Selection of chemically defined media for production of biopharmaceuticals

01-June-2018, Dirk Martens
Content

- Background industrial cell cultivation
- Serum (History)
- Chemically defined media
  - Relevance of cell volume
Products

Viral vaccines

Pharmaceutical proteins
Industrial scale

- Chinese Hamster Ovary Cell Line
- Fed-batch
- Stirred tank reactors
  - Disposable up to 2 m³
  - Stainless steel up to 20 m³
Process development

- Safety
  - No contaminants
  - Proper protein structure
- Speed
  - Predictable & robust process
  - Robust scale-up
- Volumetric productivity and product concentration
Serum

- Infectious agents (viruses, prions)
- Variable undefined composition
- High protein content problems in DSP

Serum replacement
- Growth factors
- Transferrin (Fe)
- Lipids
- Detoxification
- **Shear protection**
- .....
Scale-up: Mixing & Gas transfer

Stirred Tank Reactor
Effect of serum

(Biotechnol Bioeng. 1992 Apr 15;39(9):891-7)

![Graph showing the effect of serum concentration on relative shear sensitivity. The graph compares not serum adapted and serum adapted samples.](graph.png)
Pluronic F68

![Chemical structure of Pluronic F68](image)

![Graph showing relative shear sensitivity vs. [Pluronic] (g.dm⁻³)](image)
Media selection

- **CHO cells**: High producer and low producer
- **Fed-batch (starting day 3)**
- **12 Shake flasks (duplicate)**
Cell numbers

[Bar chart showing cell numbers in millions per liter for different categories labeled as O_EFAB, O_EFC, O_AFAB, C_EFAB, C_EFC, C_AFAB, F_EFAB, F_EFC, F_AFAB, A_EFAB, A_EFC, A_AFAB. The chart compares high producer and low producer categories.]
Volumetric productivity

High producer  Low producer

mg.dm⁻³.day⁻¹

O_EFAB  O_EFC  O_AFAB  C_EFAB  C_EFC  C_AFAB  F_EFAB  F_EFC  F_AFAB  A_EFAB  A_EFC  A_AFAB
Cell density and volume

![Graph showing cell density and volume](image)

- Total viable cells (#. reactor⁻¹)
- Total viable cell volume (ml. reactor⁻¹)

- NI Phase
- SI Phase
- Stationary Phase
- Death Phase

- Time (days):
  - 0
  - 2
  - 4
  - 6
  - 8
  - 10
  - 12
  - 14

- Total viable cells (logarithmic scale)
  - 0.0E+00
  - 2.0E+10
  - 4.0E+10
  - 6.0E+10
  - 8.0E+10
  - 1.0E+11

- Total viable cell volume (logarithmic scale)
  - 0
  - 50
  - 100
  - 150
  - 200
  - 250

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Cell dry weight

![Bar graph showing cell dry weight](image)

- **Y-axis:** pg/cell
- **X-axis:** D4, D7, D10
- **Legend:** Dry weight
Cell cycle arrest
Specific productivity

- q_p (pg/cell/day)
- q_p (pg/µl/day)

NI
SI
NI
SI
Metabolic analysis

A: NI phase

B: SI phase
Efficiency

![Bar chart showing efficiency for Moab/prot and MoAb/O2 with categories NI and SI.]

- Moab/prot: NI approximately 1.0%, SI approximately 5.0%
- MoAb/O2: NI approximately 0.0%, SI approximately 0.0%
Concluding remarks

- Chemically defined media are available.
- Clone specific
  - Effect difficult to predict
- Very specific effects
  - Cell cycle arrest and volume increase
- Cell volume is an important parameter
  - Represents biomass
  - Better efficiency
- Composition not publically available
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