Understanding and steering microbial functions in mixed culture environmental biotechnology processes

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Les **microbiotes**

et la santé humaine, animale et environnementale : Prévention et traitements du futur

Biocitech Romainville-Grand Paris

The “functional convergence” of microbiomes

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

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

Processes underpinning microbial community assembly
(Nemergut *et al.*, MMBR 2013)

Processes at play in environmental biotechnology processes ?

TABLE 2 Vellend's four processes for community assembly

Process	Description	
Diversification	Generation of new genetic variation	Minor
Dispersal	Movement of organisms across space	Major
Selection	Changes in community structure caused by deterministic fitness differences between taxa	Major
Drift	Stochastic changes in the relative abundances of different taxa within a community through time	Minor

Selection as a key tool for managing microbes in environmental biotechnology processes

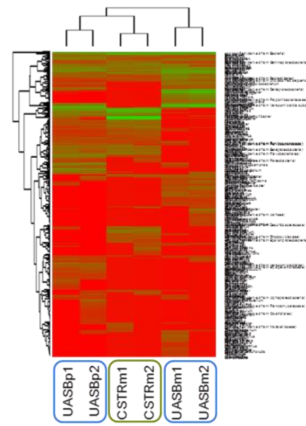
Dispersal

"open diversity systems"

Selection

Process design and operation

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



Taxonomically diverse but functionally reproducible microbial community patterns

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

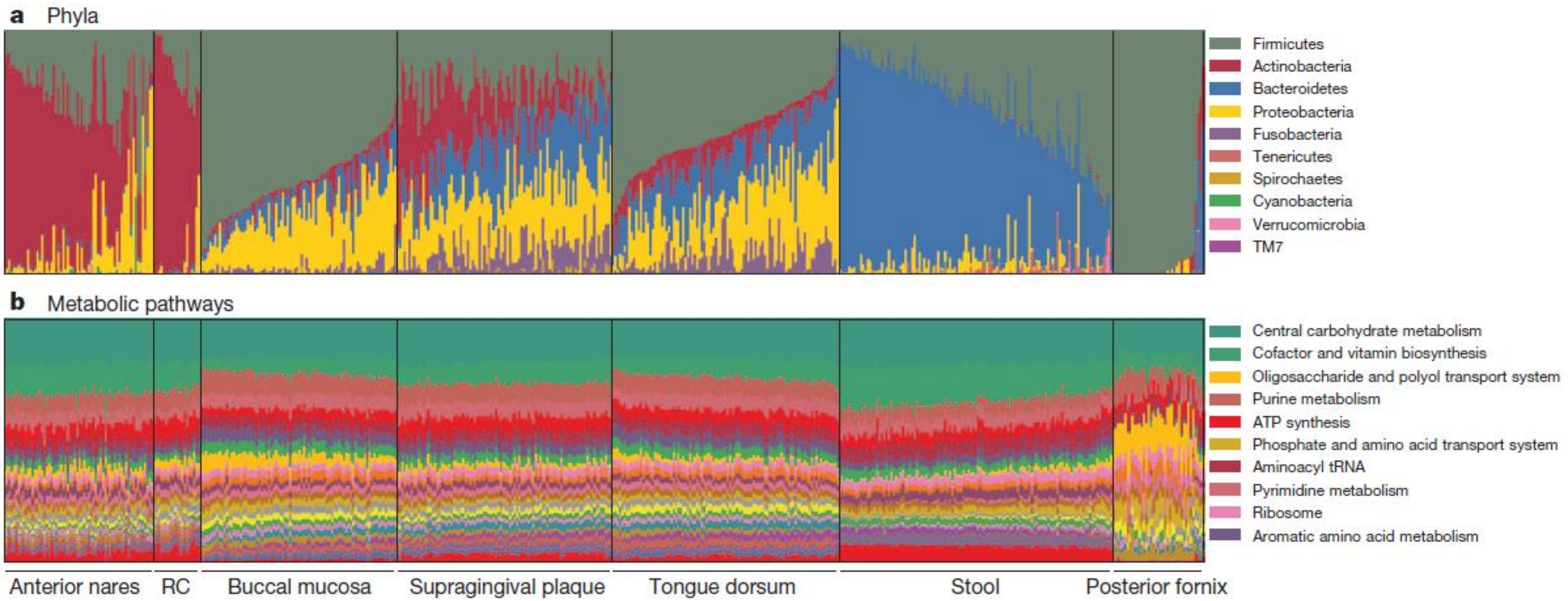
Ecological niches available => environmental filtering => fitness selection

Environmental biotechnology processes are typical "Bass Becking ecosystems"!

"Everything is everywhere, but the environment selects" Baas Becking, 1934

Diverse biotopes exhibit coherent functional assembly patterns

Healthy human microbiome



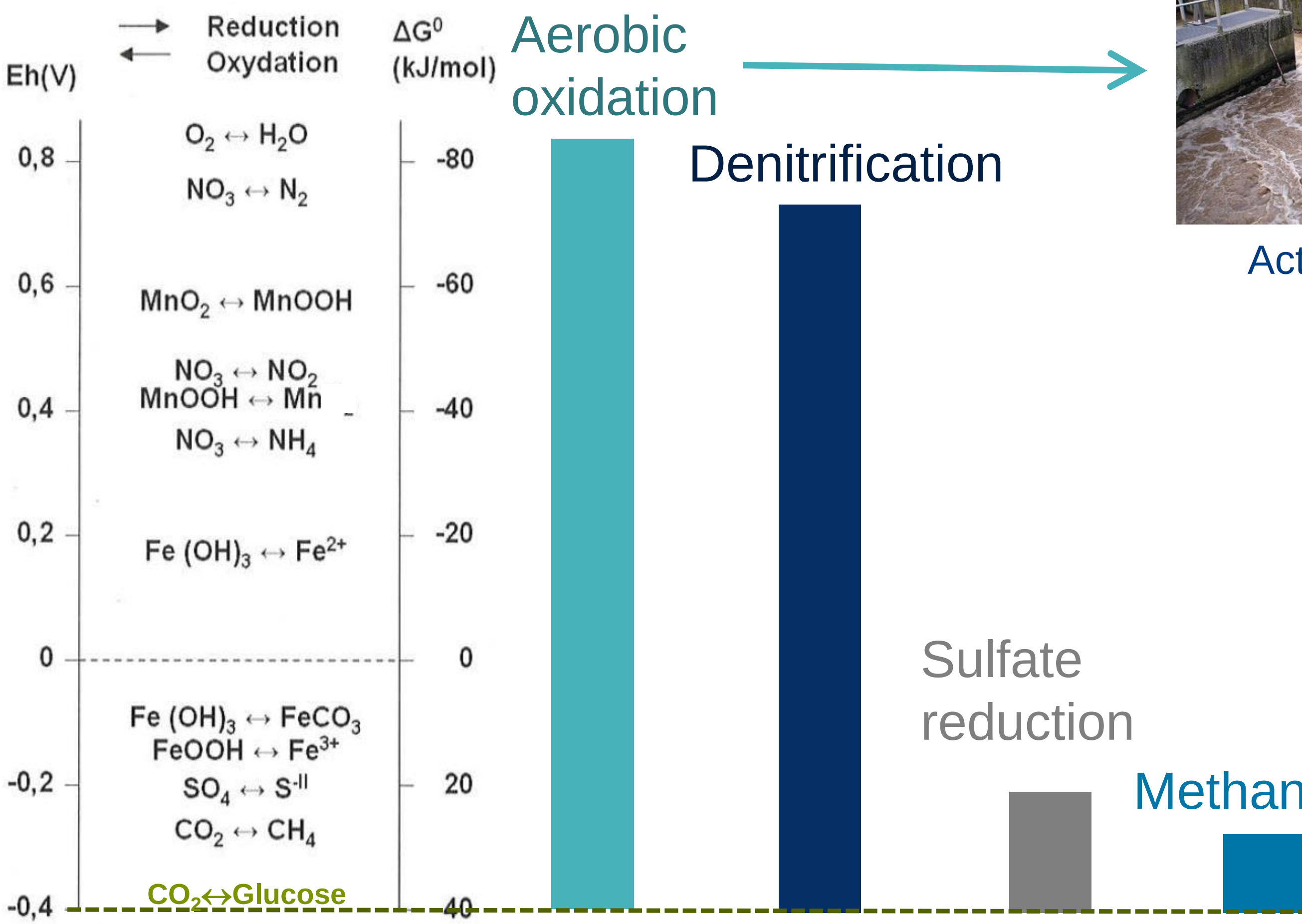
JUNE 2012 | VOL 486 | NATURE

Ocean microbiome (Raes *et al.*, 2011 MSB 7:473; MSBLouca *et al.*, 2016; Science 353: 6305)

Soil microbiome (Nelson *et al.*, 2016 PNAS 113: 29)

Plant foliage microbiome (Louca *et al.*, 2016 Nat. E&E 1:15)

Environmental biotechnology processes: selection through energy gradients



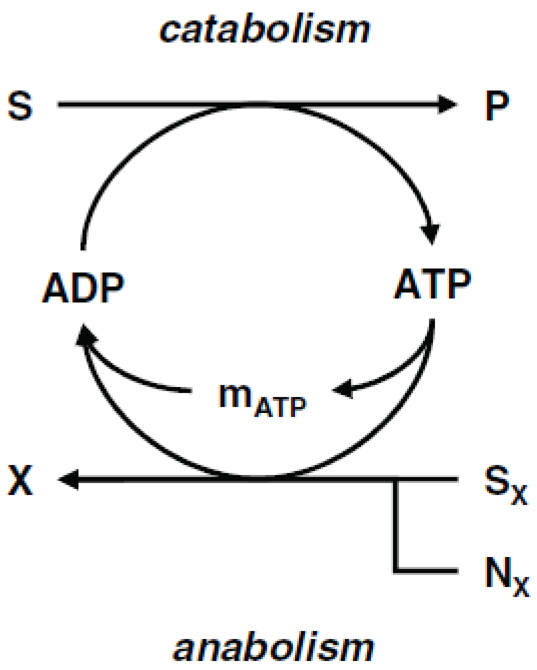
Activated sludge

Anaerobic digestion

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

A thermodynamic principle underlying functional community assembly in environmental biotechnology processes?

Thermodynamic balances of microbial growth



$\Delta_r G_{cat}$

ΔG_{an}

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

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

Introducing the exergy concept

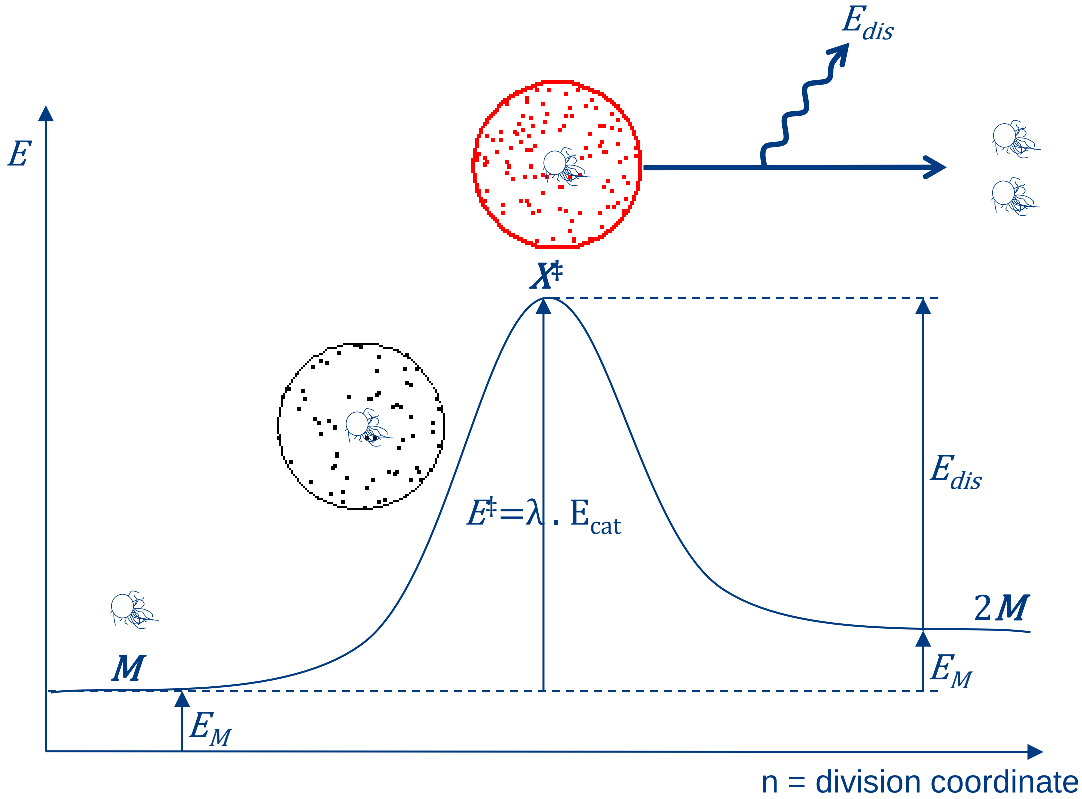
$$E_{dis} = \lambda . E_{cat} - E_M$$

From thermodynamic balances to kinetics using first principles?

To cite this Article KLEEREBEZEM, ROBBERT and VAN LOOSDRECHT, MARK C. M.(2010) 'A Generalized Method for Thermodynamic State Analysis of Environmental Systems', Critical Reviews in Environmental Science and Technology, 40: 1, 1 — 54

The Microbial "Transition State" theory (MTS)

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



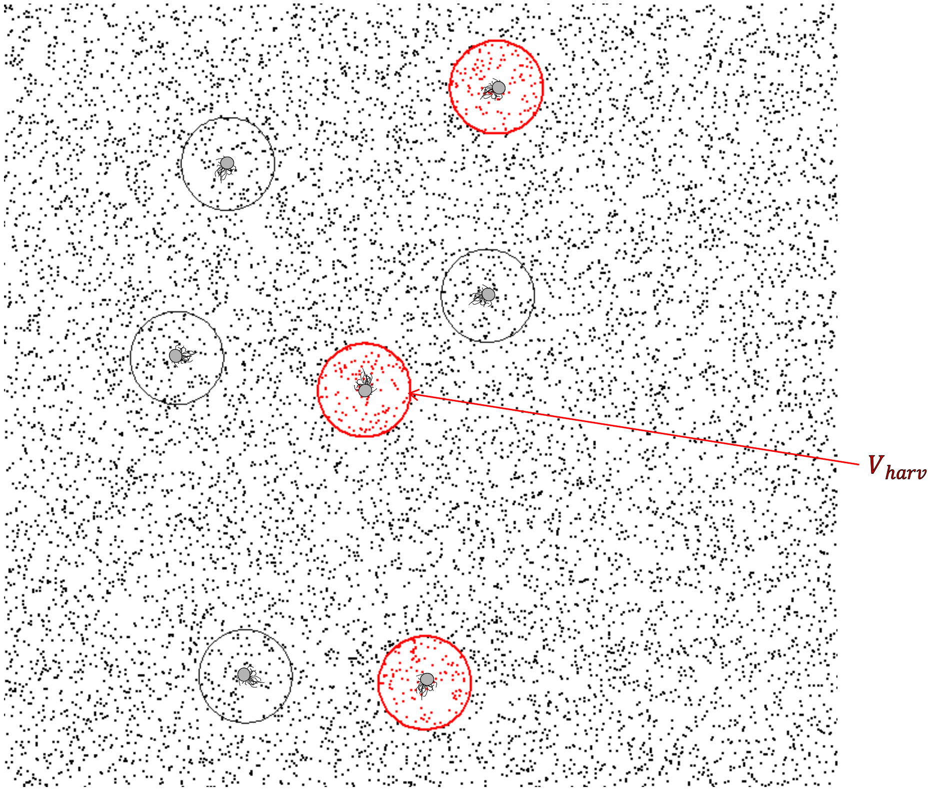
$$M \xleftrightarrow{K} X^{\ddagger} \xrightarrow{\mu_{max}} 2M$$

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

N is the number of microbes

$N^{\ddagger}$ 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

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

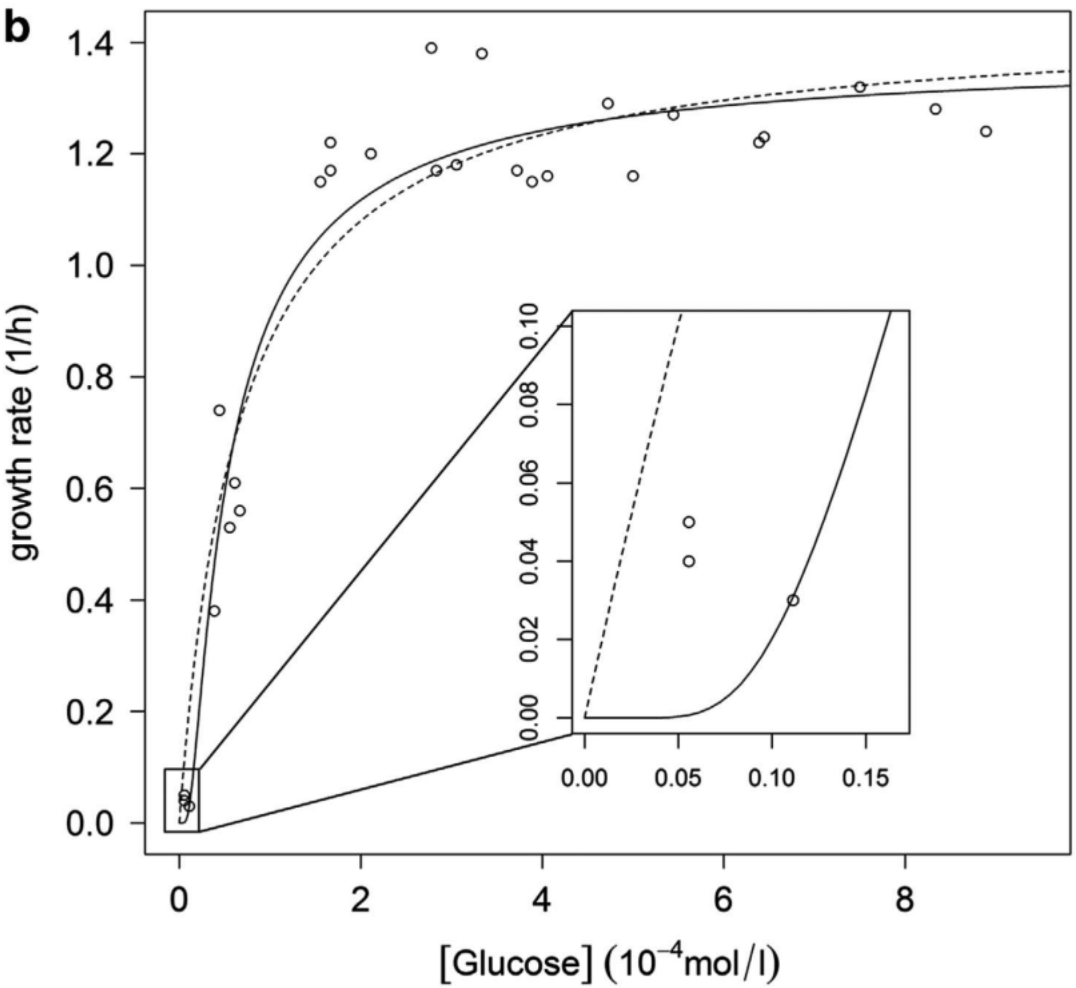
Desmond-Le Quéméner and Bouchez, The ISME-J, 2014

Growth rate as a function of substrate according to MTS theory

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

Flux: growth rate

Force: accessible energy compared to energy barrier



MTS model

Monod equation

$$\mu = \mu_{max} \cdot \frac{[S]}{K_S + [S]}$$

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

Illustrating MTS model properties

1. *Predictions in relation to the microbial isotopic fractionation phenomenon*
2. From modeling a pure culture in a minimal medium*…
3. …to mixed culture ecosystem models*

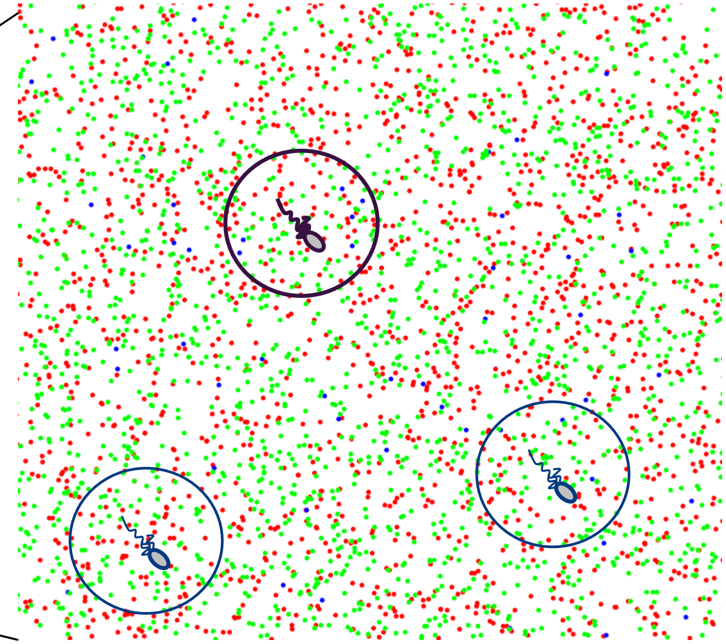
***Hadrien Delattre**
PhD
5th July in Irstea
Antony

Modeling the growth of a pure culture in a minimal medium

(Delattre et al., in revision)

Pure culture in
a minimal medium

Glucose
Oxygen
Ammonium



Anabolism

-0.167 $C_6H_{12}O_6$ -0.158 NH_4^+ + 0.430 H_2O + 0.164 H^+ + 0.00625 HCO_3^- + 1 $C_1H_{1.613}O_{0.557}N_{0.158}$

new biomass

Catabolism

+ λ (- 1 $C_6H_{12}O_6$ - 6 O_2 + 6 HCO_3^- + 6 H^+)

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

Metabolic energy coupling

MTS multi-resources growth dynamics

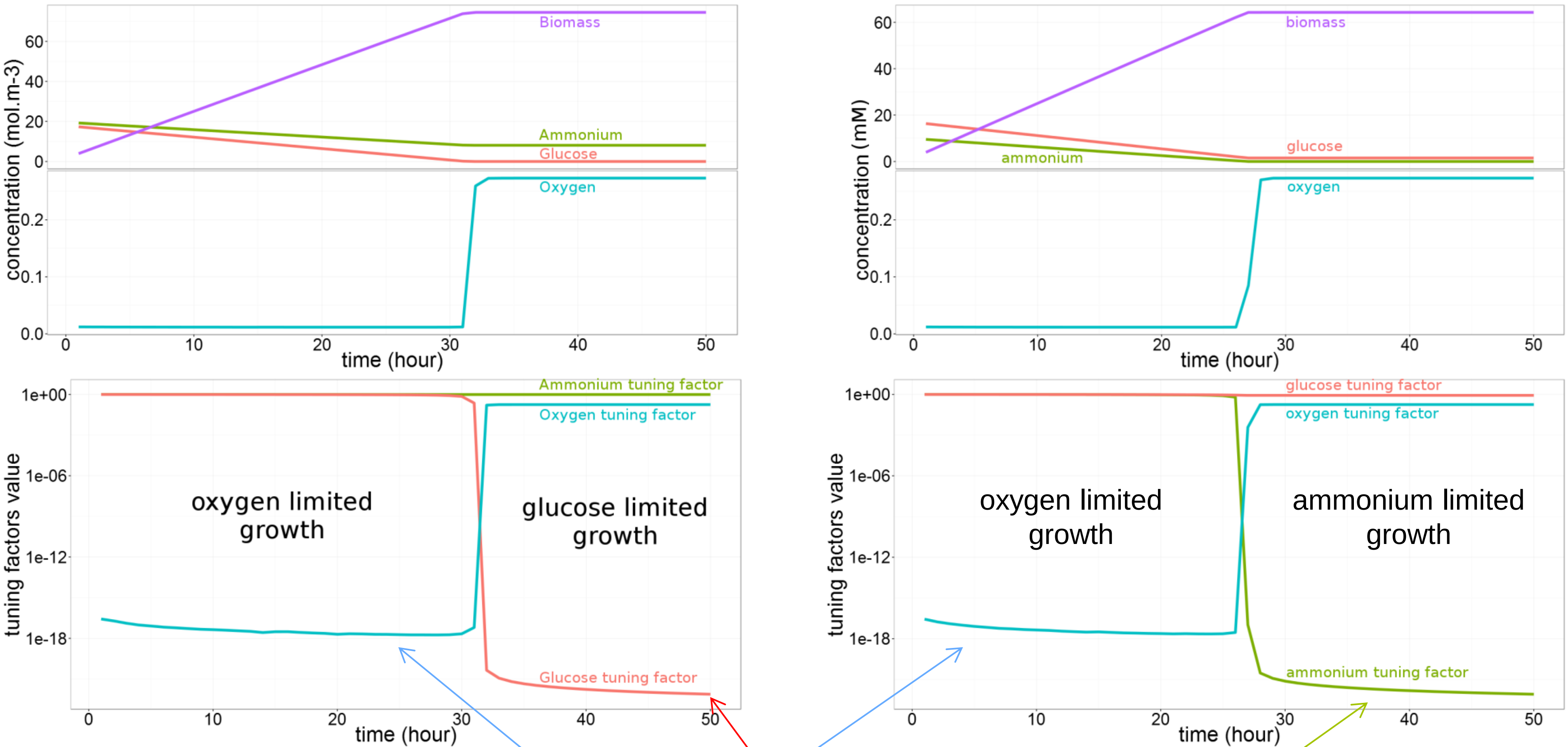
$$\mu = \mu_{max} \cdot e^{\frac{\upsilon_{Glucose}(\lambda)}{V_h \cdot [Glucose]}} \cdot e^{\frac{\upsilon_{oxygen}(\lambda)}{V_h \cdot [oxygen]}} \cdot e^{\frac{\upsilon_{ammonium}}{V_h \cdot [ammonium]}}$$

Tuning factors

Capturing the effect of all resources on anabolism and catabolism

Initial ammonium 18.7 mM

Initial ammonium 10.0 mM



$$\mu = \mu_{max} \cdot e^{\frac{v_{oxygen}(\lambda)}{V_h \cdot [oxygen]}} \cdot e^{\frac{v_{Glucose}(\lambda)}{V_h \cdot [Glucose]}} \cdot e^{\frac{v_{ammonium}}{V_h \cdot [ammonium]}}$$

➢ Growth patterns still compatible with « Liebig rule » of the single limiting substrate

Illustrating MTS model properties

1. Predictions in relation to the microbial isotopic fractionation phenomenon
2. From modeling a pure culture in a minimal medium*…
3. **…to mixed culture ecosystem models***

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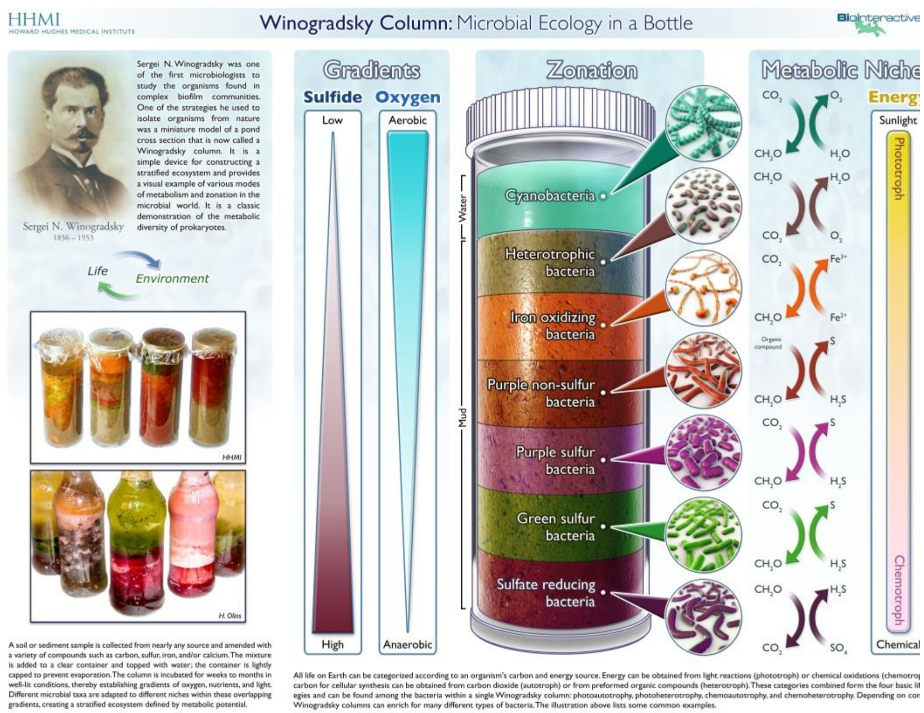
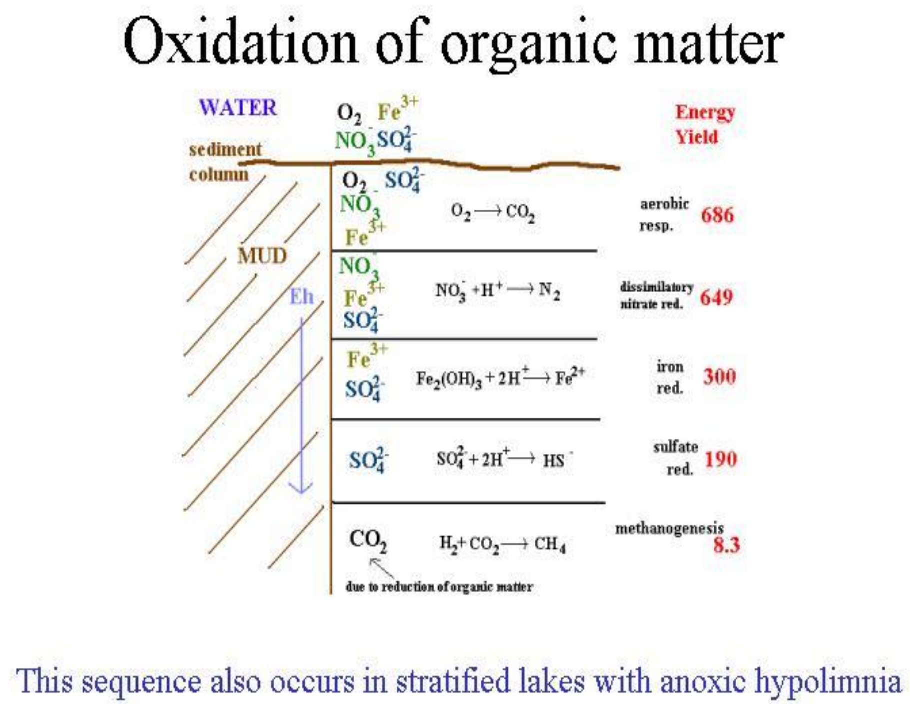
Microbial « redox towers »



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

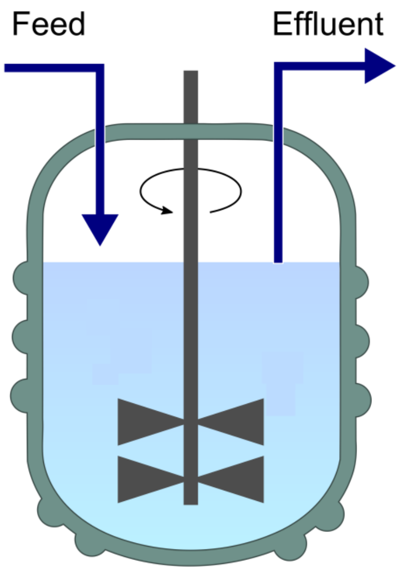


http://www.esf.edu/efb/schulz/Limnology/redox.html

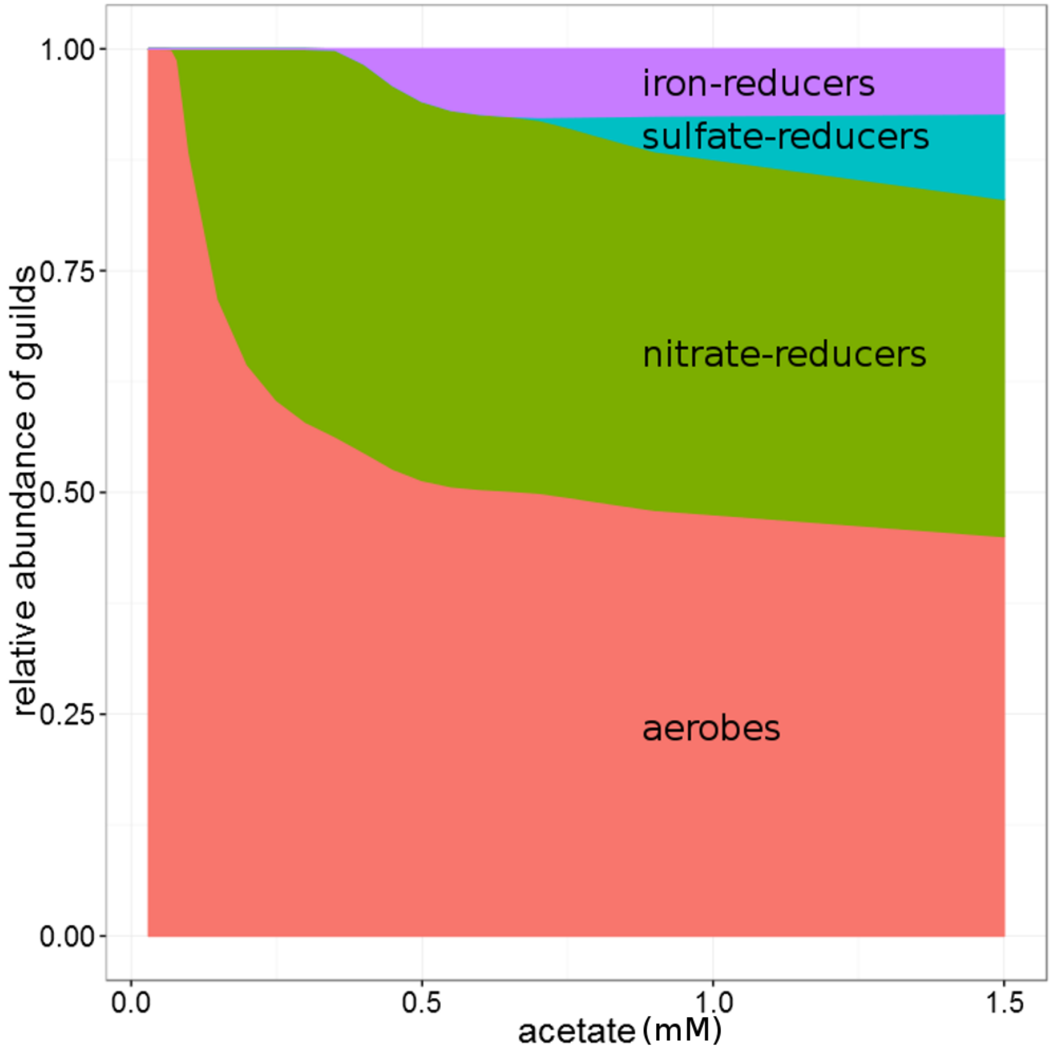
Invariant microbial functional community assembly patterns

Energy dependent competition arising without parameter adjustment

Acetate + oxygen + nitrate + sulfate + ferric iron + nutrients

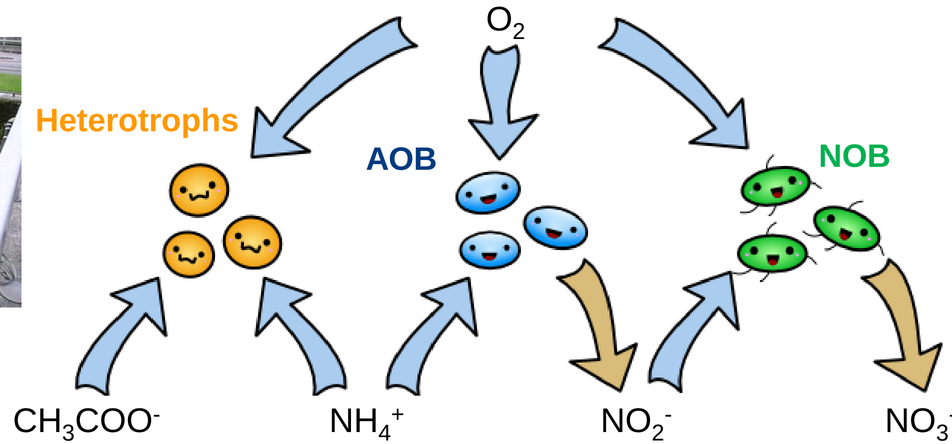


Aerobes, denitrifiers, iron reducers, sulfate reducers… all having the same fixed parameters values (μ_{max}, V_{harv})



Microbial successions according to redox tower are obtained **parsimoniously from first principles**

Modeling a simplified activated sludge batch ecosystem



$$-0.503\ C_2H_4O_2 - 0.158\ NH_4^{+} + 0.4305\ H_2O + 0.164\ H^{+} + 0.00625\ HCO_3^{-} + 1\ C_1H_{1.613}O_{0.557}N_{0.158} + \lambda(-1C_2H_3O_2^{-} - 2O_2 + 2H^{+} + 2HCO_3^{-})$$

$$-1\ HCO_3^{-} - 0{,}828\ NH_4^{+} + 1{,}101\ H_2O + 0{,}670\ NO_2^{-} + 0{,}499\ H^{+} + 1\ C_1H_{1{,}613}O_{0{,}557}N_{0{,}158} + \lambda\,(-1{,}5O_2 - 1NH_4^{+} + 2H^{+} + 1NO_2^{-} + 1H_2O)$$

$$-1\ HCO_3^{-} - 2.64\ NO_2^{-} - 1.16\ H^{+} + 0.27\ H_2O + 2.49\ NO_3^{-} + 1\ C_1H_{1.613}O_{0.557}N_{0.158} + \lambda\,(-1NO_2^{-} - 0{,}5O_2 + 1NO_3^{-})$$

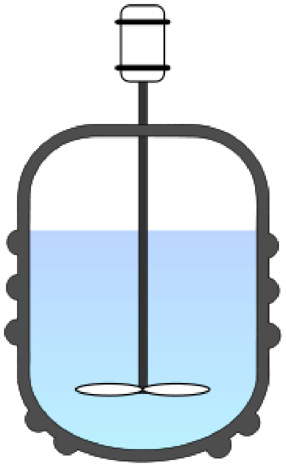
λ is dynamically adjusted using the Gibbs energy dissipation method (Kleerebezem and van Loosdrecht, 2010)

MTS derived-dynamics: $$\mu = \mu_{max} \cdot \prod_i e^{\frac{\nu_{i(\lambda)}}{V_h \cdot C_i}}$$

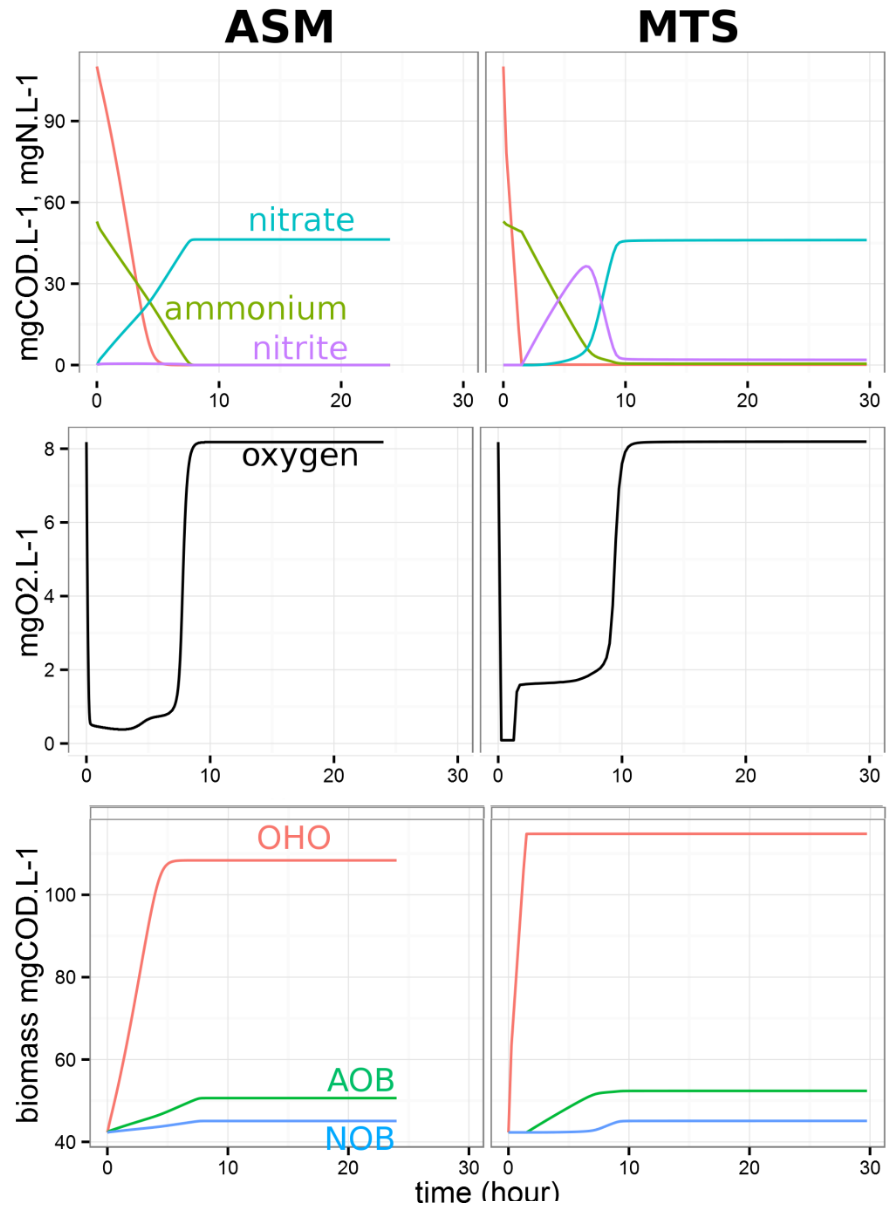
where (i) μ_{max} is fixed to $(\frac{k_B \cdot T}{h})$ and (ii) V_h is kept the same for all substrates and all groups

Modeling a simplified activated sludge batch ecosystem

(Delattre et al., submitted)



- [acetate] = 103.9 mg.L^{-1}
- [ammonium] = 68 mg.L^{-1}
- microbial inoculation: 1 mM (25e6 cell.mL^{-1})
- kla = 100 d^{-1}



Consistent dynamic patterns are obtained parsimoniously

ASM	MTS
Kinetic parameters: 9	**Kinetic parameters: 2**
Yield parameters: 3	**Yield parameters: 0**

Microbial thermodynamics and ecosystem modelling…

- In microbial ecology, scientific bottlenecks are progressively shifting from analytical methodologies to **knowledge integration** into an inclusive picture
- The development of a more **conceptual framework** is needed
- **Microbial thermodynamics**: crossing disciplinary boundaries between biology, physics and math.
- **Future perspectives**:
  - Thermodynamics driving forces and **ecosystem functional convergence**: studying the link with ecological goal functions
  - **Predictive** models for **ecological engineering** of microbial communities in environmental biotechnology processes

Many thanks to…

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

Hadrien Delattre, PhD candidate
Microbial thermodynamics
July 5th, 9h30, Irstea-Antony

Elie Desmond-Le Quéméner, INRA-LBE
Microbial thermodynamics

Agence Nationale de la Recherche
ANR

Project number ANR-16-CE04-0003-01

- **Postdoctoral position1**: MTS theory and effect of temperature - open
- **Postdoctoral position2**: MTS theory and phototrophic growth - filled
- **PhD position**: Challenging MTS theory with experiments - filled

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