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Université
de Liège

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

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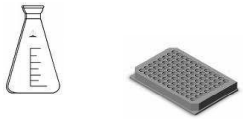
Background

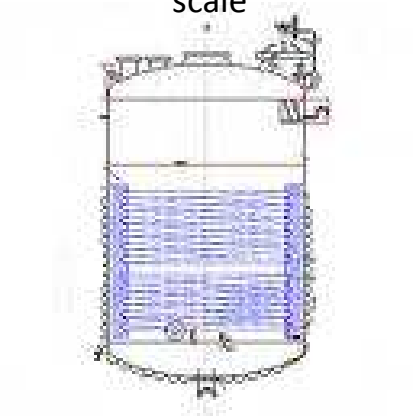
Bioprocess scale-up – general scheme

Shaken bioreactors – lab-scale



Stirred bioreactor – lab-scale

Stirred bioreactor – industrial scale



Reactor dimension (D)

Lack of efficicency compared with stirred reactors :

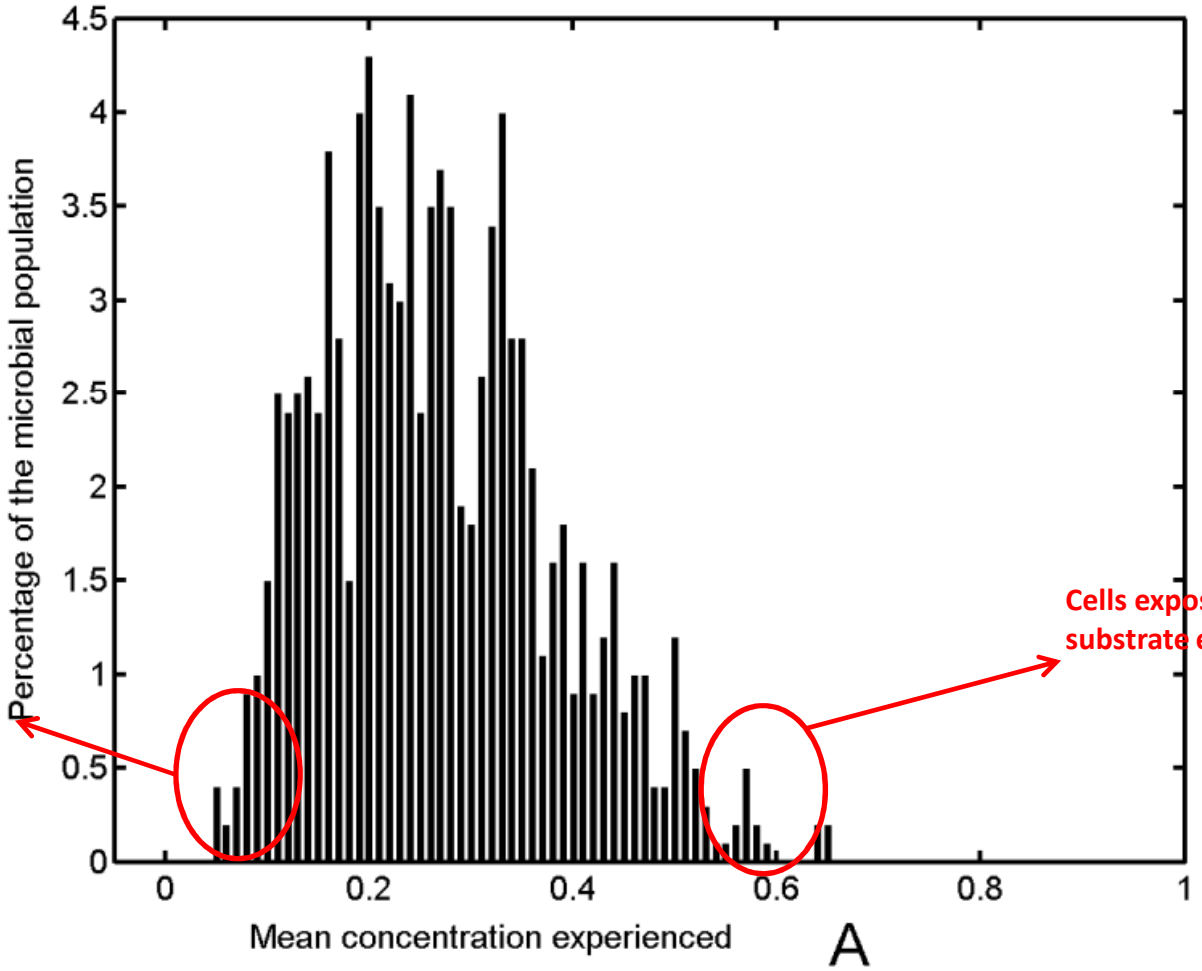
- Lower transfer efficiency
- No regulation of the main environmental variables (pH, dissolved oxygen)

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



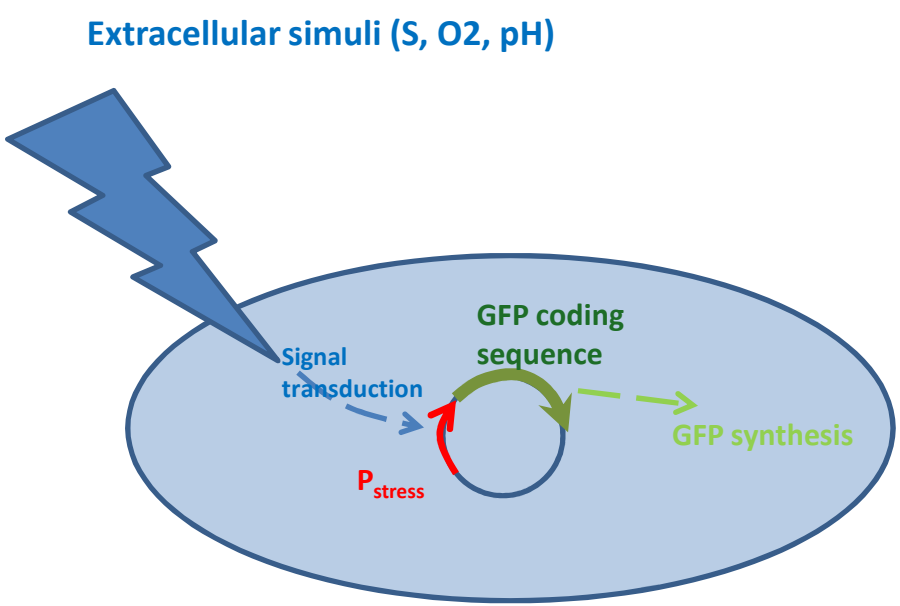
Delvigne *et al.* [2006] Chemical engineering journal

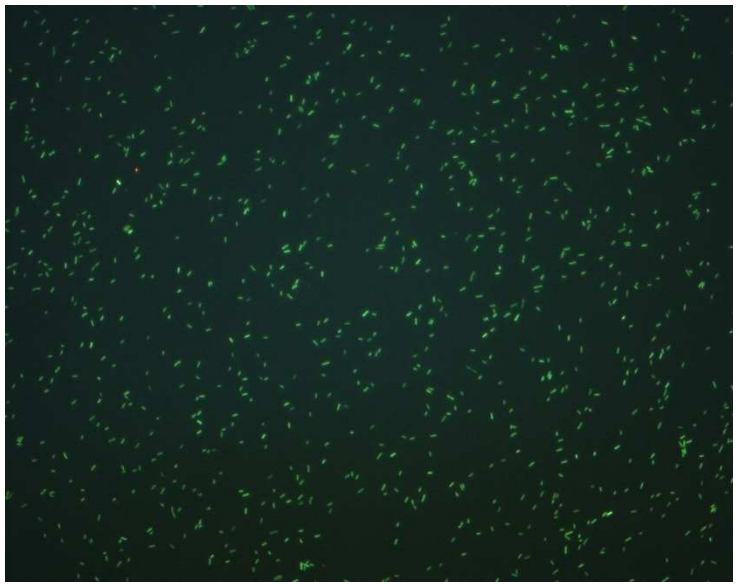
Experimental strategy

Fluorescent reporter system

Basic principle :

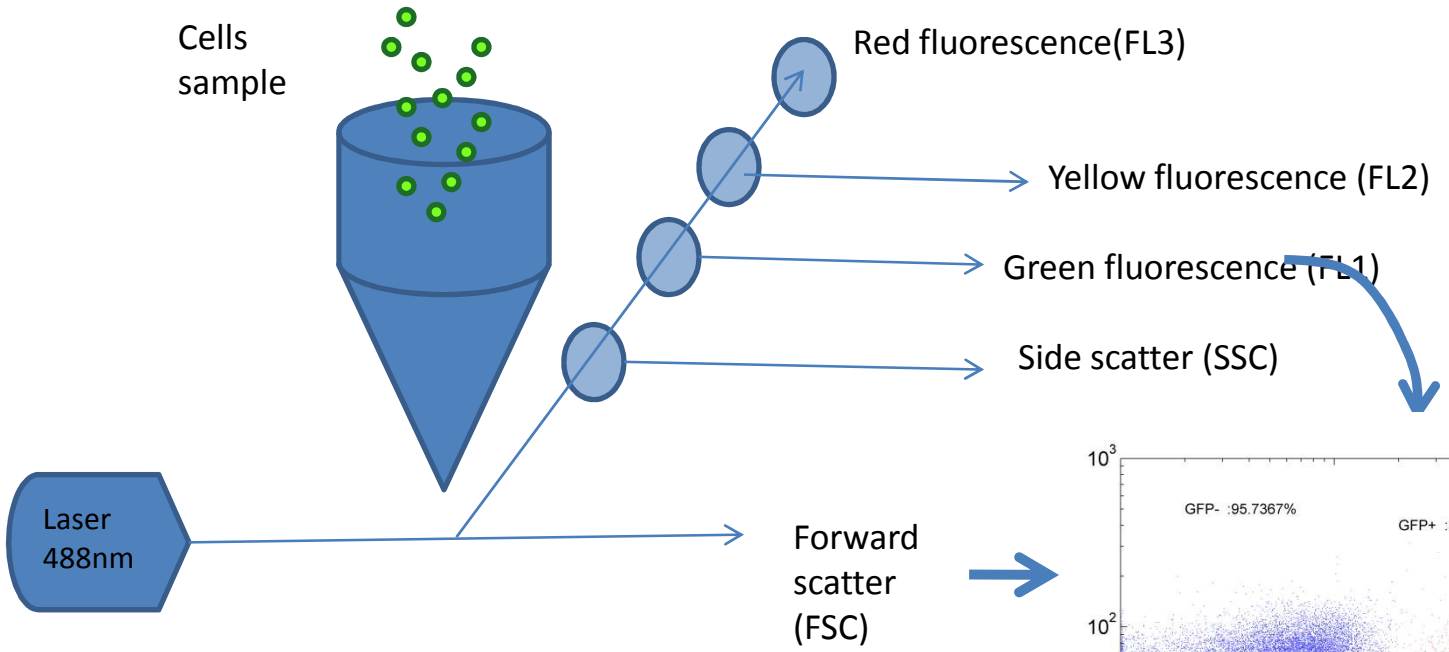
Using the microbial population as « physiological tracer » for the estimation of the bioreactor mixing and transfer efficiency (potentially capturing the stochasticity linked with the CTD)

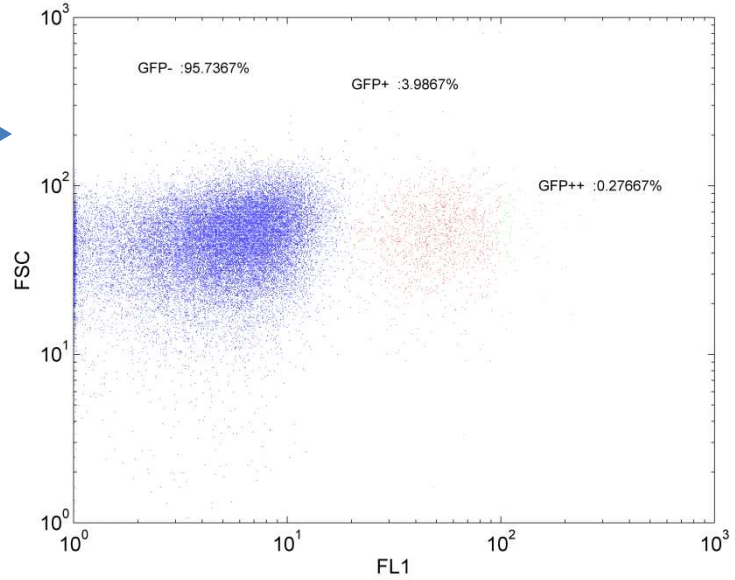




Experimental strategy

Flow cytometry – an efficient tool to characterize microbial population heterogeneity





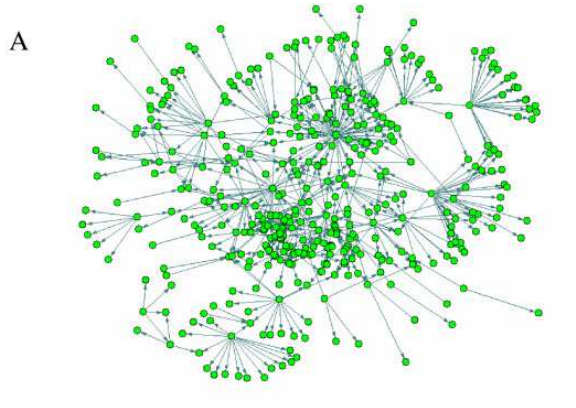
30,000 microbial cells analysed within 30 seconds

Experimental strategy

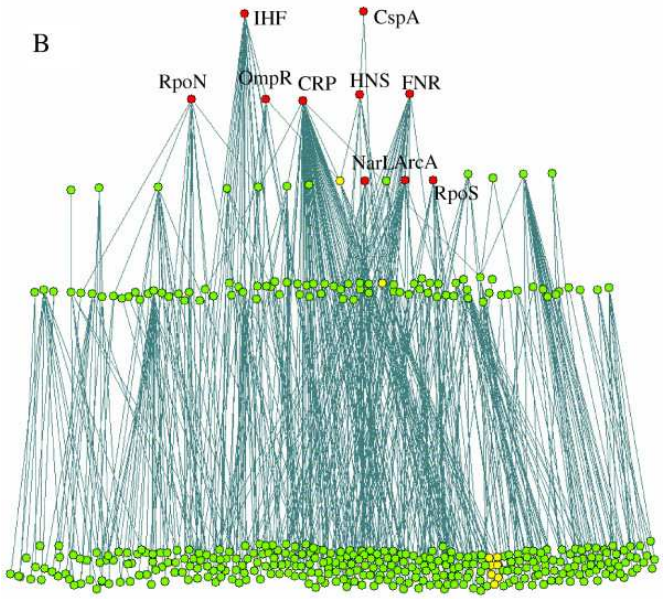
Choosing the right ORF for my application

***E. coli* : about 4000 ORFs :**

Transcriptional network



Transcriptional network – hierarchical classification



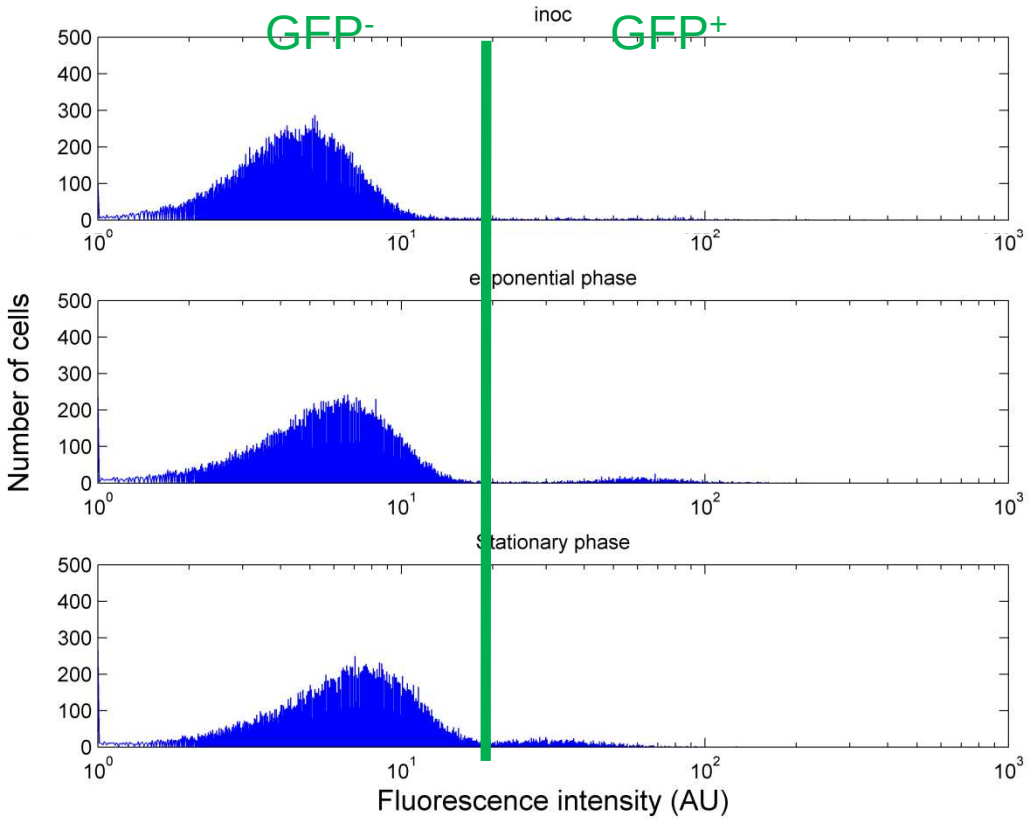
Ma *et al.* [2004] BMC Bioinformatics, **5**:199

Results

Screening among an E. coli GFP clones library

Cultivation in shake flasks on mineral medium

prpoS::gfp

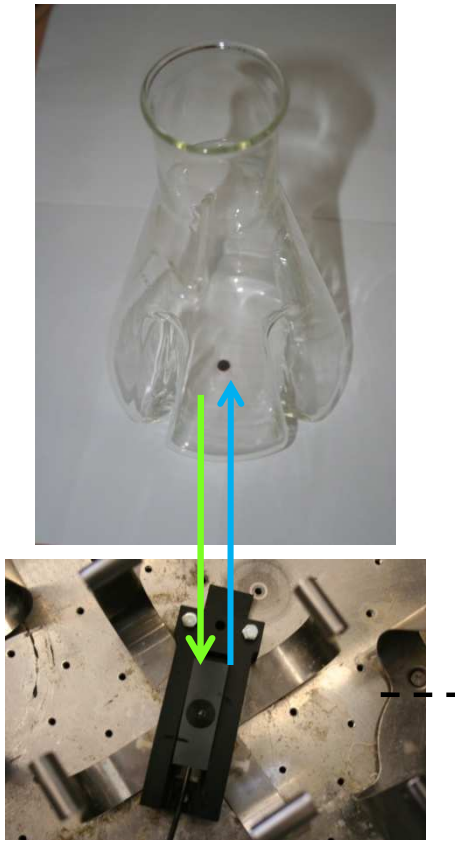


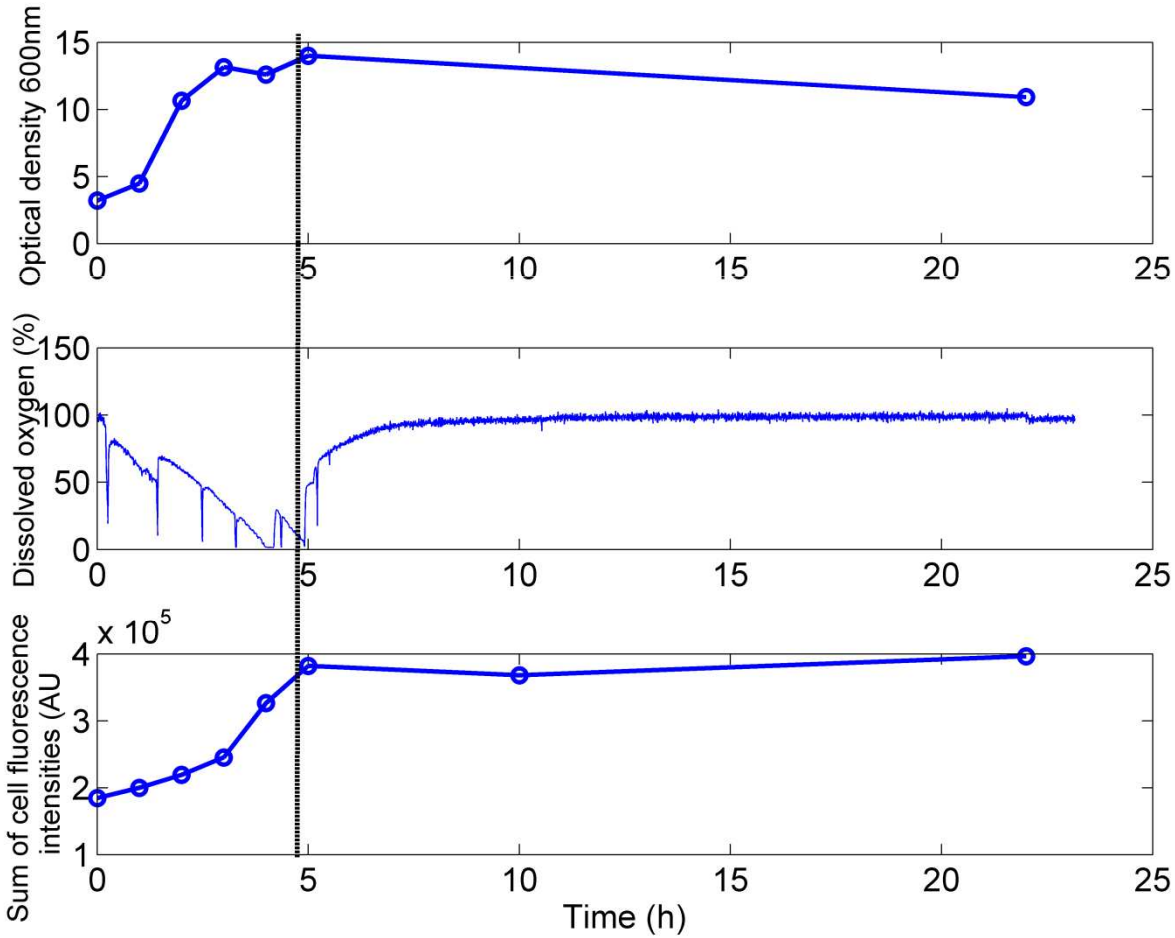
Results

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



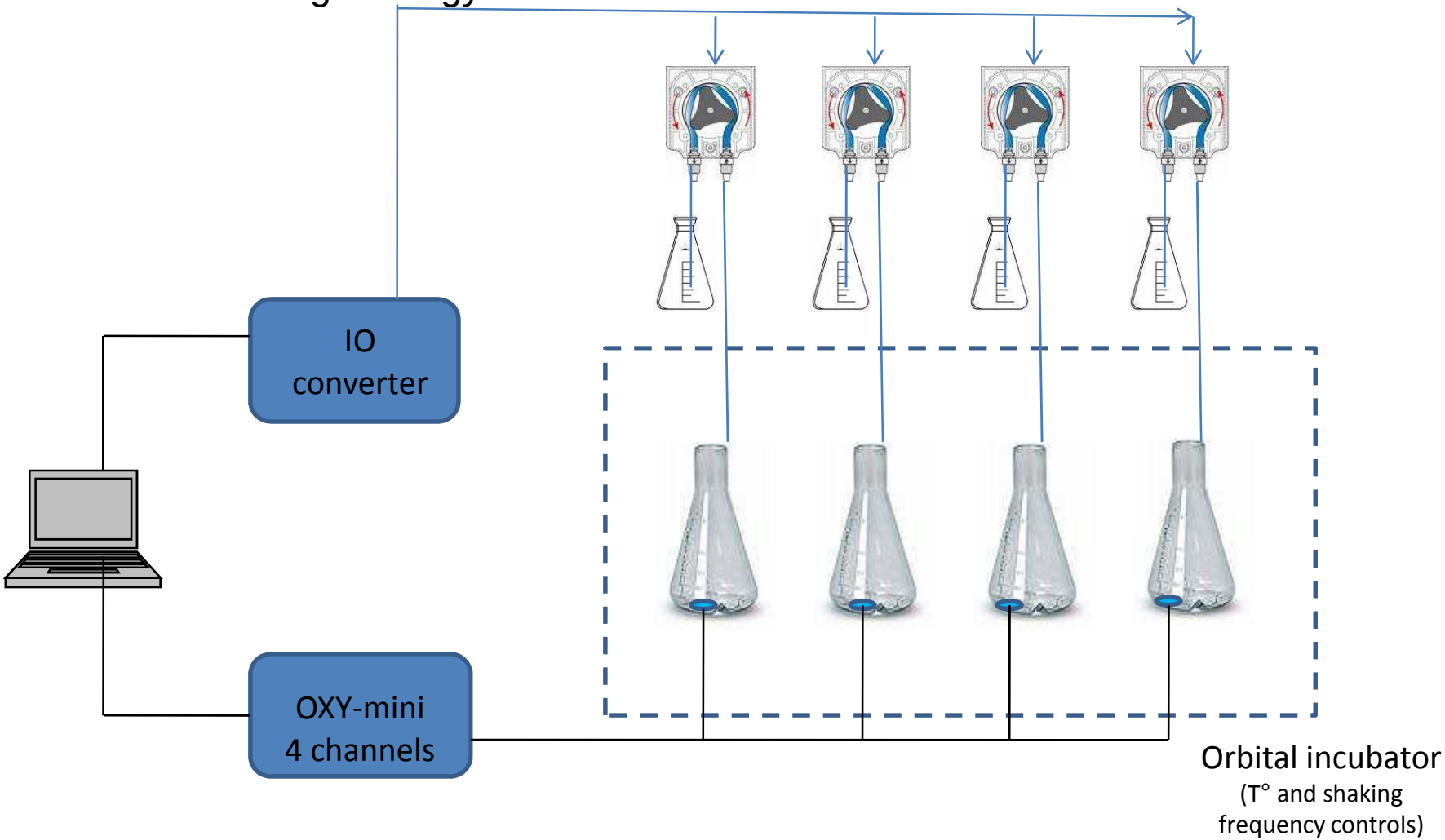


Results

Screening among an E. coli GFP clones library

Representativeness of shaken bioreactor

Intermittent feeding strategy

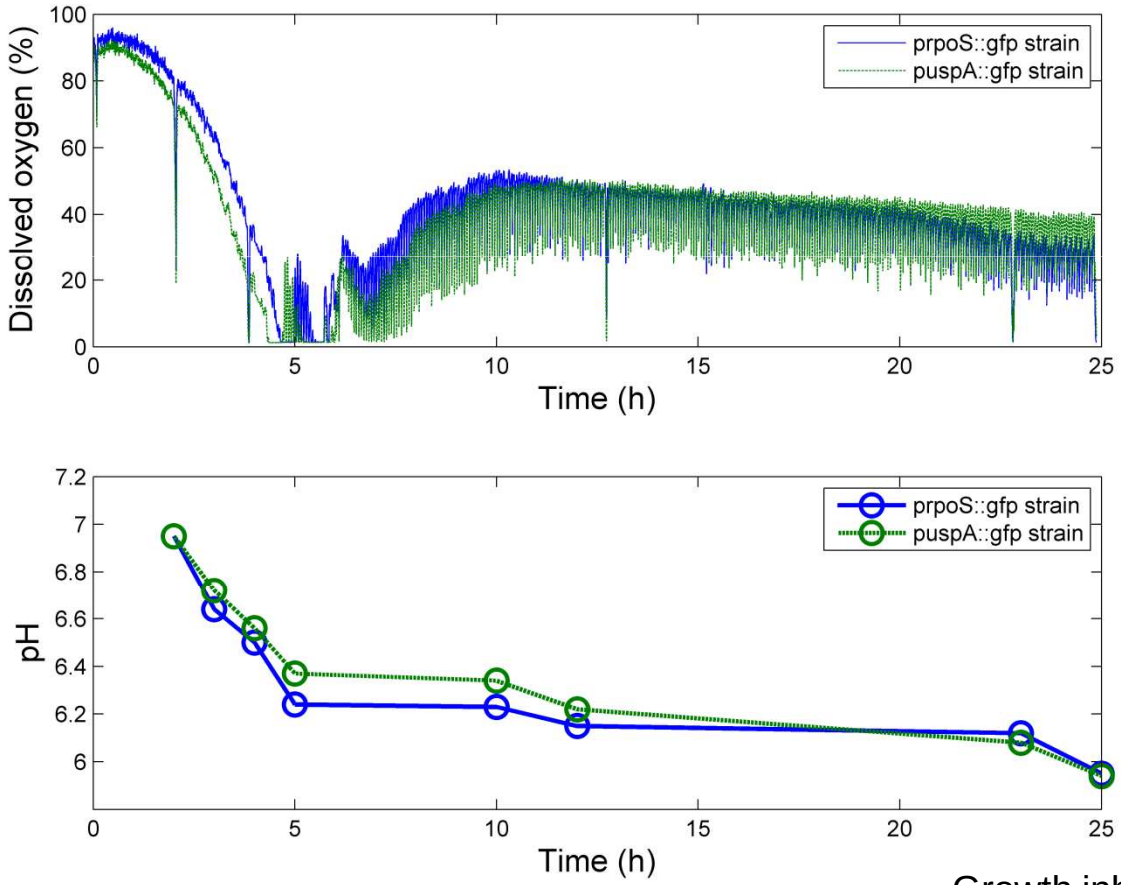


Results

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)

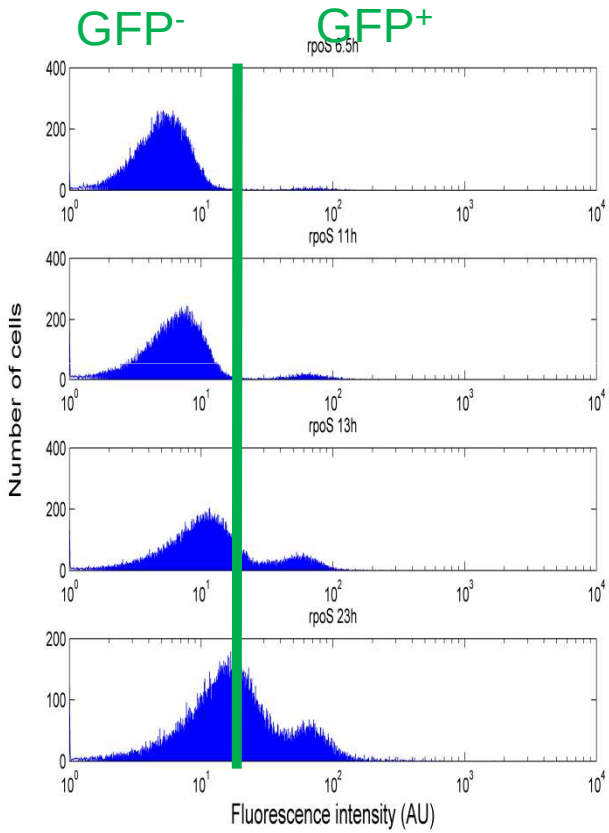


Growth inhibiting value : 4.5

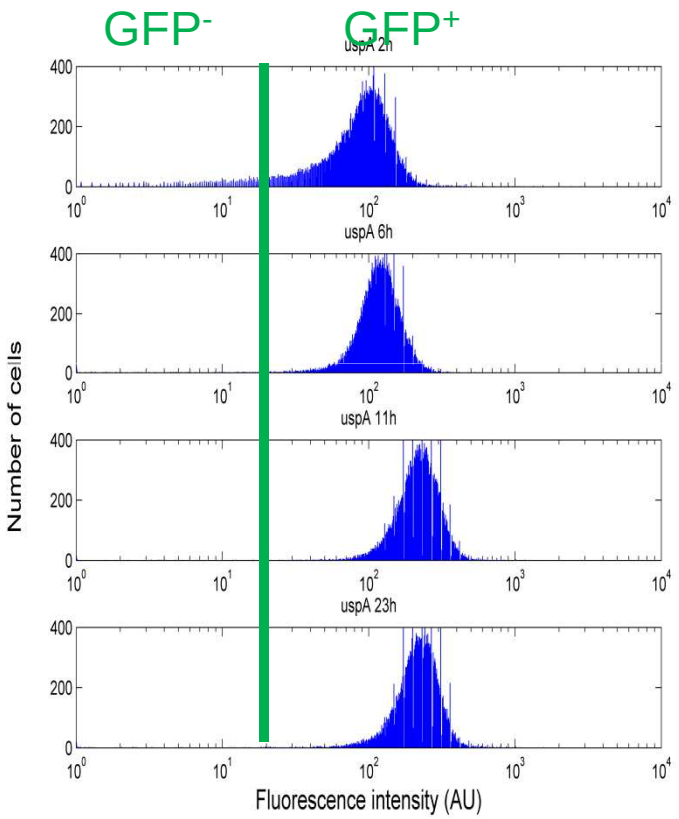
Results

Screening among an E. coli GFP clones library

Representativeness of shaken bioreactor



prpoS::gfp

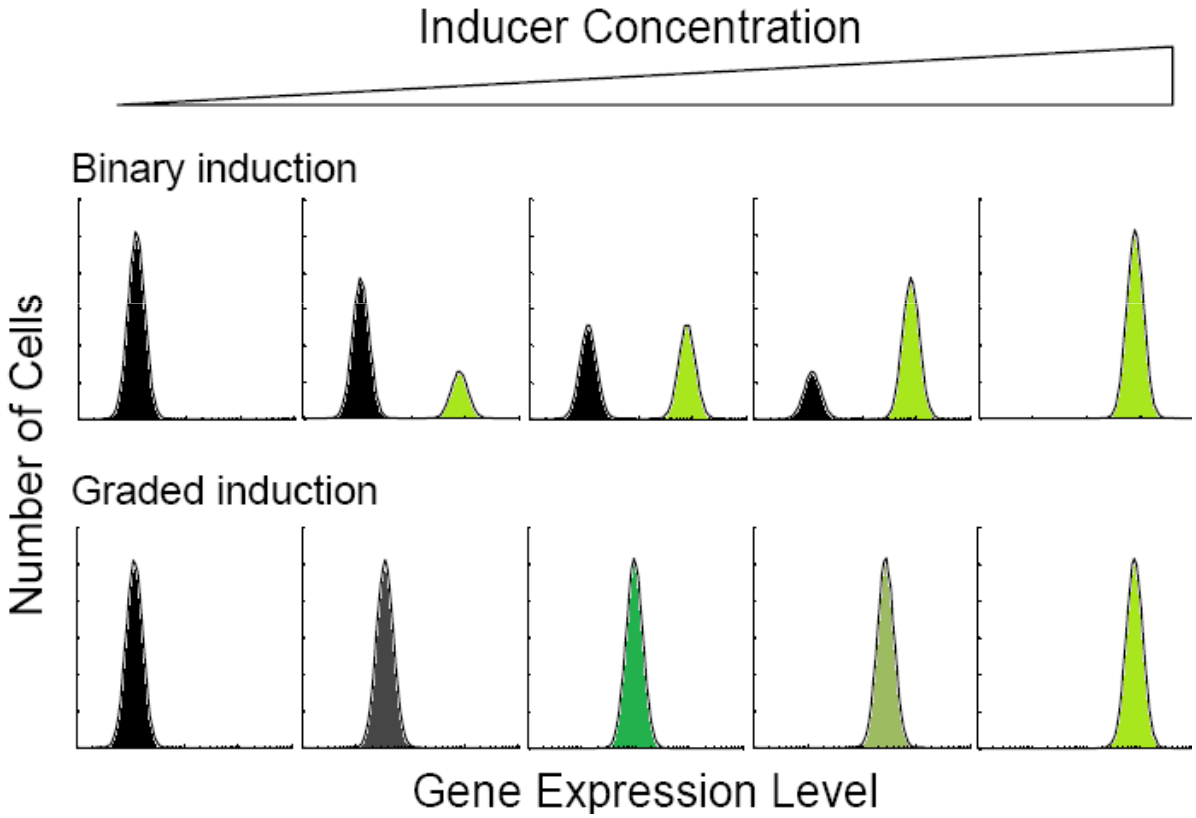


puspA::gfp

Results

Screening among an E. coli GFP clones library

Two modes of expression : binary or graded



→ rpoS

→ uspA
csiE
inaA
osmC
…

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

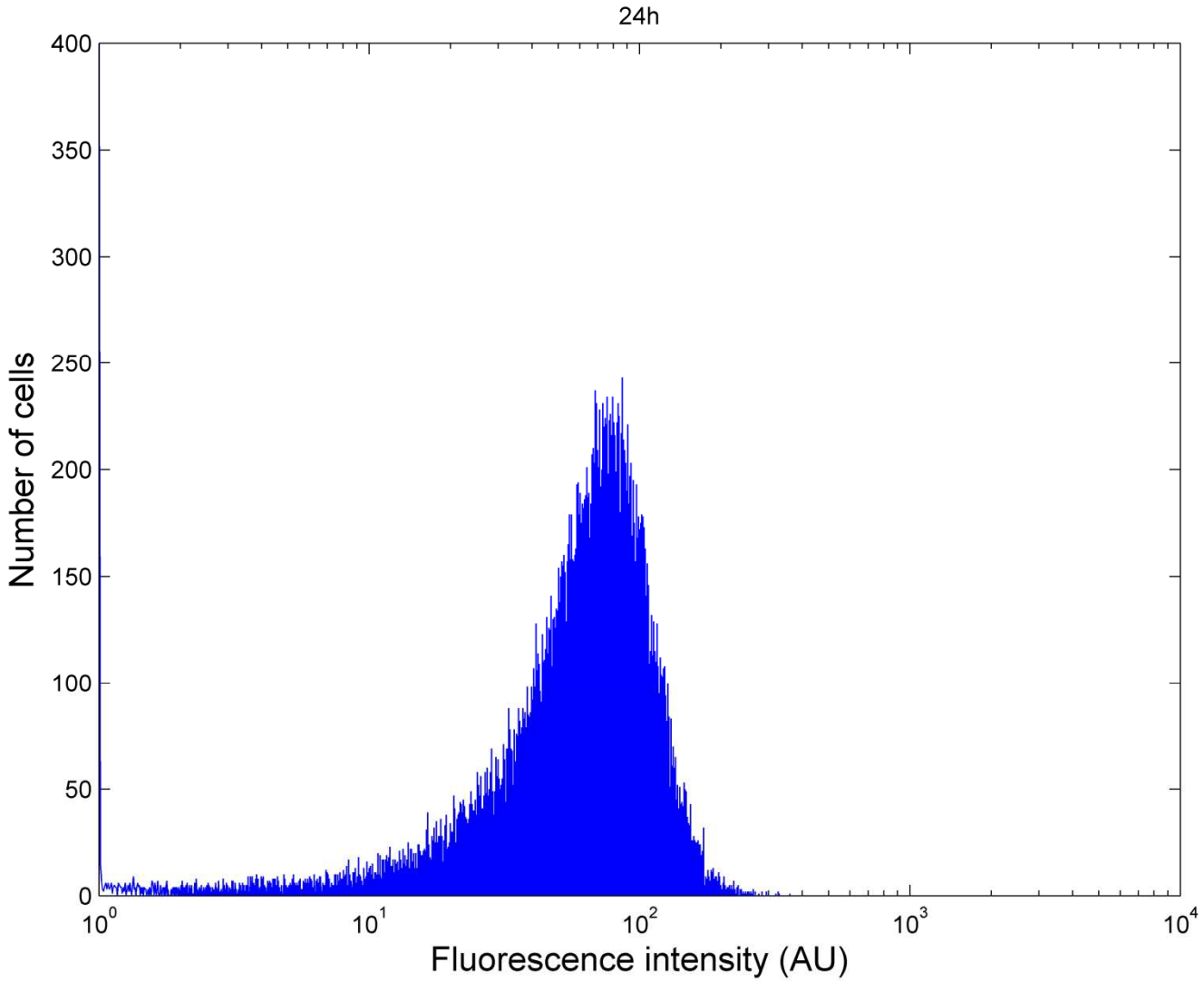
Generally not observed for GFP reporter system considering the high protein stability of this system compared with β-galactosidase and luciferase reporters

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

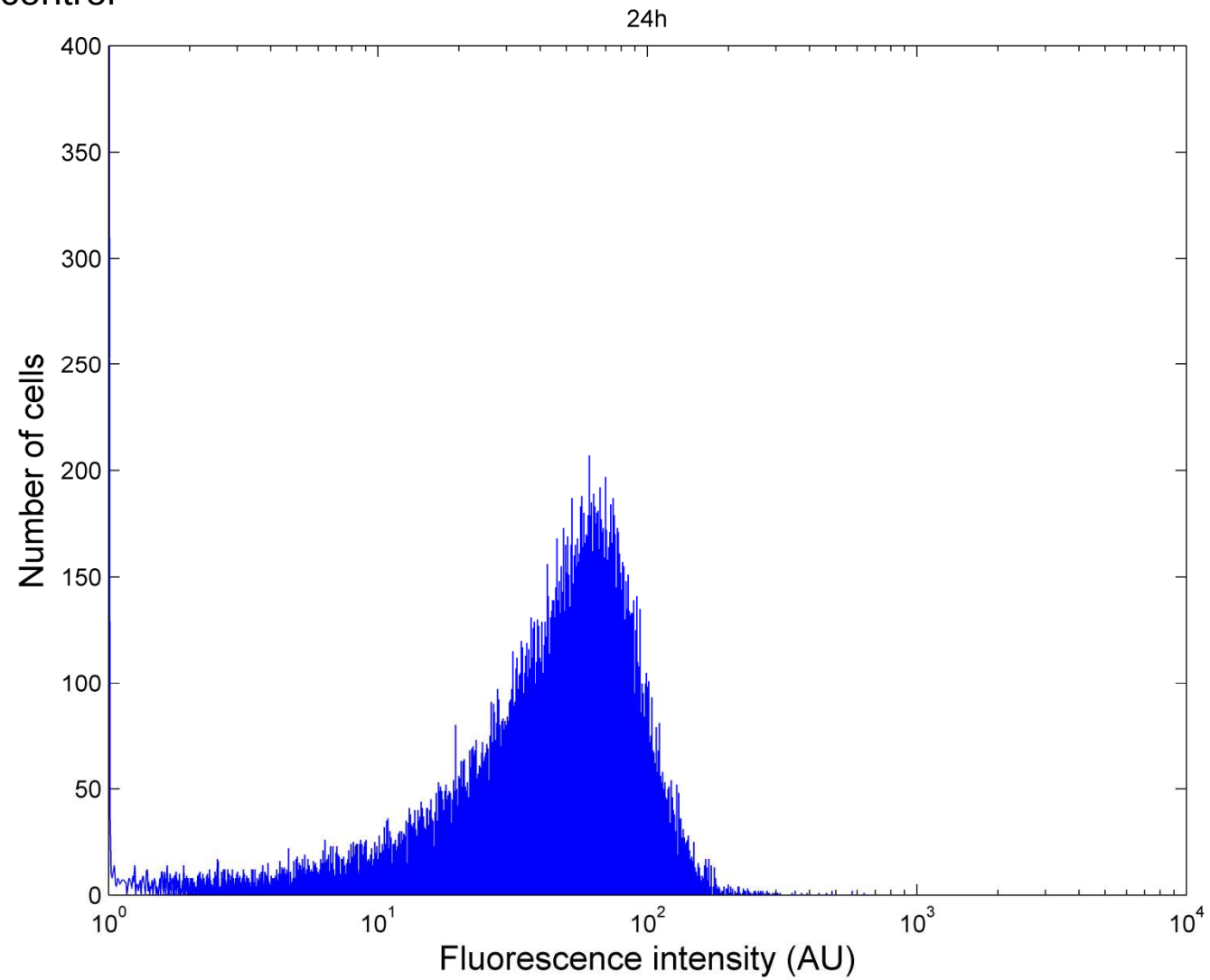
Regulation of the addition of glucose by the dissolved oxygen level (SP = 30%) PID control



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 :

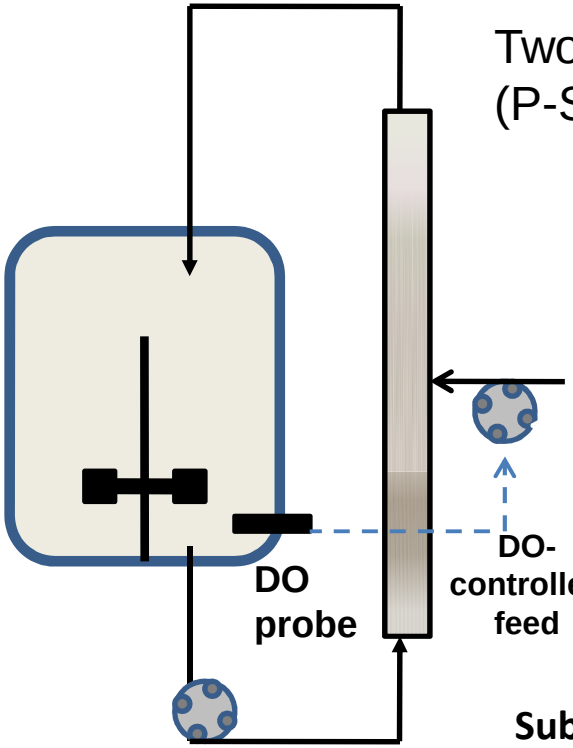
- Binary mode for GFP expression at the end of the batch phase and during the transition from batch to fed-batch phase

- 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

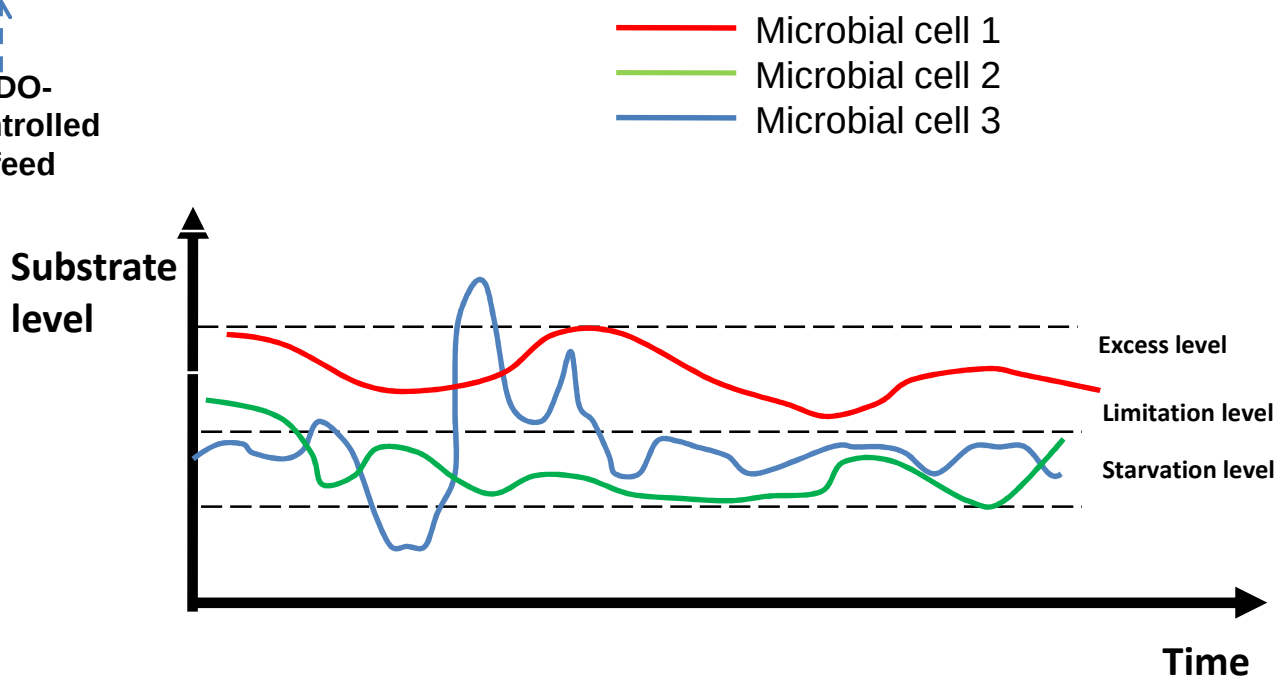
- Successive glucose excess tends to slow down the binary expression phase

Results

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



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

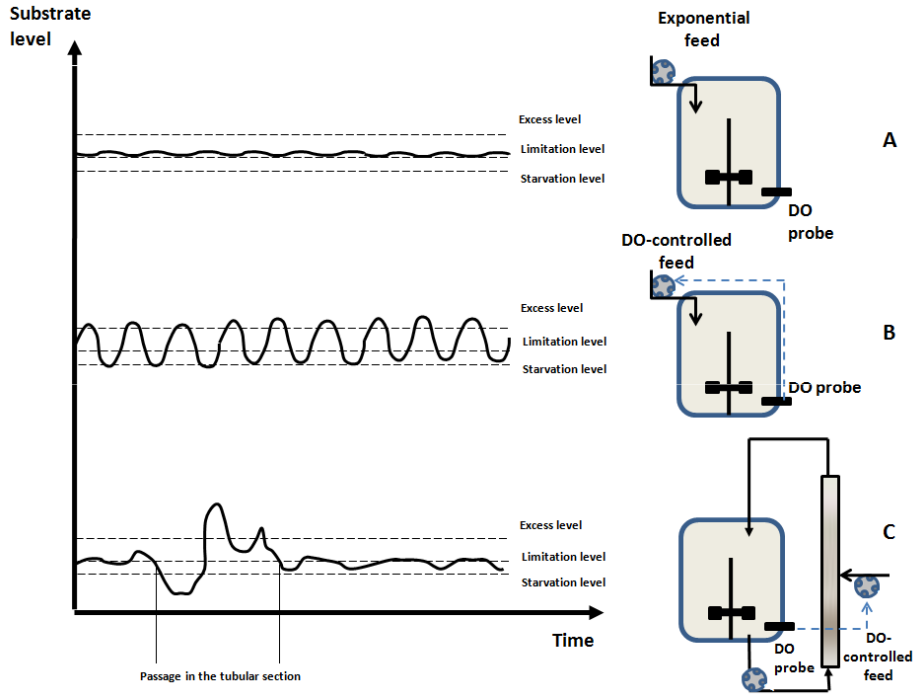


Results

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

Operating conditions :

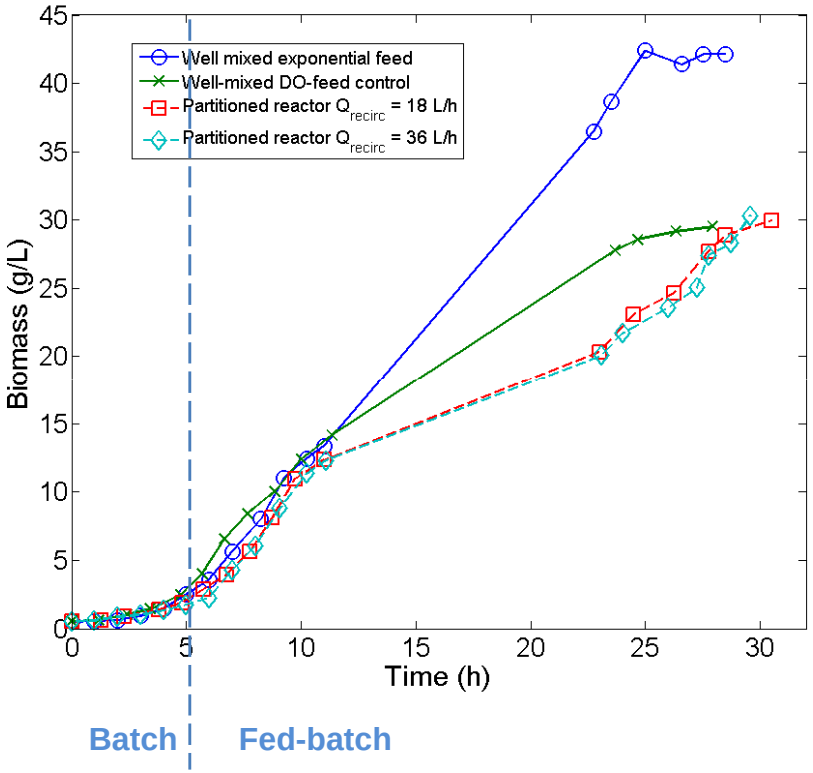
- Stirred bioreactor, working volume 10L
- Mineral medium, glucose as carbon source
- Fed-batch with exponential feed algorithm
- Scale-down approaches with DO-controlled fed-batch and partitioned reactor

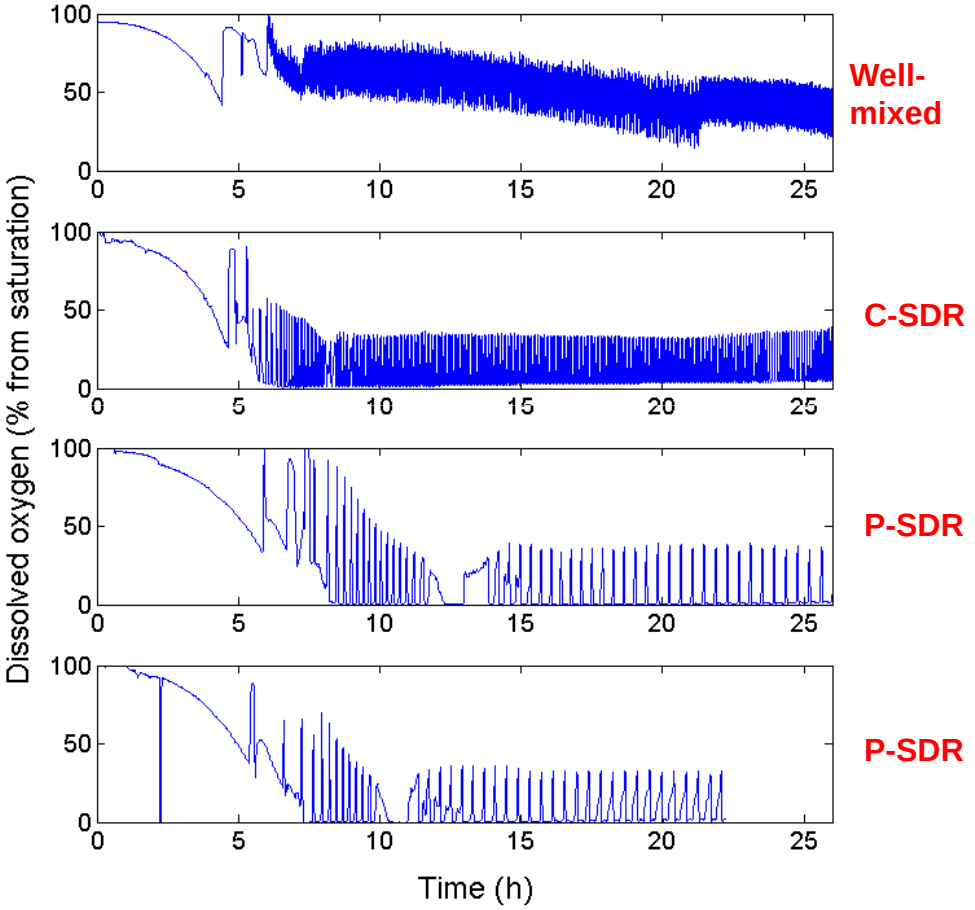


Delvigne F. *et al.* [2009] *Microbial cell factories* , **8**:15

Results

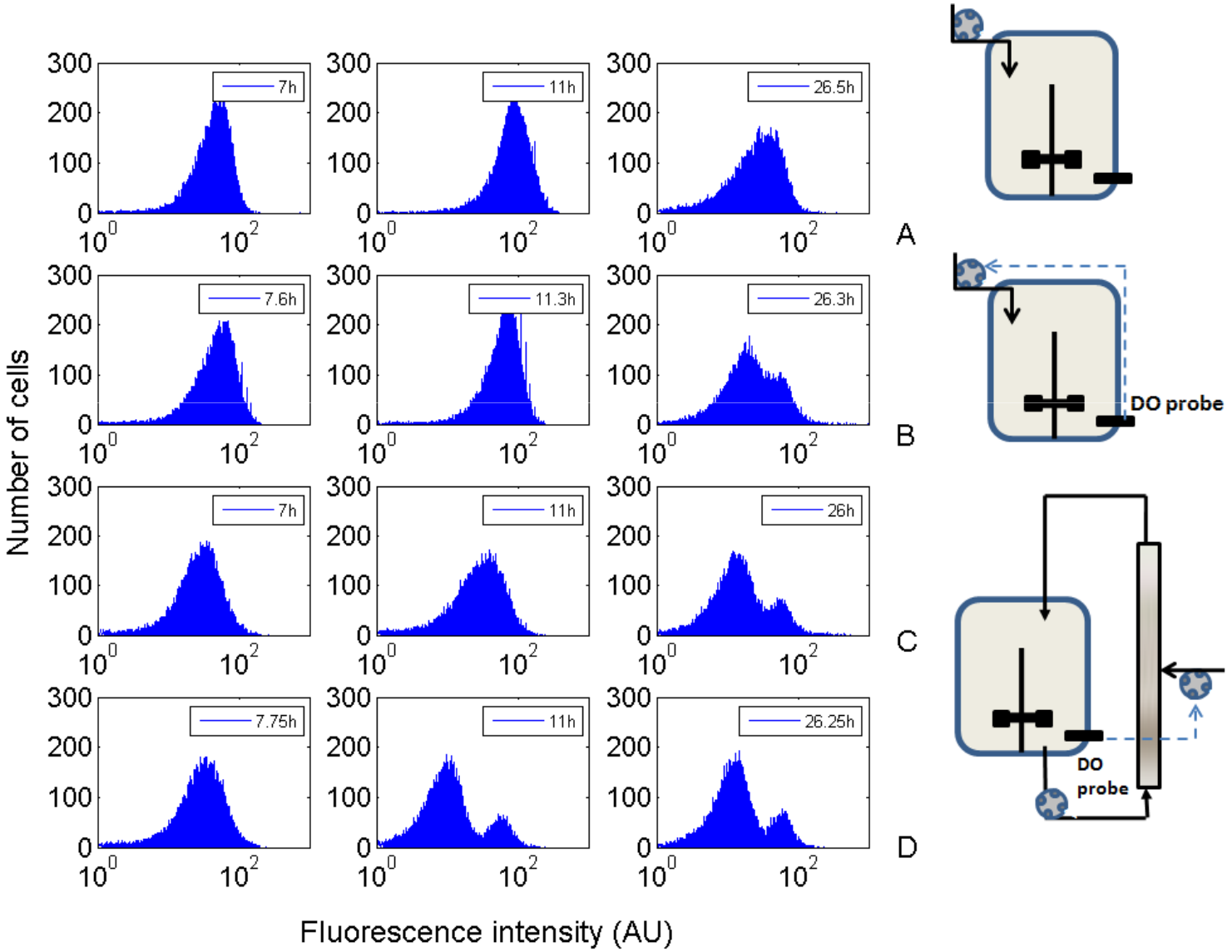
Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor





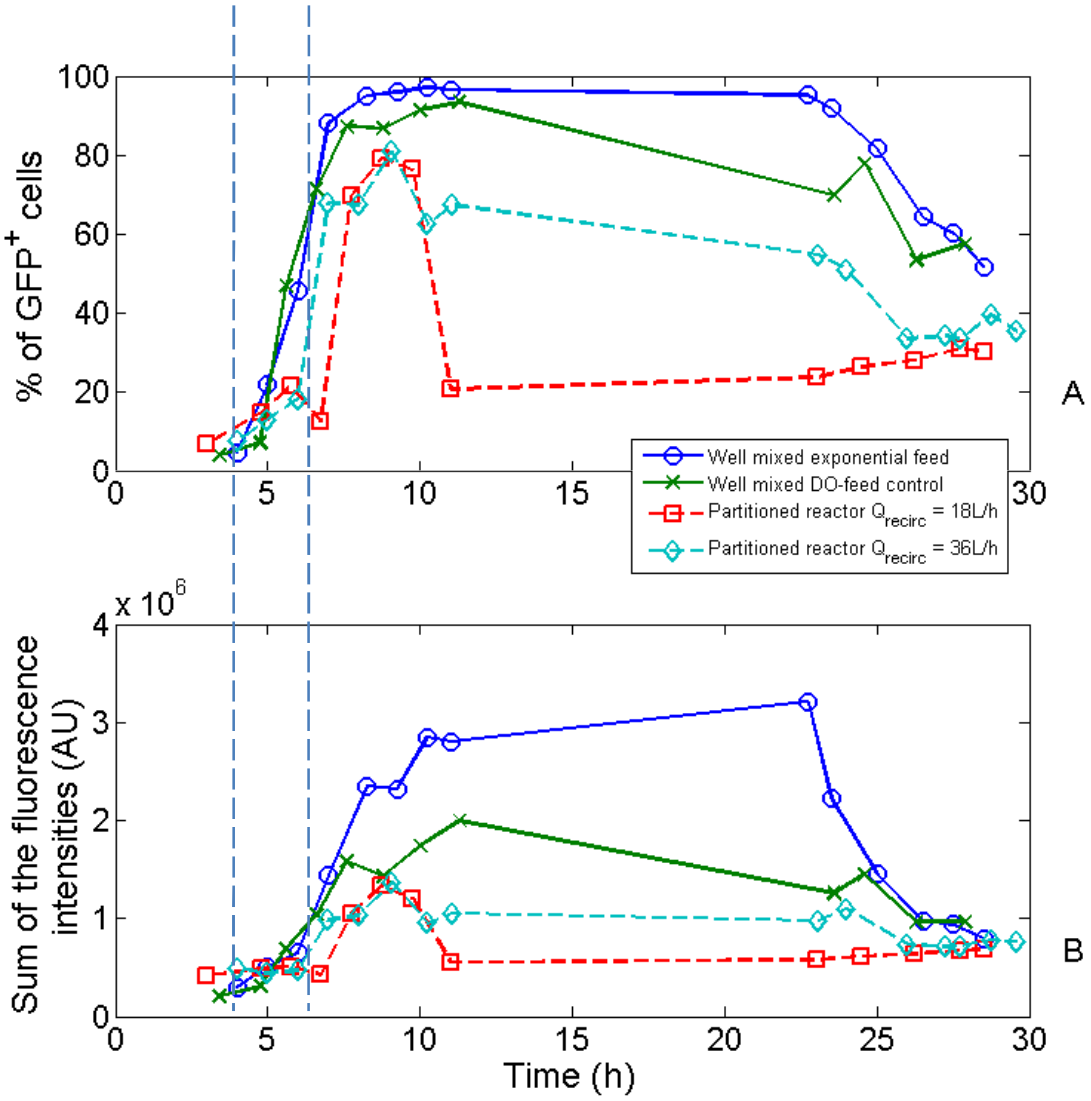
Results

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



Results

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



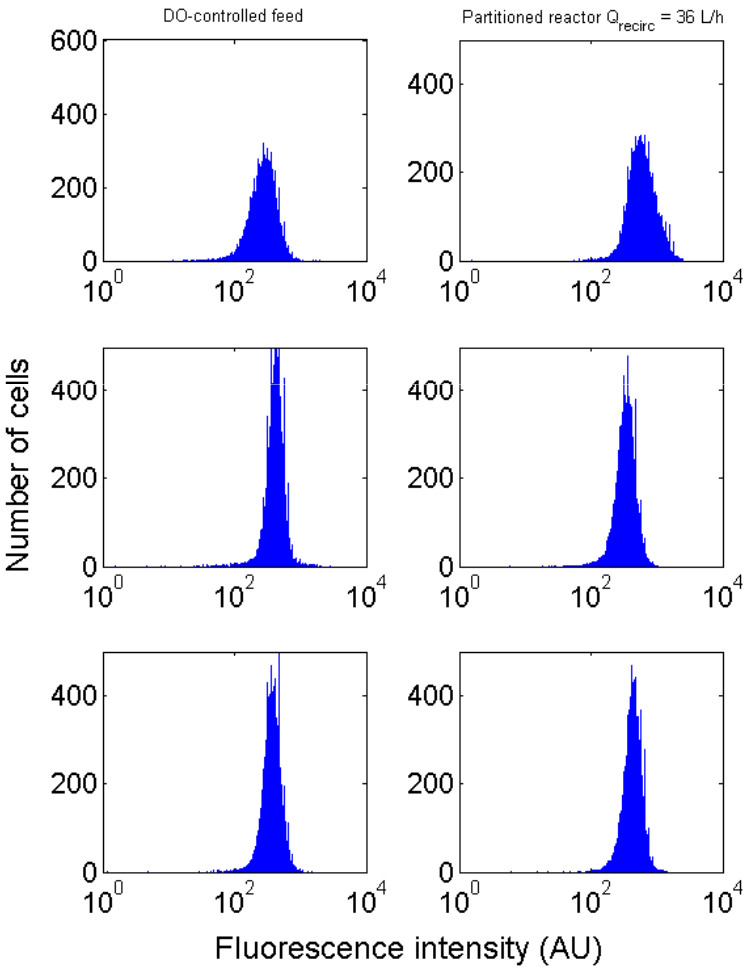
Global mixing efficiency

Global mixing efficiency

Results

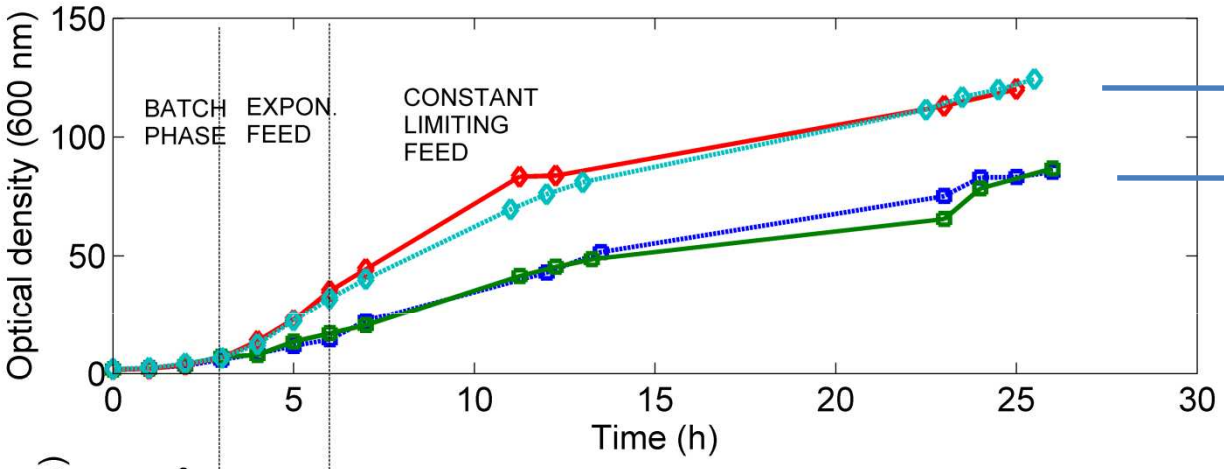
Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor

A pcya::GFPmut2 strain is not influenced by hydrodynamic conditions



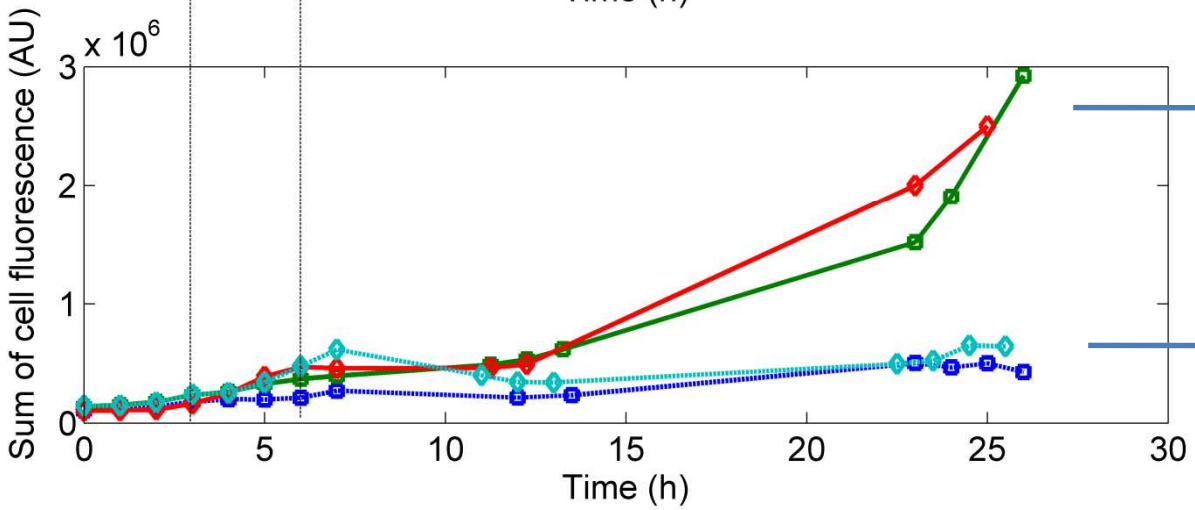
Results

Cultures performed under constant glucose feed



Constant feed at 10 g/h

Constant feed at 7 g/h

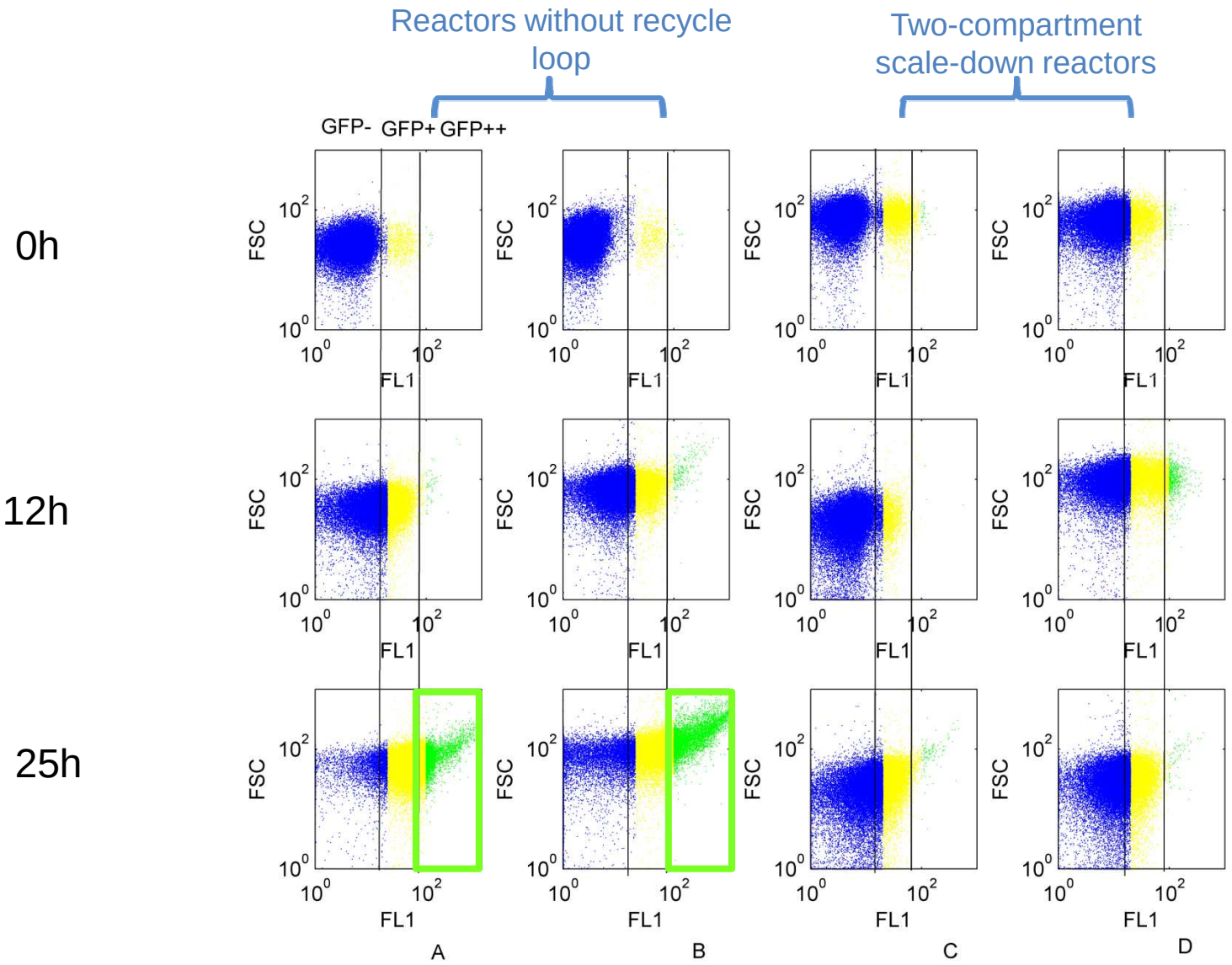


Classical bioreactors without recycle loop

Two-compatment scale-down reactors

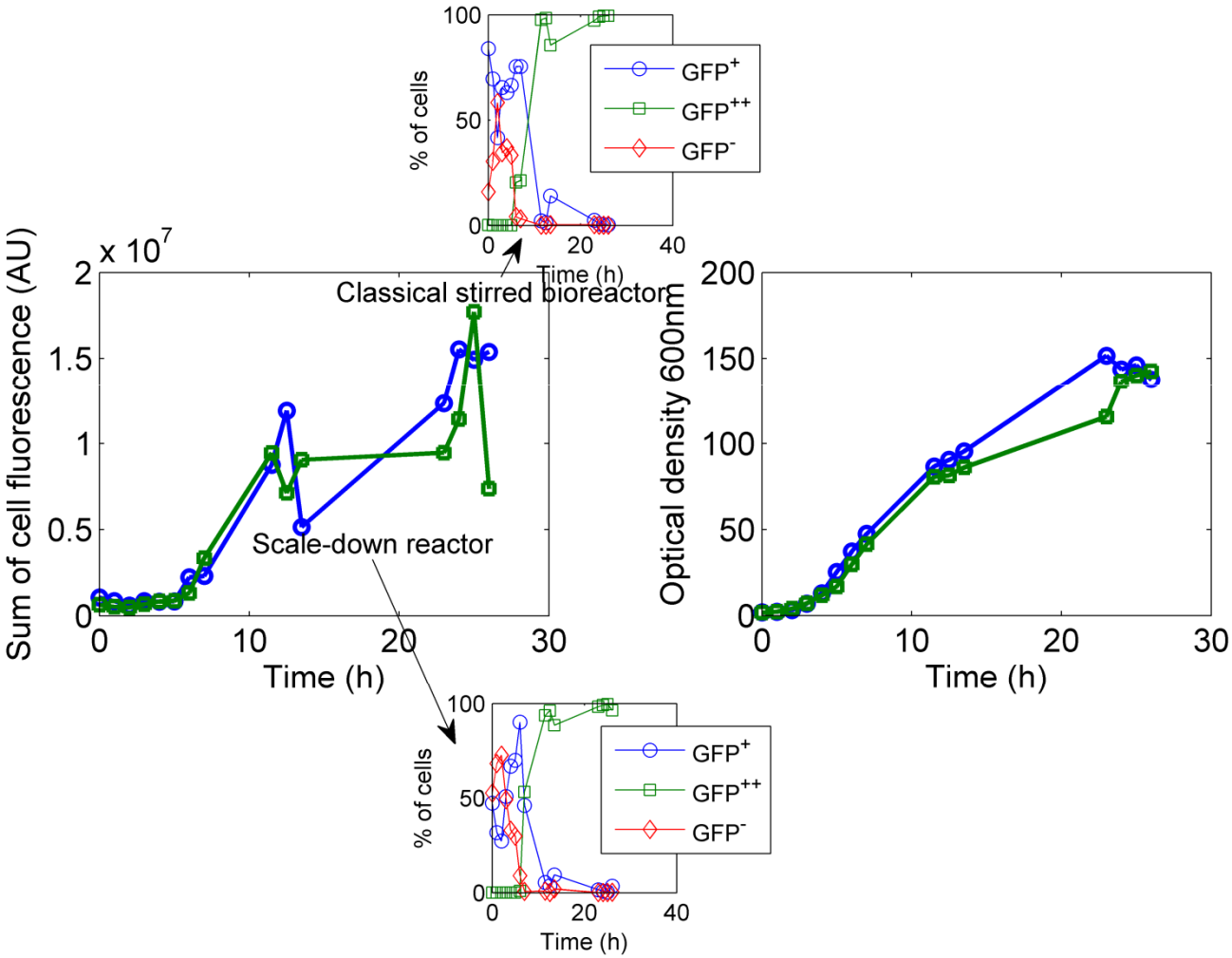
Results

Cultures performed under constant glucose feed



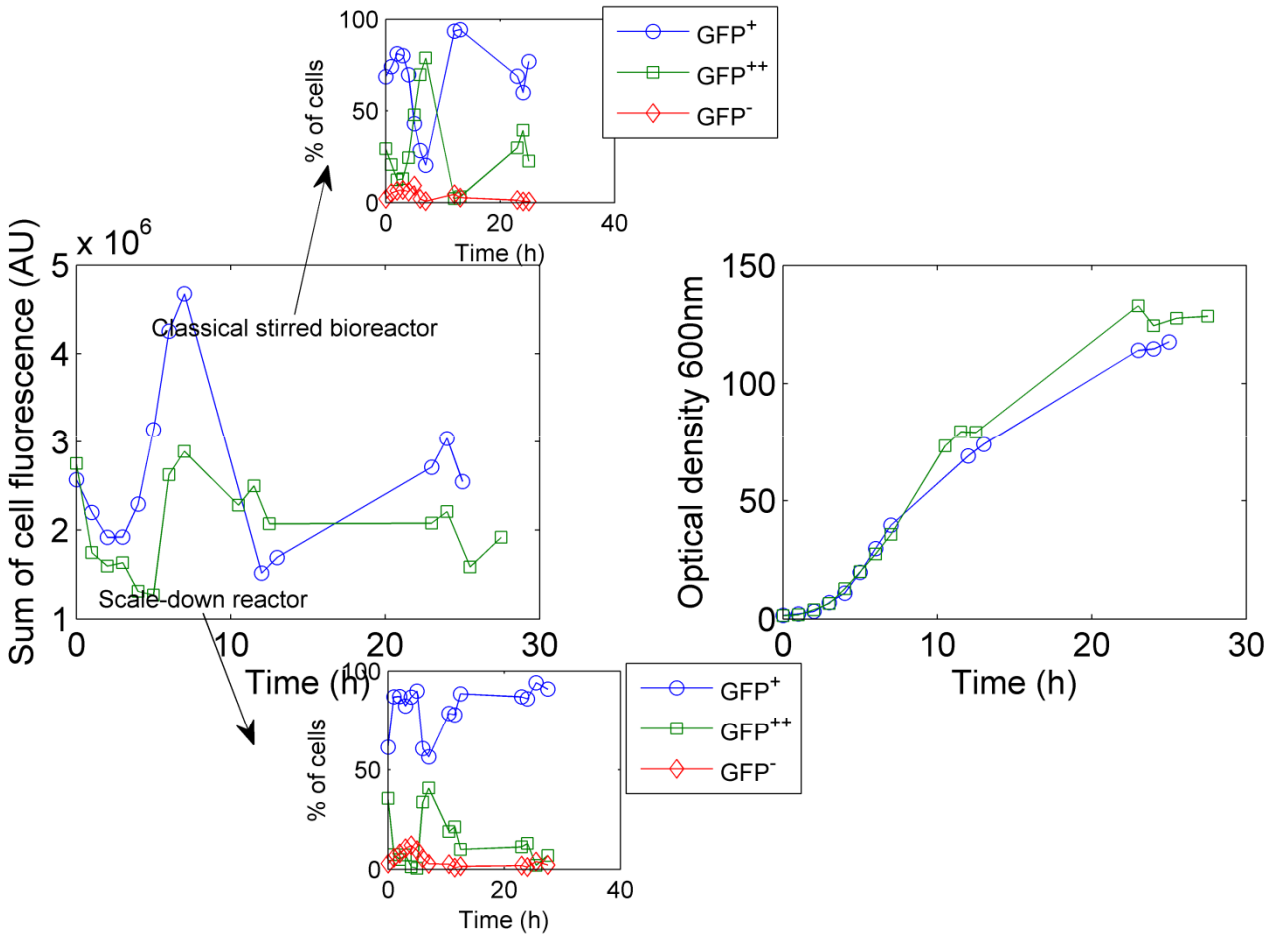
Results

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



Results

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



To be validated by using a DO-controlled feed

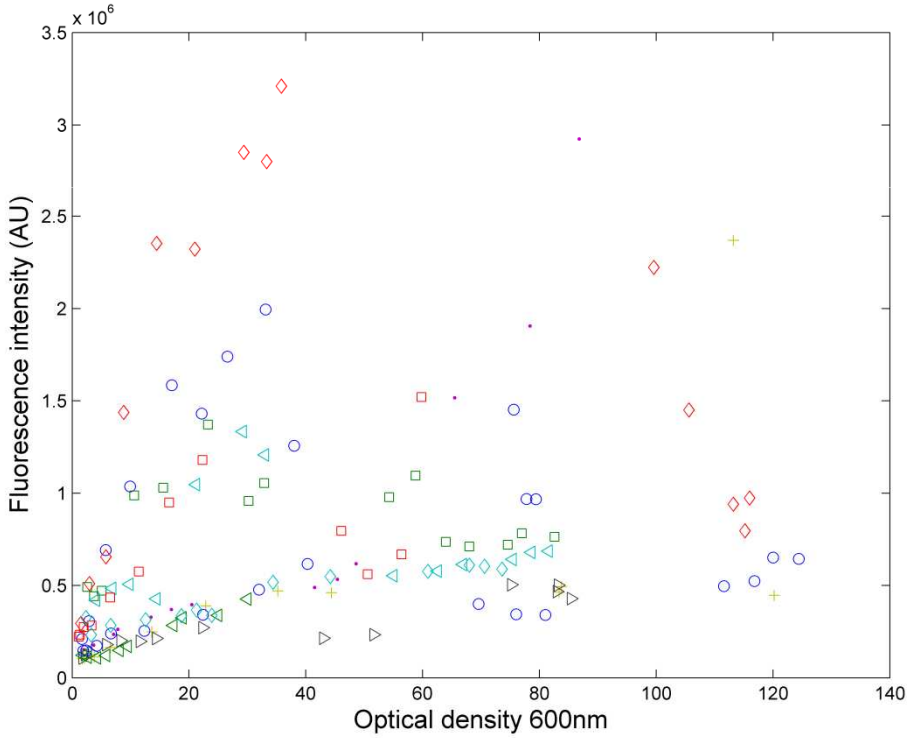
Prytz *et al* [2003] Biotech bioeng **83**:595-603

Results

Synopsis : relation between GFP expression level and cell density

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 :

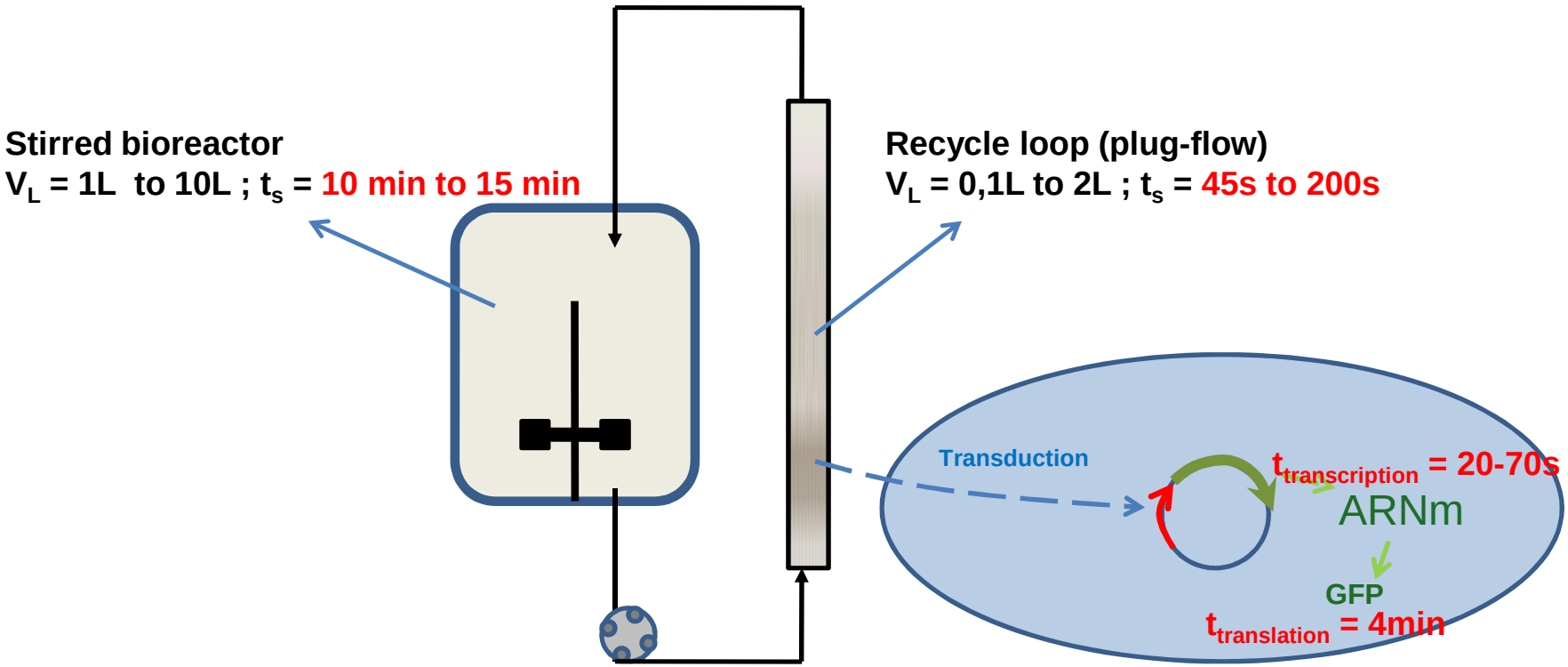
Homogenous reactor : GFP+

Inhomogenous reactor : GFP-

Perspectives and conclusion

Two questions have to be raised :

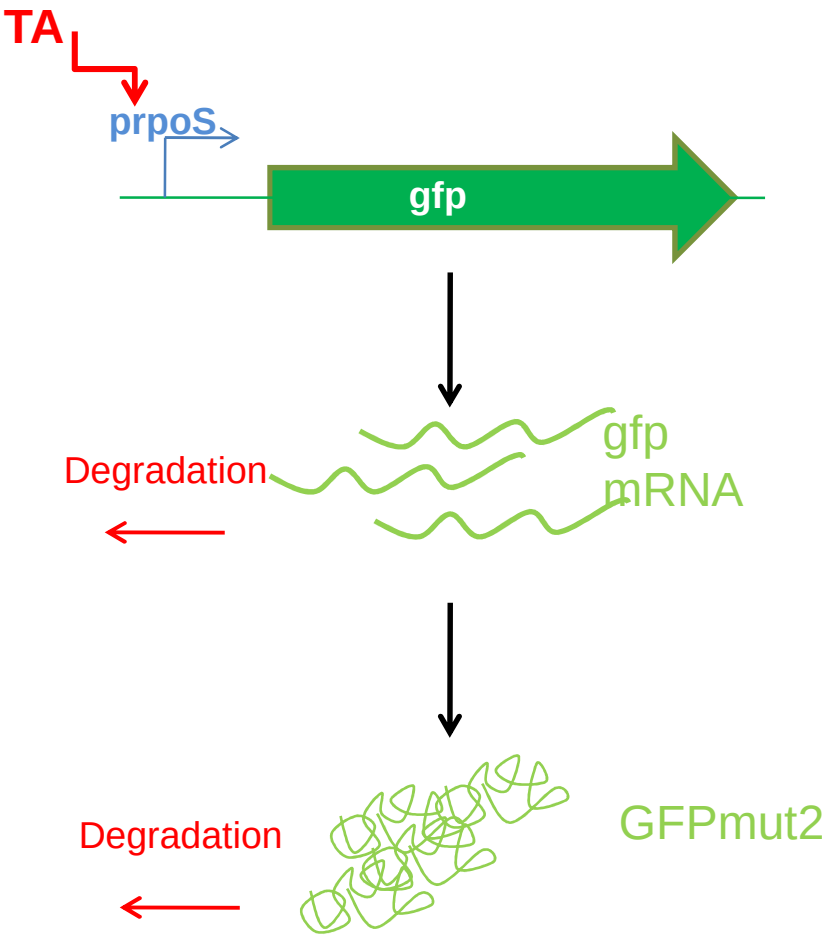
- Flow cytometry combined with P_{stress}::GFP expression → impact of extrinsic fluctuations
  What about the intrinsic fluctuations ?
- Characteristic times of hydrodynamic mechanisms compared with those of the biological processes behind GFP synthesis



Perspectives and conclusion

Complex phenomena :
- Two sources of noise (extrinsic and intrinsic)
- Very different characteristic time constants (physical and biological pocesses)

→ A model is required



$$\xrightarrow{k_1} TA$$

$$TA + DNA \xrightarrow{k_2} TA\_DNA$$

$$TA\_DNA \xrightarrow{k_3} TA + DNA$$

$$TA \xrightarrow{k_4} \emptyset$$

$$TA\_DNA \xrightarrow{k_5} TA\_DNA + RNA$$

$$RNA \xrightarrow{k_6} RNA + GFP$$

$$RNA \xrightarrow{k_7} \emptyset$$

$$GFP \xrightarrow{k_8} \emptyset$$

Perspectives and conclusion

Reaction scheme :

Exposure to glucose excess = f(t_m,t_c)

$$\xrightarrow{k_1} TA$$
$$TA + DNA \xrightarrow{k_2} TA\_DNA$$
$$TA\_DNA \xrightarrow{k_3} TA + DNA$$
$$TA \xrightarrow{k_4} \emptyset$$
$$TA\_DNA \xrightarrow{k_5} TA\_DNA + RNA$$
$$RNA \xrightarrow{k_6} RNA + GFP$$
$$RNA \xrightarrow{k_7} \emptyset$$
$$GFP \xrightarrow{k_8} \emptyset$$

Generation time : k8 = log(2)/t_g

ODEs system :

$$\frac{dTA}{dt} = k_1 - k_2.TA.DNA - k_4.TA + k_3.TA\_DNA$$
$$\frac{dTA\_DNA}{dt} = k_2.TA.DNA - k_5.TA\_DNA - k_3.TA\_DNA$$
$$\frac{dDNA}{dt} = k_3.TA\_DNA - k_2.TA.DNA$$
$$\frac{dRNA}{dt} = k_5.TA\_DNA - k_6.RNA - k_7.RNA$$
$$\frac{dGFP}{dt} = k_6.RNA - k_8.GFP$$

$$GFP_{steady-state} = RNA_{steady-state}.\left(\frac{k_6}{k_8}\right)$$

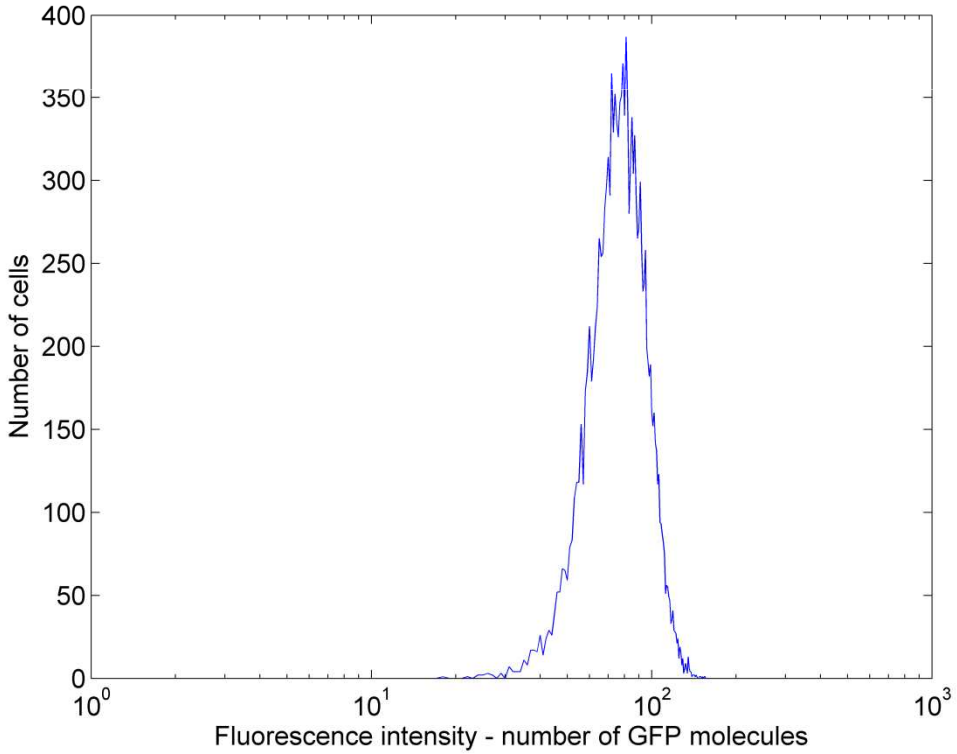
8 rates (including the characteristic time constants) to specify

Perspectives and conclusion

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

Probablity that reaction μ occurs at time τ (Gillespie algorithm)

Gillespie [1977] J. of physical chemistry, **81**:2340-2361



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

Thank you

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(postdoctoral researcher grant n°FC 65530, CGRI-FNRS grant « Tournesol »)

Special thanks to Nathalie Gorret, Stéphane Guillouet et Carole Jouve (LISBP, INSA Toulouse)