Modelling, Monitoring and Control of Biosystems

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A biological process is...

- The development (= growth) of micro-organisms (= biomass)
- By the consumption of a nutrient (= substrate)
- Under favourable environmental conditions : temperature, pH, stirring, aeration,...

Objectives :

1. Biomass production (e.g. baker's yeast)
2. Product synthesis (e.g. ethanol, biodegradable polymer, antibiotics, methane,...)
3. Waste-water treatment

R&D in biotechnology is aimed at improving productivity

3 approaches :

1. Microbiological/biochemical approach
   - selection of micro-organisms, nutrients,...
   - genetic modifications
2. Process engineering approach
   - operating modes/conditions
   - efficient techniques/processes
3. Process control/system theory approach

   productivity maximisation via an on-line optimizing operation of the bioprocess
   (on the basis of a dynamical model of the process)

There are two main obstacles for controlling bioprocesses

1. Modelling difficulties
   - How to account for the numerous factors that influence the biochemical reactions (including the growth)?
   - Nonlinear and non stationary models

2. Measuring difficulties
   - Absence of cheap and reliable sensors for the key process variables

Outline

- Introduction
- Bioprocess models and model properties
- Parameter identification (including optimal experiment design)
- State estimation
- Some typical bioprocesses
- Numerical simulation and PDE's
- Control

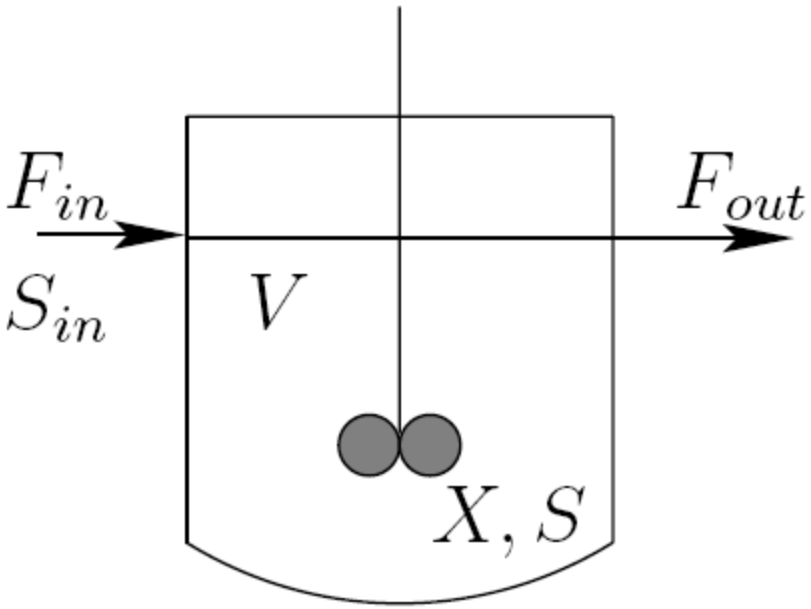
  - optimal & adaptive extremum seeking control

  - specific issues in bioprocesses

Bioprocess models

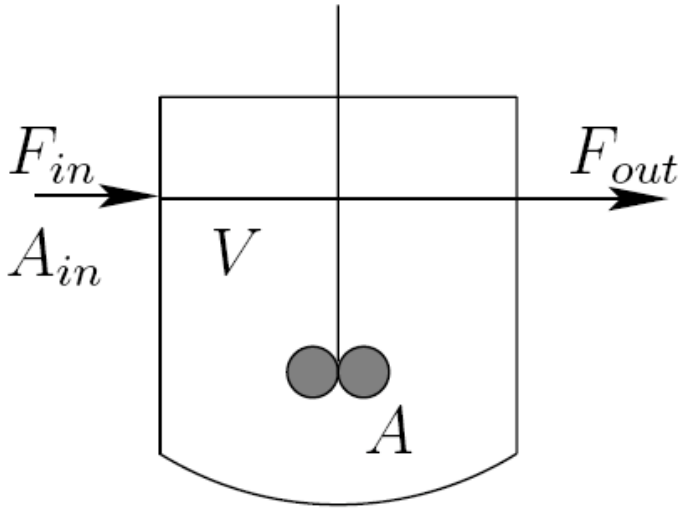
- The basic mathematical model
- The general dynamical model
- The reaction rates
- Extensions
    - link with metabolic engineering
    - population balance
    - microbial ecology
- Model properties

The basic mathematical model



- Autocatalytic reaction : S --> X
- Operating modes :
  - batch : $F_{in} = F_{out} = 0$
  - fed-batch : $F_{in} \neq 0,\ F_{out} = 0$
  - continuous : $F_{in} = F_{out},\ V$ constant

Notion of mass balance



Component A (substrate, product, biomass)

---> dynamical mass balance :

$$\left[\begin{array}{c}\text{time variation}\\ \text{of the mass}\\ \text{of A}\end{array}\right] = \left[\begin{array}{c}\text{mass inflow}\\ \text{of A into}\\ \text{the reactor}\end{array}\right] - \left[\begin{array}{c}\text{mass outflow}\\ \text{of A from}\\ \text{the reactor}\end{array}\right] - \left[\begin{array}{l}\text{mass of A}\\ \text{produced/}\\ \text{consumed}\\ \text{via reactions}\end{array}\right]$$

$$\frac{d(VA)}{dt} = F_{in}A_{in} - F_{out}A + Vr$$

Recall that :

$$\frac{d(VA)}{dt} = V\frac{dA}{dt} + A\frac{dV}{dt}$$

Therefore :

$$\frac{dA}{dt} = \frac{F_{in}}{V}A_{in} - \frac{F_{out}}{V}A - \frac{A}{V}\frac{dV}{dt} + r$$

- batch : $F_{in} = F_{out} = 0$ --> V constant and $\frac{dV}{dt} = 0$

$$\text{--> } \frac{dA}{dt} = r$$

- fed-batch : $F_{out} = 0$ and $\frac{dV}{dt} = F_{in}$

$$\text{--> } \frac{dA}{dt} = \frac{F_{in}}{V} A_{in} - \frac{A}{V}\frac{dV}{dt} + r$$

- continuous : $F_{in} = F_{out} \neq 0$ and V constant

$$\text{--> } \frac{dA}{dt} = \frac{F_{in}}{V} A_{in} - \frac{F_{in}}{V} A + r$$

- Biomass growth :

$$\frac{dX}{dt} = \mu X - DX$$

- Substrate consumption :

$$\frac{dS}{dt} = -k_1 \mu X + DS_{in} - DS$$

- growth rate : μX
  with μ : specific growth rate

- yield coefficient : k_1

- dilution rate : $D = \frac{F_{in}}{V}$

The dynamics of other components

- Liquid product (growth associated)

$$\frac{dP}{dt} = k_2 \mu X - DP$$

P : liquid product concentration

- Gaseous product

$$Q = k_3 \mu X$$

Q : gaseous product mass flowrate

- Aerobic cultures ---> (dissolved) oxygen

$$\frac{dC}{dt} = -k_4 \mu X - DC + Q_{O_2,in} - Q_{O_2,out}$$

C : dissolved oxygen concentration

$Q_{O2,in}$, $Q_{O2,out}$: gaseous oxygen inlet and outlet flowrates

If the gas-liquid transfer is limiting :

$$Q_{O_2,in} - Q_{O_2,out} = k_L a(C_S - C)$$

$k_L a$: gas-liquid transfer coefficient

C_s : oxygen saturation concentration

The general dynamical model

$$\frac{dx}{dt} = Kr(x) + F - Q - Dx$$

conversion transport dynamics

x : vector of the concentrations
K : matrix of the stiochiometric coefficients
$r(x)$: vector of the reaction rates
F : vector of feeding rates
Q : vector of the produced gas flow rates

Example #1 : simple microbial growth

S ---> X $\qquad\qquad$ $r = \mu X$

$$\begin{cases} x = \begin{bmatrix} X \\ S \end{bmatrix}, K = \begin{bmatrix} 1 \\ -k_1 \end{bmatrix}, r = [\mu X] \\ F = \begin{bmatrix} 0 \\ DS_{in} \end{bmatrix}, Q = 0 \end{cases}$$

General dynamical model

$$\frac{d}{dt}\begin{bmatrix} X \\ S \end{bmatrix} = -D\begin{bmatrix} X \\ S \end{bmatrix} + \begin{bmatrix} 1 \\ -k_1 \end{bmatrix}\mu X + \begin{bmatrix} 0 \\ DS_{in} \end{bmatrix}$$

Example #2 : two parallel reactions

Autocatalytic reaction : S ---> X $\quad r_1 = \mu X$

Catalytic reaction : $S + X$ ---> $X + P$ $\quad r_2 = \nu X$

(P in gaseous form)

Dynamical model :

$$\frac{dX}{dt} = \mu X - DX$$

$$\frac{dS}{dt} = -k_1 \mu X - k_2 \nu X + DS_{in} - DS$$

$$\frac{dP}{dt} = \nu X - DP - Q_1$$

Matrix form of the dynamical model :

$$\frac{d}{dt}\underbrace{\begin{bmatrix} X \\ S \\ P \end{bmatrix}}_{x} = -D\begin{bmatrix} X \\ S \\ P \end{bmatrix} + \underbrace{\begin{bmatrix} 1 & 0 \\ -k_1 & -k_2 \\ 0 & 1 \end{bmatrix}}_{K}\underbrace{\begin{bmatrix} \mu X \\ \nu X \end{bmatrix}}_{r} + \underbrace{\begin{bmatrix} 0 \\ DS_{in} \\ 0 \end{bmatrix}}_{F} - \underbrace{\begin{bmatrix} 0 \\ 0 \\ Q_1 \end{bmatrix}}_{Q}$$

Matrix K :

Column #1 = growth reaction

Column #2 = enzyme catalyzed reaction

+ standardisation of the yield coefficients

Example #3 : intracellular production of PHB

PHB = Poly-β-hydroxybutyric acid (biodegradable polymer)

Aerobic culture of *Alcaligenes eutrophus* in fedbatch reactor

- 2 limiting substrates :
  Source of carbone (fructose, glucose, ...)
  Source of nitrogen (NH^+_4)

- Intracellular production : 2 metabolic pathways :
  1) associated to the growth (low yield)
  2) X catalysed by an enzyme (completely inhibited by N)

- Bioreactor operation : 2 steps :
  1) growth “without” production → fed with both 2 substrates
  2) production without growth → fed only with carbon

Dynamical model (mass balance)

Step #1 : growth “without” production

$$\frac{dX}{dt} = \mu X - DX \qquad \text{biomass}$$

$$\frac{dS}{dt} = -k_1 \mu X + DS_{in} - DS \qquad \text{carbon source}$$

$$\frac{dN}{dt} = -k_2 \mu X + DN_{in} - DN \qquad \text{nitrogen}$$

$$\frac{dP}{dt} = k_3 \mu X - DP \qquad \text{PHB}$$

$$\frac{dC}{dt} = -k_4 \mu X + Q_{in} - DC \qquad \text{oxygen}$$

with

S_{in}, N_{in} : inlet concentration of S and N

Q_{in} : gaseous oxygen flowrate

Dynamical model (mass balance)(continued)

Step #2 : production without growth

$$\frac{dX}{dt} = -DX$$ biomass

$$\frac{dS}{dt} = -k_5 \nu X + DS_{in} - DS$$ carbon source

$$N = 0$$ nitrogen

$$\frac{dP}{dt} = \nu X - DP$$ PHB

$$\frac{dC}{dt} = -k_6 \nu X + Q_{in} - DC$$ oxygen

with ν : specific production rate

PHB model : matrix form

Step #1

$$x = \begin{bmatrix} X \\ S \\ N \\ P \\ C \end{bmatrix}, \; K = \begin{bmatrix} 1 \\ -k_1 \\ -k_2 \\ k_3 \\ -k_4 \end{bmatrix}, \; F = \begin{bmatrix} 0 \\ DS_{in} \\ DN_{in} \\ 0 \\ Q_{in} \end{bmatrix}$$

$$r = [\mu X], \; Q = 0$$

Step #2

$$x = \begin{bmatrix} X \\ S \\ P \\ C \end{bmatrix}, \; K = \begin{bmatrix} 0 \\ -k_5 \\ 1 \\ -k_6 \end{bmatrix}, \; F = \begin{bmatrix} 0 \\ DS_{in} \\ 0 \\ Q_{in} \end{bmatrix}$$

$$r = [\nu X], \; Q = 0$$

Example #4 : anaerobic digestion

(sequential reactions)

- Wastewater treatment with CH_4 production
- Complex process → simplified reaction scheme

1) acidogenesis : $S_1 \rightarrow X_1 + S_2 + P_1$

S_1: organic matter; X_1: acidogenic bacteria; P_1: CO_2

2) methanisation : $S_2 \rightarrow X_2 + P_1 + P_2$

S_2: fatty volatile acids; X_2: methanogenic bacteria; P_2: CH_4

Dynamical model (mass balance)

$$
\begin{aligned}
\frac{dX_1}{dt} &= \mu_1 X_1 - D X_1 \\
\frac{dS_1}{dt} &= -k_1 \mu_1 X_1 + D S_{in} - D S_1 \\
\frac{dX_2}{dt} &= \mu_2 X_2 - D X_2 \\
\frac{dS_2}{dt} &= k_3 \mu_1 X_1 - k_2 \mu_2 X_2 - D S_2 \\
\frac{dP_1}{dt} &= k_4 \mu_2 X_2 - D P_1 - Q_1 \\
\frac{dP_2}{dt} &= k_5 \mu_1 X_1 + k_6 \mu_2 X_2 - D P_2 - Q_2
\end{aligned}
$$

acidogenesis

methanisation

CO_2

Anaerobic digestion model : matrix form

$$x = \begin{bmatrix} X_1 \\ S_1 \\ X_2 \\ S_2 \\ P_1 \\ P_2 \end{bmatrix}, \; K = \begin{bmatrix} 1 & 0 \\ -k_1 & 0 \\ 0 & 1 \\ k_3 & -k_2 \\ 0 & k_4 \\ k_5 & k_6 \end{bmatrix}, \; r = \begin{bmatrix} \mu_1 X_1 \\ \mu_2 X_2 \end{bmatrix}$$

$$F = \begin{bmatrix} 0 \\ DS_{in} \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \; Q = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ Q_1 \\ Q_2 \end{bmatrix}$$

The reaction rates

The specific growth rate may depend on

- the substrate concentration S
- the biomass concentration X
- the product concentration P
- the temperature T
- the pH pH
- the dissolved oxygen concentration C
- inhibitors' concentration I
- the light intensity L
- genetic modifications, ...

- Reaction rates : (nonlinear) functions of the process variables

  A simple example : Monod microbial growth model

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

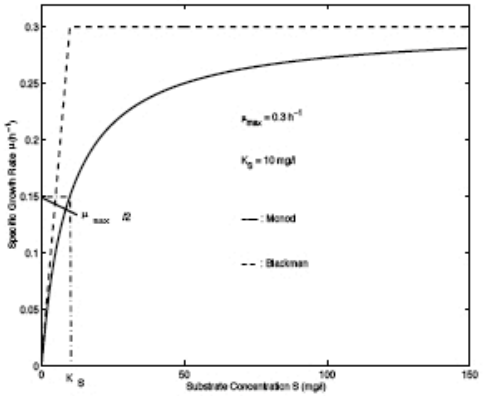
- But :
  - Choice of an appropriate model
    (more than 60 models for μ!)

  - Calibration of the model parameters on the basis of the available experimental data
    (identifiability issues)

μ depends on S, X, T, pH, …

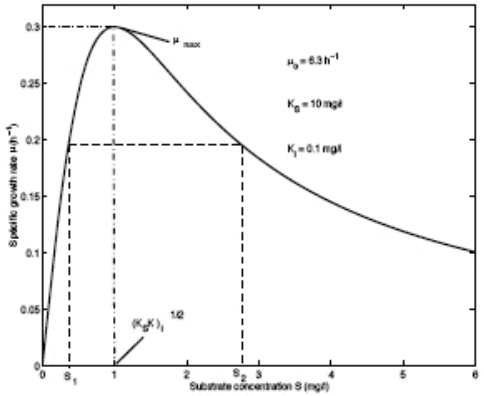
μ depends on S

Monod

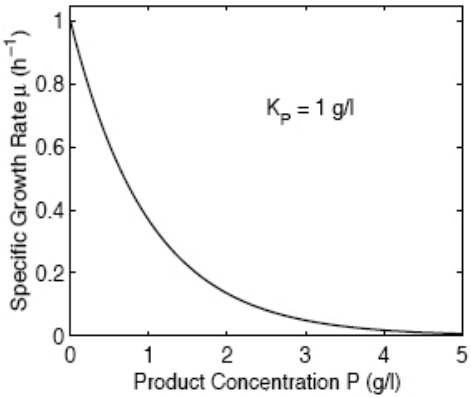


μ depends on S

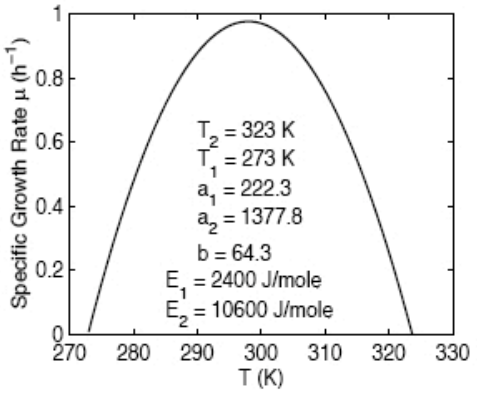
Haldane



μ depends on P



μ depends on T



Example : yeast growth

$$\mu(S,P,C) = \left(\frac{\mu_{1,max} S}{K_S + S} + \frac{\mu_{2,max} P}{K_P + P}\right)\left(\frac{C}{K_C + C} + K_1 C - K_2\right)$$

- 7 parameters : $\mu_{1,max}$, $\mu_{2,max}$, K_S, K_P, K_C, K_1, K_2
- If $S = 0$, $C = K_2/K_1$, $P \neq 0$, $X \neq 0$, $D = 0$:

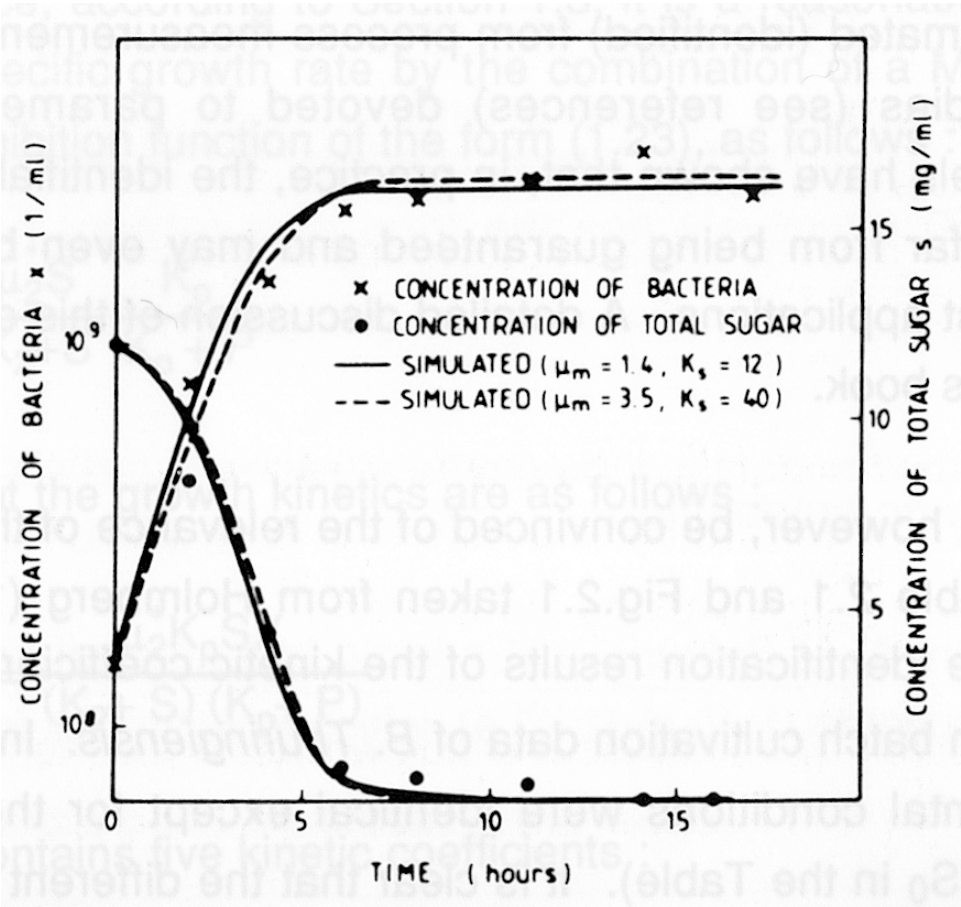
$$\frac{dS}{dt} = -k_1 \mu X + D S_{in} - D S$$

$$\longrightarrow \quad \frac{dS}{dt} = -k_1 \frac{\mu_{2,max}\, P\, C}{(K_P + P)(K_C + C)} X < 0\,!$$

physically absurd !
($S < 0$!)

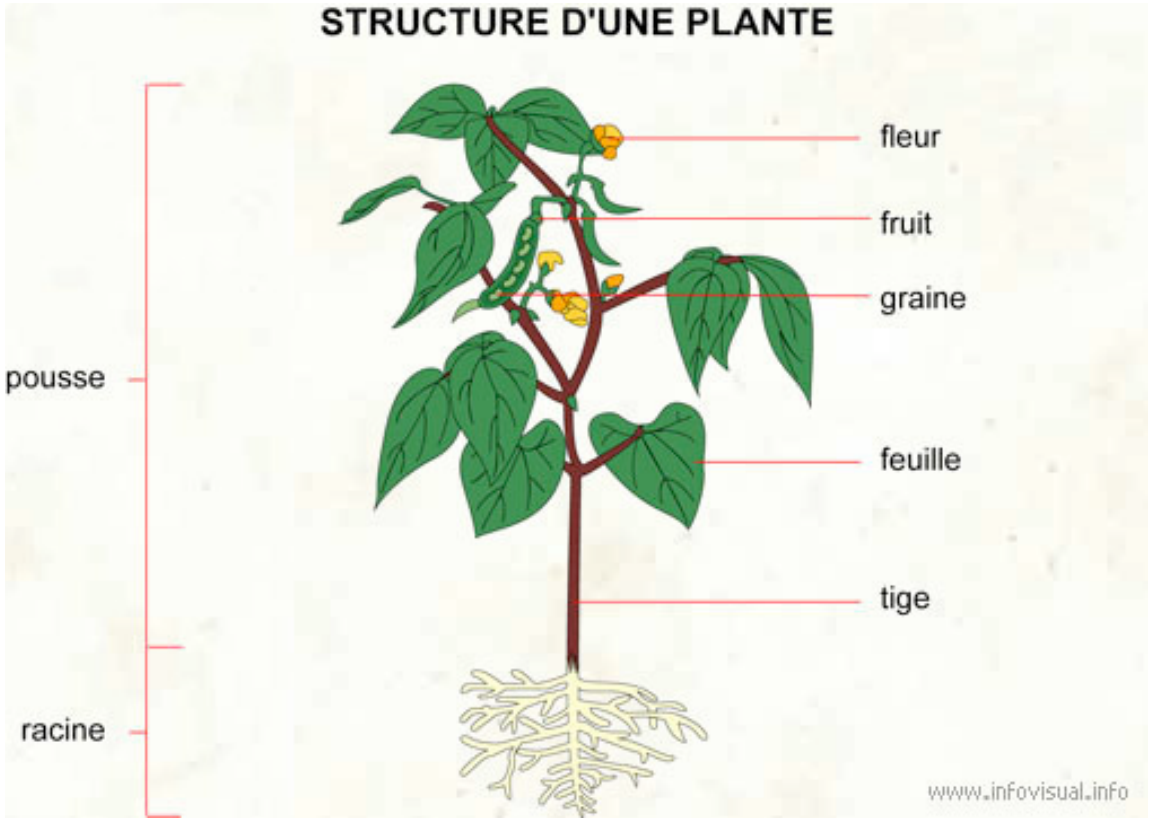
The models of biosystems are often not identifiable

Example : Monod model



$S(0)$	μ_{max}	K_S
7	1.1	1.8
11.6	1.0	6.8
18.2	0.7	12.9
25	0.3 (± 0.2)	7 (± 12)

Example : plant growth



Main reactions : photosynthesis, (photo)respiration

How to better account for the cell complexity and its interactions with the medium?

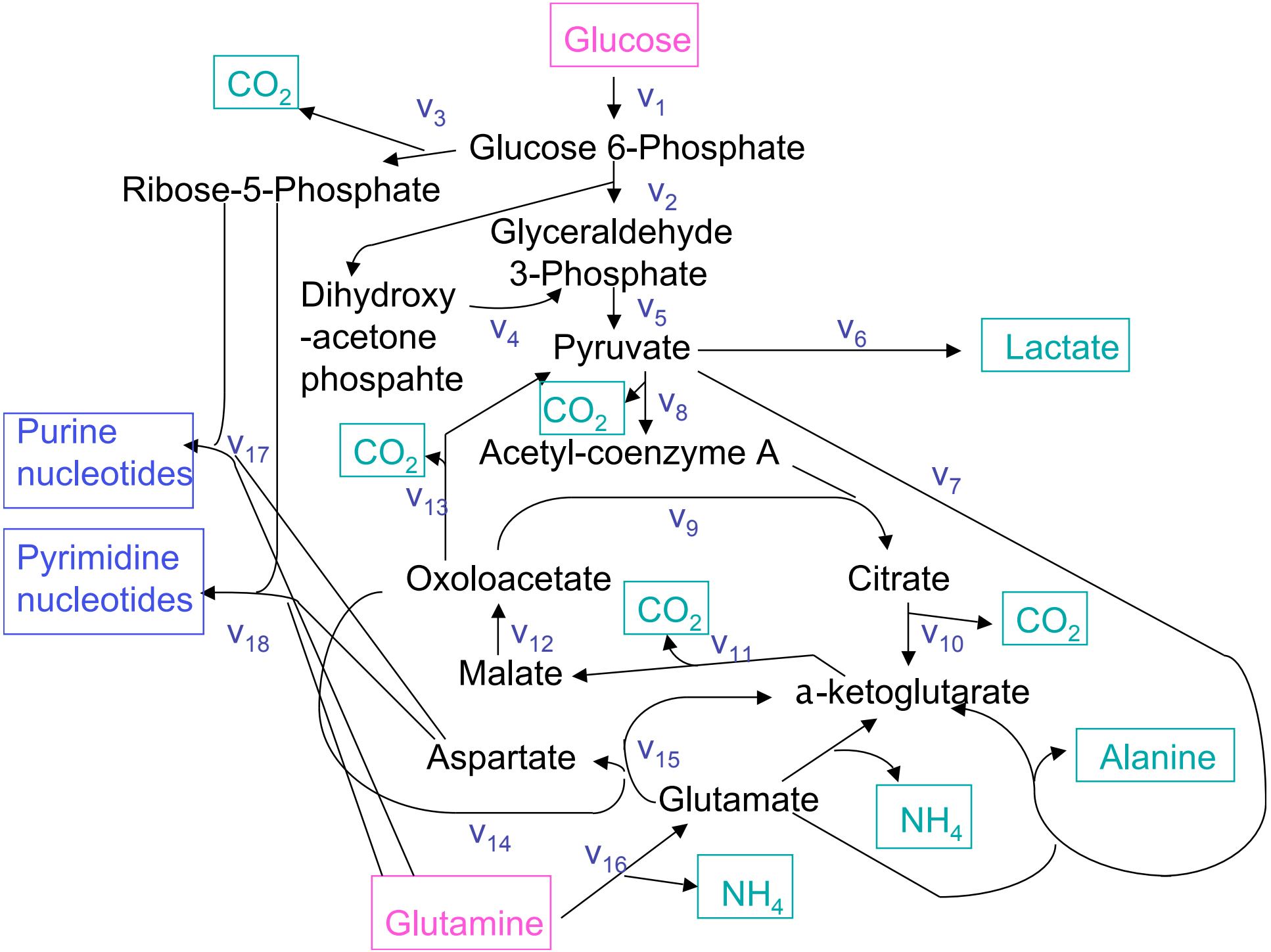
- Metabolic engineering (and “system biology”)
- Population balance
- Microbial ecology

Link betwen reaction networks and metabolic engineering

- Metabolic engineering : complex reaction networks including the cell metabolism
- Challenge : can we validate metabolic pathways on the basis of a limited number of measured components while preserving the orientation (sign) of the reactions?
- Solution : convex basis

- One (simple) example : animal cells (CHO)

- One challenge : link between the wine quality and the reaction network of the production of organoleptic components (European project CAFE)


Glucose
CO_2
v_3
v_1
Glucose 6-Phosphate
Ribose-5-Phosphate
v_2
Glyceraldehyde
3-Phosphate
Dihydroxy
-acetone
phospahte
v_4
v_5
Pyruvate
v_6
Lactate
CO_2
v_8
Purine
nucleotides
v_{17}
CO_2
Acetyl-coenzyme A
v_7
v_{13}
v_9
Pyrimidine
nucleotides
Oxoloacetate
Citrate
CO_2
CO_2
v_{18}
v_{12}
v_{11}
v_{10}
Malate
a-ketoglutarate
Alanine
v_{15}
Aspartate
Glutamate
NH_4
v_{14}
v_{16}
Glutamine
NH_4

Reaction scheme of the CHO cells

- Metabolism : utilization of 2 main energetic nutrients
  (metabolism of the amino-acids provided by the culture medium : neglected)

- 2 initial substrates : glucose and glutamine
- 4 final extracellular products : lactate, alanine, NH_4, CO_2
- 2 final intracellular metabolites :
  purine and pyrimidine nucleotides
- 12 internal metabolites

- 4 fundamental pathways : glycolysis, glutaminolysis, TCA cycle, synthesis of the nucleotides

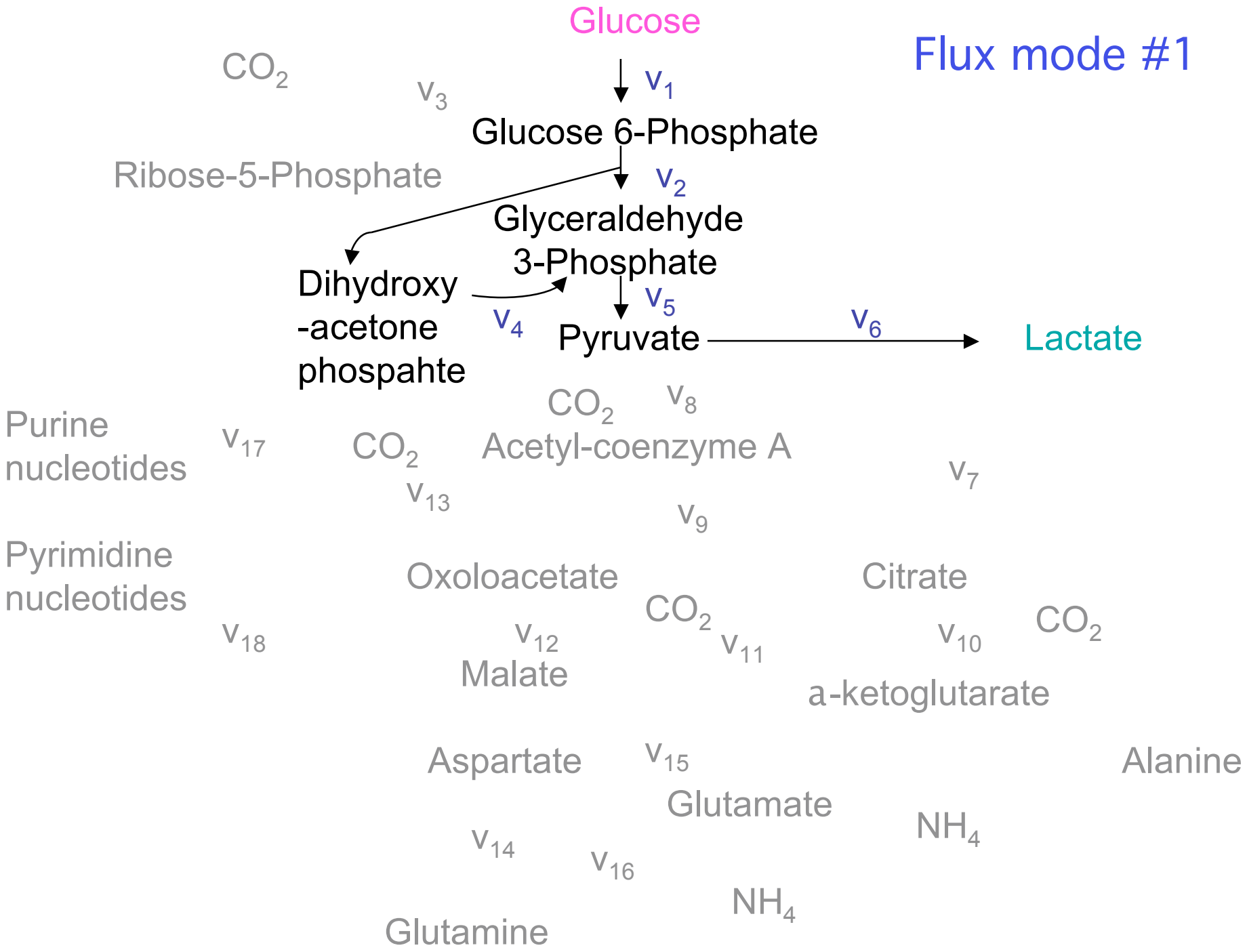
Metabolic flux analysis

- QSS approximation : $Kr = 0$ ($\dim(r) = 18$)
- reaction rates of the extracellular species (measured) : $Pr = r_m$

- Convex bases (--> values of positive fluxes) :
  here : 7

---> 7 macroscopic reactions :

1) Glucose ---> 2 Lactate
2) Glucose ---> 6 CO_2
3) Glutamine ---> Alanine + 2 CO_2 + NH_4
4) Glutamine ---> Lactate + 2 CO_2 + NH_4
5) Glutamine ---> 5 CO_2 + 2 NH_4
6) Glucose + 3 Glutamine ---> Purine + 2 CO_2 + NH_4
7) Glucose + 2 Glutamine ---> Pyrimidine + 2 CO_2 + NH_4


Flux mode #1
Glucose
CO_2
v_3
v_1
Glucose 6-Phosphate
Ribose-5-Phosphate
v_2
Glyceraldehyde
3-Phosphate
Dihydroxy
-acetone
phospahte
v_4
v_5
Pyruvate
v_6
Lactate
CO_2
v_8
Purine
nucleotides
v_{17}
CO_2
Acetyl-coenzyme A
v_7
v_{13}
v_9
Pyrimidine
nucleotides
Oxoloacetate
Citrate
CO_2
CO_2
v_{18}
v_{12}
v_{11}
v_{10}
Malate
a-ketoglutarate
Aspartate
v_{15}
Alanine
Glutamate
NH_4
v_{14}
v_{16}
NH_4
Glutamine

Flux mode #1

- 1st vector of the convex basis : $e_1 = [1\ 1\ 0\ 1\ 2\ 2\ 0\ \ldots\ 0]^T$

- In other words :

v_1 : Glucose ---> Glucose6P

v_2 : Glucose6P ---> DihydroxyacetoneP + Glyceraldehyde3P

v_4 : DihydroxyacetoneP ---> Glyceraldehyde3P

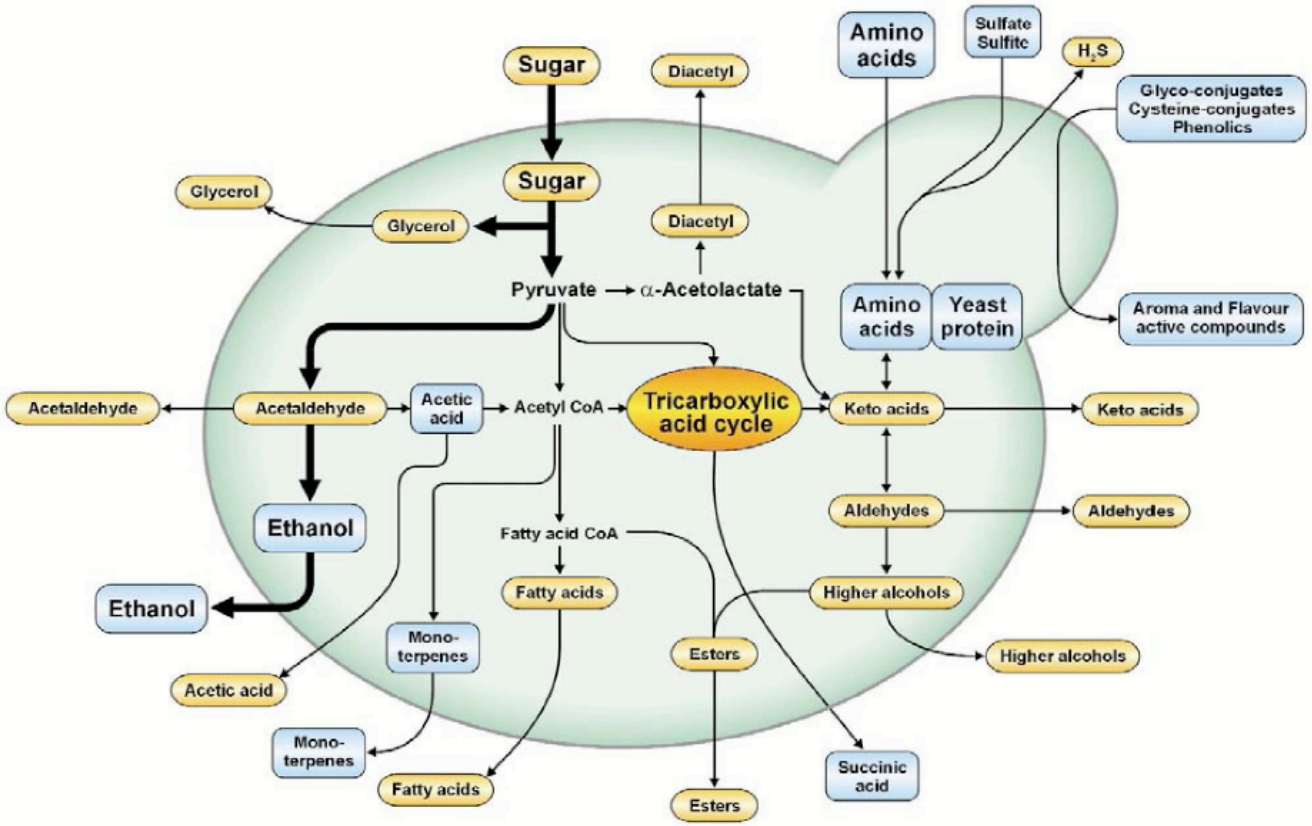
v_5 : Glyceraldehyde3P ---> 2 Pyruvate

v_6 : 2 Pyruvate ---> 2 Lactate

- Associated macroscopic reaction :

Glucose ---> 2 Lactate

A more complex application : wine production

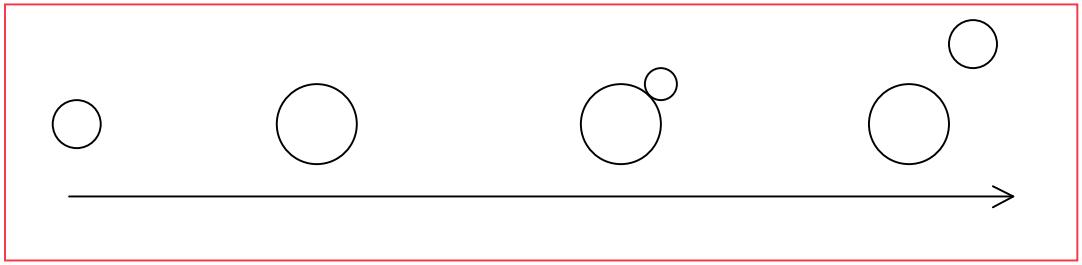


Challenge : how to transfer in simple models the synthesis of the indicators of the wine quality (amino-acids, sulfur compounds,...)

Population balance

--> Age distribution of the micro-organisms

Example : yeast



Budding (mother - daughter)

- $N(m,t)$: number of cells of mass m at time t per unit volume (cell density)

- Total number : $N_t(t) = \int_{m_{min}}^{m_{max}} N(m,t)dm$

- Total concentration : $X(t) = \int_{m_{min}}^{m_{max}} mN(m,t)dm$

- Balance equation :

growth rate — rate of cellular division

$$\frac{\partial N}{\partial t} = -\frac{\partial r(m,S)N}{\partial m} - \Gamma(m,S)N - DN + 2\int_{m}^{m_{max}} \Gamma(m',S)p(m,m',S)N dm'$$

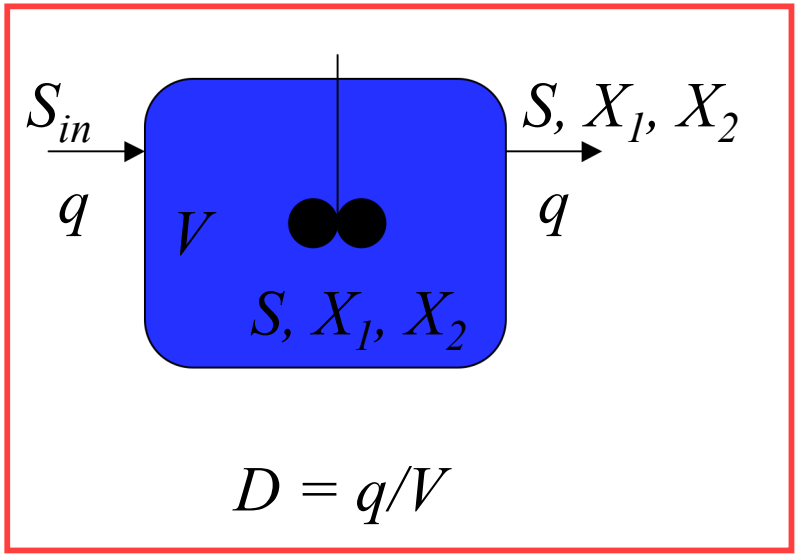
Microbial ecology

Issues and challenges

- Coexistence/competition are not just limited to ecology…
- The knowledge of the dynamical mechanisms of coexistence/competition of microbial species can be helpful for improving the running of industrial biological processes, e.g. :
  - Invasion of a culture by a contaminant
    (Can we avoid systematic re-inoculation?)
  - Mixed cultures, e.g. :
    * Lactic fermentation (*L. bulgaricus* vs *S. thermophilus*)
    * Anaerobic digestion (*thermophilic* vs *mesophilic* bacteria)

Competitive exclusion principle

Consider a continuous reactor
(« chemostat »)
with 2 species X_1 and X_2
sharing only one resource S :



$$\frac{dX_1}{dt} = \mu_1(S)X_1 - DX_1$$

$$\frac{dX_2}{dt} = \mu_2(S)X_2 - DX_2$$

$$\frac{dS}{dt} = -\frac{1}{Y_1}\mu_1(S)X_1 - \frac{1}{Y_2}\mu_2(S)X_2 + D(S_{in} - S)$$

- At equilibrium : $\overline{\mu}_1(S) = \overline{\mu}_2(S) = \overline{D}$

  (only valid for specific values of D)

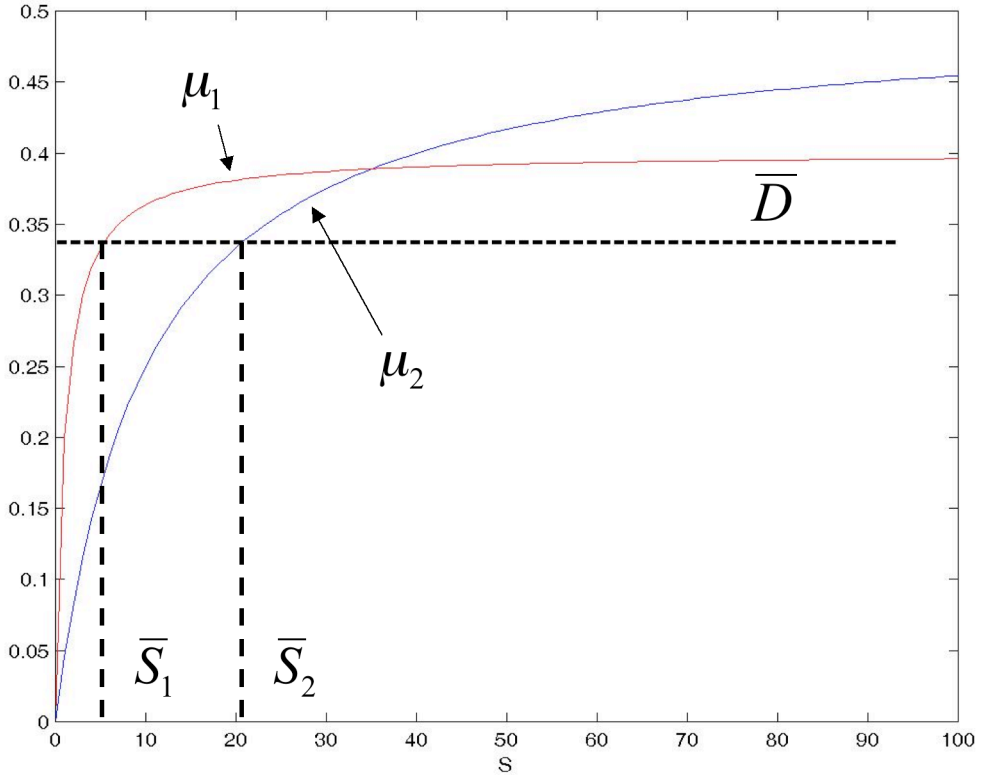
- In general, only one species «will win the competition and will survive» : --> growth curve that first intersects D

(«best affinity» or «lowest break-even concentration»)

- Here :

$$\overline{X}_1 = Y_1(S_{in} - \overline{S}), \overline{X}_2 = 0$$

(*Hardin, 1960; Butler & Wolkowicz, 1985*)



(Extension to n species and other growth curves)

Competitive exclusion principle : experimental validation

X_{A1} : E. coli (1)
X_{A2} : E. coli (2)
X_B : P. aeroginosa

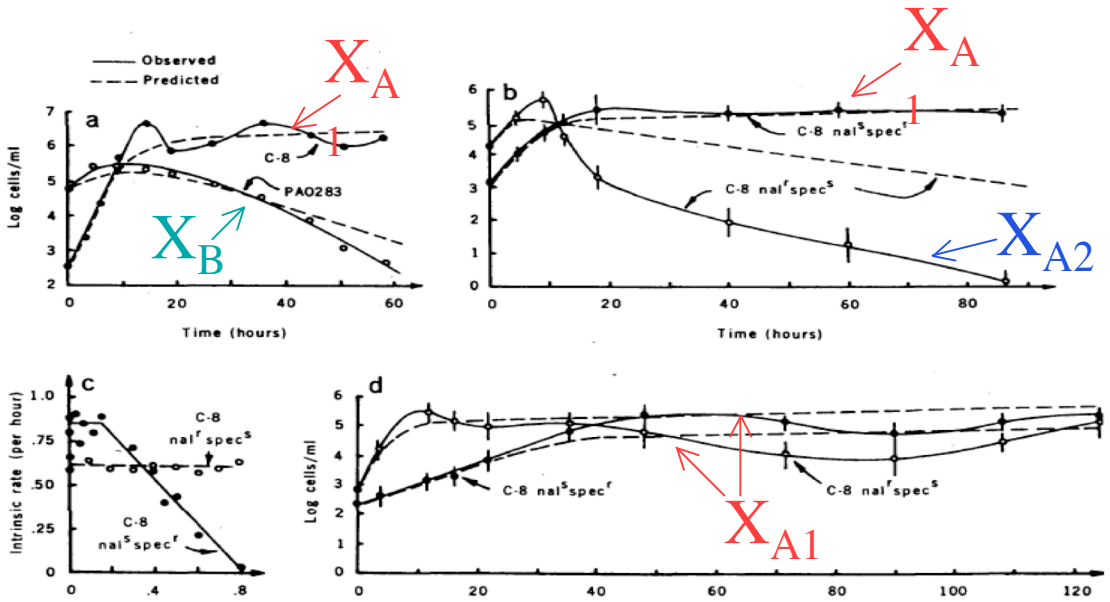
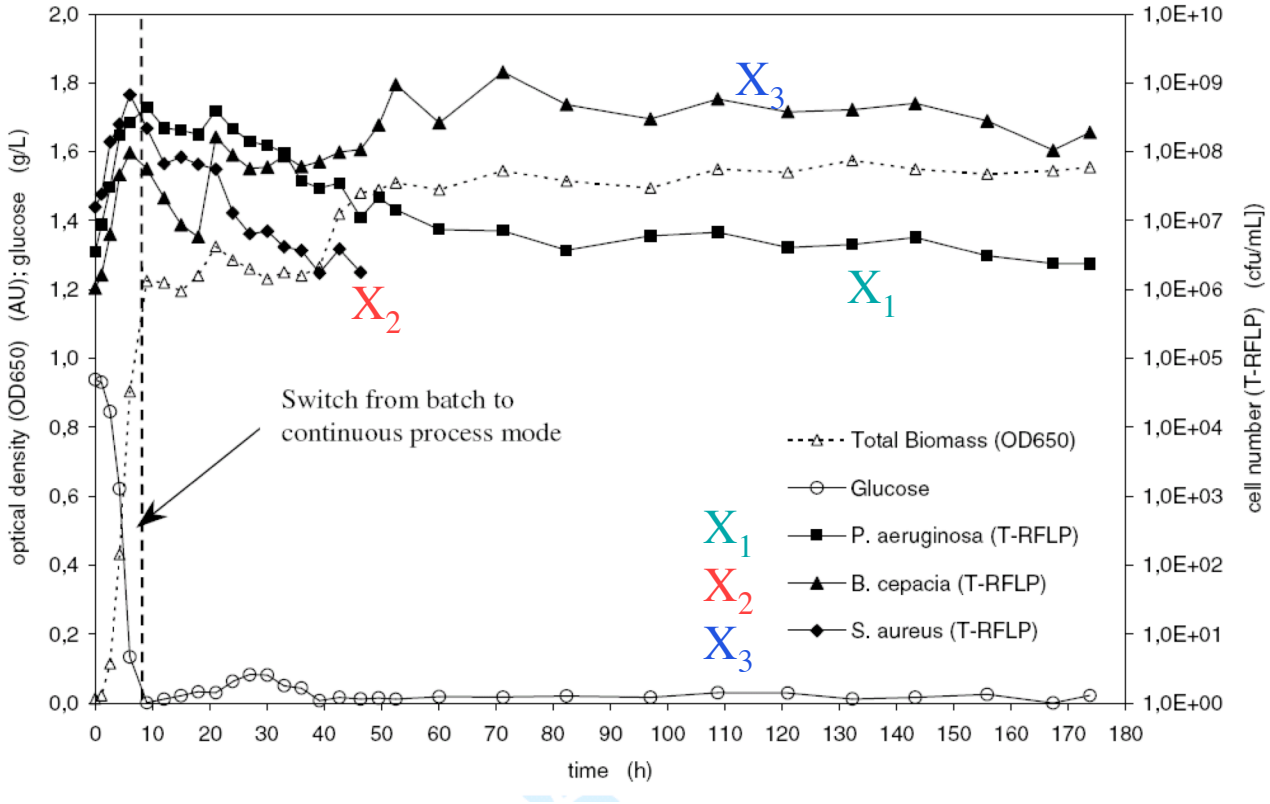


FIG. 5.2 – Validation qualitative expérimentale du comportement du modèle. Les prédictions qualitatives du modèle sont vérifiées pour : a) 2 espèces (*Escherichia coli*, souche C-8 et *Pseudomonas aeruginosa*, souche PA0283) qui diffèrent par leur constante de demi-saturation. b) 2 souches de *Escherichia coli* qui diffèrent par leur taux de croissance maximal. d) Coexistence obtenue avec 2 souches de *Escherichia coli* qui ont le même paramètre J_i. La figure c) représente l'effet de l'acide nalidixique sur le taux de croissance maximal pour les souches considérées C-8. D'après Hansen et Hubbell (1980).

S. R. Hansen S. P. Hubell, Single nutrient microbial competition, Science, 207(28), 1980, pp. 1491-1493

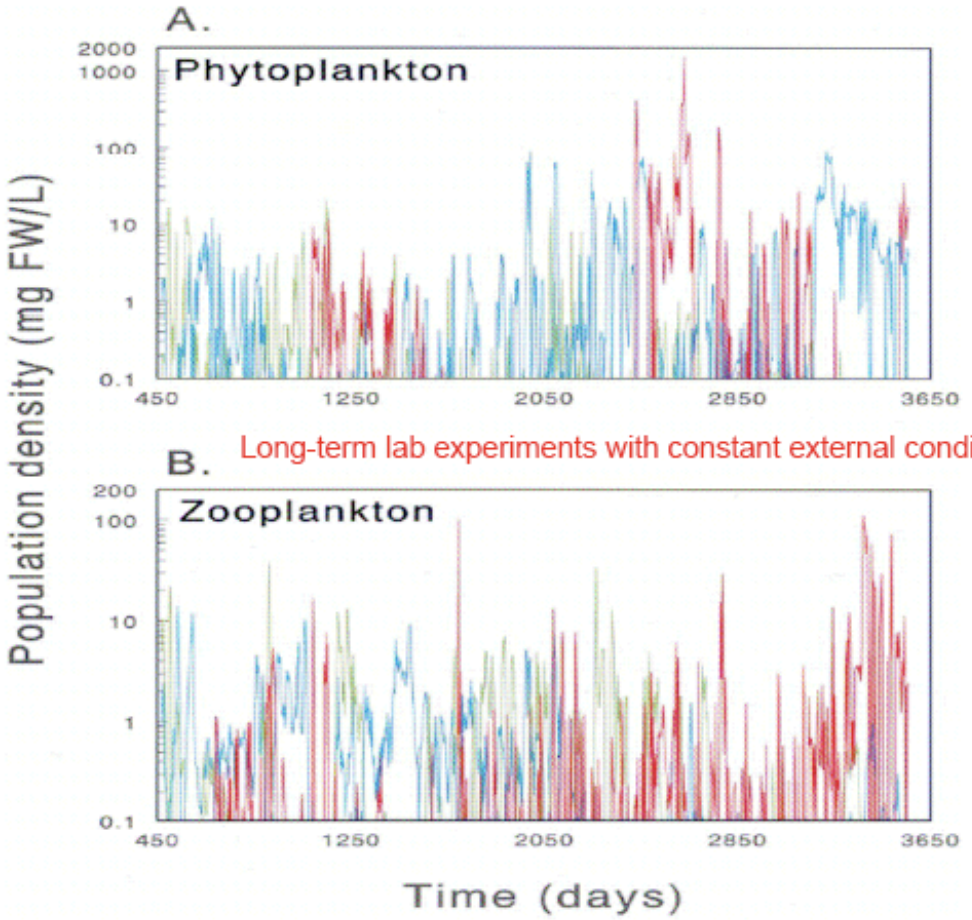
The coexistence of different species is often observed

experimental evidence :



Schmidt, J. K., B. König et U.Reichl Characterization of a three bacteria mixed culture in a chemostat: Evaluation andapplication of a quant

Dynamical persistence



Long-term lab experiments with constant external conditions!

Figure 5. Non-equilibrium dynamics observed in an experimental multispecies community. The community developed in a long-term laboratory experiment under constant external conditions, and consisted of more than 20 different species. Data show the observed time course of (A) the dominant phytoplankton groups (green = green flagellates, blue = prokaryotic pico-phytoplankton, red = the diatom *Melosira*), and (B) the dominant zooplankton groups (green = the rotifer *Brachionus*, blue = the copepod *Eurytemora*, red = protozoans). Data were kindly provided by Heerkloss (unpublished), and by Heerkloss & Klinkenberg (1998), with permission from Schweizerbartsche Verlagsbuchhandlung.

Model Properties

- Basic structural property
  - → reaction invariant
- Model reduction
  - → singular perturbations
- Stability
  - → BIBS stability
  - → unstable equilibrium points
- Observability & Controllability
- Non minimum phase (inverse response)

Basic Structural Property

Definition : p = rank(K)

→ K_a : a (pxM) full rank arbitrary matrix of K
K_b : the remaining submatrix of K
(x_a,x_b), (Q_a, Q_b) and (F_a, F_b) the partitions of x, Q and F induced by (K_a, K_b)

→
$$\begin{cases} \dfrac{dx_a}{dt} = K_a r(x_a, x_b) - Dx_a + F_a - Q_a \\ \dfrac{dx_b}{dt} = K_b r(x_a, x_b) - Dx_b + F_b - Q_b \end{cases}$$

Property

There exists a state transformation :

$$Z = A_0 x_a + x_b$$

where A_0 is the unique solution of the matrix equation :

$$A_0 K_a + K_b = 0 \qquad \dim(A_0) = (N\text{-}p)xp$$

such that the general dynamical model is equivalent to :

$$\begin{aligned} \frac{dx_a}{dt} &= K_a r(x_a, Z) - D x_a + F_a - Q_a \\ \frac{dZ}{dt} &= -DZ + F_b - Q_b + A_0(F_a - Q_a) \end{aligned}$$

Remark : dynamics of Z independent of r

Example : PHB

- «Global» model with CO_2 (P_2) as a product in both reactions

$$x = \begin{bmatrix} X \\ S \\ N \\ P \\ C \\ P_2 \end{bmatrix}, \quad K = \begin{bmatrix} 1 & 0 \\ -k_1 & -k_5 \\ -k_2 & 0 \\ k_3 & 1 \\ -k_4 & -k_6 \\ k_7 & k_8 \end{bmatrix}, \quad r = \begin{bmatrix} \mu X \\ \nu X \end{bmatrix}$$

- One possible state partition

$$x_a = \begin{bmatrix} X \\ S \end{bmatrix} \qquad x_b = \begin{bmatrix} N \\ P \\ C \\ P_2 \end{bmatrix}$$

$$\rightarrow K_a = \begin{bmatrix} 1 & 0 \\ -k_1 & -k_5 \end{bmatrix} \qquad K_b = \begin{bmatrix} -k_2 & 0 \\ k_3 & 1 \\ -k_4 & -k_6 \\ k_7 & k_8 \end{bmatrix}$$

$$
\begin{aligned}
A_0 &= \frac{1}{k_5}\begin{bmatrix} k_2k_5 & 0 \\ -k_3k_5+k_1 & 1 \\ k_4k_5-k_1k_6 & -k_6 \\ -k_5k_7+k_1k_8 & k_8 \end{bmatrix} \\
Z &= \begin{bmatrix} Z_1 \\ Z_2 \\ Z_3 \\ Z_4 \end{bmatrix} = \begin{bmatrix} k_2X+N \\ \left(k_3+\dfrac{k_1}{k_5}\right)X+\dfrac{1}{k_1}S+P \\ -\left(k_4+\dfrac{k_1k_6}{k_5}\right)X+\dfrac{k_6}{k_1}S+C \\ -\left(k_7-\dfrac{k_1k_8}{k_5}\right)X+\dfrac{k_8}{k_1}S+P_2 \end{bmatrix}
\end{aligned}
$$

Usually any partition is OK, but ...

Usually

- $\dim(K) = M$

  (OK if the reactions are independent)

- the process components are “independent”

  ⟶ any choice of state partition is OK

- but not always : e.g. PHB (RQ = 1
  --> C and P_2 are dependent))

  IWA model (activated sludge model)

IWA model (reaction network)

1. Aerobic growth of heterotrophs : $S_S + S_O + S_{NH} \longrightarrow X_{B,H}$
2. Anoxic growth of heterotrophs : $S_S + S_{NO} + S_{NH} \longrightarrow X_{B,H}$
3. Aerobic growth of autotrophs : $S_O + S_{NH} \longrightarrow X_{B,A} + S_{NO}$
4. Decay of heterotrophs : $X_{B,H} \longrightarrow X_P + X_S + X_{ND}$
5. Decay of autotrophs : $X_{B,A} \longrightarrow X_P + X_S + X_{ND}$
6. Ammonification of soluble organic nitrogen : $S_{ND} \longrightarrow S_{NH}$
7. Hydrolysis of entrapped organics : $X_S \longrightarrow S_S$
8. Hydrolysis of entrapped organic nitrogen : $X_{ND} \longrightarrow S_{ND}$

There is a loop in the reaction network

- 8 reactions, 10 components
- Loop with reactions 1, 4 and 7

→ if $x_a = [S_s, X_s, X_{B,H}, X_{B,A}, X_P, S_0, S_{NO}, S_{NH}]^T$

then K_a is not full rank

- OK if S_{ND} or X_{ND} are included in x_a

homework : check it!

IWA model (reaction network)

1. Aerobic growth of heterotrophs : $S_S + S_O + S_{NH} \rightarrow X_{B,H}$
2. Anoxic growth of heterotrophs : $S_S + S_{NO} + S_{NH} \rightarrow X_{B,H}$
3. Aerobic growth of autotrophs : $S_O + S_{NH} \rightarrow X_{B,A} + S_{NO}$
4. Decay of heterotrophs : $X_{B,H} \rightarrow X_P + X_S + X_{ND}$
5. Decay of autotrophs : $X_{B,A} \rightarrow X_P + X_S + X_{ND}$
6. Ammonification of soluble organic nitrogen : $S_{ND} \rightarrow S_{NH}$
7. Hydrolysis of entrapped organics : $X_S \rightarrow S_S$
8. Hydrolysis of entrapped organic nitrogen : $X_{ND} \rightarrow S_{ND}$

Model Reduction

- Singular perturbation : ODE --> algebraic equation
- Low solubility product P : $\frac{dP}{dt} = kr - DP - Q$

$$P = \Pi P_{sat}$$

$$\rightarrow \frac{dP}{dt} = kr - D\Pi P_{sat} - Q$$

$$P_{sat} \text{ --> } 0 \Rightarrow Q = kr$$

Substrates in fast reactions (only)

Example : two sequential reactions : $A \longrightarrow B,\ B \longrightarrow C$

Kinetics : $r_1 = \gamma_1\, A\ \alpha_1(A,B),\ r_2 = \gamma_2\, B\ \alpha_2(B,C), \qquad \gamma_1\ ,\ \gamma_2 > 0$

Assumption : reaction 2 fast and reaction 1 slow, i.e. $\gamma_1 << \gamma_2$

Define : $\epsilon = \dfrac{1}{\gamma_2}$ and $Z = C + \dfrac{k_4}{k_3} B$

$$\rightarrow \epsilon \frac{dB}{dt} = -\epsilon D B + \epsilon k_2 \gamma_1 A \alpha_1 - k_3 \alpha_2 B \qquad \varepsilon \longrightarrow 0 : B \longrightarrow 0$$

$$\rightarrow \frac{dZ}{dt} = \left(\frac{dC}{dt}\right) = -DZ + \frac{k_1 k_4}{k_3} r_1 = -DC + \frac{k_1 k_4}{k_3} r_1$$

General rule for model reduction

- $\frac{dx}{dt} = 0 \;\; \text{and} \;\; x = 0$

- Quasi-steady state (QSS) approximation

Stability Analysis

1. BIBS stability

- BIBS : bounded input bounded state
- Motivation : - is the model in accordance with the physical reality?
  - under which conditions?

- Assumptions :

  A1. $0 < D_{min} \leq D(t)$

  A2. $0 \leq F_i(t) \leq F_{max}$

  A3. Principle of Mass Conservation :

  $\exists\ \gamma > 0$ such that $\gamma^T K_j = 0$, for all j (K_j : jth column of K)

- Theorem : $0 \leq x(t) \leq x_{max}$

Rewriting of the Model Equations

- Reaction rates : $r_j(x) = \alpha_j(x) \left(\prod_{j \frown n} x_n \right), \quad 0 \le \alpha_j(x) \le \alpha_{max}$

reactants and autocatalyst in reaction j

Example : growth rate μX with a Monod model

$$\alpha_j(x) = \frac{\mu_{max}}{K_S + S}, \quad \left(\prod_{j \frown n} x_n \right) = SX$$

- Gaseous outflow rates : $Q_i = \beta_i x_i, \;\; 0 \le \beta_i, \;\; 0 \le x_i \le x_{is}$

- Feed rates (liquid phase) : $F_i = DS_{i,in}$

Proof :

a) $x_i(t) \geq 0$

$$\frac{dx_i}{dt} = \sum_j (+) k_{ij} r_j + F_i \geq 0$$

b) $x_i(t) \leq x_{max}$

Define $z = \gamma^T x$

Then : $$\frac{dz}{dt} = -Dz - \gamma^T Q + \gamma^T F \leq -Dz + \gamma^T F_{max}$$

$$\Rightarrow \quad z \leq \frac{\gamma^T F_{max}}{D_{min}} \quad \Rightarrow x_i \leq \frac{\gamma^T F_{max}}{\gamma_i D_{min}} \quad \forall i$$

Remarks :

- if $F_i = DS_{i,in}$ (liquid substrates) : $z \leq \gamma^T S_{in}$
- alternative (more complex) proofs

Example : PHB

$$K = \begin{bmatrix} 1 & 0 \\ -k_1 & -k_5 \\ -k_2 & 0 \\ k_3 & 1 \\ -k_4 & -k_6 \\ k_7 & k_8 \end{bmatrix}$$

--> one possible choice for γ : $\gamma = \begin{bmatrix} \frac{k_1 k_8}{2k_5 k_7} + \frac{k_4}{2k_3 k_6} \\ \frac{k_8}{2k_5 k_7} \\ \frac{1}{k_2} \\ \frac{1}{2k_3} \\ \frac{1}{2k_2 k_6} \\ \frac{1}{k_7} \end{bmatrix}$

2. Asymptotic Stability

Equilibrium points

- Definition : constant state such that : $\frac{d\bar{x}}{dt} = 0$

  → these are solutions of the algebraic equation :

$$-\bar{D}\bar{x} + Kr(\bar{x}) + \bar{F} - \bar{Q} = 0$$

- Specificity of (bio)chemical process models : multiple steady-state :

  One value of $(\bar{D}, \bar{F}) \Rightarrow$ multiple values for $\bar{x}$

A simple example

- $S \longrightarrow X$
- Dynamical equations in steady state :

$$\frac{d\bar{S}}{dt} = 0 \quad \rightarrow \quad -k_1 \bar{\mu} \bar{X} + \bar{D}\bar{S}_{in} - \bar{D}\bar{S} = 0 \qquad (1)$$

$$\frac{d\bar{X}}{dt} = 0 \quad \rightarrow \quad (\bar{\mu} - \bar{D})\bar{X} = 0 \qquad (2)$$

- From (2), 2 possible solutions (equilibrium points) :

1) $\bar{\mu} = \bar{D}$

2) $\bar{X} = 0 \ (\text{and } \bar{S} = \bar{S}_{in})$ (Wash-out)

(the only possible if $\bar{D} > \mu_{max}$)

- The explicit solution of 1) requires a model for μ

- Monod model : $\mu = \dfrac{\mu_{max} S}{K_S + S}$

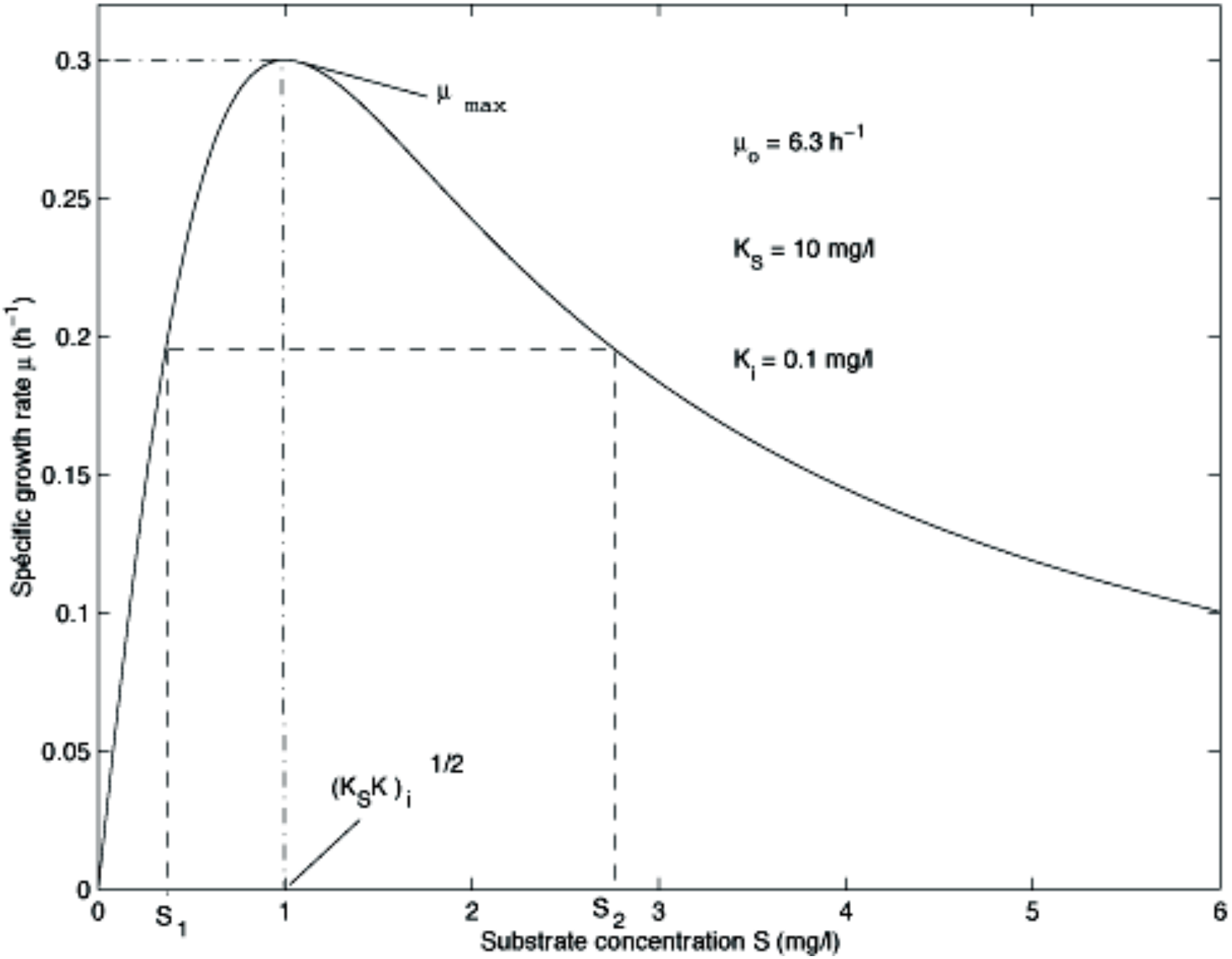
Equilibrium point 1) : $\bar{S} = \dfrac{\bar{D}}{\mu_{max} - \bar{D}}, \; \bar{X} = \bar{S}_{in} - \bar{S}$

- Haldane model (inhibition) : $\mu = \dfrac{\mu^* S}{K_S + S + \dfrac{S^2}{K_I}}$

2 possible solutions to 1) (i.e. 3 equilibrium points) :

1) $\bar{S}_1 = \dfrac{\mu^* - \bar{D}}{2\bar{D}} K_I - \dfrac{K_I}{2} \sqrt{\left(\dfrac{\mu^*}{\bar{D}} - 1\right)^2 - 4\dfrac{K_S}{K_I}}$

2) $\bar{S}_2 = \dfrac{\mu^* - \bar{D}}{2\bar{D}} K_I + \dfrac{K_I}{2} \sqrt{\left(\dfrac{\mu^*}{\bar{D}} - 1\right)^2 - 4\dfrac{K_S}{K_I}}$


0.3
0.25
0.2
0.15
0.1
0.05
0
μ max
μo = 6.3 h−1
KS = 10 mg/l
Ki = 0.1 mg/l
(KSK)i 1/2
Spécific growth rate μ (h−1)
0
S1
1
2
S2
3
4
5
6
Substrate concentration S (mg/l)

Stability of the equilibrium points

- Linearized tangent model (around equilibrium points) :

$$\frac{d}{dt}(x-\bar{x}) = A\left(\bar{x},\bar{D},\bar{F}\right)(x-\bar{x}) - \bar{x}\left(D-\bar{D}\right) + \left(F-\bar{F}\right)$$

with $A\left(\bar{x},\bar{D},\bar{F}\right) = K\left[\frac{\partial r}{\partial x}\right]_{x=\bar{x}} - \bar{D}I_N$

- Lyapunov's stability first method : eigenvalues of A ($\lambda_i(A)$) :

If $Re(\lambda_i(A)) < 0$ for all i, then the equilibrium point is stable

Example : Haldane model

$$A\left(\bar{x},\bar{D},\bar{F}\right)=\begin{bmatrix} 0 & \Omega \\ -k_1\bar{D} & -k_1\Omega-\bar{D} \end{bmatrix}$$

with $$\Omega=\frac{\mu^*\bar{X}\left(K_S-\frac{\bar{S}^2}{K_I}\right)}{\left(K_S+\bar{S}+\frac{\bar{S}^2}{K_I}\right)^2}$$

Eigenvalues of A : $Det(\lambda I - A) = \lambda^2 + \lambda(k_1\Omega + D) + k_1\Omega D = 0$

---> $\lambda_1 = -D$, $\lambda_2 = -k_1\Omega$

---> stable if $\bar{S} < \sqrt{K_S K_I}$

unstable if $\bar{S} > \sqrt{K_S K_I}$

Observability and Controllability

- Based on the linearized tangent model (sufficient conditions)

$$\frac{d}{dt}(x-\bar{x}) = \left(K \left[\frac{\partial r}{\partial x} \right]_{x=\bar{x}} - \bar{D} I_N \right) (x-\bar{x}) - \bar{x} \left(D - \bar{D} \right) + \left(F - \bar{F} \right)$$

- Observability : p measured components

  O = observability matrix

  --> $rank(\mathrm{O}) \leq min\{N, p+M\}$

- Controllability : q control inputs

  C = controllability matrix

  --> $rank(\mathrm{C}) \leq min\{N, q+M\}$ (if only feedrates F)

  --> $rank(\mathrm{C})$ increased by one with D as an additional input except at equilibrium points

Non Minimum Phase (Inverse Response)

- NMP = « unstable » zeros
- Time response : first in a direction inverse to the final response
- Already for reaction networks with two sequential reactions.

e.g. : S_1 --> $X_1 + S_2$

S_2 --> X_2

---> transfer function of the linearized tangent model between D and S_2 :

$$H(s) = \frac{s - \dfrac{k_1 k_2 \bar{\mu}_{1S} \bar{X}_1 \bar{X}_2}{\bar{S}_2}}{\left(s + k_1 \bar{\mu}_{2S} \bar{X}_1\right)\left(s + k_1 \bar{\mu}_{2S} \bar{X}_2\right)}$$

Laplace Transform

Example : reactant in a first-order reaction

$$\frac{\partial C}{\partial t} = -u\frac{\partial C}{\partial z} - k_0 C, \quad C(z=0,t) = C_{in}(t)$$

Laplace transform with respect to t :

$$s\mathcal{C}(z,s) = -u\frac{\partial \mathcal{C}}{\partial z} - k_0\mathcal{C} \quad \rightarrow \quad \frac{\partial \mathcal{C}}{\partial z} = -\frac{s+k_0}{u}\mathcal{C}$$

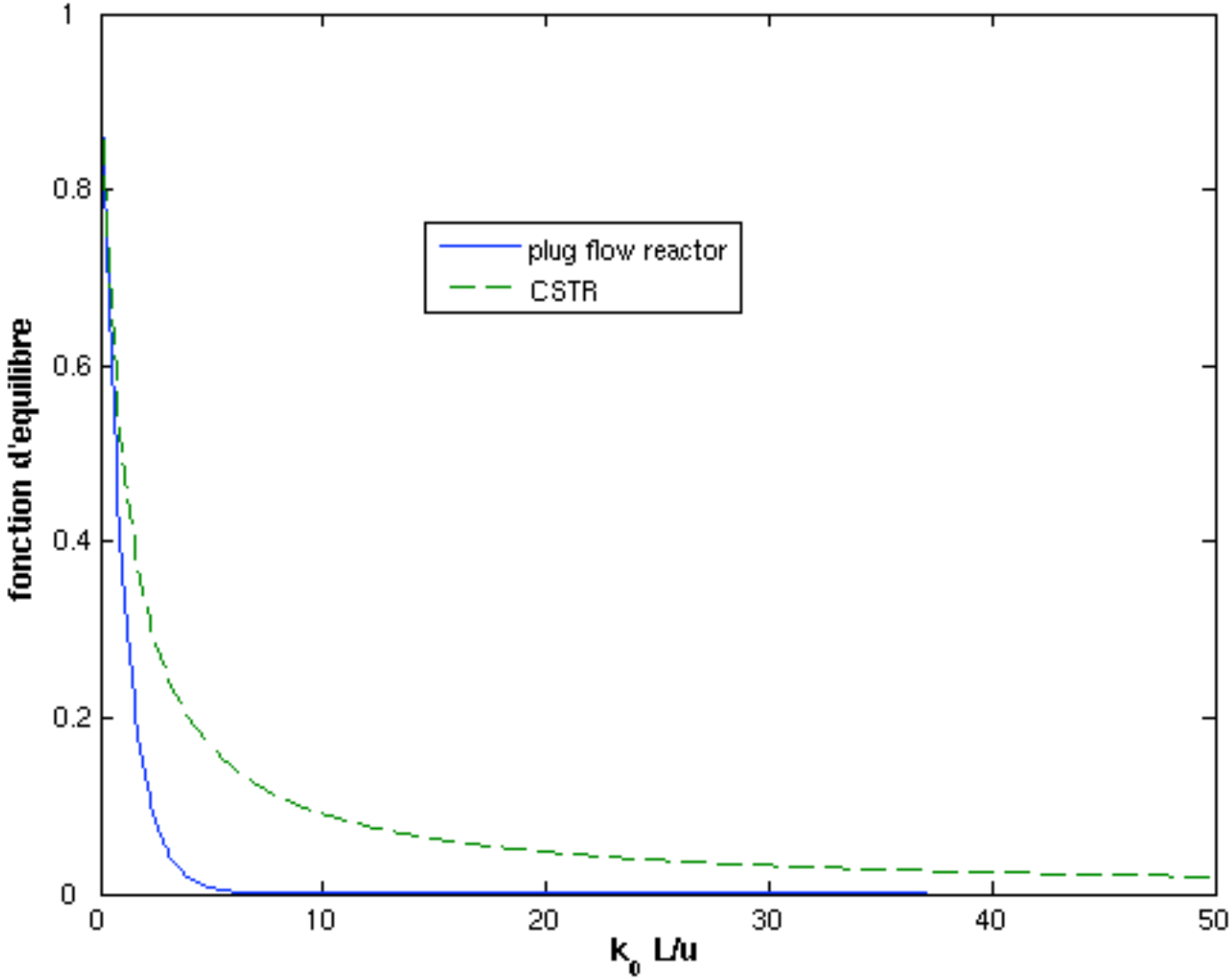
- Solution : $\mathcal{C}(z,s) = \mathcal{C}(0,s)e^{-\frac{k_0 z}{u}} e^{-\frac{sz}{u}}$

steady-state delay !

$$\mathcal{C}(0,s) = C_{in}$$

- CSTR : steady-state : $\bar{C} = \dfrac{C_{in}}{1+\dfrac{k_0}{D}} \quad \left(D = \dfrac{u}{L}\right)$

⟶ Plug flow reactor ≈ CSTR + time delay


1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
fonction d'equilibre
k0 L/u
plug flow reactor
CSTR

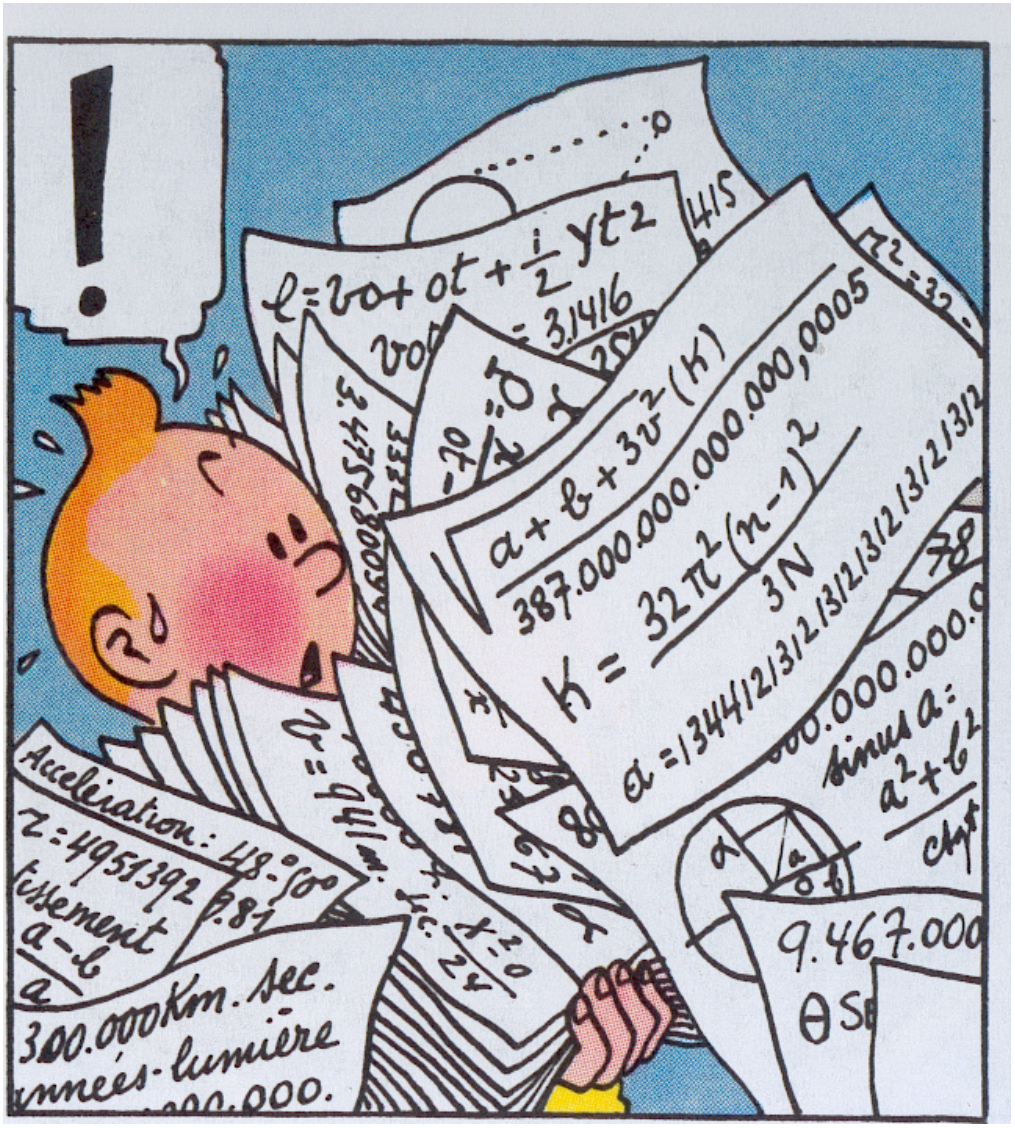
Challenges

- Complex reaction network
- Living organisms : their behaviour changes with time
- Kinetics badly known (mixture of complex biochemical kinetics and (auto-)catalytic reactions (multi-phase)

-> Complex high order nonlinear models

- Few available (on-line and off-line) measurements

-> Difficult to obtain reliable models


!
$\ell = v_0 t + \frac{1}{2} \gamma t^2$
$a + b + 3v^2 (K)$
387.000.000.000.000,0005
$K = \frac{32\pi^2 (n-1)^2}{3N}$
$a = 1344/2/3/2/3/2/3/2/3/2/3/2/3/2$
Acceleration: 48-500
$\pi = 4951392$
9.467.000
300.000Km. sec.
années-lumière