CIE4485
Wastewater Treatment

Prof.dr.ir. Jules van Lier

9. Anaerobic wastewater treatment fundamentals: Microbs

TU Delft

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CT4485 Wastewater Treatment

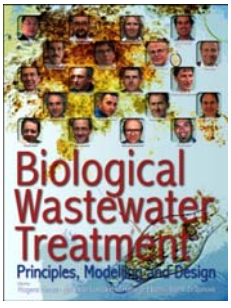
Lecture 4b: Anaerobic Wastewater Treatment
Fundamentals: Microbs

Prof.dr.ir. Jules van Lier

6 December 2012

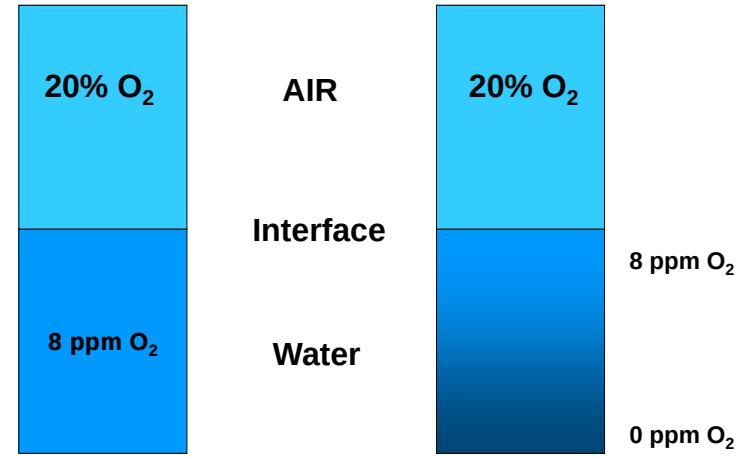
Learning objectives

- Understand principles of anaerobic wastewater treatment (AWWT)
- Understand global microbiological process and its impacts on process operation



IWA: Chapter 16
Metcalf & Eddy: Chapt.10

Natural Anaerobic Environments



Discovery of Biogas….

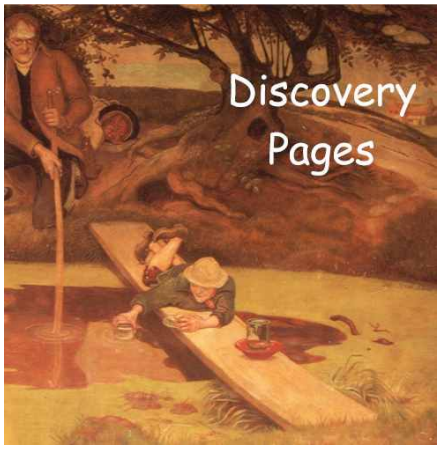
Hey, hey, there is inflammable gas escaping from the swamp!

ALESSANDRO VOLTA
Italia, 1770

Discovery of Biogas….

www.uasb.org

John Dalton (1766-1844) collecting gas samples with some students from swamps (near Manchester, UK)



Microbial aspects of anaerobic conversions

History of anaerobic microbiology

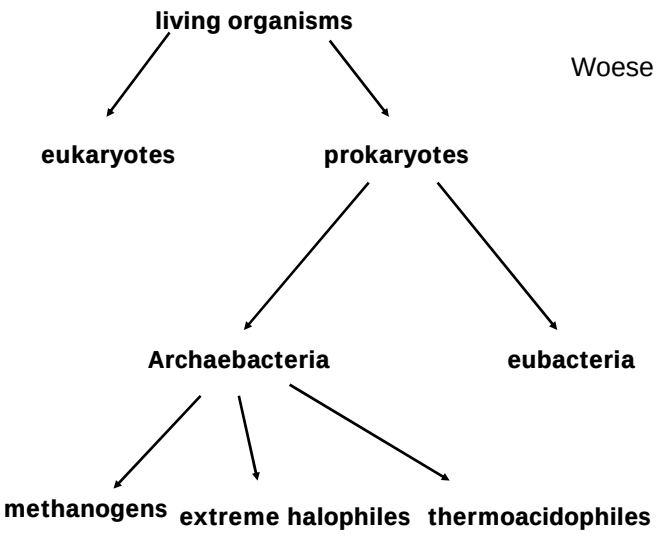
- Volta (1776) discovery of CH_4 in swamp-gas
- Early microbiologist: Béchamp (1868), Popoff (1875)
- Microbiology of methane bacteria:
  Söhngen (1906) *Methanothrix soehngenii* (in defined mix)
  (renamed *Methanosaeta soehngenii*, Patel)
  Schnellen (1947): first pure cultures (*Methanosarcina barkeri, Methanobacterium formicium*)

Bryant (1967) very important discovery:

Methanobacterium Omelianski (fermenting EtOH) exist of 2 bacteria !!
EtOH =>Acetate + H_2 (not directly to CH_4 !!)

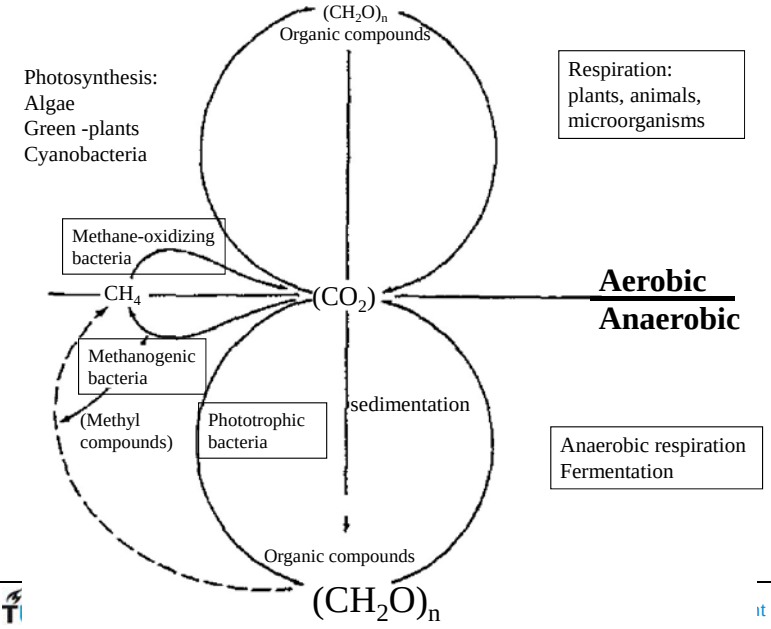
Description of the new kingdom of Archeabacteria
- methane bacteria
- sulphate reducer
- halophilic bacter,etc.

Classification of methanogens

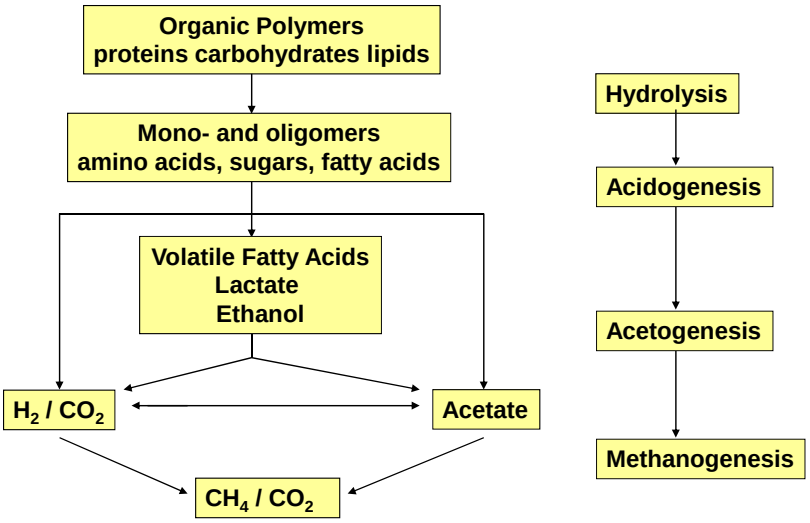


Woese et al., 1977, 1990

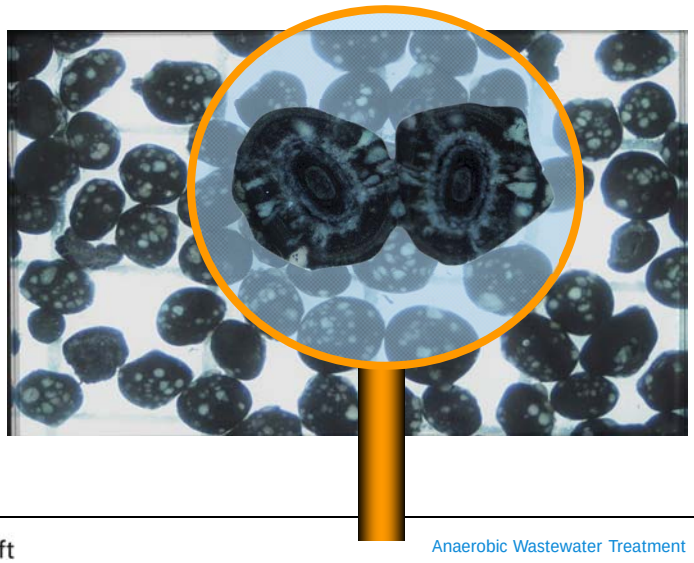
Carbon cycle



Anaerobic Conversion of Organic Matter



Anaerobic granular sludge



Hydrolysis

- slow process (generally rate limiting): $dS/dt = -K_h \cdot S$
- optimum pH = 6
- retention time and particle size are rate determining parameters
- cellulose/hemicellulose degradation depends on lignin fraction
- hydrolysis of fats hardly proceeds < 15-20 °C (rate limiting step)

  (product) inhibition by intermediates? (LCFA, NH3, amino acids, H_2)

Hydrolytic enzymes

Hydrolysis

Sugars

sucrose $(C_6H_{10}O_5)_2$ —saccharase→ glucose $C_6H_{10}O_6$

starch $(C_6H_{10}O_5)_n$ —amylase→ glucose

cellulose $(C_6H_{10}O_5)_n$ —cellulase→ glucose

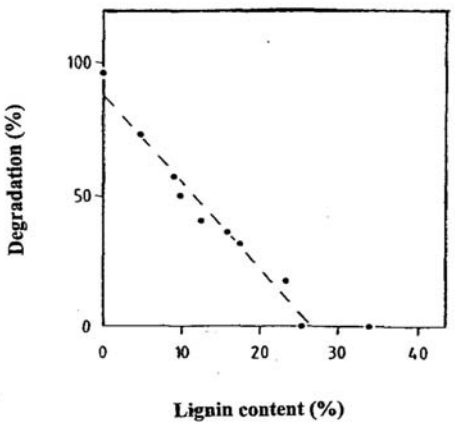
Protein

protein —protease→ VFA's+NH_3

Fat

fat —lipase→ glycerol + **Long Chain Fatty Acids (LCFA)**

Bio-degradation of ligno-cellulosic matter versus lignin content



	% lignin
Cellulose	0
Hay	5
Barley straw	9
Alfalfa	10
Bagasse	12
Rye straw	16
Reed	17
Newspaper	23
Saw dust	25
Coconut fibre	34

Acidogenesis / Fermentation - Sugars

- Release of protons (H^+) and reaction products (proton acceptors)
- H_2 formation (catalyzed by the enzyme hydrogenase)
- Performed by a very large group of bacteria (about 1% of all bacteria facultative fermenters)

$$C_{12}H_{22}O_{11} + 9\ H_2O \rightarrow 4\ CH_3COO^- + 4\ HCO_3^- + 8\ H^+ + 8\ H_2$$

$$C_{12}H_{22}O_{11} + 5\ H_2O \rightarrow 2\ CH_3CH_2CH_2COO^- + 4\ HCO_3^- + 6\ H^+ + 4\ H_2$$

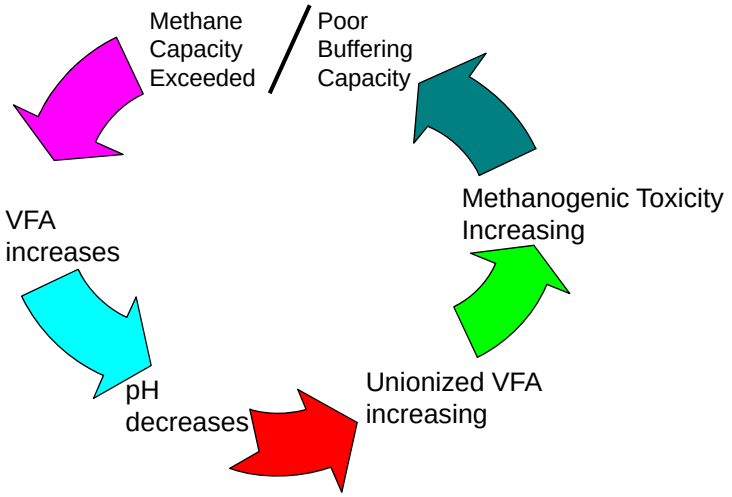
- End products depend on circumstances, e.g.
  - glucose fermentation in a two-step system: more reduced products like ethanol, lactate, propionate, butyrate, CO_2 and H_2
  - glucose fermentation in a one-step system: acetate, H_2 and CO_2
- production of acids proceeds up to pH = 4 (product inhibition)

Acidogenesis of sugars: most rapid step!

Kinetic Properties Acidifiers / Methanogens

Process	R_x gCOD/gVSS/d	Y g VSS/g COD	Ks mg COD/l	μ-max Day^{-1}
Acidogenesis	13	0.15	200	2.0
Methanogenesis	3	0.03	30	0.12
Overall	2	0.03 –0.18	-	0.12

Overloading may lead to process deterioration:



Inhibition by VFA

Concentrations of VFA that correspond to the 50% inhibition of methanogenic activity. Calculated from 16 and 6 mg COD/l of unionized acetic and propionic acids, respectively

pH	50% Inhibiting concentration	
	acetate	propionate
	--------mg COD L^{-1}----------	
4.5	9	2
5.0	27	8
5.5	86	24
6.0	272	77
6.5	859	244
7.0	2717	773
7.5	8593	2444
8.0	27172	7729
8.5	85925	24443

$$pH = pKa + \log\frac{[Ac-]}{[HAc]}$$

$$Ac- = HAc * 10^{(pH-pKa)}$$

pKa HAc: 4.77
pKa HPr: 4.89

Acidogenesis / Fermentation - Proteins

organically bound N (amino acids) is released as $NH4^+$ (stickland reaction: oxidation-reduction)

Alanine: $CH_3CHNH_2COO^- + 3\ H_2O \rightarrow CH_3COO^- + HCO_3^- + NH_4^+ + 2\ H_2$

Glycine: $2\ CH_2NH_2COO^- + 2\ H_2 \rightarrow 2\ CH_3COO^- + 2\ NH_3$

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alanine + glycine + $3\ H_2O \rightarrow$ 3 acetate + $2\ NH_3 + NH_4^+ + HCO_3^-$

($2\ NH_3 + 2\ H_2O + 2\ CO_2 \rightarrow 2\ NH_4^+ + 2\ HCO_3^-$)

Acidogenesis / Fermentation – Long Chain Fatty Acids

anaerobic degradation of LCFA proceeds via β-oxidation

CH_3-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-CH_2-COO^-

palmitic acid: CH_3-$(CH_2)_{14}$-$COO^- + 14\ H_2O \rightarrow 8\ CH_3COO^- + 7\ H^+ + 14\ H_2$

With uneven numbers: acetate + propionate is formed:

$CH_3\ (CH_2)_{14}$-$CH_2COO^- + 14\ H_2O \rightarrow 7\ CH_3COO^- + CH_3CH_2COO^-\ 7\ H^+ + 14\ H_2$

- unsaturated LCFA are firstly hydrogenated before degradation

Acetogenesis (Acetate formation)

Conversion of fermentation products into acetic acid, CO_2 , and H_2

Mainly formation of propionic acid, butyric acid and ethanol

propionate$^-$ + $3H_2O$	$\rightarrow$ acetate$^-$ + HCO_3^- + H^+ + $3H_2$	$\Delta G_0' = +76.1$ kJ/mole
butyrate$^-$ + 2H2O	$\rightarrow$ 2 acetate$^-$ + H^+ + $2H_2$	$\Delta G_0' = +48.1$ kJ/mole
ethanol + $2H_2O$	$\rightarrow$ acetate$^-$ + H^+ + $2H_2$	$\Delta G_0' = +9.6$ kJ/mole
$4\ H_2 + CO_2$	$\rightarrow\ CH_4 + 2H_2O$	$\Delta G_0' = -138.9$ kJ/mole

Need for syntrophic associations !!!

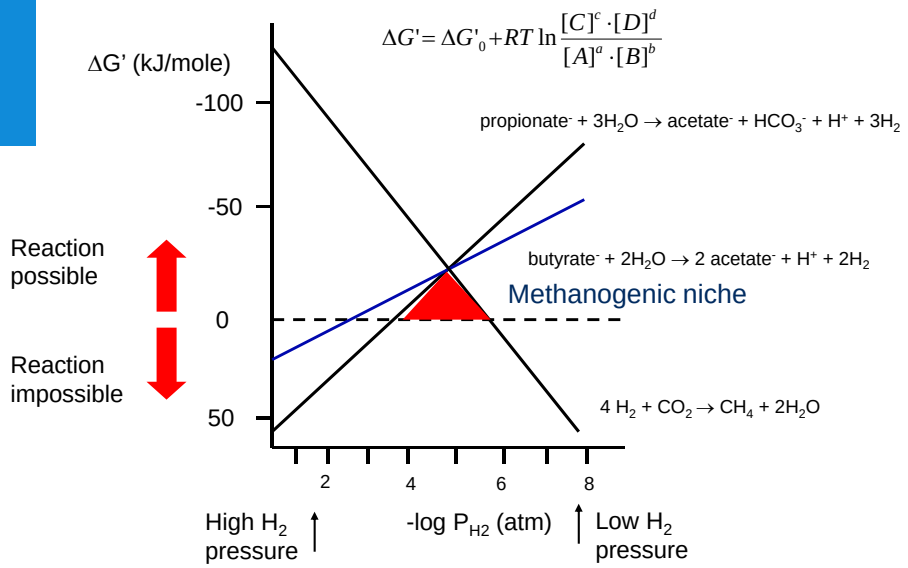
GIBB's FREE ENERGY

$$aA + bB \Leftrightarrow cC + dD$$

$$\Delta G' = \Delta G'_0 + RT \ln \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b}$$

$\Delta G'$	=	Actual Gibb's free energy change [kJ/mole]
$\Delta G'_0$	=	Standard Gibb's free energy change [kJ/mole] under standard conditions (pH = 7, T = 25 °C, p = 1 atm., the activity of all compounds present in solution is 1 mole/kg
R	=	gas constant (8.28 J)
T	=	absolute temperature [K]

Impact of $P(H_2)$ on thermodynamics



Methanogenesis

→ Substrates

		ΔG^0 (kJ/mole CH_4)
$4H_2 + CO_2$	=> $CH_4 + 2H_2O$	-130.4
$4HCOOH$	=> $CH_4 + 3CO_2 + 2H_2O$	-119.5
$4CO + 2H_2O$	=> $CH_4 + 3CO_2$	-185.5
$4CH_3OH$	=> $3CH_4 + CO_2 + 2H_2O$	-103.0
$CH_3OH + H_2$	=> $CH_4 + H_2O$	-112.5
$4CH_3NH_3 + 2\ H_2O$	=> $3CH_4 + CO_2 + 4NH_4^+$	- 74.0
$2(CH_3)_2NH_2 + 2H_2O$	=> $9CH_4 + 3CO_2 + 4NH_4^+$	- 74.0
CH_3COOH	=> $CH_4 + CO_2$	- 32.5

Most important substrates: hydrogen and acetate

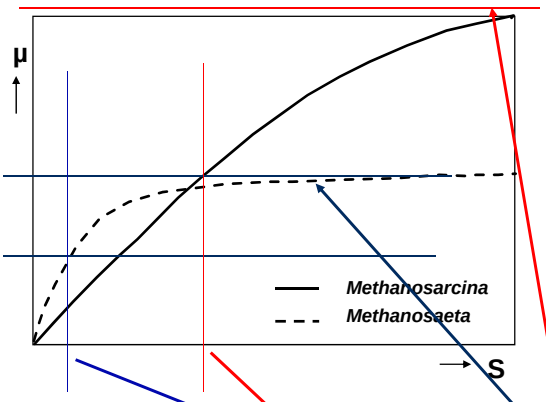
furthermore: formate, carbon monoxyde, methanol and methylamines

Kinetic parameters

Substrate	Product	μ_{max} (d^{-1})	t_d (d)	K_S (mg COD • l^{-1})
acetate*	methane	0.12 0.71	5.8 1.0	30 300
hydrogen	methane	2.85	0.2	0.06
propionate	acetate	0.22	3.2	48
butyrate	acetate	0.55	1.3	9

*** two different acetate consuming methanogens**

Impact of kinetics on bacterial selection



The Monod equation for bacterial growth:

$$\frac{dX}{dt} = \mu \cdot X$$

with:

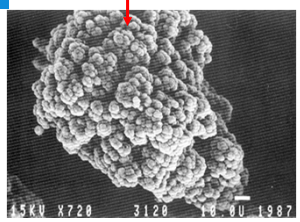
$$\mu = \mu_{max} \cdot \frac{S}{K_s + S}$$

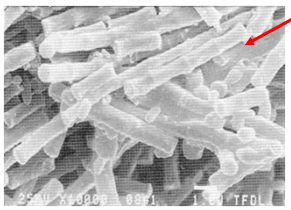
$K_S = S$ when $\mu = ½\ \mu_{max}$

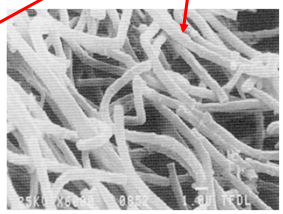
	K_S	μ_{max}
Methanosaeta	30	0.12
Methanosarcina	300	0.71

Bacterial composition of methanogenic sludge granule

1. 20-50% consist of methanogenic bacteria: acetotrophic (Methanosaeta, Methanosarcina) and hydrogenotrophic (e.g. Methanobacterium)







- Coccoid
- Excretion ECP → clumps
- Substrate: Ac^-, H_2/CO_2, MeOH, methylamines
- Low substrate affinity
- Relatively high μ

- Rod-shaped (4-10 cells) or filaments
- Hydrophobic surface
- Substrate: Acetate
- High substrate affinity
- Low μ, low Y
- Generally predominant: Granules !!

Bacterial composition of methanogenic sludge granule

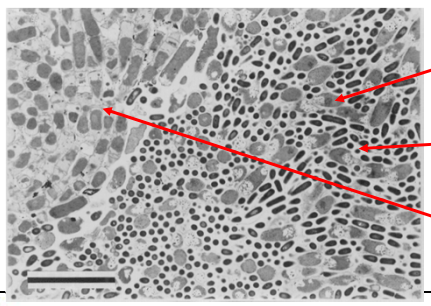
2. Close association between hydrogen producing and hydrogen consuming organisms (syntrophic associations)

$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + 3H_2 + HCO_3^- + H^+$ $\Delta G°' = +76.1$ kJ (1)

$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$ $\Delta G°' = -135.4$ kJ (2)

$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$ $\Delta G°' = -31.0$ kJ (3)

$CH_3CH_2COO^- + 0.75\ H_2O \rightarrow CH_3COO^- + 0.75CH_4 + 0.25\ HCO_3^- + 0.25H^+$ $\Delta G°' = -25.45$ kJ



(1) *Syntrophobacter*

(2) *Methanobrevibacter*

(3) *Methanosaeta*

Macro nutrients requirement of anaerobic sludge

The requirement for N and P can be calculated from the cell composition. (i.e. 10-12% N and appr. 2% P)

substrate = mixture of **volatile fatty acids**

growth yield = 0.02 - **0.05** g/g

COD : N : P = 1000 : 5 : 1

C : N : P = 330 : 5 : 1

substrate = **non-acidified carbohydrates**

⟶ growth yield = 0.10 - **0.15** g/g

COD : N : P = 350 : 5 : 1

C : N : P = 130 : 5 : 1

Level of micro-nutrients mostly sufficient in agro-industrial wastewater

Micro nutrients (heavy metals) requirement

Based on elemental composition of methane bacteria (Scherer, 1983)

Element	Concentration mg kg^{-1} dried cell	Element	Concentration mg kg^{-1} dried cell
Macronutrients:		Micronutrients:	
N	65000	Fe	1800
P	15000	Ni	100
K	10000	Co	75
S	10000	Mo	60
Ca	4000	Zn	60
Mg	3000	Mn	20
		Cu	10

Conversion factors for methane bacteria cell:
g VSS*1.4= g COD
g TS*0.825= g VSS

Important notes on microbiology

- Anaerobic microbial conversion differs from aerobic
- Ultimate COD removal via production of CH_4
- Anaerobic bacteria have a narrow substrate spectrum: complex consortia are needed for complete COD removal
- Anaerobic bacteria form bacterial aggregates (anaerobic granular sludge). Proper bacteria should be selected.
- Anaerobic bacteria use other compounds (sulfate, nitrate) as electron acceptor when present.

Anaerobic Conversion of Organic Matter with Sulfate