Microbial Ecology and Bioprocess Control : Opportunities and Challenges

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Menu

- Microbial ecology : some basic concepts
- Coexistence or competition
- Recent developments
  - Density dependent growth and coexistence
  - Coexistence in a reactor cascade
  - «Practical» coexistence
- Issues and challenges

Microbial ecology : some basic concepts

- A definition :

  Study of the interactions that determine the abundance and distribution of organisms
- A keyword : biodiversity
- A basic concept : the competitive exclusion principle

Competitive exclusion principle

Consider a CSTR (« chemostat ») with two biomasses X_1 and X_2 growing on one limiting substrate S :

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

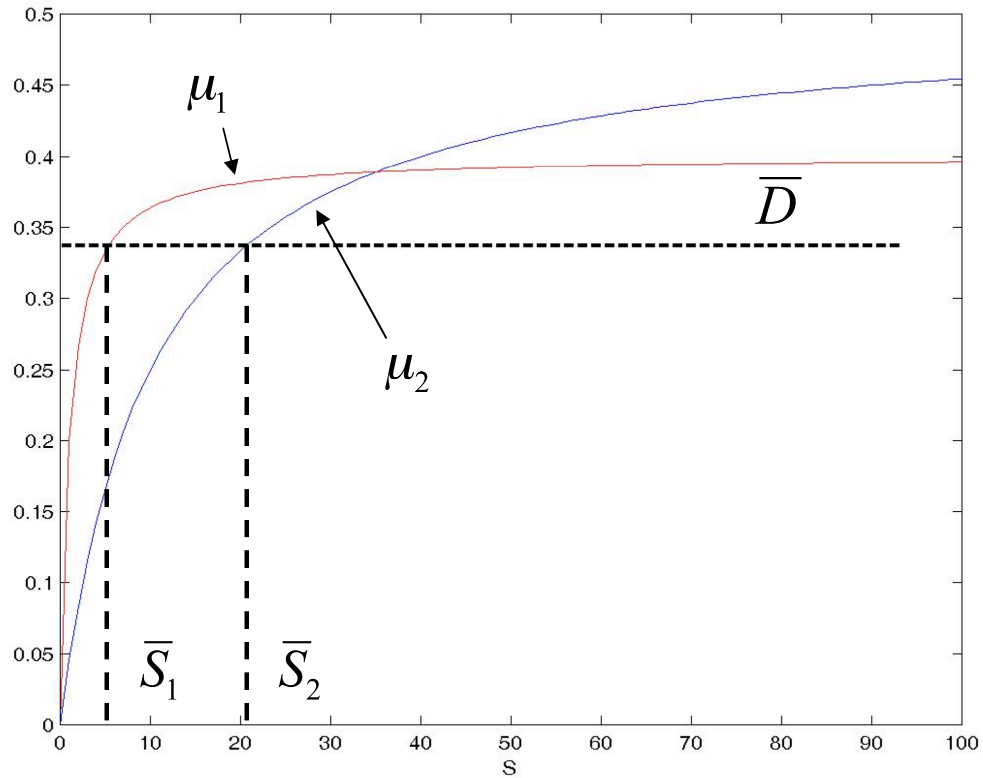
$$\frac{dX_1}{dt} = \mu_1(S)X_1 - DX_1$$

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

- At steady state : $\overline{\mu}_1(S) = \overline{\mu}_2(S) = \overline{D}$
  (only valid for specific values of D)
- In general, only one species will «win the competition and survive» : the one whose growth curve crosses first D («best affinity» or «smallest 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

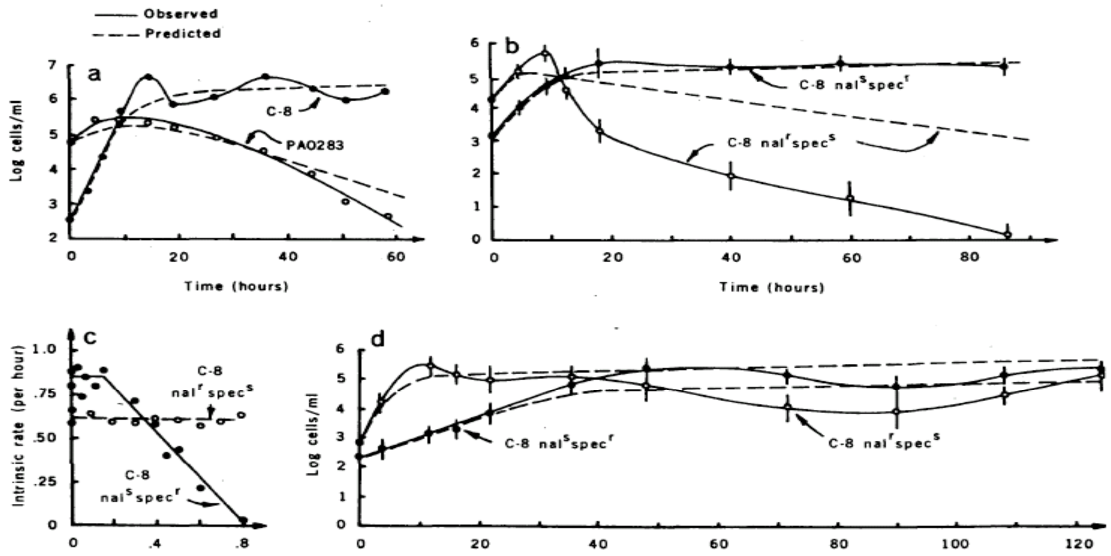
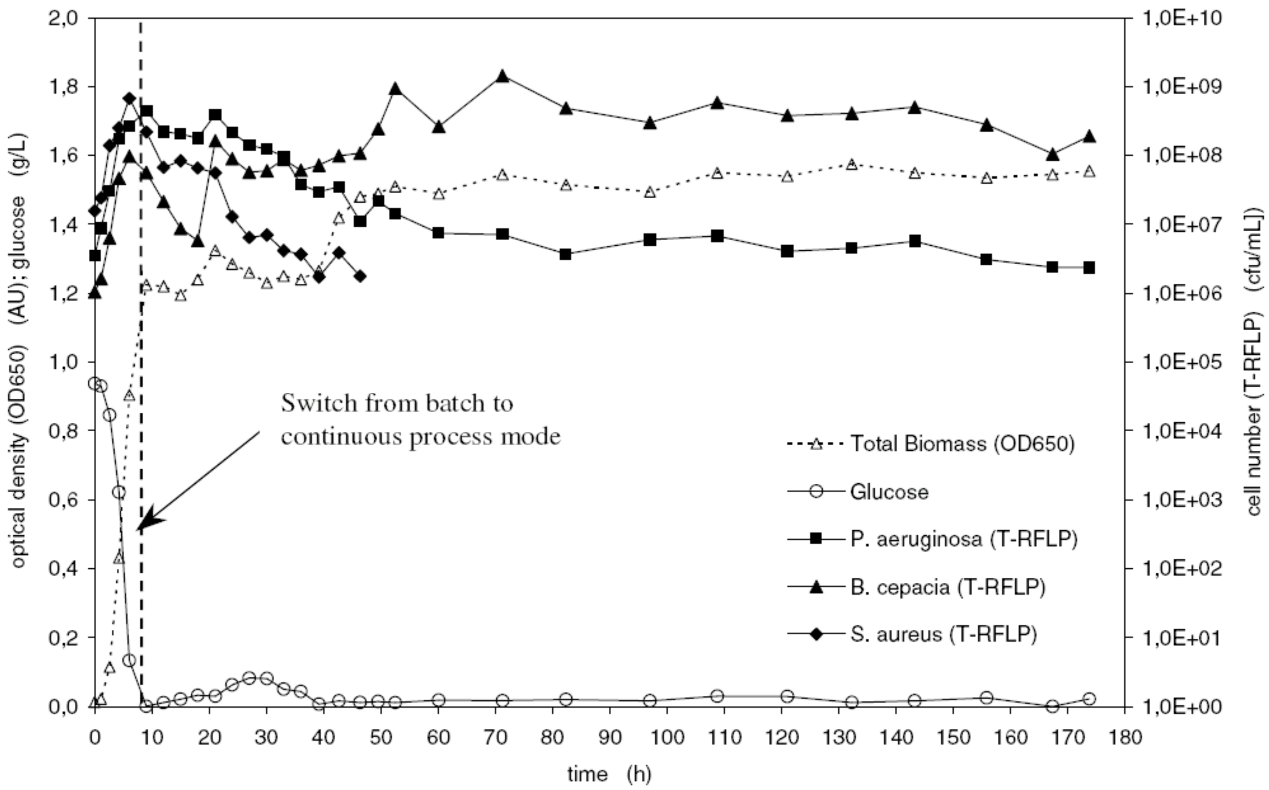


FIG. 5.2 – Validation qualitative expérimentale du comportement du modèle. Les prédictions qualitatives du modèle sont vérifiées pour : a) 2 espèces (*Escherichia coli*, souche C-8 et *Pseudomonas aeruginosa*, souche PA0283) qui diffèrent par leur constante de demi-saturation. b) 2 souches de *Escherichia 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

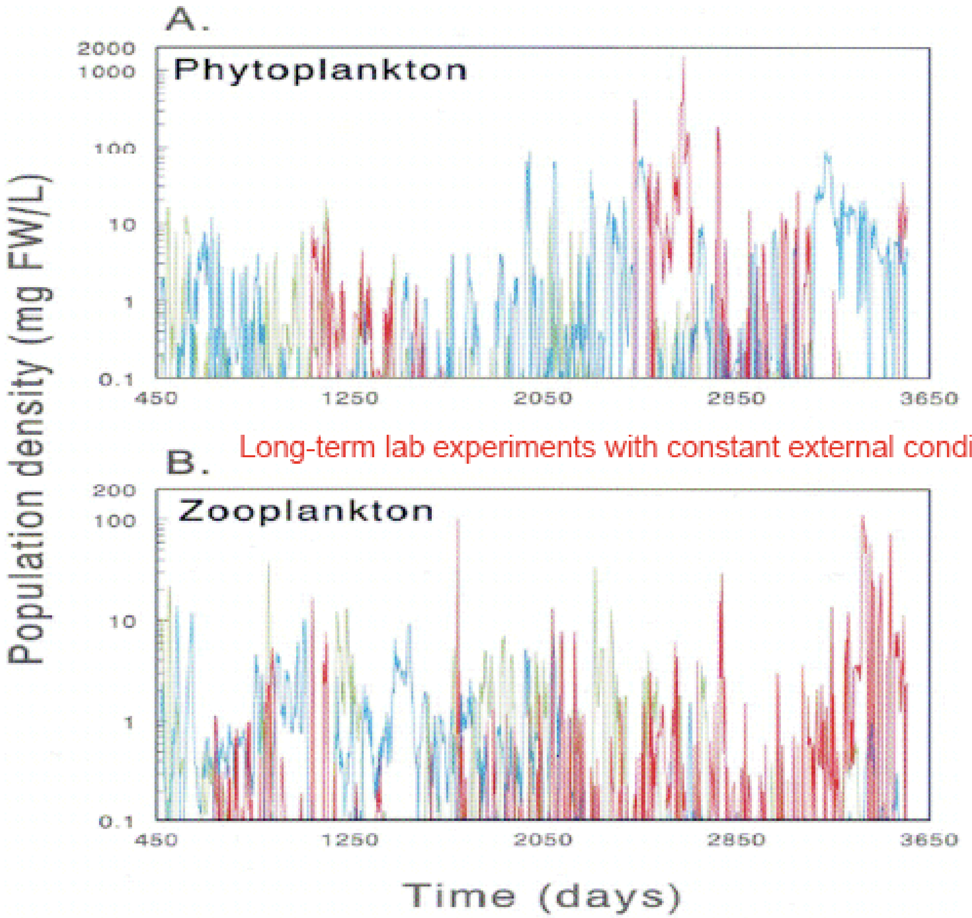
The coexistence of different species is often encountered

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.

Coexistence or competition?

- Coexistence can be mathematically emphasized for periodic inlet (D, S_{in}) conditions (e.g. *Smith, 1981*)
- Filamentous backbone theory : coexistence of floc-forming and filamentous bacteria (--> activated sludge) (*Cenens et al, 2000*)

- Recent developments (MERE team) :
  - Density dependent growth and coexistence
  - Coexistence in a cascade of reactors
  - «Practical» coexistence

1. Density dependent growth and coexistence

- Starting point : the specific growth rate depends not only on the substrate concentration S but also on the biomass concentrations X_i : $\mu_i(S, X_j)$, i, j = 1,…, n
- All species X_i compete for the same substrate S

  - $\mu_i(S,X_j)$ is a decreasing function of X_i

    e.g. the growth decreases with the size of the cells (reduced accessibility to the nutrient)

  - Inter-species vs intra-species competition :

    --> $\mu_i(S,X_j)$ is a decreasing function of X_j

- Coexistence if intra-species competition > inter-species competition

Key mathematical result

- **Dynamical model**

$$\frac{dS(t)}{dt} = D\left(S_{in} - S(t)\right) - \sum_{i=1}^{n} \mu_i\left(S(t), X_i(t)\right) X_i(t)$$

$$\frac{dX_i(t)}{dt} = \left[\mu_i\left(S(t), X_i(t)\right) - D\right] X_i(t) \qquad , \ i = 1,\ldots,n$$

$$Y_i = 1 \quad (i = 1,\ldots,n) \quad \text{(without loss of generality)}$$

- **Assumptions**

A1. $\mu_i(S,X_i) \geq 0$, $\mu_i(0,X_i) = 0$,

$\mu_i(S,X_i)$ is an increasing function of S

A2. For each i, the mapping $X_i \to \mu_i(S,X_i)$ is decreasing and tends to 0 at infinity

A3. For every i, there exists a $\tilde{S}_i < S_{in}$ such that $\mu_i(\tilde{S}_i, 0) = D$

A4. $\tilde{S} + \sum_{i=1}^{n} X_i(\tilde{S}) < S_{in}$ with $\tilde{S} = \max\{\tilde{S}_i;\ i = 1,...,n\}$

(from A2, there is a unique $X_i(S)$ such that $\mu_i(S,X_i(S)) = D$)

- **Theorem**

Under assumptions A1 to A4, there exists a unique equilibrium $(S, X_1^*, ..., X_n^*)$ such that for every i, one has $X_i^* > 0$
and it is globally asymptotically stable.
i.e. the system converges towards this equilibrium whatever the initial conditions satisfying $X_i(0) > 0$

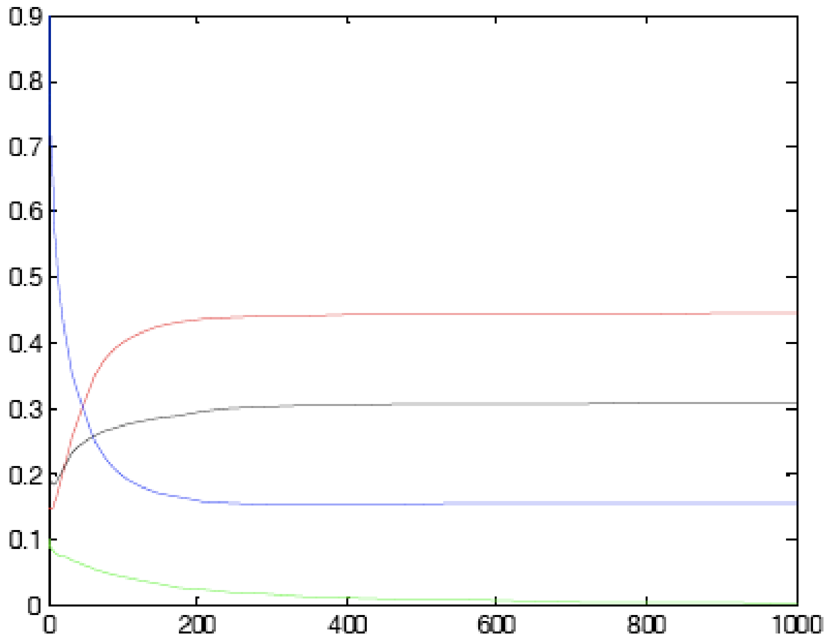
Example

$$\begin{cases} \frac{dS(t)}{dt} = D\big(S_{in} - S(t)\big) - \sum_{i=1}^{n} g\Big(X_i(t) + \lambda \sum_{j \neq i} X_j(t)\Big)\mu_i\big(S(t)\big)X_i(t) \\ \frac{dX_i(t)}{dt} = \Big[g\Big(X_i(t) + \lambda \sum_{j \neq i} X_j(t)\Big)\mu_i\big(S(t)\big) - D\Big]X_i(t) \\ \mu_i(S) = \frac{a_i S}{b_i + S} \\ g(X) = \frac{1}{1 + c\sqrt[3]{X}} \end{cases} \quad , i = 1,\dots,n$$

ratio surface/volume

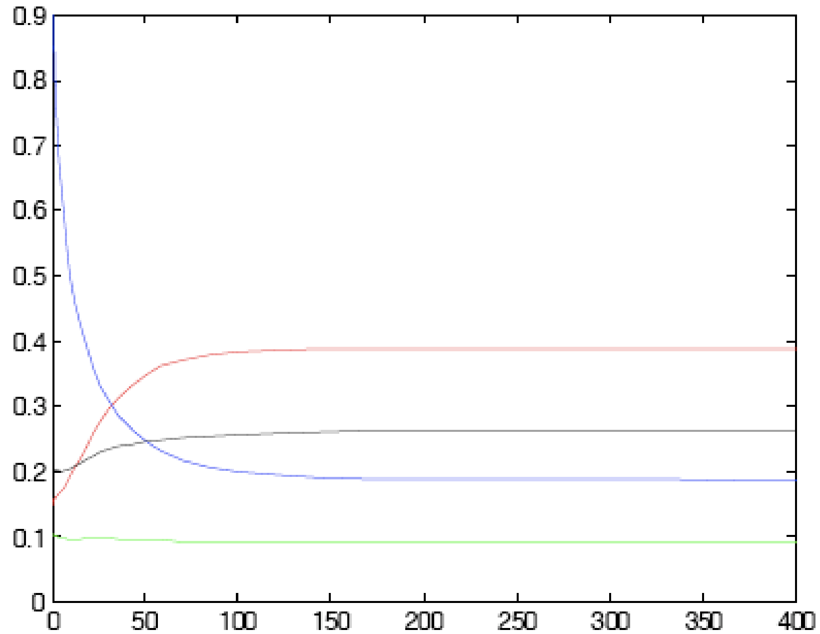
with λ as a measure of the inter-species competition
(dominates if $\lambda > 0.5$)

Exclusion



$\lambda = 0.55$

Coexistence



$\lambda = 0.1$

Various expressions of the density dependence

1) Ratio dependence, e.g. Contois model :

$$\mu = \frac{\mu_{max} S}{K_C X + S} \quad \text{which can be rewritten as :} \quad \mu = \frac{\mu_{max} \frac{S}{X}}{K_C + \frac{S}{X}}$$

2) Flocculation : $X = \sum_{n=1}^{\infty} n\, u_n$ with u_n, the density of flocs of size n

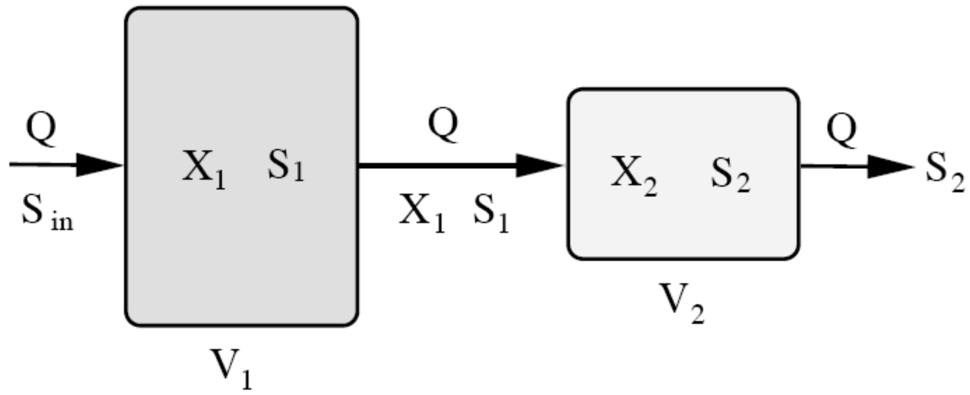
→ Mass balance equation for u_n :

$$\frac{du_n}{dt} = \left(\frac{du_n}{dt}\right)_{\text{bacterial growth}} - D u_n + \left(\frac{du_n}{dt}\right)_{\text{floc interaction}}$$

with (cell division) : $\left(\frac{du_1}{dt}\right)_{\text{bacterial growth}} = -\mu_1(S) u_1$

$$\left(\frac{du_n}{dt}\right)_{\text{bacterial growth}} = \mu_n(S) u_n - \mu_n(S) u_n \quad n \geq 2$$

2. Coexistence in a reactor cascade



2 tanks
2 species X_A, X_B

- Issue : what happens when a species B starts invading a bioprocess with originally one species A?
  - can we have coexistence/exclusion?
  - is the reactor design appropriate?

- Stephanopoulos & Frederickson (1979) : coexistence in tank 2

Assumptions (i=1,2) : $D_i = Q/V_i$

$Y_i=1$ (without loss of generality)

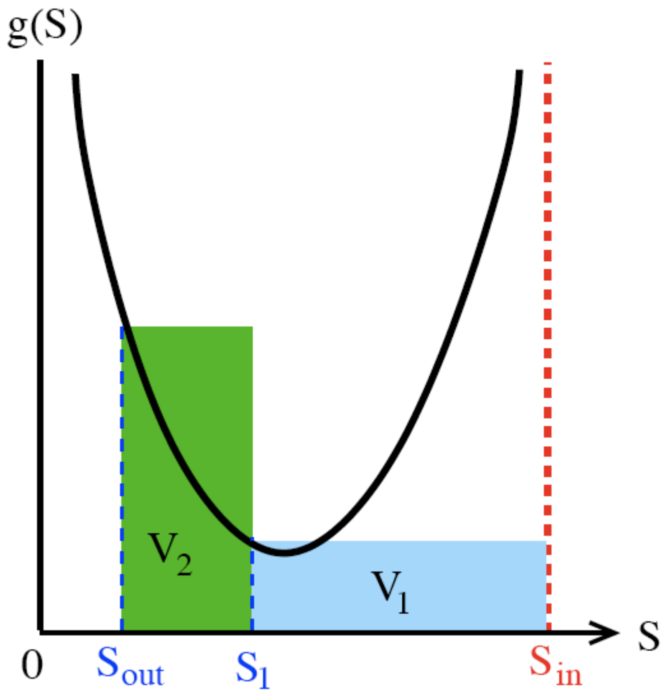
$\mu_i(S)$

→mass balance equations :

$$
\begin{cases}
\frac{dS_1}{dt} = -\mu_A(S_1)X_{A,1} - \mu_B(S_1)X_{B,1} + D_1(S_{in} - S_1) \\
\frac{dX_{A,1}}{dt} = \mu_A(S_1)X_{A,1} - D_1X_{A,1} \\
\frac{dX_{B,1}}{dt} = \mu_B(S_2)X_{B,1} - D_1X_{B,1} \\
\frac{dS_2}{dt} = -\mu_A(S_2)X_{A,2} - \mu_B(S_2)X_{B,2} + D_2(S_1 - S_2) \\
\frac{dX_{A,2}}{dt} = \mu_A(S_2)X_{A,2} + D_2(X_{A,1} - X_{A,2}) \\
\frac{dX_{B,2}}{dt} = \mu_B(S_2)X_{B,2} + D_2(X_{B,1} - X_{B,2})
\end{cases}
$$

One species in two tanks

Design based on the Modified Inverse Kinetics (MIK) function :



$$g(S) = \frac{1}{\mu(S)(S_{in} - S)}$$

- If $\mu^{-1}(S)$ is convex : MIK has a unique minimum on $]0, S_{in}[$
- If we have also $\dfrac{\mu(S)}{S}$ non-increasing : $V_1^{opt} \geq V_2^{opt}$

Invasion : two species in two tanks

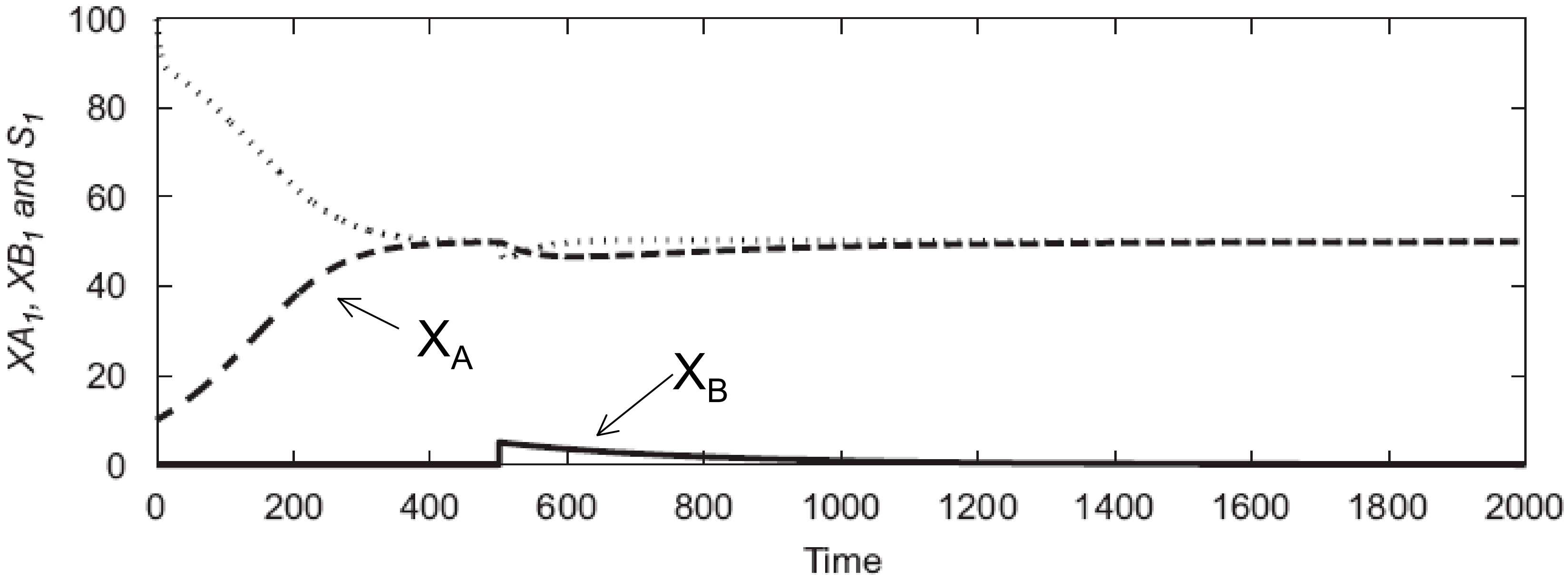
- Break-even concentration («intersection» between D and μ) :
  $\lambda_A(D)$ and $\lambda_B(D)$

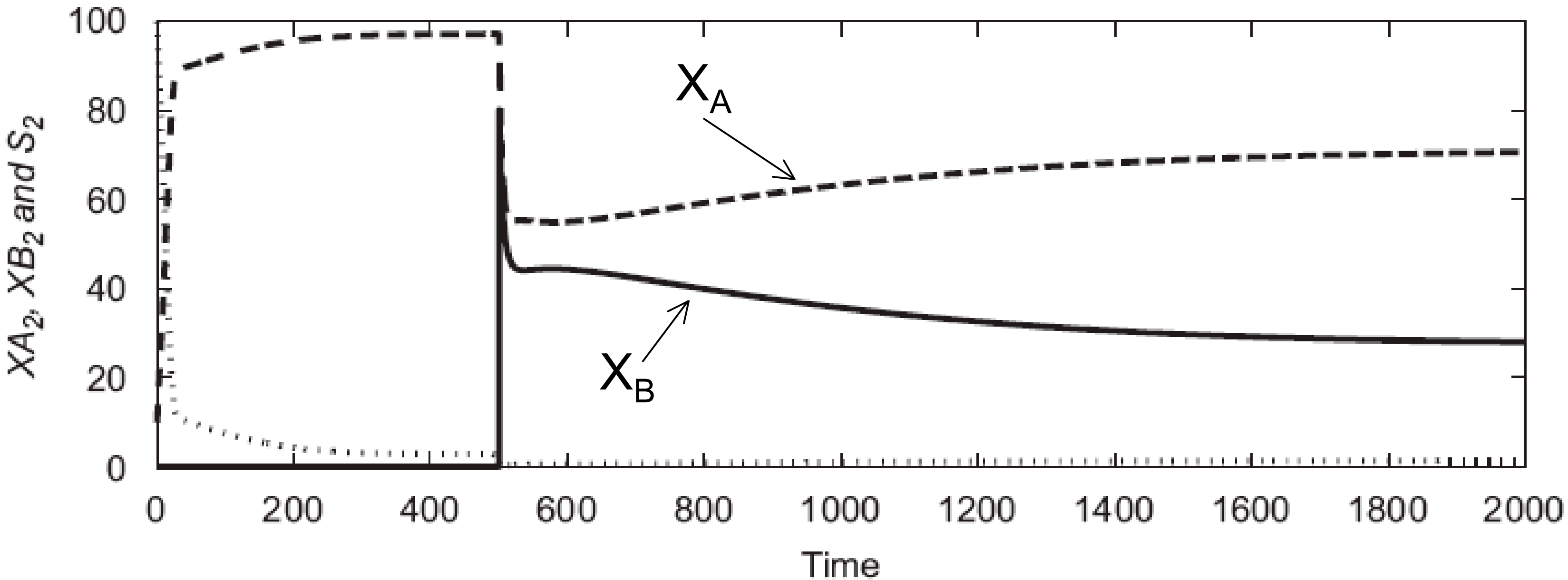
- Result :
  - $\lambda_A(D_1) < \lambda_B(D_1)$: B is washed out in tank 1
    coexistence in tank 2 if $g_A(\lambda_B(D_2))\left(\lambda_A(D_1) - \lambda_B(D_2)\right) > \frac{1}{D_2}$

  - $\lambda_A(D_1) > \lambda_B(D_1)$: A is washed out in tank 1
    coexistence in tank 2 if $g_B(\lambda_A(D_2))\left(\lambda_B(D_1) - \lambda_A(D_2)\right) > \frac{1}{D_2}$

- Coexistence only possible if $V_1 < V_2$ (non-optimal design)

Coexistence (non optimal reactor design)





3. Practical coexistence

- Competitive exclusion principle :
  predicts that only one species will survive

- But : - how long does it take for the dominant species
  to take over?
  - how will the non-dominant species survive?

- Practical coexistence : conditions for a «long» survival of
  the weak species

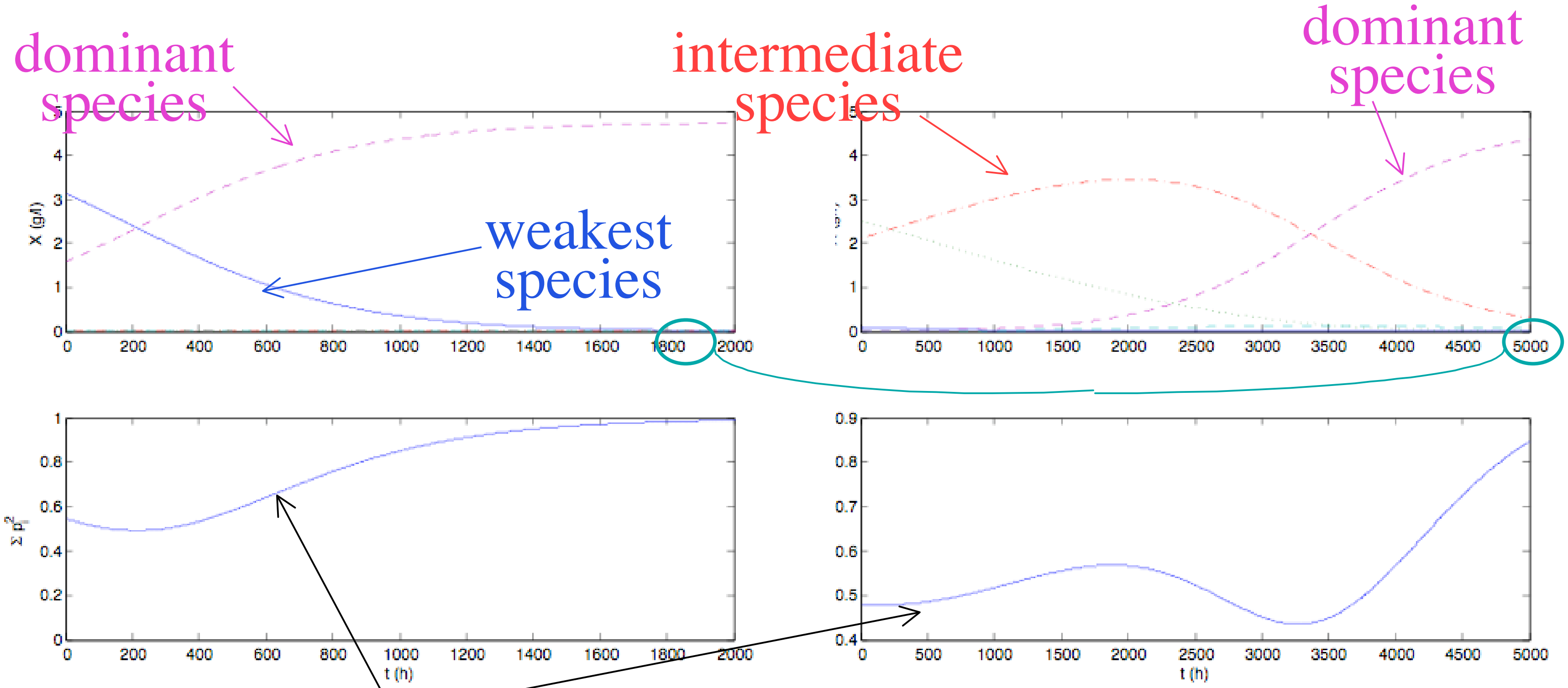
- New variable : species proportion : $p_i = \dfrac{X_i}{\sum_{j=1}^{n} X_j}$
- Dynamics of p_i :

$$\frac{dp_i}{dt} = \left(\sum_{j=1}^{n} \left(\mu_i(s) - \mu_j(s) \right) p_j \right) p_i$$

$$= \sum_{j=1}^{n} A_{ij} p_j p_i \quad \text{with } A_{ij} \text{ skew symmetric}$$

- **Results** :
  - whatever the initial conditions, the «weakest» species decreases, and the non-dominant increases
  - depending on the initial conditions, the others can possibly increase before decreasing (+ estimate of the increase duration)

Standard exclusion   Practical coexistence



Biodiversity index $\sum p_i$ (--> 1 when only species survives)

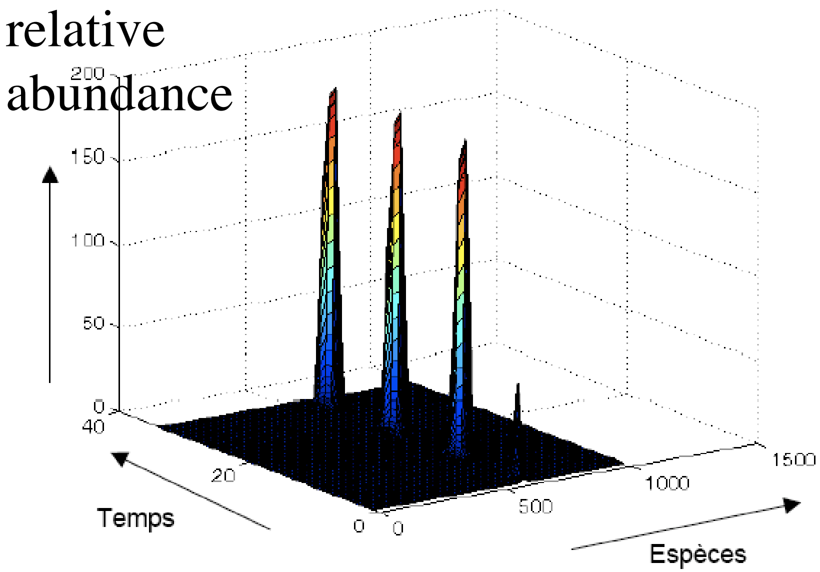
Issues and challenges

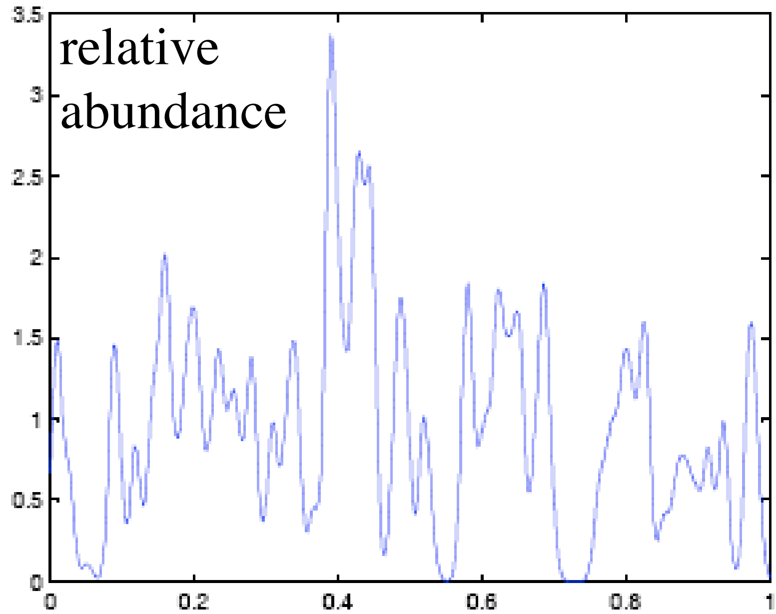
- Coexistence/competition are not just limited to ecology…

- 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 (*Tatarovsky et al*))

• Combination of theoretical/experimental studies

--> Molecular fingerprints via SSCP





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Thank you very much for your attention

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