gembloux Agro-Bio Tech

Université de Liège

Microbial bioprocesses : current states and future prospects

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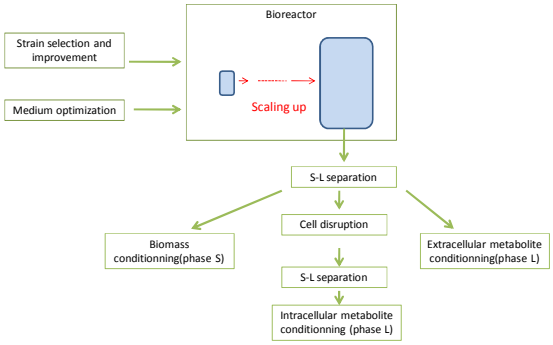
Content

(fundamental aspects and applications, estimated duration : 7 hrs)

Section I : introduction

Bioprocess : basic unit operations diagram



Category of Product	Product	Typical organism	Market value
Whole cells	Baker's yeast	*Saccharomyces cerevisiae*	
	Lactic acid bacteria	Lactic acid bacteria	
	Single cell protein	Methylotrophic bacteria	
Primary metabolites	Beer, wine	*Saccharomyces cerevisiae*	
	Ethanol	*Saccharomyces carlsbergensis*	12 billion US$
		Saccharomyces cerevisiae	
	Lactic acid	*Zymomonas mobilis*	200 million US$
		Lactic acid bacteria	
	Citric acid	*Rhizopus oryzae*	1.5 billion US$
	Glutamate	*Aspergillus niger*	1 billion US$
	Lysine	*Corynebacterium glutamicum*	500 million US$
	Phenylalanine	*Corynebacterium glutamicum*	200 million US$
		Escherichia coli	
Secondary metabolites	Penicillins	*Penicillium chrysogenum*	4 billion US$
	Cephalosporins	*Acremonium chrysogenum*	11 billion US$
		Streptomyces clavuligerus	
	Statins	*Aspergillus terreus*	9 billion US$
	Taxol	Plant cells	1 billion US$
Recombinant proteins	Insulin	*Saccharomyces cerevisiae*	3 billion US$
		Escherichia coli	
	tPA	Chinese Hamster Ovary cells	1 billion US$
	Erythropoietin	Chinese Hamster Ovary cells	3.6 billion US$
	Human growth hormone	*Escherichia coli*	1 billion US$
	Interferons	*Escherichia coli*	2 billion US$
	Vaccines	Bacteria and yeast	
	Monoclonal antibodies	Hybridoma cells	700 million US$
Enzymes	Detergent enzymes	*Bacilli, Aspergilli*	600 million US$
	Starch industry	*Bacilli, Aspergilli*	200 million US$
	Chymosin	*Aspergilli*	
Polymers	Xanthan gum	*Xanthomonas campestris*	400 million US$
	Polyhydroxyalkanoates	*Alcaligenes eutrophus*	
DNA	Vaccines	*Escherichia coli*	

Petri dish

Flasks

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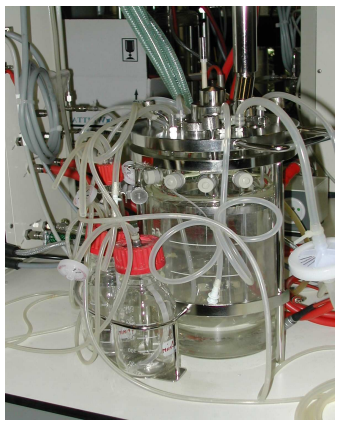
2L bioreactor



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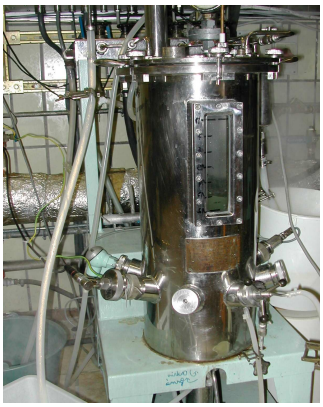
20 L bioreactor



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2000 L bioreactor

500 and 2000 L bioreactors

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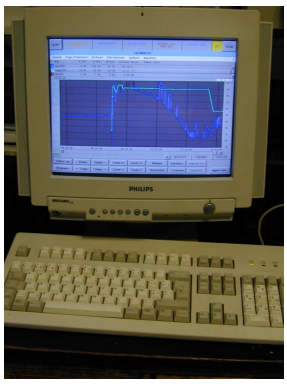
Control system



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Centrifugation

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Ultrafiltration

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Lyophilisation

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Atomization

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End product

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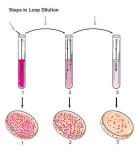
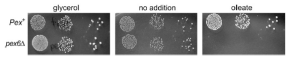
Section 2 : Microbial physiology in process conditions

- Optimisation of microbial growth and product formation
- Importance of response and adaptation to stress conditions

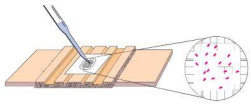
2.1. Microbial growth : basic techniques for the quantification of biomass

- Plate count



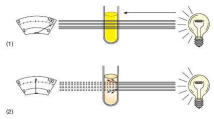


- Microscopy (direct count)



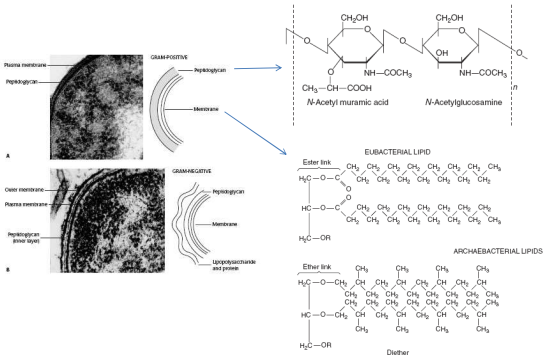
- Dry matter

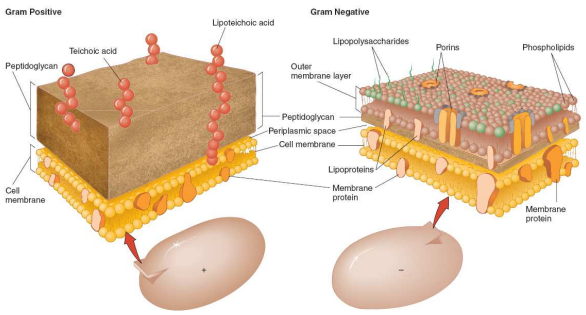
-Spectrophotometer



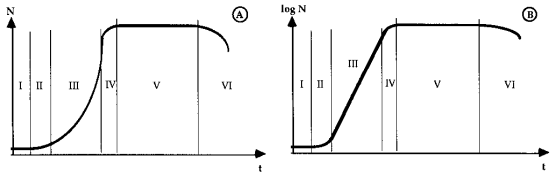
- Indirect parameters extracted from sensors (direct parameter now possible)

2.2. Interface between cellular components and the extracellular environment





Typical growth curve :



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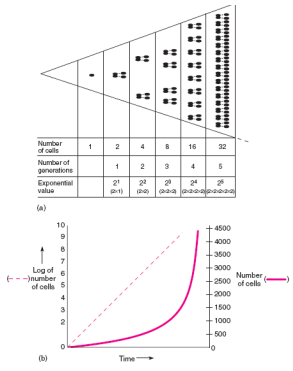
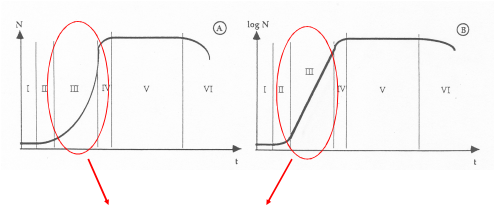


Traditional approach : growth curve



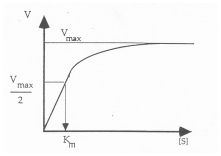
$$\frac{dX}{dt} = \mu . X$$

μ : specific growth rate (h^{-1})

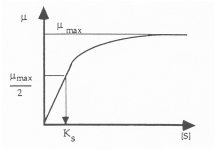
Traditional approach : Monod

Micro organisms are considered as a « pool of enzymes »

Theory of a limiting substrate → Monod



Michaelis-Menten



Monod

$$\mu = \mu_{max} \frac{S}{K_s + S}$$

$$\frac{dX}{dt} = \mu_{max} \frac{S \cdot X}{K_s + S}$$

First problem

μ_{max} and K_s varies with pH, T°, nature of the substrate, exposure to stress,…

Can be resolved by one of the numerous Monod modified expression

Monod (1942)	$\mu(S) = \frac{\mu_m S}{K_m + S}$
Tessier (1942)	$\mu(S) = \mu_m\left(1 - \exp\left(-\frac{S}{K_m}\right)\right)$
Moser (1958)	$\mu(S) = \frac{\mu_m S^\lambda}{K_m + S^\lambda}, \lambda > 0$
Contois (1959)	$\mu(S, C) = \frac{\mu_m S}{K_s C + S}$
Powell (1967)	$\mu(S) = \frac{\mu_m}{2K_m}(K_m + S - \sqrt{K_m + S^2 - 4K_m S})$
Peringer, Blachere, Corrieu and Lane (1972)	$\mu(S, A) = \mu_m^a \frac{S}{K_m + S}\left(\frac{A}{K_a + A} + \frac{1}{1 + K_a A}\right) + \mu_m^a$ with $2\mu_m^a + \mu_m^a = \mu_m$
Jackson and Edwards (1975)	$\mu(S, H^+) = \frac{\mu_m S}{\left(1 + \frac{K_2}{H^+} + \frac{H^+}{K_1}\right)(K_m + S + S^2/K_i(1 + K_s/H^+))}$
Olsson (1976)	$\mu(S, A) = \mu_m \frac{SA}{(K_m + S)(K_a + A)}$
Dourado and Calvet (1983)	$\mu(S, P) = \mu_m \frac{S}{(K_m + S + S^2/K_i)}\frac{K_p}{(K_p + P)}\left(1 - \frac{P}{P_f}\right)$
Williams, Yousefpour and Swanick (1984)	$\mu(S, A, P) = \left(\frac{K_1 S}{K_m + S} + \frac{K_2 P}{K_p + P}\right)\left(\frac{A}{K_a + A} + K_3 A - K_a\right)$

S = substrate concentration, C = cell mass concentration, P = product concentration, A = dissolved oxygen concentration, H^+ = hydrogen ion concentration, $K_{(\text{empirical})}$ = constant, μ = specific growth rate, μ_m = maximum specific growth rate.

Second problem : substrate depletion in batch system

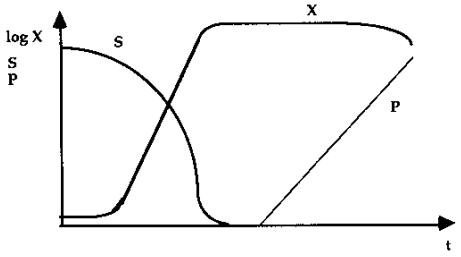
μ_{max} and K_s varies with substrate concentration (simple Monod expression)

BUT ALSO

Substrate concentration can induce a shift from a metabolic pathway to another (homofermentative shift to a heterofermentative pathway)

Solution : fed-batch system (if vessel is considered as perfectly mixed) or structured kinetic model

Growth, substrate consumption and metabolite production



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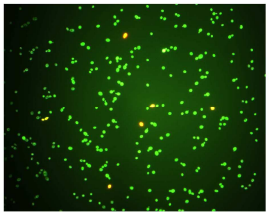
Different modelling alternatives for microbial growth :

-The simplest way to express microbial growth : Monod type equation (saturation)

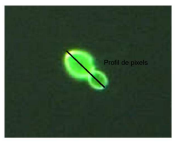
- Structured modelling : take into account the internal dynamics of the system to be studied

- Segregated modelling : take into account the heterogeneity of the microbial population

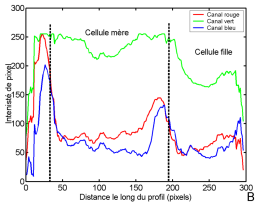


A

Image analysis

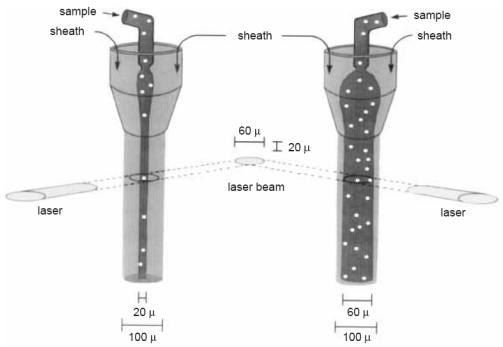


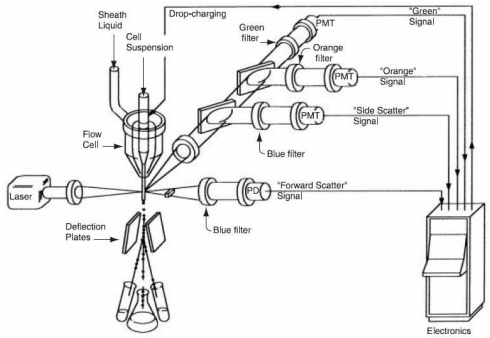
A

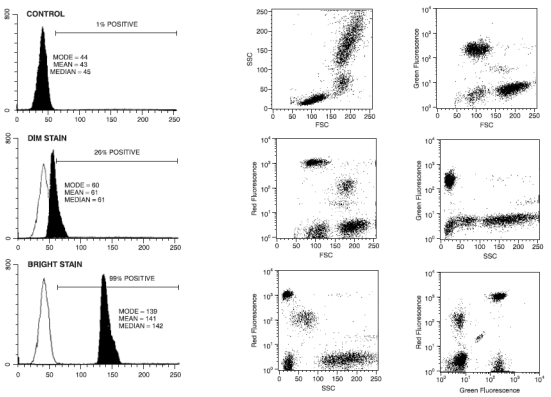


B

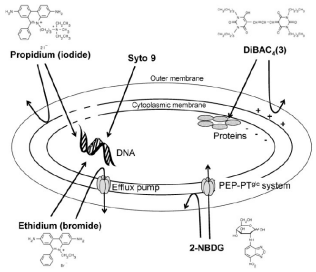
Flow cytometry

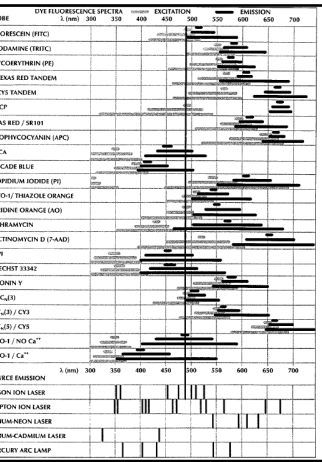






Example of application of flow cytometry : cell viability





2.3. Microbial growth : modelling in different bioreactor configurations

Mass balance

Rate of variation of a component with time = **Flow rate IN** - **Flow rate OUT** + **Production** - **Consumption**

= 0 at steady state

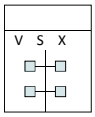
Depend on bioreactor configuration

Depend on kinetics of the reaction (in our case: Monod)

Hypothesis:
- Perfectly mixed reactor
- No cellular death
- No metabolite synthesis
- The only limiting substrate is the carbon source
- Dissolved oxygen is not limiting

In these conditions, Monod equation can be applied

$$\mu = \mu max \frac{S}{S + K_S}$$



V = working volume (m3)
S = substrate concentration (g/l)
X = biomass concentration (g/l)

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The rate of biomass variation with time (g/l.h) :

$$\frac{dX}{dt} = r_X = \mu.X$$

$$r_x = X \cdot \mu_{max} \frac{S}{S + K_s}$$

X : biomass concentration (g de cellules/litre)

S : substrate concentration (g/l)

μ_m : maximal growth rate (h^{-1})

K_s : substrate affinity constant (g/l)

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For the substrate:

$$r_s = -\frac{dS}{dt} = \frac{r_x}{Y_{x/s}} + m_s.X$$

r_s : substrate consumption rate (g/l.h)

$Y_{x/s}$: yield coefficient (g biomass per g substrate)

m_s : maintenance constant (g de substrat / g de cellules . H)

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Experimental results : batch culture of Bacillus subtilis

Durée (h)	r_X (g/l.h)	r_S (g/l.h)
0	-	-
0,5	0,003	0
1	0,008	0,02
1,5	0,011	0,04
2	0,019	0,04
2,5	0,044	0,09
3	0,083	0,20
3,5	0,142	0,27
4	0,242	0,54
4,5	0,412	0,96
5	0,698	1,43
5,5	1,158	2,34
6	1,940	4,44
6,5	3,089	7,41
7	5,00	11,10
7,5	3,811	8,89
8	0,87	0,087
8,5	0,27	0,012
9	-	-

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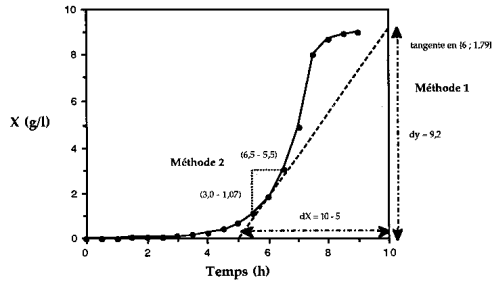

Growth curve : step method for the estimation of the rate of variation with time

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Biomass and substrate variation rates : step method

Durée (h)	r_X (g/l.h)	r_S (g/l.h)
0	-	-
0,5	0,003	0
1	0,008	0,02
1,5	0,011	0,04
2	0,019	0,04
2,5	0,044	0,09
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8	0,87	0,087
8,5	0,27	0,012
9	-	-

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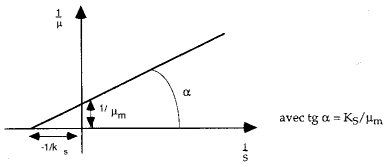
Computation of μ_m and K_s

$$\mu = \mu_m \cdot \frac{S}{K_S + S}$$

$$\frac{1}{\mu} = \frac{1}{\mu_m} \cdot \frac{K_S + S}{S}$$

$$\frac{1}{\mu} = \frac{K_S}{\mu_m} \cdot \frac{1}{S} + \frac{1}{\mu_m}$$



avec $tg\ \alpha = K_S/\mu_m$

Linewaever-Burke

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Durée (h)	1/S (g/l)	μ (h^{-1})	1/μ (h)
0	0,05	-	-
0,5	0,05	0,15	6,67
1	0,05	0,35	2,86
1,5	0,05	0,39	2,56
2	0,05	0,56	1,79
2,5	0,05	0,94	1,06
3	0,05	1,06	0,94
3,5	0,051	1,09	0,91
4	0,051	1,09	0,91
4,5	0,052	1,11	0,90
5	0,054	1,10	0,91
5,5	0,056	1,08	0,93
6	0,061	1,08	0,93
6,5	0,075	1,03	0,97
7	0,112	1,02	0,98
7,5	10,90	0,48	2,08
8	71,20	0,10	10
8,5	249,0	0,03	33,33
9	626,5	-	

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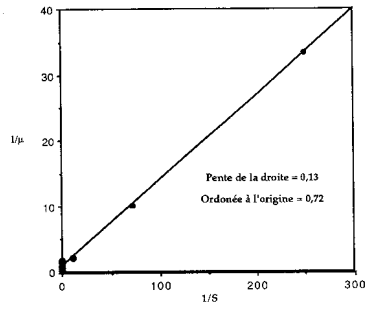

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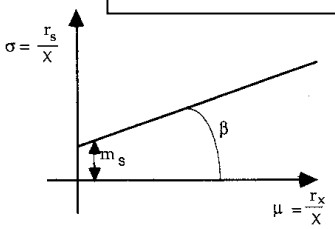
$$r_S = \frac{r_X}{Y_{X/S}} + X \cdot m_S$$

$$\frac{r_S}{X} = \frac{1}{Y_{X/S}} \cdot \frac{r_X}{X} + m_S$$

$$\frac{r_S}{X} = \frac{1}{Y_{X/S}} \cdot \mu + m_S \qquad \sigma = \frac{r_S}{X}$$



avec $tg\ \beta = \frac{1}{Y_{X/S}}$

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Durée (h)	$\mu = r_X/X$ (h-1)	$\sigma = r_S/X$ (g de glucose /g de cellules . h)
0	-	-
0,5	0,15	-
1	0,35	-
1,5	0,39	0,87
2	0,56	1,43
2,5	0,94	1,18
3	1,06	1,91
3,5	1,09	2,56
4	1,09	2,08
4,5	1,11	2,45
5	1,10	2,59
5,5	1,08	2,27
6	1,08	2,19
6,5	1,03	2,48
7	1,02	2,47
7,5	0,48	1,11
8	0,10	0,01
8,5	0,03	-
9	-	-

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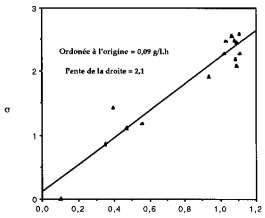

$\frac{1}{Y_{x/s}}$ = slope = 2,1

$Y_{x/s}$ = 0.48 g of cells / g de glucose

M_s = y(x=0) = 0,09 g de glucose / g of cells.h

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Productivity associated with batch reactors:

!!! Cultivation timelength + time associated with industrial operations

Total time is calculated by :

$$t_{total}=T_{exp}+t_0=\frac{1}{\mu_{max}}\ln\frac{X_f}{X_0}+t_0 \quad \text{WITH} \quad \ln\frac{X_f}{X_0}=\mu_{max}.T_{exp}$$

$$t_0=t_V+t_N+t_R+t_S+T_L+T_F$$

Amount of biomass produced :

$$X_f-X_0=Y_{X/S}.S_0$$

Productivity (*batch*) :

$$\text{Productivity}(batch)=\frac{\mu_{max}.Y_{X/S}.S_0}{\ln\frac{X_f}{X_0}+\mu_{max}.t_0}$$

Fed-batch culture : mass balance

Monod hypothesis:

- **Perfectly mixed reactor**
- **No cellular death**
- **The only limiting substrate is the carbon source**
- **Dissolved oxygen is provided in excess**

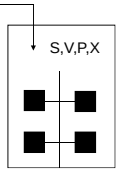
First case : metabolites synthesis

1. **Growth period: equations identical to those used for batch**

$$\frac{dX}{dt}=r_x=\mu_{max}.\frac{S.X}{K_s+S} \qquad \frac{dS}{dt}=-r_s=-\frac{r_x}{Y_{x/s}}-m_s.X \qquad \frac{dV}{dt}=0$$

2. **Metabolite synthesis period (fed-batch) :**

$\frac{dV}{dt}>0$; Q , S_a



→ **BIOMASS : cellular concentration is kept constant and $r_x = 0$. Variation at the level of the biomass concentration can only be attributed to dilution effect :**

$$\frac{dX}{dt}=-\frac{dV}{dt}\frac{X}{V}$$

→ **SUBSTRATE :** $\frac{dS}{dt}=-r_s-\frac{dV}{dt}\frac{S}{V}+Q.\frac{S_a}{V}$

Hypothesis : added substrate is immediately consumed (S ≈ 0)

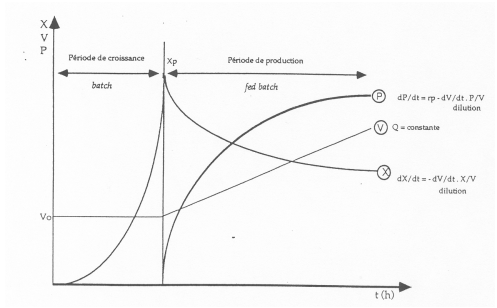
$$\frac{dS}{dt}=-r_s+Q.\frac{S_a}{V}=0$$

$$r_s=Q.\frac{S_a}{V}=m_s.X+\frac{r_p}{Y_{p/s}}$$

→ **METABOLITE :** $\frac{dP}{dt}=-r_p-\frac{dV}{dt}\frac{P}{V}$

$$r_p=\chi.X$$

→ **VOLUME :** $\frac{dV}{dt}=Q$



2nd case : biomass production.

1. **Growth period: see batch .**
2. ***Fed-batch* period:**

→ **BIOMASS :** $\frac{dX}{dt}=r_x-\frac{dV}{dt}\frac{X}{V}=\mu.X-\frac{Q}{V}X$ **with μ = cst**

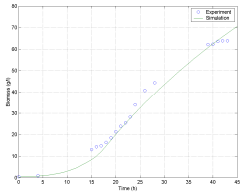
→ **SUBSTRATE :** $\frac{dS}{dt}=-r_s-\frac{dV}{dt}\frac{S}{V}+\frac{dV}{dt}\frac{S_a}{V}$

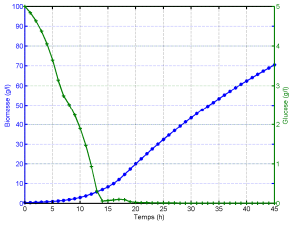
$$\frac{dS}{dt}=-r_s-Q.\frac{S}{V}+Q.\frac{S_a}{V}=-r_s+\frac{Q}{V}(S_a-S)$$

$$r_s=\frac{Q}{V}(S_a-S)=\frac{r_x}{Y_{x/s}}+m_s.X$$ **If S = cste and dS/dt = 0**

→ **VOLUME :** $\frac{dV}{dt}=Q$ **with Q being comprised between Q_{min} et Q_{max}**

Example : *P. pastoris* fed-batch culture





Fed-batch culture of S. cerevisiae with Q.R. regulated at a set point of « 1 »
(S_a = 200g/l; V0 = 10 liters; Vf = 12,88 liters)

Durées (h)	X (g/l)	S (g/l)	Q.R.	Q (l/h)	V (litre)	$S_a.(V-V_0)$ (g)*
0	0,28	0,15	-	0,02	10,00	1,6
0,83	0,3	0,21	1,22	0,016	10,013	2,6
1,83	0,39	0,19	1,3	0,009	10,022	4,4
2,83	0,42	0,18	1,76	0,003	10,025	5
3,5	0,47	0,11	1,12	0,003	10,027	5,4
4,33	0,58	0,12	1,02	0,016	10,040	8
4,83	0,66	0,11	1,13	0,016	10,048	9,6
5,33	0,97	0,14	0,97	0,072	10,084	16,8
6,33	1,52	0,14	1,04	0,063	10,147	29,4
7,33	2,42	0,13	0,97	0,104	10,251	50,2
8,33	3,12	0,06	1,02	0,129	10,38	76
9,08	4,68	0,04	0,98	0,181	10,516	103,2
10,08	5,66	0,14	1,15	0,128	10,644	128,8
23,83	16,17	0,27	1,03	0,11	12,164	432,8
25,83	18,33	0,24	0,97	0,18	12,527	505,4
26,83	19,11	0,25	1,2	0,139	12,666	533,2
28,83	**20,28**	0,28	0,9	0,107	**12,88**	**576,0**

* $S_a.(V-V_0)$ = Apport cummulé en glucose (g)

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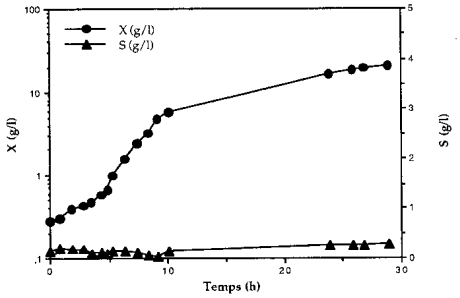

Fed-batch culture of S. cerevisiae with Q.R. regulated at a set point of « 1 »

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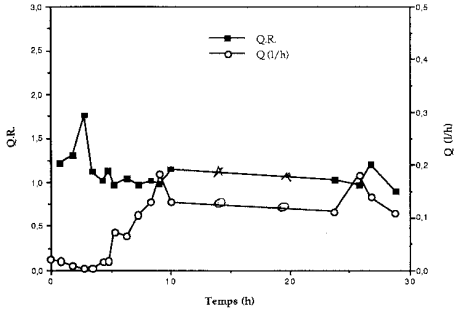

Evolution of Q.R. and« Q » (feed flow rate)
Fed-batch culture of S. cerevisiae with Q.R. regulated at a set point of « 1 »

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Control of glucose effect positive strains

Software sensor: respiratory ratio

QR > 1 → alcohol production (overflow)

QR = 1 → growth

QR < 1 → alcohol reassimilation

QR can be measured by gas balance analysis (derived method can also be used to monitor K_la)

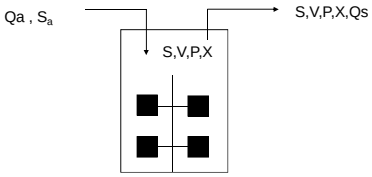
Other control procedures:

Ethanol, glucose sensores

pHstat, DOstat

Exponential feeding algorithm (if kinetics parameters are well known)

Continuous reactors : mass balance



Basic principle : maintain μ_{max} by keeping constant cellular environment during the culture

Parameters :

- **dV/dt = 0 → Qa = Qs = Q**
- **Dilution rate D = Q/V (h^{-1})**
- **Mean residence time ts = V/Q = 1/D (h)**

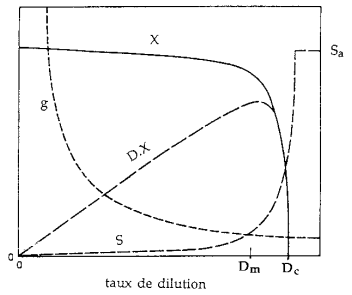
→ **BIOMASS :** $\frac{dX}{dt} = \mu \cdot X - D \cdot X = X \cdot (\mu - D)$

→**SUBSTRAT E :** $\frac{dS}{dt} = D \cdot S_a - r_s - D \cdot S = D \cdot (S_a - S) - r_s$

→**METABOLITE :** $\frac{dP}{dt} = r_p - D \cdot P$

dX/dt, dS/dt and dP/dt depend on the value of D:

- **Critical dilution rate D_c**
- **Maximal dilution rate D_m**



Effect of the dilution rate on the biomass (X) and substrate (S) concentrations (also on generation time g and productivity D.X)

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1st CASE : D > Dc

Bioreactor wash out → dX/dt < 0

2nd CASE : 0 < D < Dc

Evolution toward a steady state :

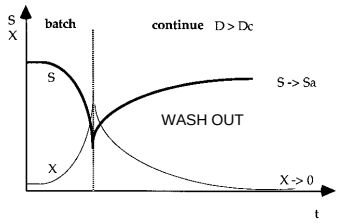
X = X_{eq} → r_x = μ.X = D.X

S = Seq → r_s = D . (Sa – S) **→ CHEMOSTAT**

P = P_{eq} → r_p = D.P

3rd CASE : D = Dc = μ_{max}

dX/dt = X . (μ_{max} - D) = 0 **→ TURBIDOSTAT**



Evolution of S and X for a continuous reactor operation in wash out conditions

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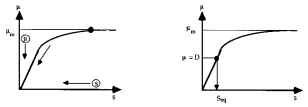
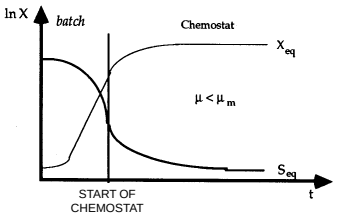


Evolution to the steady state in a continuous reactor operating in the chemostat mode



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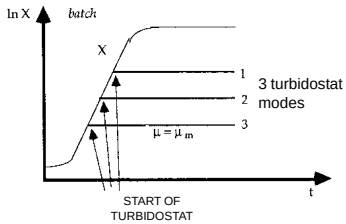

Working window in the case of a turbidostat

$$\frac{dX}{dt} = X.(\mu_m - D) = 0$$

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Chemostat : modelling

X and S mass balance equations with Monod kinetics. At steady state, μ = D :

$$S_{eq} = K_s \frac{D}{\mu_{max} - D}$$

$$X_{eq} = \frac{Y_{x/s} \cdot D}{D + m_s \cdot Y_{x/s}} \left(S_a - S_{eq} \right)$$

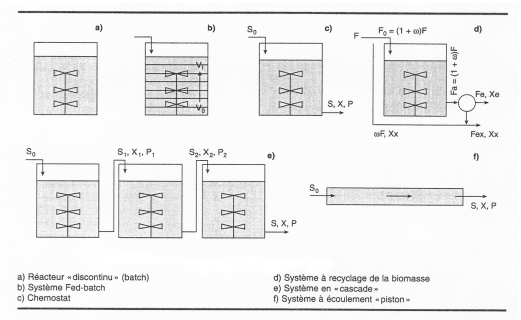
If we focus our attention on generation time and productivity :

$$g = \frac{\ln 2}{\mu_{eq}} = \frac{\ln 2}{D}$$

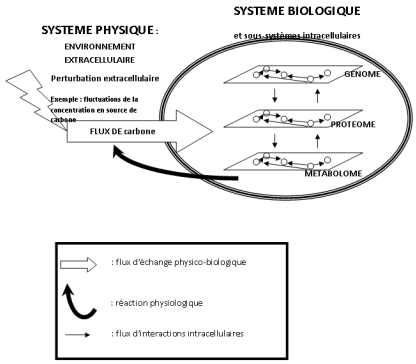
$$productivité = X_{eq} \cdot D = Y_{x/s} \cdot \left(S_a - S_{eq} \right) D$$

$$D = D_m = \mu_m \cdot \left[1 - \sqrt{\frac{K_s}{K_s + S_a}} \right]$$

Bioreactor operating mode : synopsis



2.4. Impact of process-related stresses on microbial physiology



Process-related stresses:

- Thermal shock
- Hypoxia
- pH shock
- Carbon starvation
- Carbon excess
- Nitrogen starvation
- Choc osmotique (*fed-batch*)
- Haute densité cellulaire (*fed-batch*)
- Turbohypobiosis

Physiological impact:

- Short-term : metabolic shift
- Long-term : gene induction/repression

Depending also on the intensity and the frequency of stress

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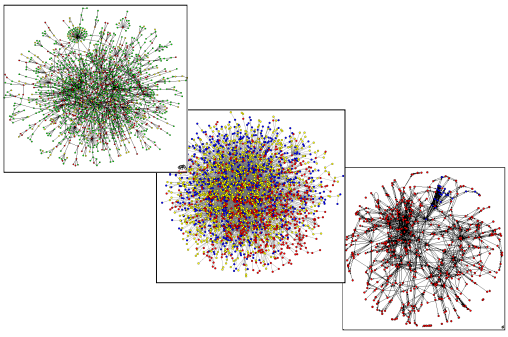
System biology

Les différents composants des systèmes (métabolome, protéome, fluxome) sont organisés en réseaux

Existence d'interactions complexes au sein de ces différents réseaux



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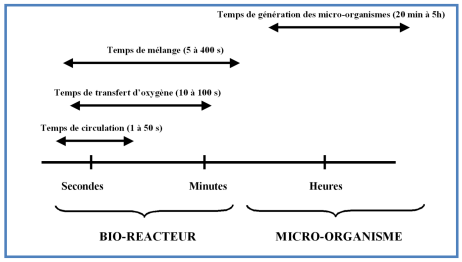
Etude des temps caractéristiques du procédé :



Temps caractéristiques pour les réactions enzymatiques du métabolisme : peuvent être inférieurs à la seconde (dépendent de la constante cinétique de réaction)

Temps caractéristiques pour la synthèse des ARN_m : quelques dizaines de secondes (dépendent de la longueur de l'ARN messager)

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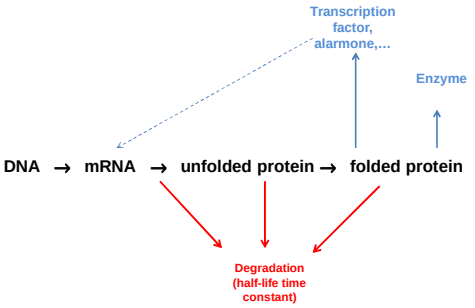

Example for *E. coli* :

	Transcription		*Translation*		*Folding*	
DNA	→	mRNA	→	Unfolded protein	→	Folded protein
Rate: 40-50 nucl./sec			15-62 nucl./sec		≈ 1 min /molecule	

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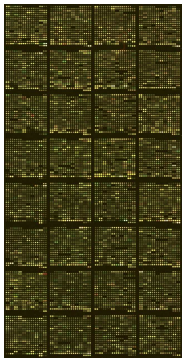
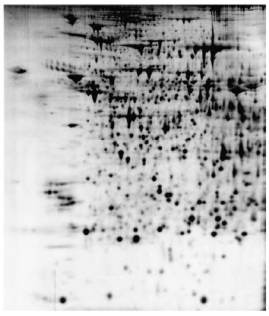
« Omics » technologies

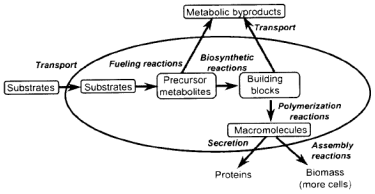
DNA microarray



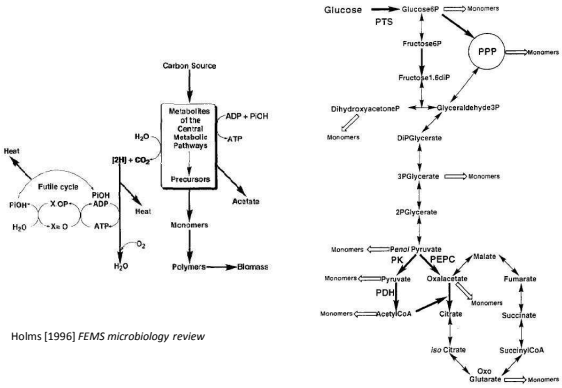
2D gel electrophoresis



Overview of the substrate assimilation pathways inside the cell



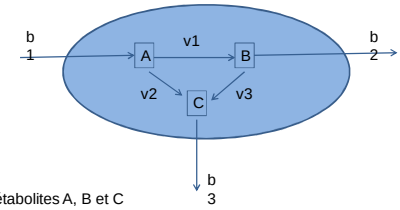
Complexity of the metabolic network : example of central carbon metabolism *in E. coli*



Holms [1996] *FEMS microbiology review*

Analyse des flux métaboliques : méthodologie

Observation de la dynamique interne d'une cellule avec un réseau métabolique simplifié



3 métabolites A, B et C
3 flux externes b1, b2 et b3
3 flux internes v1, v2 et v3

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Balance de masse dynamique : $\frac{dA}{dt} = -v1 - v2 + b1$

$\frac{dB}{dt} = v1 + v3 - b2$

$\frac{dC}{dt} = v2 - v3 - b3$

Sous forme matricielle :

$$\frac{d}{dt}\begin{bmatrix} A \\ B \\ C \end{bmatrix} = \begin{bmatrix} -1 & -1 & 0 & 1 & 0 & 0 \\ 1 & 0 & 1 & 0 & -1 & 0 \\ 0 & 1 & -1 & 0 & 0 & -1 \end{bmatrix} \cdot \begin{bmatrix} v1 \\ v2 \\ v3 \\ b1 \\ b2 \\ b3 \end{bmatrix}$$

$\frac{dX}{dt} = S.v$

S : matrice stœchiométrique
V : vecteur de flux

Balance de masse à l'équilibre :

S.v = 0

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Réseau métabolique à l'état stationnaire (turnover des intermédiaires) sous forme matricielle :

$$S.v = 0$$

On a K métabolites et J réactions

La matrice stœchiométrique S a donc les dimensions (K,J) et le vecteur de flux s a la dimension (J,1)

Le modèle reviens a un système d'équations linéaires dont le nombre de degré de liberté F = J-K

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3 cas de figure :

Si F = 0, le système est déterminé et la solution est unique
Si F < 0, le système est sur-déterminé
Si F > 0, le système est sous-déterminé et il existe plusieurs solutions

Dans la pratique, pour les réseaux métaboliques les plus complexes, le système est toujours sous-déterminé
On diminue le nombre de degré de liberté en mesurant certains flux. Le système d'équations linéaires s'écrit alors :

$$S.v = S_m.v_m + S_{nm} . V_{nm} = 0$$

La solution est alors obtenue par :

$$v_{nm} = -(S_{nm})^{-1} . S_m .v_m$$

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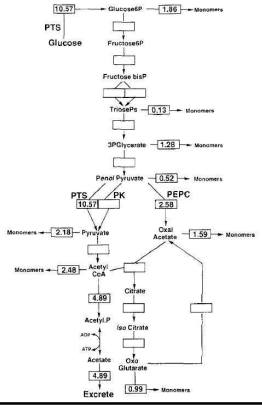
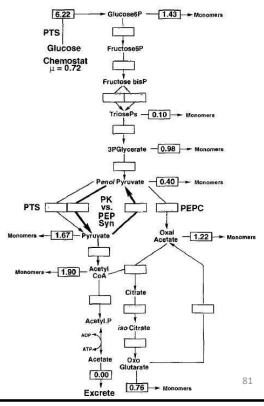
Réseau métabolique pour la croissance de *E. coli* sur glucose



Réseau métabolique pour la croissance de *E. coli* sur glucose avec limitation de la croissance



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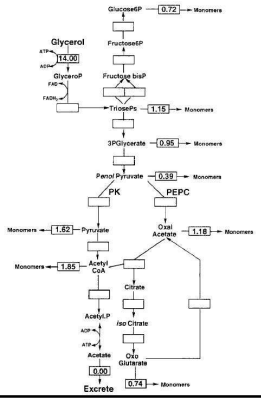
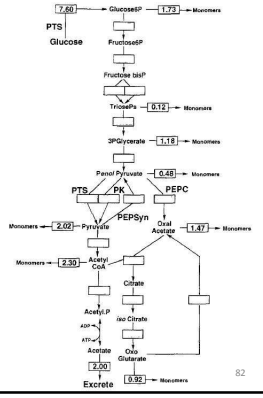
Réseau métabolique pour la croissance de *E. coli* sur glycérol



Réseau métabolique pour la croissance de *E. coli* sur glucose : surexpression de la voie PEPC



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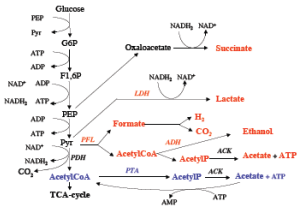
Bascule métabolique déclenchée par :
- Excès de glucose
- Manque d'oxygène (voie des acides mixtes chez certains procaryotes)

Exemples : *E. coli*



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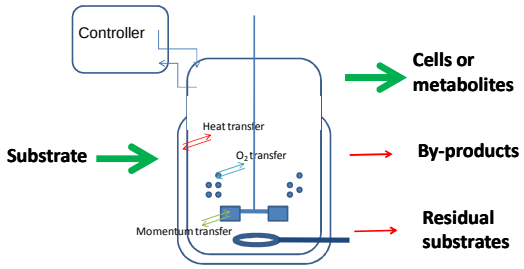
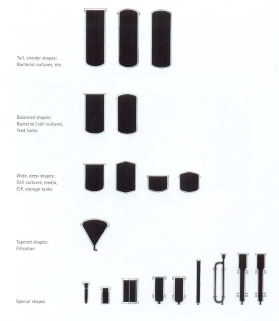
Section 3 : Basic bioreactor design

Overview of bioreactor operations



Bioreactor : system description

Bioreactor design overview



Mixing can be provided by :

- Mechanical system
- Pneumatic system

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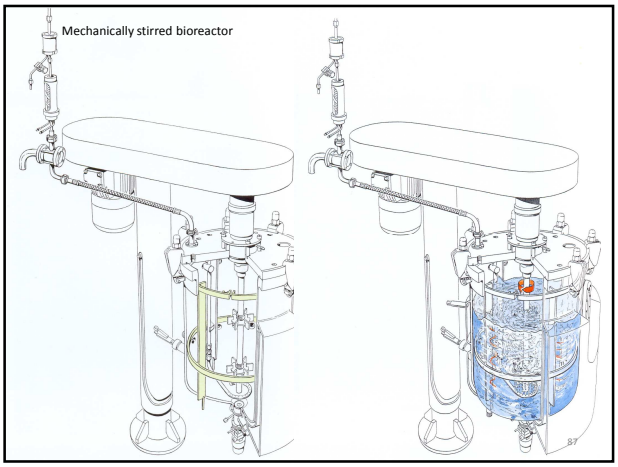
Mechanically stirred bioreactor



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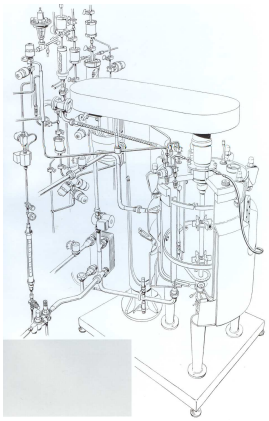

- Piping : steam, compressed air, thermal regulation
- Valves (manual and/or automatic)

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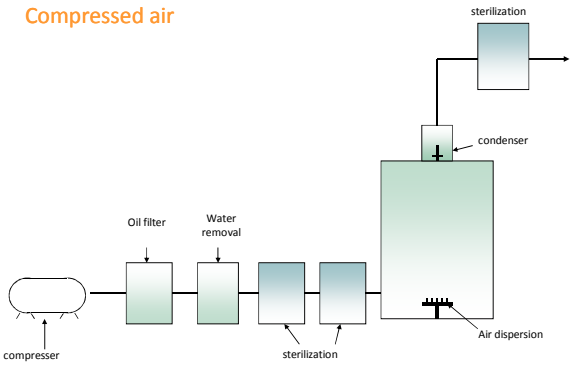
Compressed air



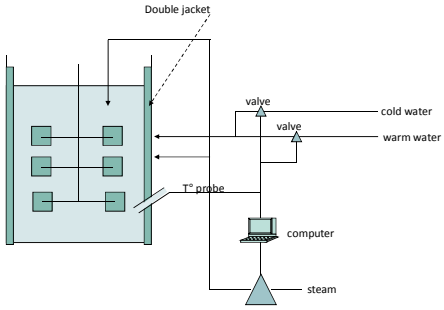
89

Filters and holders

90

Air filter

91



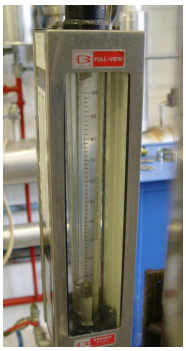
Scheme of a typical temperature regulation loop

92

Pressure

Increase the pressure in the vessel in order to :
-Avoid contaminations
-Increase oxygen solubility in water (or aqueous media)

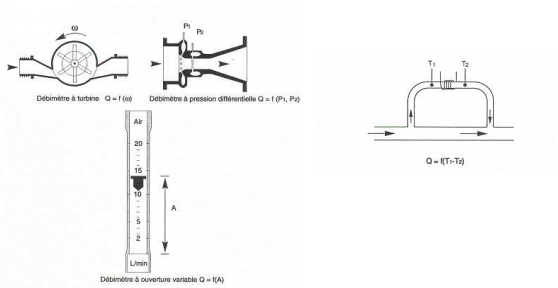
93



Classical flowmeter (ball)

94

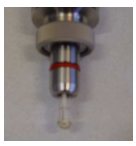
Mass flowmeter



95

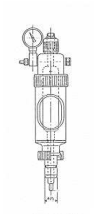
pH management

Relation between voltage and pH:
pH = a + bU
Where b depends on T°

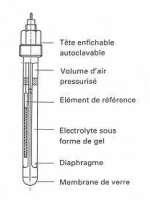


New developement : optical system

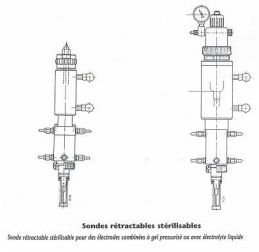
96



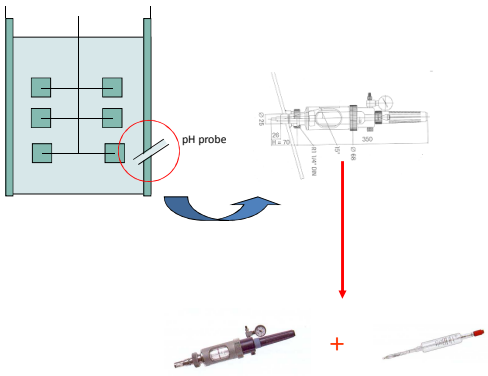
Liquid electrolyte



Solid electrolyte (gel)



97



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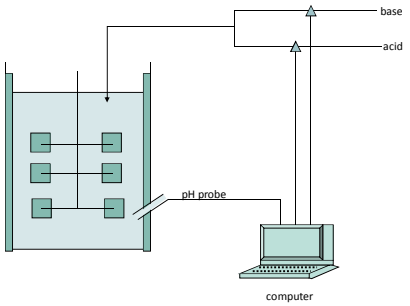
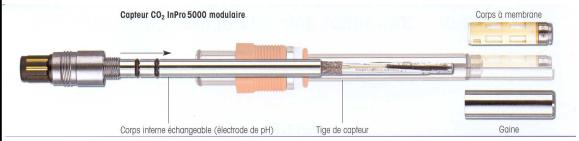
pH regulation



99

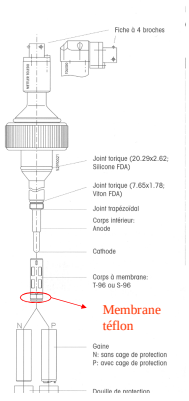
pCO_2



Actual technology not very efficient
New development : optical systems

100

pO_2

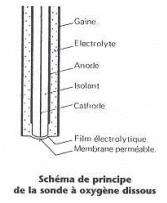


New development : optical systems

101

Schéma de la sonde

La chambre de mesure est séparée du milieu par une membrane en Teflon qui réalise l'étanchéité et est perméable aux gaz. Elle donne une information en mA (4-20 mA)



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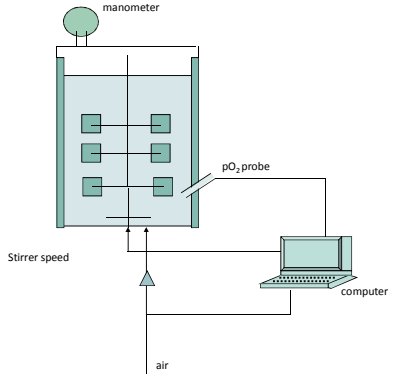
pO_2 regulation



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Gaseous phase CO_2

Gaseous phase O_2

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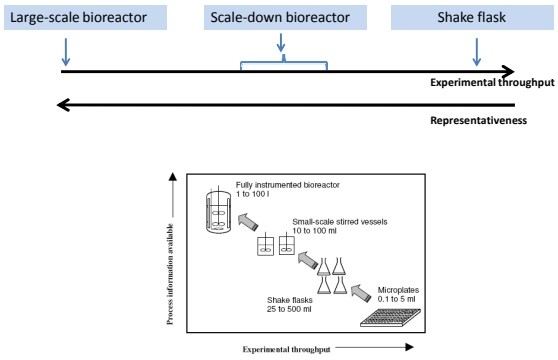
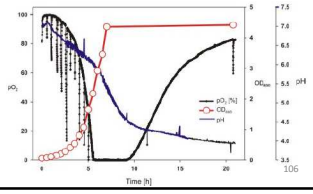
New trends : mini bioreactors



Basic screening tool (erlenmeyer): 90% of microbial culture experiments (at the academic and private level) are performed in erlenmeyer flasks

Vasala *et al.* [2006]



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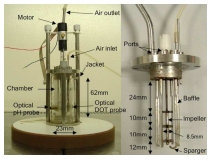
Mini-bioreactor development

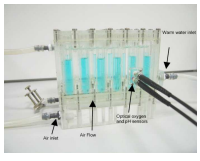
Miniaturized probes

Parallel operated SFR

Mini STR

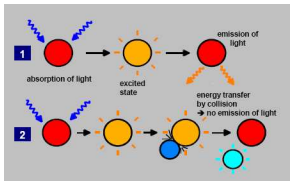


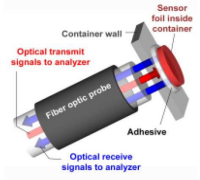
Mini bubble column



New development in sensor technology for bioprocess control : optical systems

Principle : collision between oxygen and dye molecules inducing luminescence quenching





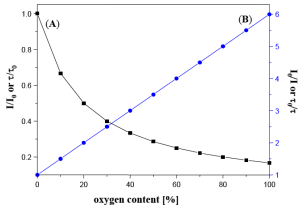
$$\frac{I_0}{I} = \frac{\tau_0}{\tau} = 1 + K_{SV} \cdot [O_2]$$

$$I = f([O_2])$$

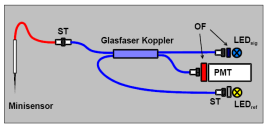
$$\tau = f([O_2])$$

2 parameters depending on oxygen concentration

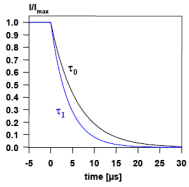
- I: Luminescence intensity in presence of oxygen
- I_0: Luminescence intensity in absence of oxygen
- τ: Luminescence decay time in presence of oxygen
- τ_0: Luminescence decay time in absence of oxygen
- K_{SV}: Stern-Volmer constant (quantifies the quenching efficiency and therefore the sensitivity of the sensor)
- $[O_2]$: Oxygen content



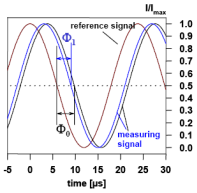
(*A*) Luminescence decrease in the presence of oxygen. (*B*) Stern-Volmer plot.



Sinusoïdal LED excitation signal and daphasage mesruement between reference PTM and measurement PTM



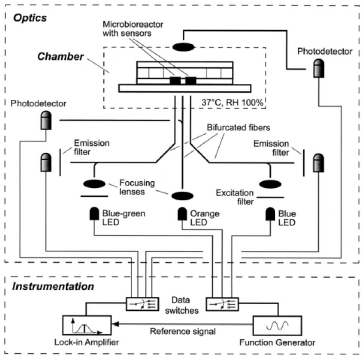
Schematic of the single exponential decay ($\tau_0 > \tau_1$).

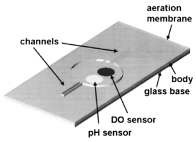


The luminophore is excited with sinusoidally modulated light. Emission is delayed in phase expressed by the phase angle F relative to the excitation signal, caused by the decay time of the excited state

Comparison with polarographic probes:
- -No polarization lag time
- -No oxygen consumption
- -No contamination with other dissolved species
- -Non invasive measurement
- -No membrane, no electolyte required
- -Insensitive to electrical interferences

Microbioreactors





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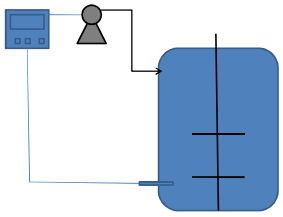
Bioprocess control

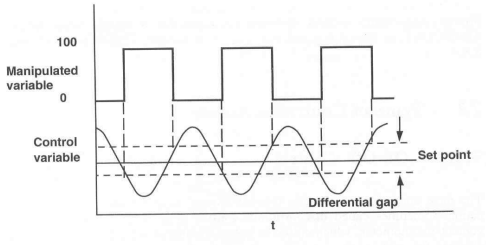
Control strategy : closed loop with *feedback*



Control algorithms

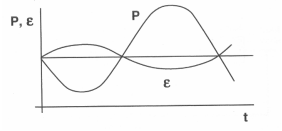
- -ON-OFF control
- -Proportionnel (P) control
- -Proportionnel-integral (PI)
- -Proportionnal-différential
- -Proportionnal-integral-différential (PID)

ON-OFF control



Possibility to introduce a dead zone where controller is never activated
Simple system, but tends to lead to oscillations of the controlled variable

Proportionnal control



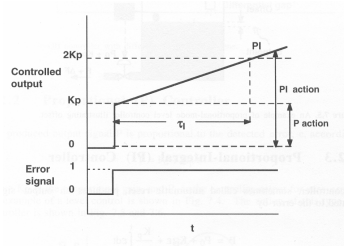
$$P = P_0 + K_p . \varepsilon$$

P : signal output (signal envoyé par le contrôleur)
ε : error (difference between the output signal,i.e. controller impulse, and the input signal, i.e. the probe signal)
K_p : proportionnal gain

Proportional-integral control

$$P = P_0 + K_p . \varepsilon + \frac{K_p}{\tau_I} . \int_0^t \varepsilon . dt$$

τ_I : integration time constant



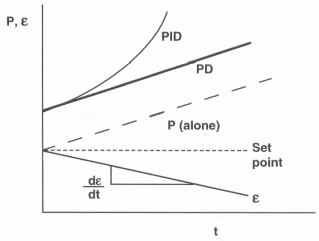
If error is constant

$$\frac{dP}{dt} = \frac{K_p}{\tau_I} . \varepsilon . t$$

Proportional-differential control

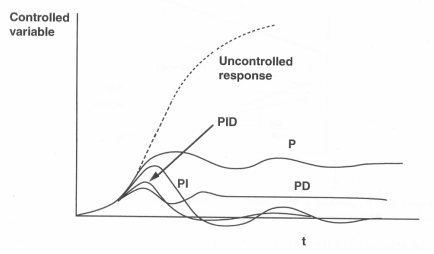
$$P = P_0 + K_p . \varepsilon + K_p . \tau_D . \frac{d\varepsilon}{dt}$$

τ_D : differential time constant



Proportional-integral-differential control (PID)

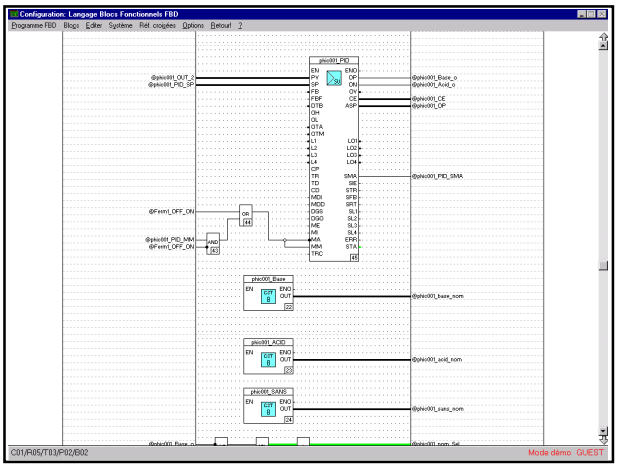
$$P = P_0 + K_p . \varepsilon + \frac{K_p}{\tau_I} . \int_0^t \varepsilon . dt + K_p . \tau_D . \frac{d\varepsilon}{dt}$$



Practical implementation of PID controller :

- At the level of transmitter

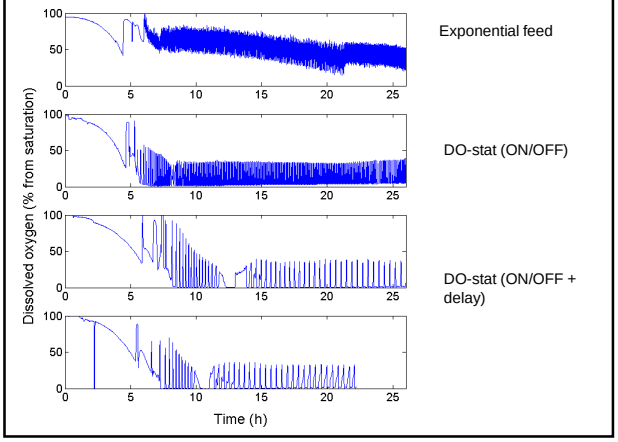
- At the level of central controller

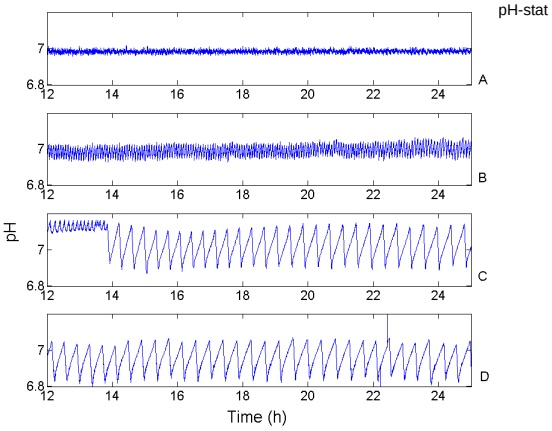


Advanced bioprocess control

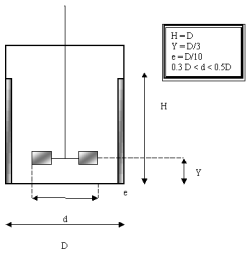
Fed-batch control :

- Exponential feed $Q = Q_0.\exp(\mu.t)$
- RQ (respiratory quotient)
- Feedback loop (biomass, ethanol or glucose probe)
- pH-stat
- DO-stat





1. Basic bioreactor design : standard geometry



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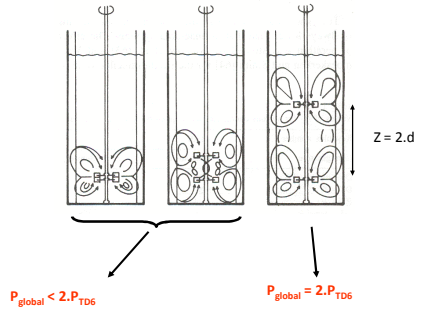
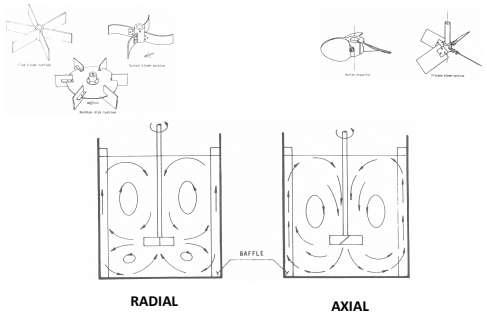


127



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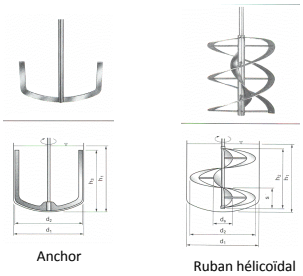
Medium to high voscosity → tangential impeller



Anchor

Ruban hélicoïdal

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Calculation of power dissipated

Three kinds of variables :

-Geometry

-Nature of the fluid

-Mixing related

Vaschy-Buckingham theorem : from 16 to 13 dimensionless variables

$N_p = k \cdot Re^x \cdot Fr^y \cdot We^z \cdot (H/d)^{x1} \cdot (D/d)^{x2} \ldots$

Reynolds number (Re) = $\rho N d^2/\mu$

Power or Newton number (N_p) = $P/\rho N^3 d^5$

Weber number (We) = $\rho N^2 d^3/\sigma$

Froude number (Fr) = $N^2 d/g$

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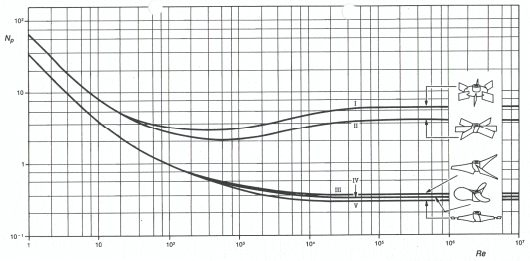
Dissipated power characteristic curve : $N_p = f(Re)$



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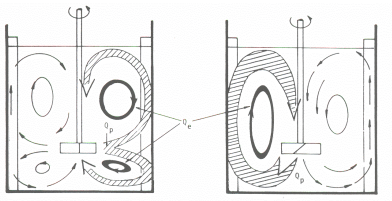
Fluid flow rates calculation



Circulation = entraînement + pompage

Pumping :

$Q_p = N_{qp}.N.d^3$

$t_p = V_l / Q_p$

$f_p = 1 / t_p$

Circulation :

$Q_c = Q_e + Q_p = N_{qc} N d^3$

$t_c = V_l / Q_c$

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Application :

Considering a standard stirred vessel (D = 0,3 m) filled with water. Mixing is promoted by a TD6 impeller at a stirring rate 400 min^{-1}.

TD6 dimensionless numbers:

N_p = 5,5

N_{qp} = 0,85

N_{qc} = 1,51

Calculate the power dissipated, peripheral speed, pumping and circulation flow rates, as well as pumping and circulation times

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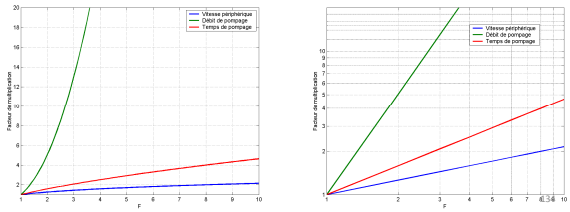
2. Scaling-up basic principle : similarities principle

$$N_{industriel} = N_{lab} \cdot (D_{industriel}/D_{lab})^{\alpha/\beta}$$
Avec $F = D_{industrial}/D_{lab}$

Example : P/V as scaling-up cirteria

$$\frac{P}{V} = \frac{N_p \cdot \rho \cdot N^3 \cdot d^5}{D^3} = \frac{N^3 \cdot D^5}{D^3} = N^3 \cdot D^2$$



Application :

The culture of an anaerobic microorganism as been set up in a pilot-scale bioreator with a working volume of 785 liters (standard vessel : D = 1m). Mixing is ensured by a TD6 impeller at a stirring rate of 180 min^{-1} in a water-like medium.

Calculate the peripheral speed, pumping and circulation flow rate, and pumping and circulation times

The cuture has to be up-scaled to a 50 m^3 bioreactor. How does the above mentionned mixing parameters vary if volumetric dissipated power is the scaling factor (i.e., is kept constant during the up-scaling).

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Answer :

Volume (m^3)	0,79	50,00	
d (m)	0,33	1,33	
N (s-1)	3,00	1,16	
P(W)	610,60	38891,45	
P/V (W/m^3)	777,83	777,83	
Vitesse périphérique (m/s)	3,14	4,84	1,54
Débit de pompage (m^3/s)	0,09	2,27	24,58
Temps de pompage (s)	8,52	22,07	2,59

Multiplicative factor = f(F)

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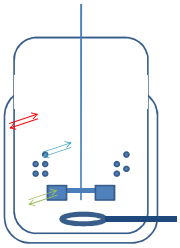
3. Transport operations

- Mass transfer
- Heat transfer
- Momentum transfer



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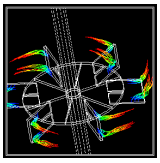
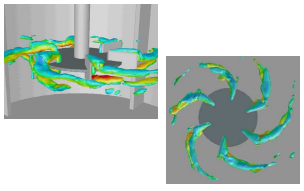
3.1. Gas-liquid dispersion and oxygen transfer



Phase L



Phase L + phase G

Bakker [2000]

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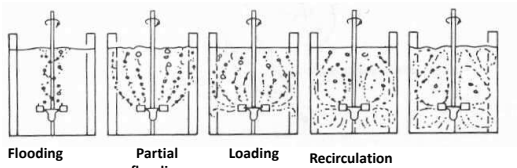
Transition between different gas-liquid flow regimes :

From left to right, the air flow rate is kept constant and stirrer speed is progressively increased

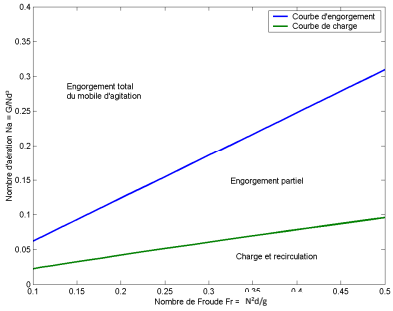


Flooding | **Partial flooding** | **Loading** | **Recirculation**

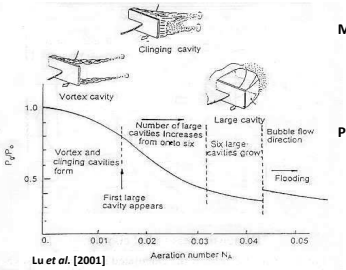
Nienow [1998]

Flow diagram (dimensionless) :

Aeration number (Na) in function of the Froude number (Fr)
Propensity of air flow rate in relation with mechanical stirring intensity



Power dissipated in G-L system : P_g decrease when the air flow rate is increased



Lu *et al.* [2001]

Michel et Miller :

$$P_g = m \cdot (P_o^2\, Nd^3 / G^{0.56})^n$$

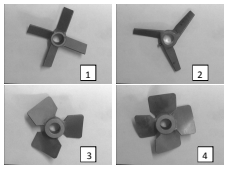
For a TD6 : m= 0,78 n = 0,45

Proportional effect on liquid flow rates:

$$Q_{p\,aéré} = (P_{aéré}/P_0) \cdot Q_p$$
$$Q_{c\,aéré} = (P_{aéré}/P_0) \cdot Q_c$$

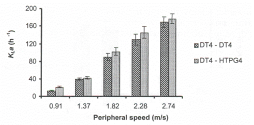
Optimization of the impeller geometry

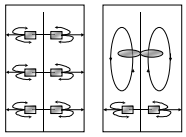
1. Profiled propellers :



1 : Pitched blade turbine
2 : Profiled propeller A310 (solidity ratio : 22%)
3 : Profiled propeller A340 (solidity ratio : 67%)
4 : Profiled propeller A315 (solidity ratio : 87%)

2. Hybrid multi-impeller system :





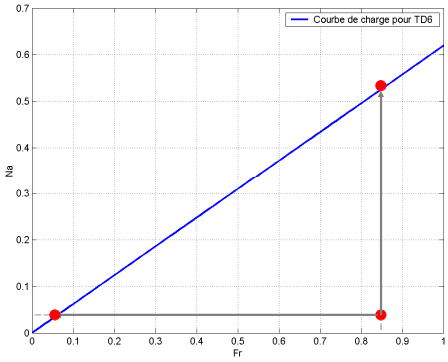
Application :

Consider a standard stirred vessel (D = 0.1m). Mixing is ensured by a TD6 impeller (N = 300 min^{-1}) in a water-like medium. Air is sparged at a flow rate of 0.5 vvm.

The loading curve for the TD6 is :

$$Na = 30 \cdot (d/D)^{3,5} \cdot Fr$$

What is the maximum flow rate acceptable in order to keep an efficient G-L dispersion (and by keeping a constant stirrer rate) ?



Application :

We consider two kinds of impeller : a TD6 and a profiled propeller (d = 0.5 m). The equations for the loading curve are the following:

TD6 : $Na = 30 \cdot (d/D)^{3,5} \cdot Fr$
Propeller : $Na = 6000 \cdot (d/D)^{1,55} \cdot Fr^{2.7}$

Considering the constraints presented in the table, determine the gas-liquid flow regime for each impeller

	N maximum for shear (s^{-1})	G minimum for Oxygen transfer (vvm)
TD6	0,51	0,13
Profiled propeller	0,83	0,19

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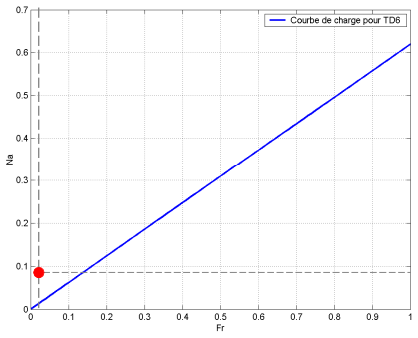
Résolution :



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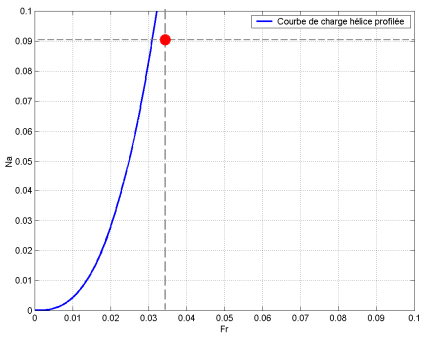
Résolution (suite) :



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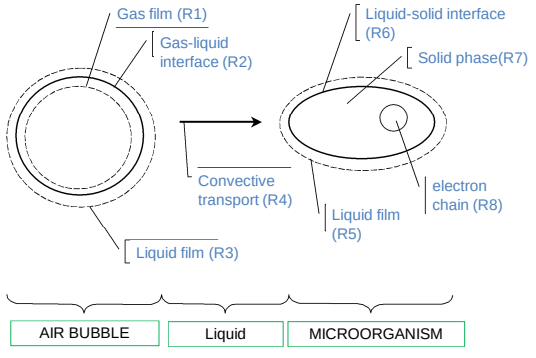
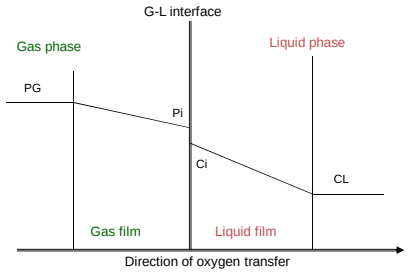
Oxygen transfer

Substrate	Concentration in liquid phase (ppm)	Critical concentration (ppm)	Consumption rate (mmoles/g biomass.h)
Glucose	10.000	100	2.6
Oxygen	7	0.8	7.7





$$q_g = k_g \cdot (p_g - p_i)$$
$$q_L = k_L \cdot (C_i - C_L)$$
$$q_g = q_L$$

$P_i = He \cdot C_i$

Concentrations at the interface are approximated : global exchange coefficients

$$q_g = K_g \cdot (p_g - p^*)$$
$$q_L = K_L \cdot (CL^0 - C_L)$$

$1/K_L = 1/k_L + 1/He.k_G$

Solubility of oxygen is very low in water, i.e. He is very

$q = k_L S(C^0_L - C_L)$

By dividing by volume :

$Q = q/V_L = k_L S/V_L (C^0_L - C_L)$

Amount of oxygen transfered per time :

$Q = k_L a(C^0_L - C_L)$

General form :

$dC_L/dt = k_L.a(C^0_L - C_L) - Q_0$

C_L : dissolved oxygen concentration in liquid phase (mg/L)
C^0_L : maximum dissolved oxygen concentration in liquid phase
$K_l a$: oxygen transfer coefficient (s-1)
Q_0 : oxygen uptake rate by the microorganisms (mg/L.h)

Optimization of the oxygen transfer rate

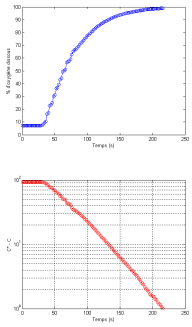
- Increase $K_l a$: nature of te medium
- Increase a : play on mixing performances
- Increase $(C^0_L - C_L)$: play on pressure/temperature to modify the G/L equilibrium

Methods for estimating $K_l a$: oxygen probe (indirect) static gassing-in gassing-out

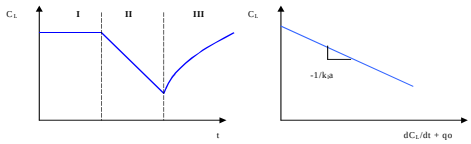
$$\frac{dC}{dt} = k_L a \cdot (C^* - C_L)$$

$$\int \frac{dC}{C^* - C_L} = k_L a \cdot t$$

$$\ln(C^* - C_L) = k_L a \cdot t$$



Methods for estimating $K_l a$: oxygen probe (direct) dynamic gassing-in gassing out



Part II :

$$\frac{dC_L}{dt} = OTR - OUR = k_L a \cdot (C^* - C_L) - q_0$$

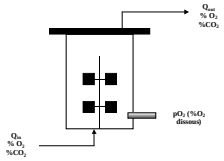
$$k_l a \cdot (C^* - C_L) = 0$$

$$\frac{dC_L}{dt} = OUR = -q_O \cdot X$$

Part III :

$$C_L = C^* - \frac{1}{k_L a} \cdot \left(\frac{dC_L}{dt} + q_0 \right)$$

Methods for estimating $K_l a$: gas balance (direct)



Biological demand for oxygen (q_0) determined by gas-balance analysis:

$$Q_{INO2} = Q_{IN} \cdot 0{,}2094$$

$$Q_{INN2} = Q_{IN} \cdot 0{,}79$$

$$\%N_{2OUT} = 100 - \%O_2 - \%CO_2$$

$$Q_{OUT} = \frac{100}{\%N_{2OUT}} \cdot Q_{INN2}$$

$$Q_{OUTO2} = \frac{\%O_2}{100} \cdot Q_{OUT}$$

At steady-state:

$$\frac{dC_L}{dt} = 0$$

$$q_0 = k_L a \cdot (C^* - C_L)$$

$$k_L a = \frac{q_0}{C^* - C_L}$$

$$k_L a = \frac{Q_{INO2} - Q_{OUTO2}}{C^* - C_L}$$

Application :

Consider a culture of *Penicillium sp*. The reactor has a volume of 50 L and aeration flow rate is of 0,16 vvm (inlet air: 20,94% d'O_2 et 79% de N_2). Culture is performed at 30°C and at atmospheric pre ssure (C^0_L oxygen = 7,6 mg/l).
The data collected from outlet gas analysis and dissolved oxygen probe:

Temps (h)	%O2	%CO2	pO2
2	20,26	0,54	71,7
20	20,1	0,84	0,3

Calculate $K_L a$ for the two culture times

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$K_L a$ calculation :

Van't Riet correlations :

- **For distilled water (coalescing) :**

$$k_l a = 0.026 \cdot (P/V)^{0.4} \cdot (G/S)^{0.5}$$

- **For medium containing electrolytes (non coalescing) :**

$$k_l a = 0.002 \cdot (P/V)^{0.7} \cdot (G/S)^{0.2}$$

General form for pneumatic reactor (without mechanical stirring):

$$k_l a = C \cdot (G/S)^{\alpha}$$

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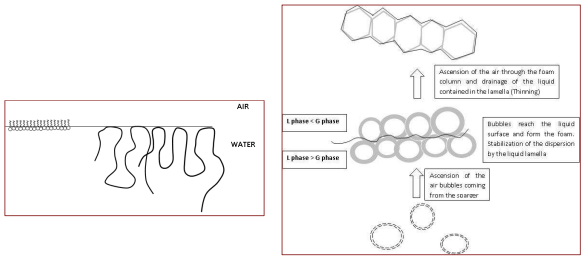
Application :

Consider a standard stirred vessel (D = 0.1m). Mixing is ensured by a TD6 impeller (N = 300 min^{-1}) in a water-like medium. Air is sparged at a flow rate of 0.5 vvm.

Calculate the power dissipated and the $K_L a$ if we consider a non-coalescing medium.

OTR limiting phenomena : foam



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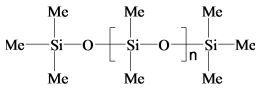
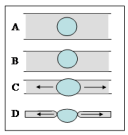
Chemical antifoam : silicon backbone





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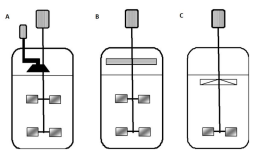
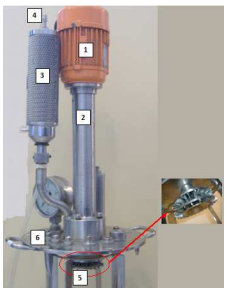
Mechanical defoamer





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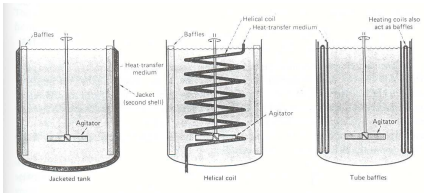
3.2. Heat transfer



$$Q = U.A_i.(T_o - T_i)$$

$$\frac{1}{U} = \frac{1}{h_i} + \frac{e}{\lambda_e} + \frac{1}{h_e}$$

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Dimensional analysis :

Variables to be considered : D, λ , μ , ρ , υ , Cp et h.

Dimensions to be considered : M, L, T, et t.

→Nusselt number : $Nu=\frac{h.D}{\lambda}$

→Reynolds number : $\mathrm{Re}=\frac{\rho.v.D}{\mu}$

→ Prandlt number : $\mathrm{Pr}=\frac{C_p.\mu}{\lambda}$

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Dimensional analysis: $Nu = C.Re^{\alpha}.Pr^{\beta}.Vi^{\gamma}$

-TD6 with heat jacket:

$$Nu = 0{,}74.\mathrm{Re}^{0{,}66}.\mathrm{Pr}^{033}.Vi^{0{,}14}$$

- Pitched blade turbine with serpentine:

$$Nu_{serp}=0{,}00752.\mathrm{Re}^{0{,}722}.\mathrm{Pr}^{0{,}4}.Vi^{0{,}2}$$

- Propeller with serpentine :

$$Nu_{serp}=0{,}011.\mathrm{Re}^{0{,}67}.\mathrm{Pr}^{0{,}37}.Vi^{0{,}2}$$

- TD6 with serpentine :

$$Nu_{serp}=0{,}0205.\mathrm{Re}^{0{,}67}.\mathrm{Pr}^{037}.Vi^{0{,}2}$$

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Relation between heat transfer and microbial growth

In intensive aerobic processes, heat released is proportional to the amount of oxygen consumed :

$$\mathbf{Q_{exo} = Y_{ho}.OTR}$$

Heat extracted by heat exchanger:

$$\mathbf{Q_{ech} = k.A.\Delta t}$$

At steady-state, heat generated by microbial growth is proportional to heat removed at the level of the exchanger:

$$\mathbf{k.A.\Delta t = Y_{ho}.OTR}$$

But, scale-up problem, as $A \sim V_L^{2/3}$

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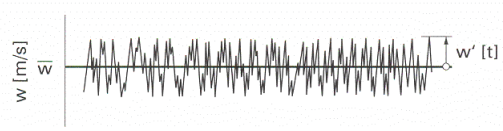
3.3. Momentum transfer

Characteristics of urbulence :



$w_i = w + w'$

$k = ½ (w'^2_x + w'^2_y + w'^2_z)$

$w'^2_x = w'^2_y = w'^2_z$

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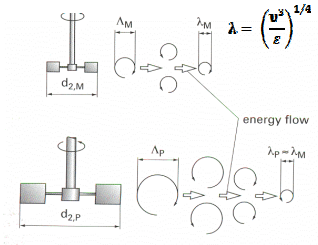
Kolmogoroff : eddies scale



$$\lambda = \left(\frac{\nu^3}{\varepsilon}\right)^{1/4}$$

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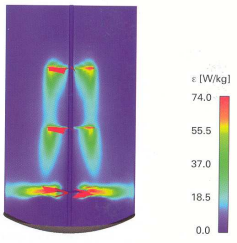

Limite de Kolmogoroff → en réalité, on a une répartition du taux de dissipation d'énergie.

La plupart de la dissipation prend place dans l'environnement direct du mobile d'agitation.

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Mixing time measurement

Temps

2 phenomena:
- Circulation
- Turbulence

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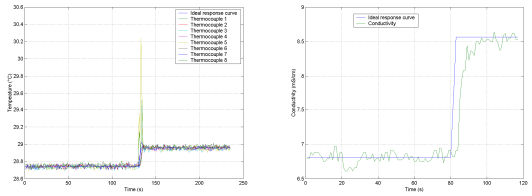
Methods involving probes:

Traceur	Sonde
Acide-base	pH
Solution saline	Conductivité
Solution chauffée	Thermocouple



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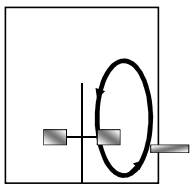
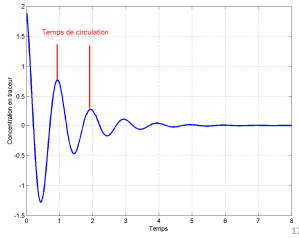
Equations based on circulation

$t_m.N = 3,9.(d/D)^{-3}.N_{qc}^{-1}$





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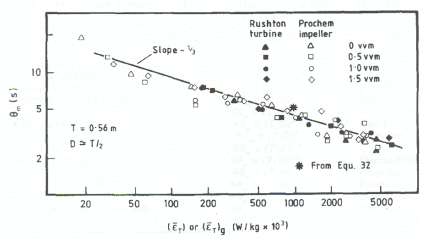
Correlation based on turbulence

$t_m = 5,9.D^{2/3}.\varepsilon_T^{-1/3}.(d/D)^{-1/3}$



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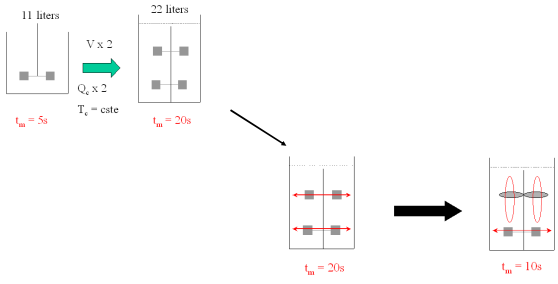
Mixing time in function of the number of agitation stages:



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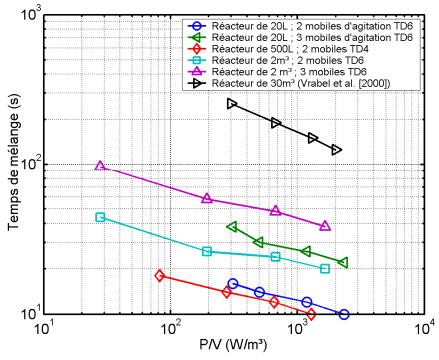
Evolution of mixing time during scale-up :



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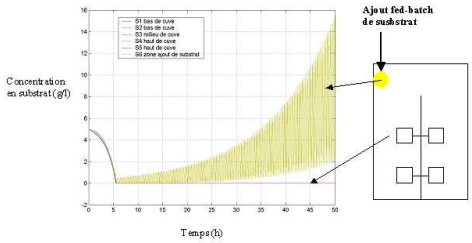
Example of the impact of mixing time on process efficiency : concentration gradient during a fed-batch process



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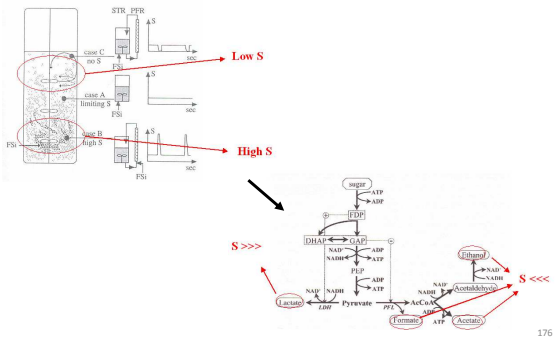
Effect of concentration gradient on cell physiology : example of *Lactococcus lactis*



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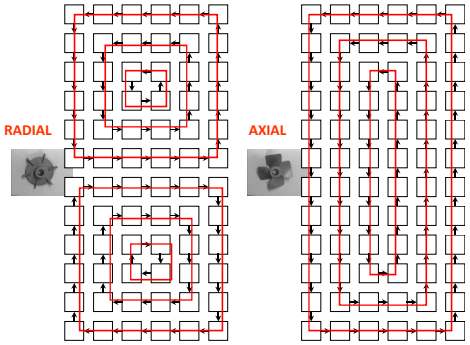
Structured model for bioreactor hydrodynamics

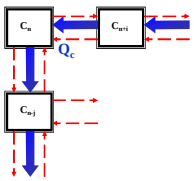
- Compartment model
- Network-of-zones



177

Mathematical basis

Evolution of concentration with time for each compartment :



The evolution of C_n is described by an ordinary differential equation (ODE):

$$V \cdot \frac{dC_n}{dt} = Q_c \cdot (C_{n+i} - C_n) + Q_e \cdot (C_{n+i} + C_{n-j} - 2C_n)$$

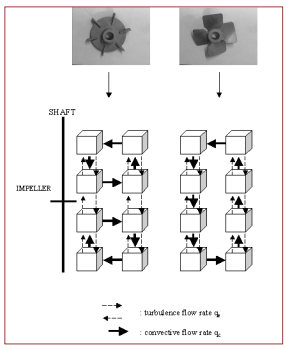
Convective fluxes (Q_c) are calculated (circulation flow rate) and the turbulence flow rates are estimated on the basis of mixing time experiments

System of ODEs is numerically resolved by a Runge-Kutta routine (e.g., ode45 routine in MatLab)

178

Application :

A stirred bioreactor (V = 500L) is equiped with a TD6 or a profiled propeller. The hydrodynamics can be modelled by the compartment principle, leading to the model structure shown on the figure.
Write the ODEs system for the evolution of the concentration of a specie C in the compartments



179

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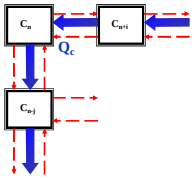

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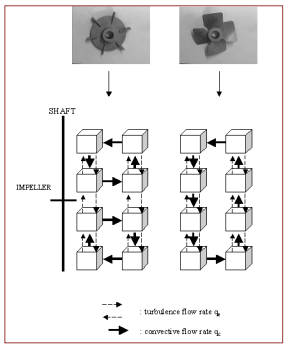
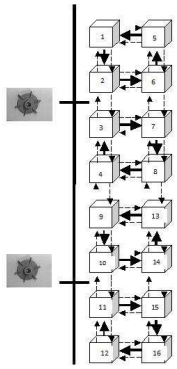


181

Application :



Consider a two-staged stirred bioreactor (TD6-TD6) as shown on the figure
If qc = 0.1 m³/s et qe = 0.2 m³/s
Model and simulate the mixing time when a pulse is added at the level of the first compartment

182

Resolving the ODEs system with MatLab :

First file.m :
- function y = f(t,y)
- Constant
- Algebraic equations
- Ordinary differential equations, ODEs (in matrix form)

Second file.m :
- Solver ode
- function plot

183

Application of structured modelling procedure to oxygen transfer

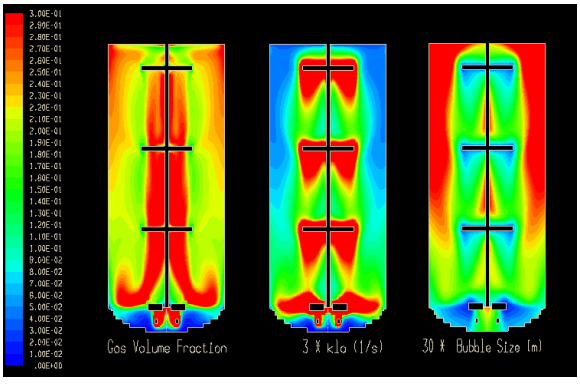
2 scaling-up criteria:

→ Air flow rate: G/V (en v.v.m.) ou G/S (en m/s)

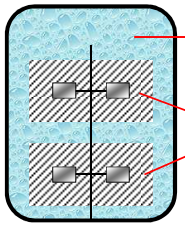
→Agitation rate : πNd ou P/V

But doesn't necessarily lead to reliable results, considering the spatial distribution of basic hydrodynamic parameter inside the vessel

184



185



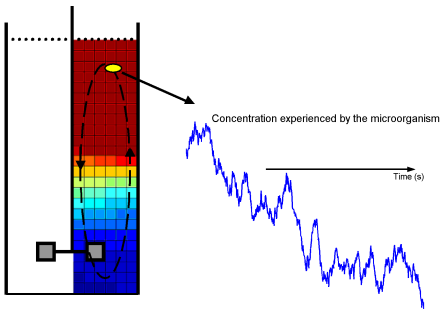
Bubble region → $k_la = a \cdot (G/S)^{\alpha}$

Well mixed regions → $k_la = a \cdot (P/V)^{\alpha} \cdot (G/S)^{\beta}$

Oosterhuis N.M.G., Kossen N.W.F. [1993]

186

Bioreactor hydrodynamic structured model : stochastic framework



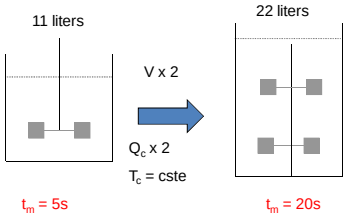
Case study 1 : modelling approaches for bioreactor hydrodynamics

Traditional approach

Scale-up

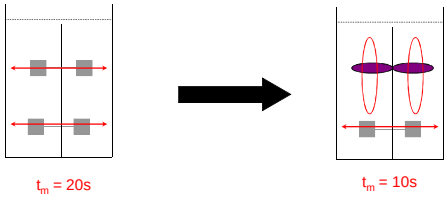
Problem 2

Evolution of the performance criteria for multi-agitated system



Traditional approach

Scale-up



Model is needed to understand the evolution of the mixing mechanism

4. Bioreactor hydrodynamics : local approach

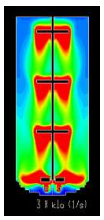
FIRST : homogenisation process

Mechanical constraints → P/V limitation → increase of the mixing time

Consequences : gradients establishement (substrate, pH, O_2,…)

Fed-batch process

Enfors *et al.* [2001] Journal of biotechnology



Oxygen transfer

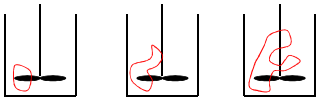
Bakker [2003]

SECOND : circulation process

Increase of the broth volume → increase of the variability (the randomness) of the circulation paths taken by the microorganisms

→ this phenomena is not often considered

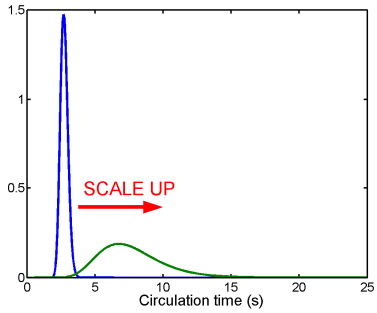
If we observe three distincts circulation paths followed by a microorganism :



There is not a single value of circulation time, but a CTD

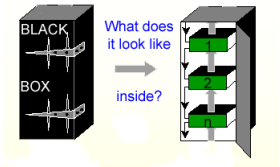
Namdev *et al.* [1992] Biotechnology and bioengineering

Circulation time distribution (CTD)

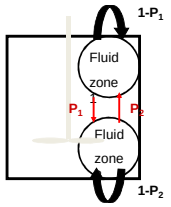


→ **Increase of the randomness at the level of the circulation paths followed by microorganisms**

Compartment modelling concept (shift from a black box to a grey box)



Stochastic compartment modelling concept



Probability matrix or transition matrix

$$T=\begin{bmatrix} 1-P_1 & P_1 \\ P_2 & 1-P_2 \end{bmatrix}$$

Mathematical implementation of stochastic models :

Markov chain

Let S be the discrete state vector of the previous system, the following equation allow us to calculate the discrete time evolution of the system:

$$\begin{bmatrix} S_1 \\ S_2 \end{bmatrix}_t \times \begin{bmatrix} 1-P_1 & P_1 \\ P_2 & 1-P_2 \end{bmatrix} = \begin{bmatrix} S_1 \\ S_2 \end{bmatrix}_{t+1}$$

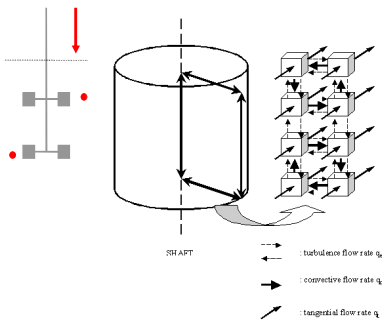
This equation can be generalised :

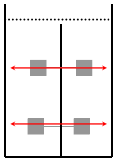
$$S_t = S_0 . T^t$$

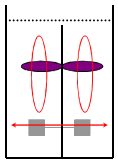
Don't keep track of the particle history, but computationaly ligth

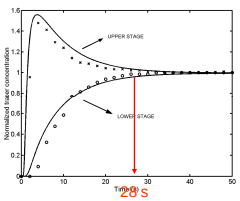
→ **Well suited for the simulation of the homogenisation of a solute (large amount of molecules, particles)**

Markov chain mixing model

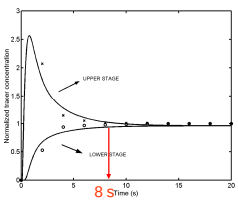








28 s

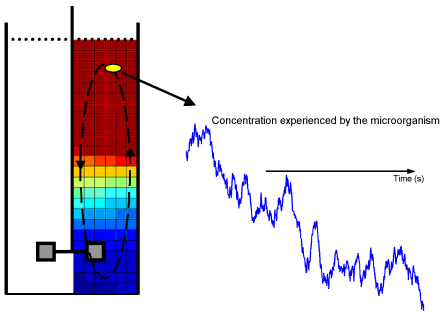


8 s

Hydrodynamic modelling of bioreactors

Superimposition of the two stochastic formulations

« Microorganisms view »



5. Microbial growth in bioreactors : physiological variables

Case study : fed-batch culture of *S. cerevisiae* in large scale bioreactor

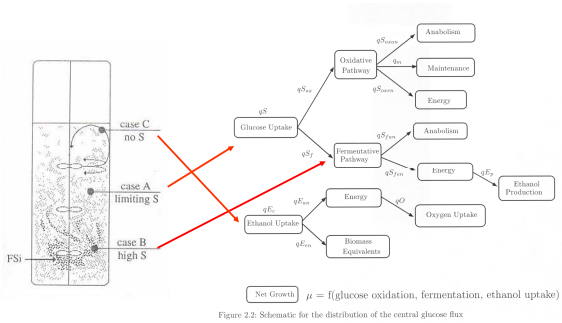
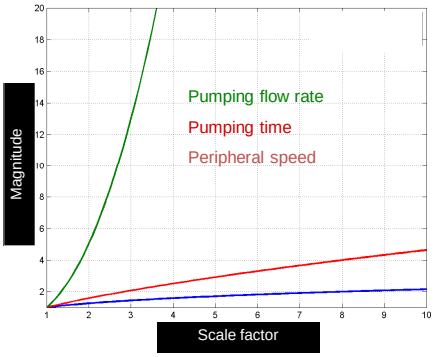
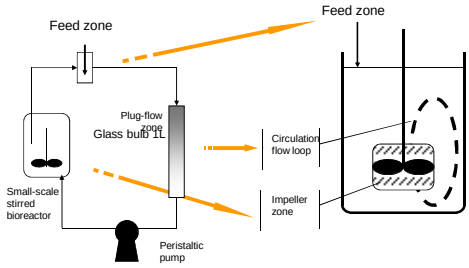


Figure 2.2: Schematic for the distribution of the central glucose flux





SCALE-DOWN REACTOR

LARGE-SCALE REACTOR

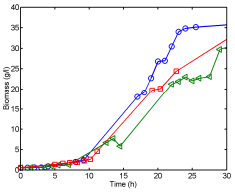
Microbial kinetics :

20L stirrered bioreactor (RTD6 ; working volume 10L + glass bulb for SDRs)

Regulation : pH 5.5 ; T°30°C ; pO $_2$ 30% ; air flow rate (no regulation for the nonmixed part of the SDR)

Exponential feed of glucose (start after 5 hours) : $F = F_0 \exp(\mu t)$

($F0 = 0.086$ ml/min; $\mu = 0.005$ min^{-1})



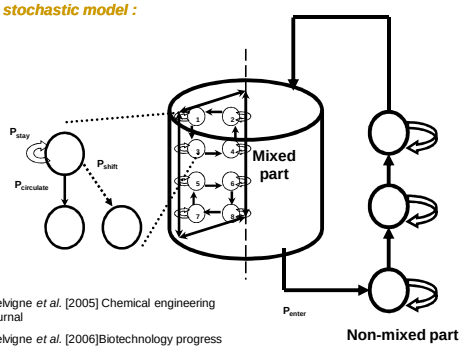
20L stirred bioreactor

SDR Q_{recirc} = 39 L/h

SDR Q_{recirc} = 18 L/h

Reactor	Biomass yield Y_{xs}
Classical bioreactor	0.48
SDR recirculation flow rate Q = 18 l/h	0.36
SDR recirculation flow rate Q = 39 l/h	0.45

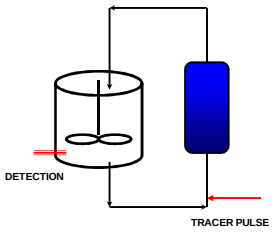
SDR stochastic model :

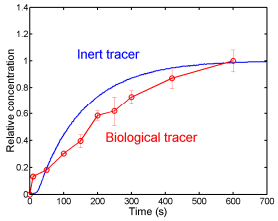


Delvigne *et al.* [2005] Chemical engineering journal

Delvigne *et al.* [2006]Biotechnology progress

Model validation :





- **Inert tracer (NaCl solution)**
- **Biological tracer (CFDA SE stained cells)**

6. Perspective : modelling the microbial response to bioreactor environmental fluctuations

We have described the physical parameters

BUT

What about the biologicals implications

What is the microbial response in front of these environmental fluctuations ?

TO MAKE THE LINK

What about the environmental sensing capabilities of microorganisms ?

In the case of S. cerevisiae :

- Cells react very rapidly in front of glucose fluctuations
- Glucose sensing mechanisms are well documented but no quantified data available

Rolland *et al.* [2005] FEBS journal

Özcan *et al.* [1999] Microbiology and molecular biology reviews

Roland *et al.* [2002] FEMS yeast research

Johnston *et al.* [2005] Biochemical society trans.

- The cell don't sense all the environmental fluctuations (sensors and some specific metabolic pathways act as intracellular homeostasis devices) c

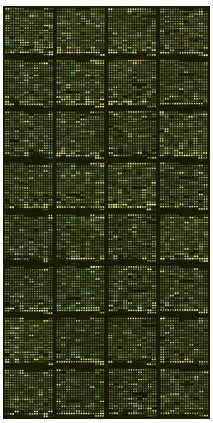
Wolf *et al.* [2005] Journal of theoretical biology

Rao *et al.* [2002] Nature

Important in order to make the link between the physical and the biological parameters

Investigation tool : DNA microarray

Transcriptomic response to gradient stress (superimposition of a « perfectly mixed » small scale bioreactor with a scale-down reactor)



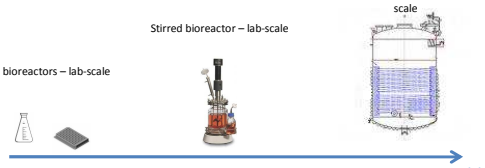
Case study 2 : Whole cell biosensors for the detection of mixing imperfections

Background
Bioprocess scale-up – general scheme

Stirred bioreactor – industrial scale

Stirred bioreactor – lab-scale

Shaken bioreactors – lab-scale

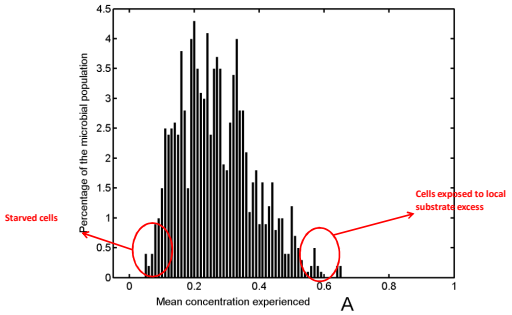


Reactor dimension (D)

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

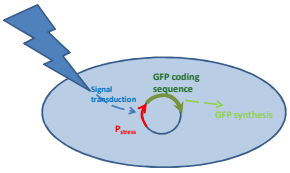


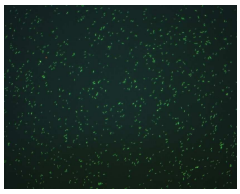
Delvigne *et al.* [2006] Chemical engineering journal

Experimental strategy
Fluorescent reporter system

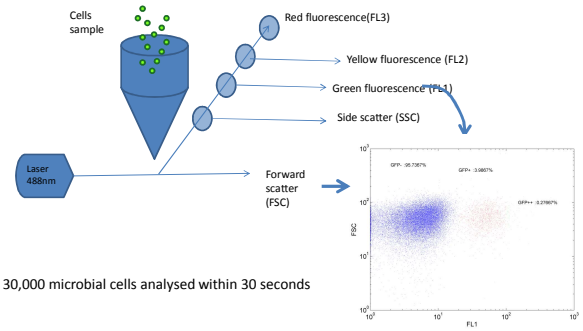
Basic principle :
Using the microbial population as « physiological tracer » for the estimation of the bioreactor mixing and transfer efficiency (potentially capturing the stochasticity linked with the CTD)

Extracellular simuli (S, O2, pH)





Experimental strategy
Flow cytometry – an efficient tool to characterize microbial population heterogeneity

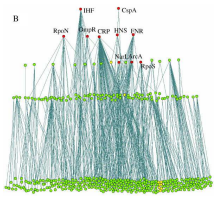


30,000 microbial cells analysed within 30 seconds

Experimental strategy
Choosing the right ORF for my application

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

Transcriptional network



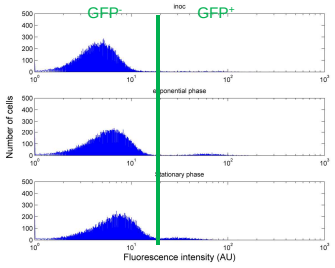
Transcriptional network – hierarchical classification

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

Results
Screening among an E. coli GFP clones library

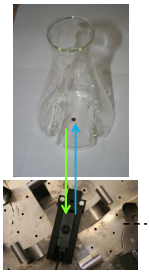
Cultivation in shake flasks on mineral medium

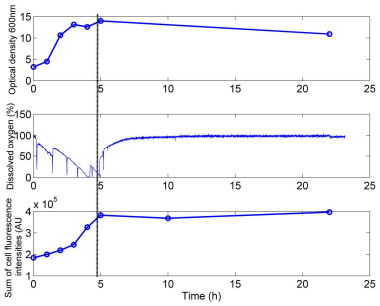
prpoS::gfp



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

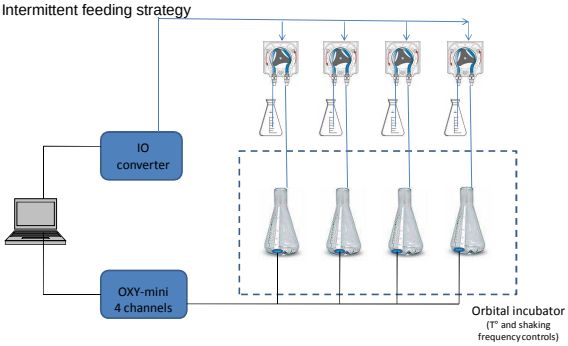
Shake flask : easy to handle, well suited to perform parallel cultures, but lack of representativeness compared to the performances of stirred bioreactors





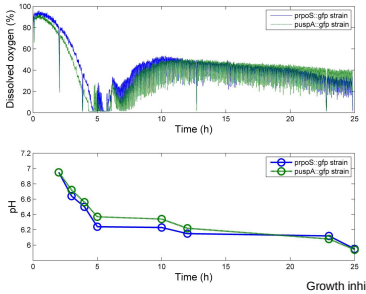
Results
Screening among an E. coli GFP clones library
Representativeness of shaken bioreactor

Intermittent feeding strategy



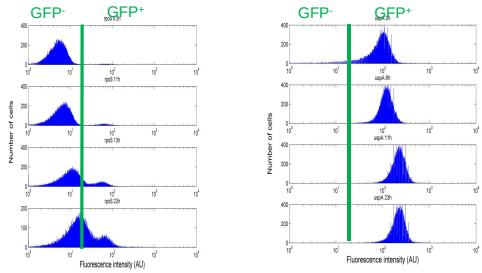
Results
Screening among an E. coli GFP clones library
Representativeness of shaken bioreactor

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

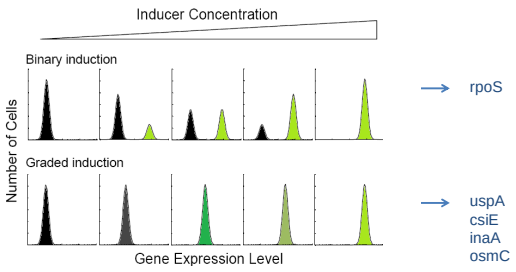
Results
Screening among an E. coli GFP clones library
Representativeness of shaken bioreactor



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

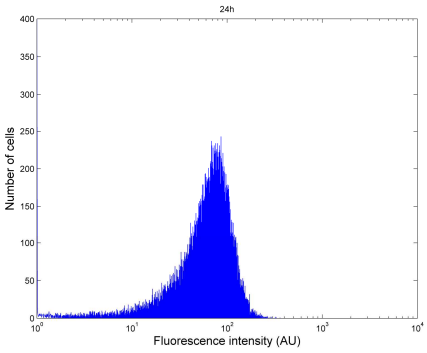
Binary mode of gene expression → sources :
-Short mRNA and protein half-lives
-High sensitivity for the detection of the reporter protein

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

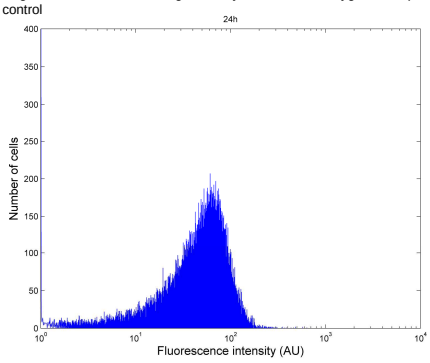
Results
Behaviour of prpoS::gfp strain in fed-batch stirred bioreactor

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

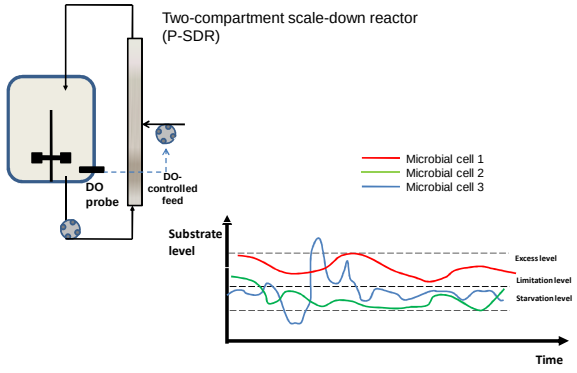


Results
Behaviour of prpoS::gfp strain in fed-batch stirred bioreactor

Basic observations :

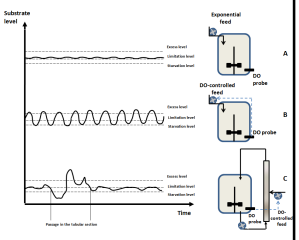
- Binary mode for GFP expression at the end of the batch phase and during the transition from batch to fed-batch phase
- After the induction of the major part of the population (all the cells are in the GFP+ state), graded mode of GFP expression is observed
- Successive glucose excess tends to slow down the binary expression phase

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



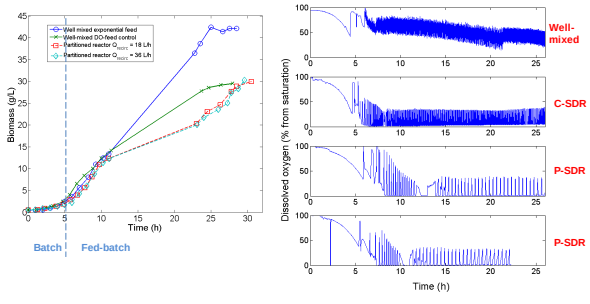
Results
Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor

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



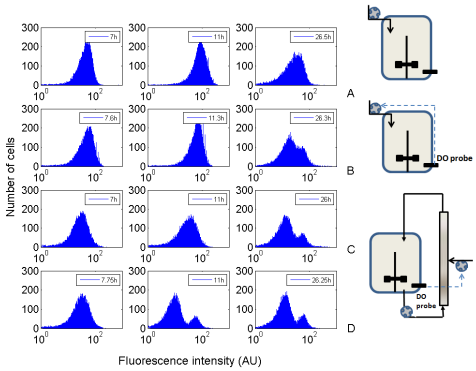
Delvigne F. *et al.* [2009] *Microbial cell factories* , **8**:15

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



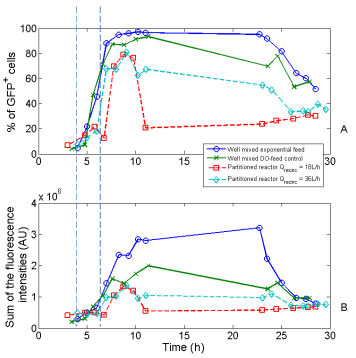
Results

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



Results

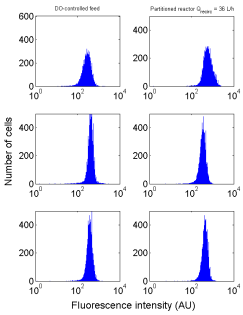
Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor



Results

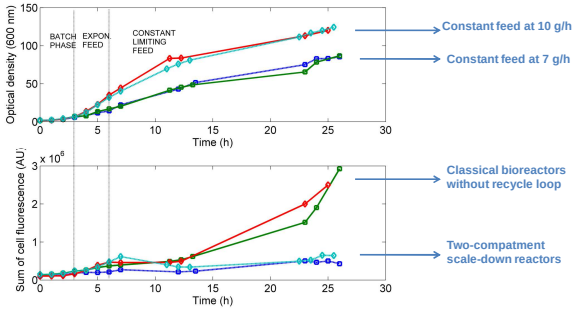
Behaviour of prpoS::gfp strain in two-compartment scale-down bioreactor

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



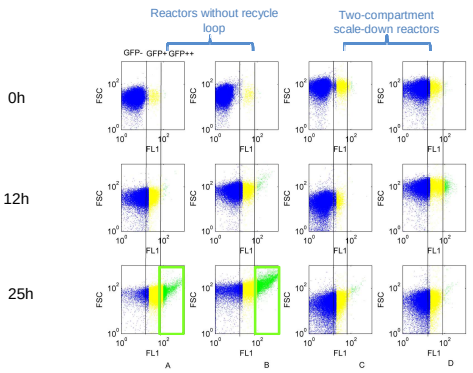
Results

Cultures performed under constant glucose feed



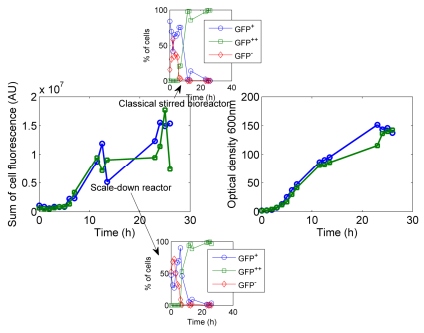
Results

Cultures performed under constant glucose feed



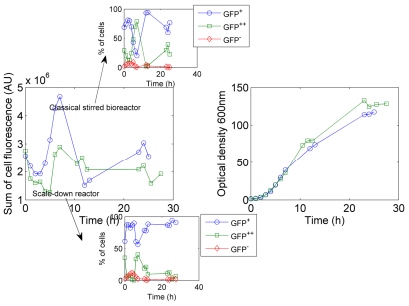
Results

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



Results

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



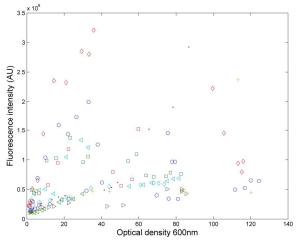
To be validated by using a DO-controlled feed

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

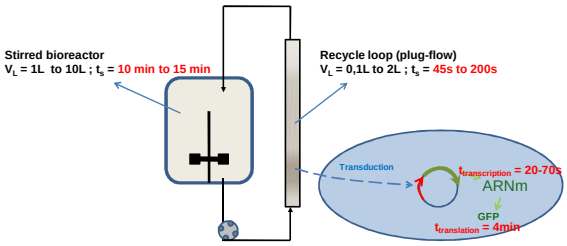
prpoS::GFP strains seems to react to the degree of homogeneity inside the bioreactor :

Homogenous reactor : GFP+

Inhomogenous reactor : GFP-

Perspectives and conclusion

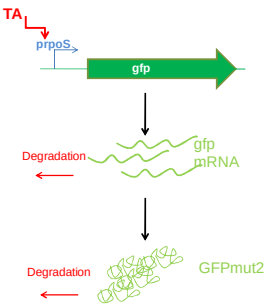
Two questions have to be raised :
- Flow cytometry combined with P_{stress}::GFP expression → impact of extrinsic fluctuations
  What about the intrinsic fluctuations ?
- Characteristic times of hydrodynamic mechanisms compared with those of the biological processes behind GFP synthesis



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_{mi},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 :

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

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

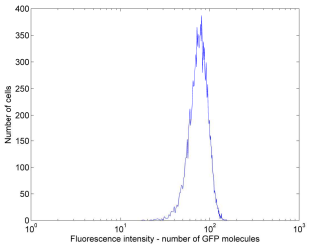
8 rates (including the characteristic time constants) to specify

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