Syntrophic Cooperations in Methanogenic Degradation

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Overview

- Concept of interspecies electron transfer
- Syntrophic cooperations and
- Energetical implications
- Alternative electron carriers
- Anaerobic methane oxidation

- Conclusions

The „Microbial Redox Tower“

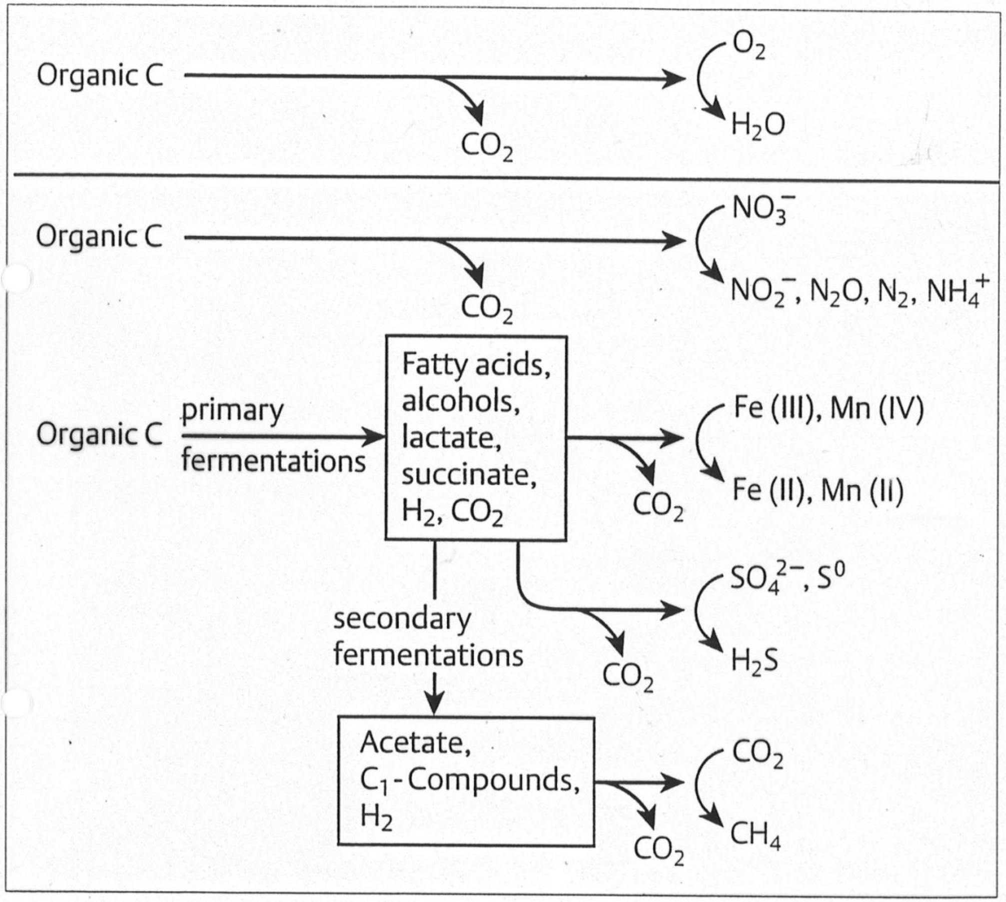

Fig. 30.**4** **Sequence of redox processes coupled to mineralization of organic matter**

Major carriers	E_o' (mV)
O_2/H_2O	+ 810
NO_3^-/N_2	+ 751
NO_3^-/NO_2^-	+ 430
NO_3^-/NH_4^+	+ 363
MnO_2/Mn^{2+}	+ 600
$FeOOH/Fe^{2+}$	+ 150
SO_4^{2-}/H_2S	- 218
$S°/H_2S$	-240
CO_2/CH_4	- 244
$2 H^+/H_2$	- 414
CO_2/<CH_2O>	- 434

Sediment core from
Lake Constance,
Profundal,
about 80 m water depth

LETTERA PRIMA.

Al Padre Carlo Giuſeppe Campi
C. R. S.

CARISSIMO AMICO.

Como, *li* 14 *Novembre*, 1776.

QUANDO mi ſcriveſte primamente della ſorgente d' aria infiammabile da voi ritrovata ſul principio dell' autunno, e quindi converſammo alcuni giorni inſieme, vi ricorderà quanti diſcorſi, e quante congetture ſi fecero tra noi ſul ſoggetto ſempre più maraviglioſo ed intereſ-

Alessandro Volta, 1776 :
„aria infiammabile“

Energetics of Glucose Degradation

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

$\Delta G°' = -2870$ kJ per mol

$C_6H_{12}O_6 \rightarrow 3\ CH_4 + 3\ CO_2$

$\Delta G°' = -390$ kJ per mol

$3\ CH_4 + 6\ O_2 \rightarrow 3\ CO_2 + 6\ H_2O$

$\Delta G°' = -2480$ kJ per mol

Energetics of ATP formation

ADP + P_i → ATP + H_2O $\Delta G^{0'}$ = +32 kJ /mol rct.

with [ATP], [P_i] = 10^{-2} M; [ADP] = 10^{-3} M: $\Delta G'$ = + 49 kJ /mol rct.

Heat loss in irreversible reactions: 10-20 kJ/ mol rct.

→ + 60-70 kJ/mol ATP

(F_1/F_o) ATPase:

Synthesizes or hydrolyses ATP at the cytoplasmic membrane

The reaction is coupled to a proton (or Na^+ ion) flux across the charged membrane (pmf = -180 - -200 mV).

If 3-4 protons (Na^+ ions) cross the membrane per ATP the smallest energy quantum exploitable by a biochemical process for ATP synthesis is equivalent to 1/3 – 1/4 ATP, i. e.

→ + 15-20 kJ/mol H^+ (Na^+) translocated

Energetics of glucose degradation

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

$\Delta G°' = -2870$ kJ per mol

= 38 ATP per mol glucose

→ - 75 kJ per mol ATP

$C_6H_{12}O_6 \rightarrow 3\ CH_4 + 3\ CO_2$

$\Delta G°' = -390$ kJ per mol

= ca. 5 ATP per mol

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With 70 kJ per ATP, [glucose] could go down to 10^{-35} M before the system would become energy-limited.

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(= <1 molecule in Lake Constance (50 km^3 water)

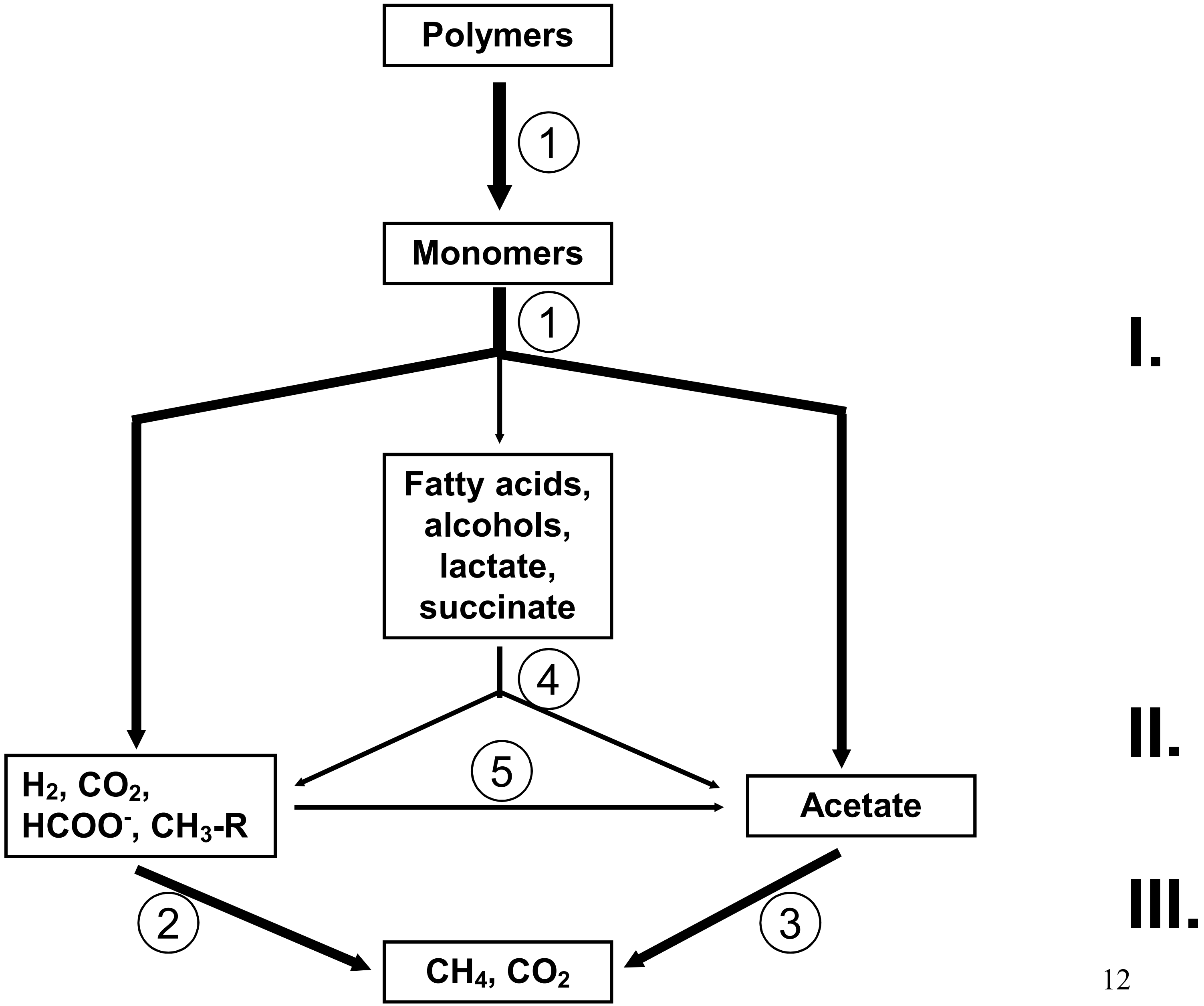
Energetics of glucose degradation

$C_6H_{12}O_6 \rightarrow 3\ CH_4 + 3\ CO_2$

$\Delta G°' = -390$ kJ per mol
= ca. 5 ATP per mol

With 70 kJ per ATP, [glucose] could go down to
10^{-7} M (100 nM) before the system would become energy-limited.

Electron flow in a methanogenic microbial community



Metabiotic glucose fermentation

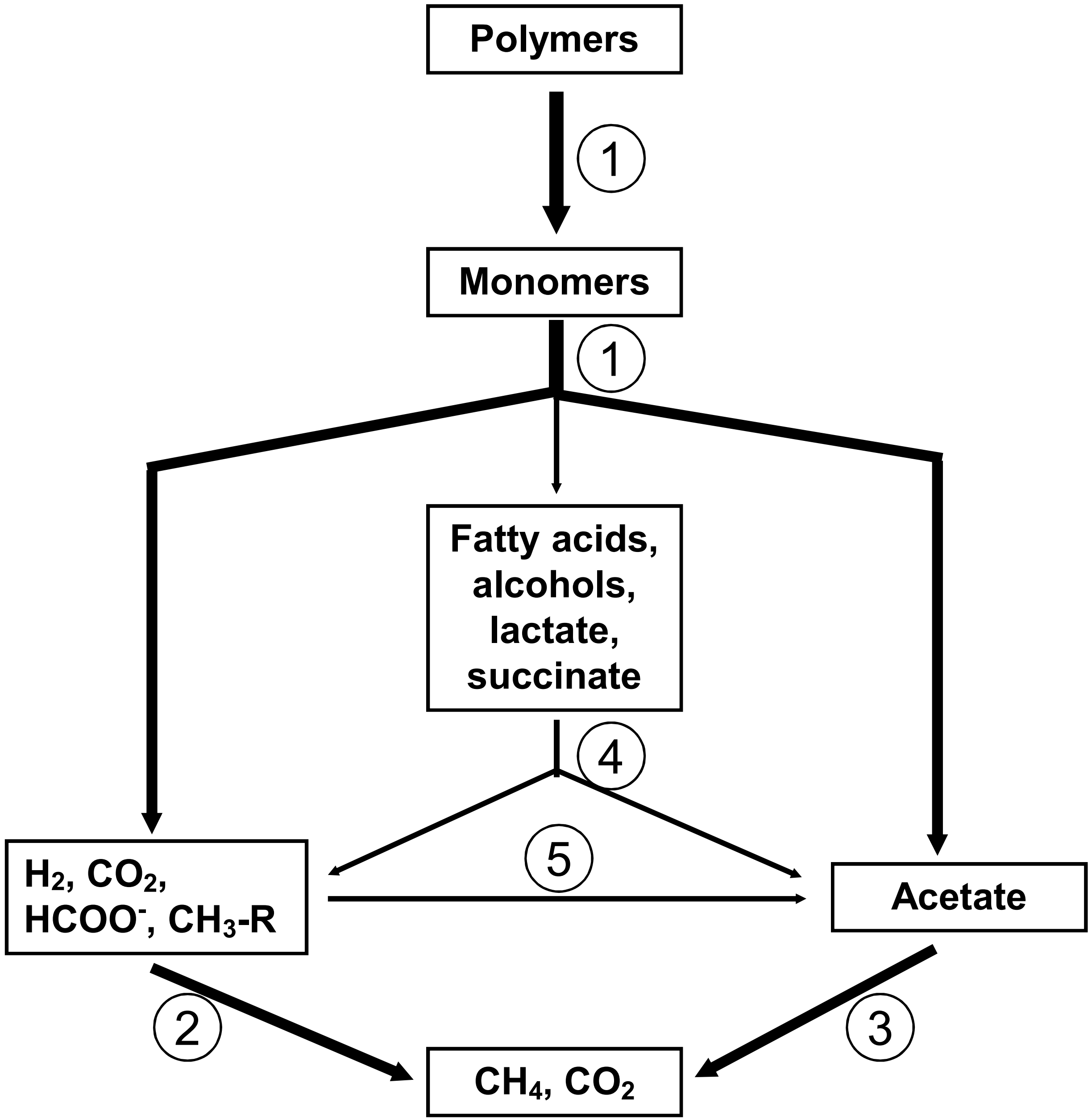
$$\text{Glucose} \longrightarrow 3\ CH_3COO^- + 3\ H^+ \longrightarrow 3\ CH_4 + 3\ CO_2$$

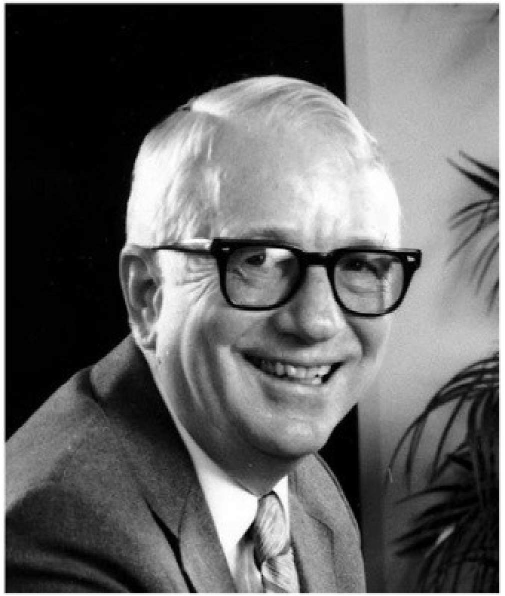
Acetobacterium woodii *Methanosarcina barkeri*

(Winter and Wolfe, 1979)

Secondary Fermentations
„Syntrophic“ Cooperations

Electron flow in a methanogenic microbial community





Marvin Bryant

Archiv für Mikrobiologie 59, 20—31 (1967)

Methanobacillus omelianskii, a Symbiotic Association of Two Species of Bacteria*

M. P. Bryant, E. A. Wolin, M. J. Wolin, and R. S. Wolfe

Departments of Dairy Science and Microbiology, University of Illinois, Urbana, Illinois

Received April 20, 1967

Methanobacillus omelianskii

2 ethanol + CO_2 → 2 acetate + CH_4 $\Delta G^{0\prime}$ = - 112 kJ

S-organism

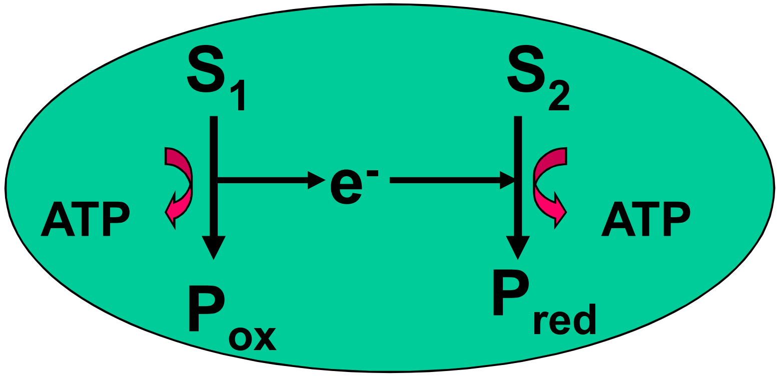
2 ethanol + 2 H_2O → 2 acetate + 4 H_2 $\Delta G^{0\prime}$ = + 20 kJ

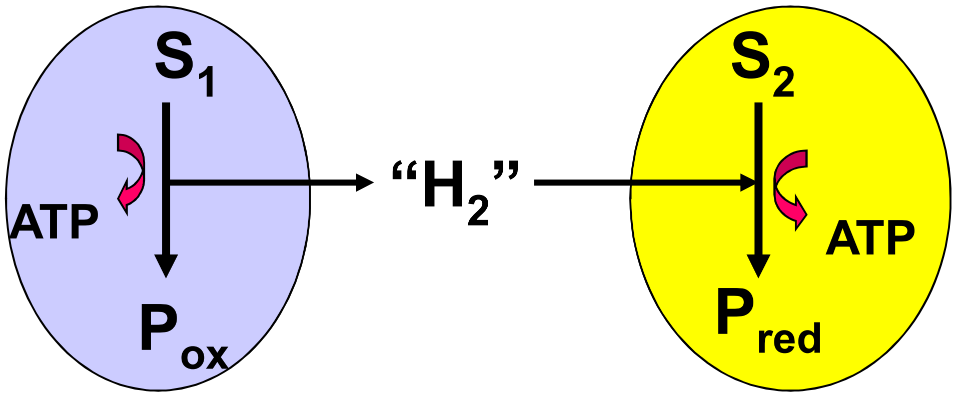
Methanogen

4 H_2 + CO_2 → CH_4 + 2 H_2O $\Delta G^{0\prime}$ = -131 kJ

Bryant et al (1967)

Electron transfer in energy metabolism





Syntrophic oxidation reactions

	$\Delta G_o'$ (kJ per mol rct)
Primary alcohols	
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2\,H_2$	+ 9.6
Fatty acids	
$CH_3CH_2CH_2COO^- + 2\,H_2O \rightarrow 2\,CH_3COO^- + 2\,H^+ + 2\,H_2$	+ 48.3
$CH_3CH_2COO^- + 2\,H_2O \rightarrow CH_3COO^- + CO_2 + 3\,H_2$	+ 76.0
$CH_3COO^- + H^+ + 2\,H_2O \rightarrow 2CO_2 + 4\,H_2$	+ 95.0
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2\,H_2O \rightarrow$ $3\,CH_3COO^- + 2\,H^+ + H_2$	+ 25.2
Benzoate	
$C_6H_5COO^- + 6\,H_2O \rightarrow 3\,CH_3COO^- + 2\,H^+ + CO_2 + 3\,H_2$	+ 49.5

Arch. Microbiol. 122, 129–135 (1979)

Archives of Microbiology

Anaerobic Bacterium that Degrades Fatty Acids in Syntrophic Association with Methanogens

Michael J. McInerney[1], Marvin P. Bryant[1], and Norbert Pfennig[2]

[1] Departments of Dairy Science and Microbiology University of Illinois, Urbana, IL 61801, U.S.A.
[2] Institut für Mikrobiologie der Gesellschaft für Strahlen- und Umweltforschung mbH München in Göttingen, Federal Republic of Germany

Abstract. A new species of anaerobic bacterium that degrades the even-numbered carbon fatty acids, butyrate, caproate and caprylate, to acetate and H_2 and the odd-numbered carbon fatty acids, valerate and heptanoate, to acetate, propionate and H_2 was obtained in coculture with either an H_2-utilizing methanogen or H_2-utilizing desulfovibrio. The organism could be grown only in syntrophic association with the H_2-utilizer and no other energy sources or combination of electron donor and acceptors were utilized. It was a Gram-negative helical rod with 2 to 8 flagella, about 20 nm in diameter, inserted in a linear fashion about 130 nm or more apart along the concave side of the cell. It grew with a generation time of 84 h in co-culture with *Methanospirillum hungatii* and was present in numbers of at least 4.5×10^{-6} per g of anaerobic digestor sludge.

Key words. Anaerobic degradation – Methanogenesis – Sludge – Syntrophic association – H_2 transfer – Butyrate – Propionate – Acetate – H_2.

products were degraded by a complex of methanogenic species per se to CO_2 and methane. However, Bryant et al. (1967, 1977) showed that the fermentation of ethanol as carried out by *Methanobacillus omelianskii* [Eq. (a)]

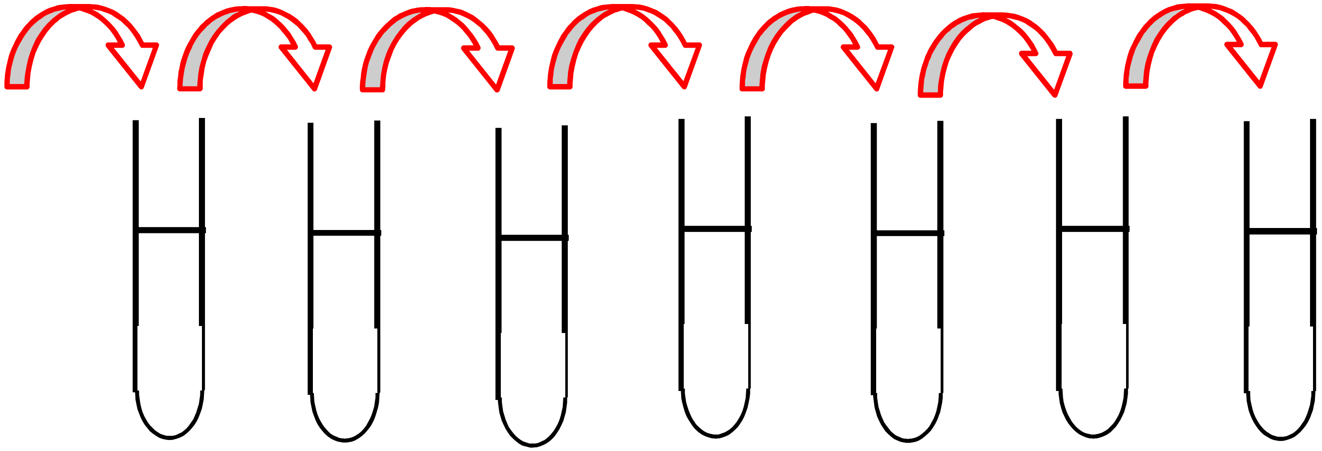
$$2\,CH_3CH_2OH + HCO_3^- \rightleftharpoons CH_3COO^- + H^+ + CH_4 + H_2O \quad \Delta G^{0\prime} = -116.4\ \text{kJ/reaction, pH 7 (Thauer et al., 1977)} \qquad \text{(a)}$$

is in fact carried out by a syntrophic association of two species. Nonmethanogenic species fermented ethanol according to Eq. (b).

$$2\,CH_3CH_2OH + 2\,H_2O \rightleftharpoons 2\,CH_3COO^- + 4\,H_2 + 2\,H^+ \qquad \text{(b)}$$
$$\Delta G^{0\prime} = 19.2\ \text{kJ/reaction}$$

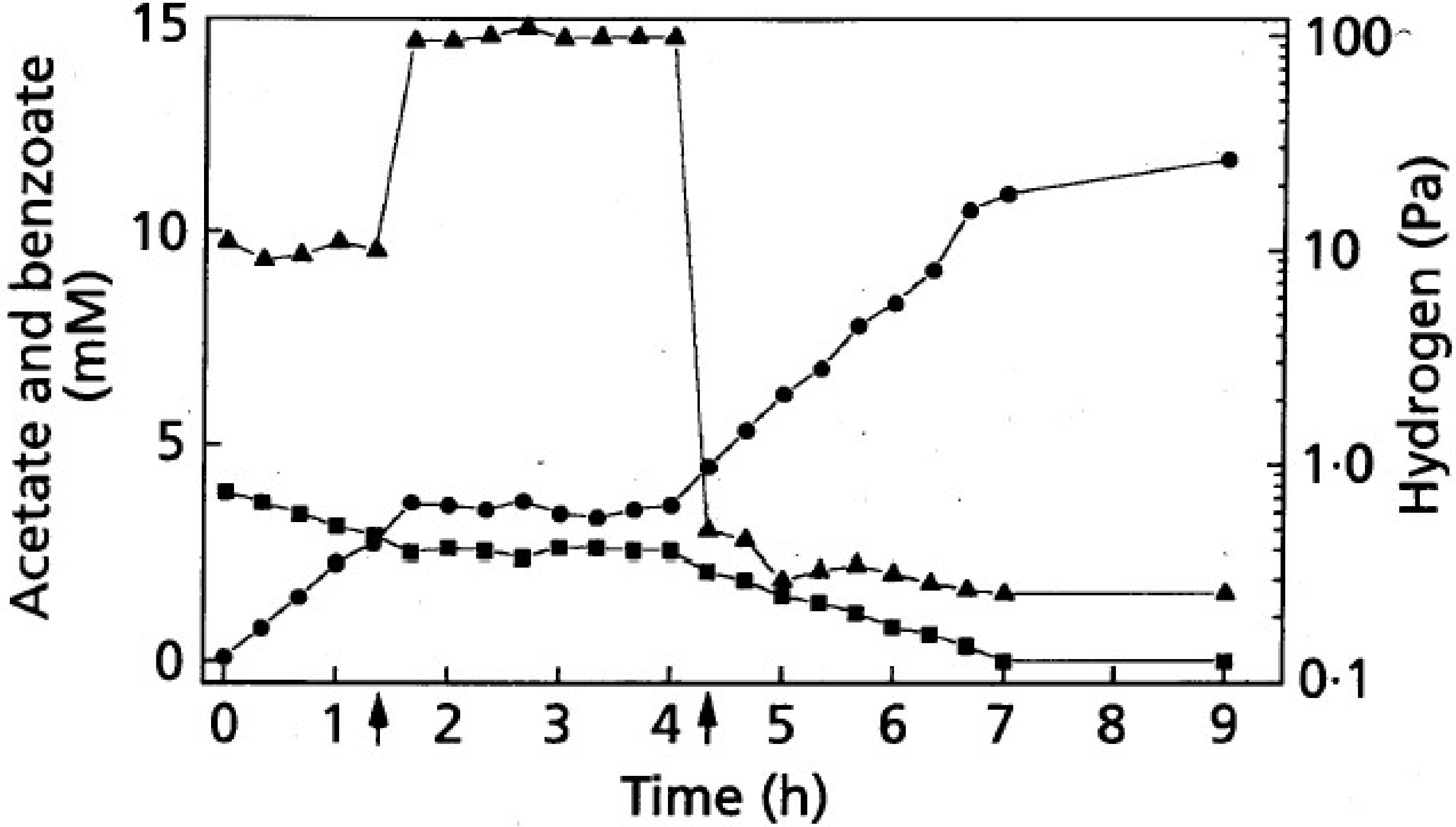
This fermentation is thermodynamically unfavorable unless the partial pressure of H_2 is maintained at a low level by the second species, a methanogenic bacterium that obtains energy for growth via reduction of CO_2 with H_2 [Eq. (c)].

Agar Shake Dilution Technique
for Purification of Strictly Anaerobic Microbes



Growth medium with 1% Agar

Syntrophic benzoate degradation by *Syntrophus gentianae* in coculture with *Methanospirillum hungatei*



First arrow: Addition of BES,
Second arrow: Addition of *Desulfovibrio desulfuricans* (+ sulfate)

Energy sharing in a ternary syntrophic coculture converting butyrate to CH_4 and CO_2

$$2\ CH_3CH_2CH_2COO^- + H^+ + 2\ H_2O \rightarrow 5\ CH_4 + 3\ CO_2$$

$\Delta G°' = - 177$ kJ per mol

with 10 μM butyrate: $\Delta G' = - 140$ kJ per mol

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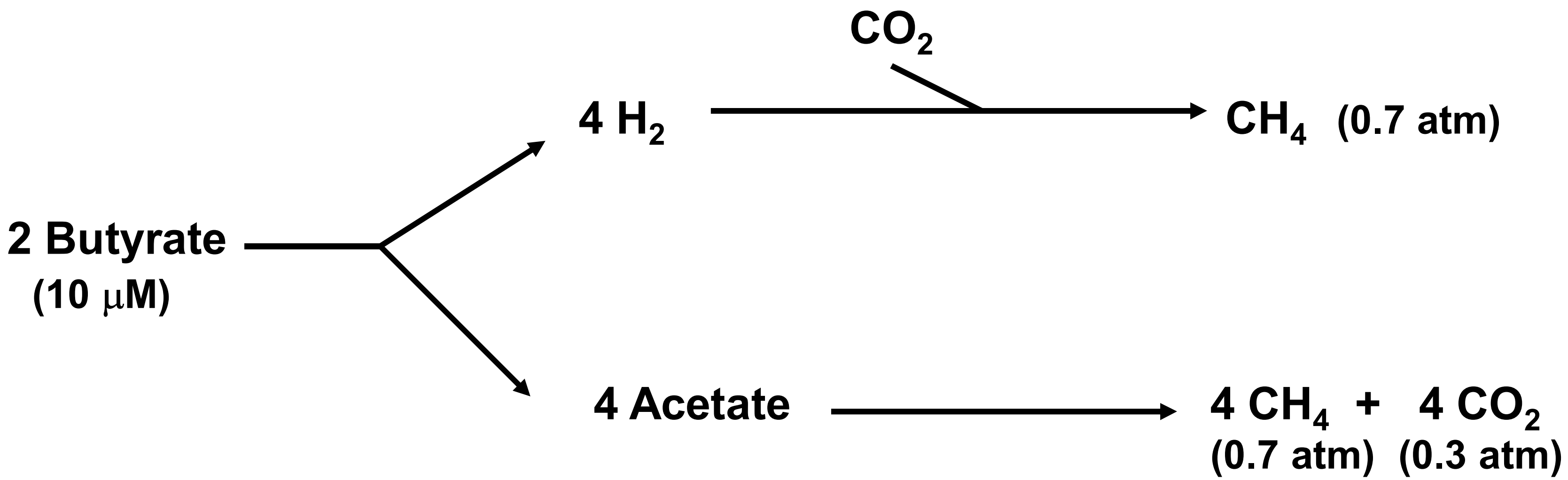

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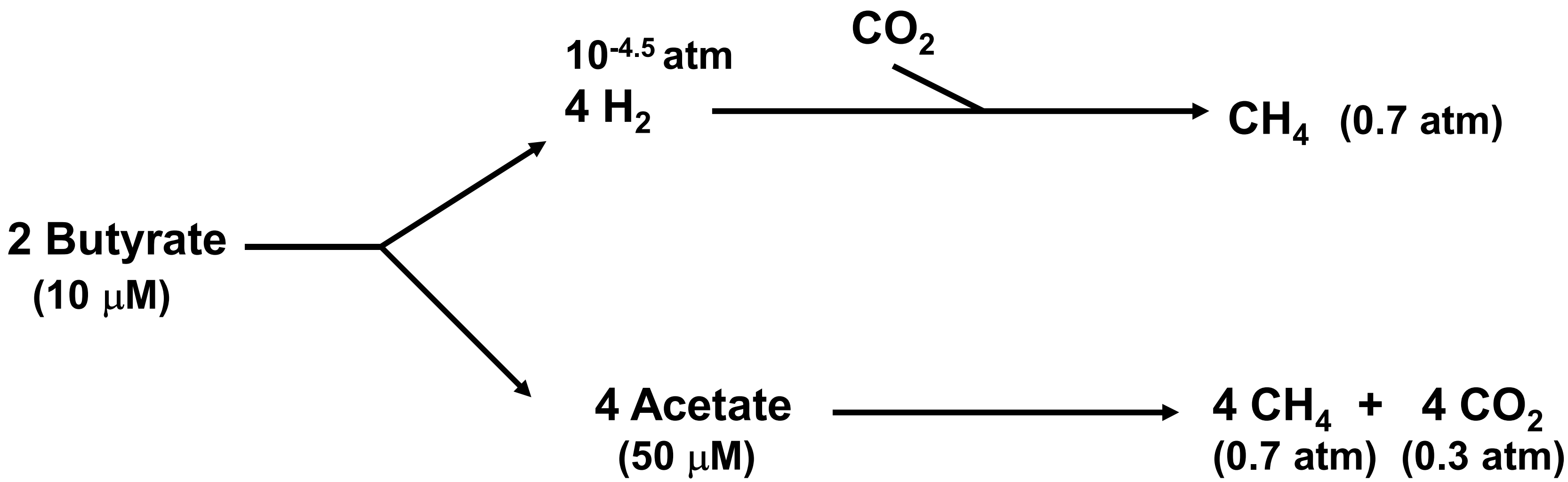

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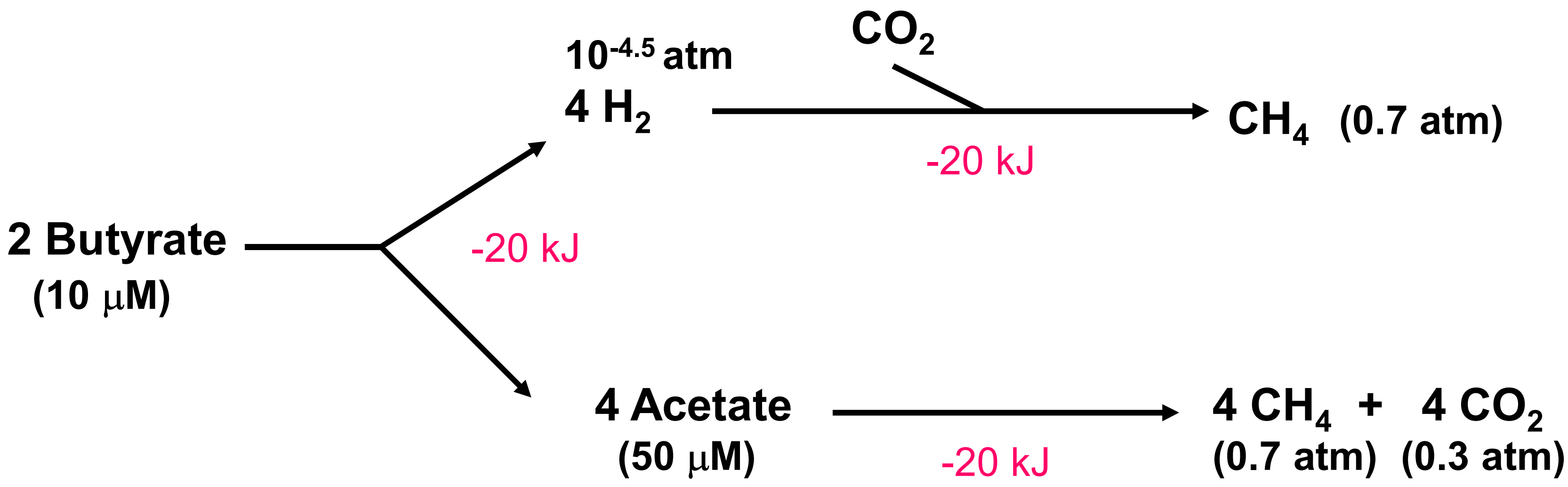
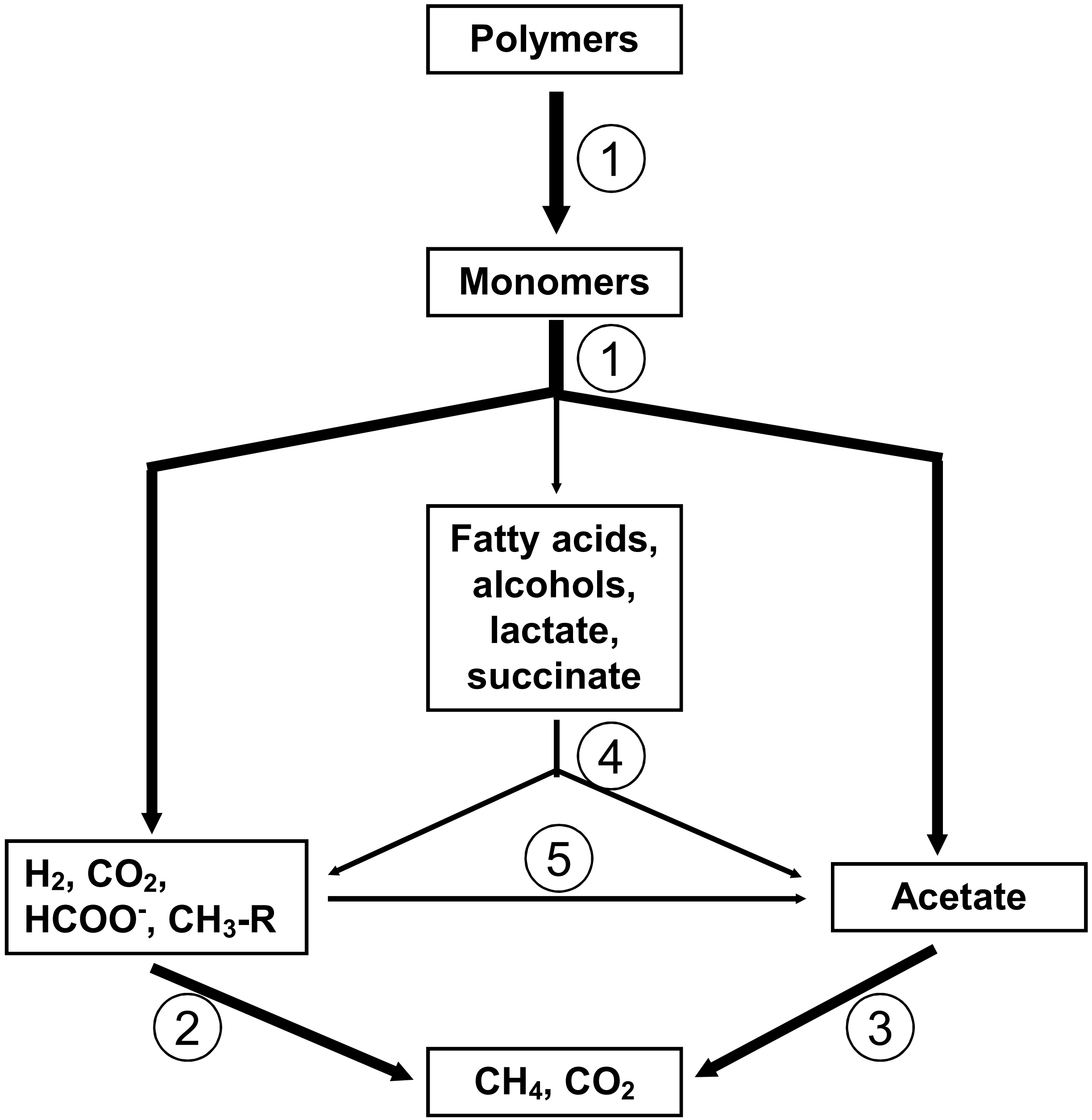
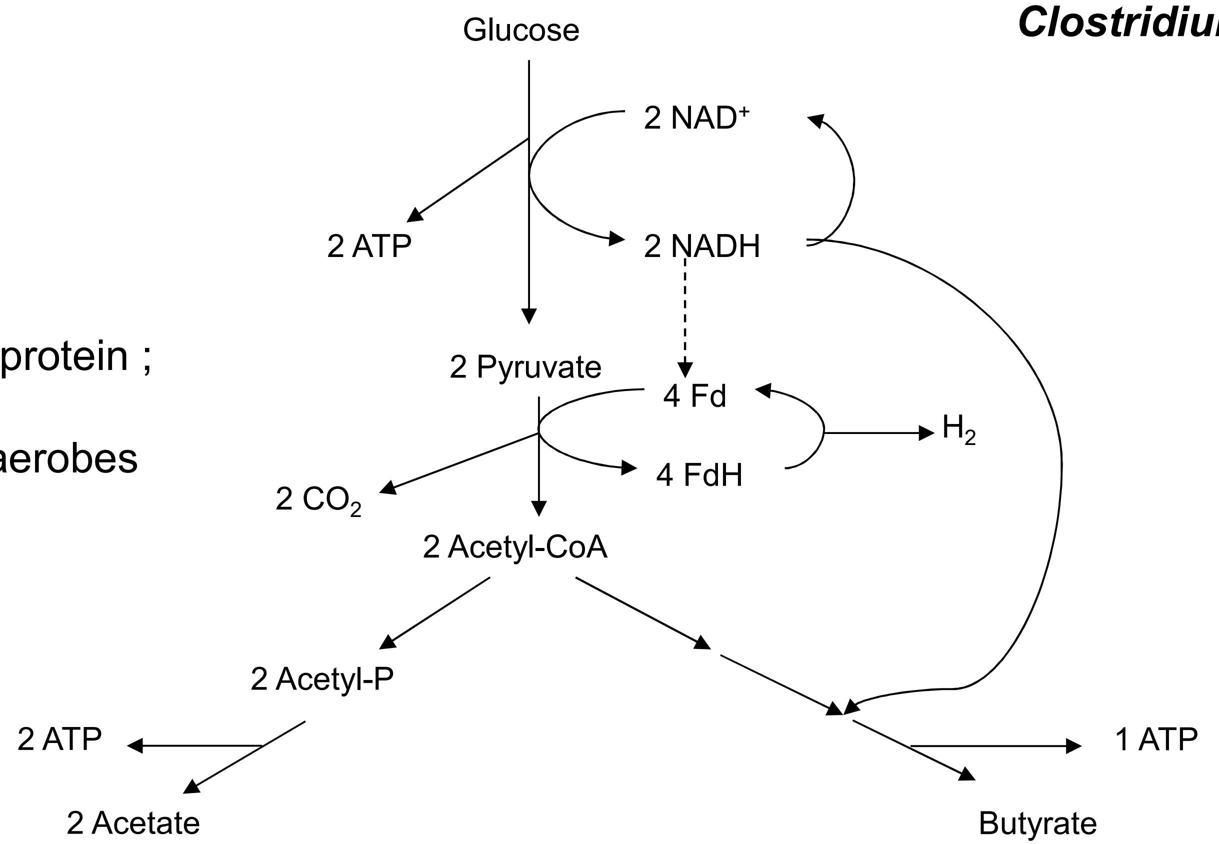


Electron flow in a methanogenic microbial community



Butyric acid fermentation

Clostridium butyricum



Fd = Ferredoxin

Small iron-sulfur protein ;
Redox carrier
in fermenting anaerobes

Alternatives:

(1) Glucose → Butyrate + 2 CO_2 + 2 H_2 **$\Delta G_0'$ = -247 kJ/mol → 3 ATP**

(2) Glucose → 2 Acetate + 2 CO_2 + 4 H_2 **$\Delta G_0'$ = -216 kJ/mol ↛ 4 ATP**

Observed:

(3) Glucose → 0.7 Butyrate + 0,6 Acetate + 2.6 H_2 + 2 CO_2 **$\Delta G_0'$ = -233 kJ/mol → 3.3 ATP**

Energetics of glucose fermentation by *Clostridium butyricum*

In pure culture:

glucose + 2 H_2O → 0.7 butyrate$^-$ + 0.6 acetate$^-$ + 1.3 H^+ + 2 CO_2 + 2.6 H_2

$\Delta G_0'$ = -233 kJ per mol, yielding 3.3 ATP per glucose

Alternatively:

glucose + 2 H_2O → 2 acetate$^-$ + 2 H^+ + 2 CO_2 + 4 H_2 ;

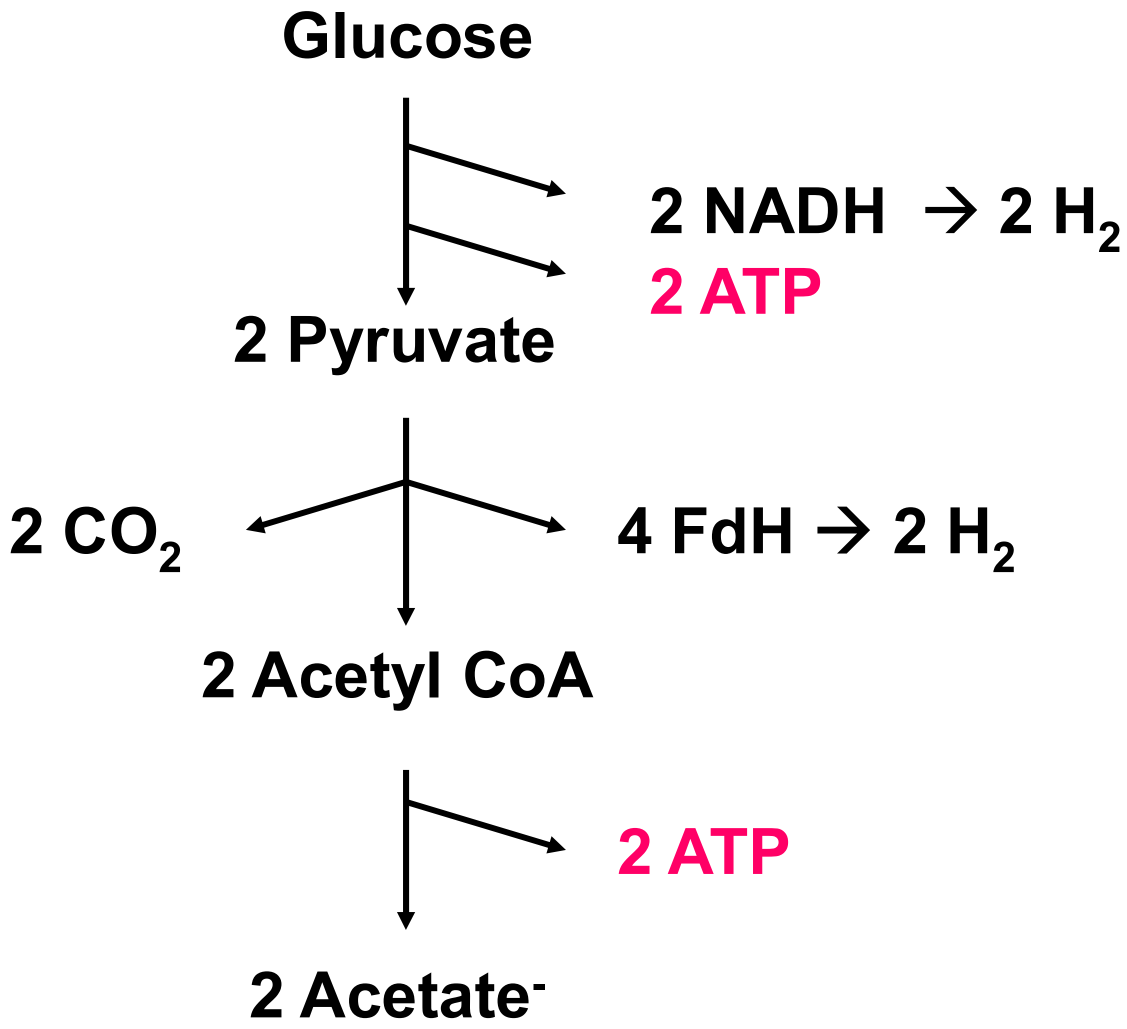
$\Delta G_0'$ = -216 kJ per mol glucose

60-70 kJ needed per mol ATP; ➔ too little energy to form 4 ATP!

At [H_2] = 10^{-4} atm, the overall energetics changes to

$\Delta G'$ = -307 kJ, allowing for 4 ATP per glucose.

Glucose fermentation to acetate only



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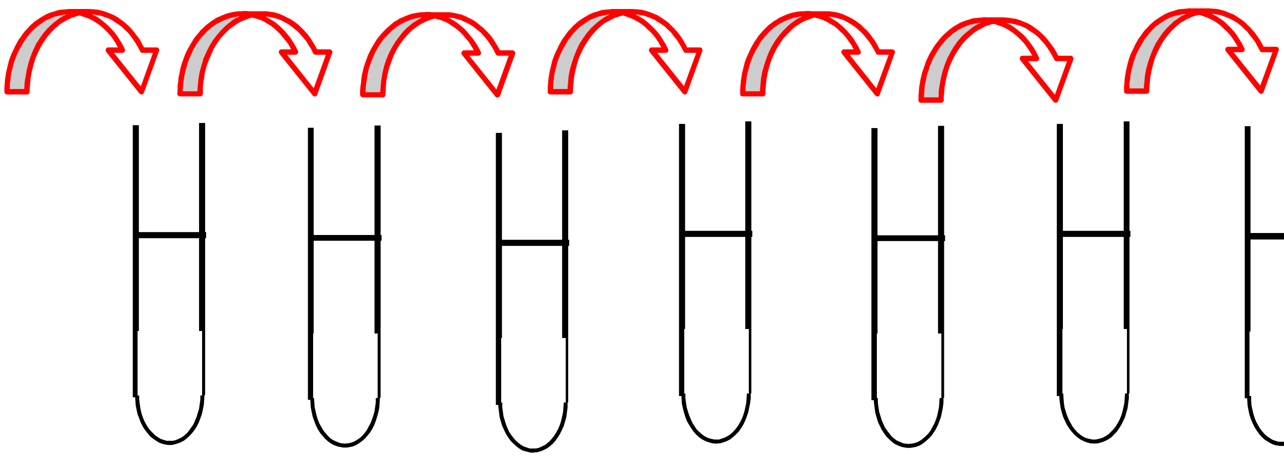


Growth medium with 1% Agar

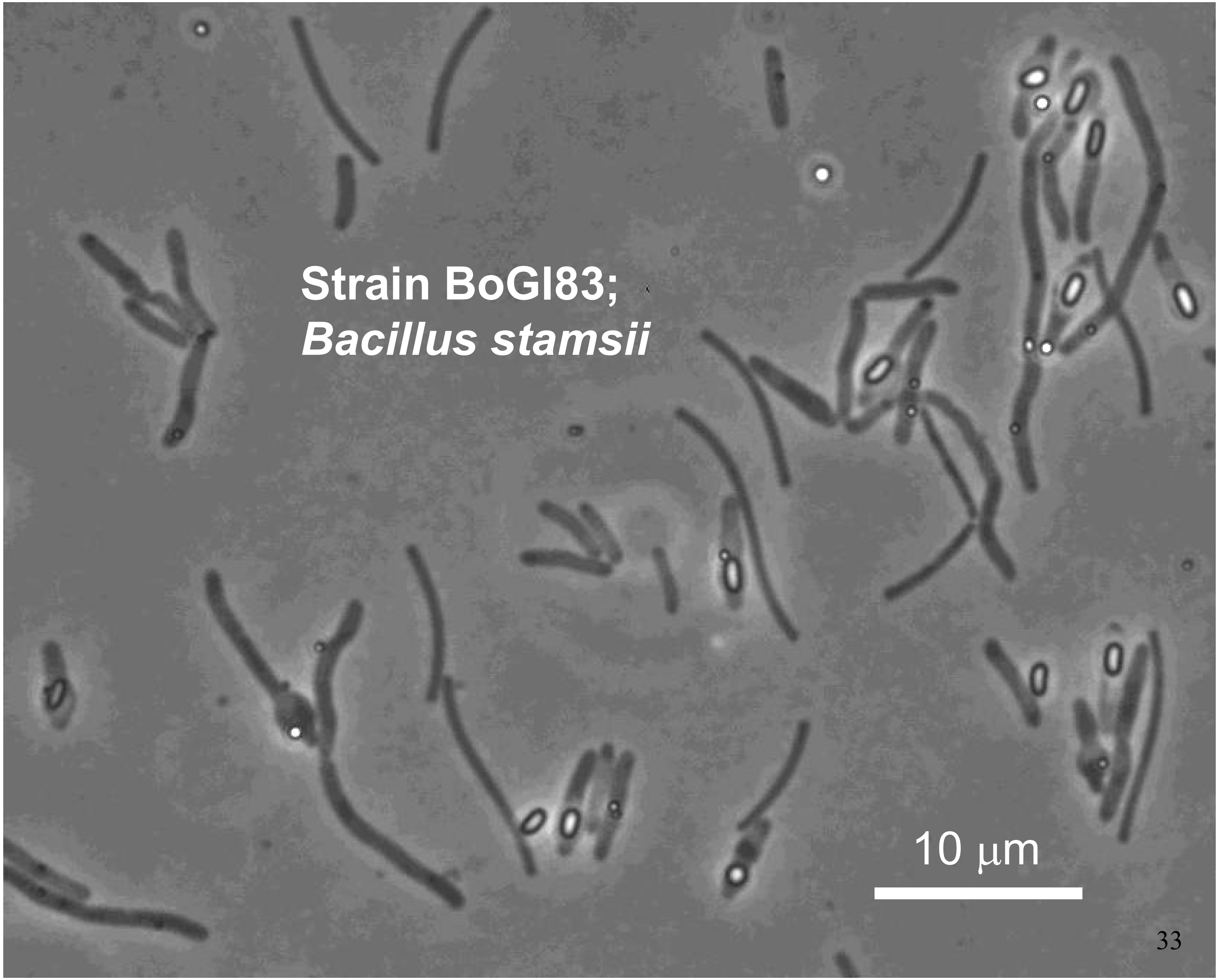
Direct dilution cultivation with Lake Constance profundal sediment

Substrate	Cultivation conditions	CFU per ml
Glucose	- *Msp. hungatei*	$1.2 \cdot 10^5$
	+ *Msp. hungatei*	$1.4 \cdot 10^8$
Starch	- *Msp. hungatei*	$2.0 \cdot 10^5$
	+ *Msp. hungatei*	$4.2 \cdot 10^7$
Sucrose	- *Msp. hungatei*	$8.0 \cdot 10^4$
	+ *Msp. hungatei*	$1.6 \cdot 10^7$

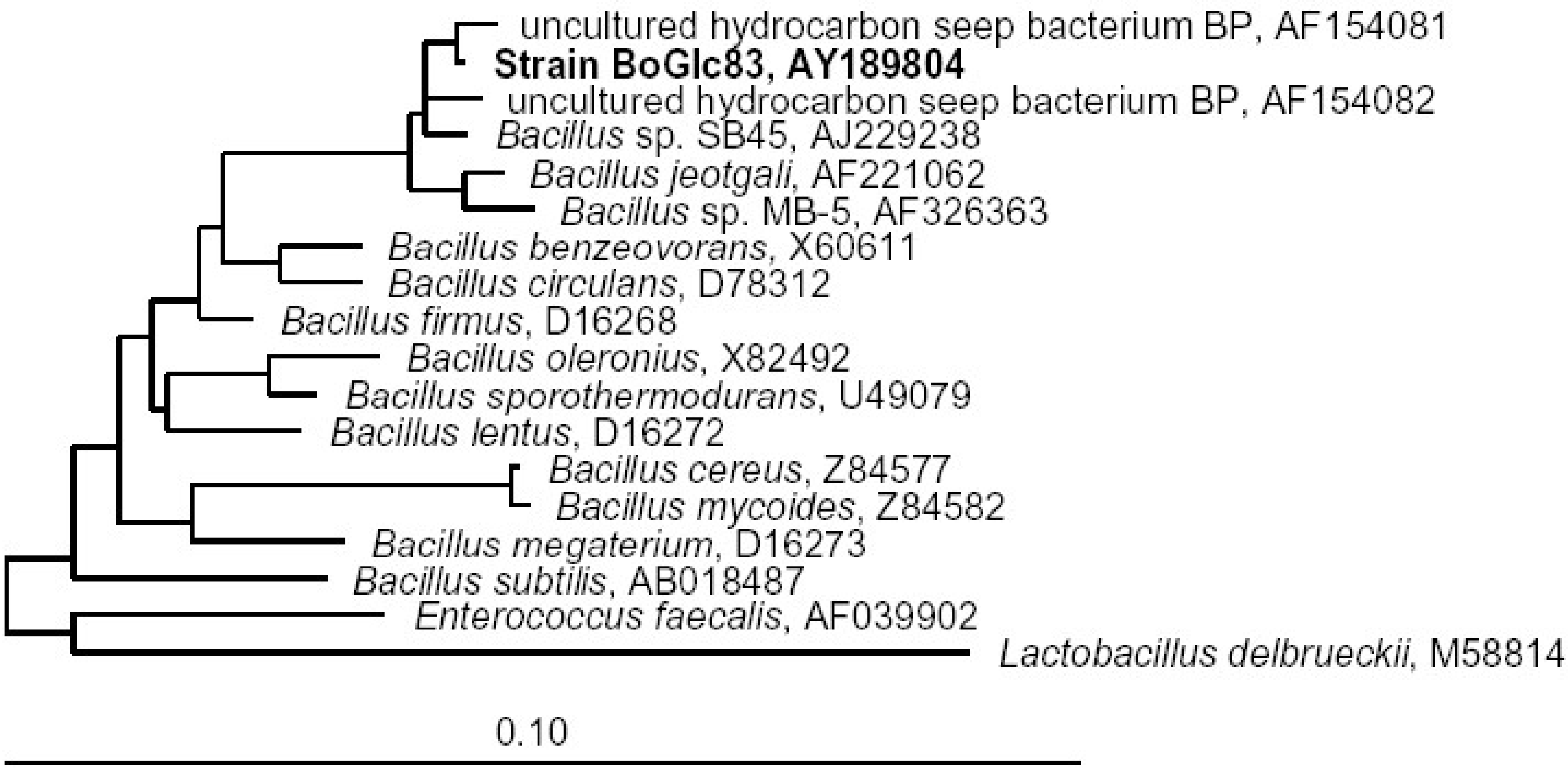
Total count (DAPI): 8.7×10^7 cells per ml sediment

Incubation at 28°C for 2 months.

Predominant colonies show “satellites”.


Strain BoGI83;
Bacillus stamsii
10 µm

16S rRNA sequence comparison of strain BoGlc83



Six strains of defined cocultures isolated from direct dilution series:

- Short rods, 2.0 x 0.5 μm in size
- Gram-positive, forming subterminal spores
- Facultatively aerobic
- Glucose degradation inhibited by 5 mM BES
- Glucose concentrations >5 mM inhibitory, 2 mM optimal
- Fermentation pattern of the defined cocultures:

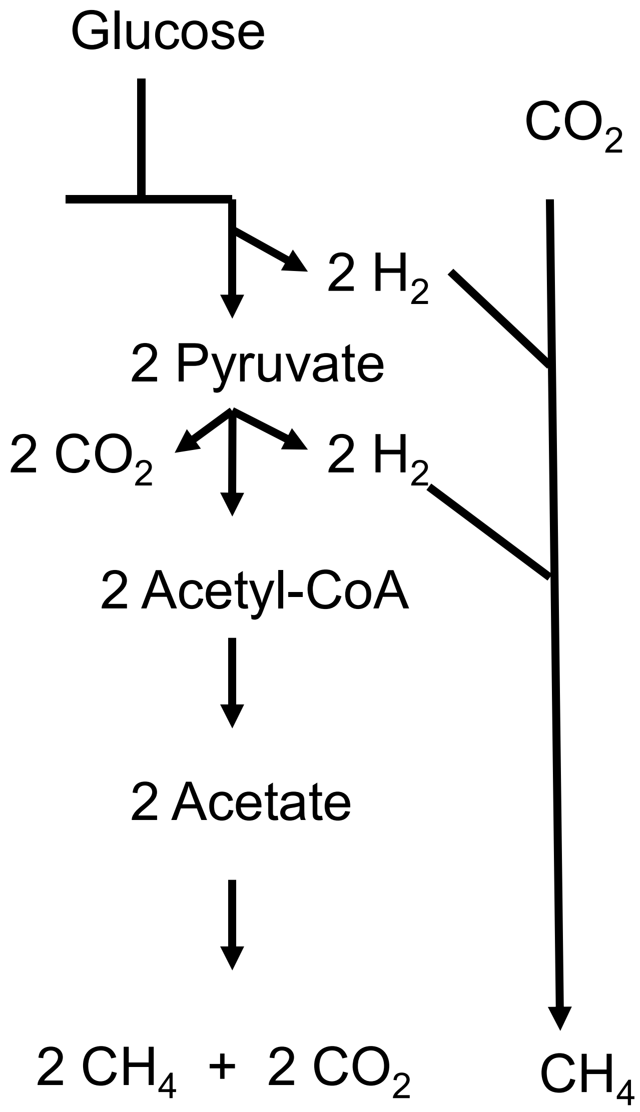
  Glucose → 2 Acetate + CH_4 + CO_2
- Not (yet) found in wastewater biogas reactors

Müller, N., U. Stingl, B. M. Griffin, B. Schink: Environ. Microbiol. 10, 1501-1511 (2008)
Müller, N., F. D. Scherag, M. Pester, B. Schink: Syst. Appl. Microbiol. 38, 379-389 (2015)

"It's Bob, all right . . . but look at those vacuous eyes, that stupid grin on his face — he's been domesticated, I tell you."

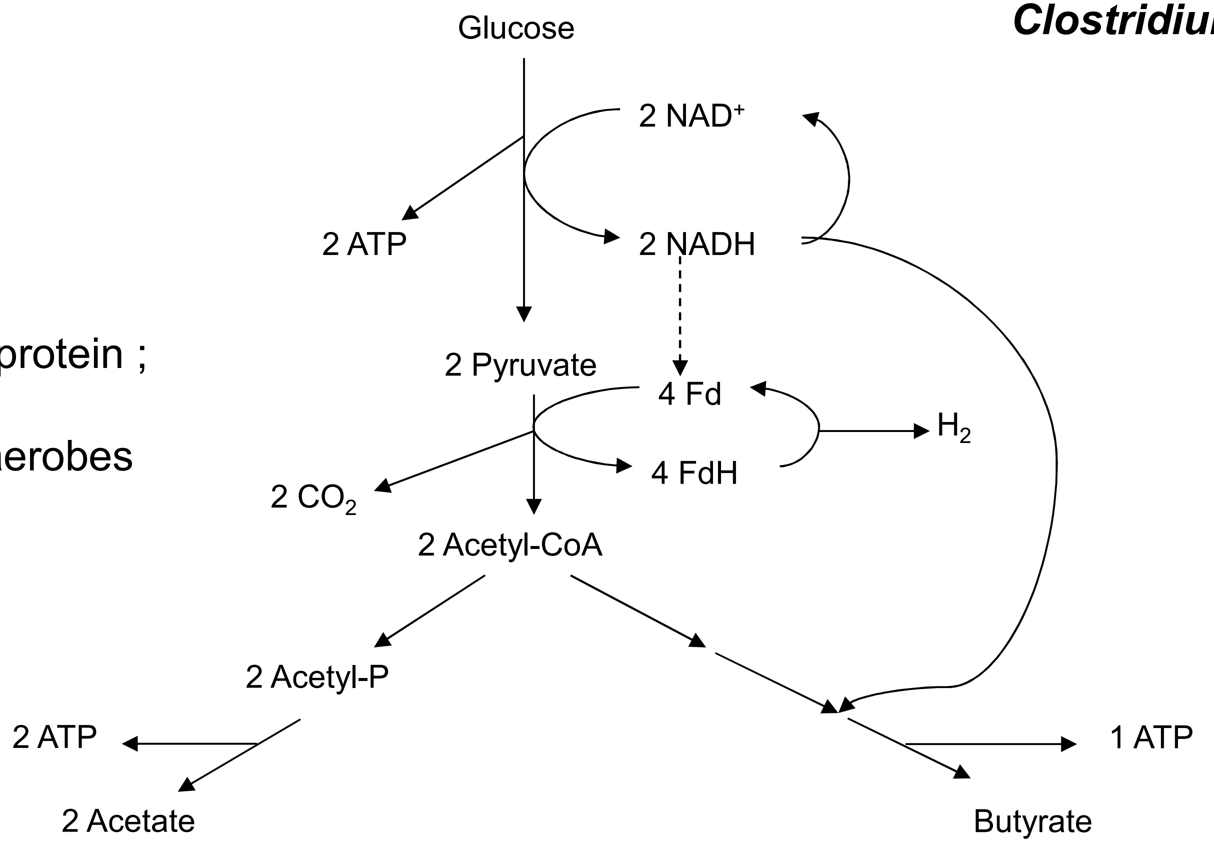
Gary Larson

Importance of Acetate vs. H_2 as Electron Carriers



Butyric acid fermentation

Clostridium butyricum



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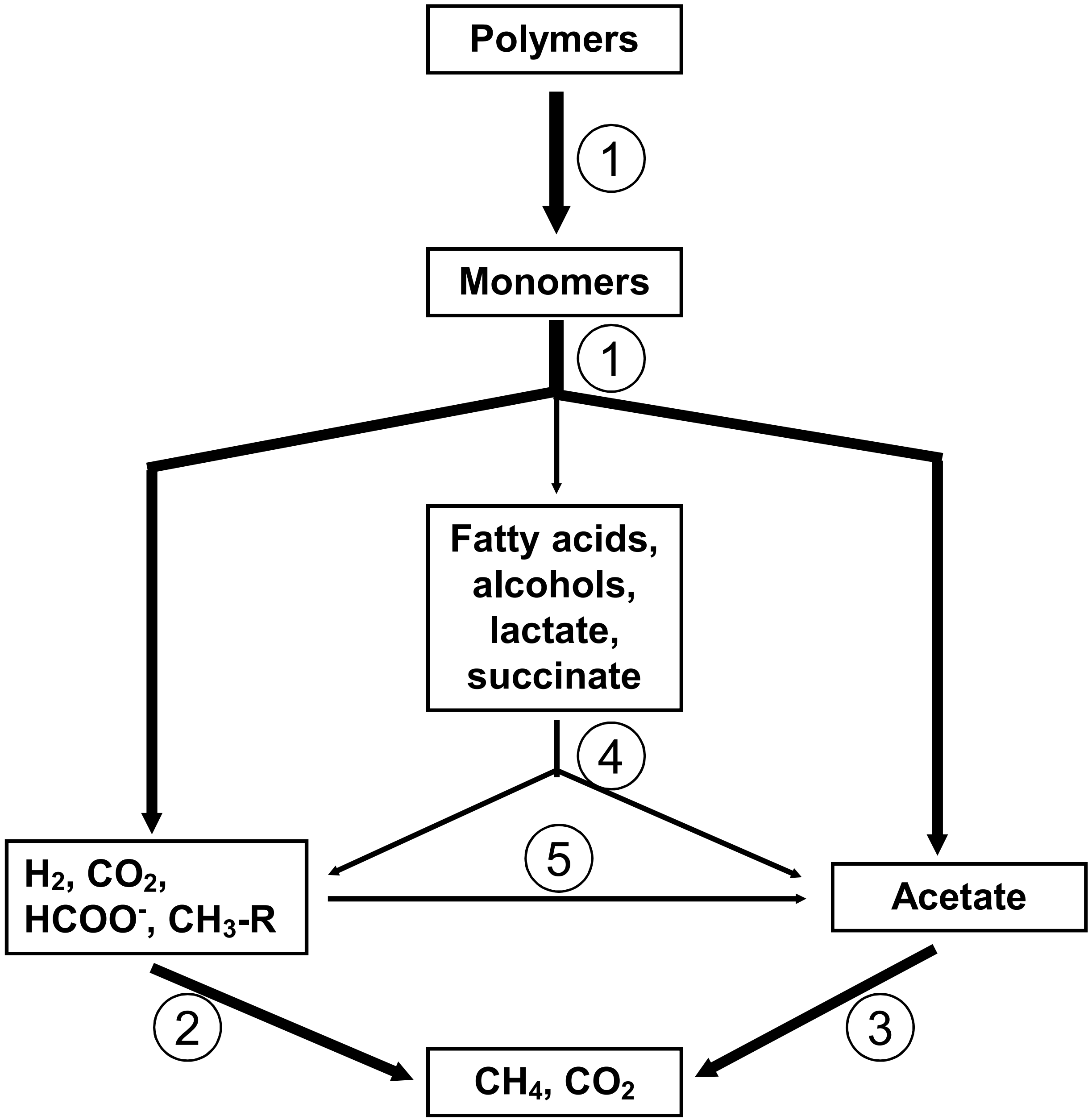

Properties of methanogenic habitats

Habitat	Temperature (°C)	Detention time (d)	[Acetate] (µM)	[Propionate] (µM)	[Butyrate] (µM)	[Hydrogen] (Pa)
Eutrophic Marine Sediment	4 - 10	„unlimited“	1 - 660	1 - 24	0.1 - 22	<1
Eutrophic Freshwater Sediment	4 - 10	„unlimited“	1 - 160	1 - 20	1 - 10	<2
Anaerobic Sewage Sludge	30 - 35	15 - 30	5 - 6000	1 - 500	1 - 500	1 - 10
Rumen of a Cow	37 - 39	0.5 - 2	60 000	20 000	10 000	20 - 5000

(modified after Schink, B. Naturwissenschaften 76, 364-373 (1989))

Sewage Sludge Digestor,
Sewage Plant, Constance
ca. 5000 m^3

Biogas Reactors

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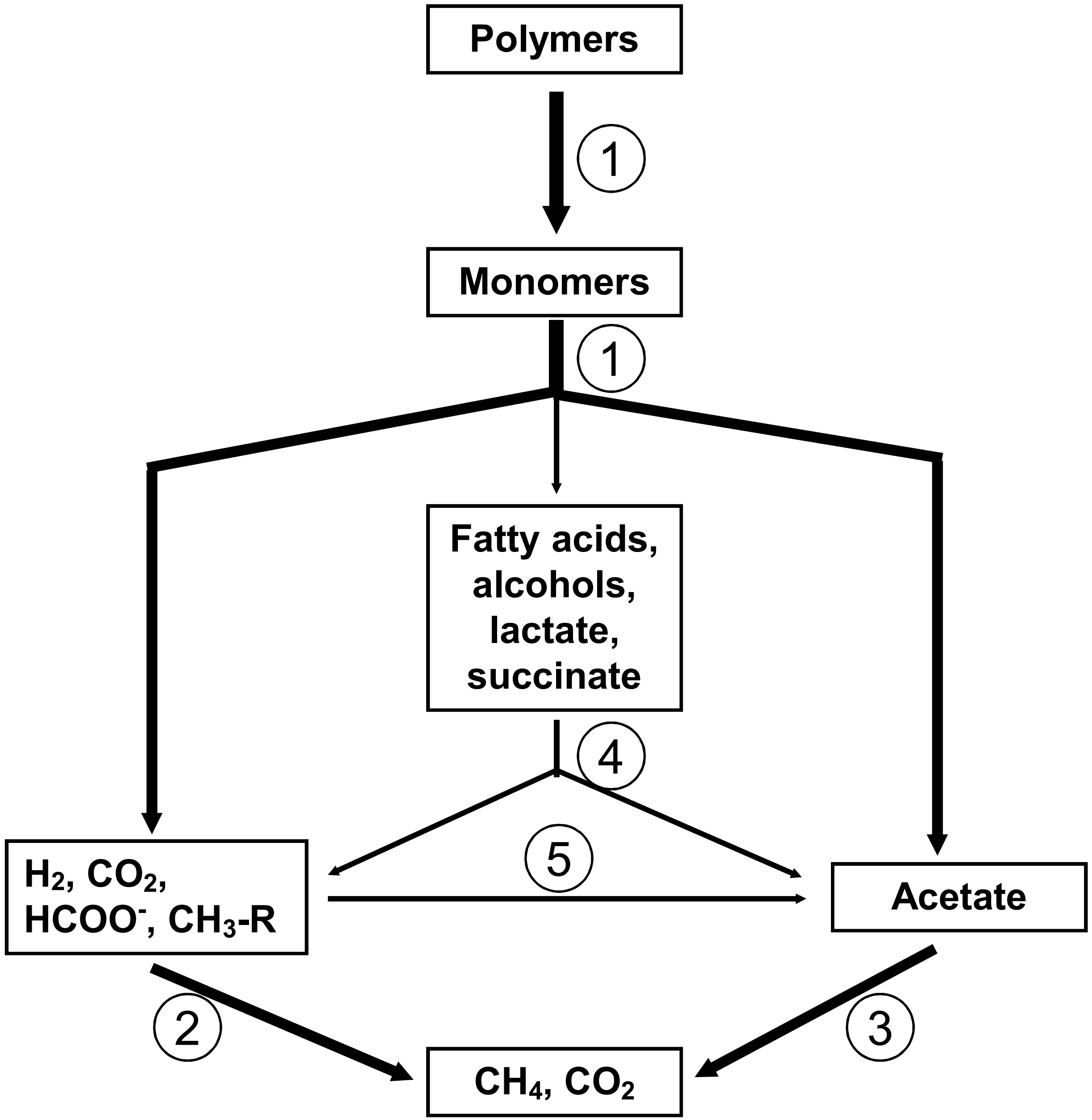
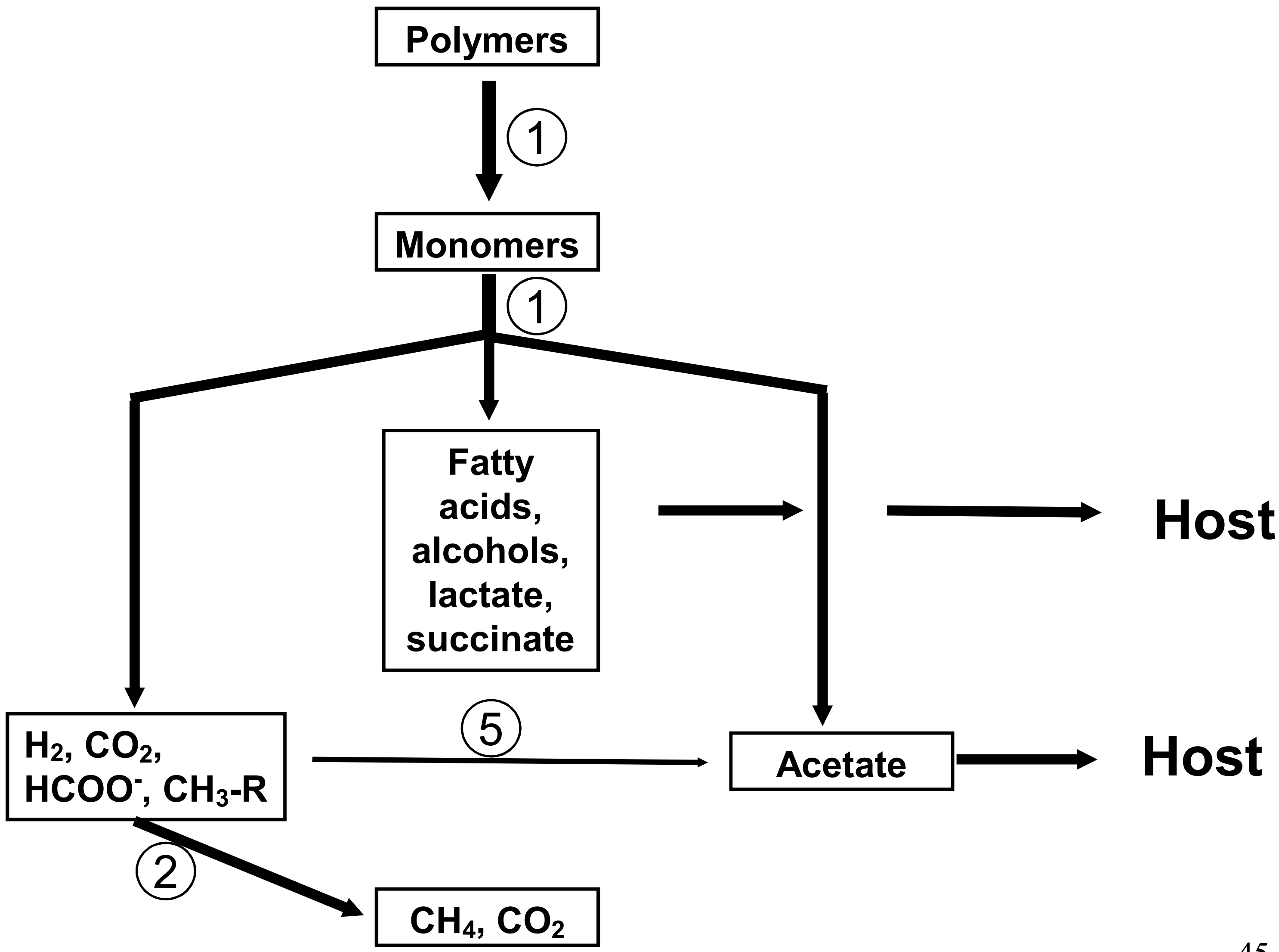


Electron flow in a methanogenic microbial community inside a ruminant



Digestion of Termites

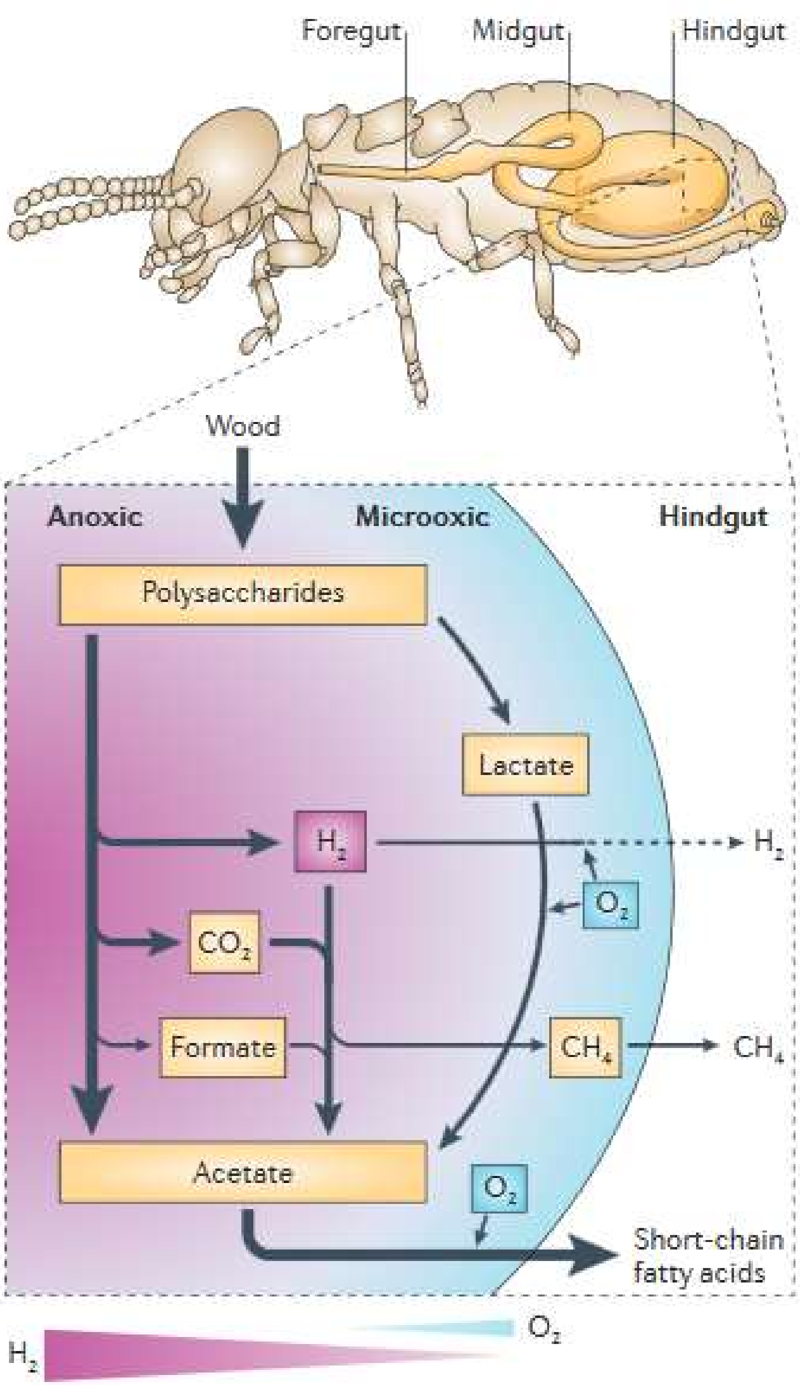
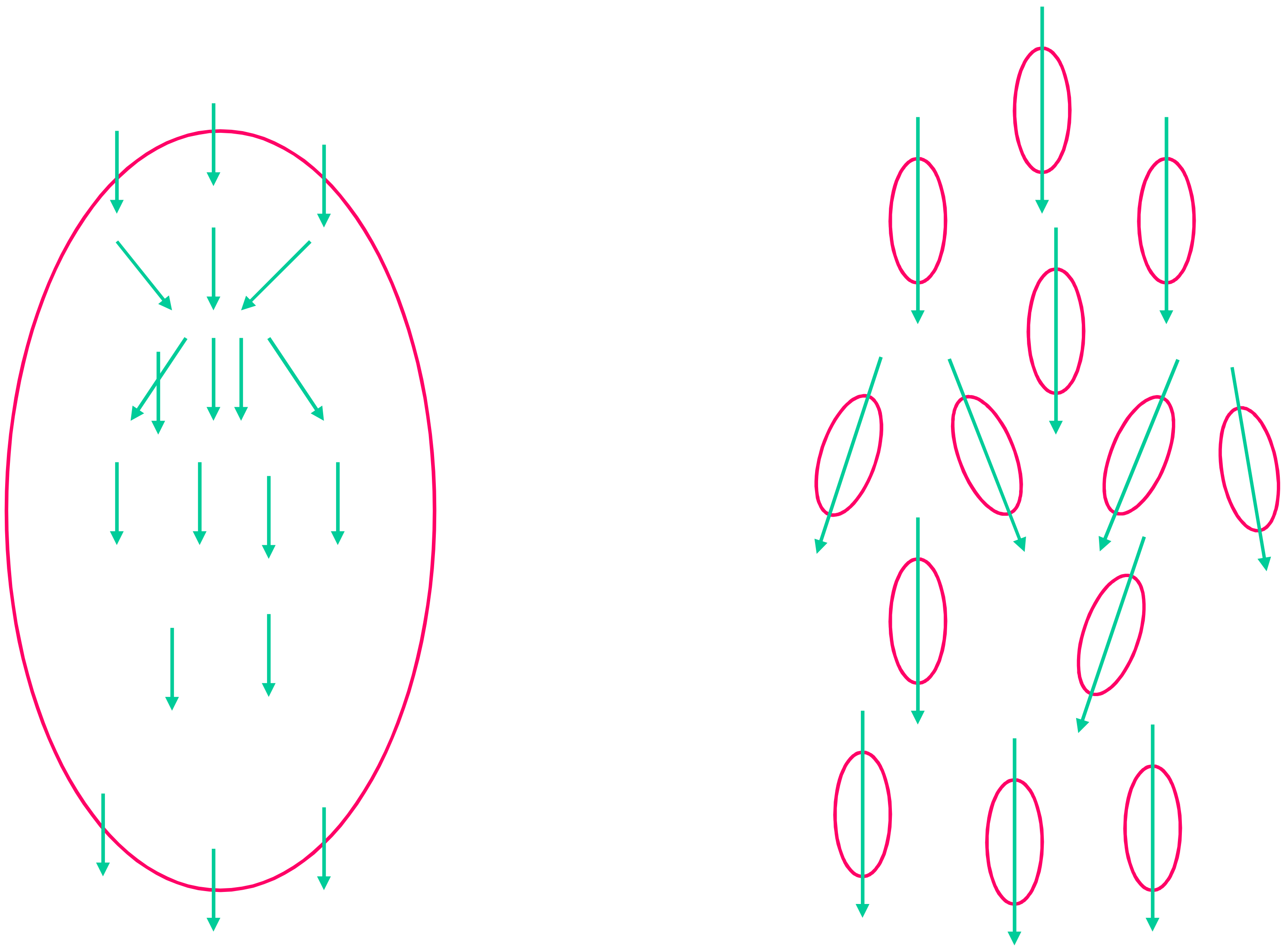


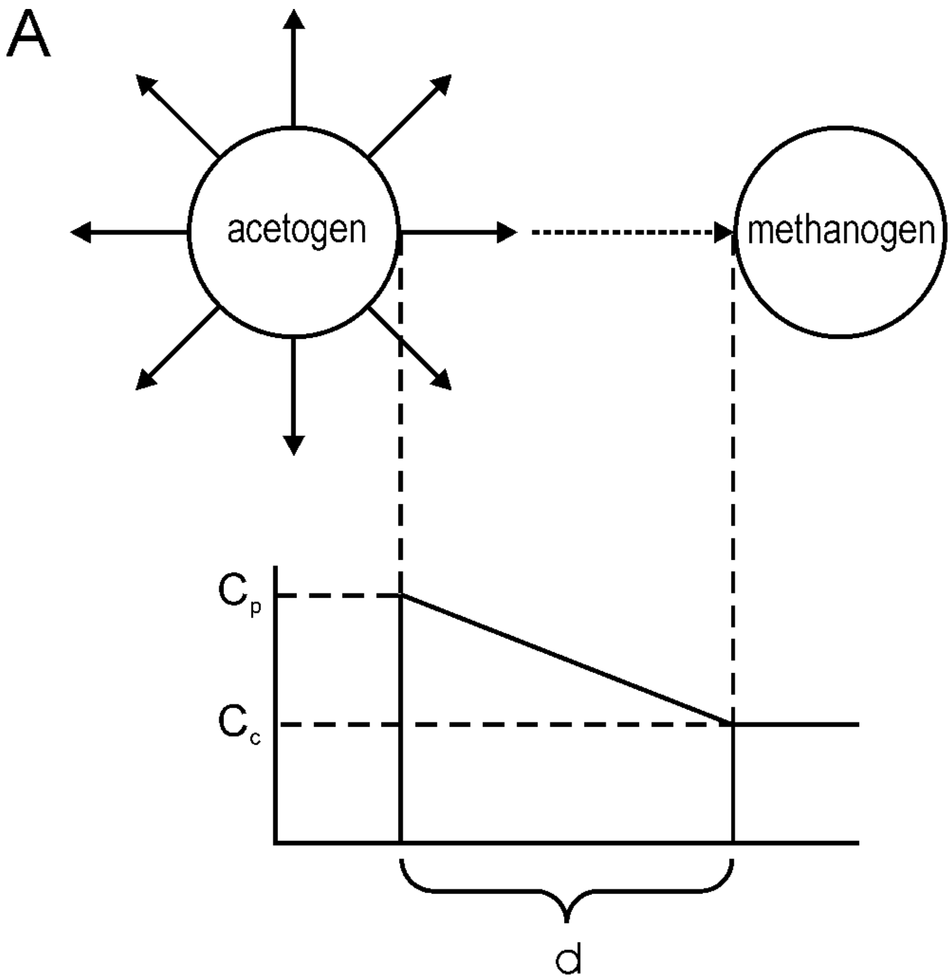
Figure 4 | **Major microbial processes in the hindgut of lower termites.** The fermentation of wood polysaccharides by the gut flagellates yields acetate and other short-chain fatty acids, which are resorbed by the host. Hydrogen is an important intermediate that drives the reduction of CO_2, which yields additional acetate (via homoacetogenesis) and some methane[14]. Although H_2 may strongly accumulate at the gut centre, most of it is consumed before it can escape from the gut[13,72]. The high surface-to-volume ratio of the microlitre-sized hindgut compartment causes an enormous influx of oxygen across the gut wall. Its efficient removal by the gut microbiota within fractions of a millimetre results in steep gradients in the hindgut periphery[11,70]. Oxygen is consumed by both microaerobic and anaerobic bacteria and methanogenic archaea that use acetate, lactate or hydrogen as the electron donor[71,72,100,104].

Brune, A. Nature Rev. 2014

Integrative versus modular organisation of metabolic reaction chains



Effect of intermicrobial distances

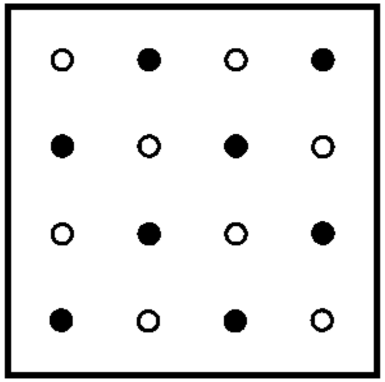


$$\text{Flux} = A \cdot D \cdot (C_p - C_c) / d \quad \text{mol sec}^{-1}$$

Schink and Thauer
1987

B

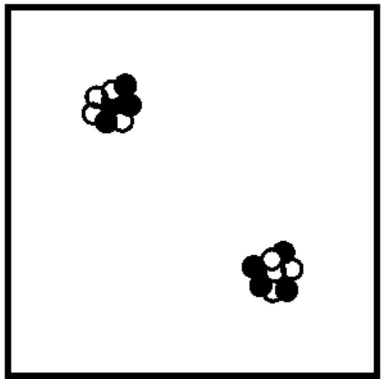
equal distribution



r = 1 μm
d = 8 μm ←
C_p = 0.1 mM
C_c = 0.01 mM

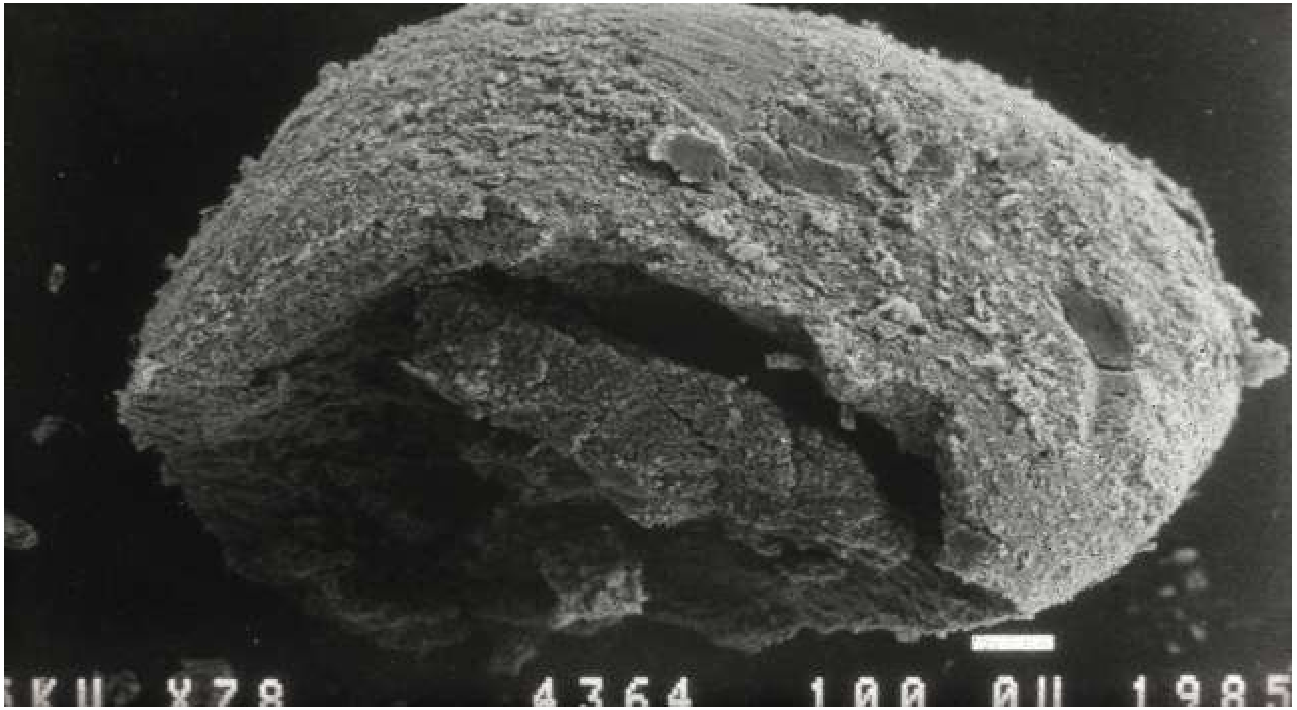
Flux = 20 nmol ml^{-1} min^{-1}

cluster formation

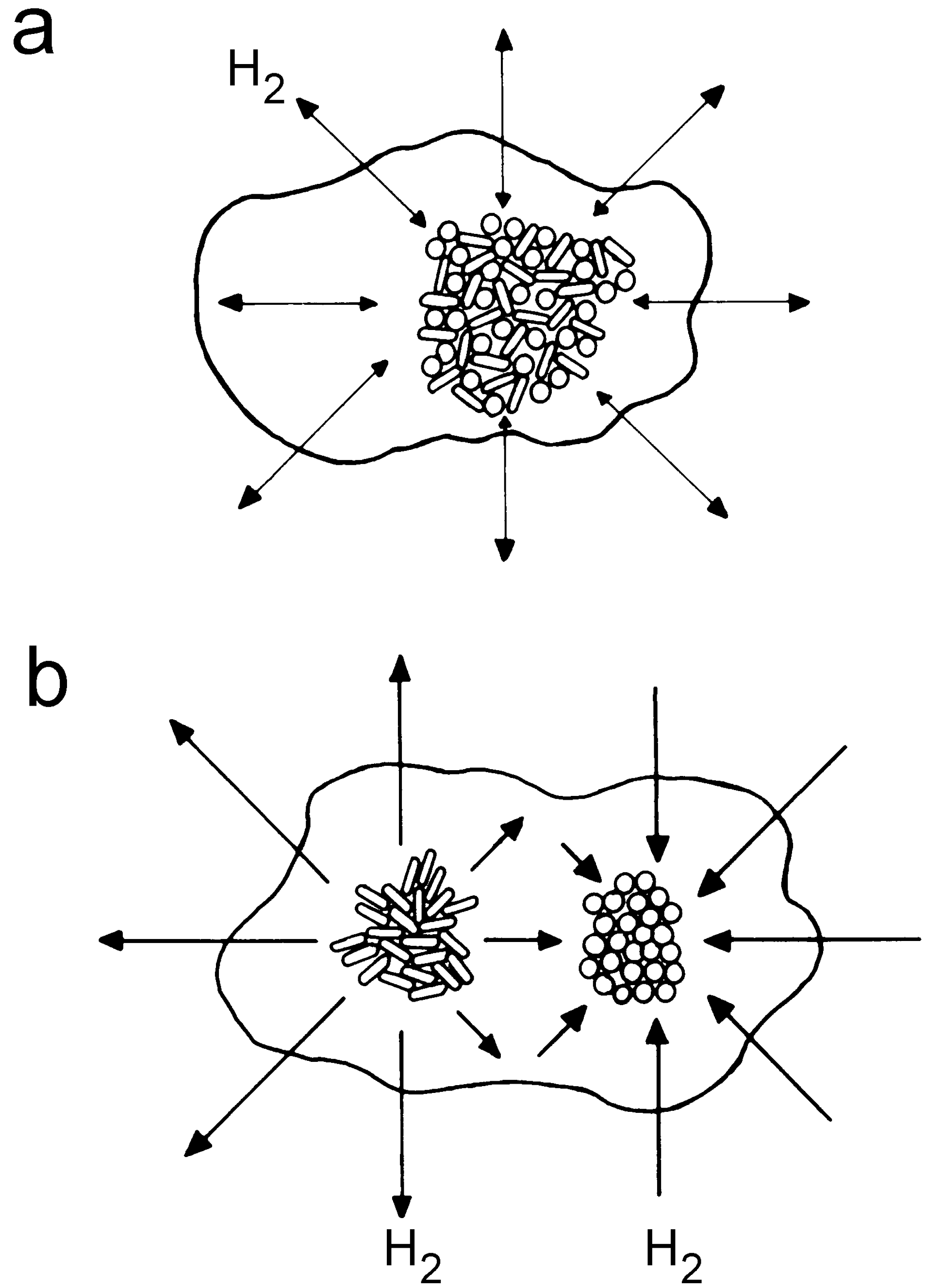


r = 1 μm
d = 0.08 μm ←
C_p = 0.1 mM
C_c = 0.01 mM

Flux = 2000 nmol ml^{-1} min^{-1}



Granules in an UASB Reactor,
G. Lettinga, Wageningen, NL



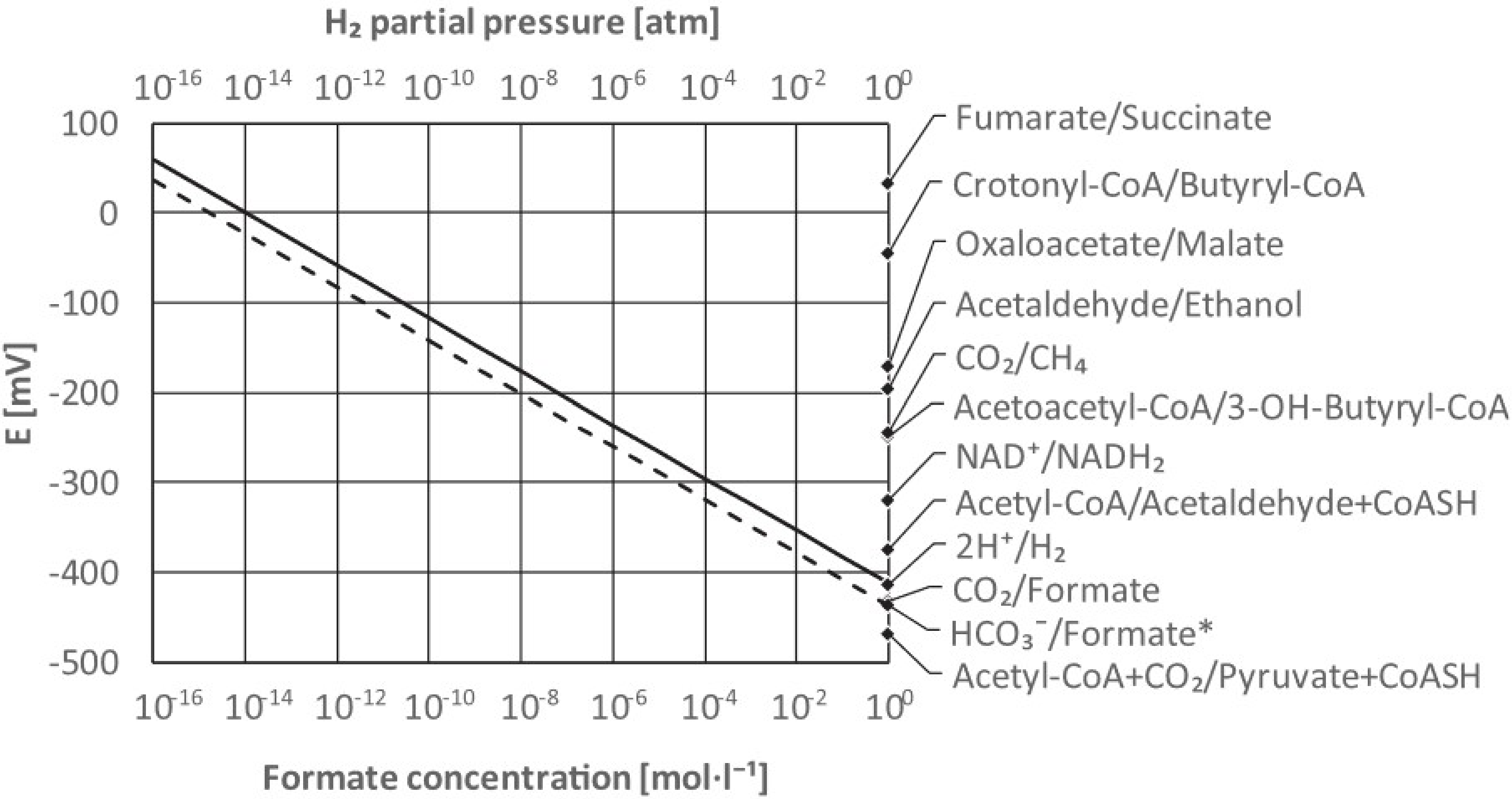
Growth of a syntrophic community in, e. g., a sewage digestor sludge floc

Formate or hydrogen?

- Indications for interspecies formate transport in syntrophic oxidation of butyrate, propionate, and ethanol
- Redox potentials nearly identical (E°‘ = -430 vs. -414 mV);

  in situ ca. -300 mV
- Diffusion kinetics nearly equivalent
- Solubility in water quite different
- Energetically equivalent?

Hydrogen vs. Formate as Electron Shuttle in Syntrophic Cooperations

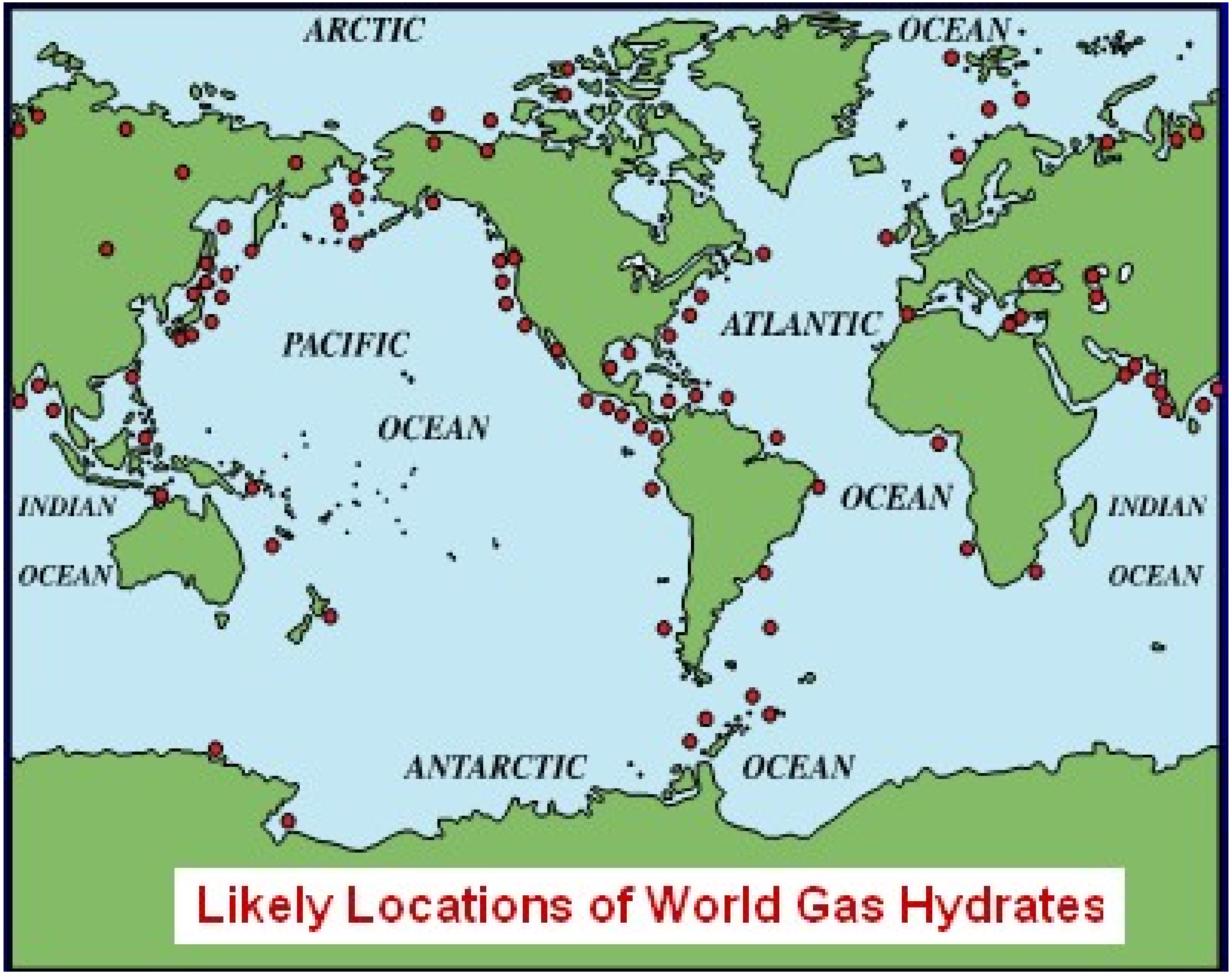


Schink, Montag, Keller, Müller, 2017

Anaerobic oxidation of methane

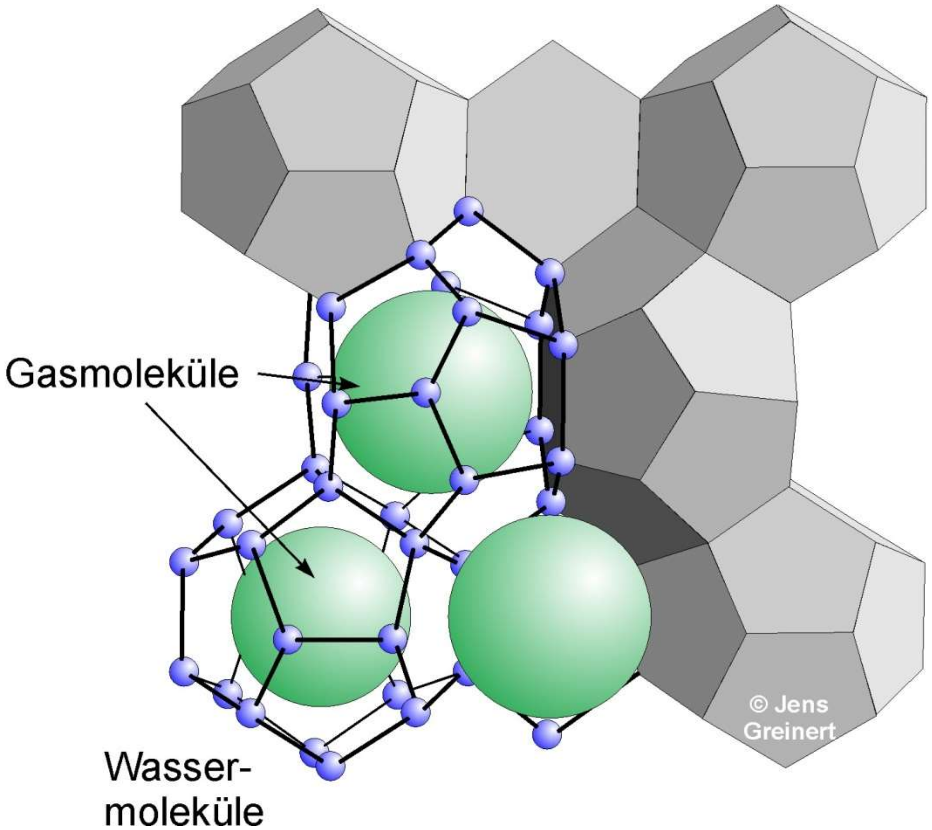
$$CH_4 + SO_4^{2-} + 2\ H^+ \rightarrow CO_2 + H_2S + 2\ H_2O$$

$$\Delta G°' = -21\ \text{kJ per mol}$$



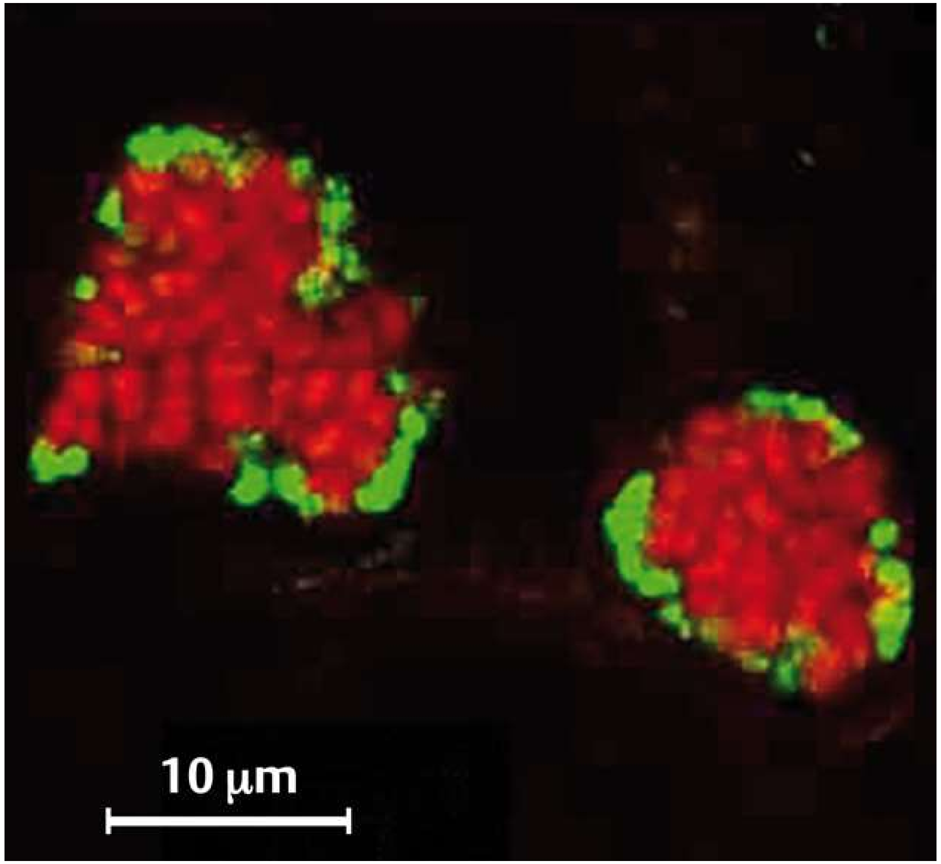
Global amount ca. 10^{19} g; Kvenvolden and Lorenson, 2013

Gas hydrates



Gas hydrate („Methane Ice“)

Anaerobically methane-oxidizing aggregates



Aggregates were harvested
off the coast of Oregon
at about 1000 m water depth
above methane hydrates

(*Photo:*
K. Knittel, A. Boetius, Bremen)

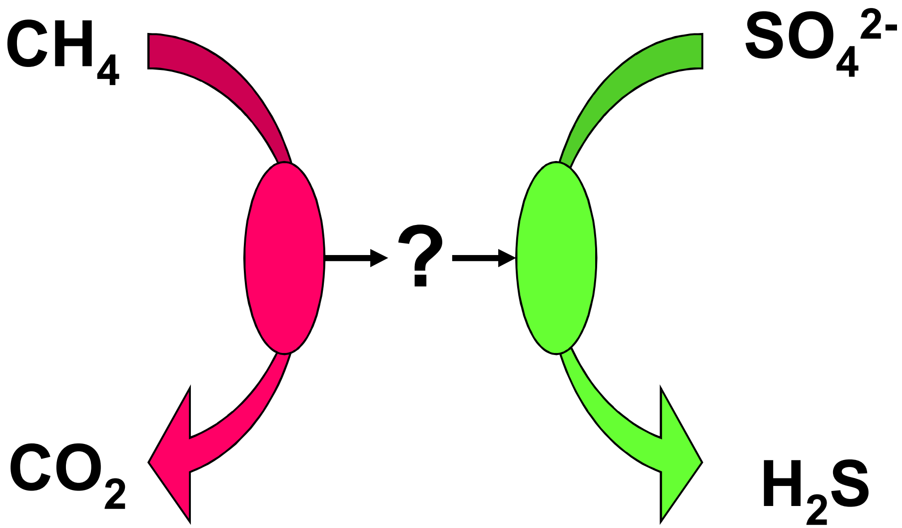
Anaerobic oxidation of methane

$CH_4 + SO_4^{2-} + 2\ H^+ \rightarrow CO_2 + H_2S + 2\ H_2O$

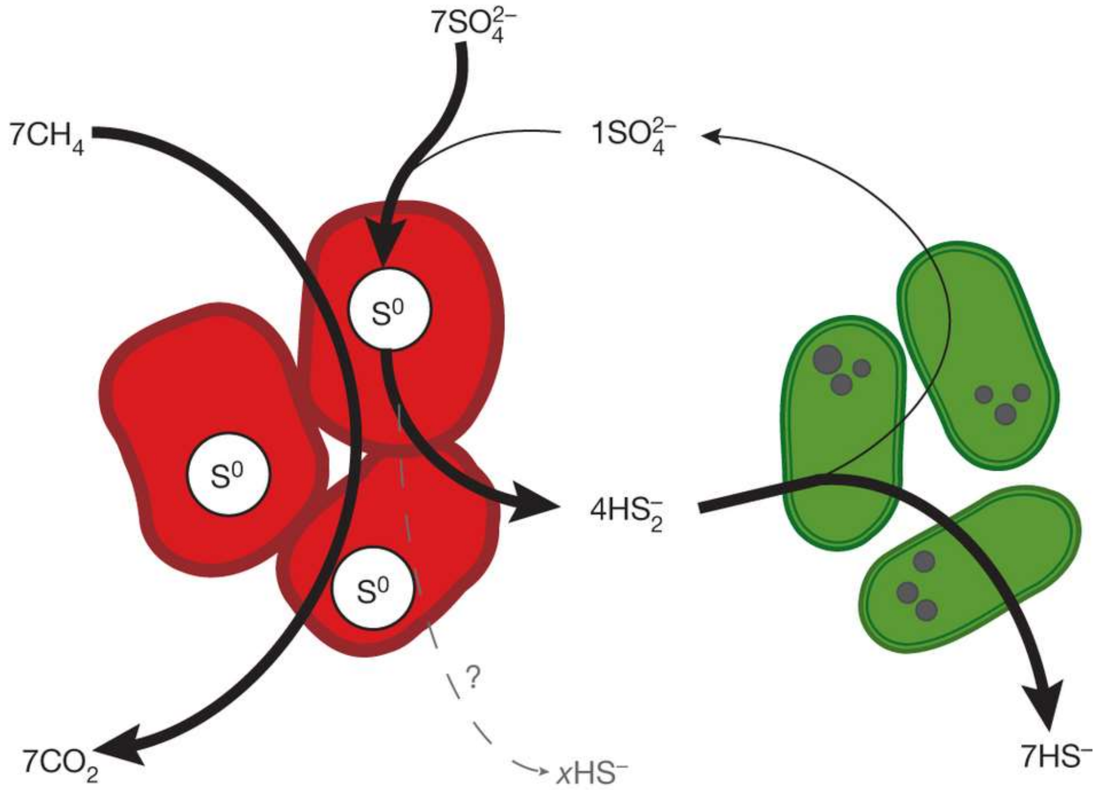
$\Delta G°' = -\ 21$ kJ per mol

at 100 atm CH_4 , 20 mM SO_4^{2-}, 2 mM H_2S:

$\Delta G' = -\ 35$ kJ per mol



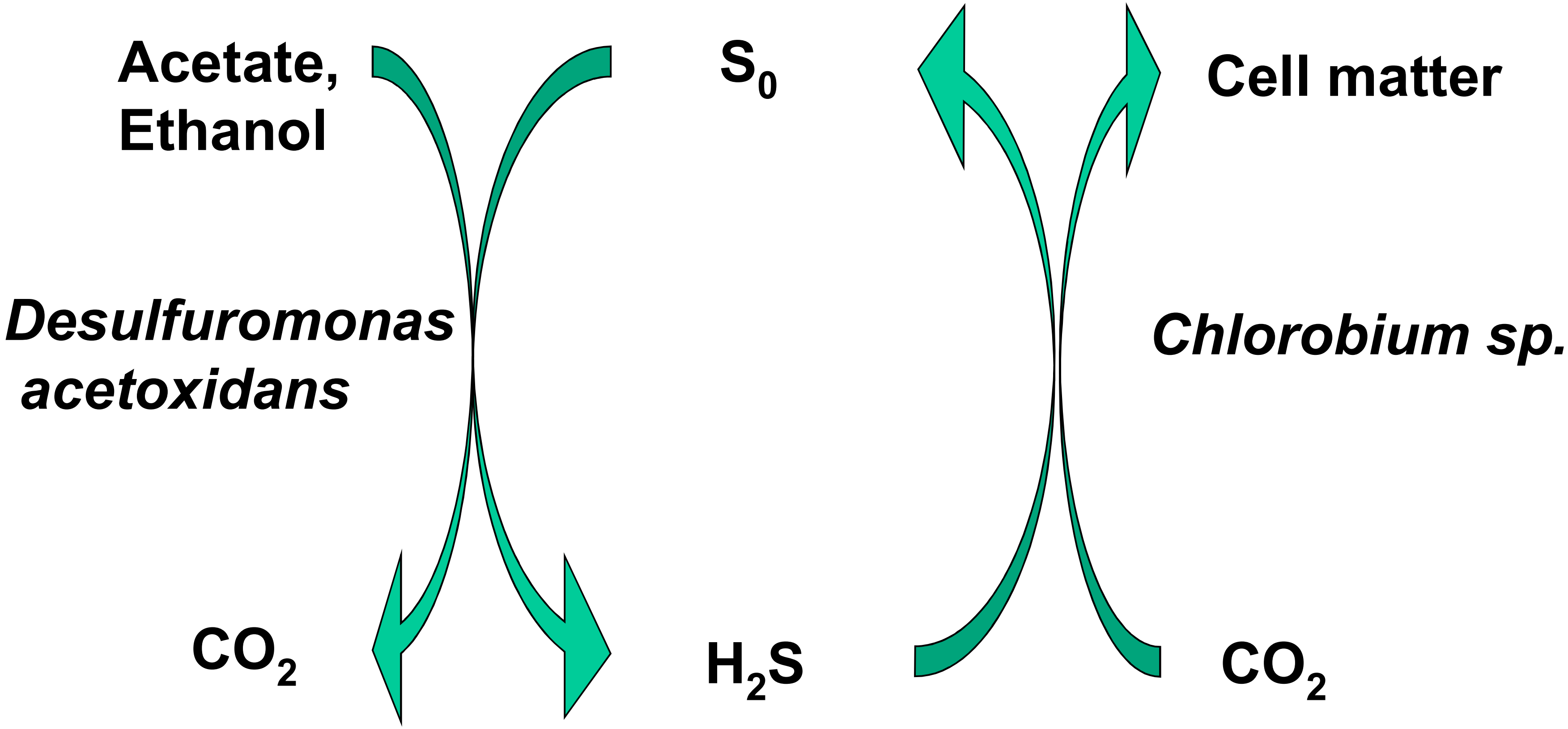
Scheme of electron flow in sulfate-dependent anaerobic methane oxidation



Milucka, Widdel, Kuypers et al., Nature 491, 541-546 (2012)

„*Chloropseudomonas ethylica*“ (Pfennig and Biebl, 1976)

Interspecies electron transfer ("syntrophy") in a coculture of *Desulfuromonas acetoxidans* and *Chlorobium sp.* (Pfennig and Biebl, 1976)



Conclusions

- In anoxic environments, interspecies interactions are much more common than in the oxygen-supplied world.
- Beyond hydrogen or formate, also sulfur compounds (and others?) may shuttle electrons between partner organisms.
- Also degradation of sugars and other complex substrates, e. g. amino acids, may proceed in nature in the presence of partner organisms in a different mode than in pure culture in the lab.
- The energy available to the partners in methanogenic degradation is often at the lowermost limit that can be converted into metabolic energy (ca. 20 kJ per mol).

Universität Konstanz

Biochemistry of Syntrophic Oxidations in Methanogenic Degradation

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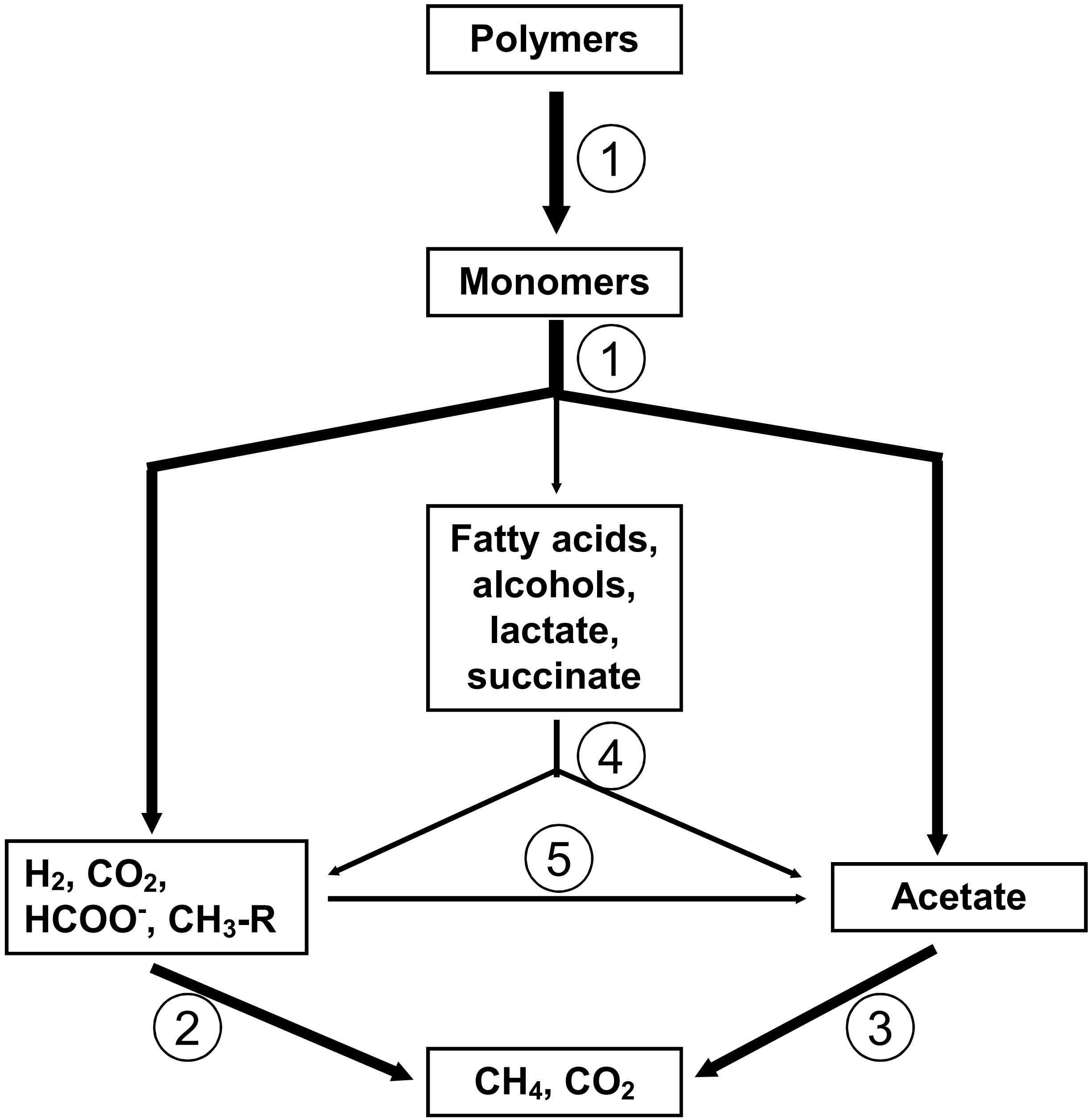
Overview

- Syntrophic oxidation of butyrate
- Syntrophic oxidation of acetate
- Syntrophic oxidation of ethanol
- Conclusions

Electron flow in a methanogenic microbial community

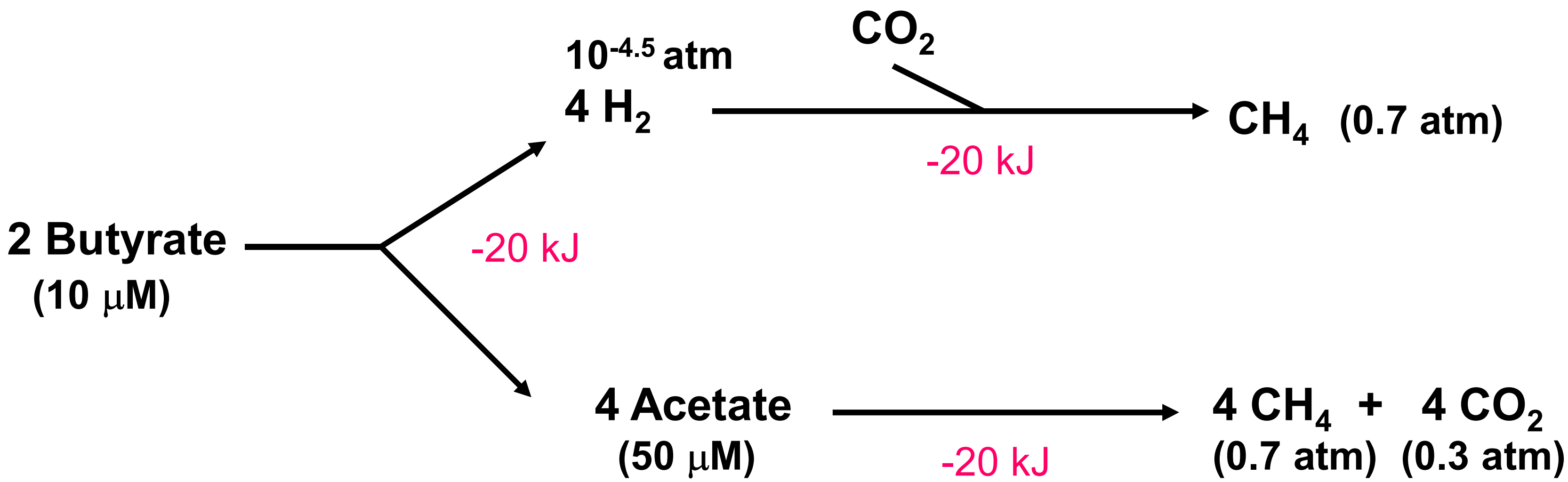


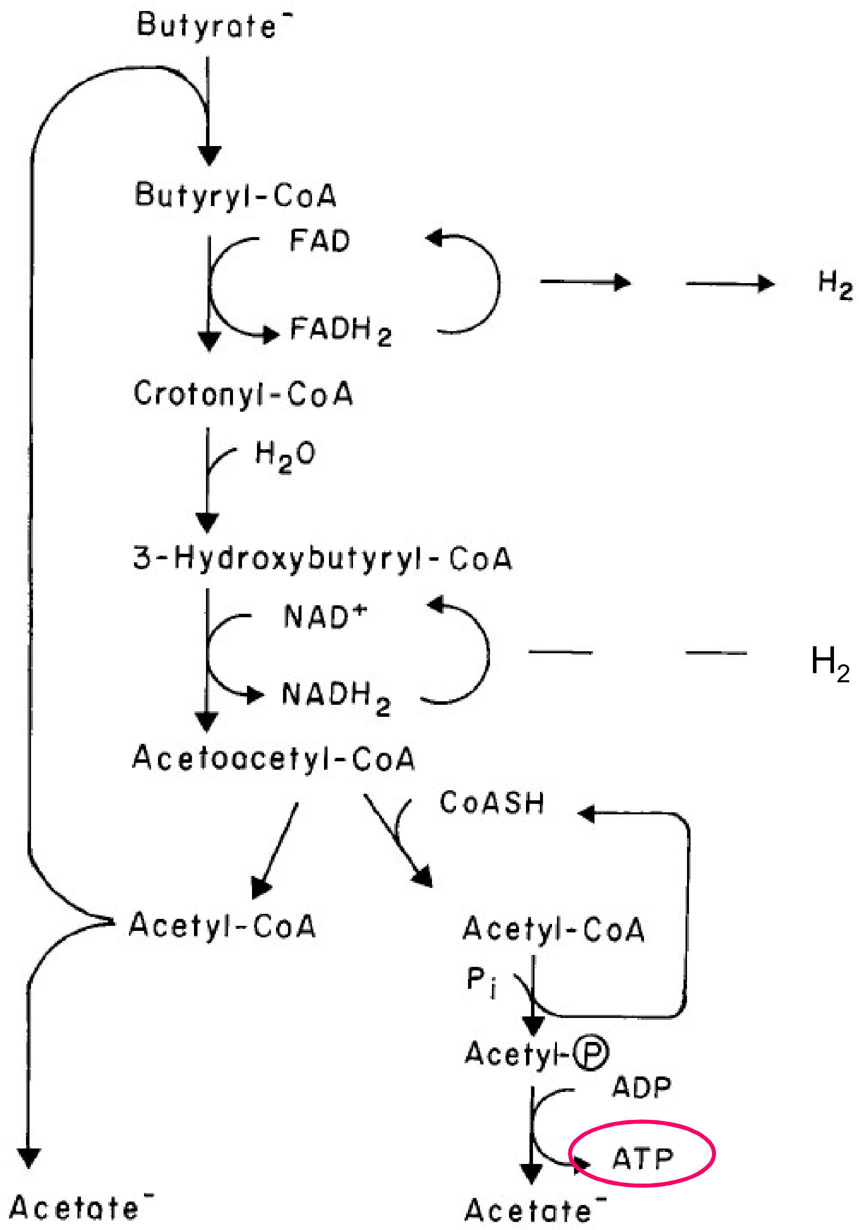
Energy sharing in a ternary syntrophic coculture converting butyrate to CH_4 and CO_2

$$2\ CH_3CH_2CH_2COO^- + H^+ + 2\ H_2O \rightarrow 5\ CH_4 + 3\ CO_2$$

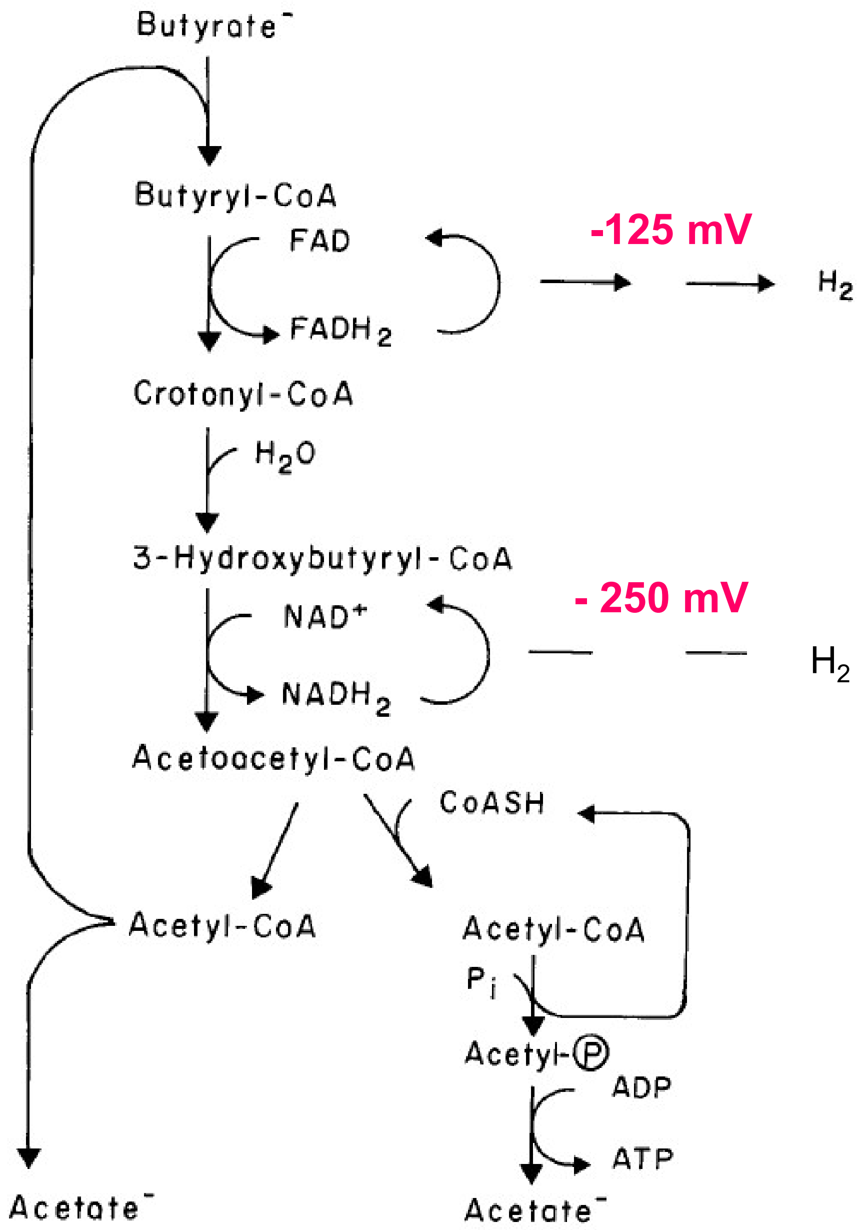
$\Delta G°' = -177$ kJ per mol

with 10 µM butyrate: $\Delta G' = -140$ kJ per mol



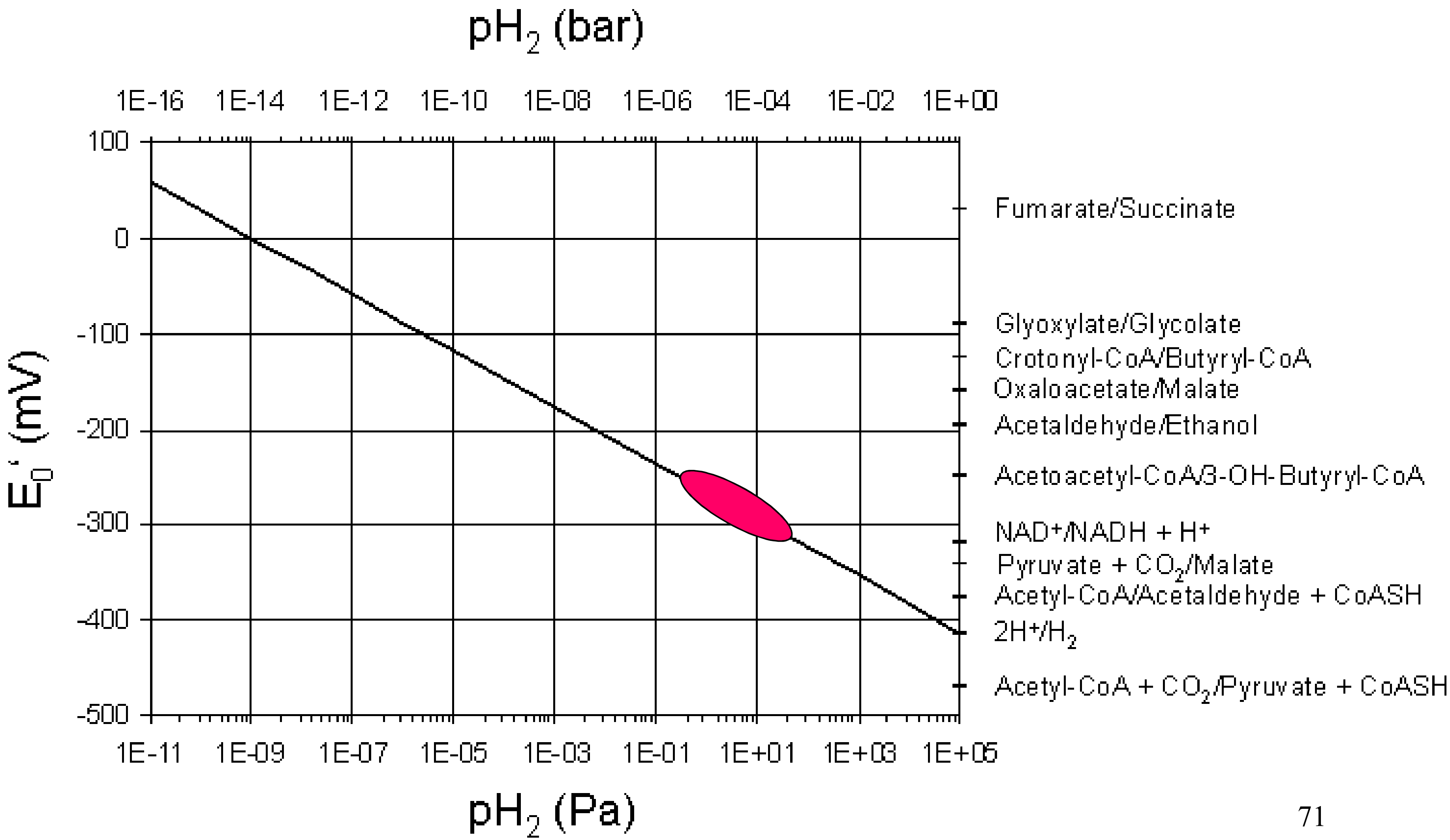


Pathway of syntrophic butyrate oxidation (Wofford et al., 1986)

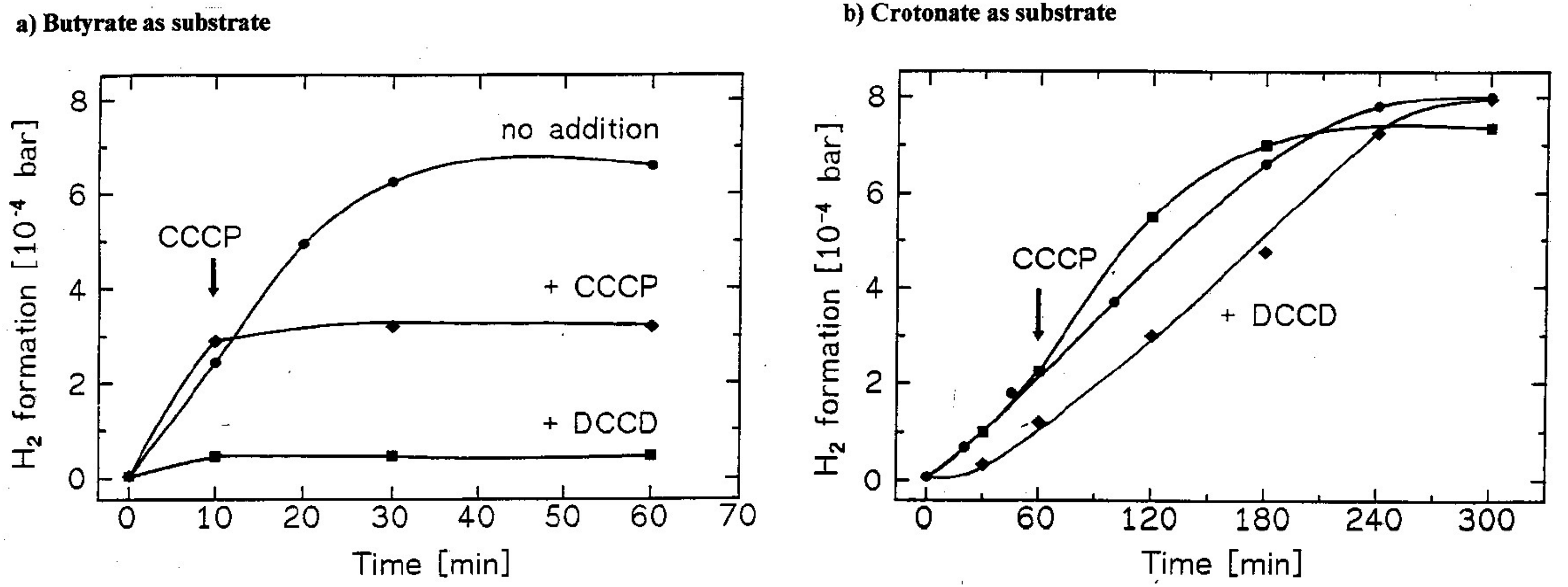


Pathway of syntrophic butyrate oxidation (Wofford et al. 1986)

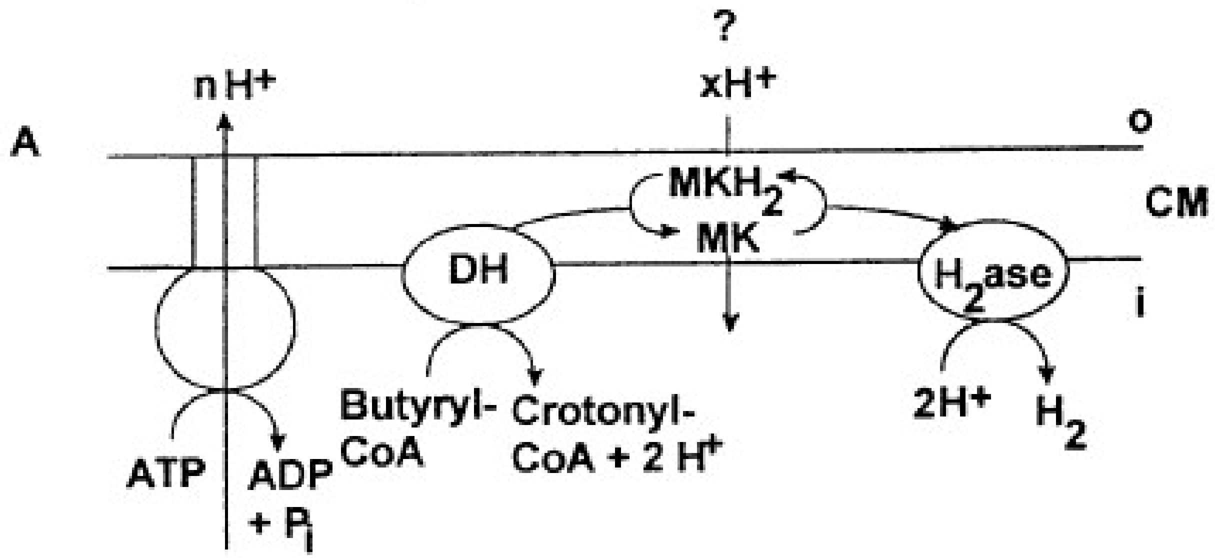
Hydrogen partial pressures and corresponding redox potentials in syntrophic oxidation reactions

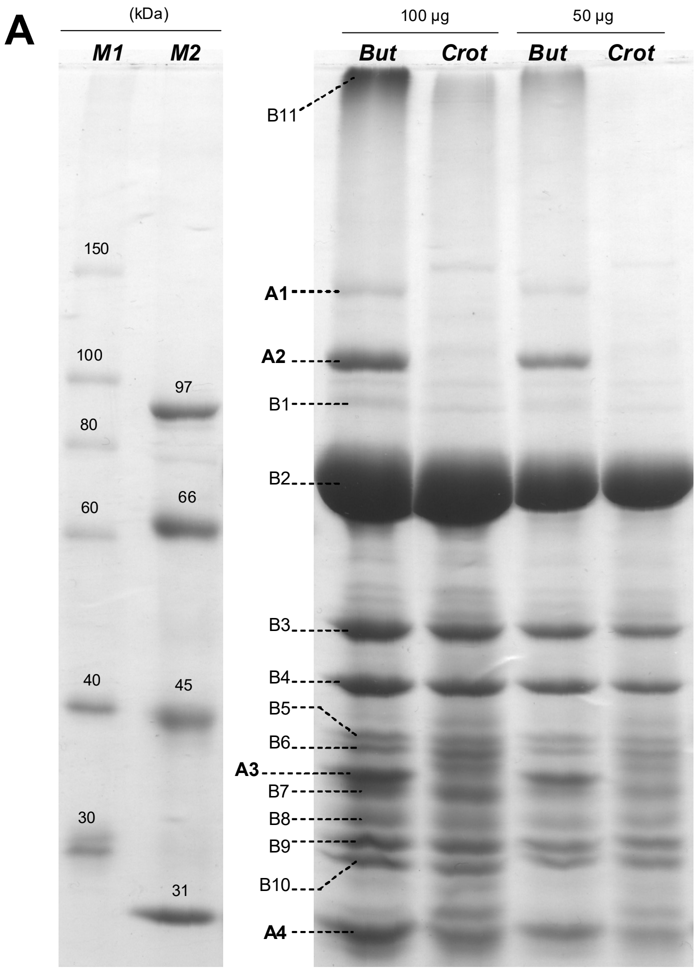


Hydrogen release in a BES-inhibited coculture of *Syntrophomonas wolfei* and *Methanospirillum hungatei*



Hypothetical concept of reversed electron transport in syntrophic butyrate oxidation





Prominent membrane proteins of *Syntrophomonas wolfei* expressed after growth with either butyrate or crotonate

A1 hypothetical outer membrane protein (invasin/intimin/lectin-like)

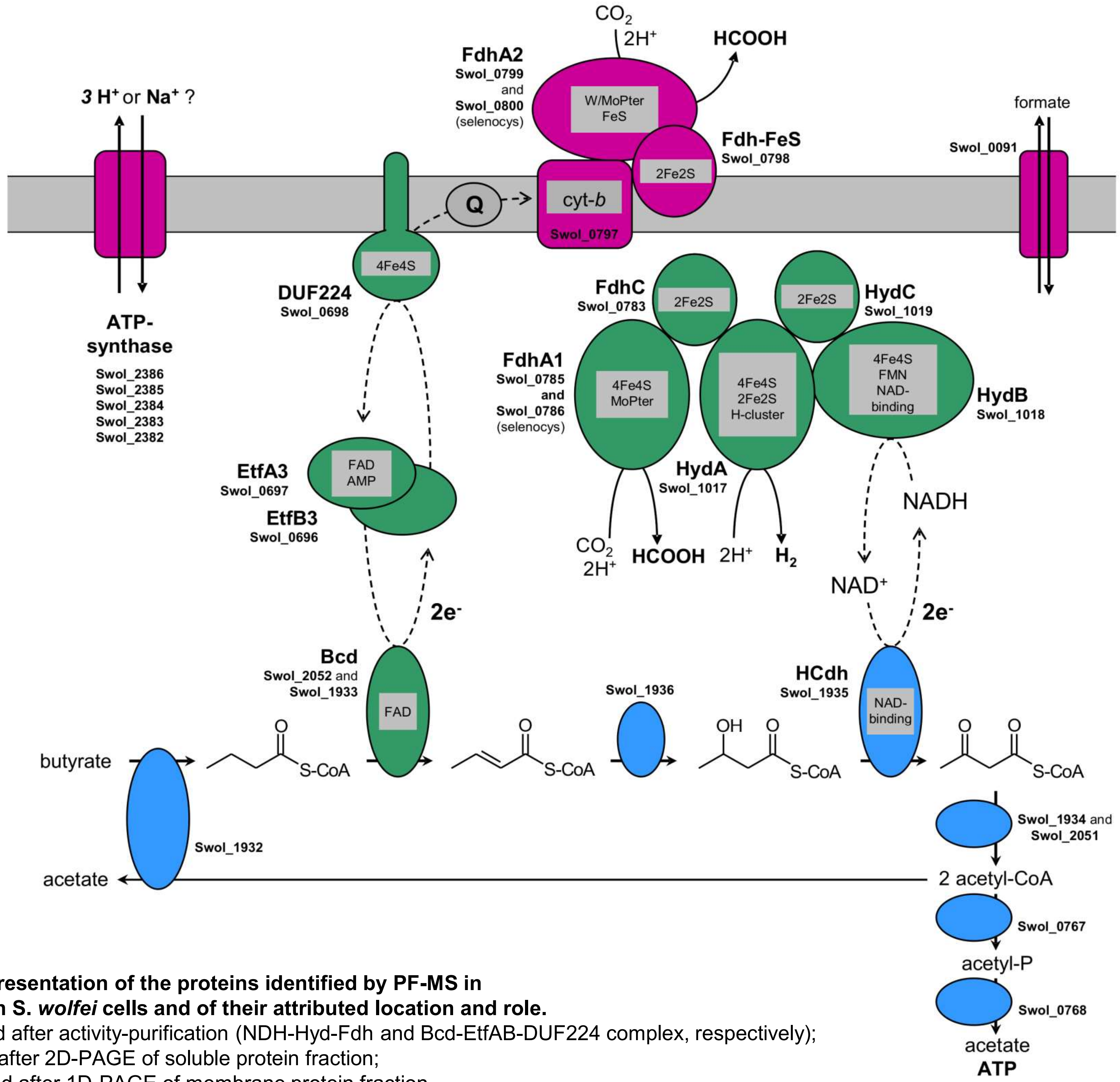
A2 formate dehydrogenase *alpha*-subunit, molybdopterin-binding;
formate dehydrogenase major subunit, selenocysteine-containing

A3 ABC-type transport permease protein (tungstate uptake)

A4 formate dehydrogenase iron-sulfur subunit (cytochrome, quinone?);
ABC-type transport substrate-binding protein (metal uptake)

B3 ATP synthase, sodium translocating, F1 *alpha*-subunit
B4 ATP synthase, sodium translocating, F1 *beta*-subunit
B9 ATP synthase, sodium translocating, F1 *gamma*-subunit
B12 ATP synthase, sodium/proton translocating F0F1-type, F0 subunit *a*
B13 ATP synthase, sodium/proton translocating, F1 *delta*-subunit
B14 ATP synthase, sodium/proton translocating F0F1-type , F0 subunit *b*
B15 ATP synthase, sodium/proton translocating , F1 *epsilon*-subunit

Schmidt, Müller, Schink, Schleheck (2013)



Schematic representation of the proteins identified by PF-MS in butyrate-grown S. *wolfei* cells and of their attributed location and role.
green, identified after activity-purification (NDH-Hyd-Fdh and Bcd-EtfAB-DUF224 complex, respectively);
blue, identified after 2D-PAGE of soluble protein fraction;
purple, identified after 1D-PAGE of membrane protein fraction.

Schmidt, Müller, Schink, Schleheck (2013)

Syntrophic conversion of acetate to $CH_4 + CO_2$

	$\Delta G^{0'}$
$CH_3COO^- + H^+ + 2\ H_2O \rightarrow CO_2 + 4\ H_2$	+ 95 kJ /mol rct.
$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	- 131 kJ /mol rct.
$CH_3COO^- + H^+ + \rightarrow CH_4 + CO_2$	- 35 kJ /mol rct.

at 60°C: $\Delta G'$ = - 42 kJ /mol rct.

"*Reversibacter*" — Zinder and Koch, 1984 ff.

Thermacetogenium phaeum — Hattori et al., 2000

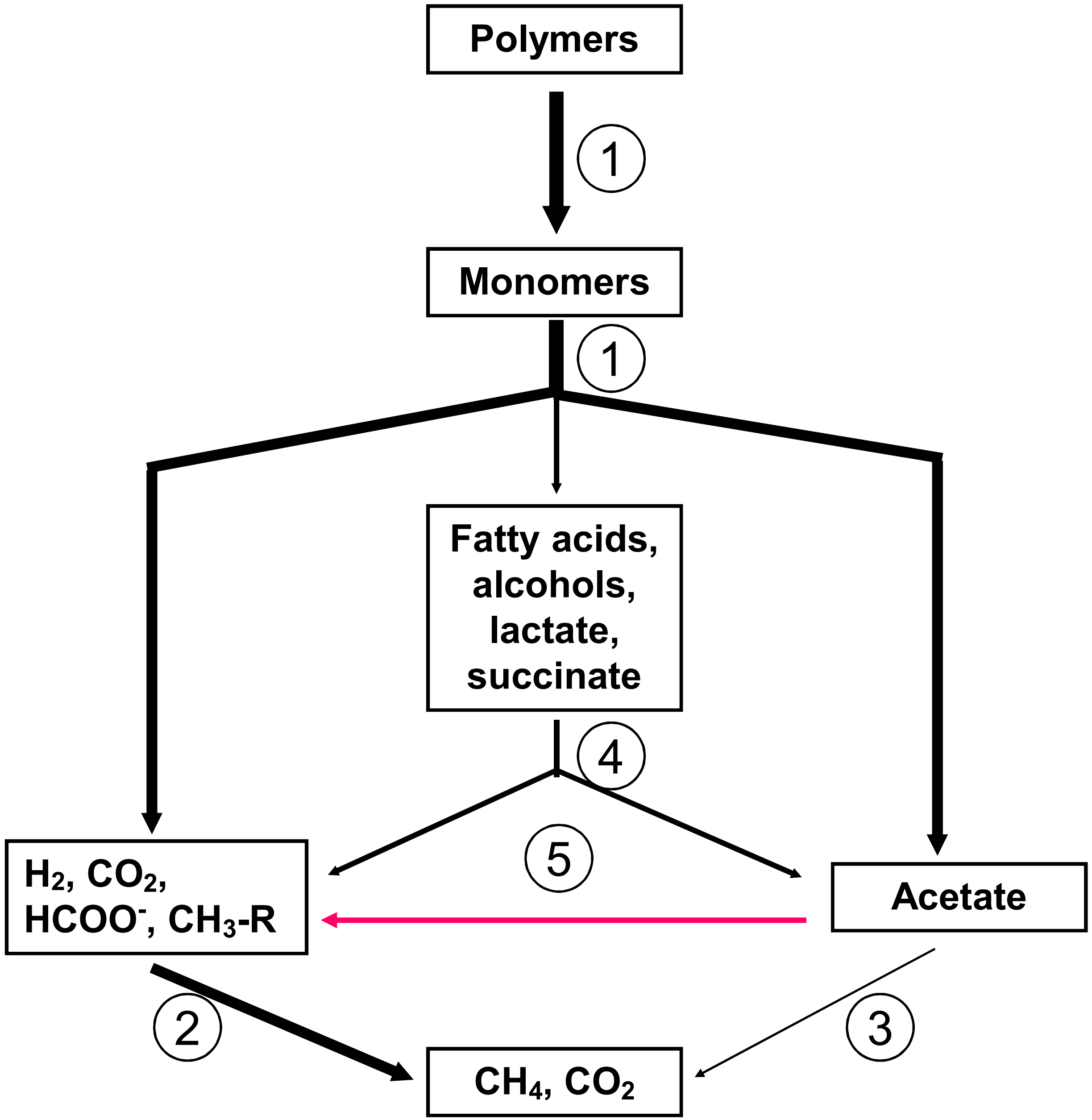
t_d: 8-10 days; $[H_2]$ = 5-10 Pa (10^{-5} - 10^{-4} atm)

at 35°C: $\Delta G'$ = - 38 kJ /mol rct.;

Clostridium ultunense — Schnürer et al., 1996

t_d: 4-6 weeks; $[H_2]$ = 5-10 Pa (10^{-5} - 10^{-4} atm)

Electron flow in a thermal methanogenic microbial community



Energetics of fermentative formation and degradation of acetate

$4\ H_2 + 2\ CO_2 \rightarrow CH_3COO^- + 2\ H_2O$

$\Delta G°' = -95$ kJ per mol

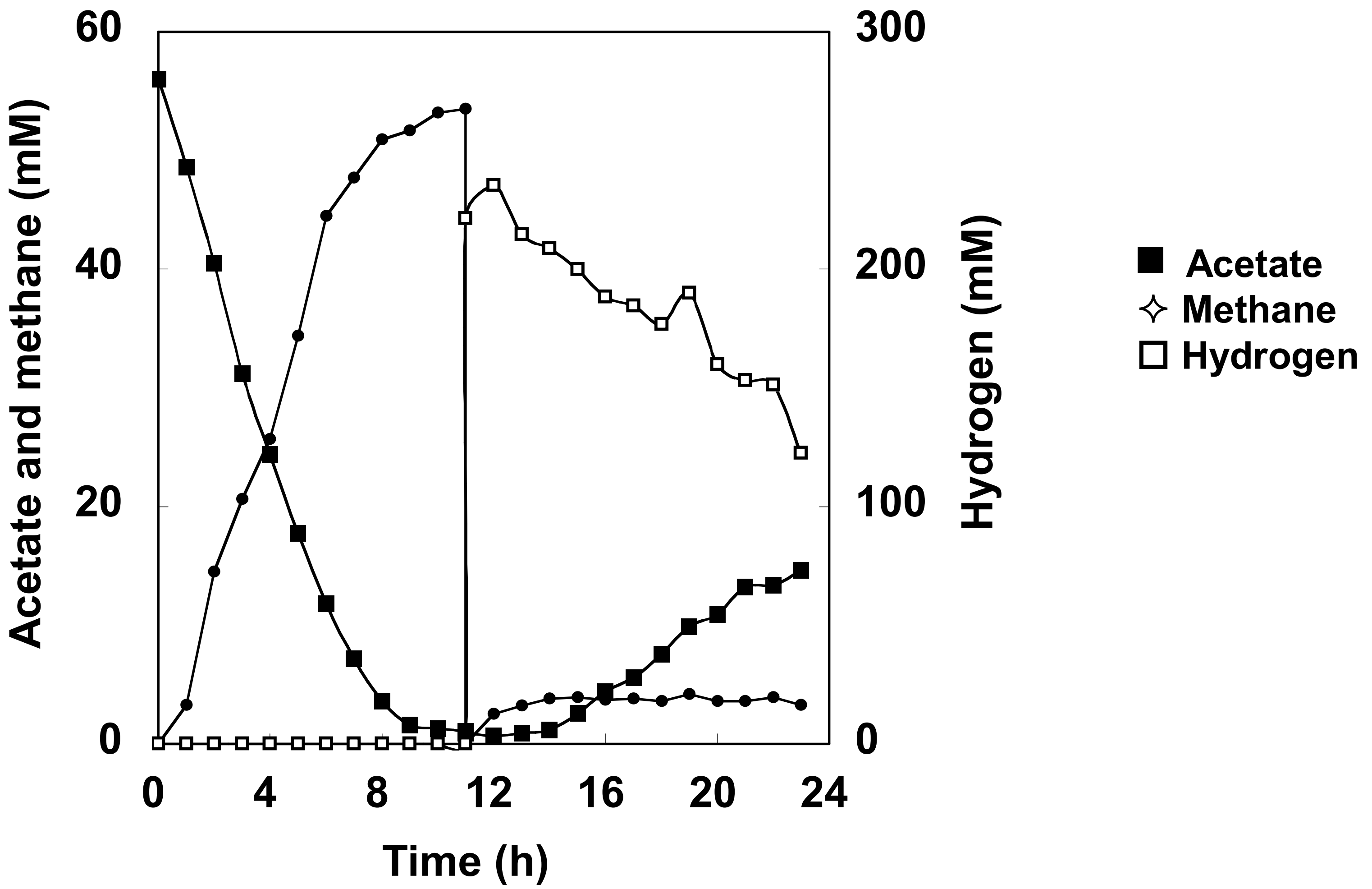
at $[H_2] = 10^{-4}$ atm., [Ac] = 100 μM, T = 25°C

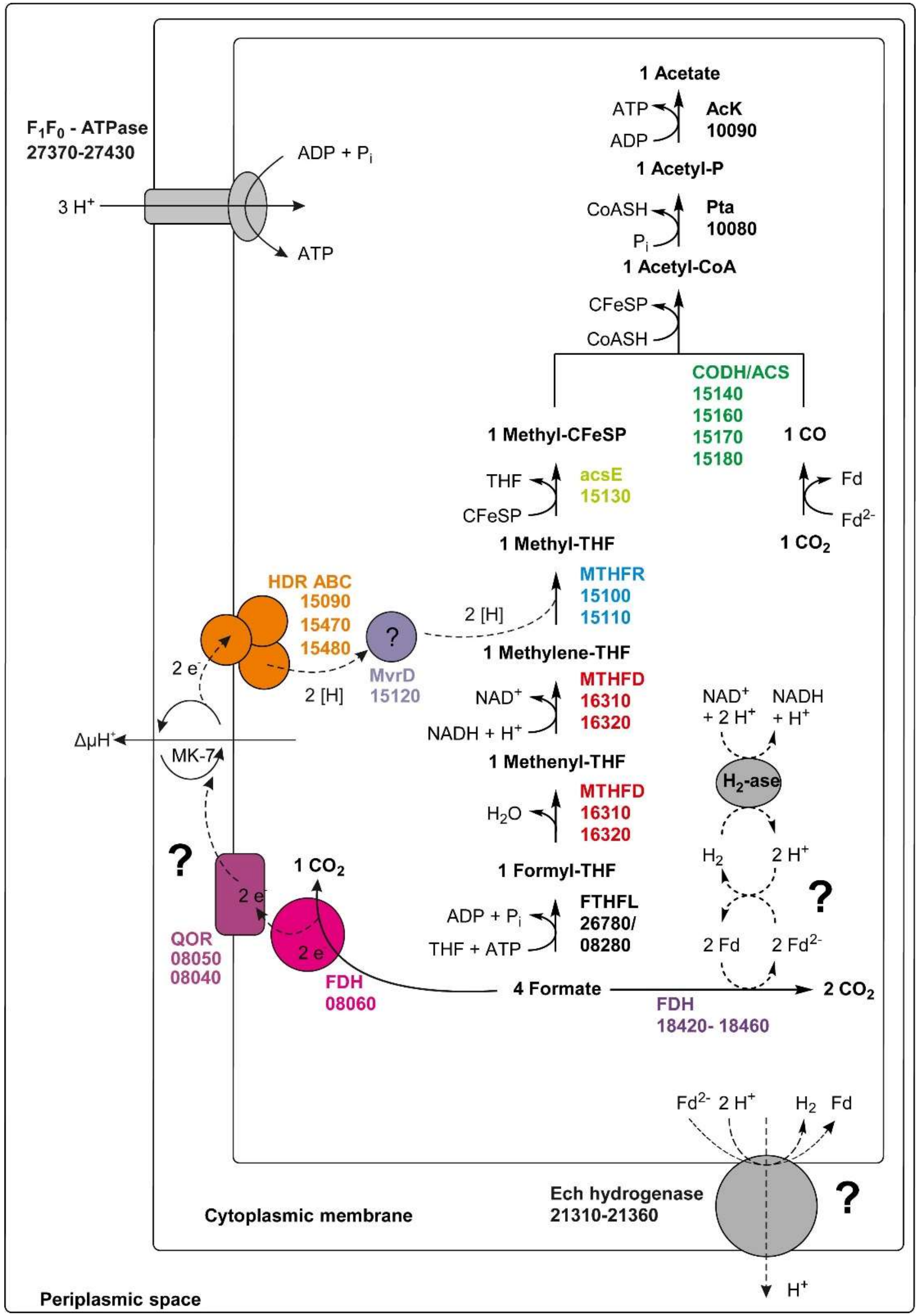
$\Delta G' = -20$ kJ per mol

at $[H_2] = 10^{-5}$ atm., [Ac] = 10 mM, T = 60°C

$\Delta G' = +20$ kJ per mol

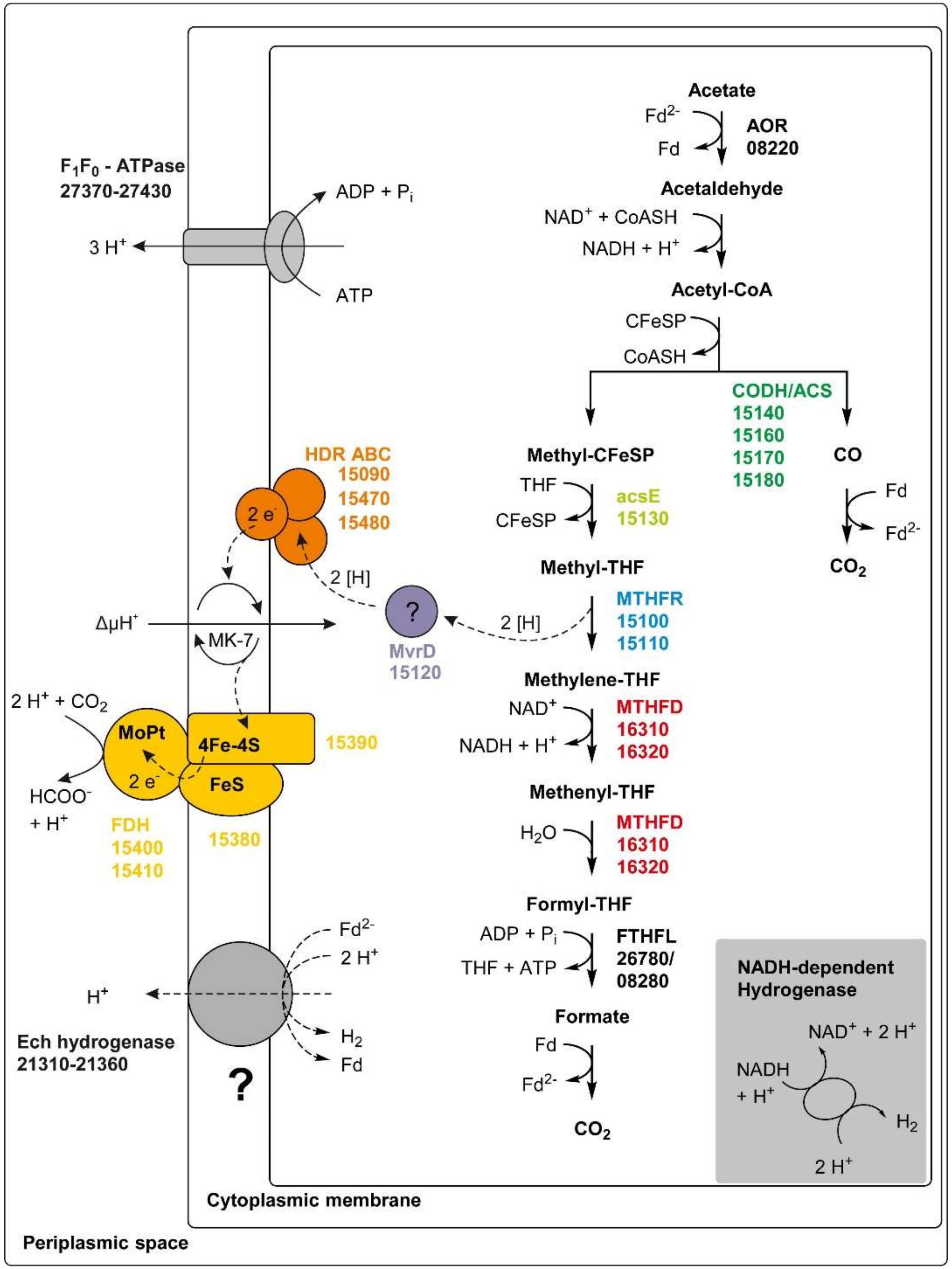
Acetate oxidation and formation by *Thermacetogenium phaeum* at 60°C (plus *Methanothermobacter thermautotrophicus*)





Acetate formation from formate or hydrogen by *Thermacetogenium phaeum*

Keller et al., 2019



Acetate oxidation by *Thermacetogenium phaeum*

Keller et al., 2019

Biomass degradation in technical reactors
BMBF Project BioPara

Table 1 - Technical process parameters and substrate composition; HRT: hydraulic retention time; DM: dry matter; oDM: organic dry matter; Nm³: normal m³ (at 1 atm)

	WWTP	BGR 1		BGR 2		BGR 3	
Sample taken	17.11.2014	12.01.2015		21.01.2015		04.02.2015	
Temperature (°C)	39	40		40		47	
Reactor size [m^3]	4500	2800		2500		1650	
HRT [h]	690	81		86.2		55	
Volume load [kg DM/m^3·d]	1.73	4.27		4.11		7.57	
Volume load [kg oDM/m^3·d]	1.44	3.93		3.82		6.47	
biogas formation rate [Nm^3/d]	3560	6373		6024		7680	
Methane formation rate [mmol/kg·h]	0.831	1.92		2.03		3.92	
Substrates [t dry matter per day]	sewage sludge						
Maize silage		8.24	68.9%	6.08	59.1%	4.80	38.4%
Green rye		0	0%	0.96	9.3%	2.24	17.9%
Grain of wheat		0	0%	0	0%	3.48	27.9%
Cow manure		2.36	19.8%	0.26	2.6%	0	0%
Cattle slurry		0	0%	0.12	1.2%	0.88	7.0%
Horse manure		0	0%	0.27	2.6%	1.09	8.7%
Dried chicken feces		1.35	11.3%	2.59	25.2%	0	0%
NH_4 - nitrogen	1.1	3.1		4.5		1.5	

Montag, D., B. Schink: Appl. Microbiol. Biotechnol. 100, 1019-102 (2016)

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Pool sizes of fatty acids and other parameters measured in samples from a wastewater treatment plant (WWTP) and three biogas reactors (BGR1-3).

Compound	WWTP	BGR1	BGR2	BGR3
Formate [$mmol \cdot l^{-1}$]	0.015 ±0.000	0.001 ±0.001	0.011 ±0.002	0.001 ±0.000
Acetate [$mmol \cdot l^{-1}$]	0.010 ±0.001	0.180 ±0.012	10.103 ±0.703	0.948 ±0.057
Propionate [$mmol \cdot l^{-1}$]	0.078 ±0.005	0.016 ±0.005	1.315 ±0.156	0.097 ±0.015
Butyrate [$mmol \cdot l^{-1}$]	< 0.001	< 0.001	0.597 ±0.042	0.034 ±0.002
pH	7.4	8.2	8.3	8.1
Hydrogen [ppm]	44.7 ±0.5	18.2 ±0.7	29.1 ±0.2	10.9 ±0.7
Carbon monoxide [ppm]	18.0 ±0.2	17.7 ±0.5	10.2 ±0.1	1.8 ±0.3
Methane [bar]	0.67 ±0.02	0.55 ±0.01	0.50 ±0.02	0.50 ±0.01

Gibbs' free energy changes of conversion reactions in equilibrated digested sludge systems (waste water treatment plant and biogas reactors); the complete conversion reactions are given in Table 1. *) calculation based on estimated butyrate concentrations below the detection limit: WWTP: 10 nM and BGR1: 100 nM

Step	Eq. #	WWTP [$kJ \cdot mol^{-1}$]	BGR1 [$kJ \cdot mol^{-1}$]	BGR2 [$kJ \cdot mol^{-1}$]	BGR3 [$kJ \cdot mol^{-1}$]
Butyrate oxidation	(1)	-21.4*	-20.0*	-20.0	-28.6
Propionate oxidation	(2)	-19.3	-13.8	-10.9	-17.9
Acetate oxidation	(3)	+8.8	-3.5	-7.7	-13.2
H_2 to CH_4	(4)	-9.6	-10.4	-15.8	-5.6
Acetate to CH_4	(5)	-18.4	-13.9	-23.5	-18.8
Formate to $H_2 + CO_2$	(6)	+2.6	-5.9	-2.0	-4.3

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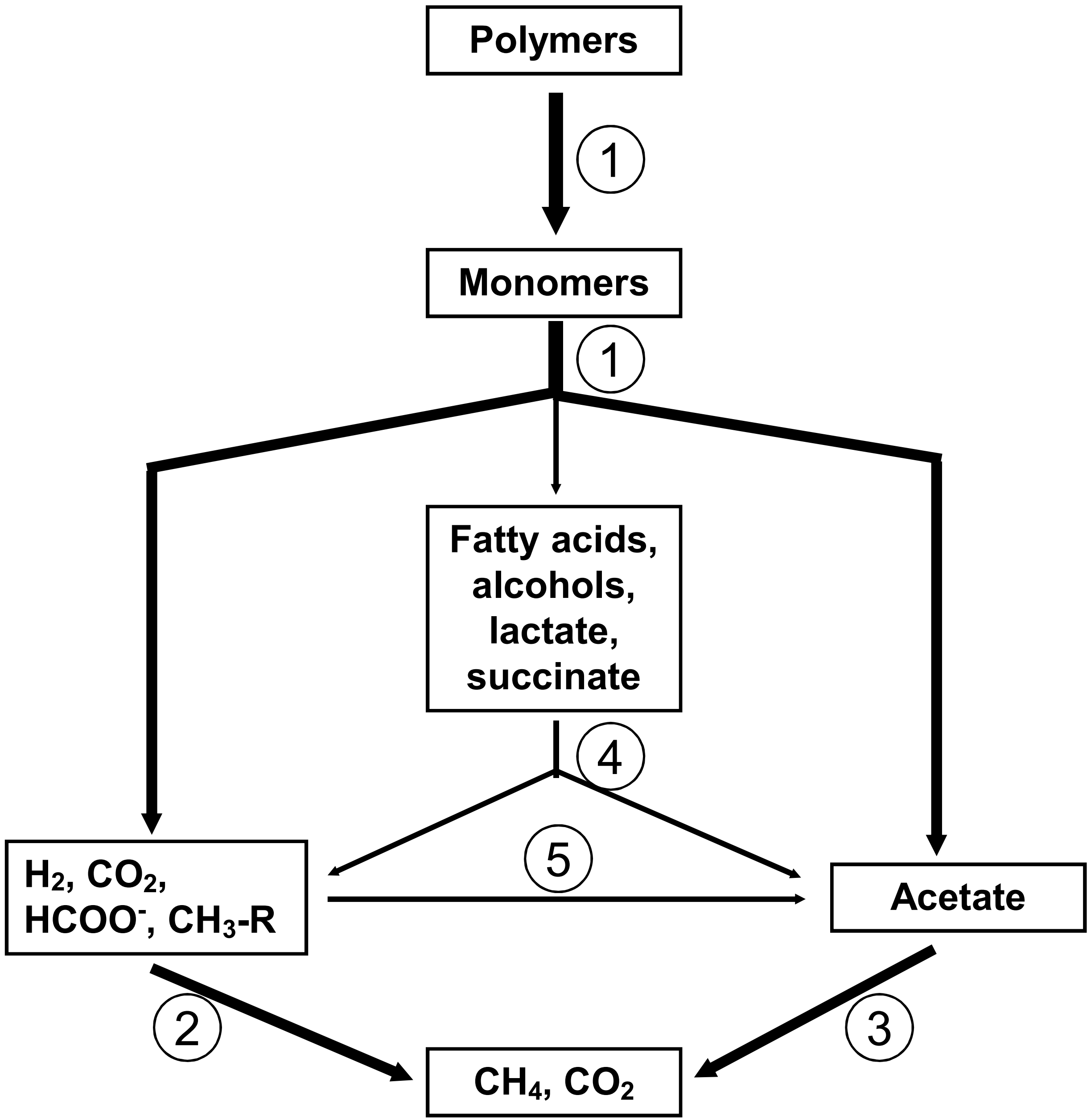
Electron flow in a methanogenic microbial community



Conclusions

- **The energy available to the partners in methanogenic degradation is often at the lowermost limit that can be converted into metabolic energy (~20 kJ per mol).**
- **In all cases of syntrophic oxidations studied so far, ATP is formed via substrate-level phosphorylation**
- **In most cases, evidence of energy-dependent reversed electron transport or of other energy-consuming membrane-bound reactions has been obtained.**
- **With syntrophic butyrate oxidation and syntrophic acetate oxidation by *Thermacetogenium phaeum*, menaquinone-linked reversed electron transport systems have been identified.**
- **In syntrophic ethanol oxidation by *Pelobacter spp*., the present working concept includes a Na^+-driven Rnf complex and a confurcating hydrogenase or formate dehydrogenase.**

A great „Thank You“ to

- Michael Friedrich
- Christina Wallrabenstein
- Jan Kaden
- Ben Griffin
- Nicolai Müller
- David Schleheck
- Dirk Oehler
- Alexander Schmidt
- Alfons Stams, Caroline Plugge, Wageningen, NL
- Mike McInerney, Oklahoma State University, OK, USA

...and to you for your attention!

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