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Usefulness of molecular biology tools in the operation and control of the anaerobic digestion process

Jean-Philippe Steyer

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XIII Latin American Symposium on Anaerobic Digestion

“An alternative energy, environmental and social innovation”

October 21 - 24, 2018. Medellín. Colombia.



Usefulness of Molecular Biology Tools in the Operation and Control of the Anaerobic Digestion Process

Jean-Philippe Steyer

Laboratoire de Biotechnologie de l'Environnement, INRA Narbonne

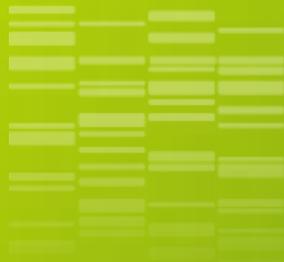


Usefulness of Molecular Biology Tools

➤ Microbial Resource Management towards Ecological Engineering ?

From « Who is present ? » « Who is doing what ? » « With whom ? »....

... to « **why** are they doing it together? »



Study and control of electro-assisted fermentation in mixed cultures: The role of engineering of microbial interactions

Javiera Toledo Alarcón
PhD Candidate



Supervisor: Nicolas Bernet

Co-Supervisor: Eric Trably



Metabolic Pathways

✓ H_2 to release e^- excess

✓ Acetate route



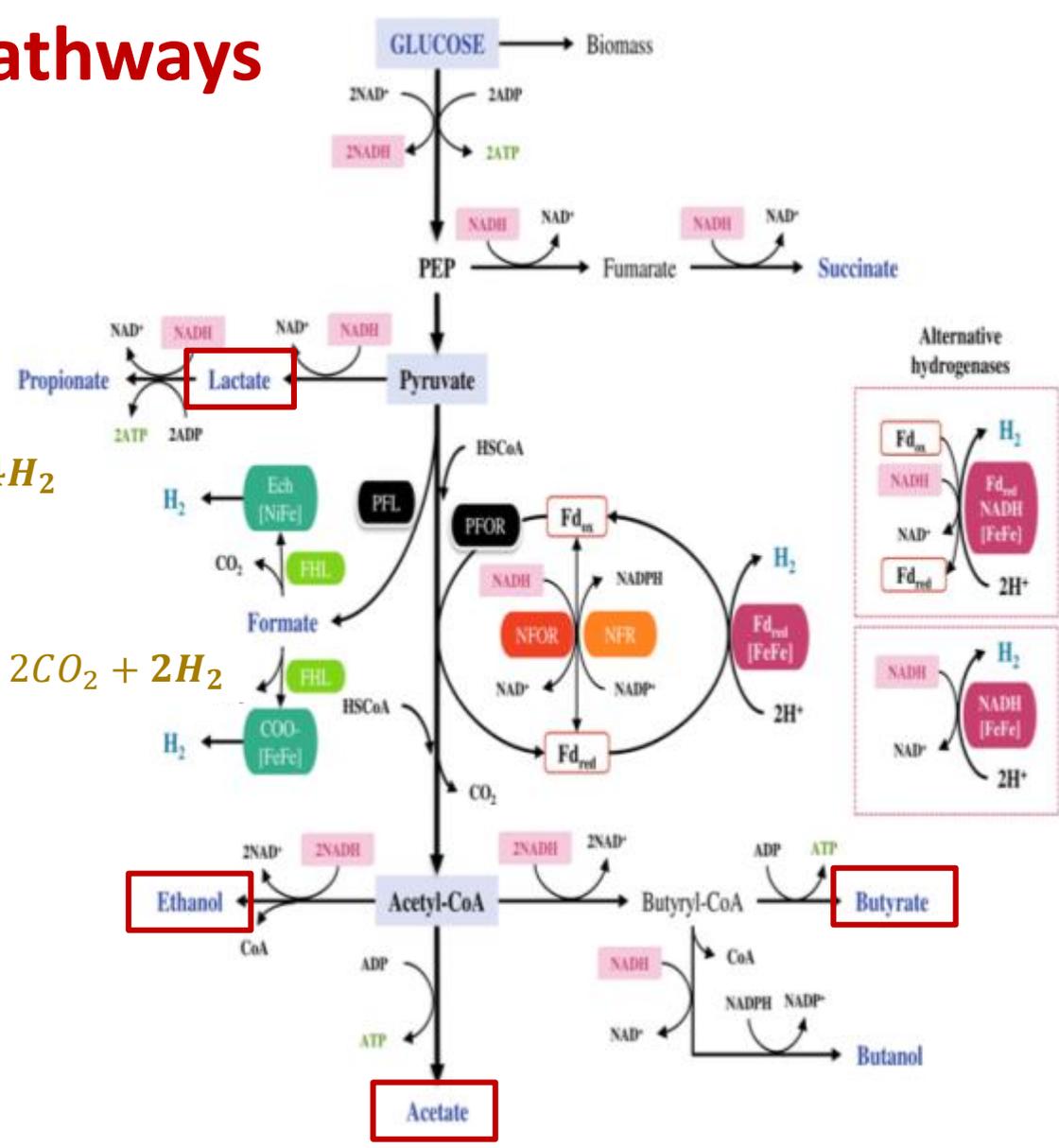
✓ Acetate + ethanol route



✓ Butyrate route

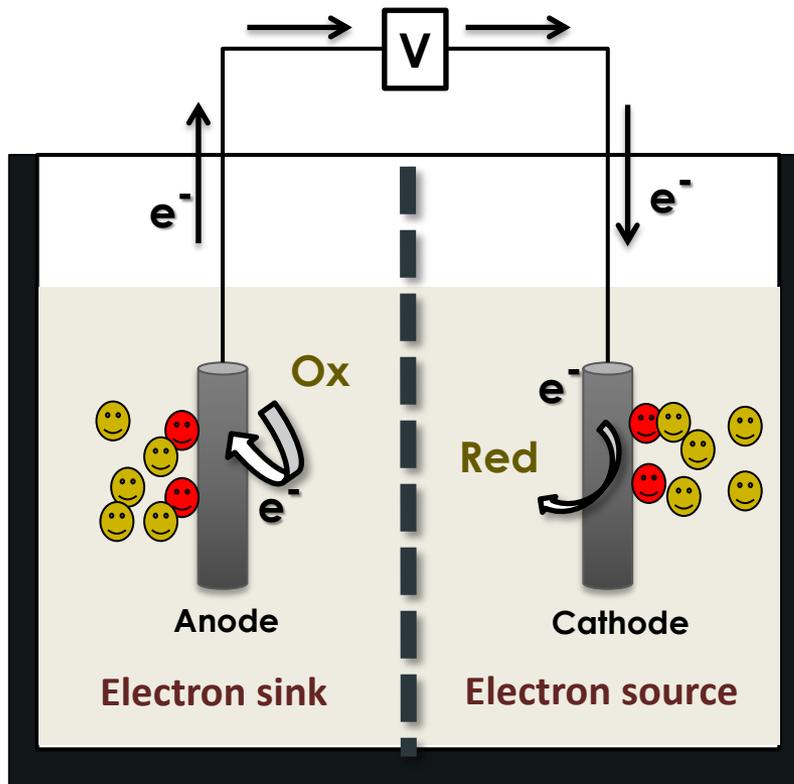


✓ Lactate route



Electro-Fermentation

“**Novel** process that consists of electrochemically **controlling** microbial **metabolism** with **electrodes**”



- Voltage applied on working electrode
- **Anodic EF:** partial electron sink
- **Cathodic EF:** additional electron source
- Microbial interaction between species and electrode surface
- Microbial interaction between species

Experimental methodology



Control
(*n*=5)

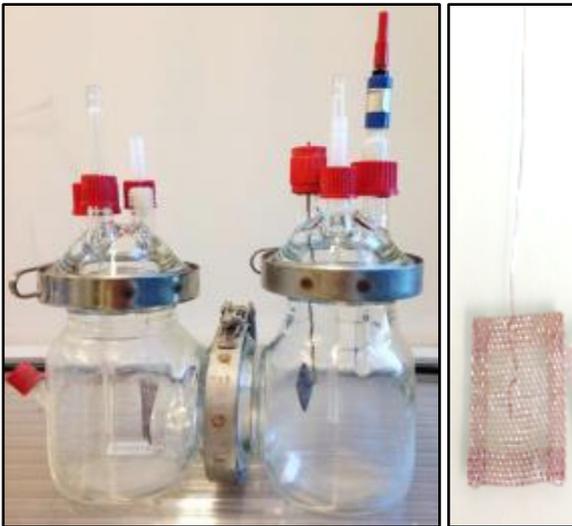
Glucose (5 g.l⁻¹) & Other Nutrients: **Starkey**

pH_{initial}: **6.0** (MES Buffer)

Temperature: **37 °C**

Operation: **Batch x 20 h**

Inoculum: **HT anaerobic sludge** sampled from a lab-scale AD treating sewage sludge



Electro-Fermentation

- **0.9 V** vs SCE (*n*=2)

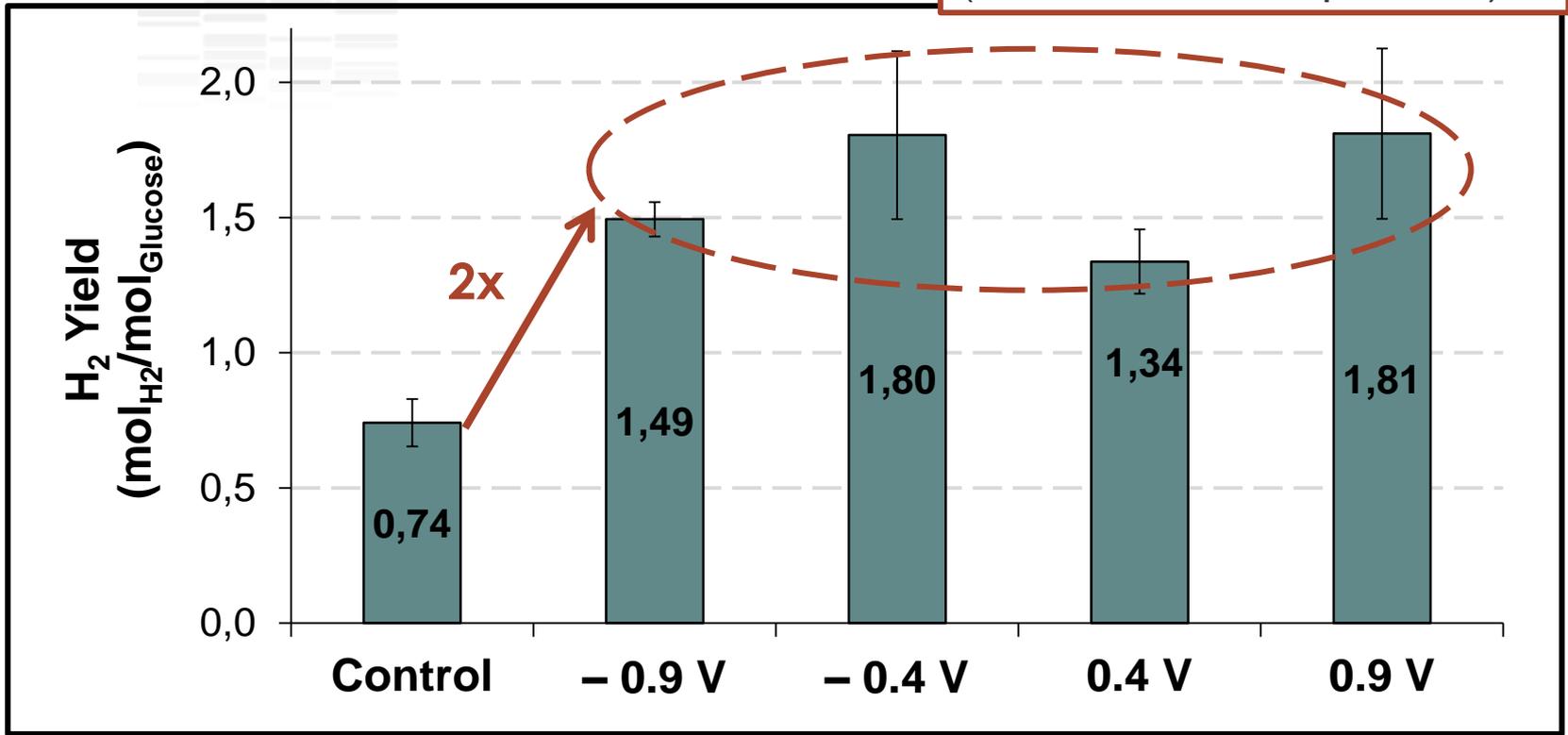
- **0.4 V** vs SCE (*n*=2)

+ **0.4 V** vs SCE (*n*=3)

+ **0.9 V** vs SCE (*n*=3)

H₂ production

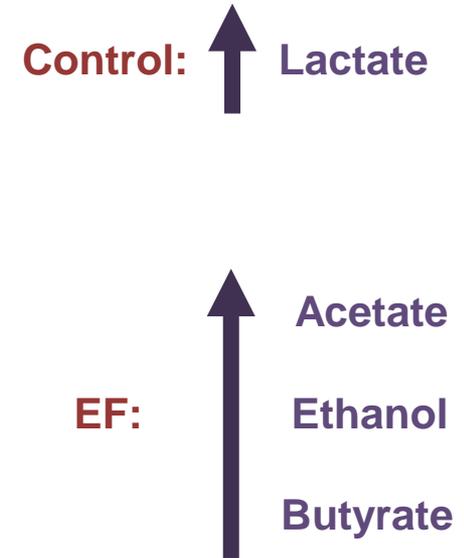
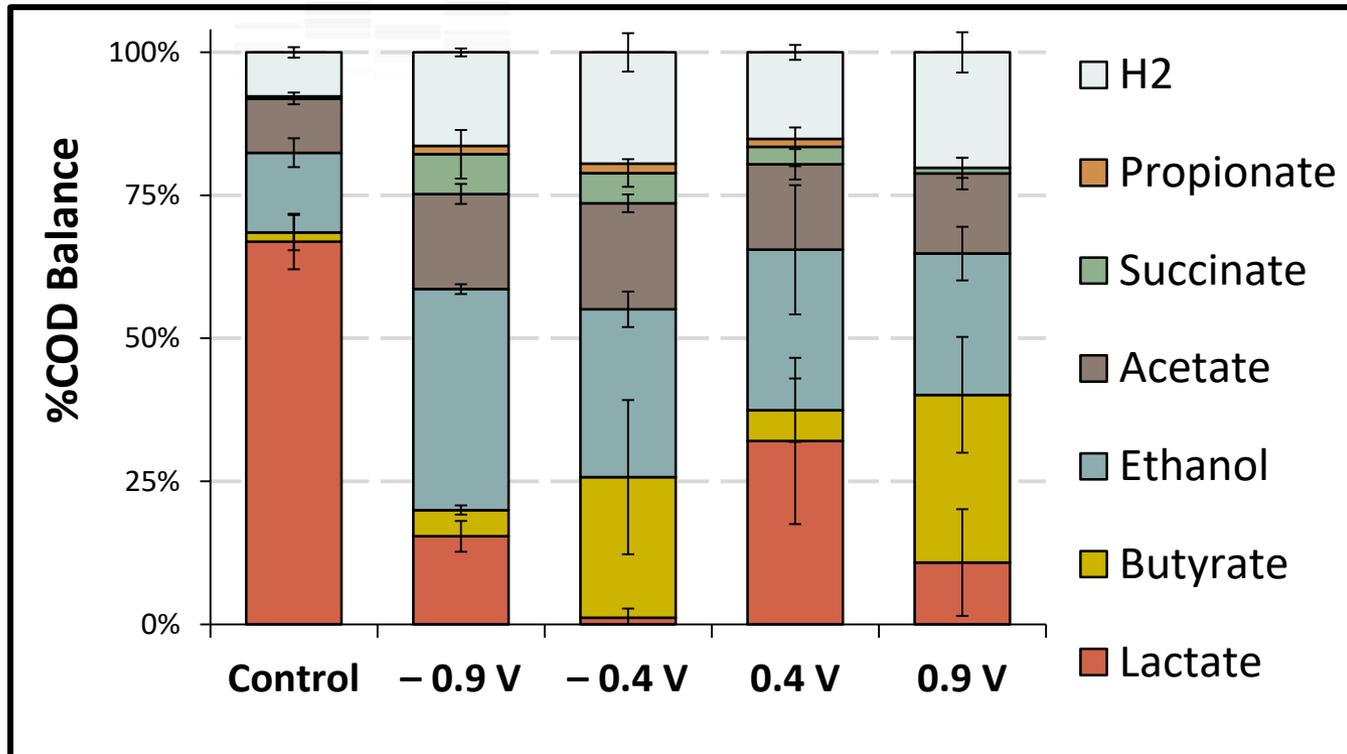
(Anova, F=20.68, p=0.001)



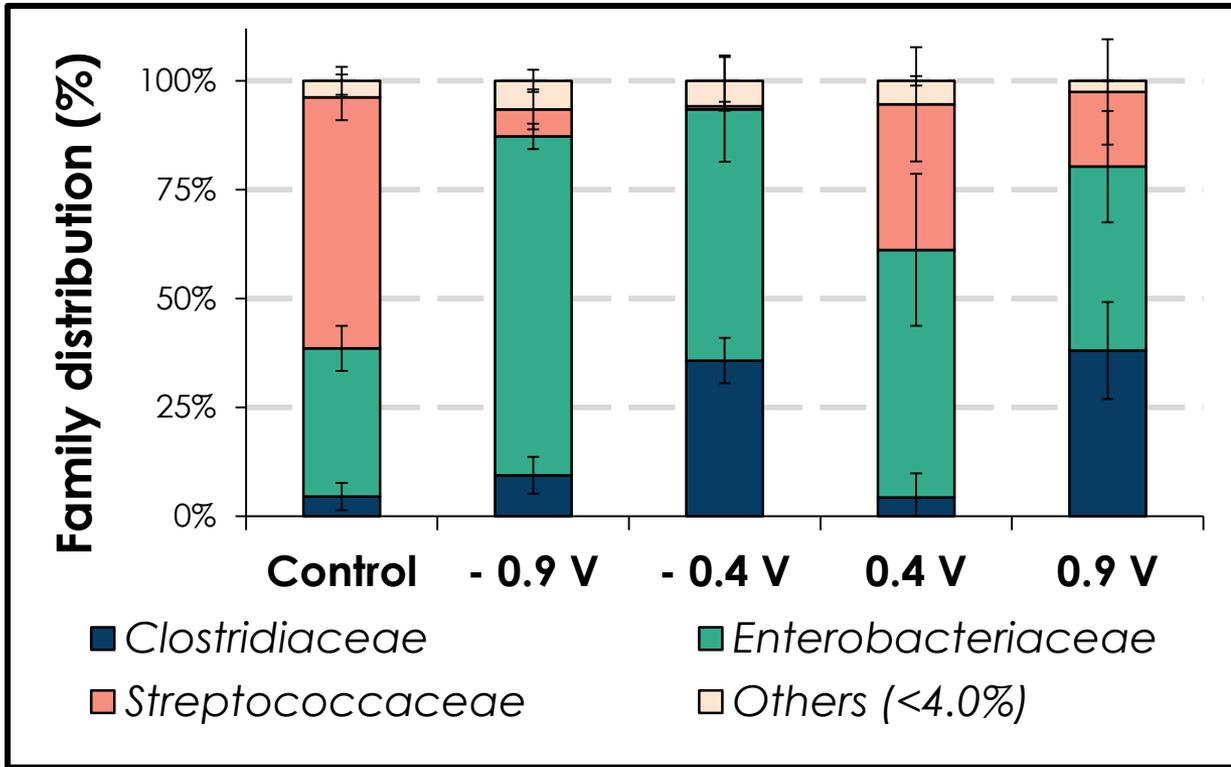
↑ H₂ production during Electro-Fermentation
(Not correlated with the voltage applied)

Small current → High impact

Metabolite distribution



Microbial community

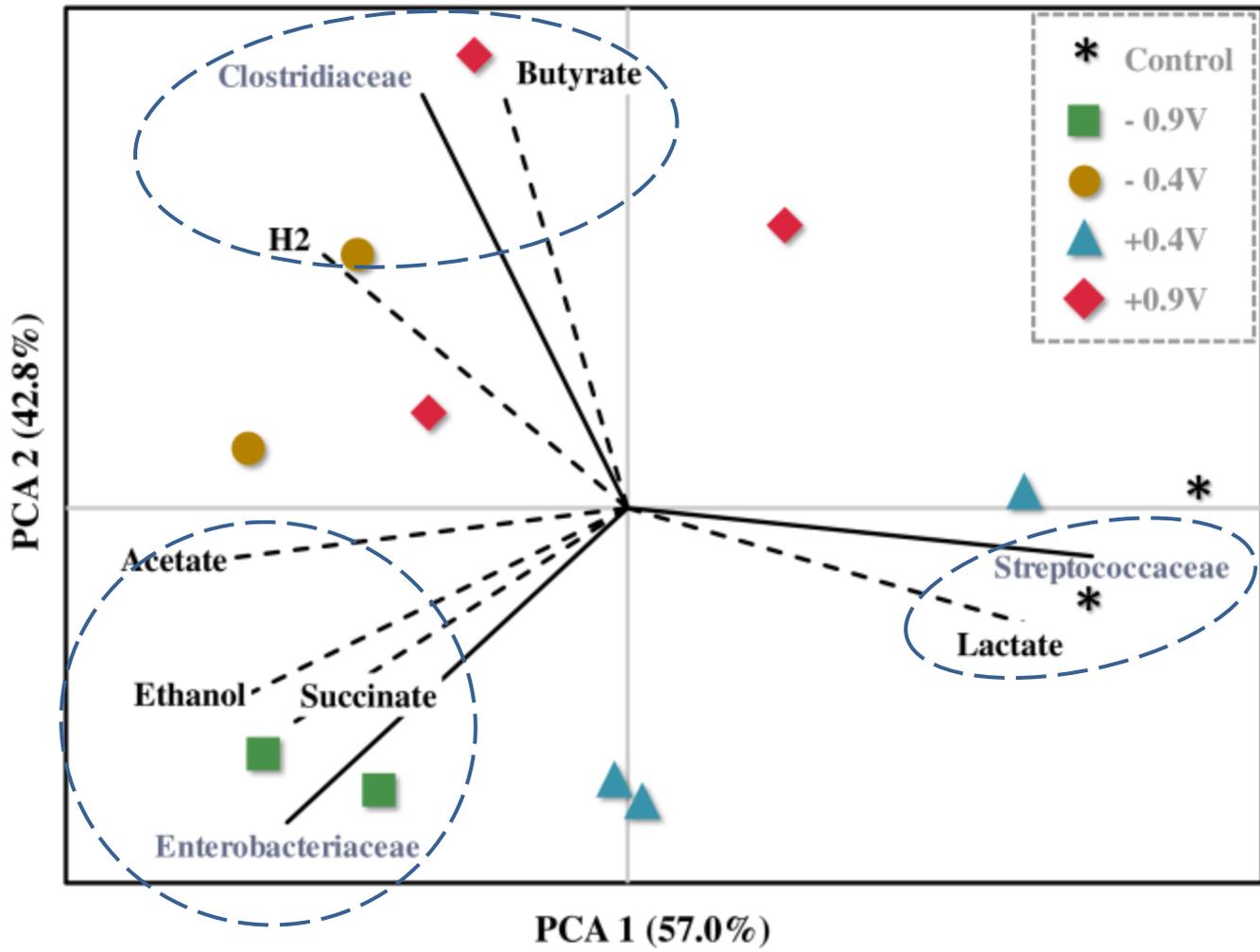


↓ *Streptococcaceae*

↑ *Enterobacteriaceae*

↑ *Clostridiaceae*

Analysis of Metabolic patterns & microbial community



Streptococcaceae

✚ Lactate

■ H₂

Clostridiaceae

✚ Butyrate

✚ H₂

■ Lactate

Enterobacteriaceae

✚ Acetate

✚ Ethanol

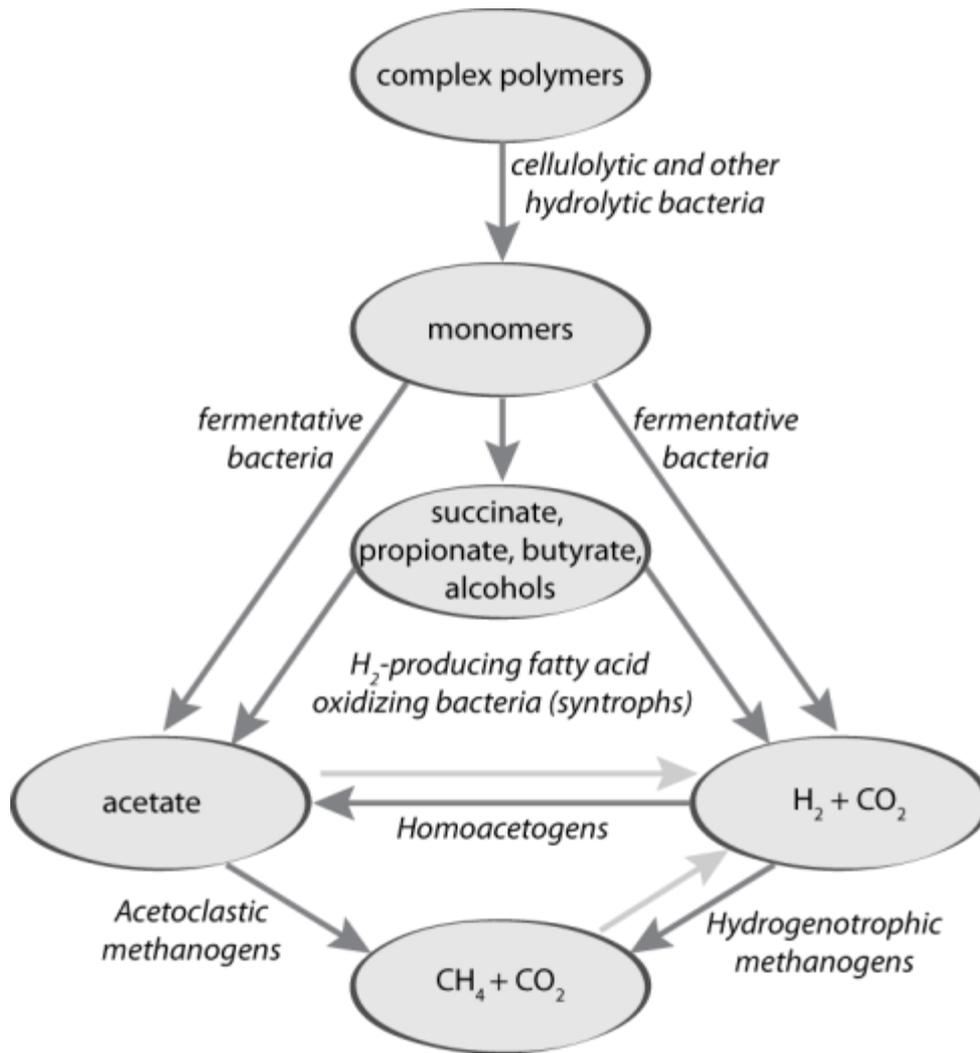
✚ Succinate

Usefulness of Molecular Biology Tools

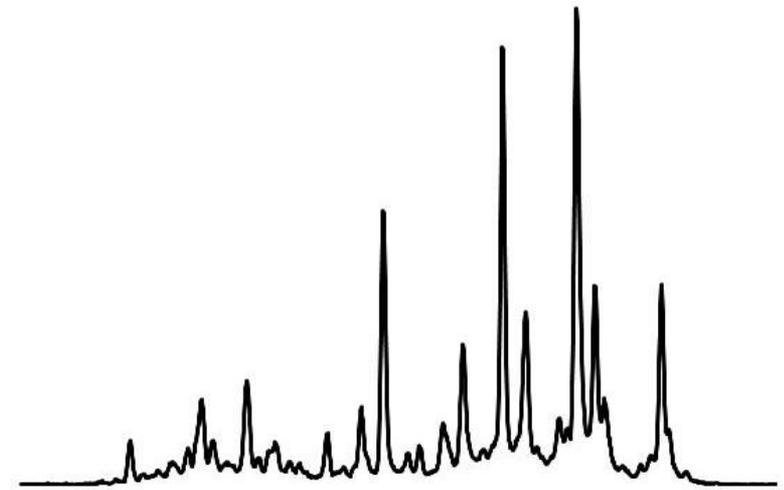


Could these tools help us
to optimize process performances?

Anaerobic digestion

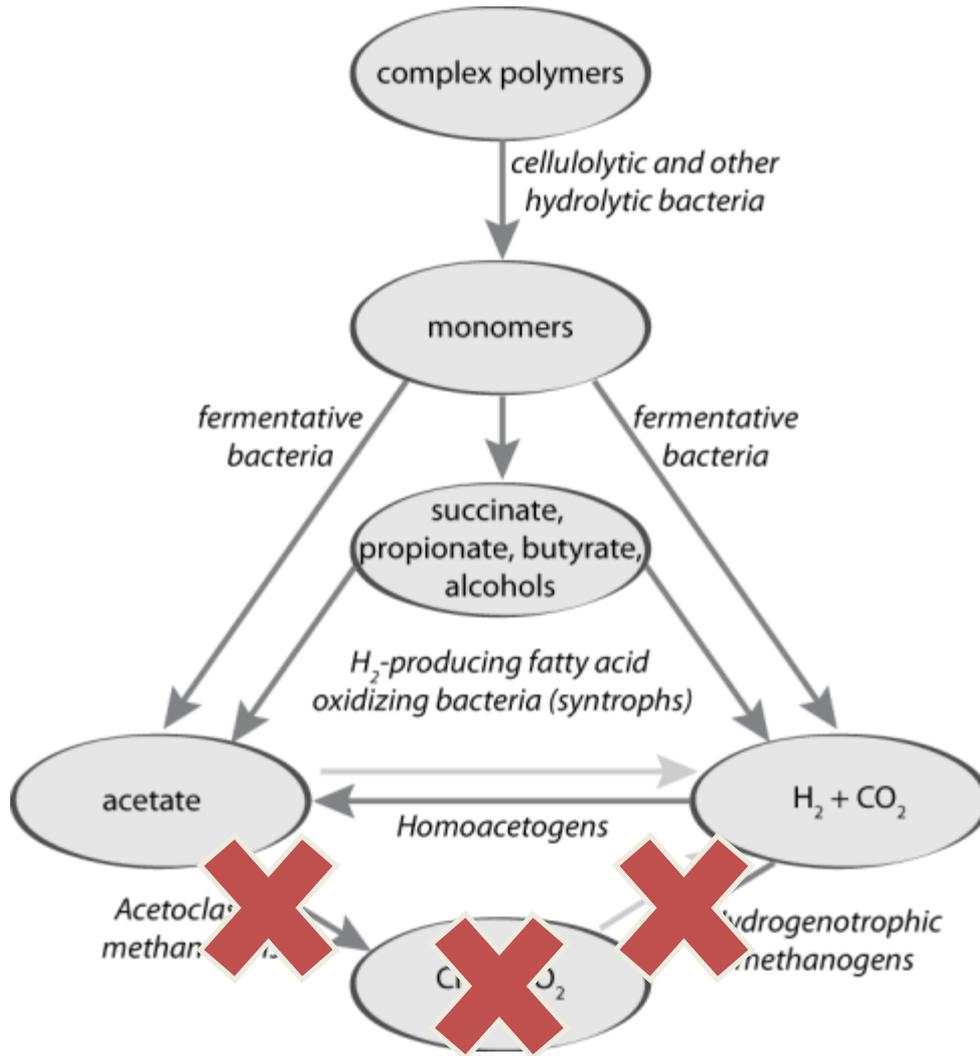


Complex ecosystem – High diversity



SSCP fingerprint

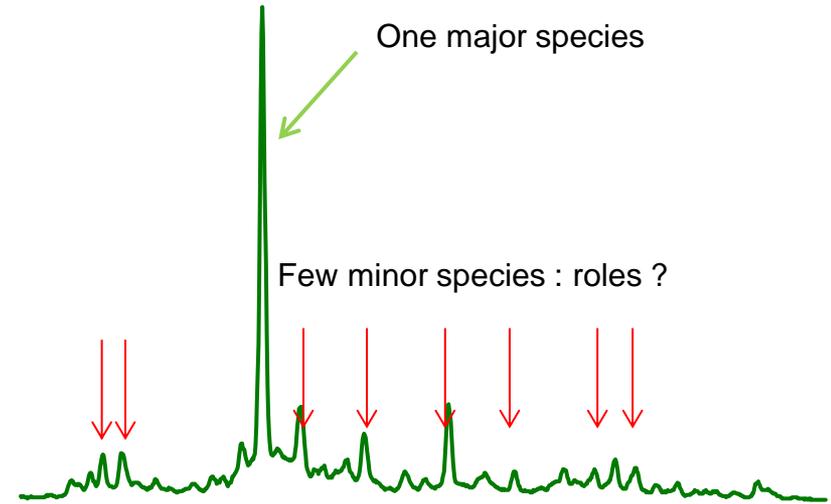
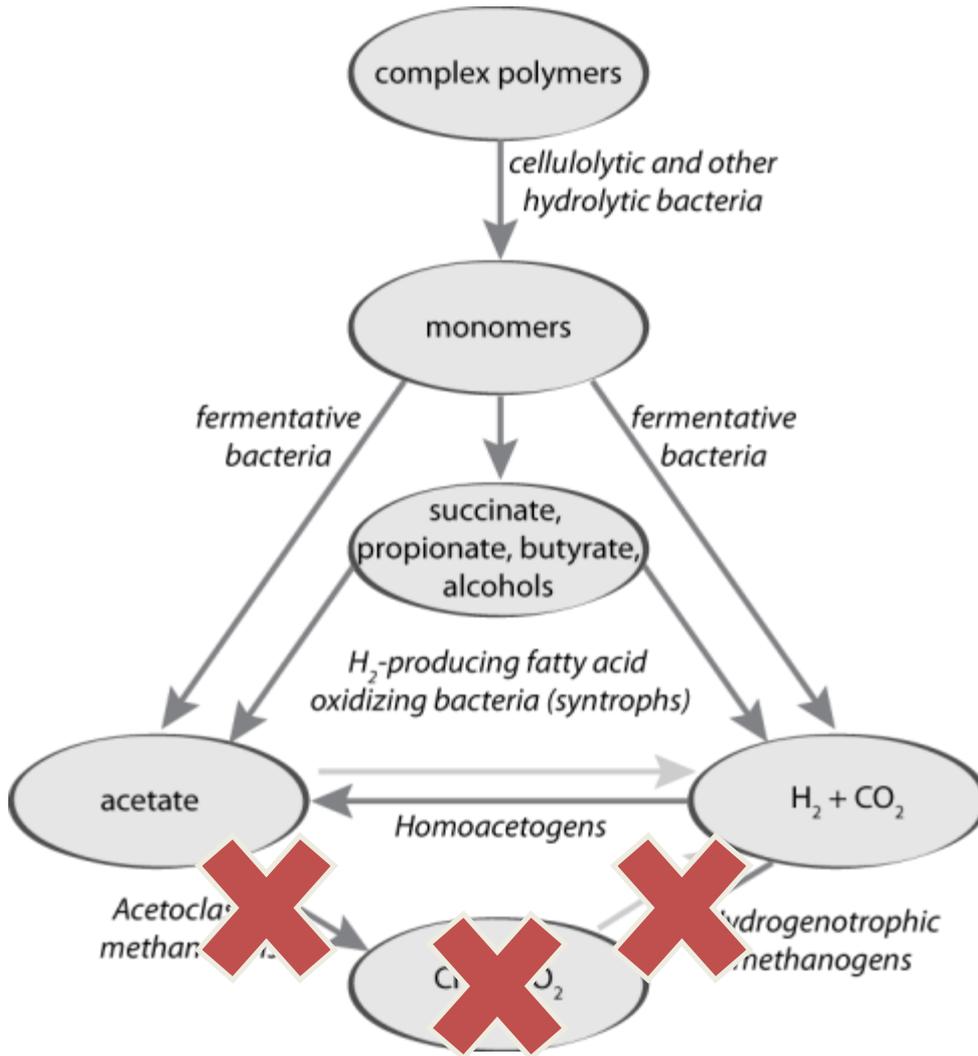
Anaerobic digestion



Under specific operating conditions
(pre-heating, low pH, short HRT)

No biogas but bioH₂ and biomolecules

Anaerobic digestion



Ecological Engineering for biotic control

In each reactor, same operating conditions
(feed=glucose, HRT=10h, T=37°C, pH=5.5)



heated

raw



heated

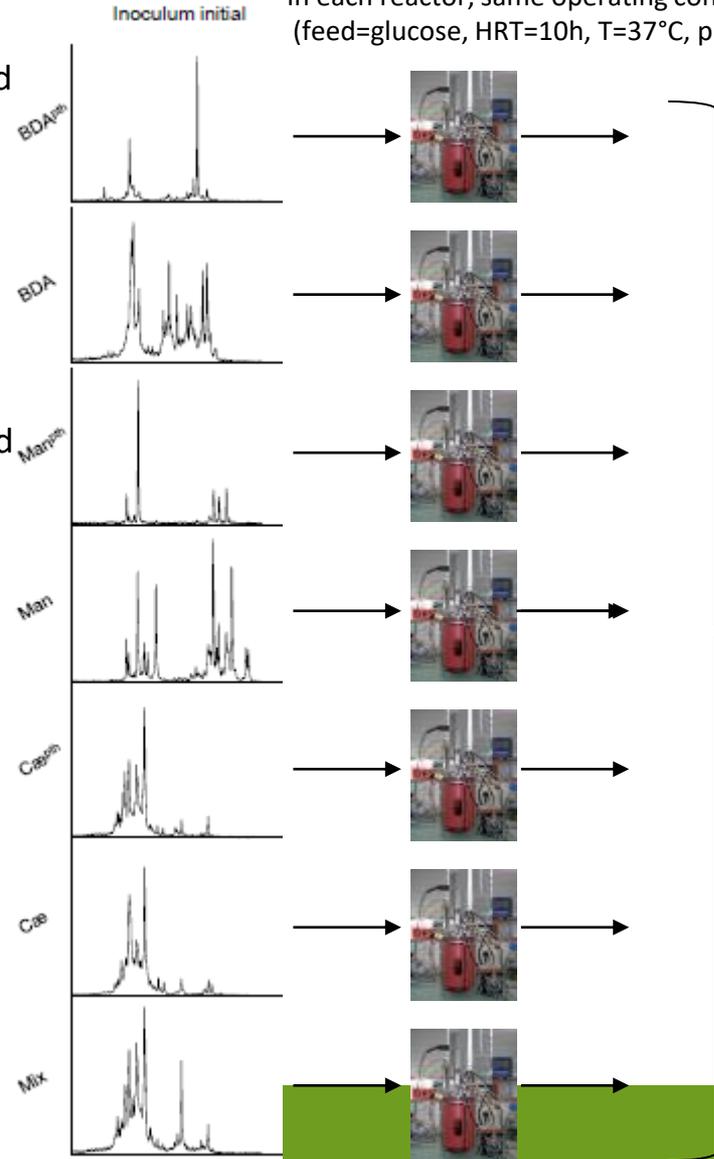
raw



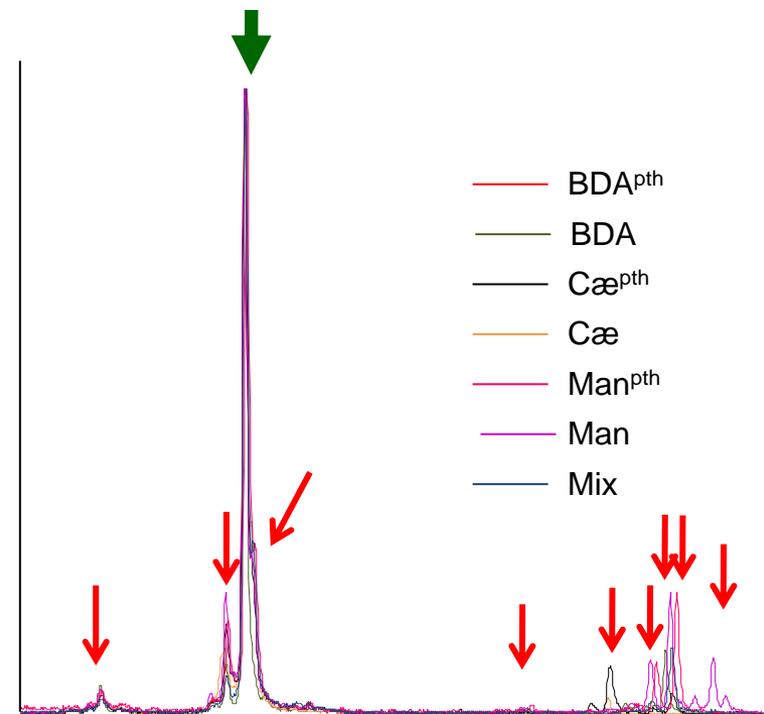
heated

raw

Mix of all



Relative Auondance of bacteria

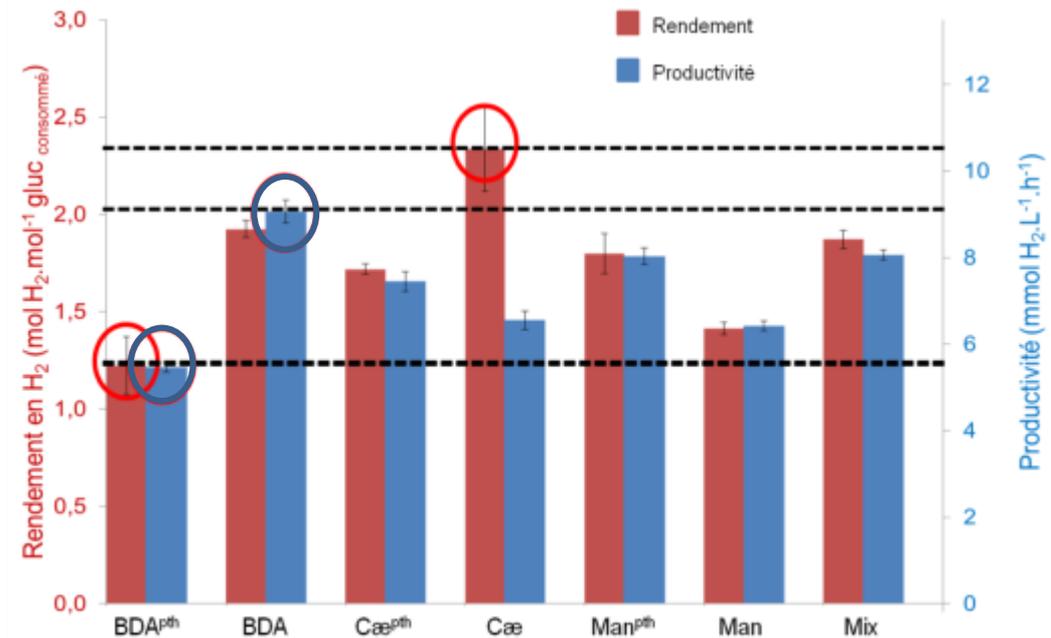
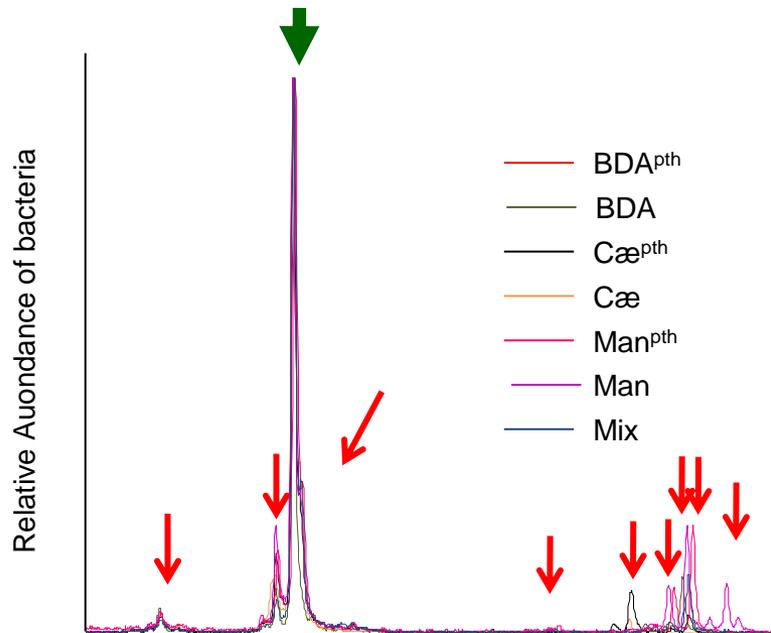


In steady state in each reactor
(after several HRTs),
only one major specie
(difference only in minor species)

Ecological Engineering for biotic control

Identical major bacteria,
so identical performance, isn't it ?....

NO !!!



**Link between
structure and function
of the ecosystem**

INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 38 (2013) 4975–4985

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/he

Sub-dominant bacteria as keystone species in microbial communities producing bio-hydrogen

Yan Rafrafi^a, Eric Trably^{a,*}, Jérôme Hamelin^a, Eric Latrille^a, Isabelle Meynial-Salles^b, Saida Benomar^c, Marie-Thérèse Giudici-Ortoniconi^c, Jean-Philippe Steyer^a

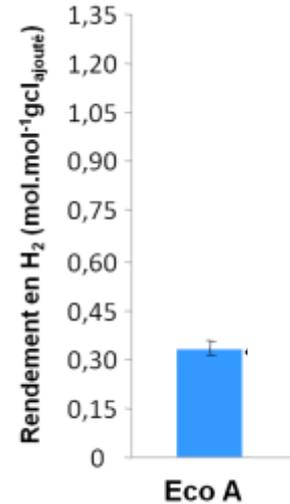
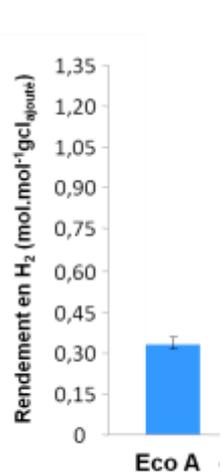
Ecological Engineering for biotic control

To act on the structure of the ecosystem to influence the function

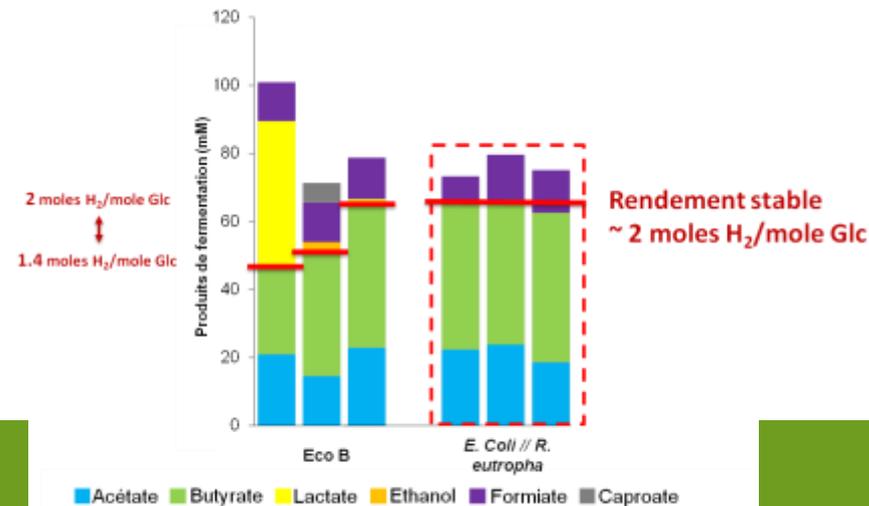


**Biotic control
of the metabolism**

✓ **PERFORMANCES**

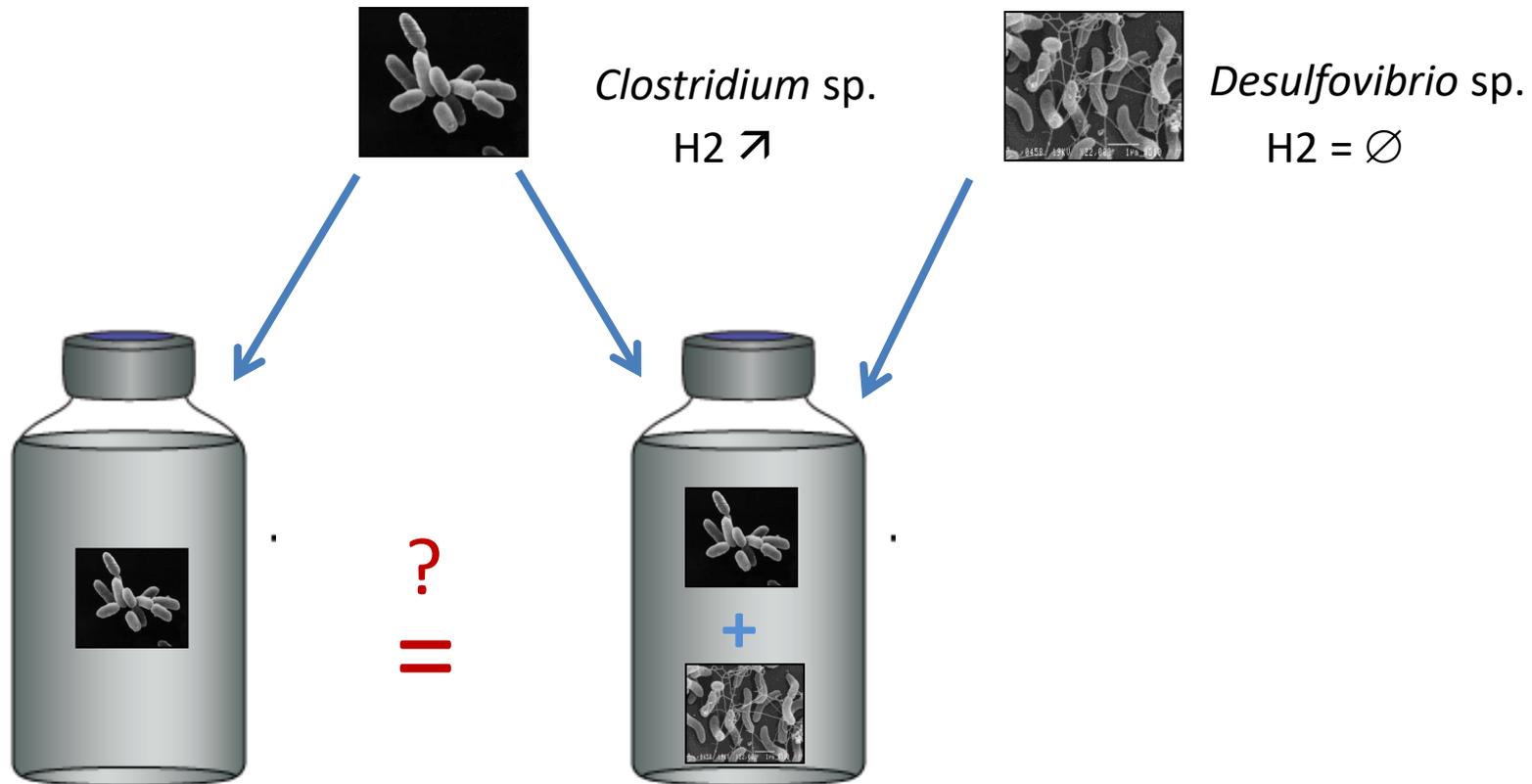


✓ **STABILITY**

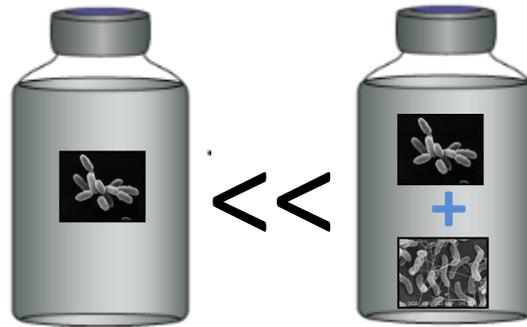


Ecological Engineering for biotic control

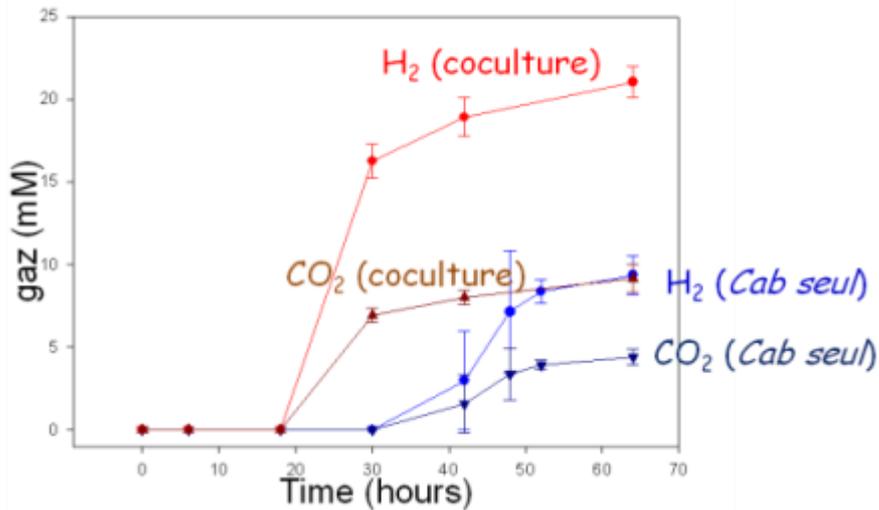
Study of the *interactions* : a model



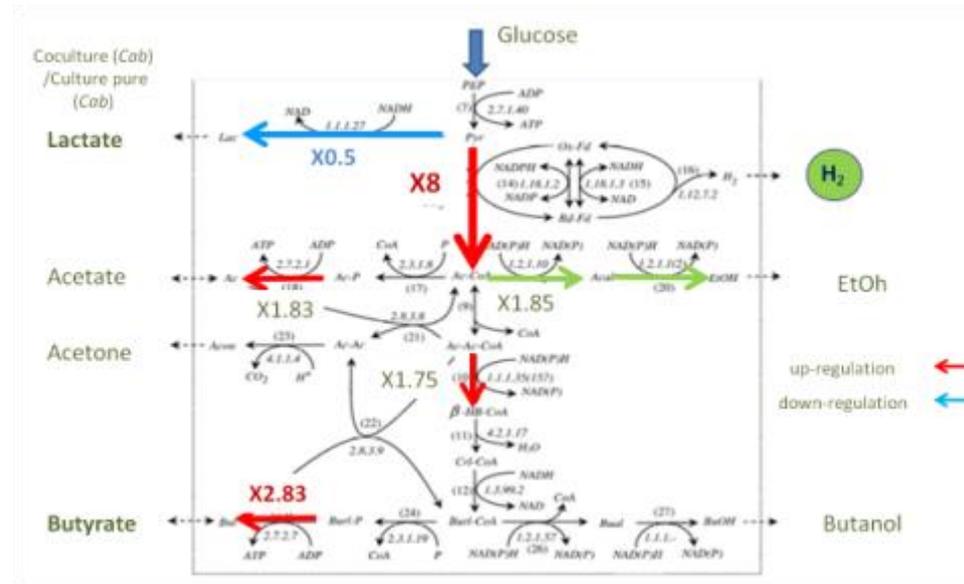
Ecological Engineering for biotic control



influences the metabolism of !!!



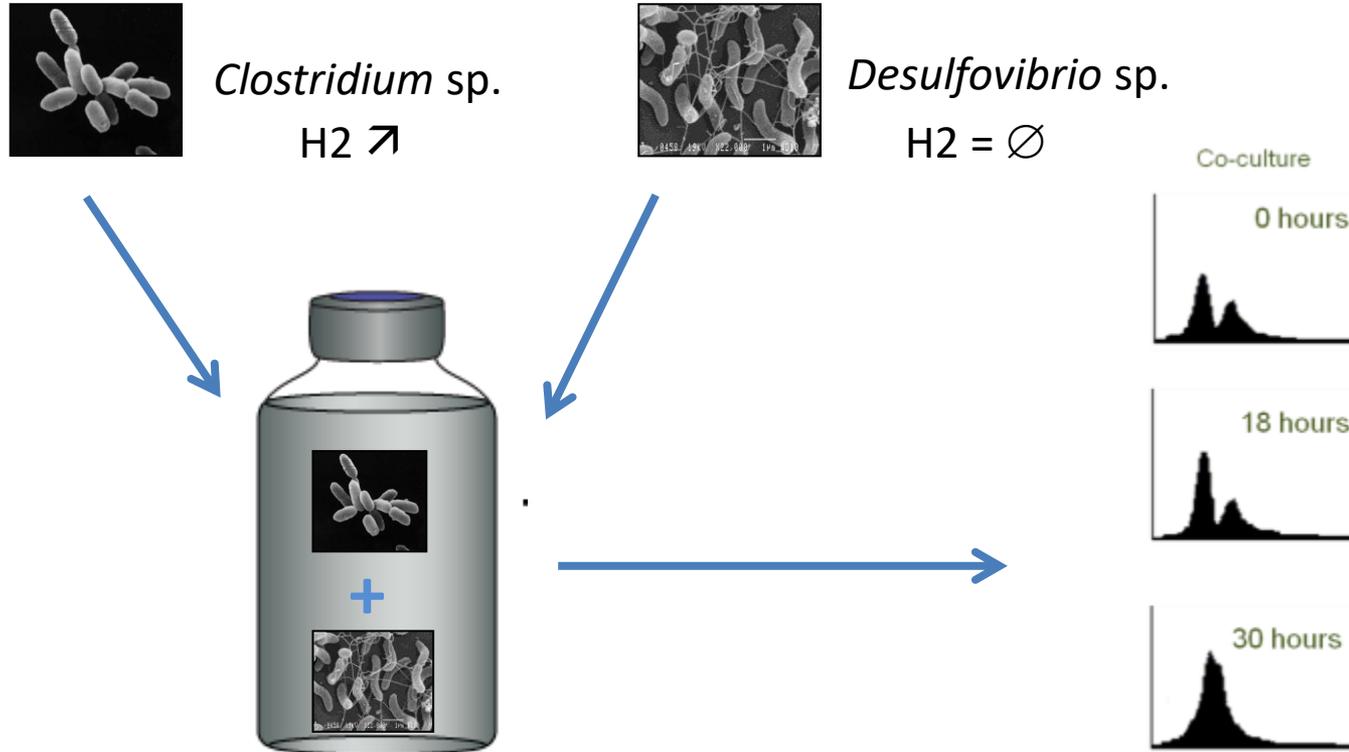
[H₂] x 2.5 ! And faster !



Change in metabolic flux

Ecological Engineering for biotic control

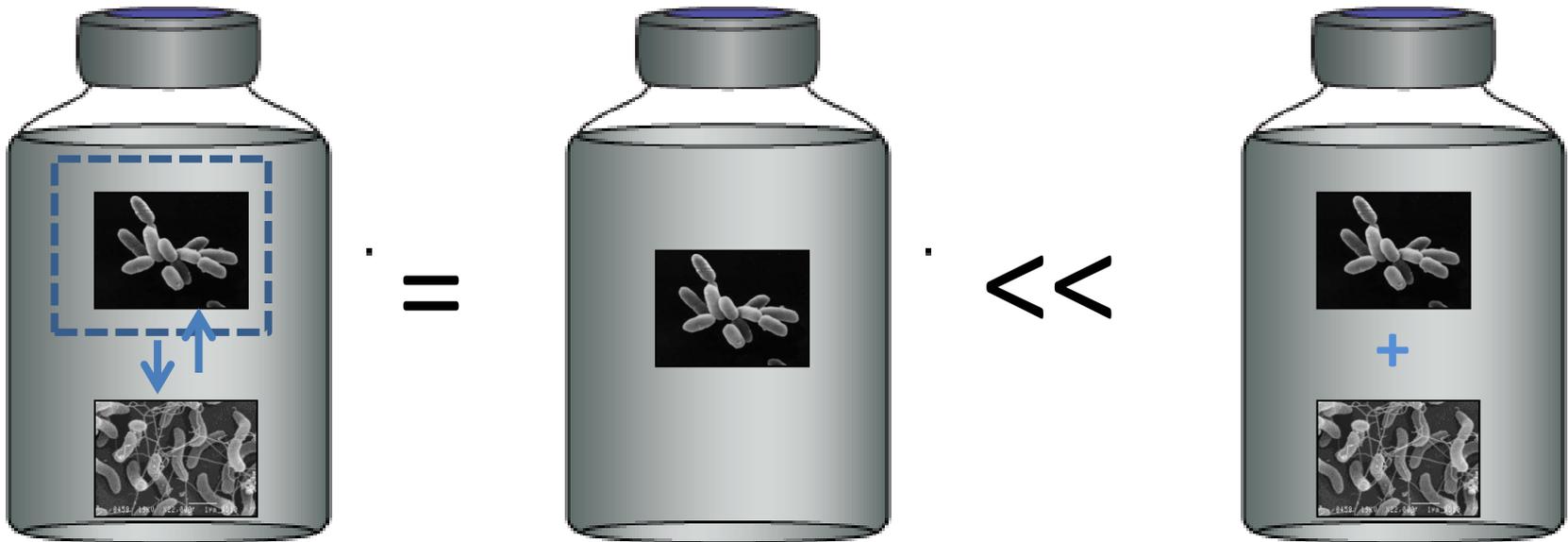
Study of the *interactions* : a model



Aggregation of the two organisms

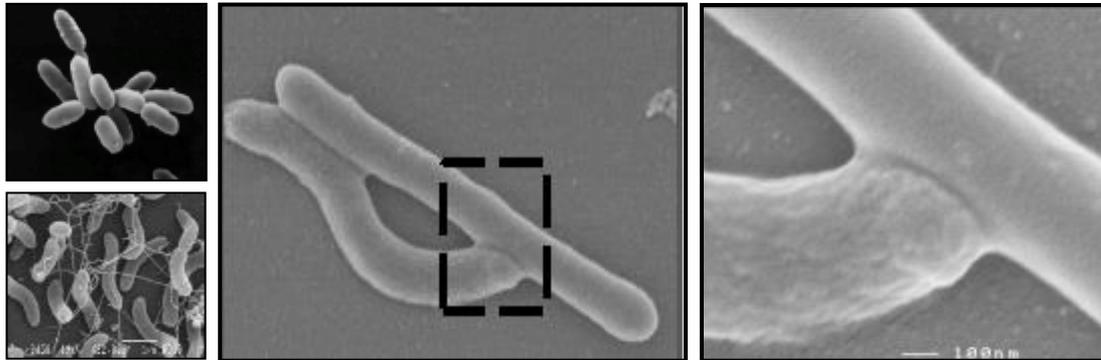
Ecological Engineering for biotic control

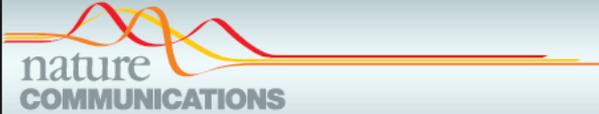
In addition....



A physical contact is mandatory !

New microbial interactions !




nature
COMMUNICATIONS

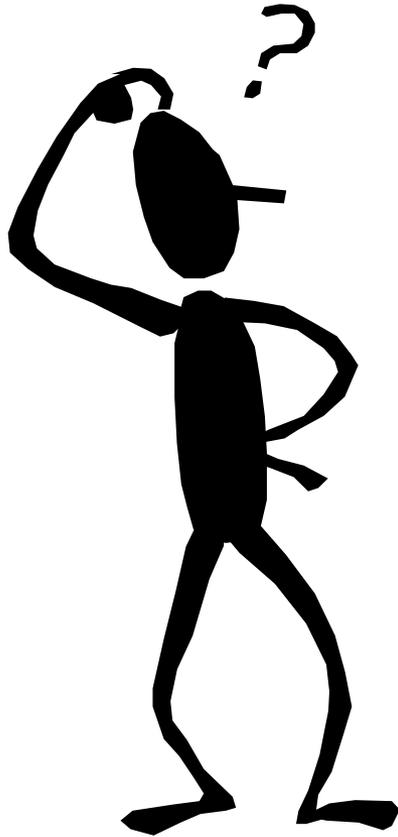
ARTICLE

Received 30 Jun 2014 | Accepted 12 Jan 2015 | Published xx xxx 2015 DOI: 10.1038/ncomms7283

Nutritional stress induces exchange of cell material and energetic coupling between bacterial species

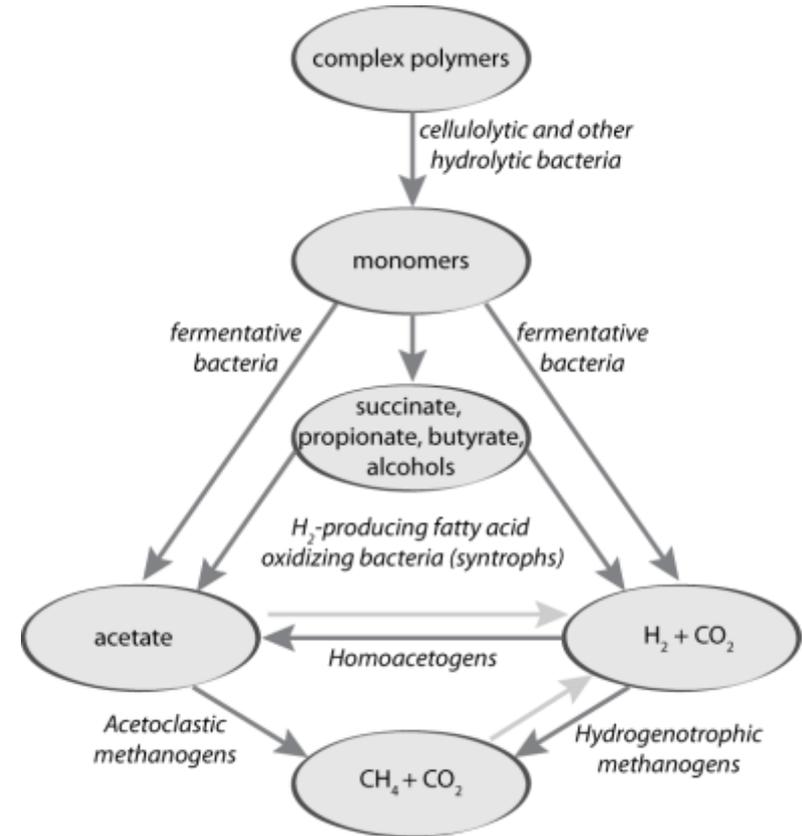
Saida Benomar^{1,*}, David Ranava^{1,*}, María Luz Cárdenas¹, Eric Trably², Yan Rafrafi², Adrien Ducret³, Jérôme Hamelin², Elisabeth Lojou¹, Jean-Philippe Steyer² & Marie-Thérèse Giudici-Orticoni¹

Usefulness of Molecular Biology Tools



Is microbial diversity an advantage?

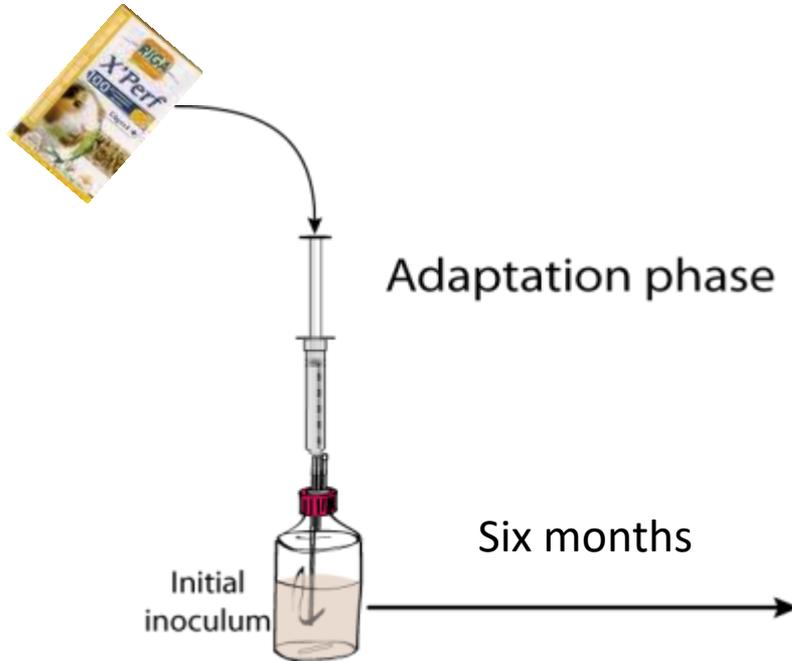
Role of the inoculum and microbial diversity



- ✓ 17 different inocula (from real digesters réels or natural environments)
- ✓ Same complex substrate for all digesters
- ✓ Same stable conditions for hundreds of days

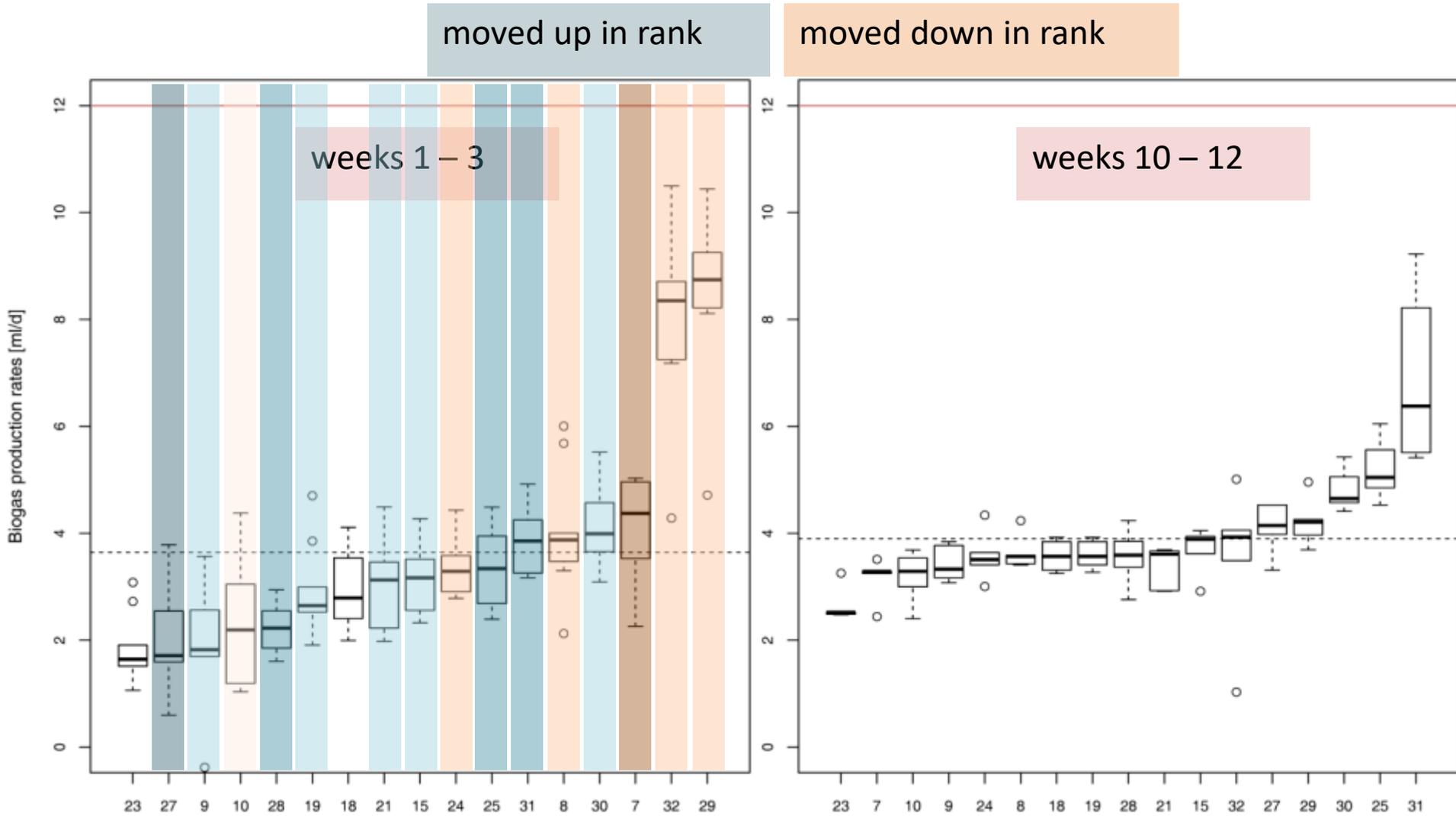
Role of the inoculum and microbial diversity

occasional substrate
addition as fed-batch



The 17 inocula : soil, compost, freshwater sediments, digestors...

Hypothesis: Divergence of ecosystem performance

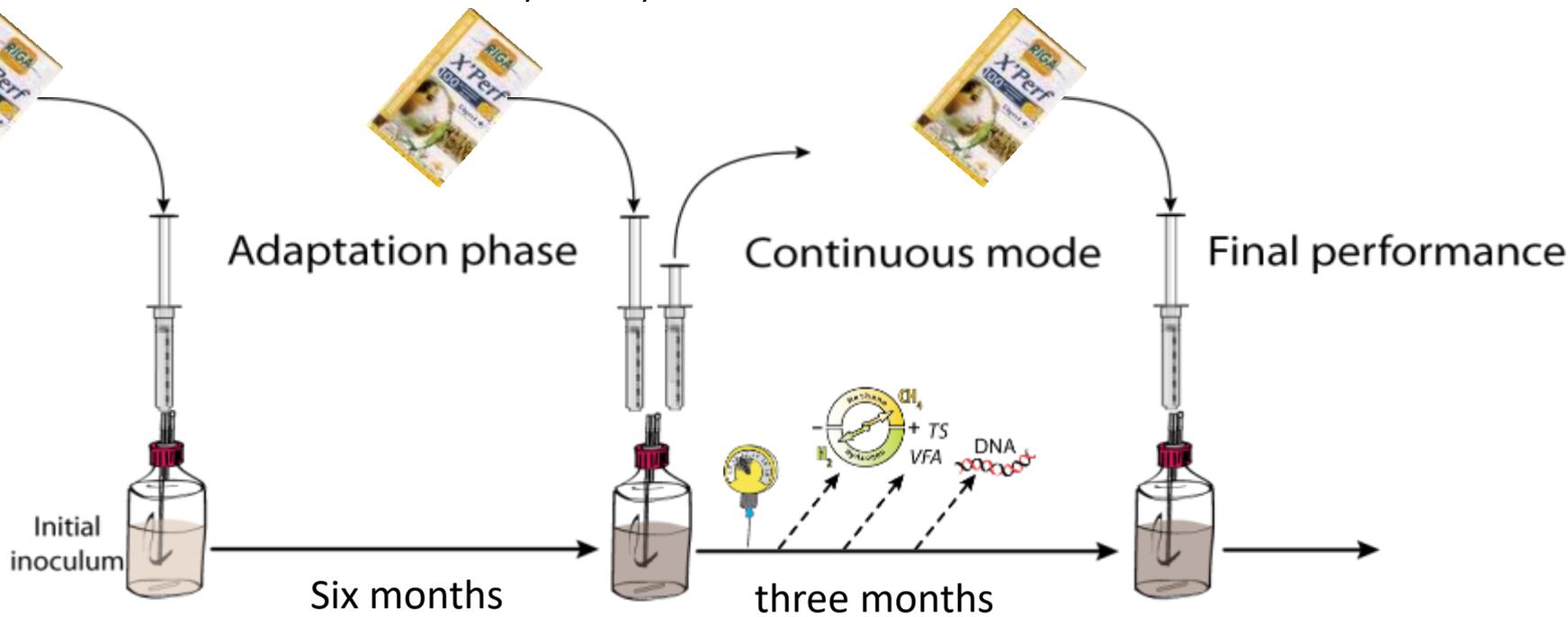


Role of the inoculum and microbial diversity

occasional substrate addition as fed-batch

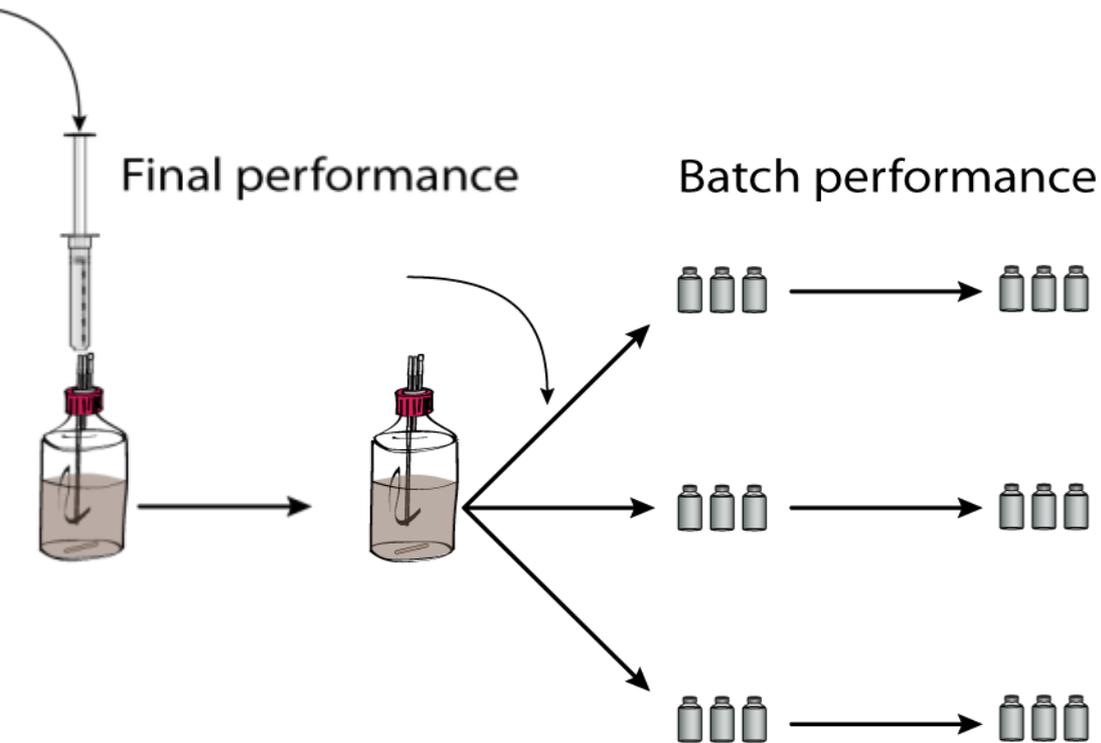
feeding/withdrawing every two day

final substrate pulse(s) in batch, BMP-like



The 17 inocula : soil, compost, freshwater sediments, digestors...

Role of the inoculum and microbial diversity



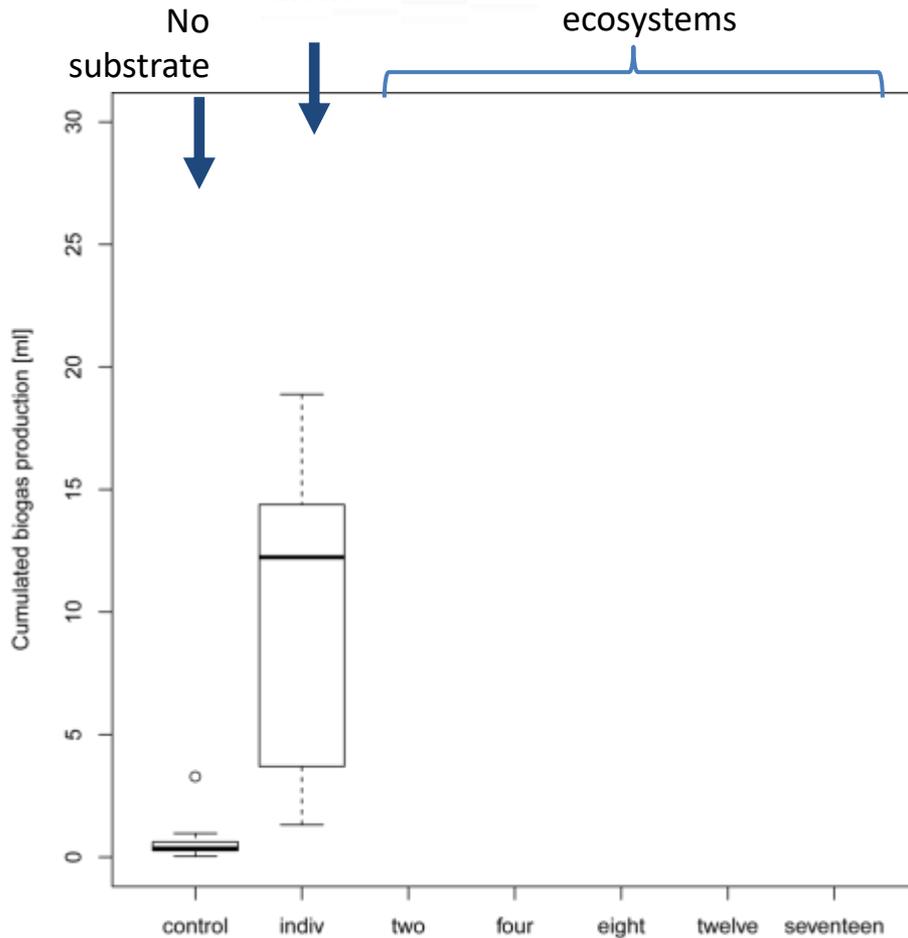
From 17 continuous processes to 256 batch reactors !



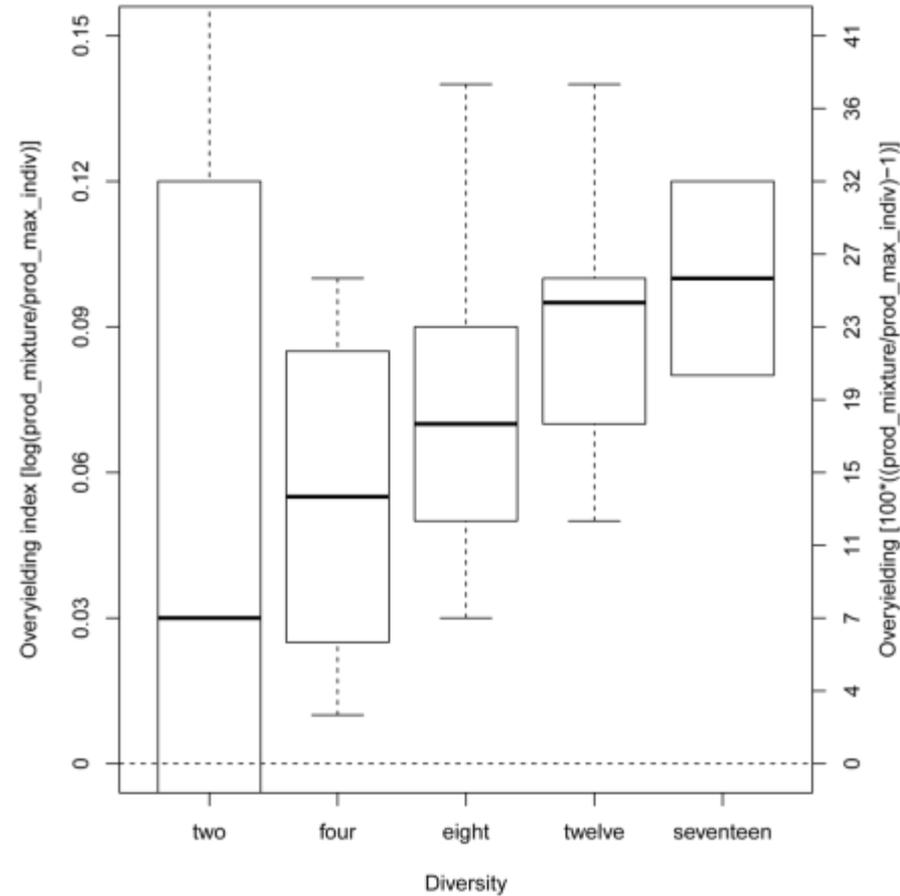
Role of the inoculum and microbial diversity

The 17 ecosystems alone

After mixing of the 17 ecosystems

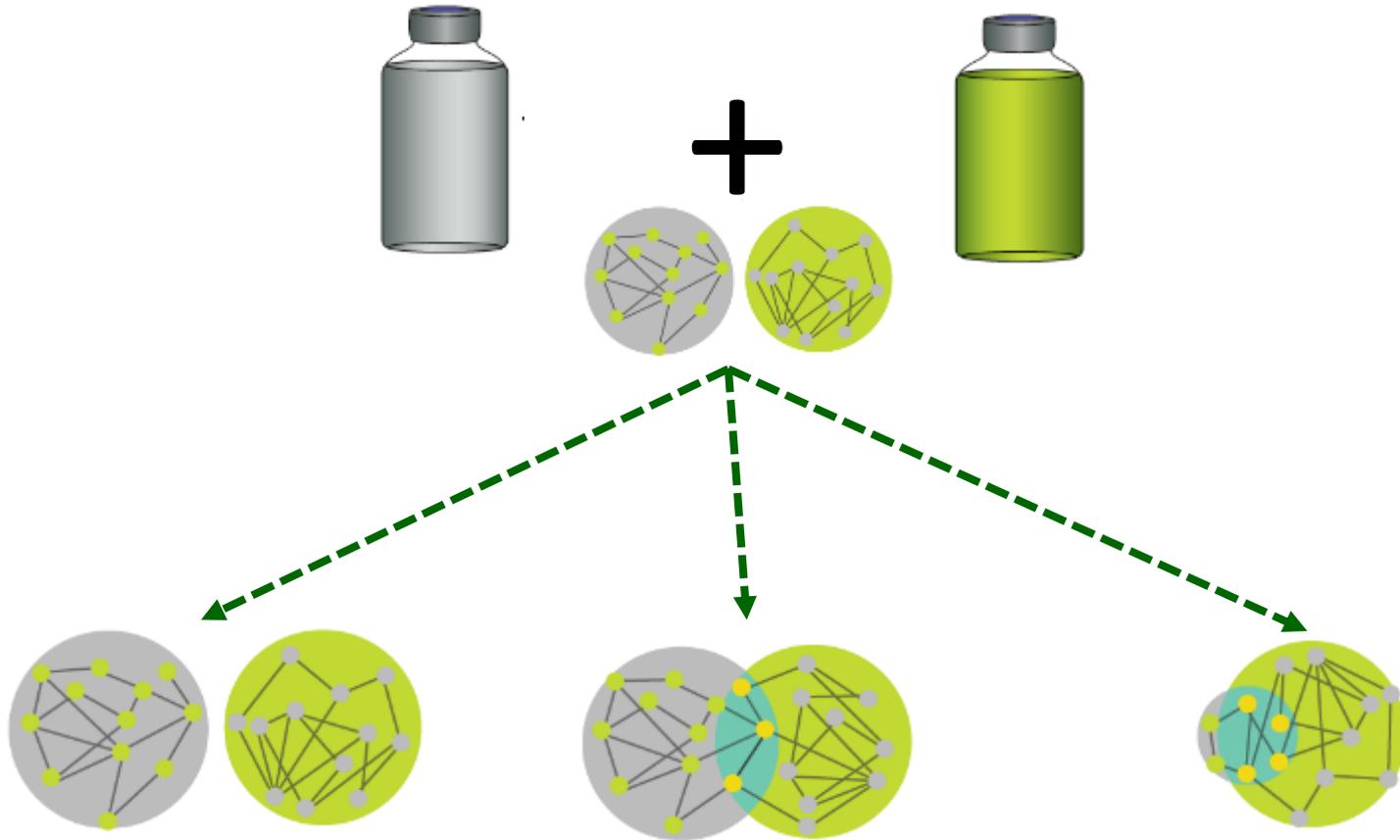


“Overyielding”



Role of the inoculum and microbial diversity

syntrophic networks
with different community structures

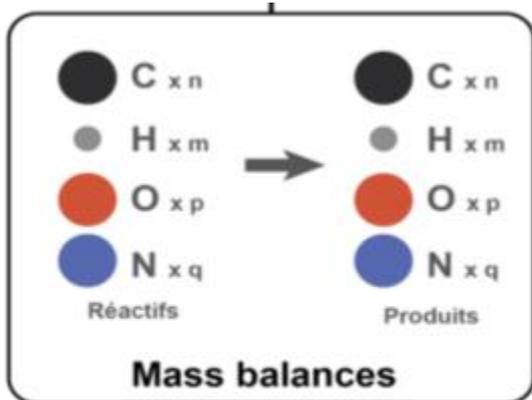
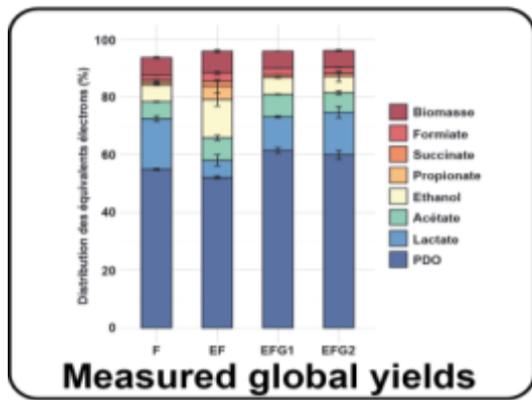
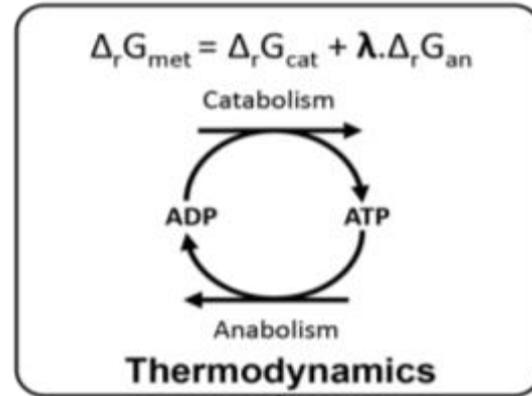
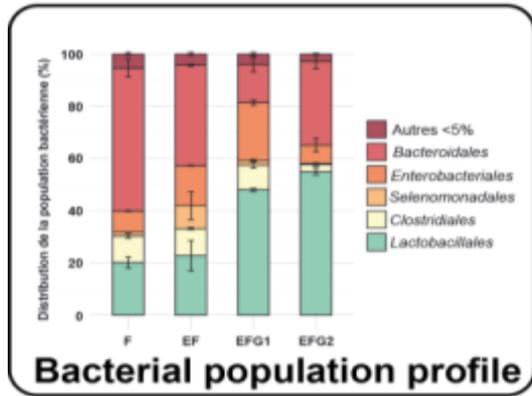


Usefulness of Molecular Biology Tools



**How to integrate all the information
in mathematical models?**

Modeling of microbial ecosystems



Data

Model constraints

Microbial diversity or functional distribution?

The ISME Journal (2014), 1–5
© 2014 International Society for Microbial Ecology All rights reserved 1751-7362/14
www.nature.com/ismej

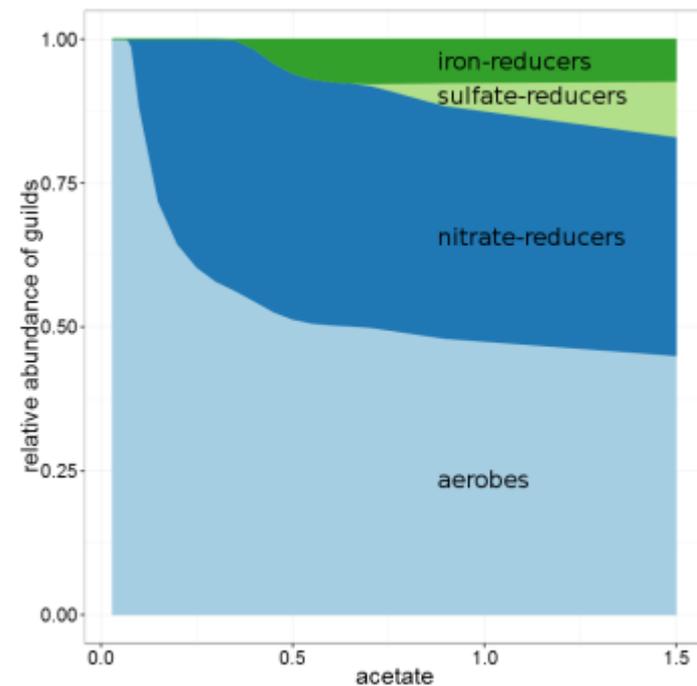


SHORT COMMUNICATION

A thermodynamic theory of microbial growth

Elie Desmond-Le Quéméner and Théodore Bouchez
Irstea, UR HBAN, Antony, Centre d'Antony, Antony, France

Modelling competition between
multiple populations:
emergence of the
"redox tower of microbial metabolism"



Microbial diversity or functional distribution?

Growth rate formulation:

$$\text{From } \mu = \mu_{\max} \frac{S}{K_S + S} \quad \text{to} \quad \mu = \mu_{\max} \prod_{i=1}^{\text{card}(C)} e^{\frac{v_i^{\text{MET}}}{V_h [C]_i}}$$

$$\text{with } \mu_{\max} = \frac{k_B T}{h} f(T, \Delta S, k_B, h)$$

$$v_i^{\text{MET}} = v_i^{\text{AN}} + \lambda(C, T) \cdot v_i^{\text{CAT}}$$

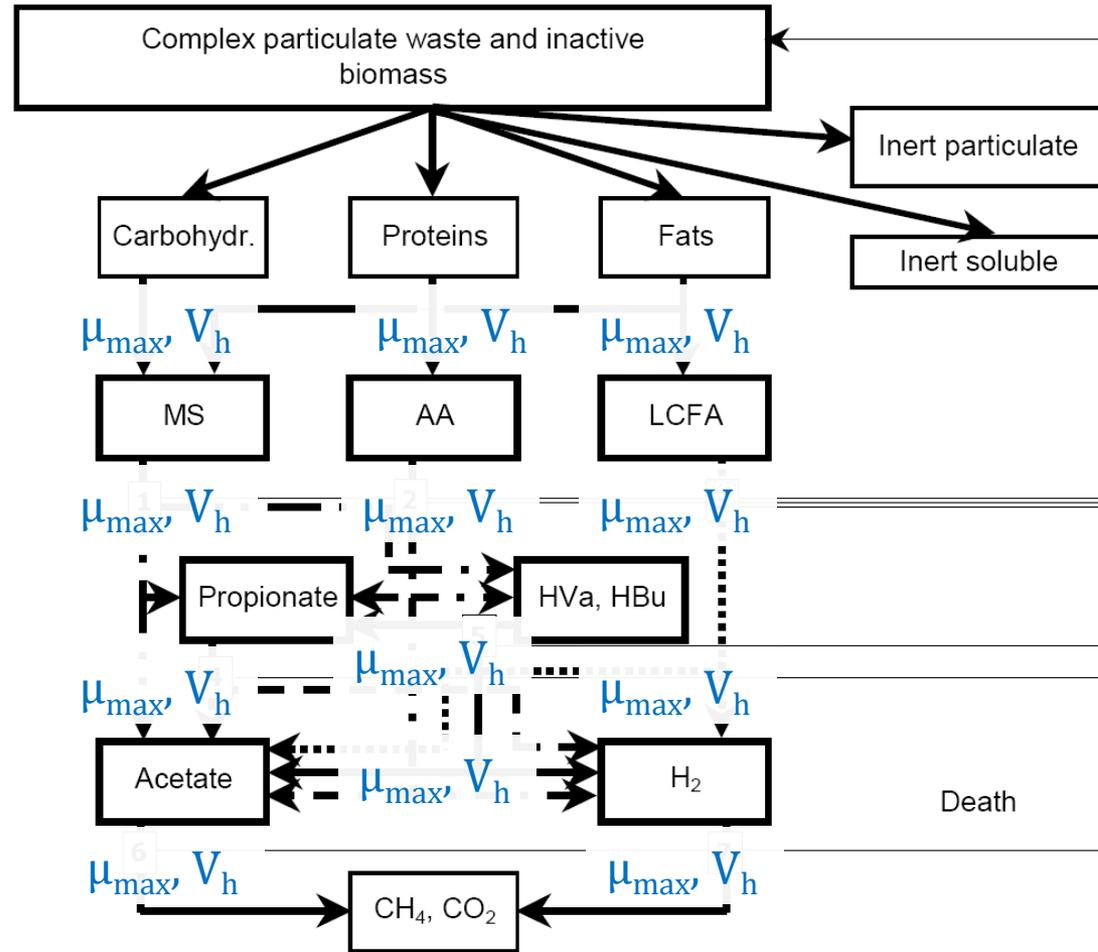
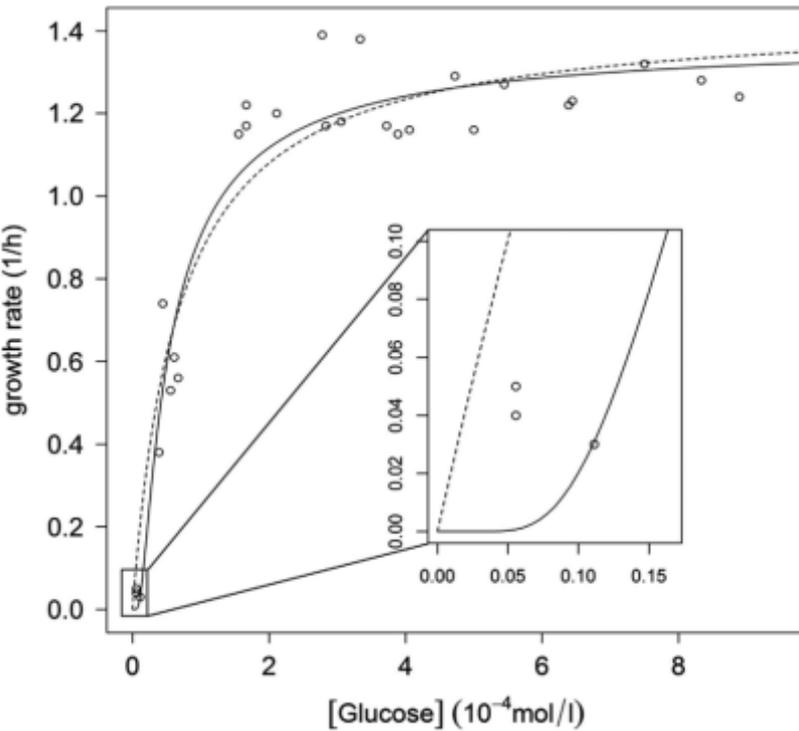
$$\lambda(C, T) = \frac{\Delta G_{\text{an}}(C, T) - \Delta G_{\text{dis}}(N_0 C_{C_S}, \gamma_{C_S})}{-\Delta G_{\text{cat}}(C, T)}$$

Only tuning parameter !  V_h : harvest volume

$[C]_i$: concentration of chemical specie #i

Microbial diversity or functional distribution?

Comparing with classical Monod equation

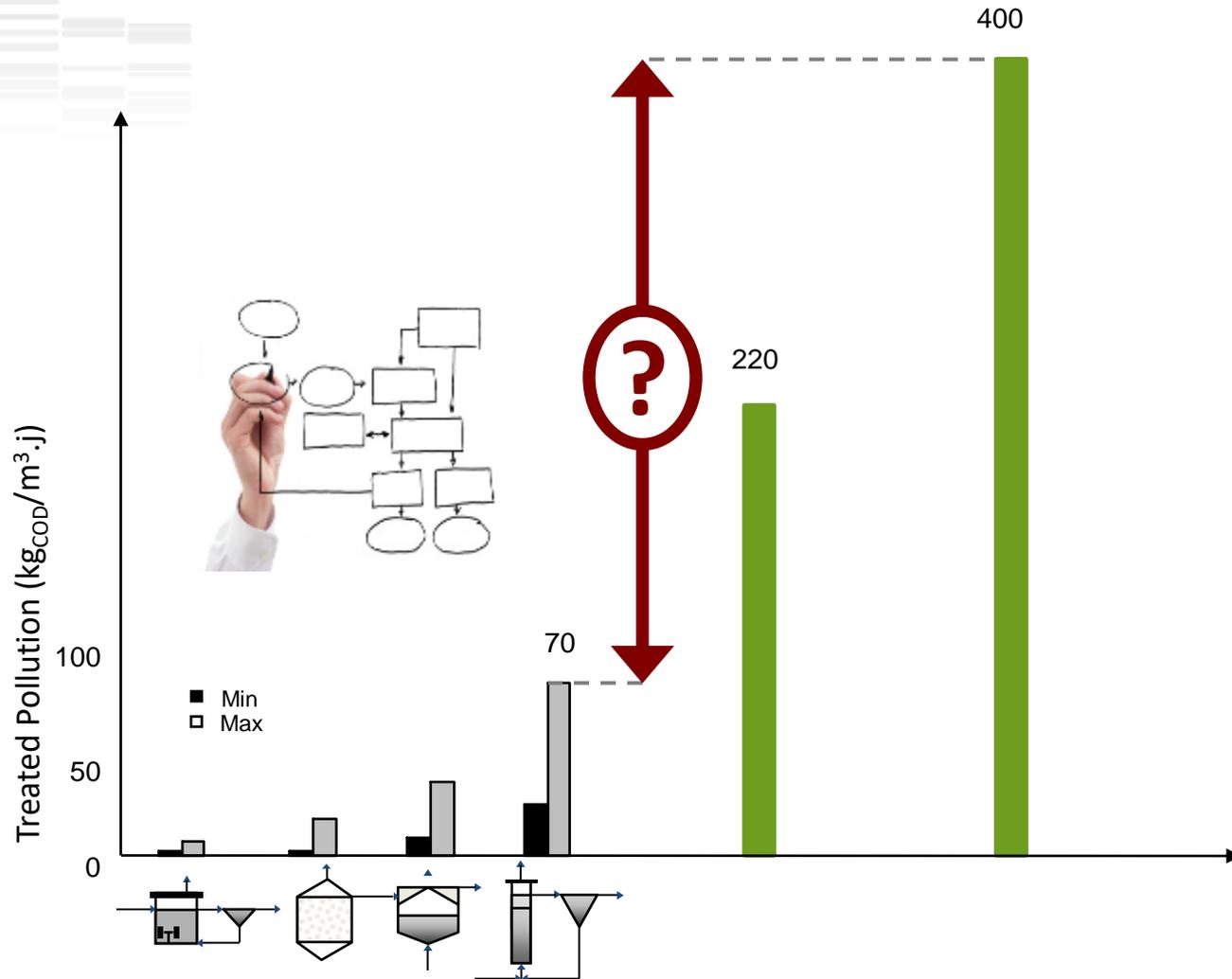


Usefulness of Molecular Biology Tools



Examples of remaining
open questions

Never forget Mother Nature !



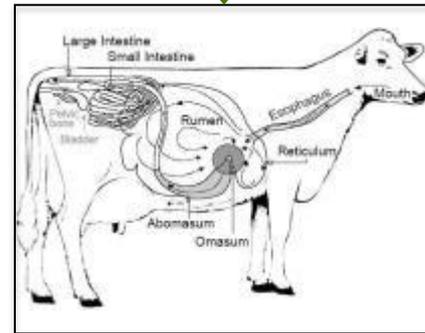
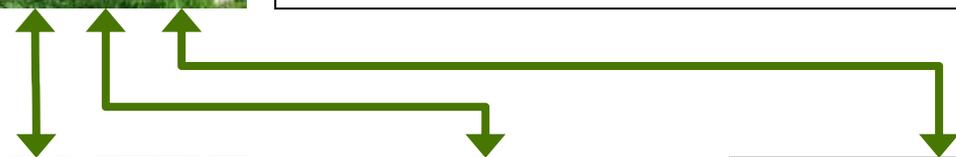
Never forget Mother Nature !



Bioenerg. Res. (2013) 6:1063–1081
DOI 10.1007/s12155-013-9539-y

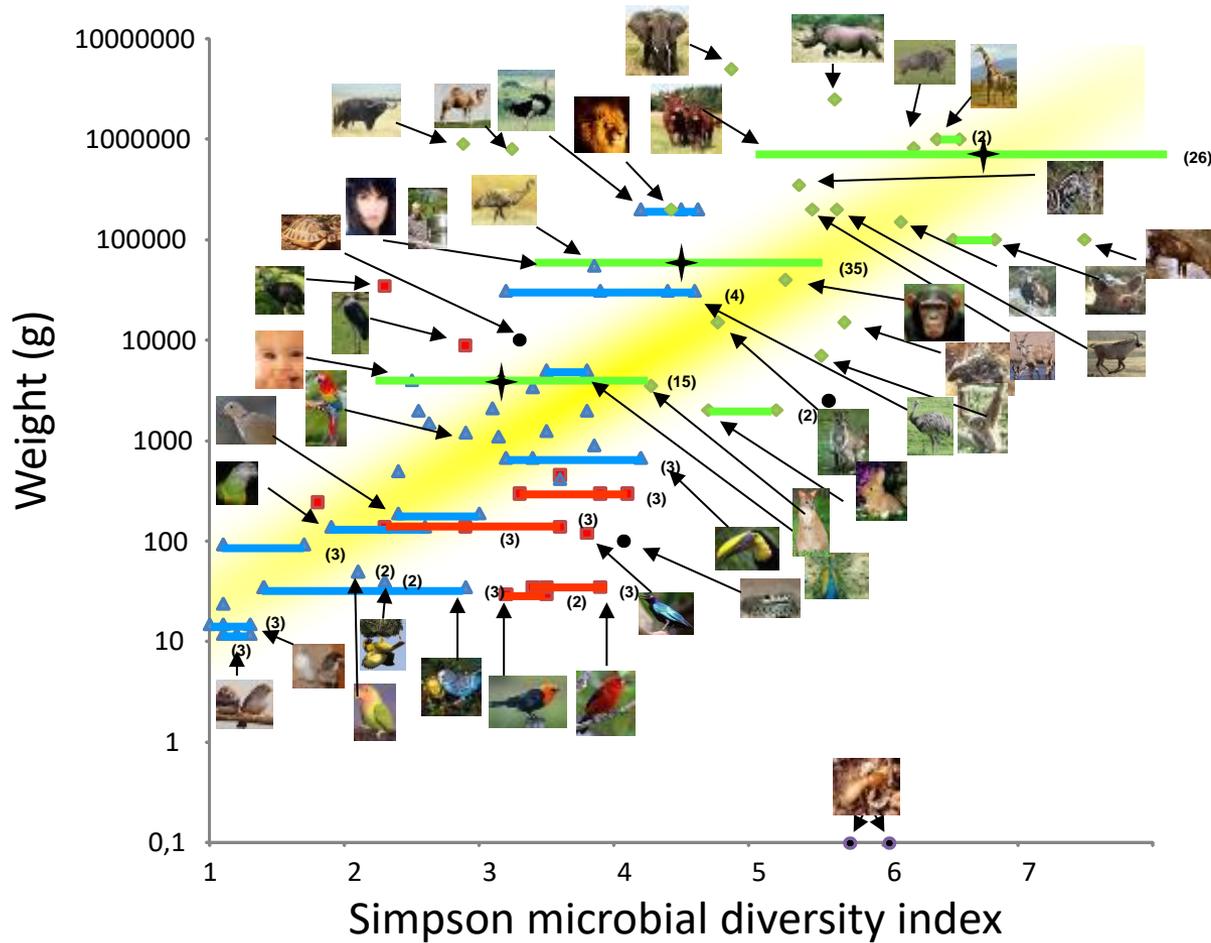
Overview of the Oldest Existing Set of Substrate-optimized Anaerobic Processes: Digestive Tracts

Jean-Jacques Godon • Laure Arcemishèbère •
Renaud Escudé • Jérôme Harmand • Edouard Miambi •
Jean-Philippe Steyer

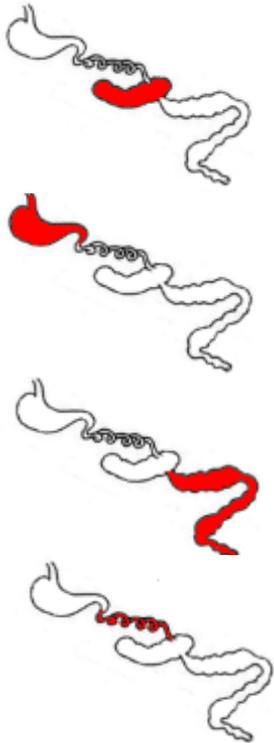


Never forget Mother Nature !

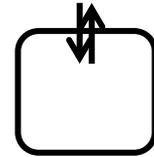
From the analysis of 190 digestive tracts



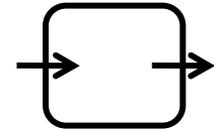
Never forget Mother Nature !



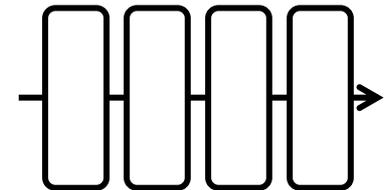
Batch reactor



Continuous stirred tank reactor (CSTR)



CSTRs in serie

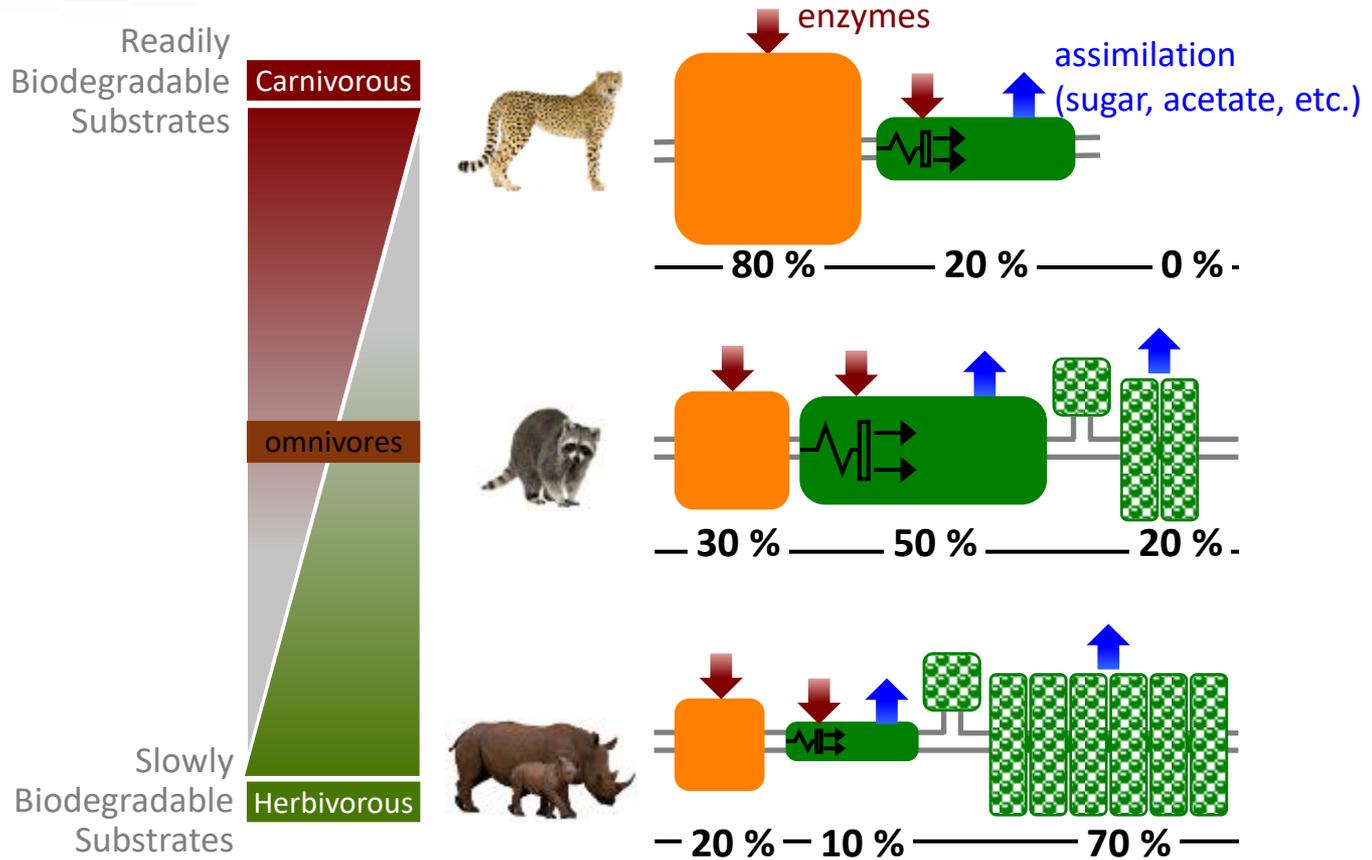


Plug-flow reactor

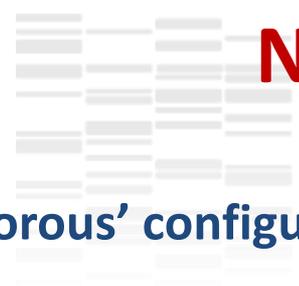


Never forget Mother Nature !

In terms of volume



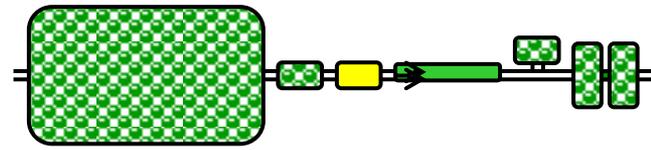
Never forget Mother Nature !



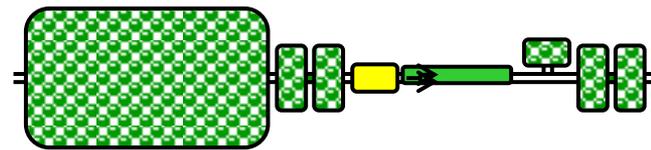
The 'herbivorous' configuration



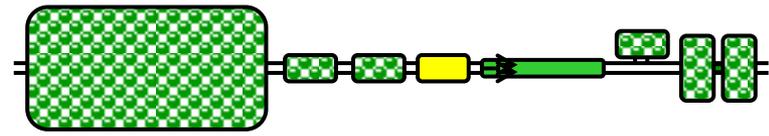
Lama



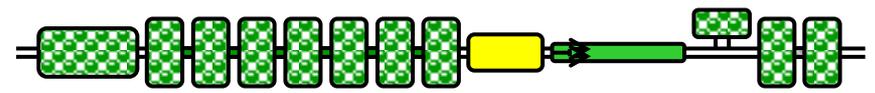
Hoazin



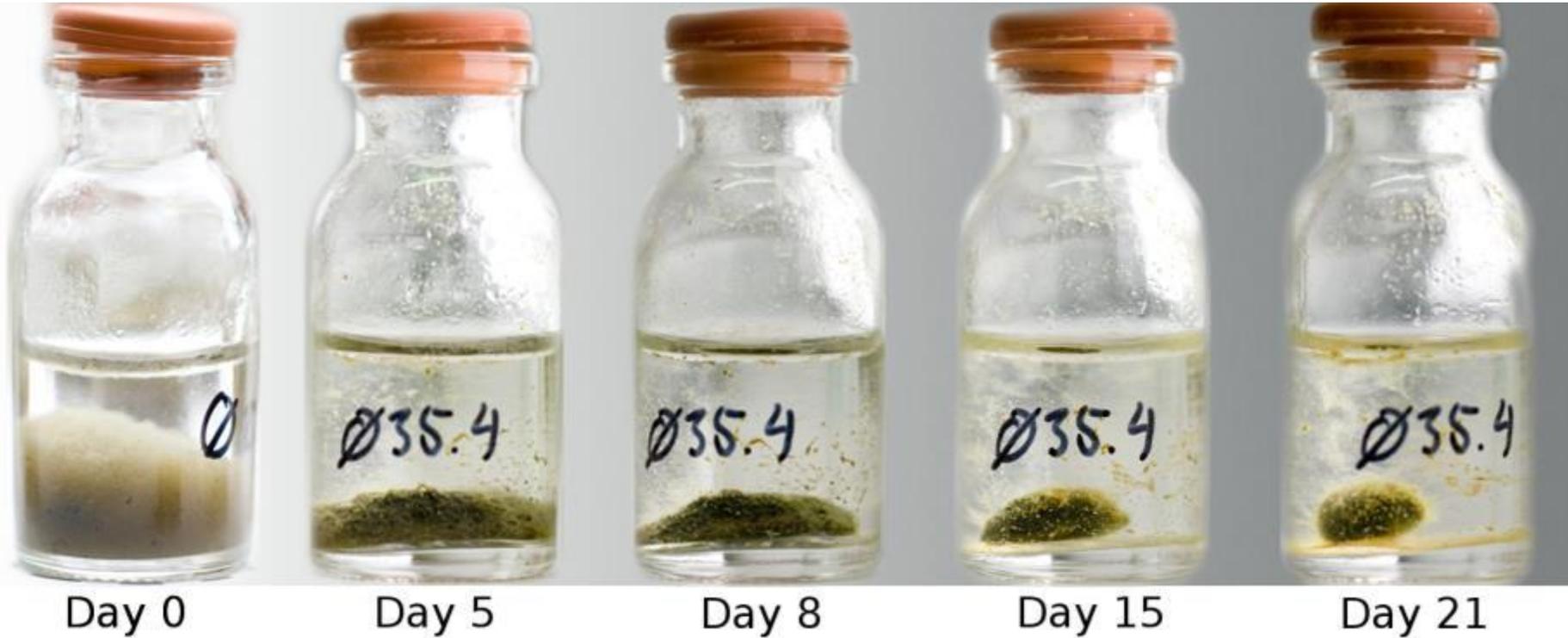
Cow



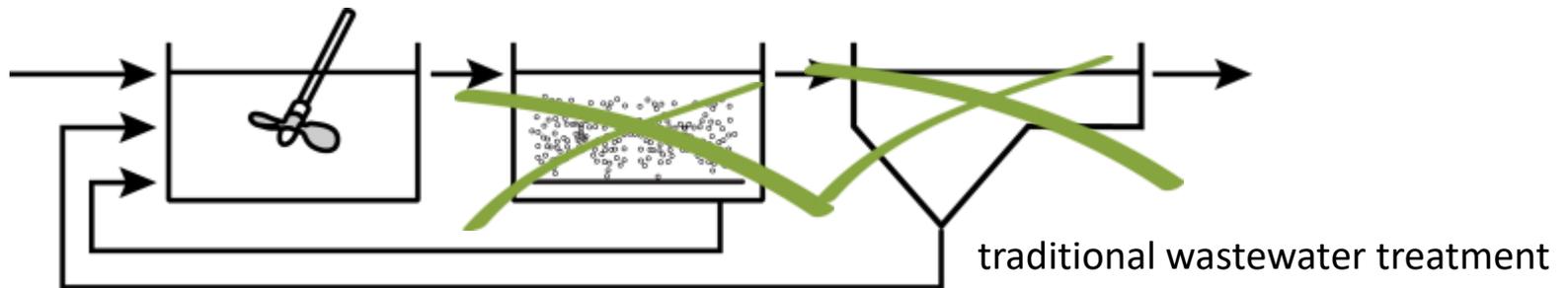
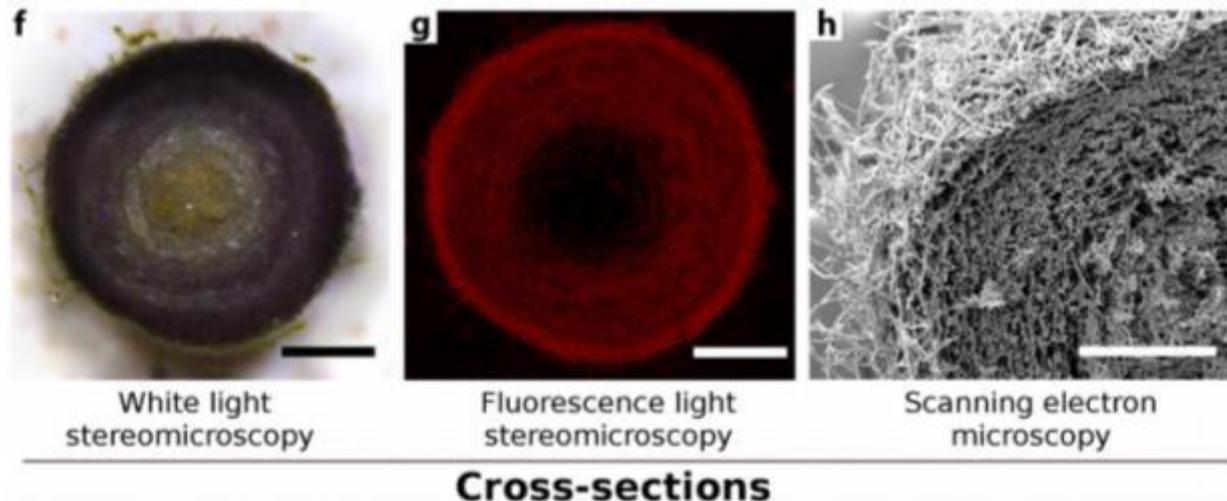
Kangaroo



New opportunities for wastewater treatment ?

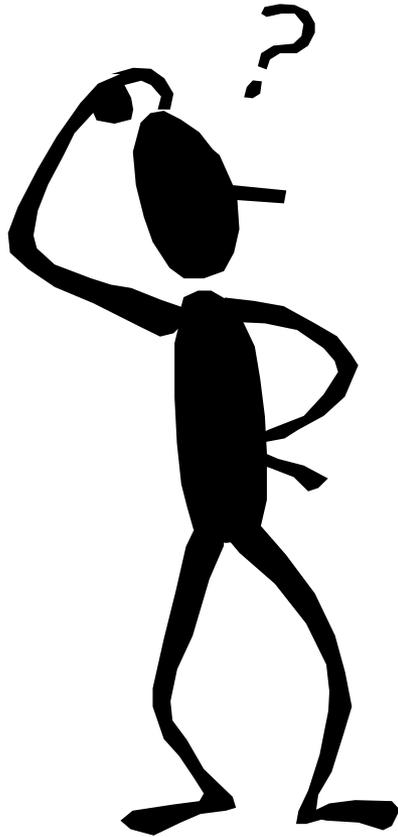


New opportunities for wastewater treatment ?



- Granules settle very well: Largely reduce size of settler (space and operation = \$\$)
- Phototrophs produce oxygen: reduced need for external oxygen supply (aeration = \$\$)
- When digested, phototrophic granules produces 30% more CH_4 (\$\$)

Usefulness of Molecular Biology Tools



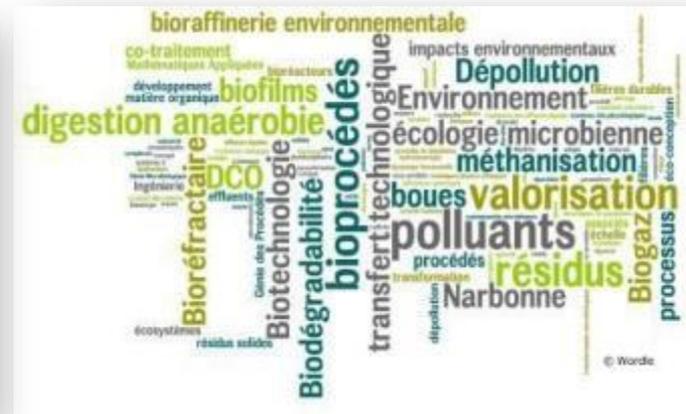
As a conclusion...

Usefulness of Molecular Biology Tools

Demonstrated at the industrial scale !



Thank you for your attention



<http://www.montpellier.inra.fr/narbonne>

jean-philippe.steyer@inra.fr