Bioprocess scale-up – Tracking the informations relevant for scaling-up by GFP reporter strains

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Background
Bioprocess scale-up – general scheme

Shaken bioreactors – lab-scale

Stirred bioreactor – lab-scale

Stirred bioreactor – industrial scale

Reactors dimension (D)

Lack of efficiency compared with stirred reactors:
- Lower transfer efficiency
- No regulation of the main environmental variables (pH, dissolved oxygen)

Drop of mixing efficiency when D↑ at constant P/V
Generation of heterogeneities (substrate, dissolved oxygen, pH, temperature,...)
Background
Exposure to spatial heterogeneities – hydrodynamic aspects

Delvigne et al. [2006] Chemical engineering journal
**Experimental strategy**

**Fluorescent reporter system**

**Basic principle:**
Using the microbial population as « physiological tracer » for the estimation of the bioreactor mixing and transfer efficiency (potentially capturing the stochasticity linked with the CTD)

- Extracellular stimuli (S, O2, pH)
- Signal transduction
- GFP coding sequence
- GFP synthesis
- Stress (P<sub>stress</sub>
Experimental strategy

Flow cytometry – an efficient tool to characterize microbial population heterogeneity

30,000 microbial cells analysed within 30 seconds
Experimental strategy
Choosing the right ORF for my application

*E. coli*: about 4000 ORFs:

Transcriptional network

Transcriptional network – hierarchical classification

Ma et al. [2004] BMC Bioinformatics, 5:199
Results
Screening among an E. coli GFP clones library

Cultivation in shake flasks on mineral medium

\[ \text{prpoS::gfp} \]
Results

Screening among an E. coli GFP clones library
Representativeness of shaken bioreactor

**Shake flask**: easy to handle, well suited to perform parallel cultures, but lack of representativeness compared to the performances of stirred bioreactors.
Results

Screening among an E. coli GFP clones library
Representativeness of shaken bioreactor

Intermittent feeding strategy

IO converter

OXY-mini 4 channels

Orbital incubator
(T°C and shaking frequency controls)
Results

Screening among an E. coli GFP clones library
Representativeness of shaken bioreactor

Cultures of GFP clones in shaken bioreactors (1L baffled shake flask: initial working volume: 200mL; final working volume: 400 mL)

Growth inhibiting value: 4.5
Results

Screening among an E. coli GFP clones library
Representativeness of shaken bioreactor

\[ \text{prpoS::gfp} \]

\[ \text{puspA::gfp} \]
Results

Screening among an E. coli GFP clones library

Two modes of expression: binary or graded

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Zhang et al. (2006) Theoretical biology and medical modelling, 3:18
Results

Screening among an E. coli GFP clones library

Binary mode of gene expression → sources:
- Short mRNA and protein half-lives
- High sensitivity for the detection of the reporter protein

Generally not observed for GFP reporter system considering the high protein stability of this system compared with β-galactosidase and luciferase reporters.

This mechanism of gene induction give rise to differentially expressed phenotypes at the protein level. Can potentially be used to gain more sensitivity about the impact of extracellular fluctuations.
Results

Behaviour of prpoS::gfp strain in fed-batch stirred bioreactor

Regulation of the addition of glucose by the dissolved oxygen level (SP = 30%) PID control
Results

Behaviour of prpoS::gfp strain in fed-batch stirred bioreactor

Regulation of the addition of glucose by the dissolved oxygen level (SP = 30%), ON/OFF control
Results

Behaviour of prpoS::gfp strain in fed-batch stirred bioreactor

Basic observations:

- Binary mode for GFP expression at the end of the batch phase and during the transition from batch to fed-batch phase.

- After the induction of the major part of the population (all the cells are in the GFP+ state), graded mode of GFP expression is observed.

- Successive glucose excess tends to slow down the binary expression phase.
Results

Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor

Two-compartment scale-down reactor (P-SDR)

Substrate level

Excess level

Limitation level

Starvation level

Microbial cell 1

Microbial cell 2

Microbial cell 3

Time
Results

Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor

Operating conditions:
- Stirred bioreactor, working volume 10L
- Mineral medium, glucose as carbon source
- Fed-batch with exponential feed algorithm
- Scale-down approaches with DO-controlled fed-batch and partitioned reactor

Delvigne F. et al. [2009] Microbial cell factories, 8:15
Results

Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor
Results

Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor
Results

Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor

![Graphs showing the behavior of prpoS::gfp strain in a two-compartment scale-down bioreactor.](image)

- **Global mixing efficiency**

  - Figure A: % of GFP+ cells over time.
  - Figure B: Sum of the fluorescence intensities (AU) over time.

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**Legend**:
- Blue circles: Well mixed exponential feed
- Green crosses: Well mixed DO-feed control
- Red squares: Partitioned reactor Q_{ref_0} = 10 L/h
- Cyan diamonds: Partitioned reactor Q_{ref_0} = 36 L/h
A pcyA::GFPmut2 strain is not influenced by hydrodynamic conditions.

**Results**

*Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor*
**Results**

* Cultures performed under constant glucose feed

- Constant feed at 10 g/h
- Constant feed at 7 g/h
- Classical bioreactors without recycle loop
- Two-compartment scale-down reactors
Results

Cultures performed under constant glucose feed

Reactors without recycle loop

Two-compartment scale-down reactors

0h

12h

25h
Results

_Cultures performed under constant glucose feed_: pcsI::gfp strain
Results

Cultures performed under constant glucose feed: puspA::gfp strain

To be validated by using a DO-controlled feed

Prytz et al [2003] Biotech bioeng 83:595-603
Two main mechanisms proposed to regulate rpoS in high cell density cultures:

- Cell density
  DeLisa and Bentley [2002] Microbial cell factories, 1:5
- Decreasing growth rate
  Ihssen and Egli [2004] Microbiology, 150:1637:1648
Perspectives and conclusion

prpoS::GFP strains seems to react to the degree of homogeneity inside the bioreactor:

- Homogenous reactor: GFP+
- Inhomogenous reactor: GFP-
Two questions have to be raised:
- Flow cytometry combined with $P_{\text{stress}}$::GFP expression $\rightarrow$ impact of extrinsic fluctuations
  What about the intrinsic fluctuations?
- Characteristic times of hydrodynamic mechanisms compared with those of the biological processes behind GFP synthesis

**Perspectives and conclusion**

Stirred bioreactor
$V_L = 1L$ to $10L$ ; $t_s = 10\text{ min to } 15\text{ min}$

Recycle loop (plug-flow)
$V_L = 0,1L$ to $2L$ ; $t_s = 45s$ to $200s$

Transduction
$t_{\text{transcription}} = 20-70s$

ARNm

GFP
$t_{\text{translation}} = 4\text{min}$
Perspectives and conclusion

Complex phenomena:
- Two sources of noise (extrinsic and intrinsic)
- Very different characteristic time constants (physical and biological processes)
→ A model is required

\[ \begin{align*}
T_A & \xrightarrow{k_1} T_A \\
T_A + DNA & \xrightarrow{k_2} T_A\_DNA \\
T_A\_DNA & \xrightarrow{k_3} T_A + DNA \\
T_A & \xrightarrow{k_4} \emptyset \\
T_A\_DNA & \xrightarrow{k_5} T_A\_DNA + RNA \\
RNA & \xrightarrow{k_6} RNA + GFP \\
RNA & \xrightarrow{k_7} \emptyset \\
GFP & \xrightarrow{k_8} \emptyset
\end{align*} \]
Perspectives and conclusion

Reaction scheme:

Exposure to glucose excess = f(t_m,t_c)

\[ \begin{align*}
    & k_4 \rightarrow TA \\
    & TA + DNA \xrightarrow{k_5} TA\_DNA \\
    & TA\_DNA \xrightarrow{k_6} TA + DNA \\
    & TA \xrightarrow{k_7} \emptyset \\
    & TA\_DNA \xrightarrow{k_8} TA\_DNA + RNA \\
    & RNA \xrightarrow{k_9} RNA + GFP \\
    & RNA \xrightarrow{k_{10}} \emptyset \\
    & GFP \xrightarrow{k_{11}} \emptyset \\
\end{align*} \]

Generation time:
\[ k_8 = \log(2)/t_g \]

ODEs system:

\[ \begin{align*}
    \frac{dTA}{dt} &= k_1 - k_2.TA.DNA - k_4.TA + k_3.TA\_DNA \\
    \frac{dT_{TA\_DNA}}{dt} &= k_2.TA.DNA - k_5.TA\_DNA - k_3.TA\_DNA \\
    \frac{dDNA}{dt} &= k_3.TA\_DNA - k_2.TA.DNA \\
    \frac{dRNA}{dt} &= k_5.TA\_DNA - k_6.RNA - k_7.RNA \\
    \frac{dGFP}{dt} &= k_4.RNA - k_8.GFP
\end{align*} \]

\[ GFP_{steady-state} = RNA_{steady-state} \times \left( \frac{k_6}{k_8} \right) \]

8 rates (including the characteristic time constants) to specify
Perspectives and conclusion

These equations can be used in the classical deterministic formalism (ODEs solver), but more interestingly in the stochastic formalism:

Probability that reaction $\mu$ occurs at time $\tau$ (Gillespie algorithm)


Example: simulation of 30,000 cells after 6 hours of induction
Thank you

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