

Lonza group

# Optimization and Scale-up of Protein-free Processes for Antibody Production

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## Why protein-free?

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- Increasing emphasis from regulatory authorities on removal of animal-derived raw materials from antibody production processes
  - Potential source of adventitious agents and product contaminants
- Sourcing becoming increasingly difficult as demand increases due to expanding worldwide production capacity
  - Potentially a similar issue with components used as protein replacements e.g. hydrolysates

## Why chemically defined?

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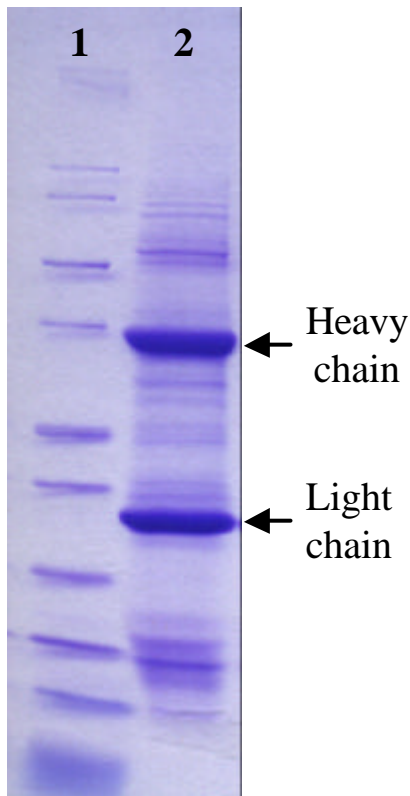
- Raw material lot to lot variability reduces manufacturing process robustness and consistency
  - Issue associated with both proteins and hydrolysates
  - May necessitate extensive raw material testing to identify 'good' raw material lots
- Chemical definition alleviates these issues

## Additional benefit

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- Simplification of downstream processing
  - Reduced protein contaminant levels
  
- Increased harvest material purity
  - GS-NSO in protein-containing and protein-free culture
    - ◆ Protein-containing culture <30% purity
    - ◆ Protein-free culture 62% - 76% purity

# Harvest material purity



- Harvest material from protein-free (chemically defined medium)  
GS-NSO process - Lane 2
  - Lane 1 MWt markers
- Major components are antibody associated
- Minor bands are host cell proteins
- Improved purity prior to purification

# Process optimization

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- Purpose of process optimization
  - Increase manufacturing-scale facility productivity
  - Minimize COG
- Considerations
  - Fermentation process productivity
    - ◆ Maintenance of product quality
  - Downstream process yields
    - ◆ Maintenance of product quality
  - Facility throughput
    - ◆ Balance increased yield and process duration

# Generic processes

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- **Basis for process optimization is efficient generic processes**
  - **Well defined with proven performance**
  - **Use allows rapid cell line evaluation and early phase product supply**
  - **Provide some of the information required for subsequent process optimization**
  - **Updated as technology advances**

# Platform technology

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- Generic processes need to be based on a sound platform
  - Effective, robust and operable at manufacturing-scale
  - Unconstrained by raw material supply and regulatory issues
  
- Fermentation process example
  - Selection of basal medium formulation for CHO based processes

## Medium selection

Medium	Maximum viable cell conc. (10 <sup>6</sup> /mL)	Cumulative cell time (10 <sup>9</sup> cell h/L)	Product concentration (mg/L)	Specific production rate (mg/10 <sup>9</sup> cells/h)
A	1.8	260	255	0.98
B	2.2	359	213	0.59
C	1.3	260	253	0.97
D	3.2	509	225	0.44
<b>E</b>	<b>3.6</b>	<b>603</b>	<b>315</b>	<b>0.52</b>
F	3.3	458	223	0.49
G	2.5	382	136	0.36
H	2.1	303	89	0.30

- Medium E selected as platform for generic process
  - Maximum growth and volumetric productivity
  - Chemically defined protein-free with acceptable supply chain for large-scale operation

# Fermentation process optimization

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## ■ Aims

- Maximize productivity
  - ◆ Maintain product quality
- Minimize impact on process duration
  - ◆ Assessment of effect on facility throughput
- Compatibility with manufacturing-scale operations

# Fermentation process optimization

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## ■ Considerations

- Cell line and expression system
- Fermentation process
  - ◆ Physicochemical environment
  - ◆ Nutritional environment

## High expression cell lines

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- Creation of highly expressing cell lines critical to improving process productivity
- GS expression system facilitates rapid creation of highly productive NSO and CHO cell lines
  - High specific production rate coupled with good growth characteristics
  - Ease of selection using glutamine-free medium
  - No requirement for amplification for NSO cell lines

## GS expression system

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- Widely utilised with NS0 cell lines for production of monoclonal antibodies
  - Product concentrations up to 2 g/L at Lonza
  - Higher productivities reported
    - ◆ >3.5 g/L

## Improving expression

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- Improvements in vectors, transfection, cell line cloning and selection procedures
  - Increase probability of selecting a highly productive cell line
- Metabolic engineering
  - e.g. apoptosis resistance

# Fermentation process

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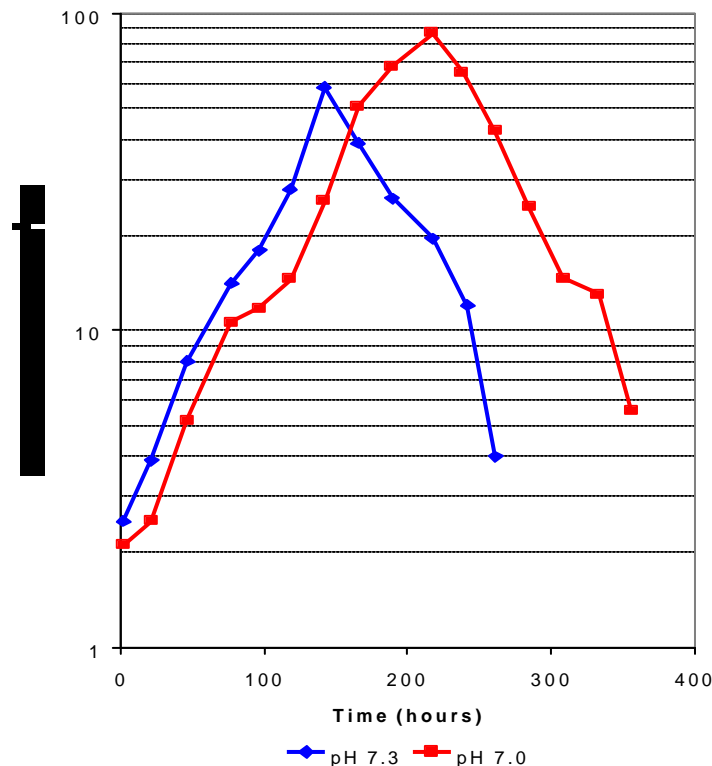
## ■ Physicochemical environment

- Culture pH in particular can have dramatic effect on cell growth and productivity
  - ◆ Responses are cell line specific

## ■ Nutritional environment

- Processes typically operated in fed-batch mode
  - ◆ Higher productivity than batch processes
- Development of improved basal medium, feed formulations and feed addition strategies

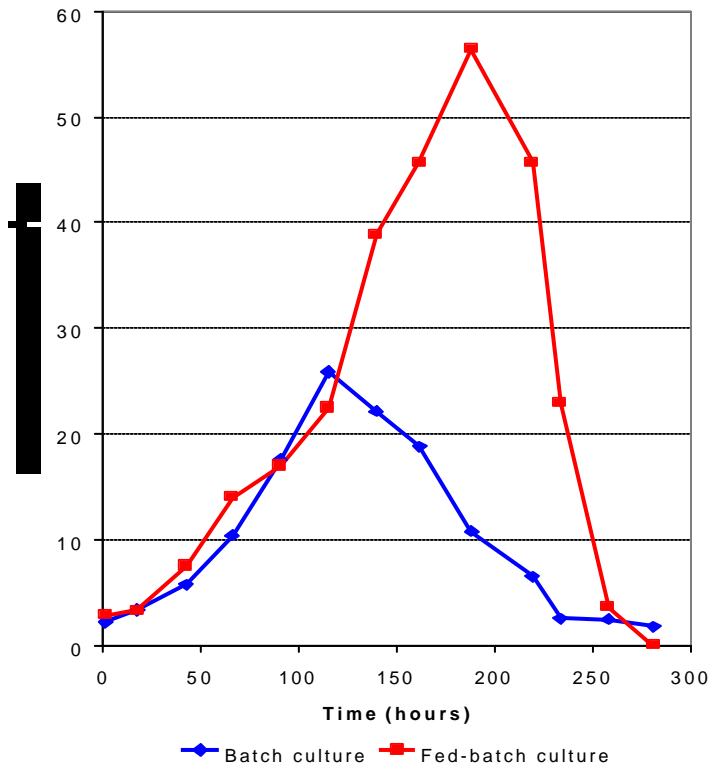
# Effect of culture pH



■ Reduction of culture pH for a protein-free (chemically defined medium) GS-NSO process

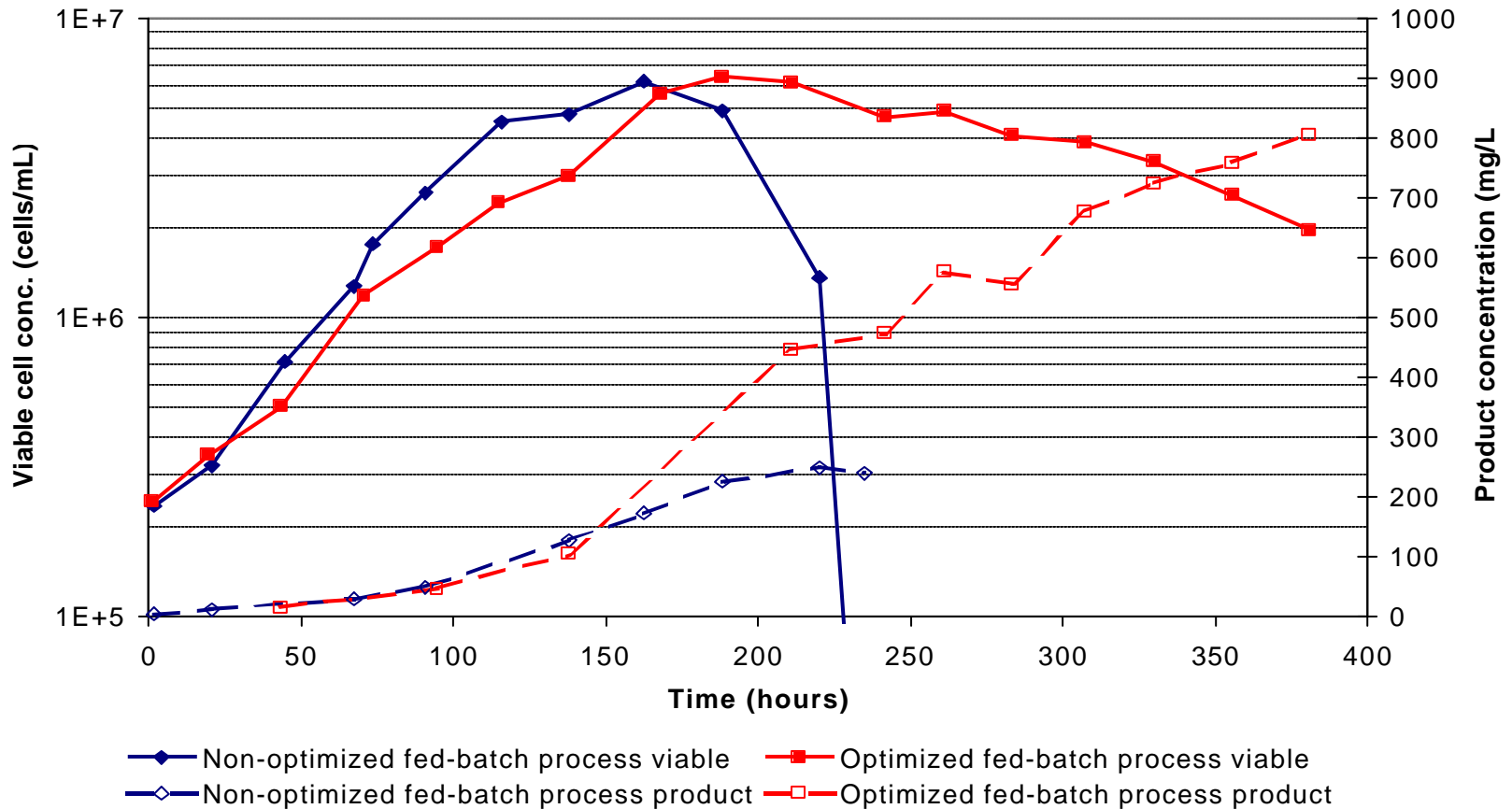
- Increased maximum viable cell concentration
- Increased culture duration
- Increased integral viable cells
- Increased productivity
  - ◆ 590 mg/L compared with 240 mg/L

# Fed-batch culture



- Fed-batch culture of protein-free (chemically defined medium) GS-NS0
  - Increased maximum viable cell concentration
  - Increased integral viable cells
  - Increased productivity
    - ◆ 300 mg/L compared with 175 mg/L in batch culture

# Improved fermentation process

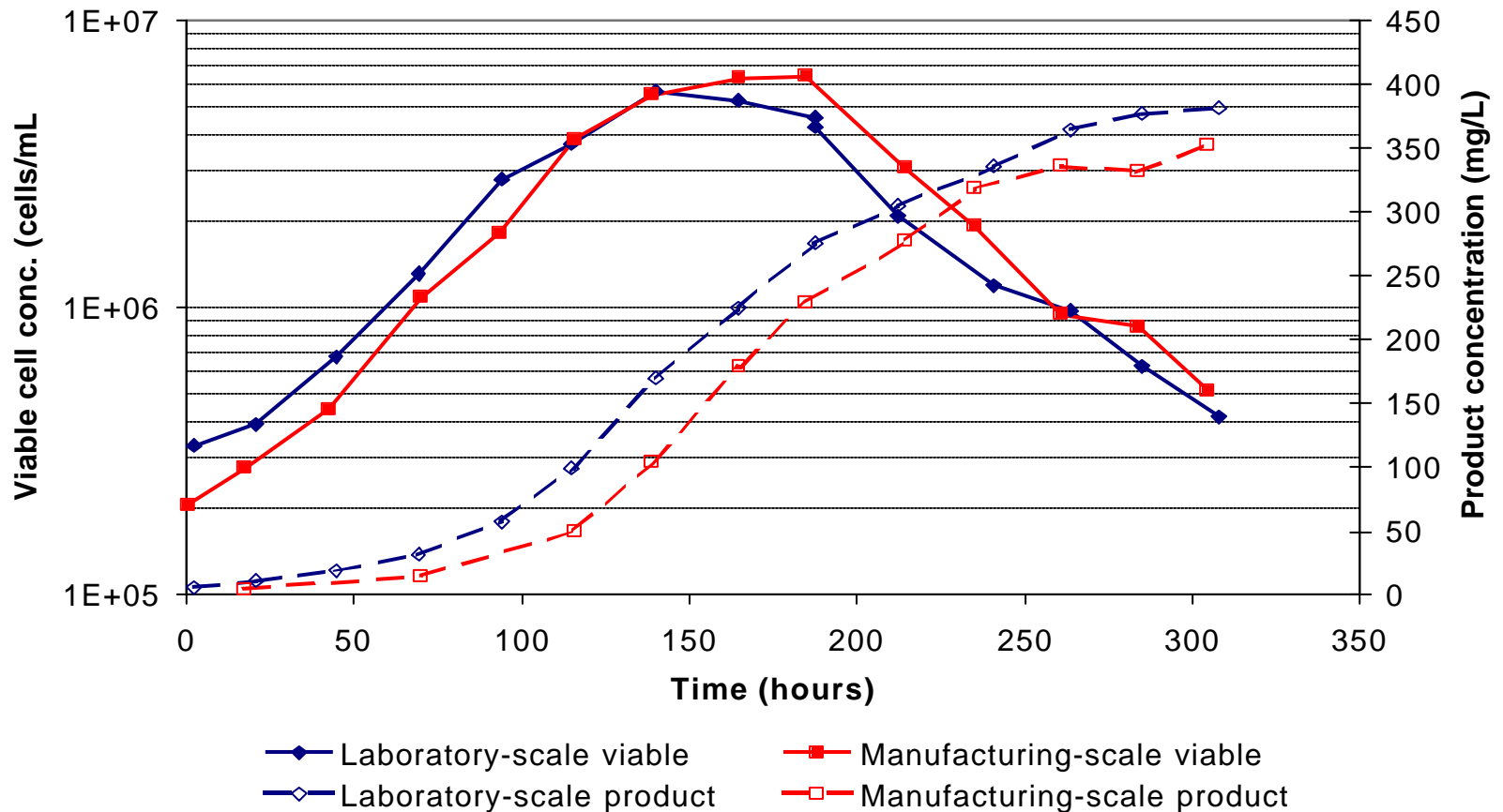


# Fermentation process scale-up

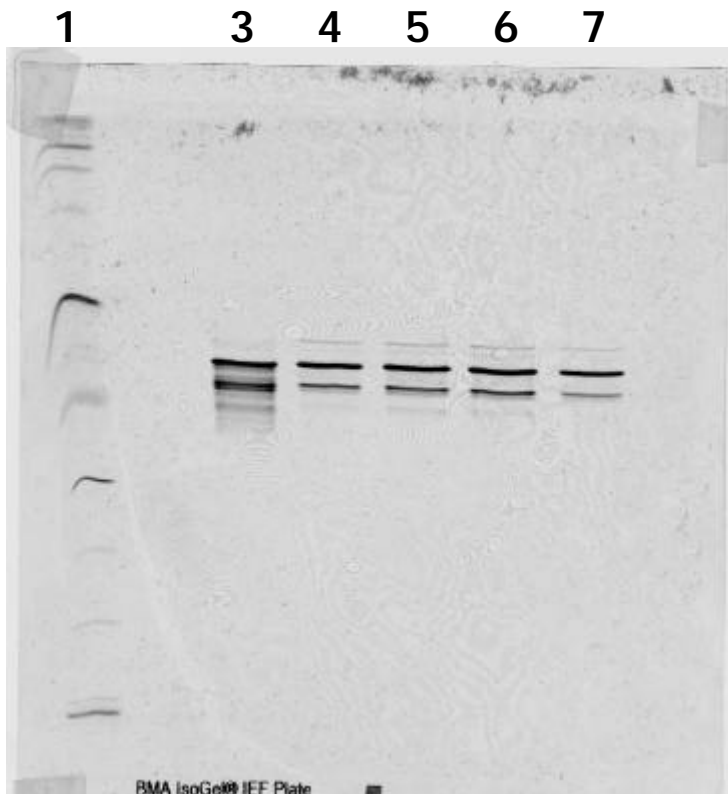
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- Development and use of appropriate scale-down models eases process scale-up
  - Reactor design based on defined scaling parameters
    - ◆ Geometric, mass transfer etc.
  - Consistent operation procedures between scales
  
- Use of comparable (identical) raw materials

# Scale-up of a protein-free GS-NSO process



# Product quality - IEF analysis



- Lane 1: pI markers
- Lane 3: Standard from protein-containing culture
- Lanes 4,6,7: Laboratory-scale
- Lane 5: Manufacturing-scale
- Product equivalence confirmed by additional assays

# Facility throughput

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- Facility throughput is key to achieving improved per annum productivity
- Aim is to increase process productivity whilst minimising impact on process duration
  - e.g. Two fold increase in productivity with process duration increased from 12 to 15 days

# Maximising output

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- Development of process models is essential in achieving maximum facility productivity
  
- Example - modeling of a GS-NSO process
  - Productivity improved by 100%
  - Process duration increased by 25%
  
- Per annum productivity increased by »70%
  - Compromise between increased per batch productivity and increased batch duration

## Summary

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- Generic processes form the basis of process optimization strategies
- Fermentation optimization can result in significant increases in process productivity
  - Additional increases can be achieved through downstream process optimization
- The goal is increased productivity from the manufacturing-scale facility
  - Compromise between process productivity and process duration

## Summary

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- **Process scale-up is eased through the development of appropriate scale-down models**
  
- **Chemically-defined protein-free processes are the preferred route**
  - **Regulatory compliance**
  - **Improved process robustness**
  - **Simplification of downstream processing**