Comprendre et modéliser le fonctionnement des écosystèmes microbiens dans les procédés de biotechnologie environnementale

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affirmer
ses missions,
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devient Irstea

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Chaire Modélisation Mathématique et Biodiversité

Ecole Polytechnique, le 22 septembre 2017

Irstea-Antony

National Research Institute of Science and Technology for Environment and Agriculture

Environmental research through a multidisciplinary, action-oriented approach

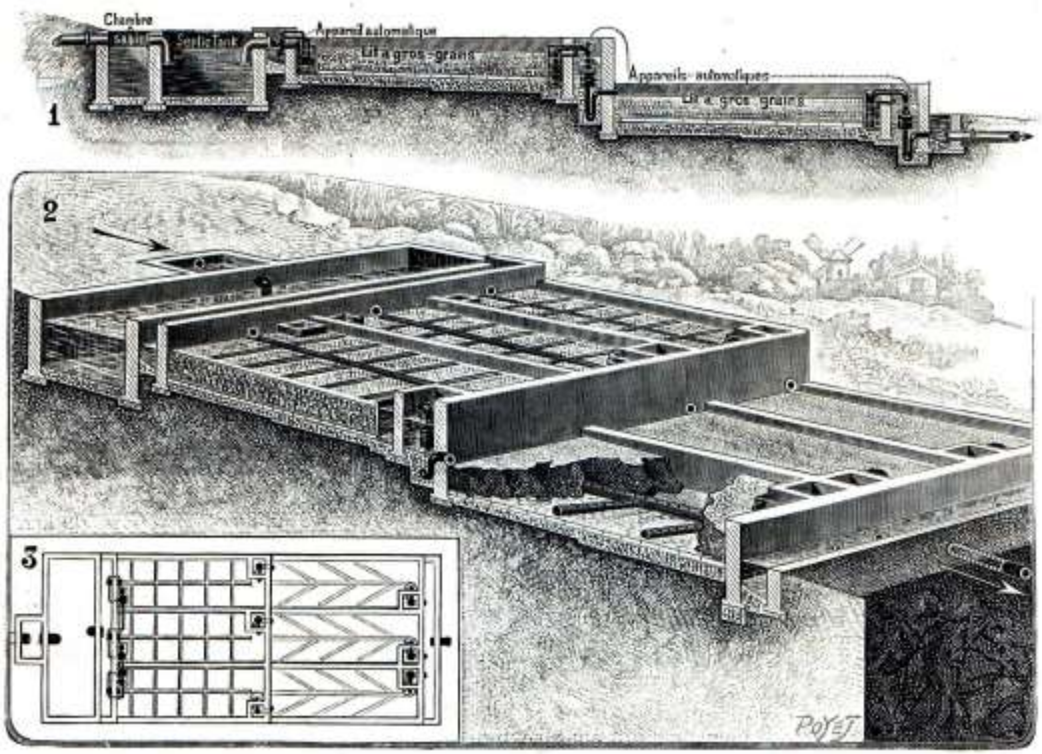
- 1500 collaborators, 113 M€ budget (27% from contracts)
- 3 scientific departments: Waters, Ecotechnologies, Territories
- 9 locations

Facing the sanitary threat…

With the help of our microscopic allies!

$$\color{red}{C_5H_7O_2N} + 7\ O_2 \rightarrow 2\ H_2O + 5\ CO_2 + NO_3^- + H^+ + H_2O$$

Granular beds of Dr Calmette



Installation de traitement biologique des eaux d'égouts. — 1. Coupe longitudinale. — 2. Disposition et tuyauterie des bassins successifs. 3. Plan général.

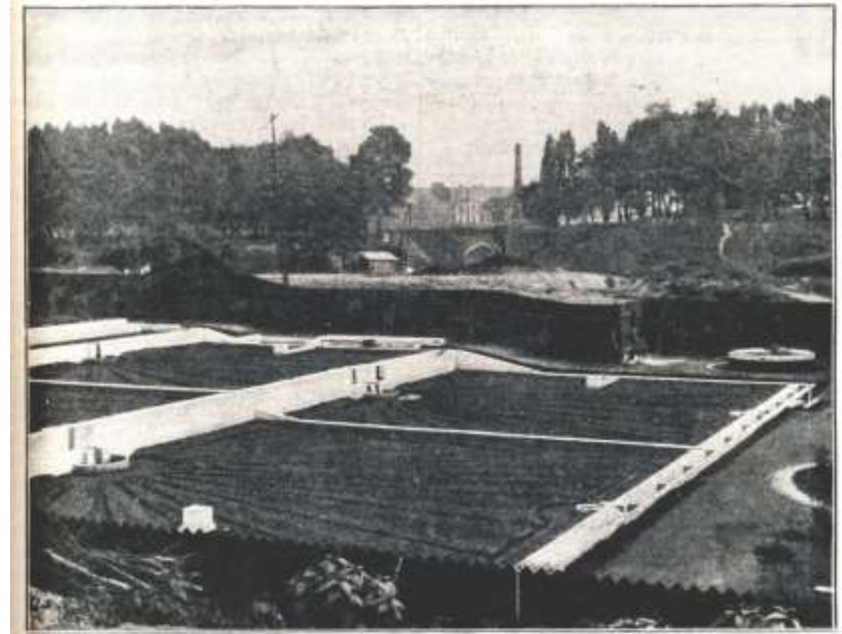


Fig. 5. — Vue générale des lits bactériens de premier et de second contact à la station expérimentale de la Madeleine.

Experiments in Madeleine-Les-Lille, 1904

Source : http://sciences.gloubik.info/spip.php?article679

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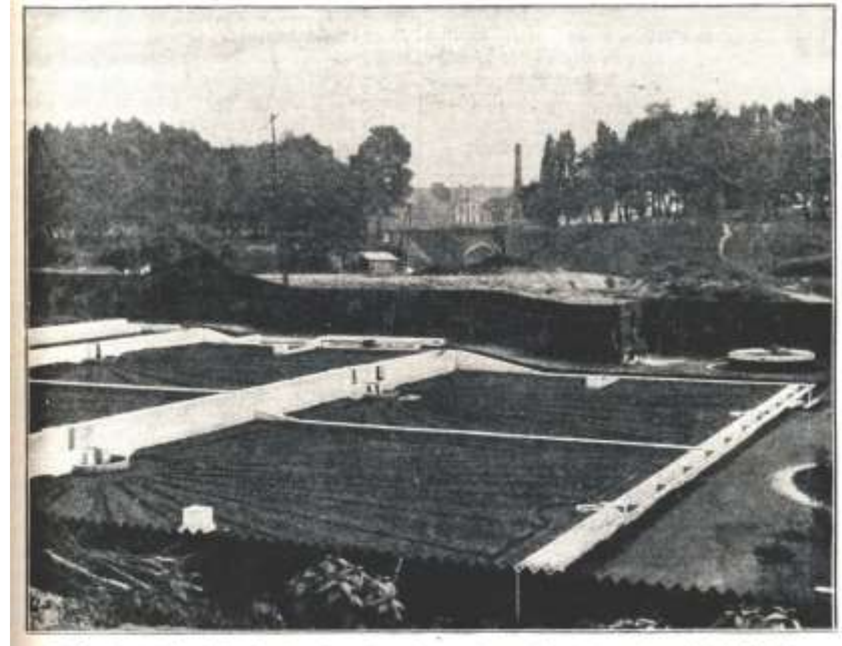

Experiments in Madeleine-Les-Lille, 1904

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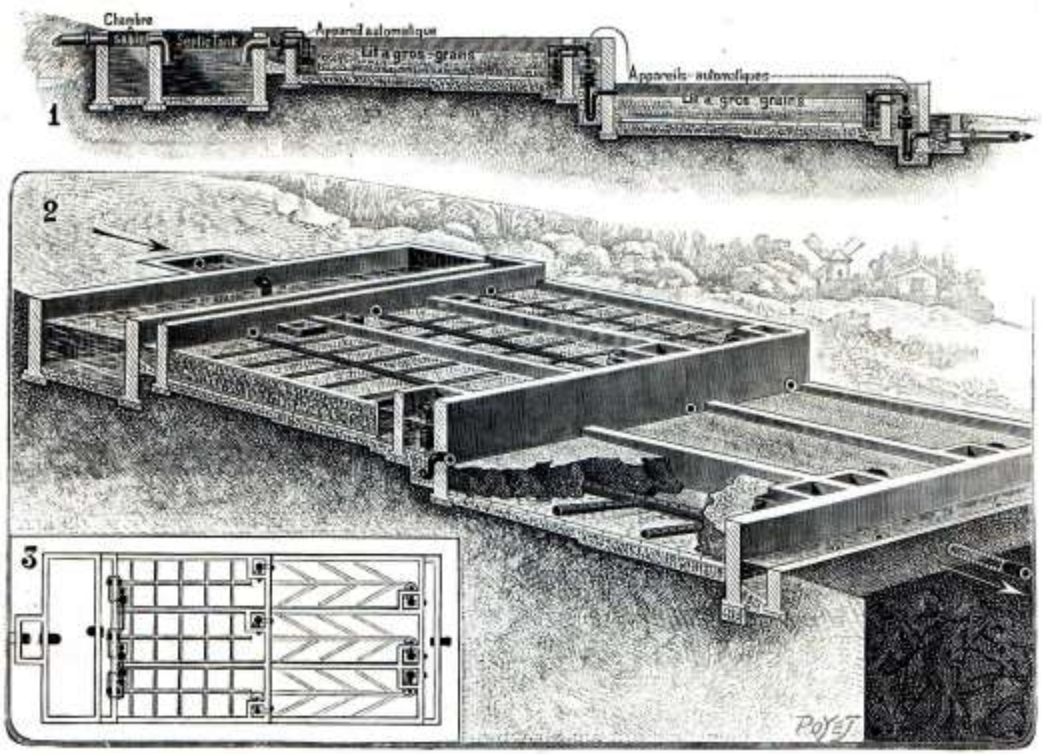
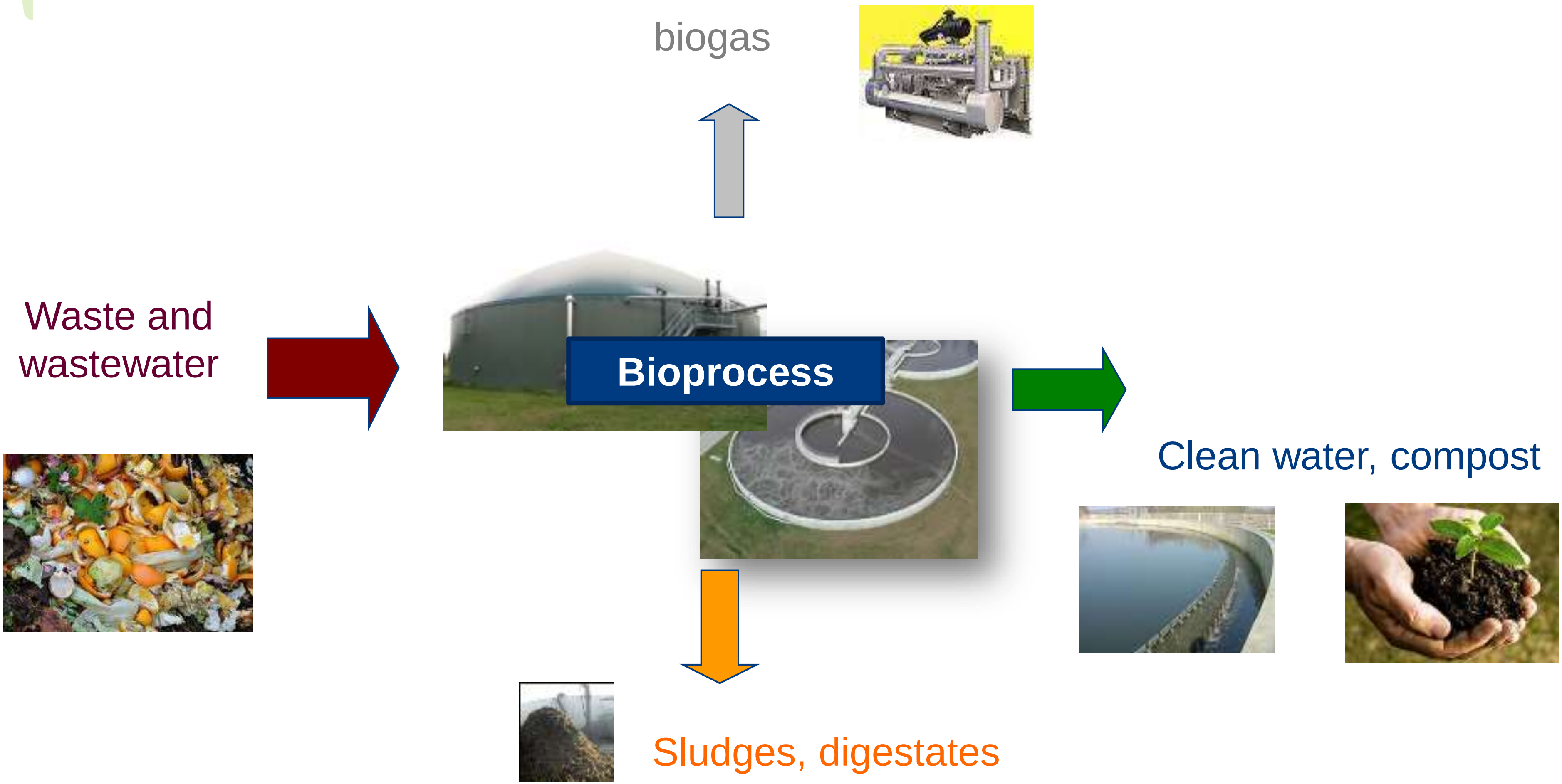


Installation de traitement biologique des eaux d'égouts. — 1. Coupe longitudinale. — 2. Disposition et tuyauterie des bassins successifs. 3. Plan général.

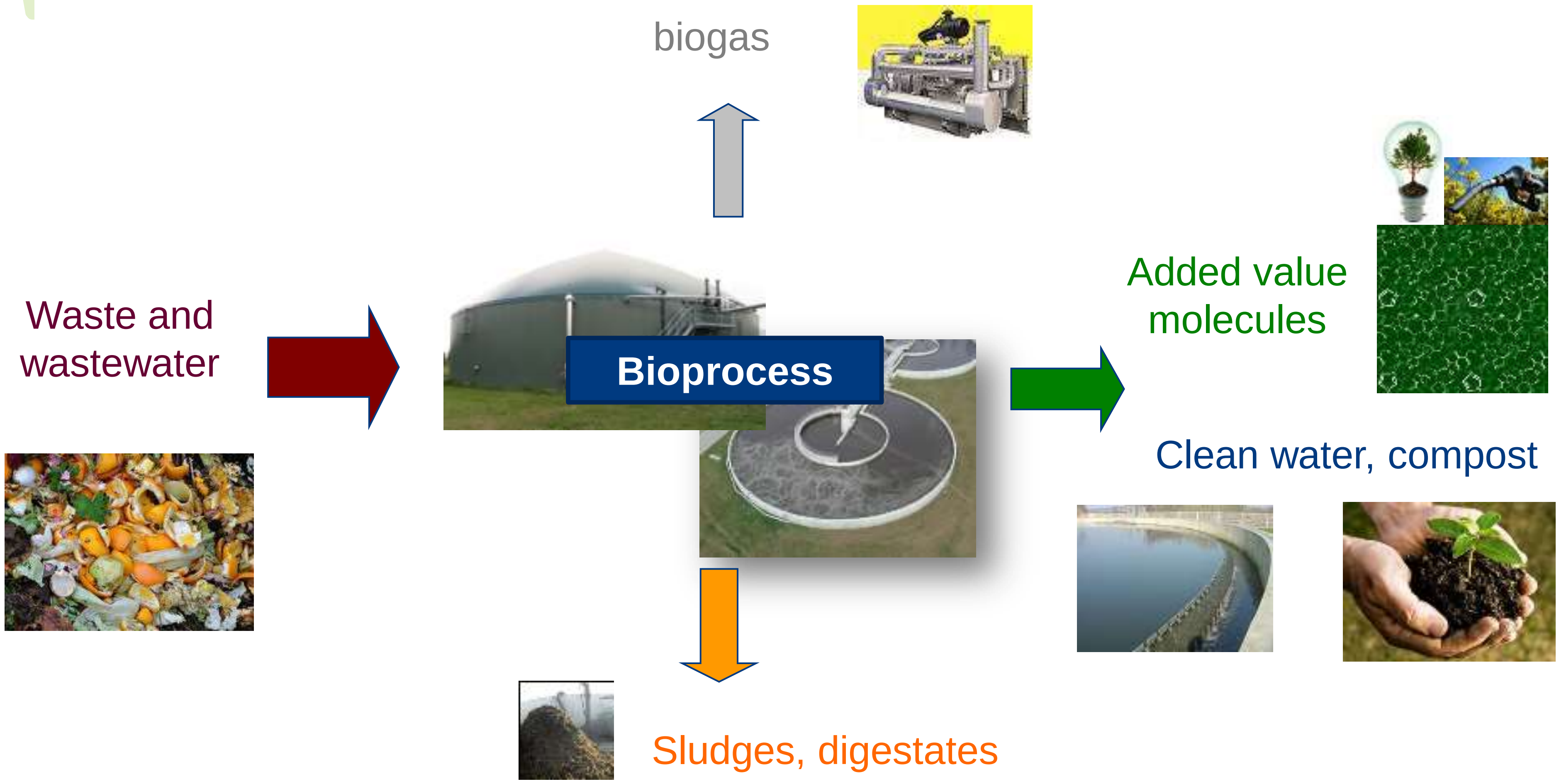
Environmental biotechnologies

Source : http://sciences.gloubik.info/spip.php?article679

Environmental biotechnologies



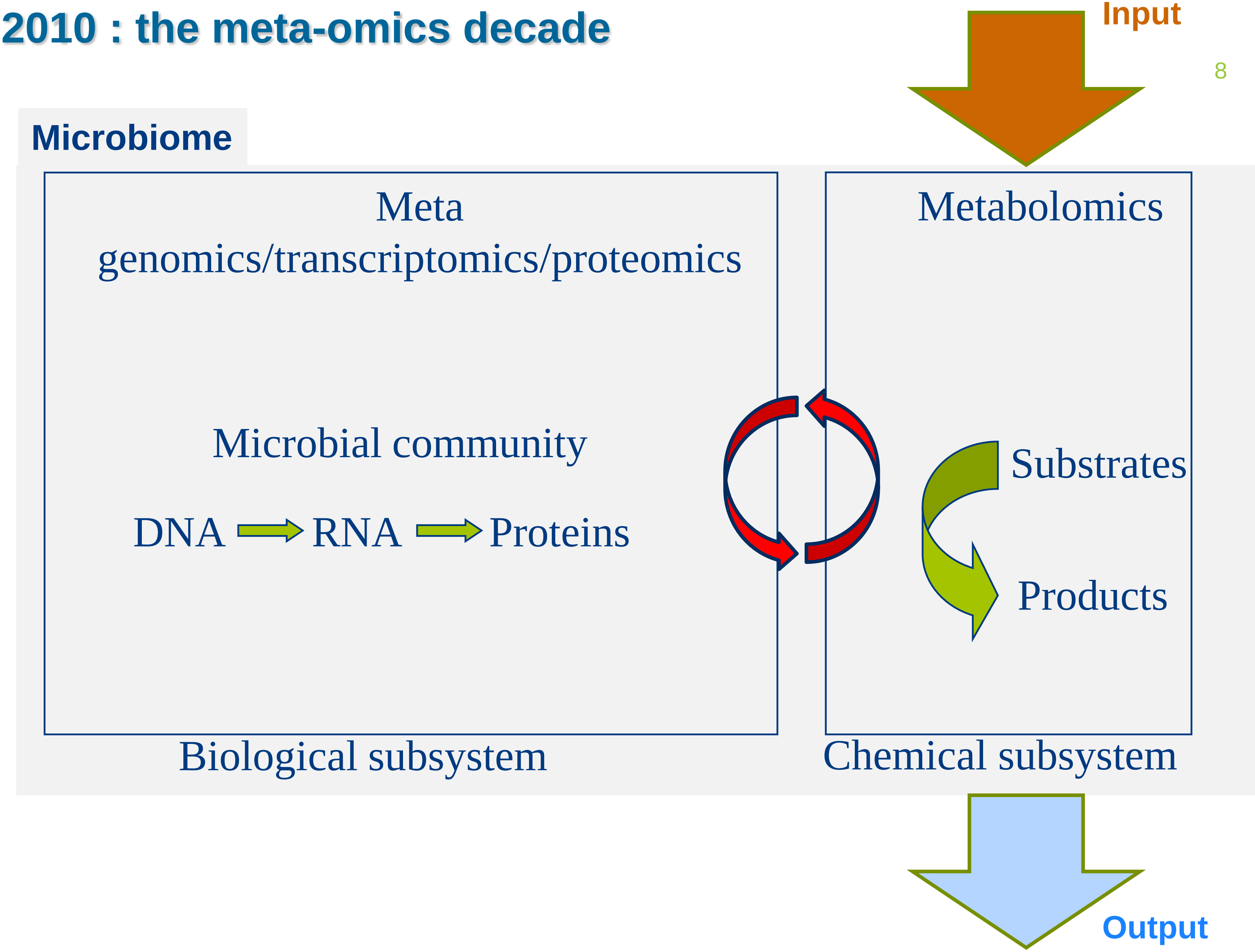
Environmental biotechnologies



Harvest, manage and exploit the natural conversion abilities of the earth microbiome

= process engineering + microbial ecology

2010 : the meta-omics decade



Shotgun metaproteomics of thermophilic anaerobic digestion of cellulose (Lü *et al.*, The ISME-J, 2014, 8, 88–102)

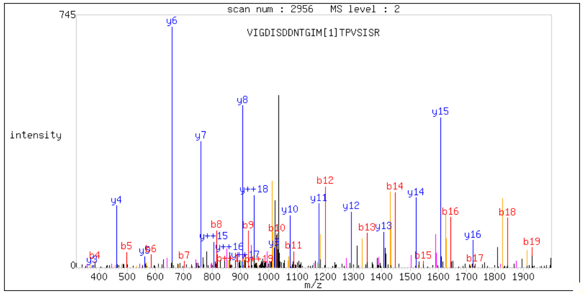
Protein extraction

Fractionation

digestion

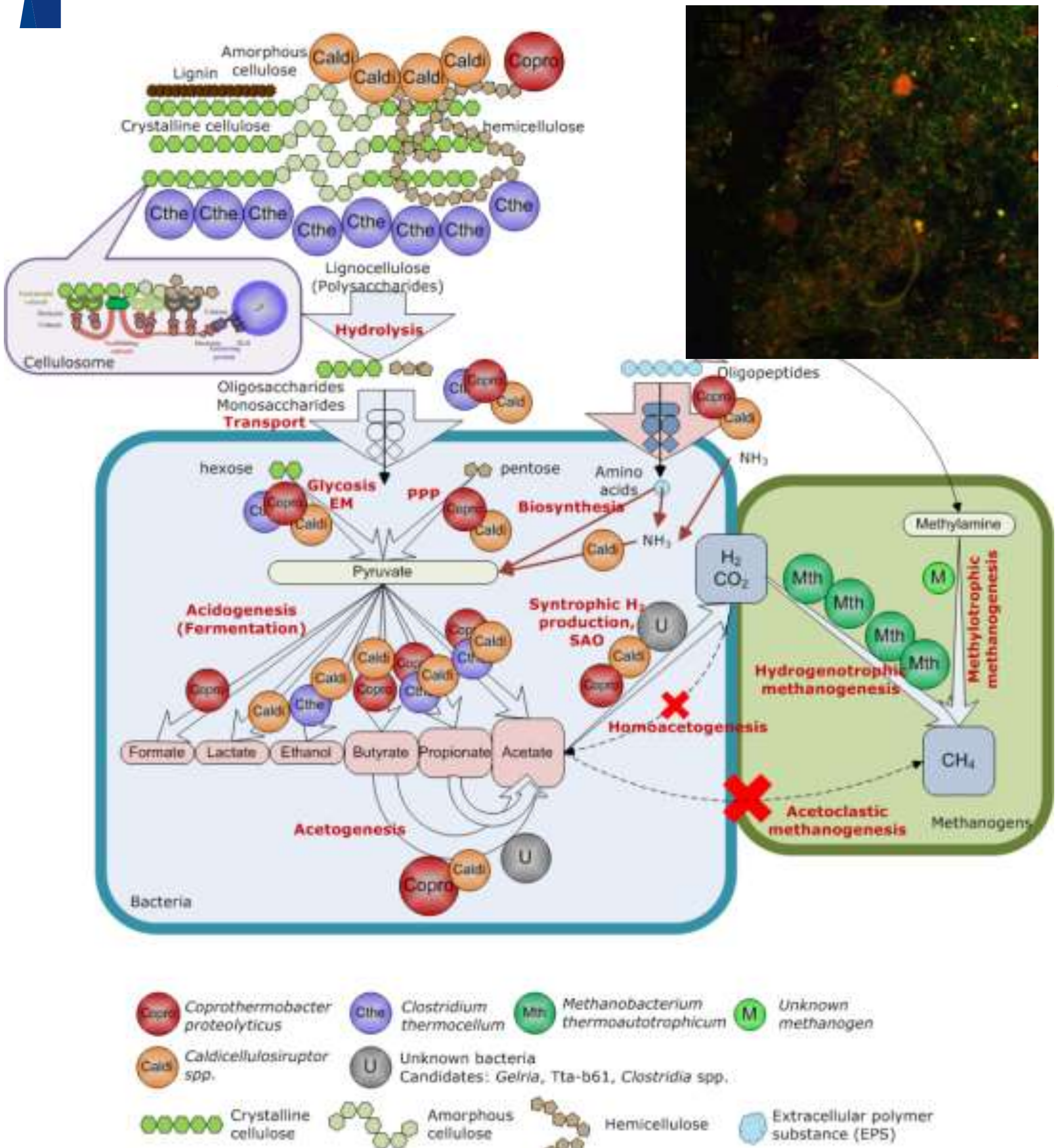
NanoLC-MS analysis (Orbitrap)

Spectra analysis using public databases (Uniprot)



Mass spectra

Confirmations, findings and…surprises!



The Coprothermobacter proteolyticus surprise:

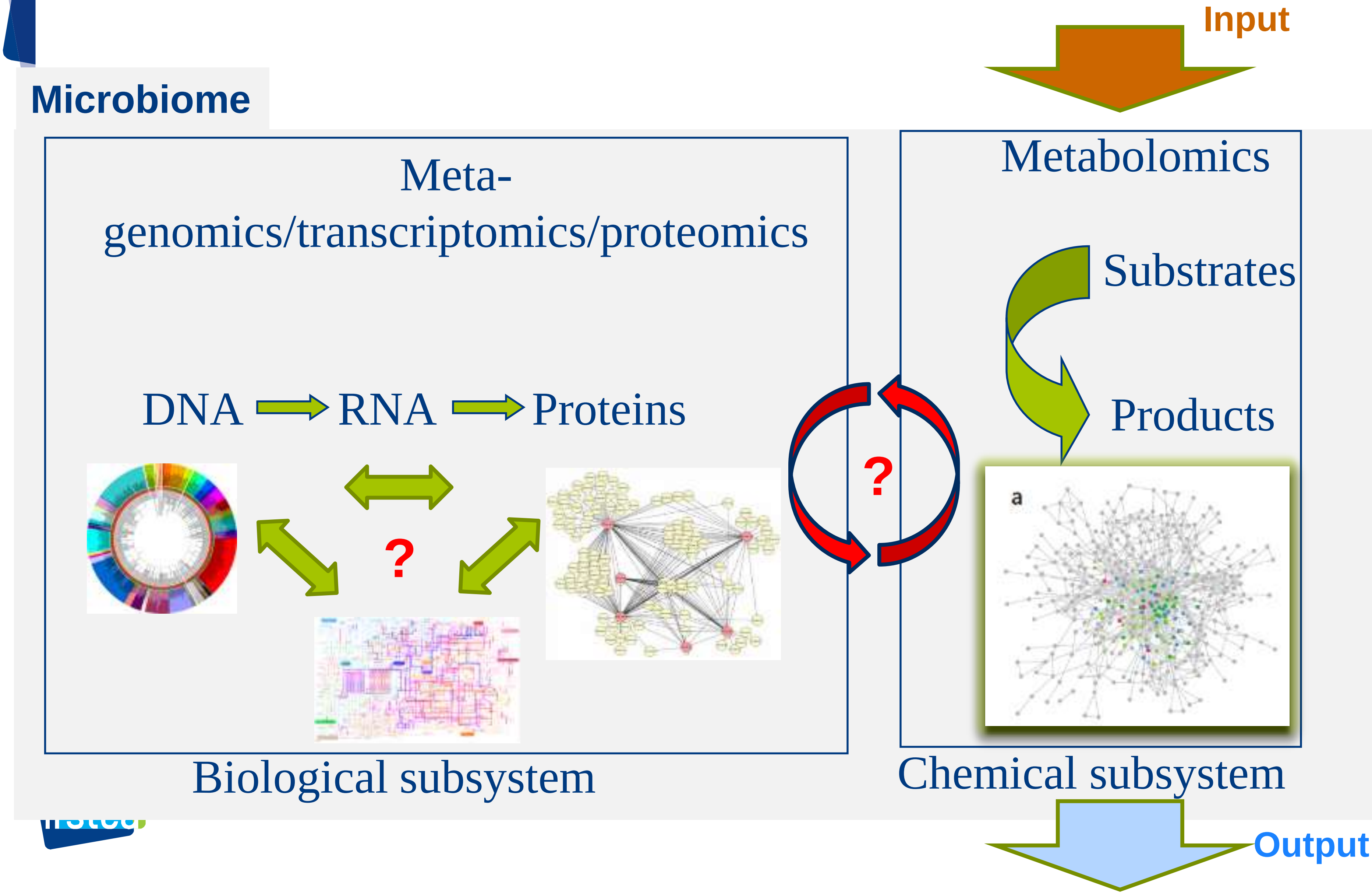
- 22 **peptidases**
  - membrane bound extracellular peptidases
  - 3 **Microcins**
- 7 **ABC transporters** among which 3 are specific for peptides

⇒ **Evidencing new and unexpected competitive interaction between community members**

⇒ **Consequences on process performances require further studies**

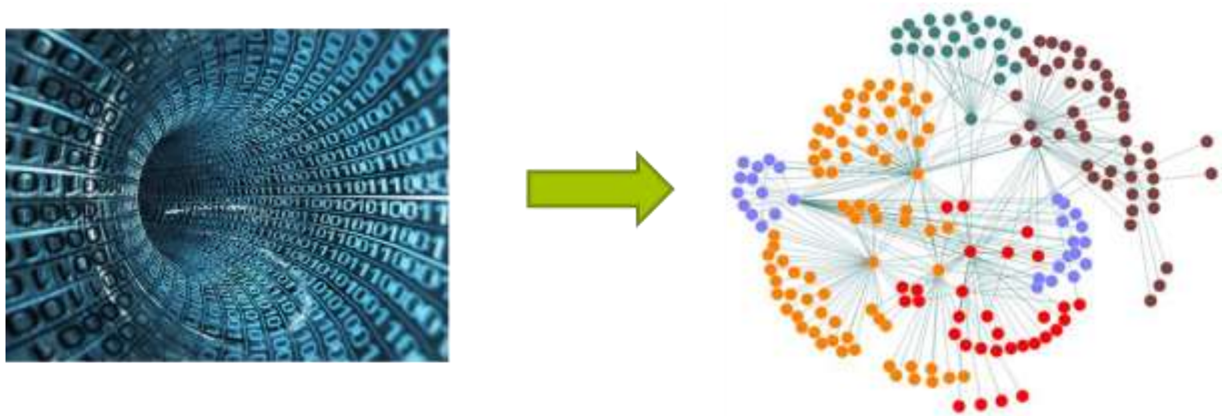
Lü *et al.*, The ISME Journal (2014) 8, 88–102

From a blackbox to a flood of data…



The next challenge: making sense out of the data…

- **Data driven approaches**



❖ Organizing data-derived knowledge into specific databases
❖ Computational and statistical approaches

- **The need for new abstractions** (P. Medawar, 1982)

"…an epoch in the growth of a science during which facts accumulate faster than theories can accommodate them
[…]
As science advances, particular facts are comprehended within, and therefore in a sense annihilated by, **general statements** of steadily increasing explanatory power… "

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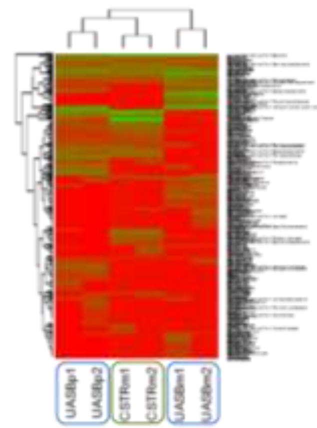
Selection as a key tool for managing microbes in environmental biotechnology processes

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

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

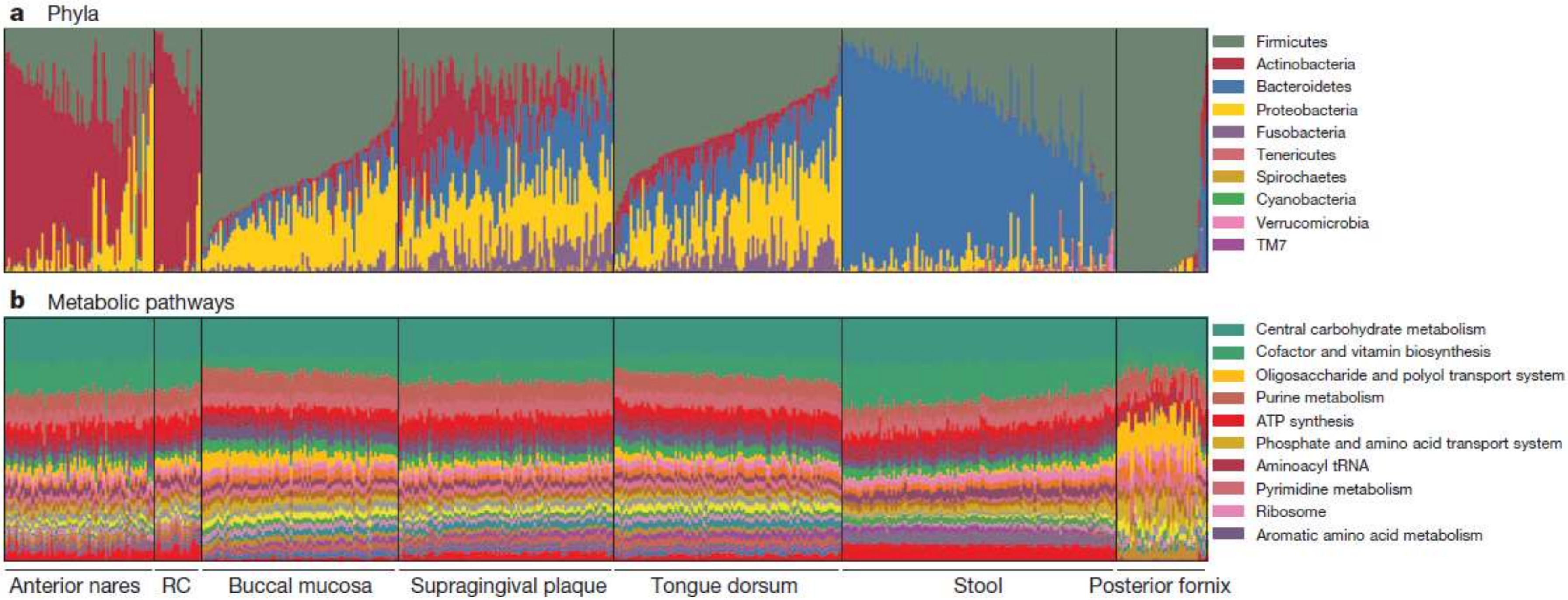
Ecological niches available => environmental filtering => fitness selection

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

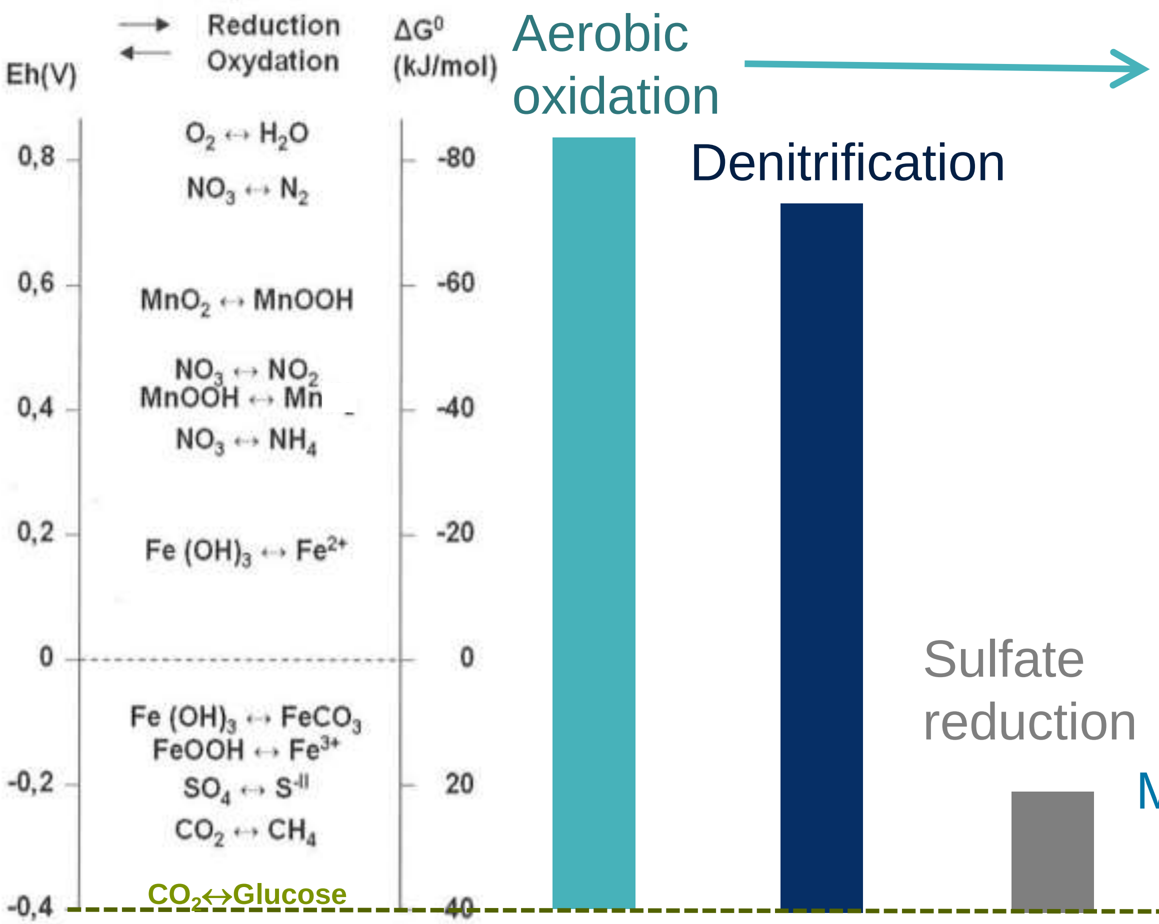
Healthy human microbiome



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



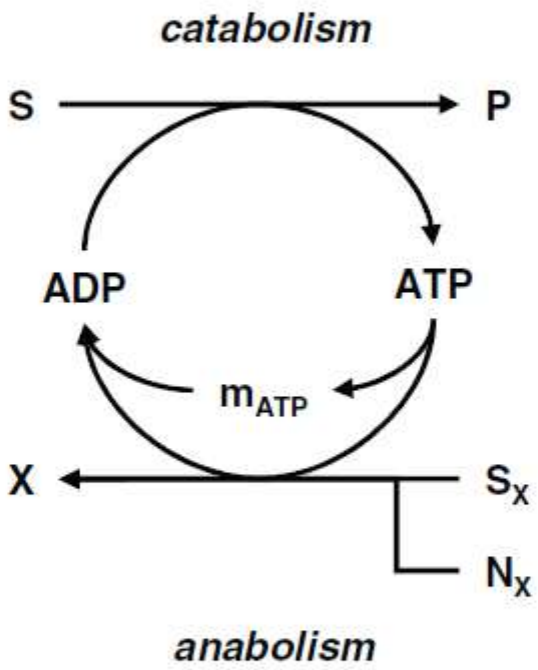
Activated sludge

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

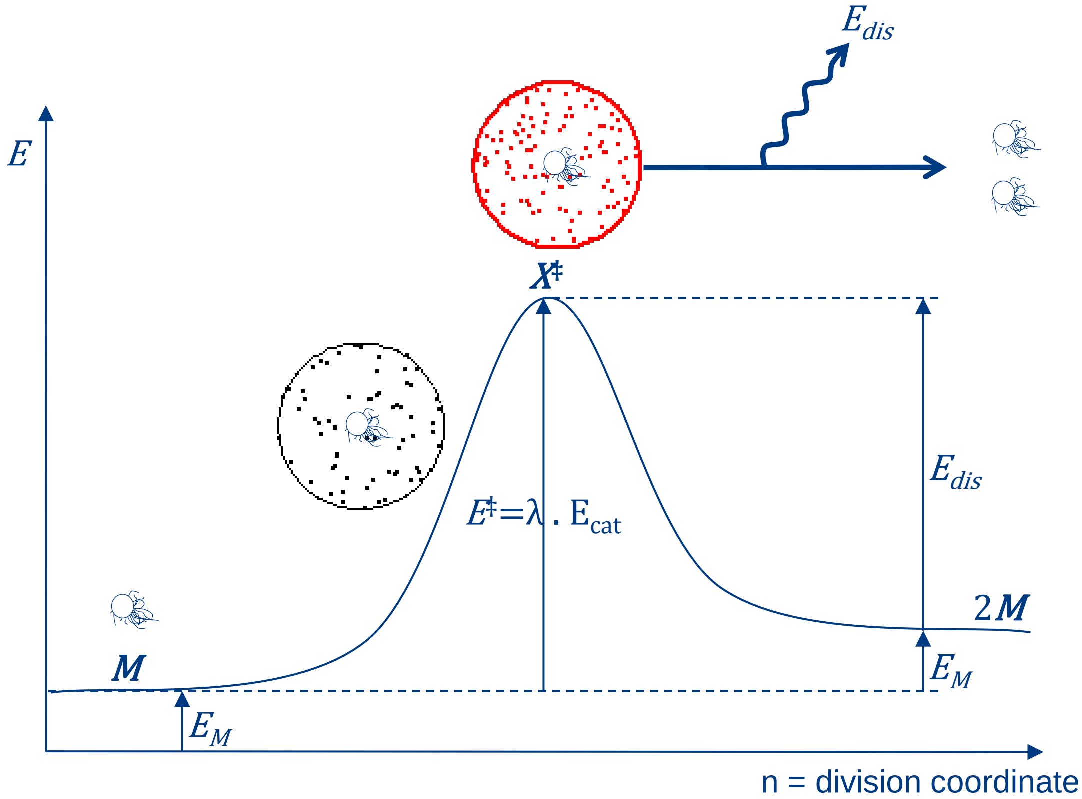
$$E_{dis} = \lambda . E_{cat} - E_M$$

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



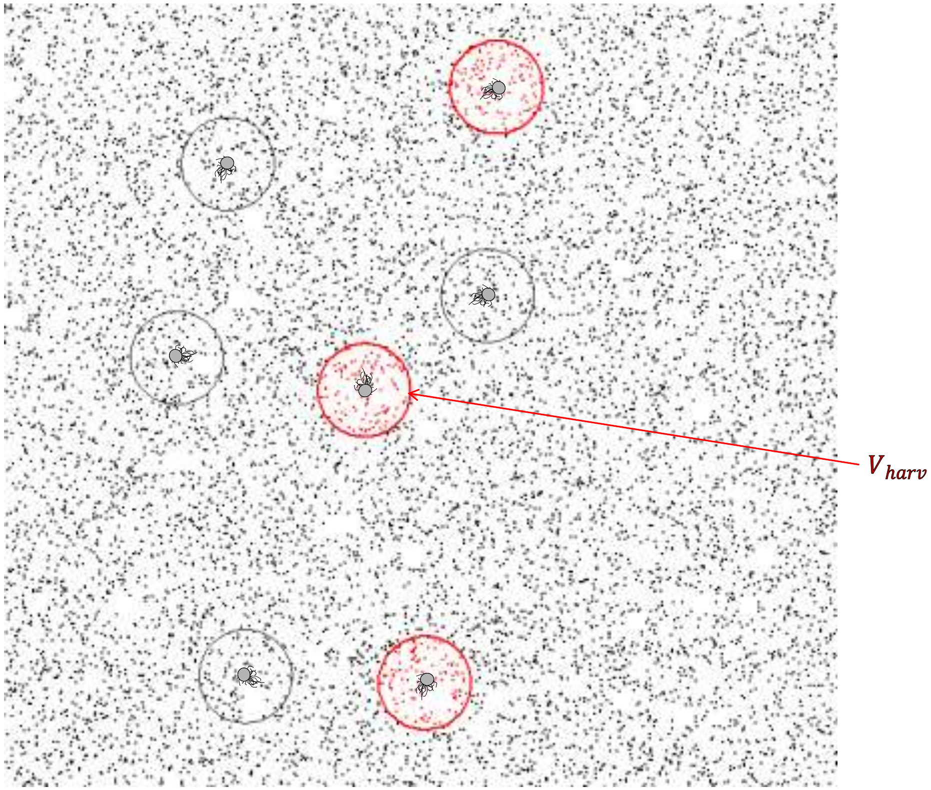
$$M \xleftrightarrow{K} X^‡ \xrightarrow{\mu_{max}} 2M$$

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

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

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

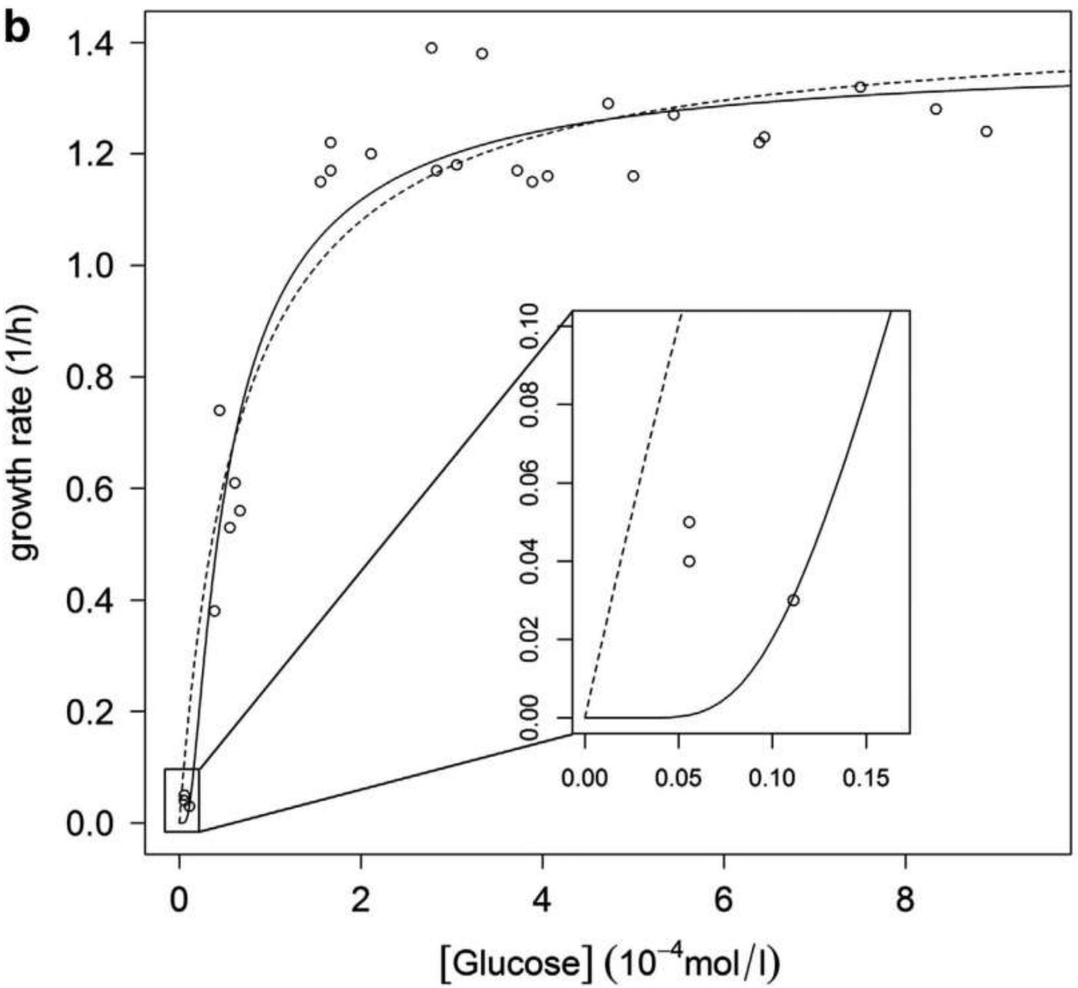
Desmond-Le Quéméner and Bouchez, The ISME-J, 2014

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

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

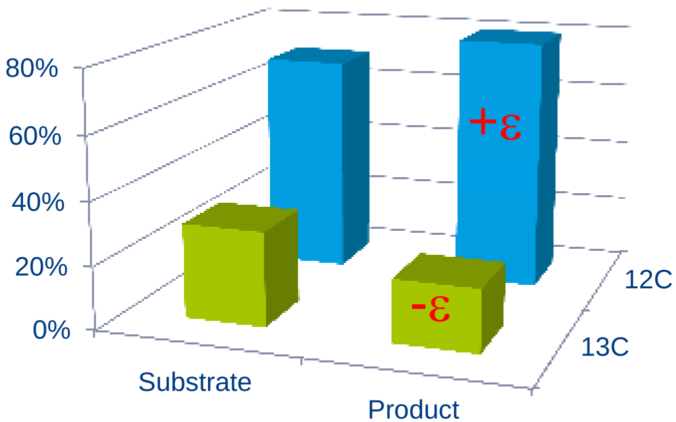
Desmond-Le Quéméner and Bouchez, The ISME-J, 2014

Illustrating MTS model properties

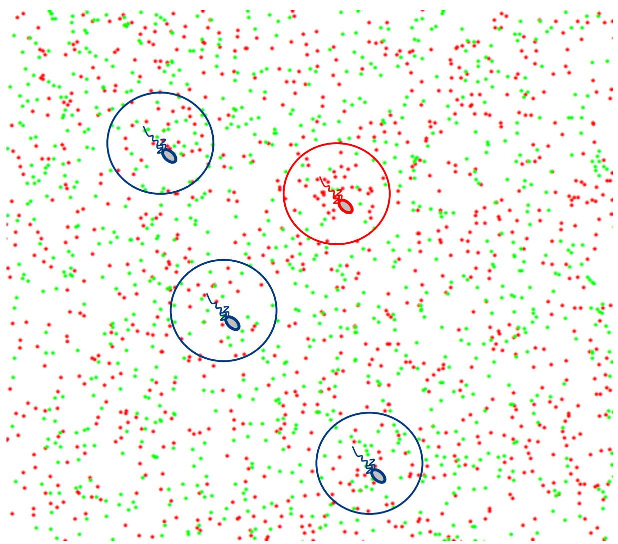
1. **Predictions in relation to the microbial isotopic fractionation phenomenon**
2. From modeling a pure culture in a minimal medium*…
3. …to mixed culture ecosystem models*

***Hadrien Delattre**
PhD

Analyzing the microbial isotopic fractionation phenomenon with MTS theory

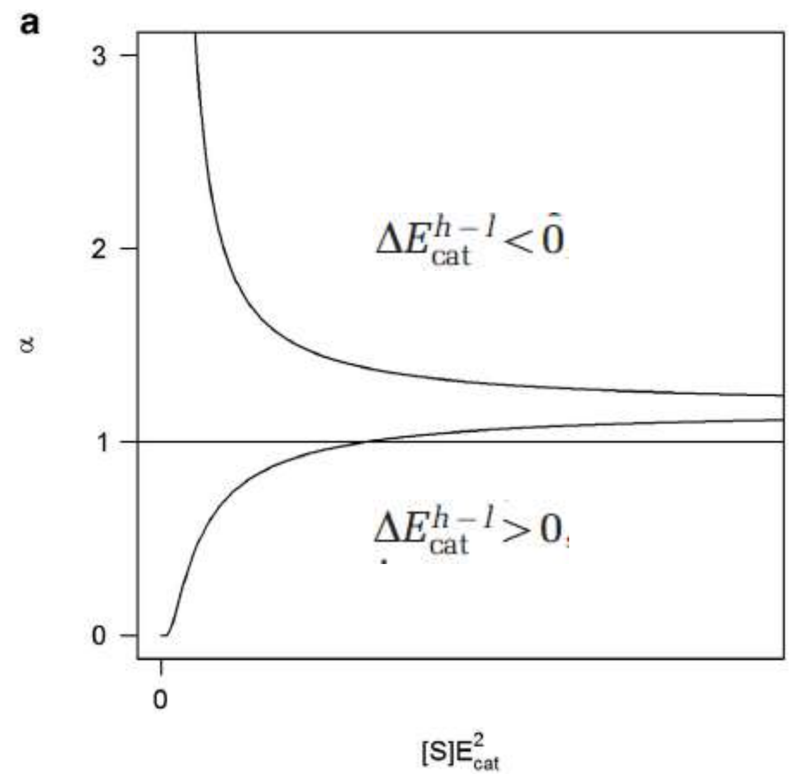


Isotopomer have different energy!
⇒ Should lead to different rates!



Light ressource (^{12}C, ^{14}N,…)
Heavy ressource (^{13}C, ^{15}N,…)

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



Challenging these predictions with real datasets…

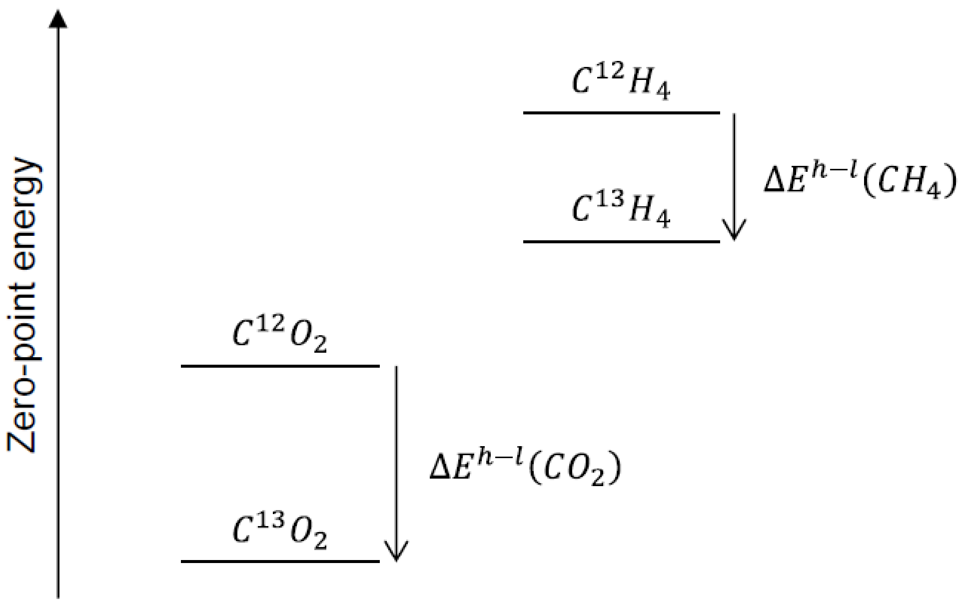
Desmond-Le Quéméner and Bouchez, The ISME-J, 2014

Challenging thermodynamic growth model's predictions with actual isotopic data

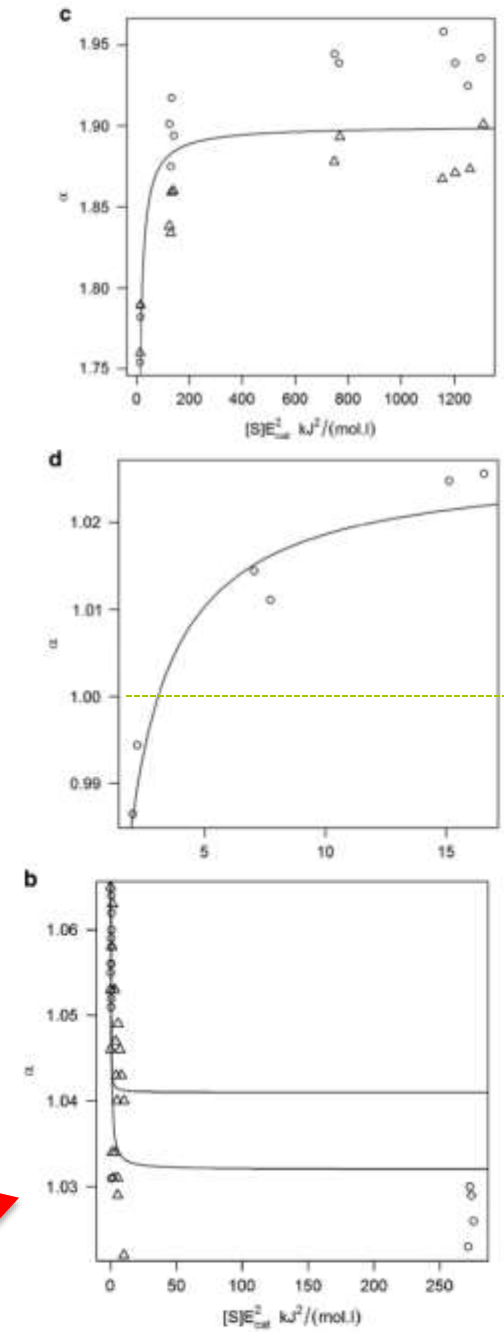
$$\alpha_{S/P} = \alpha_0 \cdot \exp\left(-\frac{E_M + E_{dis}}{V_{harv} \cdot [S_{lim}] \cdot E_{cat}^2} \Delta E_{cat}^{h-l}\right)$$

Hydrogenotrophic methanogenesis

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



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



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

Illustrating MTS model properties

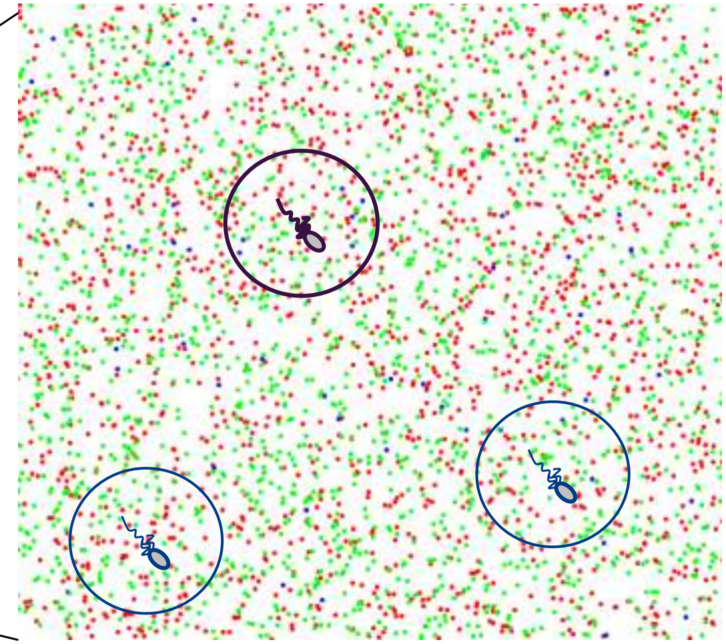
1. Predictions in relation to the microbial isotopic fractionation phenomenon
2. **From modeling a pure culture in a minimal medium*…**
3. …to mixed culture ecosystem models*

***Hadrien Delattre PhD**

Modeling the growth of a pure culture in a minimal medium

(Delattre et al., submitted)

Pure culture in
a minimal medium



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

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

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

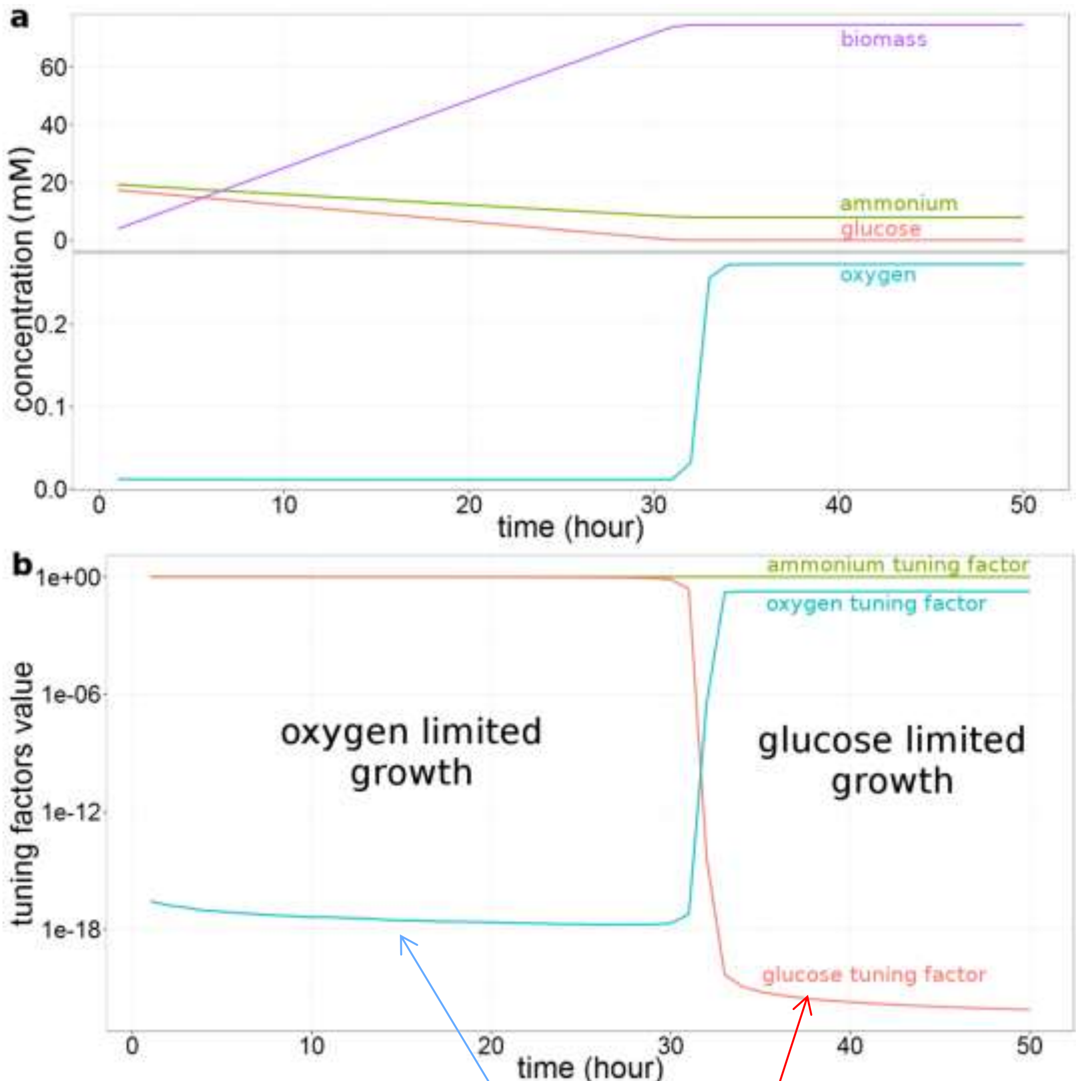
Metabolic energy coupling

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

Dynamics arising from MTS theory for a pure culture

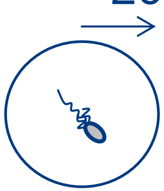


Fixed parameters

$$\mu_{max} = \frac{k_B \cdot T}{h}$$

$$V_h = 1\, m^3 \cdot C - mol^{-1}$$

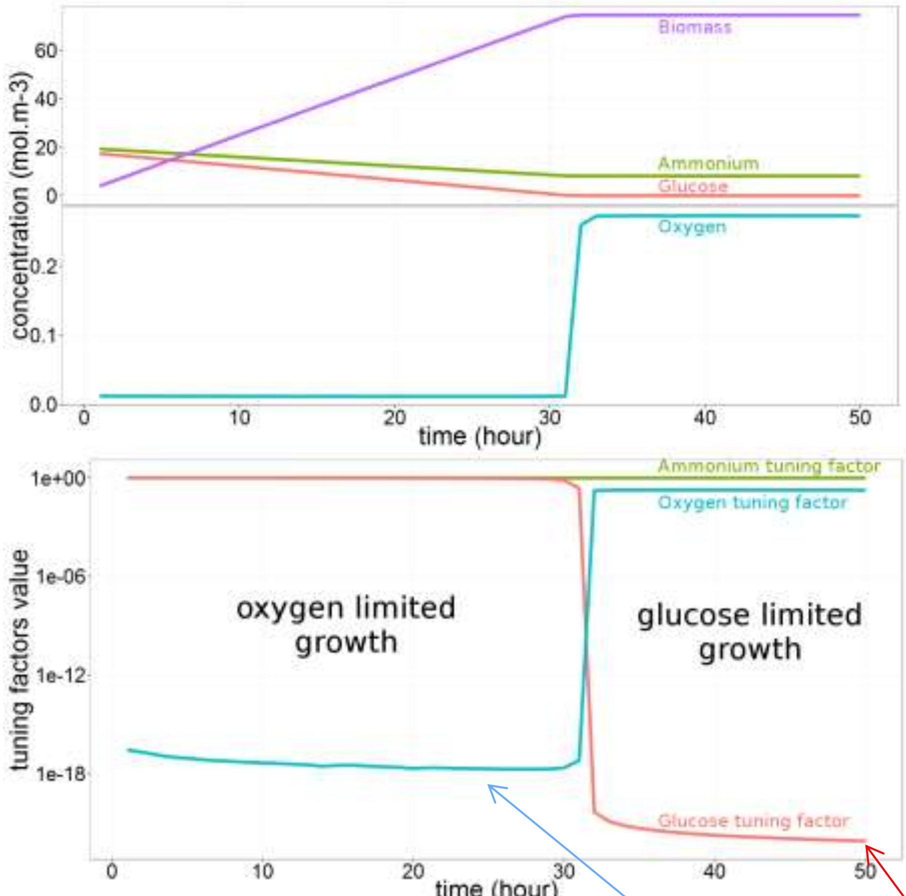
20 µm



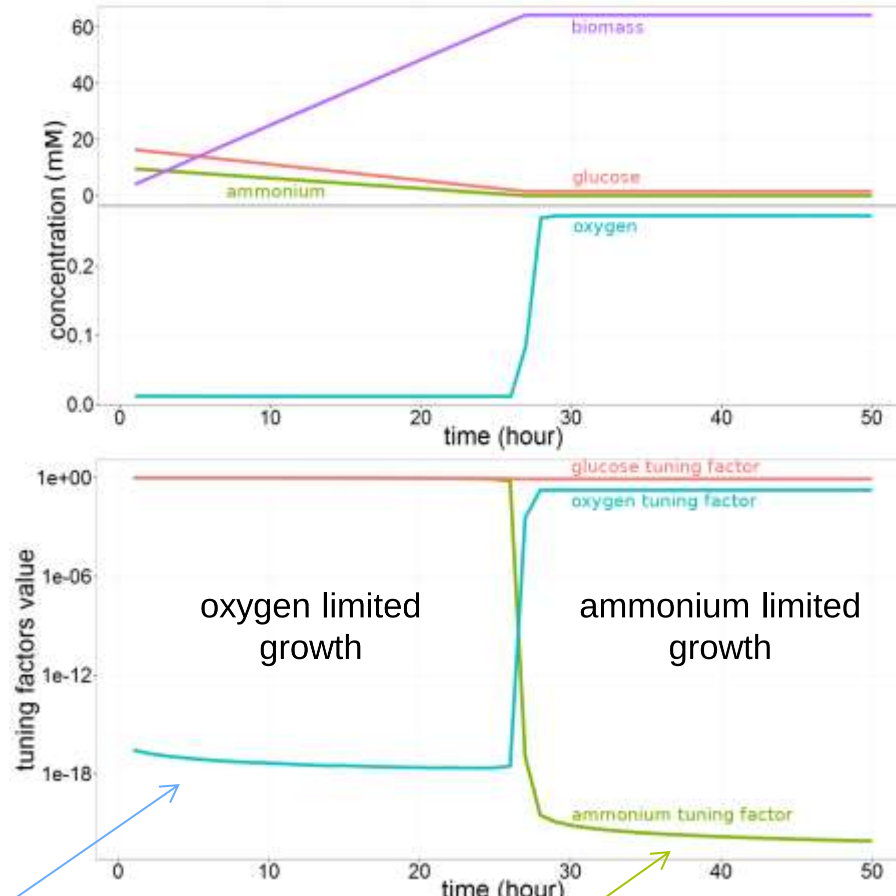
$$\mu = \mu_{max} \cdot e^{\frac{\upsilon_{oxygen}(\lambda)}{V_h \cdot [oxygen]}} \cdot e^{\frac{\upsilon_{Glucose}(\lambda)}{V_h \cdot [Glucose]}} \cdot e^{\frac{\upsilon_{ammonium}}{V_h \cdot [ammonium]}}$$

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

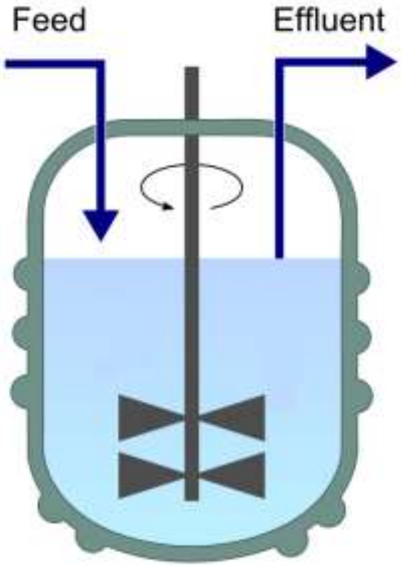
Illustrating MTS model properties

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***

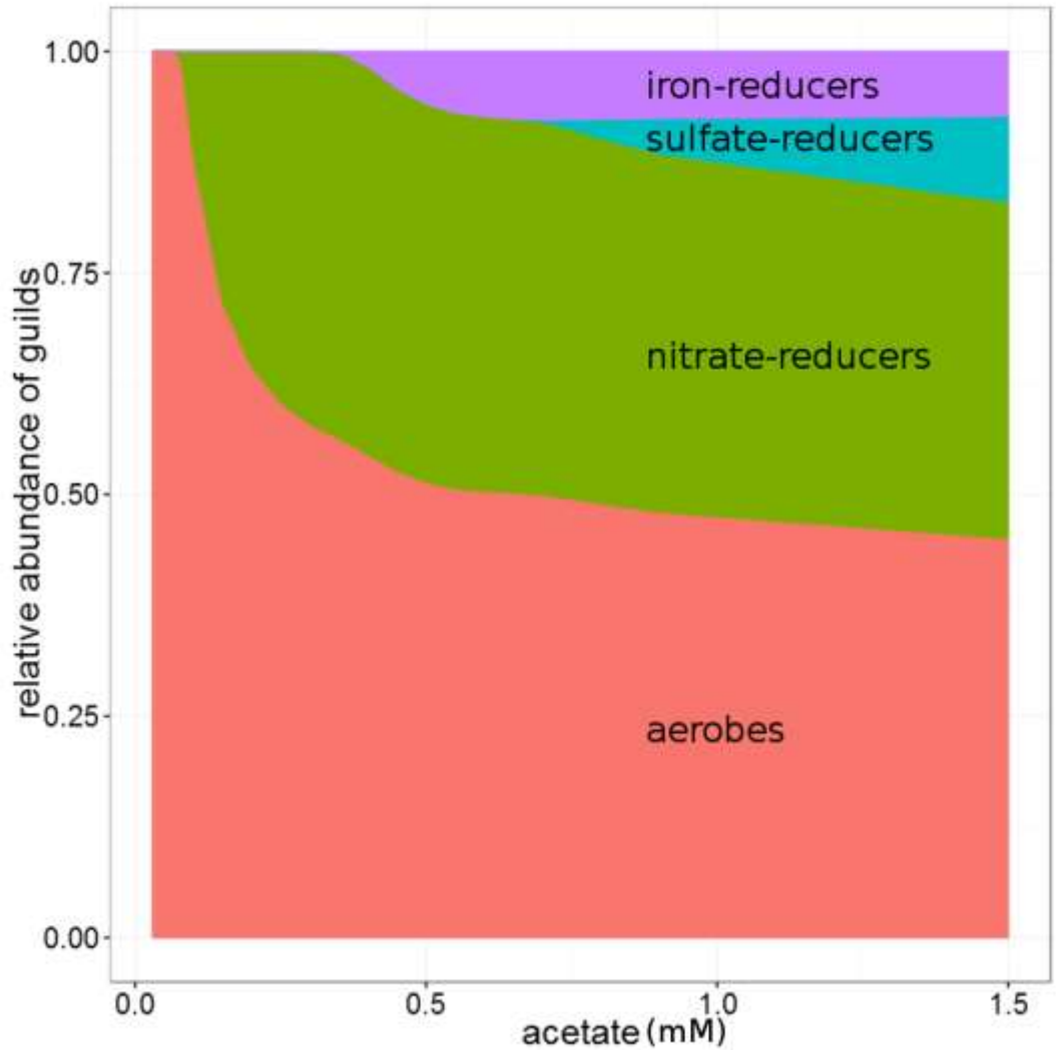
***Hadrien Delattre PhD**

Energy dependent competition arising without parameter adjustment

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



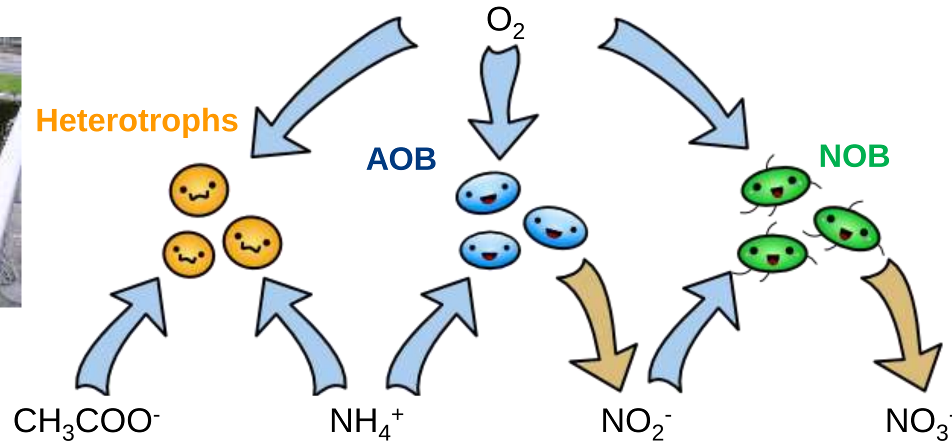
Aerobes, denitrifiers, iron reducers, sulfate reducers… all having the same fixed parameters values (μ_{max}, V_{harv})



Hadrien Delattre PhD

Microbial successions according to redox tower are obtained parsimoniously

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)

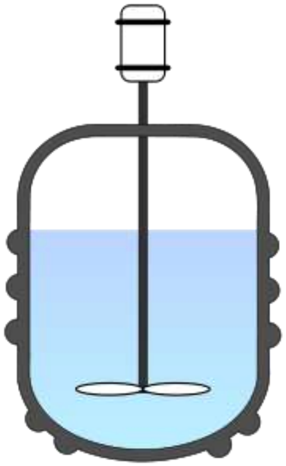
MTS derived-dynamics:

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

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

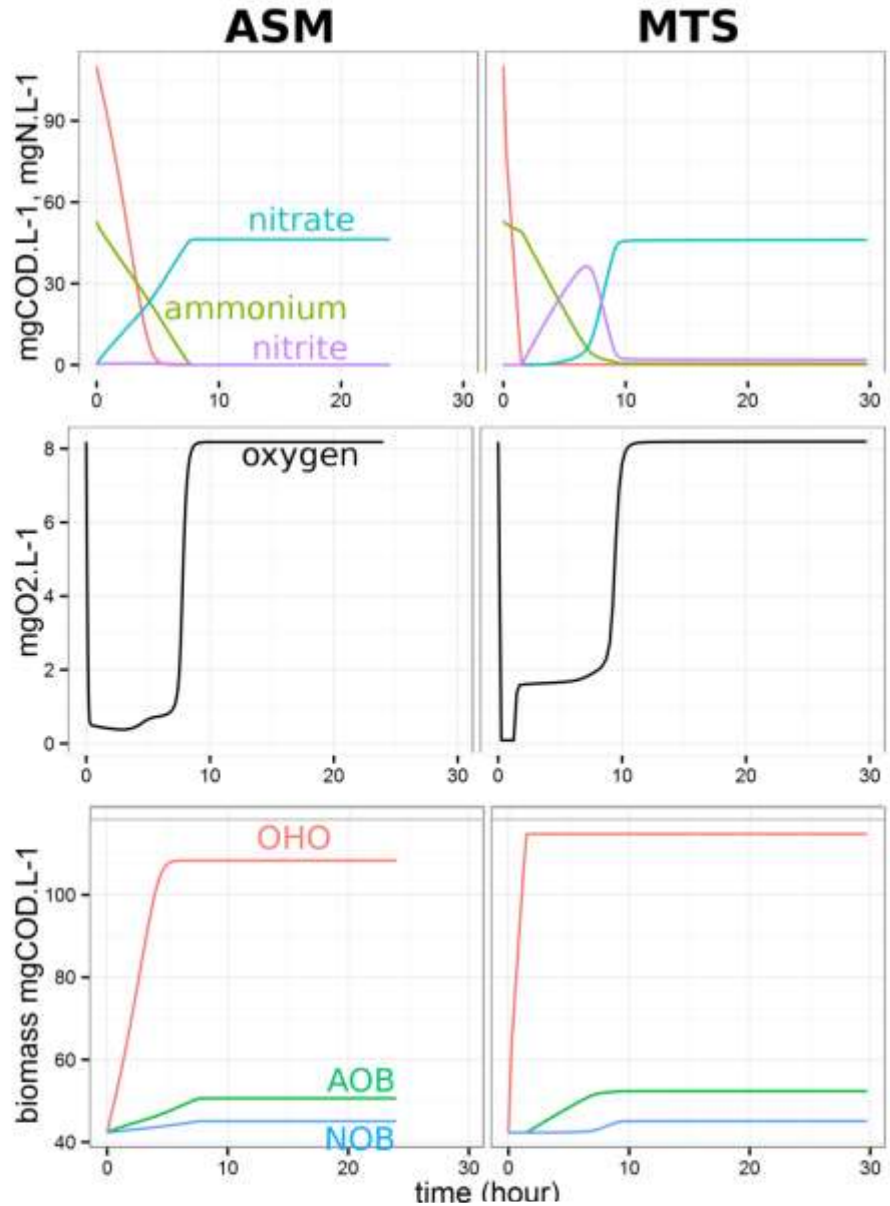
Modeling a simplified activated sludge batch ecosystem

(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



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

ANR-THERMOMIC : general objectives and workpackages

Establish and assess a general THERMOdynamic framework for modelling MICrobial growth and community dynamics (2016-2020, ANR 16-CE04-0003)

1. Refine the theory and check concepts with experiments (WP1 : coord. Irstea-HBAN)
2. Explore the mathematical properties of the MTS model (WP2 : coord. INRA-LBE)
3. Assess MTS approaches for environmental biotechnology applications (WP3 : coord. INSA-LISBP)

3 positions currently opened

- **Postdoctoral position 1**: Studying the effect of temperature on growth rates with MTS
- **Postdoctoral position 2**: MTS theory and phototrophic growth
- **PhD position**: Challenging MTS theory with experiments

email to theodore.bouchez@irstea.fr

Thermodynamics and microbial community management in environmental biotechnology processes

- In microbial ecology, scientific bottlenecks are progressively shifting from analytical methodologies to **knowledge integration** into an inclusive picture
- In addition to data integration, the development of a more **conceptual framework** is needed
- **Generic** abstractions to grasp a **whole category** of phenomena
- Microbiology is a fertile thinking ground for **crossing disciplinary boundaries** between biology, physics and math
- Linking **thermodynamics** and **growth kinetics** to could ultimately give rise to more **generic** (less parameter dependent) and **predictive** models to manage microbial community in environmental biotechnology processes

Many thanks to…

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

Hadrien Delattre, PhD candidate
Microbial thermodynamics

Elie Desmond-Le Quéméner, INRA-LBE
Microbial thermodynamics

Project number ANR-16-CE04-0003-01

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