Dipartimento di ingegneria civile e ambientale- DICA

Sezione Ambientale

POLITECNICO DI MILANO

Energy conversion routes - anaerobic digestion for biogas production and dark fermentation for biohydrogen production

Elena Ficara

elena.ficara@polimi.it

Table of content

Terminology/abbreviations

Total solids (TS) = Total solids are a measure of the suspended (particles) and dissolved (salts) solids in water.

Total Suspended Solids (TSS) = Suspended solids are those that can be retained on a water filter (silt, clay, plankton, organic wastes, and inorganic precipitates)

Total Dissolved Solids (TDS) = Dissolved solids are those that pass through a water filter (some organic materials, as well as salts, inorganic nutrients, and toxins).

Volatile Solids (VS) = as well as TS, but referred to organic matter

Volatile Suspended Solid (VSS)

Determination:

TS = Weight after evaporation to dryness in the oven at 105ºC.

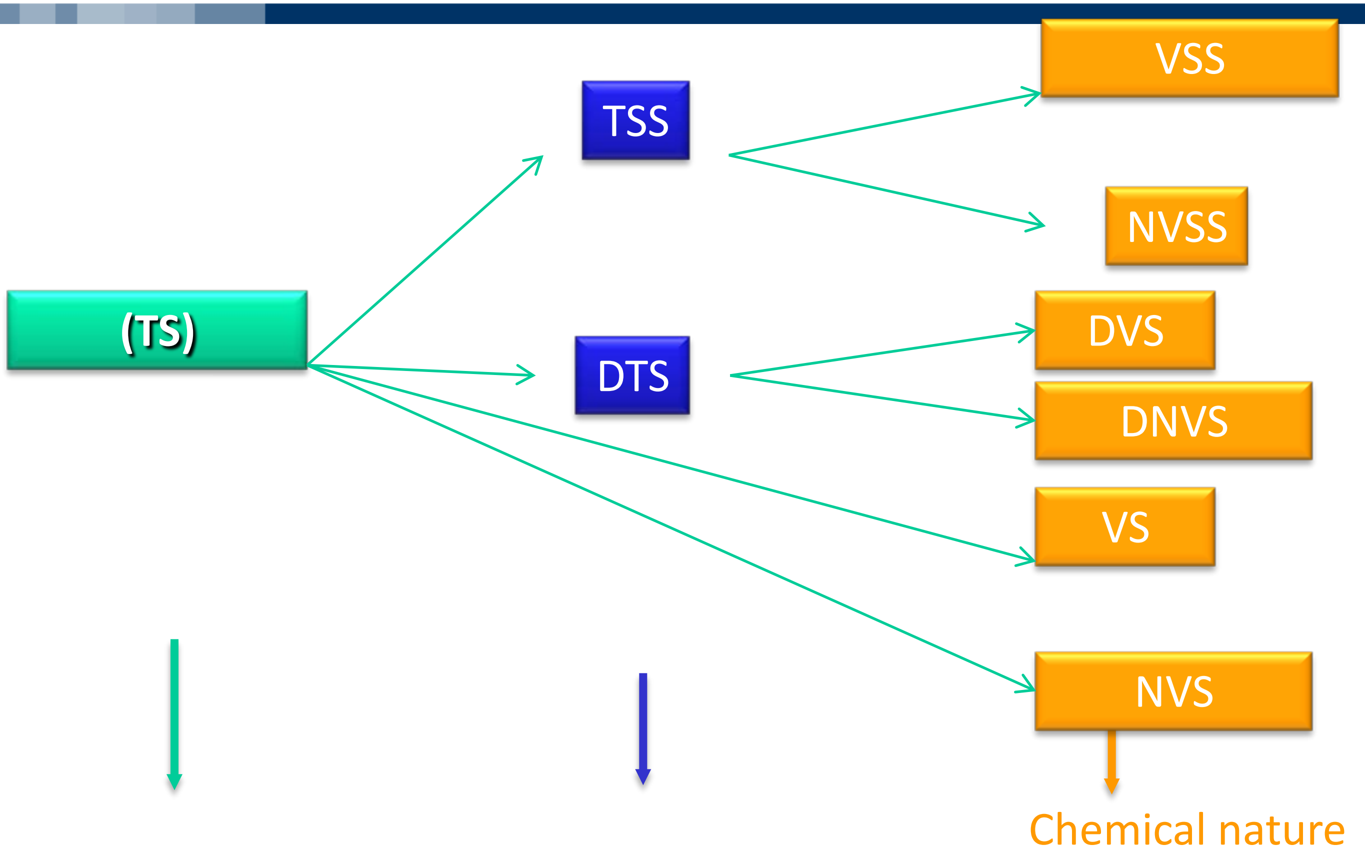
TSS = after filtration and evaporation to dryness in the oven at 105ºC.

Volatile Solids (VS) = Volatile solids are those solids lost apon ignition (heating to 600°C)

Volatile Suspended Solid (VSS) = as well as VS but after filtration

Terminology/abbreviations

Terminology/abbreviations



Evaporation a 105°C

Filtration on a 0,45 µm filter

Ignition 600°C

Terminology/abbreviations

Chemical Oxygen Demand (**COD**) test is commonly used to indirectly measure the amount of organic matters (suspended and dissolved) in water.

The COD test is performed by oxidizing all organic matter to carbon dioxide by using a strong oxidizing agent under acidic conditions.

The COD quantifies the amount of oxygen required to oxidize the organic matter in the sample

The COD is a measure of the chemical energy content of the organic matter, theoretically available to the bacteria (to be transfer to methane during AD)

Terminology/abbreviations

The COD of an organic compound $C_nH_aO_b$ can easily be calculated on the basis of the chemical oxidation reaction, assuming a complete oxidation:

$$C_nH_aO_b + \frac{1}{4}(4n+1-2b)O_2 \rightarrow nCO_2 + (a/2)H_2O \quad (16.15)$$

Eq. 16.15 shows that 1 "mol" of organic material demands 1/4(4n+a-2b) moles O_2 or 8(4n+a-2b) gO_2. Hence the theoretical oxygen demand of organic material can be expressed as:

$$COD_t = 8(4n + a - 2b)/(12n + a + 16b)$$

$(gCOD/gC_nH_aO_b)$ (16.16)

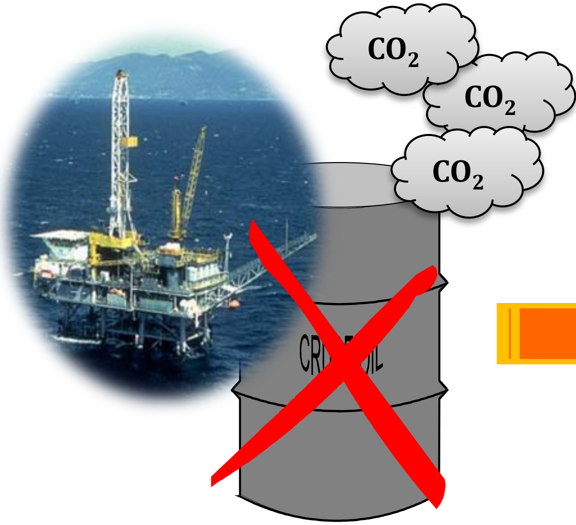
Obviously, with nitrogen containing compounds (proteins and amino acids) Eq. 16.16 needs to be corrected for the number of electrons that will stay with N and the total weight of N in the compound.

$$COD_t = 8(4n + a - 2b - 3d)/(12n + a + 16b + 14d)$$

$(gCOD/gC_nH_aO_bN_d)$ (16.17)

Introduction – Renewable energies

Fossil energies: 85%



Kyoto 1997

Renewable energy directive (2009)

« Food vs Fuel »

Biofuel generations from biomass

(i.e. biodiesel, bioethanol, biomethane, biohydrogen)

1st

seeds, sugars

2nd

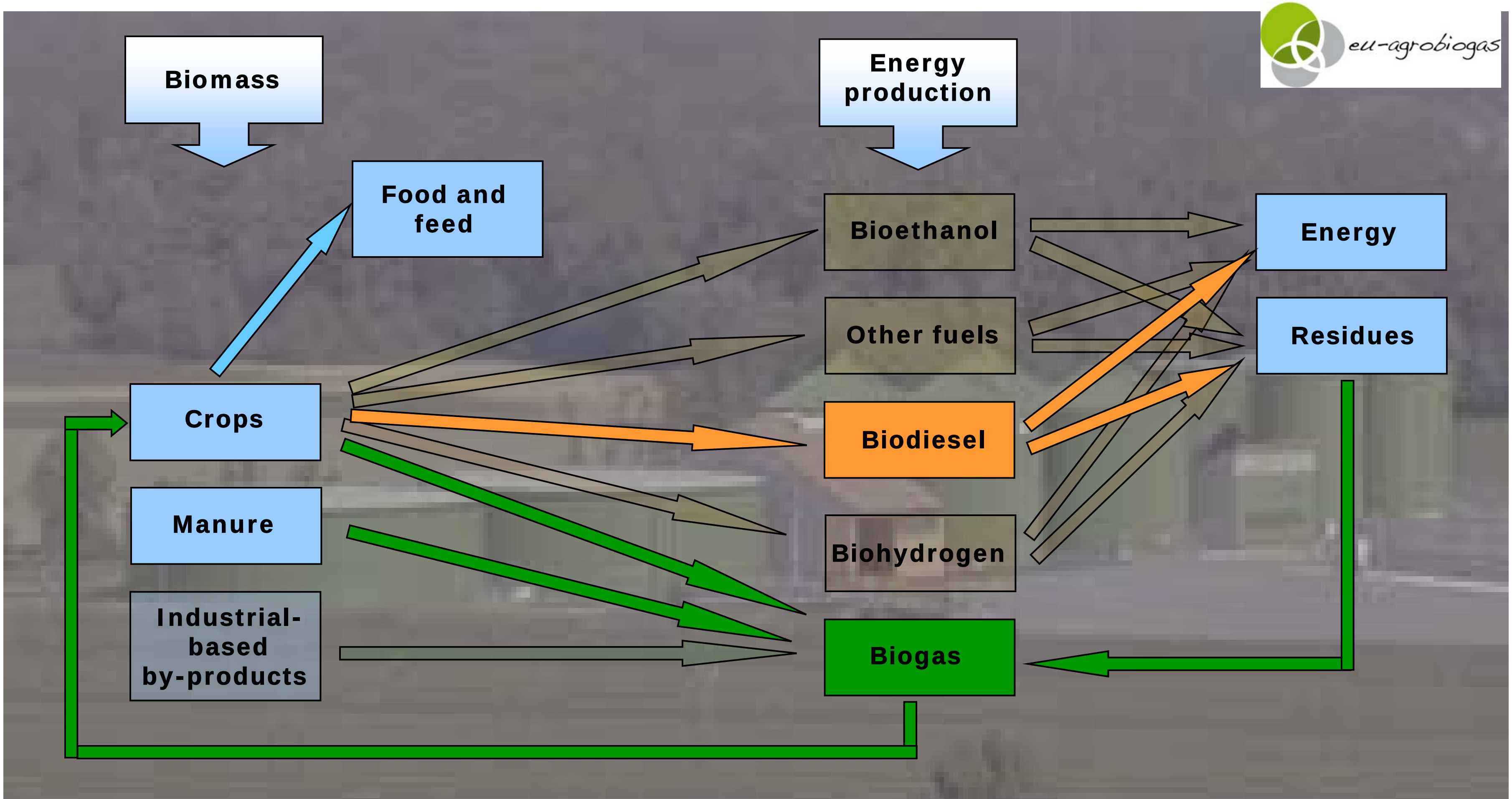
lignocellulosic biomass

3rd

algal biomass

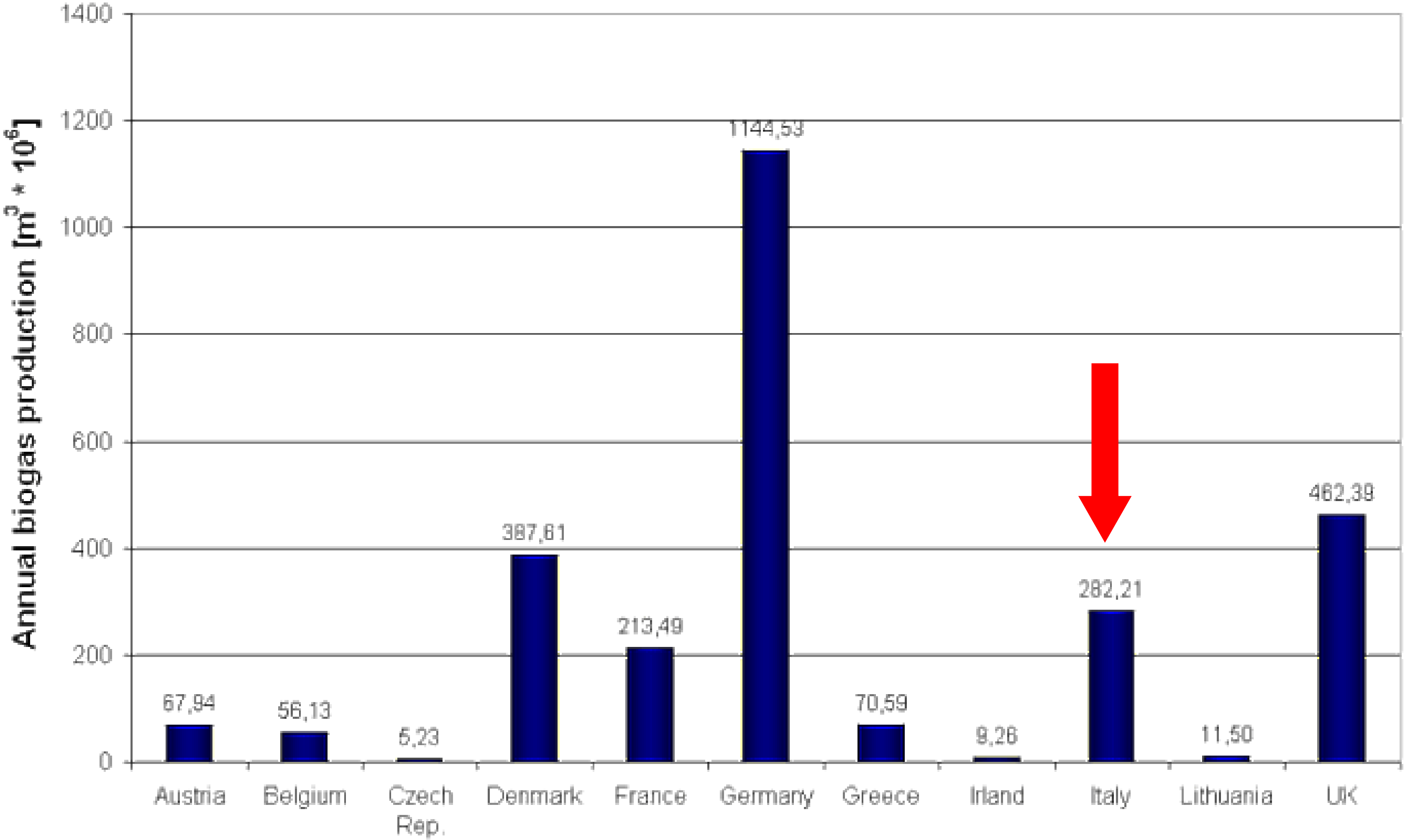
Introduction– Biofuels-Based-Biorefinery

Universität für Bodenkultur Wien



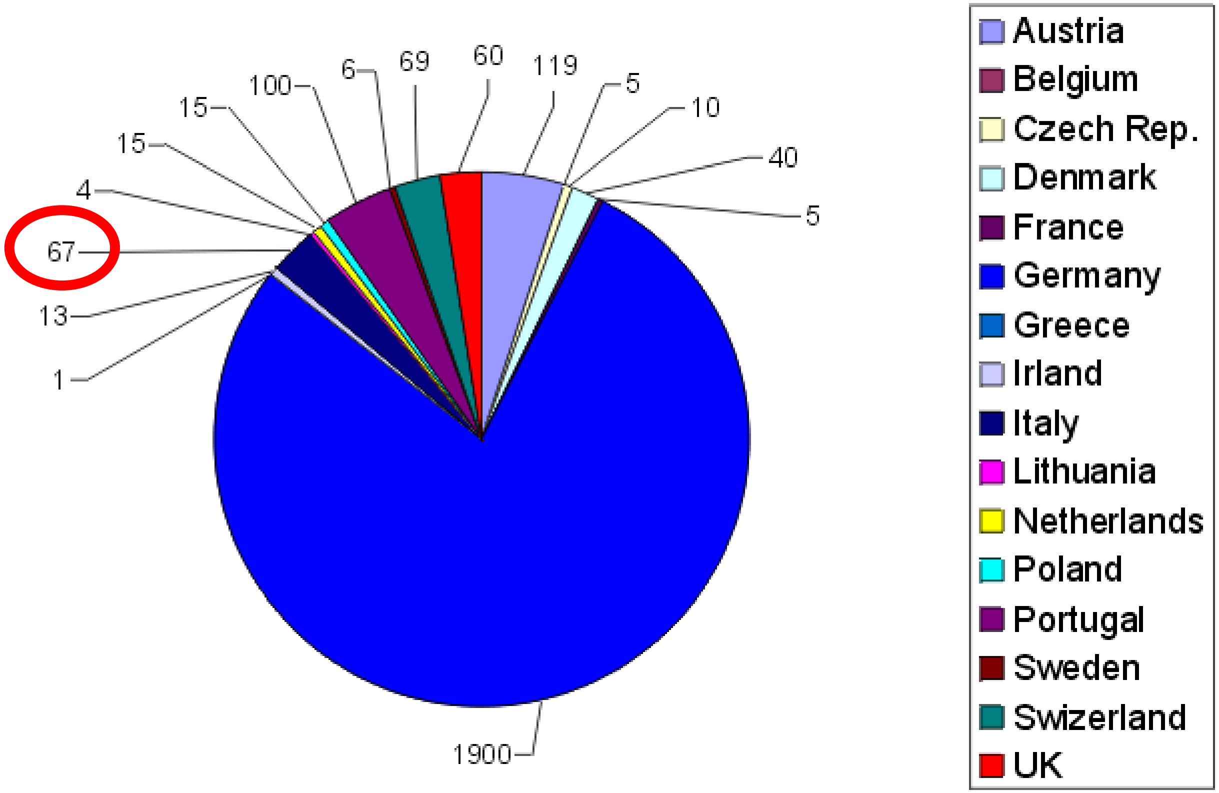
Biogas from anaerobic digestion

EU Gas production



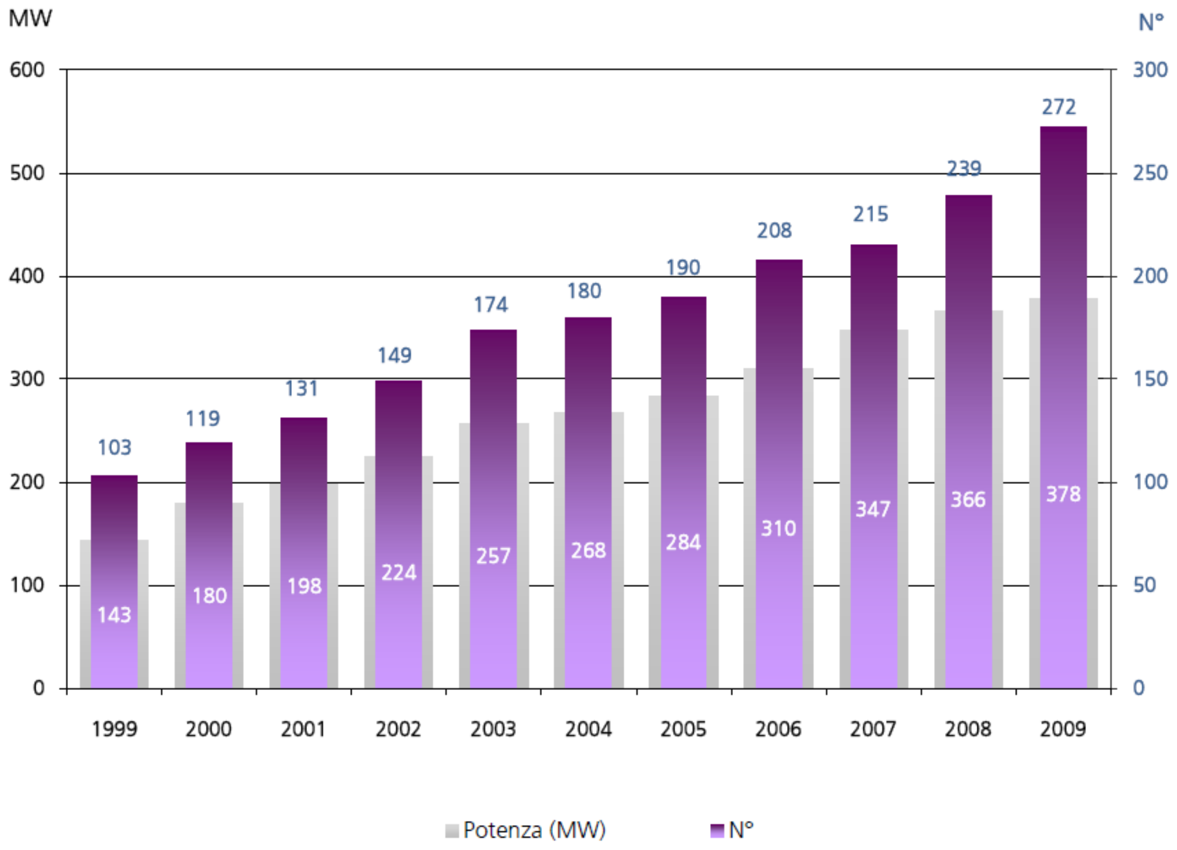
(Ref: http://www.adnett.org/ al 2005)

Farm Biogas Plants in EU



(Ref: http://www.adnett.org/ al 2005)

Trend of biogas plants in Italy



GSE, 2011

GSE 2012: approx. 1000 AD plants Pel = 770 MW, (accounting for the 4% of the overall renewable energy national production)

Of which approx. 300 MW in Lombardy

What's Anaerobic Digestion?

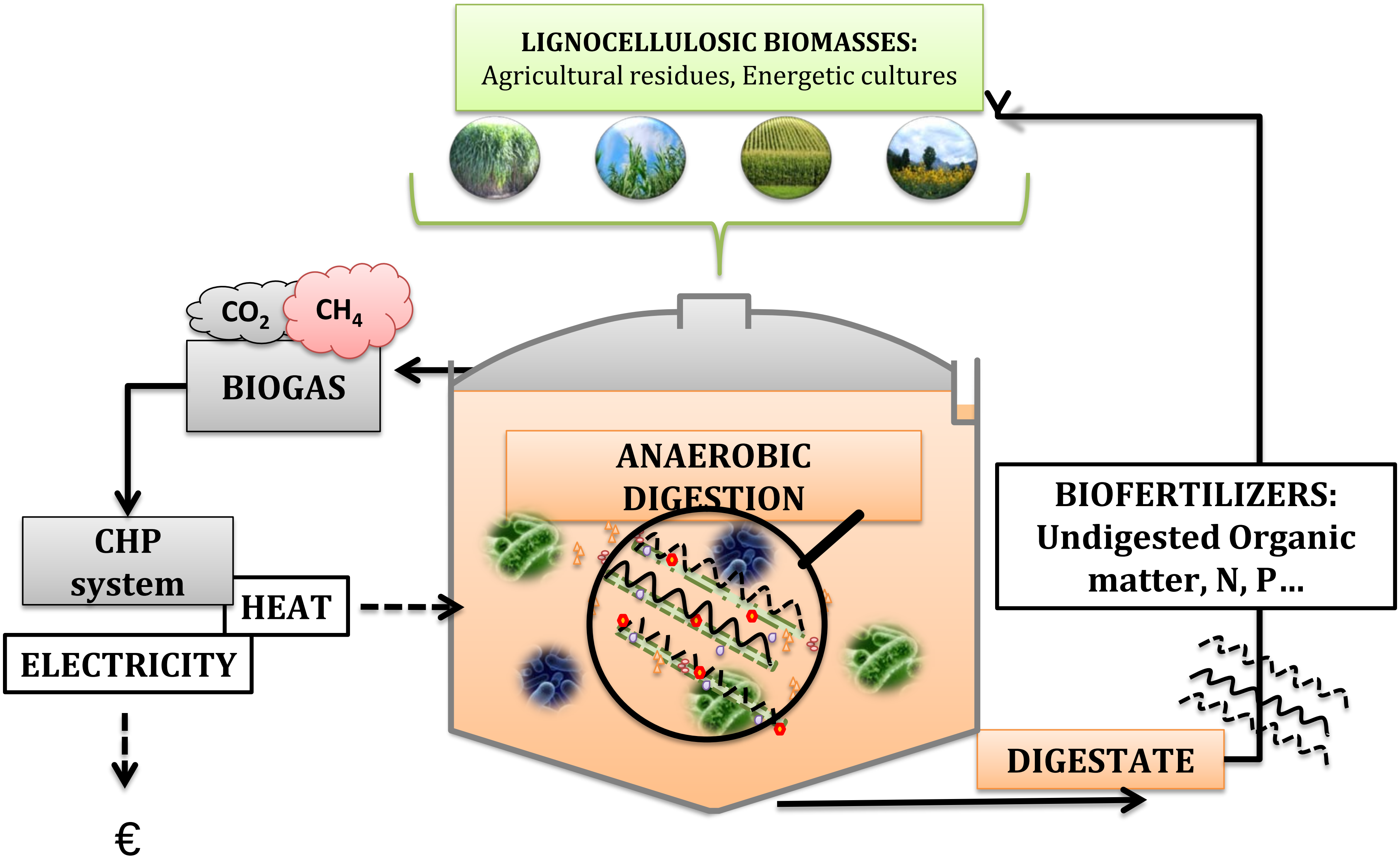
Anaerobic digestion is a biological process carried out in the absence of O_2 coverting the organic materials into methane and carbon dioxide

Organic materials $\rightarrow$ CO_2 + CH_4 + *gas in trace (+undegraded material)*

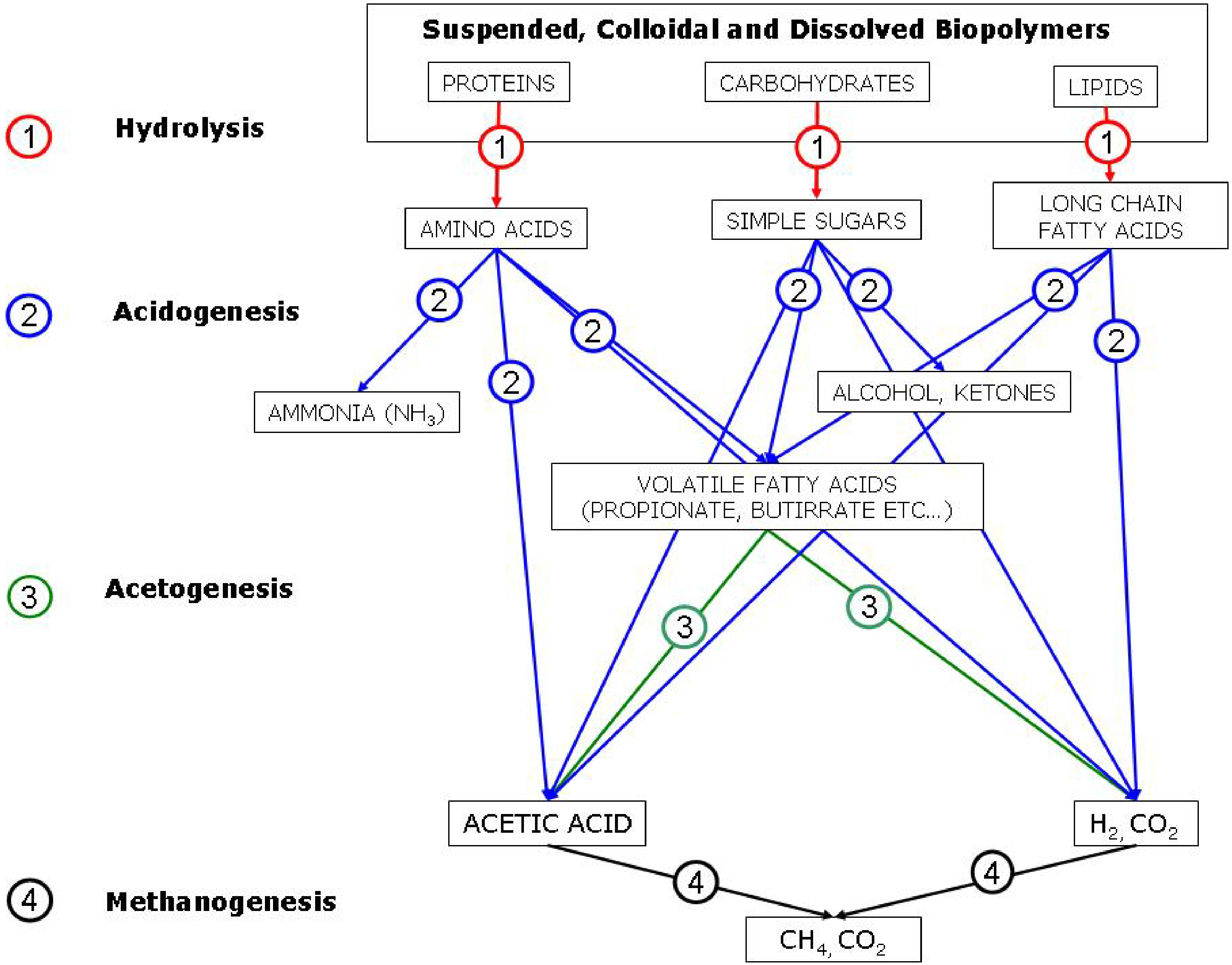
The anaerobic degradation of complex organic matter is carried out by a number of bacteria and archea that operate according to coordinated interactions.

The process may fail if a part of these organisms are inhibited.

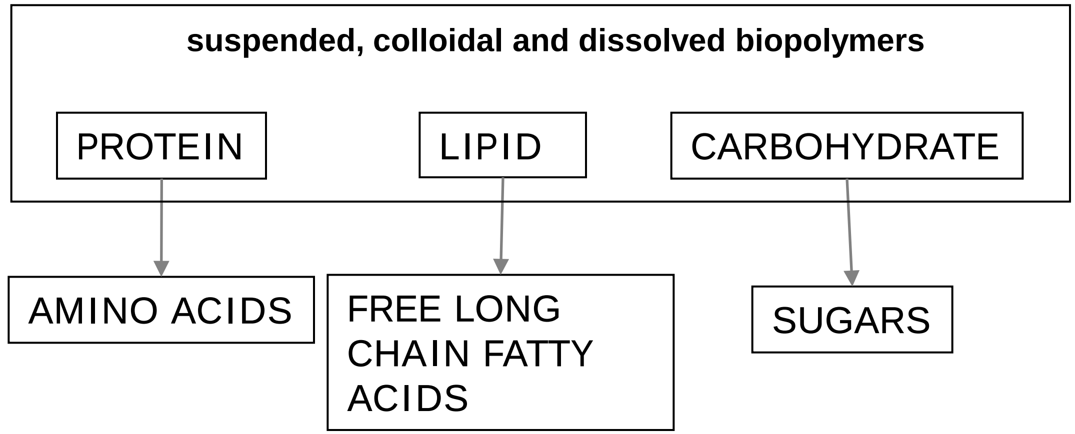
Introduction – Biogas from anaerobic digestion



Anaerobic degradation process



Anaerobic degradation process

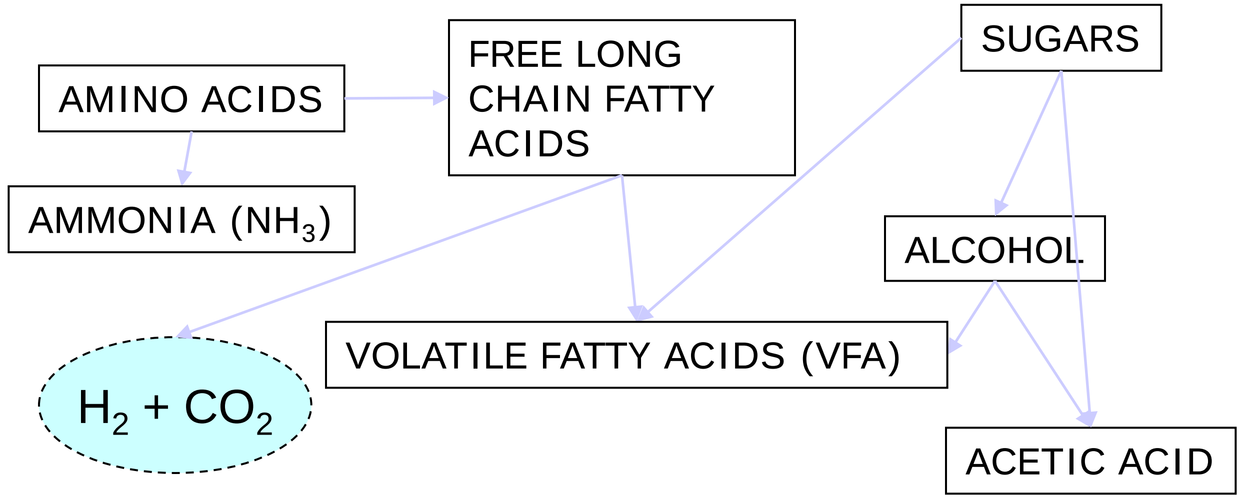


① **hydrolysis**

- ❑ Complex organic molecules (proteins, lipids and carbohydrates) are broken down into simple sugars, amino acids, and fatty acids.
- ❑ Hydrolytic bacteria are facultative anaerobes and they hydrolyze the substrate with extracellular enzymes (cellulases, hemicellulases, proteases, lipases, amylases)

Anaerobic degradation process

2 **acidogenesis**



- Primary fermenting bacteria uptake the products of hydrolysis and convert them into VFA, hydrogen and alcohols. These microorganisms are both obligate and facultative anaerobes.

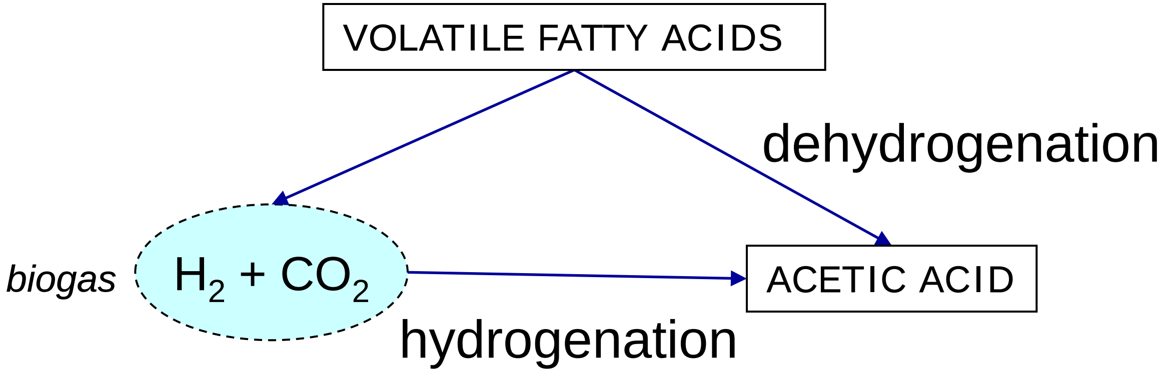
- **Optimal conditions:** bacteria produce mainly acetic acid, hydrogen and carbon dioxide, used directly as substrates by methanogenic microorganisms.

- **non-optimal conditions (excess of supply of substrate, present of toxic compounds,…):** increase of the concentration of hydrogen and formation of intermediates (VFA, alcohols).

- pH tends to decrease

Anaerobic degradation process

3 acetogenesis



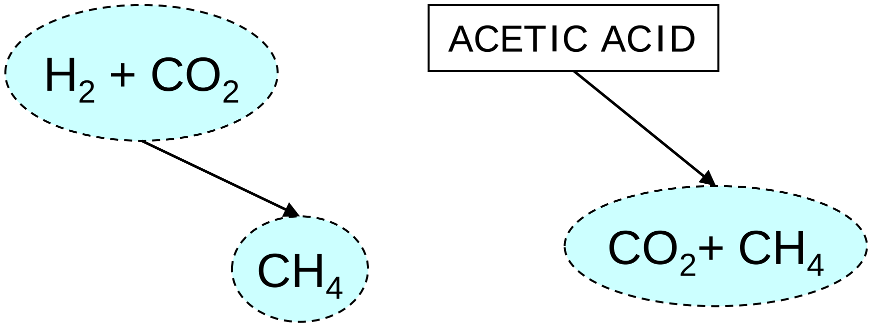
❑ Although some acetic acid (20%) and H_2 (4%) are directly produced by acidogenic fermentation of sugars, and amino acids, both products are primarily derived from the acetogenesis and dehydrogenation of higher volatile fatty acids.

❑ Secondary fermenting bacteria convert VFA and alcohols into acetic acid, hydrogen and carbon dioxide. These microorganisms are obligate hydrogen producing bacteria.

❑ The acetic acid, hydrogen and carbon dioxide produced during acidogenesis and acetogenesis are the substrates for the methanogenesis step.

Anaerobic degradation process

④ methanogenesis



- *Methane is produced through two processes:*
  - ***Aceticlastic methanogenesis** (almost 70%):*

  acetic acid → methane and carbon dioxide
  - ***Hydrogenotrophic methanogenesis** (almost 30%):*

  oxidation of hydrogen produced by the secondary fermenting bacteria and reduction of carbon dioxide to methane.
- *Methanogenic microorganisms are:*
  - *Obligate anaerobic*
  - *Slow growth*
  - *Sensitive to environmental conditions*

Environmental factors

The successful operation of anaerobic reactor depends on maintaining the environmental factors accommodating the need of all microorganisms involved in the process.

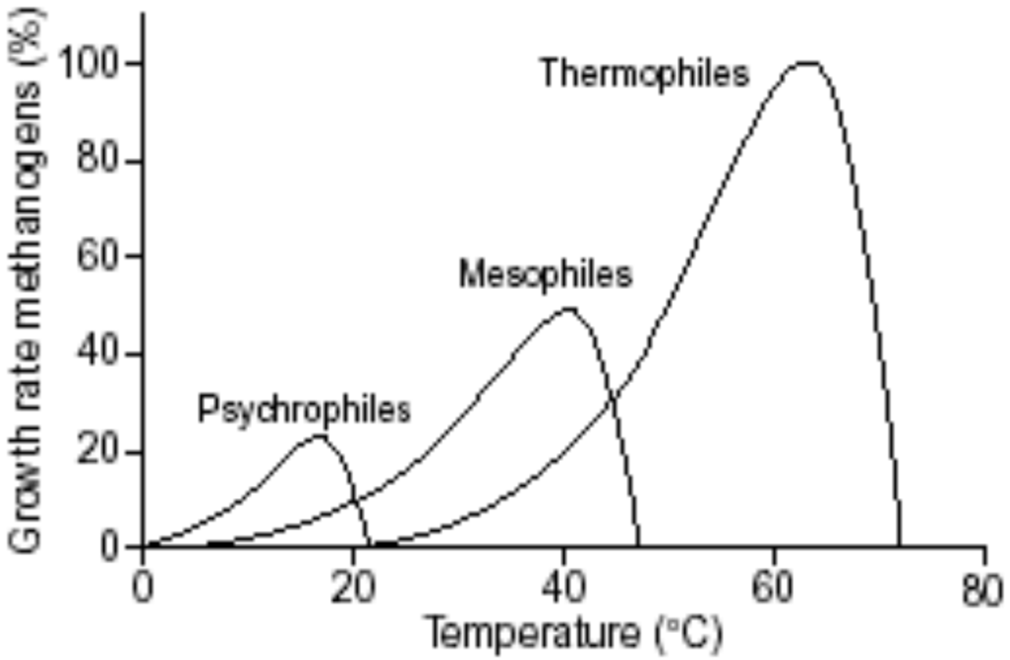
1. Temperature
2. pH/buffering
3. Nutrients and trace metals
4. Toxic/inhibitor compounds

1. Temperature

- Anaerobic processes like other biological processes operate in certain temperature ranges

- In anaerobic systems: three optimal temperature ranges:

  - Psychrophilic (5-15 °C)
  - Mesophilic (35-40 °C)
  - Thermophilic (50-55 °C)



2. pH

There exist two microbial domains in terms of pH optima namely acidogens and methanogens.

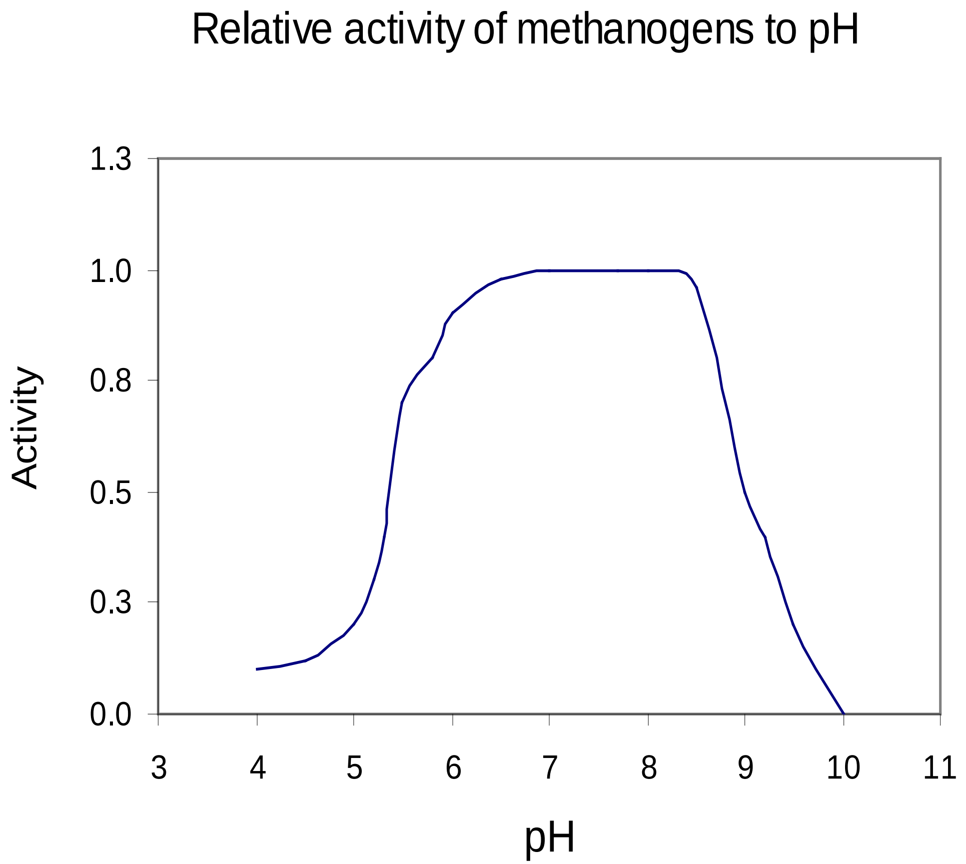
The best pH range for acidogens is 5.5 – 6.5 and for methanogens is 7.8 – 8.2.

The operating pH for combined cultures is 6.8-7.4 with neutral pH being the optimum.

Low pH reduces the activity of methanogens causing accumulation of VFA and H_2. At higher partial pressure of H_2, propionic acid degrading bacteria will be severely inhibited thereby causing excessive accumulation of higher molecular weight VFAs such as propionic and butyric acids and the pH drops further. If the situation is left uncorrected, the process may eventually fail.

Remedial measures: Reduce the loading rates and supplement chemicals to adjust the pH: alkaline chemicals such as $NaHCO_3$, NaOH, Na_2CO_3, quick lime (CaO), slaked lime [$Ca(OH)_2$], limestone (or softening sludge) $CaCO_3$, and NH_3 can be used.

pH dependence of methanogens



Natural buffering

An anaerobic treatment system has its own buffering capacity against pH drop because of alkalinity produced during waste treatment: e.g. the degradation of protein present in the waste releases NH_3, which reacts with CO_2 forming ammonium carbonate as alkalinity.

$$NH_3 + H_2O + CO_2 \rightarrow NH_4HCO_3$$

Sulfate and sulfite reduction also generate alkalinity.

$$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + HCO_3^- + 3H_2O$$

When pH starts dropping due to VFA accumulation, the alkalinity present within the system neutralizes the acid and prevents further drop in pH. If the alkalinity is insufficient to buffer the system pH, external addition is needed.

3. Nutrients and trace metals

All microbial processes including anaerobic require macro (N, P and S) and micro (trace metalsm, Ni, Co, Fe, Mo, Se etc) nutrients in sufficient concentration to support biomass synthesis.
The nutrients and trace metals requirements for anaerobic process are much lower as only 4 - 10% of the COD removed is converted to biomass.

COD:N:P = 350:7:1 (for highly loaded system) 1000:7:1 (lightly loaded system)

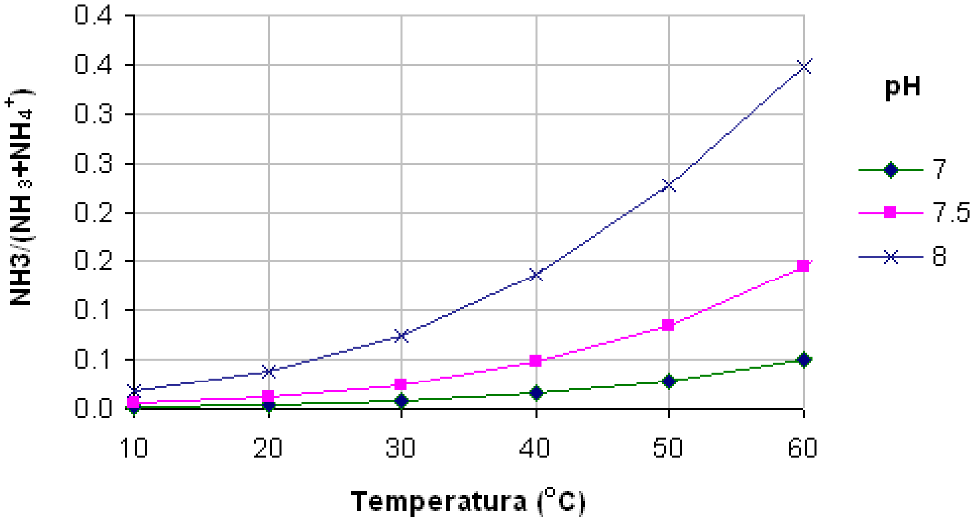
4. Inhibition/Toxicity

Inhibitory substances are often found to be the leading cause of anaerobic reactor upset and failure since they are present in substantial concentrations in wastewaters and sludges. A material may be judged inhibitory when it causes an adverse shift in the microbial population or inhibition of bacterial growth. Inhibition is usually indicated by a decrease of the steady-state rate of methane gas production and accumulation of organic acids (Chen et al., 2007).

Example: pH and Ammonia Inhibition

- Methanogens are sensitive to the presence of ammonia
- Ammonia derives from proteins hydrolysis
- The presence of ammonia depends on pH and temperature

$$NH_4^+ \xleftrightarrow{k_b} NH_3 + H^+$$



- Tollerance level of methanogens ~ hundreds of mg/L (limits are not determined)
- Inhibition is reversible in presence of adaptation

Essential conditions for efficient anaerobic treatment

- ❑ Avoid excessive air/O_2 exposure

- ❑ No toxic/inhibitory compounds present in the influent

- ❑ Maintain pH between 6.8 –7.2

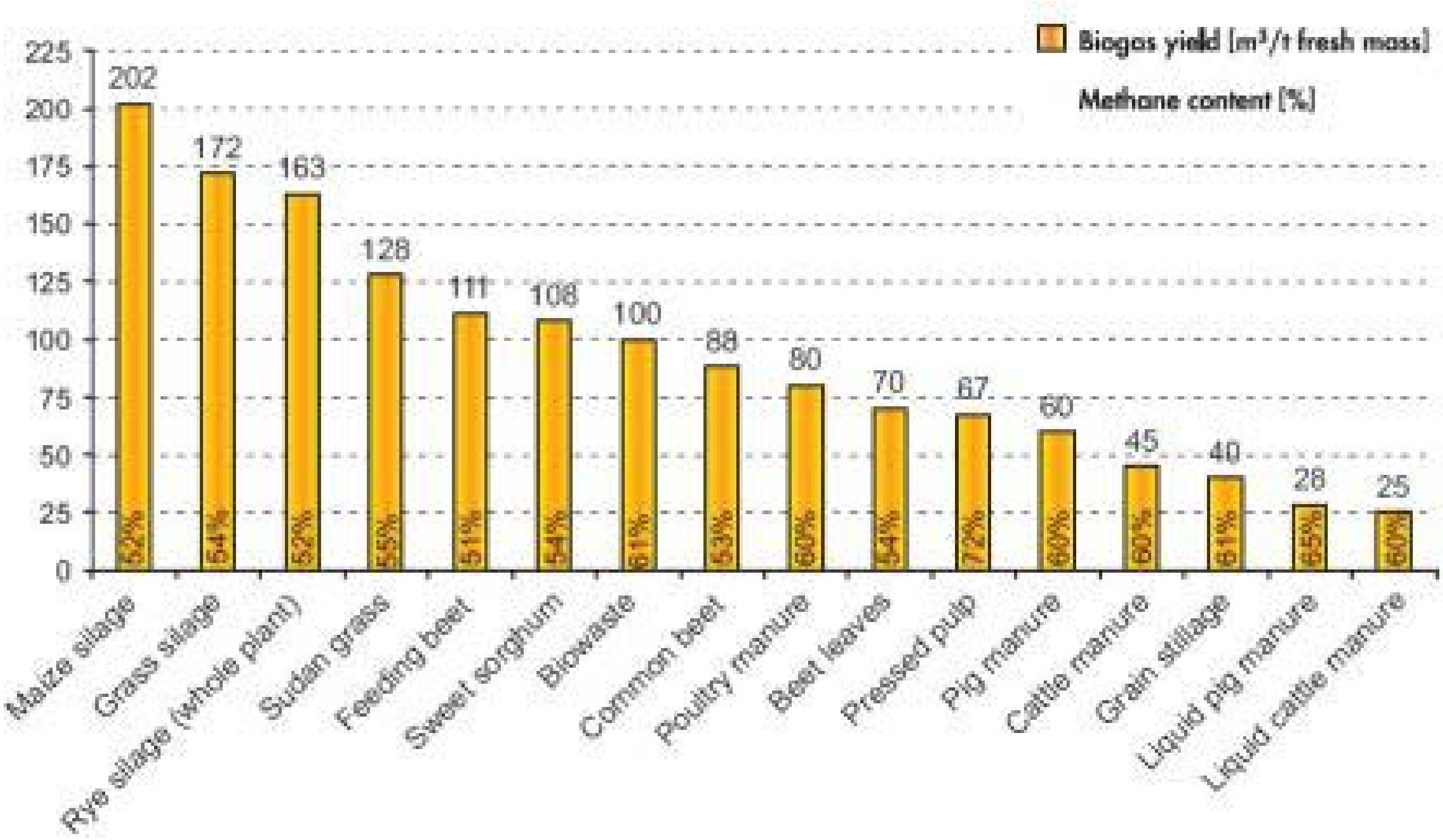
- ❑ Sufficient alkalinity present (mainly bicarbonates)

- ❑ Low volatile fatty acids (VFAs)

- ❑ Temperature around mesophilic range (30-38 ^{o}C)

- ❑ Enough nutrients (N & P) and trace metals especially, Fe, Co, Ni, etc.
  COD:N:P = 350:7:1 (for highly loaded system) 1000:7:1 (lightly loaded system)

Substrates for biogas production



BIOGAS

Biogas typically refers to a gas produced by the biological breakdown of organic matter in the absence of oxygen.

Biogas comprises primarily methane (CH_4) and carbon dioxide (CO_2) and may have small amounts of hydrogen sulphide (H_2S), moisture and siloxanes.

Biogas production depends on:

- ❑ Source and degradability of substrate
- ❑ Process condition (organic loadings, retention times, environmental factors)

Biogas components	**%**
CH_4	50–75
CO_2	25–50
N_2	0–10
H_2	0–1
H_2S	0–3
O_2	0–2

Not all COD (organic matter) is completely degraded. The fate of COD during anaerobic treatment process can be viewed as

- COD diverted to biomass synthesis (5 ed il 10%)
- Residual COD (in effluent)
- COD converted to CH_4 gas
- COD utilized for sulfate/nitrate reduction (if present)

Biochemical Methane Potential

Biochemical Methane Potential

Biochemical methane potential (BMP) is a procedure developed to determine the methane production of a given organic substrate during its anaerobic decomposition.

- Theoretical methane potential (BMP_{th})
- Experimental methane potential (BMP_{exp})

Theoretical methane potential (BMP_{th})

The theoretical methane potential is widely used to predict the methane production of a specific organic substrate.

It is frequently expressed as mL_{CH4} at normal or standard temperature and pressure conditions per amount of organic material added (VS or COD basis), although it can also be expressed per organic material removed.

Theoretical methane potential (BMP_{th})

There are different ways to calculate this parameter:

1. Traditionally BMP_{Th} has been calculated when the atomic or the organic fraction compositions are known

2. COD analysis permits the calculation of BMP_{Th}. Theoretically, 0.350 L of methane at 0°C or 0.395 L at 35 °C and 1 atm can be obtained from 1 g COD removed (CODrem).

Theoretical methane potential (BMP_{th})

Elementary composition (Buswell e Boruff)

$C_aH_bO_cN_d + \alpha_1\ H_2O \rightarrow \alpha_2\ CH_4 + \alpha_3\ CO_2 + \alpha_4\ NH_3$

$$\begin{cases} \alpha_1 = \left(a - \dfrac{b}{4} - \dfrac{c}{2} + \dfrac{3d}{4}\right) \\ \alpha_2 = \left(\dfrac{4a + b - 2c - 3d}{8}\right) \\ \alpha_3 = \left(\dfrac{4a - b + 2c + 3d}{8}\right) \\ \alpha_4 = d \end{cases}$$

Hypothesis:

- *Biomass synthesis (negligible)*
- *The reaction is assumed to be complete*

$$B_{0,CH_4}\left[\frac{m_n^3}{kg_{VS}}\right] = \frac{\dfrac{4\cdot a + 1\cdot b - 2\cdot c - 3\cdot d}{8}\cdot 22{,}415}{12\cdot a + 1\cdot b + 16\cdot c + 14\cdot d}$$

$$B'_{0,CH4}\left[\frac{m_n^3}{kg_{COD}}\right] = \frac{\dfrac{4\cdot a + 1\cdot b - 2\cdot c - 3\cdot d}{8}\cdot 22{,}415}{2\,\dfrac{4\cdot a + 1\cdot b - 2\cdot c - 3\cdot d}{8}\cdot 32} = 0{,}35$$

All COD (organic matter) is completely transformed to methane

Theoretical methane potential (BMP_{th})

Elementary composition (Buswell e Boruff)

Substrato	Elementary composition	COD/SV ($g_{COD}\ g_{SV}^{-1}$)	B,CH_4 $Nm^3\ kg_{SVbio}^{-1}$	% CH_4
Carbohydrates	$(C_6H_{10}O_5)_n$	1,19	0,415	50
Proteins	$C_5H_7O_2N$	1,42	0,496	63
Lipids	$C_{57}H_{104}O_6$	2,90	1,014	70
Ethanol	C_2H_6O	2,09	0,730	75
Acetic Acid	$C_2H_4O_2$	1,07	0,373	50
Propionic Acid	$C_3H_6O_2$	1,51	0,530	58
Butyric Acid	$C_4H_8O_2$	1,82	0,64	63
Primary sludge	$C_{3.65}H_7O_2N_{0.196}$	1,59	0,556	62

The theoretical methane potential is widely used to predict the methane production of a specific substrate. Traditionally it has been calculated when the atomic or the organic fraction compositions are known.

Theoretical methane potential (BMP_{th})

If the organic fraction composition (lipids, proteins, and carbohydrates) is known, methane yield can be estimated using the following general equation:

$$BMP_{th}\,(m^3/kgVS) = 0.415\cdot Carbohydrates\ (\%VS) + 0.496\cdot Proteins\ (\%VS) + 1.014\cdot Fats\ (\%VS)$$

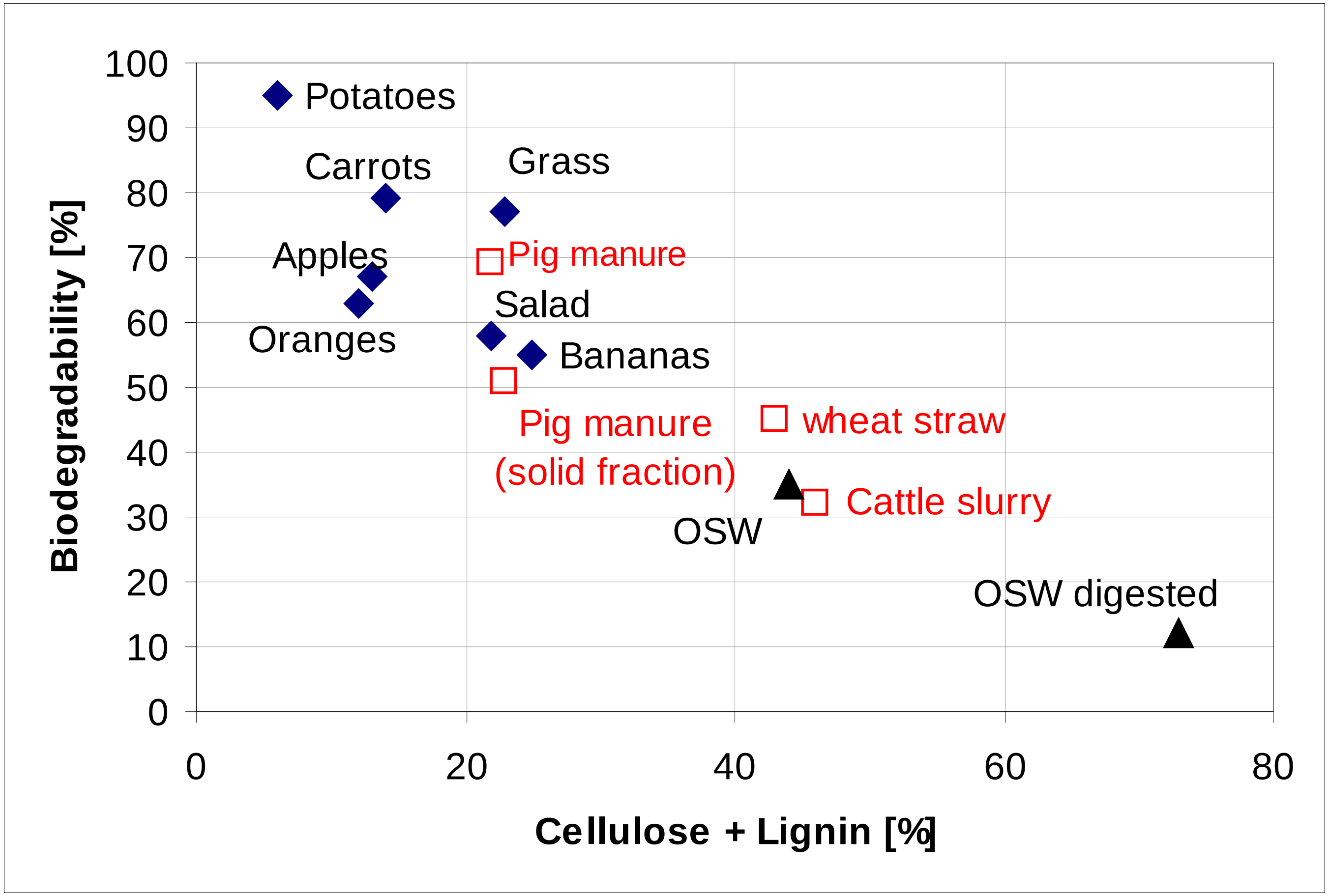
where the different fractions must be quantified by analytical composition measurements of the organic matter. The coefficients in this equation are derived from stoichiometric conversion of model compounds representing average formulae for carbohydrates, proteins, and lipids.

Recently, some authors have proposed more sophisticated multiple regression models to predict the methane yield of organic matter from their chemical composition.

Example, for mais (Amon et al., 2007):

$$\begin{aligned} B_{0,CH_4}\left[\frac{m_n^3}{kg_{VS}}\right] &= 19{,}05\cdot proteins(\%TS) + \\ &+ 27{,}73\cdot fats(\%TS) + \\ &+ 1{,}80\cdot cellulose(\%TS) + \\ &+ 1{,}70\cdot hemicelluloses(\%TS) \end{aligned}$$

Degradability of different substrates



Experimental methane potential (BMPexp)

The BMP assay has proved to be a relatively simple and reliable method to obtain the extent and rate of organic matter conversion to methane.

Biochemical Methane Potential (BMP) test is a laboratory test assessing the potential biogas yield of a feedstock.

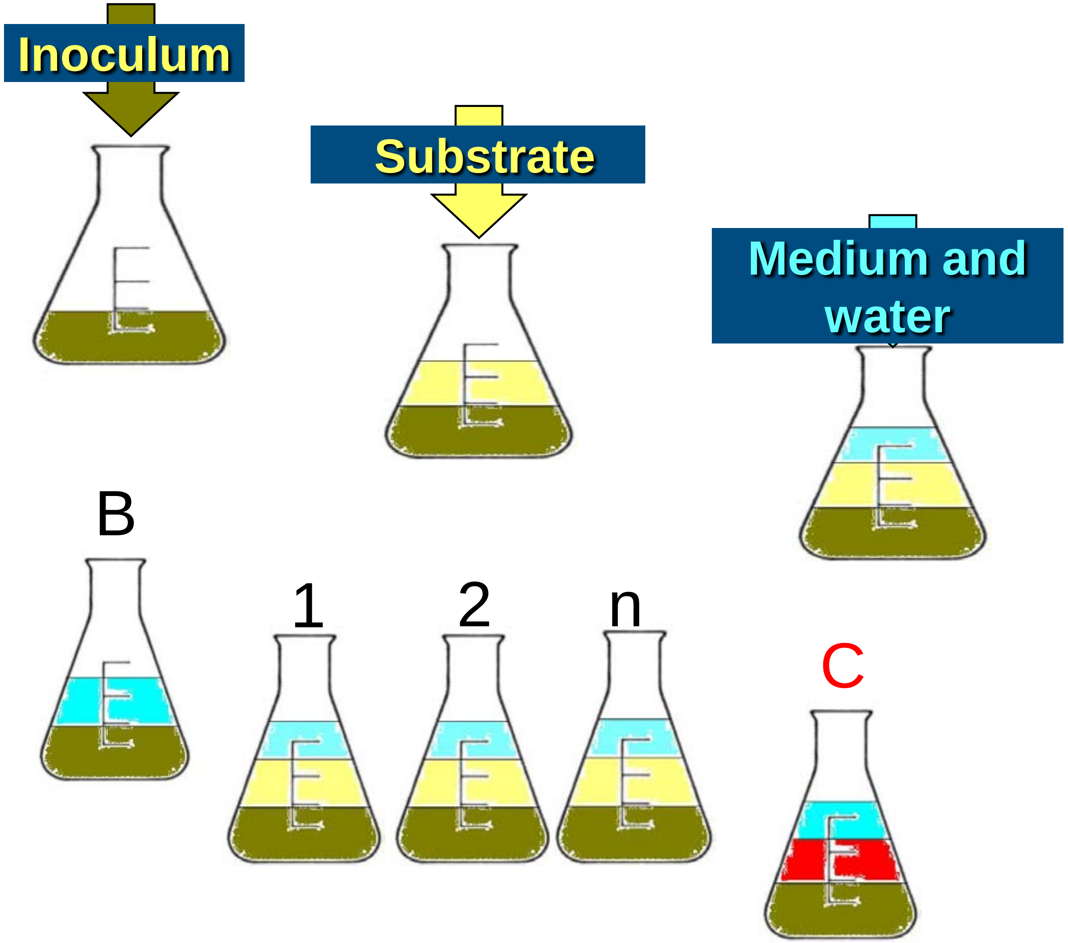
BMP testing allows the identification of the most appropriate feedstocks (waste, organic material, crops), pre-treatments, nutrient requirements etc. to achieve optimum biogas yield.

ASSESSMENT

SAMPLE PREPARATION

TEST

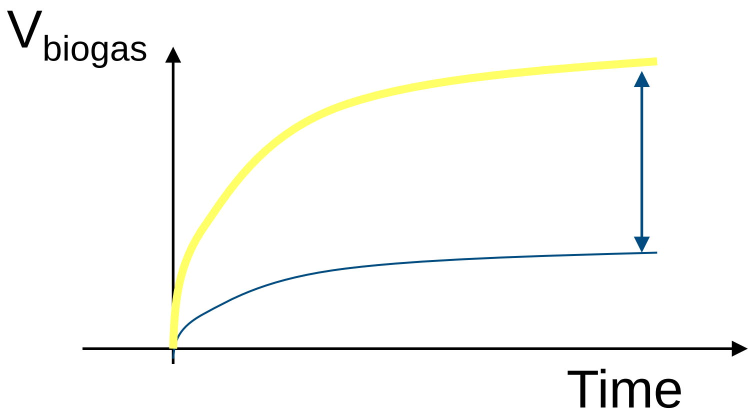
•Blank samples (B)
•Samples (1,2,n)
•Controls (C)

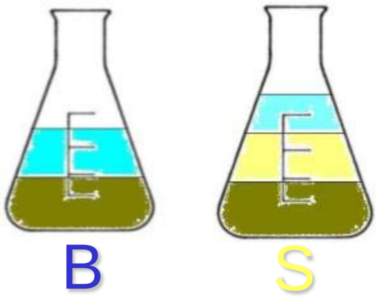


Experimental methane potential (BMPexp)

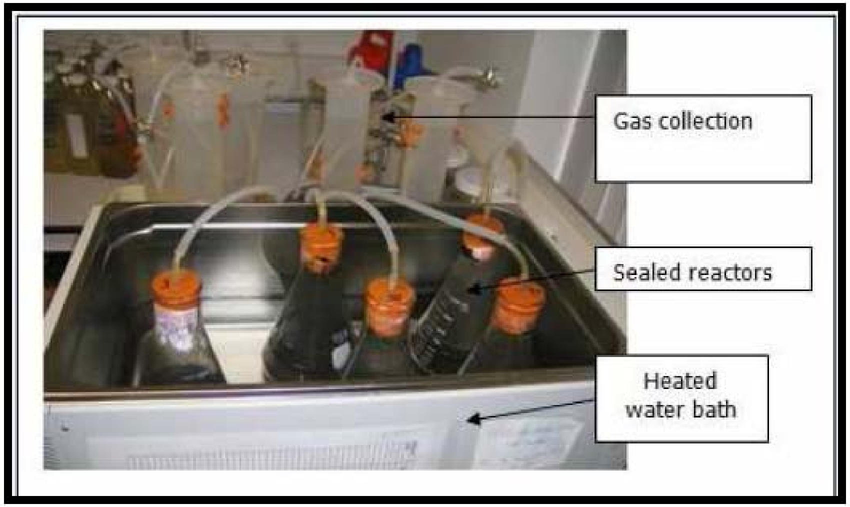
The BMP is compute

The BMP is computed add the difference between the volume of methane produced by the sample and by the blank (aspecific gas production)





Laboratory instruments – Gas measuring

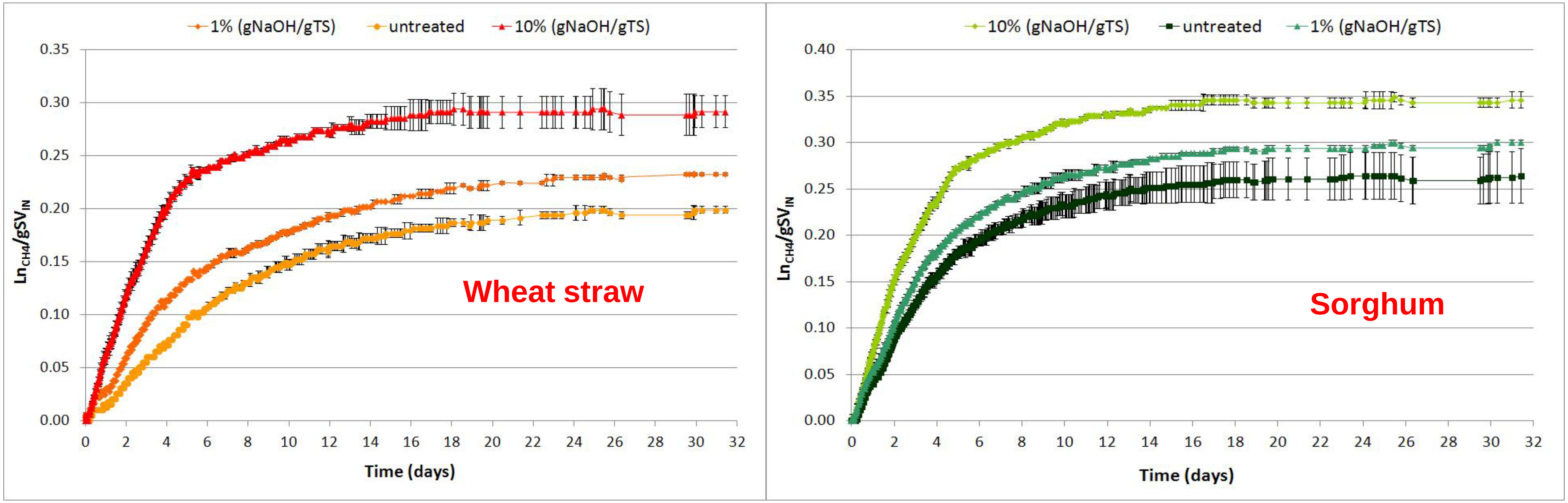


Raposo et al., 2010

Manometric devices

Volumetric devices

Experimental methane potential (BMPexp)



	L_nCH_4/gVS
untreated sorghum	0.26
1% (gNaOH/gTS)	0.30
10% (gNaOH/gTS)	0.35
untreated wheat straw	0.20
1% (gNaOH/gTS)	0.23
10% (gNaOH/gTS)	0.29

Sambusiti et al., 2011

- **Simplified models**

A range of models have been developed for modelling anaerobic digestion processes. Early models were steady state and assumed a rate-limiting step *(Lawrence, 1971).* Based on reports in the literature there is evidence of a number of multi-species models that are based upon different assumptions and have different configurations *(Angelidaki et al., 1999; Pavlostathis and Gossett, 1986; Siegrist et al., 1993).*

- **Complex models**

Requiring:

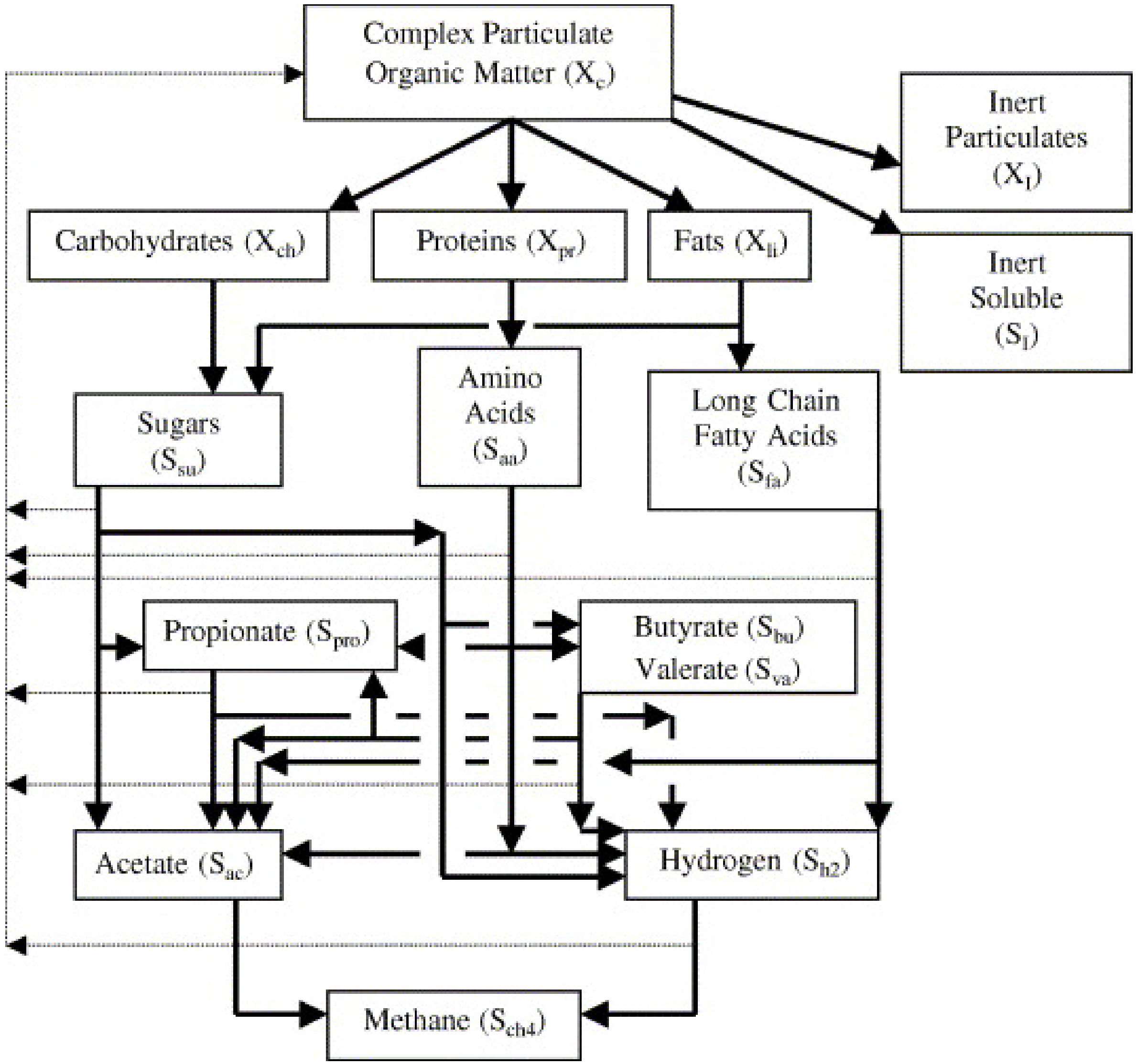
  - Knowledge of bioprocesses
  - Characteristics of substrate

Useful for monitoring/control and forecast

- In 2002: The anaerobic digestion model number 1 was issued by Task Group for Mathematical Modelling of Anaerobic Digestion Processes, of the the International Water Association's (IWA)

D.J. Batstone, J. Keller, I. Angelidaki, S.V. Kalyuzhnyi, S.G. Pavlostathis, A. Rozzi, W.T.M. Sanders, H. Siegrist and V.A. Vavilin, The IWA Anaerobic Digestion Model No 1 (ADM1), Water Science and Technology Vol 45 No 10 pp 65–73.*

ADM1 (Batstone et al., 2002)



ADM1 (Batstone et al., 2002)

19 biological processes and **24 components (12 solubles and 12 particulates).**

Biological processes:

- Hydrolysis of Complex Particulate Organic Matter, Proteins, Carbohydrates and Fats;
- Acidogenesis from Sugars, Amino Acids and Long Chain Fatty Acids;
- Acetogenesis from Long Chain Fatty Acids, Propionate, Butyrate and Valerate;
- Acetoclastic and Hydrogenotrophic methanogenesis;
- Endogenous decay processes

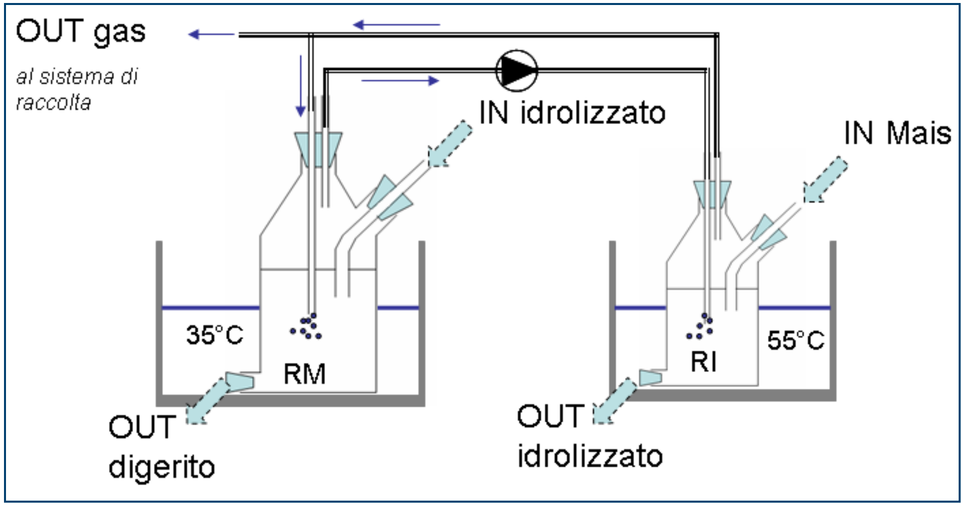
Chemical-physical processes:

- Liquid–liquid reactions (i.e. acid/base equilibria: NH_4^+/NH_3 (pKa = 9.25), CO_2/HCO_3^- (pKa = 6.35), and VFA/VFA^- (pKa~4.8)).
- Liquid–gas mass transfer of gaseous components (methane, carbon dioxide and molecular hydrogen)

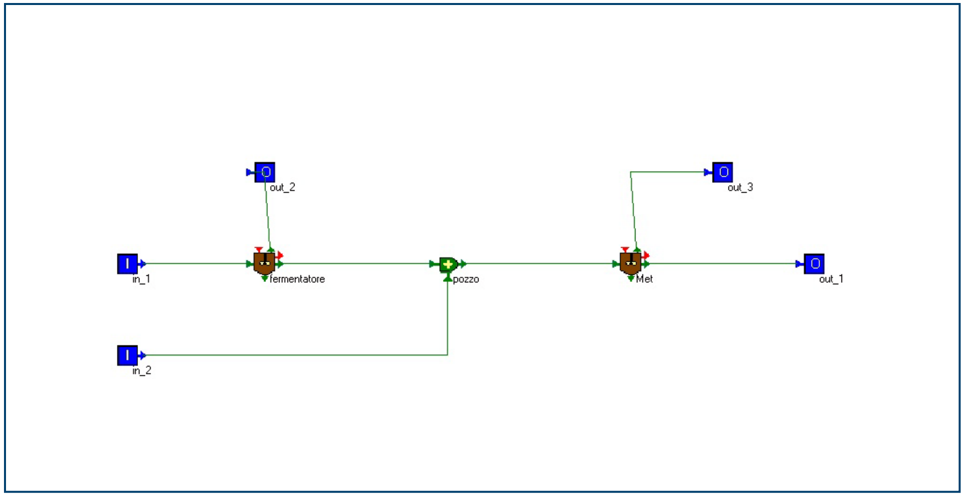
Example - optimization of anaerobic digestion of maize silage

Step 1: laboratory test to define model parameters

Laboratory scale

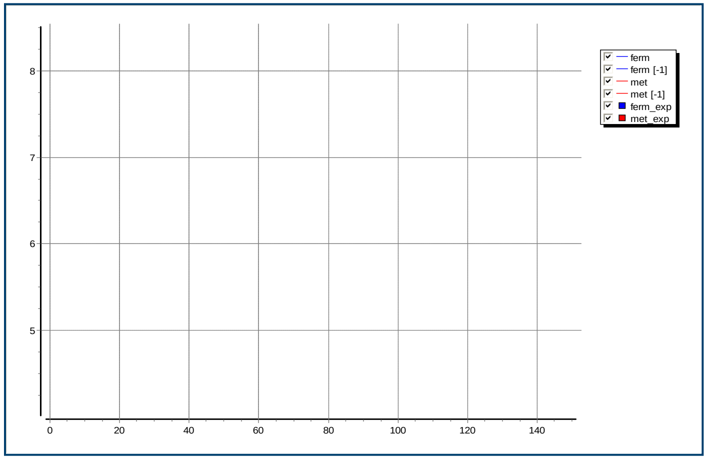


Simulations

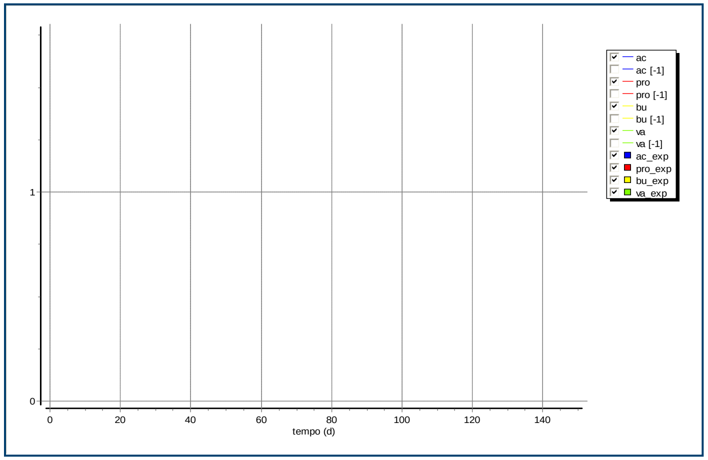


Step 1: Comparison between experimental and estimated results

Example: pH

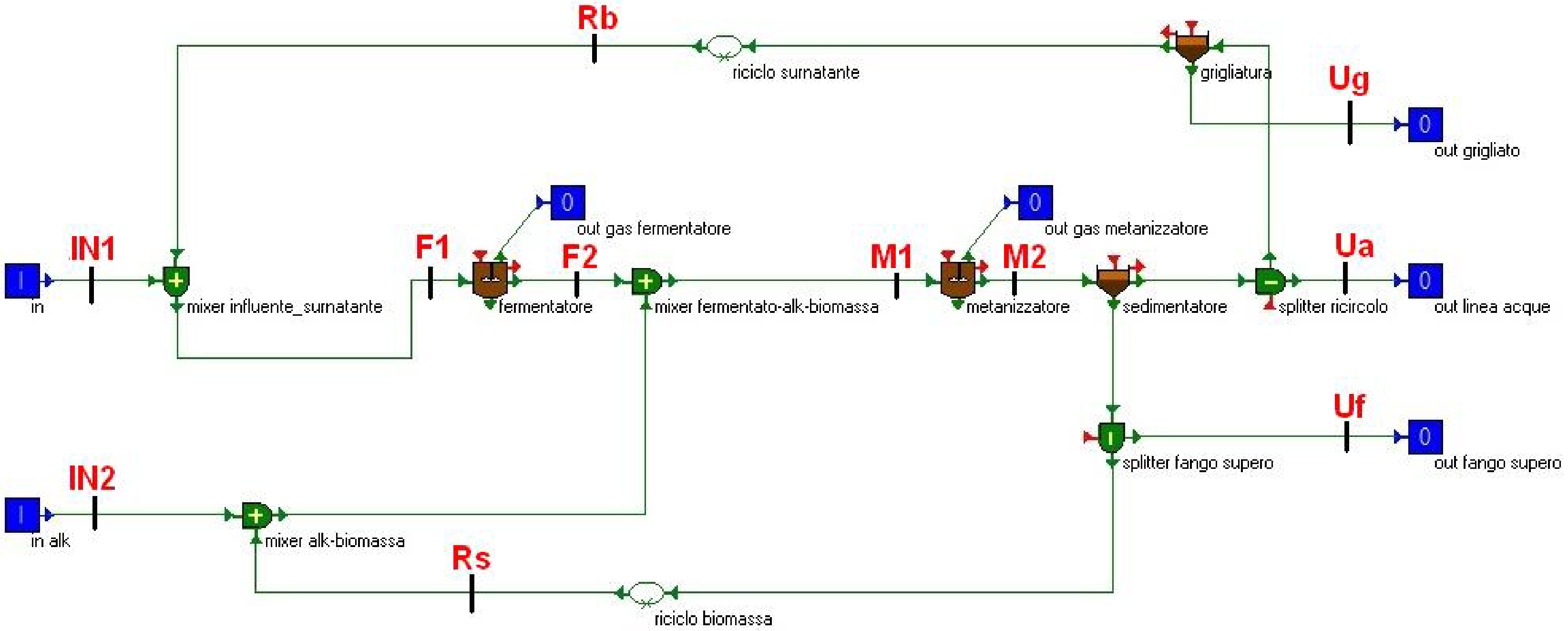


Example: Volatile acids



Example - optimization of anaerobic digestion of maize silage

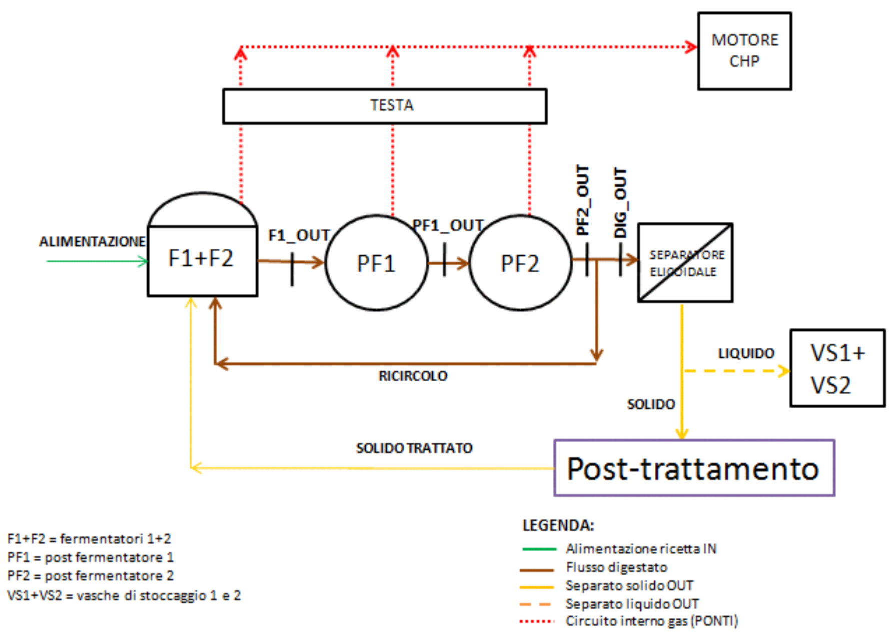
Step 2: Analysis of a complex scenario



Model evaluation of the recycle effect and the maximum organic load

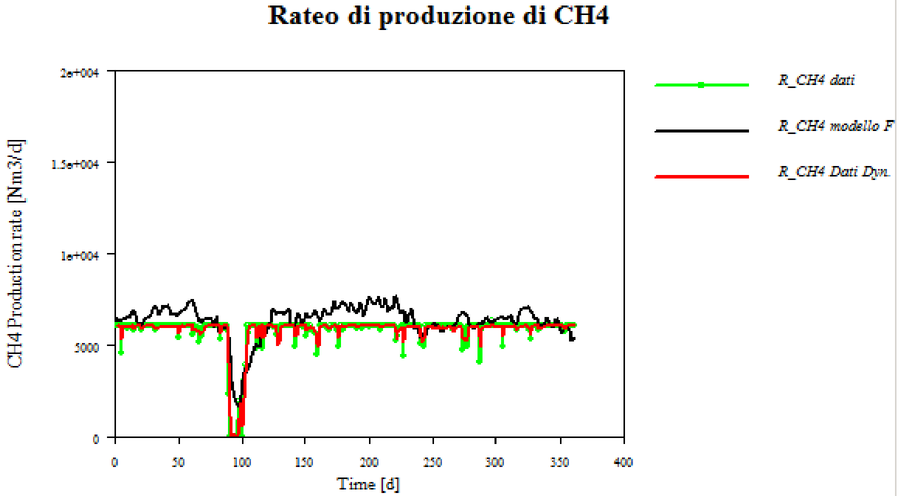
Example – simpler model, hydrolysis limited

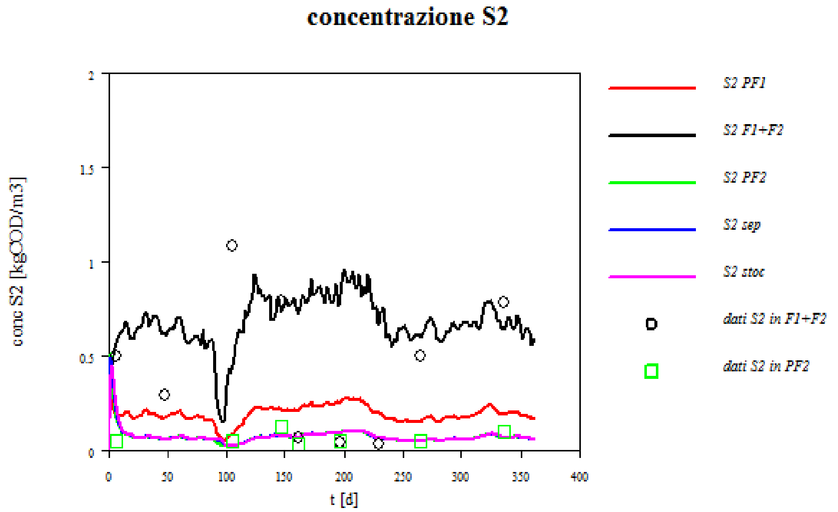
	$X_0 i$	X_{nd}	X_{nd}^{Post}	X_1	X_2	S_1	S_2	CH_4	Rate
	g-VS L^{-1}					g-COD L^{-1}		mol L^{-1}	
$P_0 i$	-1	$+k_{nd} i$				$+k_0 i$			$X_1 \cdot K_{hydr}\, i$
P_1				+1		$-k_1$	$+k_2$		$\mu_1^{max} \cdot \frac{S_1}{S_1 + K_{S1}} \cdot X_1$
P_2					+1		$-k_3$	$+k_4$	$\mu_1^{max} \cdot \frac{S_2}{S_2 + K_{S2} + {S_2}^2/K_I} \cdot X_1$
P_3				-1					$K_{d1} \cdot X_1$
P_4					-1				$K_{d2} \cdot X_2$
P-PT*		-1	$1-k_{PT}$			k_{PT}			equilibrium
P_5			-1			+1			$X_{nd}^{Post} \cdot K_{hydr}^{Post}$

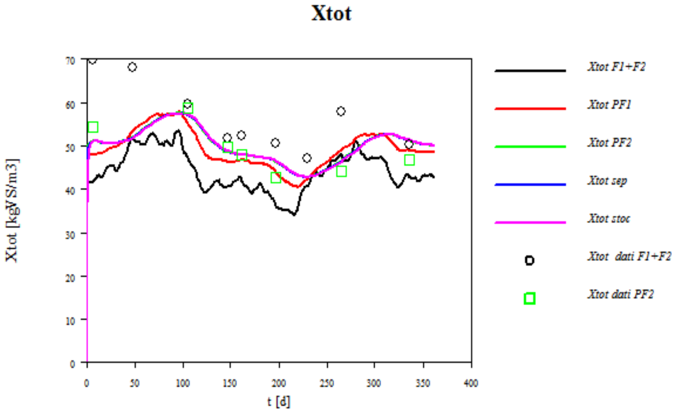


Example - optimization of anaerobic digestion of maize silage

Simpler models, to be used for scenario analyses







Biohydrogen from dark fermentation

Introduction – Why Biohydrogen?

- Lightest element on the periodic table.
- Hydrogen gas is highly flammable and burns in air at a very wide range of concentrations between 4% and 75% by volume.
- energy density is very high per unit of mass but very low per unit of volume
- Highly reactive and highly flamable

- High energy convertion efficiency
- Combustion produces only H2O

- Safeaty issues in storage, production and use

Applications

- **Energy production**
  - ✓ Hydrogen fuel cells and batteries for the production of electric energy (transport, industry, …) (http://www.hydrogencarsnow.com; Audi A2H2 car)

- **Industrial uses**
  - ✓ Chemical industry
    - NH_3 production
    - Oil refineries

      (desulphurization of gasoline and diesel)
    - Methanol production
  - ✓ Many industrial applications

Introduction – hydrogen Origin

- **Actual hydrogen sources**

World's production of H_2: 550 billions of Nm^3/years

- ✓ 96% from fossil fuels
- ✓ 4% from water electrolysis

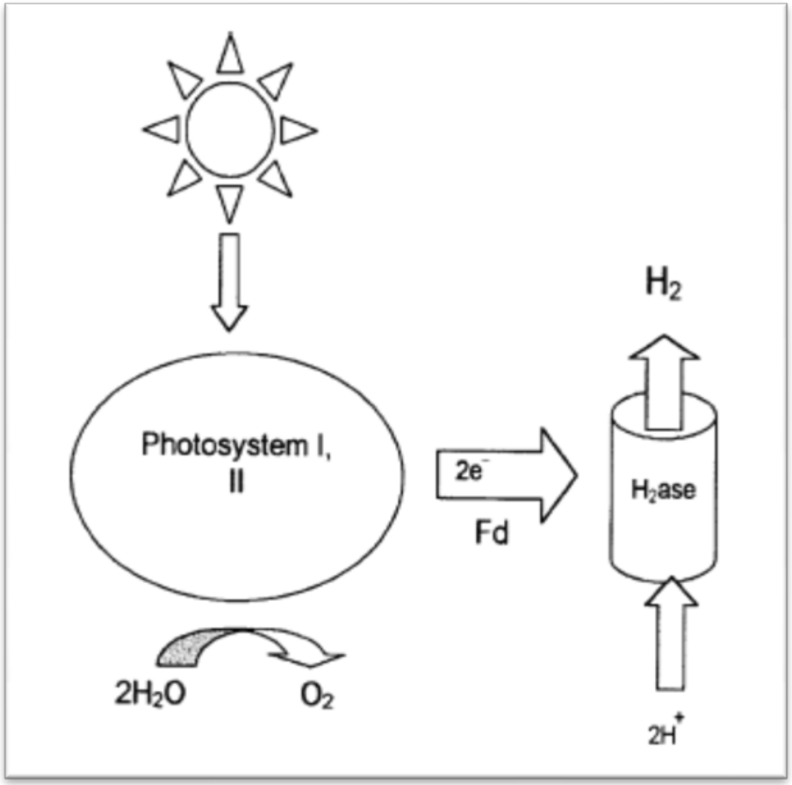
- **Biotechnologies**

Renewable sources

- ✓ Biophotolysis
- ✓ Photo-fermentation
- ✓ Dark fermentation

✓Biophotolysis

$$2\ H_2O + \text{light} \rightarrow 2\ H_2 + O_2$$



Based on **microalgal photosynthesis** (e.g. *Chlamydomonas reinhardtii)*

(+) Autotrophic production

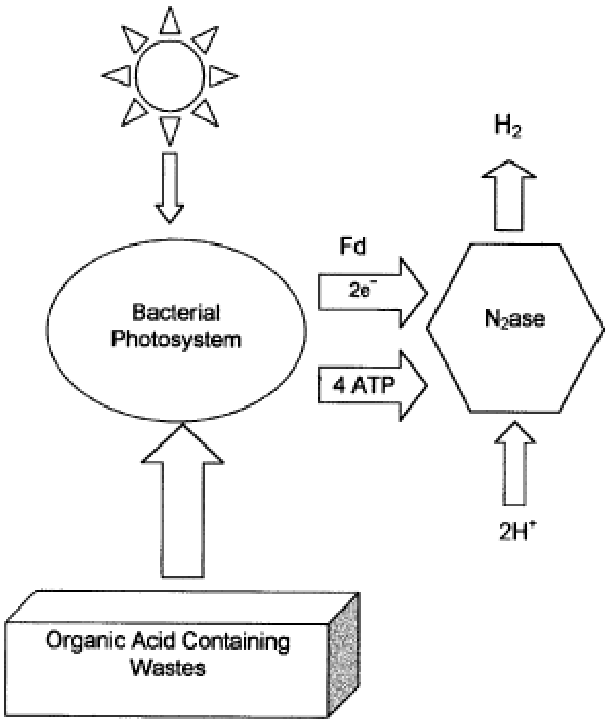
(-) inhibition of hydrogenase by O_2

(-) Low energy efficiency (max. 3%)

Hallenbeck et al. (2002) Biological Hydrogen Production:fundamentals and limiting processes. *IJHE* **27:** 1185
Tamagnini et al. (2002) Hydrogenases and hydrogen metabolism of cyanobacteria. *Microbiol.Mol.Biol.Rev.* **66:** 1
Reith et al.(2004) *Biomethane and Biohydrogen.* Edited by Reith, Wijfells & Barten
Nath et al. (2004) Improvment of fermentative hydrogen production: various approaches. *Appl.Microbiol.Biotechnol.* **65:** 520
Bartacek et al. (2007) Developments and constraints in fermentative hydrogen production. *Biofuels, bioprod, Bioref.* **1:** 201

✓Photo-fermentation

$$CH_3COOH + 2H_2O + light \rightarrow 4H_2 + 2\ CO_2$$



Based on **bacterial photosystem** (e.g. *Rhodobacter)*

(+) Effluent utilisation

(-) inhibition of hydrogenase by O_2

(-) Low energy efficiency (max. 1%)

Hallenbeck et al. (2002) Biological Hydrogen Production:fundamentals and limiting processes. *IJHE* **27:** 1185

Tamagnini et al. (2002) Hydrogenases and hydrogen metabolism of cyanobacteria. *Microbiol.Mol.Biol.Rev.* **66:** 1

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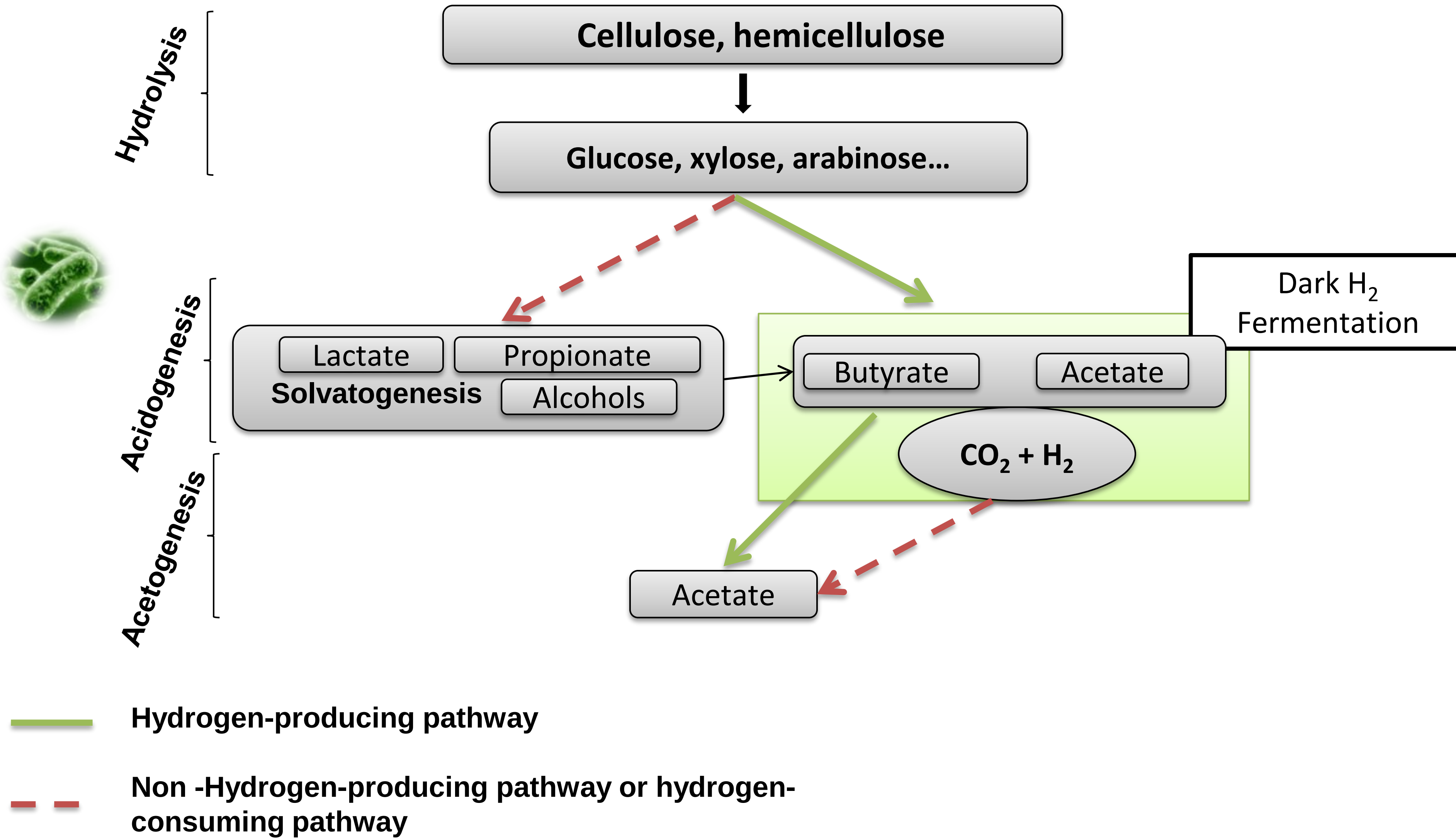
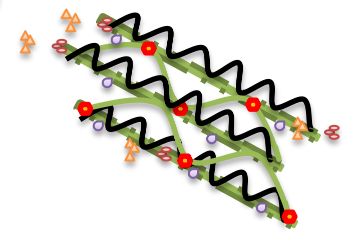
Dark fermentation



Dark fermentation

Lignocellulosic substrate



Hydrolysis

Cellulose, hemicellulose

Glucose, xylose, arabinose…

Acidogenesis

Lactate

Propionate

Alc

Acetogenesis

Dark H_2 Fermentation

Hydrogen-producing path

Non -Hydrogen-producing pathway or hydrogen-consuming pathway

- **Dark H_2 fermentation**
- **Acetate pathway:**

$C_6H_{12}O_6 + 4H_2O \rightarrow 2CH_3COOH + 2CO_2 + \mathbf{4H_2}$

- **Butyrate pathway :**

$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + \mathbf{2H_2}$

Mixed culture[1] :

$C_6H_{12}O_6 + \frac{1}{2}H_2O \rightarrow \frac{3}{4}\ CH_3CH_2CH_2COOH + \frac{1}{2}\ CH_3COOH + 2CO_2 + \mathbf{2.5H_2}$

Dark fermentation

- **Microbial species**
  - ✓Clostridia (e.g. pasteurianum, butyricum, beijerinkii)
  - ✓Enterobacteria
  - ✓Thermoanaerobacteria
- **Substrates**

Sugars (glucole, lactose)

Sugar rich organic substrates:

- molasses
- cheesy whey
- lignocellulosic biomasses
- OFMSW

- **Main environmental parameters**
  - ✓pH (acidic range 5-6, with 5,5 as optimum)
  - ✓Temperature ranges:
    - Mesophilic (20-40°C),
    - Thermophilic (40-65°C),
    - Iper-thermophilic (65-80°C).
  - ✓H_2 partial pressure: DF is very sensitive to H_2 concentrations ; with increasing pH_2 (above 50 kPa) H_2 synthesis decreases and metabolic pathways shift to production of more reduced substrates such as lactate, ethanol, acetone, butanol, or alanine.

Dark fermentation

Limit of DF: no more than 30% of COD in the substrate can be converted into H2 → 2 stage process → **bioHythane**

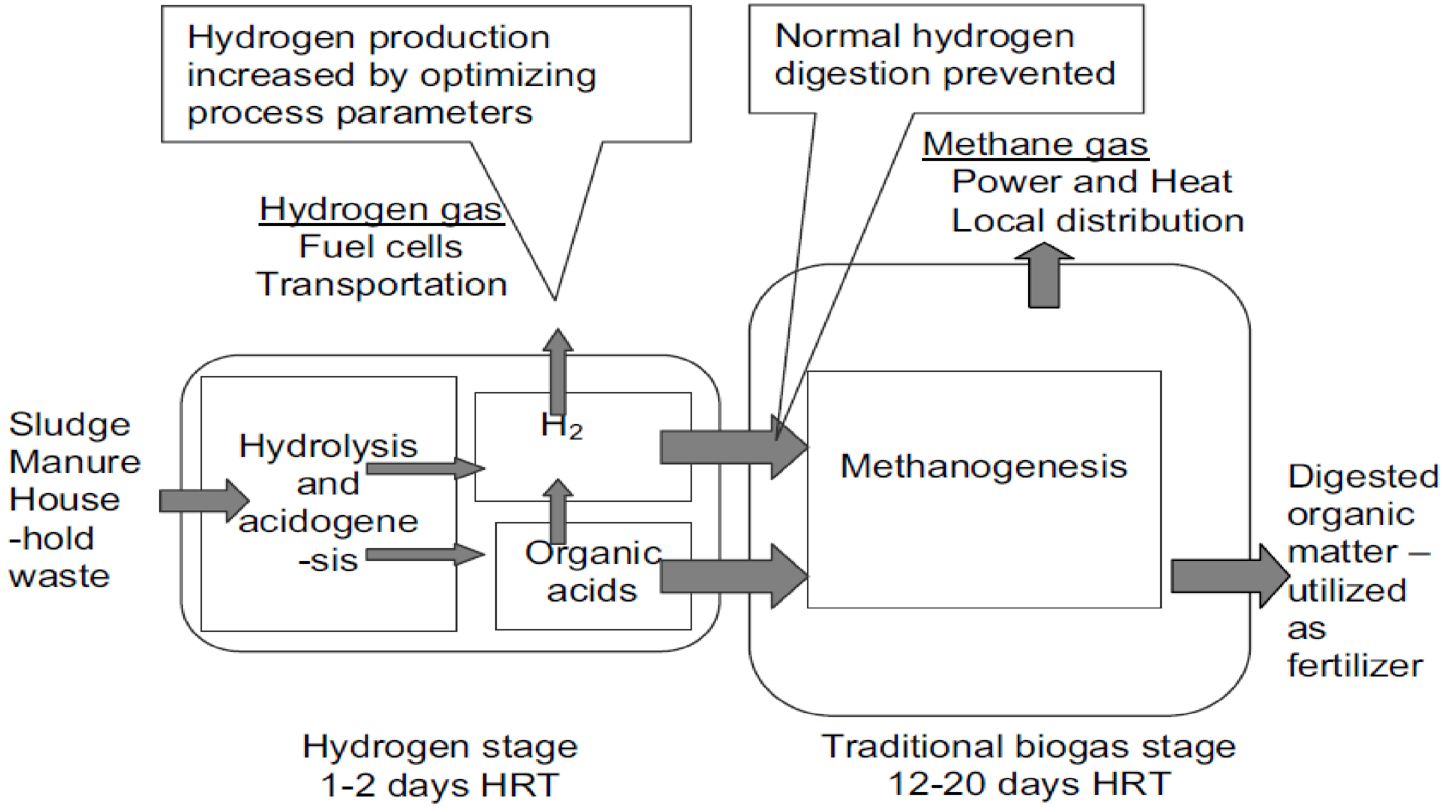


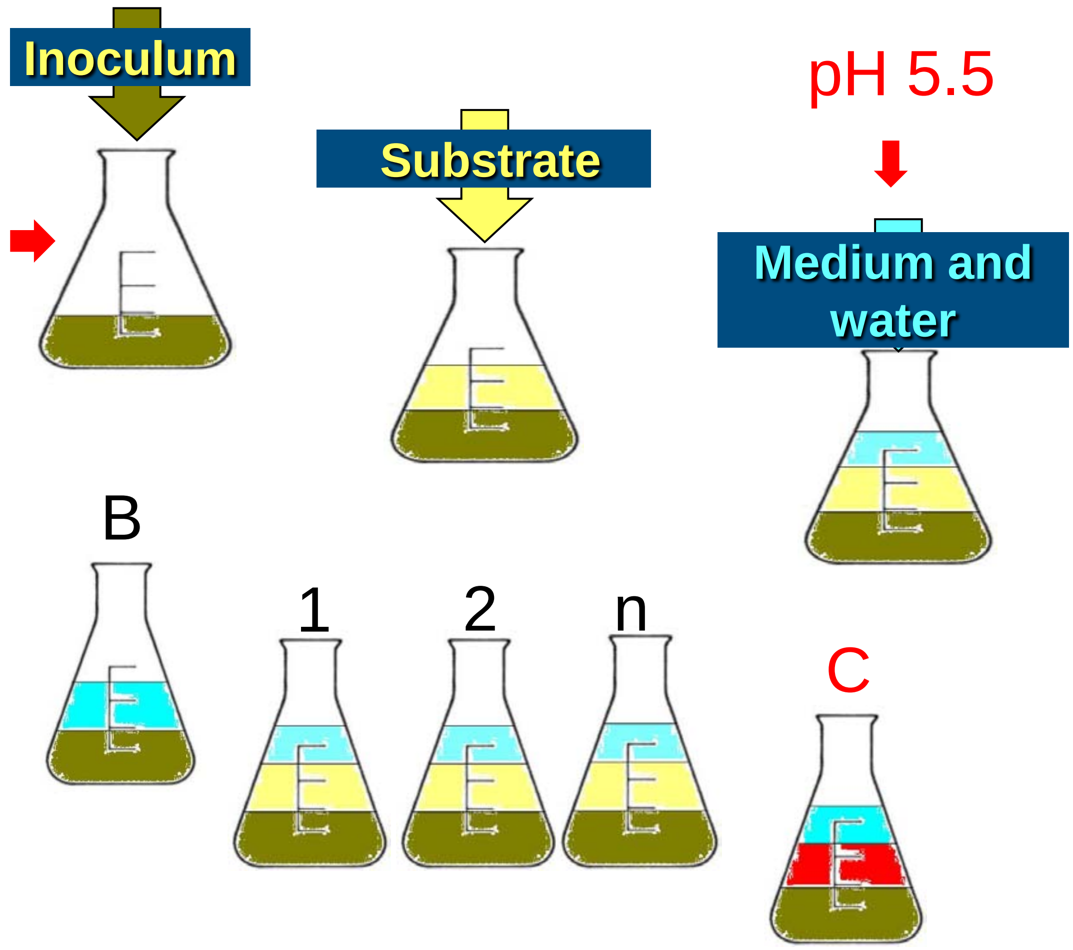
Figure 1. Principle diagram of two-stage process for hydrogen and methane production

Dawei, 2008

Biochemical Hydrogen Potential (BHP) tests ASSESSMENT

SAMPLE PREPARATION

Temperature or chemical shock to inhibit methanogenic activity



TEST

- Blank samples (B)
- Samples (1,2,n)
- Controls (C)

Biochemical Hydrogen Potential (BHP) results - EXAMPLES