inside of the shaker. This may result in differences in the growth, viability and productivity and can lead to wrong decisions during processes development. To take the oxygen concentration into account, a novel shaker (LT-XC with O_2 control) was developed by the Kühner AG. It offers the possibility to control the temperature, humidity, carbon dioxide concentration, shaking frequency (shaking diameter is changeable) and the concentration of oxygen simultaneously.

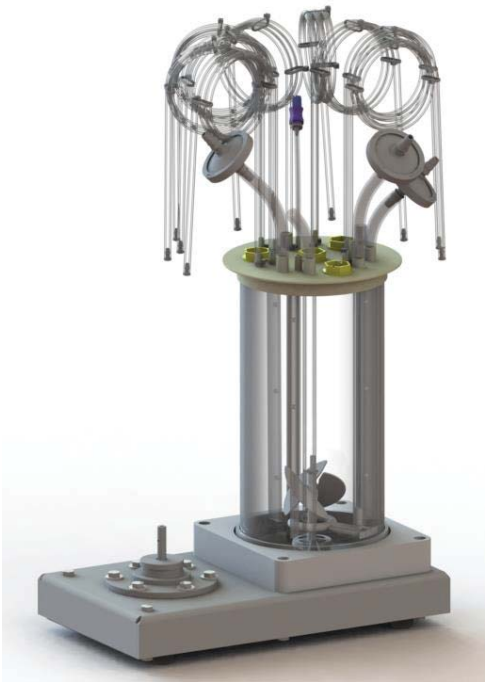
The aim of this work was to characterize mass transfer phenomena of shaking flasks with atmospheric and reduced oxygen conditions. Therefore, disposable baffled Erlenmeyer flasks (250 mL, Corning) with a vent cap (0,2 µm) were equipped with a dissolved oxygen sensor and valves, to flush the shaking flask with nitrogen or pressured air. The volumetric oxygen transfer coefficient k_La was determined by the dynamic gassing out method for shaking rates between 20 – 300 rpm. In addition, the mass transfer coefficient through the vent cap $k_{vent\text{-}cap}$ was determined. $k_{vent\text{-}cap}$ was further used to estimate the liquid saturation time until 90 % of the adjusted oxygen concentration (set-point shaker) was reached in the shaking flask liquid. $K_{vent\text{-}cap}$ was significant smaller than the k_La and is regarded as the limiting mass transfer resistance during medium saturation. Afterwards, the effect of sampling in hypoxic shaking flasks under atmospheric oxygen concentrations was investigated. Even if the gas composition inside the shaker has readjusted quickly, a fluctuation of the oxygen level inside the shaking flask was observed. Depending on the cell line, this may lead to oxidative stress.

In summary, a novel shaker was characterized and the mass transport phenomena in disposable shaking flasks under atmospheric and reduced oxygen concentrations were evaluated. Furthermore, the effect of sampling on the dissolved oxygen concentration was determined for shaking flask cultivations with reduced oxygen concentrations.

PerfuseCell at BioTech2016

Title:
Perfusion-Single-Use-Bioreactor integrating both Single-Use-Pump, Single-Use-Sensors and Single-Use cell retention devices.





Abstract:
Both www.cercell.com and www.perfusecell.com develop and manufacture customizable Single-Use-Bioreactors (SUB) integrating various Single-Use-Sensors (SUS). PerfuseCell take one step further and integrate both Single-Use-Pumps (SUP) and Single-Use cell retention concepts such as Porous-Scaffold-Support (PSS) and Cross-Flow-Filters (CFF).

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3. Single-Use Cross-Flow-Filter, SU-CFF for cell retention in Pulsating-Tangential-Flow (PTF) setup.
4. Single-Use-Sensor, SUS for a range of analysts
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Poster No. 22

Online monitoring of fermentation processes via contactless microwave sensors

Thomas Nacke[1], Brian P. Cahill[1], Mike Eggli[2], Ralf Kluks[3], Robert Kühler[1], Doreen Lisiki[1], Daniel Martin[1], Yahor Zaikou[1]

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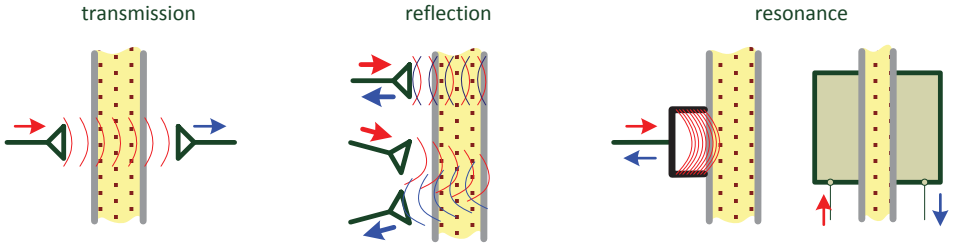
[3] IRK, Dresden, DE

Keywords: microwave, contactless conductivity and permittivity detection

The presentation provides a short discussion of the present state of theory of microwave impedance sensors, the principle design and the application for monitoring of fermentation processes. There is a widespread need for highly-sensitive robust sensors that operate without direct fluid contact for analysis in bioprocess technology and especially for single use technologies. Contamination-free process measurements are essential for efficient monitoring of fermenters.

Measuring the variation of dielectric properties (conductivity and permittivity) in the microwave frequency band can be used as an approach to investigate biological and chemical matter and processes such as, cell growth, cell metabolism and the concentration of large aqueous based molecules. The number of cells, consumption of nutrients and cell metabolism directly influence the passive dielectric properties.



This presentation describes a microwave impedance sensor that combines detection in macro- or microfluidic networks with quick and precise analysis. The contactless nature of these sensors enables the cost-effective production of microwave sensors that can avoid sample contamination.

Numerical simulation with high-frequency full wave electromagnetic software is necessary for optimization of the development of these sensors. Reproducible measurements require high precision in the mechanical production of the geometry of each sensor: obviously microfabrication offers such specifications.

Together with reasonably priced high frequency electronic circuits this technique opens up the possibility of simpler automation and miniaturization of routine applications and in a wide variety of sample pretreatments.

Poster No. 23

Cultivation of the marine heterotrophic algae *Crypthecodinium cohnii* in single use bioreactors – potentials and scalability

Peter Neubauer[1], *Anna Maria Marbà Ardébol*[1], *Olivia Zakrzewski*[1], *Stefan Junne*[1]

[1] *Technische Universität Berlin, Berlin, DE*

Corrosion by the high chloride concentration exhibits a major challenge for the cultivation of marine organisms in steel bioreactors. Also shear sensitivity can be crucial when cells are cultivated in stirred tank reactors. The application of single-use bioreactors (SUB) offers an alternative to traditional bioreactors in order to circumvent these problems, when the initial stage of process development does not allow the investment in process-specific infrastructure. Additionally, marine production processes can demand for high gas mass transfer rates, e.g. in the case of Crypthecodinium cohnii, a heterotrophic algae applied for the production of docosahexaenoic acid (DHA), an important polyunsatured fatty acid (PUFA). These cells are highly sensitive to shear forces and oxygen limitation. In order to provide methodologies for the cultivation of this class of microorganisms in various scales, procedures are presented, which include the use of the oxygen carrier perfluorodecalin in the microwell plate scale (1), the application of suitable shake flasks and finally the utilization of a wave-mixed scalable single-use bioreactor (SUB) system. The 2-dimensionally rocking CELL-tainer® is well suited for the application with marine cultures, since it achieves a high volumetric oxygen gas mass transfer of k_La = 400 h^{-1} (up to 40 fold higher than in conventional wave-mixed SUBs) while comparably low shear forces are maintained (2,3).

The suitability of the presented concepts is proven based on cultivation performances of C. cohnii in the different developmental scales. Process and physiological parameters are determined in order to compare the results obtained with those of traditional stirred tank reactors (4).

The suitability of single use systems is proven by the physiological and morphological constitution of the cells up to the 120 L scale: the cell viability and the intracellular concentration of DHA are increased in the SUBs as revealed with gas chromatography, flow cytometry and holographic microscopy. Such processes show an enhanced DHA yield, because of cells, which have shifted from the growth to a fatty acid production stage, are competent to synthesize DHA over a longer time. The presented methodology has also relevance for the development of other marine cultivation processes, e.g. marine bacteria, while contributing to reduced development times and costs in early product and process development.

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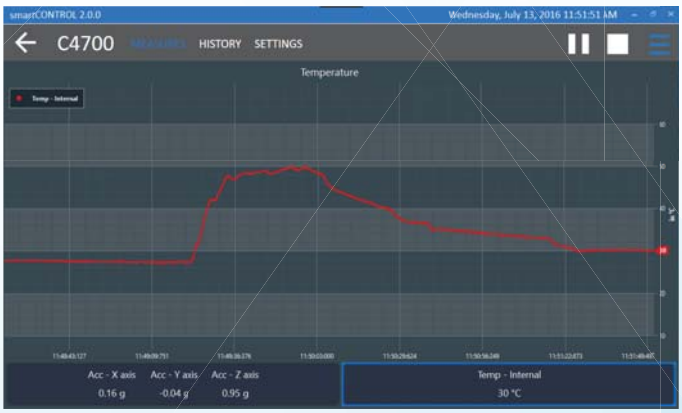

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Poster No. 24

FLEXMAG - Accurate flow measurement in single use bioprocessing applications

Christine Perfetti[1]

[1] *KROHNE, Romans sur Isère, FR*

The last 10 years have seen a massive growth in the use of Single Use Systems for the production of biopharmaceuticals. Many components of a process are now available on the market (reactors, tubes, connectors...).

The new generation of Single Use Systems is now designed to provide process parameters. The wish there is to reach the same level of performance as with standard stainless steel process equipment. The market is waiting for accurate and reliable single use sensors.

KROHNE is a worldwide leader of instrumentation for industrial processes, and specifically known for flow measurement of various technology.

KROHNE pioneered a single use flowmeter, the FLEXMAG. FLEXMAG contributes to the benefit of eliminating cleaning requirements and claims performances close to those of standards processes.

The FLEXMAG is based on the electromagnetic principle (according to Faraday's law), which is very convenient and therefore widely used in industrial processes. The measurement is directly linked to the velocity, so the flow of the fluid. It is independent from the process and fluid parameters. No need to compensate the variation of pressure or temperature. The measurement is not affected by a change in viscosity. The FLEXMAG is installed at the filtration and purification steps of downstream bioprocessing applications.

The FLEXMAG is made of two parts: the fix part is creating the magnetic field, collecting and filtering the signal and transmitting the flow value. The single use part is a tube which is simply installed in a specific location of the fix part. The fluid is in contact only with the disposable tube while the fix part remains on the machine (TFF, Chromatography…).

The innovative FLEXMAG is designed by KROHNE experts and using KROHNE's patents. The flow value is measured with an accuracy of 1%, even by avoiding any wet calibration: The operator received a clean and dry single use tube, which is manufactured in a clean room ISO 7.

The single part is made of material compatible with ISO 10993. Its hardness make possible accurate measurement even at high pressure like 3 bars. This flowmeter doesn't cause pressure loss due to its full bore shape.

Because of the last improvement of KROHNE in the design of hardware, the FLEXMAG is small enough to hold in one hand. This flowmeter meets the highly specialized demands of the biopharmaceutical market, and contribute to the bioprocessing optimization.

Poster No. 25

Process Raman spectroscopy for in-line monitoring of mammalian cell cultures in real time

Alexander Pitters[2], Maryann Cuellar[1], Pat Wiegand[1], Sean Gilliam[1]

[1] Kaiser Optical Systems, Inc., Ann Arbor, US

[2] Kaiser Optical Systems, SARL, Ecully, FR

Mammalian cell cultures are complex processes where cells are cultivated under highly controlled conditions using media with a very high number of components. Current effort is focused on obtaining a better understanding of mammalian cell cultures by cultivating predominantly CHO cells for therapeutic protein production. To ensure a healthy progression of the cell culture, it is important to understand and monitor the stages of the biologic manufacturing.

In order to build quality into a process a primary step is to analyze the process, understand what the critical quality attributes are, and monitor or rather control those factors. Consequently, there is a significant interest and value in techniques that provide instantaneous response for monitoring and analyzing biopharmaceutical processes. Molecular techniques - such as Raman spectroscopy - are widely used for PAT applications, because they provide in-situ information in real-time.

Kaiser Raman spectroscopy is a method by which multiple bioprocess assays can be measured in situ within the bioreactor or fermenter environment. Raman is a fundamental vibrational spectroscopic technique that provides chemical and physical information that can be used to generate multi-component qualitative and quantitative predictive models. The presence of water does not interfere with the spectrum as it does with other spectroscopic methods such as NIR and mid-IR. Thus, Raman is well-suited for a host of upstream and downstream bioprocess applications.

Real-time measurements within Biopharma are achieved for Glucose, Glutamine, Glutamate, Lactate, Ammonium, Viable Cell Density, Total Cell Density, Osmolality, Viability, Titer and Buffer. The analyzer software enables a fully integrated bioprocess management and the instruments allow to control 4 bioreactors from a working distance of 1 to 1000 m.

In recent years, Kaiser Raman has opened up new avenues to bioprocess analytics by demonstrating a technology that is robust, scaleable, provides in situ knowledge, and is transferable between cell lines, media feedstocks, and process conditions. During process development, Raman is now crucial for adopting QbD principles to define manufacturing design spaces and demonstrate holistic process and quality understanding. During production, a single Raman sensor can be used to monitor and enable control of several critical parameters in real time. Compared to traditional off-line analytical methods, in situ Raman reduces cost, consumables, sterility risk, equipment maintenance, and operator overburden. The wealth of bioprocess information enabled by in situ, real-time Raman is being realized by many companies to deploy leaner, continuous, and hybrid biopharmaceutical manufacturing.

Analyzers can be used to study solids, liquids or gas without sampling accessories or preparation. Kaiser Optical Systems is the leader in Raman instrumentation and advanced holographic components for spectroscopy. Products and services are positioned in pharmaceutical and chemical manufacturing around the world.

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Poster No. 26

Measurement of hairy root biomass by impedance spectroscopy

Iris Poggendorf[1], Joel Varonier[1], Thomas Nacke[2], Caspar Demuth[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

[2] Institut für Bioprozess- und Analysenmesstechnik e.V., Heilbad Heiligenstadt, DE

In this poster we describe the online measurement of growth of hairy root cultures by means of dielectric spectroscopy. This technique is well-known for monitoring single cell suspension cultures, however, it has hardly ever been used to measure biomass growth of plant organ cultures (e.g. hairy roots).

Hairy Roots are transformed organ cultures that can produce a variety of natural or recombinant products. They can be cultured stable without the addition of plant hormones and therefore are a very interesting target for production of pharmaceuticals or natural substances of interest for the cosmetic industry.

The online bioprocess control is essential and provides important insights for monitoring and controlling of cultivations in biotechnology. In recent years more and more online measurement systems have replaced the traditional sampling and offline sample analysis. For hairy root cultures the market offers currently still no online monitoring systems for quantification of biomass in traditional or single-use bioreactors.

The root mass was cultured in modified Tubespin 50 (TPP, Trasadingen, Schweiz) which had been adapted with a 4-electrode measuring cell (Sy-Lab, Neupurkersdorf, Austria). Each day the dielectric measurement was done and after that the tube was harvested to determine the wet and dry biomass by the standard offline method. The data obtained by means of a self-built impedance analyser system showed that the calculated capacitance correlated well with the root biomass derived from offline measurements. Therefore, the method developed in this work proved to be a valuable tool to monitor biomass growth in plant organ cultures without the need to perform offline sampling.

Poster No. 27

Investigation of factors influencing the intracellular accumulation and secretion of porcine trypsinogen from *Pichia pastoris*

Hana Raschmanová[1,2], Leona Paulová[1], Barbora Branská[1], Zdeněk Knejzlík[1], Iwo Zamora[2], Karel Melzoch[1], Karin Kovar[2]

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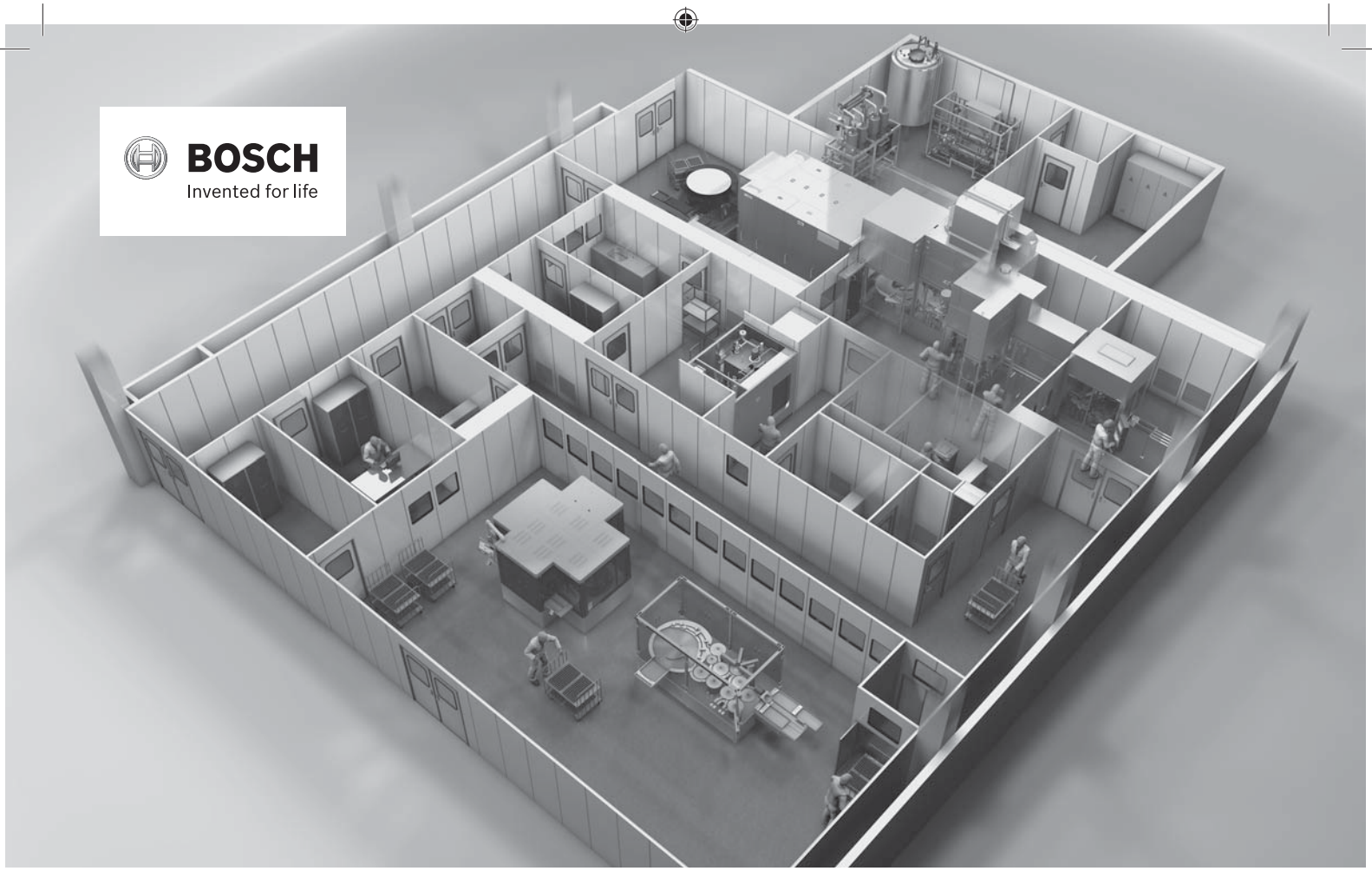
[2] Zurich University of Applied Sciences, Wädenswil, CH

Trypsinogen is the precursor of trypsin, a protease of high economic relevance that is widely used in science, industry and medicine. Trypsin is traditionally isolated from animal pancreases, but in recent years there has been an increasing interest in the commercial production of recombinant proteins by microbial systems, as they promise to provide products of pharma-compliant quality at a reasonable production cost. A popular host for the production of such high-quality recombinant proteins is the methylotrophic yeast *Pichia pastoris* (recently reclassified as *Komagataella pastoris*). Since the quality and quantity of synthesised protein is greatly affected by cultivation/process conditions, we investigated the influence of pH, specific growth rate, and oxygen availability on the production and secretion of porcine trypsinogen from *Pichia pastoris* in fed-batch bioreactor cultures.

Trypsinogen was *N*-terminally fused with Enhanced Green Fluorescent Protein (EGFP) and cloned under the control of the methanol-induced alcoholoxidase 1 promoter (AOX1). The EGFP fusion tag was to monitor intracellular accumulation of trypsinogen in a non-invasive and efficient manner when utilising flow cytometry to quantify the green fluorescence, as a method typically referred to in other studies. Surprisingly, the *N*-terminal EGFP-tag was cleaved from trypsinogen under all culture conditions tested, and therefore was not suitable to facilitate trypsinogen tracking in the cell. This cleavage was, however, not caused by trypsin, but most likely by an endogenous protease. We therefore recommend exercising caution when using EGFP for monitoring target recombinant protein production by *P. pastoris*. Simultaneously, flow cytometry was used to assess cell viability, as determined by membrane permeability to propidium iodide. Four fed-batch cultivations were performed, differing in pH of the cultivation medium (5.0 or 5.9), specific growth rate of cells with methanol (0.02 or 0.04 h^{-1}), and oxygen availability (inlet gas with or without addition of pure oxygen). The pH during cultivation did not affect biomass growth, but significantly influenced the specific productivity of trypsinogen, where 1.7 times higher product concentration was achieved at pH 5.9 compared to pH 5.0. The maximum concentration of trypsinogen (64 mg l^{-1}) was achieved at pH 5.9 and at a constant specific growth rate of 0.02 h^{-1} as controlled by an exponential methanol feed rate. The cultures were capable of utilising methanol, growing and producing at a specific growth rate of 0.04 h^{-1} (with methanol) only when aeration was enriched with oxygen; in contrast, oxygen limitation during the production phase led to methanol accumulation of up to 30 g l^{-1}. Extracellular methanol negatively affected both the synthesis and secretion of fusion protein, but only to a marginal extent where 10% of cells were dead.

This systematic study revealed the operational limits of the *P. pastoris* system when used to produce trypsinogen. It also confirmed the results of previous studies showing that high concentrations of methanol did not severely compromise cell viability as long as the pH was within the physiological optimum for the strain and the cells were not further stressed due to protein overproduction.



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Poster No. 28

Validation of a Tecan needle and determination of k_La in an automated mini-bioreactor handling system

Lisa Reinold[1,2], Bettina Weiss[1], Tobias Maucher[1]

[1] University of Applied Sciences, Esslingen, DE

[2] Festo AG, Esslingen, DE

A high number of parallel experiments in bench-scale are necessary for process development and optimization in the upstream process of cell cultures. Therefore, orbitally shaken mini-bioreactors are used because of their simple structure and easy handling. The University of Applied Sciences Esslingen develops an automated handling system in cooperation with the Festo AG (Esslingen) to increase reproducibility and to reduce contamination risk in the cultivation of CHO cells. Pipetting must be very precise for the preparation of analyses and for feeding the cells. The objective of this study is the validation of the Tecan needle used for pipetting in an automated tube spin handling system and the identification of k_La values in two different media with CHO-ES1 cells.

For the validation of the Tecan needle pipetting of four different volumes (1000 µL, 500 µL, 100 µL, and 20 µL) in three speeds has been tested. The viscosity of the solutions differed but did not influence the pipetting significantly. The biggest discrepancy between the supposed volume and the actual volume was observed when using the slowest speed and the smallest volume as droplets remained on the needle. The slowest speed S400R should only be used if shear forces of the other speeds are too high for the cells. For routine pipetting the middle speed S100R is most appropriate and can be used for small volumes as well.

Analysing solutions must be homogenous to give proper values but mixing with a Vortexer cannot be implemented in the automated handling system. This is why it was tested to pipette and mix a glucose solution only with the Tecan needle. The concentration of glucose was tested in a glucose analyzer after pipetting 20 µL of three different glucose concentrations. The solution was mixed in two ways: pipetting three times 200 µL up and down and no mixing at all. The measured values were compared to manual pipetting and mixing with a Vortexer. This shows that mixing with the Tecan needle is necessary as it improves the reproducibility and precision of the glucose concentration.

The oxygen supply of cells is the major problem by the implementation of bioreactors as only dissolved oxygen in the media can be used. Therefore, the cultivation is limited by the oxygen transport which is determined by the oxygen transfer coefficient k_La. In this study the dissolved oxygen concentration pO_2 was measured by an optical sensor when shaking the orbitally shaken minibioreactors has stopped. In comparison, the oxygen supply is better in Hyclone CDM4PerMAb ($kLa = 32\ h^{-1}$) than in Lonza ProCHO5 ($kLa = 24\ h^{-1}$) and another study showed that also the production of antibodies by CHO-ES1 cells is higher in CDM4PerMAb. In conclusion, CDM4PerMAb is recommended for cultivating CHO cells.

Poster No. 29

In-line glucose measurement in single-use bioreactors

Diego Reyes-Romero[1], *Isabella Moser*[2], *Gerhard Jobst*[2], *Caspar Demuth*[3], *Fabienne Seiler*[3], *Irène Stutz*[3], *Iris Poggendorf*[3], *Karin Hasler*[1]

[1] *Innovative Sensor Technology IST AG, Ebnat-Kappel, CH*
[2] *Jobst Technologies GmbH, Freiburg im Breisgau, DE*
[3] *Zurich University of Applied Sciences, Wädenswil, CH*

Development and manufacturing of drugs is increasingly relying on cell cultures, especially animal cells [1]. These cells are engineered to produce therapeutic proteins. Product quality and yield can be optimized by controlling the growing conditions in bioreactors [2]. Monitoring of nutrients, like glucose, plays a vital role in the feeding strategy of a cell culture. Enzymatic sensors offer high specificity. Conventional (on-line) glucose monitoring requires a fluidic-dilution stage that has to be outside the culture vessel. Such systems show problems like increased system's complexity, long latency, and large footprint. The membrane technology developed by Jobst Technologies combines the specificity of enzymatic sensors with a robust construction allowing for weeks-long continuous glucose monitoring either in- or on-line. Sensors can also be gamma sterilized and stored for months before utilization.

A CHO cell line was used for a batch culture in a conventional 1-liter glass bioreactor. The glucose concentration was continuously monitored with an in-line glucose probe. For the control measurements, a Nova Bioprofile Analyzer and a HPLC system were used. A single calibration at t = 5h provides accurate readings throughout the entire cultivation lasting days. During this time, a measurement point is acquired every second without requiring any manual work as the used reference systems do. Figure 1 depicts the glucose concentration over time. The in-line probe provided a smooth signal and, compared to the off-line reference methods, did not show the errors that result from the necessary sample handling and pre-treatment.

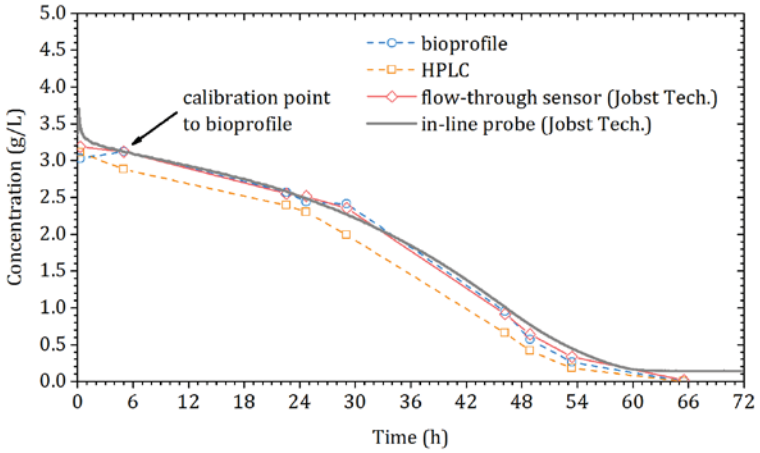

Figure 1: Measured glucose concentration in a CHO batch culture. A calibration point has done 5 hours after cultivation start.

[1] M. Butler, *Appl. Microbiol. Biotechnol.* (2005) 68: 283-291
[2] A. G. McAtee, N. Templeton, J. D. Young, *Pharm. Bioprocess.* (2014) 2(1): 63-74

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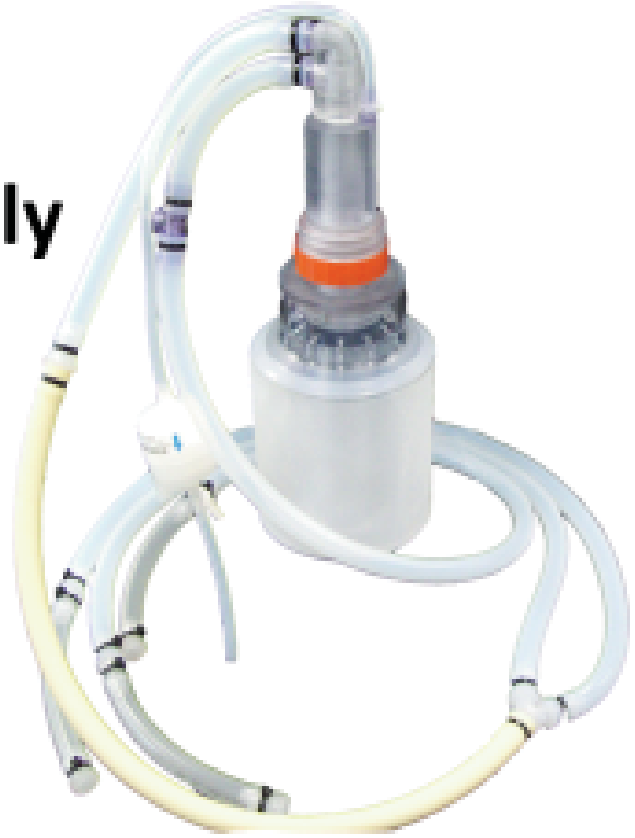

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Poster No. 30

CHO harvest of a large scale single-use bioreactor using the UniFuge® single-use disposable centrifuge

David R. Richardson[1]

[1] *Pneumatic Scale Angelus, Clearwater, US*

Production of Chinese Hamster Ovary proteins can be collected by single-use centrifuge methods for harvest. The centrifuge is very efficient from several perspectives. First the high cell density and protein rich solution can be separated from the SUB broth with the elimination of cells and debris by using a single use centrifuge. At the same time, the process is gentle on the solubilized proteins.

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Poster No. 31

CHO fed-batch process transfer from a single-use process development to a production system

Sebastian Ruhl[1], Ute Husemann[1], Christian Zahnow[1], Christoph Zehe[2], Gerhard Greller[1]

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[2] Sartorius Stedim Cellca GmbH, Laupheim, DE

KEYWORDS:

Single-use bioreactors, Stirred tank reactors, Process transfer, CHO fed-batch process, Process Development

Small bioreactor systems are the base of a good process development procedure in modern mammalian cell culture. A variety of process parameters is tested in these systems to define a first version of the production process. The transfer from a process development system to production scale is a challenging step. The agitation principle of process development systems most often differs from conventional production bioreactors that are commonly designed in accordance to stirred tank reactors, well-defined and characterized. To facilitate a direct process transfer from process development to production scale a complete single-use bioreactor family from 0.25 to 2,000 L with comparable geometrically rations and impeller design was developed.

The applied CHO fed-batch process in this trial was established in the full range from 0.25 - 2,000 L. The aim was to achieve comparable results in terms of viable cell density and viability trend, product quantity and quality. A complete process engineering characterization was performed for all used systems. The critical process parameters like mixing time, k_La and shear stress were considered and scaling criteria were defined with a design space approach. As a reference system a multi-use 5 L glass bioreactor was used.

The process parameters were defined to fit the design space for all scales. Cell densities of up to 20×10^6 cells/mL and product titers of up to 4.5 g/L were reached. Overall, the process was established successfully in all scales and similar results were achieved. All results were comparable to prior performed cultivations in reference bioreactors.

The achieved results represent a major step in terms of process development in single-use bioreactors. In the future this new process development bioreactor system makes it easy to transfer new processes from process development to production scale.

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Poster No. 32

Online biomass quantification – Paving the way to broader implementation

Jana Rupp[1], Angela Babst[1,2], Matthias Barmettler[1], Marcel Straumann[1], Marlene Frank[2], Karin Kovar[1], Caspar Demuth[1], Lukas Neutsch[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH
[2] Hamilton Bonaduz AG, Bonaduz, CH

The development of accurate and reliable methods for online estimations of biomass concentrations in cultivations is a central objective in the practical implementation of PAT and QbD concepts in biotechnological manufacturing. Improved process control and a deeper understanding of physiological principles and interdependencies are two of the alluring prospects to be gained by having available detailed, real-time information on the amount and physiological state of cells. However, the technical challenges associated with this analytical task are not trivial. Accuracy over a large dynamic range, cross-platform transferability, robustness with regard to interferences and changing process conditions, ease of use, compatibility with sterilization protocols and high information content are some of the performance attributes that should be met by an ideal biomass sensor.

Among the panel of technologies that have so far been evaluated for this purpose, the in-situ determination of total cell density via optical turbidity measurements and viable cell density by permittivity are the most frequently adopted methods. Both are orthogonal with regard to the underlying measurement principle and allow for concluding on different, physiology-related variables of the culture. Their potential in bioprocess monitoring has been demonstrated on multiple occasions and in many different settings, but some of the basic determinants for measurement accuracy and information extraction still remain to be fully elucidated. Currently, considerable efforts are being directed towards implementing this technology for a broader setting of production setups including disposable bioreactors, which entails new challenges in sensor mounting and positioning.

Against this backdrop, an interdisciplinary research project at ZHAW seeks to explore in further detail the parameters that influence sensor performance and refine existing strategies for data analysis. Here, we present the first results of this study and highlight caveats that should be respected when defining the optimal system configuration. The potential for interfacing multiparametric cell density data with advanced statistical analysis methods to extract additional information on cell physiology is illustrated, and future perspectives for integrated control strategies are pointed out.

Poster No. 33

Detection of enzymatic activities and kinetics using a 3D-printed smartphone-spectrometer

Matthias Schäfer[1], *Karmela Marinčić*[1], *Tomislav Ujdur*[1], *Roland Ulber*[1]

[1] *University of Kaiserslautern, Kaiserslautern, DE*

Smartphones are by now everyday life devices containing a broad variety of sensors. While physical parameters such as sound or acceleration are often detected with these sensors, the detection of biological parameters is usually done by using only the camera. For the detection and quantification of enzymatic activities and non-biological reaction kinetics by absorbance a lens-free smartphone spectrometer is development. For the measurement of absorbance in the smartphone spectrometer the flashlight LED with an inhomogeneous, diffuse emitted light spectrum of 450 – 650 nm and the ambient light sensor is used. No additional electronic equipment is required. To optimize the detection the light spectrum of the LED flashlight is reduced by low cost filter foils.

As an example an urease based colorimetric enzyme assay with an absorption maximum at 550 nm is used. This assay can be used as a bioassay for the detection and measurement of heavy metal ions in water samples in biologically relevant, micro molar scale. The detection of enzyme activity and the measurement of enzyme kinetics is possible in colorimetric assays with absorption maxima of 440 ± 20 nm, 550 ± 50 nm and 650 ± 10 nm depending on the used type of smartphone. In addition the system can be used for the determination of optical density of slurry. The correlation between the slurry dry mass concentration and optical density is comparable to commercially available laboratory spectrometers.

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Poster No. 34

Cell debris removal by single-use diatomaceous earth (DE) filtration

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Many biotechnological products are obtained by the help of native or recombinant microbial cells in fermentation processes. The desired product is formed by the metabolism of the cells used. In order to get intracellular target molecules, like proteins, the cells have to be destructed. This is done with cell rupture techniques (e.g. high-pressure homogenization) which generate a suspension containing cell debris and cell contents (proteins, DNA, mRNA, etc.). The first step in the following purification sequence is the removal of the cell debris. One possibility to do this is the use of depth filters and filter aids.

The removal of cell debris from an *E.coli* strain, which produces the enzyme Cytochrom P 450 Monooxygenase, was performed with the aid of single use filter capsules (FILTRODISC™ BIO SD) from FILTROX AG. A sufficient filtration rate in such filter capsules can only be achieved by using a filter aid like diatomaceous earth (DE). On the one hand, filter aid supported filtrations were executed. On the other hand, filtrations were performed in which an additional pre-coat layer (also consisting of diatomaceous earth) is formed first on the filtration media before the filter aid supported filtration starts. Both filtration methods were carried out in comparison. The quality of the filtrate was determined by measurements of the optical density and the protein content (Bradford test).

The results, which will be presented, show that the quality of the obtained filtrates is formidable if an additional pre-coat layer is used. In this case, the values of the optical density were below 0.01, which points out, that almost all cell debris were removed. At the same, time more than 80 % of the proteins were detected in the filtrate. The removal of the cell debris using FILTRODISC™ BIO SD can be realized without further addition of flocculants and without adjusting the pH value. Therefore, the cell debris removal by single-use diatomaceous earth filtration is surely of great interest for the usage in biotechnological scopes.

Poster No. 35

Orbitally shaken goes automation

Tamara Schlienz[1], *Bettina Weiss*[1], *Tobias Maucher*[1]

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For the production of biopharmaceuticals animal cells are nowadays indispensable. 70 % of those recombinant proteins are produced by utilizing CHO (Chinese hamster ovary)-cells. Due to the fact that every cell line has different requirements to the cultivation process (eg. media composition) and there is limited time for process development the use of high-throughput technologies is required. Compared with conventional spinner flasks small scale orbitally shaken reactors for volumes of 15 and 50 mL enable more parallel experiments to be performed on one shaker. This technology provides an easy handling and no complex built-in elements are needed. A major advantage is the homogeneous distribution during shaking by not damaging the cells. Furthermore the tubes can directly be used for centrifugation during sample preparation.

By taking samples for process monitoring the tubes need to be removed out of the incubator. That results in a change of important process parameters like the temperature, DO or the pH value. In order to avoid the risk of contamination and to guarantee a continuously monitored and reproducible process the University of Applied Science Esslingen develops an automatized handling system for 50 ml orbitally shaken minibioreactors in corporation with a company in Esslingen, Festo AG & Co. KG.

To have a point of reference for demonstrating that the system exhibits an improvement over the manual application, additionally it was necessary to perform an optimization in the conventional way. During the project thesis different media and feed-strategies with the focus on the antibody production were compared. Subsequent the most promising strategies were used to verify the transferability in larger scales of 400 ml.

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Poster No. 36

BioSpeckle- An innovative, non-invasive, photonic technology to characterize heterogeneous biomass-structures

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The increasing shortage of fossil raw material is one reason why alternative biotechnological processes are urgently required to produce natural agents and additives. Plant cells, tissue cultures, fungi and algae are common natural resources and therefore quite interesting for science and industry.

For example, plant cells and tissue cultures bare a wide range of nutritional, physiological and pharmaceutical relevant secondary metabolites. However, conventional production is dependent on a number of biotic and abiotic influences. The in vitro production of secondary metabolites in plant cell and tissue cultures (e.g. hairy roots) represents a suitable alternative to conventional techniques and allows a year-round cultivation in the bioreactor under optimal conditions with consistent quality and quantity.

The development and monitoring of biotechnological processes requires the determination of biomass characteristics and their growth- and reaction kinetics. One of the most important parameter is the biomass formation.

In hairy root cultures, which form heterogeneous, network-like structures, the sampling and direct quantification of biomass is complicated and in some case not possible. The quantification of hairy roots with indirect methods is very laborious and time-intensive. Therefore an alternative rapid method is required.

The poster presents the capabilities of the Laser-Speckle-Photometry as an alternative and rapid method for determination and characterization of biomass-structure like hairy root cultures or other filamentous, network-like structures like fungi or macro-algae. The photonic technique of time-resolved Laser-Speckle-Photometry is based on the analysis of the variation of speckle-patterns. Dynamic behavior in biological structures can be detected by this technology. The temporal and spatial intensity variations of time resolved speckle-patterns contain information about the biological system.

This very rapid, photonic method allows the non-destructive, contactless measurement without sampling. The innovative, non-invasive method for the characterization of heterogeneous biomass-structures based on Laser-Speckle-Photometry is adaptable to conventional cultivation systems.

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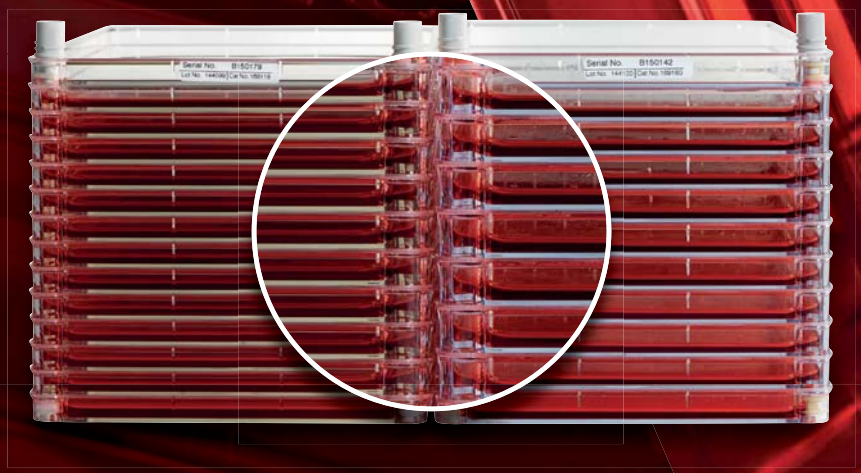

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Poster No. 37

Ultra-fast pH-gradient ion exchange chromatography for the separation of monoclonal antibody charge variants

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[4]Thermo Fisher Scientific, Germering, DE

Charge variants of monoclonal antibodies (mAbs) are prone to modifications such as sialylation, deamidation or C-terminal lysine truncation. Traditionally, salt gradient cation exchange chromatography has successfully been used for the assessment of the mAb charge variant profile. However, a significant effort is often required to tailor the salt gradient method to each individual mAb and generally long run times are needed to achieve the desired resolution. In the fast-paced drug development environment, a rapid and robust platform method is desirable that accommodates the majority of the mAbs analyzed. In this study the charge variant profile of top-selling mAbs were analyzed by strong cation exchange with a linear pH gradient method utilizing next generation UHPLC technology. This pH gradient method serves as a platform method for the mAb charge variant analysis, covering the pH range from 5.6 – 10.2 and also allows to determine the pI value of the charge variants when combined with an on-line pH monitor. Bevacizumab, Cetuximab, Infliximab, and Trastuzumab were analyzed on a small particle MAbPac SCX-10 column using a full pH gradient of only 10 min. Satisfactory separations of multiple charge variants are observed for all mAbs analyzed. Additional improvement of the resolution and a significant shortening of the analysis time was achieved by optimizing the utilized pH range for each mAb in conjunction with the application of an elevated flow rate to further decrease the applied gradient slope. This easy and fast method optimization approach allowed the registration of the charge variant profile for each mAb in less than 5 min while retaining the high-resolution separation normally only associated with longer gradient runs.

Poster No. 38

Modular Sampling Systems as a Tool for Innovative PAT Solutions

Sunjeet Singh[1], Andreas Riklin[2], Matthias Barmettler[1], Marcel Straumann[1], Karin Kovar[1], Caspar Demuth[1], Lukas Neutsch[1]

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State-of-the-art PAT concepts in biotechnology strongly focus on the time-resolved measurement of critical process variables to allow for better control, enhanced process understanding, higher efficiency and shorter overall development times. The panel of variables available in real-time is constantly being expanded, and to an increasing extent involves descriptors of cell physiology such as biomass and substrate concentrations, metabolites, or the secreted product itself. A major challenge associated with these innovative PAT concepts is that additional analytical devices need to be interfaced to the bioreactor, while maintaining the sterile barrier and respecting other constraints such as limited sample volumes, and ample changes in biomass levels, gas bubble or surfactant content. Depending on the question in focus, cell suspensions or cell-free supernatant, with or without additional conditioning or reaction steps, have to be transferred to the analyser in a fully automated mode.

These requirements illustrate the need for highly flexible and customizable solutions, which nevertheless should be robust, and easy to implement and maintain. Most importantly, the technical hardware should be seamlessly integrated into the process control software to best possibly extract the relevant information from the expanded datasets and make it utilizable for real-time monitoring and QbD-control strategies.

Here, we present the first results obtained via a modular sampling system for advanced PAT solutions (Numera®, Securecell AG, Schlieren) which can be adapted to different analytical tasks and interfaced to a wide range of bioreactor types. The system allows for sampling of small volumes (<2 ml) with a multiplexer interface and fully automated variable processing steps, such as dilution, reagent addition and filtration. Via a standardized preparation process, samples can be drawn at high frequency for prolonged periods of time (> 3 days), providing data with higher validity and consistency. Moreover, an outlook is given to the novel, integrated online control tools and additional analytical devices that may now be implemented for e.g. physiology-based feed profiles.

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Poster No. 39

Soft-sensor assisted bioprocess development for single-use systems

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During the last decade, single-use technologies managed the leap into the biopharmaceutical industry were they got a lot of attention. Disposable systems entail many advantages compared to conventional technologies, i.e. lower capital costs, a reduced contamination risk and a higher manufacturing flexibility. However, some conventional sensor equipment suffers from serious drawbacks, is too expensive, or cannot be used in combination with single-use technologies at all. Here, soft-sensors are a valid alternative to conventional "hard-type" sensors.

A soft-sensor in general is a piece of software processing easily measurable data to estimate difficult to access information. Starting with only one measured input variable and a simple mathematical conversion, a soft-sensor can become arbitrarily complex and convert a plurality of measured inputs via a mechanistic or statistical model to the parameter of interest. Here we focus on two different soft-sensor implementations.

One of the most mature soft-sensors is a soft-sensor for the fed-batch phase of microbial fermentations, the predominant process form in the biopharmaceutical industry. In the development and manufacturing of microbial processes the biomass concentration, as well as all derived parameters like yield coefficients and specific rates, are key parameters to monitor and control the process. The soft-sensor for the fed-batch phase allows to estimate the biomass as well as all derived variables (μ, qS, YX/S). This can be done without calibration and non-invasive by using already available raw data from feed signals and off-gas analysis.

The second soft-sensor was developed for mammalian processes. Here, samples are taken in a regular interval by the operator and analyzed off-line. Measured parameters are concentrations like glucose, lactate, glutamine or the cell number. The soft-sensor uses the off-line values as inputs to for the underlying physiological model. The model predicts the concentrations of the variables of interest in real-time.

In three case studies we show how the mentioned soft-sensors were successfully tested under conditions similar to single-use ones, i.e. low feeding and off-gas outflow rates. The mentioned soft-sensors can be used for process monitoring, analysis and control. For process monitoring, the soft-sensors can be used to get real-time information of unmeasurable or rarely measured process variables. When using the soft-sensor for process data analysis, the framework works off-line as information mining tool to extract valuable information from different runs. The mined information is input data for multivariate statistical methods. In the last case study we show that the soft-sensors allows it to establish process control strategies based on physiological data.

Poster No. 40

New business opportunities in microbial biotechnology: An innovative educational environment

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The full potential for microbial biotechnology in the development of products for many, diverse markets, including biopharmaceuticals, biosimilars, chemicals, dietary supplements, cosmetics, foods, and animal feed has, to date, been poorly exploited. Compared to traditional processes of chemical synthesis or extraction from natural materials, the development of biotechnological production processes is typically too slow or too costly. The challenge is to substitute established technologies with more environmentally sustainable, more cost effective biotechnologies.

Novel technologies, which have the potential to supersede traditional technologies, are now being evaluated through the New Business Opportunity (NBO) programme, which was launched in 2010 and received the Best Teaching Award 2015 from the ZHAW. This new teaching/learning environment was developed for students in the Master's programme in Pharmaceutical Biotechnology, and is designed to encourage cross-disciplinary analysis and to develop students' competencies in the initiation, evaluation and implementation of viable new ideas for products or production processes in the field of bio-manufacturing utilising bacteria, yeasts, fungi, or microalgae.

The innovative educational programme facilitates collaboration between academia and industry and stimulates the development of 'green technologies' for the Swiss economy. Due to the close interaction between students and industry practitioners, who are topic owners and/or act as coaches for the student teams, unparalleled opportunities for networking and discussion of novel ideas are created. Industry partners become inspired by a multi-perspective analysis of the opportunities, encompassing the key trends and drivers and the technical, economic and ecological feasibility, in addition to the societal impacts, and the legal and ethical issues.

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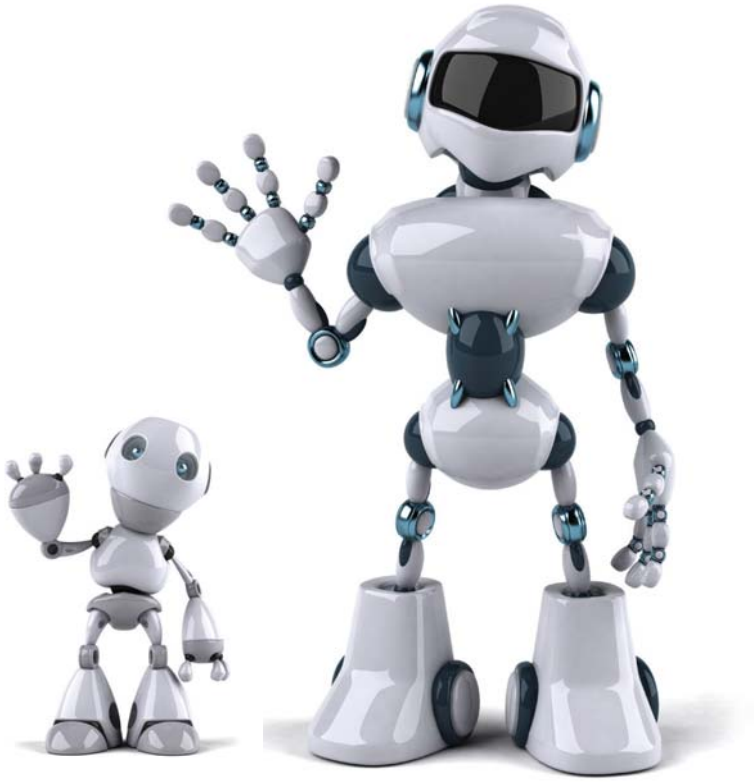
- Do you have a novel idea but lack appropriate resources for its evaluation and development?

Three collaborative, industry-academia research projects, which were originally conceived based on NBO-analyses, are already being supported by the Swiss Commission of Technology and Innovation (CTI).

For further information, please do not hesitate to contact us at: www.zhaw.ch/icbt/nbo



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Poster No. 41

Low-cost disposable sensors for biotechnology applications

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Printed electronics is a technology adaptable to the most diverse applications, thanks to its cost-effectiveness, the vast choice of materials and its flexibility in the design. The Swiss Center of Electronics and Microtechnology (CSEM) has been active in the development of printed electronics for many years, one of the field application being the integration of printed electrochemical sensors in systems for biological markers monitoring. The functional elements realized, integrating sensors, fluidics and data acquisition, were mostly for (semi-)disposable or single-use detection cartridges.

An example of development of a monitoring system for an in-vitro hepatic tissue model system has been performed in the framework of the European project HeMiBio (hemibio.eu). Within the HeMiBio project a system for alternative repeated dose toxicity studies was conceived. The system is envisioned to test the effects of chronic exposure to chemicals, in particular cosmetic ingredients, thus limiting the use of animal models. The monitoring system was designed to automatically detect changes in pH, glucose, lactate, pO2 and ALT, a cell death marker specific for hepatic cells.

The printed electronics technology was employed for the realization of disposable cartridges for biomarkers detection in saliva. Proof of concept of detection of ESAT-6, a marker of tuberculosis, or of monitoring of pH and uric acid, for the renal failure patients, have been performed. The saliva monitoring device is now being adapted for direct in-mouth monitoring of pH.

Collaborations with industrial partners are ongoing to adapt the printed electrochemical sensing technology to the real-time bioprocess monitoring in disposable bioreactors. The development of a platform for pH, glucose, lactate and pO_2 control is envisioned.

Poster No. 42

Biological bioreactor characterization by means of an *Escherichia coli* model process

Damian von Blarer[1], Yannick Senn[1], Klaus Kienle[1], Cedric Schirmer[1], Katharina Blaschczok[1], Ute Husemann[2], Marco Leupold[2], Gerhard Greller[2], Jens Rupprecht[2], Rainer Glöckler[3], Dieter Eibl[1]

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Nowadays, a wide variety of single-use and conventional stainless steel bioreactors are provided by several manufacturers. As different designs and properties may lead to deviations in biological performance, characterization and qualification of bioreactors is essential. So far, bioreactor characterization has mainly been based on bioengineering parameters, such as oxygen transfer, mixing time or power input. In order to guarantee reliable comparison and evaluation of different production systems, standardized biological methods are also needed. For this reason, DECHEMA`s expert group on single-use technology has developed guidelines providing a set of uniform procedures which allow cross-system comparison independent of the manufacturer's data, applicable for re-usable as well as single-use systems.

In this study, bioengineering and biological characterization were carried out according to DECHEMA's guidelines [1][2] for two stainless steel stirred tank bioreactors with working volumes of 30 and 100 L. The biological performance of the bioreactors was assessed by means of batch fermentations with *Escherichia coli* W3110 and Biener's medium. Process parameters were set to the aeration rate and stirrer speed which resulted in the highest k_La values, no oxygen regulation was used, and the process was terminated at 0 % dissolved oxygen. The seed cultures for the 30 L and 100 L bioreactors were produced in 10 L and 20 L wave-mixed single-use bioreactors respectively, and were inoculated from a 1 L Erlenmeyer flask. Dissolved oxygen regulation in the wave-mixed bioreactor was realized by increasing addition of pure oxygen. pH was regulated with ammonium hydroxide solution.

Reproducible results were obtained for the batch fermentations with average optical densities of OD_{600} = 39.2 ± 6.1 for the 30 L bioreactor und 26.5 ± 1.5 for the 100 L bioreactor. In addition, a highly demanding fed-batch process was performed to evaluate the limits of each bioreactor. Here, optical densities of 293 (30 L bioreactor) to 315 (100 L bioreactor) were obtained.

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Poster No. 43

Engineering characterization and cultivation of fast growing plant suspension cells of the GE Healthcare's ReadyToProcess WAVE™ 25 bioreactor system

Sören Werner[1], Mischa Stalder[1], Christina Vontobel[1], Andreas Castan[2], Dieter Eibl[1], Regine Eibl[1]

[1] Zurich University of Applied Sciences, Wädenswil, CH

[2] GE Healthcare Life Sciences, Uppsala, SE

The ReadyToProcess WAVE 25 bioreactor system from GE Healthcare can be equipped with bags in the sizes of 2 L, 10 L, 20 L, and 50 L with 1 L, 5 L, 10 L, and 25 L maximum working volumes, respectively. Mixing is achieved by a rocking motion, at which the rocking speed, angle, and the sinusoidal profile can be adjusted. To design and scale-up biological processes, it is essential to determine the main engineering parameters of the bioreactor system and this was the objective for this investigation.

The mixing time was measured with the decolourization method and the specific oxygen mass transfer coefficient was determined by the gassing out method, both in accordance with the guidelines of DECHEMA. In order to determine the specific power input and to investigate the fluid flow pattern, including shear and normal forces and the Kolmogorov microscale of turbulence, numerical investigations utilizing computational fluid dynamics were carried out.

The mixing time was in a range which is suitable for cell cultures and the oxygen mass transfer coefficient was found to be up to 12 h^{-1}. The specific power input ranged between 50 and 500 W m^{-3}.

Based on these findings, mass propagations of fast growing *Nicotiana tabacum* BY-2 suspension cells were realized. The triplicate cultivation run delivered a reproducible cell growth. A maximum cell fresh weight of 350 g L^{-1} could be achieved, which is comparable to data found in the literature (1). Although the viscosity of the culture broth strongly changed during the cultivation, oxygen limitations were not observed.

Thus, the new ReadyToProcess WAVE 25 bioreactor system with its 20 L bag is characterized regarding the main important engineering parameters. The successful cultivation of plant cells demonstrated the system's ability for this specific type of application.

Literature

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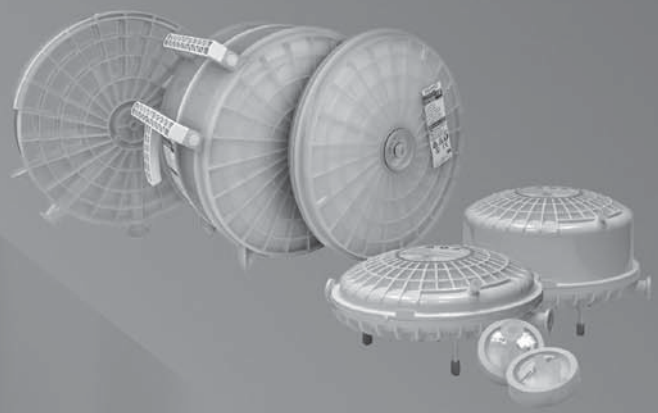

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Poster No. 44

Superior scalability of a single-use bioreactor family from 0.25 to 2000 L

Christian Zahnow[1], *Neil Bargh*[2], *Thomas Dreher*[1], *Adrian Stacey*[2], *Ute Husemann*[1], *Sebastian Ruhl*[1], *Gerhard Greller*[1]

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[2] *Sartorius Stedim Biotech Ltd., Royston, UK*

KEYWORDS:

Single-use bioreactor, process engineering, stirred tank reactor, scale-up, QbD

The use of single-use bioreactors has increased in the biopharmaceutical industry over the past decades, due to several advantages in comparison to multi-use systems. These include safety factors like a reduced risk of cross contaminations and time saving factors like shorter lead times. Due to different shapes, agitation principles and gassing strategies the scale-up and scale-down in single-use bioreactors can be challenging. As a result, due to their geometrical properties and the long experience of process engineering knowledge, multi-use bioreactors are regarded as the gold standard up to now.

Based on these difficulties a stirred 250 mL system for process development and a bioreactor family for scaling-up to production scales were developed with geometrical ratios similar to multi-use systems. These bioreactors are characterized by process engineering methods to allow a quality by design approach. To generate a control space for cell cultivations the k_La, mixing times and specific power input were determined for 0.25 to 2000 L scale according to DECHEMA guidelines.

The achieved data are comparable to established multi-use cell culture bioreactors and are suitable for cultivations of mammalian cells. Scale-up or scale-down process transfers can be performed based on the control space approach generated by the process engineering data. This shows the advantages of the presented bioreactors for single-use technology over a broad range of scales.

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