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

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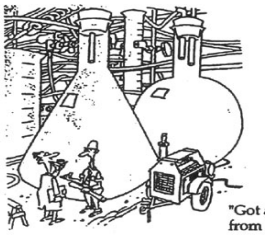
Your (or our) engineers are no magicians - despite of what they may claim.



"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

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## 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**

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## 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

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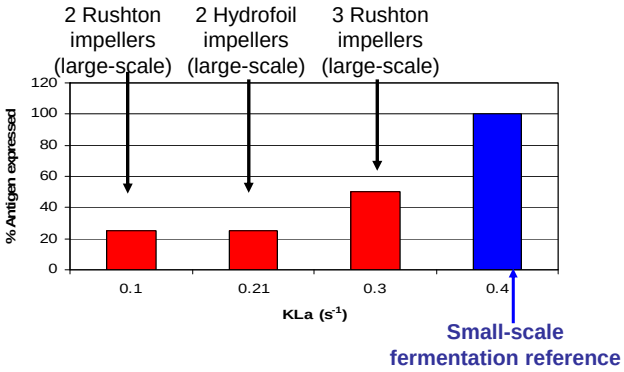
## The effect of KLa on protein yields upon scale-up




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

$$K_{La} = 0.002\left(\frac{P_g}{V}\right)^{0.7}(U_g)^{0.2}$$

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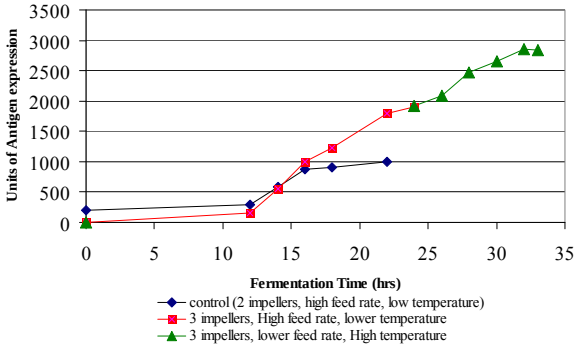
## Optimizing substrate feed and temperature to improve productivity (Large scale)




- Optimization at large-scale: 6 batches & 2 months
- Costly in time and resources

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## 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**

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## Where the µ-reactor fits


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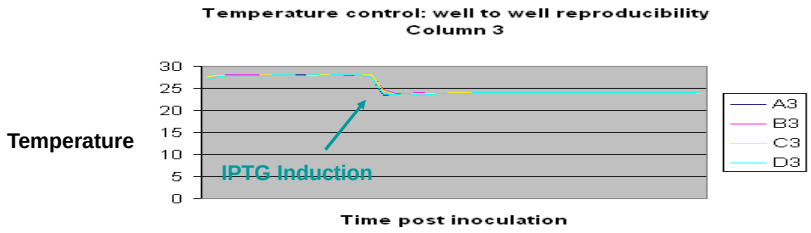
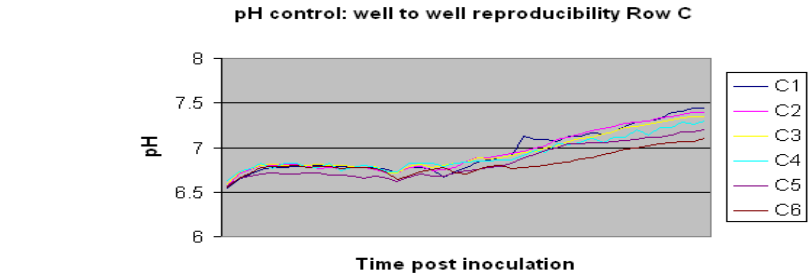
# Temperature and pH Control

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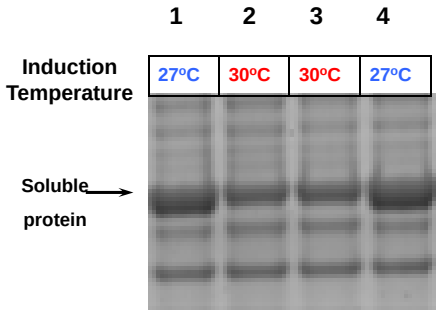
# Soluble protein Expression

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

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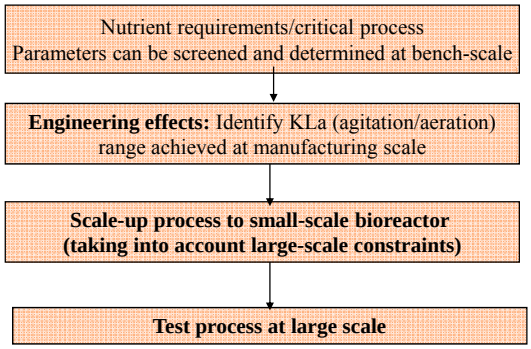
## Lessons Learnt:

## " Scale-down" your final manufacturing process

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## Upstream recovery optimization for bacterial proteins

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## Considerations in protein recovery for Intracellular proteins

*E.coli cells grow in fermentor* → **Cell broth Separation**

→ Homogenize → Clarification → 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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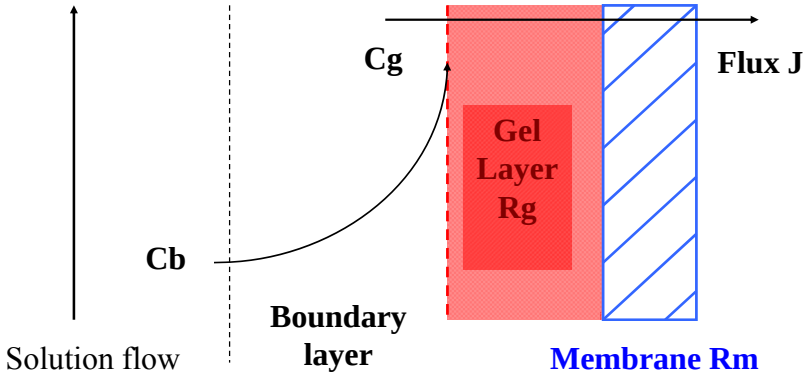
## Gel Polarization (Darcy's law) for X-flow Microfiltration




**Flux α (Driving Force)/(resistance)**

Rg = gel resistance, Rm = membrane resistance, J= flux,

$\Delta P_{TM}$ = Transmembrane pressure

$$J \propto \frac{\Delta P_{TM}}{\mu(R_g + R_m)}$$

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## Parameters affecting the build-up of the gel layer ($R_g$)

$$R_g = \frac{\alpha \mu \mathbf{c}}{2\mathbf{A}^2\mathbf{P}_{\mathbf{TM}}}$$

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

→ Filtration flux (LMH)

→ Product recovery (product permeation)

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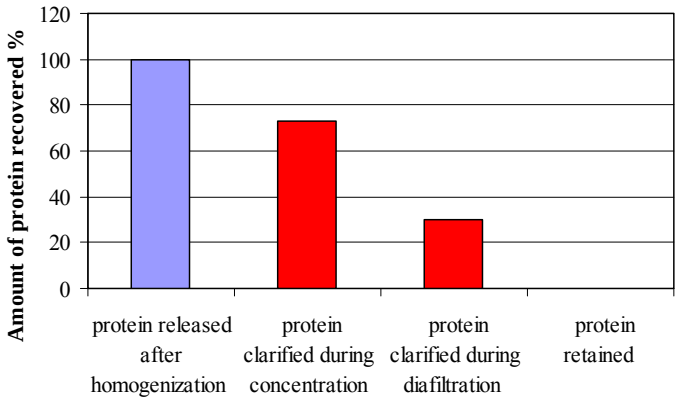
## Clarification efficiency using cross-flow filtration

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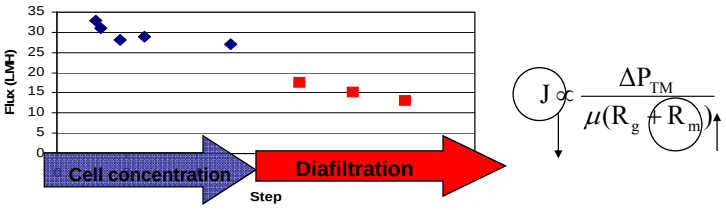
## Things to consider: Irreversible fouling during concentration/Diafiltration using E.coli cells




◆ 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**

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## 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**

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## 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**

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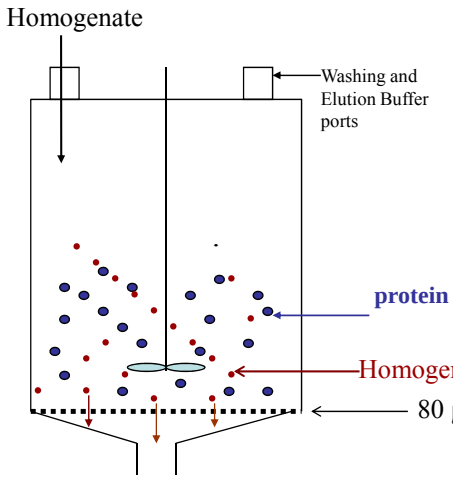
## Binding and washing steps of the batch clarification process




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

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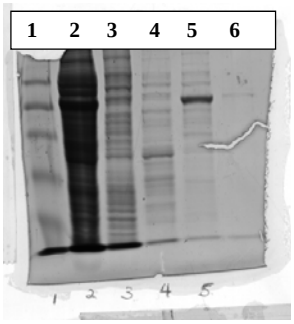
## Large-scale process results

| | Protein 1 | | Protein 2 | |
|---|---|---|---|---|
| Lot# | 1 | 2 | 1 | 2 |
| Amount protein produced at fermentation (%) | 100 | 100 | 100 | 100 |
| % Clarification recovery | 90% | 90% | 75% | 73% |




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

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## 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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