Population balance and hydrodynamic models for bioreactors

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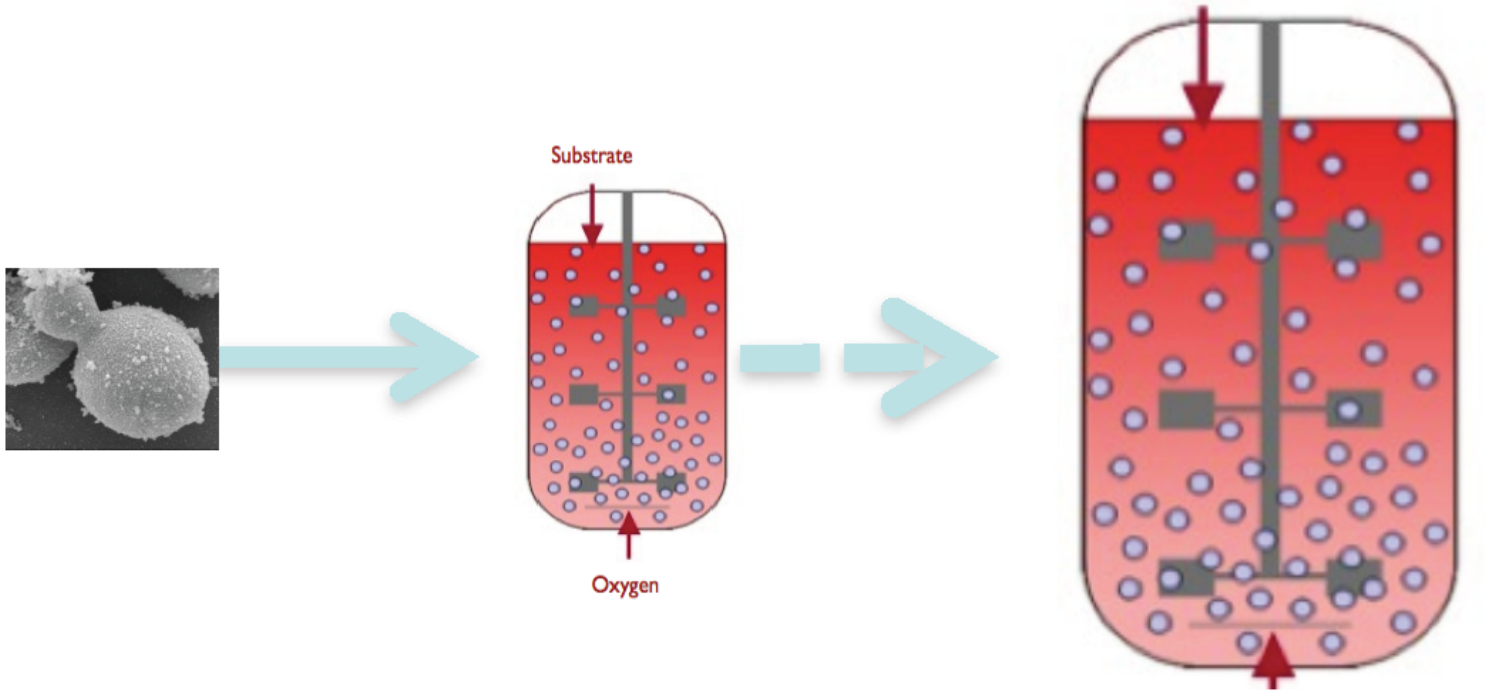
Primary Objective: Bioreactor Scale-Up



Cell factories
d≃μm

Laboratory scale
d≃0.1 m

Industrial scale
d≃10 m

Space/time gradients in concentrations, shear, etc. are unavoidable at industrial scale

Stirred Tank Scale-Up Constraints[1]

Interdependence of Scale-Up Parameters

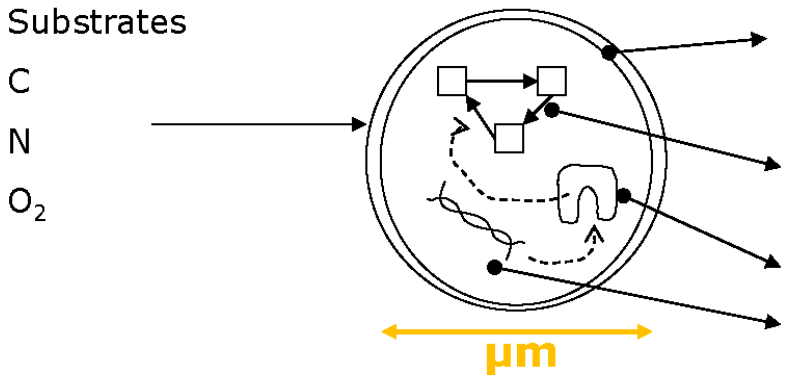
Scale-up criterion[a]	Designation	80-L fermenter	Production fermenter, 10,000 L			
			Constant Po/V	Constant N	Constant N Di	Constant Re
Energy input	Po	1	125	3125	25	0.2
Volumetric energy input	Po/V	1	1	25	0.2	0.0016
Impeller rotation number	N	1	0.34	1	0.2	0.04
Impeller diameter	Di	1	5	5	5	5
Pump rate of impeller	Q	1	42.5	125	25	5
Circulation time[b,c]	V/Q, tc	1	2.94	1	5	25
Maximum impeller tip speed	NDi	1	1.7	5	1	0.2
Reynolds number	$NDi^2\rho/\mu$	1	8.5	25	5	1

[a]Scale-up criterion indicates the variable conserved constant between the two scales.
[b]Calculated as the inverse of the volumetric pump rate of impeller.
[c]tc ≈ tm/4, where tm is mixing time.
Adapted from Oldshue *(1)*.

Macroscale, mesoscale and microscale mixing times cannot all be held constant during scale up

[1]Lara et al. Molecular Biotechnology (2006)

Time Scales for Biological Processes



CO_2

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

Intracellular Metabolites(→X, 1000 s)

Enzymes

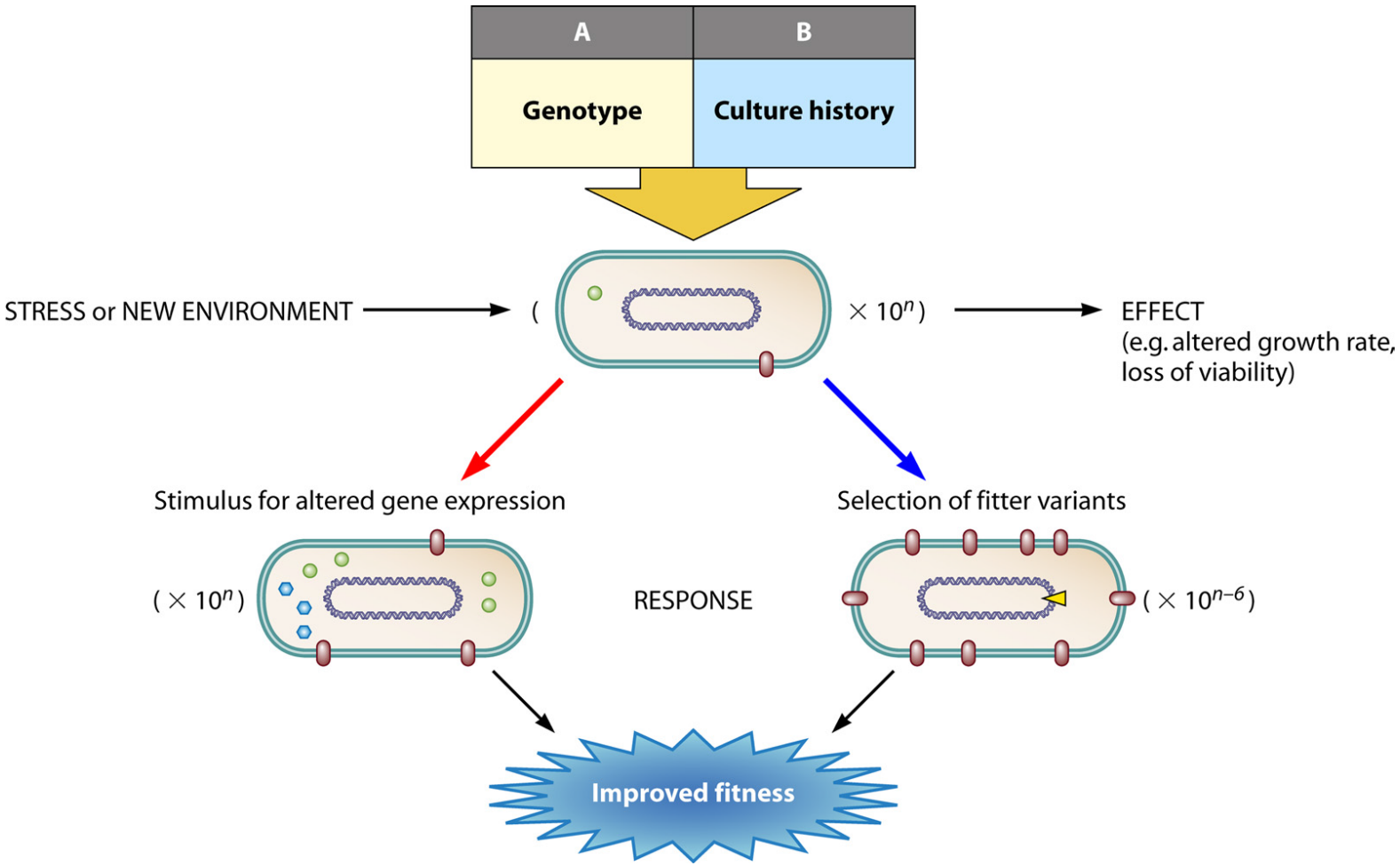
Genes

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

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

Ratio of mixing to biological time scales determines which processes may change during scale up

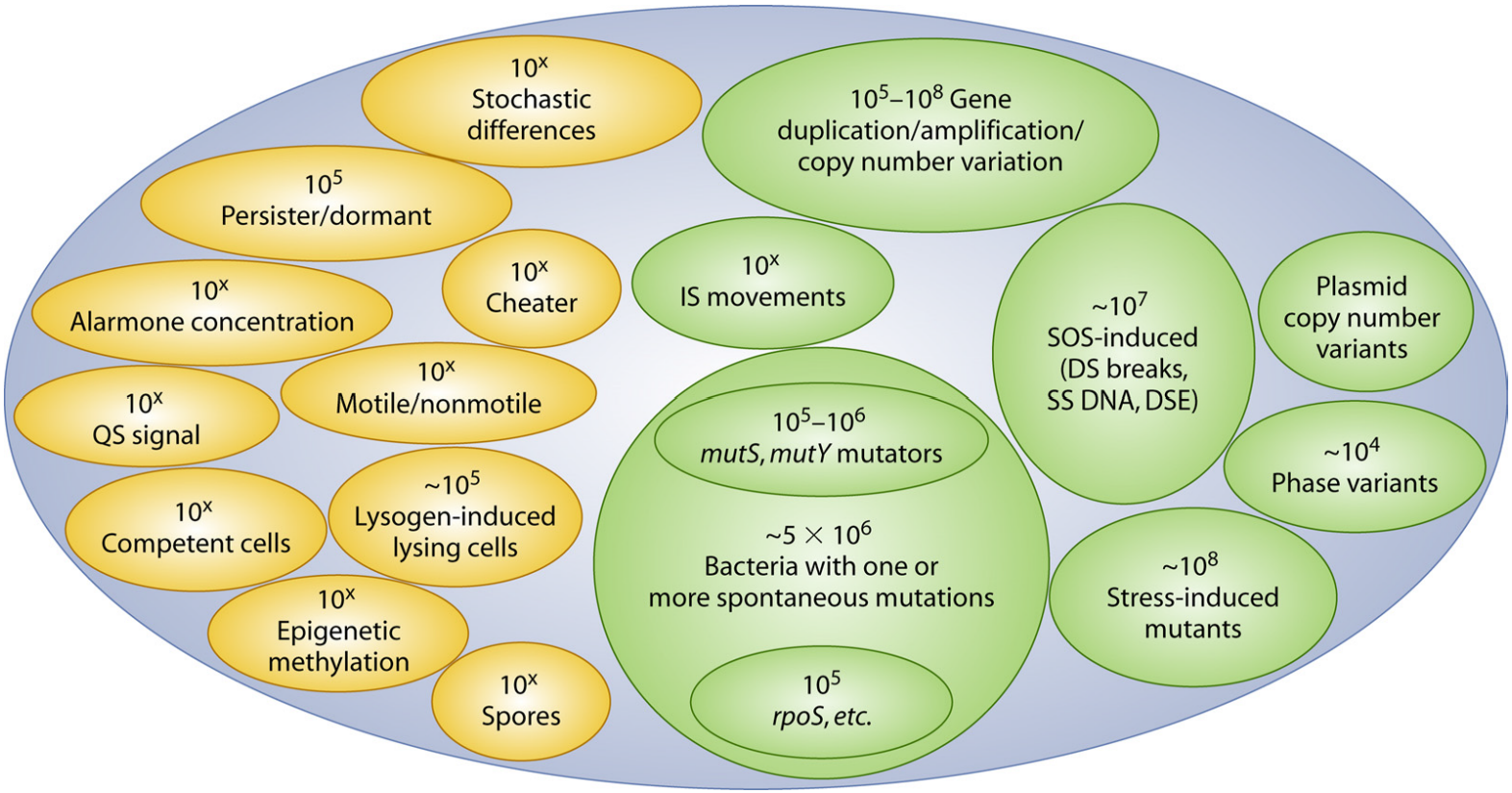
Adaptation to External Heterogeneity[2]



Unlike chemical systems, biological systems can adapt (i.e., change their time scales)

[2]Ryall et al. Microbiology and Molecular Biology Reviews (2012)

Variations in Microbial Population[3] (10^9 cells)

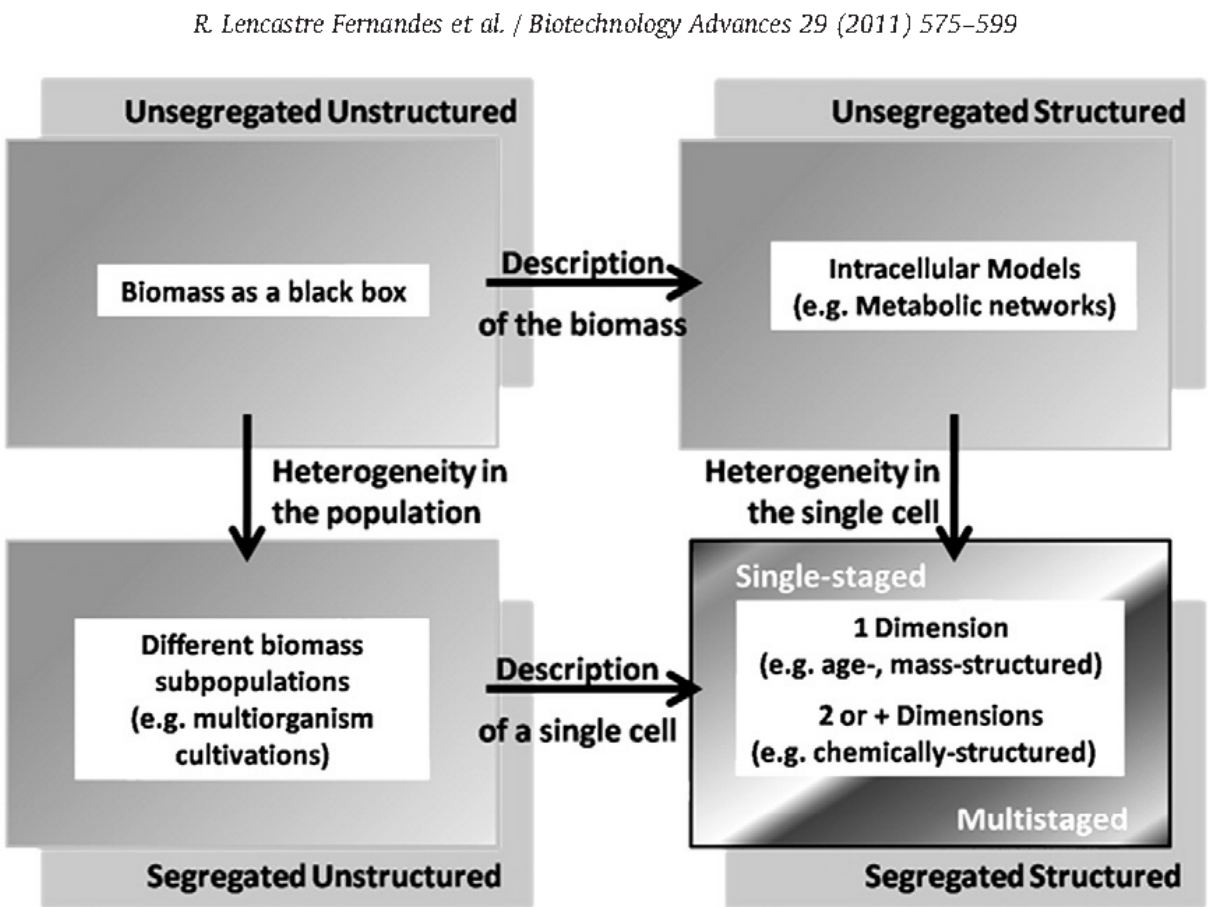


Yellow: different phenotype. Green: different genes

Microbial population composed of individuals with very different properties (e.g. growth rates)

[3] Ryall et al. Microbiology and Molecular Biology Reviews (2012)

Models to Account for Microbial Heterogeneity[4]

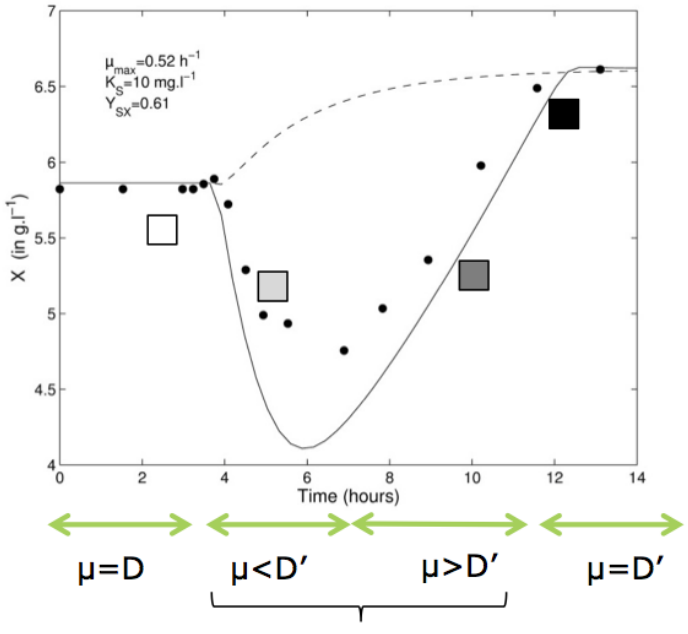


Population balances with one or more internal variables needed to capture cell-to-cell variations

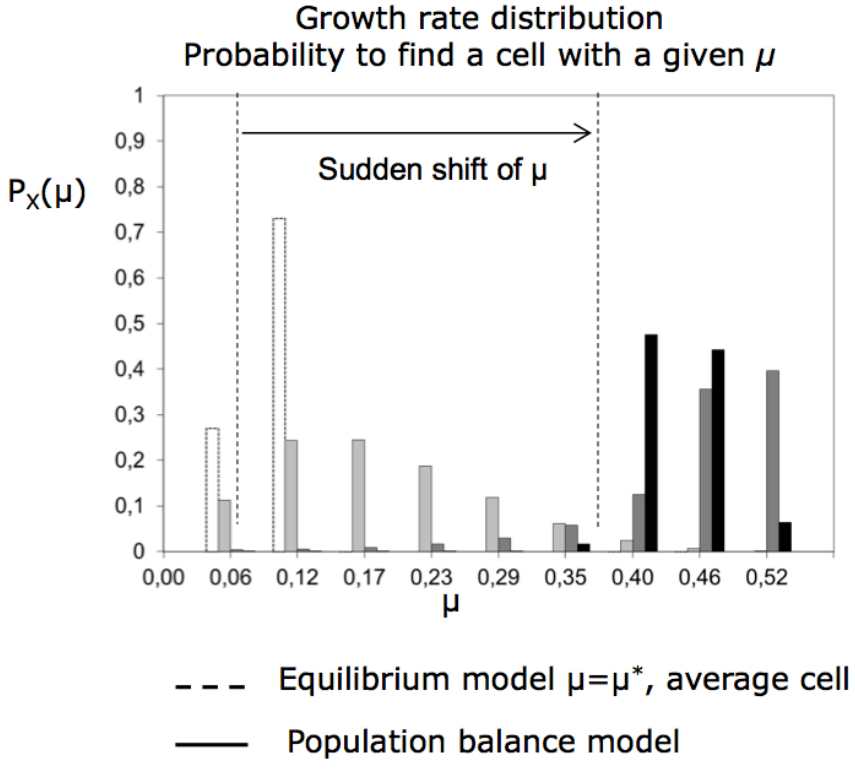
[4]Lencastre Fernandes et al. Biotechnology Advances (2011)

Population Balance Model for Growth Rate Distribution

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)



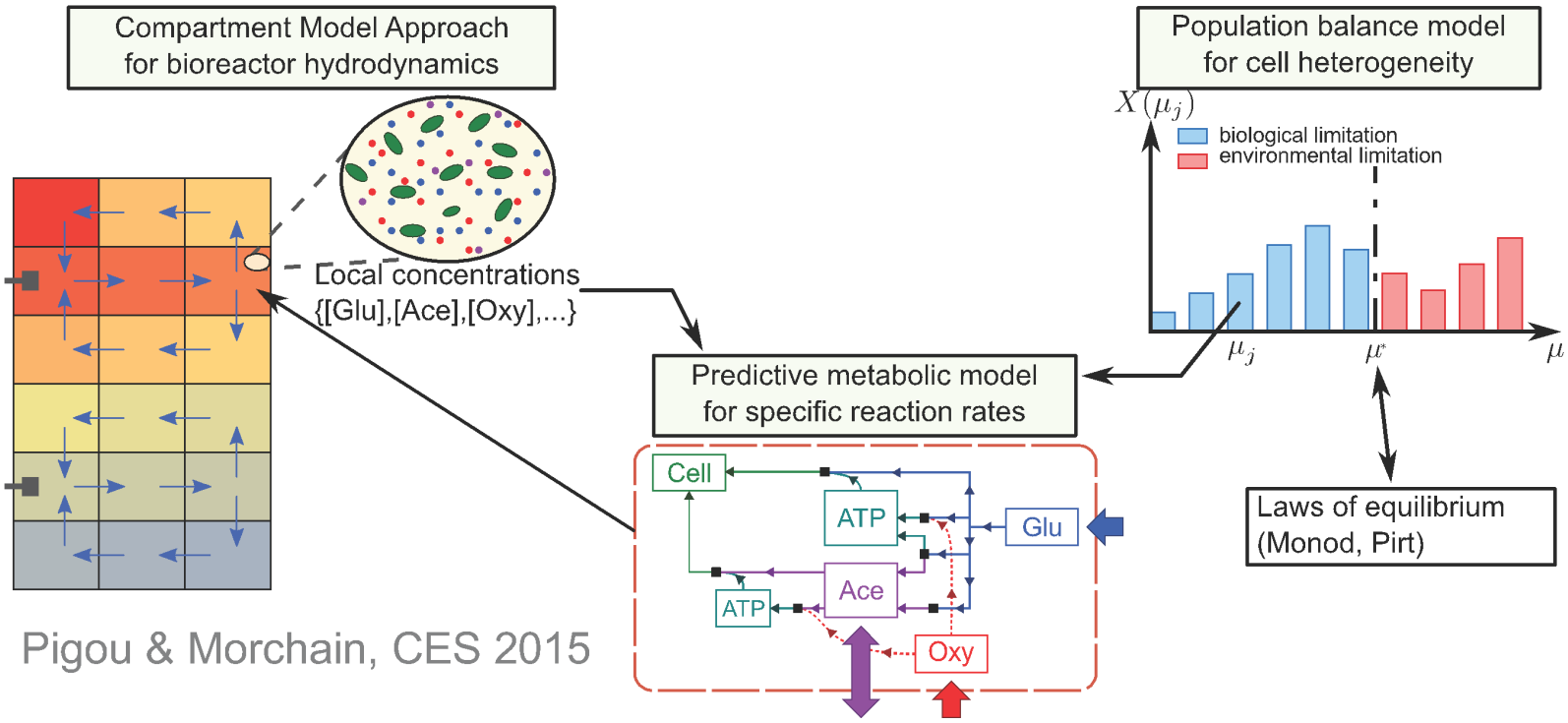
$$\mu^{pop} \neq \mu^*$$



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

Growth rate $\mu(t)$ distributed around equilibrium μ^*

Coupling to PBM with Computational Fluid Dynamics

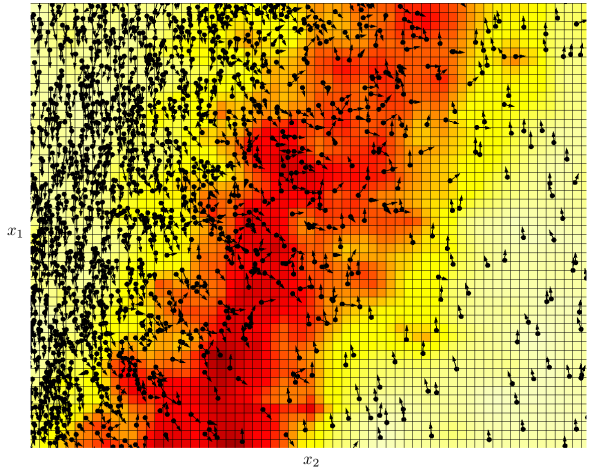


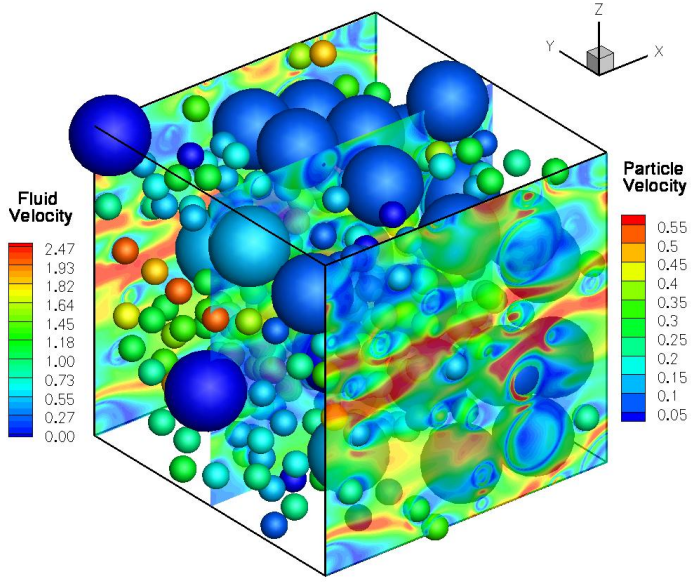
Equilibrium μ^* depends on *local* concentrations, temperature, shear rate, etc. in reactor

What is Computational Fluid Dynamics?

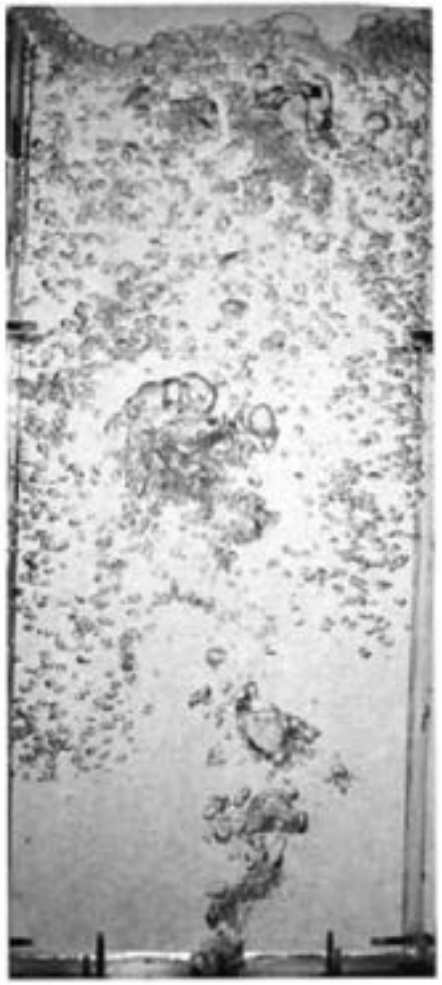
Principal steps:

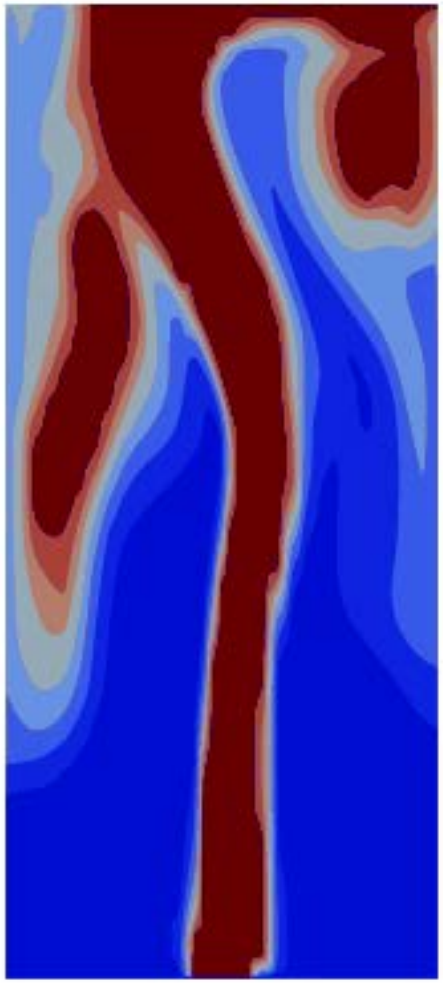
1. Computational grid for bioreactor
2. Select conservation eqns and closures
   - (Bio)mass
   - Chemical species (internal/external)
   - Momentum (gas/liquid)
   - Energy (temperature)
   - multiphase, population balance, ...
3. Discretize conservation equations
   - Finite volume for space
   - ODE solver for exchange/reactions
   - Moment method for population balance
   - Lagrangian method for cells
4. Solve discretized equations
5. Post-process results (lifelines, etc.)

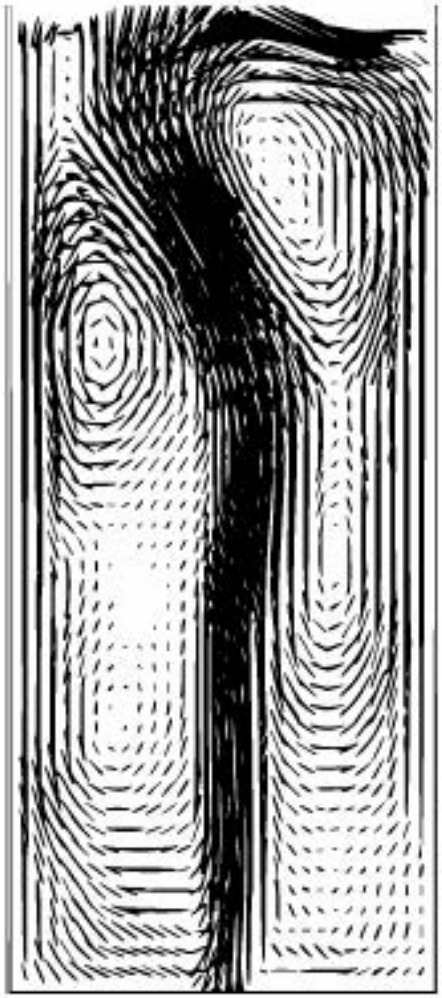




CFD for Polydisperse Bubbly Flows







Bubble size/concentration distribution

CFD Model for Bubbly Flow

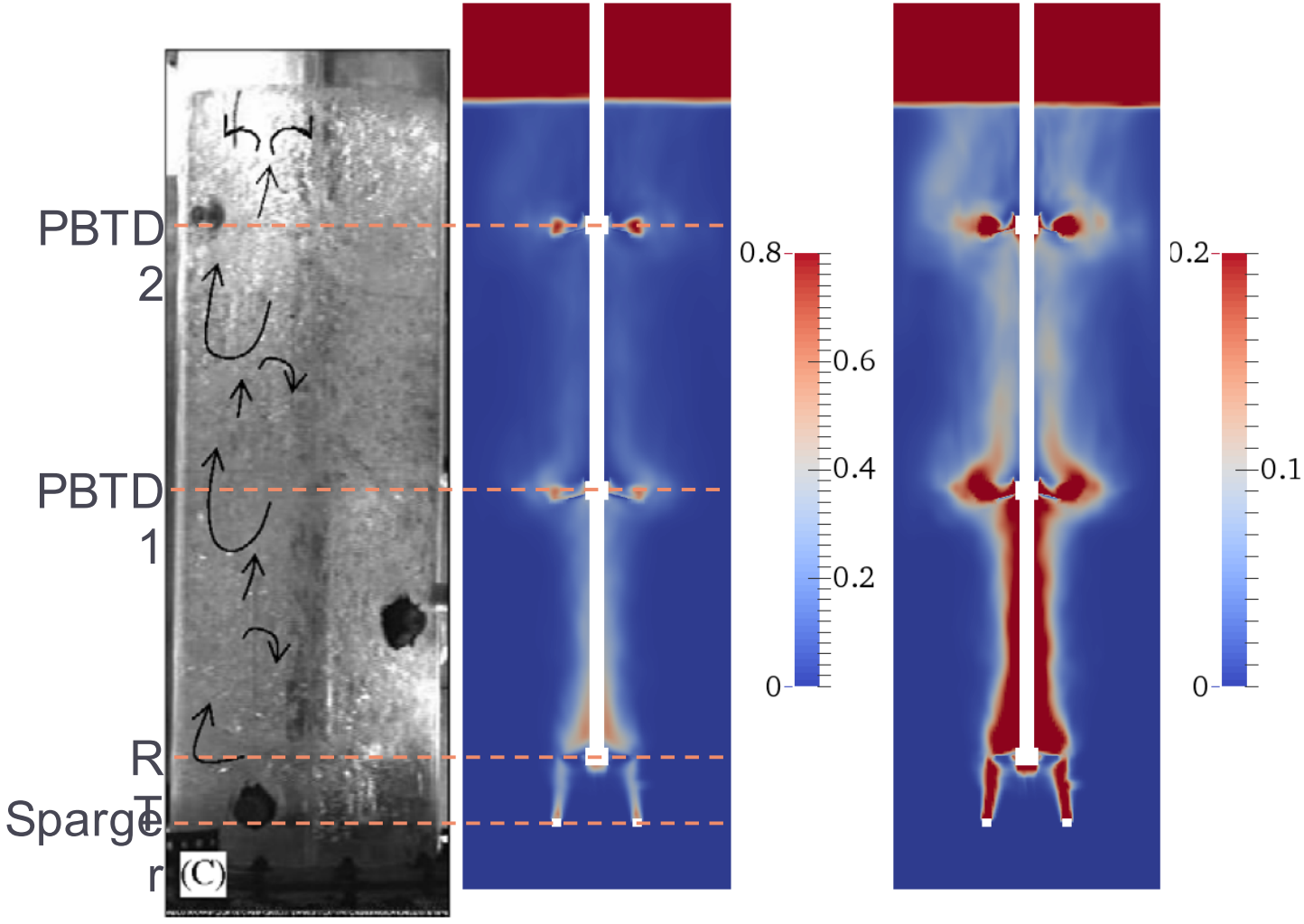
Two phases: gas and liquid

- Mass & momentum balances
  - Buoyancy & drag forces
  - Added mass & lift forces
  - Strong phase coupling
  - Flow regimes (flooded/dispersed/etc.)

Bubble size/concentration distribution

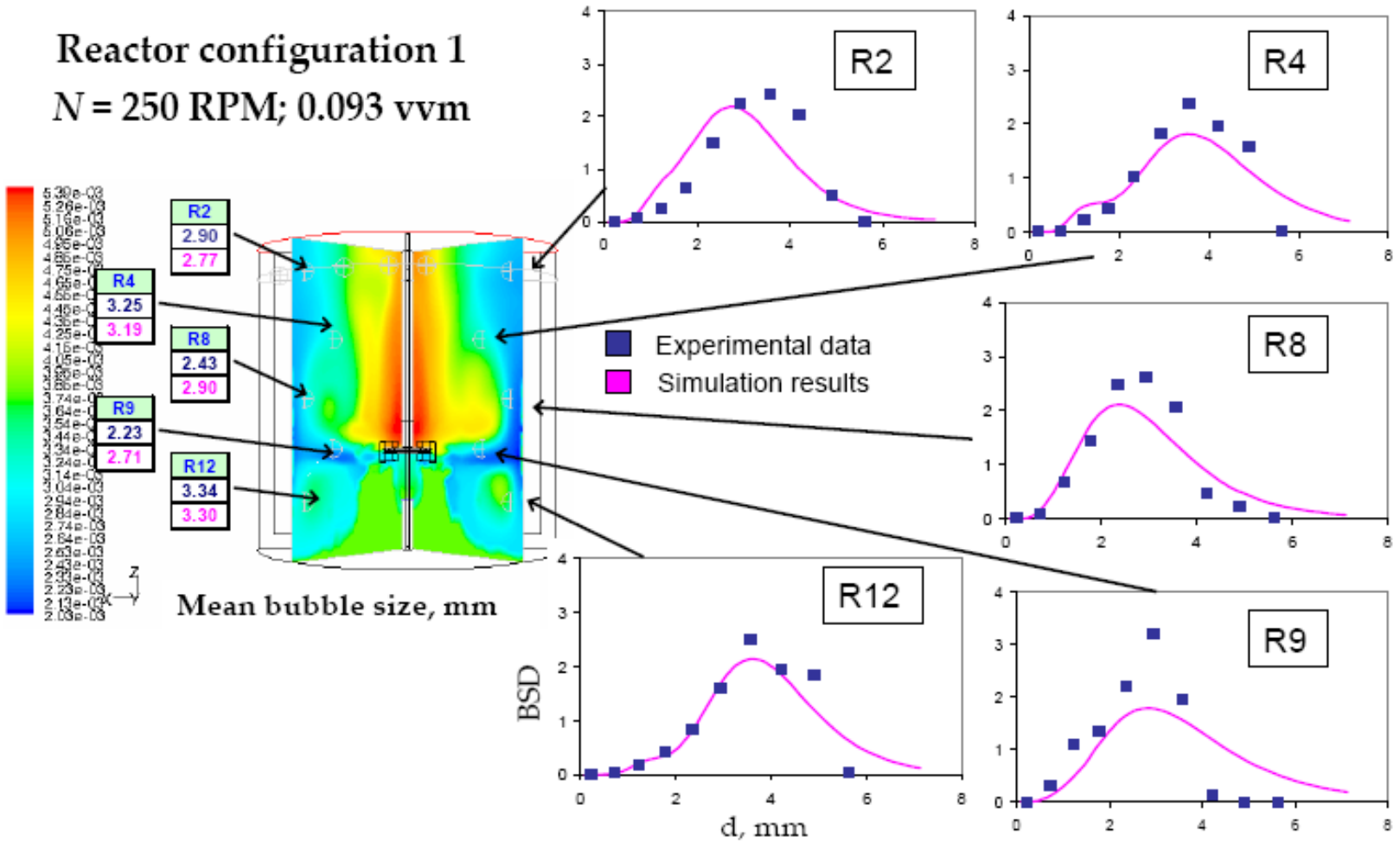
- Population balance equation
  - Solve for number density $n(v, \xi)$
  - Size-conditioned bubble velocity
  - Mass transfer gas/liquid
- Moment methods
  - Solve for moments of number density
  - Close by reconstructing $n(v, \xi)$

CFD for Stirred Reactor with Gas Sparger



Impeller flooding with high gas flow

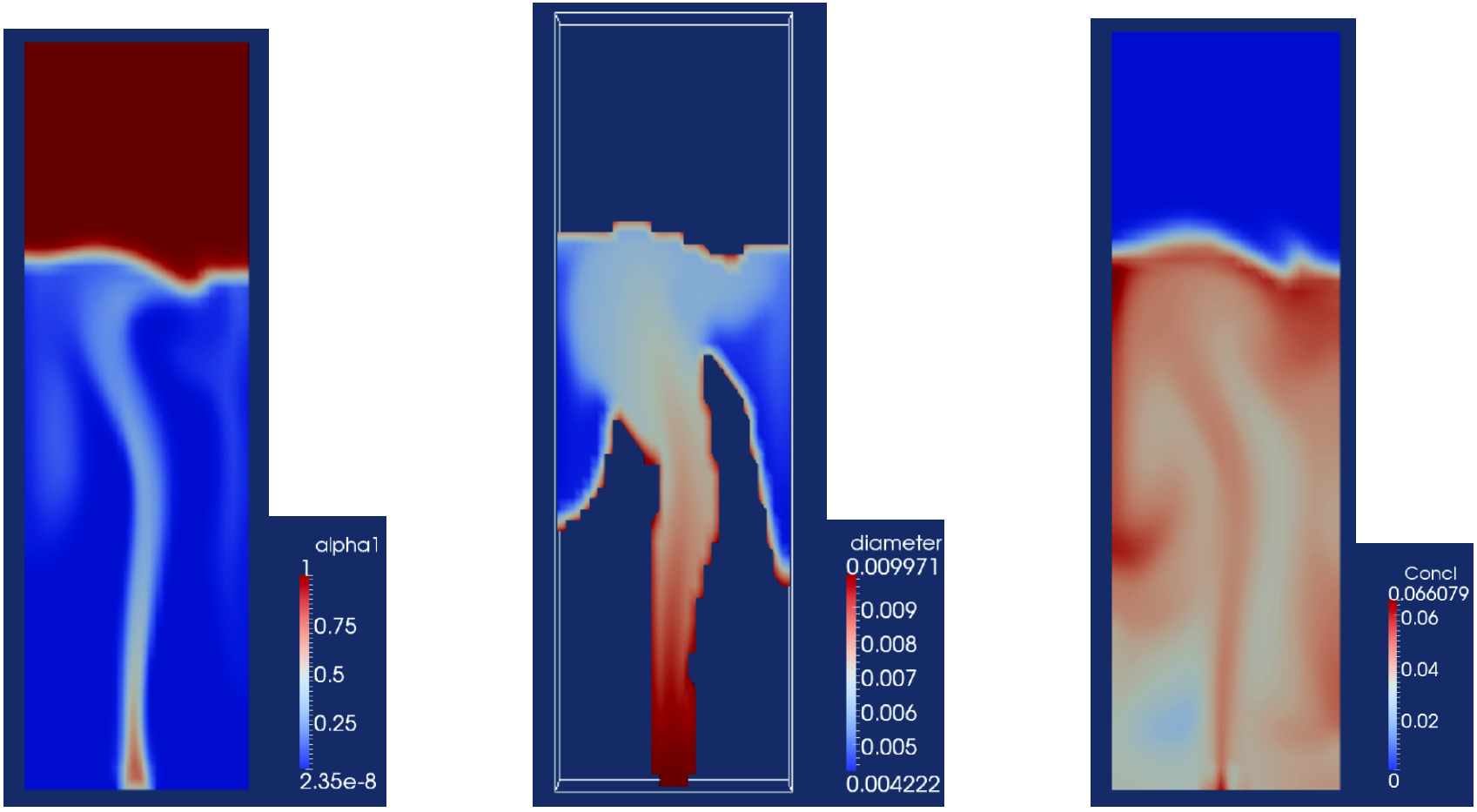
CFD for Stirred Reactor with Gas Sparger



Good agreement between CFD and experiments

Bubble Column with Oxygen Transfer

Gas vol. frac. / Sauter dia. (m) / Oxy. conc. liq. (mol/m^3)



Existing CFD models are adequate for bioreactors

Population Balance Models for Bioreactors[5]

Batch culture PBE: (spatially homogeneous environment)

$$\frac{\partial n(t,\xi)}{\partial t}+\frac{\partial}{\partial \xi}\left[\mu(\xi,\phi)n(t,\xi)\right]+k(\xi,\phi)n(t,\xi)=2\int p(\xi,\xi')k(\xi',\phi)n(t,\xi')\mathrm{d}\xi'$$

- $n(t,\xi)$ number concentration of cells in state ξ
- $\mu(\xi,\phi)$ growth rate in state ξ and environment ϕ
- $k(\xi,\phi)$ rate of cell division (binary) in state ξ and environment ϕ
- $p(\xi,\xi')$ probability of daughter in state ξ given mother in state ξ'

Key Question

How should cell states and growth rates be chosen to account for external heterogeneity?

[5] Fredrickson et al., Mathematical Biosciences (1967)

Population Balance Models for Bioreactors[6]

Batch culture PBE: cell state determined by specific growth rate

$$\frac{\partial n}{\partial t} + \frac{\partial}{\partial \mu}\left[\zeta(\mu,\phi)n\right] + k(\mu,\phi)n = 2\int p(\mu,\mu')k(\mu',\phi)n(t,\mu')\mathrm{d}\mu'$$

- $\mu \in [\mu_{min}, \mu_{max}]$ specific growth rate
- $n(t,\mu)$ number concentration of cells with μ
- $\zeta(\mu,\phi)$ adaptation rate in environment ϕ
- $k(\mu,\phi)$ rate of cell division (binary) in environment ϕ
- $p(\mu,\mu')$ probability of daughter with μ for mother with μ'

Average growth rate: $\langle\mu\rangle := \frac{\int \mu n \,\mathrm{d}\mu}{\int n \,\mathrm{d}\mu} \longrightarrow$ not same as μ_{ext}

Adaptation time scales introduced in $\zeta(\mu,\phi)$ are much shorter than average growth time scale

[6] Morchain et al., AIChE Journal (2013)

Coupling Population Balance Models to CFD

Spatially inhomogeneous PBE:

$$\frac{\partial n}{\partial t} + \frac{\partial}{\partial \mathbf{x}} \cdot (\mathbf{u}_l n) - \frac{\partial}{\partial \mathbf{x}} \cdot \Gamma_l \frac{\partial n}{\partial \mathbf{x}} = RHS$$

- $\mathbf{u}_l$ liquid-phase velocity
- Γ_l liquid-phase effective diffusivity
- $RHS \longrightarrow$ all other terms in PBE

Direct solution of RHS to find $n(t, \mathbf{x}, \xi)$ too expensive

Use moment method: $M_k := \int \xi^k n \, \mathrm{d}\xi$ for $k = 0, 1, \ldots, N$

$$\frac{\partial M_k}{\partial t} + \frac{\partial}{\partial \mathbf{x}} \cdot (\mathbf{u}_l M_k) - \frac{\partial}{\partial \mathbf{x}} \cdot \Gamma_l \frac{\partial M_k}{\partial \mathbf{x}} = \int \xi^k RHS \, \mathrm{d}\xi$$

CFD code handles LHS, Quadrature-based moment method for source terms

Key Points for CFD/PBE Models for Bioreactors

1. For purpose of scale up, multiphase CFD provides detailed information on spatial/temporal gradients in local environment
2. Cell adaptation model is required to predict how individual cells will react to changes in local environment
3. Single-cell data incorporated into a population balance model should be able account for cell-to-cell variations
4. Using moment methods, the PBE can be solved in context of CFD (or even compartmental models)

At present, weakest link is cell adaptation modeling (Steps 2 & 3)

Specific Challenges for Biological Systems

1. Number of potential state variables for cell growth is enormous: which state variables are pertinent?

2. Choice of state variables depends on modeling objectives:
   - predict growth in transient regime
   - predict behavior such as oscillations in steady-state regime
   - predict metabolic response to external disturbances

3. Constitutive laws are difficult to obtain at single-cell level:
   - formulate an hypotheses and see what PBE predicts
   - solve inverse problem to find particle-scale rates that yield observed population-scale distribution

Conclusions & Perspective

- Time scales for hydrodynamics and cell growth are well separated at lab scale, but not on larger scales
- Sensitivity of cell population to operating conditions at scale up may be due to cellular response to short-lived fluctuations in local environment
- Computational fluid dynamics can be applied to bioreactors to predict the local fluid environment (concentrations, temperature, shear rate, etc.) in which cells live
- Population balance models describe how cell population evolves in a given fluid environment

Population balance must contain "internal variables" that mimic sensitivity of growth rate to external disturbances

Merci pour votre attention !

Questions ?