The chemostat: a suitable environment to design and validate physiological models both at steady state and in dynamical conditions

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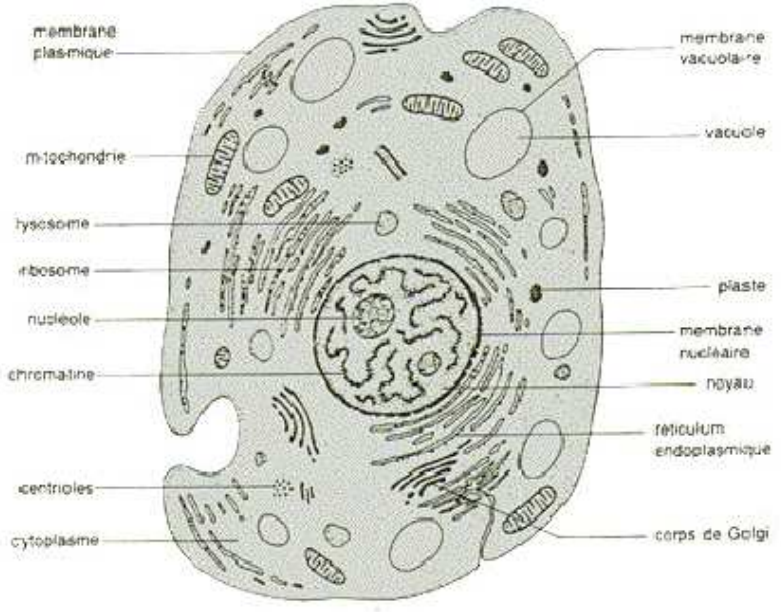
Introduction

- System modelling: difficult task
- Difficulty even higher for biological systems
- 3 main reasons...

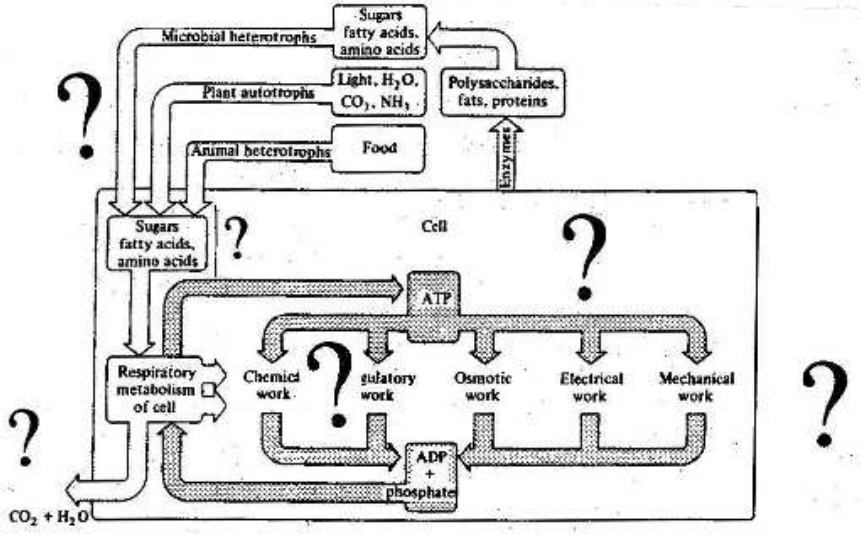
Introduction

Biological systems are **complex**



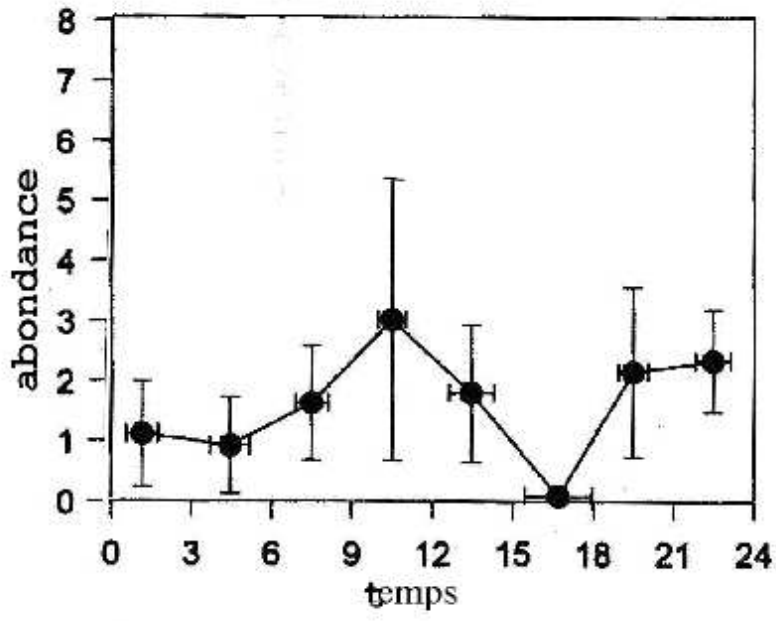
Introduction

Biological systems are **poorly known**



Introduction

Biological systems are **not well measured**

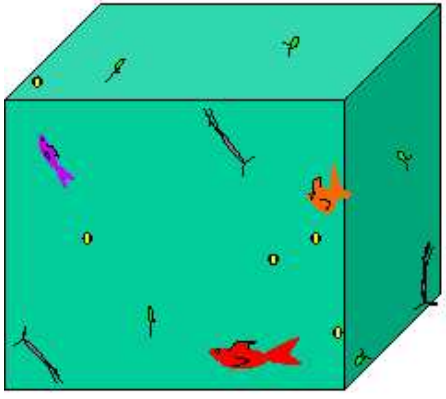


Introduction

- The chemostat: an experimental framework more suitable for modelling
- Sort the hypotheses assumed during model development
- Test these hypotheses with the principle of maximum uncoupling

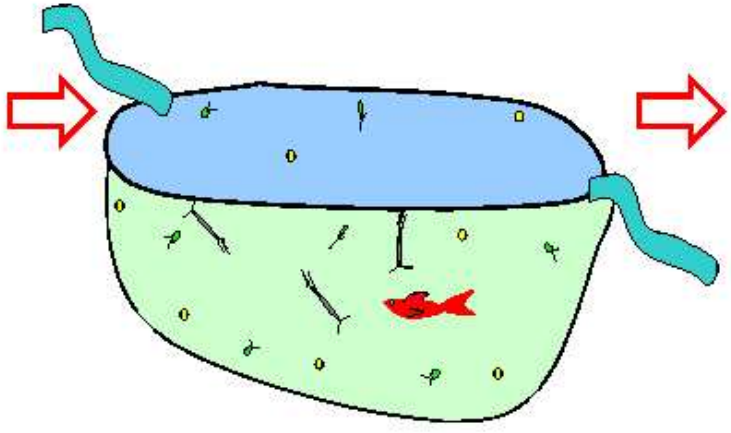
I. Simplification of the framework

Marine Ecosystem



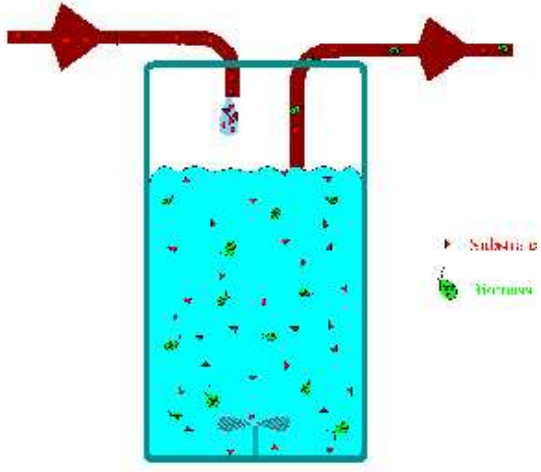
- Strong influence of hydrodynamics
- A large amount of interacting species
- Poorly known inputs

Pond



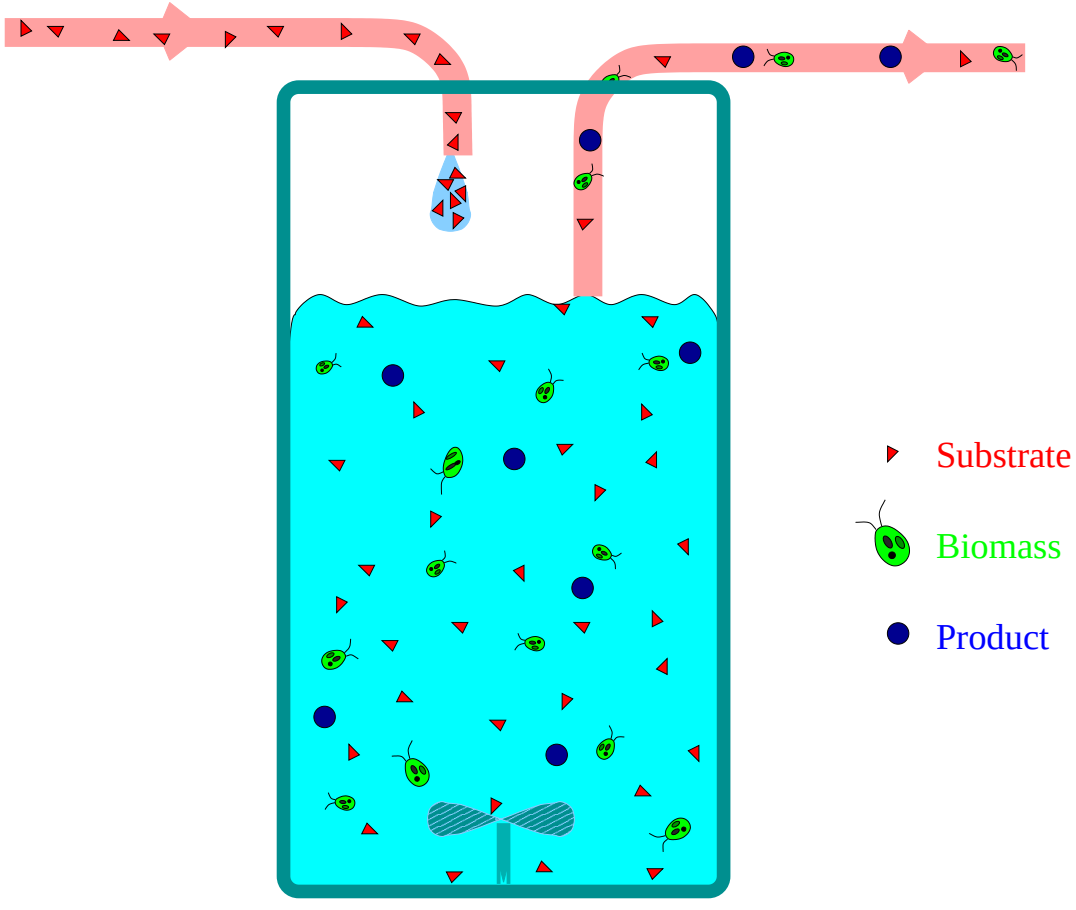
- Lower effect of spatial heterogeneity
- Inputs better measured

Chemostat



- homogeneous medium
- Inputs measured and controlled
- Low species number

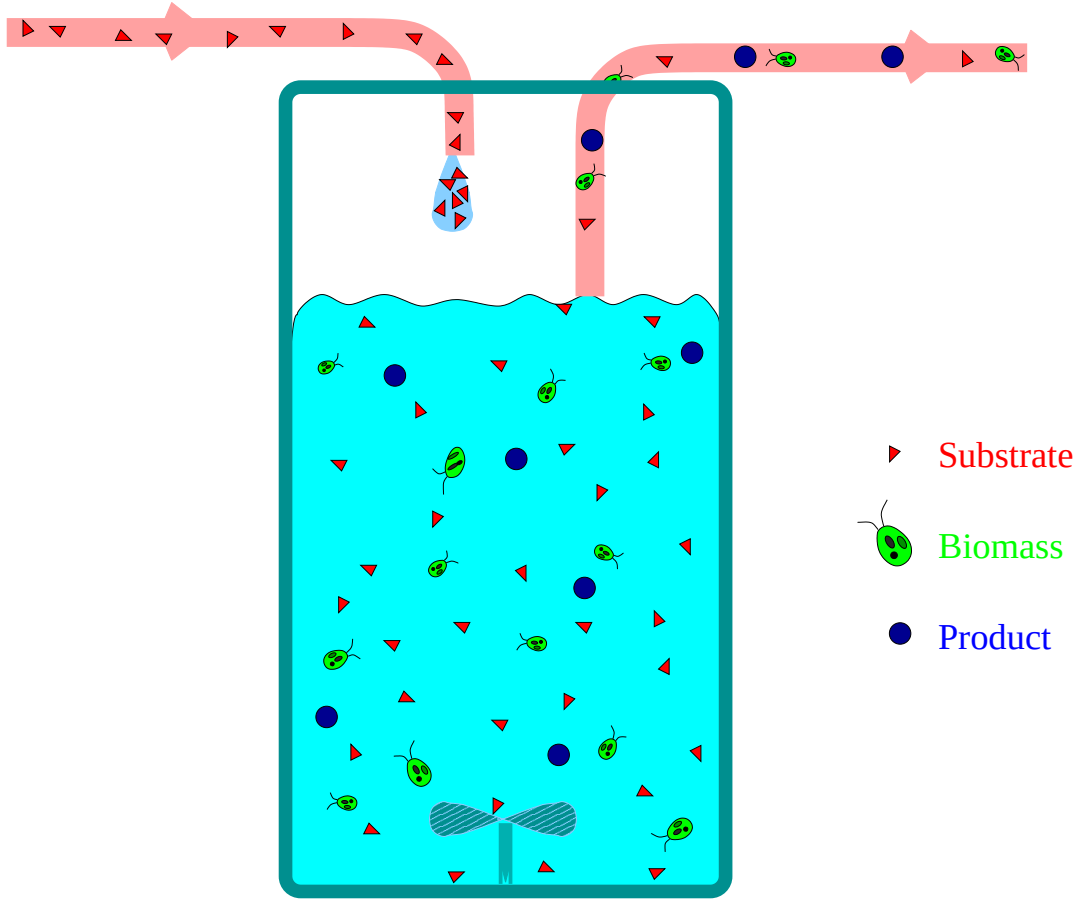
The chemostat



The chemostat

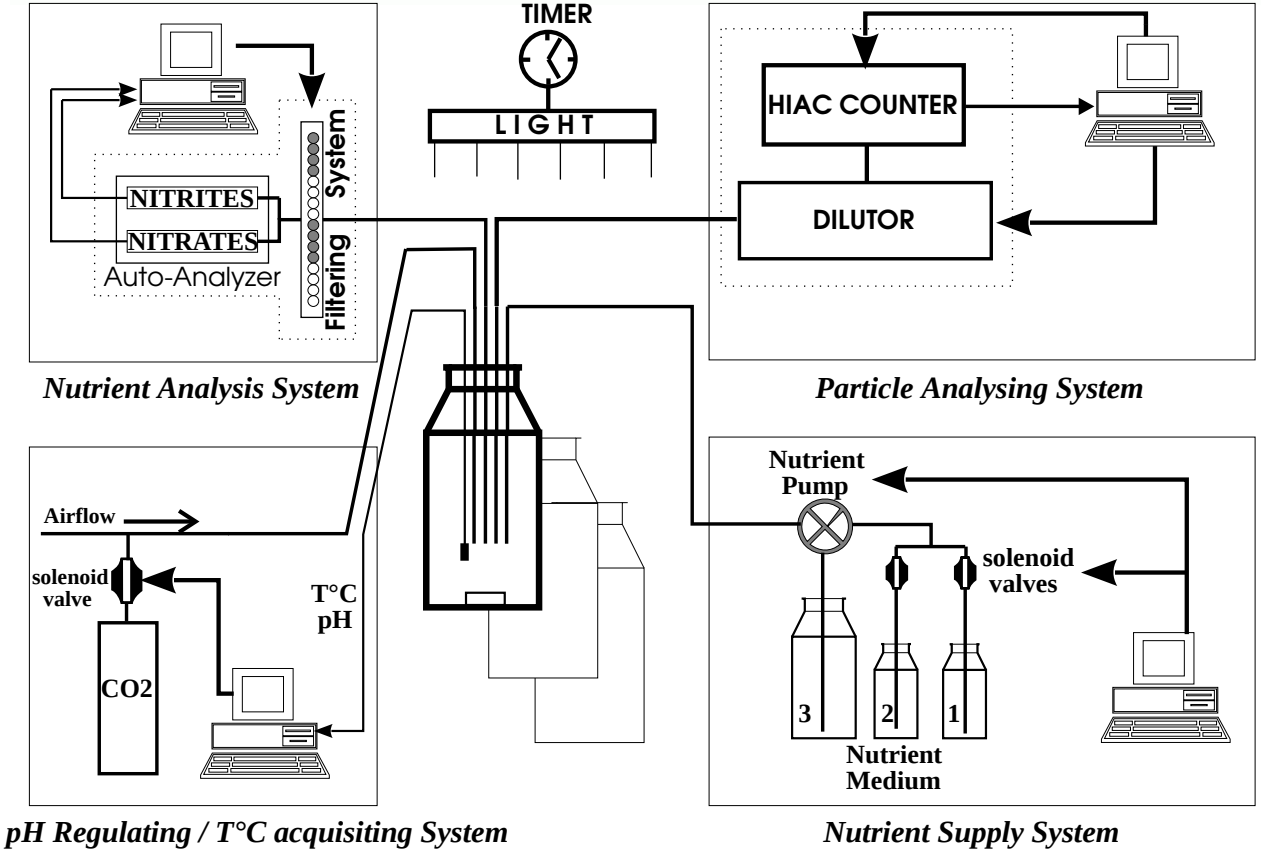
D, ξ_{in}

ξ



The chemostat

The chemostat



II. model design

1 - Proposition of a mass balance

2 - Determination of the biological kinetics

3 - Identification of the model parameters

1. Proposition of a mass balance

Example

Reaction network : example of coccolithophorids

$$6\,CO_2 + 12\,H_2O \longrightarrow C_6H_{12}O_6 + 6\,O_2 + 6\,H_20$$

$$Ca^{2+} + 2\,HCO_3 \longrightarrow CaCO_3 + CO_2 + H_2O$$

$\Rightarrow$ Are growth and calcification coupled ?

1. Proposition of a mass balance

Example

YES

$$N \quad \xrightarrow{\rho(.)X} \quad V$$

$$\frac{1-\alpha}{\alpha}S + \frac{1}{\alpha}C \quad \xrightarrow{\mu(.)X} \quad \frac{1-\alpha}{\alpha}P + X$$

1. Proposition of a mass balance

Example

YES

$$N \xrightarrow{\rho(.)X} V$$

$$\frac{1-\alpha}{\alpha}S + \frac{1}{\alpha}C \xrightarrow{\mu(.)X} \frac{1-\alpha}{\alpha}P + X$$

$$\begin{cases} \dot{N} &= D(N_{in} - N) - \rho(.)X \\ \dot{C} &= D(C_{in} - C) - \frac{1}{\alpha}\mu(.)X - qC \\ \dot{Q} &= \rho(.) - \mu(.)Q \\ \dot{X} &= -DX + \mu(.)X \\ \dot{P} &= -DP + \frac{1-\alpha}{\alpha}\mu(.)X \\ \dot{S} &= D(S_{in} - S) - \frac{1-\alpha}{\alpha}\mu(.)X \end{cases}$$

1. Proposition of a mass balance

Example

NO

$$
\begin{aligned}
N &\xrightarrow{\rho(.)X} V \\
C &\xrightarrow{\mu(.)X} X \\
S + C &\xrightarrow{\sigma(.)X} P
\end{aligned}
$$

1. Proposition of a mass balance

Example

NO

$$
\begin{array}{rcl}
N & \xrightarrow{\rho(.)X} & V \\
C & \xrightarrow{\mu(.)X} & X \\
S + C & \xrightarrow{\sigma(.)X} & P
\end{array}
$$

$$
\left\{
\begin{array}{rcl}
\dot{N} & = & D(N_{in} - N) - \rho(N)X \\
\dot{Q} & = & \rho(N) - \mu(.)Q \\
\dot{X} & = & -DX + \mu(.)X \\
\dot{P} & = & -DP + \sigma(.)X \\
\dot{C} & = & D(C_{in} - C) - \mu(.)X - \sigma(.)X - qC \\
\dot{S} & = & D(S_{in} - S) - \sigma(.)X
\end{array}
\right.
$$

2. Proposition biological kinetics

Example

Expression of the growth rates and of the calcification rate

$$\mu(.) = \mu(N, CO_2) \quad ?$$

$$\mu(.) = \mu(Q, HCO_3^-) \quad ?$$

$$\mu(.) = \mu(Q, CO_3^{--}) \quad ?$$

$$\sigma(.) = \sigma(CO_2) \quad ?$$

$$\sigma(.) = \sigma(CO_3^{--}) \quad ?$$

II. General structure

$$\dot{\xi} = Kr(.) + D(\xi_{in} - \xi) - Q(\xi)$$

- ξ : state vector
- ξ_{in} : vector of influent concentrations,
- $r(.)$: vector of the reaction rates
- K : pseudo stoichiometric matrix
- $Q(\xi)$: exchanges liquid/gas
- D : dilution rate.

III. Model validation

1 - Validation of the reaction network

2 - **Qualitative** validation of static properties

3 - **Qualitative** validation of dynamical properties

4 - Global quantitative model validation

Introduction

- Most important step in biological modelling!!!!

... but most of the time the most neglected step.

- We will see how to test **separately** the various hypotheses

III.1 Validation of the reaction network

Property 1 (Reaction invariant) *The $n \times k$ matrix K $(n > k)$ has a left kernel with dimension at least $n - k$. There exists $n - k$ independent vectors $v_i \in \mathbf{R}^n$ such that:*

$$v_i^t K = 0_{1 \times k}$$

Consequence : the real variable $w_i = v_i^t \xi$ verifies:

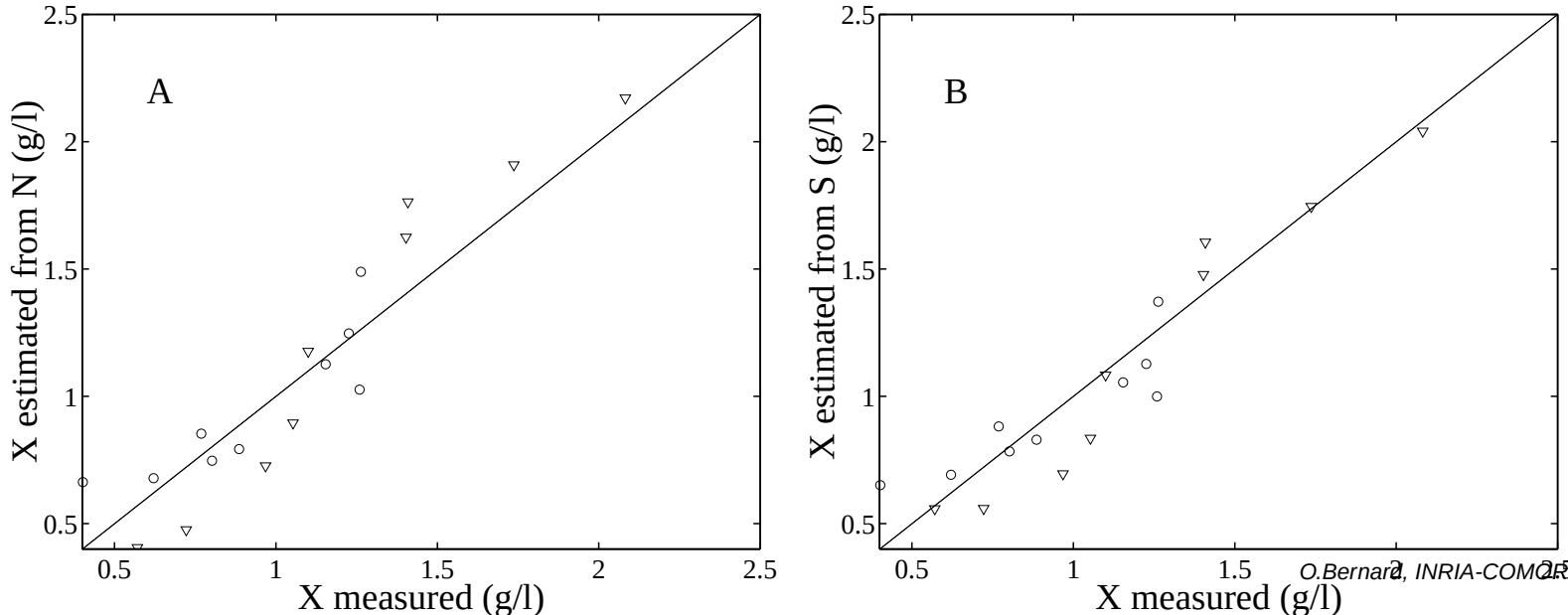
$$\frac{dw_i}{dt} = D(w_{i\,in} - w_i) - v_i^t Q(\xi) \qquad (1)$$

with $w_{i\,in} = v_i^t \xi_{in}$.

Example : growth of Pycnoporus cinnabarinus

Growth of the filamentous fungi *Pycnoporus cinnabarinus* (X) on a carbon source C and a nitrogen source N.

$$k_1 N + k_2 C \longrightarrow X$$



III.2. Qualitative Validation of the model at steady state

- How to test the model from a qualitative point of view using the available experimental data ?

  - Asymptotic behaviour obtained for constant inputs (steady states, limit cycles, chaos,...) in agreement with experiments ?
  - How do these properties evolve when the forcing inputs fluctuate ?
  - Qualitative input-output behaviour

Competition between 2 bacterial species in the chemostat

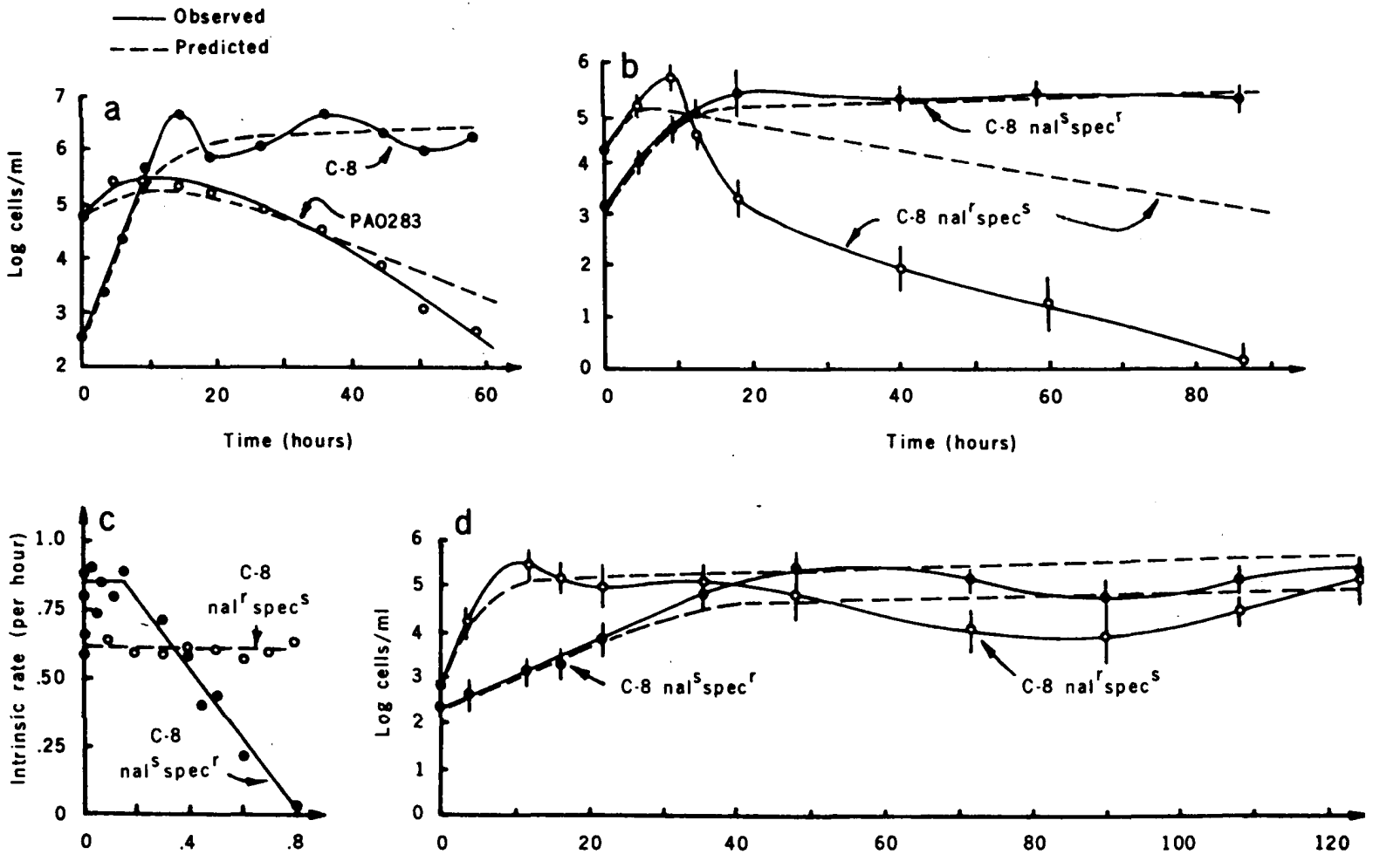
$$k_1\, S \longrightarrow X_1$$

$$k_2\, S \longrightarrow X_2$$

$\Longrightarrow$ The winner of the competition depends on the dilution rate; species with the lowest ratio J_i :

$$J_i = \frac{K_{s\,i}}{\mu_{max\,i} - D}$$

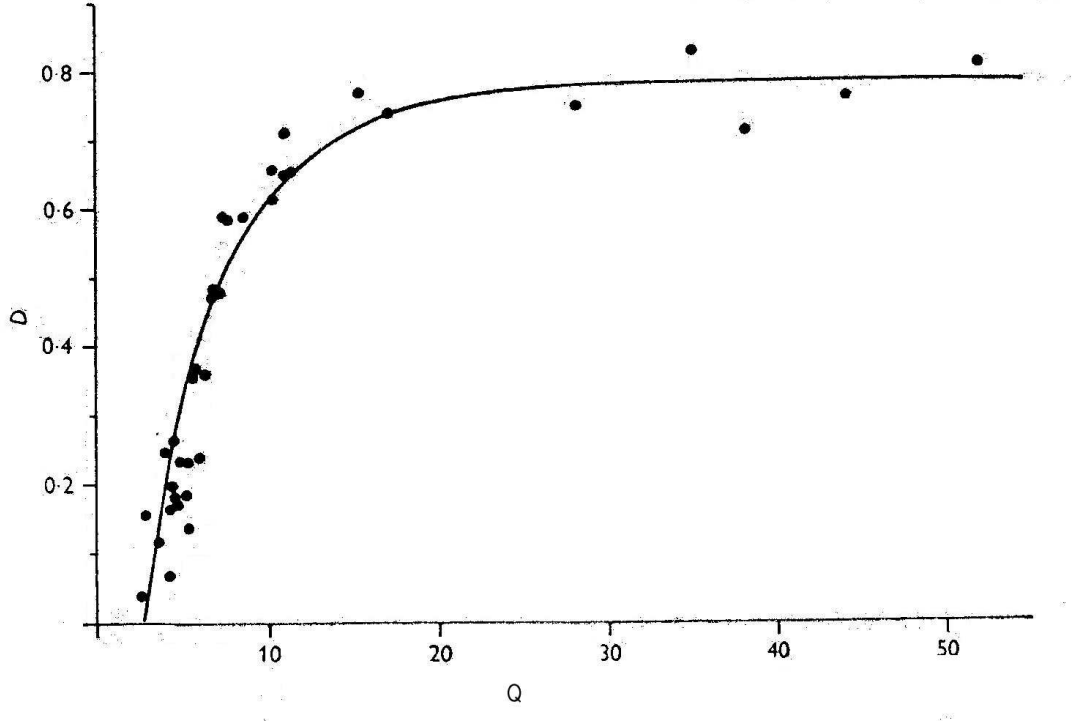
Competition between 2 bacterial species in the chemostat



From Hansen and Hubbell (1980)

Qualitative input-output behaviour

- How do AT STEADY STATE the model variables change in response to a variation of the chemostat inputs (dilution rate, limiting nutrient concentration, etc) ?



Internal quota of vitamin B_{12} at chemostat steady state with respect to the dilution rate

Phytoplankton growth limited by nitrogen/light

Thesis L.Pawlowski

Variable at steady state	Light intensity	dilution rate
$C^\star$	+	+
$L^\star$	-	+
$N^\star$	+	-
$\frac{N^\star}{C^\star}$	-	+
$\frac{L^\star}{C^\star}$	- & +	+

Trend at steady state with respect to inputs variation.

(+: increasing, -:decreasing) : model and experience are in agreement

Growth and calcification

	CI			UI								
	HCO_3^-	CO_2	CO_3^{2-}	HCO_3^- HCO_3^-	HCO_3^- CO_2	HCO_3^- CO_3^{2-}	CO_2 HCO_3^-	CO_2 CO_2	CO_2 CO_3^{2-}	CO_3^{2-} HCO_3^-	CO_3^{2-} CO_2	CO_3^{2-} CO_3^{2-}
C*	↗	↗	↗	↗	↗	↗ ?	↗	↗	↗ ?	↗ ?	↗ ?	↗ ?
Cc*	↗	↗	↘	↗	↗	↗	↗	↗	↗	↘	↘	↘
Cd*	↗	↗	↘	↗	↗	↘	↗	↗	↘	↗	↗	↘
X*	↗	↗	↘	↗	↗	↗	↗	↗	↗	↘	↘	↘
Q*	↘	↘	↗	↘	↘	↘	↘	↘	↘	↗	↗	↗
N*	↘	↘	↗	↘	↘	↘	↘	↘	↘	↗	↗	↗
P*	↗	↗	↘	↗	↗	↘ ?	↗	↗	↘ ?	↗?	↗ ?	↘
$\frac{P^*}{X^*}$	→	→	→	↗	↗	↘	↗	↗	↘	↗	↗	↘
Φ^*	↗	↗	↘	↗	↗	?	↗	↗	?	?	?	↘
ϕ_p^*	→	→	→	→	→	→	→	→	→	→	→	→
ϕ_c^*	→	→	→	↗	↗	↘	↗	↗	↘	↗	↗	↘
$\frac{\Phi_p^*}{\Phi_c^*}$	→	→	→	↘	↘	↗	↘	↘	↗	↘	↘	↗

Qualitative variations of the state variables at steady-state after an elevation of pCO_2 for the 12 considered models (Coupled (CI) or uncoupled (UI) processes)

III.3 Qualitative dynamical validation of the model

- In some cases, the transient behaviour of the model can be completely determined by a mathematical analysis
- Possible succession of the state variables extrema
- Position of the variables with respect to a reference value

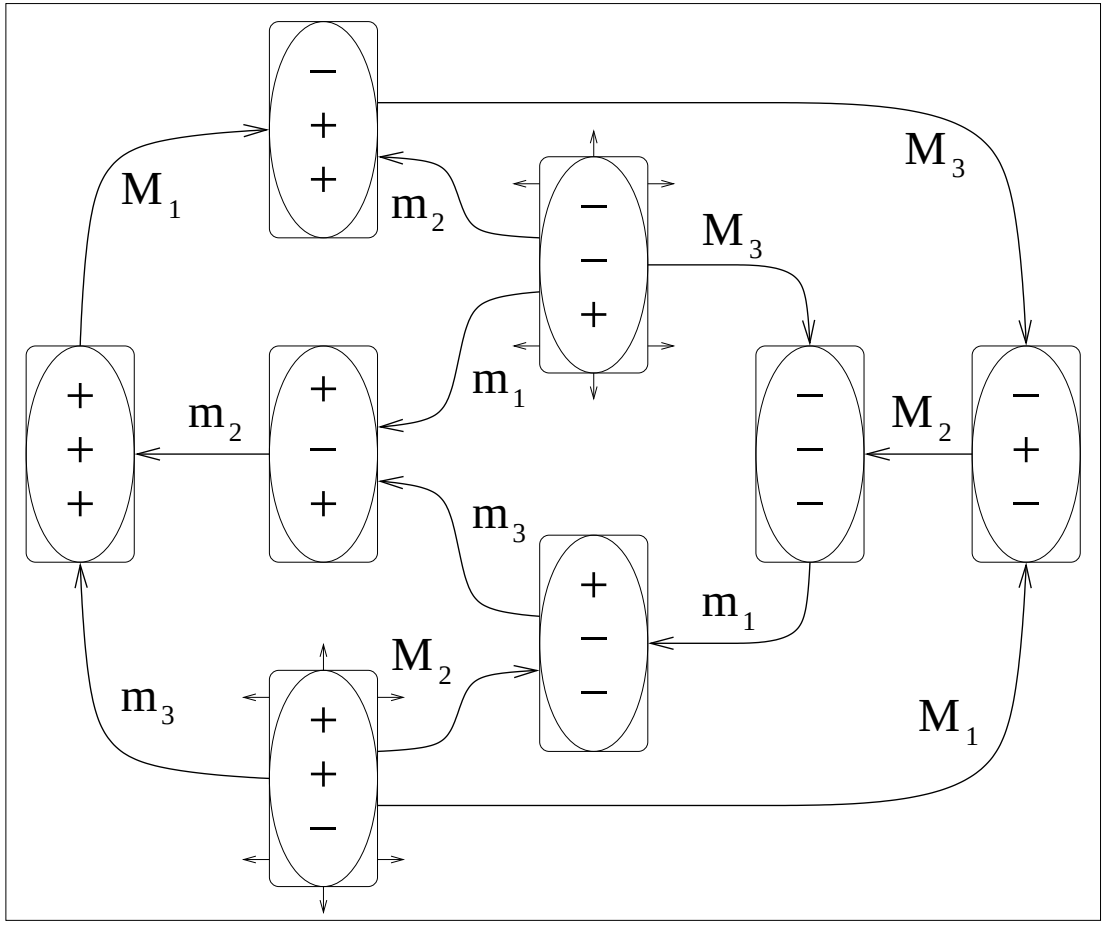
Example: analysis of Droop's model

Growth of algae (X) in the chemostat limited by NO_3 (S)

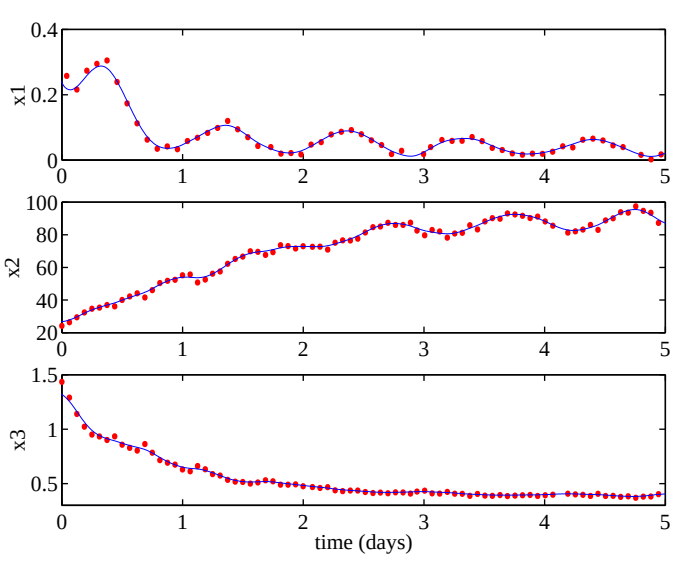
$$\begin{cases} \dot{X} = \mu_m(1 - \frac{K_Q}{Q})X - DX \\ \dot{Q} = \rho_{max}\frac{S}{K_\rho + S} - \mu_m(Q - K_Q] \\ \dot{S} = D[S_{in} - S] - \rho_{max}\frac{SX}{K_\rho + S} \end{cases}$$

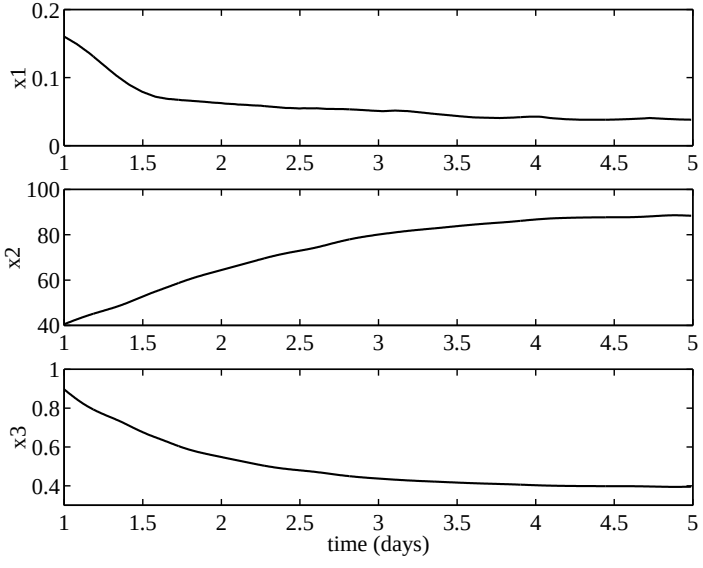
Example: response of an algal population to a periodic fluctuation of a nitrogen source

- Theoretical dynamical behaviour



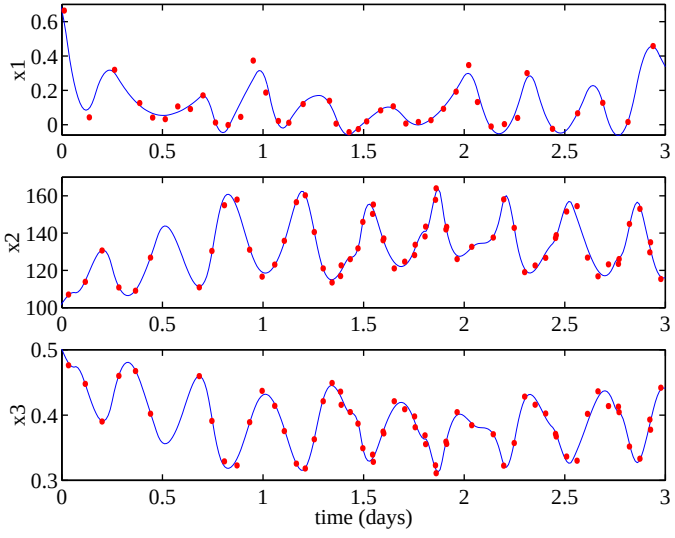
Transition graph for the models with Droop structure

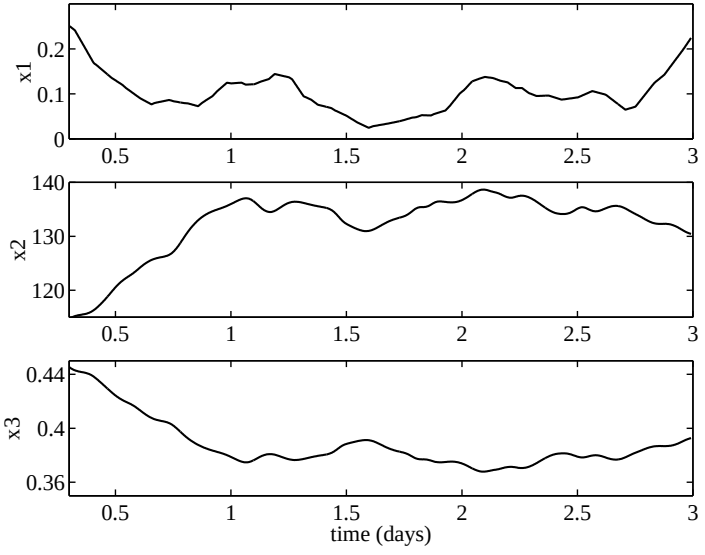




Dunaniella tertiolecta in chemostat with periodic nitrate limitation (period: 24 h).

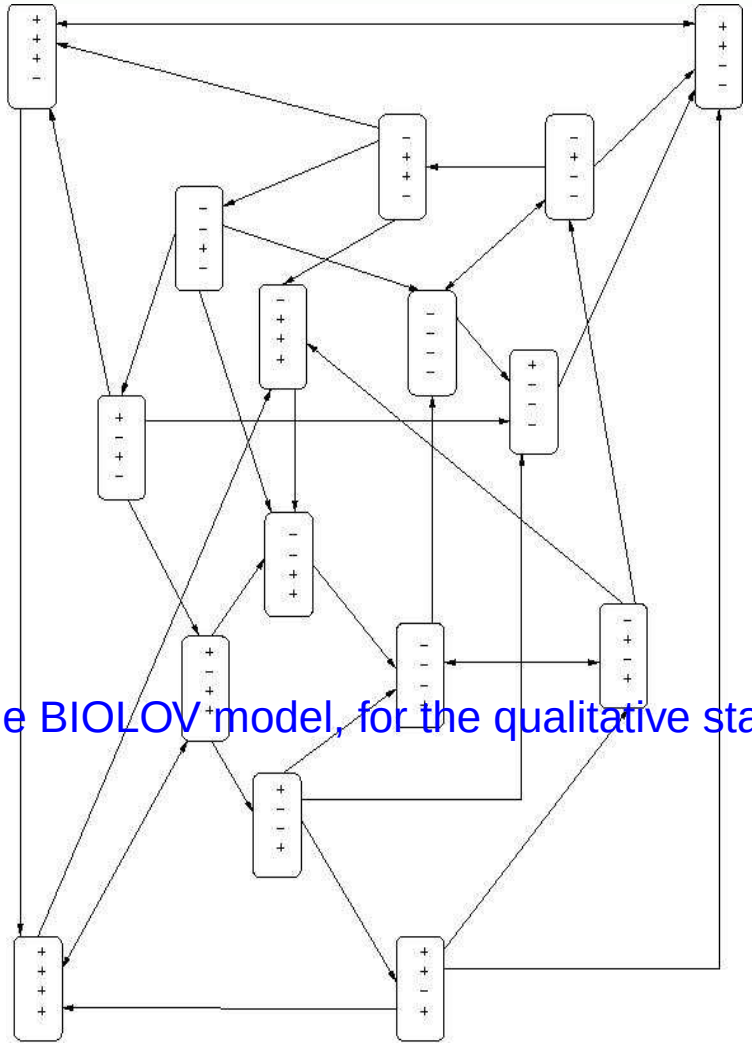
Transition graph is respected





Dunaniella tertiolecta in chemostat with periodic nitrate limitation (period: 8 h).

Transition graph is NOT respected



Transition graph for the BIOLOV model, for the qualitative state $(\dot{S}, \dot{Q}, \dot{Q}_l, \dot{C})$.

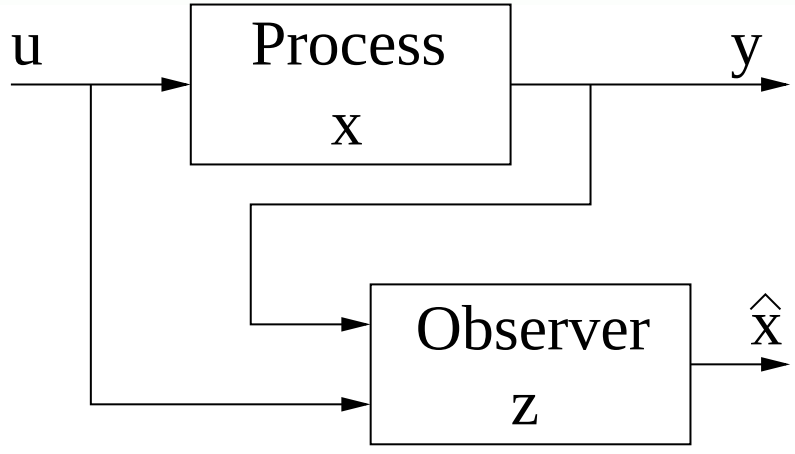
III.4. Global model validation

i. Known model with uncertain parameters (interval)

ii. Data driven known model with known parameters

iii. Known model with known parameters

ii. Data driven simulations



Observer principle

- Model and available (partial) measurements are combined to estimate the non measured variables

Example: growth of phytoplankton with Droop model

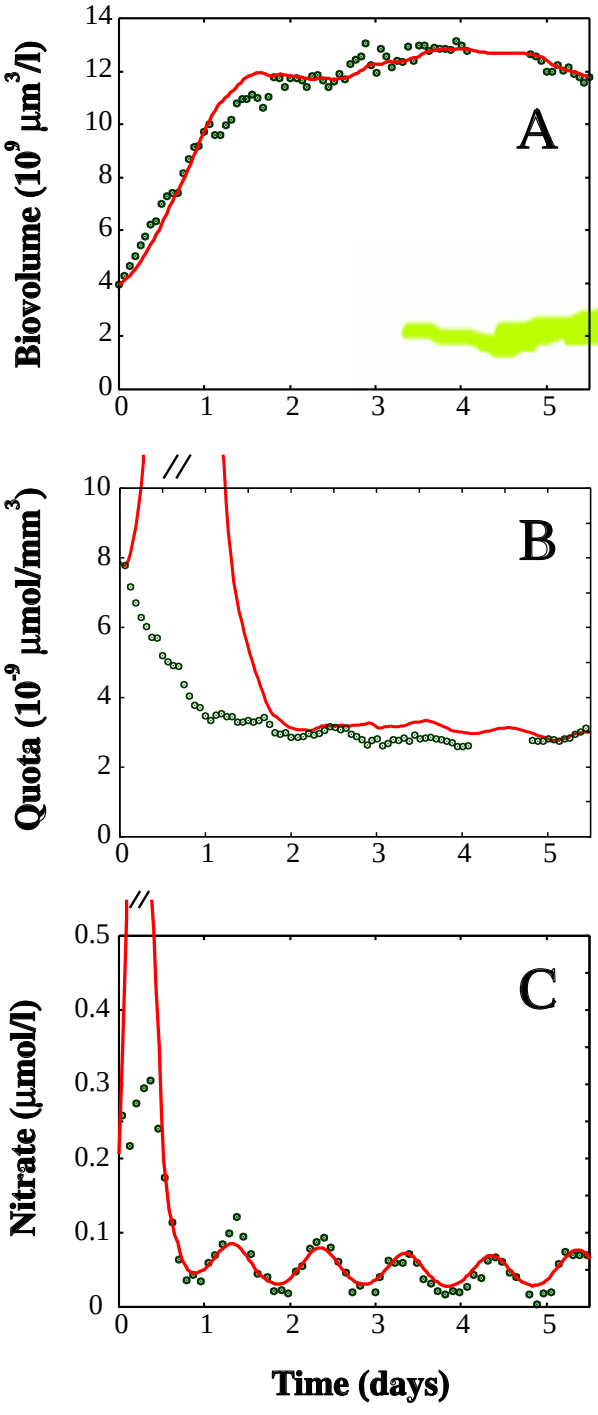
Growth of *Dunaliella tertiolecta* in the chemostat limited by NO_3 (S)

$$\begin{cases} \dot{X} = \mu_m(1 - \frac{K_Q}{Q})X - DX \\ \dot{Q} = \rho_{max}\frac{S}{K_\rho + S} - \mu_m(Q - K_Q] \\ \dot{S} = D[S_{in} - S] - \rho_{max}\frac{SX}{K_\rho + S} \end{cases}$$

Biomass is measured $y = X$,

the high gain observer estimates S and Q.

Data driven simulations for validation

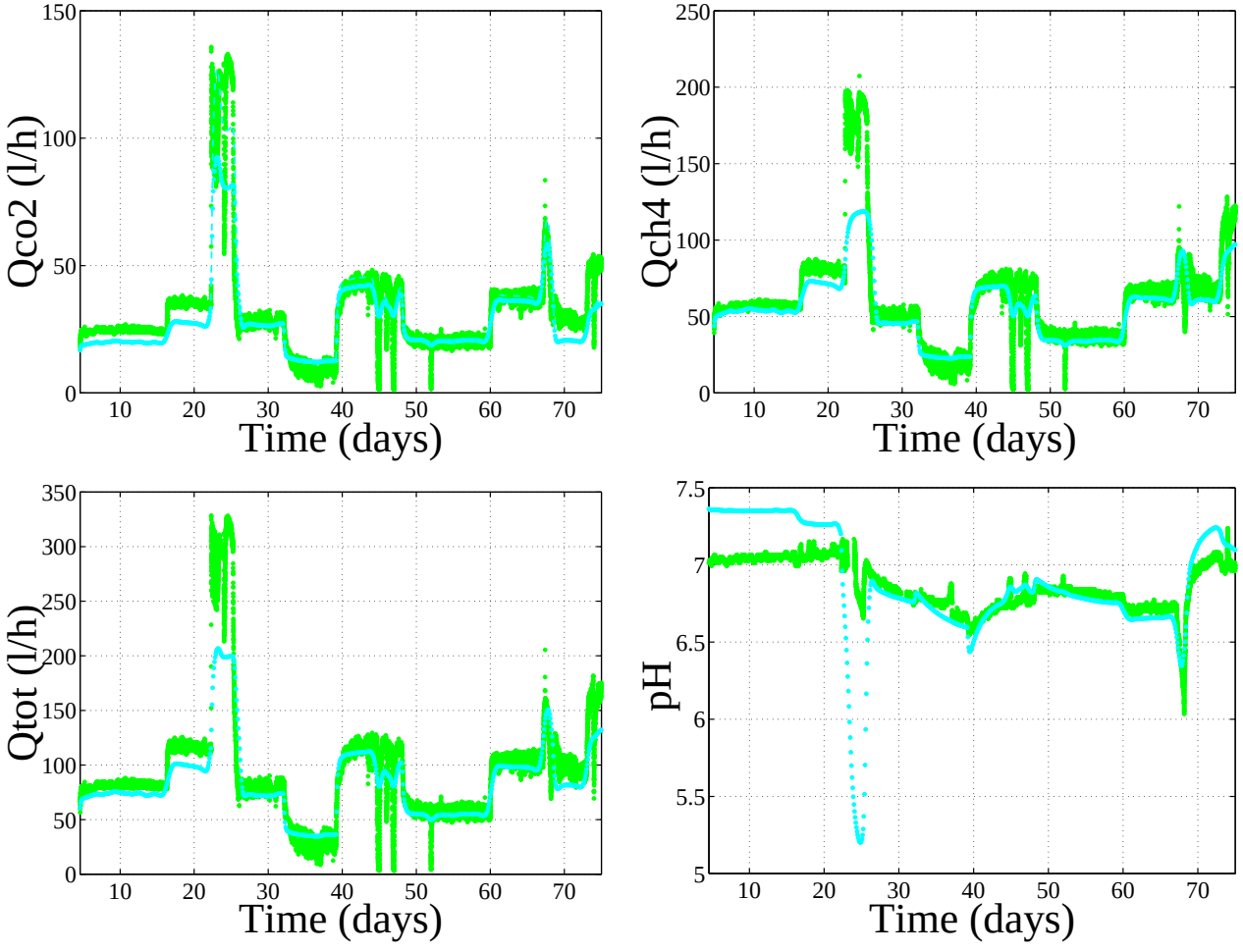


Direct measurements (●),

Observer predictions (—)

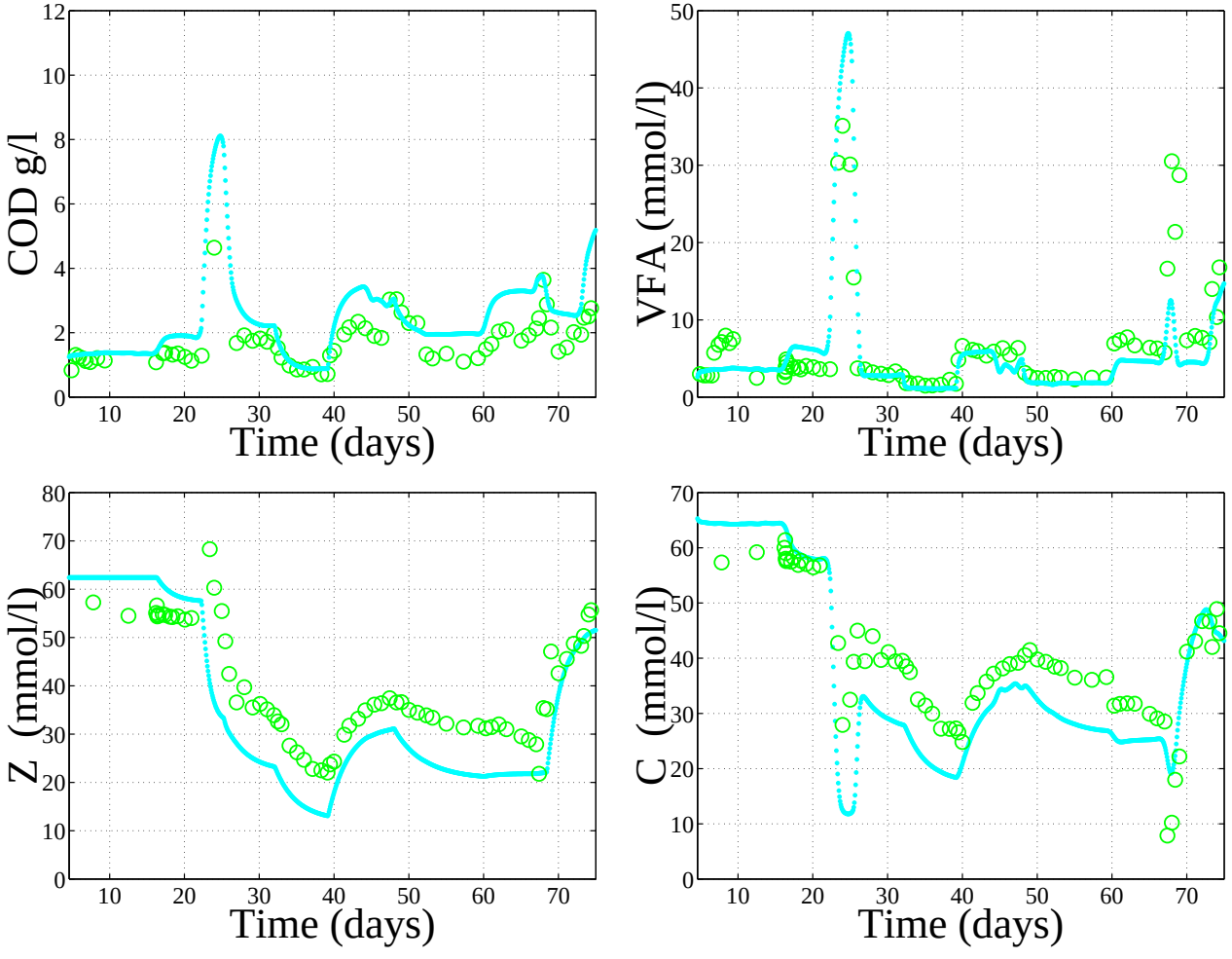
iii. ***Validation of the whole model***

Anaerobic digestion



iii. **Validation of the whole model**

Anaerobic digestion



Conclusion on modelling-validation

- Models with various hypothesis levels
- Models validated up to a given threshold
- Models validated with respect to SPECIFIC experiments (steady states, periodic forcing...).

Conclusion

- Validate the models in the conditions for which they are used
- Permanent model revisiting from available data
- The chemostat: an ideal framework

Thank you...