Bioreactor Process Control – Principles from Lab to Industrial Scale

Daniel Egger & Manfred Zinn
Agenda

What is industrial production
Scale up importance
Classic scale up principles
Problems in industrial scale
Scale down systems and PAT
The Universities of applied sciences with activities in Life Sciences

- École d'ingénieurs et d'architectes de Fribourg
  - Chemistry

- École d'ingénieurs de Changins
  - Oenology

- Haute école du paysage, d'ingénierie et d'architecture
  - Natural Sciences

- HES-SO/Valais, Haute Ecole d’ingénierie
  - Life Technologies
Institute of Life Technologies at HES-SO Valais//Wallis

- 19 Professors (Food Technology, Biotechnology, Analytical Chemistry)
- 36 Scientific collaborators (PhD, MSc, BSc, Engineers) and technicians
- 4 PhD students
- 12 Apprentices
Projects are carried out by research groups of principal investigators and senior research associates.
Research activities of Life and Bioresource Technologies and my contributions

- Bioresources
  - Liquid and gaseous substrates
  - White and Red Biotechnology
  - Bioprocess design
  - Scale-up from shake flasks to 300 L

- Bioanalytics
- Chemical Biotechnology
- Biomaterials
- Bioengineering
- Chemical Synthons
Our company is true to its roots and to our traditional Swiss values. We combine perfection with passion. We work on each solution with scrupulous care and imaginative flair until everything fits like clockwork. We are instinctively quality-conscious and place a high value on top-quality materials and expertly trained employees. Our stable corporate culture ensures that we remain firmly grounded and determinedly independent. To us, our Swiss heritage means that we also act sustainably – in environmental, social and economic terms.
The INFORS HT Group
History

1965  Founding of Hawrylenko Technique by Alexander Hawrylenko, 1st patent
1967  First shaker for high-speed applications
1968  First 75 L bioreactor
Today Complete solution portfolio for cultivations from 1 mL up to 1000 L; Microbial, cell culture, biofuel 2nd and 3rd gen. and specialised bioprocess platforms
Strong bioprocess platforms enhanced by powerful software
Product development

Clone screening ➔ Process development ➔ Pilot scale ➔ Production
What defines the industrial scale?
Biotech products: price and volume

Market volume $\rightarrow$ determines scale $\rightarrow$ determines price
Examples: price and volume

- Annual output from one facility
  - 400 Tonnes - Cephalosporin

- Annual output from one facility
  - 8 grams - Therapeutic proteins e.g. antibodies

→ Approx. equal value
Different requirements → different solutions

Industrial priorities influence the scale up

- **High Yield**
  - Genetic engineering
  - Media development
  - Process development

- **High Quality**
  - Process development
  - Process understanding

- **Fast time to market**
  - Fast development and scale up (patents run out!)

→ Genetic engineering helps but is not the end of the story (process development, media development)

→ Bulk production vs. pharmaceutical production: different priorities

→ Parallel production still has importance (e.g. roller bottles or vaccine production in eggs)
One simple solution
Traditional product development

Considerations:
- Simple growth conditions (in shake flasks at first)
- Media Composition
- Simple product isolation
- Biological Activity
- Toxicity

Considerations:
- Media optimisation
- Process optimisation
- Device SOP’s
- Clinical trial material
- Downstream processing

Considerations:
- Suitability of existing plant for process
- Validation
- Process SOP
- Safety/Risk assessment
- Downstream processing
  - Recovery
  - Recycling
  - Waste disposal
Traditional product development

Scale up parameters mostly:
- Vessel geometry
- Power input
- $K_La$ & mixing time
Practical operating boundaries for aerated, agitated bioreactors
Scale up

5 L Bioreactor

? 

500 L Bioreactor
Simple methods for (lucky) scale up

- Constant $pO_2$
- Constant tip speed
- Constant gassed power / volume

$\rightarrow$ Similar vessel geometry, gassing rates & equivalent $k_La$ assumed
Constant $pO_2$

\[ pO_{2(1)} = pO_{2(2)} \]
Constant tip speed

\[ N_2 = N_1 \left( \frac{D_1}{D_2} \right) \]

\( N \) = rotation speed \((\text{min}^{-1})\)

\( D_i \) = impeller diameter \((\text{m})\)
Examples: tip speed

<table>
<thead>
<tr>
<th>Rotation speed (min⁻¹)</th>
<th>Impeller tip speed (m s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minifors 5 L</td>
</tr>
<tr>
<td>0 0</td>
<td>0.0</td>
</tr>
<tr>
<td>200 3.3</td>
<td>0.6</td>
</tr>
<tr>
<td>400 6.7</td>
<td>1.1</td>
</tr>
<tr>
<td>600 10.0</td>
<td>1.7</td>
</tr>
<tr>
<td>800 13.3</td>
<td>2.3</td>
</tr>
<tr>
<td>1000 16.7</td>
<td>2.8</td>
</tr>
<tr>
<td>1200 20.0</td>
<td>3.4</td>
</tr>
</tbody>
</table>
Constant gassed Power / Volume

\[ N_2 = N_1 \left( \frac{D_1}{D_2} \right)^{2/3} \left( \frac{Q_2}{Q_1} \right)^{5/42} \]

This equation provides the relationship between impeller top speeds for bioreactors of different scales, assuming a constant \( k_{La} \)

- \( N \) = rotation speed (RPM)
- \( D_i \) = impeller diameter (m)
- \( Q \) = volumetric gas flow (m\(^3\) s\(^{-1}\))
Example: constant gassed power

Assume:  
(1) Constant $k_La$  
(2) Gas flow = 1.5 vvm  
(3) Constant gassed power for a range of RPM

<table>
<thead>
<tr>
<th>Minifors 5 L min⁻¹</th>
<th>Techfors-S 42 L min⁻¹</th>
<th>Techfors 300 L min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>200</td>
<td>113</td>
<td>61</td>
</tr>
<tr>
<td>400</td>
<td>226</td>
<td>122</td>
</tr>
<tr>
<td>600</td>
<td>339</td>
<td>183</td>
</tr>
<tr>
<td>800</td>
<td>451</td>
<td>244</td>
</tr>
<tr>
<td>1000</td>
<td>564</td>
<td>305</td>
</tr>
<tr>
<td>1200</td>
<td>677</td>
<td>366</td>
</tr>
</tbody>
</table>
Comparison of different strategies for up-scaling:
(volumetric: factor 125)

<table>
<thead>
<tr>
<th>Strategy</th>
<th>$P_0$</th>
<th>$P_0/V$</th>
<th>$k_L a$</th>
<th>$N$</th>
<th>$t_m$</th>
<th>$N D$</th>
<th>$N D^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_0/V =$const</td>
<td>125</td>
<td>1</td>
<td>1</td>
<td>0.34</td>
<td>2.9</td>
<td>1.7</td>
<td>8.5</td>
</tr>
<tr>
<td>$N =$const</td>
<td>3125</td>
<td>25</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>$v =$const</td>
<td>25</td>
<td>0.2</td>
<td>0.4</td>
<td>0.2</td>
<td>5</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>$Re =$const</td>
<td>0.2</td>
<td>0.002</td>
<td>0.04</td>
<td>0.04</td>
<td>25</td>
<td>0.2</td>
<td>1</td>
</tr>
</tbody>
</table>

Increase of volume: factor 125 (at constant geometric ratio)
Most used scale up models in the industry (Rule of thumb)

<table>
<thead>
<tr>
<th>% of industry</th>
<th>Scale-up criterion used</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>P/V</td>
</tr>
<tr>
<td>30</td>
<td>(k_{i,a})</td>
</tr>
<tr>
<td>20</td>
<td>Tip speed</td>
</tr>
<tr>
<td>20</td>
<td>Oxygen tension</td>
</tr>
</tbody>
</table>
Scale up is easy!? 

“Got a few problems going from lab scale to full scale commercial”

Source: www.fda.gov
Special scale up problems - examples
What happens if geometries are very different?

- Bioprocess
  - Tubes
  - Bags
  - Bioreactor
  - Shake flasks
Computerised Flow Dynamics (CFD)

Can be used to estimate energy input, turbulence, etc. in simulation.

Source: Eibl et al.
Computerised flow dynamics (CFD) and bioreactors

Building the CFD model
From the geometry of a bioreactor to its oxygen distribution

Poor mixing
Low nutrients/pO₂
Different physiology & gene expression
Crabtree effect (S. cerevisiae)
Crabtree effect (*S. cerevisiae*)

I: Glucose consumption (EtOH fermentation and cell respiration)
II: Oxidation of EtOH (pyruvate consumption)
III: Oxidation of EtOH (acetate consumption)
IV: Oxidation of EtOH
V: Acetate consumption
Nitrogen (ammonia)-limited batch culture

Comments:

- Acetate is produced
- All glucose is consumed and the culture is N- plus glucose-limited (residual carbon?)
- DOC indicates that a lot of carbon remains in the medium as products that are only slowly utilized (or not at all?).
- Cell composition changes slightly before limitation is reached

Source: Batch growth of *Klebsiella pneumoniae* in synthetic medium with ammonia as limiting substrate.

(from Wanner & Egli, 1990)
Understanding complex relationships: PCAs & MVAs

Typical combination of problems at large scale:
- pH-control
- Mixing time
- Gradients
- Energy input (e.g. aeration and cooling)

PCAs and MVAs are a good way to identify critical parameters.

Source: http://www.bytefish.de
Online tools help to optimize scale-up further

Using data from a standard process signals of a bioreactor for optimization
PAT-based closed loop control: Cellphysiostat

- PAT Controlled Feed Dosage
- Bioreactor
- PAT Analyzers
- PAT Data of > 50 process variables and quality attributes

- Feed Strategy
- Controller settings

Process Information Management System
Scale Up / Scale Down

Scale up: Increasing development costs
Scale down: Increasing speed
Think big (and small)!
obrigado Dank U mahalo Köszí
cracušo Grazie mauruuru Takk
Gracias Dziękuję Děkuju danke Kiitos