The importance of biogeochemical cycles in waste valorization bioprocesses

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Environmental Chemistry Module

The importance of biogeochemical cycles in waste valorization bioprocesses

Isotopic Biogeochemistry and case studies

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Isotopes definition

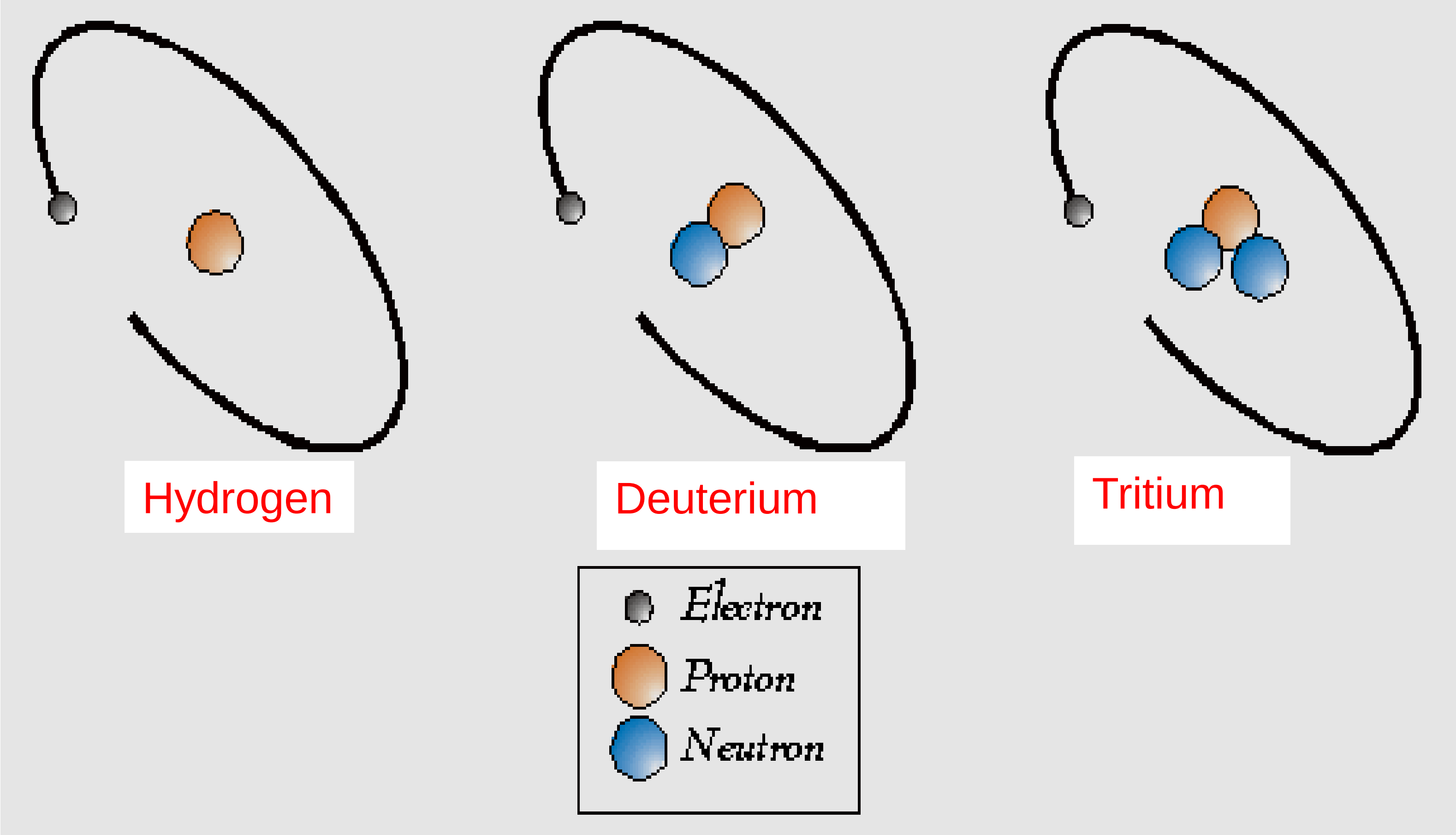
$$^{A=Z+N}X_Z$$

- **Same atomic number Z**

  (Z= number of protons)

- number of neutrons N (then mass number A = Z+N) different

Hydrogen isotopes



$$^{A=Z+N}X_{Z}$$

Exemple: **Carbon C**

- **6 protons → atomic number Z= 6**

- **6, 7, ou 8 neutrons (N)**

  →Atomic mass A = Z+N = 12, 13, ou 14

Notation **: ^{12}C, ^{13}C, ^{14}C**

Two types of isotopes

-Radioactive = Transformation into isotopes of another element

$^{147}Sm \rightarrow ^{143}Nd$

$^{238}U \rightarrow ^{206}Pb$

$^{87}Rb \rightarrow ^{87}Sr$

- **Stables = the others**

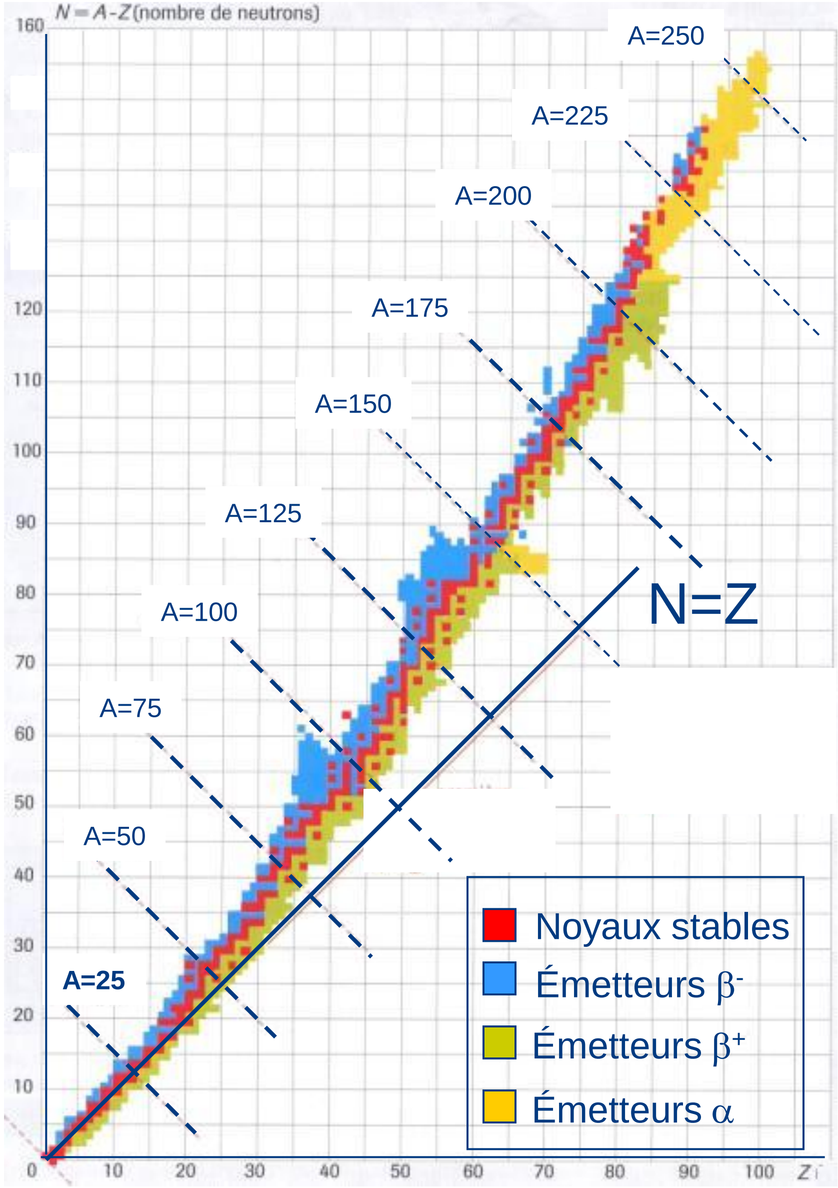
No transformation as a function of time

Légers: ^{1}H, ^{2}H, ^{12}C, ^{13}C, ^{16}O, ^{17}O, ^{18}O, ^{14}N, ^{15}N

Lourds: ^{63}Cu, ^{65}Cu, ^{54}Fe, ^{56}Fe, ^{57}Fe, ^{58}Fe

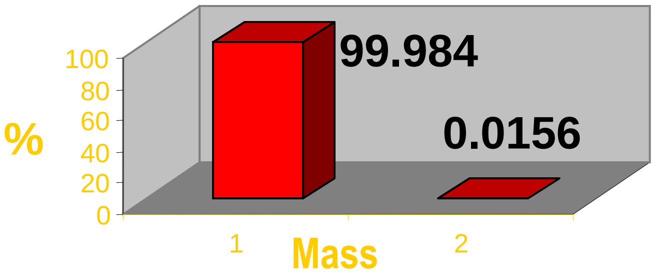
^{66}Zn, ^{68}Zn, ^{70}Zn

Nucleus stability

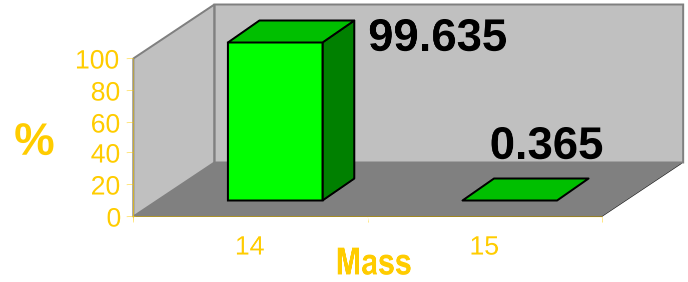


Isotope natural abundancy

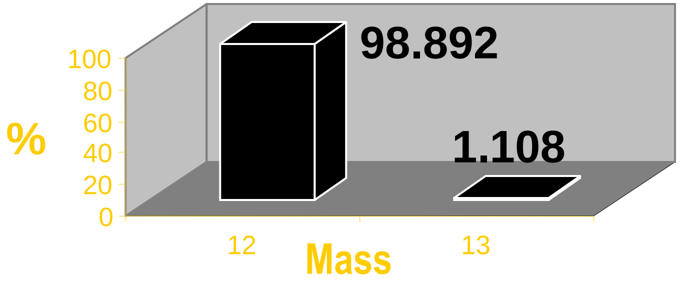
Hydrogen



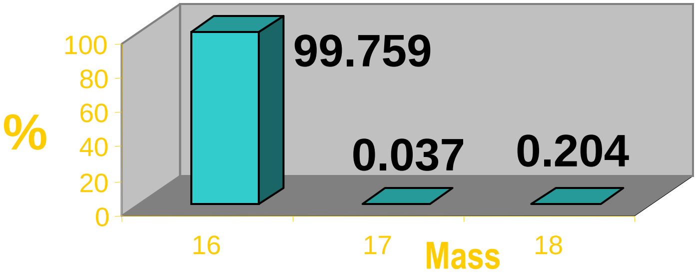
Nitrogen



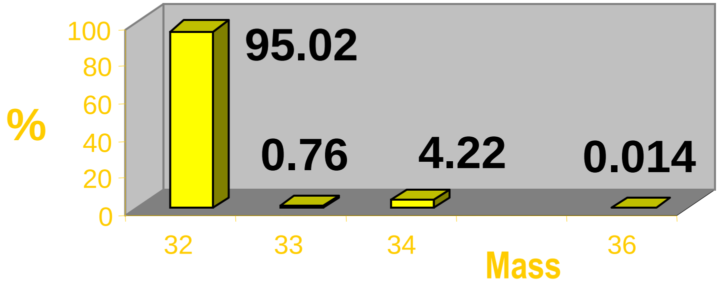
Carbon



Oxygen



Sulphur

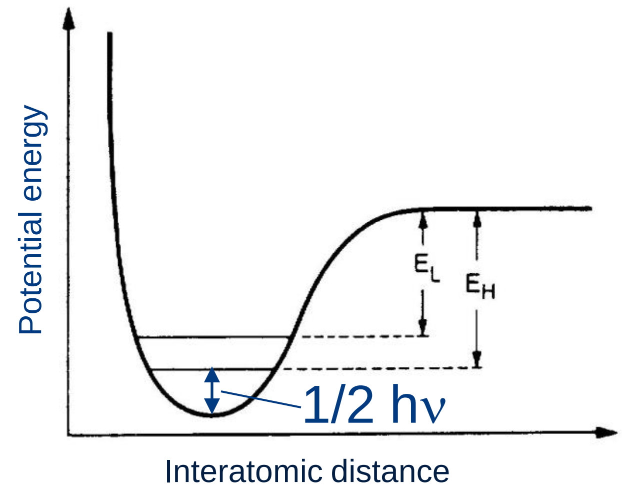


ISOTOPES:
Comparable chemical properties
(identical electronic structure**)**

BUT

Mass difference

Bonds energy ≠



Merging Temperatures ≠
Viscosity ≠
Reaction rate ≠

Constantes	$H_2{}^{16}O$	$D_2{}^{16}O$	$H_2{}^{18}O$
Densité (20°C, en g cm^{-3})	0.9979	1.1051	1.1106
Température de plus grande densité (°C)	3.98	11.24	4.30
Point de fusion (°C)	0.00	3.81	0.28
Point d'ébullition (°C)	100.00	101.42	100.14
Pression de vapeur (100°C, en Torr)	760.00	721.60	
Viscosité (à 20°C en centipoises)	1.002	1.247	1.056

ISOTOPIC FRACTIONATION

Evaporation:

T(boiling): H_2O= 100 °C
D_2O= 101.4 °C

Preferential evaporation of H_2O
Preferential condensation of D_2O

Chemical reaction:

The light isotope forms slightly weaker bonds

↓

The products of the reaction will be enriched in light isotope
The reagents will be enriched in heavy isotopes

THE CONCEPT OF ISOTOPIC COMPOSITION

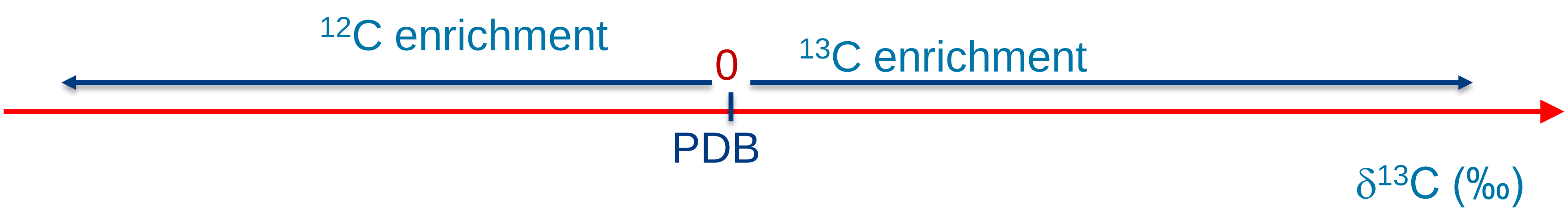
In nature, slight variations in the order of ‰ of the ^{13}C / ^{12}C ratio.

Variations measured using the isotopic composition:

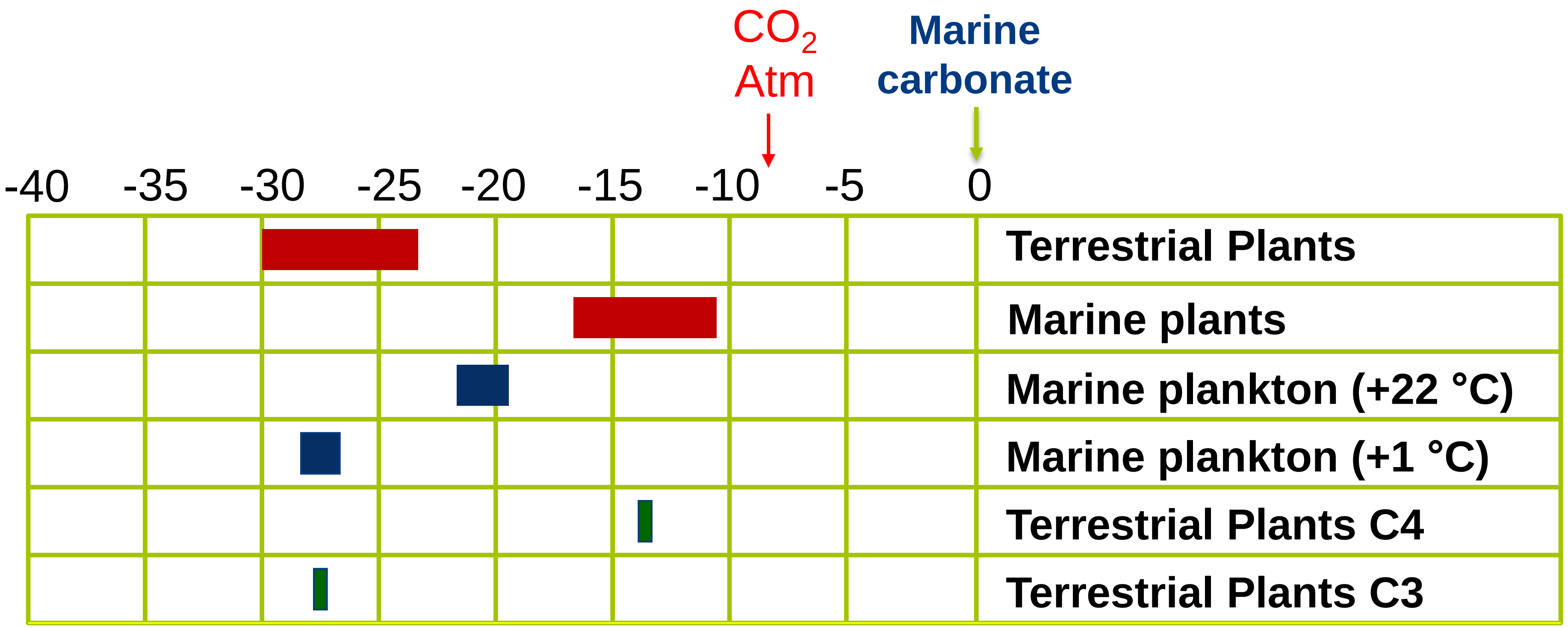
$$\delta^{13}C = [(Rs - Ris)/Ris] \times 1000 \text{ ‰}$$

With Rs = $^{13}C/^{12}C$ of the sample

Ris = $^{13}C/^{12}C$ of the international standard (PDB)



VARIATION OF THE $^{13}C/^{12}C$ RATIO



The ^{13}C / ^{12}C ratio depends on:

- Carbon origin
- Environmental conditions
- Biosynthetic cycle

C4 (Hatch et Slack)

C3 (Calvin)

TWO TYPES OF APPLICATION

NATURAL ISOTOPIA:

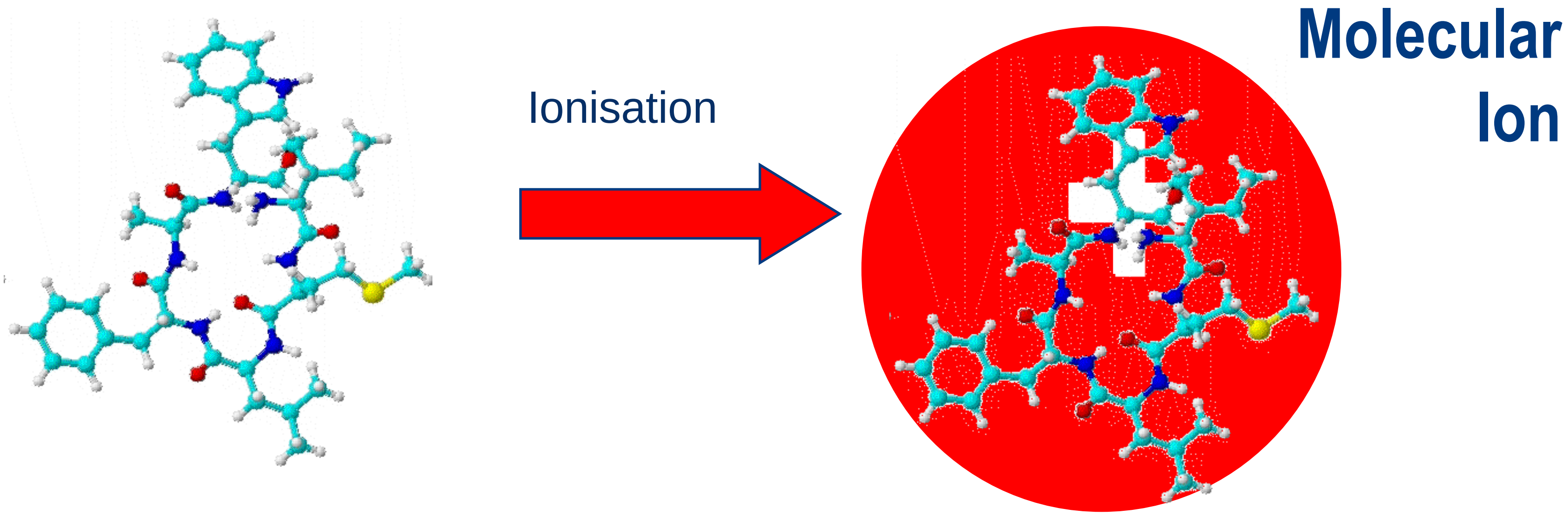
- Compounds origin
- Compounds fate

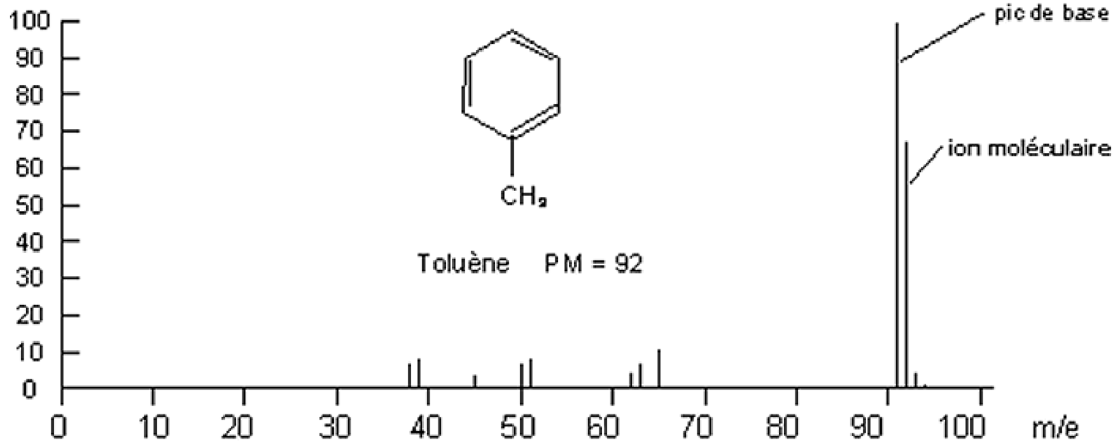
ISOTOPIC TRACING:

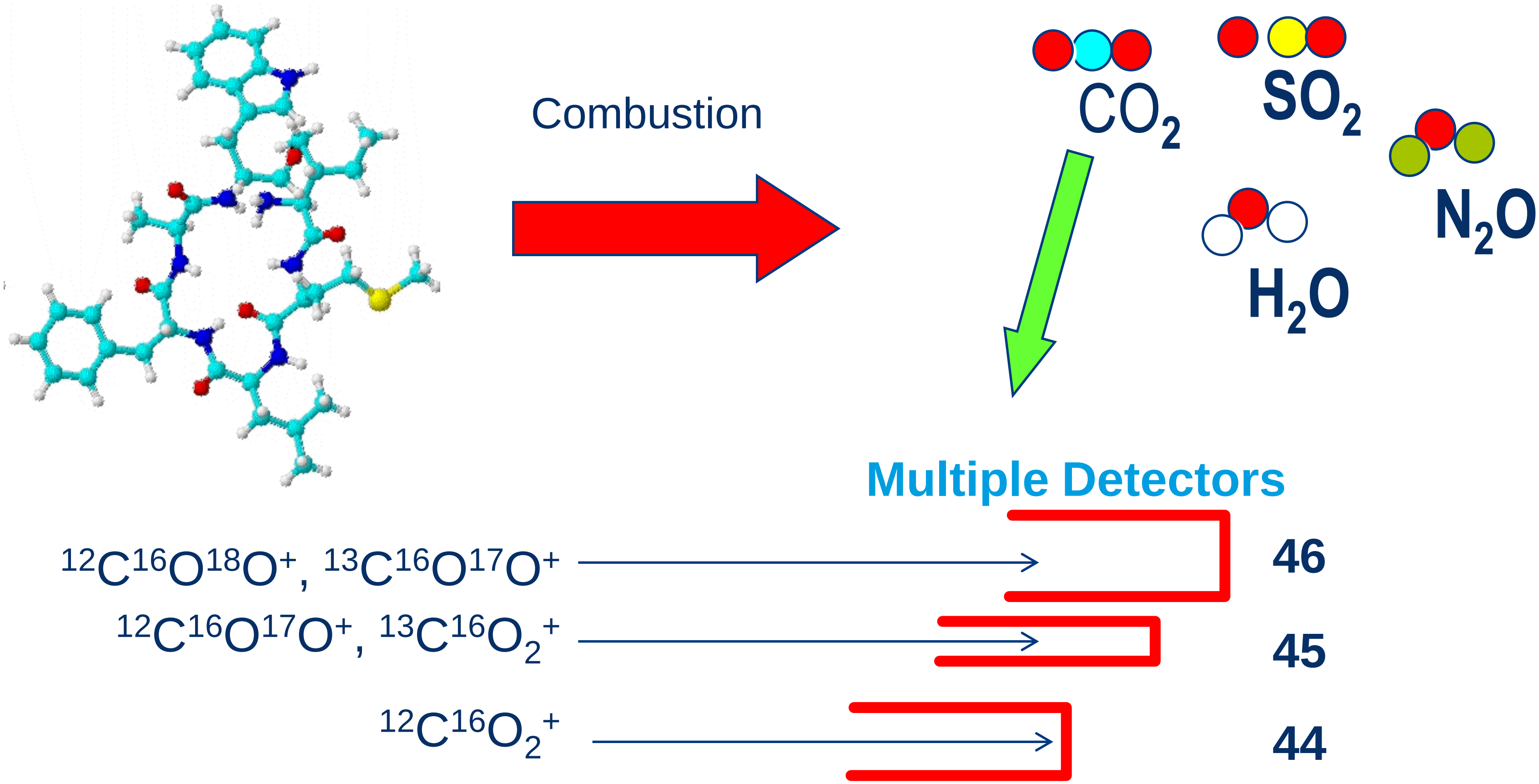
Use of artificially enriched compounds in D, ^{13}C, ^{15}N, ^{18}O

↓

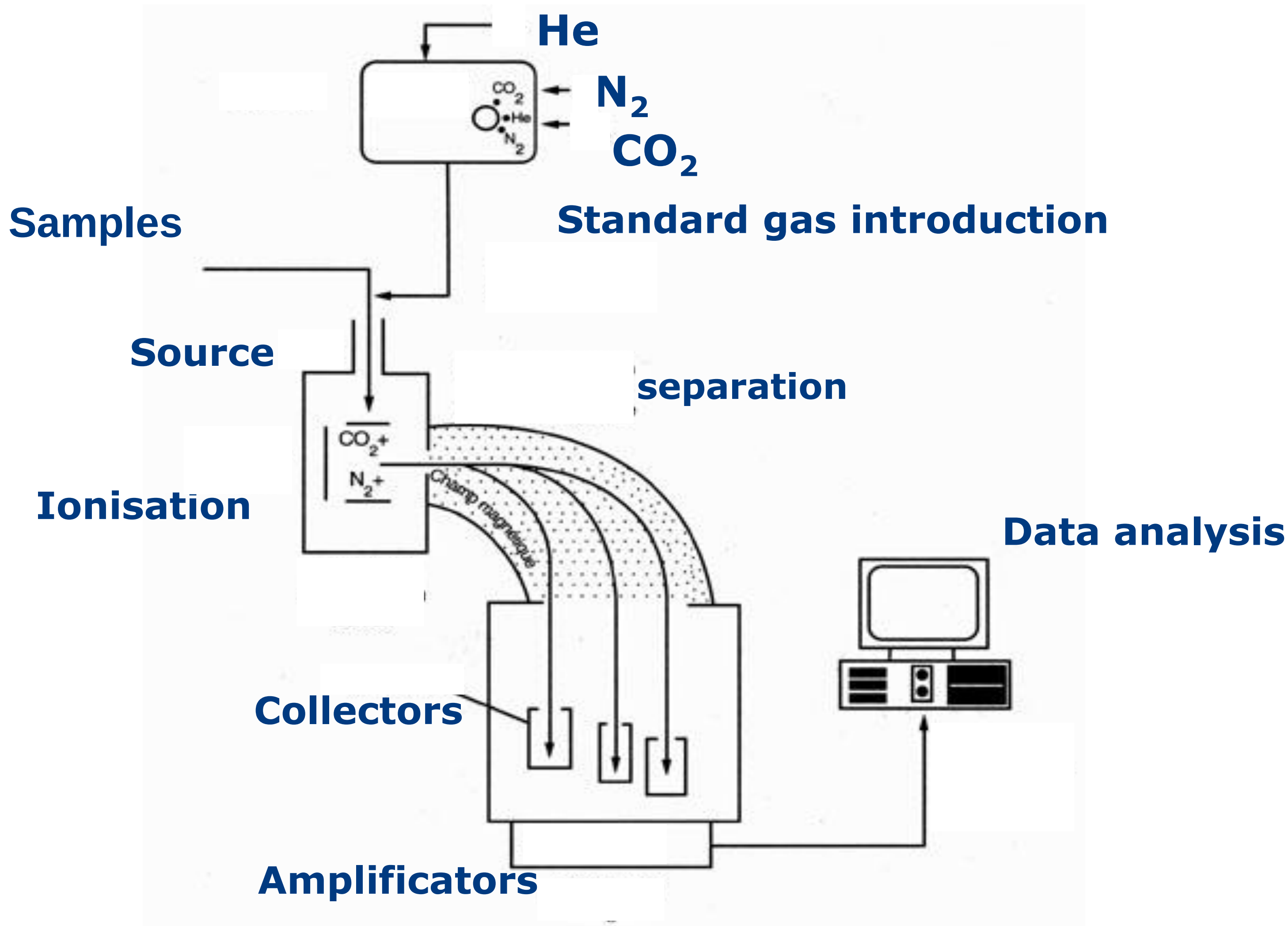
Compounds fate (Transfer, degradation…)


Ionisation
Molecular Ion

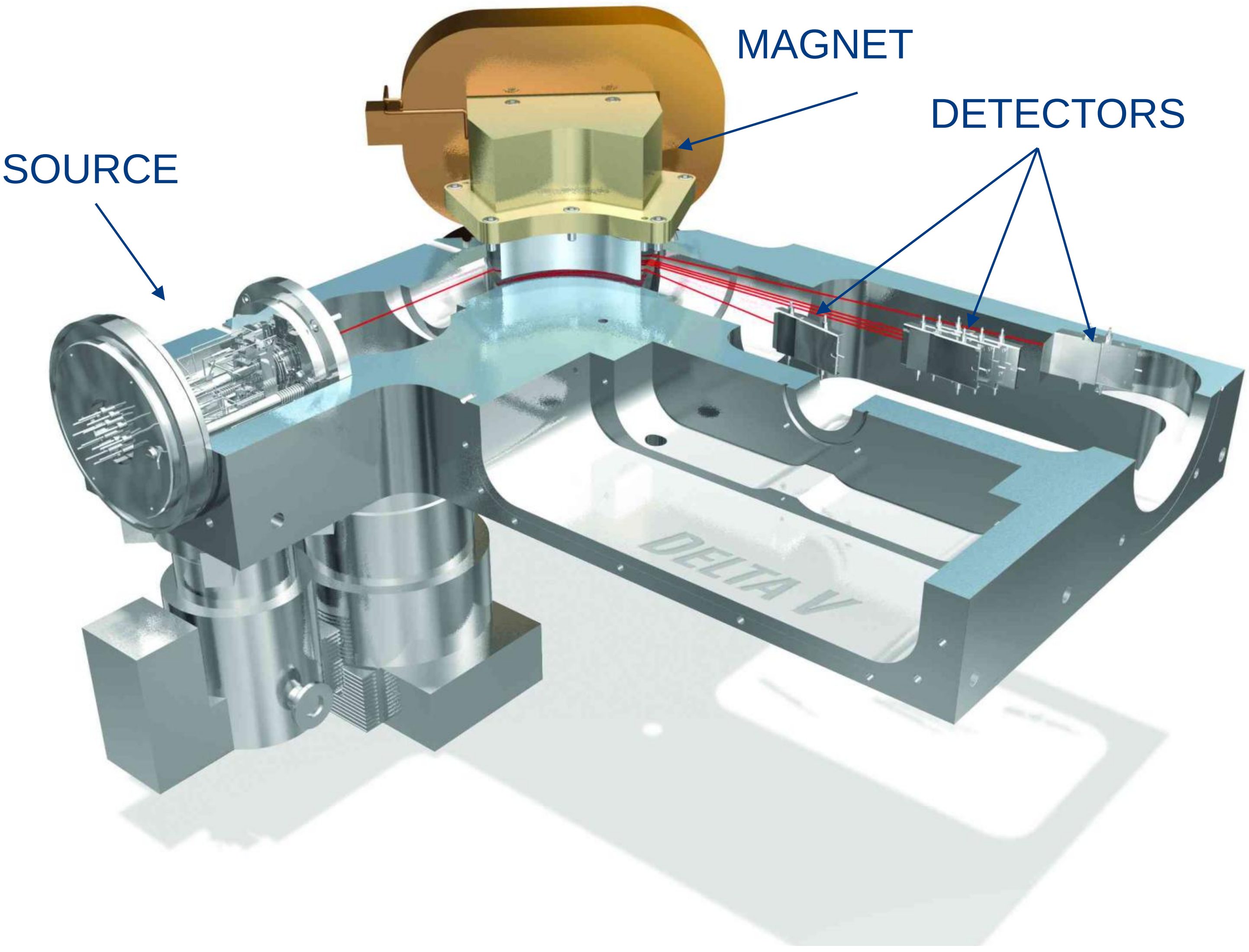

100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
m/e
CH₃
Toluène PM = 92
pic de base
ion moléculaire


Combustion
CO_2
SO_2
N_2O
H_2O
Multiple Detectors
$^{12}C^{16}O^{18}O^{+}$, $^{13}C^{16}O^{17}O^{+}$
46
$^{12}C^{16}O^{17}O^{+}$, $^{13}C^{16}O_2^{+}$
45
$^{12}C^{16}O_2^{+}$
44

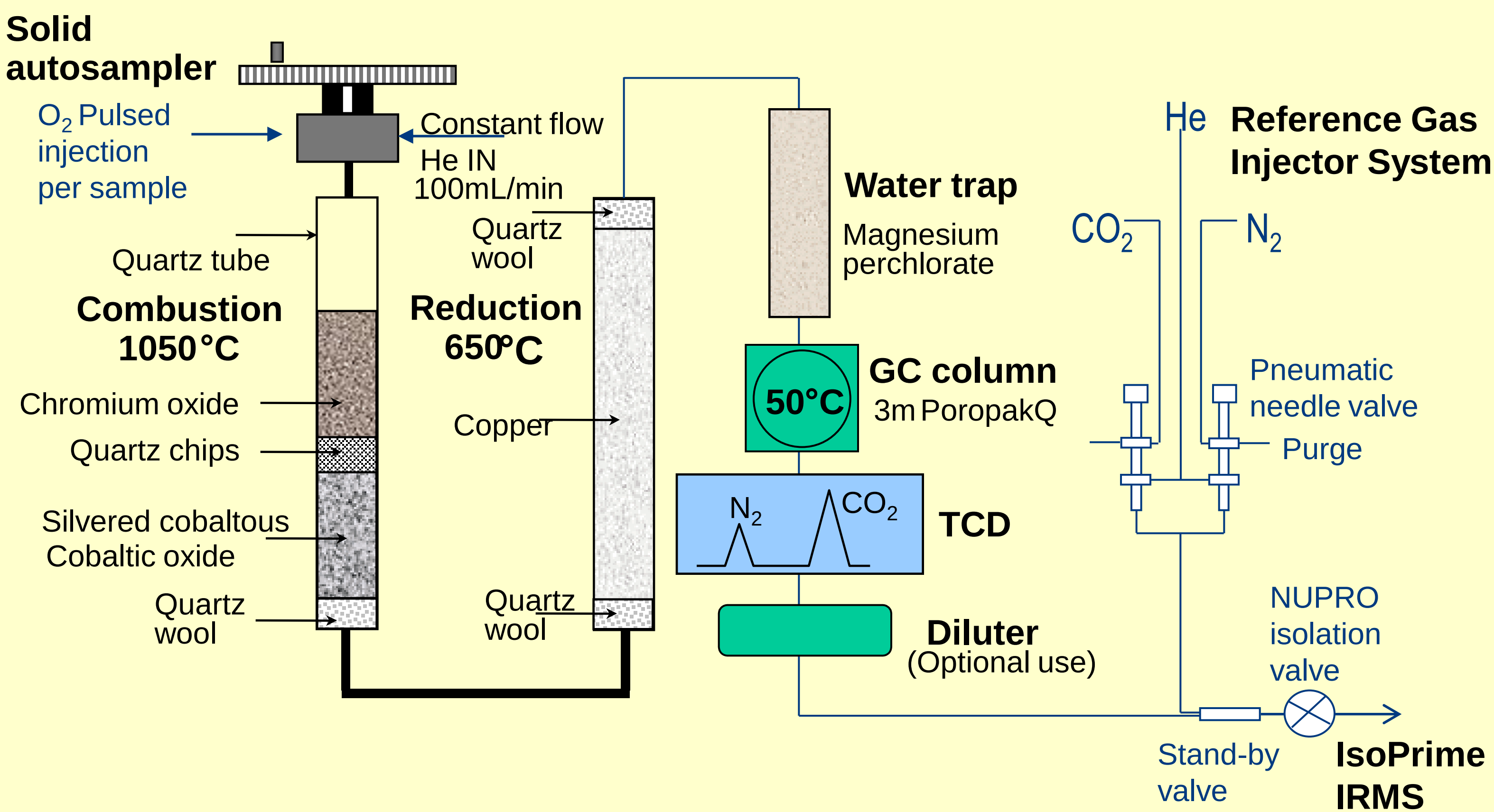
ISOTOPIC MASS SPECTROMETRY



SCHEMES OF AN IRMS

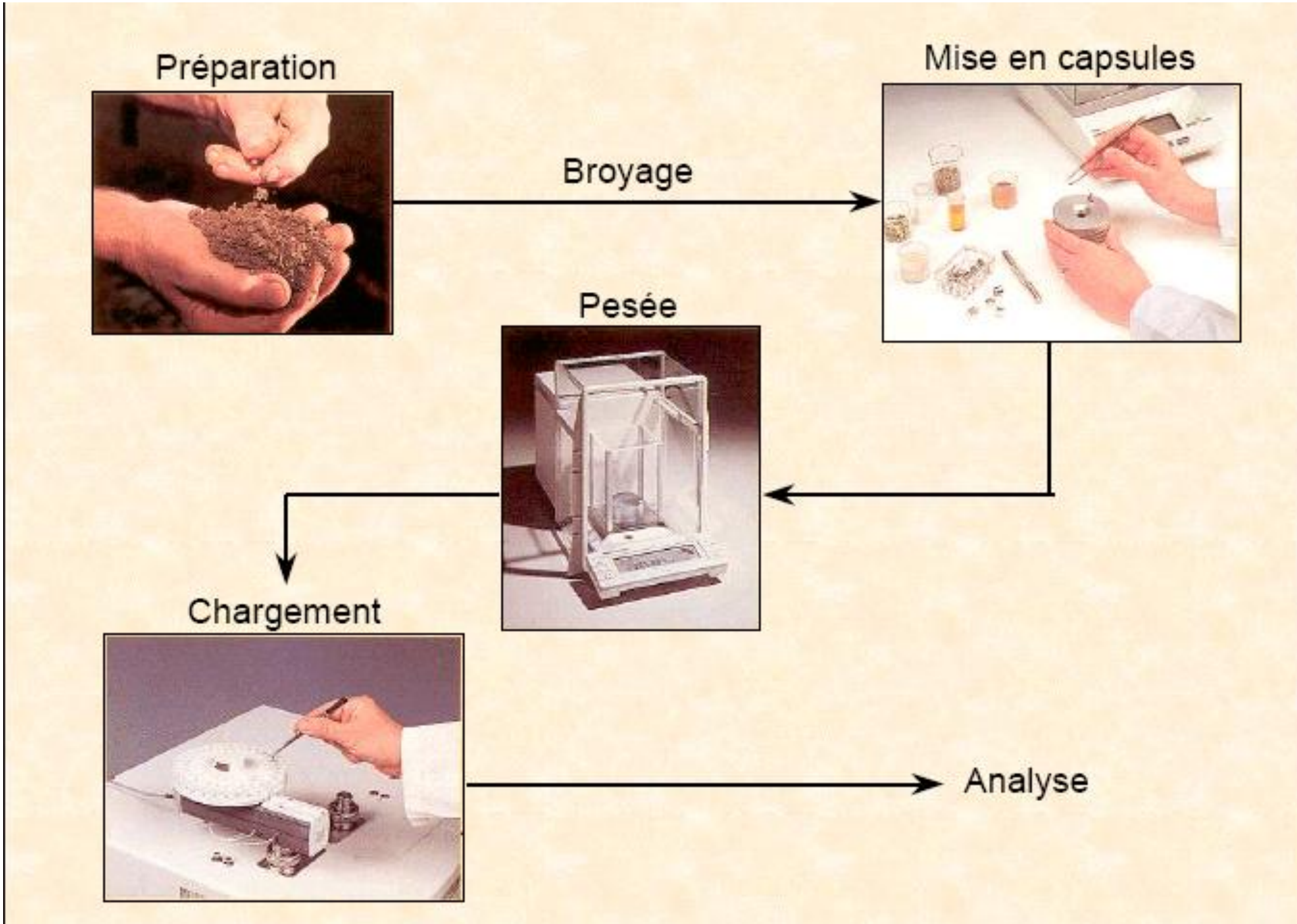


EA Carbon and Nitrogen combustion - solids

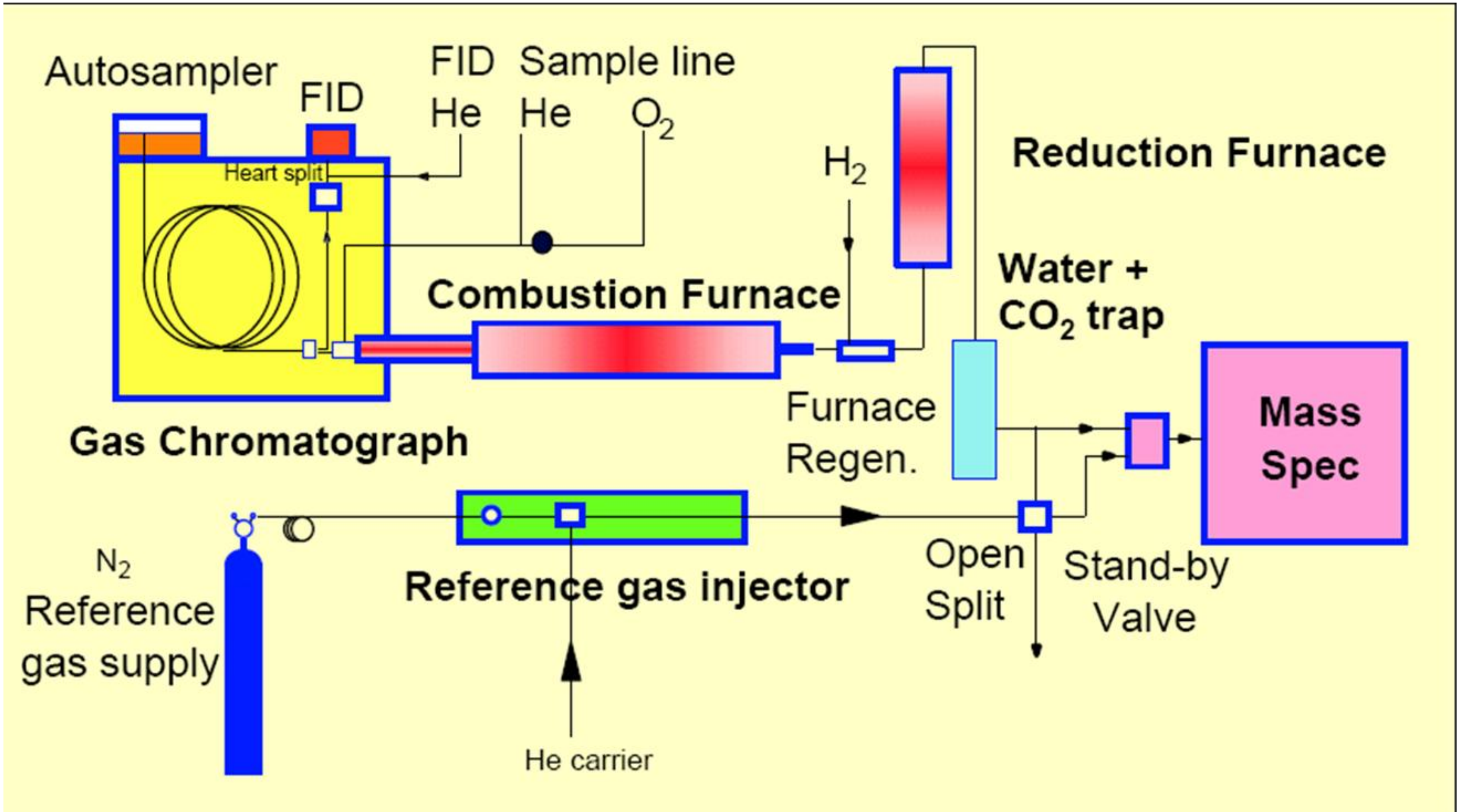


Amount of carbon needed : 50 µg

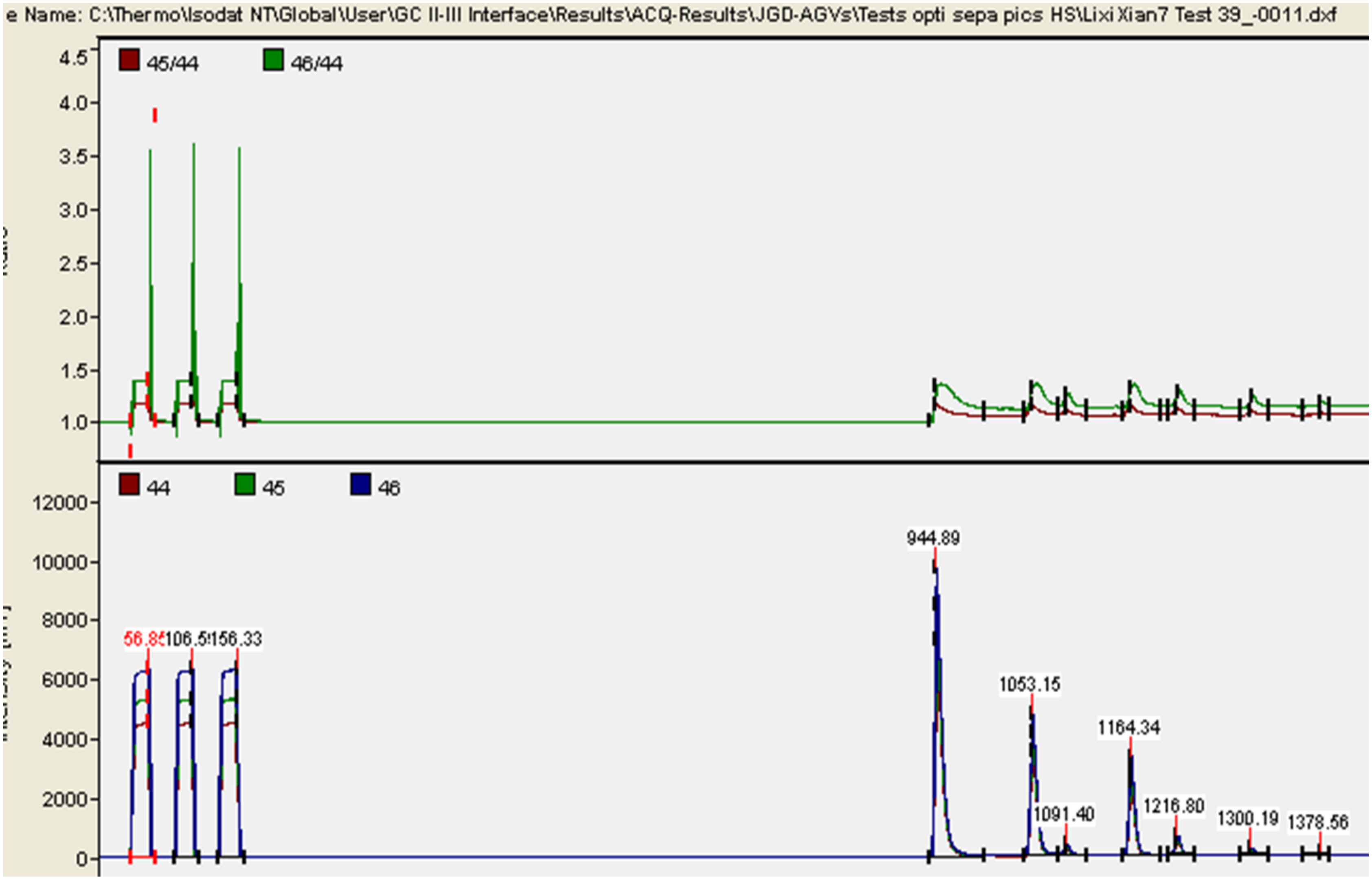
EA-IRMA SAMPLE PREPARATION



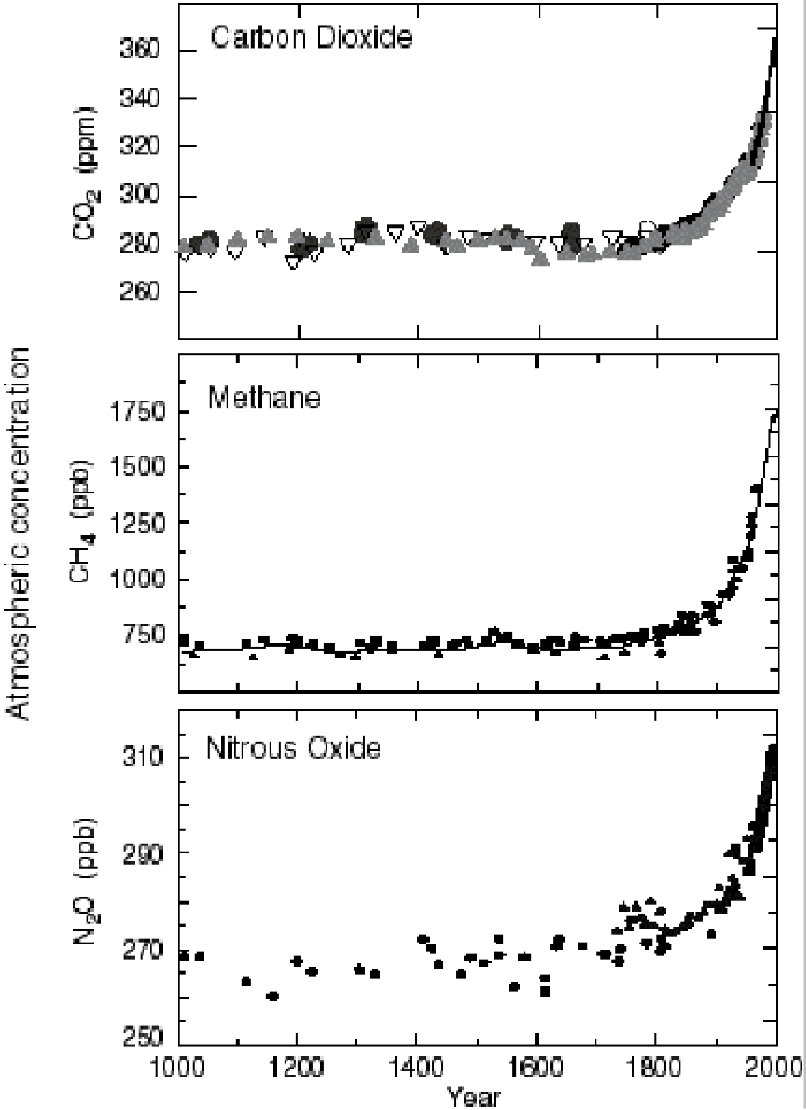
COUPLING WITH GAS CHROMATOGRAPHY



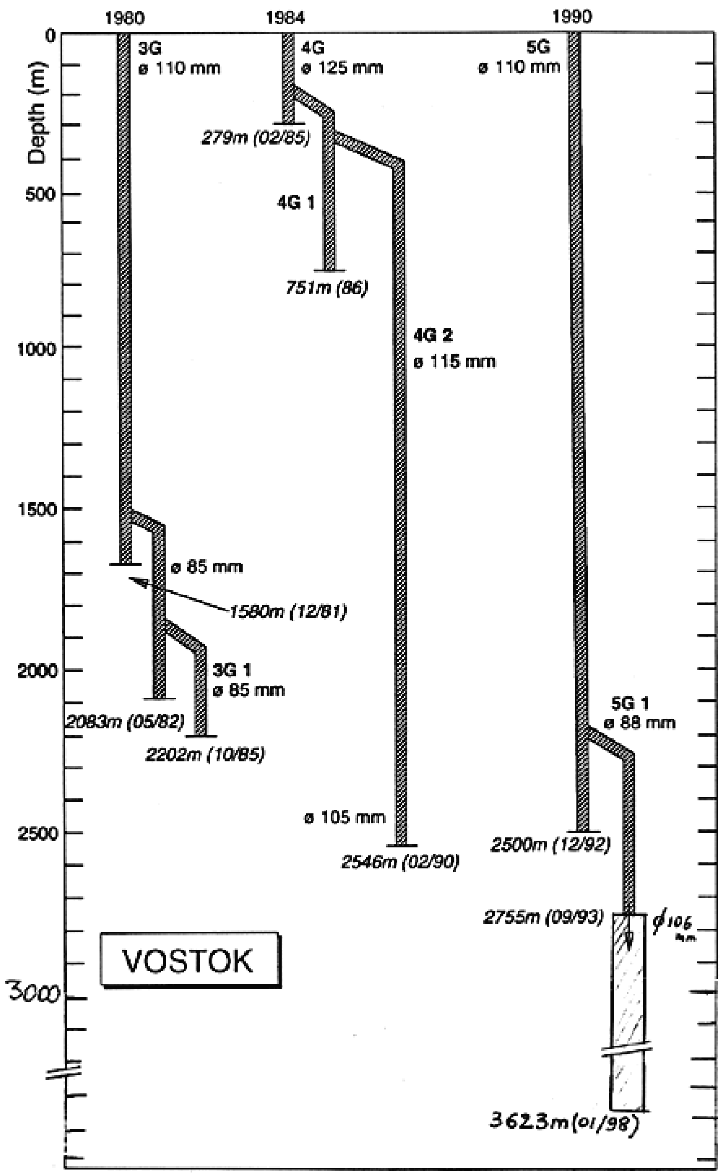
EXAMPLE OF CHROMATOGRAM



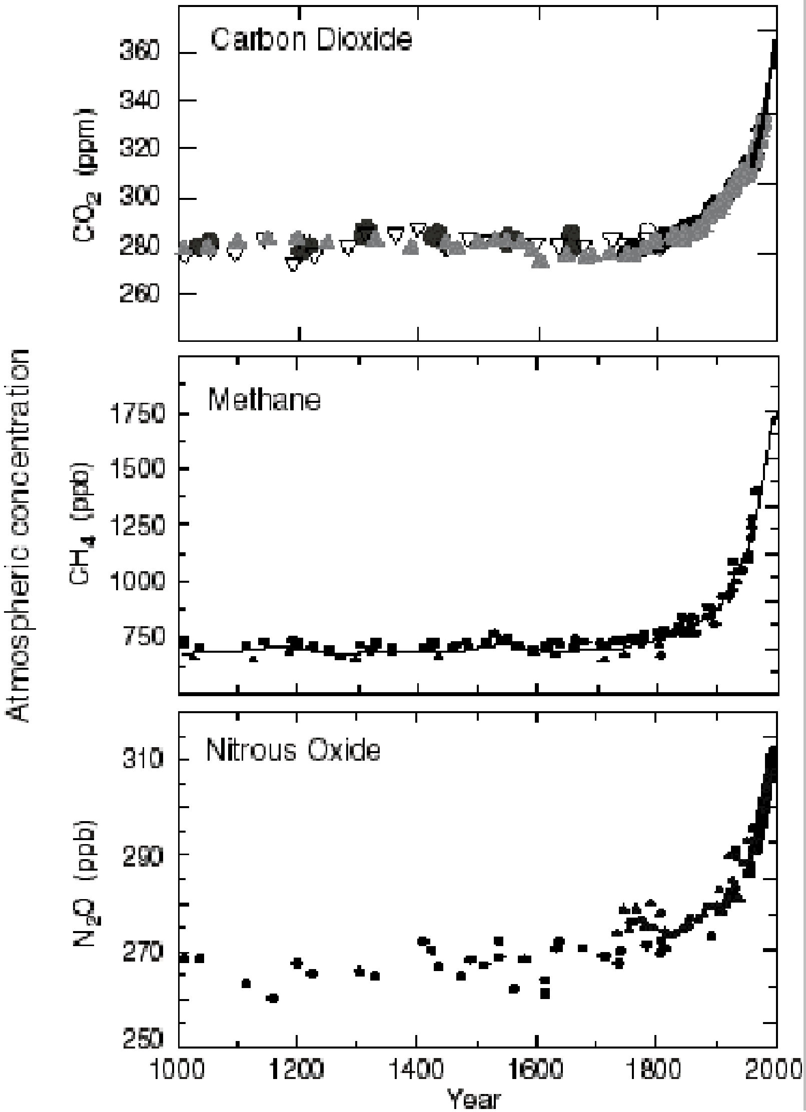
INCREASE IN GHG CONCENTRATION

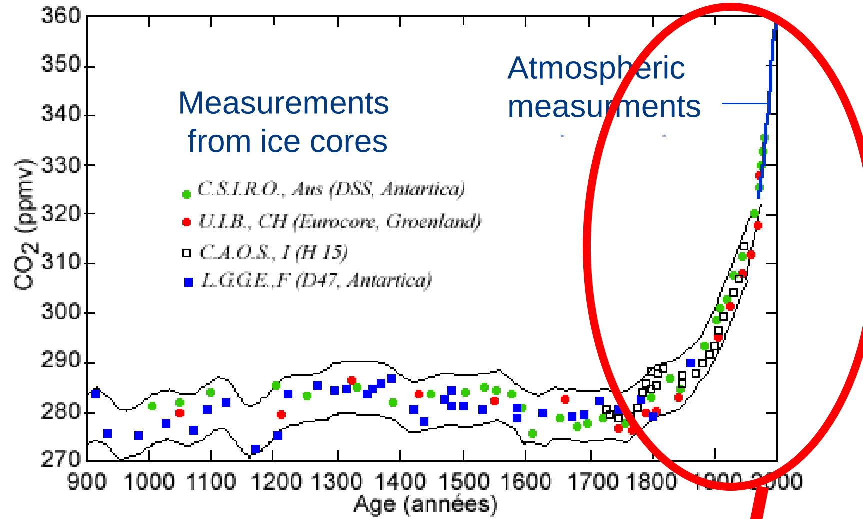


Ice : greenhouse gas archives

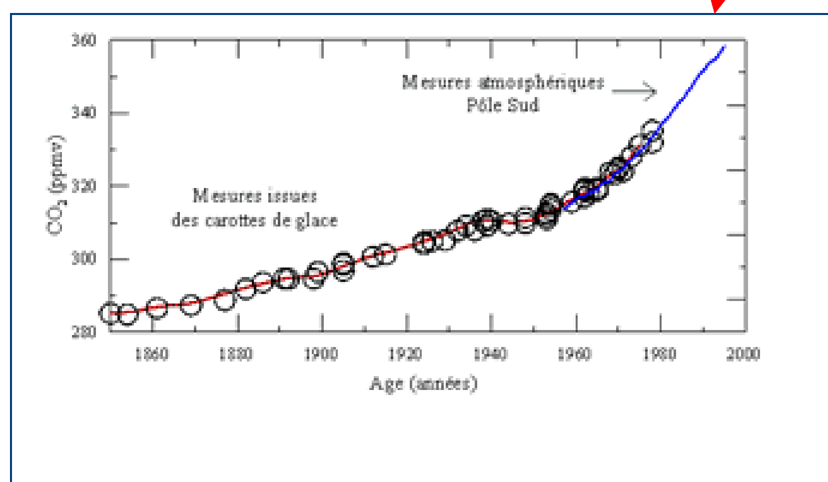


INCREASE IN GHG CONCENTRATION

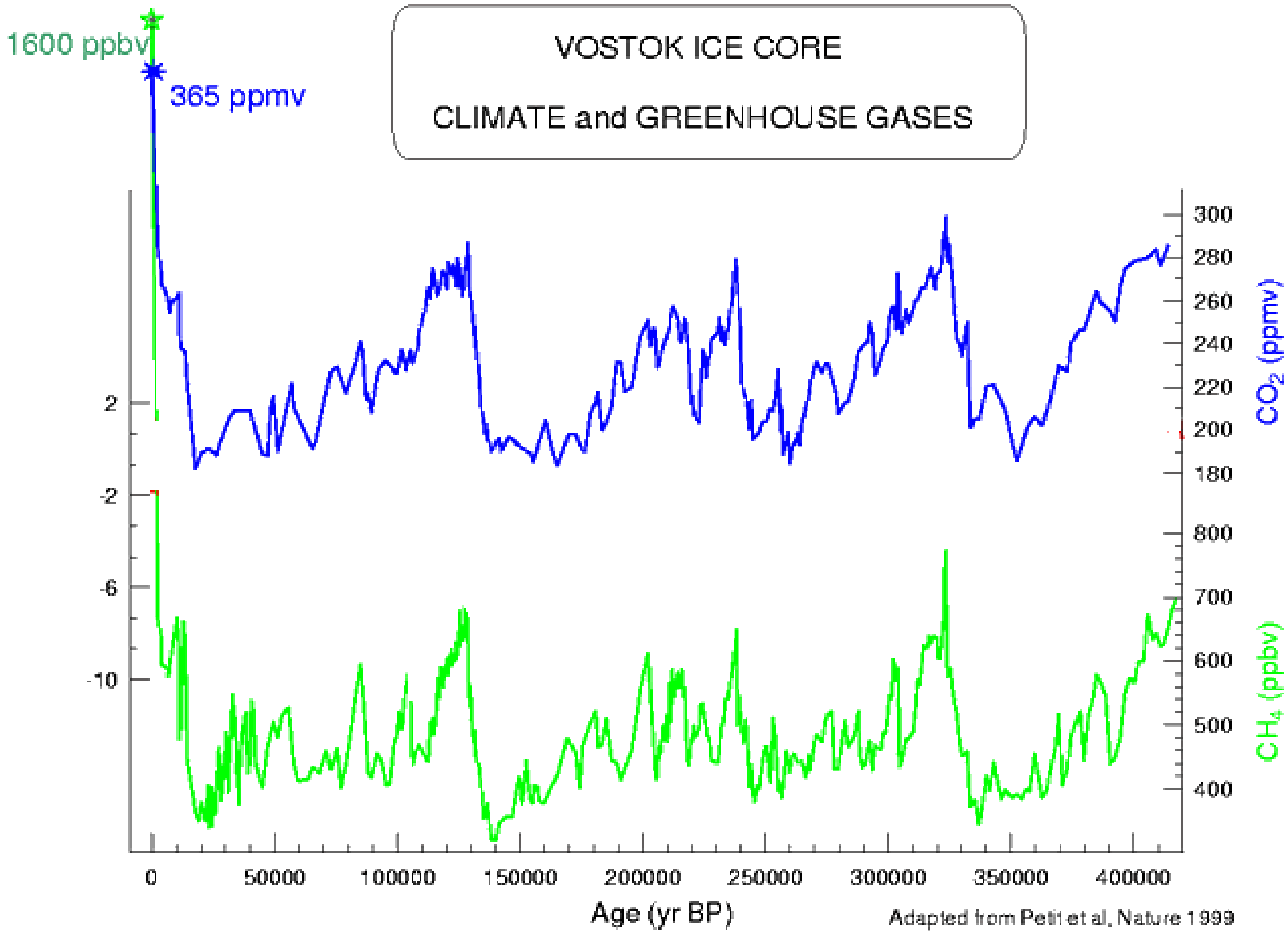




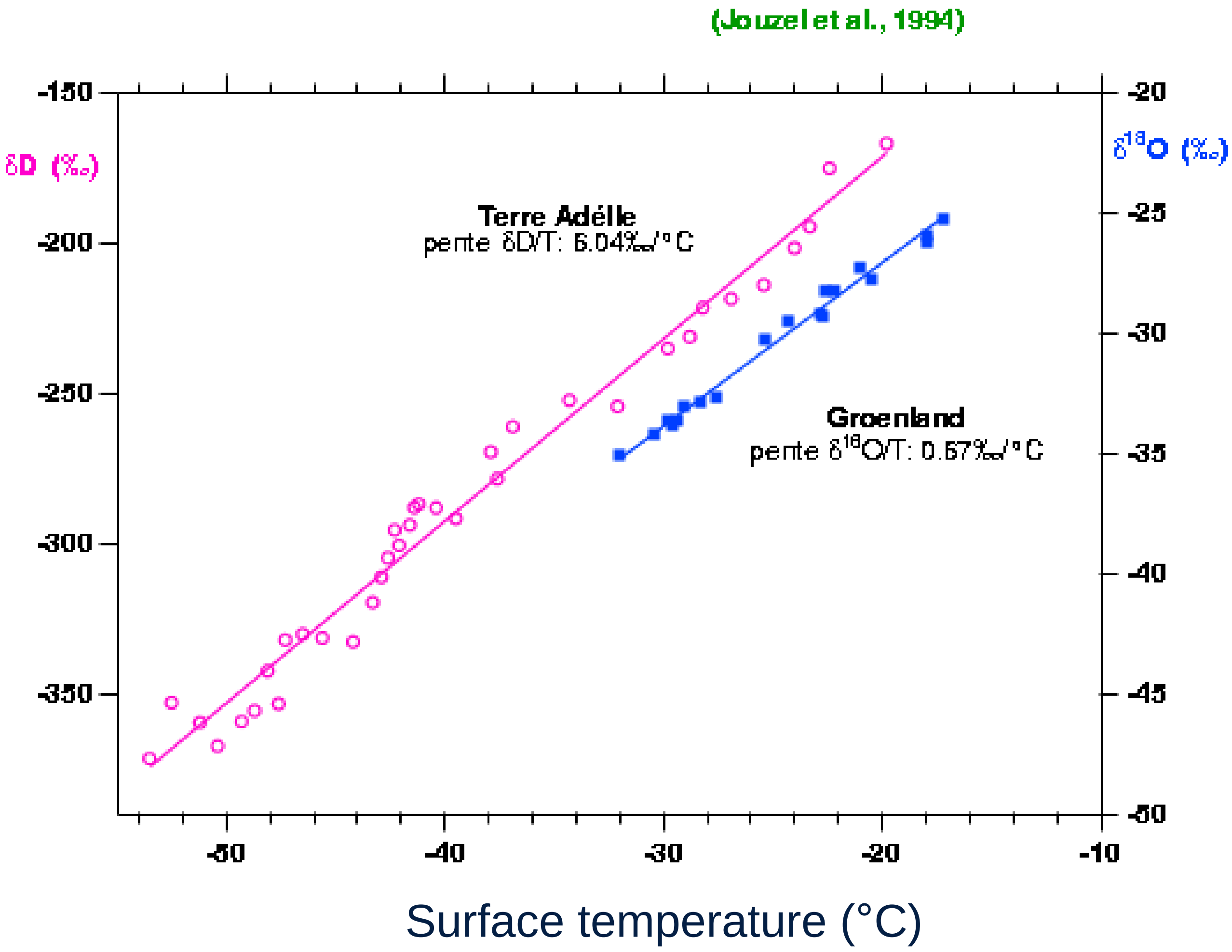
Compilation d'aprés J.M. Barnola et J. Chappelaz (LGGE), figure extraite et modifiée à partir du site http://www.balzan.it/english/pb2001/lorius/sintesi.htm

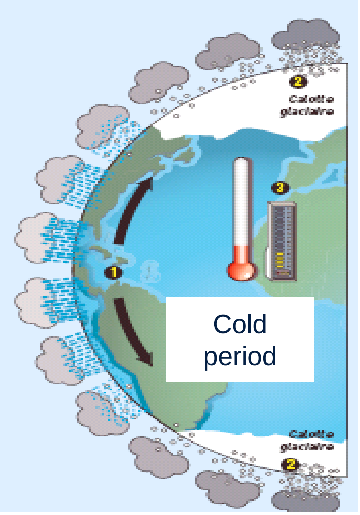


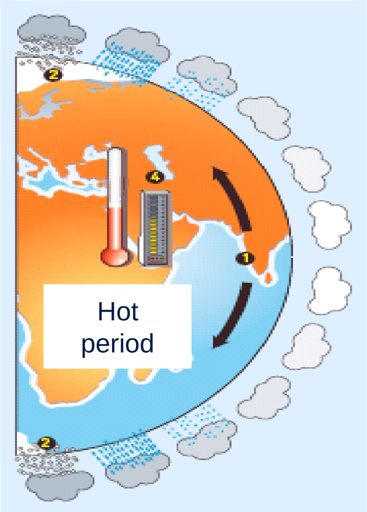
GHG concentration evolution



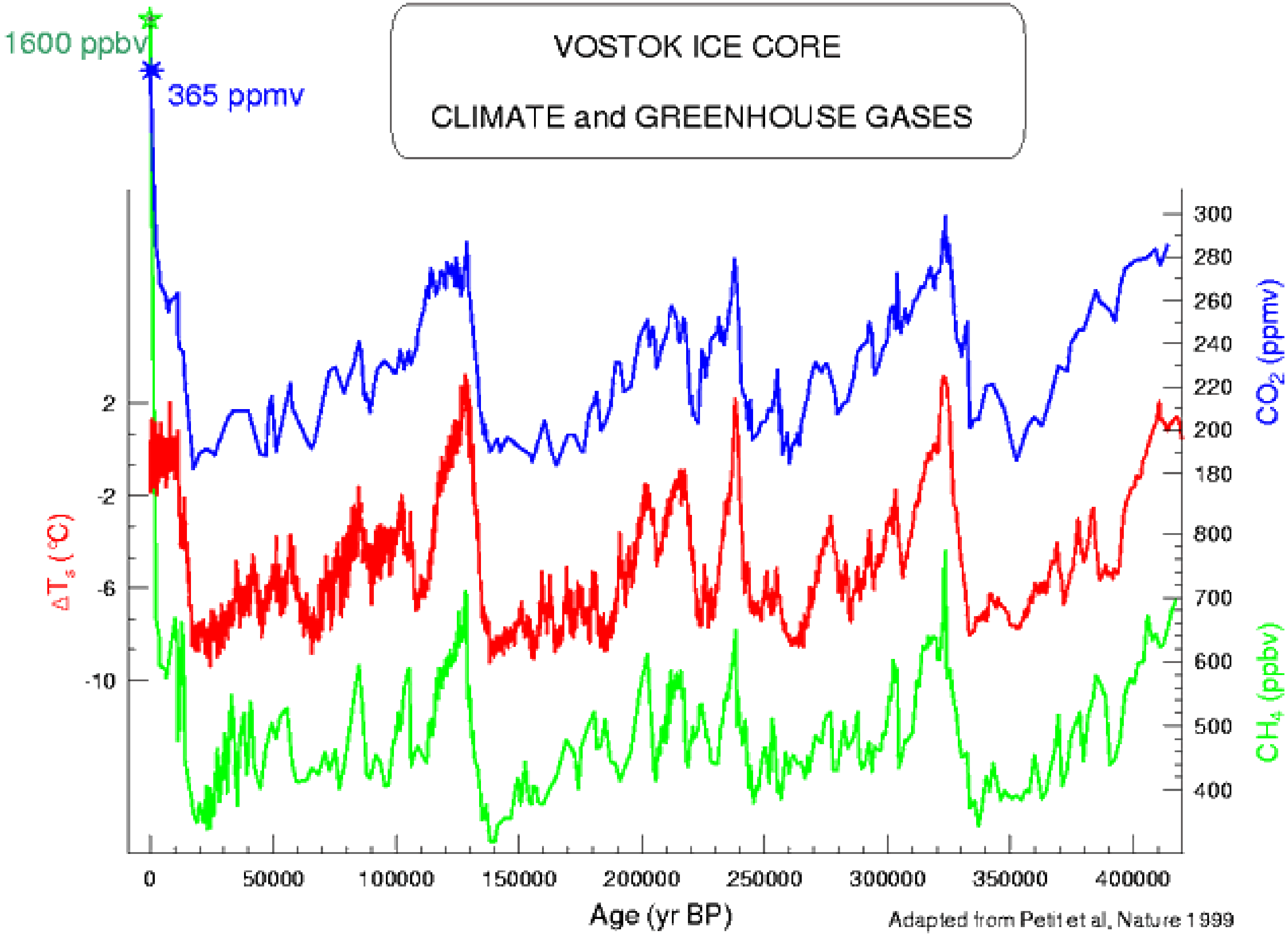
δ D et δ ^{18}O: isotopic thermometer







Temperature evolution



Adapted from Petit et al, Nature 1999

Origin of CO_2

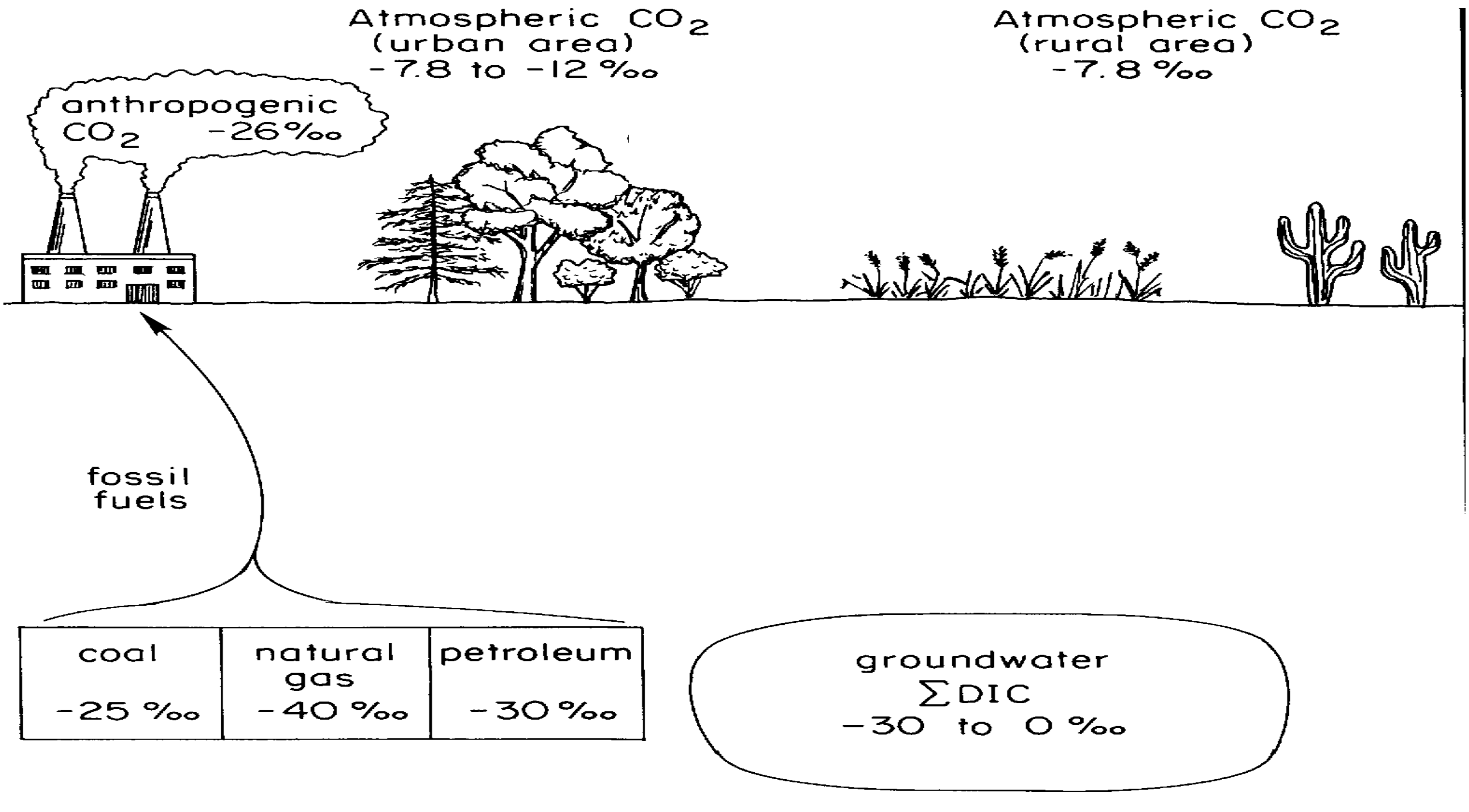
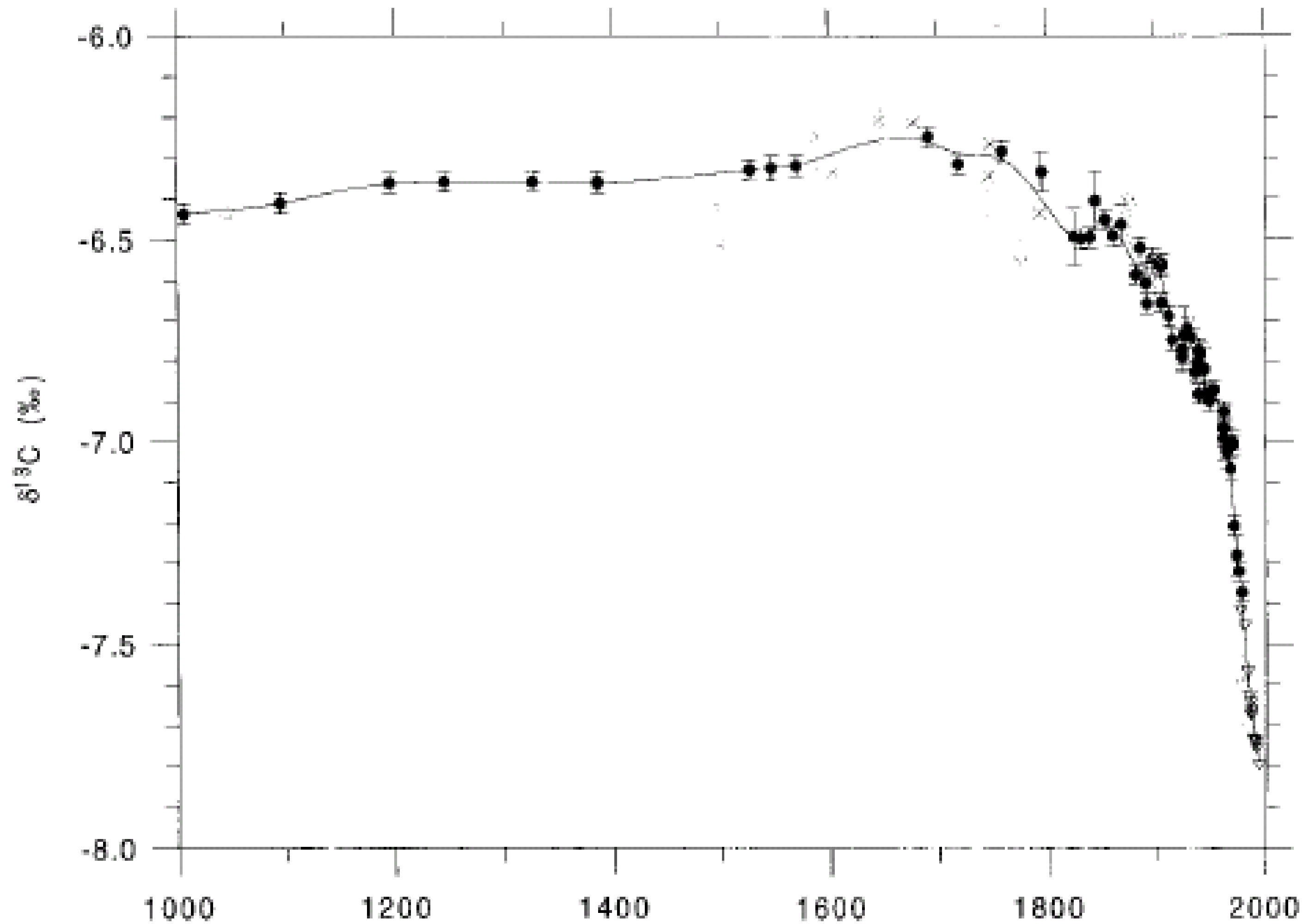


Fig. 1 Stable carbon isotope ratios of major components of terrestrial ecosystems

Evolution of the $\delta^{13}C$ atmospheric CO_2



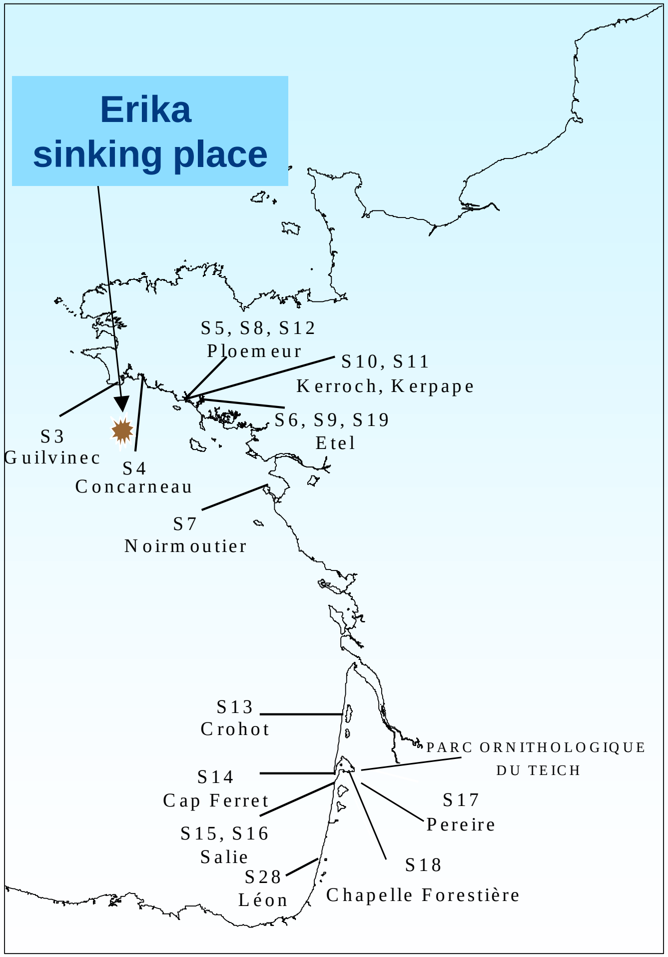
Samples collected after the Erika oil spill

Oil carried by the oil tanker Erika (S1)

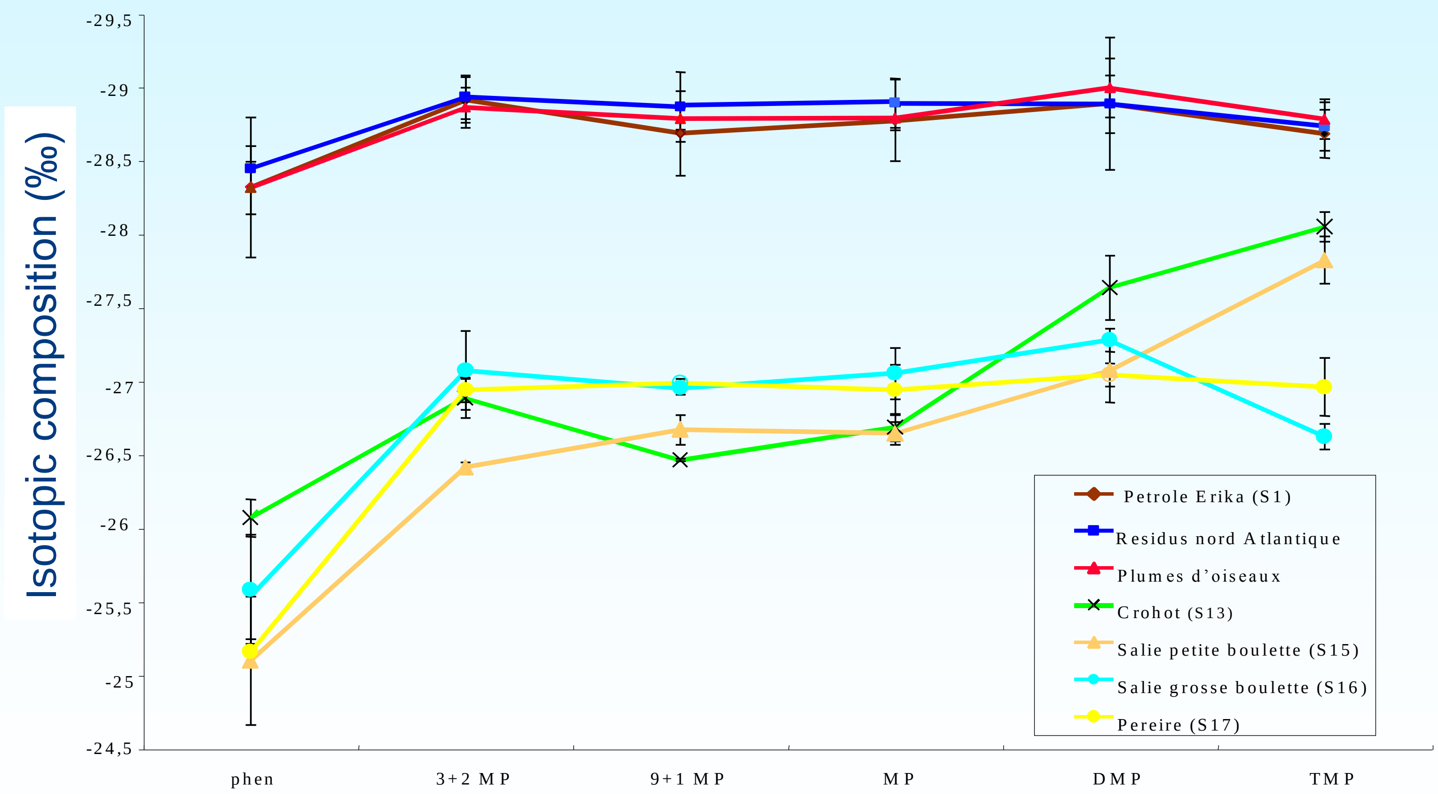
1 sample from an oil slick

16 oil residues (S3 to S18)

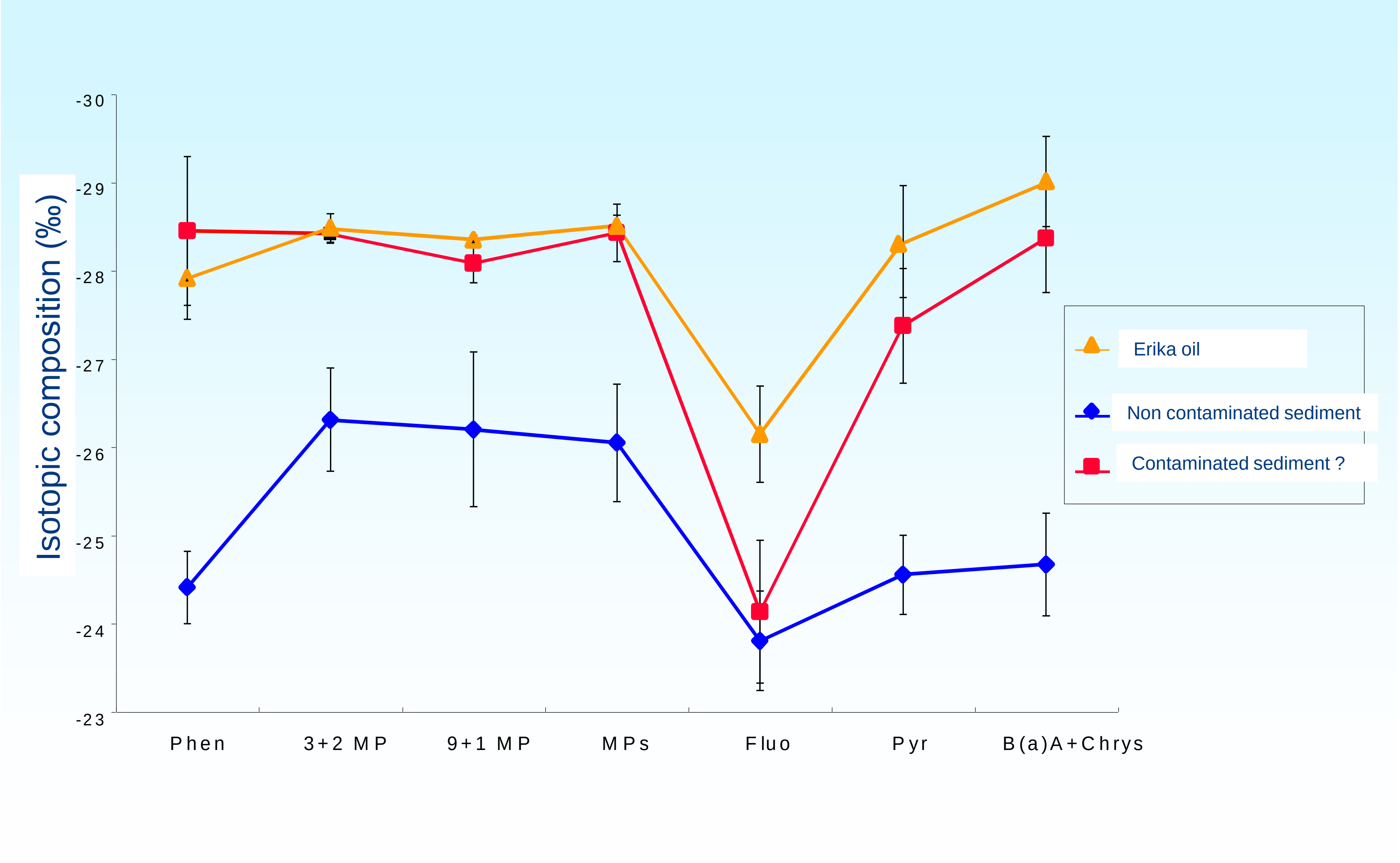
10 Oiled bird feather (S19 to S28)



Phenanthrenic isotopic compositions

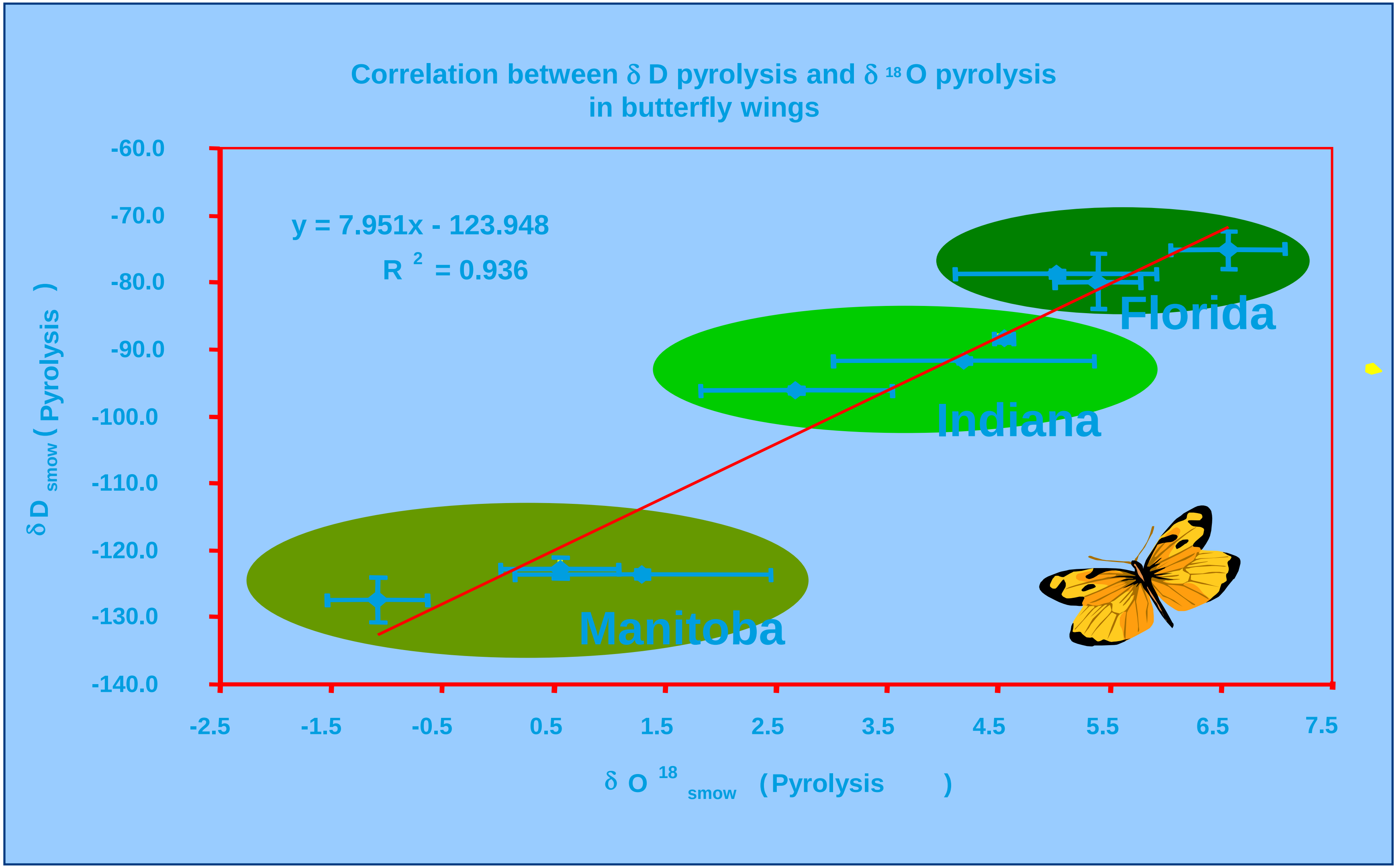


Sediment contaminated by the Erika oil ?



MIGRATION OF BUTTERFLIES

MIGRATION OF BUTTERFLIES

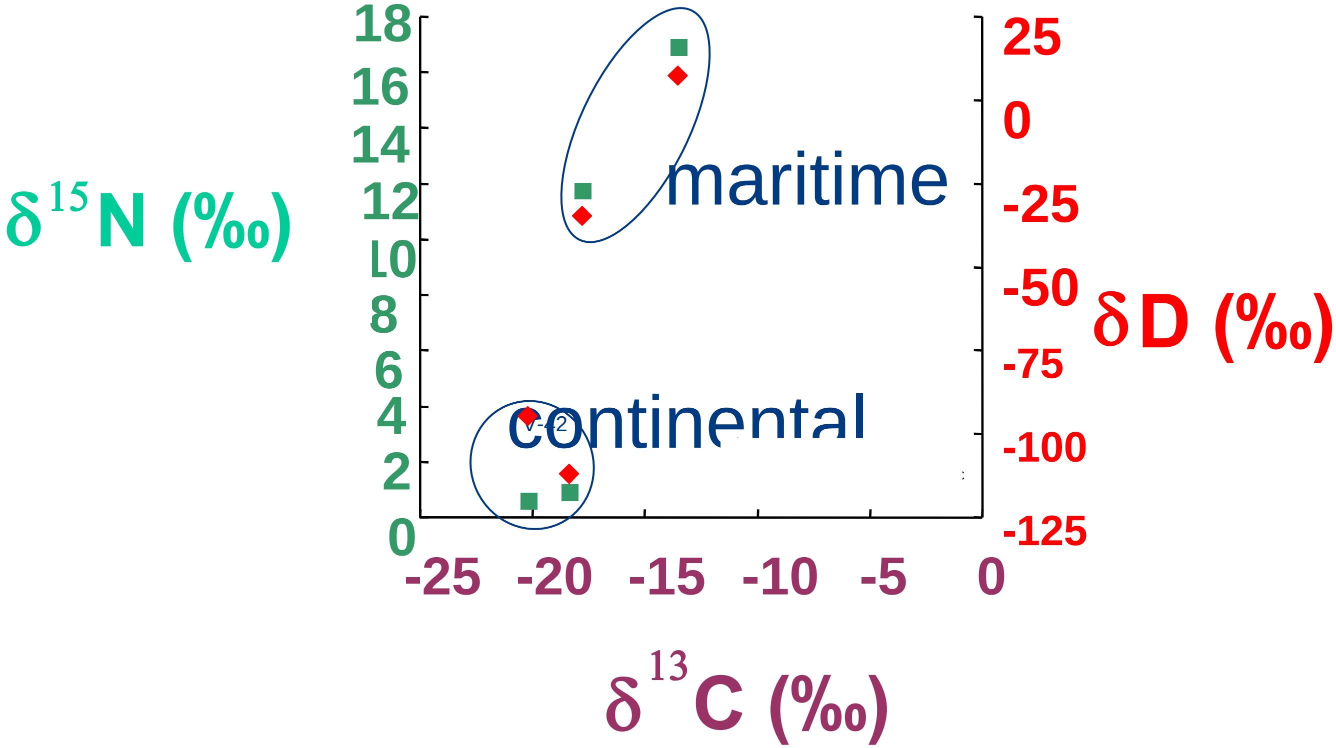


You are what you eat !

Isotopic analysis of Viking fingernails

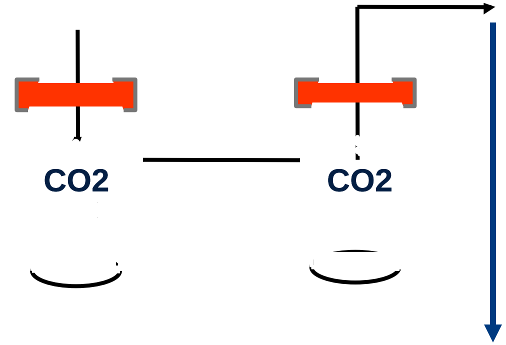
$\delta^{15}N$ $\delta^{13}C, \delta D$

TC/EA & EA

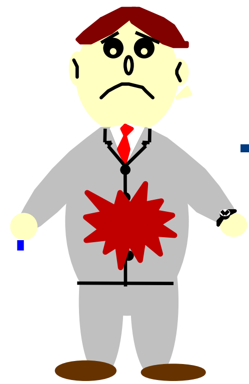


Respiratory isotopic test

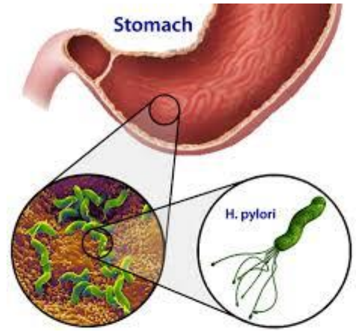
Sampling of exhaled air

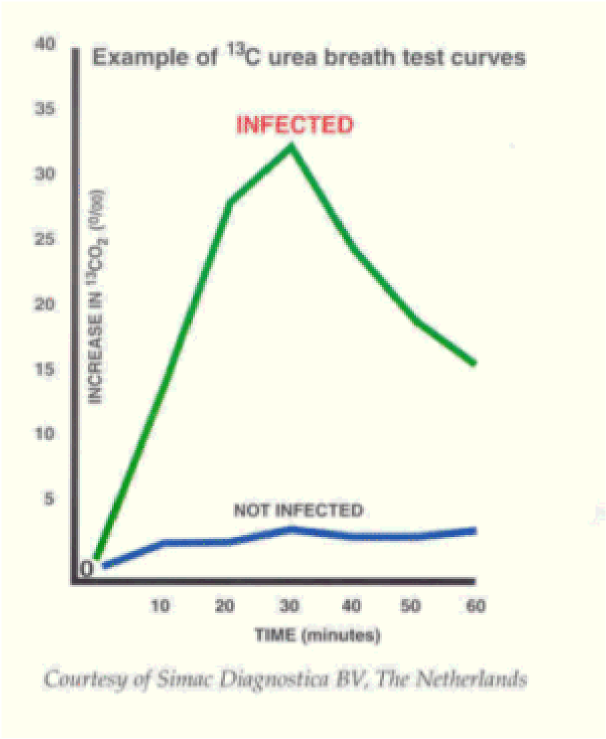


Stressed ?



ulcer ?

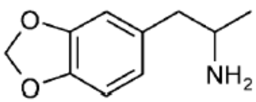




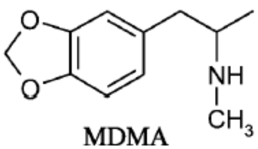
^{13}C enriched compound
(Urea)

DETERMINATION OF THE ORIGIN OF EXTASIA

Isotopic analysis of Ecstasy



MDA



MDMA

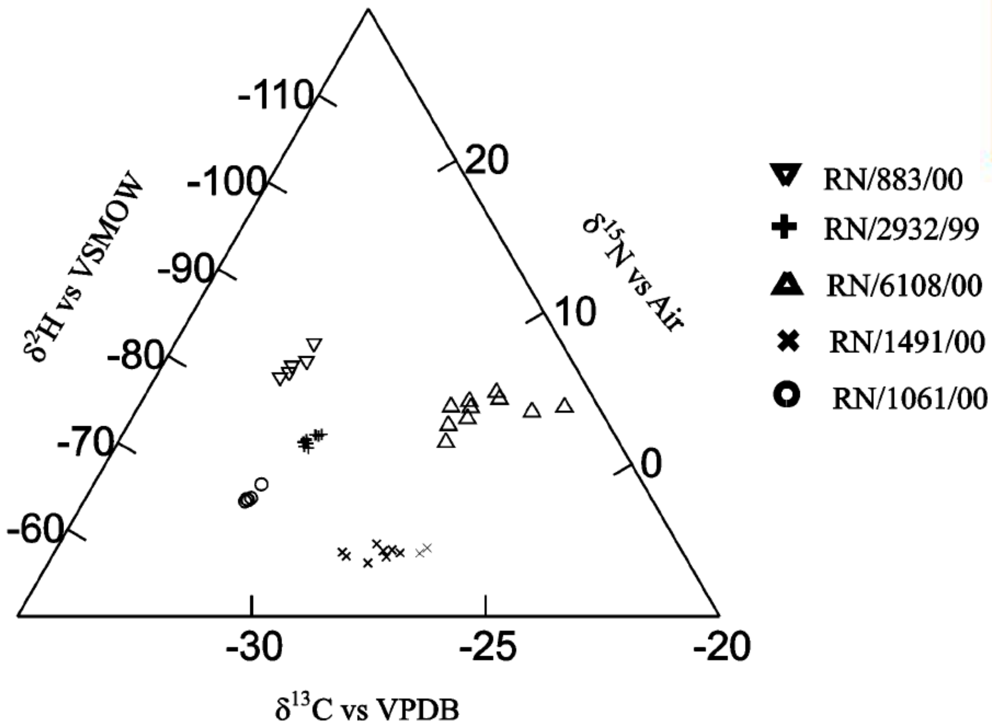
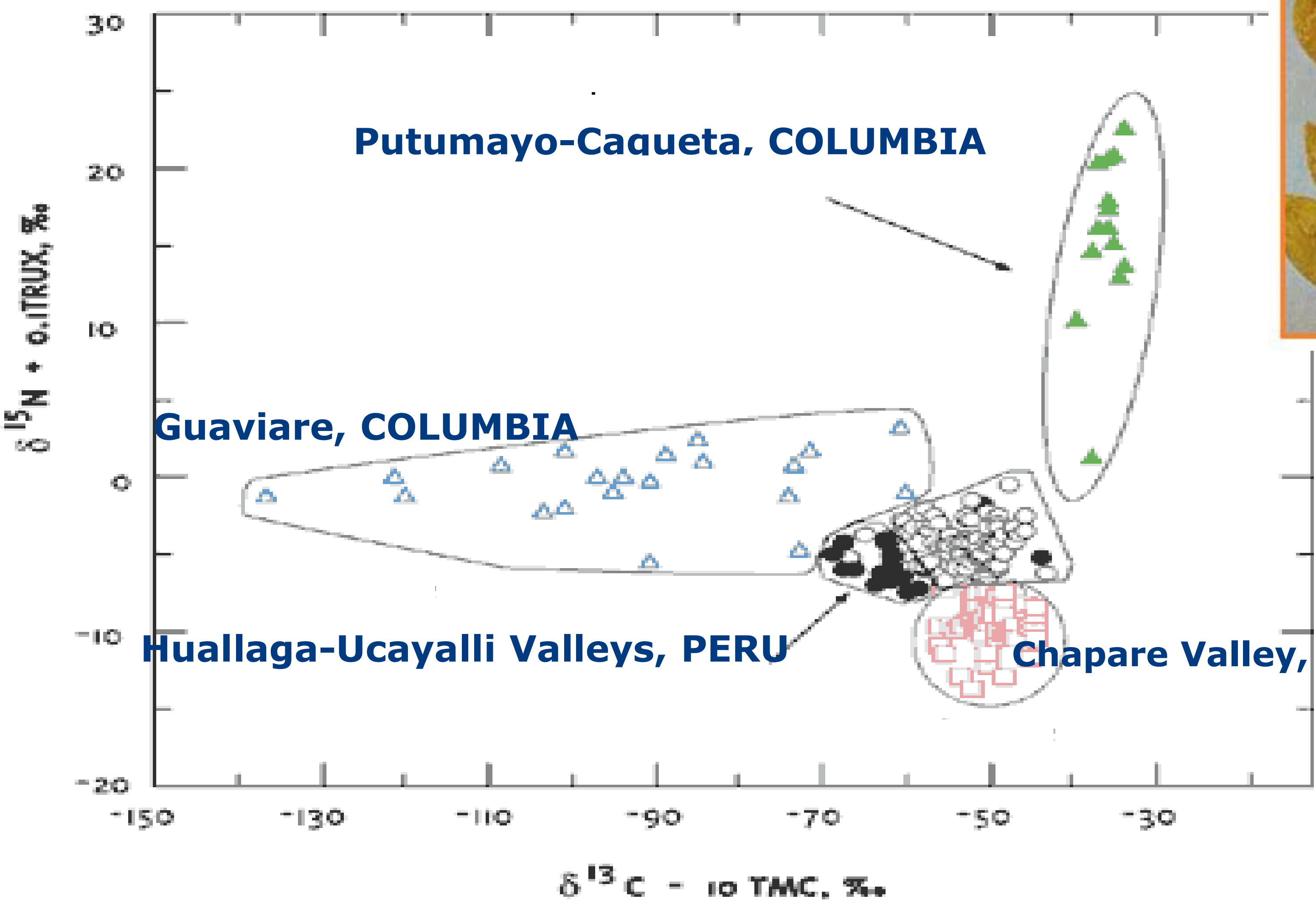


Fig. 3 Combined isotopic data for five tablet batches.

James F. Carter et al., Analyst, 2002, 127, 830–833

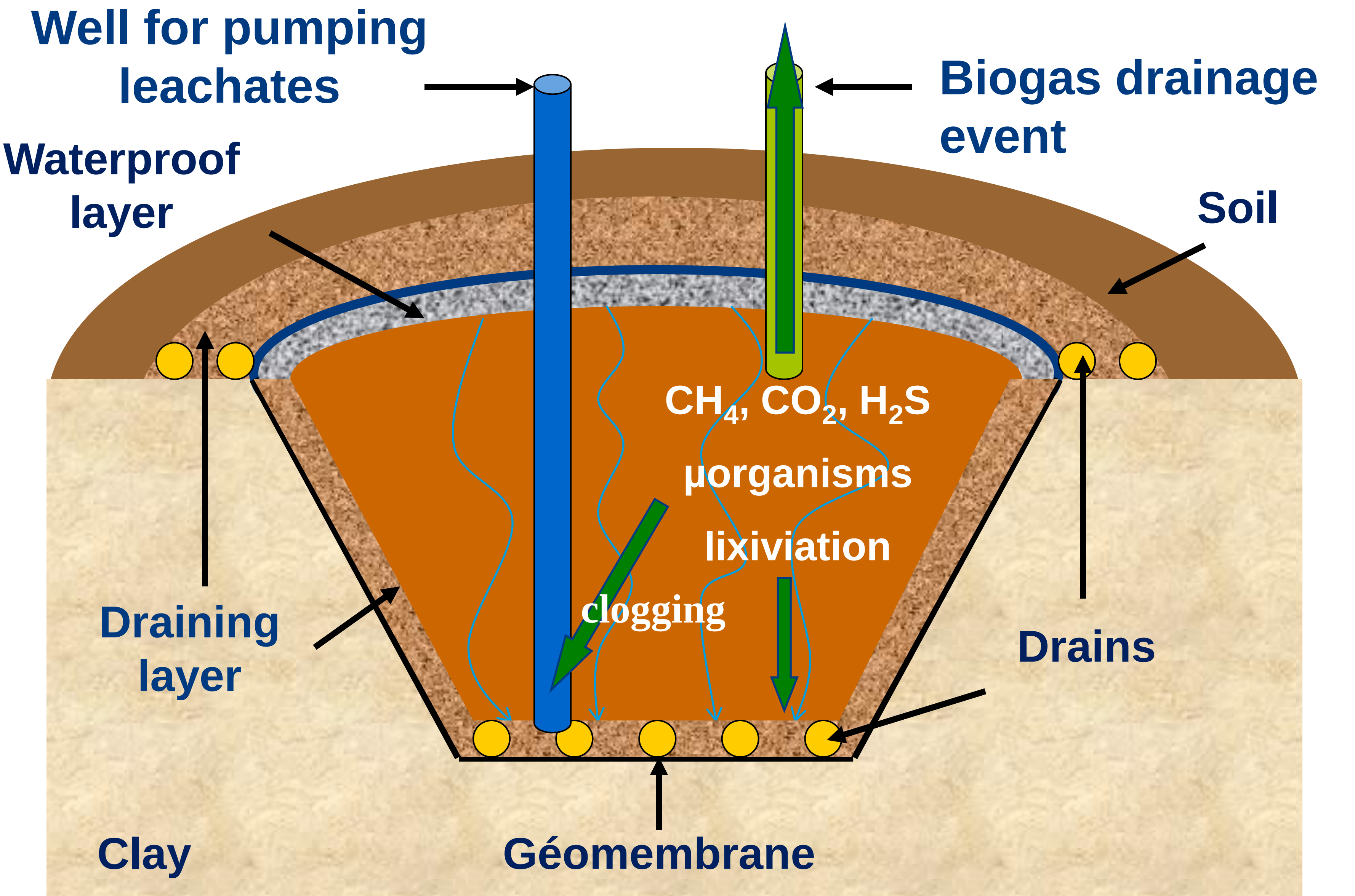
ORIGIN OF THE COCAINE



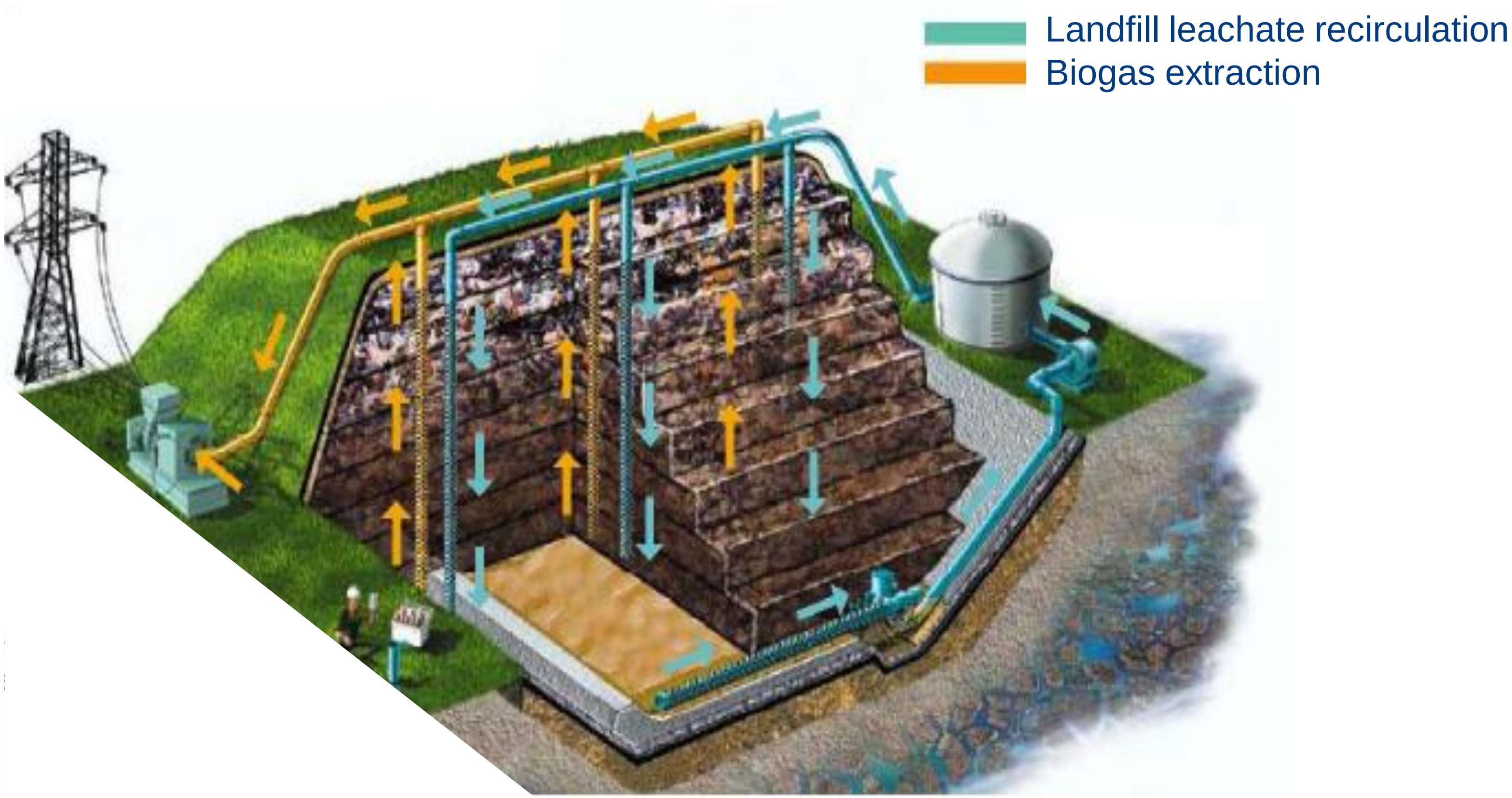
Application to environnemental bioprocesses

Municipal solid waste landfill

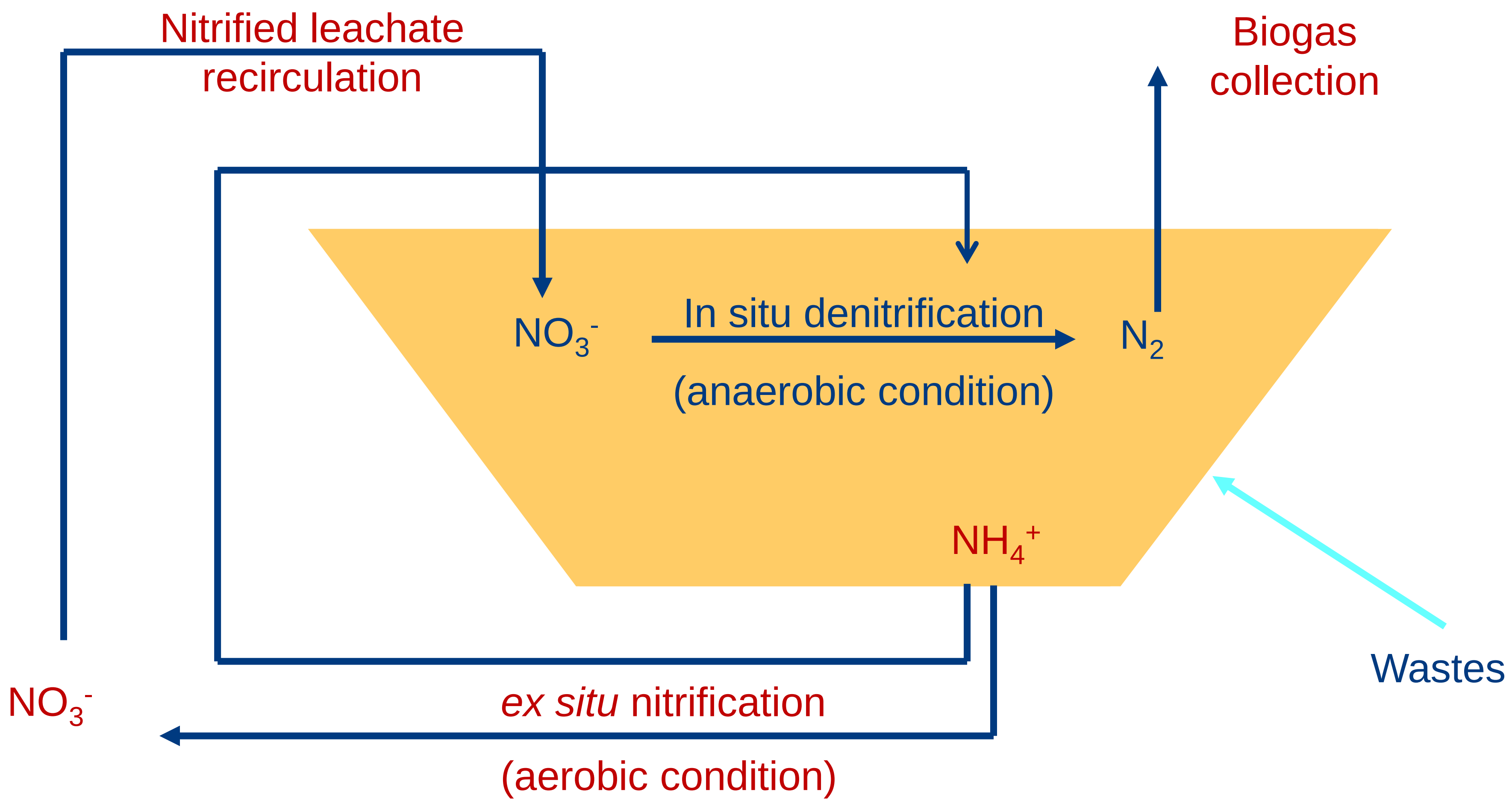
Municipal solid waste landfill



Bioreactor Municipal solid waste landfill



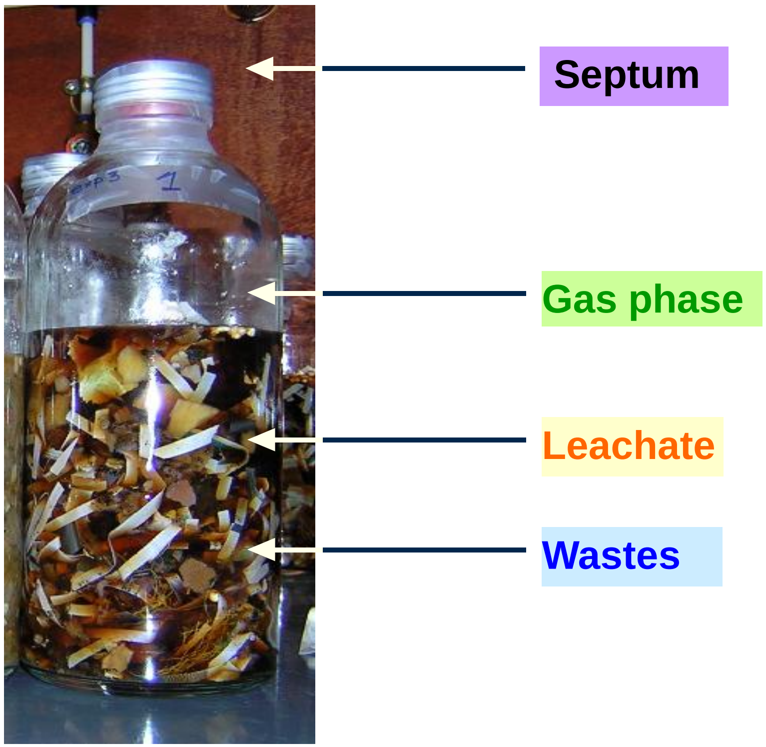
Bioreactor Municipal solid waste landfill



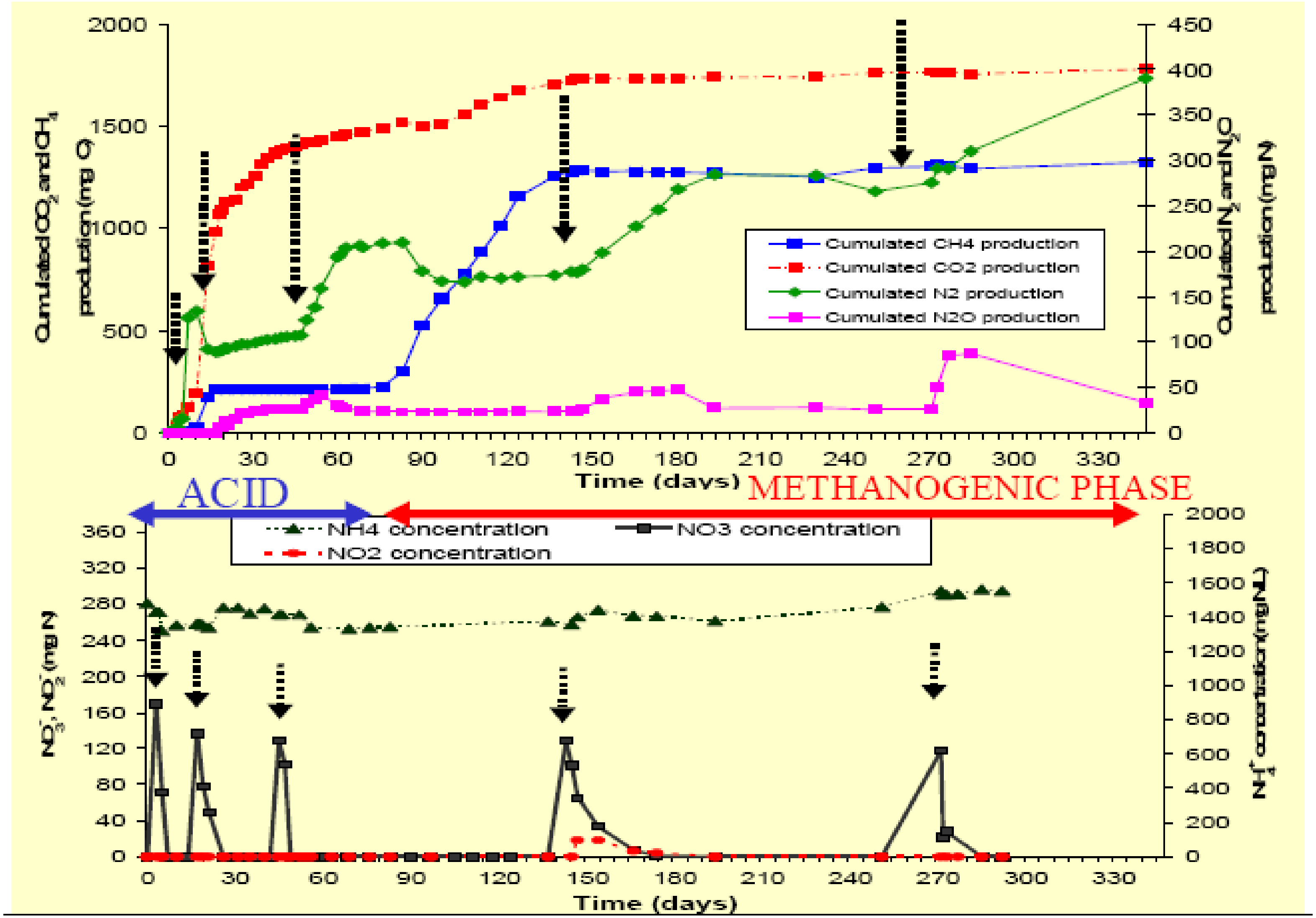
Strategy: leachate pretreatment before recirculation

Experimental set up

Anaerobic batch experiment



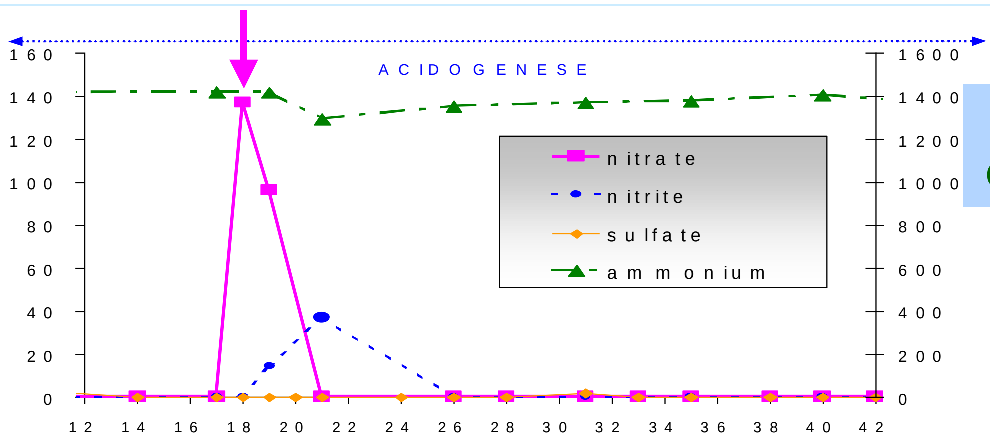
Experimental design



First Case

NO_3^- and NO_2^- content (mg of N)

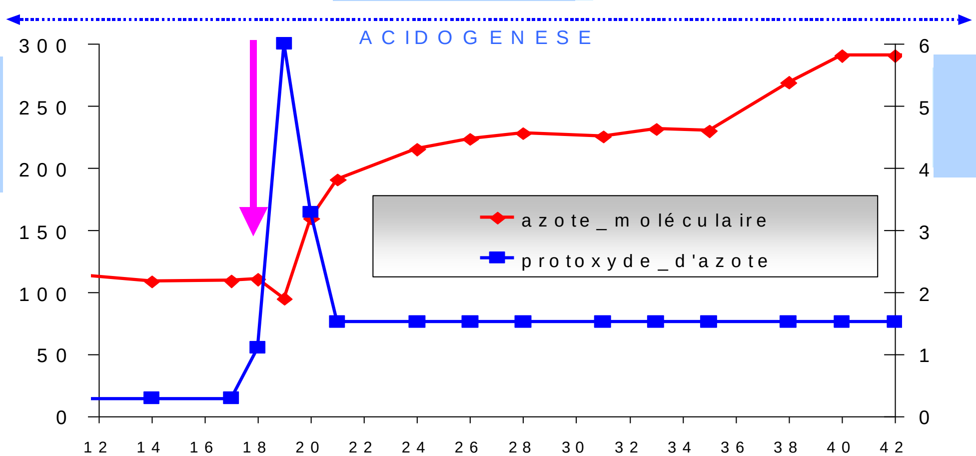
SO_4^{2-} concentration (mg.l^{-1})



NH_4^+ concentration (mg.l^{-1})

Time (Days)

N_2 cumulated production (mg of N)



N_2O cumulated production (mg of N)

Time (Days)

Case 1 Results interpretation

N_2 production = denitrification

Presence of organic matter

→ Heterotrophic denitrification

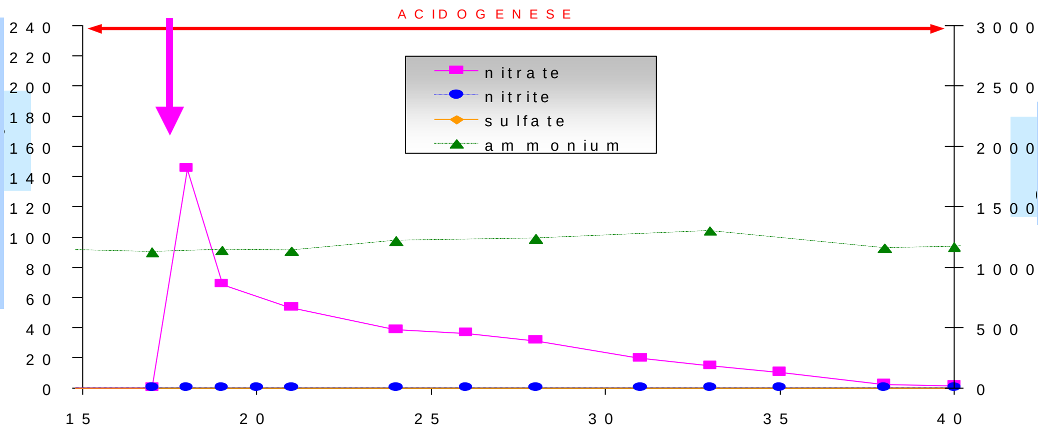
$$0{,}625\ CH_3COOH + NO_3^- \rightarrow HCO_3^- + 0{,}25\ CO_2 + 0{,}75\ H_2O + 0{,}5\ N_2$$

Observed in 13 cases upon 20

Second Case

NO$_3^-$ and NO$_2^-$ content (mg of N)

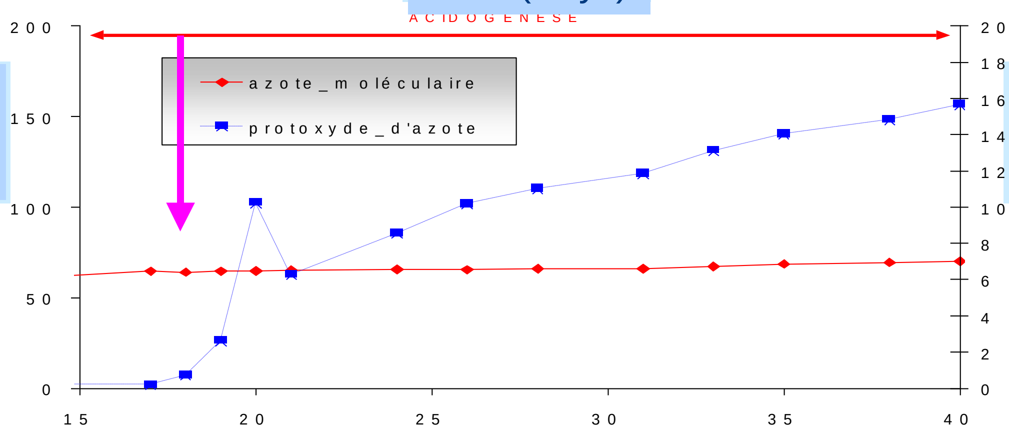
SO$_4^{2-}$ concentration (mg.l^{-1})



NH$_4^+$ concentration (mg.l^{-1})

Time (Days)

N$_2$ cumulated production (mg of N)



N$_2$O cumulated production (mg of N)

Time (Days)

Case 2 Results interpretation

No N_2 production = Nitramonification?

$$NO_3^- + 4\ H_2 + 2\ H^+ \rightarrow NH_4^+ + 3\ H_2O$$

Observed in 4 cases upon 20

Identification of the shifting parameter

Statistical analysis :

$$N_2 \text{ production} = 0{,}5245 + 0{,}1059\ [\text{acétate}] - 6{,}2153\ [H_2S] \qquad R^2 = 0{,}801$$

Positive effect on N_2 production

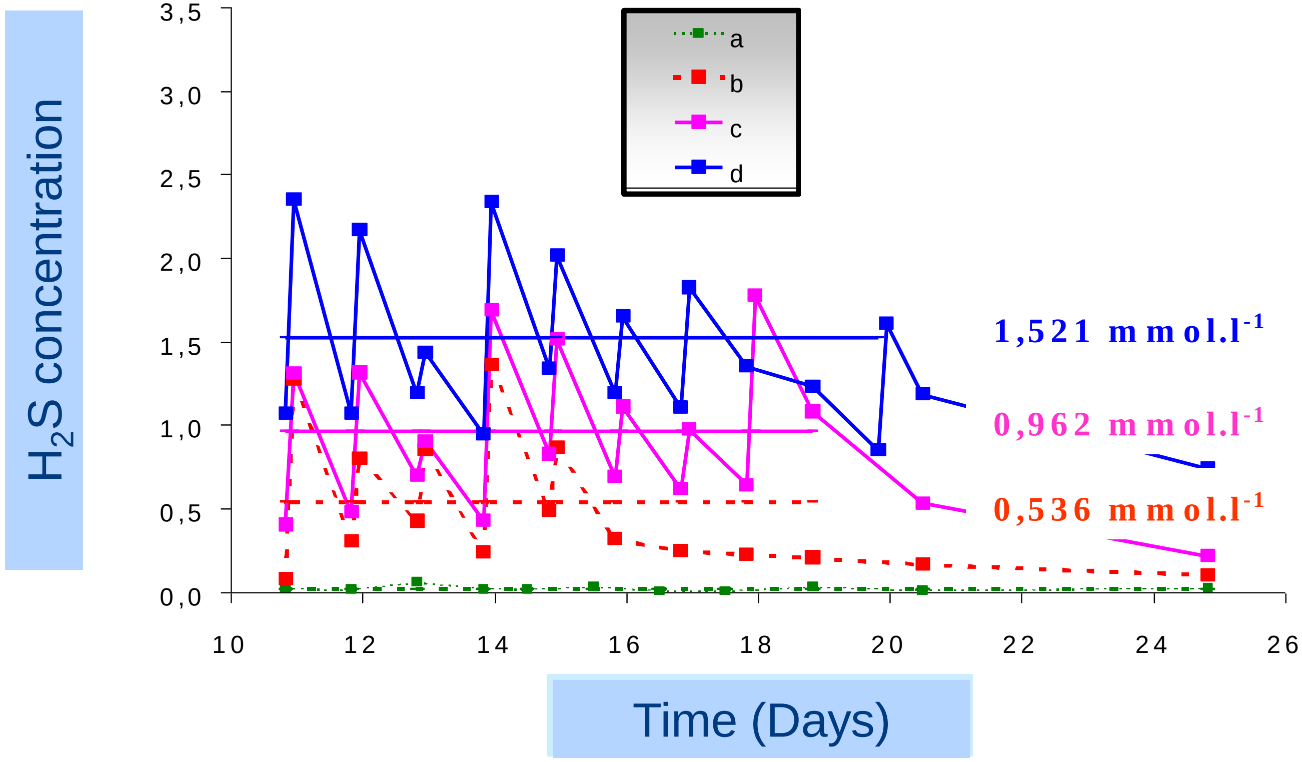
(Positive coefficient)

Negative effect on N_2 production

(Negative coefficient)

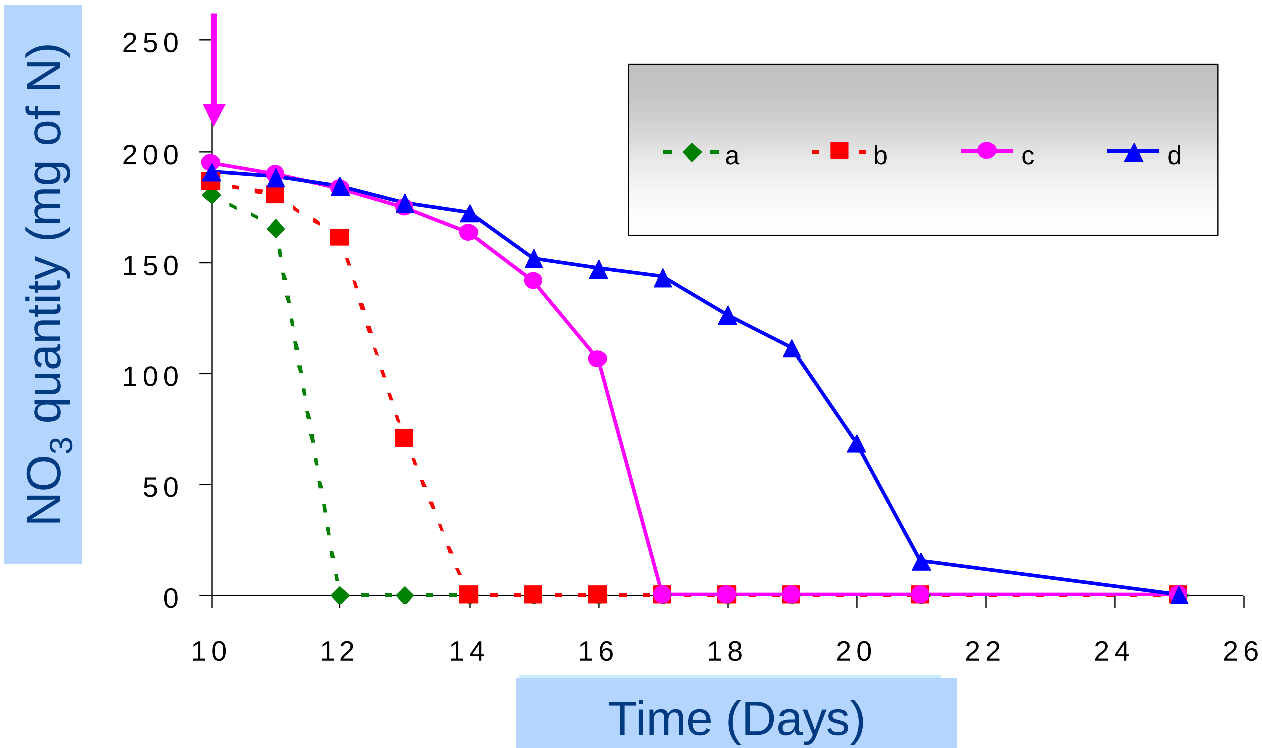
Evaluation of H_2S effect

Injection of H_2S at different concentration together with nitrate



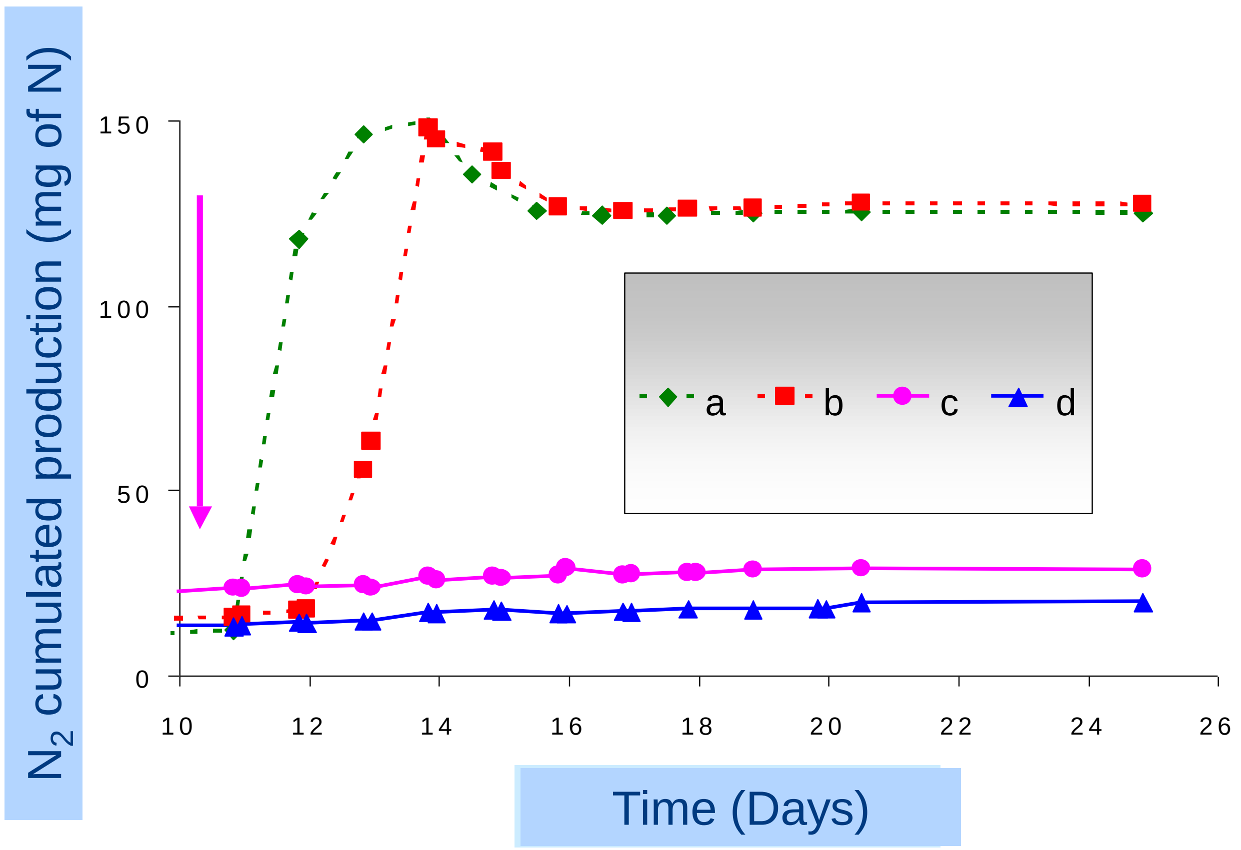
Evaluation of H_2S effect

NO_3 reduction kinetic in the four reactors



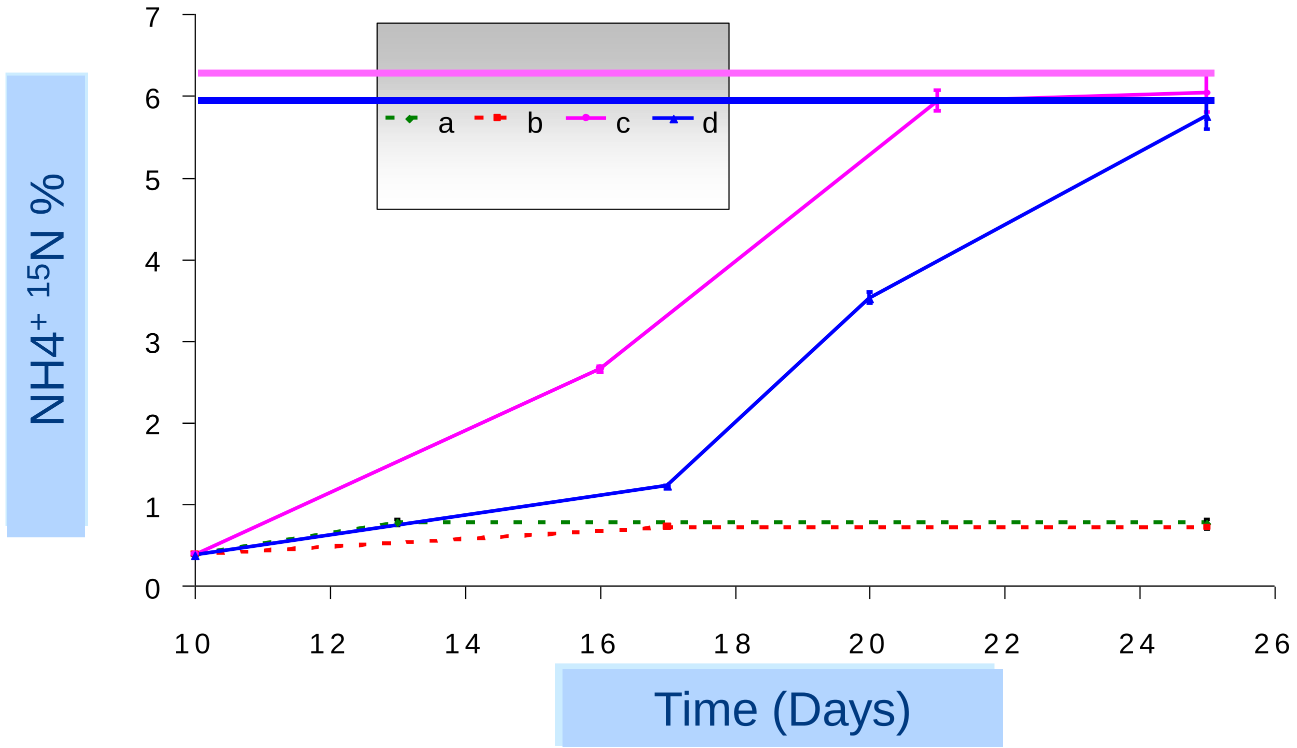
Evaluation of H_2S effect

N_2 production in the four reactors

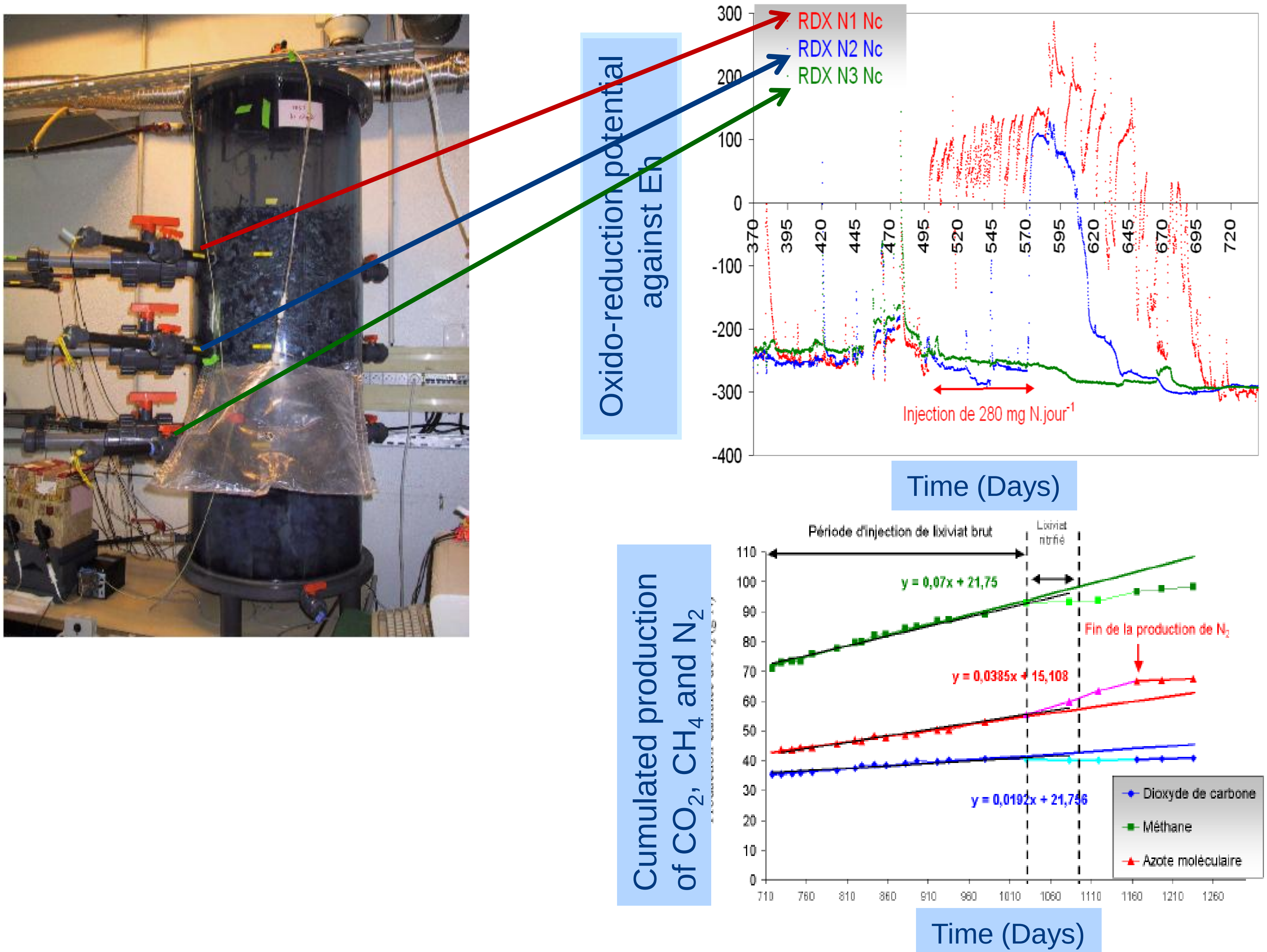


Evaluation of H_2S effect

Is nitramonification happening?

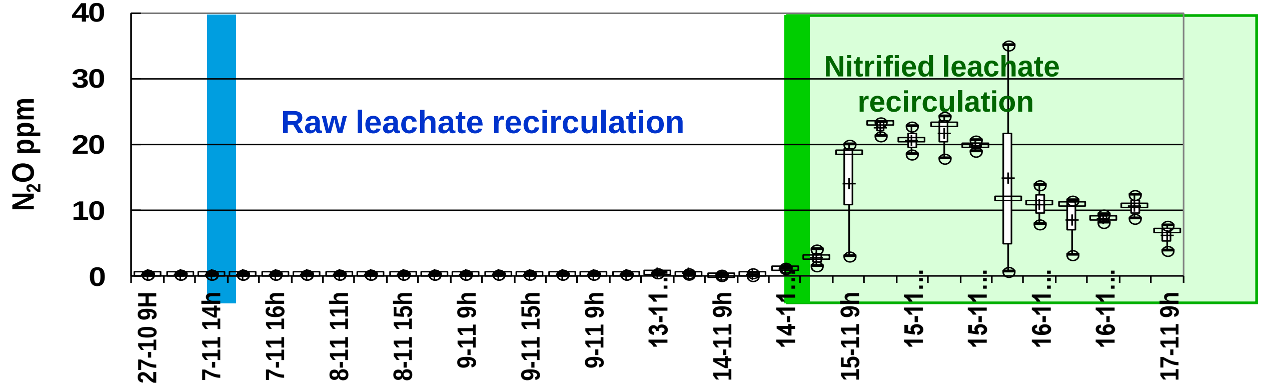


Mesocosm experiment

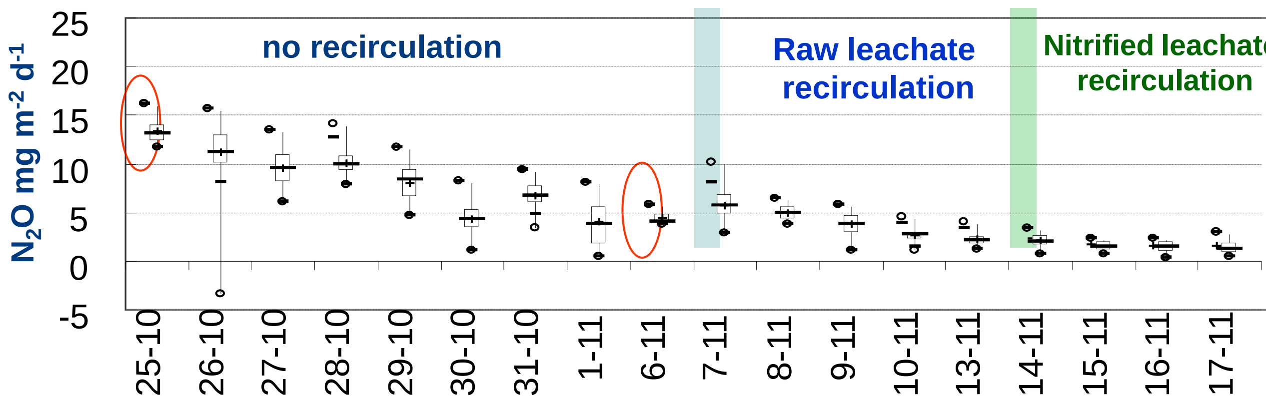


Field experiment

N_2O concentration in biogas collecting system

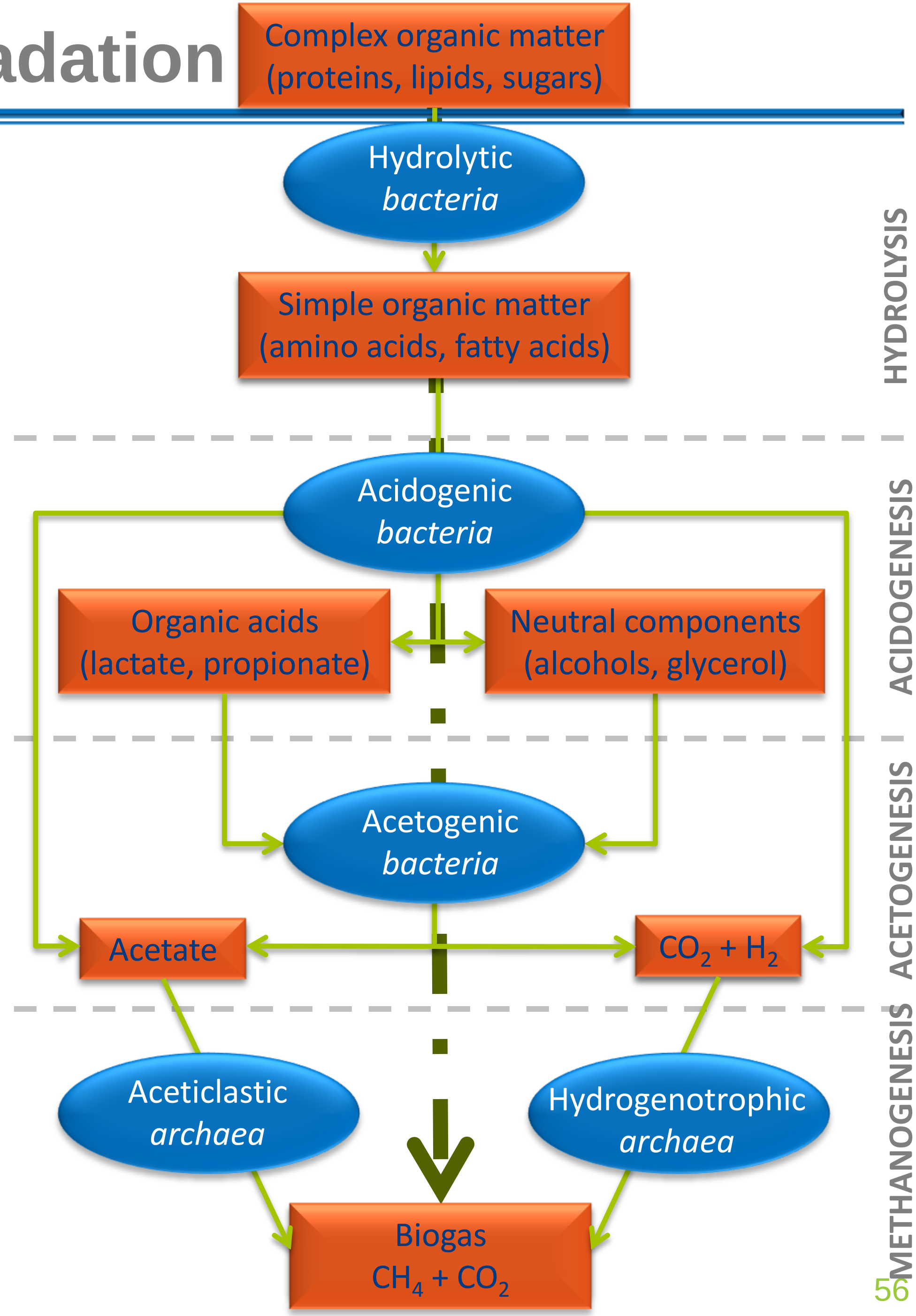


Surface N_2O emission

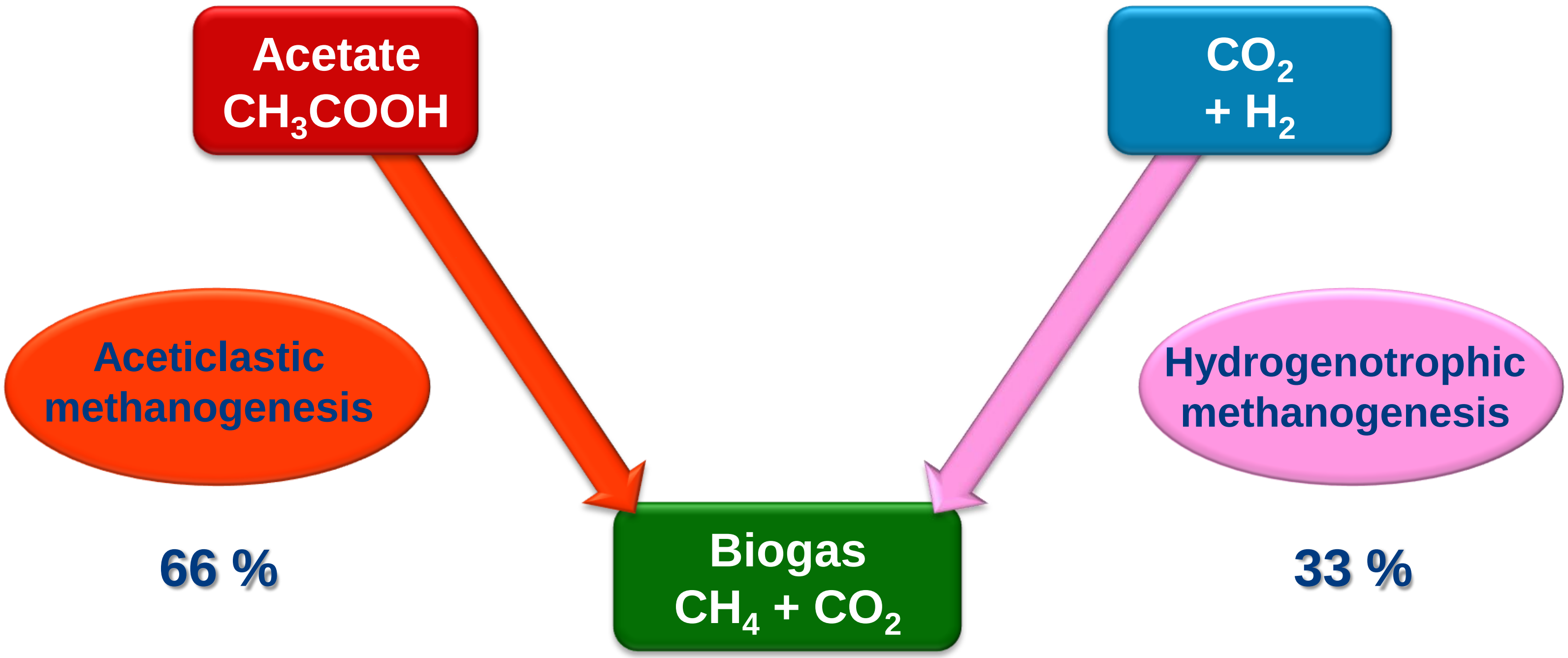


Anaerobic OM degradation

- **Numerous reactions**
  - **Hydrolysis**
  - **Acidogenesis**
  - **Acetogenesis**
  - **Methanogenesis**
- **Numerous microorganisms are involved**
- **Complex process**
- **Two main reactions can lead to methane production**



During OM degradation



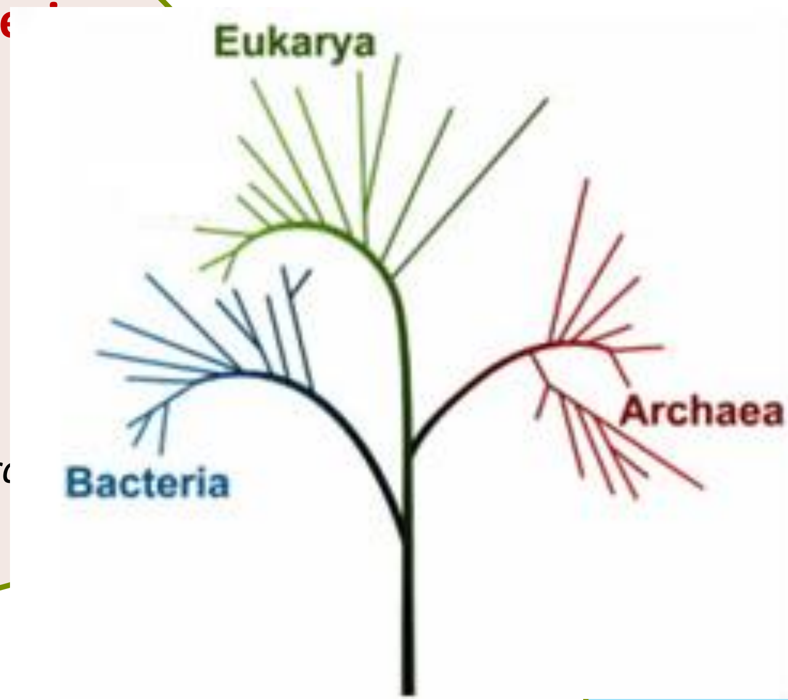
Aceticlastic methanogenesis : $CH_3COOH \rightarrow CH_4 + CO_2$
Hydrogenotrophic methanogenesis : $4H_2 + CO_2 \rightarrow CH_4 + H_2O$

Archaea

Aceticlastic
methanogene

Methanosaeta

Methanosarc



Life phylogenetic tree (C. WOESE)

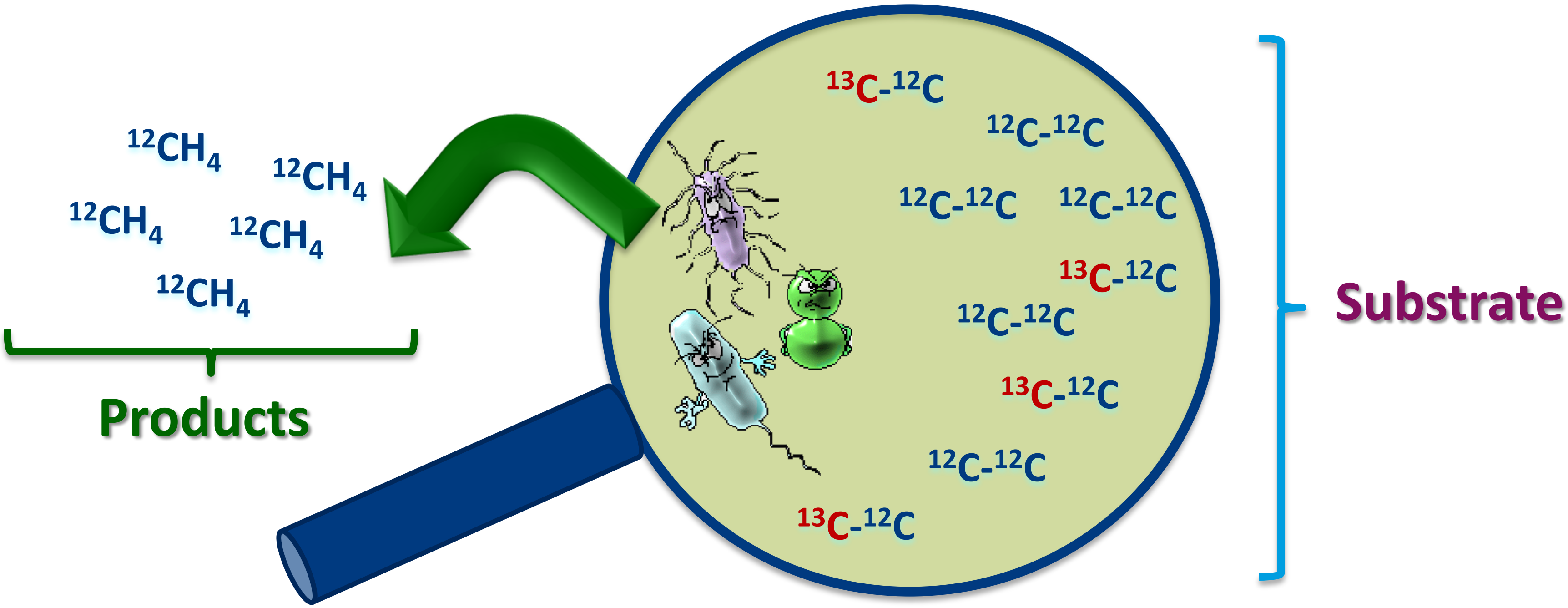
ethanosarcina

noculleus
anobacterium,
Methanobrevibacter,
Methanosphaera,
Methanothermobacter, …

Hydrogenotrophic
methanogenesis

Isotopic fractionation

- Molecules containing heavy isotopes undergo the same chemical or biological reactions than light molecules, but simply because chemical bonds involving heavy isotopes are stronger, they have slower reaction rates



- Due to these tiny differences in reaction rates the products of reactions have different isotope ratios than the source materials

Isotopic composition

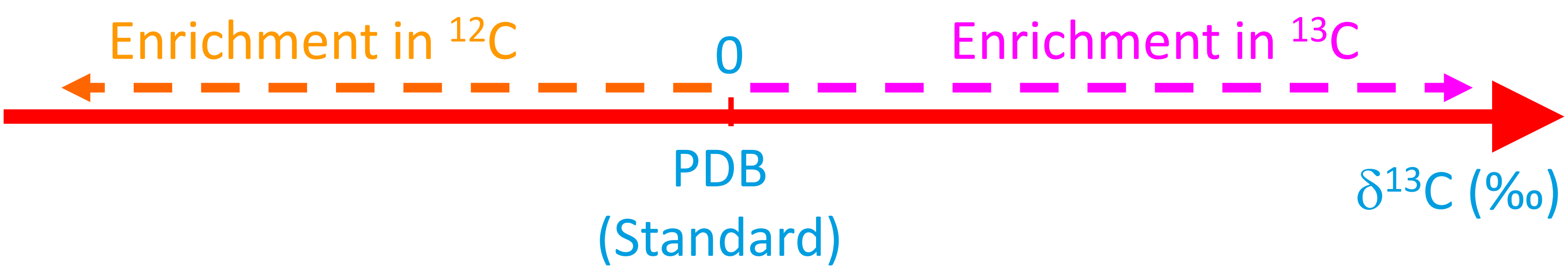
Delta notation is a way to express isotopic composition of a sample relative to an international standard (marine carbonate, PDB)

$$\delta^{13}C = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \quad [{}^{0}\!/_{00}]$$

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

➔ When $\delta^{13}C$ is positive, the sample is enriched in ^{13}C / Standard

➔ When $\delta^{13}C$ is negative, the sample is enriched in ^{12}C / Standard



Stable isotopic approach

Isotopic composition is generall different
between C , and Ac e

M

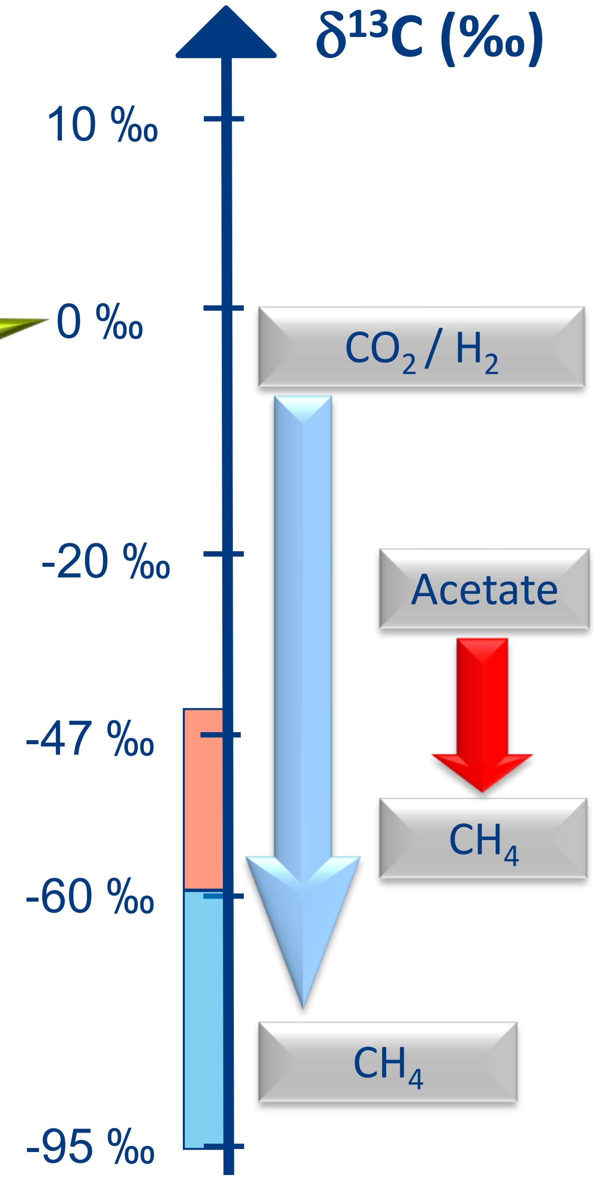
Mic

ed

is e important for the
h genot phic methanogenesis

The stable isotopic approach is already used for the study of natural methanogenic environnement

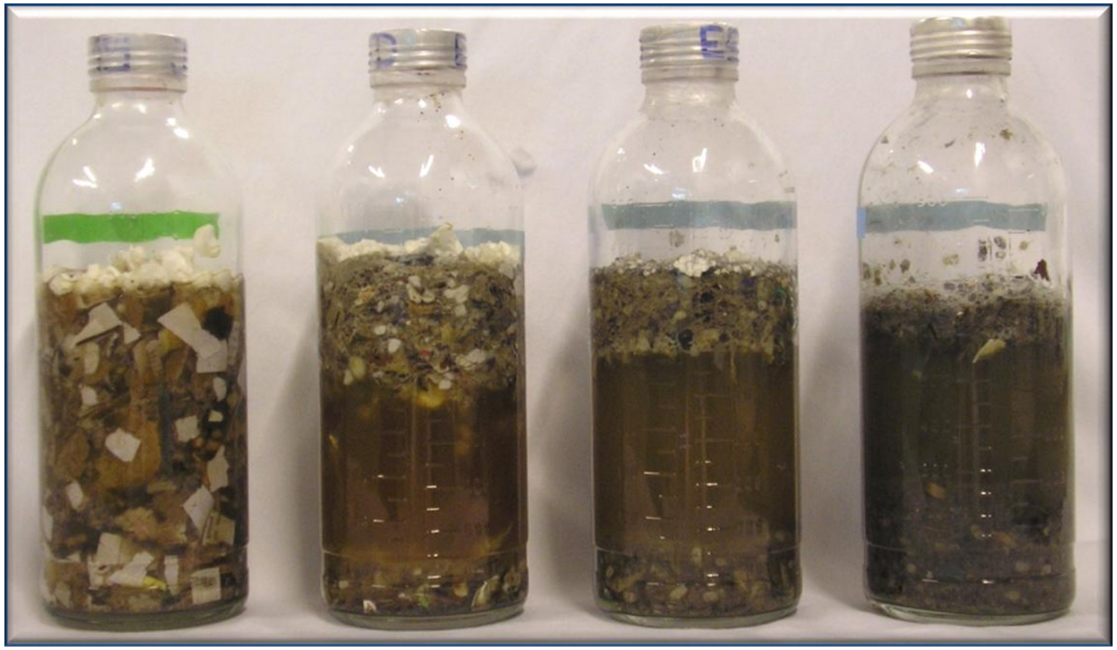
Using bibliographic data, it's possible to determine the methanogenic pathway by using methane stable isotopic composition



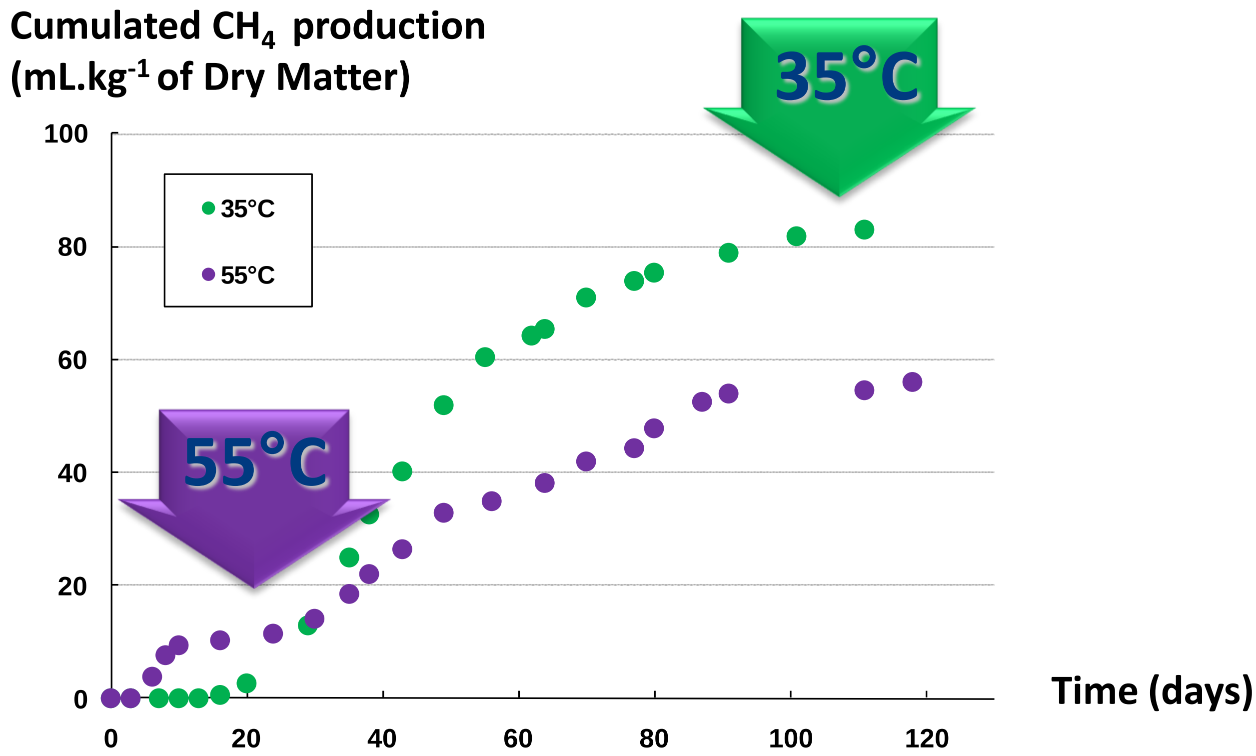
Waste incubations (35°C vs 55°C)

Batch reactors

Inoculum: « Landfill leachate »
Substrate: Reconstituted MSW
Liquid medium : Carbonated solution

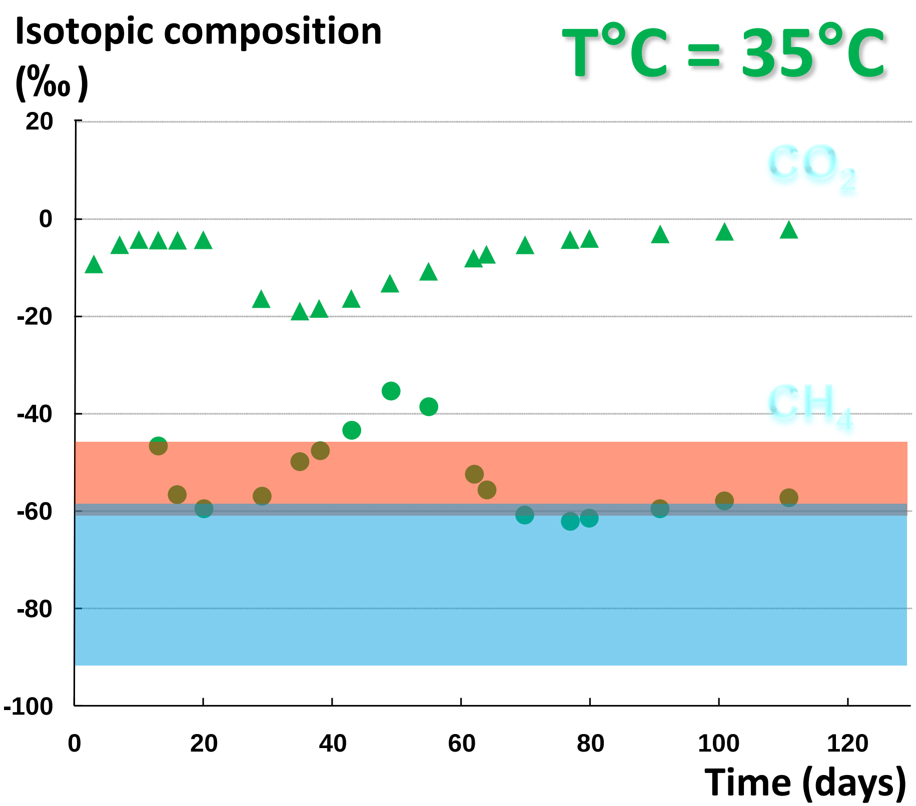


Methane production - Waste incubations (35°C vs 55°C)



- At 55°C, Methanogenesis startup is enhanced compare to 35°C
- At 35°C, final cumulated methane production is more importante compare to 55°C

Isotopic composition - Waste incubations (35°C vs 55°C)



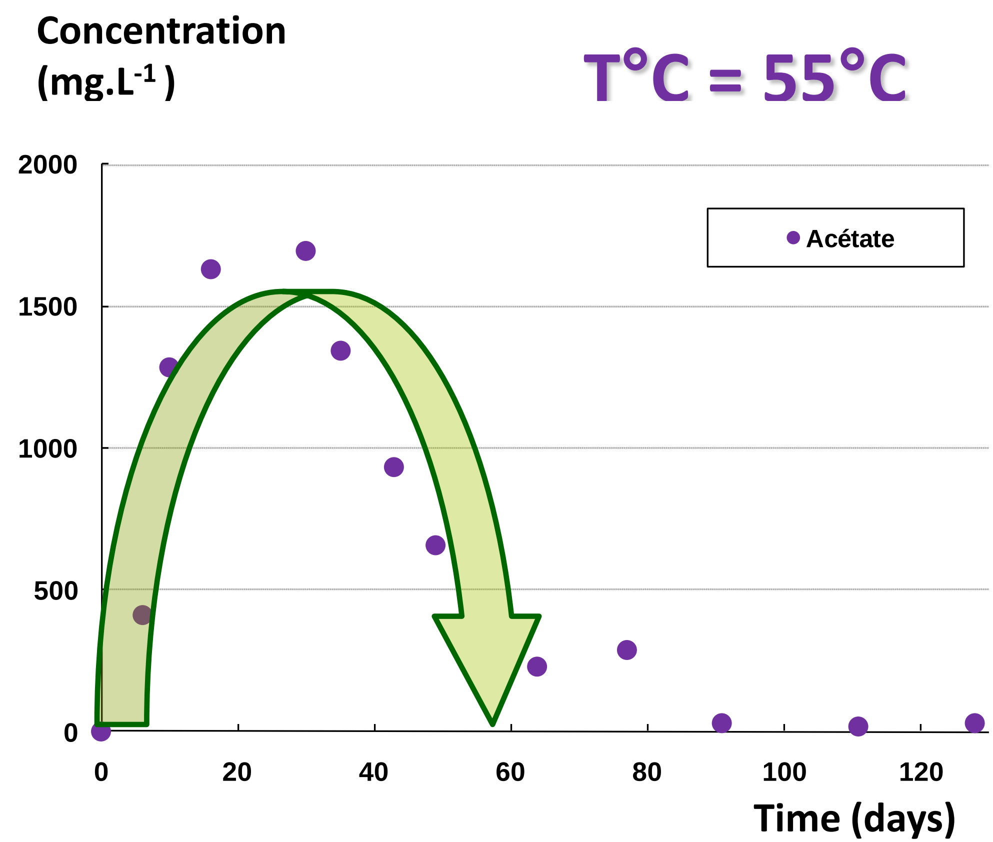
- Firstly, we have a decrease of $\delta^{13}CH_4$
- During active methane production phase , methane appears to be produced by the aceticlastic pathway
- And finally methane appears to be produced by both metabolisms (syntrophic propionate oxydation)

Hydrogenotrophic pathway

Aceticlastic pathway

Isotopic composition - Waste incubations (35°C vs 55°C)

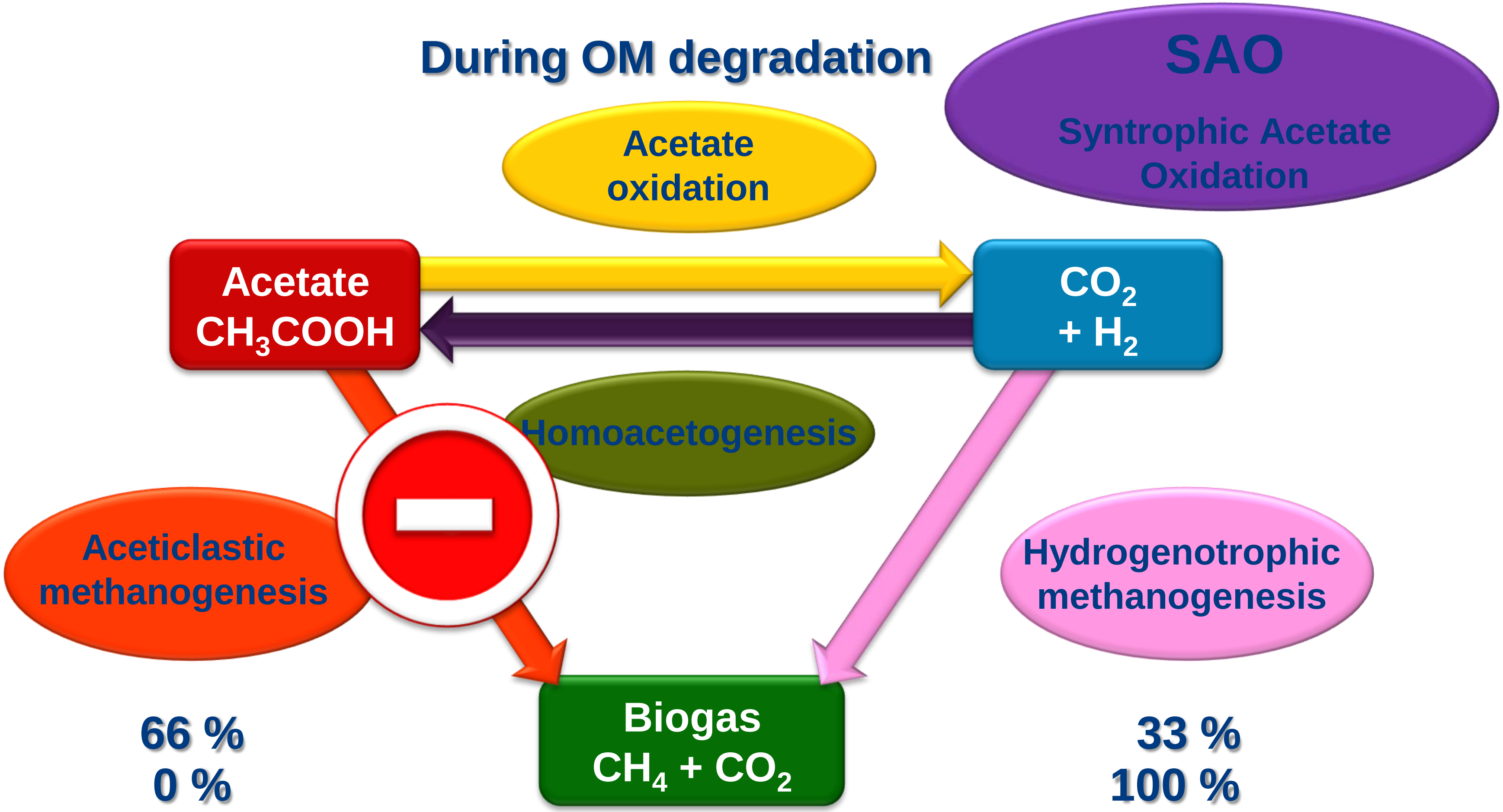
- Methane is produced by hydrogenotrophic pathway
- But acetate is produced and consumed
- SAO reaction (Syntrophic Acetate Oxidation)



Hydrogenotrophic pathway

Aceticlastic pathway

Syntrophic Acetate Oxidation



Aceticlastic methanogenesis :	$CH_3COOH \rightarrow CH_4 + CO_2$
Hydrogenotrophic methanogenesis :	$4H_2 + CO_2 \rightarrow CH_4 + H_2O$
Homoacetogenesis :	$4H_2 + CO_2 \rightarrow CH_3COOH + 2H_2O$
Acetate oxidation :	$CH_3COOH + 2H_2O \rightarrow 4H_2 + CO_2$

Sequencing results - Waste incubations (55°C)

	Domain	Order	Percentage*
55°C	*Archaea*	*Methanomicrobiales*	84 %
		Thermoplasmatales	14 %
		*Putative chimera***	02 %

* Number of sequences identified in regard to the number analyzed
** Sequences originating from 2 or more distinct DNA templates

At 55°C, *Methanomicrobiales* is the dominant *archaeal* order present

Sequencing result agree with previous interpretation at 55°C (SAO)

67

Syntrophic acetate oxidation

A two steps reaction :

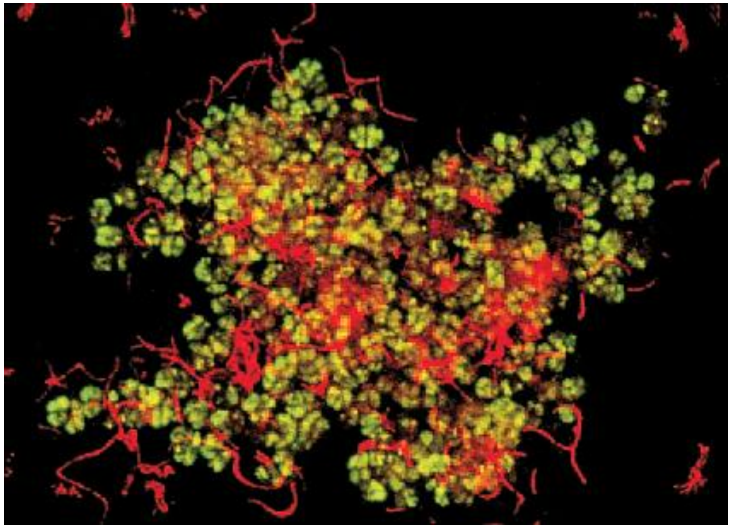
1) $CH_3COOH + 2H_2O \rightarrow 4H_2 + 2CO_2$ Bacteria

!

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

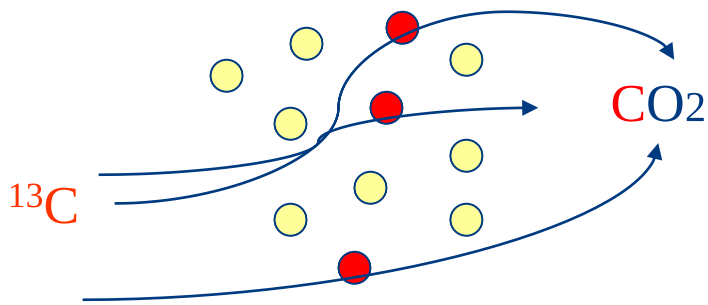
Syntrophic relationship between

bacteria and archaea



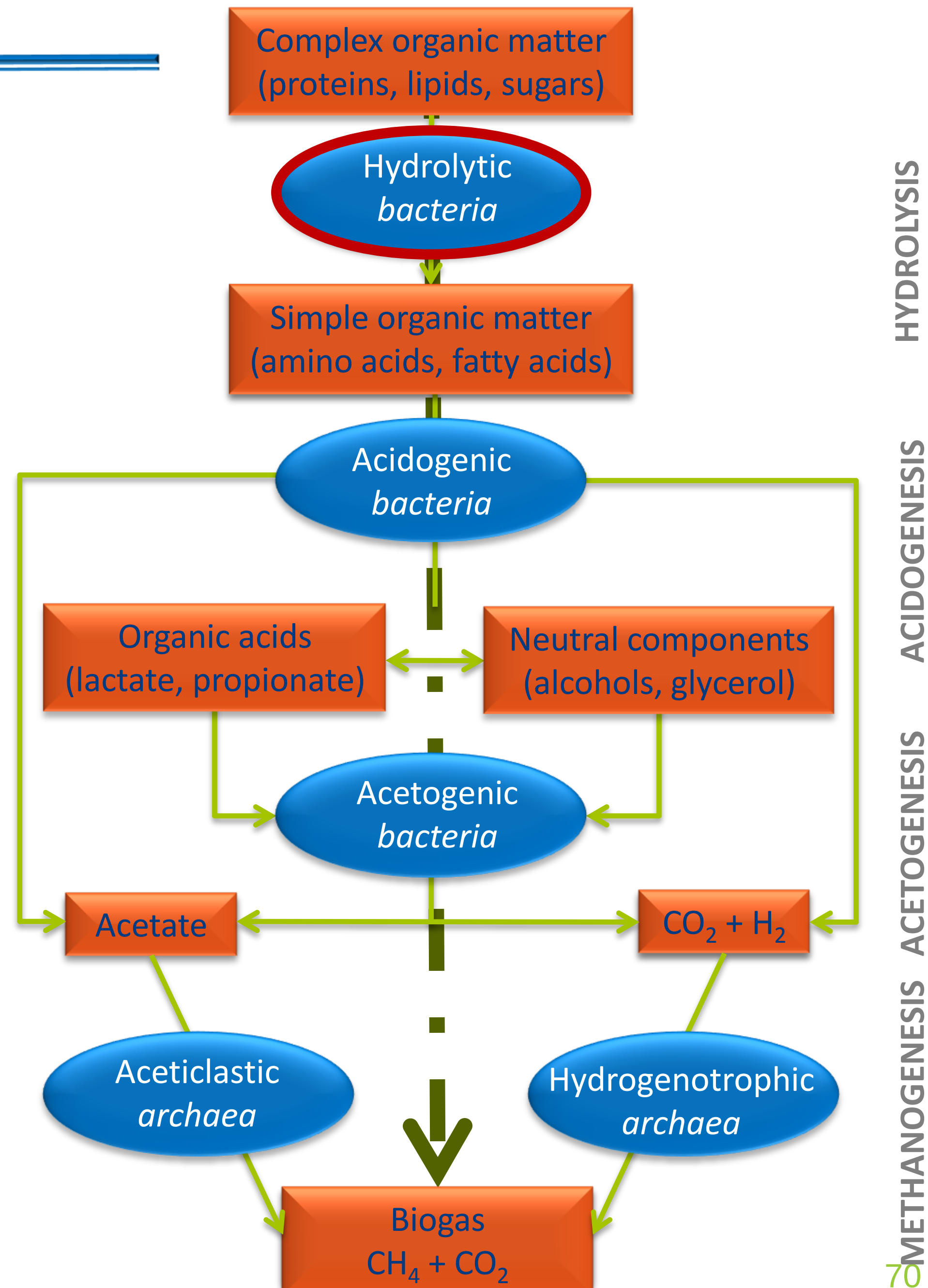
Identification of functional microorganism

Complexe
ecosystem



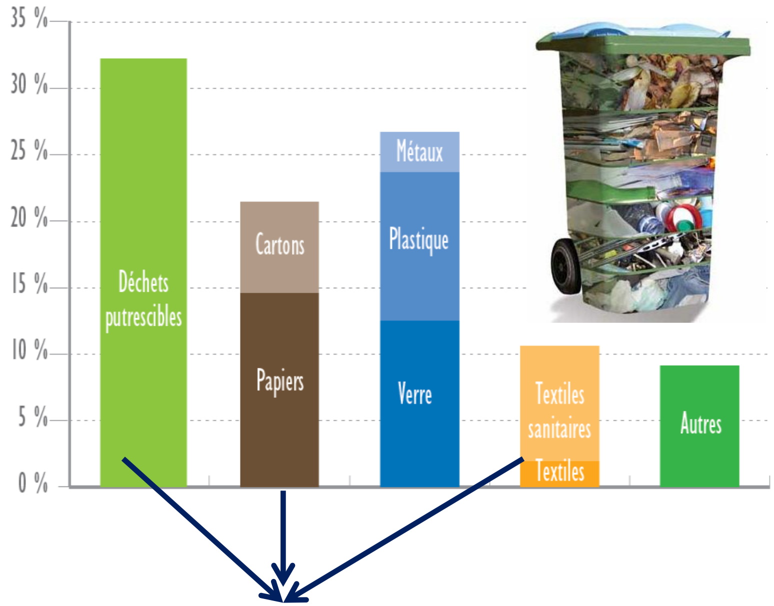
^{13}C enriched nucleic
acid recovery

Cellulose hydrolysis



Why it is important to improve cellulose anaerobic digestion?

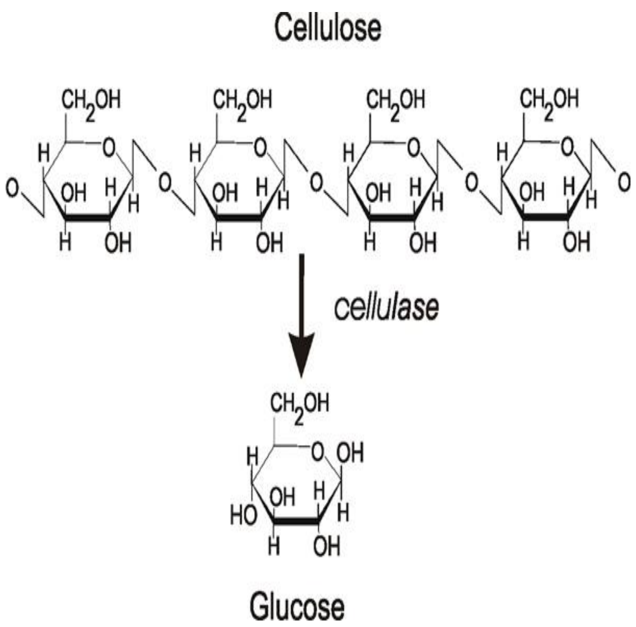
Municipal solid waste composition



Cellulose

80 % of the methane potential

Cellulose Hydrolysis

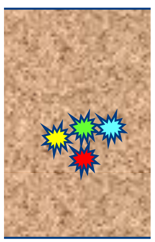


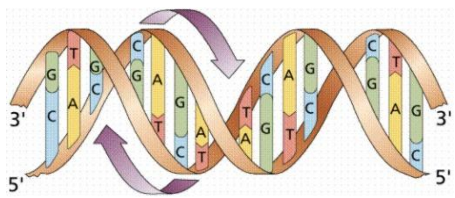
Kinetic limiting step

Identification of microorganisms involved in cellulose hydrolysis

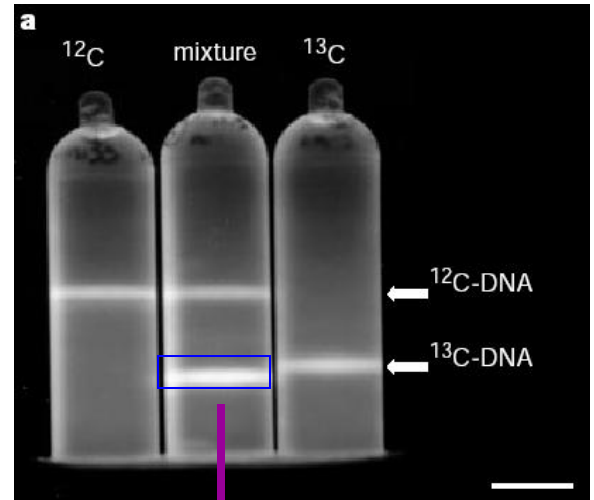
Stable isotope probing (SIP)

^{13}C enriched substrat





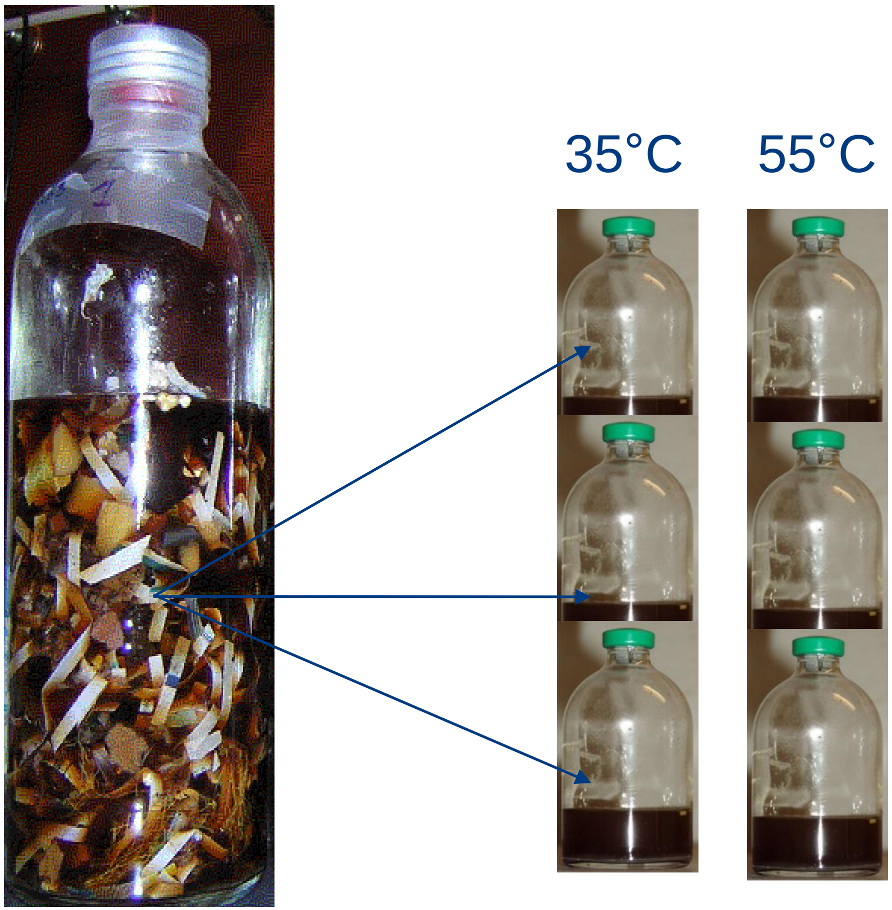
DNA extraction



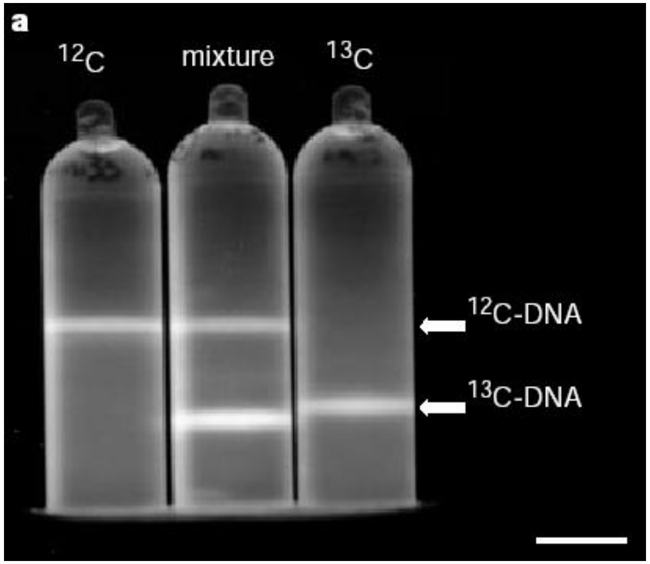
Sequencing

Presumed Fonctional microbial group identification

Incubations

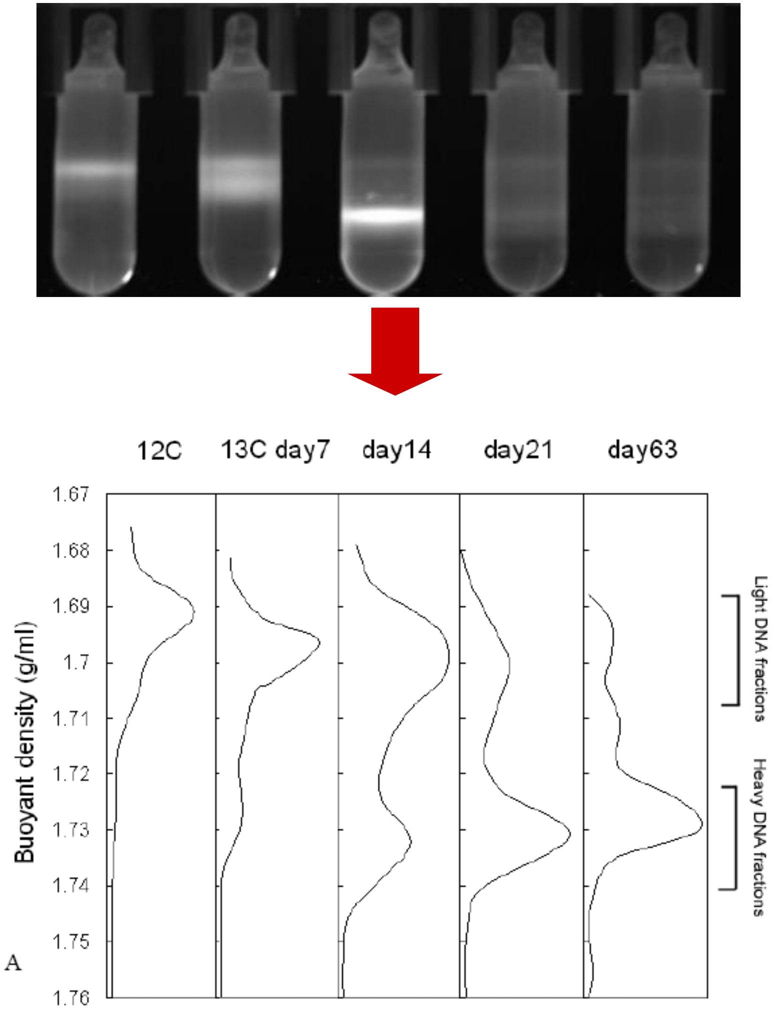


Stable isotope probing (SIP)



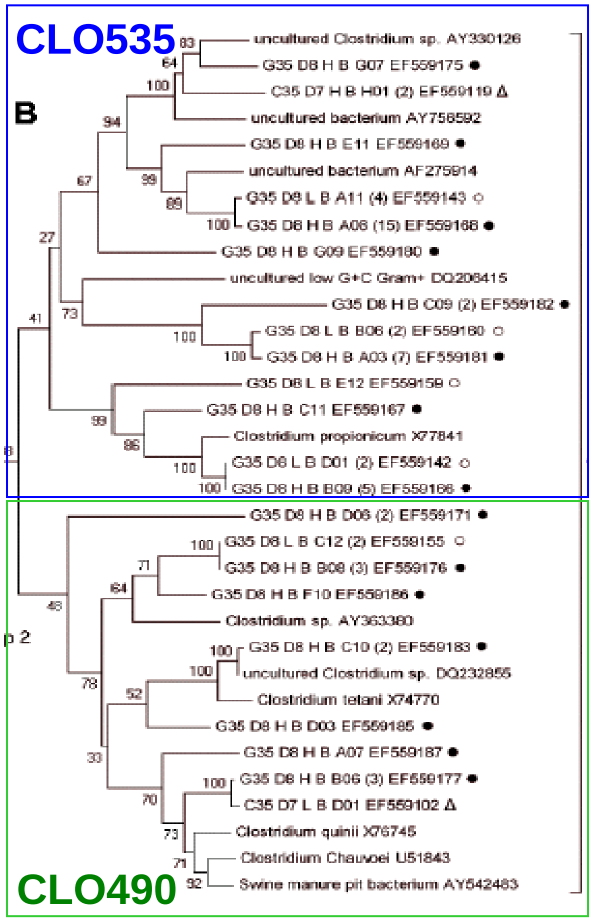
Radajewski, Nature, 2000

SIP during cellulose degradation



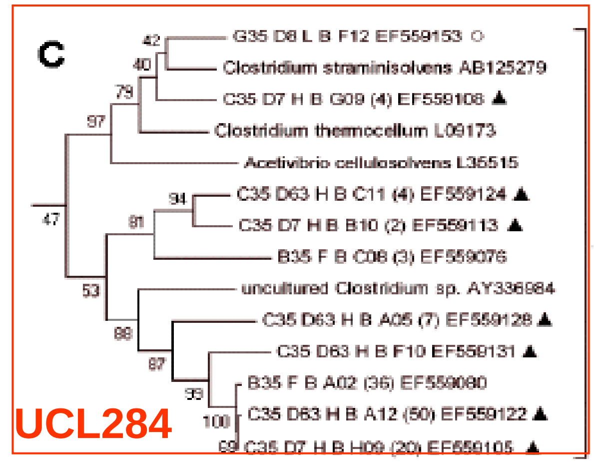
Identification of involved microbial group

Clostridium



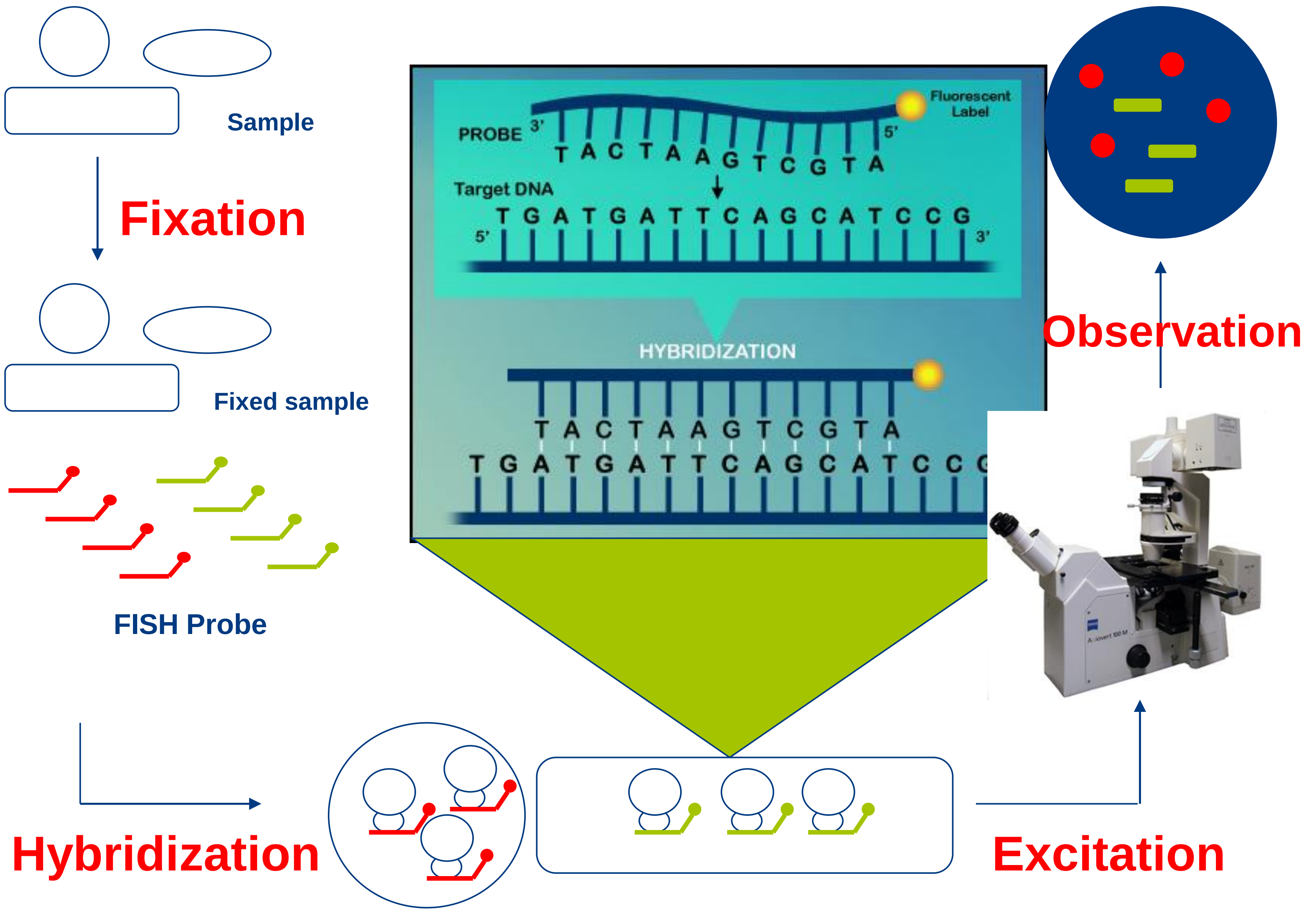
Involved in glucose degradation at 35°C

Acetivibrio

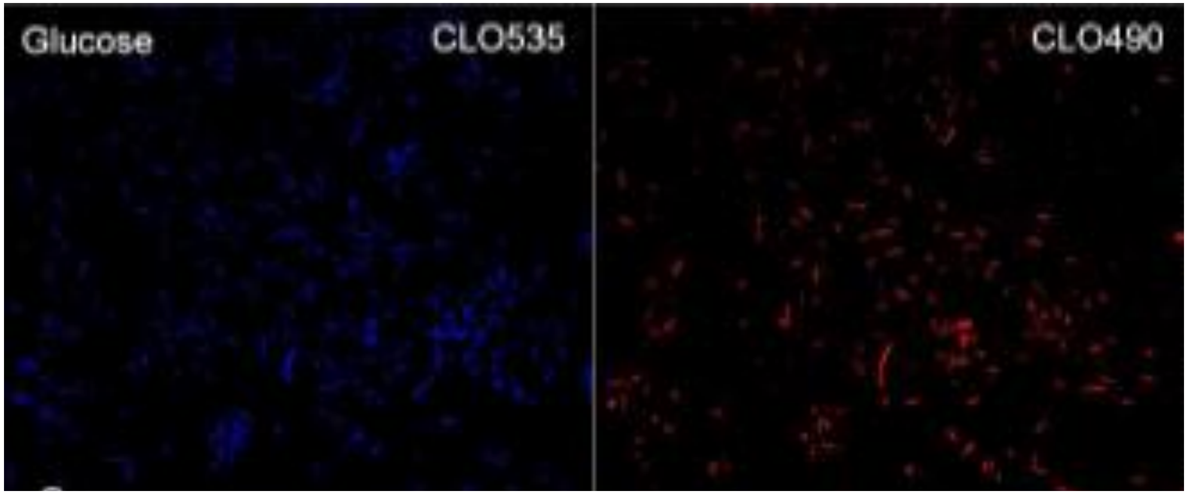


Involved in the first step of cellulose degradation à 35°C

FISH (Fluorescence In Situ Hybridization)



Spatial distribution of microorganisms

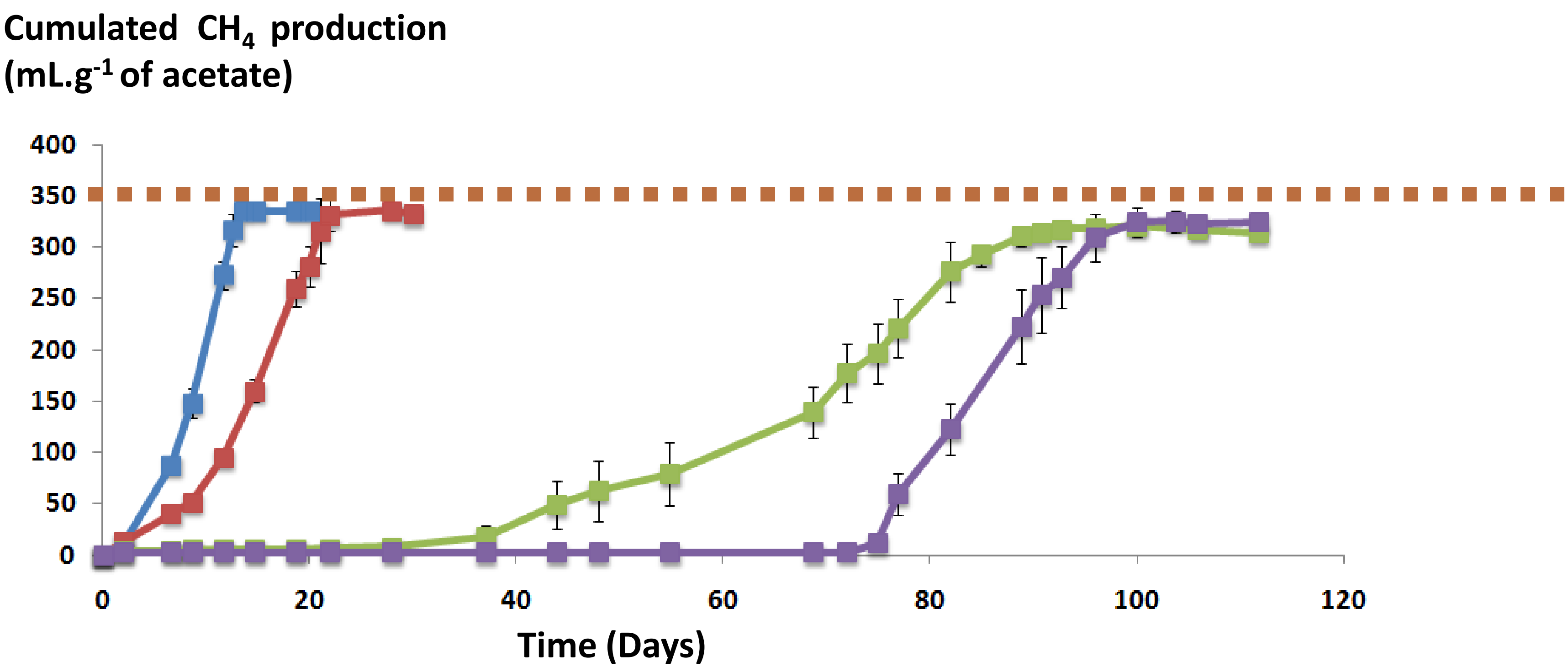


Clostridium in glucose incubation

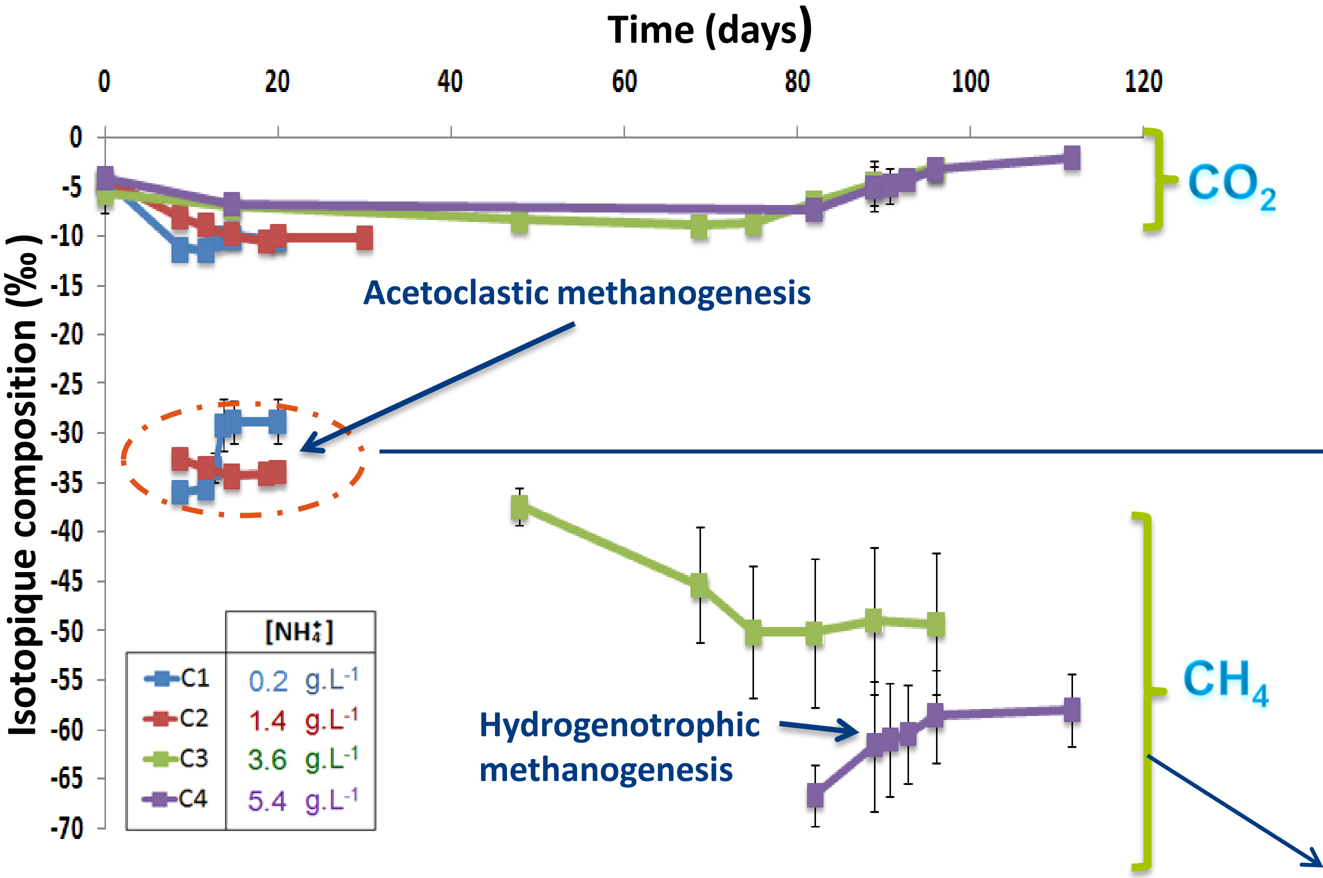
AMMONIA EFFECT

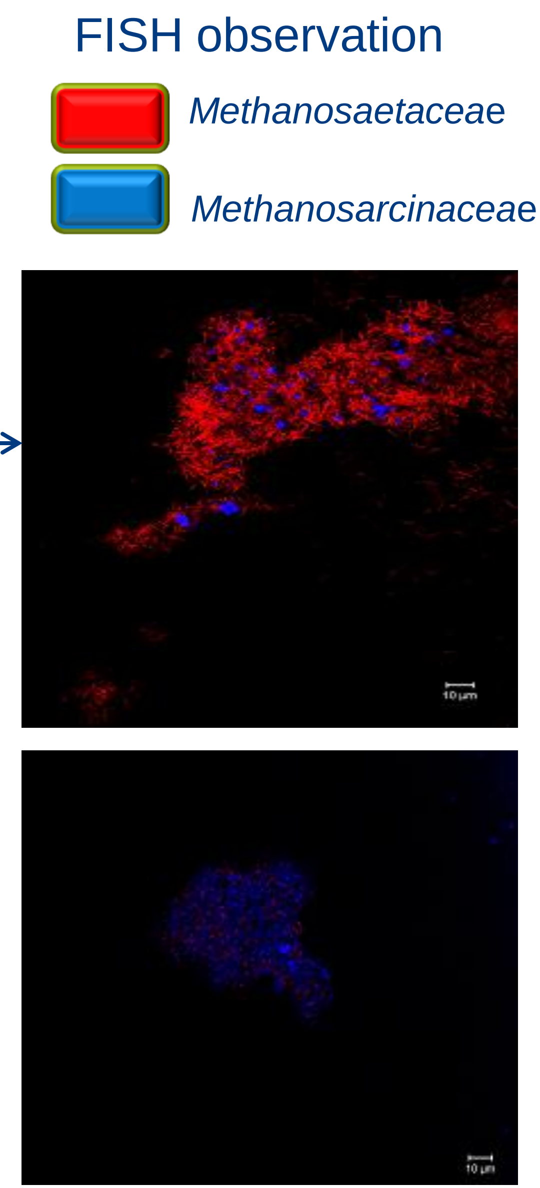
- C1 & C2: Immediate start of methanogenesis
- C3 & C4: High latency
- Similar level of methane production at the end

	$[NH_4^+]$
C1	0.2 $g.L^{-1}$
C2	1.4 $g.L^{-1}$
C3	3.6 $g.L^{-1}$
C4	5.4 $g.L^{-1}$



ISOTOPIC COMPOSITION





- C1& C2: Acetoclastic methanogenesis
- C4: Syntrophic acetate oxidation

79

Syntrophic acetate oxidation

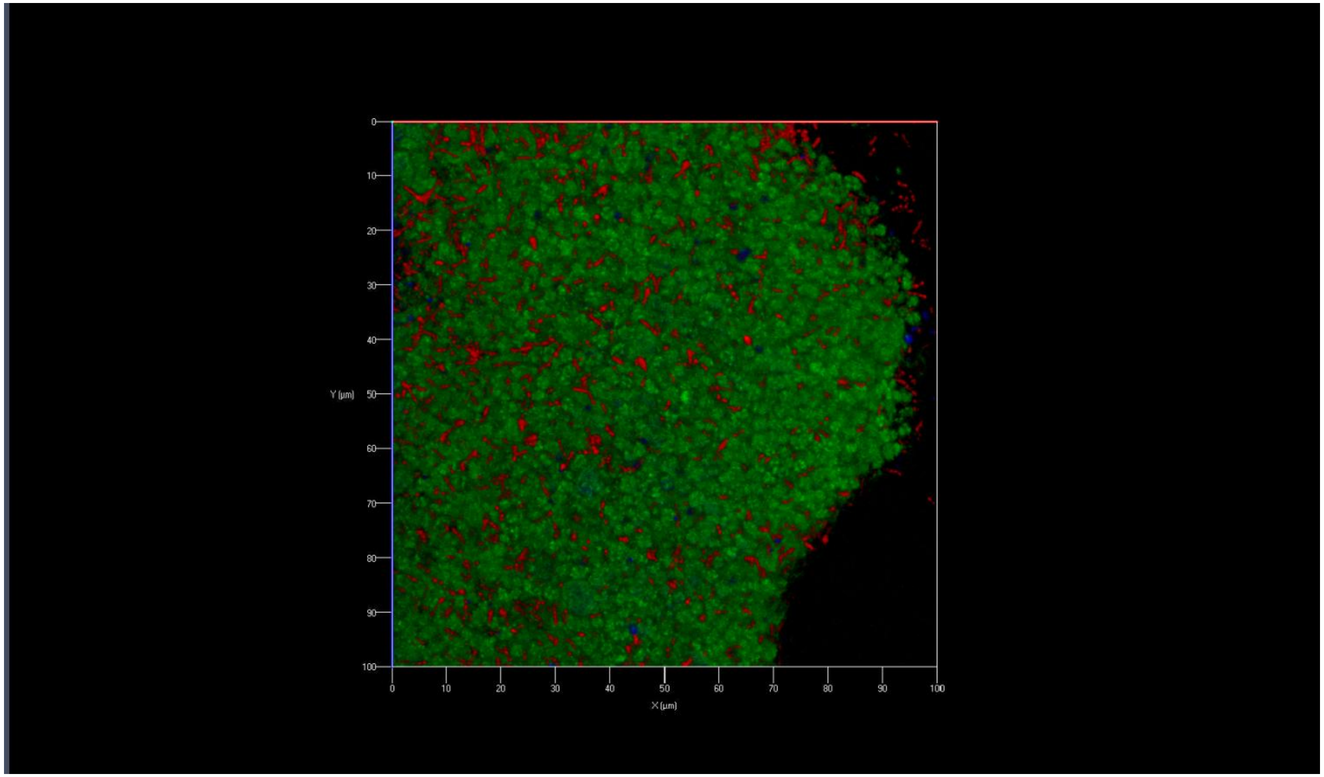


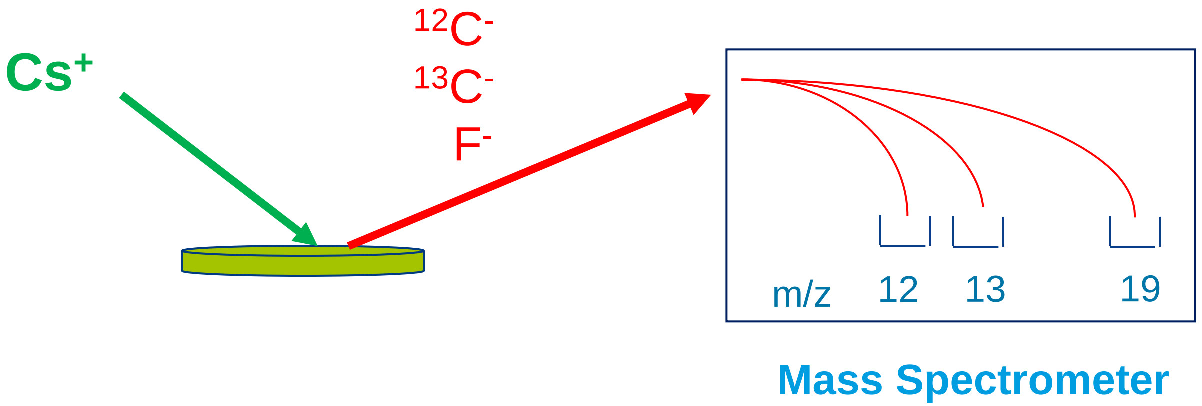
Table 1. Reactions involved in acetate and hydrogen metabolism

Process	Reaction			$\Delta G^{0\prime}$ (kJ/mol)
(1) Aceticlastic methanogenesis	$^*CH_3COO^- + H_2O$	$\rightarrow$	$^*CH_4 + HCO_3^-$	–31.0
(2) Syntrophic acetate oxidation	$^*CH_3COO^- + 4H_2O$	$\rightarrow$	$H^*CO_3^- + 4H_2 + HCO_3^- + H^+$	+104.6
(3) H_2-consuming methanogenesis	$4H_2 + HCO_3^- + H^+$	$\rightarrow$	$CH_4 + 3H_2O$	–135.6
(4) sum (2)+(3)	$^*CH_3COO^- + H_2O$	$\rightarrow$	$H^*CO_3^- + CH_4$	–31.0
(5) H_2-consuming acetogenesis	$4H_2 + 2HCO_3^- + H^+$	$\rightarrow$	$CH_3COO^- + 4H_2O$	–104.6

Asterisks (*) represent the fate of the methyl group carbon of acetate. It was assumed that 100% of the labeled carbon was converted to CH_4 (reaction 1) or HCO_3^- (reaction 4). The standard Gibbs free energy change ($\Delta G^{0\prime}$) values were calculated from reference 75.

Hattori, 2008

Surface analysis technique



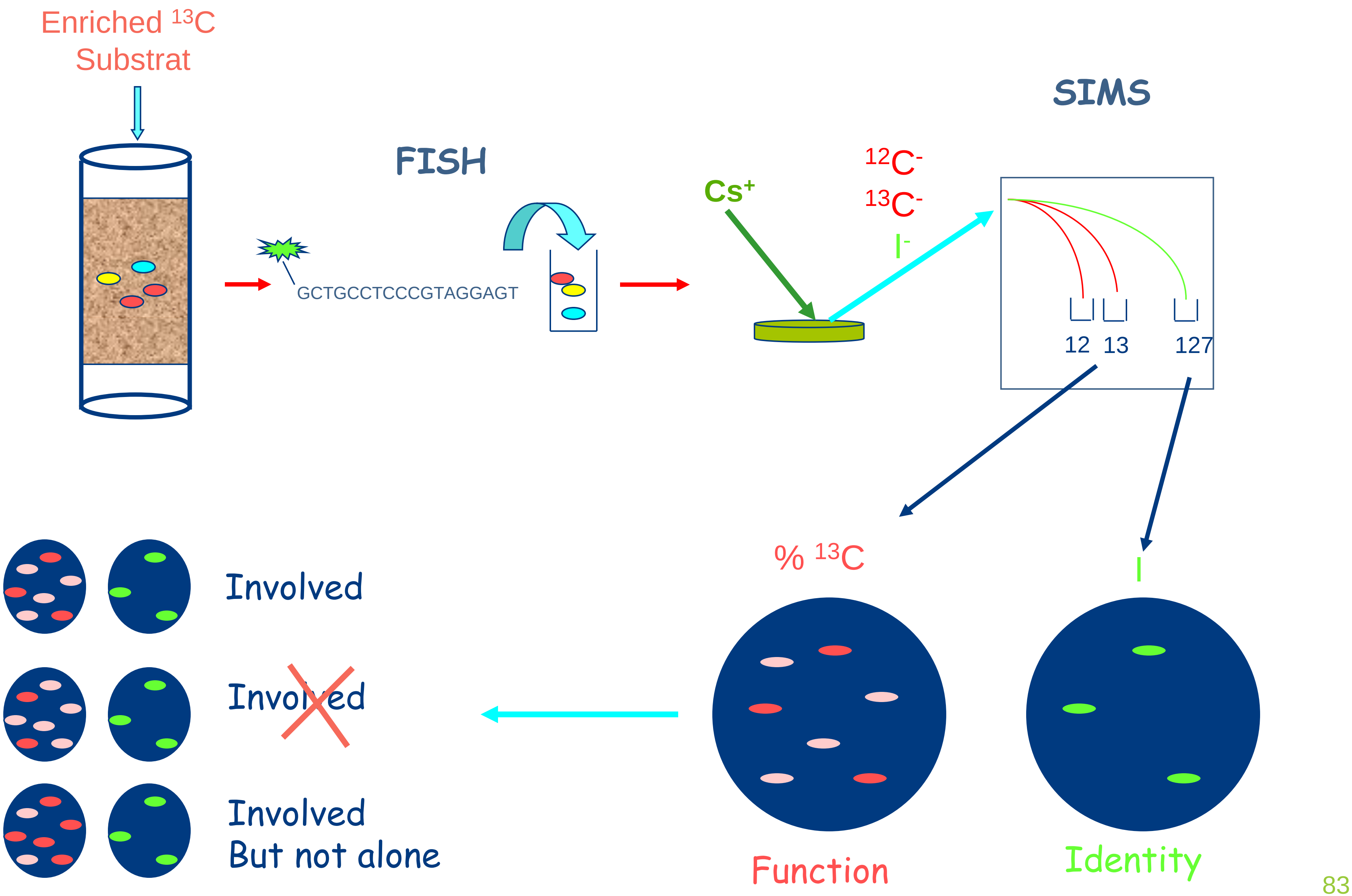
Two-dimensional mapping of elemental and isotopic composition the surface of a sample

Nano sims

- haute résolution latérale (~50nm en césium)
- détection parallèle de cinq images ioniques distinctes
- Sensibilité de l'ordre du ppm

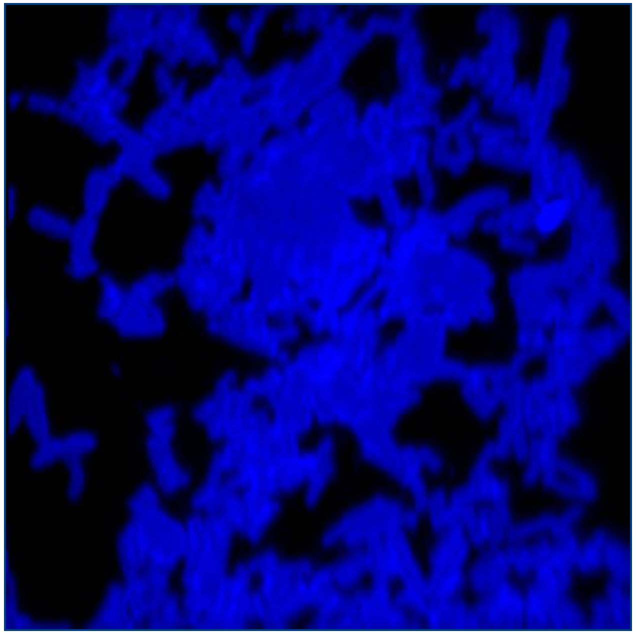
(JL. Kerguin-Kern, Alain Croisy, Ting-Di Wu)

Visualization of function and identity by FISH-SIMS



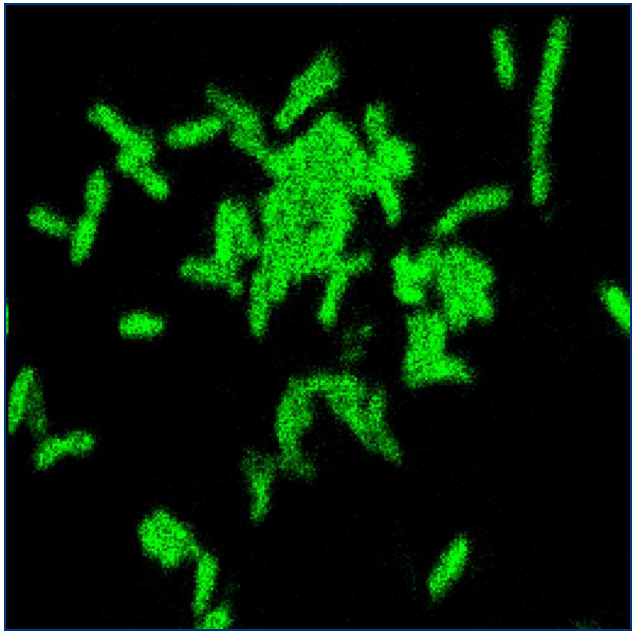
Bacillus subtilis + *E. coli* 100% ^{13}C hybridized by iodine probe

S



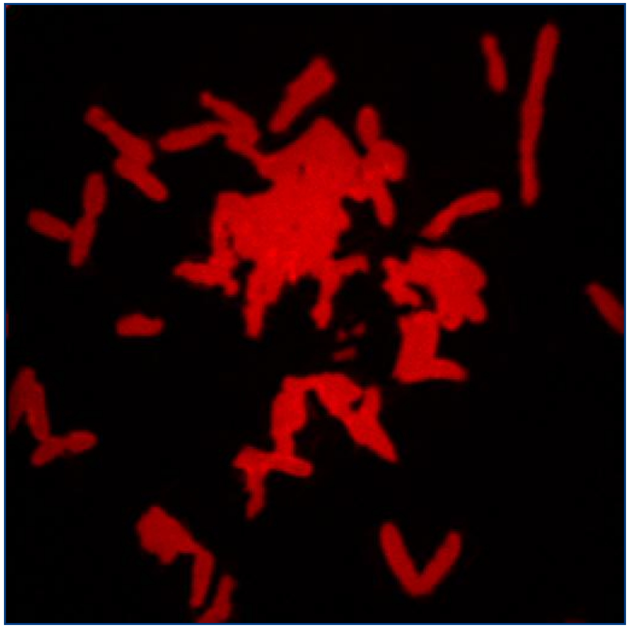
Visualization of the whole biomass

I



Visualization of the targeted strain

^{13}C

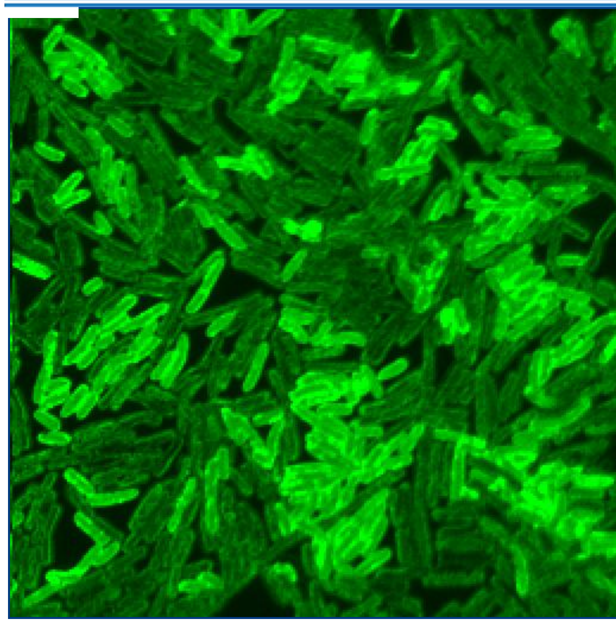


Visualization of the isotopic enrichment

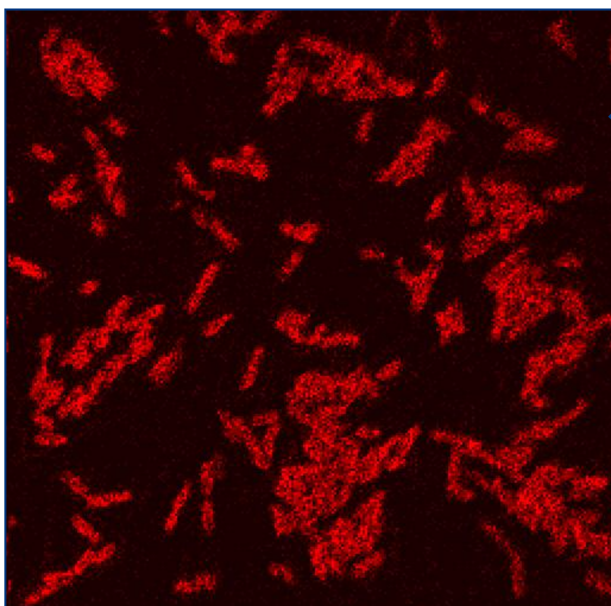
Co-localisation

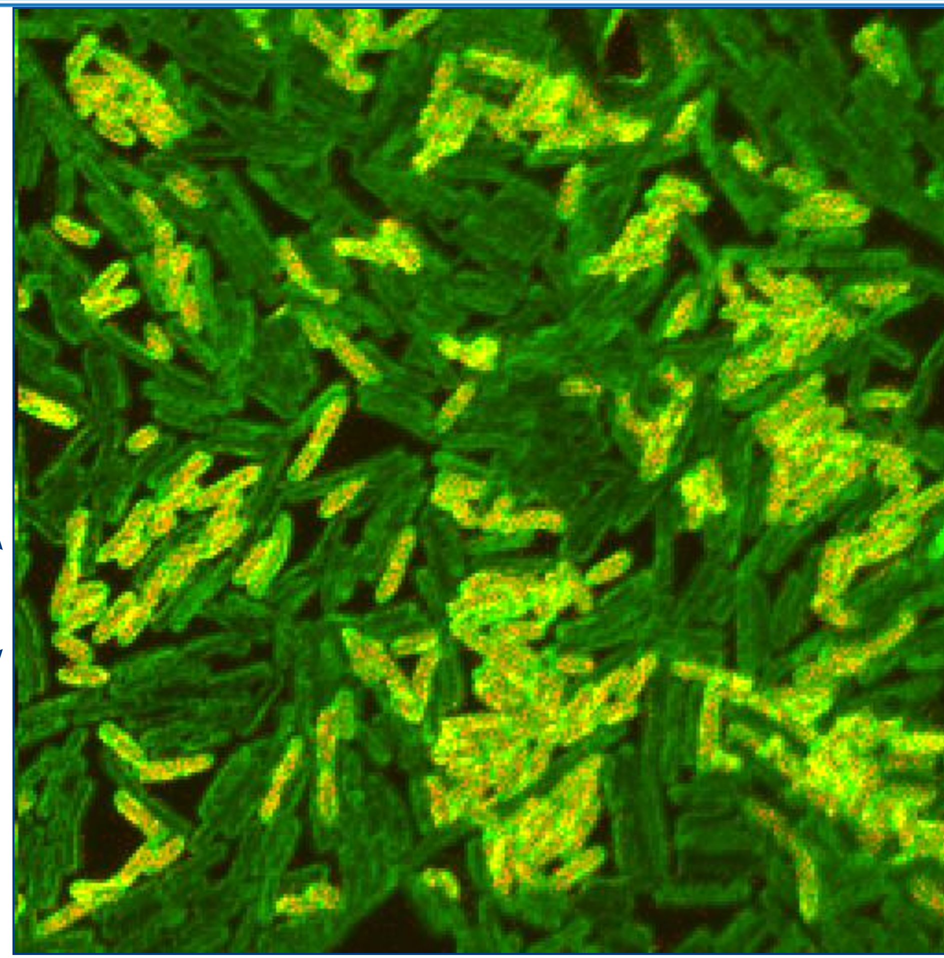
E.coli 40% ^{13}C EubI + B.subtilis 10% ^{13}C

^{13}C



I



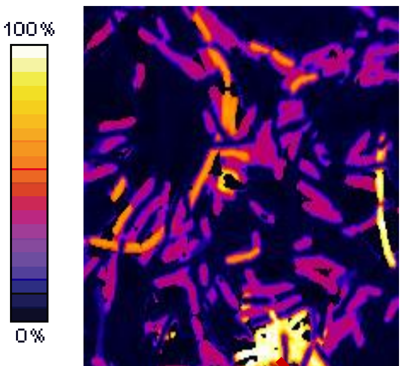


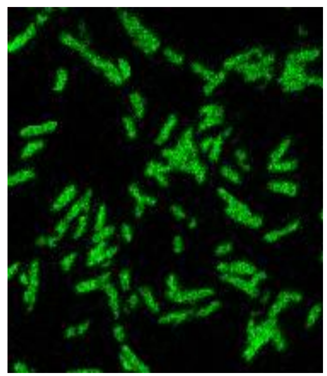
^{13}C
+
I

Possibility of distinguishing different levels of enrichment

CARBONE

%, 9%, 58%, 90% en ^{13}C +
E. coli 34% en ^{13}C, hybridized by the probe EubI (Iode)

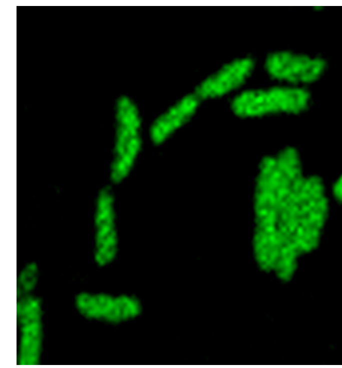




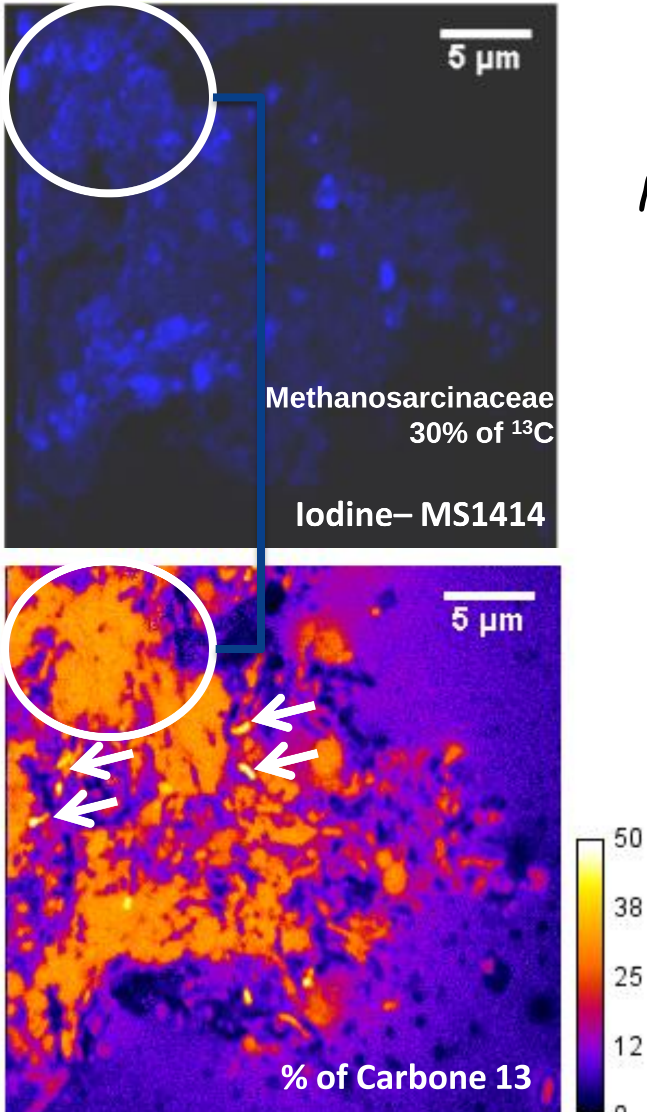
% ^{13}C IRMS	% ^{13}C SIMS
1,10 ± 0,00	1,1 ± 0,03
8,86 ± 0,09	9,6 ± 0,17
33,99 ± 0,08	32,6 ± 0,22
58,12 ± 0,47	58,0 ± 0,41

NITROGEN

E. coli 1%, 9%, 63%, 95% en ^{15}N
+
E. coli 37% en ^{15}N, hybridized by the probe EubI (Iode)



% ^{13}C IRMS	% ^{13}C SIMS
9,25 ± 0,10	10,1 ± 0,17
36,46 ± 0,21	38,9 ± 0,19
63,99	66,5 ± 0,13



SIMSISH image

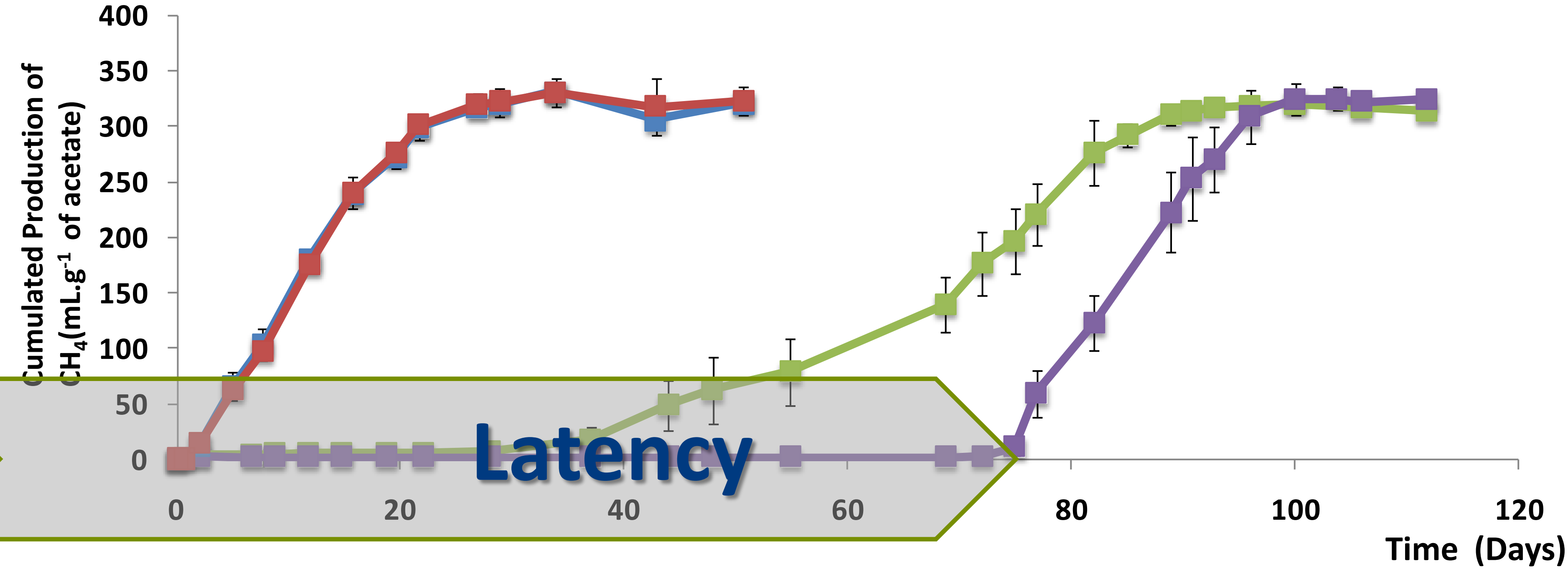
The isotopic enrichment of members of the Methanosarcinaceae family shows their involvement in the syntrophic oxidation of acetate.

in addition

Bacterium-like structures also exhibit non-natural isotopic enrichment (40%)

The OSA appears to be made by a syntrophic relationship Bacterium-Methanosarcinaceae

ACETATE REINJECTION



- No latency
- Synthrophic acetate oxidation

Importance of inoculum acclimatation

From the black box to the grey box

Tools for exploring metabolic pathways

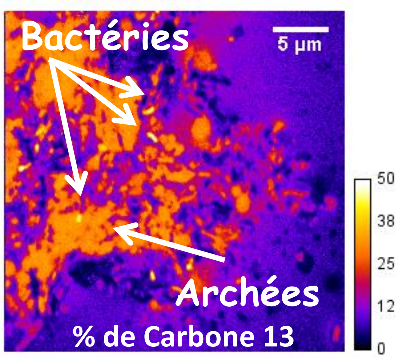
Functional microbiota identification tools

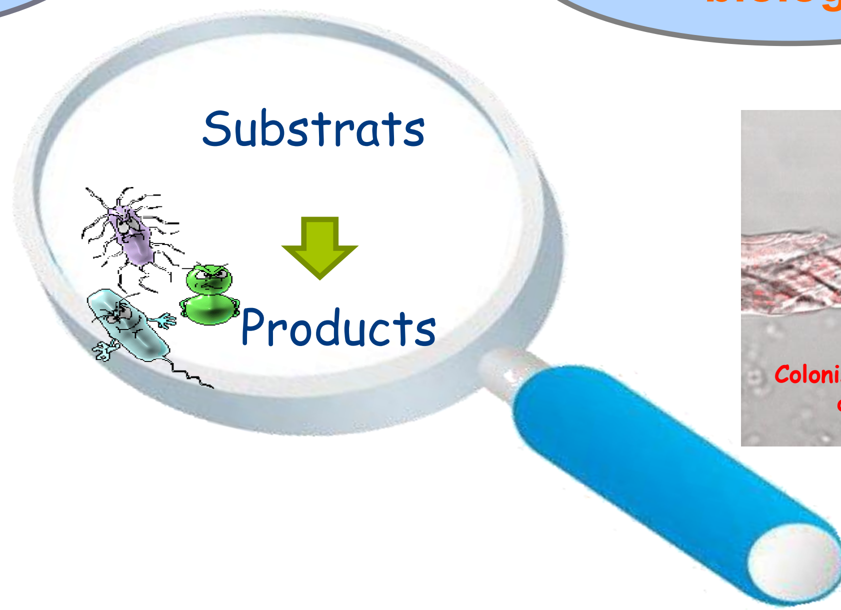
Isotopic Biogeochemistry ⟷ **Molecular biology**

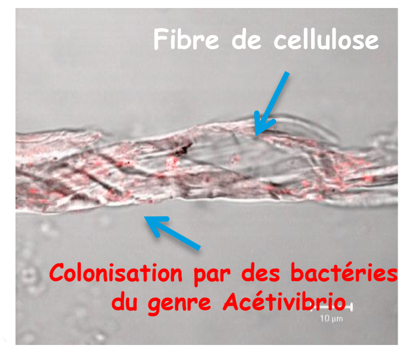
Acetoclastic methanogenesis

SAO

SIMSISH







- **Demonstration of the inhibition of certain metabolic pathways**
- **Identification of the responsible microorganisms**
- **Bio-augmentation strategies often show limitations**
- **Relationship between diversity, structure and ecosystem functions**

METAOMIC APPROACHES