

# BIOCHEMICAL ENGINEERING AND CELL CULTURE TECHNIQUE

ACTIVITIES and REFERENCE LIST

## CONTACT

### **Prof. Dr. Ing. Regine Eibl**

Head of Cell Cultivation Techniques

regine.eibl@zhaw.ch

zhaw  
Departement Life Sciences und Facility Management  
Institut für Biotechnologie  
Grüental  
Postfach  
CH-8820 Wädenswil

Tel. +41 58 934 57 13  
Tel. Zentrale +41 58 934 50 00  
Fax Zentrale +41 58 934 50 01

[www.lsfm.zhaw.ch](http://www.lsfm.zhaw.ch)  
[www.zellkulturtechnik.ch](http://www.zellkulturtechnik.ch)

### **Prof. Dr. Ing. Dieter Eibl**

Head of Biochemical Engineering

dieter.eibl@zhaw.ch

zhaw  
Departement Life Sciences und Facility Management  
Institut für Biotechnologie  
Grüental  
Postfach  
CH-8820 Wädenswil

Tel. +41 58 934 57 11  
Tel. Zentrale +41 58 934 50 00  
Fax Zentrale +41 58 934 50 01

[www.lsfm.zhaw.ch](http://www.lsfm.zhaw.ch)  
[www.bioverfahrenstechnik.ch](http://www.bioverfahrenstechnik.ch)

## TRAINING FOR STUDENTS AT ZHAW

- Biotechnology: Introduction (1<sup>st</sup> term, field of study: Biotechnology)
- Biotechnology: Introduction (4<sup>th</sup> term, field of study: Food technology)
- Plant cell cultivation technique (3<sup>rd</sup> term, field of study: Biotechnology)
- Cell- and tissue cultures for pharmaceutical and medical application (4<sup>th</sup> term, field of study: Environmental engineering)
- Specialization: Cell cultivation technique (5<sup>th</sup> and 6<sup>th</sup> term, field of study: Biotechnology)
- Technical equipment and installation I and II (1<sup>st</sup> and 2<sup>nd</sup> term, field of study: Biotechnology)
- Project week: Systems engineering (3<sup>rd</sup> term, field of study: Biotechnology)
- Biochemical engineering: Basic training I and II (3<sup>rd</sup> and 4<sup>th</sup> term, field of study: Biotechnology)
- Biotechnological processes (5<sup>th</sup> and 6<sup>th</sup> term, field of study: Biotechnology)
- Specialization: Design of biotechnological production facilities (5<sup>th</sup> and 6<sup>th</sup> term, field of study: Biotechnology)
- Course “Disposables in biomanufacturing”, Master study “Life Sciences” of the Universities of Applied Sciences in Switzerland

## COURSES IN FURTHER EDUCATION

- Science week (4.-8. August 2014, 3.-7. August 2015, ZHAW and partners)
- Advances training course: Cell expansion and protein expression: An introduction for standard and single-use bioreactors (6.-23. August 2013, 12.-29. August 2014, 11—28. August 2015, ZHAW and partners)
- Mammalian cell-based protein expression: Upstream- and downstream-processing: From cell to bioactive substance (22. -26. April 2013 and 7.-11. April / 16.-20. June 2014, 13.-17. April 2015 and 20.-24. April / 8.-12. June 2015, Swiss BiotechNet/Hoffmann-La Roche Ltd., Basel)
- Single-use technology (18.-19. April 2013, 9. June 2015, Master training, TU Berlin)
- Single-use systems used in biopharmaceutical production processes (8. January 2014, 13. November 2014, Master training, BOKU Vienna)
- The modern biotechnological manufacturing facility: Plastics instead glass or stainless steel (11. January 2013, 17. January 2014, 16. January 2015, Aprentas Switzerland)
- Trends in cell cultivation techniques, cell culture bioreactors and selection criteria, bioreactor modelling (22.-24. Mai 2013, 7.-9. July 2014, 23.-26. June 2015, Master training, University of Applied Sciences Anhalt/Köthen)

## RESEARCH

- Directly financed projects by companies
  - Characterization and optimization of novel disposable bioreactors (Sartorius)  
Cooperation contract
  - Cultivation studies with cell culture bioreactors (Eppendorf/DASGIP)  
03/2014-12/2016
  - Characterization of a new wave-mixed bioreactor system (GE Healthcare)  
08/2014-07/2015
  - Optimization of plant cell bioreactors (Phyton)  
07/2014-06/2016
  - PhD thesis St. Kaiser (Finesse)  
4/2011-6/2014
- CTI projects
  - Intelligent SCADA expert system for user friendly process control (Infors)  
11/2014-10/2017
  - Development of a single-use nano dose system (ReseaChem, Berner Fachhochschule BFH)  
04/2013-05/2015
  - A robust CHO cell-based process platform for rapid manufacture of novel fully human bispecific monoclonal antibodies with importance for preclinical studies, immuno- and cancer therapies (Novimmune, Cell Culture Technologies and University of Zurich)  
05/2013-10/2014
  - Platform for rapid manufacture of novel fully human bispecific mabs (NovImmune)  
06/2013-11/2014
  - Magnetic single-use centrifugal pump (Levitronix)  
1/2012-6/2013
  - Process optimization of starter cultures (Haya)  
04/2013-4/2014
  - Development of a platform for scalable production of therapeutic relevant stem cells (Lonza)  
1/2012-1/2014
  - Process optimization with a human hybridoma cell line for the production of a h mab for treatment of MRSA infections (Kenta)  
2/2012-4/2013
  - CitsENS, a wireless disposable sensor array for disposable bioreactors (C-CIT, no lead)  
7/2012-6/2014
  - Scale-up factors for orbitally shaken bioreactors (Infors AG)  
06/2011-05/2012
- EU projects
  - BIOCOTES: Pesticide production with *in vitro* cultivated insect cells  
1/2014-12/2017
  - glucoCell: Single-use glucose sensor for single-use bioreactors (Eurostar)  
4/2012-10/2013
  - Smartcell/Green Factory (platform lead)  
1/2009-6/2014
  - Contained Molecular Farming (ComoFarm)  
1/2009-12/2012
  - BIOCOTES  
submitted

- Small projects supported by ZHAW
  - A new production approach for insect cell-based, biotechnological production processes (Kühner AG)  
from 07/2015
  - Immortalized hADSCs: An alternative to primary hADSCs for expansion and shear stress studies  
from 05/20015
  - Serum-free expansions and differentiations of hMSCs for therapeutical use (Cardiocentro Ticino)  
from 05/2014
  - Modelling of fluid flow in scaffolds used to grow epithelial cells and hMSCs (Universität Mainz)  
05/2014-04/2015
  - Hairy root cultivations aimed at production of bioactive compounds (ROOTec)  
5/2014-11-2014
  - Investigation of the influence of light on growth and secondary metabolism of plant suspension cells in single-use bioreactors (VTT)  
5/2013-12/2013
  - Micromixing and cells (TU Hamburg-Harburg)  
09/2013-08/2014
  - Development of strategies for comparison of different bioreactors for the cultivation of hairy roots. (ROOTec)  
5/2014-11-2014
  - Influence of light on growth and secondary metabolism of plant suspension cells in single-use bioreactors (VTT)  
5/2013-12/2013
  - Development of a rapid test for bag materials to evaluate leachables and extractables with cell toxicity (DECHEMA)  
4/2012-12/2012
  - Development of an integrated, scalable cell cultivation process based on single-use bioreactors (Book chapter)  
4/2012-2/2013
  - MaquiSelect: Preservation of wild growing *Aristotelia chilensis* and sustainable production of Maqui berries in Chile by efficient phytochemical and activity-guided selection methods (no lead, co-operation phytopharmacy group of the IBT)  
10/2012-10/2013
  - Mass propagation of *Helianthus annuus* and *Salvia officinalis* suspension cultures (TU Dresden)  
4/2012-12/2012
  - Development of a taxol production process based on hazelnut suspension cells and single-use bioreactors (University Barcelona)  
9/2012-8/2013
  - Optimizing relevant factors for a medicinal aerosol which influence efficient segregation at the human olfactory epithelium by usage of CFD (UAS Biberach)  
9/2012-3/2012

2015

- V. Jossen, C. Schirmaier, G. T. John, D. Eibl, R. Eibl (2015) On-line-pH- und DO-Messungen in Mikrocarrier-basierter hMSC-Kultur. *BIOspektrum* 4:422-424.

**Abstract**

Spinner flasks are often used for microcarrier-based cultivations of human mesenchymal stem cells (hMSCs). Normally, they are not equipped with pH and dissolved oxygen (DO) probes. This application note describes the cultivation of hMSCs in single-use spinner flasks equipped with optical pH (SP-HP8) and DO (SP-PSt3) sensors for the first time. While reaching peak cell numbers between  $4.1 \times 10^7$  cells and  $5.9 \times 10^7$  cells in two cultivation runs, reliable DO and pH data were delivered.

- I. Dittler, W. Dornfeld, R. Schöb, J. Cocke, J. Rojahn, M. Kraume, D. Eibl (2015) A cost effective and reliable method to predict mechanical stress in single-use and standard sumps. *J. Vis. Exp.*, doi:10.3791/53052 (2015).

**Abstract**

Pumps are mainly used when transferring sterile culture broths in biopharmaceutical and biotechnological production processes. However, during the pumping process shear forces occur which can lead to qualitative and/or quantitative product loss. To calculate the mechanical stress with limited experimental expense, an oil-water emulsion system was used, whose suitability was demonstrated for drop size detections in bioreactors<sup>1</sup>. As drop breakup of the oil-water emulsion system is a function of mechanical stress, drop sizes need to be counted over the experimental time of shear stress investigations. In previous studies, the inline endoscopy has been shown to be an accurate and reliable measurement technique for drop size detections in liquid/liquid dispersions. The aim of this protocol is to show the suitability of the inline endoscopy technique for drop size measurements in pumping processes. In order to express the drop size, the Sauter mean diameter  $d_{32}$  was used as the representative diameter of drops in the oil-water emulsion. The results showed low variation in the Sauter mean diameters, which were quantified by standard deviations of below 15 %, indicating the reliability of the measurement technique.

- R. Eibl, D. Eibl (2015) Single-Use (Disposable) Systems in Biopharmaceutical Processing: Quo Vadis? *ACHEMA online – Trend report No. 13*, <http://www.achema.de/en/press/service-for-journalists/trend-reports/trend-report-nr-13-single-use-disposable-systems-in-biopharmaceutical-processing-quo-vadis.html>, 19.06.2015.

**Abstract**

Single-use systems (SUS) are now used in the majority of biopharmaceutical processes involving animal cell cultures. Single-use filters, plastic storage bags, single-use mixers and single-use bioreactors for upstream processing (USP) in pre-clinical and clinical sample production are the items most commonly used. However the biopharmaceutical industry is also making greater use of SUS for downstream processing (DSP), formulation and filling. Stirred-tank single-use bioreactors for microorganisms are one of the current development priorities. There is also a need for single-use equipment to process stem cells and T-cells.

- B. Badertscher (2015) A contribution to the *in vitro* production of a gypsy moth baculovirus biopesticide. *A JOUR* 1: 11-13.

**Abstract**

The gypsy moth (*Lymantria dispar*), an invasive defoliator, is controlled by a biological insecticide: the baculovirus *Lymantria dispar* multicapsid nuclear polyhedrosis virus (LdMNPV). The baculovirus is currently commercially produced *in vivo*. An *in vitro* virus production process would bring a variety of advantages. Establishing an efficient biotechnological production based on cell culture techniques was the focus of the Bachelor's thesis, and contributes to the EU project BIOCAMES ([www.biocomes.eu](http://www.biocomes.eu)).

- A. Gallego, I. Imseng, M. Bonfill, R.M. Cusido, Palazon, R. Eibl, E. Moyano (2015) Development of a hazel cell culture-based paclitaxel and baccatin III production process on a benchtop scale. *Journal of Biotechnology*, 195:93-102.

**Abstract**

The growing demand for the antitumorous agent paclitaxel and the difficulty in increasing its production by genetic engineering has prompted a search for new sources of taxanes. It has been reported that taxanes can be extracted from the angiosperm *Corylus avellana* L. Our aim was to improve taxane production by scaling up the process from mL-level to benchtop bioreactors, optimizing culture conditions and comparing the effect of two elicitors, 1  $\mu$ M coronatine (Cor) and 100  $\mu$ M methyl jasmonate (MeJA). Orbitally shaken flask cultures achieved a maximum fresh cell weight of 11.54 gDCW/L under control conditions, and MeJA- and Cor-treatment produced a statistically significant reduction in growth to 4.28 gDCW/L and 5.69 gDCW/L, while increasing the taxane content 3- and 27-fold, respectively. The enhancing effect of these elicitors on taxane production, despite affecting growth, was confirmed in orbitally shaken TubeSpin® Bioreactors 50, where the highest taxane content (8583.3 g/L) was obtained when 1  $\mu$ M Cor was used and elicitation took place at a packed cell volume of 50%. Two benchtop stirred bioreactors, BIOSTAT® B plus and UniVessel® SU, were compared, the latter providing a higher biomass of *C. avellana* cell suspension

cultures. Transferring the established optimum culture conditions for taxane production to the UniVessel®SU resulted in a total taxane content of 6246.1 g/L, a 10-fold increase compared with shake flask experiments.

2014

- C. Fritz, R. Eibl, D. Müller, D. Eisenkrätzer, J. Bär, D. Eibl (2014) Single-use Bags in Zellkulturprozessen (in press)
- S. Werner, J. Greulich, K. Geipel, J. Steingroewer, T. Bley, D. Eibl (2014) Mass propagation of *Helianthus annuus* suspension cells in orbitally shaken bioreactors: Improved growth rate in single-use bag bioreactors. Engineering in Life Sciences, online. DOI:10.1002/elsc.201400024

**Abstract**

Stirred tank-bioreactors made of glass or steel, wave-mixed and orbitally shaken bag bioreactors have all proven to be suitable for the rapid development and commercial production of bioactive compounds with plant cell suspensions. Although these bag bioreactors are characterized by reduced foam formation and less flotation in comparison to stirred systems, their power input is limited. Engineering parameters such as mixing time, oxygen transfer and power input are dependent on the viscosity of the liquid and thus, investigations with plant cell suspensions are necessary. However, to save time and achieve better controllability, sodium carboxymethyl cellulose (Na-CMC) solutions in concentrations ranging from 1 to 20 g·L<sup>-1</sup>, with viscosities of between 0.005 and 0.4 Pa, were identified as appropriate model systems for mimicking plant cell suspensions with packed cell volumes (PCV) of between 30 and 70 % and similar viscosities. The current study has shown that it is possible to transfer a *Helianthus annuus* cell suspension process from an orbitally shaken CultiBag RM 1 L to a CultiBag RM with a 10 L working volume by adjusting the operating parameters to achieve a constant  $k_L a$  value. A maximum specific growth rate  $\mu_{max}$  of around 0.25 d<sup>-1</sup> was achieved, which corresponds to optimized data for shake flasks and even exceeds the growth rate for stirred glass-bioreactors.

- V. Jossen, S. C. Kaiser, C. Schirmaier, J. Herrmann, A. Tappe, D. Eibl, A. Siehoff, C. van den Bos, R. Eibl (2014) Modification and qualification of a stirred single-use bioreactor for the improved expansion of human mesenchymal stem cells at benchtop scale, Pharmaceutical Bioprocessing, online, DOI: 10.4155/pbp.14.29

**Abstract**

To improve cultivation conditions for human bone-marrow-derived mesenchymal stem cells, we redesigned the commercially available UniVessel® SU bioreactor using results obtained from computational fluid dynamics. The goal was to produce  $\geq 1 \times 10^9$  cells and to achieve expansion factors  $\geq 30$ . Screening studies suggested that microcarrier solid fractions of at least 0.3% are required to reach the appropriate cell densities. The fluid flow pattern found in the most promising modification (#2) was altered by increasing the impeller blade angle and lowering the off-bottom clearance. As a result, the maximum required specific power input was reduced by a factor of 2.2–4.6, depending on the microcarrier concentration, and peak cell densities were 3.4-times higher than in the standard version. The peak cell number of nearly  $1.1 \times 10^9$  cells (expansion factor = 35), which was achieved in our low-serum cultivations, indicates an improvement in the redesigned UniVessel® SU configuration for bone marrow-derived mesenchymal stem cell expansions.

- W. Klöckner, C. Lattermann, F. Pursche, S. Werner, D. Eibl, J. Büchs (2014) Time efficient way to calculate oxygen transfer areas and power input in cylindrical disposable shaken bioreactors. Biotechnology Progress, online. DOI:10.1002/btpr.1977

**Abstract**

Disposable orbitally shaken bioreactors are a promising alternative to stirred or wave agitated systems for mammalian and plant cell cultivation, because they provide a homogeneous and well-defined liquid distribution together with a simple and cost-efficient design. Cultivation conditions in the surface-aerated bioreactors are mainly affected by the size of the volumetric oxygen transfer area ( $a$ ) and the volumetric power input ( $P/V_L$ ) that both result from the liquid distribution during shaking. Since Computational Fluid Dynamics (CFD)—commonly applied to simulate the liquid distribution in such bioreactors—needs high computing power, this technique is poorly suited to investigate the influence of many different operating conditions in various scales. Thus, the aim of this paper is to introduce a new mathematical model for calculating the values of  $a$  and  $P/V_L$  for liquids with water-like viscosities. The model equations were derived from the balance of centrifugal and gravitational forces exerted during shaking. A good agreement was found among calculated values for  $a$  and  $P/V_L$ , CFD simulation values and empirical results. The newly proposed model enables a time efficient way to calculate the oxygen transfer areas and power input for various shaking frequencies, filling volumes and shaking and reactor diameters. All these parameters can be calculated fast and with little computing power.

- N. Imseng, N. Steiger, D. Frasson, M. Sievers, A. Tappe, G. Greller, D. Eibl, R. Eibl (2014) Single-use wave-mixed versus stirred bioreactors for insect-cell/BEVS-based protein expression at benchtop scale, Engineering in Life Sciences, 14 (3): 264–271, DOI: 10.1002/elsc.201300131

**Abstract**

Spodoptera frugiperda-9 (Sf-9) cells used in conjunction with the baculovirus expression vector system (BEVS) represent a

promising platform for the rapid development and manufacture of protein complexes and virus-like particle (VLP) products. Several studies have described the superiority of single-use wave-mixed bioreactors although reusable stirred and, more recently, single-use stirred bioreactors have also been successfully applied. Due to their bioengineering characteristics (more homogeneous energy dissipation, reduced foam formation), wave-mixed systems are often preferred. However, a direct comparison of the influence of single-use wave-mixed and single-use stirred bioreactors on cell growth and protein expression in Sf-9/BEVS-based production processes was still lacking. We investigated Sf-9 cell growth and expression of a recombinant secreted alkaline phosphatase (rSEAP) in the wave-mixed BIOSTAT® RM as well as the stirred UniVessel R SU and a serum-free culture medium. Irrespective of the bioreactor system, comparable growth, substrate, and metabolite courses as well as peak cell densities ( $>1.2 \times 10^7$  cells mL<sup>-1</sup>) were observed in Sf-9 cell expansions performed in batch mode. Additionally, identical rSEAP quality and maximum rSEAP activities were found in biphasic productions in both bioreactor systems. Concluding, comparability of single-use wave-mixed and stirred bioreactors for insect cell culture processes was demonstrated for the first time.

- I. Dittler, St. C. Kaiser, K. Blaschczok, C. Löffelholz, P. Bösch, W. Dornfeld, R. Schöb, J. Rojahn, M. Kraume, D. Eibl (2014) A cost effective and reliable method to predict mechanical stress in single-use and standard pumps, *Engineering in Life Sciences*, DOI: 10.1002/elsc.201300068

**Abstract**

The suitability of oil-water emulsions to predict shear forces in stirred bioreactors under cost-effective and time-saving conditions has been demonstrated several times, but no application to pumps has been described so far. In this report, the drop sizes in a model oil-water system were determined for the Levitronix PuraLev® MU series (PuraLev® 200MU and PuraLev® 600MU), a peristaltic pump (Masterflex® I/P Easy Load), and a 4-piston diaphragm pump (Quattroflow 1200-SU) using inline endoscopy. It was determined that the Sauter mean diameter could be used as a comparison criterion to estimate mechanical stress in pumps. The investigation showed that PuraLev® MU pumps are characterized by up to 59 % larger Sauter mean diameters than their counterparts at comparable operational conditions. This indicates lower hydrodynamic stress in the PuraLev® MU pumps. Using computational fluid dynamics (CFD), a well-streamlined fluid flow and low turbulent energy dissipation rates (TEDR) were found in the PuraLev® MU pumps, which correlated well with experimental results. A calculation model was used to predict the Sauter mean diameter by combining both experimental and CFD data. Good agreement with deviations below 13 % was determined between model predictions and experimental data.
- A. Ritala, L. Dong, N. Imseng, T. Seppänen-Laakso, N. Vasilev, S. van der Krol, H. Rischer, H. Maaheimo, A. Virkki, J. Brändli, S. Schillberg, R. Eibl, H. Bouwmeester, K. M. Oksman-Caldentey (2014) Evaluation of tobacco (*Nicotiana tabacum* L. cv. Petit Havana SR1) hairy roots for the production of geraniol, the first committed step in terpenoid indole alkaloid pathway, *Journal of Biotechnology*, 176C:20-28, DOI: 10.1016/j.jbiotec.2014.01.031

**Abstract**

The terpenoid indole alkaloids are one of the major classes of plant-derived natural products and are well known for their many applications in the pharmaceutical, fragrance and cosmetics industries. Hairy root cultures are useful for the production of plant secondary metabolites because of their genetic and biochemical stability and their rapid growth in hormone-free media. Tobacco (*Nicotiana tabacum* L. cv. Petit Havana SR1) hairy roots, which do not produce geraniol naturally, were engineered to express a plastid-targeted geraniol synthase gene originally isolated from *Valeriana officinalis* L. (VoGES). A SPME-GC-MS screening tool was developed for the rapid evaluation of production clones. The GC-MS analysis revealed that the free geraniol content in 20 hairy root clones expressing VoGES was an average of 13.7 µg/g dry weight (DW) and a maximum of 31.3 µg/g DW. More detailed metabolic analysis revealed that geraniol derivatives were present in six major glycoside forms, namely the hexose and/or pentose conjugates of geraniol and hydroxygeraniol, resulting in total geraniol levels of up to 204.3 µg/g DW following deglycosylation. A benchtop-scale process was developed in a 20-L wave-mixed bioreactor eventually yielding hundreds of grams of biomass and milligram quantities of geraniol per cultivation bag.
- N. Vasilev, C. Schmitz, L. Dong, A. Ritala, N. Imseng, S.T. Häkkinen, S. van der Krol, R. Eibl, K. M. Oksman-Caldentey, H. Bouwmeester, R. Fischer, S. Schillberg (2014) Comparison of plant-based expression platforms for the heterologous production of geraniol, *Plant Cell, Tissue and Organ Culture* 117(3), 373-380, DOI : 10.1007/s11240-014-0446-z

**Abstract**

We compared the ability of different plant-based expression platforms to produce geraniol, a key metabolite in the monoterpenoid branch of the terpenoid indole alkaloid biosynthesis pathway. A geraniol synthase gene isolated from *Valeriana officinalis* (VoGES) was stably expressed in different tobacco systems. Intact plants were grown *in vitro* and in the greenhouse and were used to generate cell suspension and hairy root cultures. VoGES was also transiently expressed in *N. benthamiana*. The highest geraniol content was produced by intact transgenic plants grown *in vitro* (48 µg/g fresh weight, fw), followed by the transient expression system (27 µg/g fw), transgenic plants under hydroponic conditions in the greenhouse and cell suspension cultures (16 µg/g fw), and finally hairy root cultures (9 µg/g fw). Differences in biomass production and the duration of cultivation resulted in a spectrum of geraniol productivities. Cell suspension cultures achieved a geraniol production rate of 1.8 µg/g fresh biomass per day, whereas transient expression produced 5.9 µg/g fresh biomass per day (if cultivation prior to agroinfiltration is ignored) or 0.5 µg/g fresh biomass per day (if cultivation prior to agroinfiltration is included). The superior

productivity, strict process control and simple handling procedures available for transgenic cell suspension cultures suggest that cells are the most promising system for further optimization and ultimately for the scaled up production of geraniol.

- C. Schirmaier, V. Jossen, S.C. Kaiser, F. Jüngerkes, S. Brill, A. Safavi-Nab. A. Siehoff, C. van den Bos, D. Eibl, R. Eibl (2014) Scale-up of adipose tissue-derived mesenchymal stem cell production in stirred single-use bioreactors under low-serum conditions, *Engineering in Life Sciences* 14(3), 292-303, DOI: 10.1002/elsc.201300134

**Abstract**

Suspension cultures, in which human mesenchymal stem cells (hMSCs) are cultivated on microcarriers in scalable single-use stirred bioreactor types, have been shown to be a promising alternative to planar flask cultures. However, stirred single-use bioreactors were originally developed for production processes with robust, permanent cell lines. hMSCs are adherent primary cells and thus expanding them in such bioreactor systems imposes more stringent requirements on bioreactor systems. For low-serum conditions (5%) and different types of stirred single-use bioreactors, a suspension criteria-based approach for expanding human adipose tissue-derived mesenchymal stem cells (hASCs) from mL- to pilot scale was successfully developed. For process scale-up, experimental and numerical investigations were performed to (1) predict optimum impeller speeds, (2) determine the main engineering parameters (local shear stress, turbulent dissipation rate, Kolmogorov microscale), and (3) verify suspension criteria  $N_{S1}$  and  $N_{S1u}$  for rapid process transfer from 100 mL to 2 L and 35 L cultures. Using optimized medium/microcarrier combinations as well as  $N_{S1}$  and  $N_{S1u}$  as scale-up factors, total hASC quantities between  $3 \cdot 10^7$  (100 mL scale) and  $1 \cdot 10^{10}$  (35 L scale) were obtained. The cell quantities obtained are the highest reported to date for scalable single-use bioreactors under low-serum conditions.

- S. Werner, S. C. Kaiser, M. Kraume, Dieter Eibl (2014) Computational fluid dynamics as a modern tool for engineering characterization of bioreactors, *Pharmaceutical Bioprocessing* 2(1), 85-99, DOI: 10.4155/pbp.13.60

**Abstract**

Since design, construction and evaluation of bioreactors for large-scale production is costly and time consuming, computational methods may give some insights into the fluid mechanics within bioreactors. Thus, critical limiting factors, such as insufficient mixing as well as inhomogeneous nutrient and oxygen mass transfer, may be identified early in the design process. Although advanced experimental techniques such as laser Doppler anemometry and particle image velocimetry are also reliable, they are too time consuming to characterize the complete flow pattern in industrial scales and rely on optical accessibility. Therefore, the knowledge of flow characteristics provided by computational fluid dynamics (CFD) is indispensable for the rational design of bioreactors. Based on previously published reviews, the present work summarizes the latest publications on the usage of CFD to characterize and scale-up bioreactors used in biotechnological processes. Selected models that are used to predict the fluid flow pattern and key engineering parameters of commonly used bioreactors are described. Related issues, such as grid dependency of CFD results and the requirement for experimental verification are also addressed. Finally, an overview of proposed but not yet feasible CFD applications is presented, including fluid-structure interaction, the use of direct numerical simulation and the coupling fluid flow and chemical reactions.

- K. Blaschczok, C. Löffelholz, R. Eibl, D. Eibl (2014,) Combining single-use stirred bioreactor with standard cross-flow technology in biphasic protein production processes at pilot scale, *Engineering in Life Science*, DOI: 10.1002/elsc.201300069

**Abstract**

The increasing implementation of single-use bioreactors arrived hand-in-hand with the development of new technologies contributing to increased productivity, process flexibility, and additional savings in time and costs. As a result, hollow fiber technology has recently gained renewed interest in upstream processing. Using a Chinese hamster ovary cell line in a biphasic protein production process with chemically defined minimal culture media, we combined Sartorius Stedim's BIOSTAT STR 50 L with GE Healthcare Life Sciences' reusable Hollow Fiber Cartridge CFP-6-D-55A. After a 3-day feeding growth phase, secretion of the model protein secreted alkaline phosphatase (SEAP) was introduced by replacing the growth medium with production medium using cross-flow filtration. The process was then continued and harvested as a batch with temperature shift. High cell densities exceeding  $1 \times 10^7$  cells mL<sup>-1</sup> were achieved 5 days post inoculation and maximum secreted alkaline phosphatase activities of 24 U mL<sup>-1</sup> 11 days post inoculation. Our results showed that a further decrease in processing time is possible by reducing the number of diafiltration steps from three to two.

- C. Löffelholz, S. C. Kaiser, M. Kraume, R. Eibl and D. Eibl (2014) Dynamic Single-Use Bioreactors Used in Modern Liter- and m<sup>3</sup>-Scale Biotechnological Processes: Engineering Characteristics and Scaling Up, *Advances in Biochemical Engineering/Biotechnology* 138, 1-41, DOI: 10.1007/10\_2013\_187

**Abstract**

During the past 10 years, single-use bioreactors have been well accepted in modern biopharmaceutical production processes targeting high-value products. Up to now, such processes have mainly been small- or medium-scale mammalian cell culture-based seed inoculum, vaccine or antibody productions. However, recently first attempts have been made to modify existing



single-use bioreactors for the cultivation of plant cells and tissue cultures, and microorganisms. This has even led to the development of new single-use bioreactor types. Moreover, due to safety issues it has become clear that single-use bioreactors are the “must have” for expanding human stem cells delivering cell therapeutics, the biopharmaceuticals of the next generation. So it comes as no surprise that numerous different dynamic single-use bioreactor types, which are suitable for a wide range of applications, already dominate the market today. Bioreactor working principles, main applications, and bioengineering data are presented in this review, based on a current overview of greater than milliliter-scale, commercially available, dynamic single-use bioreactors. The focus is on stirred versions, which are omnipresent in R&D and manufacturing, and in particular Sartorius Stedim’s BIOSTAT family. Finally, we examine development trends for single-use bioreactors, after discussing proven approaches for fast scaling-up processes.

- C. van den Bos, S. R. Keefe, C. Schirmaier, M. McCaman (2014), Therapeutic human cells: Manufacture for cell therapy/regenerative medicine, *Advances in Biochemical Engineering/Biotechnology* 138, 61-97, DOI: 10.1007/10\_2013\_233

**Abstract**

Human primary cells (e.g. adult stem cells) as well as differentiated cells, including those of the immune system, have been found to be therapeutically useful and free of ethical concerns. Several products have received market authorization and numerous promising clinical trials are underway. We believe that such primary therapeutic cells will dominate the market for cell therapy applications for the foreseeable future. Consequently, production of such cellular products warrants attention and needs to be a fully controlled pharmaceutical process. Thus, where possible, such production should change from manufacture towards a truly scalable industrialized process for both allogeneic and autologous products. Here, we discuss manufacturing aspects of both autogeneic and allogeneic products, review the field, and provide historical context.

- R. Eibl, N. Steiger, S. Wellnitz, T. Vicente, C. John and D. Eibl (2014) Fast Single-Use VLP Vaccine Productions Based on Insect Cells and the Baculovirus Expression Vector System: Influenza as Case Study, *Advances in Biochemical Engineering/Biotechnology* 138, 99-125, DOI: 10.1007/10\_2013-186

**Abstract**

During the last few years virus like particles (VLPs) have become increasingly interesting for the production of vaccines. This development is explained by their excellent safety profile as well as a significant number of clinical studies showing strong and long-lasting protection. A further reason is the possibility of speeding up VLP vaccine manufacturing by implementing single-use (SU) technology in the case of mammalian and insect-cell-based processes, for which a multitude of SU devices up to middle-volume scale already exist. After briefly introducing the vaccine types and expression systems currently in use, this chapter turns to VLP vaccines and the insect cell/baculovirus expression vector system (IC/BEVS). Based on the main process characteristics and typical process flow of IC/BEVS-based VLP vaccine productions, suitable SU devices and their implementation are addressed. We subsequently report on the successful development of a fast, scalable benchtop production process generating a four-protein component influenza A H1N1 VLP vaccine candidate. This process is based on *Spodoptera frugiperda* (Sf)-9 cells and combines Redbiotec’s rePAXTM technology with obtainable SU devices for upstream (USP) and downstream processing (DSP).

## 2013

- J. Olownia, S. Werner, D. Eibl (2013), Plant and Animal Suspension Cell Cultures: Scaleup of Geometrically Dissimilar Orbitally Shaken Single-Use Bioreactor, *Genetic Engineering & Biotechnology News* 33(21)
- S.C. Kaiser, D. Eibl (2013) Single-use Pumpen in der Prozesstechnologie. *ChemieXtra*, 10-2013. 30-31
- S.C. Kaiser, K. Blaschczok, D. Eibl (2013) Novel CHO Suspension Cell Cultivation: Studying Cellular Growth and SEAP Expression in the Finesse SmartGlass Bioreactor, *Genetic Engineering & Biotechnology News* 33(14), 32 – 33, DOI:10.1089/gen.33.14.17
- S.C. Kaiser, K. Blaschczok, D. Eibl (2013) Cultivation of CHO Suspension Cells and SEAP Expression in the Finesse Glass Bioreactor, *BioProcess International* 11(7), 30
- Milen I. Georgiev, Regine Eibl, Jian-Jiang Zhong (2013) Hosting the plant cells in vitro: recent trends in bioreactors, *Applied Microbiology and Biotechnology*, DOI: 10.1007/s00253-013-4817-x

**Abstract:**

Biotechnological production of high-value metabolites and therapeutic proteins by plant in vitro systems has been considered as an attractive alternative of classical technologies. Numerous proof-of-concept studies have illustrated the feasibility of scaling up plant in vitro system-based processes while keeping their biosynthetic potential. Moreover, several commercial processes have been established so far. Though the progress on the field is still limited, in the recent years several bioreactor configurations has been developed (e.g., so-called single-use bioreactors) and successfully adapted for growing plant cells in

vitro. This review highlights recent progress and limitations in the bioreactors for plant cells and outlines future perspectives for wider industrialization of plant in vitro systems as “green cell factories” for sustainable production of value-added molecules.

- N. Lehmann, H. Rischer, D. Eibl, R. Eibl (2013) Wave-Mixed and Orbitally Shaken Single-Use Photobioreactors for Diatom Algae Propagation, *Chemie Ingenieur Technik* 85 (1-2), 197 – 201, DOI: 10.1002/cite.201200137  
**Abstract**  
Although vertical column, flat-plate, tubular, bubble column and airlift photobioreactors are widely used for the cultivation of diatoms, it has been shown that bubble-induced hydrodynamic stress created in these types of bioreactor can damage cells. Therefore, three single-use surface-aerated bag bioreactors, known for their outstanding results in cultivating shear-sensitive mammalian cells, were for the first time investigated for their suitability in growing the model microalgae *Phaeodactylum tricornutum*. All of the systems, which were additionally aerated with CO<sub>2</sub> and equipped with illumination systems providing different light qualities, guaranteed a 22- to 43-fold increase in cell density within seven days, without any addition of cell protection agents or changes in cell morphology. Maximum cell density and dry biomass were achieved in the orbitally shaken 2D-bag by using cool-white fluorescent tubes.
- N. Steiger, R. Eibl (2013) Interlaboratory Test for Detection of Cytotoxic Leachables arising from Single-Use Bags, *Chemie Ingenieur Technik* 85 (1-2), 26 – 28, DOI: 10.1002/cite.201200171  
**Abstract**  
An interlaboratory test for detection of cytotoxic leachables arising from single-use bags was established and performed. Results from cultivations with two bag materials indicate that leachables influencing cell growth and metabolism were secreted. For the other seven bag materials a migration of leachables can be excluded.
- C. Löffelholz, U. Husemann, G. Greller, W. Meusel, J. Kauling, P. Ay, M. Kraume, R. Eibl, D. Eibl (2013) Bioengineering Parameters for Single-Use Bioreactors: Overview and Evaluation of Suitable Methods, *Chemie Ingenieur Technik* 85 (1-2), 40 – 56, DOI: 10.1002/cite.201200125  
**Abstract**  
During the past five years, the number of single-use bioreactors used in biopharmaceutical research and production has increased tremendously. This increase has been particularly associated with mammalian cell culture processes from small- to medium-scale volumes. Even though nowadays customers can choose from a multitude of 2nd and 3rd generation single-use bioreactors, ranging from mL- up to m<sup>3</sup>-scale, there is a lack of knowledge of their engineering parameters. Different approaches have been applied to characterization investigations, resulting in an inability to compare different single-use bioreactors with each other and their reusable counterparts, creating an obstacle to a systematic approach to scaling-up the process. This article describes parametric, experimental and computer-based numeric methods for biochemical engineering characterization of single-use bioreactors, which have already been used successfully for the characterization of their reusable counterparts. For the first time, these methods have been evaluated in terms of their practical application.
- S.C. Kaiser, M. Kraume, D. Eibl (2013) Development of the Travelling Wave Bioreactor – A Concept Study, *Chemie Ingenieur Technik* 85 (1-2), 136 – 143, DOI: 10.1002/cite.201200127  
**Abstract**  
This study presents the concept of the travelling wave single-use bioreactor, based on an orbitally shaken, annular-shaped vessel. A numerical model was developed for early design studies in order to reduce the number of prototypes. The flow characteristics in two different vessel shapes were investigated. It was shown that the orbital motion combined with the toroidal shape of the bioreactor create the desired wave characteristics in the contents of the vessel.
- S. Werner, J. Olowonia, D. Egger, D. Eibl (2013) An Approach for Scale-Up of Geometrically Dissimilar Orbitally Shaken Single-Use Bioreactors, *Chemie Ingenieur Technik* 85 (1-2), 118 - 126, DOI: 10.1002/cite.201200153  
**Abstract**  
An initial approach for scaling up geometrically dissimilar orbitally shaken bioreactors is presented. A novel ShakerBag Option for Multitron Cell shaking incubators allows a rapid increase in working volume from small TubeSpin bioreactors or shake flasks up to 10 L, thus offering a simple and cost-efficient alternative to present systems for scale-up. The engineering parameters for scale-up of the orbitally shaken single-use bags were determined using traditional methods. Modern computational fluid dynamics based methods were used to gain a deeper insight into the fluid flow behavior. Furthermore, mass propagation of plant cell suspensions (*Nicotiana tabacum* and *Vitis vinifera*), as well as cell expansion and production of protein complexes using insect cells (SF-9), show the potential of orbitally shaken single-use bags.
- K. Blaszczyk, S.C. Kaiser, C. Löffelholz, N. Imseng, J. Burkart, P. Bösch, W. Dornfeld, R. Eibl, D. Eibl (2013) Investigations on Mechanical Stress Caused to CHO Suspension Cells by Standard and Single-Use Pumps, *Chemie Ingenieur Technik* 85 (1-2), 144 – 152, DOI: 10.1002/cite.201200135  
**Abstract**  
Mechanical stress caused to transfected Chinese hamster ovary (CHO) suspension cells by reusable and single-use magnetically

levitated, bearingless centrifugal pumps was investigated. Cell death rates were determined for different pump speeds and compared with data from a peristaltic and a 4-piston diaphragm pump. Furthermore, the fluid distribution inside the PuraLev® 200 pump was modeled using computational fluid dynamics. The results reveal considerably lower mechanical stress to CHO cells caused by the magnetically levitated bearingless centrifugal pumps than by the peristaltic and diaphragm pump.

- S.C. Kaiser, V. Jossen, C. Schirmaier, D. Eibl, S. Brill, C. van den Bos, R. Eibl (2013) Fluid Flow and Cell Proliferation of Mesenchymal Adipose-Derived Stem Cells in Small-Scale, Stirred, Single-Use Bioreactors, *Chemie Ingenieur Technik* 85 (1-2), 95 - 102, DOI: 10.1002/cite.201200180

**Abstract**

The fluid flow and suspension characteristics inside small-scale, stirred, single-use bioreactors were investigated experimentally and by means of computational fluid dynamics. The required impeller speeds for homogenous suspension were determined for two microcarrier types. The shear stress level and turbulence distribution were predicted using a numerical model, which was verified by particle image velocimetry measurements. In subsequent cultivations of primary mesenchymal adipose-derived stem cells, up to 31.4-fold expansion in cell number was achieved for serum concentrations as low as 5 %.

## 2012

- I. Bauer, I. Poggendorf, S. Spichiger, U.E. Spichiger-Keller, G.T. John (2012) Novel Single-Use Sensors for Online Measurement of Glucose, *BioProcess International* 10(8), 56-60
- N.C. Bögli, C. Ries, G. Greller, D. Eibl, R. Eibl (2012) Large-scale, insect-cell-based vaccine development, *BioProcess International* 10(5), 2-7
- S. Rothe, C. Ries, D. Eibl, K. Walsh, W. Hohenauer (2012) Method for preserving cell density and viability in two-phase, fed-batch CHO culture, *BioProcess International* 10(4), 1-6
- T. Backoff, J. Malig, S. Werner, D. Eibl, G. T. John (2012) Oxygen Go? -  $k_L a$  Measurement in Bioreactors, *G.I.T. Laboratory Journal* 9-10, 20-21
- J. Brändli, M. Müller, N. Imseng, S. Schillberg, R. Eibl (2012) Antikörperproduktion in Pflanzenzellen: Prozessentwicklung und -übertragung vom 50mL auf den 10L Massstab, *Biospektrum* 2, 216-217

## 2011

- R. Eibl, D. Eibl (2011) Single-Use Cell Culture Systems Arrive: Tracking the evolution and development of an increasingly used technology, *Genetic Engineering News* 31(17), 59-61, DOI: <http://dx.doi.org/10.1089/gen.31.17.15>
- S.C. Kaiser, R. Eibl, D. Eibl (2011) Engineering characteristics of a single-use stirred bioreactor at benchtop scale: The Mobius CellReady 3 L bioreactor as case study, *Engineering in Life Sciences* 11(4), 359-368, DOI: <http://dx.doi.org/10.1002/elsc.201000171>  
**Abstract**  
 Stirred single-use bioreactors can be used as substitutes for their conventional counterparts made of glass or stainless steel in the development and production of biopharmaceuticals wherever possible. Various studies have confirmed their comparability in cell growth as well as in product quantity and quality. However, information about their engineering characteristics is still rare. This study focuses on the stirred Mobius® CellReady 3L bioreactor. The main engineering parameters for typical operation conditions used in animal cell cultivations are presented for the first time. Numerical simulations with a commercial CFD package (Fluent 6.3) were accomplished to obtain data on the single- and multi-phase fluid flow, power input, mixing time and oxygen mass transfer. The results, which were compared with data from experiments and from the literature, reveal the suitability of the Mobius® CellReady 3L bioreactor for cell expansion and protein production with animal cell cultures. Furthermore, the data enable comparisons with other single-use and reusable cell culture bioreactors at bench-scale.
- R. Eibl (2011) Zellkultivierung in einem wellendurchmischten, DO-regulierten Einwegbioreaktor, *BioForum* 34(1), 22-23

## 2010

- S. Werner, R. Eibl, C. Lettenbauer, M. Röhl, D. Eibl, M. DeJesus, X. Zhang, M. Stettler, S. Tissot, C. Bürki, G. Broccard, M. Kühner, R. Tanner, D. Hacker, F. M. Wurm (2010) Innovative, non-stirred bioreactors in scales from milliliters up to 1000 L for suspension cultures of cells using disposable bags and containers - a Swiss contribution, *Chimia* 64(11), 819-823, DOI:

<http://dx.doi.org/10.2533/chimia.2010.819>

**Abstract:**

Innovative mixing principles in bioreactors, for example using the rocking of a platform to induce a backwards and forwards 'wave', or using orbital shaking to generate a 'wave' that runs round in a cylindrical container, have proved to be successful for the suspension cultures of cells, especially when combined with disposable materials. This article presents an overview of the engineering characteristics when these new principles are applied in bioreactors, and case studies covering scales of operation from milliliters to 1000 liters.

- S. C. Kaiser, C. Löffelholz, S. Werner, D. Eibl (2010) Numerische Fluidodynamik in der Biotechnologie, Nachrichten aus der Chemie 58(9), 897-899, DOI: <http://dx.doi.org/10.1002/nadc.201075806>

**Abstract:**

Simulationsverfahren helfen, Prozesse zu optimieren, weil sie verfahrenstechnische Parameter liefern, die experimentell nur mit großem Aufwand ermittelbar sind. Dabei sind die Grenzen der Modelle zu beachten, um fehlerhafte Interpretationen zu vermeiden.

- R. Eibl, S. Kaiser, R. Lombriser, D. Eibl (2010) Disposable bioreactors: the current state-of-the-art and recommended applications in biotechnology, Applied Microbiology and Biotechnology 86(1), 41-49, DOI: <http://dx.doi.org/10.1007/s00253-009-2422-9>

**Abstract:**

Disposable bioreactors have increasingly been incorporated into preclinical, clinical, and production-scale biotechnological facilities over the last few years. Driven by market needs, and, in particular, by the developers and manufacturers of drugs, vaccines, and further biologicals, there has been a trend toward the use of disposable seed bioreactors as well as production bioreactors. Numerous studies documenting their advantages in use have contributed to further new developments and have resulted in the availability of a multitude of disposable bioreactor types which differ in power input, design, instrumentation, and scale of the cultivation container. In this review, the term "disposable bioreactor" is defined, the benefits and constraints of disposable bioreactors are discussed, and critical phases and milestones in the development of disposable bioreactors are summarized. An overview of the disposable bioreactors that are currently commercially available is provided, and the domination of wave-mixed, orbitally shaken, and, in particular, stirred disposable bioreactors in animal cell-derived productions at cubic meter scale is reported. The growth of this type of reactor system is attributed to the recent availability of stirred disposable benchtop systems such as the Mobius CellReady 3 L Bioreactor. Analysis of the data from computational fluid dynamic simulation studies and first cultivation runs confirms that this novel bioreactor system is a viable alternative to traditional cell culture bioreactors at benchtop scale.

- C. Ries, G. John, C. John, R. Eibl, D. Eibl (2010) A shaken disposable bioreactor system for controlled insect cell cultivations at millilitre-scale, Engineering in Life Sciences 10(1), 75-79, DOI: <http://dx.doi.org/10.1002/elsc.200900040>

**Abstract:**

While wave-mixed and stirred bag bioreactors are common devices for rapid, safe insect cell culture-based production at liter-scale, orbitally shaken disposable flasks are mainly used for screening studies at milliliter-scale. In contrast to the two aforementioned bag bioreactor types, which can be operated with standard or disposable sensors, shaker flasks have not been instrumented until recently. The combination of 250 mL disposable shake flasks with PreSens's Shake Flask Reader enables both pH and dissolved oxygen to be measured, as well as allowing characterization of oxygen mass transfer. Volumetric oxygen transfer coefficients ( $k_L a$ -values) for PreSens 250 mL disposable shake flasks, which were determined for the first time in insect cell culture medium at varying culture volumes and shaker frequencies, ranged between 4.4 and 37.9/h. Moreover, it was demonstrated that online monitoring of dissolved oxygen in shake flasks is relevant for limitation-free growth of insect cells up to high cell densities in batch mode ( $1.6 \times 10^7$  cells/mL) and for the efficient expression of an intracellular model protein.

- R. Eibl, S. Werner, D. Eibl (2010) Bag bioreactor based on wave-induced motion: characteristics and applications, Advances in Biochemical Engineering/Biotechnology 115, 55-87, DOI: [http://dx.doi.org/10.1007/10\\_2008\\_15](http://dx.doi.org/10.1007/10_2008_15)

**Abstract:**

Today wave-mixed bag bioreactors are common devices in modern biotechnological processes where simple, safe and flexible production has top priority. Numerous studies that have been published on ex vivo generation of cells, viruses and therapeutic agents during the last 10 years have confirmed their suitability and even superiority to stirred bioreactors made from glass or stainless steel for animal as well as plant cell cultivations. In these studies the wave-mixed bag bioreactors enabled middle to high cell density and adequate productivity in laboratory and pilot scale. This mainly results from low-shear conditions and highly efficient oxygen transfer for cell cultures, as demonstrated for the widely used BioWave<sup>®</sup>. Starting with an overview of wave-mixed bag bioreactors and their common operation strategies, this chapter delineates engineering aspects of BioWave<sup>®</sup>, which like Wave Reactor<sup>™</sup> and BIOSTAT<sup>®</sup> CultiBag RM originates from the prototype of a wave-mixed bag bioreactor introduced in 1998. Subsequently, the second part of the chapter focuses on reported BioWave<sup>®</sup> applications. Conditions and results from cultivations with animal cells, plant cells, microbial cells and nematodes are presented and discussed.

## 2009

- R. Eibl, D. Eibl (2009) Disposable bioreactors in cell culture-based upstream processing, *BioProcess International* 7 (Supplement 1), 18-23

- R. Eibl, S. Werner, D. Eibl (2009) Disposable bioreactors for plant liquid cultures at Litre-scale, *Engineering in Life Sciences* 9(3), 156-164, DOI: <http://dx.doi.org/10.1002/elsc.200800102>

**Abstract:**

Driven by the demands of the market and the manufacturing industry, disposable bioreactors have gained in importance in cell culture-based processes during the last 10 years. Today they are widely accepted in R&D and also in manufacturing where process simplicity, safety and flexibility have top priority. Although disposable bioreactors are mainly used for cell expansions, glycoprotein secretions and virus generations realised with mammalian and insect cell lines, there are several reports delineating their suitability for the cultivation of plant cell and tissue cultures. This review describes the current disposable bioreactor types suitable for growing plant cell suspensions and organ cultures (hairy roots, meristematic clusters, somatic embryos) at Litre-scale. Based on a definition of the term "disposable bioreactor", a categorisation of the prevalent types for plant liquid cultures is presented. We describe the bioreactor regimes, working principles and bioengineering parameters of mechanically and pneumatically agitated bag bioreactors, which have advantages of process scalability and efficiency. Furthermore, results from the literature and data from our own research (obtained during production of undifferentiated bioactive cells, expressions of secondary metabolites and glycoproteins, and micropropagations of plant tissues) are discussed.

- S. Werner, C. Löffelholz, D. Eibl (2009) Einsatz von CFD zur Bioreaktorcharakterisierung. *Transfer* 3
- C. Ries, R. Eibl, C. John (2009) Einwegbioreaktoren für die Prozessentwicklung mit Insektenzellen, *Bioforum* 3, 11-13
- R. Eibl, D. Eibl (2009) Application of disposable bag-bioreactors in tissue engineering and for production of therapeutic agents, *Advances in Biochemical Engineering/Biotechnology*, 112, 183-207, DOI: [http://dx.doi.org/10.1007/978-3-540-69357-4\\_8](http://dx.doi.org/10.1007/978-3-540-69357-4_8)

**Abstract:**

In order to increase process efficiency, many pharmaceutical and biotechnology companies have introduced disposable bag technology over the last 10 years. Because this technology also greatly reduces the risk of cross-contamination, disposable bags are preferred in applications in which an absolute or improved process safety is a necessity, namely the production of functional tissue for implantation (tissue engineering), the production of human cells for the treatment of cancer and immune system diseases (cellular therapy), the production of viruses for gene therapies, the production of therapeutic proteins, and veterinary as well as human vaccines. Bioreactors with a pre-sterile cultivation bag made of plastic material are currently used in both development and manufacturing processes primarily operating with animal and human cells at small- and middle-volume scale. Because of their scalability, hydrodynamic expertise and the convincing results of oxygen transport efficiency studies, wave-mixed bioreactors are the most used, together with stirred bag bioreactors and static bags, which have the longest tradition. Starting with a general overview of disposable bag bioreactors and their main applications, this chapter summarizes the working principles and engineering aspects of bag bioreactors suitable for cell expansion, formation of functional tissue and production of therapeutic agents. Furthermore, results from selected cultivation studies are presented and discussed.

## 2007

- R. Eibl, D. Eibl (2007) Einwegbioreaktoren für Zellkulturen: Status quo und Trends, *Process PharmaTEC* 4,14-15
- R. Eibl, D. Eibl (2007) Disposable bioreactors for cell culture based-bioprocessing, *Achema Worldwide News* 2, 8-10
- R. Eibl, D. Eibl (2007) Design of bioreactors suitable for plant cell and tissue cultures, *Phytochemistry Reviews* 7(3), DOI: <http://dx.doi.org/10.1007/s11101-007-9083-z>

**Abstract:**

Plant cell suspension cultures and hairy roots are potential sources of secondary metabolites and recombinant proteins. In contrast to traditionally grown "whole wild plants" or "whole transgenic plants", their production in bioreactors guarantees defined controlled process conditions and therefore minimizes or even prevents variations in product yield and quality, which simplifies process validation and product registration. Moreover, bioreactors and their configuration significantly affect cultivation results by accomplishing and controlling the optimum environment for effective cell growth and production of bioactive substances. This review highlights the main design criteria of the most widely used bioreactor types, both for plant cell suspension cultures and for hairy roots, and outlines suitable low-cost disposable bioreactors which have found increasing acceptance over the last 10 years.

- S. Werner, M. Nägeli (2007), Good Vibrations: Charakterisierung eines neuen Einweg-Bioreaktorsystems, *BioTec* 3, 22–23
- M. Bonfill, S. Bentebibel, E. Moyano, J. Palazón, R.M. Cusidó, R. Eibl, M.T. Piñol (2007) Paclitaxel and baccatin III production induced by methyl jasmonate in free and immobilized cells of *Taxus baccata*, *Biologia Plantarum* 51(4), 647-652, DOI: <http://dx.doi.org/10.1007/s10535-007-0137-2>  
**Abstract:**  
 The effects of 100 and 200 µM methyl jasmonate (MJA) on cell proliferation and paclitaxel and baccatin III production were investigated in free and alginate immobilized cells of *Taxus baccata* growing in a selected product formation culture medium. The greatest accumulation of paclitaxel (13.20 mg dm<sup>-3</sup>) and baccatin III (4.62 mg dm<sup>-3</sup>) occurred when 100 µM MJA was added to the culture medium of cells entrapped using a 1.5 and 2.5 % alginate solution. The effects of different treatments on the viability of cultured cells and their capacity to excrete both taxanes into the surrounding medium were considered.
- S. Cuperus, R. Eibl, T. Hühn, R. Amado (2007) Plant cell culture based platform investigating biochemical processes in wine production, *BioForum Europe* 6, 2-4

## 2005

- R. Eibl, D. Eibl (2005) Einwegkultivierungstechnologie für biotechnische Pharma-Produktionen, *BioWorld* 3 (Beilage)
- F. Zuchuat, A.C. Luisier, S. Crelier, D. Eibl, J. Müller (2005) Starterkulturen und Schutzhüllen für bioactive Stoffe, *BioWorld* 4 (Beilage)
- S. Bentebibel, E. Moyano, J. Palazón, R.M. Cusidó, M. Bonfill, R. Eibl, M.T. Piñol (2005), Effects of immobilization by entrapment in alginate and scale-up on paclitaxel and baccatin III production in cell suspension cultures of *Taxus baccata*, *Biotechnology and Bioengineering* 89(6), 647-655, DOI: <http://dx.doi.org/10.1002/bit.20321>  
**Abstract:**  
 Paclitaxel and baccatin III-producing cells of *Taxus baccata* were immobilized within Ca<sup>2+</sup>-alginate beads. Under established optimum conditions for the biosynthesis of both taxanes, the yields of paclitaxel and baccatin III in shake-flask cultures of free cells increased by factors of up to 3 and 2, respectively, in the corresponding cultures of immobilized cells. Although the scale-up from shake-flask to bioreactor culture usually results in reduced productivities when both free and immobilized cells were grown in the same optimum conditions in three different bioreactor types (Stirred, Airlift, and Wave) running for 24 days in a batch mode and with the system optimized in each case, there was a considerable increase in the yields of paclitaxel and baccatin III. Among the reactors, the Stirred bioreactor was the most efficient in promoting immobilized cell production of paclitaxel, giving a content of 43.43 mg.L<sup>-1</sup> at 16 days of culture, equivalent to a rate of 2.71 mg.L<sup>-1</sup>.day<sup>-1</sup>. To our knowledge, the paclitaxel productivity obtained in this study is one of the highest reported so far by academic laboratories for *Taxus* species cultures in bioreactors.

## 2003

- R. Eibl, K. Rutschmann, L. Lisica, D. Eibl (2003), Kostenreduktion bei der Säugerzellkultivierung durch Einwegbioreaktoren?, *BioWorld* 5, 22-23
- J. Palazón, A. Mallo, R. Eibl, C. Lettenbauer, R.M. Cusidó, M.T. Piñol (2005) Growth and ginsenoside production in hairy root cultures of *Panax ginseng* using a novel bioreactor, *Planta Medica* 69, 344-349, DOI: <http://dx.doi.org/10.1055/s-2003-38873>  
**Abstract:**  
 We tested the effect of three variables: the bioreactor system (Wave or Spray reactor), medium exchange and culture period, on the capacity of a selected hairy root line of *Panax ginseng* to produce ginsenosides. Among the reactors, the Wave bioreactor appeared to be the most efficient in promoting hairy root line growth. Periodic exchanges of the medium and a longer culture period increased the growth rate of cultured hairy root line and, consequently, its capacity to produce ginsenosides. Under established optimum conditions (medium exchange every 14 days over a culture period of 56 days using the Wave bioreactor), the initial root fresh weight was enhanced more than 28-fold, giving a root biomass of 284.9 g L<sup>-1</sup> and a ginsenoside content of 145.6 mg L<sup>-1</sup>. It is noteworthy that this ginsenoside production exceeded by almost 3-fold that obtained during the shake flask culture of our hairy root line, although it often happens that the scale-up from shake flask to a bioreactor culture results in reduced productivities. To our knowledge this is the first time that a Wave bioreactor has been used for hairy root culture.

## 1991-2002

- R. Eibl. (2002), Fermentative Herstellung bioaktiver Wirkstoffe, BioWorld 6 (Beilage)
- A. Viviani, R. Eibl (2001), Rote Biotechnologie, BioWorld 4 ( Beilage)
- R. Eibl, J. Brändli, C. Lettenbauer, D. Eibl (2000), Kultivierung pflanzlicher und animaler Zellkulturen, BioWorld 5, 48-50
- Eibl, R; Lettenbauer, C; Eibl, D; Röhl, M (1999), Experiences in the application of the Wave Bioreactor, BioForum Europe 3, 110-112
- R. Eibl, D. Hans, S. Warlies, C. Lettenbauer, D. Eibl (1999), Zellkulturtechnik: Einsatz eines Taumelreaktorsystems mit interner Beleuchtung, BioWorld 2, 10-12
- D. Eibl (1999) Konstruktive und hygienetechnische Anforderungen an Anlagen der Lebensmittelindustrie und Biotechnologie, Lebensmittel-Technologie 32 (9), 266-268
- D. Eibl, D. Jenny, H.P. Meier (1996), Einsatz eines Magnetrührwerkes in der Lebensmittel- und Pharmaindustrie, Bioworld 5, 53 - 55
- R. Eibl (1996) Pflanzenzellkulturtechnik an der Ingenieurschule Wädenswil, BioWorld 5, 3-7
- B. Kühne, H. Feller, R. Imhof, D. Eibl (1995), CIP-Reinigung und Einsatz von Additiven in der Milchindustrie, Teil 3, Lebensmittel-Technologie 28(7-8), 258-262
- B. Kühne, H. Feller, D. Eibl (1995), CIP-Reinigung und Einsatz von Additiven in der Milchindustrie, Teil 2, Lebensmittel-Technologie 28(5), 150-156
- B. Kühne, H. Feller, D. Eibl (1995), CIP-Reinigung und Einsatz von Additiven in der Milchindustrie, Teil 1, Lebensmittel-Technologie 28(4), 95-101
- D. Eibl (1993); Biotechnologie in der Lebensmittelproduktion, Lebensmittel-Technologie 26(7-8), 199-206

## 2015

- V. Jossen, R. Eibl, D. Eibl (2015) Numerische Strömungsuntersuchungen für die Massstabsübertragung der Expansion von humanen mesenchymalen Stammzellen in gerührten Single-Use Bioreaktoren. 17. Köthener-Rührer-Kolloquium 2014, 29- 43, ISBN 978-3-86011089-8

**Abstract**

Aufgrund des hohen Potentials von humanen mesenchymalen Stammzellen (hMSC) für therapeutische Anwendungen werden neben geeigneten Kultivierungssystemen Methoden für die schnelle Massstabsübertragung (Scale-Up) benötigt. Dabei liegt der aktuelle Fokus auf gerührten Single-Use Bioreaktoren, in welchen die hMSC auf Microcarriern (MC) expandiert werden. In der vorliegenden Arbeit wurden 2 Vertreter von gerührten Single-Use Bioreaktoren, der Corning® Spinner und der BIOSTAT® STR 50L, mittels numerischer Fluidodynamik (Computational Fluid Dynamics CFD) verfahrenstechnisch untersucht, und geeignete Bedingungen für die Kultivierung mesenchymaler Knochenmarksstammzellen (hBM-MSC) definiert. Diese Untersuchungen bilden die Grundlage für die nachfolgend realisierte Massstabsübertragung vom Spinner auf den gerührten Pilotbioreaktor. Die numerischen Untersuchungen zeigten, dass bei den ermittelten NS1- und NS1u- Kriterium in beiden Kultivierungssystemen vergleichbare verfahrenstechnische Bedingungen (Scherbelastung, spezifischer Leistungseintrag) vorlagen, wobei nach 7 Tagen Kultivierungen mit einer einmaligen Feedzugabe eine Gesamtlebendzellzahl von  $3.58 \cdot 10^{10}$  hMSC pro Batch (V 50L) erreicht wurde. Dies entspricht einer Lebendzellichte von  $7.16 \cdot 10^5$  Zellen mL<sup>-1</sup>, die zu den höchsten bisher in der Fachliteratur beschriebenen Werten für hMSC-Produktionen mit MC bei nur 5% Serumeinsatz gehört. Eine Zelldifferenzierung konnte durch flowzytometrische Untersuchungen ausgeschlossen werden.

- S. C. Kaiser, D. Eibl, R. Eibl (2015) Single-use bioreactors for animal and human cells. In: Al-Rubeai M (ed.), Animal cell culture. Cell Engineering 9, 445-499, Springer Cham: DOI: 10.1007/978-3-319-10320-4

**Abstract**

Single-use (SU) bioreactors are being increasingly used in production processes based on animal (i.e. mammalian and insect) and human cells. They are particularly suitable for the production of high-value products on small and medium scales, and in cases where fast and safe production is a requirement. Thus, it is not surprising that SU bioreactors have established themselves for screening studies, cell expansions, and product expressions where they are used for the production of pre-clinical and clinical samples of therapeutic antibodies and preventive vaccines. Furthermore, recent publications have revealed the potential of SU bioreactors for the production of cell therapeutics using human mesenchymal stem cells (hMSCs). This chapter provides a perspective on current developments in SU bioreactors and their main applications. After briefly introducing the reader to the basics of SU bioreactor technology (terminology, historical milestones and characteristics compared to their reusable counterparts) an overview of the categories of currently available SU bioreactor types is provided. SU bioreactor instrumentation is then examined, before discussing well-established and novel applications of SU bioreactors for animal and human cells. This includes descriptions of the engineering characteristics of often-used types of SU bioreactors, covering wave-mixed, stirred, orbitally shaken systems and fixed-bed systems. In this context, the scaling-up of geometrically and non-geometrically similar SU bioreactors is also addressed.

## 2014

- V. Jossen, R. Pörtner, S. Kaiser; M. Kraume, D. Eibl, R. Eibl (2014) Mass production of mesenchymal stem cells – Impact of bioreactor design and flow conditions on proliferation and differentiation. In: Eberli D (ed.), Regenerative Medicine and Tissue Engineering, InTech. ISBN 978-953-51-4114-3
- N. Lehmann, I. Dittler, M. Lämse, A. Ritala, H. Rischer, D. Eibl, K.M. Oksman-Caldentey, R. Eibl (2014) Disposable bioreactors for the cultivation of plant cell cultures. In: Paek KY, Murthy HN, Zhong JJ (eds.), Production of biomass and bioactive compounds using bioreactor technology, 17-46, Springer Berlin. DOI: 10.1007/978-94-017-9223-3\_2

**Abstract**

The trend for using disposable bioreactors in modern biotechnological processes has also been adopted for plant cell cultivations. In fact, plant cell cultures are now being grown in disposable bioreactors with volumes up to 400 L. This trend has been witnessed for both the development and commercial manufacture of therapeutic proteins, secondary metabolite-based pharmaceuticals and cosmetic compounds. Prominent examples of commercial products are Protalix's ELEYSO and Mibelle Biochemistry's Phyto Cell Tech-derived bioactive compounds.

This chapter discusses the current state of disposable bioreactor technology for plant cell cultures. After a brief introduction to the general fundamentals of disposable bioreactors (relevant technical terms, advantages and limitations of disposable bioreactors) a current overview of disposable plant cell bioreactors and their instrumentation will be provided. We will describe the working principles and engineering characteristics of disposable bioreactor types that are scalable and successfully being used for the cultivation of plant cell suspension and hairy root cultures. In addition, we will provide selected application examples focusing on the cultivation of geraniol producing tobacco cells. The chapter will end with perspective on future developments of disposable bioreactor technology for plant cell cultures.



- R. Eibl, N. Steiger, C. Fritz, D. Eisenkrätzer, J. Bär, D. Müller, D. Eibl (2014) Recommendation for leachables studies: Standardized cell culture test for the early identification of critical films for CHO cell lines in chemically defined culture media. DECHEMA. ISBN: 978-3-89746-149-9  
[http://www.dechema.de/dechema\\_media/SingleUse\\_Leachables\\_2014\\_en-p-4734.pdf](http://www.dechema.de/dechema_media/SingleUse_Leachables_2014_en-p-4734.pdf)
- N. Imseng, S. Schillberg, C. Schürch, D. Schmid, K. Schütte, G. Gorr, D. Eibl, R. Eibl (2014) Suspension culture of plant cells under heterotrophic conditions. In: Meyer HP, Schmidhalter DR (eds.) Industrial scale suspension culture, 225-257, Wiley Blackwell. DOI: 10.1002/9783527683321.ch07  
**Abstract**  
Plant cell culture technology (PCCT) provides an environmentally friendly and controlled method to produce secondary metabolites, and recombinant proteins (e.g. enzymes, antibodies and vaccines). These molecules having importance in medicine, food industry and cosmetics are expressed in heterotrophic growing plant cell suspension cultures in the majority. After presenting an overview of commercial products based on plant cell culture processes, this chapter will focus on plant cell suspension cultures, process and bioreactor designs which have proven to be useful for successful growing them up to large scale. Two case studies describing the usage of the plant stem cell line PhytoCellTec™Malus Domestica cv. Uttwiler Spätlauber for cosmetic purposes and the manufacture of the pharmaceutical molecule paclitaxel highlight latest improvements in PCCT and discuss costs and regulatory aspects from manufacturer's view. At the end a view on the development of heterotrophic plant cell fermentation will be given.
- R. Eibl, C. Löffelholz, D. Eibl (2014) Disposable bioreactors for inoculum production and protein expression. In: Pörtner R (ed.), Animal cell biotechnology: Methods and protocols, methods in molecular biology. Vol. 1104, 265-284, Humana Press. DOI: 10.1007/978-1-62703-733-4\_18  
**Abstract**  
Disposable bioreactors have been increasingly implemented over the past ten years. This relates to both R & D and commercial manufacture, in particular, in animal cell-based processes. Among the numerous disposable bioreactors which are available today, wave-mixed bag bioreactors and stirred bioreactors are predominant. Whereas wave-mixed bag bioreactors represent the system of choice for inoculum production, stirred systems are often preferred for protein expression. For this reason, the authors present protocols instructing the reader how to use the wave-mixed BIOSTAT CultiBag RM 20 L for inoculum production and the stirred UniVessel SU 2 L for recombinant protein production at benchtop scale. All methods described are based on a Chinese hamster ovary (CHO) suspension cell line expressing the human placental secreted alkaline phosphatase (SEAP).

## 2013

- D. Eibl, R. Eibl (eds.) (2013), Disposable Bioreactors II, In series: Advances in Biochemical Engineering/Biotechnology, Vol. 138, Springer, Berlin  
ISBN: 978-3-642-45157-7, DOI: 10.1007/978-3-642-45158-4

## 2012

- R. Eibl, J. Brändli, D. Eibl (2012) 6.58.4.8 Plant cell bioreactors, In: *Encyclopedia of life support systems, Biotechnology*, Vol. 6, (Horst W Doelle, Stefan Rokem, Martin Berovic (eds.)),  
<http://www.eolss.net/ebooklib/> (ISBN 978-1-84826-710-7)

## 2011

- S.C. Kaiser, C. Löffelholz, S. Werner, D. Eibl (2011) CFD for Characterizing Standard and Single-use Stirred Cell Culture Bioreactors, In: *Computational Fluid Dynamics*, Igor Minin (ed.), InTech - Open Access Publisher DOI: <http://dx.doi.org/10.5772/23496>
- D. Eibl, T. Peuker, R. Eibl (2011) Single-use equipment in biopharmaceutical manufacture: A brief introduction, In: *Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 4-11, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch1>
- N. Riesen, R. Eibl (2011) Single-use bag systems for storage, transportation, freezing and thawing, In: *Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 14-20, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch2>

- S. Werner, M. Kraume, D. Eibl (2011) Bag mixing systems for single-use, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 22-32, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch3>
- R. Eibl, C. Löffelholz, D. Eibl (2011) Single-use bioreactors – an overview, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 34-51, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch4>
- S. Rothe, D. Eibl (2011) Systems for coupling and sampling, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 54-65, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch5>
- T. Peuker, D. Eibl (2011) Biopharmaceutical manufacturing facilities integrating single-use systems, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 146-158, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch12>
- J. Kocourek, L. Herrmann, L. Lisica, I. Bauer, R. Eibl (2011) Disposable SuperSpinner: Characteristics and typical applications, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 200-213, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch16>
- C. Ries, C. John, R. Eibl (2011) A new scale-down approach for the rapid development of Sf21/BEVS-based processes - a case study, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 208-213, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch17>
- N. Raven, S. Schillberg, J. Kirchhoff, J. Brändli, N. Imseng, R. Eibl (2011) Growth of BY-2 suspension cells and plantibody production in single-use bioreactors, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 252-261, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch21>
- C. Löffelholz, S.C. Kaiser, S. Werner, D. Eibl (2011) CFD as tool to characterize single-use bioreactors, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 264-279, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch22>
- N. Franscini, I. Poggendorf, C. Cavelier, J.M. Kelm, W. Moritz and U. Graf-Hausner (2011), Automated Disposable Systems: Application reports, *In: Single-use technology in biopharmaceutical manufacture*, R. Eibl, D. Eibl (eds), John Wiley & Sons, Hoboken, New Jersey, 281-294, (ISBN 978-0-470-43351-5), DOI: <http://dx.doi.org/10.1002/9780470909997.ch23>
- N. C Bögli, C. Ries, I. Bauer, T. Adams, G. Greller, R. Eibl and D. Eibl (2011), Bag-based rapid and safe seed-train expansion method for *Trichoplusia ni* suspension cells, *BMC Proceedings* 2011, 5(Suppl 8):P124, doi:10.1186/1753-6561-5-S8-P124
- 

## 2010

- R. Eibl, D. Eibl (2010) Antibody manufacture: Disposables, *Series: Encyclopedia of Industrial biotechnology: Bioprocess, bioseparation and cell technology*, M.C. Flickinger, St.W. Drew, R.E. Spier (eds.), Wiley, 344-351, DOI: <http://dx.doi.org/10.1002/9780470054581.eib628>

## 2009

- R. Eibl, D. Eibl, H. Rischer, A. Ritala, K.M. Oksman-Caldentey, (2009) Culture establishment, plant cell, *Series: Encyclopedia of industrial biotechnology: Bioprocess, bioseparation and cell technology*, M.C.

Flickinger (ed), John Wiley & Sons, Hoboken, New Jersey, 247, (ISBN 978-0-471-79930-6), DOI: <http://dx.doi.org/10.1002/9780470054581.eib247>

**Abstract:**

Alternatively to whole plants, plant cell cultures are used to produce bioactive substances for food industry, cosmetics and pharmacy. This mainly concerns secondary metabolites and recombinant proteins (so-called plant made proteins, PMPs). Among the employed culture types (which comprise suspension cultures, root cultures and shoot cultures), plant cell suspensions induced via callus cultures dominate. This fact can be explained by the suspension culture's morphology being less complex than those of root and shoot cultures, which allows easier cell banking and cultivation up to m3-scale. Based on a summary of semi- and commercial plant cell-derived products the preferable culture types and their establishment are described in more detail. Finally, most common indirect and direct methods of gene transformation (Agrobacterium- and virus-mediated transformation, particle bombardment, polyethylene glycol method, electroporation, microinjection) and of cell banking (slow growth storage, cryopreservation) are discussed for highly productive cell lines of plant origin.

- R. Eibl, D. Eibl (2009) Plant cell-based bioprocessing, *In: Cell and tissue reaction engineering*, R. Eibl, D. Eibl, R. Pörtner, G. Catapano, P. Czermak (eds), Springer, Heidelberg, Berlin, S. 315-356, (ISBN 978-3-540-68175-5), DOI: [http://dx.doi.org/10.1007/978-3-540-68182-3\\_8](http://dx.doi.org/10.1007/978-3-540-68182-3_8)

**Abstract:**

Plant cell-based bioprocessing is the use of plant cell and tissue cultures for the production of biologically active substances (low molecular secondary metabolites and recombinant proteins). The most significant advantage of plant cell culture over the traditionally grown whole wild plant or engineered transgenic plant is the sterile production of metabolites under defined controlled conditions independent of climatic changes and soil conditions, which means that variations in product yield and quality can be better avoided. Furthermore, regulatory requirements such as the cGMP standards, which have to be adhered to in the early stages of pharmaceutical production, are more easily met. Moreover, plant cells are capable of performing complex posttranslational processing, which is a precondition for heterologous protein expression. When compared with mammalian cells, which currently dominate in the commercial protein manufacture, plant cell cultures as alternative expression systems guarantee safer processes because there is a lower risk of contamination by mammalian viruses, pathogens, and toxins. In addition to this considerable advantage, the process costs can also be substantially reduced. This is due to the fact that plant cell culture medium is very simple in composition and therefore relatively inexpensive. This chapter provides an overview of culture types, techniques, and suitable bioreactors used to produce secondary metabolites and recombinant proteins in plant cells. We describe plant cell culture basics, discuss key topics relevant to plant cell bioreactor engineering with application examples, and give an overview of approaches to improving productivity of plant cell-based processes.

- G. Catapano, P. Czermak, R. Eibl, D. Eibl, R. Pörtner (2009) Bioreactor design and scale-up, *In: Cell and tissue reaction engineering*, R. Eibl, D. Eibl, R. Pörtner, G. Catapano, P. Czermak (eds), Springer, Heidelberg, Berlin, S. 173-259, (ISBN 978-3-540-68175-5), DOI: [http://dx.doi.org/10.1007/978-3-540-68182-3\\_5](http://dx.doi.org/10.1007/978-3-540-68182-3_5)

**Abstract:**

Design and selection of cell culture bioreactors are affected by cell-specific demands, engineering aspects, as well as economic and regulatory considerations. Mainly, special demands such as gentle agitation and aeration without cell damage, a well controlled environment, low levels of toxic metabolites, high cell and product concentrations, optimized medium utilization, surface for adherent cells, and scalability have to be considered. This chapter comprises engineering aspects of bioreactor systems (design, operation, scale-up) developed or adapted for cultivation of mammalian cells, such as bioreactors for suspension culture (stirred-tank reactors, bubble columns, and air-lift reactors), fixed bed and fluidized bed reactors, hollow fiber and membrane reactors, and, finally, disposable bioreactors. Aspects relevant for selection of bioreactors are discussed. Finally, an example is given of how to grow mammalian suspension cells from cryopreserved vials to laboratory and pilot scale.

- D. Eibl, R. Eibl (2009) Bioreactors for mammalian cells: General overview, *In: Cell and tissue reaction engineering*, R. Eibl, D. Eibl, R. Pörtner, G. Catapano, P. Czermak (eds), Springer, Heidelberg, Berlin, S. 55-82, (ISBN 978-3-540-68175-5), DOI: [http://dx.doi.org/10.1007/978-3-540-68182-3\\_3](http://dx.doi.org/10.1007/978-3-540-68182-3_3)

**Abstract:**

For the development and manufacturing of biotechnological medicines, the in vitro cultivation of animal cells has now become an accepted technology. In fact, about 50% of all commercial biotechnological products used for in vivo diagnostic and therapeutic purposes today are made using procedures based on animal cells. In addition to products from cells that are glycoproteins (drug products, e.g., cytokines, growth hormones, hematopoietic growth factors and antibodies, and viral vaccines, see Chaps. 1 and 2), cells as products for regenerative medicine, namely cellular therapies and tissue engineering, have been successfully investigated in clinical trials and introduced on the market. Bioreactors from small (milliliter range up to 10L) to large scale (above 500L) have been developed over the past 50 years for animal-cell-culture-based applications. Suitable cell and tissue culture types displaying similar characteristics and specific differences (Chap. 2) have resulted in a variety of bioreactor types and their modifications, manufactured from plastics, glass, or steel. Although no universal bioreactor suitable for all cell and tissue culture types exists, it is obvious that conventional stirred bioreactors from stainless steel are the gold standard and dominate both in R&D and manufacturing. This chapter aims to present a general overview of suitable bioreactor types for animal cells. On the basis of differentiation between static and dynamic bioreactors as well as methods of power input

and primary pressure, we attempt to categorize the most frequently used cell culture bioreactor types, explain their typical working principles, and deduce possible fields of application. Furthermore, cell culture bioreactor trends for R&D and manufacturing, and special features of bioreactors for 3D tissue formation and stem cell cultivation are summarized.

- D. Eibl, R. Eibl, R. Pörtner (2009) Mammalian cell culture technology: An emerging field, *In: Cell and tissue reaction engineering*, R. Eibl, D. Eibl, R. Pörtner, G. Catapano, P. Czermak (eds), Springer, Heidelberg, Berlin, S. 3-11, (ISBN 978-3-540-68175-5), DOI: [http://dx.doi.org/10.1007/978-3-540-68182-3\\_1](http://dx.doi.org/10.1007/978-3-540-68182-3_1)

**Abstract:**

Mammalian cell culture technology has become a major field in modern biotechnology, especially in the area of human health and fascinating developments achieved in the past decades are impressive examples of an interdisciplinary interplay between medicine, biology and engineering. Among the classical products from cells we find viral vaccines, monoclonal antibodies, and interferons, as well as recombinant therapeutic proteins. Tissue engineering or gene therapy opens up challenging new areas. Bioreactors from small- (ml range up to 10L) to large-scale (up to 20m<sup>3</sup>) have been developed over the past 50 years for mammalian cell culture-based applications. In this chapter we give a definition of mammalian cells and a brief outline of the historical development of mammalian cell culture technology. Fields of application and products from mammalian cells, as well as future prospects, are discussed.

## 2007

- R. Eibl, D. Eibl (2007) Disposable bioreactors for animal cell based mass propagation and protein expression, *In: Animal cell biotechnology: Methods and protocols*, R. Pörtner (ed.), Humana Press, Totowa, New Jersey, S. 321-335, (ISBN 1-58829-660-1), DOI: [http://dx.doi.org/10.1007/978-1-59745-399-8\\_15](http://dx.doi.org/10.1007/978-1-59745-399-8_15)

## 2006

- R. Eibl, D. Eibl (2006) Design and use of the Wave bioreactor for plant cell culture, *In: Plant tissue culture engineering*, S. Dutta, Y. Ibaraki (eds), Springer, 203-227, (978-90-481-6906-1), DOI: [http://dx.doi.org/10.1007/1-4020-3694-9\\_12](http://dx.doi.org/10.1007/1-4020-3694-9_12)

**Abstract:**

For plant cell and tissue cultures, disposable bioreactors such as the Wave provide an efficient alternative to standard glass or steel bioreactors. Its application in process development as well as in small and middle volume commercial production processes can increase process safety and reduce time as well as process costs. For example, time-intensive cleaning and sterilisation procedures as well as intermediate steps for inoculum production can be omitted. Biomass as well as secondary metabolite production in Wave bioreactors is comparable or even higher than in traditional laboratory reactors. This is a consequence of optimum hydrodynamic characteristics for hairy roots, suspension cultures and embryogenic cultures. High shear stress can be countered by high filling volume, minimum rocking rates and angles. Because of these characteristics and, in addition, its scale-up capability, the Wave has enormous potential for efficient commercial production processes based on plant cells. We expect this potential to be verified in the near future.

## 2003

- R. Eibl, D. Eibl, D. (2003) Bioreactors for plant cell and tissue cultures, *In: Plant biotechnology and transgenic plants*, K.M Oksman-Caldentey, W. Barz, (eds.), Marcel& Dekker Inc., New York, S. 163-199, (ISBN 0-8247-0794-X), DOI: <http://dx.doi.org/10.1201/9780203910849.ch8>

## 2001

- C. Lettenbauer, R. Eibl (2001) Application of the wave bioreactor system 20 for hairy root cultures, *In: Trends in medicinal plant research*, E. Wildi, M. Wink, (eds.), Romneya-Verlag, Dossenheim, 139-142

## PH.D. THESIS

2015

- S. Kaiser (2015) Characterization and optimization of single-use bioreactors and biopharmaceutical production processes using Computational Fluid Dynamics, Ph.D. thesis, TU Berlin, Germany

2014

- C. Löffelholz (2014) CFD as a tool for engineering characterization of single-use bioreactors and for scaling-up processes for establishing and producing biotherapeutics, Ph.D. thesis, BTU Cottbus, Germany

## ORAL PRESENTATIONS WITH PROCEEDINGS

2014

2013

2012

- C. Löffelholz, S. C. Kaiser, S. Werner, P. Ay, D. Eibl (2012) Beitrag zur Charakterisierung und zum Einsatz des 50 L S.U.B. in der biopharmazeutischen Industrie, *In: Vortragsband zum 14. Köthener Rührer-Kolloquium*, FH Anhalt/VDI (eds), Köthen 2011

2011

- C. Ries, V. Wasem, D. Karrer, C. John, R. Eibl (2011) A new approach for rapid development of *Spodoptera frugiperda*/BEV-based processes, *In: Proceedings of the 21<sup>st</sup> ESACT meeting*, June 2009, Dublin, Irland, Springer (in press)
- S.C. Kaiser, C. Löffelholz, S. Werner, P. Ay, M. Kraume, D. Eibl (2011): Beitrag zur Charakterisierung und Optimierung von Zellkulturreaktoren für die biopharmazeutische Industrie mit Hilfe von CFD, *In: Vortragsband zum 13. Köthener Rührerkolloquium*, FH Anhalt/VDI (eds), Köthen 2010, 77 - 86

2010

- C. Löffelholz, S. Werner, D. Eibl, P. Ay (2010) Untersuchungen zum Strömungsverhalten des Einweg-Vibromix-Bioreaktors, *In: 18. Fachtagung Lasermethoden in der Strömungsmesstechnik – Neue Entwicklungen und Anwendungen*, C. Egbers, B. Ruck, A. Leder, & D. Dopheide (eds), Cottbus, Germany, S. 26/1–26/8
- D. Eibl, R. Eibl (2010) Sensortechnik für Einwegzellkulturbioreaktoren: Anforderungen in F&E und der biopharmazeutischen Produktion: Ein Kurzreview aus Anwendersicht, *In: Technische Systeme für Biotechnologie und Umwelt*, D. Beckmann, M. Meister (eds), iba, Heiligenstadt-2010, S.9-17 (ISBN 978-3-00-032249-5)

2007

- R. Eibl, D. Eibl: Disposable bioreactors for pharmaceutical research and development, *In: Proceedings of 2<sup>nd</sup> International Conference on Bioreactor Technology in Cell, Tissue Culture and Biomedical Applications*, S. Sorvari, O. Toldi (eds), Lapland-2006, S. 12-21

2004

- P. Blum, D. Eibl, R. Eibl: Einsatz und Qualifizierung des on-line Analysensystems ANTRIS für zellkulturtechnische Applikationen, *In: Technische Systeme für Biotechnologie und Umwelt*, D. Beckmann, M. Meister (eds), iba, Heiligenstadt-2004, S.237-251 (ISBN 3-00-015042-0)
- D. Eibl, R. Eibl: Reaktorsysteme für die Herstellung von Proteinwirkstoffen: Quo vadis, *In: Technische Systeme für Biotechnologie und Umwelt*, D. Beckmann, M. Meister (eds.), iba, Heiligenstadt-2004, S.271-278 (ISBN 3-00-015042-0)

1991-2002

- D. Eibl, R. Eibl (2002) Entwicklungsstand und –trends in der Reaktortechnologie für Zellkulturen, *In: Technische Systeme für Biotechnologie und Umwelt – Biosensorik und Zellkulturtechnik*, D. Beckmann, M. Meister, S. Heiden, R. Erb, R. (eds.), Erich Schmidt Verlag, Berlin, 255-263 (ISBN 3-503-066645-4)
- D. Eibl, H. Schindler: Magnetrührwerke in der Biotechnologie, *In: Vortragsband zum 4. Köthener Rührer-Kolloquium*, FH Anhalt/VDI (eds), Köthen-2001, 102-108
- R. Eibl, D. Eibl, M. Sievers: Klassische und moderne Biotechnologie an der Hochschule Wädenswil, HSW (eds), Wädenswil-2000
- D. Grob, C. Lettenbauer, R. Eibl, H.P. Meier: Vergleich verschiedener Verfahren und Apparate zur Sterilisation von Zellkulturmedien. *In: Technische Systeme für Biotechnologie und Umwelt*, D. Beckmann, M. Meister (eds), iba, Heiligenstadt-1998, S.133-137
- P. Blum, H.P. Meier, G. Bafelli, D. Eibl: Einsatz von Flüssigkeitssonotroden in der Aufarbeitungstechnik, *In: Technische Systeme für Biotechnologie und Umwelt*, D. Beckmann, M. Meister (eds), iba, Heiligenstadt-1998, S.124-127
- R. Eibl, D. Eibl: Das Taumelreaktorsystem und seine Applikationen in der Biotechnologie, *In: Technische Systeme für Biotechnologie und Umwelt*, D. Beckmann, M. Meister (eds), iba, Heiligenstadt-1998, S.105-108

## ORAL PRESENTATIONS

### 2015

- V. Jossen (2015) Successfully employing single-use bioreactors for different expression systems, ECI Conference, Leesburg VA (USA), 10/2015
- I. Dittler (2015) Evaluierung der mechanischen Beanspruchung von Pumpen auf ein nicht-biologisches Modellsystem, Rührerkolloquium Köthen „Mischvorgänge in Bioprozessen“, Köthen (Germany) 06/2015
- S. Werner (2015) Characterization of single-use bioreactors: Possibilities, limitations and recommendations from an engineering point of view, A3P Bioproduction International Congress, Lausanne (Switzerland), 05/2015
- I. Dittler, W. Wolfgang (2015) New low shear force single-use pump system enables high cell viabilities, protein activities and a safe, pulsation free conveyance of sensitive biotech media, Biopharmaceutical Development & Production (BDP), Los Angeles (USA), 03/2015
- S. Werner, D. Eibl (2015) Numerische und experimentelle Untersuchung zum Sedimentationsverhalten von Pflanzenzellen in gerührten Bioreaktoren, ProcessNet Jahrestreffen der Fachgruppen Extraktion und Mischvorgänge, Heidelberg (Germany), 03/2015
- R. Eibl, V. Jossen, D. Eibl (2015) Mass production of hMSCs: An approach based on stirred single-use bioreactors, ECI Conference, San Diego (USA), 01/2015

### 2014

- V. Jossen (2014) Scale-up of hMSC cultivation processes in stirred, single-use bioreactors, Informa conference “Cell therapy manufacturing”, Brussels (Belgium), 12/2014
- D. Eibl (2014) Single-Use Technologien in der biopharmazeutischen Produktion: Vom USP bis Fill&Finish, 7. Konferenz Single-Use-Disposables 2014, Concept Heidelberg, Heidelberg (Germany), 12/2014
- R. Eibl, V. Jossen (2014) Stammzellen zur Behandlung von Augenerkrankungen der Netzhaut, Preceptorship der Firma Novartis im Stadtspital Triemli, Zurich (Switzerland), 11/2014
- R. Eibl (2014), Single-use (disposable) bioreactors: Current state and trends, BIOCAMES meeting, Graz (Austria), 11/2014
- K. Blaszczyk, C. Löffelholz, N. Steiger, L. Lisica, R. Eibl, D. Eibl (2014) Integrierter Zellexpansions- und Proteinexpressionsprozess für gerührte Einwegbioreaktoren im Pilotmassstab, 17. Heiligenstädter Kolloquium “Technische Systeme für die Lebenswissenschaften”, Heiligenstadt (Germany), 09/2014
- N. Perepelitsa, S. Kaiser, R. Eibl, D. Eibl (2014) Der neuartige “travelling wave” Bioreaktor: Verfahrenstechnische Untersuchungen und Kultivierungsergebnisse, 17. Heiligenstädter Kolloquium “Technische Systeme für die Lebenswissenschaften”, Heiligenstadt (Germany), 09/2014
- R. Eibl, V. Jossen, A. Siehoff, S. Brill; A. Safavi-Nab, F. Jüngerkes; C. van den Bos, D. Eibl (2014) A new approach for expanding human mesenchymal stem cells in clinically relevant doses, 41<sup>st</sup> Annual ESAO Congress, Rome (Italy), 09/2014
- V. Jossen, S. Kaiser, A. Ott, A. Siehoff, S. Brill, G. Greller, A. Tappe, R. Eibl, D. Eibl (2014) Optimization of a bench-top single-use bioreactor for the expansion of adipose-tissue and bone marrow derived human mesenchymal stem cells, 41<sup>st</sup> Annual ESAO Congress, Rome (Italy), 09/2014
- V. Jossen, S. Kaiser, A. Siehoff, C. van den Bos, D. Eibl, R. Eibl (2014) A successful approach in order to rapidly scaling-up microcarrier-based expansions of human mesenchymal stem cells for allogeneic



therapies, 3<sup>rd</sup> International Conference on Tissue Science & Regenerative Medicine, Valencia (Spain) 09/2014

- T. Dreher, C. Löffelholz, G. Greller, U. Husemann, D. Eibl (2014) Charakterisierung und Einsatz der gerührten single-use Bioreaktor-Baureihe BIOSTAT STR in der pharmazeutischen Industrie, Rührerkolloquium Köthen „Massstabsübertragung in gerührten Behältern“, Köthen (Germany), 06/2014
- V. Jossen, S. C. Kaiser, R. Eibl, D. Eibl (2014) Numerische Strömungsuntersuchungen für die Expansion und Maßstabsübertragung von humanen mesenchymalen Stammzellen in gerührten single-use Bioreaktoren, Rührerkolloquium Köthen „Massstabsübertragung in gerührten Behältern“, Köthen (Germany)06/2014
- S.C. Kaiser, V. Jossen, C. Schirmaier, A. Siehoff, S. Brill, G. Greller, A. Tappe, R. Eibl, D. Eibl (2014) Optimization of a single-use bioreactor for the expansion of human mesenchymal stem cells at bench-top scale by means of CFD: a QbD approach, 3rd BioProScale Symposium "Inhomogeneities in large-scale bioprocesses: System biology and process dynamics", Berlin (Germany), 04/2014
- V. Jossen, R. Eibl (2014) Development of a technology platform for a scalable production of therapeutically relevant stem cells, Cell Therapy Quarterly Review Meeting, Visp (Switzerland), 03/2014
- D. Eibl (2014), Computational Fluid Dynamics as tool to optimize single-use bioreactors and to realize scaling-up, BOKU, Department of Biotechnology, Vienna (Austria), 01/2014

## 2013

- D. Eibl (2013) Einwegkultivierungstechnologie im Biomanufacturing, 6. Konferenz Single-Use-Disposables 2013, Concept Heidelberg, Heidelberg, 12/2013
- C. Löffelholz (2013) Characterisation and scale-up of single-use bioreactors, Biological Production Forum 2013: Using robust process development strategies to facilitate upstream and downstream processes, Neuss, 10/2013
- D. Eibl, R. Eibl (2013) Single-use Bioreaktoren für tierische und humane Zellen, Expertendialog der Gesellschaft für Industrielle Zelltechnik, Technische Universität Hamburg-Harburg, 09/2013
- S. Werner (2013) Engineering Characterisation For Single-Use Bioreactors With Modern Methods: The Potential Of Computational Fluid Dynamics In Biomanufacturing, Cell Culture Asia Congress, Singapore, 09/2013
- S. C. Kaiser, R. Eibl, D. Eibl (2013) Single-use equipment in biomanufacturing: a current overview. Biopharma 2013, Budva, Montenegro, 09/2013
- V. Jossen (2013) Theoretische und experimentelle Untersuchungen zur Expansion von Stammzellen in Microcarrier-Kulturen. SGVC Preisverleihung, Sarnen, Switzerland, 06/2013
- C. Löffelholz (2013) CFD as tool for engineering characterization of single-use bioreactors and scaling-up of biopharmaceutical production processes, BTU Cottbus, Cottbus, Germany, 04/2013
- S. Werner (2013) OpenFOAM in Biotechnology: A Tool for Bioreactor Development and Optimisation, OpenFOAM User Conference, Frankfurt, Germany, 04/2013
- S. Werner, S.C. Kaiser, M. Kraume, D. Eibl (2013) Neue Bioreaktor-Konzepte mit orbital-geschüttelter Bewegung, ProcessNet Jahrestreffen der Fachgruppen Kristallisation und Mischvorgänge, Magdeburg, Germany, 03/2013

## 2012

- R. Eibl, Usage of single-use bioreactors in modern biotechnological production processes: Status quo and trends, VTT BioChem Science Seminar, Finland, 12/2012
- S.C. Kaiser, C. Löffelholz, N. Imseng, K. Blaschczok, N. Lehmann, P. Bösch, W. Dornfeld, D. Eibl, Investigations on mechanical stress caused to CHO suspension cells by standard and single-use pumps,ACHEMA Congress, 06/2012
- C. Löffelholz, S. Werner, S.C. Kaiser, U. Husemann, A. Tappe, G. Greller, D. Eibl, Comparative studies of single-use stirred bioreactors by means of traditional methods, CFD and cultivation experiments,ACHEMA Congress, 06/2012
- C. Ries, S. Wellnitz, C. John, R. Eibl, Development of a disposable influenza VLP production process based on wave-mixed bag bioreactors and insect cells,ACHEMA Congress, 06/2012
- S. Werner, D. Egger, J. Olownia, D. Eibl, 2D orbitally shaken single-use bags: Engineering characterization and cell culture application examples,ACHEMA Congress, 06/2012
- S. Werner, D. Egger, J. Olownia, D. Eibl, 2D orbitally shaken single-use bags: Engineering characterization and cell culture application examples,ACHEMA INFORS HT Innovation Day, 06/2012
- R. Eibl, Development of a fast influenza VLP vaccine production, Bioprocessing and Stem Cell, London, 06/2012
- S. Werner, D. Egger, J. Olownia, D. Eibl, Verfahrenstechnische Charakteristik und Scale-up-Faktoren für geometrisch nicht-ähnliche, orbital geschüttelte Einwegbioreaktoren, GVC/DECHEMA Vortrags- und Diskussionstagung Biopharmazeutische Produktion, Freiburg, 05/2012
- S. Werner, An Approach for Characterization and Optimization Single-use Bioreactors for Cell Cultures by Using Computational Fluid Dynamics (CFD), PepCon-2012, Beijing, China, 03/2012
- D. Eibl, Single-Use Technologien im USP und DSP, Webinar-Reihe des temporären Arbeitskreises "Single-Use-Technologien in der biopharmazeutischen Produktion" der DECHEMA, 02/2012

## 2011

- R. Eibl, Trends für Einwegbioreaktoren und ihren Einsatz bei der Entwicklung und Herstellung pharmazeutischer Produkte, Heidelberg Concept, Heidelberg, Germany, 11/2011
- D. Eibl, Aktueller Stand, Vorteile und Limitationen der Einwegkultivierungstechnologie im Biomanufacturing, Heidelberg Concept, Heidelberg, Germany, 11/2011
- C. Löffelholz, D. Eibl, Characterization and scalability of the S.U.B. systems, Single-Use Technology, Copenhagen, ThermoFisher Scientific, 10/2011
- S.C. Kaiser, R. Eibl, D. Eibl, Disposables in biomanufacturing: An overview, 1<sup>st</sup> Biberach Pharmaceutical Biotechnology Meeting, Biberach, Deutschland, 10/2011
- C. Ries, S. Rothe, D. Eibl, Method for improved productivity in a two-phase, fed-batch culture, Seminar GE Healthcare, Uppsala, Sweden, 09/2011
- R. Eibl, Single-use bioreactors and their application in mammalian cell-based processes, Workshop Merck Millipore, Basel, Switzerland, 09/2011
- C. Löffelholz, D. Eibl, Characterization and scalability of the S.U.B. systems, Webinar, ThermoFisher Scientific, 09/2011
- C. Löffelholz, S. Werner, D. Eibl, P. Ay, Untersuchungen zum Strömungsverhalten des Einweg-Vibromix-Bioreaktors. Fachtagung Lasermethoden in der Strömungsmesstechnik, Cottbus, 09/2011
- S. Werner, Computational Fluid Dynamics, Summerschool Swiss BioteCHnet, Berlin, Germany, 08/2011

- S. Werner, Disposable bioreactors and their characterisation by Fluid Dynamics. Egemin, Life Science Division, Zwijndrecht, Belgium, 07/2011
- C. Löffelholz, S.C. Kaiser, S. Werner, P. Ay, D. Eibl, Beitrag zur Charakterisierung und zum Einsatz des 50 L Single-Use Bioreactor (S.U.B.) in der biopharmazeutischen Industrie, Rührerkolloquium, Köthen, Germany, 06/2011
- R. Eibl, Disposables in biomanufacturing – an overview, Symposium “Single-use solutions in bioprocess technology”, Nijmegen, Netherlands, 06/2011
- D. Eibl, Sensortechnik für Einwegzellkulturreaktoren, Symposium Endress+Hauser Conducta, Gerlingen, Germany, 4/2011

## 2010

- R. Eibl, D. Eibl, Developments in cell cultivations, Workshop Infors, Bottmingen, Switzerland, 12/2010
- R. Eibl, Trends für Einwegbioreaktoren und ihren Einsatz bei der Entwicklung und Herstellung pharmazeutischer Produkte, Heidelberg Concept, Heidelberg, Germany, 10/2010
- D. Eibl, Aktueller Stand, Vorteile und Limitationen der Einwegkultivierungstechnologie im Biomanufacturing, Heidelberg Concept, Heidelberg, Germany, 10/2010
- D. Eibl, Single-use Technologie: Stand und Perspektive, Meeting of the DECHEMA working group „Cell cultivation techniques“, Hamburg, Germany, 10/2010
- D. Eibl, Messen und Regeln in Einwegbioreaktoren: Anforderungen aus Anwendersicht, Meeting of the DECHEMA working group „Measurement and control“, Göttingen, Germany, 10/2010
- D. Eibl, R. Eibl, Sensortechnik für Einwegzellkulturbioreaktoren: Anforderungen in F&E und der biopharmazeutischen Produktion: Ein Kurzreview aus Anwendersicht, 19. Heiligenstädter Kolloquium, Germany, 9/2010
- I. Bauer, D. Eibl, R. Eibl, Cell cultivation in single-use bioreactors: ThermoFisher’s S.U.B. as a case study, Workshop ThermoFisher Scientific, Frankfurt, Germany, 9/2010
- C. Löffelholz, D. Eibl, Characterization of the 50 L S.U.B. by using CFD, Workshop ThermoFisher Scientific, Frankfurt, Germany, 9/2010
- C. Löffelholz, S. Werner, D. Eibl, P. Ay, Untersuchungen zum Strömungsverhalten des Einweg-Vibromix-Bioreaktors, 18. GALA-Fachtagung „Lasermethoden in der Strömungsmechanik“, Cottbus, Germany, 9/2010
- S. Werner, Interactive Panel Discussion: Single-use technologies – Cost comparison and flexible manufacturing strategies. BioProcess AsiaPacific, Singapore, 07/2010
- S. Werner, D. Eibl, Single-use bioreactors for cell cultures: An approach for characterization and optimization by using CFD, BioProcess AsiaPacific, Singapore, 07/2010
- S.C. Kaiser, C. Löffelholz, S. Werner, P. Ay, M. Kraume, D. Eibl, Beitrag zur Charakterisierung und Optimierung von Zellkulturreaktoren für die biopharmazeutische Industrie mit Hilfe von CFD, Workshop „Stirring“, UAS Köthen/Anhalt, Germany, 6/2010
- D. Eibl, Einwegkultivierungstechnologie in der biopharmazeutischen Produktion: Status quo und Trends, DECHEMA Jahrestagung, Nürnberg, Germany, 5/2010
- S. Werner, Disposable Bioreactors and their Characterization by Fluid Dynamics. Lecture for Executive MBA in Management of Technology – Mot at EPFL, Lausanne, Switzerland, 05/2010
- D. Eibl, Disposable Technologien für die Entwicklung und Produktion biopharmazeutischer Produkte, Pharmakongress 2010, Düsseldorf

- R. Eibl, D. Eibl, Use of single-use bioreactors in plant and animal cell-based production studies, Applikon Workshop, Delft, Netherlands, 3/2010

## 2009

- R. Eibl, D. Eibl, Influence of various disposable upstream systems towards product expression and product quality, Invited lecture, APV conference “Lessons learned in disposable biopharmaceutical development and manufacturing”, Dessau, Germany, 11/2009
- S. Werner, Modelling of wave-mixed bioreactors. RAFT Meeting, San Diego, USA, 11/2009
- R. Eibl, Disposable or single-use bioreactors: Types, characteristics and main applications, Summer School, Basel, Switzerland, 10/2009
- S. Werner, D. Eibl, Wave-mixed bioreactors: Characterisation and scaling-up by using CFD, BioProduction 2009, Barcelona, Spain, 10/2009
- R. Eibl, D. Eibl, Disposables in upstream processing: An overview, Innovation day GE Healthcare; Bern, Switzerland, 10/2009
- S. Werner, M. Kraume, D. Eibl, Investigations of hydrodynamics in wave-mixed bioreactors using Computational Fluid Dynamics (CFD), Innovation day GE Healthcare; Bern, Switzerland, 10/2009
- R. Eibl, D. Eibl, Biotechnologische Herstellung von bioaktiven Substanzen für die Pharma- und Kosmetikindustrie, Bioengineering AG, Wald (Switzerland), 07/2009
- S. Werner, M. Kraume, D. Eibl, Characterisation and optimisation of single-use bioreactors for cell cultures by using Computational Fluid Dynamics, Werkgroependag, NBV Dutch Biotechnology Society, Ede, Netherlands, 04/2009
- S. Werner, M. Kraume, D. Eibl, Untersuchung des Strömungsverhaltens in Einwegbioreaktoren zur Optimierung von Zellkulturprozessen unter Einsatz von CFD, Jahrestreffen der Fachausschüsse Computational Fluid Dynamics, Mischvorgänge und Extraktion, ProcessNet, Fulda, Deutschland, 03/2009
- R. Eibl, Einwegbioreaktoren und ihr Einsatz in der Prozessentwicklung, Heidelberg Concept, Mannheim, 03/2009
- D. Eibl, Disposables in der Biopharmazeutikaproduktion: Eine Übersicht, Heidelberg Concept, Mannheim, 03/2009

## 2008

- D. Eibl, Disposable cultivation technology: An overview, Prague, 12/2008
- R. Eibl, Disposable bioreactors for cell expansion and production of bioactive agents, Prague, 12/2008
- R. Eibl, Disposable bioreactors for production of cells and biologically active substances: A comparative study, pharmaIQ, London, 11/2008
- S. Werner, D. Eibl, CFD investigations focusing on bioreactor design and characterization. Roche Weiterbildung, Basel, Switzerland, 10/2008
- R. Eibl, D. Eibl, Wave-mixed and stirred bag bioreactors for production of cells and therapeutic agents, Roche, Basel, 09/2008
- S. Werner, D. Eibl, CFD investigations focusing on bioreactor design and characterization, Roche, Basel, 09/2008
- R. Eibl, D. Eibl, Monitoring and control in cell culture bioreactors: Requirements in R&D and manufacturing; Chemical Sensors Forum, Zürich, 09/2008

- R. Eibl, Disposable bioreactors for production of cells and biologically active substances; Sartorius Stedim, Seminar in Munich, 06/2008
- R. Eibl, D. Eibl, Use of disposable bioreactors in process development; DECHEMA, Frankfurt, 03/2008

## APPLICATION NOTES AND SCIENTIFIC REPORTS

- C. Löffelholz, N. Imseng, R. Eibl, D. Eibl, S. Rosemann, K.H. Scheibenbogen (2015) CHO cell-based production of a secreted model protein using the novel BIOSTAT A (in press).
- V. Jossen, A. Ott, A. Siehoff, C. van den Bos, D. Eibl, R. Eibl (2015) Microcarrier-based expansion of hBM-MSCs in the BIOSTAT CultiBag STR 50L: An approach for allogeneic therapies (in press).
- V. Jossen, C. Schirmaier, D. Eibl, V. Luginbühl, T. Merseburger, R. Eibl, S. Michels, M. Becker (2014) Stem cells for the treatment of retinal diseases.
- C. Löffelholz, K. Blaschczok, I. Dittler, N. Lehmann, S.C. Kaiser, D. Eibl, P. Bösch, R. Schöb (2014) Investigations into Mechanical Stress Caused to CHO Suspension Cells by Single-use Magnetically Levitated, Bearingless Centrifugal Pumps.
- K. Blaschczok, C. Löffelholz, I. Dittler, N. Lehmann, S.C. Kaiser, D. Eibl, P. Bösch, R. Schöb (2014) Mechanical stress exerted on proteins by magnetically levitated single-use centrifugal pumps.
- I. Dittler, K. Blaschczok, C. Löffelholz, N. Lehmann, S.C. Kaiser, D. Eibl, P. Bösch, R. Schöb (2014) Evaluation of mechanical stress on an oil-water emulsion system in magnetically levitated single-use centrifugal pumps.
- N. Perepelitsa, S.C. Kaiser, D. Eibl (2014). Cultivation of Sf-9 Insect Cells and rSEAP Expression in the Wave-Mixed Single-Use Finesse SmartRocker Bioreactor.
- N. Perepelitsa, S.C. Kaiser, D. Eibl (2014). Cultivation of CHO Suspension Cells and SEAP Expression in the Wave-mixed Single-use Finesse SmartRocker Bioreactor.
- N. Imseng, S.C. Kaiser, N. Perepelitsa, D. Eibl (2014) Mass propagation of *Nicotiana tabacum* cv. BY-2 suspension cells in the Finesse SmartRocker bioreactor.
- V. Jossen, C. Schirmaier, G. John, D. Eibl, R. Eibl (2014) Online pH and DO measurement in microcarrier-based hMSC cultivations performed in single-use spinner flasks.
- I. Dittler, G. John, R. Eibl (2014) Online pH measurements in iTube pH bioreactors.
- S.C. Kaiser, K. Blaschczok, D. Eibl (2013). Cultivation of CHO suspension cells and SEAP expression in the Finesse glass bioreactor.
- S.C. Kaiser, I. Dittler, N. Steiger, D. Eibl (2013). Cultivation of Sf-9 insect cells and SEAP expression in the Finesse SmartGlass™ bioreactor.
- S.C. Kaiser, L. Lisica, N. Imseng, D. Eibl (2013). Cultivation of *Nicotiana tabacum* cv. BY-2 suspension cells and IgG antibody production in the Finesse SmartGlass™ bioreactor.
- N. Perepelitsa, S.C. Kaiser, D. Eibl (2013). Application of the Finesse glass bioreactor for fermentation of *Saccharomyces cerevisiae*.
- S.C. Kaiser, F. Fietz, N. Steiger, D. Eibl (2013) Optrode Dual connects Single-Use Bioreactors to Conventional Controllers - Monitoring of CHO Cell Cultivation with Chemical Optical Sensors.
- N. Imseng, R. Eibl (2012) Propagation of hairy roots delivering bioactive compounds for pharmaceutical industry and cosmetics in a 2L orbitally shaken single-use 2D-bag
- T. Beltrametti, N. Bögli, C. Ries, G. Greller, T. Adams, R. Eibl (2011) Protocol for rapid expansion of non-infected High Five suspension cells in the BIOSTAT CultiBag RM 20 optical (batch mode, DO controlled, 1 L culture volume)

## REVIEWS FOR

- Applied Microbiology and Biotechnology
- Engineering in Life Sciences
- Bioresource Technology
- Biotechnology Progress
- Biotechnology and Bioengineering
- Biotechnology and Applied Biochemistry
- Phytochemistry Reviews
- Plant Cell Reports
- Journal Biotechnology