# Analyse des couplages entre mélange, transfert et réaction dans les bioréacteurs

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**Equipe : Transferts Interfaces Mélange**

**Journée : Utilisation de la CFD en génie des procédés. Gestion de la complexité des systèmes**

25 novembre 2014

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IMFT INSTITUT DE MECANIQUE DES FLUIDES DE TOULOUSE

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# Industrial Bioreactors

Large scale system : aerated, stirred, biological transformation

Industrial production of

Pharmaceuticals

Energy (Bio fuels)

Water

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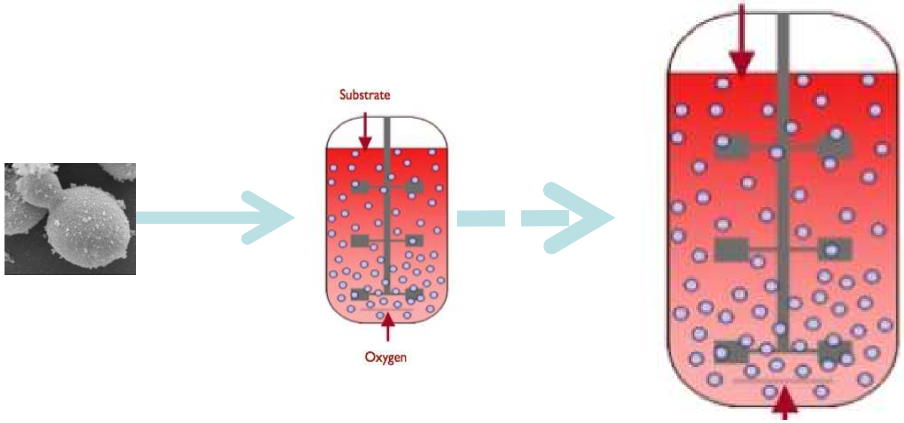
# Modeling of Bioreactors




Cell factories d≃µm | Laboratory scale d≃0.1 m | Industrial scale d≃10 m

Strongly coupled phenomena
Large range of scales
Macro [1-5] and micro[6-8] mixing issues

Industrial challenges:

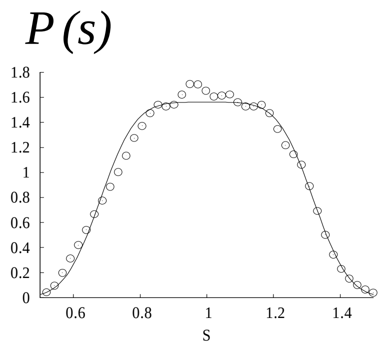
- Design
- Performances

Academic challenges:

- Hydrodynamics
- Multiphase
- Mixing
- Mass transfer
- Heat transfer
- Bioreaction
- *Adaptation* of microorganisms

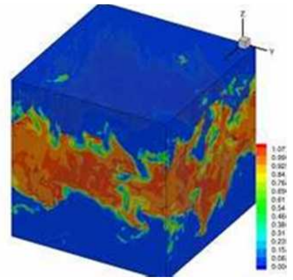

[1] Larsson et al., 1996; [2] Bylund et al., 1998; [3] Enfors et al., 2001[4] Senn et al., 1994; [5] Lin and Neubauer, 2000
[6] Dunlop and Ye, 1990; [7] Al-Homoud and Hondzo, 2008; [8] Garcia et al., 2009

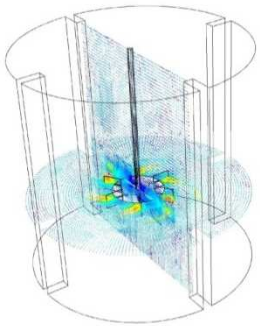
# CFD Tools

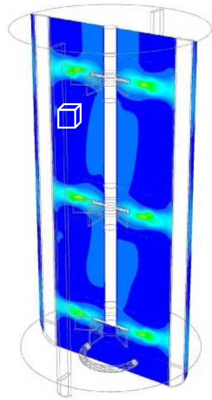



Concentration distribution

DNS Numerical experiments

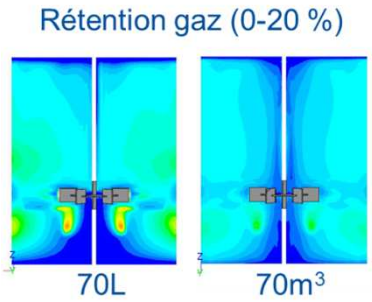




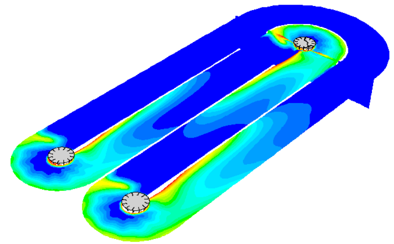


LES, RANS
Experimental validation
PIV
PIV-HF (IMFT, Fermat)

Industrial cases

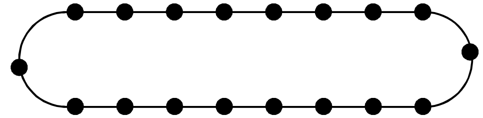



Eulerian
Multiphase
Scale-up




1D Model

*C(z,t)*



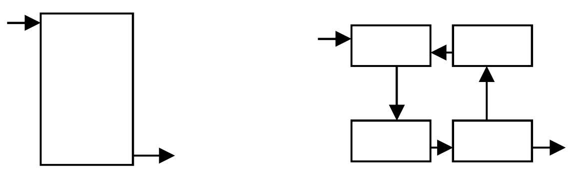
$$\frac{\partial C}{\partial t} + U\frac{\partial C}{\partial Z} - \frac{1}{Pe}\frac{\partial^2 C}{\partial Z^2} = R(C)$$

# Limitations of existing approaches

## Chemical Reactor Engineering

Ideal reactor [9] (CSTR, PFR)

Compartment Model [10]



## Biological reaction model

Kinetic (unstructured, structured)

Metabolic

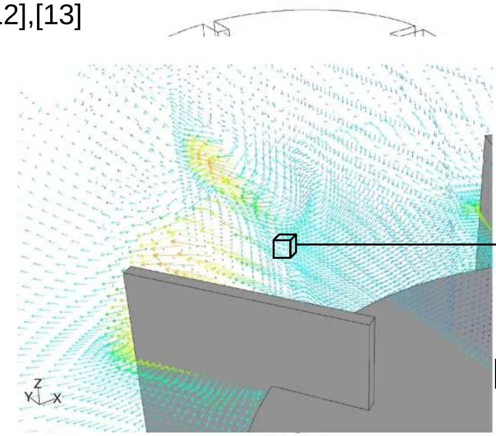
Uptake rate $\Phi(S)$ $g_S g_X^{-1} h^{-1}$

## Computational Fluid Mechanics

RANS [11],[12],[13]

LES [12]

DNS[14], [15]



[12]

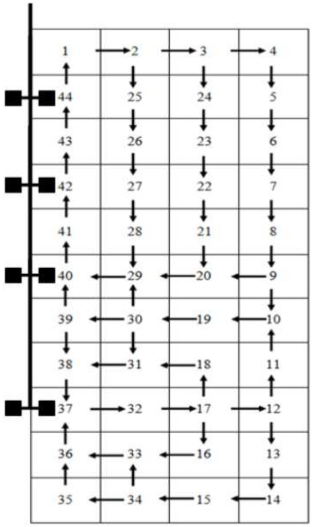
## Coupling

Conservation equation for the substrate over a *volume of control*

$$\frac{\partial S}{\partial t} + U\nabla S - \nabla(D\nabla S) = -\Phi(S)X$$

[9] Villermaux, 1995; [10] Delvigne et al., 2005; [11] Schmalzriedt et al., 2003; *[12] Delafosse et al., 2008*; [13] Lapin et al., 2006. ; *[14] Douaire 2010; [15] Linkès 2012*

# Should work but …

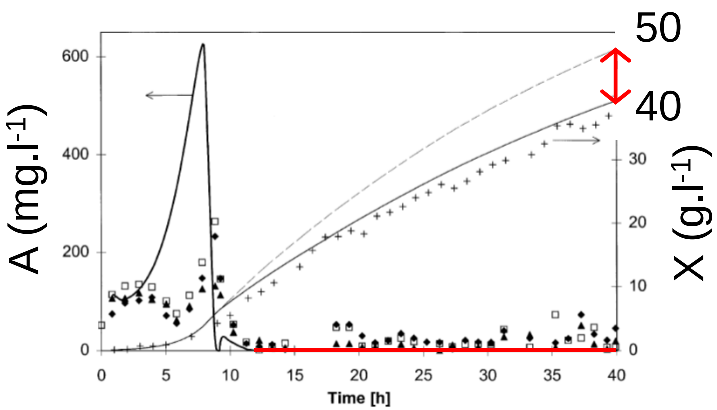


Compartment Model Approach (Vràbel et al. 2001) + Metabolic model *E. coli* (Xu et al. 1999)

Based on a known flow map $Q_{i,j}$

Cell concentration over predicted
By product formation underpredicted

Scale dependent Yield constants
$Y_{\text{large scale}} = Y_{\text{lab scale}} * \mathbf{0.75}$

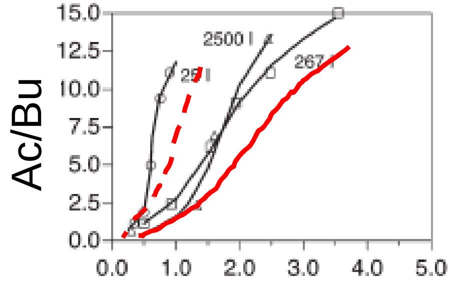



3D - Two phases CFD RANS (Schmaldzriedt et al. 2003)
+ Kinetic Model *B. subtilis* (Moes et al. 1985, Griot 1987)

Increasing the power input favours Acetoïn production
→ Sensitivity to mixing/transfer

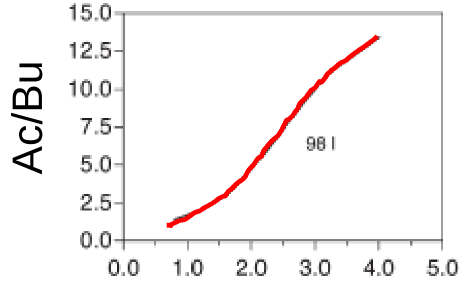
The simulated ratio is out of the expected range
→ Kinetic model is scale dependent …

experiments




power input (W/kg)

simulation




power input (W/kg)

# Restart Analysis

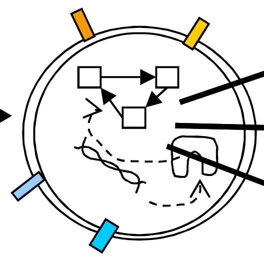
Through scale-up

$\tau_{bio} \approx \tau_{\varphi} \nearrow$ ⟶ Competition

Mixing/Transfer/Bioreaction

Time scale estimation ?

$\Phi_p$ ⟶



Anabolism

Catabolism

Overflow

Multiple parallel pathways

Regulatory mechanisms

Dynamic system

***Adaptation*** to concentration fluctuations experienced

# Characteristic time scales of mixing

Table 1: Time (in seconds) and length scales of mixing in two bioreactors (lab-scale 3L, industrial scale 20 $m^3$) equipped with a Rushton turbine. Average power input $\epsilon_V = 1.3\,kW.m^{-3}$ in both cases. $t_C$ : macromixing time, $t_S$ mesomixing time, $t_E$ : micromixing time due to engulfment, $t_Ds$ micromixing time due to diffusion, $\Lambda$ : Taylor Macro scale, $eta_k$ : Kolmogorov scale, $\eta_B$ : Batchelor scale

| Mecanisms | Time scale | Length scale | Labscale | Industrial (1/0.05) |
|---|---|---|---|---|
| Macromixing | $t_C = \frac{V}{1.8\,N_{Q_P}\,N\,D^3}$ | $V^{1/3}$ | 0.8 | 13 |
| Mesomixing | $t_S = 2\left(\frac{\Lambda^2}{\epsilon}\right)^{\frac{1}{3}}$ | $\Lambda = w\ \Lambda = \frac{1}{2}\frac{k^{3/2}}{\epsilon}$ | 0.03 | 0.2/0.55 |
| Micromixing (Engulfment) | $t_E = 17\left(\frac{\nu}{\epsilon}\right)^{\frac{1}{2}}$ | $\eta_K = \left(\frac{\nu^3}{\epsilon}\right)^{\frac{1}{4}}$ | 0.015 | 0.015/0.07 |
| Micromixing (Diffusion) | $t_{Ds} = 2\left(\frac{\nu}{\epsilon}\right)^{\frac{1}{2}} \text{arcsinh}(0.05Sc)$ | $\eta_B = \left(\frac{\nu\,D_m^2}{\epsilon}\right)^{\frac{1}{4}}$ | 0.008 | 0.008/0.0026 |

Macro and meso mixing times increase with reactor size


A. Delafosse, PhD, 2008
B. M. Linkès, M. Martins Afonso, P. Fede, J. Morchain, P. Schmitz, Numerical study of substrate assimilation by a microorganism exposed to fluctuating concentration, Chemical Engineering Science. 81 (2012) 8–19.

# Characteristic time scales of bioreactions

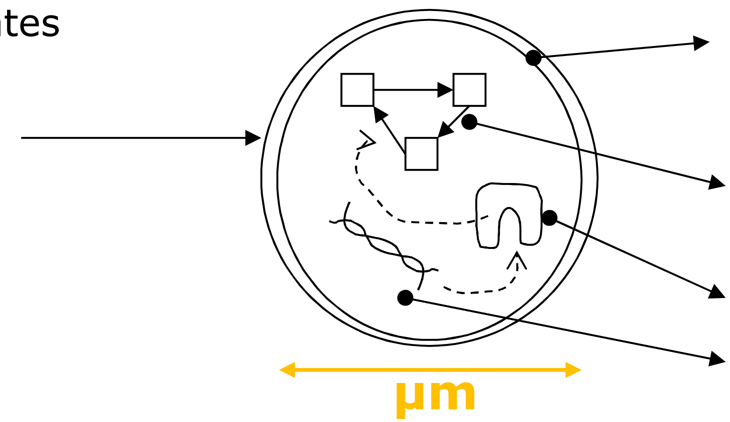
$$\frac{dC}{dt} = \frac{C}{\tau_C}$$

Substrates

C

N

$O_2$




$CO_2$

Products : Excreted Metabolites *(1 à 100 s)*

Intracellular Metabolites($\rightarrow$X, 1000 s)

Enzymes

Genes

Cell $$\frac{dX}{dt} = \mu_{max}\frac{S}{K_S+S}X\,,$$

Substrate $$\frac{dS}{dt} = -q_{max}\frac{S}{K_S+S}X.$$

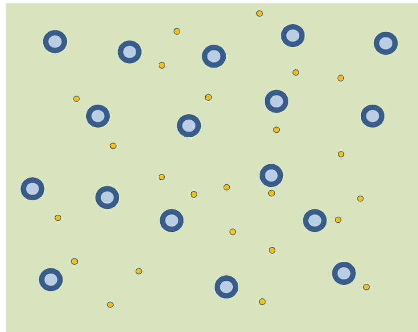
$$\tau_X = \frac{K_S+S}{\mu_{max}S}\,,$$ Growth: 1000 s

$$\tau_S = \frac{K_S+S}{\mu_{max}X} \approx \frac{S}{\mu_{max}X}$$ Uptake : 1-10 s

Growth and Uptake obey to different time scales
Uptake characteristic time decresase as the cell concentration increases

# Mixing times and substrate uptake time

$$\tau_S = \frac{K_S + S}{\Phi_{max} X} \qquad X(t) \nearrow$$



$$\mathbf{C_k} \equiv \{\mathbf{c_k}(\mathbf{x},t)\}_k$$

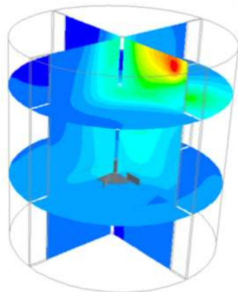
$$\varepsilon_\mathbf{k} \equiv \langle \alpha_\mathbf{k}(\mathbf{x},t) \rangle_V$$

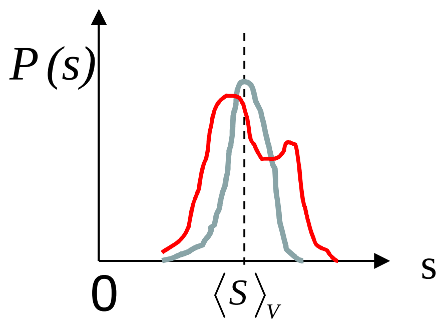
Macromixing

$\tau_M / \tau_S$

Homogeneous Reactor (Perfectly mixed)

Macroscopic gradients



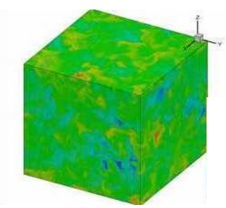


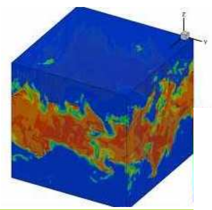

Mesomixing

Local concentration distribution

$\tau_m \ll \tau_S$

Non premixed reactants
near the feed point





# Specificity of biological systems : Two-way coupling

**Direct coupling**

Velocity field/ Mixing
Local gaz hold up
Bio-Reactions =f(cell.state,t) → Concentration fields

**Inverse coupling**

Is the cell at equilibrium with the liquid phase ?

Is the « average cell » approach sufficient ?

Is the concentration homogeneous within the volume of control ?

# Specificity of biological systems : Two-way coupling

**Direct coupling**

Velocity field/ Mixing
Local gaz hold up
Bio-Reactions =f(cell.state,t)

→ Concentration fields

**Inverse coupling**

3D hydrodynamics and transfer : Multiphase CFD

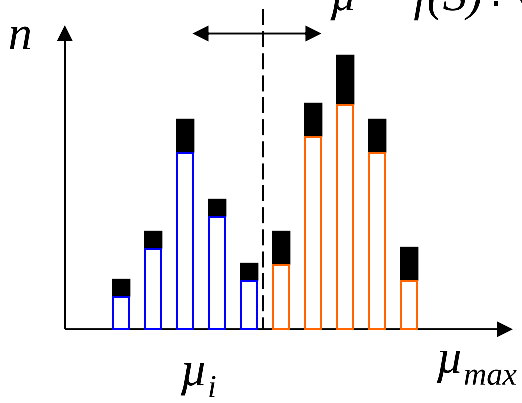
Equilibrium : Local reaction rates =f(local concentrations, *cell state*)

Describe and couple physical and biological heterogeneities :

PBM for Biological populations in an Eulerian framework

$$\frac{\partial n(\xi,x,t)}{\partial t}+u_i\frac{\partial n(\xi,x,t)}{\partial x_i}-\frac{\partial}{\partial x_i}\left(\Gamma\frac{\partial n(\xi,x,t)}{\partial x_i}\right)=-\frac{\partial}{\partial \xi}\left(u_\xi n(\xi,t)\right)+h(\xi,t)$$

$\mu^* = f(S)$ : equilibrium growth rate in the environment $\mu^* = \mu_{\max}\cdot\frac{[S]}{K_S+[S]}\cdot\frac{[O_2]}{K_{O_2}+[O_2]}$




Cells can be out of equilibrium

$$\mu_i \neq \mu^*$$

$$\mu^{pop} = \frac{1}{X_T}\sum_i \mu_i X_i$$

Rate of change in the number of individuals in each class

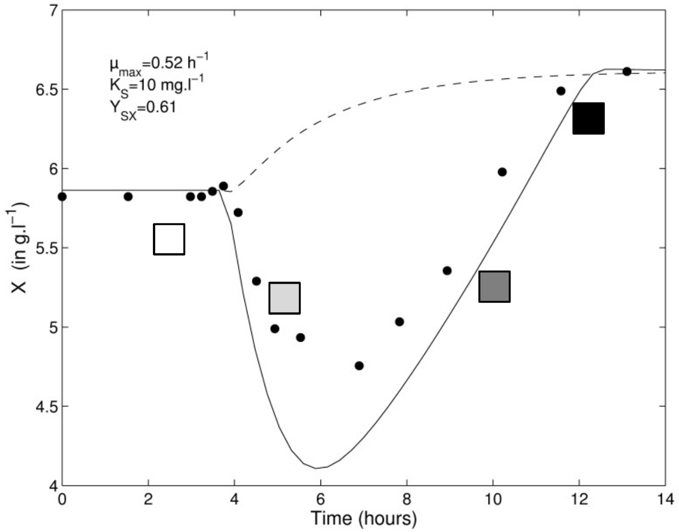
- growth (within each class) $h_i = \mu_i$
- adaptation $u_\xi^A = \left(\frac{1}{T_A}+\mu_i\right)\frac{\mu^*-\mu_i}{\mu_{\max}}$
- limitation $u_\xi^L = \left(\frac{1}{T_A}+\mu_i\right)\frac{\mu_i-\mu^*}{\mu_{\max}}$

Population heterogeneity
Inertia
ξ :mass, size, intracellular component, reaction rate

$$T_A \approx \frac{1}{\mu_{\max}} \approx 1h$$

# PBM → Out of Equilibrium …

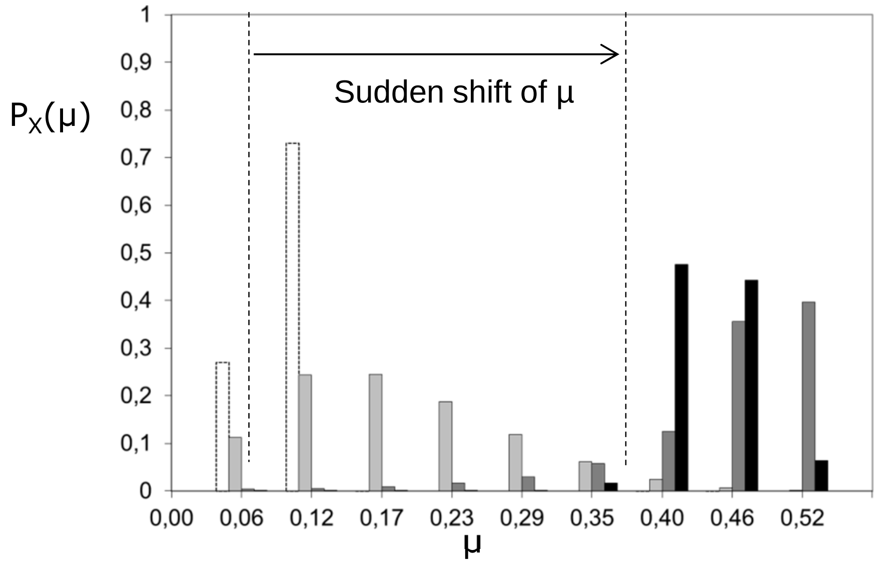
Experimental evidence: shift of the dilution rate (D=Q/V), perfectly homogeneous reactor
D=0.1 $h^{-1}$ to D'= 0.4 $h^{-1}$ at t =4 h (Käterrer et al. 1986)




μ=D μ<D' μ>D' μ=D'

$$\mu^{pop} \neq \mu*$$

Growth rate distribution
Probability to find a cell with a given *μ*




- - - Equilibrium model μ=μ*, average cell

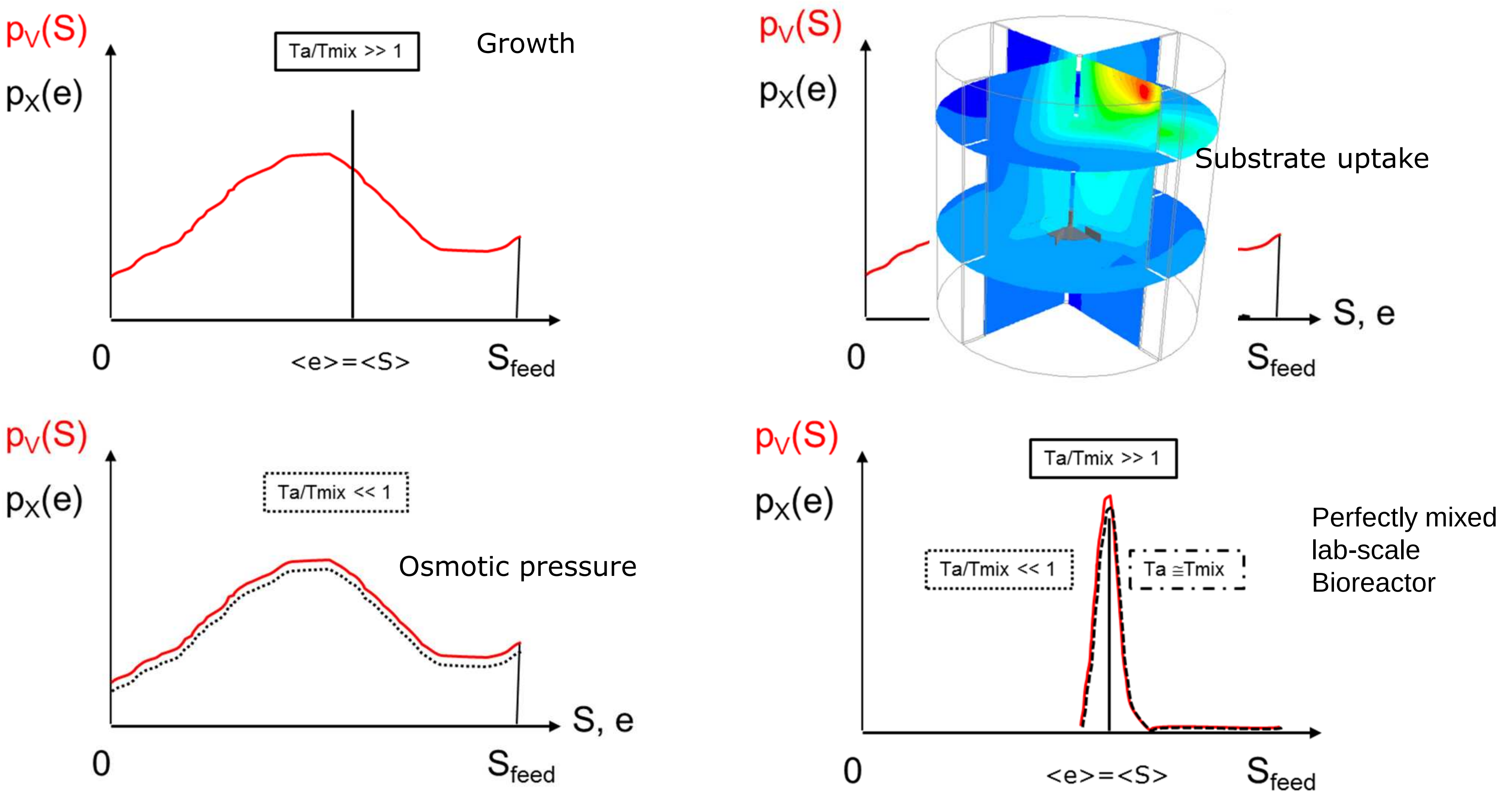
—— Population balance model

Morchain J, Fonade C. AIChE Journal. 2009, Morchain et al. AIChE Journal 2012

$P_V(S)$ : probability to find a volume of liquid at the concentration S

$P_X(e)$ : probability to find a cell at the internal concentration e

# Time scale ratio and consequences



# Effect of feed point location on population disequilibrium

70 m³ aerated fermentor, kinetic reaction, G-L transfer

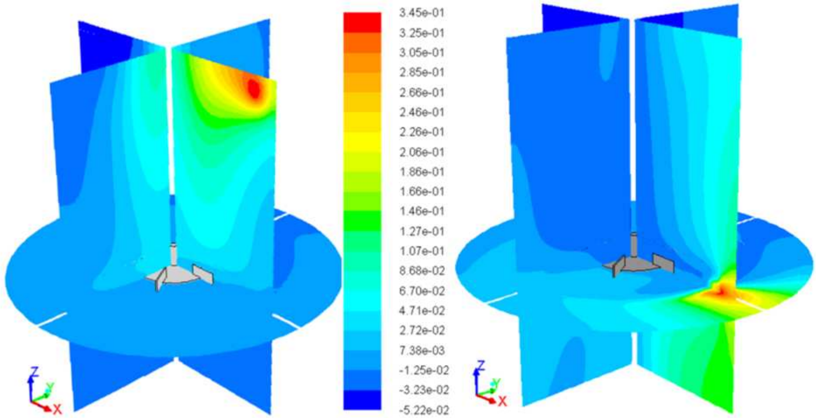
$\tau_M / \tau_S$ → *macrosopic gradients*

## 1°) Growth ?

$$\mu^* = \mu_{max} \cdot \frac{[S]}{K_S + [S]} \cdot \frac{[O_2]}{K_{O_2} + [O_2]}$$ Spatially heterogeneous

*Ta=T*$_{growth}$ *>> Tmix* → $\mu^{pop}$ Spatially homogeneous

$\mu^* - \mu^{pop}$




Local differences between the equilibrium growth rate based on local concentrations and the actual population growth rate
but….
$<\mu^*>=\mu^{pop}$

A Coupled Population Balance Model and CFD Approach for the Simulation of Lab-scale and Industrial Bioreactors, 2013, AIChE

# Effect of feed point location on population disequilibrium

70 $m^3$ aerated fermentor, kinetic reaction, G-L transfer

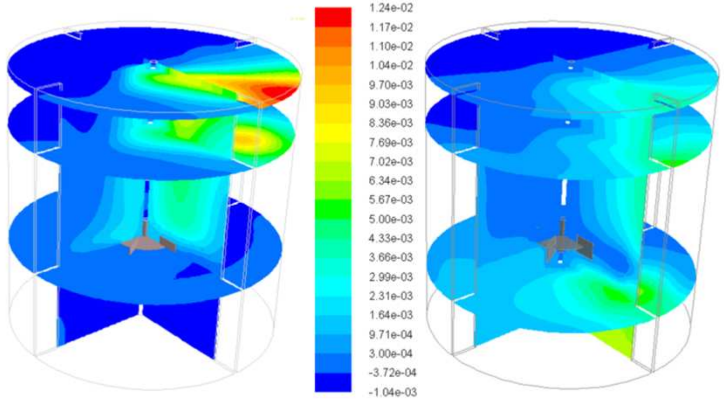
$\tau_M / \tau_S$ → *macrosopic gradients*

$\mu^{pop}$ Spatially homogeneous

## 2°) Uptake ?

*Ta=T*$_{uptake}$ *~ Tmix* → $\Phi^{pop}$ Spatially dependent

$\Phi^{pop} - \mu^{pop}$

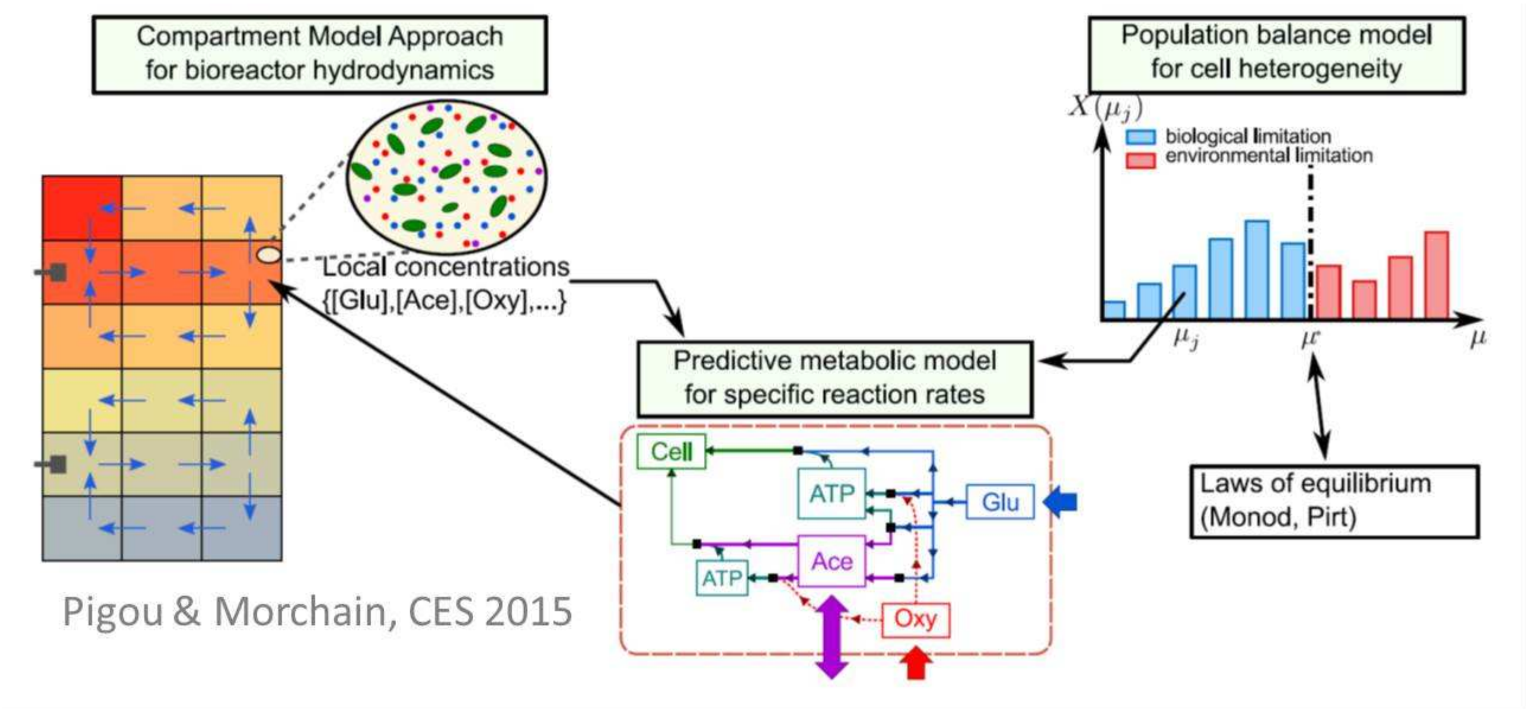



« Uptake rate-growth rate »

Local disequilibrium between Uptake rate-growth rate induce perturbation on cell metabolism : Consequences on bioreactions ???

A Coupled Population Balance Model and CFD Approach for the Simulation of Lab-scale and Industrial Bioreactors, 2013, AIChE

# Full Two-way coupling with « ADENON »




Investigating the interactions between physical and biological heterogeneities in bioreactors using compartment, population balance and metabolic models, Pigou and Morchain, CES, Accepted paper 2015

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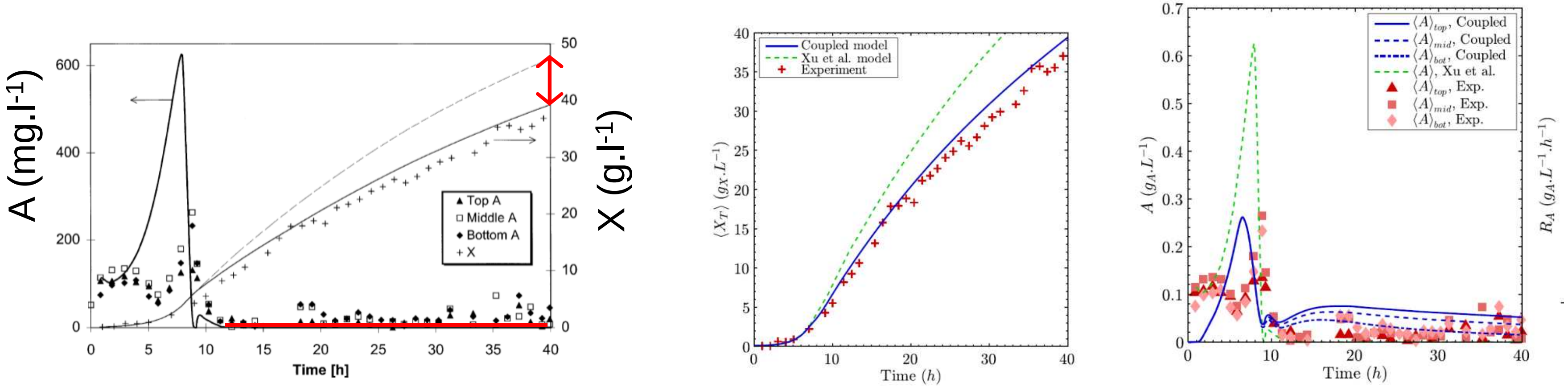
# Full Two-way coupling with « ADENON »

ADENON : User Code devellopped by Morchain and Pigou

Batch and Fed-batch test cases from litterature studies :

Compartment Model Approach (Vràbel et al. 2001) + Metabolic model *E. coli* (Xu et al. 1999)




Accurate prediction of cell and by-product formation in time and space

Investigating the interactions between physical and biological heterogeneities in bioreactors using compartment, population balance and metabolic models, Pigou and Morchain, CES, Accepted paper 2015

# Thank you for your attention

Questions and Answers

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