Microbial heterogeneity affects bioprocess robustness
Future of dynamic single-cell analysis for large-scale bioprocess control

Frank Delvigne
Université de Liège, Gembloux Agro Bio Tech
Passage des Déportés, 2
5030 Gembloux BELGIUM
F.Delvigne@ulg.ac.be
1. Introduction: microbial phenotypic heterogeneity, two fields, two views

2. Single cell analysis in process conditions: rpoS response

3. Single cell analysis in process conditions: membrane permeability and protein leakage

4. What’s next?
Impact of microbial phenotypic heterogeneity on process productivity
Accepted picture: only a fraction of the population (non productive phenotypes) affects the global production profile
Single cell studies are of great importance both from a fundamental and from an applied perspective:

Two fields with two distinct perceptions of microbial phenotypic heterogeneity:

1. **Chemical engineering**: focused on the effect of external perturbations on biological noise

2. **System biology**: focused on the intrinsic and extrinsic source of noise

Each discipline integrates only a fraction of the knowledge and have evolved by considering distincts (cultivation and analytical) tools for the determination of microbial phenotypic heterogeneity.
(Bio)chemical engineering approach:

Distribution of fluorescence among cell population

Time

GFP coding sequence

Promoter

GFP synthesis
Flow cytometry (FCM): different modes of operation

Manual sampling

On-line

Real time

Alison Brognaux (L20)
System biology approach:

Massively parallelized micro-cultivation devices

Clone number

Time

Distribution of fluorescence among cell population


Inter-scale comparison: biology vs chemical engineering

Biological systems...

... cultivation devices
Integrating microfluidics (and single cell microfluidics) in the scale-up/down loop?

BUT, flow regime is not the only difference between microfluidic-based micro-bioreactors and large-scale bioreactors

Some examples reported from the litterature :

- **Cell density effect (Quorum sensing)**
  

- **Competition for metabolically efficient phenotypes**
  

- **Differences in growth rate**
  

- **Transition to « solid-culture » phenotypes (biofilm, floculation)**
  

- ...
But gives useful information at the level of history-dependent mechanisms.

Recombinant protein secretion is cell-cycle dependent (switch between producer/non-producer state).

Impossible to point out this phenomenon in process conditions (history independent techniques)
Single cell analysis in process conditions

_E. coli K12 MG1655 with fluorescent reporters_

Well-mixed vs Scale-down

Feed

Substrate level

- Excess level
- Limitation level
- Starvation level

Time

Microbial cell 1
Microbial cell 2
Microbial cell 3
Batch Fed-batch

Well-mixed

C-SDR

P-SDR

Bioproscale conference 2014
Link between mixing quality and population segregation at the level of the \textit{rpoS} response:

Trade-off effect - microbial cells have to choose between:

- Improvement of the nutritional level – growth rate
- Stress sensitivity

\[\rightarrow \text{Well-mixed, lab-scale bioreactors}\]

- Improvement of stress resistance
- Lower nutritional status– growth rate

\[\rightarrow \text{Scale-down bioreactors}\]
Extracellular perturbations increases cell viability


Analysis performed by flow cytometry with propidium iodide exclusion test and thus related to cell membrane permeability
Intermediate PI uptake:

Fed-batch, well-mixed reactor
Propidium iodide (PI) uptake and appearance of an intermediate subpopulation with reduced red fluorescence

**Hypothesis**: accumulation of PI in the periplasm when cells are exposed to substrate limitation (increased expression of porins)
PI exclusion test

Ref. bioreactor

SDR bioreactor, $t_R = 38s$

SDR bioreactor, $t_R = 79s$
Extracellular proteome analysis of the impact of bioreactor performances
Well-mixed/scale-down comparative analysis: 2-D differential in-gel electrophoresis

Fold ratio: well-mixed reactor compared to scale-down reactor

Interesting results but without any control at the level of the substrate limitation and growth (batch)

Validation is needed (chemostat)

What’s next

Limitation of the actual fluorescent reporter system: global view of physiology. There is a need to be closer to the metabolism:

- By appropriately choosing promoter sequences
- By using new generation of promoter independent fluorescent reporter
- Subpopulations omics

Subpopulations → Metabolically relevant? Switch between subpopulations?
Challenges:

Going beyond the need of fluorescent tags: single cell omics technologies

Metabolic engineering aiming at controlling microbial phenotypic heterogeneity

Insertion of microfluidics bioreactor in the scaling-up/down loop

**Technical staff**
Samuel Telek
Benoît Massaux

**Post-docs**
Alison Brognaux Tambi Kar

**PhD students**
Quentin Zune
Jonathan Baert
Romain Kinet
Michel Musoni
Thanh Huu Nguyen
Naila Boudjelal Annick Lejeune

**Collaborations:**
INSA Toulouse
Tech Univ Denmark
Copenhagen Univ
UNA-Cuajimalpa
Leiden Univ
GSK biologicals
Puratos/Beldem
Sequip

Lunchtime! But don’t forget to ask for some questions
Thank you