



## Challenges within a CMO Purification Process A Case Study of Crossflow Micro- and Ultrafiltration

6<sup>th</sup> European Downstream Technology Forum  
September 7, 2010, Sartorius College, Goettingen



## Agenda

- I. Introduction
- II. Overview applications for CFF at RHB
- III. Case study I & II
- IV. Strategies to maintain quality

# Richter-Helm offers services for (co-)development and manufacture of biopharmaceuticals

## *Business Units and Services*



**In- & Out-Licensing  
Marketing & Sales**



Business Development Team



**Contract development  
Contract manufacturing**



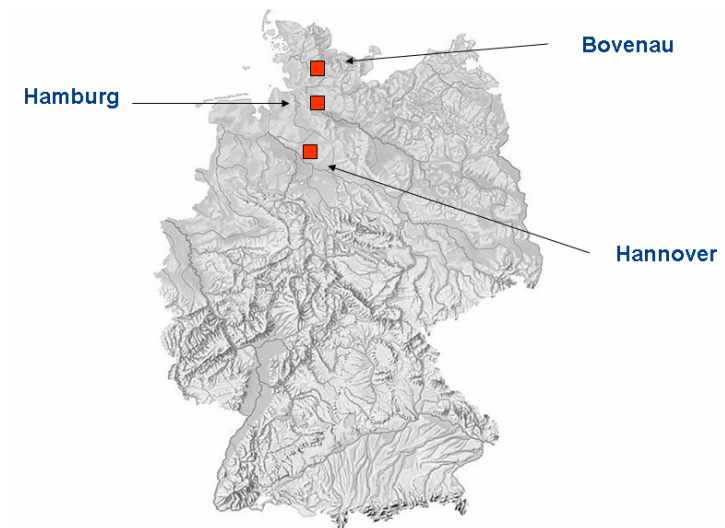
GMP-Production Facility, Bovenau

# Richter-Helm was one of the first companies to produce recombinant proteins in accordance to GMP-guidelines

## History of Richter-Helm

Timeline	
1987	Foundation of Pharma Biotechnologie Hannover and start of GMP production
1989	Entering contract GMP manufacturing
1993	Broadening service portfolio / establishment of contract development services
1998	Building up of development centre in Hamburg
2000	Planning and start of construction of the new large scale GMP facility in Bovenau
2001	First long-term commercial development and supply agreement for large scale facility signed
2003	Initiation of GMP production in Bovenau
2007	Acquisition by Gedeon Richter Plc and HELM AG
2007	Start of in-house development projects
2009	Fully remodelled Hannover facility in operation
2010	Capacity enlargement in Bovenau

### 3 development and production locations



More than 20 years of experience in biopharmaceuticals production

# Streamlining their biotechnology activities Gedeon Richter Plc. and HELM AG formed the dynamic joint venture

## Owners structure



Gedeon Richter Plc.,  
Budapest, Hungary

- Founded 1901, **Richter** sells APIs and finished form drugs to nearly one hundred countries around the world
- Largest Hungarian pharmaceutical company, today
- Turnover in 2008 was ~940 million € generated with 10,500 employees
- Traditionally strong brand name and a well established sales network in Hungary as well as in Central and Eastern European and CIS countries
- In the USA and the 'traditional' 15 EU countries **Richter's** products are marketed under a framework of strategic partnerships and long-term supply agreements

[www.richter.hu](http://www.richter.hu)



HELM AG, Hamburg,  
Germany

- HELM is one of the world's leading independent chemicals and pharmaceuticals marketing enterprises with branches and sales offices in more than 30 countries.
- HELM world-wide sales in 2008 were 9.1 billion €
- Business Unit Pharma:
  - Global sourcing and marketing of active pharmaceutical ingredients (APIs) and finished products
  - Chemical and pharmaceutical development of generic APIs and finished products of conventional dosage forms

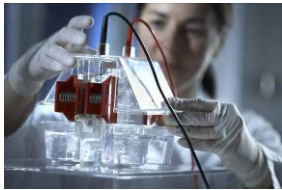
[www.helmag.com](http://www.helmag.com)



# Richter-Helm offers development and manufacturing from small to commercial scale

## *Locations and capabilities*

### Development centre, Hamburg



- Strain development
- Process development in down scale model of manufacturing equipment:
  - Multifermenter systems
  - 15 L fermenters
  - Primary recovery equipment
  - Chromatography skids
  - Filtration units
- Bioassay development
- Analytical development in state of the art equipment

### GMP pilot plant, Hannover



- GMP compliant multipurpose facility
- Manufacturing in up to 300 L fermenter scale
- State of the art DSP equipment
- Clean room facilities category D – A in B



### GMP production plant, Bovenau



- GMP compliant multipurpose facility
- Manufacturing in up to 1500 L fermenter scale
- 4500 m<sup>2</sup> production area
- Manufacturing license for proteins, DNA and vaccines

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# Applications for CFF at RHB

- Harvest of supernatant
- Filtration of protein solutions
- Buffer exchange after refolding/or chromatography
- Aggregate removal

➔ Big differences in product streams with respect to amount, physico/chemical properties and purity

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# Case study I: original process



High cell density fermentation (E.coli)



Harvest of supernatant by CFF (0.2 $\mu$ m) ⚡



Concentration of clarified supernatant  
by CFF (10kDa) factor 3 ⚡



Adjustment of pH and conductivity by dilution



0.2 $\mu$ m dead end filtration ⚡



Capture chromatography



# Challenges

Different components of product stream lead to low performance.

High particular load

High viscosity

Antifoam



Low Flux

Fouling of membranes

# Improvements I

## Improvements without changing techniques

~~Viscosity of feed stream by time of harvest~~

Preclarification by separator and/or depth filter

~~Different membrane types~~

~~Adaptation of pore size~~

Adaptation of parameter (CFF)

## Case study I: modified process

Preclarification by separator



Harvest of supernatant by CFF (0.2 $\mu$ m)



Concentration of clarified supernatant  
by CFF (10kDa) factor 3



Adjustment of pH and conductivity by dilution



0.2 $\mu$ m dead end filtration



Capture chromatography

# Improvements II

## Improvements introducing new techniques

- EBA Chromatography
- Inline dilution
- Filter aid
- Benzonase® treatment

## Improved Process:

Preclarification by separator



Dilution of clarified supernatant



EBA-Chromatography



EBA eluate, adjustment  
of conductivity by dilution

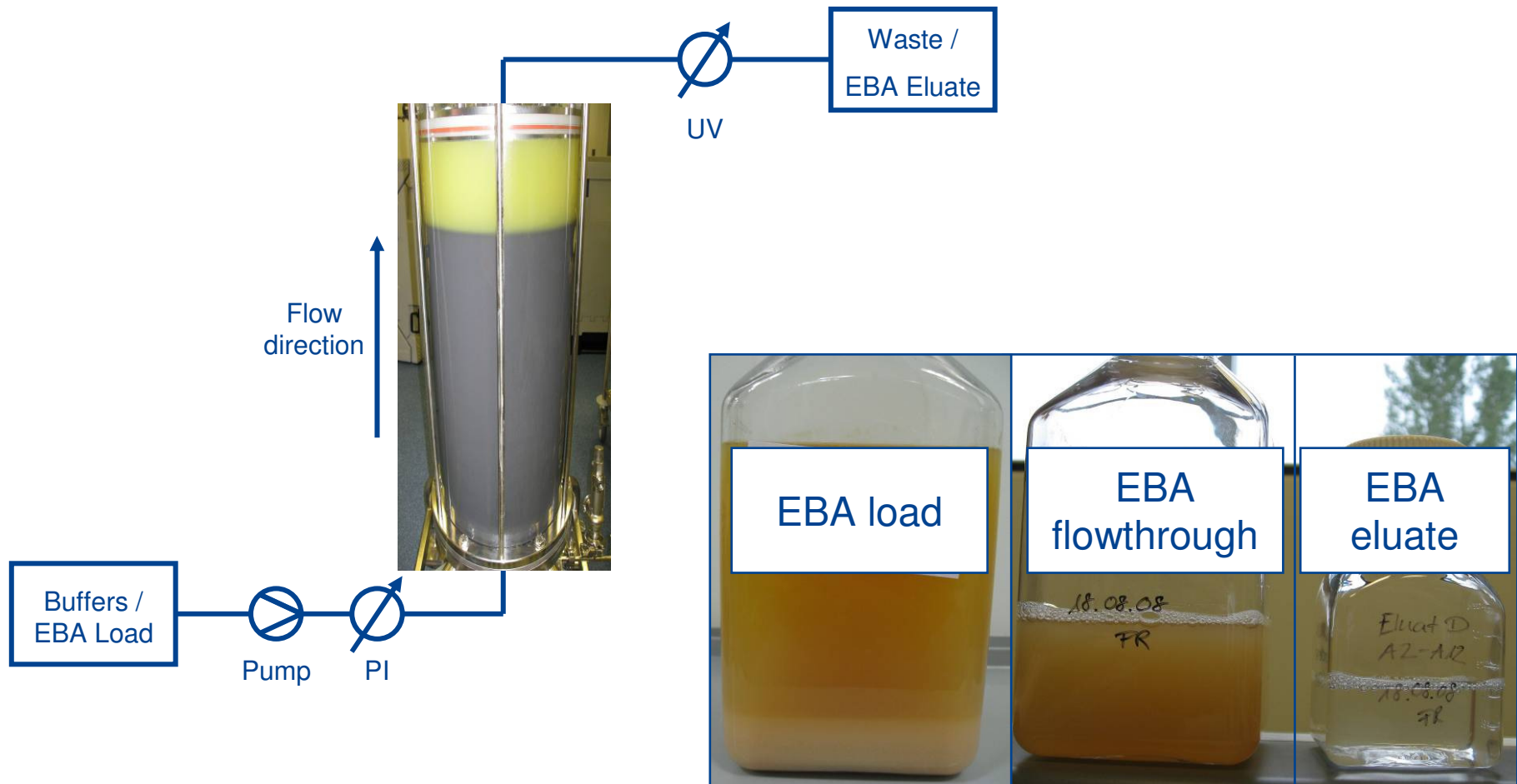


0.2µm dead end filtration



Capture chromatography

# EBA operation set-up



# Results

## Results of Improvements

- Reduction of time by 50%
- Elimination of 2 CFF steps
- Reduction of filter surface by 50%
- No loss of product
- Same quality

## Case study II: original process

Dilution of denaturated protein, pH adjustment



Depth filtration



Concentration by CFF (20 x)



10 fold buffer exchange by CFF



0.2µm dead end filtration



Capture chromatography

# Challenges

One component of feed stream induces low performance.

High viscosity

High salt concentration



Low Flux

High buffer consumption

# Improvements

Limitations implementing new techniques  
because of:

Large volume

High salt concentration



Inline dilution

Direct capture

# Improvements

## Improvements without changing techniques

- ~~Different membrane types~~
- Pore size (5 to 10kDa)
- Better temperature control (lower viscosity)
- Smaller volume during diafiltration (60%)
- Rinsing of filter after concentration
- Better control of continuous diafiltration

# Results

## Robust and efficient process

- Reduction of membrane area by 20 %
- Reduction of buffer consumption by 50%
- Process time unchanged
- No loss of product

Small adjustments lead to big effect.

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# Quality aspects

Limited information concerning:

- Membrane life time
- Cleaning procedures
- Long time storage of membranes

# Quality: Cleaning

Measurements to guarantee efficient cleaning:

- General procedures for cleaning and storage
- Extensive testing of cleaning by:
- Determination of TOC, Endotoxin & Bioburden
- Determination of NWP

## Quality: Membrane life time

Ongoing verification of long time storage and membrane life time:

- Determination of NWP before use
- Integrity test
- Testing of Bioburden (rinse and storage solution)

# Quality: Strategy

Rely on known procedures and materials.

Strategy:

- General procedures
- Same filter material type originating from manufacturer
- High quality of filters

Filters have to cover a broad range of applications.



**Thank you!**

[www.richter-helm.eu](http://www.richter-helm.eu)

