Trouble-shooting Fermentation and Primary recovery manufacturing issues in order to optimize antigen expression for the Vaccine business

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Your (or our) engineers are no magicians - despite of what they may claim.

"Got a few problems going from lab scale to full-scale commercial."

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Agenda

- Fermentation manufacturing issues in antigen expression
  - Parameters (i.e. physical & nutritional) to consider
  - Large-scale limitations
  - Scale-down methodology in finalizing a large-scale process

- Antigen recovery issues in manufacturing
  - Process parameters for consideration
  - Large-scale limitations and solutions for antigen recovery
  - Future considerations
Process considerations during fermentation scale-up

- Physical parameters to maintain
  - Shear rate – turbulence
  - Bulk flow – mixing time
  - $K_La$ – mass transfer of oxygen
  - Power/Volume ratio
  - pH
  - Temperature

- Nutritional requirements to control
  - Substrate feeding concentration

- Output: Productivity, dissolve oxygen and pH profile

Problems associated with scaling-up to large bioreactors

- Oxygen transfer is less effective at the larger scale.
- To achieve similar mixing time of nutrients as the smaller scale bioreactor is not achievable

- Bioreactor Constraints
  - Aeration and Agitation

Solutions

- Increase the number of impellers (i.e. Rushton impeller) during operation to increase power and improve oxygen transfer
- Try different combination of impellers (ie. Rushton and Hydrofoil impellers) to improve mixing
The effect of KLa on protein yields upon scale-up

- Hydrofoil impellers didn't improve protein expression
- Improving mass-transfer by additional impeller improve the protein yield in large-scale reactor

\[ K_{La} = 0.002 \left( \frac{P}{V} \right) \left( \frac{t}{U_L} \right) \]

KLa relates to gas velocity and power input to stirrer for stirred fermentors containing non-coalescing non-viscous media (Doran, 1995)

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Optimizing substrate feed and temperature to improve productivity (Large scale)

- Optimization at large-scale: 6 batches & 2 months
- Costly in time and resources
Scale-down process to Millilitres to determine key process parameters

- Benchtop, computer controlled fermentation system
  - massive screening/testing
    - Key Media components,
    - Process conditions: Temperature, pH, Dissolved oxygen concentration, aeration
  - single use 24-reactor cassette
  - independently control and monitored
    - Gas supply, temperature, pH, Dissolve oxygen
  - 24 simultaneous experiments

- Reduce cost
- Improve productivity

Where the µ-reactor fits
Temperature and pH Control

Temperature control: well to well reproducibility

Column 3

- Temperature
- IPTG Induction

pH control: well to well reproducibility Row C

Soluble protein Expression

- 27°C
  - Reproducible expression
- 30°C
  - Reproducible expression
- ↓ Induction Temp.
  - ↑ soluble protein expression

Induction Temperature

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Lessons Learnt:
“Scale-down” your final manufacturing process

- Nutrient requirements/critical process parameters can be screened and determined at bench-scale.
- Engineering effects: Identify KLa (agitation/aeration) range achieved at manufacturing scale.
- Scale-up process to small-scale bioreactor (taking into account large-scale constraints).
- Test process at large scale.

Upstream recovery optimization for bacterial proteins.
Considerations in protein recovery for Intracellular proteins

\( E. coli \) cells grow in fermentor \( \rightarrow \) Cell broth Separation

\( \rightarrow \) Homogenize \( \rightarrow \) Clarification \( \rightarrow \) Purification

- **Parameters affecting Homogenization**
  - Pressure
  - Number of passes
  - Cell concentration
  - Process fluid variables (viscosity, temperature)

- **Cell broth Separation / Clarification**
  - Clarification method (filtration/centrifugation)
  - Processing time, unit operation and product recovery
  - Process fluid variables (cell and protein characteristics, viscosity)

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**Gel Polarization (Darcy’s law) for X-flow Microfiltration**

\[
\text{Flux } J \propto \frac{\Delta P_{\text{TM}}}{\mu (R_g + R_m)}
\]

- \( R_g \) = gel resistance, \( R_m \) = membrane resistance, \( J \) = flux,
- \( \Delta P_{\text{TM}} \) = Transmembrane pressure

\( \text{Flux } \alpha \text{ (Driving Force)}/(\text{resistance}) \)
Parameters affecting the build-up of the gel layer ($R_g$)

$$R_g = \frac{\alpha \mu c}{2A^2 P_{TM}}$$

- Specific cake resistance ($\alpha$)
- Membrane (A)
- Transmembrane Pressure ($P_{TM}$)
- Crossflow Rate
- Cell concentration ($c$)
- Viscosity ($\mu$)

Clarification efficiency using cross-flow filtration

- Filtration flux (LMH)
- Product recovery (product permeation)
Things to consider: Irreversible fouling during concentration/Diafiltration using E.coli cells

- Cell concentration/cell broth separation
- Diafiltration using buffer
  - Subsequent diafiltration doesn’t restore flux due to lysis of cells onto the membrane
  - Age of the fermentation cells does play a role in filtration
  - Need to stop fermentation before death phase

Problems associated using cross-flow filtration for product clarification

Protein characteristics may affect adhesion to membrane and thereby decrease recovery

- Surface charge densities (i.e. pH, solution ionic strength) (Baruah & Belfort, Biotech Bioeng., Vol.87, 2004)
- Cell surface chemistry
  - Cell surface adhesion causing membrane fouling
- Nature of the protein

Static Filtration
Difficulty in static filtration

- High product recovery at 20 L scale
- Not scalable at the large scale (200 L and beyond)
  - Difficult in handling
- Disposable costs may be high when scaled to manufacturing scale
- Not as robust as cross-flow filtration where the process is dependent on the upstream fermentation and homogenization conditions

Direct Adsorption method

Binding and washing steps of the batch clarification process

1. Bind homogenate with beads
2. Wash beads with buffer
3. Wash beads with low-salt buffer
4. Elute protein

protein binds Big Beads
Homogenate debris and proteins
80 µm filter mesh
Large-scale process results

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<tr>
<th>Lane</th>
<th>Protein 1</th>
<th>Protein 2</th>
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<tr>
<td>Amount protein produced at fermentation (%)</td>
<td>100</td>
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<tr>
<td>% Clarification recovery</td>
<td>90%</td>
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Lane 1: marker
Lane 2: Homogenized supernatant
Lane 3: Unbound debris
Lane 4: Wash with low salt buffer
Lane 5: Eluted protein
Lane 6: High salt strip

Future of Clarification

- **Metal Affinity/ Ion Exchange Membranes**
  - Available in strong Anionic and Cationic forms, as well as in a metal ion complex form
  - Disposable (single-use)
  - Increase availability of affinity ligands will help increase its use

- **Multi-modal adsorbents**
  - More selectivity (i.e. directly bind proteins from high ionic strength feedstocks) and higher capacity
  - Streamline Direct CST – ion exchanger (Biotech BioEng Vol.94, no.6, 2006, 1155-1163)
  - Used for Expanded bed chromatography
  - Possibility remains for stirred tank applications
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