Nordics BioProcess Improvement Seminar 1

STOCKHOLM March 28, 2012

Innovation in cell culture process development & production

Interactive seminars for and by industrial and academic experts on the latest relevant developments in bioprocess innovation, with special focus on single-use technology and process intensification in upstream and downstream bioprocessing.

The low threshold opportunity for idea sharing, learning, networking and new partnerships building on bioprocessing improvement.

Cell tainer OPTIMAL MASS TRANSFER

CEFFORT

AL PRODUKTER AB LIFE SCIENCES

BioProcess INTERNATIONAL

CELS

Nordics BioProcess Improvement Seminar 1

Time & Venue

Wednesday, 28 March, 2012
Royal Institute of Technology, Albanova, Roslagstullsbacken 21, Stockholm, SWEDEN (room FB42)

Talks & Breaks

09.15-09.50	**Registration with coffee**
09.50-10.00	**Welcome**
10.00-10.30	❶ **Dr. Veronique Chotteau, Royal Institute of Technology, Stockholm** *Very high cell density perfusion of CHO cells in disposable bioreactor, challenge or reality*
Short break	
10.35-11.05	❷ **Ryan Hicks, AstraZeneca** *How AZ looks upon single-use and cell culture process development*
11.10-11.40	❸ **Dr. Andreas Castan, GE Healthcare Life Science** *Application of disposable bioreactors for biopharmaceutical production*
11.45-12.15	❹ **Dr. Wian de Jongh, ExpreS2ion** *Development of a vaccine production process using a rapid single-use non-viral insect cell expression platform*
12.20-13.30	**Lunch**
13.30-14.00	❺ **Dr. ir. Nico Oosterhuis, CELLution Biotech** *Mixing, mass transfer and bioprocess scaling up and down in new generation single-use bioreactors*
14.05-14.30	❻ **Bjarne Rask Poulsen, Novo Nordisk** *Continuous cell cultures in shake flasks*
14.35-15.05	❼ **Kristina Lae, Cobra Biologics** *Implementation of a Micro Bioreactor System for Platform Cell Culture Process Development*
Short break	
15.10-15.35	❽ **Prof. Dr. Peter Neubauer, Technical University of Berlin** *Potential, challenges and needs for the use of disposable bioreactors in microbial processes*
15.40-16.10	❾ **Prof. Carl Fredrik Mandenius, Linköping University** *Biomechatronic design for bioprocesses*
16.15	**Coffee (or drinks) and general discussion**

Organizers: CELS Network/CEFFORT AB, ANL Life Sience, Cellution Biotech, Bioprocess International

1

Very high cell density perfusion of CHO cells in disposable bioreactor, challenge or reality

Dr. Veronique Chotteau, Royal Institute of Technology, Stockholm

In perfusion mode, the cultivation medium is continuously renewed while the cells are retained in the bioreactor. This mode has advantages: smaller size of the bioreactor, constant environment for the cells, favourable harvest conditions for unstable proteins and drawbacks: higher technical and sterility challenge. Robust, disposable and simple perfusion systems can alleviate these drawbacks rending this operation mode very attractive.

Different new perfusion technologies based on filtration were studied in our group: WAVE Bioreactor™ equipped with tangential flow filtration, WAVE Bioreactor™ equipped with alternating tangential flow filtration (ATF) and CerCoreTM matrix (CerCell). Very high viable cell densities of $2*10^8$ viable cells/ml could be achieved, making perfusion at cell densities of 1 to $1.5 * 10^8$ viable cells/ml a feasible reality. These results will be presented and discussed.

Veronique Chotteau is researcher, group leader of the Cell Technology group, CETEG, at the Div. of Bioprocess, School of Biotechnology, KTH. Her research is focused on actual problems met in the biopharmaceutical industry in the development of animal cell cultivation processes and on stem cell cultivation processes. Veronique has worked more than ten years at Biovitrum/Pharmacia in Sweden before she joined KTH. She has more than twenty years of experience with animal cell cultivation in suspension and adherence including an expertise in mammalian cell-based process development, fed-batch and perfusion, small and pilot scale(clinical and commercial production), pilot scale/upscaling.

2

How AstraZeneca looks upon single use and cell culture process development

Dr. Ryan Hicks, AstraZeneca

Generating bioreagents to support a large and diverse research organization gives us some exciting challenges and places high demands on the flexibility and adaptability of the methods and technologies we utilize. This is especially true when the required bioreagents range from peptides and purified recombinant proteins to assay-ready cells. To balance time, cost and quality demands we use a range of single use systems and perform cell culture process development to different extents. In this presentation an overview will be presented on some of the processes we use to support different projects, with some examples of the tools we have available today to be able to provide high quality bioreagents throughout AstraZeneca.

Ryan Hicks is the Associate Director leading a Cellular Reagents and Assay Development group within Discovery Sciences at AstraZeneca. His focus within AstraZeneca over the past 10 years has been on cell culture and assay development, supporting different research areas across the business, leading global groups in the UK and Sweden.

3

Application of disposable bioreactors for biopharmaceutical production

(Dr. Andreas Castan, GE Healthcare Life Science)

Biopharmaceutical production is currently facing several challenges: Among these is the need to drive out production costs, to reduce the complexity of the manufacturing process and to create flexible manufacturing plants. Disposables have a key role to play as a platform technology in order to handle these challenges. In this presentation, disposable bioreactor solutions are presented for biopharmaceutical production. Examples are given on the use of disposable bioreactor systems for the intensification of fed-batch processes. Furthermore, cell-based vaccine production using adherent cells in disposable bioreactors as well as scale-up strategies are presented.

Andreas Castan is Senior Scientist at GE Healthcare Life Sciences R&D. After studying chemical engineering at the Technische Universität Hamburg-Harburg (TUHH), Germany, he received a Ph.D. in Biochemical Engineering at the Royal Institute of Technology (KTH), Stockholm. His research was focused on the characterization and scale-up of processes for the production of recombinant therapeutic proteins in high cell density cultures. Before joining GE, Andreas was Manager Upstream Development at Swedish Orphan Biovitrum AB, working with expression system development, process development of microbial and mammalian cell based processes and scale-up to c-GMP manufacturing scale. During the last 17 years, Andreas has been working in various positions within biopharmaceutical development including project and line management as well as manufacturing.

4

Development of a vaccine production process using a rapid single-use non-viral insect cell expression platform

Dr. Wian de Jongh, ExpreS2ion

The Drosophila S2 expression system will be discussed with focus on development of the expression vectors and production processes up to phase II clinical trials. Furthermore, a case study for the application of the S2 system to a Placental Malaria vaccine development program using disposable technology will be discussed.

Wian de Jongh, PhD, Vice President, Product Development, dr. de Jongh (South African) obtained a Bachelor degree followed by a M.Sc. (with Honours) in Chemical Engineering from the University of Stellenbosch, South Africa. Thereafter, he was awarded a doctorate in Biotechnology from DTU in 2006. Dr. de Jongh has six years' experience in the pharmaceutical industry in molecular biology, upstream process development; process scale-up; production document preparation and process transfer to cGMP manufacturing.

5

Mixing, mass transfer and bioprocess scaling up and down in new generation single-use bioreactors *Dr. ir. Nico Oosterhuis, CELLution Biotech*

The application of single-use equipment is common practice now in the biopharmaceutical industry. Compared to the traditional glass or stainless steel stirred tanks, single-use bioreactors offer clear advantages: a quicker turnaround time; minimal utilities required; greatly reduced potential of cross contamination; greater operational flexibility; reduced validation requirements.

The CELL-tainer® single-use bioreactor creates a superior oxygen mass-transfer compared to other single-use bioreactors, making the system suitable for high density mammalian cell cultures, but especially also for microbial and viscous mycelia cultures.

Comparison of the CELL-tainer® performance with the standard stirred bioreactor and of cultivation results of different cell-lines like CHO-cells, PER.C6®-cells, and also microbial cultures like *E.coli* and *Pichia* shows the opportunity to design high-performance processes in single-use equipment.
As the $k_l a$ value in the CELL-tainer® can be controlled, the equipment also can be used for process development.
The CELL-tainer® bioreactor opens a new area for bioprocesses optimization in a single-use system and using the advantages thereof. As a single-use bioreactor is less sensitive to contamination, the system could also be applied in seed trains for large-scale bulk fermentations.

Dr. ir. Nico Oosterhuis is CTO/CSO and co-owner of CELLution Biotech. Started the company in 2005, he has been involved in the development from the start. Before, Dr. ir. Oosterhuis has had several project management and R&D management positions in both the bio-pharmaceutical as food processing companies, Dr. ir. Oosterhuis is also a consultant to several companies in the biotech industry and as such involved in a large-scale biotechnology project in Russia. Dr. Oosterhuis achieved his PhD at the TU Delft on "Scale-up of bioreactors" and studied food process technology before at the Wageningen University.

6

Continuous cell cultures in shake flasks

Bjarne Rask Poulsen, Novo Nordisk

The continuous cell cultures in shake flasks are simple, inexpensive continuous cultures, where the dilution rate, which is the ratio between flow of fresh medium into the culture and volume of the culture, is maintained constant at the specific growth rate μ by starting the flow at μ times the starting volume and increasing it exponentially with a rate constant of μ. The volume therefore also increases exponentially with a rate constant of μ. When the maximum working volume of the shake flasks has been reached after a few days dependent on starting volume and μ or when a culture sample is needed, a certain volume

is removed from the culture and the flow adjusted to the new starting volume, whereby a steady-state is not disturbed. In this way, only the stability of the cell line limits the time that a steady-state can be maintained. These cultures have the advantage of no continuous effluent flow. There is to our knowledge no simple setup for continuous and controlled removal of culture liquid from shake flasks to maintain a constant volume as in a conventional chemostat. The disadvantage or challenge is that a controlled pump able to increase a certain flow exponentially at relatively low flow is required. The big difference in this set-up compared to a conventional chemostat is of course that the volume increases with time. However, we have reached steady states with this system, where the concentration of substrate, cells, metabolites etc. was constant in time for many days. We have shown that for certain experiments, where batch cultures give non-reproducible results because of high dependency on sampling time, the well-defined conditions of the continuous cultures are a prerequisite for qualified conclusions. We are currently setting up experiments to test different medium compositions. One of our main goals is to use the continuous shake flask cultures as a scale-down model for continuous perfusion cultures using the flow of fresh medium per cell per time as the scaling factor.

Bjarne Rask Poulsen is senior scientist in the department Cell Culture Technology at Novo Nordisk A/S. The three main focus areas in the department are cell line evaluation, production of the first protein for non-clinical testing in animal models to obtain proof of concept and early process development for production of protein in mammalian cells. Furthermore, the department focuses on medium development for mammalian cells. Bjarne has 20 years of experience in bioreactor and bioprocess design from lab- to industrial scale for filamentous fungi, algae and mammalian cells.

7

Implementation of a Micro Bioreactor System for Platform Cell Culture Process Development *Kristina Lae, Cobra Biologics*

The presentation covers the implementation of Ambr Micro Bioreactor system for use in cell line development and process development at Cobra Biologics. The use of a micro bioreactor system gives the opportunity to develop a suitable process early in a development project in small scale. This enables the possibility to find a robust and reliable process suitable for biopharmaceutical manufacturing.

Kristina Lae is a scientist in the cell culture services team at Cobra Biologics with a MSc degree in Engineering Biology from Linköping University. She has several years of experience of cell line development, cell culture GMP manufacturing and cell culture process development from AstraZeneca, RecipharmCobra and Cobra Biologics.

8

Potential, challenges and needs for the use of disposable bioreactors in microbial processes *Prof. Dr. Peter Neubauer, Technical University of Berlin*

Currently disposable bioreactors are predominantly used for the cultivation of higher eukaryotic cells. However we believe that polymer based cultivation systems could be interesting also for microbial cultivation systems.

Within the presentation two exemplary applications will be discussed: (i) a high cell density process for production of a recombinant protein with the Gram negative bacterium *Escherichia coli* and (ii) production of an unsaturated fatty acid by a dinoflagellate. The efficiency of both processes is dependent on a good oxygen transfer and they are performed as fed-batch processes. The second process is challenging as the cultivation lasts for 2 to 3 weeks and the final process will be performed as a repeated fed-batch procedure, i.e. the cultivation time will be in the order of months.
The processes were evaluated in different commercial disposable bioreactors. Generally the results are very promising. High cell densities above 30 g/L of cell dry weight can be obtained in state-of-the-art disposable reactors and the fed-batch technology can be applied either as an internal delivery system (EnBase) or by traditional feeding procedures.

Challenges are clearly the mechanical stability and costs of the bags, but also high oxygen transfer rates and the stability of the sensors.

Dr. Peter Neubauer is Professor for Bioprocess Engineering, at the Technische Universität Berlin, Germany. Co-Founder of ScanBEC (2000) and BioSilta (2007). His research focus on methods development for faster and parallel bioprocess development by integrating computational tools, up-to-date screening systems, advanced controlled growth strategies, and state of the art analytical methods.

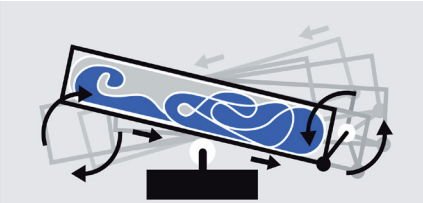
9

Biomechatronic design for bioprocesses

Prof. Carl Fredrik Mandenius, Linköping University

Biomechatronic design combines mechanical and electric product design principles with biotechnology. Systematic conceptual design, used for decades in product development for mechanical products is here adapted to cope with the complexity of biological systems and processes. Typical examples are bioreactors and biosensors. The biomechatronic design facilitates and coordinates the product development work by using a few simple procedures, tools and models. In the presentation these tools and models are discussed on the design of the bioprocessas well as the sensor systems used for monitoring and controlling the production.

Carl Fredrik Mandenius is professor of Technical Biology at Linköping University. His main research interests involve industrial biotechnology, biosensors and design of bioprocesses.



Binding Registration

SEMINAR 1 - STOCKHOLM KTH ALBANOVA
Innovation in cell culture process development & production
Wednesday 28th March 2012

SEMINAR 2 - COPENHAGEN DTU
Process optimization from parallel micro bioreactors to large-scale bio-manufacturing
Thursday 26th April 2012

Registration fees

Stockholm meeting registration (2012-03-09) 80€ (750 SEK)
Copenhagen meeting registration (2012-03-09) 80€

* *VAT (25%) will be added for Swedish participants and participants without an international VAT registration number*
** *Lunch and coffee the and snacks is included*

Payment to Handelsbanken:
IBAN SE 6000 0000 0003 5200
SWIFT: HANDSESS
Bankgiro: 5672-7688. *MARK: Seminar 1 or Seminar 2*

Needed information

- Company / University / Organisation
- Name, e-mail, telephone and address of Participant
- International VAT reg number (non Swedish participant)
- Mark participating in seminar 1 or seminar 2

Register by email

Send e-mail with the information to:
ake@anl.se
Question on registration: Call +46 8 99 00 90

Note: *The number of participants are limited. ´First registered are first served´ applies.*

Confirmation of Meeting and Registration (Disclaimer)
A formal confirmation of the meeting will be sent via email to participant. The organizers take no responsibility for travel and other costs in the case an unconfirmed meeting is moved to a different venue, cancelled or postponed.

ANL PRODUKTER AB LIFE SCIENCES

ANL Life Sciences
P.O Box 26, SE-125 21 Älvsjö, Sweden
www.anl.se

Cell tainer OPTIMAL MASS TRANSFER
CEFFORT
ANL PRODUKTER AB LIFE SCIENCES
BioProcess INTERNATIONAL
CELS