

# 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



www.inrae.fr

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Master Environmental Engineering and Sustainability Management





$$A=Z+NX_Z$$

Same atomic number Z
(Z= number of protons)

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







# $A=Z+N\chi_{Z}$ Exemple: Carbon C $A=Z+N\chi_{Z}$ $A=Z+N\chi_{Z}$

# . 6, 7, ou 8 neutrons (N) ->Atomic mass A = Z+N = 12, 13, ou 14

*Notation* : <sup>12</sup>C, <sup>13</sup>C, <sup>14</sup>C



## -Radioactive = Transformation into isotopes of another element

<sup>147</sup>Sm →<sup>143</sup>Nd <sup>238</sup>U →<sup>206</sup>Pb <sup>87</sup>Rb →<sup>87</sup>Sr

- Stables = the others		
	No transformation as a function of time	
Légers:	<sup>1</sup> H, <sup>2</sup> H,	<sup>12</sup> C, <sup>13</sup> C, <sup>16</sup> O, <sup>17</sup> O, <sup>18</sup> O, <sup>14</sup> N, <sup>15</sup> N
Lourds:	<sup>63</sup> Cu, <sup>65</sup> Cu, <sup>54</sup> Fe, <sup>56</sup> Fe, <sup>57</sup> Fe, <sup>58</sup> Fe	
<sup>66</sup> Zn, <sup>68</sup> Zn, <sup>70</sup> Zn		







## **Isotope natural abundancy**

## Hydrogen



Carbon



Nitrogen





**Sulphur** 





### ISOTOPES: Comparable chemical properties

#### (identical electronic structure)



Pression de vapeur (100°C, en Torr)

Viscosité (à 20°C en centipoises)

## **ISOTOPIC FRACTIONATION**

H<sub>2</sub>18O

1.1106

4.30

0.28

760.00

1.002

721.60

1.247

100.14

1.056



# Evaporation:

T(boiling):  $H_2O= 100 \ ^{\circ}C$  $D_2O= 101.4 \ ^{\circ}C$ 

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



## In nature, slight variations in the order of ‰ of the <sup>13</sup>C / <sup>12</sup>C ratio.

Variations measured using the isotopic composition:

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

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







The <sup>13</sup>C / <sup>12</sup>C ratio depends on:

- Carbon origin
- Environmental conditions
- Biosynthetic cycle

C4 (Hatch et Slack)







#### NATURAL ISOTOPIA:

- Compounds origin
- Compounds fate

#### **ISOTOPIC TRACING:**

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Use of artificially enriched compounds in D, <sup>13</sup>C, <sup>15</sup>N, <sup>18</sup>O
```

Compounds fate (Transfer, degradation...)











## **ISOTOPIC MASS SPECTROMETRY**







IRMS COUPLING WITH AN ELEMENTARY ANALYZER

## EA Carbon and Nitrogen combustion - solids



Amount of carbon needed : 50 µg











e Name: C:\Thermo\lsodat NT\Global\User\GC II-III Interface\Results\ACQ-Results\JGD-AGVs\Tests opti sepa pics HS\LixiXian7 Test 39\_-0011.dxf









## Ice : greenhouse gas archives







## **INCREASE IN GHG CONCENTRATION**



23

**GHG concentration evolution** 



# $\delta$ D et δ <sup>18</sup>O: isotopic thermometer















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



























## **Respiratory isotopic test**



Courtesy of Simac Diagnostica BV, The Netherlands






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





## Application to environnemental bioprocesses









# Bioreactor Municipal solid waste landfill



## Bioreactor Municipal solid waste landfill





#### **Anaerobic batch experiment**













## N<sub>2</sub> production = denitrification

## **Presence of organic matter**

## → Heterotrophic denitrification

 $0,625 \text{ CH}_{3}\text{COOH} + \text{NO}_{3}^{-} \rightarrow \text{HCO}_{3}^{-} + 0,25 \text{ CO}_{2} + 0,75 \text{ H}_{2}\text{O} + 0,5 \text{ N}_{2}$ 

**Observed in 13 cases upon 20** 







## No N<sub>2</sub> production = Nitramonification?

$$NO_{3}^{-} + 4H_{2} + 2H^{+} \rightarrow NH_{4}^{+} + 3H_{2}O$$

**Observed in 4 cases upon 20** 

# () Identification of the shifting parameter

#### **Statistical analysis :**





#### Injection of H<sub>2</sub>S at different concentration together with nitrate





#### NO<sub>3</sub> reduction kinetic in the four reactors





#### N<sub>2</sub> production in the four reactors





#### Is nitramonification happening?









N<sub>2</sub>0 concentration in biogas collecting system





Surface N<sub>2</sub>O emission







#### **During OM degradation**









 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)

$$\delta^{13}C = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \quad [\overset{0}{_{00}}] \qquad R = {}^{13}C/{}^{12}C$$

→ 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





## **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 δ<sup>13</sup>CH<sub>4</sub>
- 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)





#### 65

() 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)







#### Aceticlastic pathway





Aceticlastic methanogenesis : Hydrogenotrophic methanogenesis : Homoacetogenesis : Acetate oxidation :  $\begin{array}{c} {\sf CH}_3{\sf COOH} \ -> {\sf CH}_4 + {\sf CO}_2 \\ \\ {\sf 4H}_2 + {\sf CO}_2 \ -> {\sf CH}_4 + {\sf H}_2{\sf O} \\ \\ {\sf 4H}_2 + {\sf CO}_2 \ -> {\sf CH}_3{\sf COOH} + {\sf 2H}_2{\sf O} \\ \\ {\sf CH}_3{\sf COOH} + {\sf 2H}_2{\sf O} \ -> \ {\sf 4H}_2 + {\sf CO}_2 \end{array}$ 

# **Sequencing results** - Waste incubations (55°C)



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



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

**Mathematical Syntrophic acetate oxidation** 

A two steps reaction :

1)  $CH_3COOH + 2H_2O -> 4H_2 + 2CO_2$ 

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

**Bacteria** 

Archeae

Syntrophic relationship between

bacteria and archaea



Montparticities and the second structure of the sec







## Why it is important to improve cellulose anaerobic digestion?

#### Municipal solid waste composition



#### **Cellulose Hydrolysis**

Cellulose



#### 80 % of the methane potential

**Kinetic limiting step** 

Identification of microorganisms involved in cellulose hydrolysis


#### <sup>13</sup>C enriched substrat







**DNA** extraction



# Sequencing Presumed Fonctional microbial group identification









Radajewski, Nature, 2000

#### SIP during cellulose degradation



# Identification of involved microbial group

#### Clostridium



#### Acetivibrio



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

Involved in glucose degradation at 35°C



### FISH (Fluorescence In Situ Hybridization)





## Spatial distribution of microorganisms







- C1 & C2: Immediate start of methanogenesis
- C3 & C4: High latency

Cumulated CH<sub>4</sub> production

Similar level of methane production at the end

	[NH4]	
- <b>-</b> C1	0.2 g.L <sup>-1</sup>	
<b></b> C2	1.4 g.L <sup>-1</sup>	
- <b>-</b> C3	3.6 g.L <sup>-1</sup>	
<b></b> C4	5.4 g.L <sup>-1</sup>	





#### ISOTOPIC COMPOSITION



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

10 µm 79







 Table 1.
 Reactions involved in acetate and hydrogen metabolism

Process	Reaction			$\Delta G^{0'}$ (kJ/mol)
(1) Aceticlastic methanogenesis	*CH <sub>3</sub> COO <sup>-</sup> + H <sub>2</sub> O	$\rightarrow$	*CH <sub>4</sub> + HCO <sub>3</sub> <sup>-</sup>	-31.0
(2) Syntrophic acetate oxidation	$*CH_3COO^- + 4H_2O$	$\rightarrow$	$H^*CO_3^- + 4H_2 + HCO_3^- + H^+$	+104.6
(3) H <sub>2</sub> -consuming methanogenesis	$4H_2 + HCO_3^- + H^+$	$\rightarrow$	$CH_4 + 3H_2O$	-135.6
$(4) \operatorname{sum}(2) + (3)$	$*CH_3COO^- + H_2O$	$\rightarrow$	$H^*CO_3^- + CH_4$	-31.0
(5) H <sub>2</sub> -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<sub>4</sub> (reaction 1) or HCO<sub>3</sub><sup>-</sup> (reaction 4). The standard Gibbs free energy change ( $\Delta G^{0}$ ) values were calculated from reference 75.



#### 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)



Enriched <sup>13</sup>C





S



# Visualization of the whole biomass

Visualization of the targeted strain

Visualization of the isotopic enrichment

**Co-localisation** 



T

### E.coli 40% <sup>13</sup>C EubI + B.subtilis 10% <sup>13</sup>C



13**C** 



100%

0.9

#### CARBONE

%, 9%, 58%, 90% en <sup>13</sup>C + E. coli 34% en <sup>13</sup>C, hybridized by the probe EubI (Iode)

% <sup>13</sup> C IRMS	% <sup>13</sup> 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 <sup>15</sup>N, hybridized by the probe EubI (Iode)

% <sup>13</sup> C IRMS	% <sup>13</sup> C SIMS
9,25 ± 0,10	10,1 ± 0,17
36,46 ± 0,21	38,9 ± 0,19
63,99	66,5 ± 0,13





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 nonnatural 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





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



