



# Microbiologie et transition énergétique, l'exemple de la production de bioH<sub>2</sub> à partir de déchets ménagers



Institut  
Microbiologie,  
Bioénergies et  
Biotechnologie  
Aix-Marseille Université

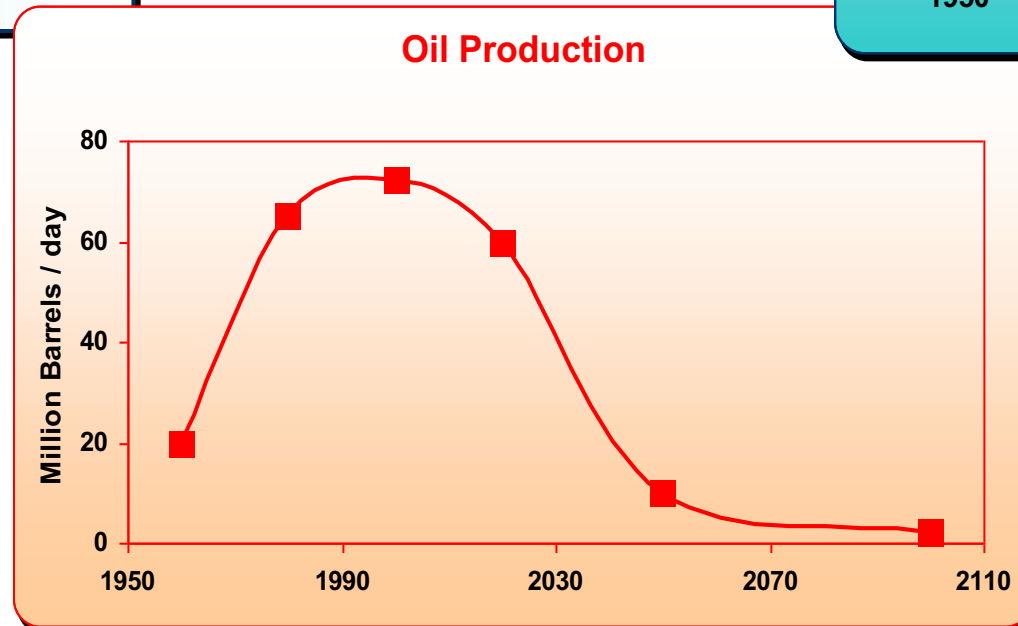
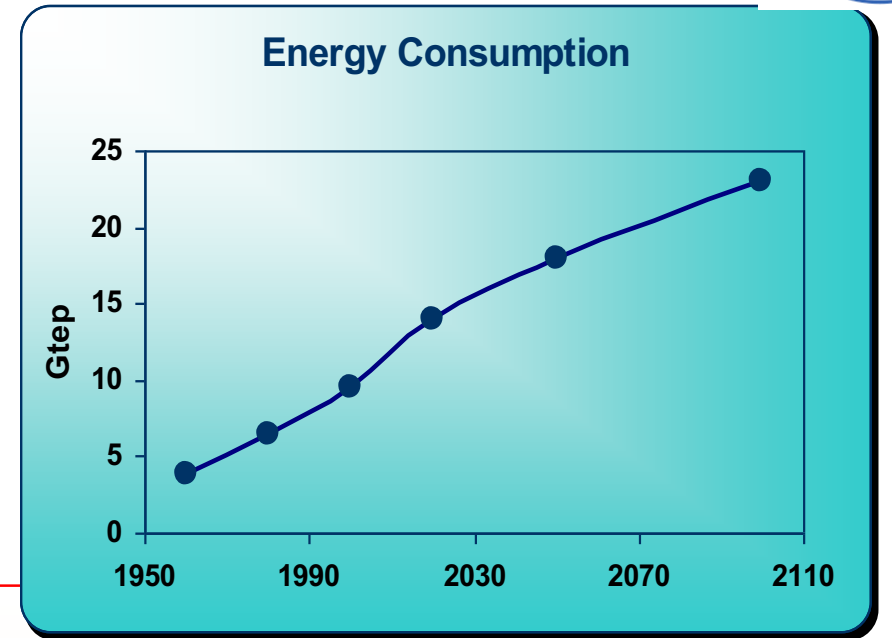
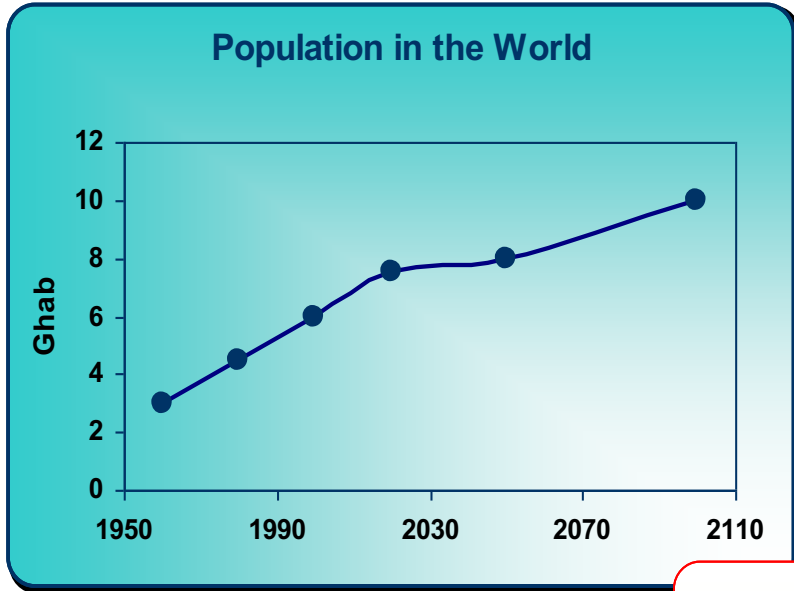
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Institut de Microbiologie, bioénergies et biotechnologies

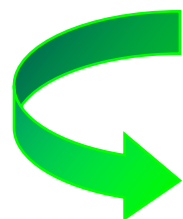


Séminaire 2023 de l'OHM BMP  
et Transition énergétique en région Sud-PACA  
Meyreuil, 9 et 10 Novembre 2023

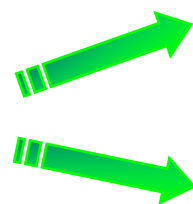
# Our present and our future...



# Our future...



alternative energies to limit



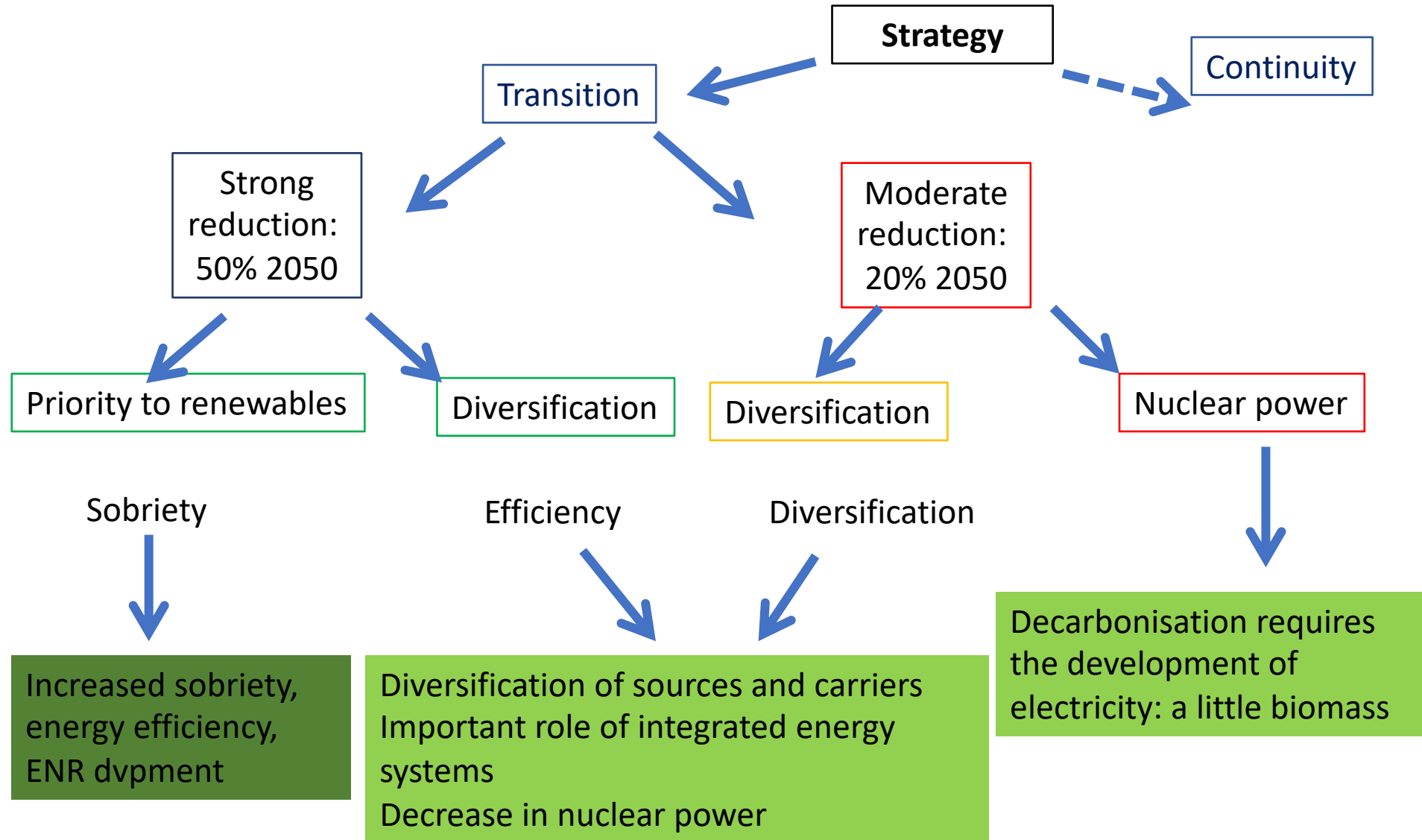
Dependence on fossil fuels

Greenhouse gas emissions





# Examples of Scenarii for the energy transition (source report for energy transition)



# H2: a green energy

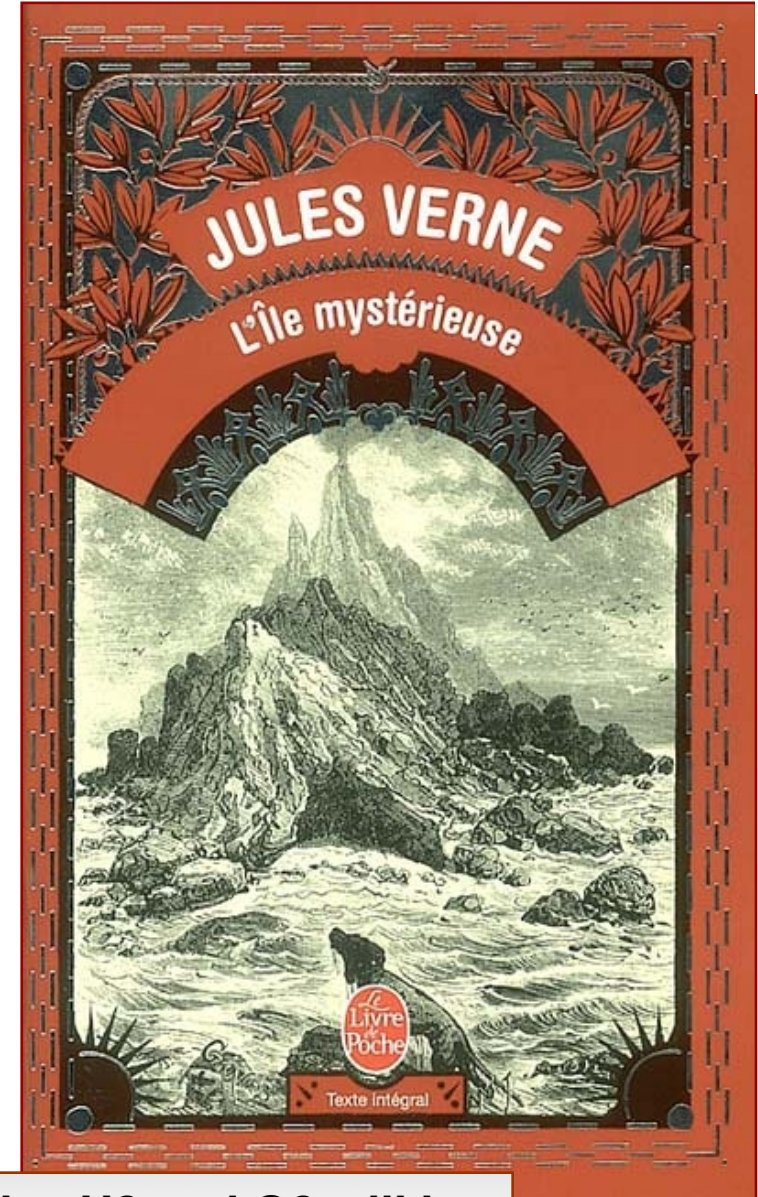
Most common element in the world  
but not in a 'free' form

Non-toxic, invisible and odourless gas,

Lowest density  
(1 l of petrol = 4.6 l H2 compressed to  
700 bars)

But the most energetic  
(3 times more than petrol)

Its combustion releases only heat and  
water



1874: « one day H2 and O2 will be  
a source of light and heat »

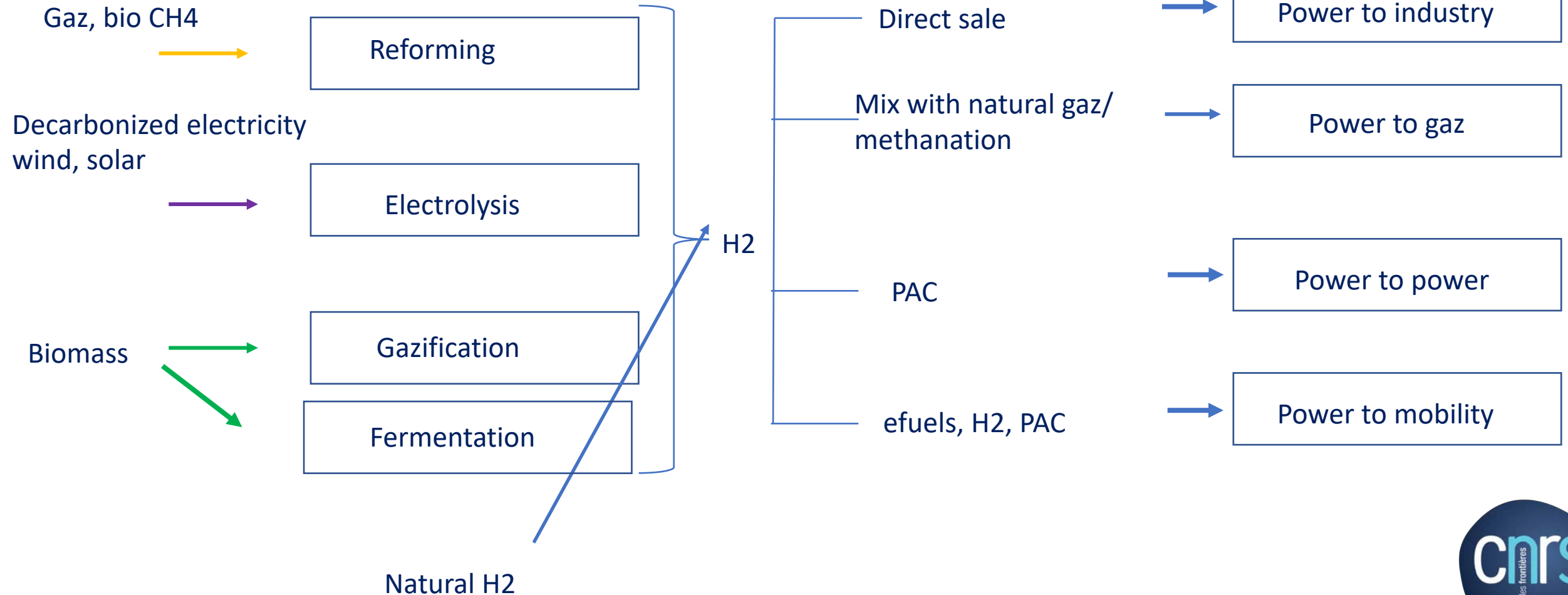


# H2: an energetic vector



## Production/conversion/stockage

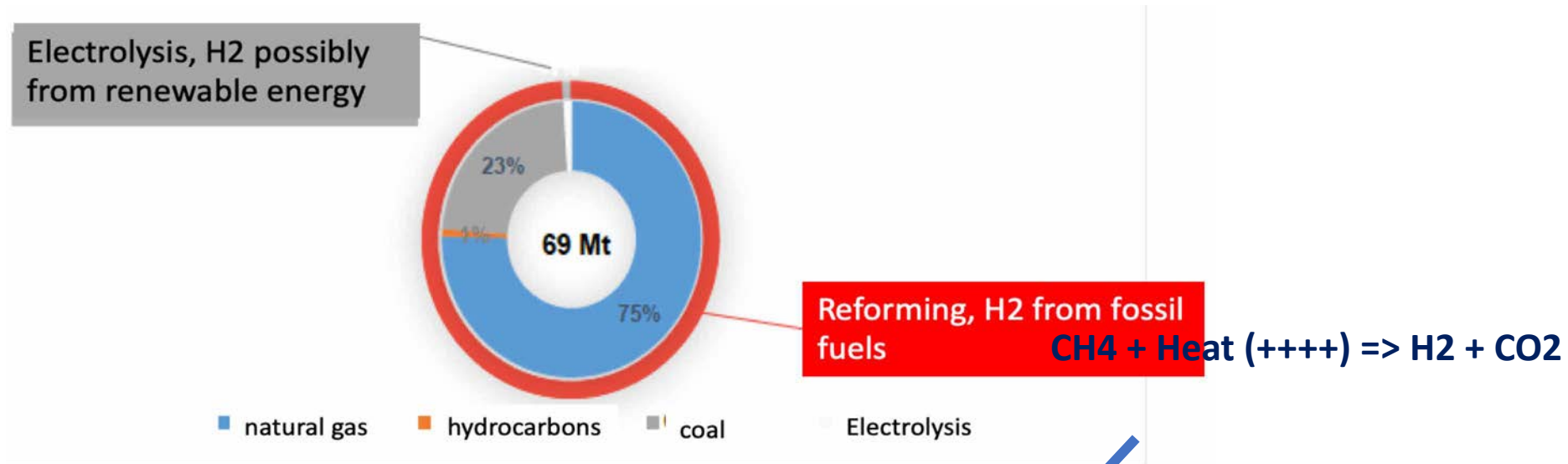
## Use





# H2: an energetic vector

## Primary energy source for H2 production worldwide



Source : EY d'après AIE 2019, Hydrogen Council



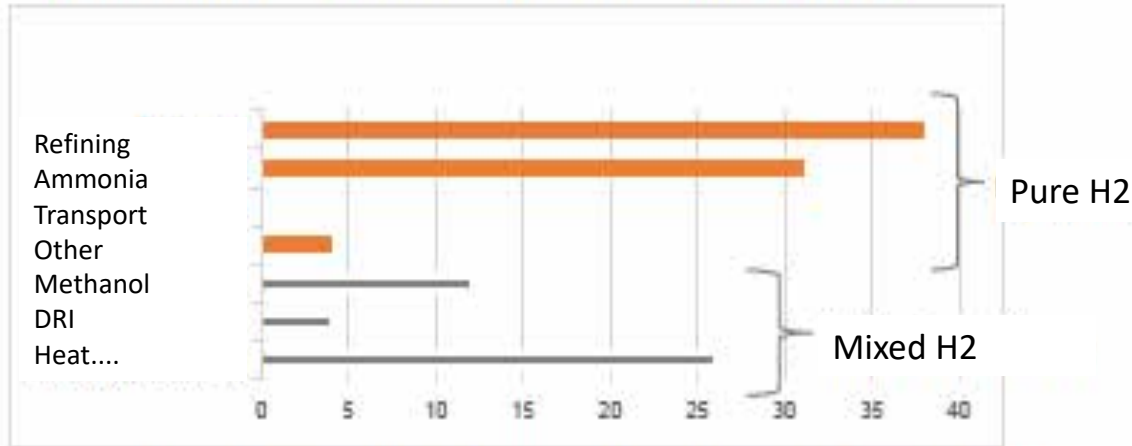
Currently the most economical way to produce H2 (from 1.5 to 2.5 €/kg H2)  
However, this process generates 10kg of CO2 per kg of H2 and 1 to 2% of total French emissions is due to this H2 production



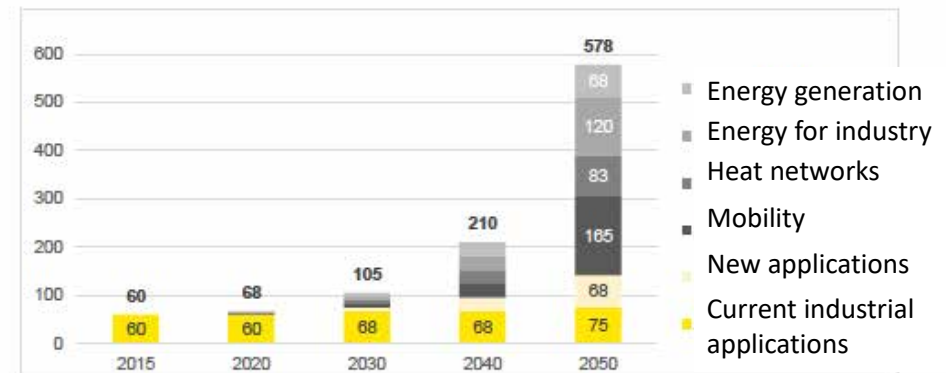


# H2: an energetic vector

## Global H2 consumption by sector (Mt H2)



## Request of pure H2, by application (Hydrogen Council)





# H2: an energetic vector



	Context and budget	Ambition for mobility
China	Recent but massive involvement	2020: 6200 FCEV 2025: 50 000 FCEV 2030: 1 million FCEV
US	California, a pioneer region	2020: 9000 FCEV 2030: 1M FCEV
Japan	2019 budget: 510M€ Industry mobilised	2020: 3800 FCEV 2025 :200000 FCEV 2030: 800000 FCEV
South Korea	2020 Budget: 386 M€; Several H2 cities	2020: 9000 2030: 850000
Germany	Historical H2 country in Europe Budget 2016-2026: 1400 M€ + H2 Plan 9Md€	2020: 630+ 2 Trains 2025: 90 Trains
France	National plan 7.2 Md€	2020: 400 FCEV 2028 : > 20000 FCEV

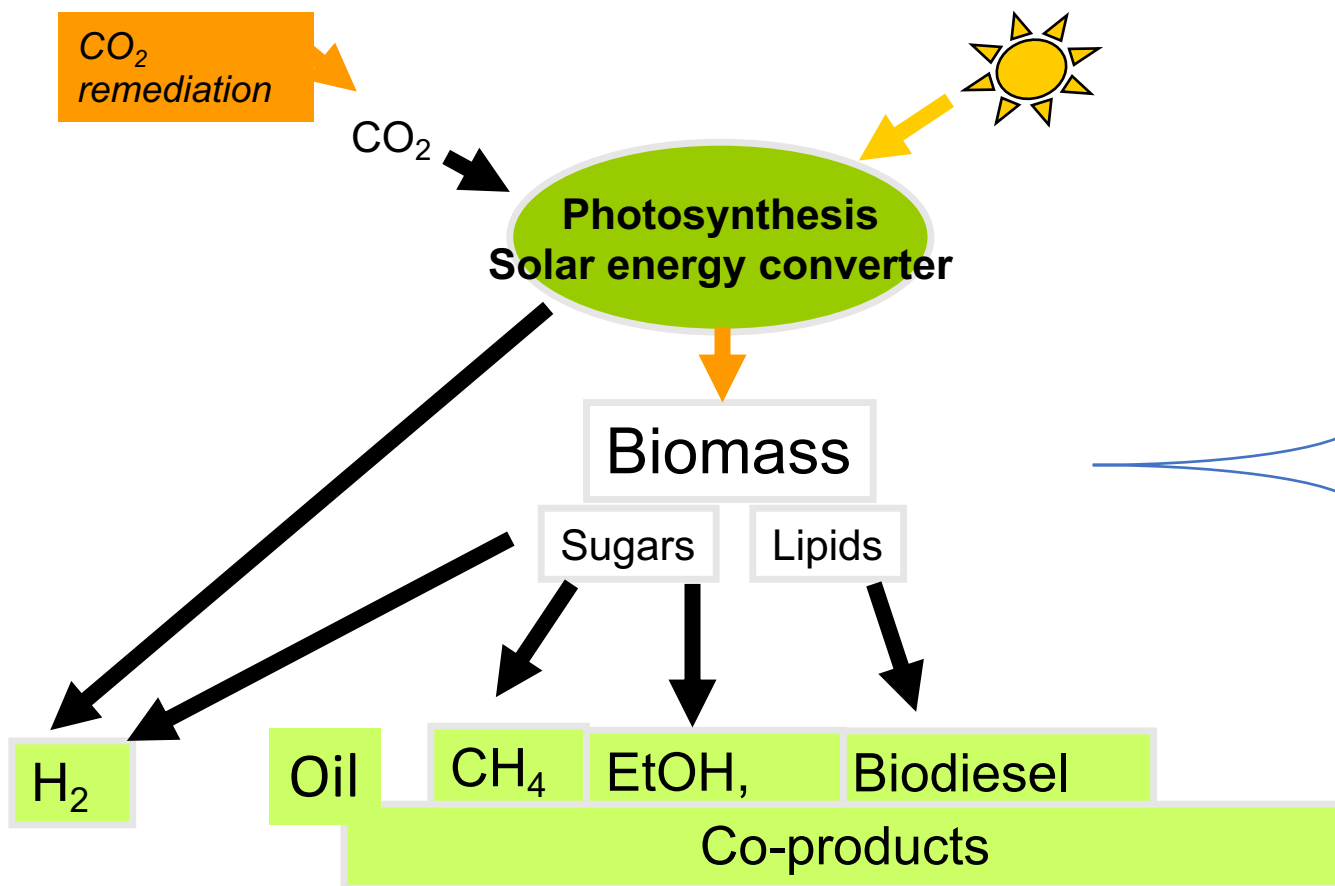
In 2020, following the Covid crisis, many countries have put in place national recovery plans which almost all include an H2 plan. This is the case in Europe with a focus on green H2



# Biomass for energy



Produce energy from biomass, consists of recovering the energy released during the degradation of biomass into CO<sub>2</sub> and H<sub>2</sub>O, and making it available in a form that can be used by humans.



This section provides visual examples of biomass sources and waste types. On the left, a blue bracket groups **Forestry resources** (a forest), **Aquatic resources** (a field of colorful algae), and **Agricultural resources** (a field of hay bales). On the right, a vertical stack of images shows different waste categories: **industrial** (a truck dumping waste), **green** (a pile of leaves), **Organic** (a pile of food waste), and **Waste water** (a wastewater treatment plant). The **cnrs** logo is at the bottom right, with the tagline "dépasser les frontières".

# Biofuels: Energy from biomass



## why biofuels?

- ✓ Historical drivers
  - Promote employment in rural areas
  - Develop energy independence
  - Contribute to the reduction of greenhouse gas emissions in transportation
  
- ✓ Main current drivers
  - Contribute to the reduction of CO<sub>2</sub> emissions in transport
  - Geopolitics

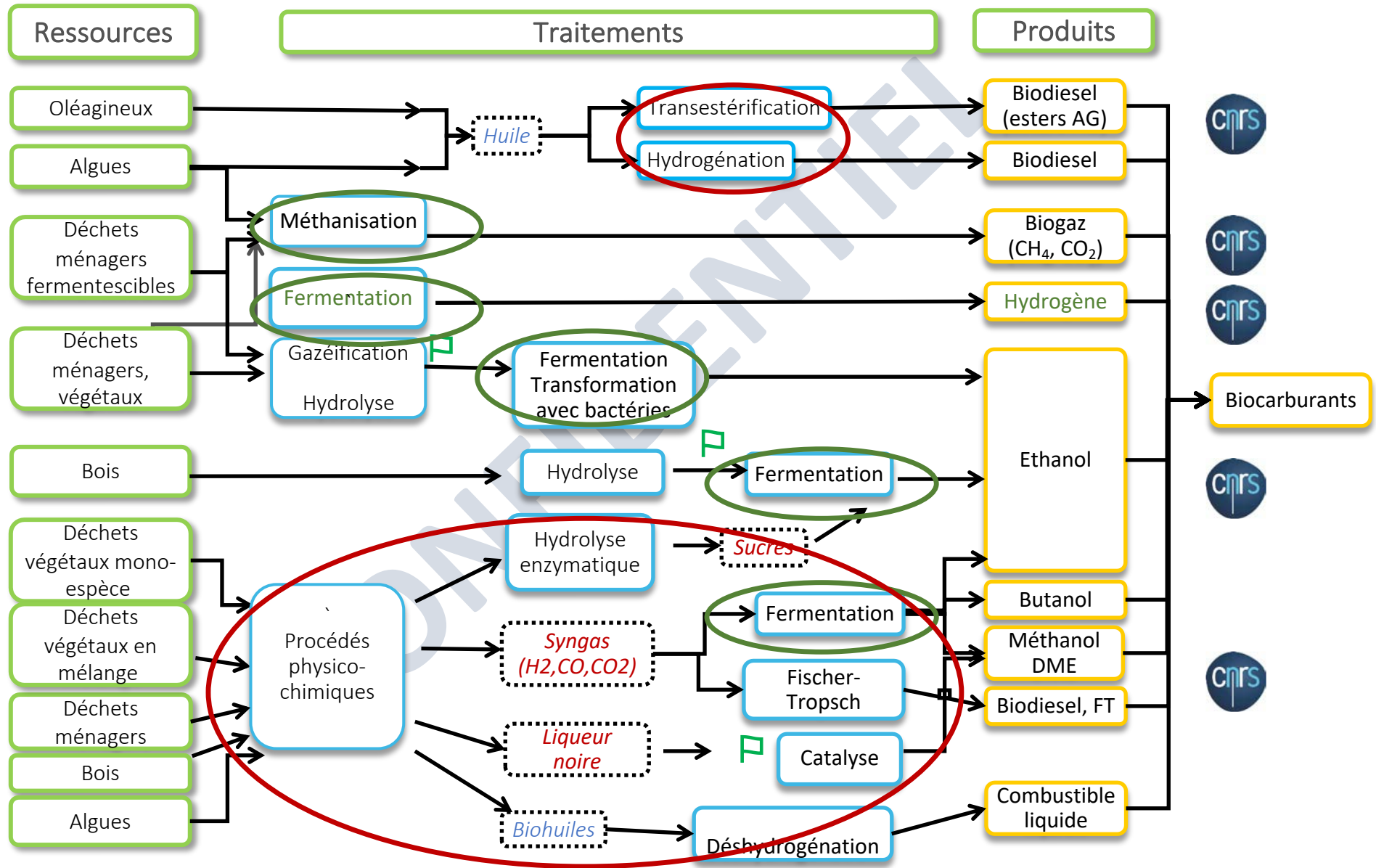
# Biofuels: Energy from biomass



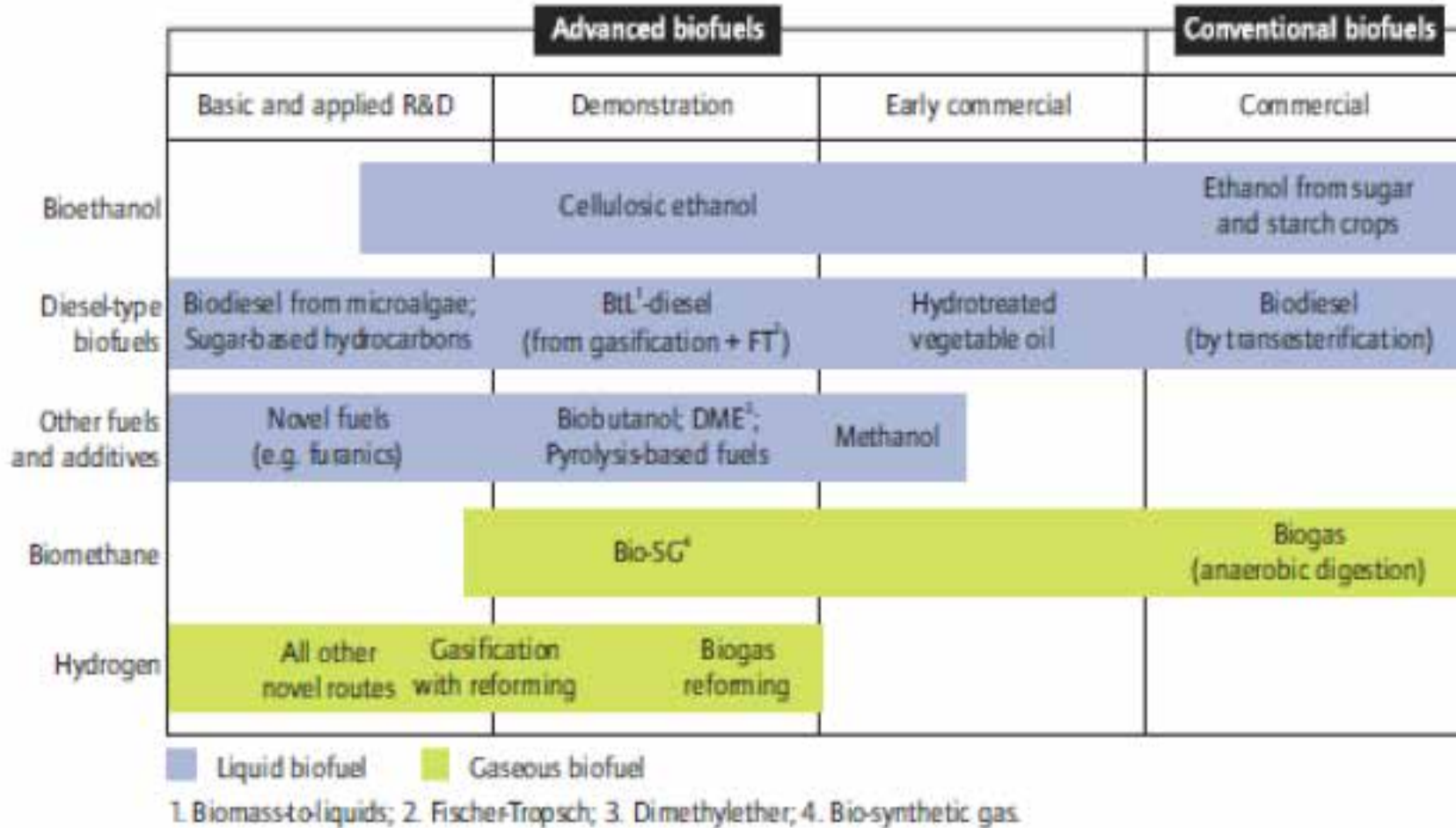
- 1st generation: Oil, ethanol from the transformation of biomass (wheat, beet, etc.). Represents from 7% to 12% of fuels.
  - In competition with water, food: Environmental and societal problem.
- Advanced biofuels: 2G and 3G: no competition with water and food, derived from biomass and therefore from CO<sub>2</sub> fixation.
  - 2G: Ethanol, biomethane: production by anaerobic fermentation (without oxygen) from dedicated crops, waste... (2020-2050)
  - 3G Hydrogen and biodiesel: production from biomass. (2050....)



# Biofuels: the world of possibilities



# Maturity of the main biofuel production technologies

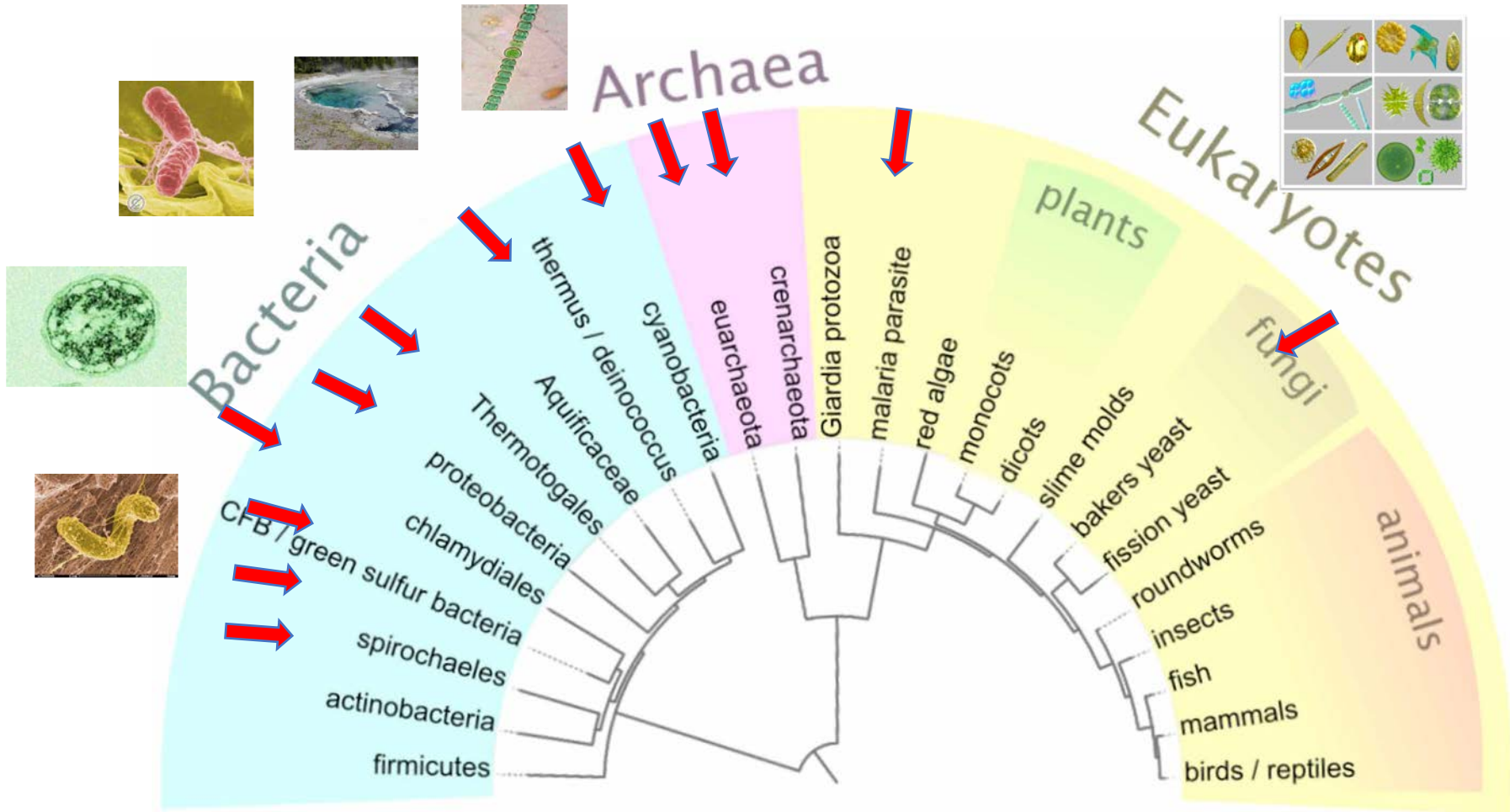


fuel eq/l	Decrease CO2
0,66	59%
0,91	47%
0,5	50%
1kg= 1,6 fuel= 1,3 gazoil	f(resource)
1kg= 4l fuel	F(resource)

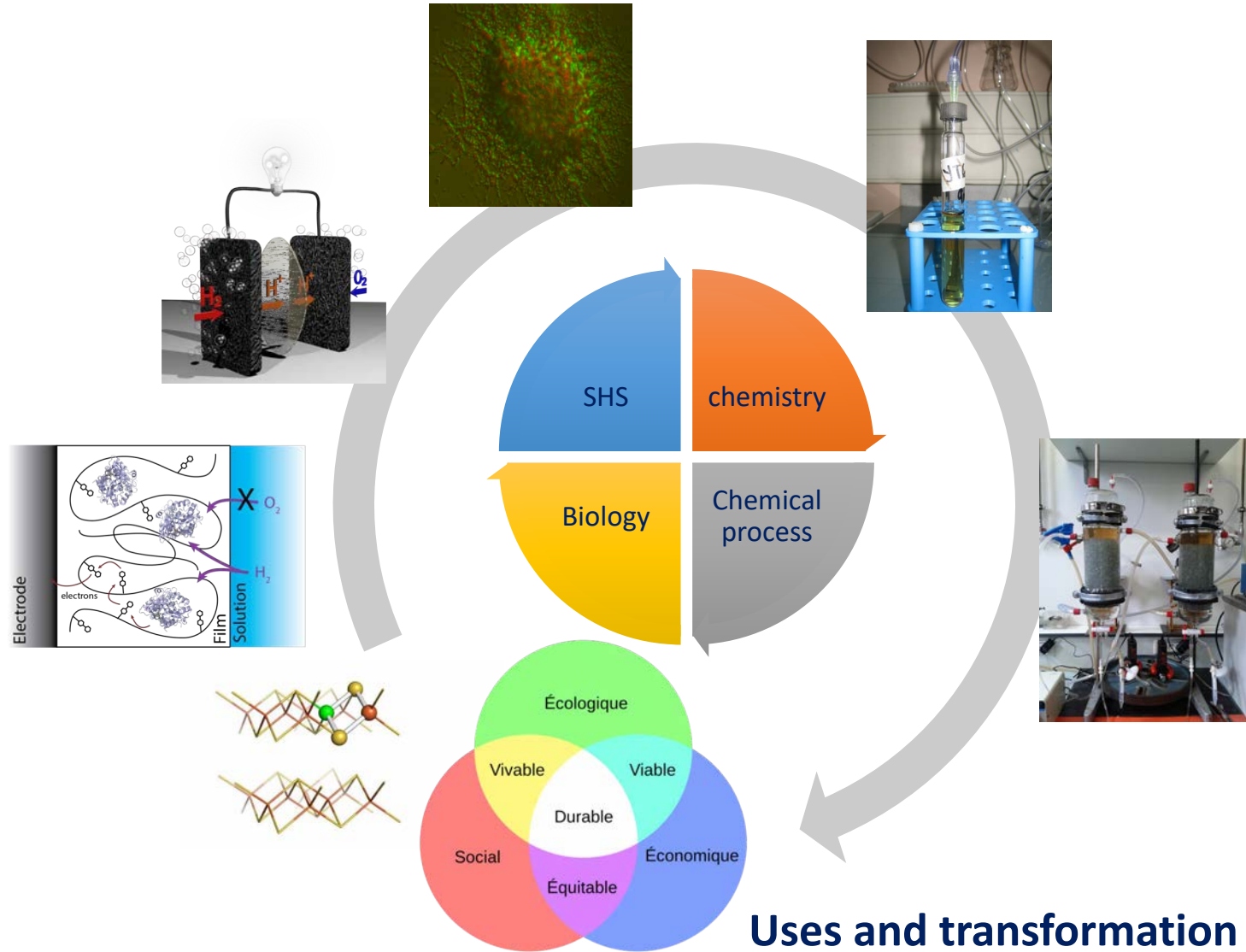
Most of the advanced technologies are at the R&D or demonstration stage



# H2: the energy carrier of life



# A multi-scale and interdisciplinary approach for BIO-H2

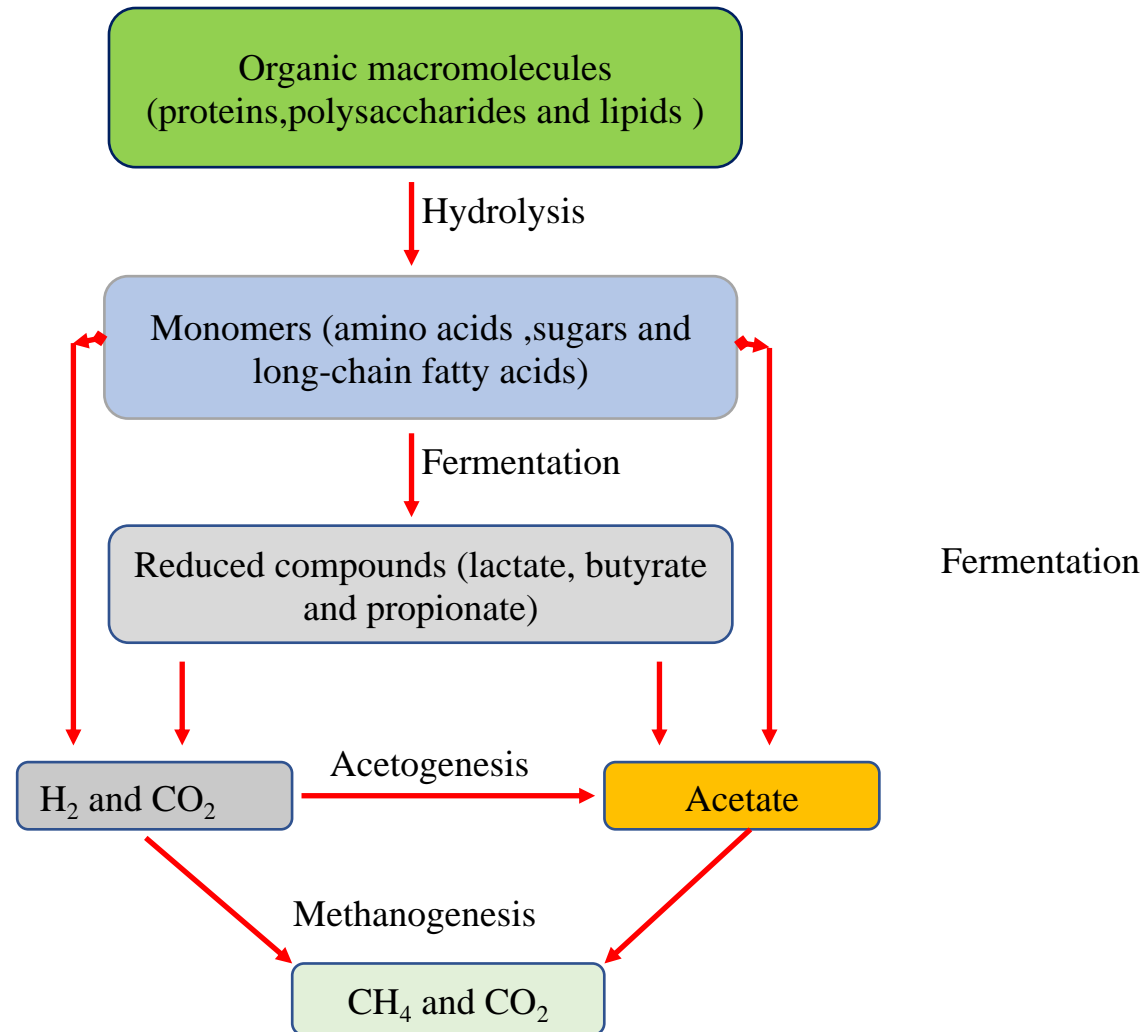




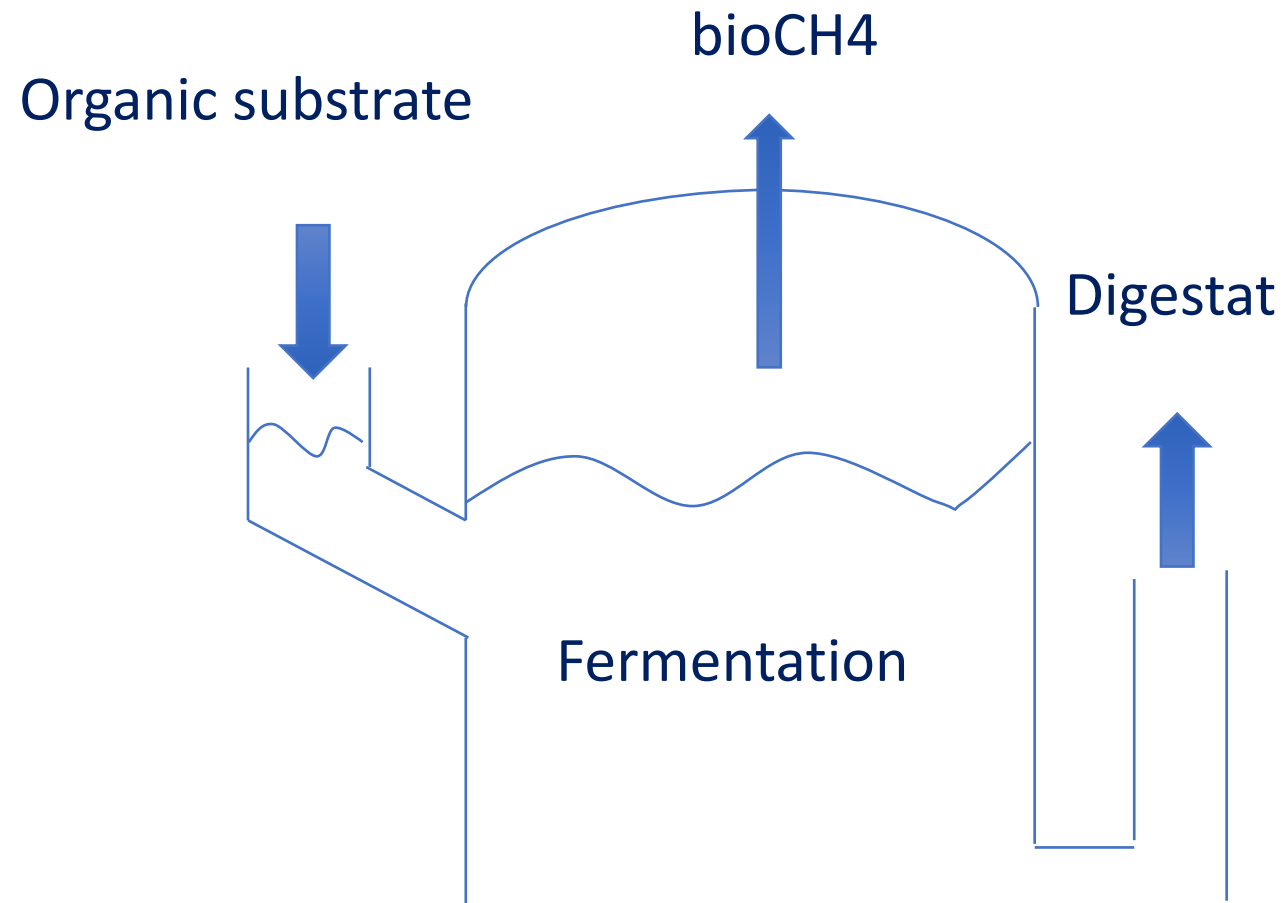


# H2: the energy carrier of life

## Syntrophic Biomass degradation



# BioH2 from mature technologies to innovation



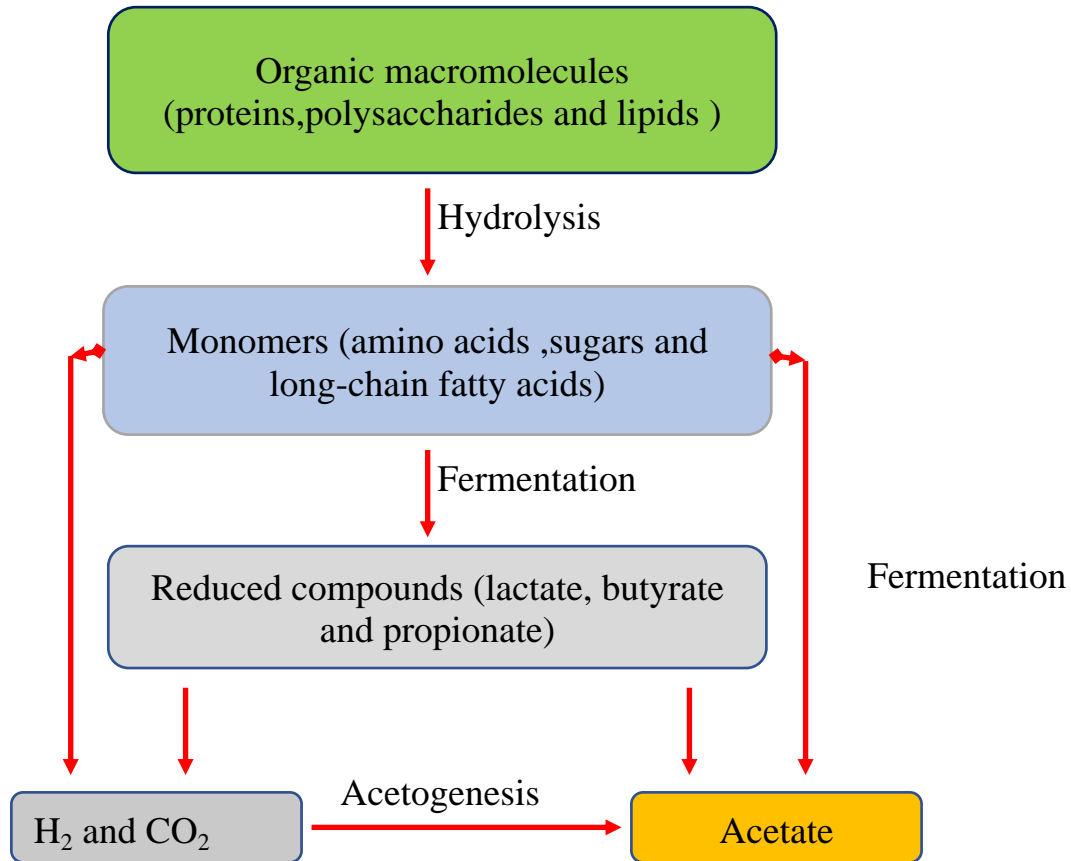
BioCH4 production:

- Mature technology
- Biological process not controlled
- In France in 2020, 214 installations injected biomethane into the natural gas networks. Their capacity amounts to 3.9 TWh/year, an increase of 73% compared to the end of 2019

# H<sub>2</sub>: a green energy

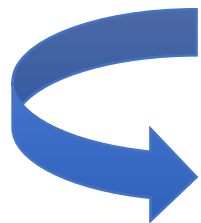
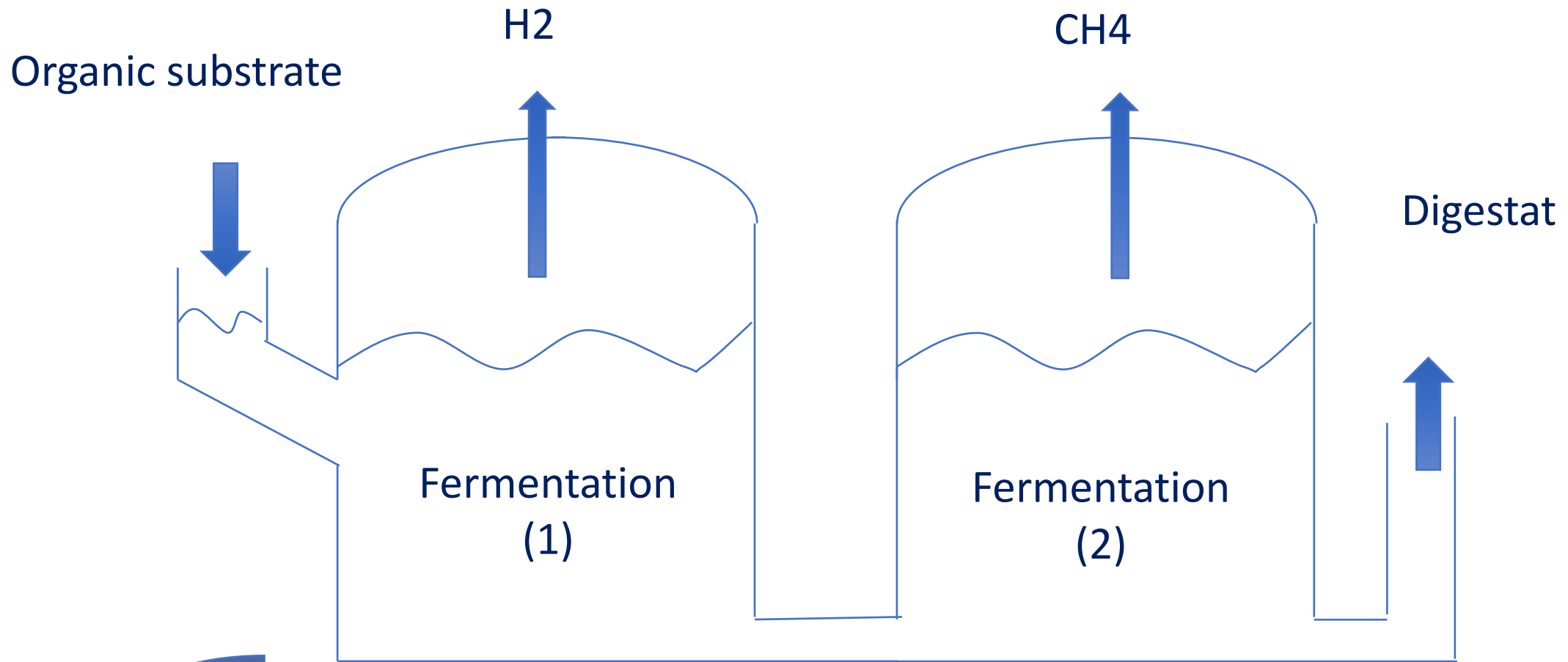


## Syntrophic Biomass degradation and H<sub>2</sub> metabolism



- Requires reverse electron transfer, physical proximity and metabolic synchronization of the partners *via* biofilm formation.

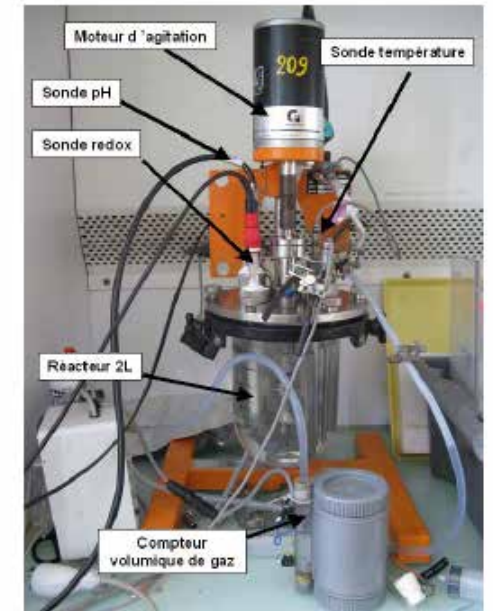
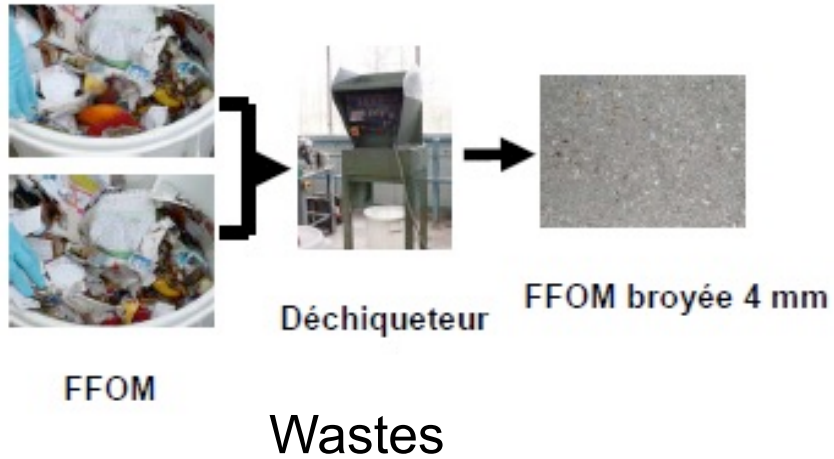
# Advanced biofuels based new biological concepts



**Better energetic biomass valorisation**

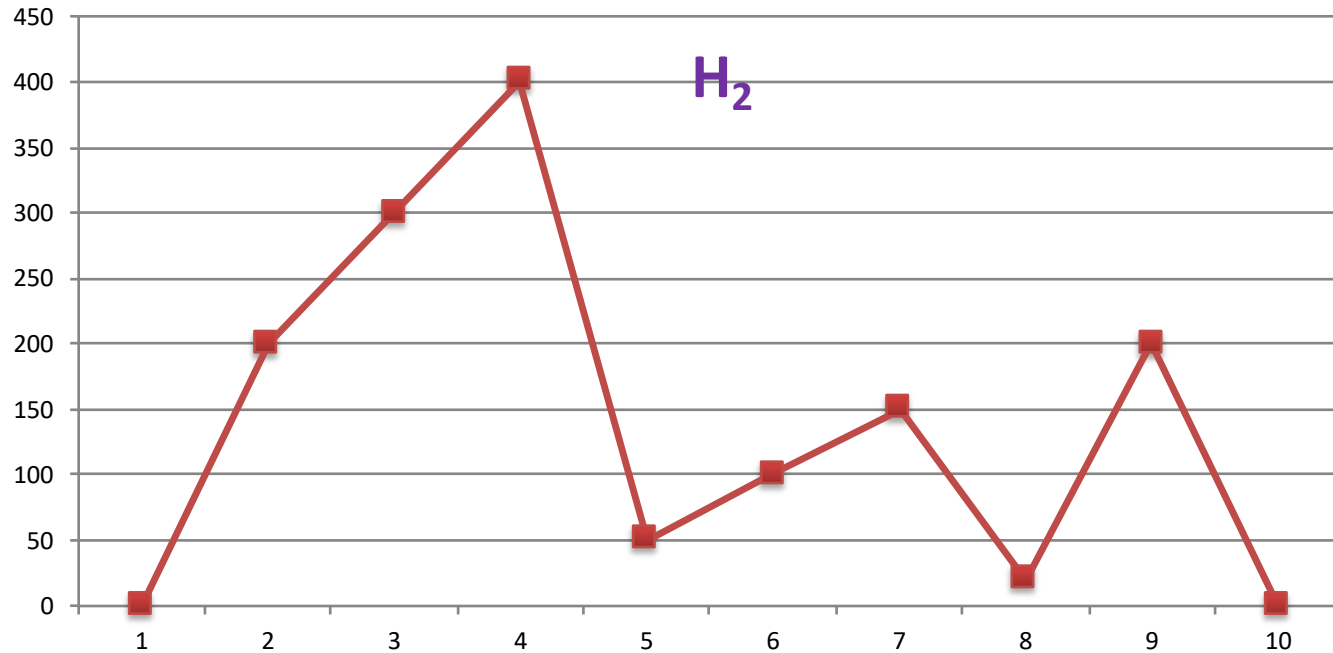
# bioH<sub>2</sub> based new biological concepts

## BIOH<sub>2</sub> production from organic wastes



Cultivation, determination of hydrogen, methane, microbial and enzymatic potential  
no need to inoculate the waste with a specific exogenous inoculum  
Yield of 1.5 to 7 lH<sub>2</sub>/kg FFOM (~ 20 kWh/kg FFOM)

# bioH2 based new biological concepts



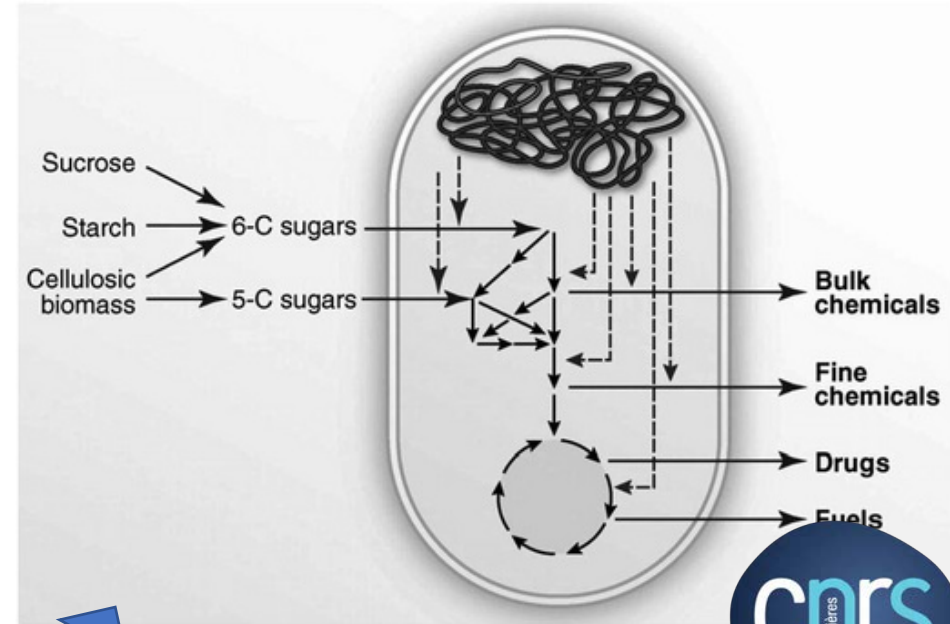
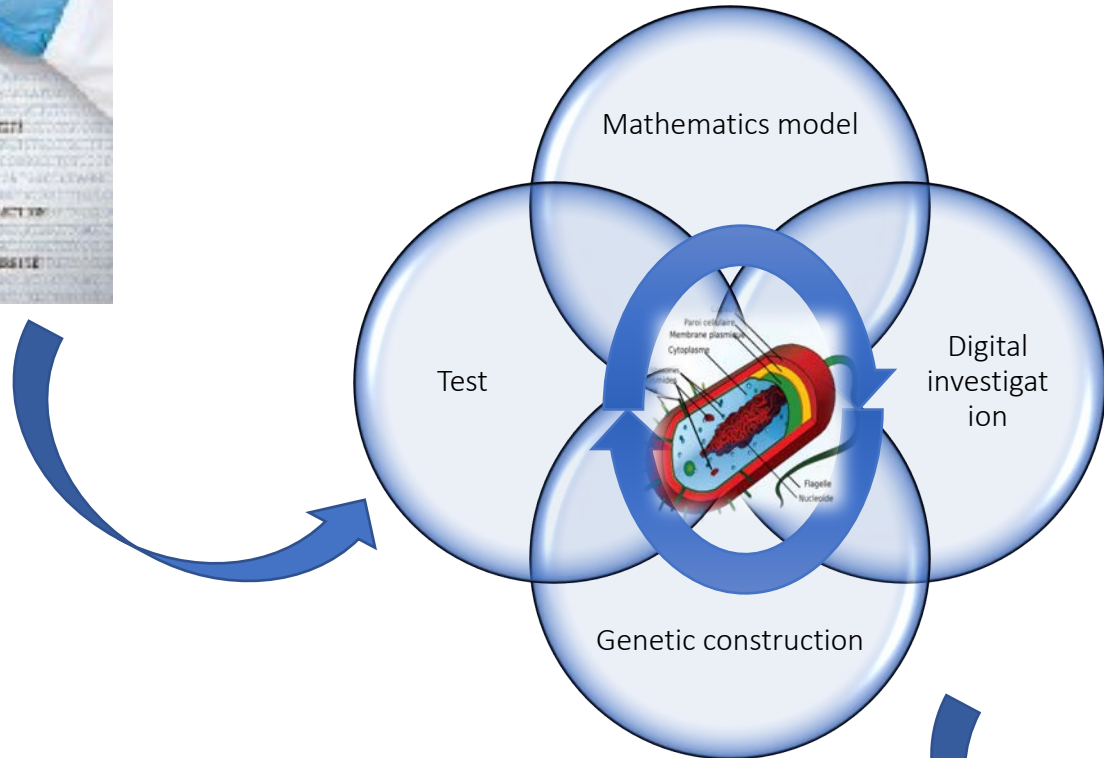
**Abiotic conditions**  
(pH, ° C, TSH)



**Biotic conditions**  
intra/inter species  
interactions



# BioH2 based on metabolic engineering and synthetic biology



# Advanced biofuels based on metabolic engineering and synthetic biology



Program funded by U.S. Department of Energy's

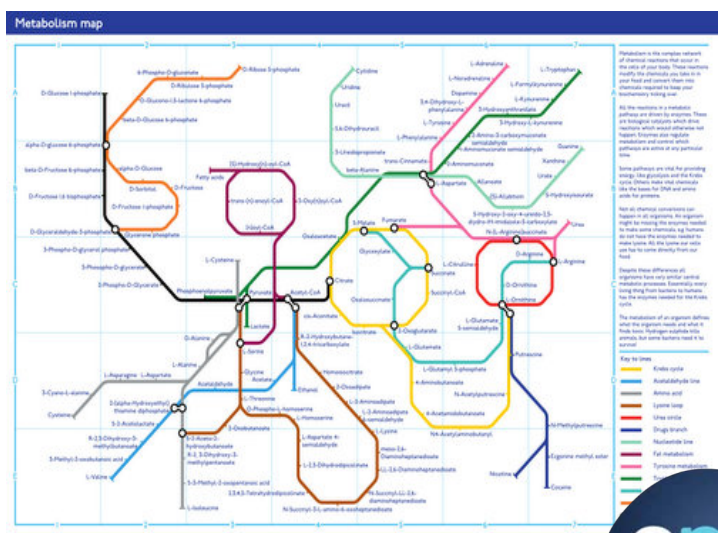
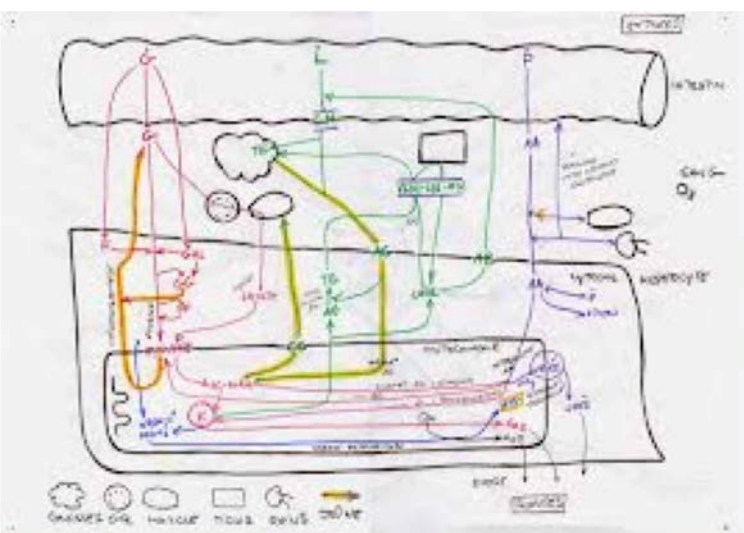
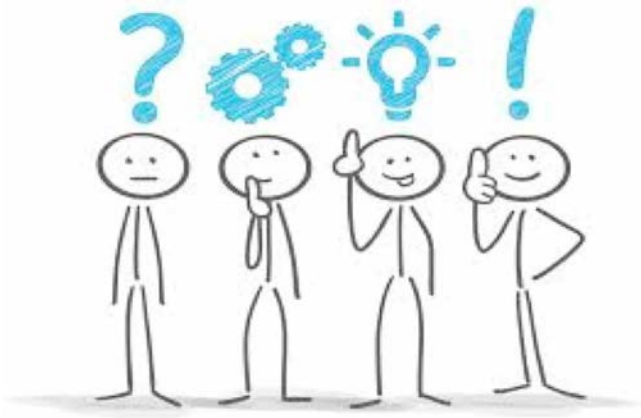
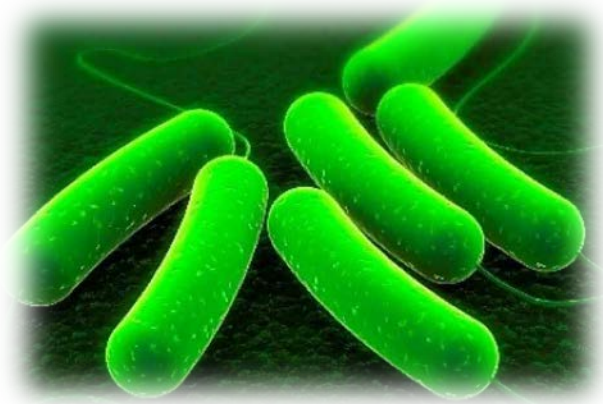
Developing emerging model microorganisms and/or microbial communities with unique or enhanced capabilities to produce H<sub>2</sub> and advanced biofuels and/or bioproducts.

Understanding novel microbial functional capabilities and biosynthetic pathways relevant to the production of H<sub>2</sub> and advanced biofuels and bioproducts.





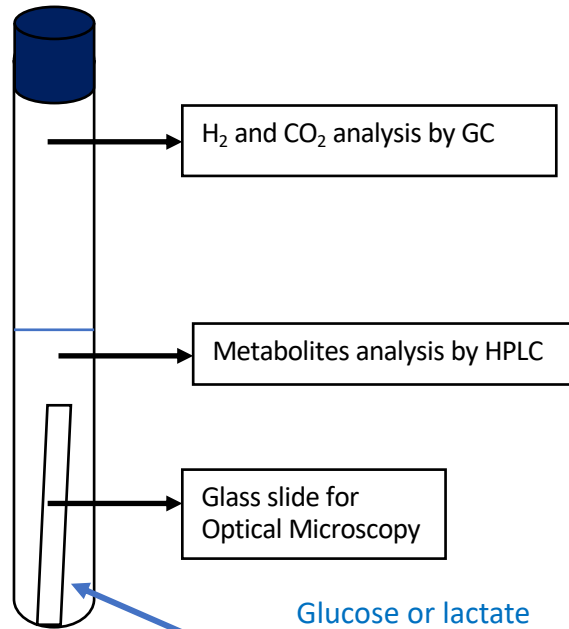
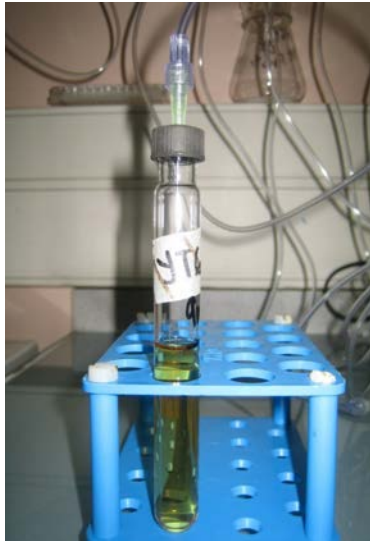
# Advanced biofuels based on metabolic engineering and synthetic biology



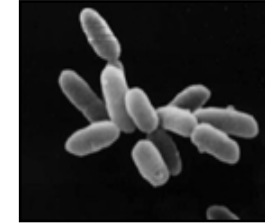


# bioH2 based new biological concepts

Microbial consortium = simplified ecosystem



Glucose or lactate  
& nutrients  
Inoculii  
*C. acetobutylicum*  
or/and *D. vulgaris*  
Hildenborough



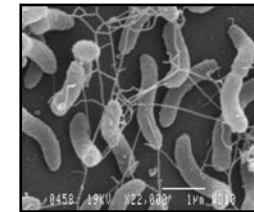
Fermentative bacterium

Large range of  
carbon source

H<sub>2</sub> prod.

*C. acetobutylicum* (wt)

Gram +



Sulfate reducing  
bacterium

Lactate

H<sub>2</sub> prod/cons.

*D. vulgaris* (wt)

Gram -

Both involved in complex biomass degradation

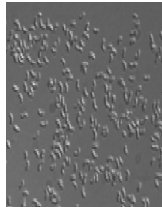
# BioH<sub>2</sub> production by synthetic consortium



## Hydrogen production: batch results

### Pure culture

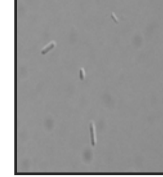
*D. vulgaris*



Lactate/sulfate medium

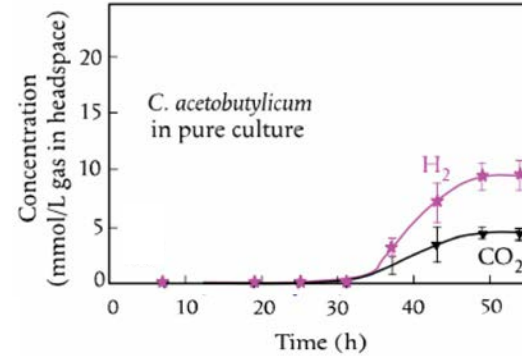
→ No H<sub>2</sub>

*C. acetobutylicum*



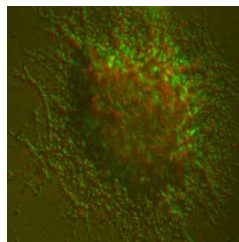
Glucose medium

→



No pH buffer  
[glucose] = 14 mmol/L

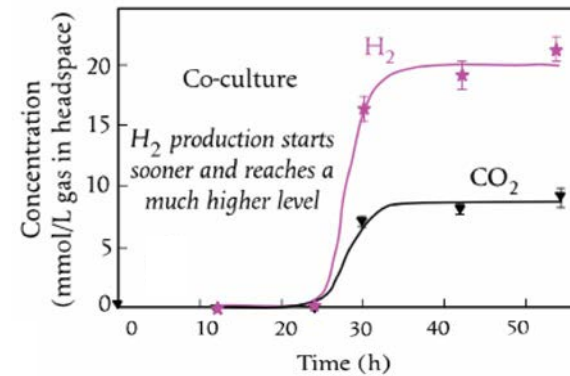
### Co culture: consortium



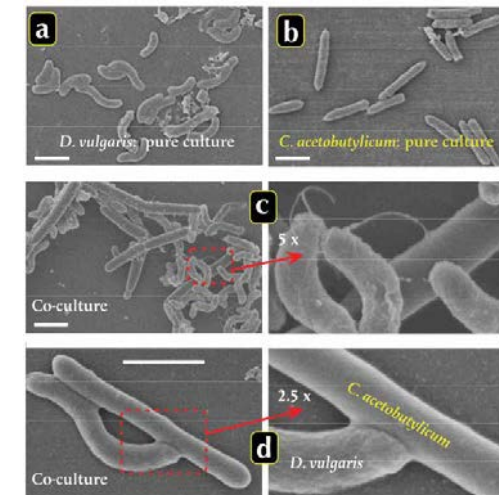
Glucose medium

**Biofilm formation**

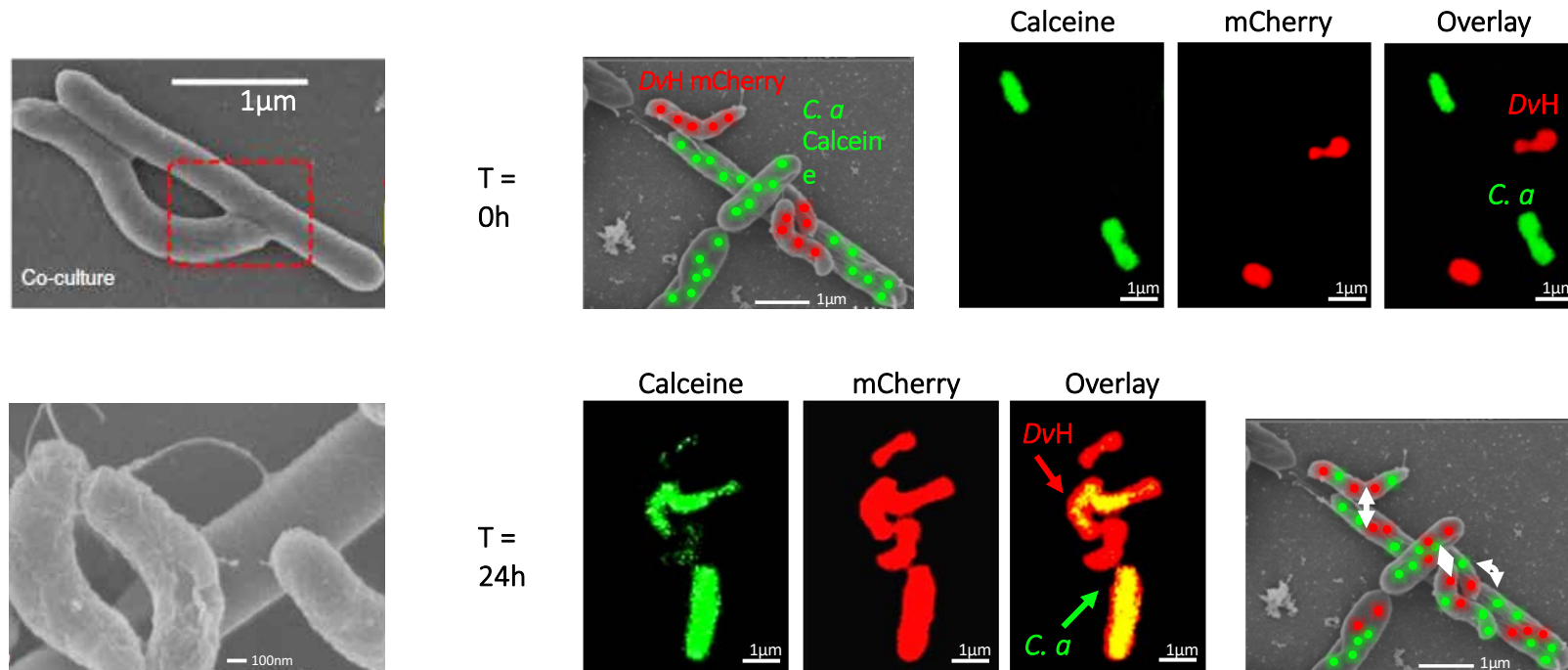
→



**Increase of hydrogen evolution**

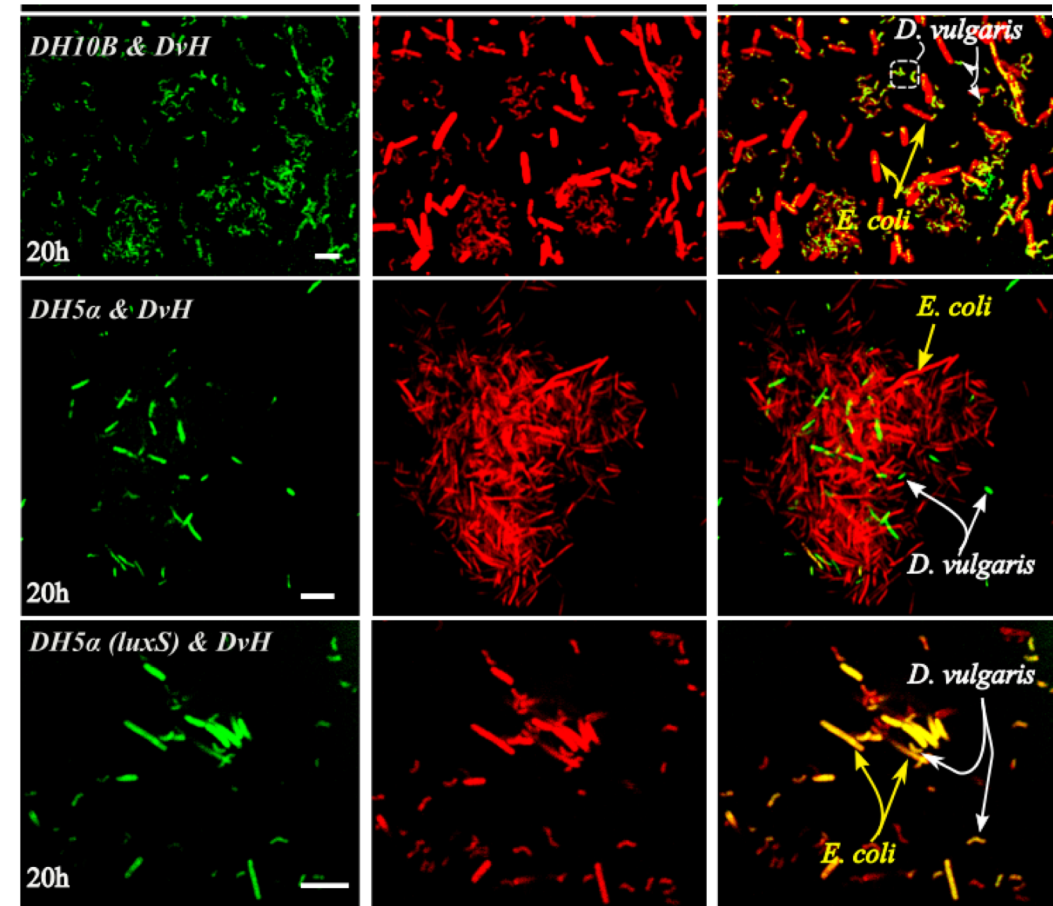
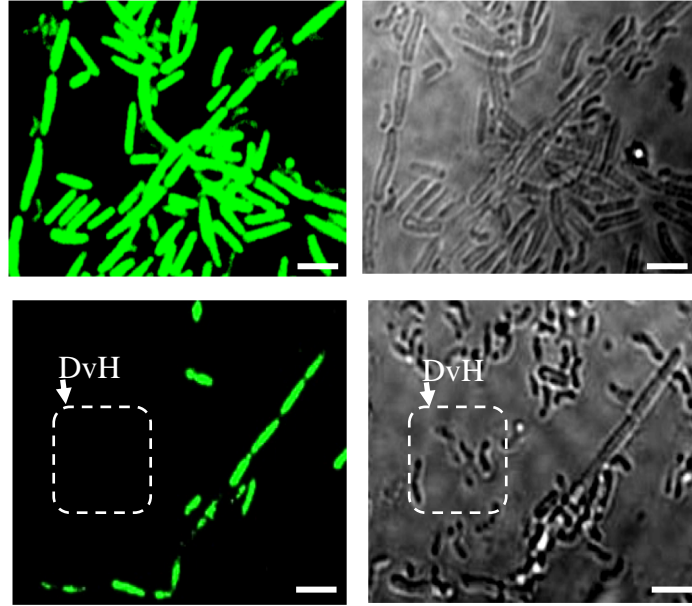


# BioH<sub>2</sub> production by synthetic consortium: role of physical interactions



- Bidirectional exchange of cytoplasmic material between *C. acetobutylicum* and *D. vulgaris* associated with higher H<sub>2</sub> production (better yield)

# BioH<sub>2</sub> production by synthetic consortium: role of physical interactions

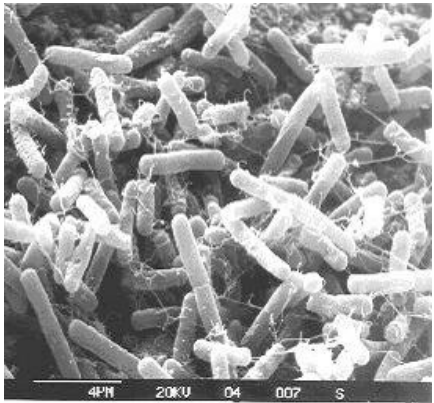


Link between nutritional stress and bacterial communication

QS and nutritional stress are necessary

**Stress + QS = Increase in H<sub>2</sub> production**

# Metabolic and cellular coupling model

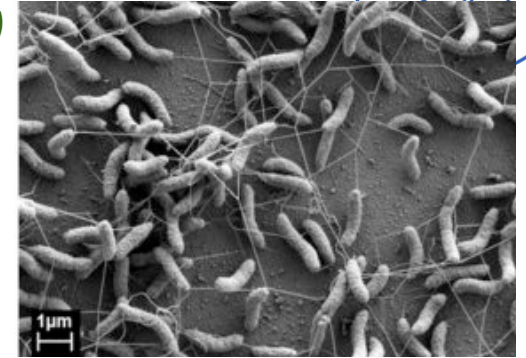


*Clostridium acetobutylicum (Cab)*

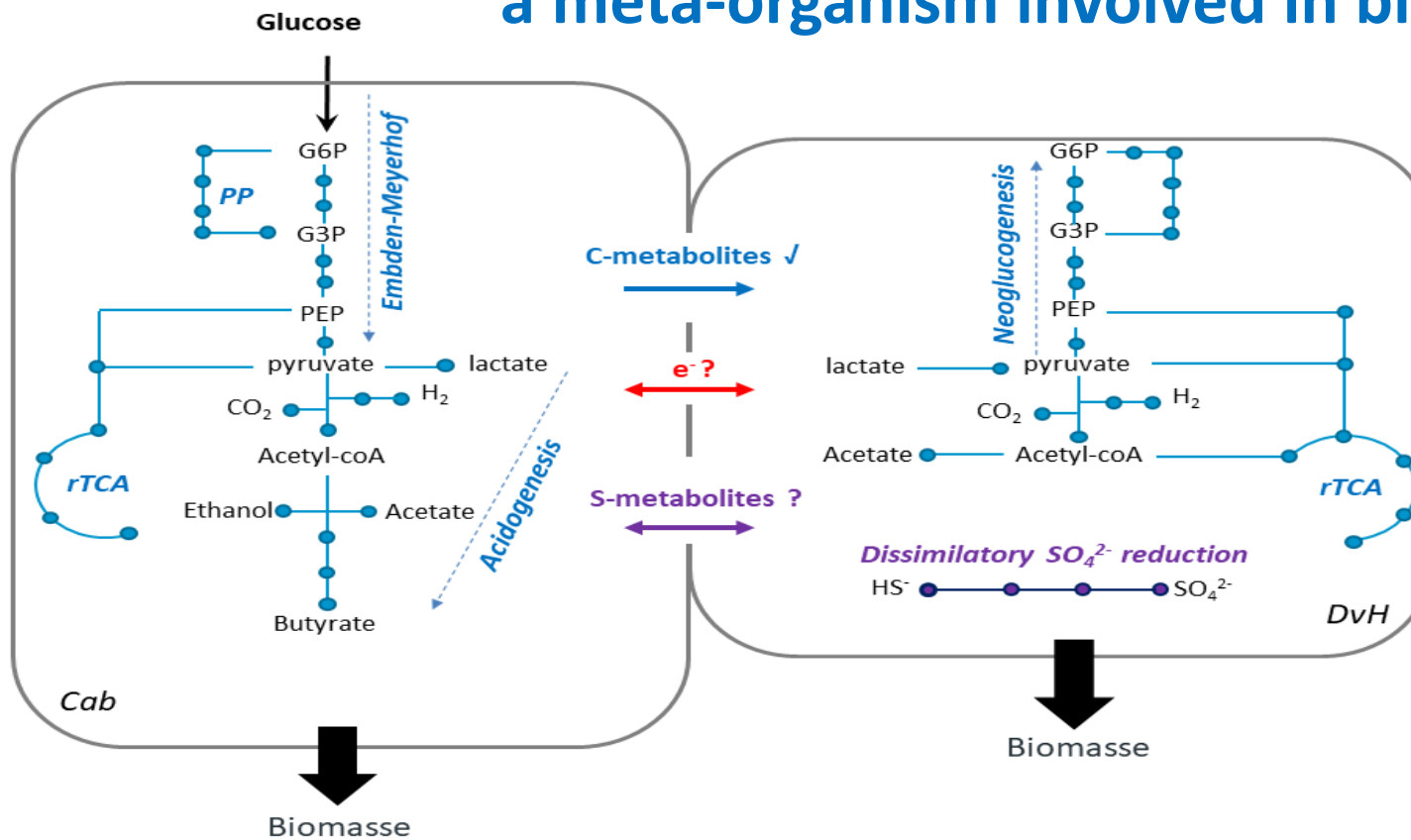
Glucose fermentation  
 Acidogenesis ->  
H<sub>2</sub>-producing

*Desulfovibrio vulgaris Hildenborough (DvH)*

Lactate/Pyruvate respiration (SO<sub>4</sub><sup>2-</sup>)?  
 Lactate/Pyruvate/Formiate  
 Fermentation ?  
H<sub>2</sub>-producing/consuming



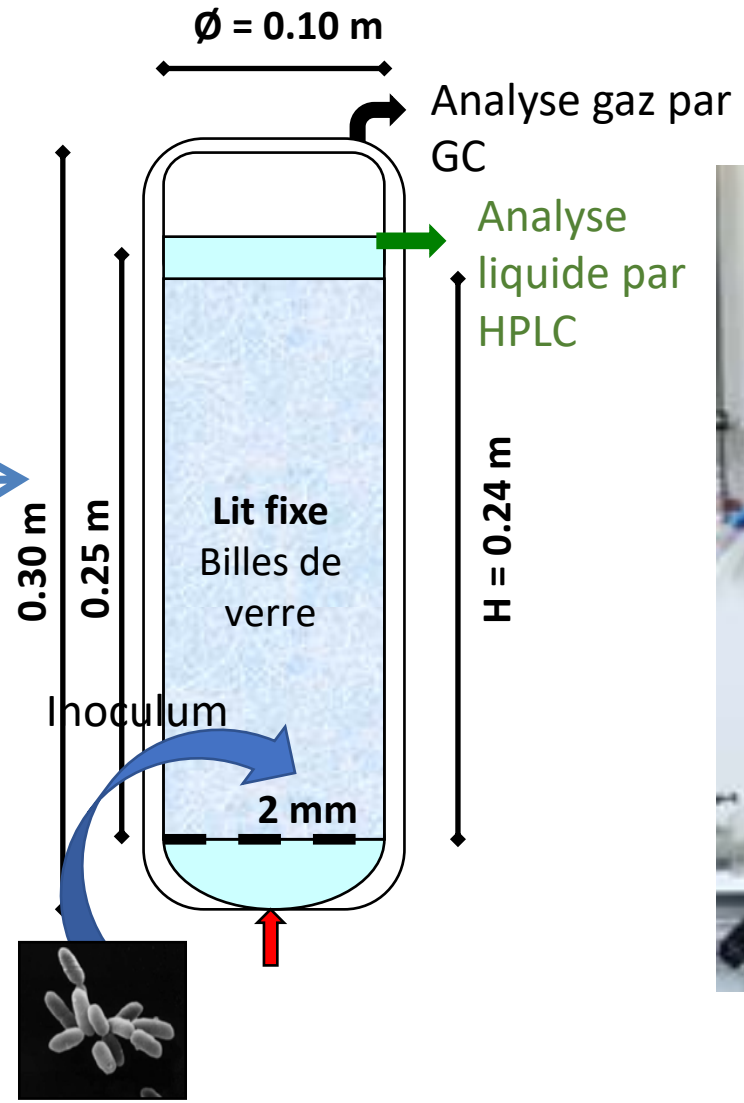
a meta-organism involved in bioH<sub>2</sub> production



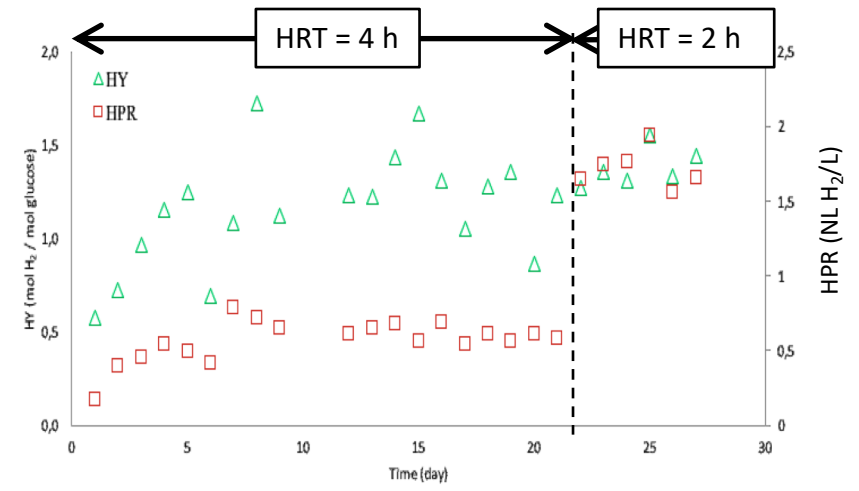
# Scale up: Design



10.10<sup>-3</sup> L batch



Système non stérile, dé-oxygéné, eaux « sales » synthétiques

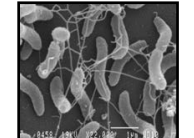


0.01 < 0.1 < 7.4 NL H<sub>2</sub>/h/L (réacteur)

Production stable et continue d'hydrogène

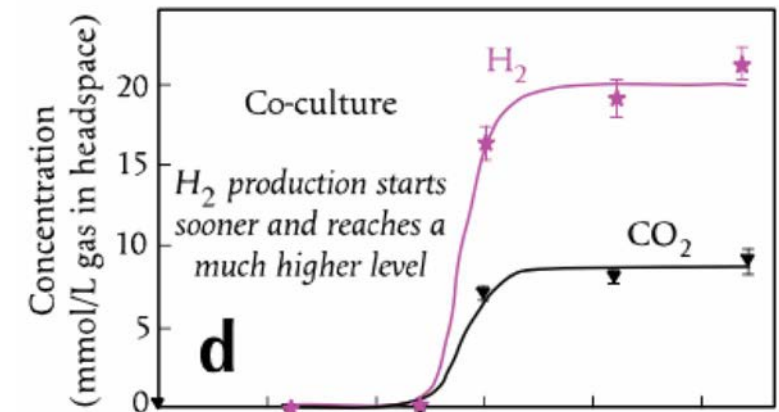
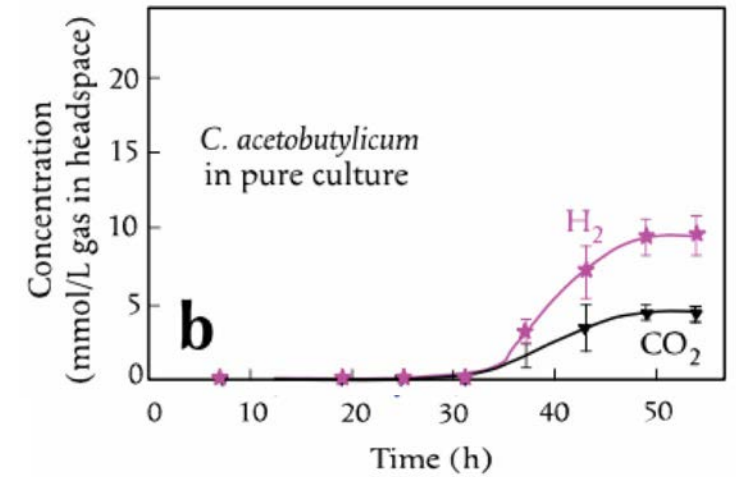
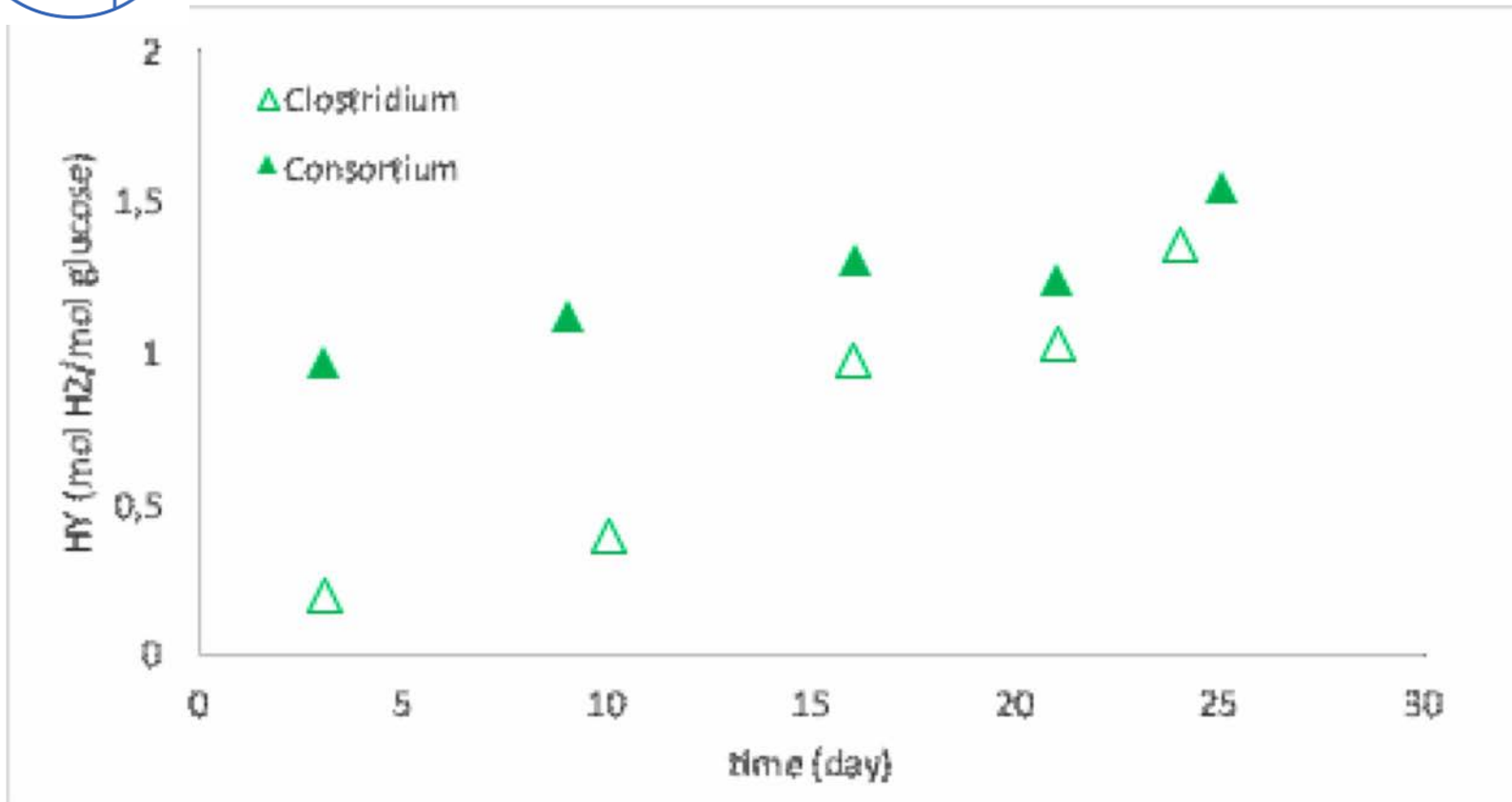
Bactéries toujours présentes

Rendement constant = voies métaboliques





# H<sub>2</sub> Yield



Etude en continu confirme les résultats batch

## Continuous

$0.5 < HY_{C.ab} < 1.3$  mol H<sub>2</sub>/mol glu

$1 < HY_{C.ab+D.vH} < 1.45$  mol H<sub>2</sub>/mol glu

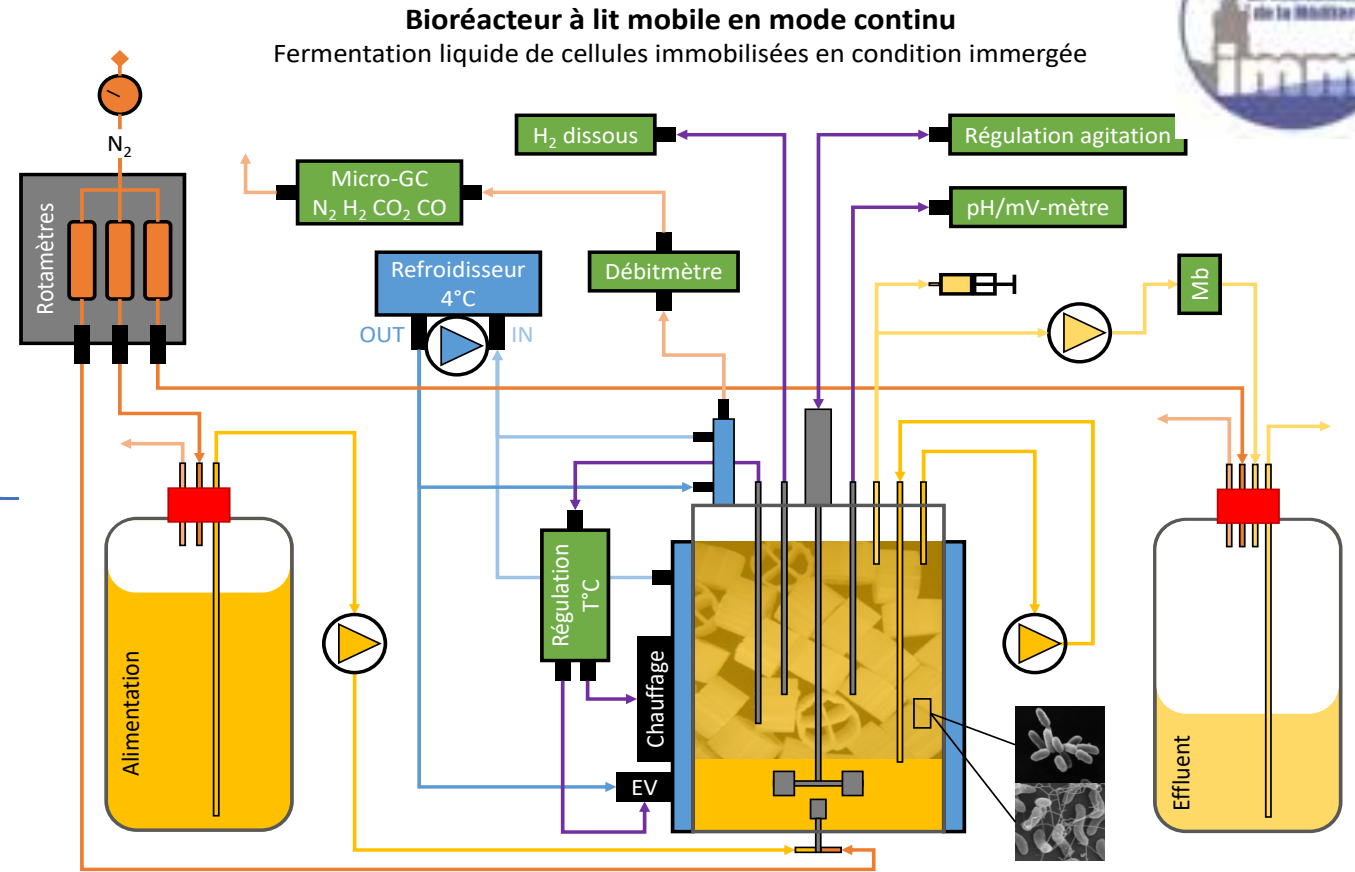
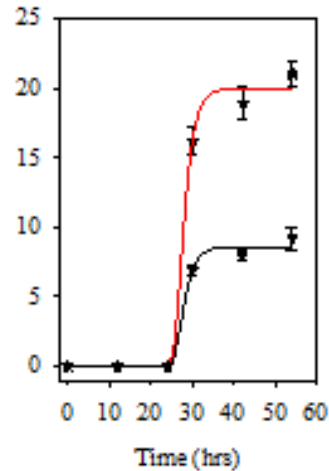
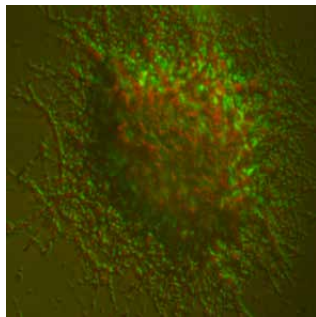
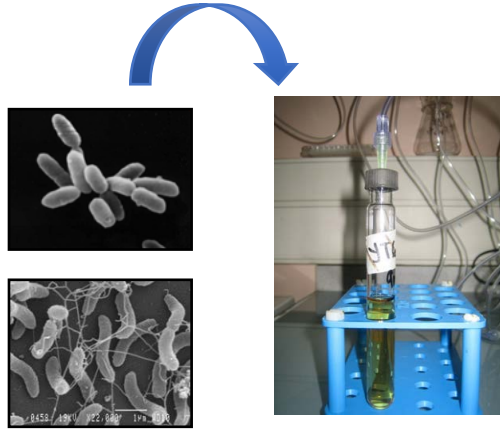
## Batch

$HY_{C.ab} = 0.5$  mol H<sub>2</sub>/mol glu

$HY_{C.ab+D.vH} = 1.43$  mol H<sub>2</sub>/mol glu

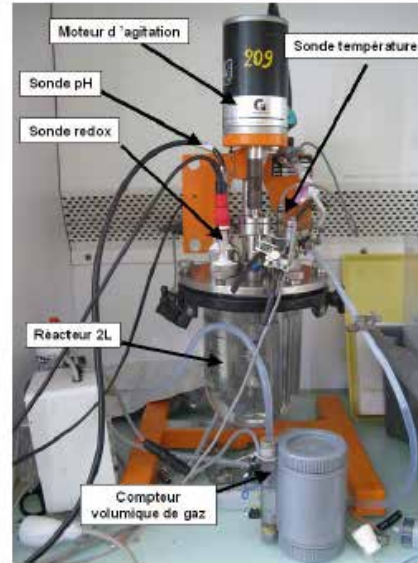
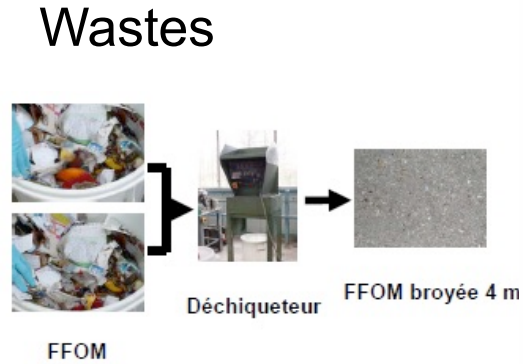


# Advanced biofuels based on new biological concepts



Ecological engineering: meta organism concept

# Production d'Hydrogène à partir de déchets ménagers



Le pilote PROMETHEE : le DIGESTRON



Alimentation séquentielle de déchets solides.  
2 réacteurs en série de 22 litres et 358 litres.



Déchets solides

Mise en culture, détermination du potentiel hydrogène, méthane et potentiel microbien et enzymatique  
pas nécessaire d'ensemencer les déchets avec un inoculum exogène spécifique

Rendement de 1.5 à 7 l H<sub>2</sub>/kg FFOM ( ~ 20 kWh/kg FFOM)



# To take home: Biofuels need integrated researches

