A thermodynamic theory of microbial growth and its perspectives for modelling environmental biotechnology processes

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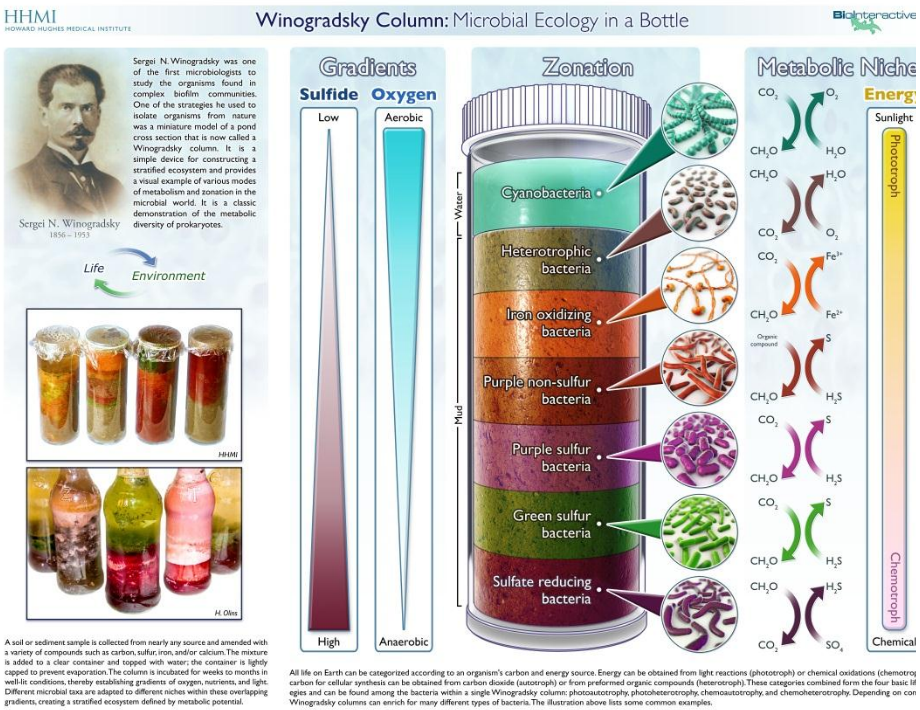
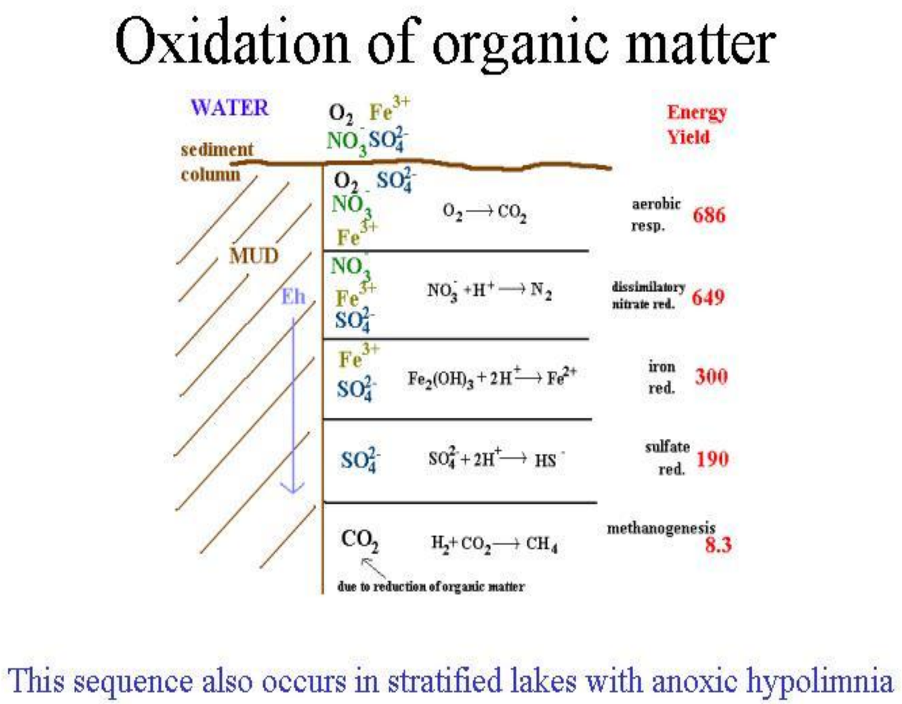
MELiSSA WORKSHOP

8-9 June 2016, Lausanne Switzerland

Microbial successions and energy gradients



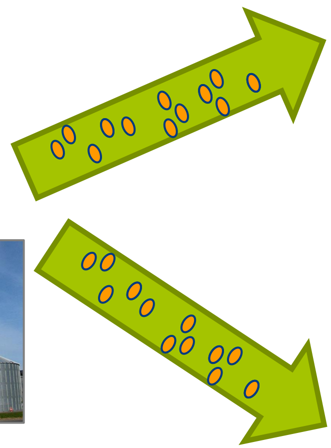
http://www.hhmi.org/biointeractive/poster-winogradsky-column-microbial-evolution-bottle



http://www.esf.edu/efb/schulz/Limnology/redox.html

Existence of a functional convergence phenomenon of microbial communities in environmental bioprocesses

« Open diversity systems »



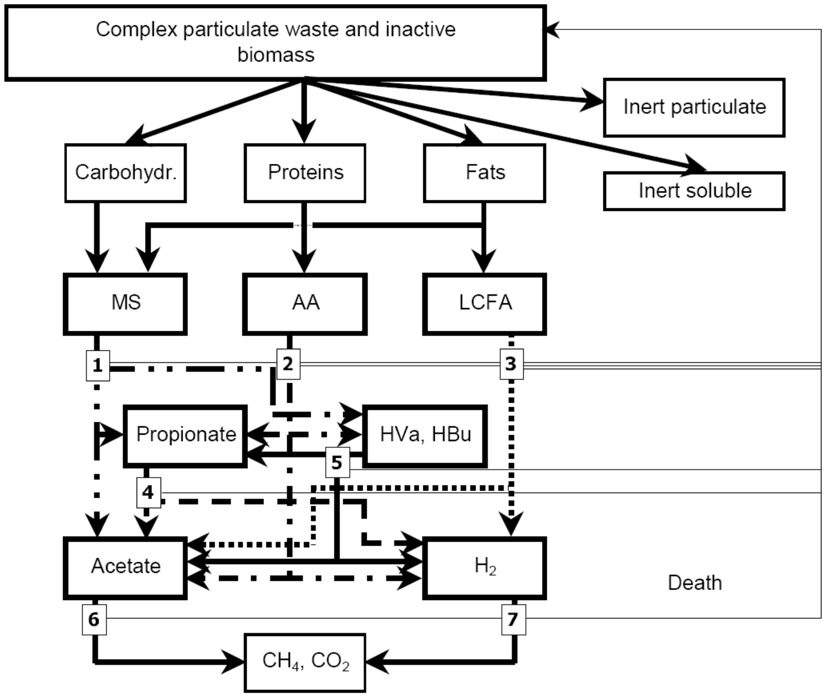
$C_5H_7O_2N + 3\ H_2O \rightarrow 2{,}5\ CH_4 + 2{,}5\ CO_2 + NH_3$

$C_5H_7O_2N + 7\ O_2 \rightarrow 2\ H_2O + 5\ CO_2 + NO_3^- + H^+ + H_2O$

How could all these complex ecological interactions lead to such a reproducible functional convergence?

What do models tell us about this convergence?

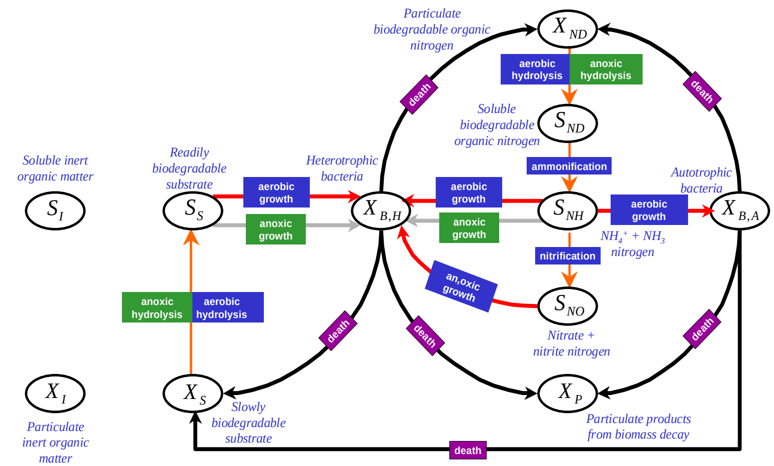
Anaerobic Digestion Model N°1



$$\frac{dB}{dt} = \mu.B$$ and $$\mu = \mu_{\max}\frac{[S]}{K_s + [S]}$$ …n_1 times

$\neq$

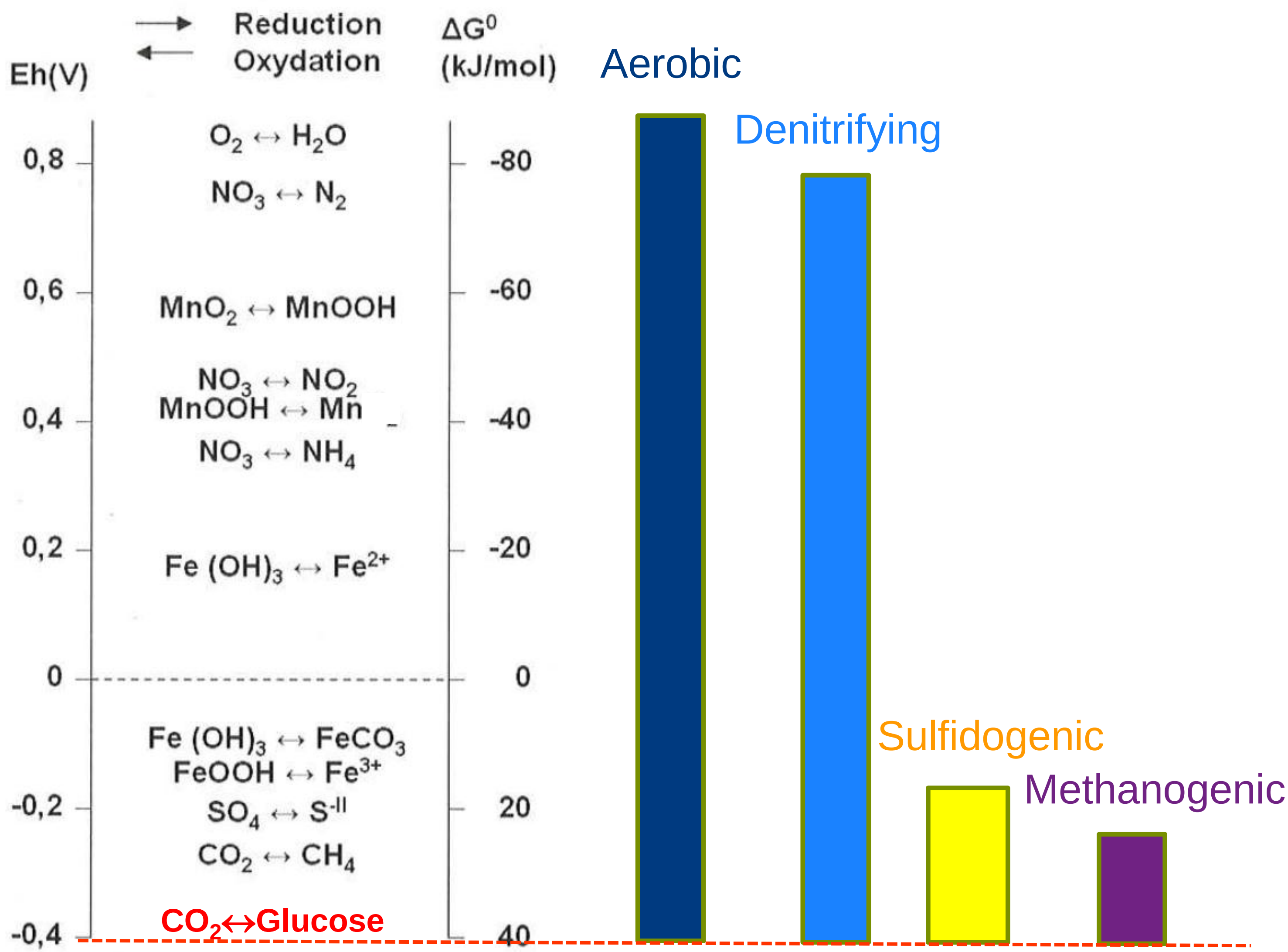
Activated Sludge Model N°1



$$\frac{dB}{dt} = \mu.B$$ and $$\mu = \mu_{\max}\frac{[S]}{K_s + [S]}$$ …n_2 times

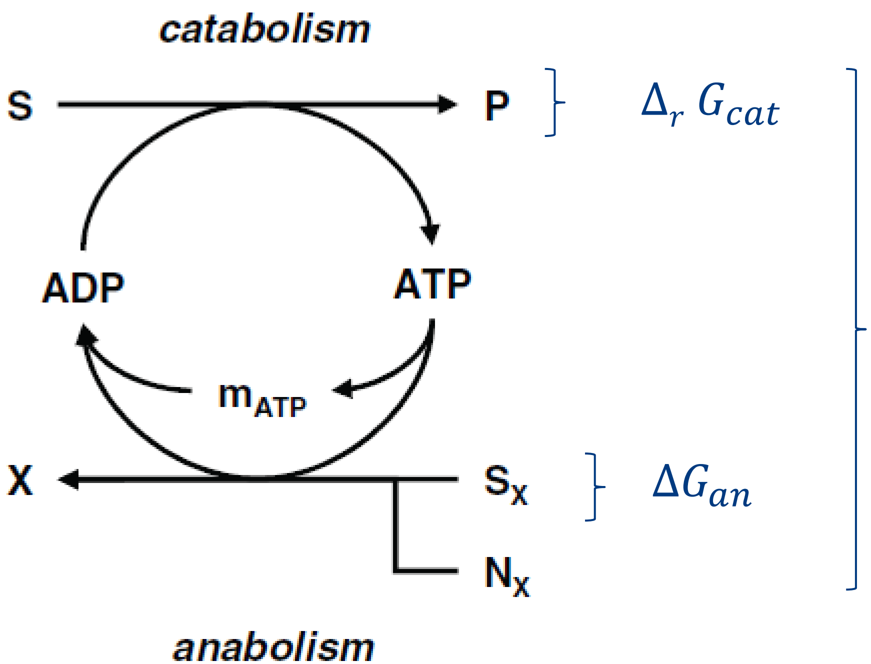
=> Current models are not appropriate to handle this question

Observed metabolic successions and thermodynamics



$$|\Delta_r G_{cat}| > |\Delta_r G_{cat}| > |\Delta_r G_{cat}| > |\Delta_r G_{cat}|$$

Thermodynamic balances of microbial growth



$$\Delta G_{met} = \Delta G_{an} + \lambda .\ \Delta_r G_{cat}$$

$$\Delta G_{met} = \Delta G_{an} + \lambda .\ \Delta_r G_{cat} = \Delta G_{dis} = f\ (\text{substrate})$$

Introducing the exergy concept

$$E_{dis} = \lambda\ .E_{cat} - E_M$$

From thermodynamic balances to kinetics?

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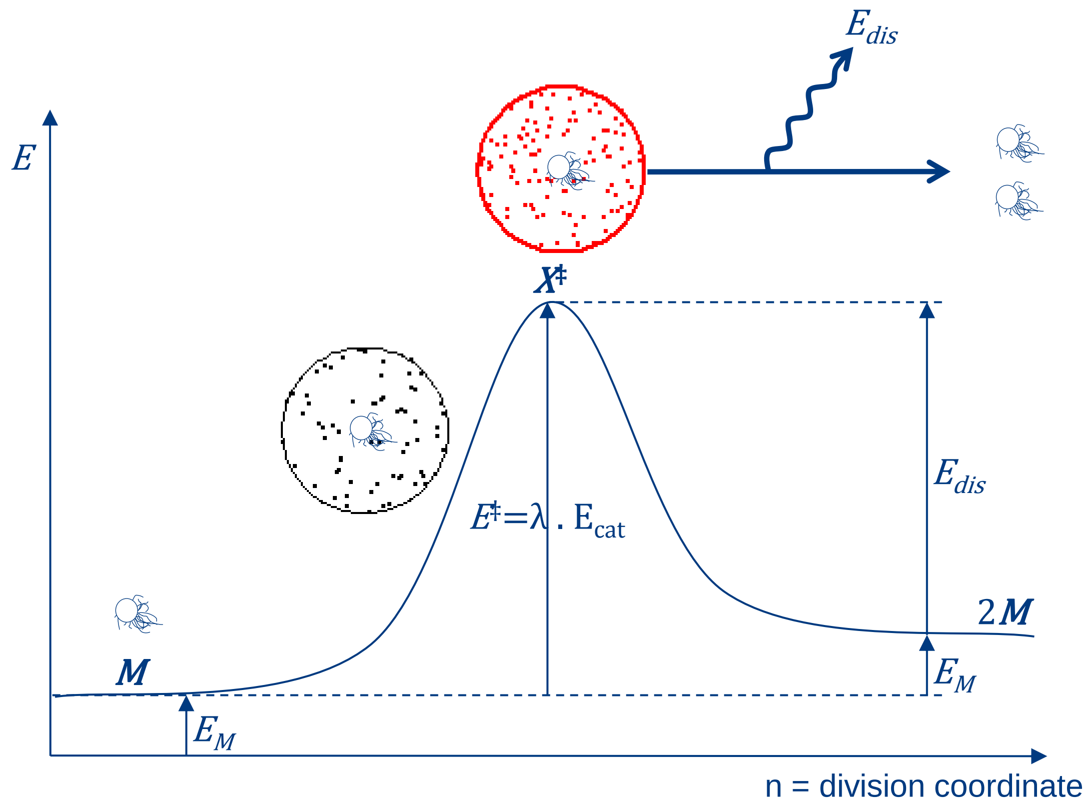
A microbial "transition state" theory

Desmond-Le Quéméner and Bouchez, The ISME-J, 2014



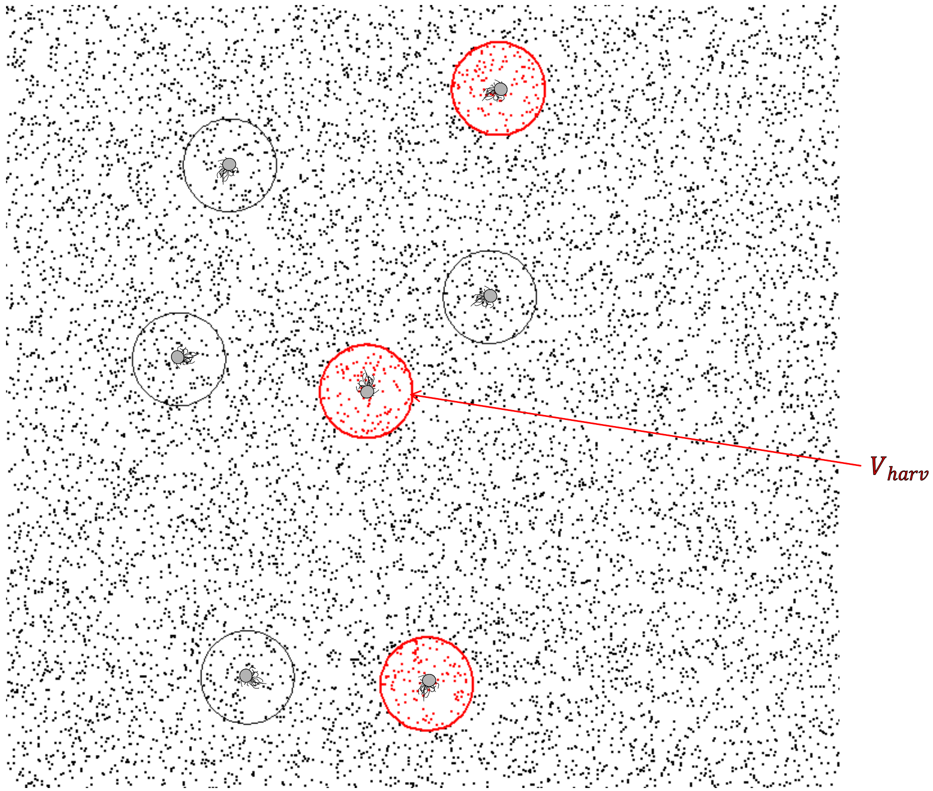
$$M \overset{K}{\longleftrightarrow} X^{‡} \xrightarrow{\mu_{max}} 2M$$

$$K = \frac{[X^{‡}]}{[M]} = \frac{N^{‡}}{N} \quad \text{and} \quad \frac{dN}{dt} = \mu_{max} \cdot N^{‡}$$

N is the number of microbes

$N^{‡}$ is the number of activated microbes

Resource allocation among microbes: a statistical question



- Define the spatial distribution of molecules in the medium
- Introduce V_{harv} « the harvesting volume »
- Compute the distribution of molecules in the various harvesting volumes

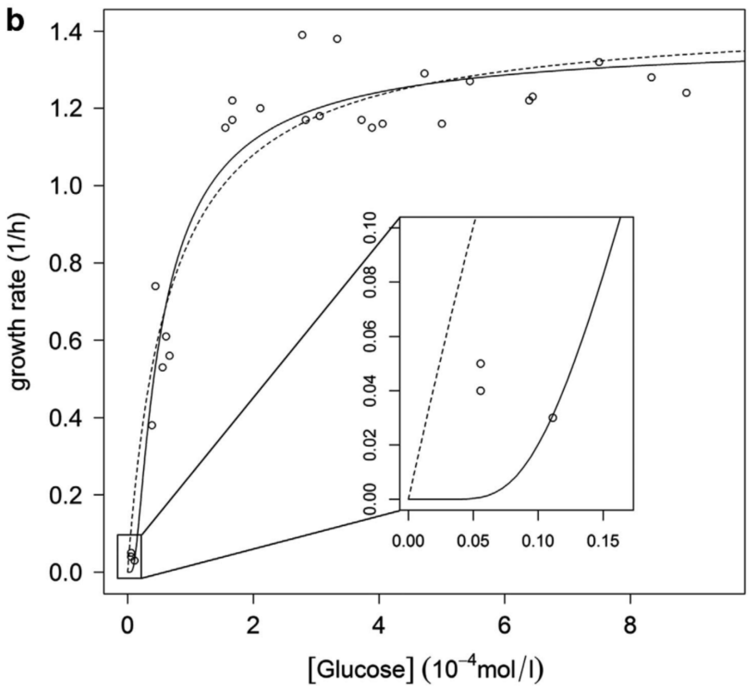
$\Rightarrow N^{\ddagger}$ can be deduced from this calculation

$$\frac{N^{\ddagger}}{N} = \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}}\right)$$

Desmond-Le Quéméner and Bouchez, The ISME-J, 2014

A microbial growth equation and its consequence on isotopic fractionation

$$\mu = \mu_{max} \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}}\right)$$



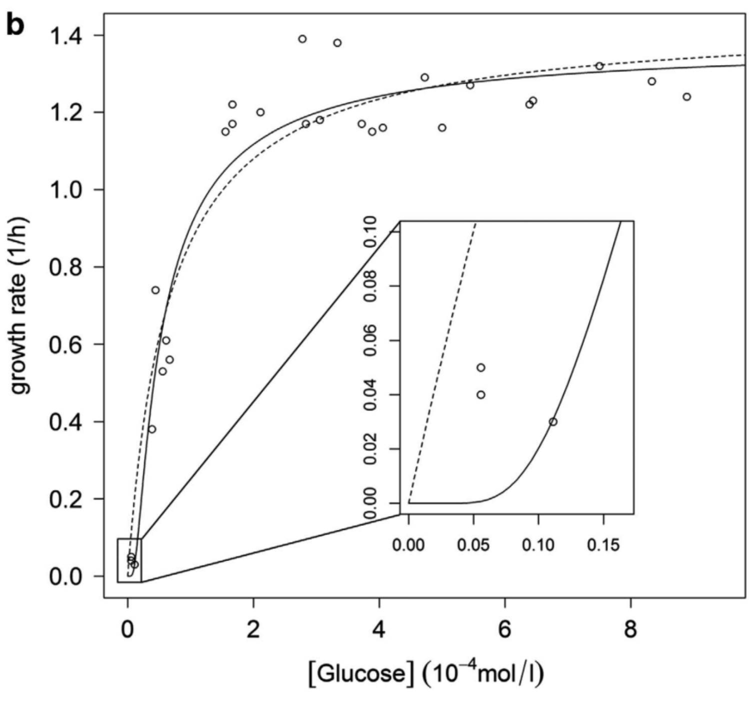
- Any further predictions?

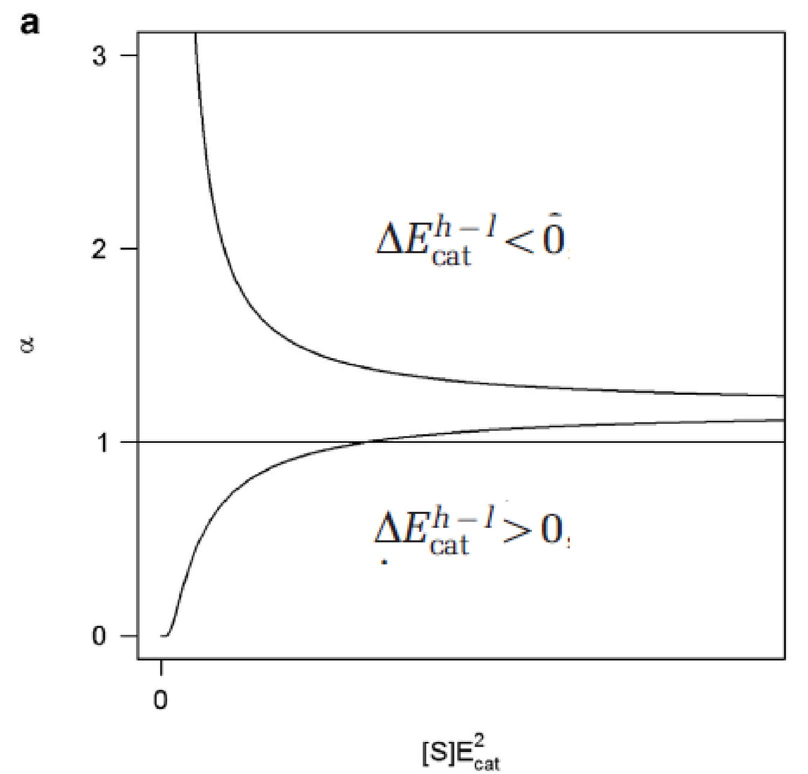
Desmond-Le Quéméner and Bouchez, The ISME-J, 2014

A microbial growth equation and its consequence on isotopic fractionation

$$\mu = \mu_{max} \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}}\right)$$

$$\alpha_{S/P} = \alpha_0 \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S_{lim}] \cdot E_{cat}^2}\Delta E_{cat}^{h-l}\right)$$





- Any further predictions?

Challenging these predictions with real datasets…

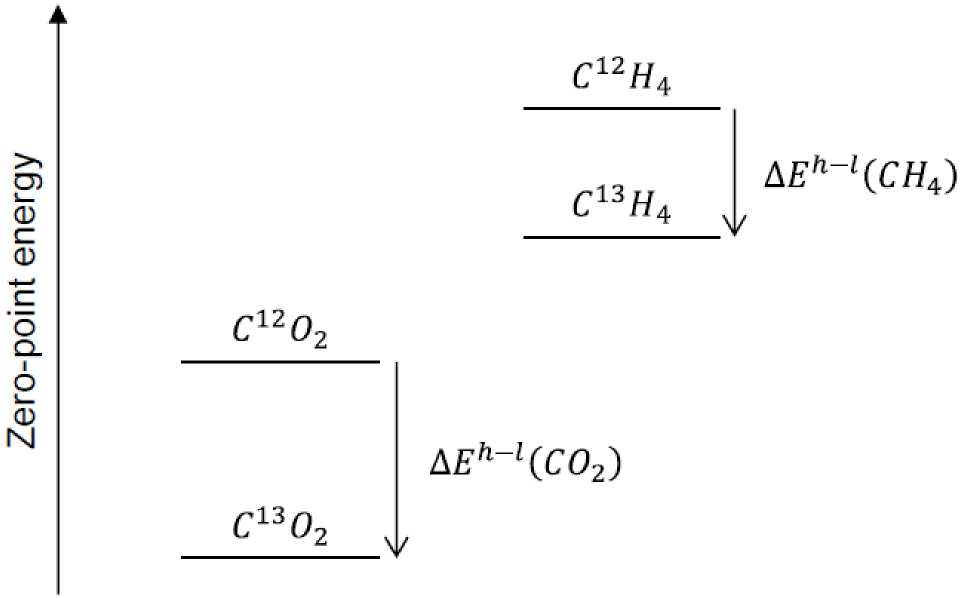
Desmond-Le Quéméner and Bouchez, The ISME-J, 2014

Challenging thermodynamic growth model's predictions with actual isotopic data

$$\alpha_{S/P} = \alpha_0 \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S_{lim}] \cdot E_{cat}^2} \Delta E_{cat}^{h-l}\right)$$

Hydrogenotrophic methanogenesis

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$



$$\Delta E_{cat}^{h-l} = \Delta E^{h-l}(CH_4) - \Delta E^{h-l}(CO_2) < 0$$

Challenging thermodynamic growth model's predictions with actual isotopic data

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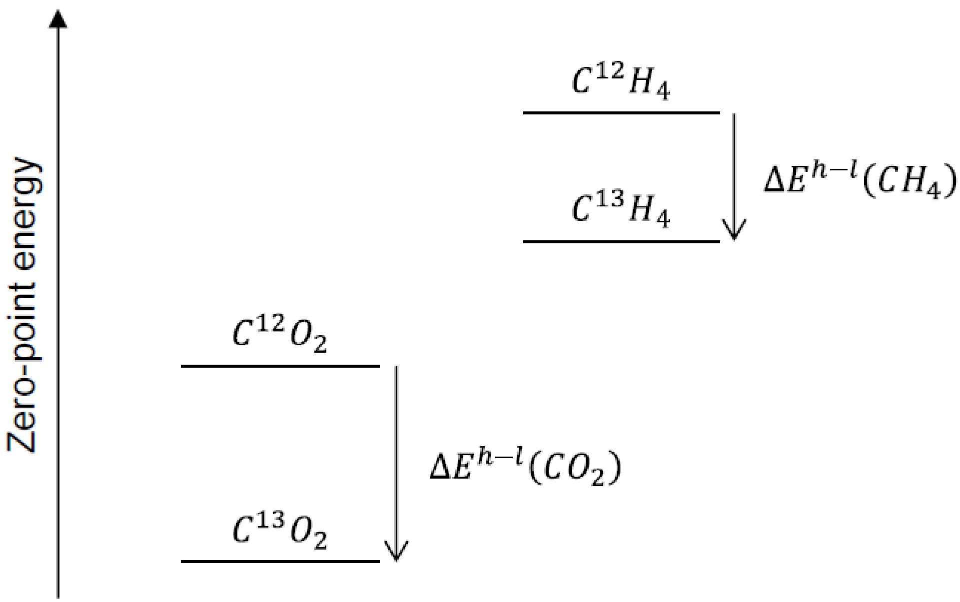

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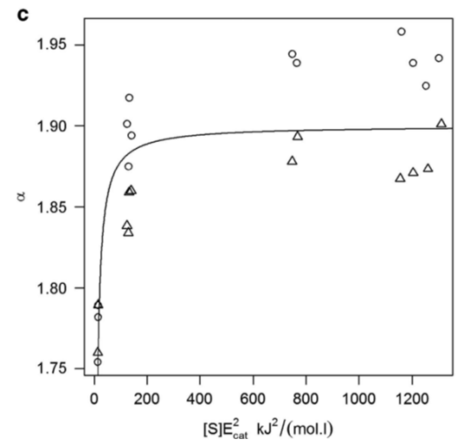
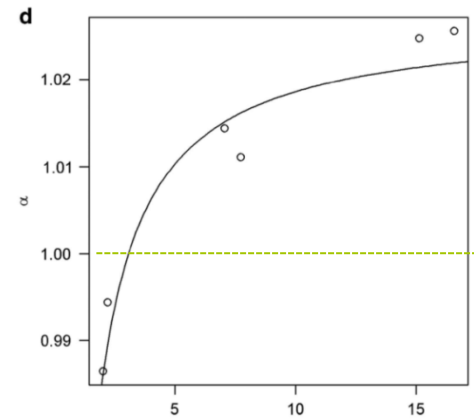


D/H

Aerobic phenol Degradation

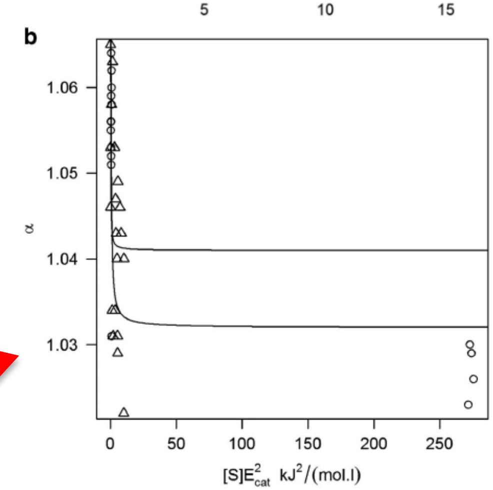
Kampara *et al.*, 2008



$^{13}C/^{12}C$

Acetoclastic Methanogenesis

Goevert and Conrad, 2009



$^{13}C/^{12}C$

Hydrogenotrophic Methanogenesis

Valentine *et al.*, 2004

Penning *et al.*, 2005

How are communities shaped by thermodynamic gradients?

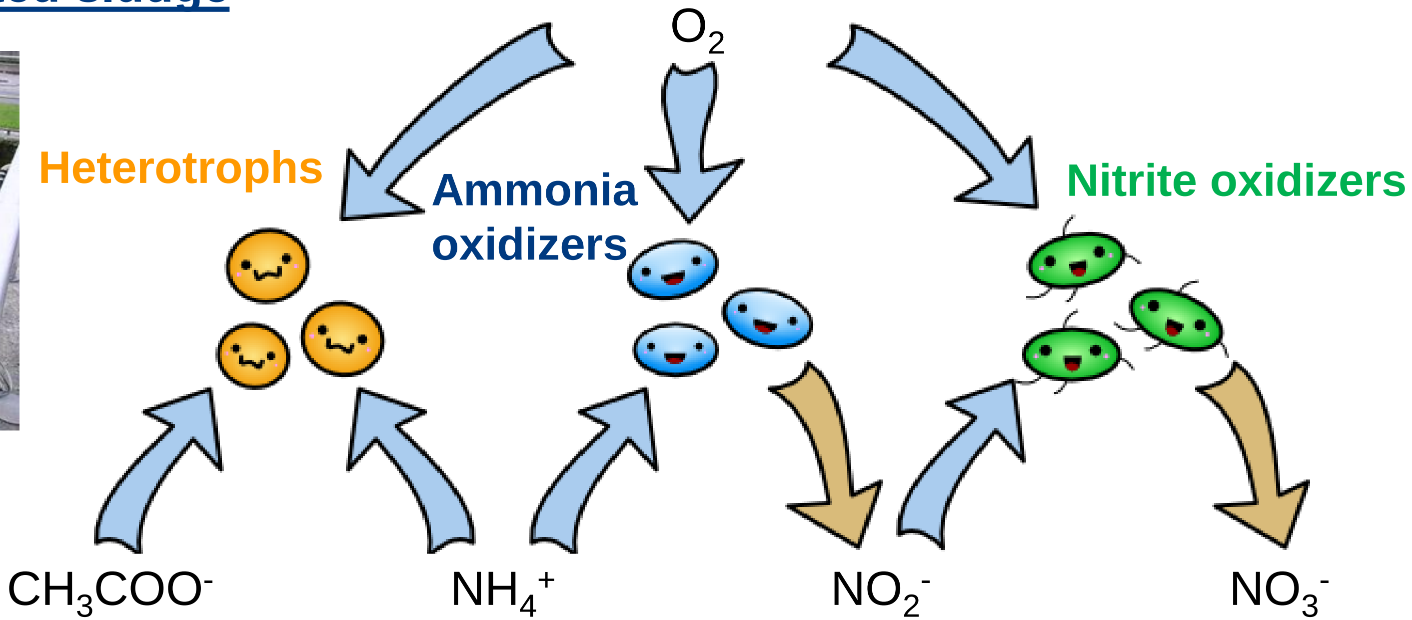
Hadrien Delattre's PhD Project

$$\mu = \mu_{max} \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S] \cdot E_{cat}}\right)$$

Flux: microbial growth rate

Force: catabolic exergy density

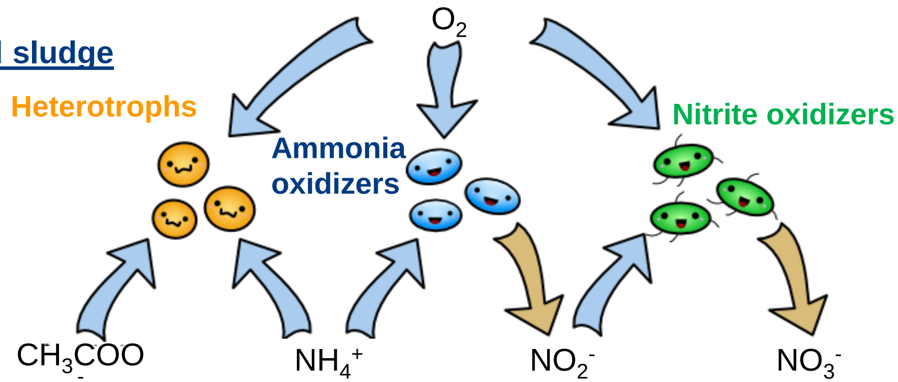
Application to activated sludge



Coupled modelling of energy balances, stoichiometry and microbial dynamics

Hadrien Delattre's PhD Project

Application to activated sludge



$$-0{,}525C_2H_3O_2^- - 0{,}2NH_4^+ - 0{,}325H^+ + 0{,}45H_2O + 0{,}05CO_2 + 1C_1H_{1,8}O_{0,5}N_{0,2} + \lambda(-1C_2H_3O_2^- - 2O_2 - 1H^+ + 2CO_2 + H_2O)$$

$$-0{,}2NH_4^+ - 0{,}6H_2O - 1CO_2 + 1{,}05O_2 + 0{,}2H^+ + 1C_1H_{1,8}O_{0,5}N_{0,2} + \lambda(-4{,}5O_2 - 2NH_4^+ - 2H^+ + 2NO_2^-)$$

$$-0{,}2NO_2^- - 0{,}8H_2O - 1CO_2 - 0{,}2H^+ + 1{,}35O_2 + 1C_1H_{1,8}O_{0,5}N_{0,2} + \lambda(-1NO_2^- - 0{,}5O_2 + 1NO_3^-)$$

Coupled modelling of energy balances, stoichiometry and microbial dynamics

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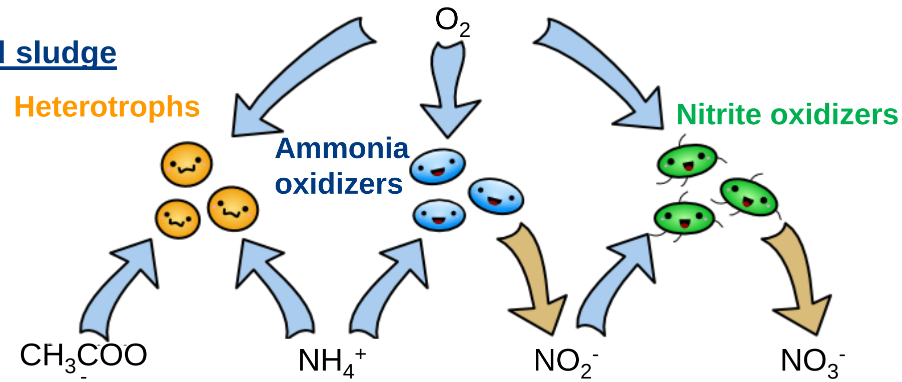
Application to activated sludge



$$-0{,}525C_2H_3O_2^- - 0{,}2NH_4^+ - 0{,}325H^+ + 0{,}45H_2O + 0{,}05CO_2 + 1C_1H_{1,8}O_{0,5}N_{0,2} + \lambda(-1C_2H_3O_2^- - 2O_2 - 1H^+ + 2CO_2 + H_2O)$$

$$-0{,}2NH_4^+ - 0{,}6H_2O - 1CO_2 + 1{,}05O_2 + 0{,}2H^+ + 1C_1H_{1,8}O_{0,5}N_{0,2} + \lambda(-4{,}5O_2 - 2NH_4^+ - 2H^+ + 2NO_2^-)$$

$$-0{,}2NO_2^- - 0{,}8H_2O - 1CO_2 - 0{,}2H^+ + 1{,}35O_2 + 1C_1H_{1,8}O_{0,5}N_{0,2} + \lambda(-1NO_2^- - 0{,}5O_2 + 1NO_3^-)$$

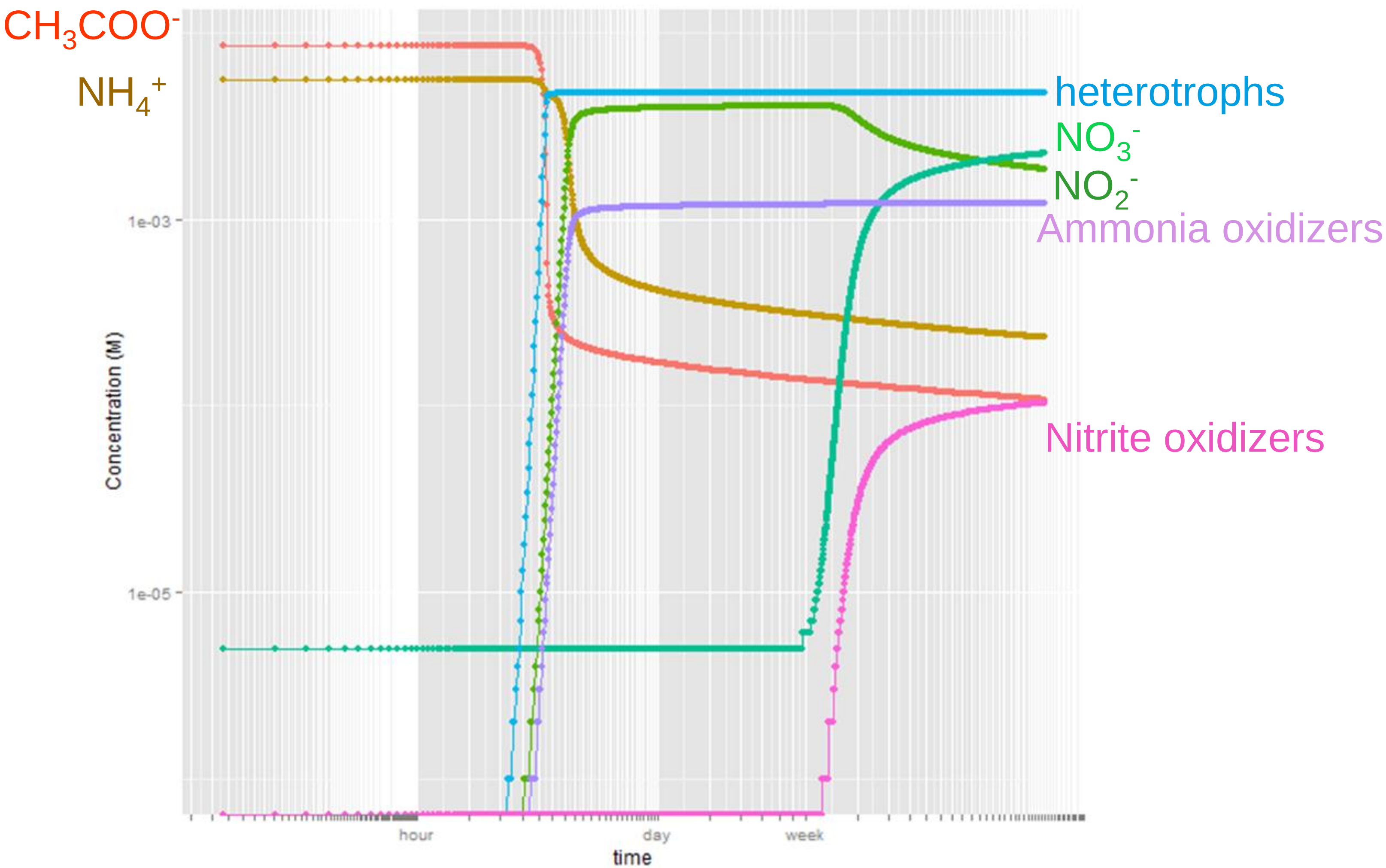
Multisubstrate rate law expression for all groups:

$$\mu = \mu_m \prod_i e^{-\frac{\nu_{i(\lambda)}}{V_h \cdot S_i}}$$

λ is calculated from thermodynamic balances of microbial growth

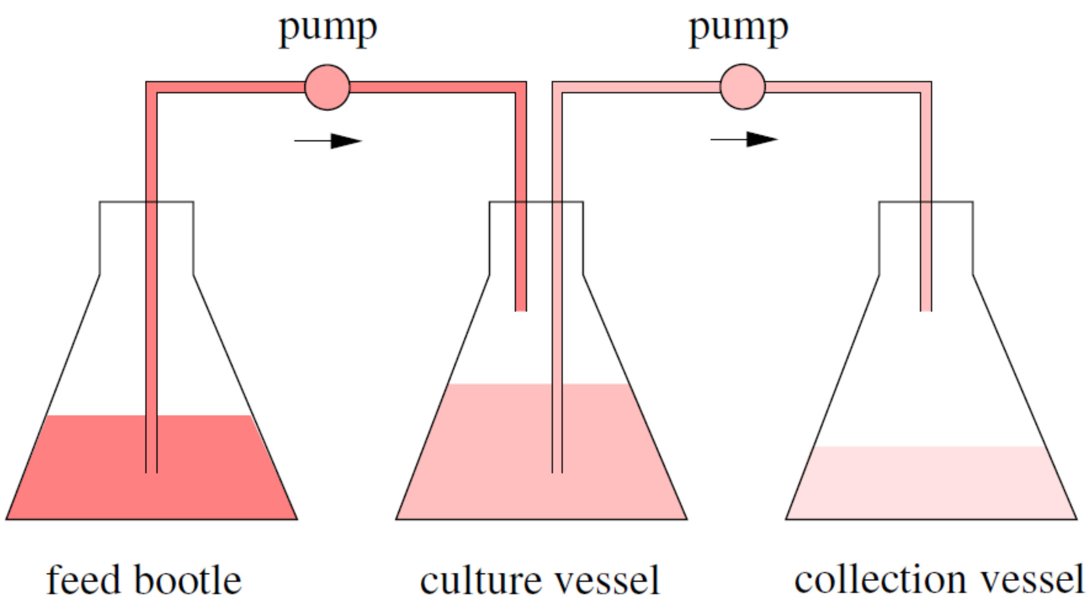
Simulating activated sludge batchs fed with wastewater

Simulation conditions: constant supply of air, default ASM chemical species and microbial composition used for initialization, same default *E.coli* growth parameters for all groups



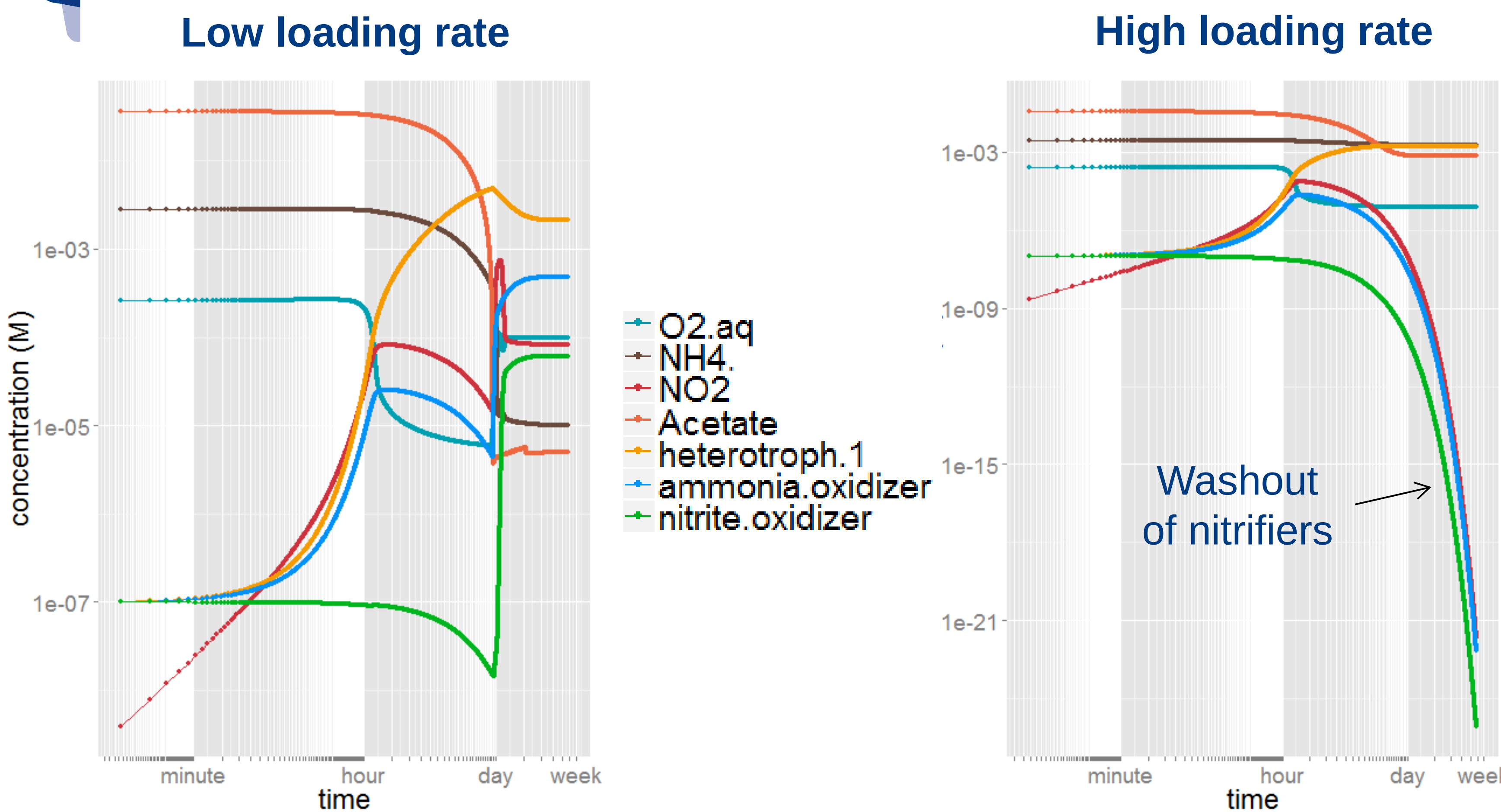
=> Proper metabolic succession obtained without parameter fitting

Simulating « chemostats »



Monod 1950 – Novick & Szilard 1950

Varying the loading rate in virtual activated sludge chemostats



- Nitrification
- No nitrification

=> Engineering rules as emerging properties of thermodynamic models

Take home messages…

- A consistent framework for coupled modelling of energy balance, stoichiometry and microbial dynamics
- Revisiting the importance of energy drivers for shaping microbial community structures
- Reproducing microbial successions without parameter fitting
- Towards more **predictive** models?

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Many thanks to…

All the BIOMIC team members in Irstea-Antony
http://www.irstea.fr/la-recherche/themes-de-recherche/ted/biomic

Elie Desmond, postdoc
Microbial thermodynamics

Hadrien Delattre, PhD, Thermodynamic simulation of microbial ecosystems

Christian Duquennoi, multiphysic models

Ahlem Filali, Activated sludge models

Agence Nationale de la Recherche ANR

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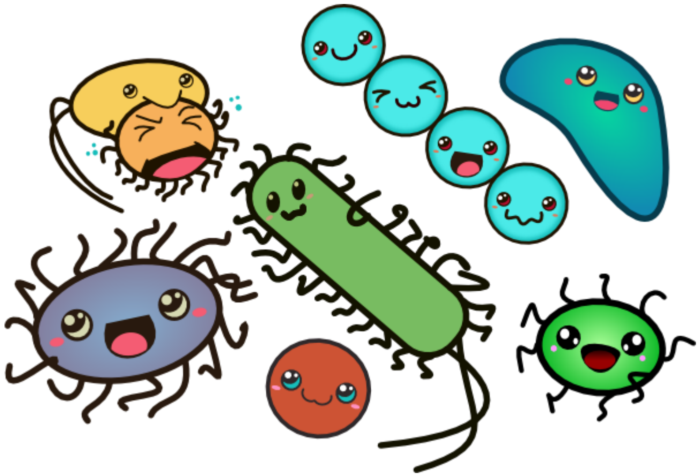
INVESTISSEMENTS D'AVENIR

BIORARE project
ANR-10-BTBR-02

Thanks for you attention



Choice of a generic biomass formula

- Hoover and Porges' biomass: $C_5H_7O_2N$
  - Widely used in wastewater bioprocesses modelling
  - Gibbs energy of formation is unknown
  - Composition appears to be unrealistic
- Heijnen's biomass: $C_1H_{1.8}O_{0.5}N_{0.2}$
  - Infered from growth data of multiple microbes on multiple substrates
  - Gibbs energy of formation is known
  - Composition appears to be unrealistic
- Battley's biomass: $C_1H_{1.613}O_{0.557}N_{0.158}$
  - Experimentally measured from Saccharomyces cerevisiae cultures
  - Gibbs energy of formation directly measured by calorimetry
  - Composition seems to be more realistic

- Hoover S.R. and Porges N. (1952). Assimilation of dairy wastes by activated sludge. II. The equations of synthesis and rate of oxygen utilization. Sew. Indus. Wastes J., 24, 306-312.
- Heijnen J. and Dijken J. (1991). In Search of a Thermodynamic Description of Biomass Yields for the Chemotrophic Growth of Microorganisms. biotechnology and bioengineering, 39(8), 833-858.
- Battley E. (1998). The development of direct and indirect methods for the study of the thermodynamics of microbial growth. Thermochimica Acta, 309, 17-37.

Estimation of the harvesting volume (V_h)

$$\mu = \mu_m e^{\frac{\nu}{V_h S}}$$

$$V_h = \frac{\nu}{S(\ln \mu - \ln \mu_m)}$$

V_h estimated for E Coli growing on various substrates ;

- Glycerol : 0.42 $m^3.C\text{-}mol^{-1}$ (sphere of radius 14.3 µm around each cell)
- Glucose : 0.74 $m^3.C\text{-}mol^{-1}$ (sphere of radius 19.2 µm around each cell)
- Sorbitol : 0.79 $m^3.C\text{-}mol^{-1}$ (sphere of radius 17.7 µm around each cell)

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Data is from Taheri-Araghi S. *et al.* (2015). Cell-size control and homeostasis in bacteria. Current Biology, 25(3), 385-391.

Lambda factor computation

$\bar{\bar{S}}$: chemical species concentrations vector (Cx1)

$\overline{\overline{\nu_{cat}}}$, $\overline{\overline{\nu_{an}}}$: raw stoichiometric coefficients vectors (1xC)

$$\Delta G_{cat} = \Delta G^{0}{}_{cat} - RT\overline{\overline{\nu_{cat}}} * \ln \bar{\bar{S}}$$

$$\Delta G_{an} = \Delta G^{0}{}_{an} - RT\overline{\overline{\nu_{an}}} * \ln \bar{\bar{S}}$$

ΔG_{dis}: energie to dissipate(constant depending on the carbon source)

$$\lambda = \frac{\Delta G_{an} + \Delta G_{dis}}{-\Delta G_{cat}}$$

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Dissipated energy calculation

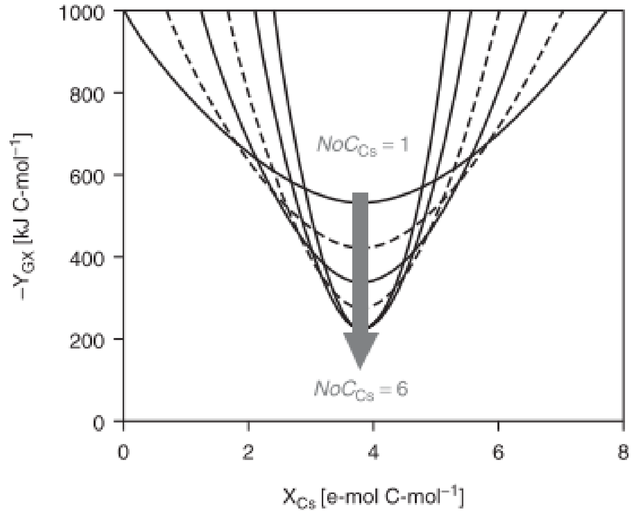

Figure 6. Minimum Gibbs energy dissipation for biomass production (Y_G^{max} kJ/cmol X) as a function of the degree of reduction (NoC_{Cs}) and the carbon chain length of the carbon source (γ_{Cs}) according to Equation 19.

Dissipated energy is a constant whose value depends on the anabolic carbon source, according to Heijnen and Kleerebezem

Heijnen J. and Kleerebezem R. (1999). Bioenergetics of microbial growth. Encyclopedia of Bioprocess Technology.