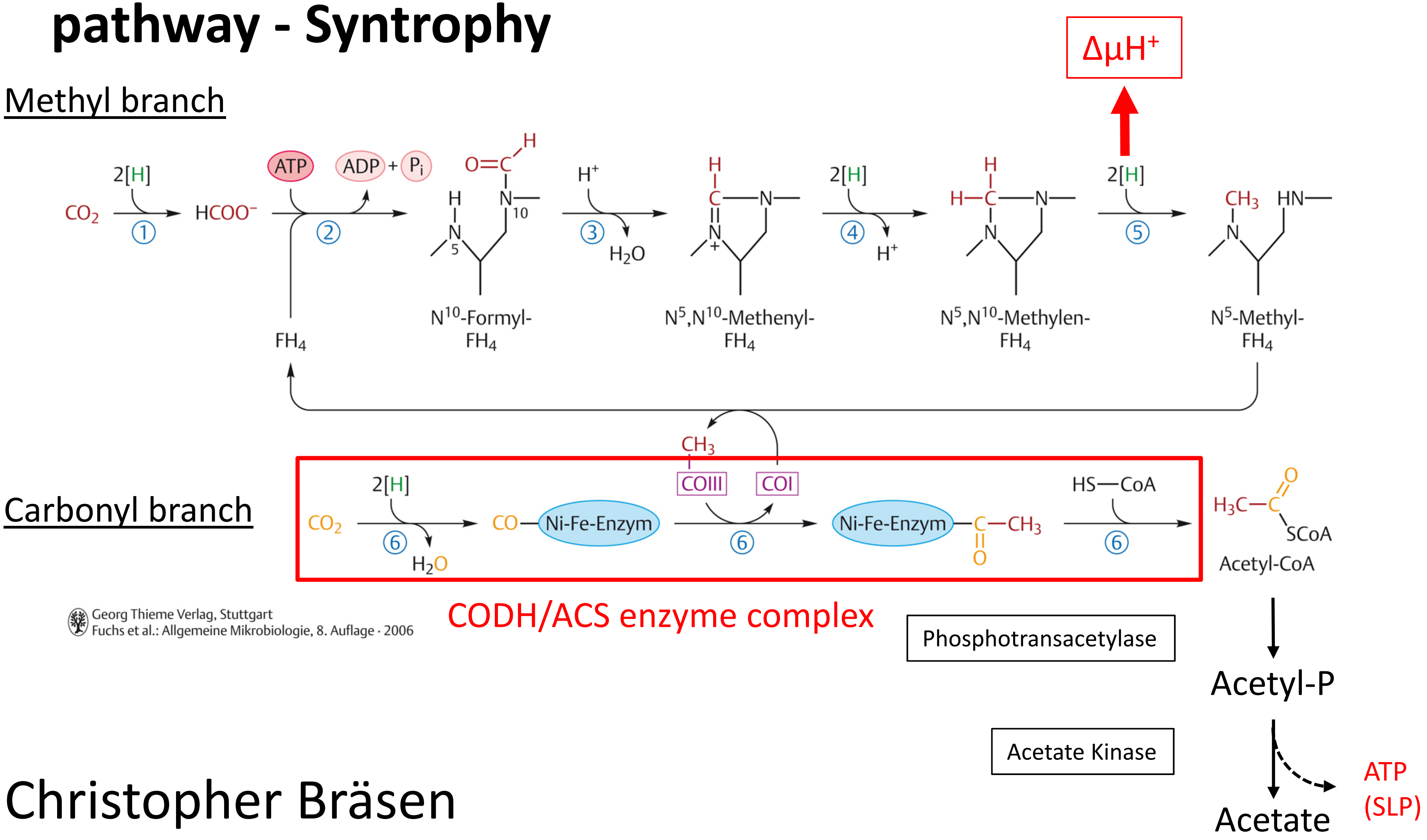
Microbiology II

Fermentations – Acetogenesis and the CODH/ACS pathway - Syntrophy



Methyl branch

Carbonyl branch

CODH/ACS enzyme complex

Phosphotransacetylase

Acetyl-P

Acetate Kinase

Acetate

ATP (SLP)

Georg Thieme Verlag, Stuttgart
Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage · 2006

Christopher Bräsen

Lecture Plan

09.10.2018	**Keine Vorlesung**	
16.10. 2018	Mikrobielle Physiologie I - Energetik	Bräsen
23.10. 2018	Mikrobielle Physiologie II – Einige Prinzipien und Mechanismen im zentralen Kohlenstoffmetabolismus	Bräsen
30.10. 2018	**Keine Vorlesung**	
06.11. 2018	Mikrobielle Physiologie III – Nitrat-Atmung	Bräsen
13.11. 2018	Mikrobielle Physiologie IV – Acetogenese und der Acetyl-CoA/Kohlenmonoxid Dehydrogenase-Weg	Bräsen
20.11. 2018	Mikrobielle Physiologie V – Anaerobe Nahrungskette und Methanogenese	Bräsen
27.11. 2018	Mikrobielle Physiologie VI – Sulfate Reduktion	Bräsen
04.12. 2018	Antibiotika (Penicillium notatum)	Meckenstock
11.12. 2018	Mikroorganismen in der Umwelt (Geobacter metallireducens)	Meckenstock
18.12. 2018	Mikrobielles Wachstum (Elusimicrobium minutum)	Meckenstock
08.01. 2019	Mikrobielle Fortbewegung (Thioploca)	Meckenstock
15.01. 2019	Viren (T4)	Meckenstock
22.01. 2019	Geschichte der Mikrobiologie	Meckenstock/Bräsen
29.01.2019	**Wrap up/Ausweichtermin**	Meckenstock/Bräsen

Questions 3

- What means thermodynamic hierarchy of electron acceptors?
- What are the two main mechanisms of nitrate respiration?
- What is denitrification, what are the intermediates? Examples?
- What is the difference to dissimilatory nitrate reduction to ammonia? Examples for the latter?
- Energy of both mechanisms? Compare to O_2.
- What are the differences to the nirate assimilation?

Fragen 3

- Was bedeutet „thermodynamische Hierarchie“ der Elektronenakzeptoren?
- Was sind die beiden Hauptmechanismen der Nitratatmung?
- Was ist Denitrifikation, Was sind die Intermediate? Beispielorganismen?
- Was ist der Unterschied zur dissimilatorischen Nitratreduktion zu Ammonium? Beispielorganismen für letztere?
- Was ist in etwa die Energiebilanz beider Mechanismen? Vergleichen Sie mit O_2.
- Was ist bei der Nirat assimilation anders?

Questions 3

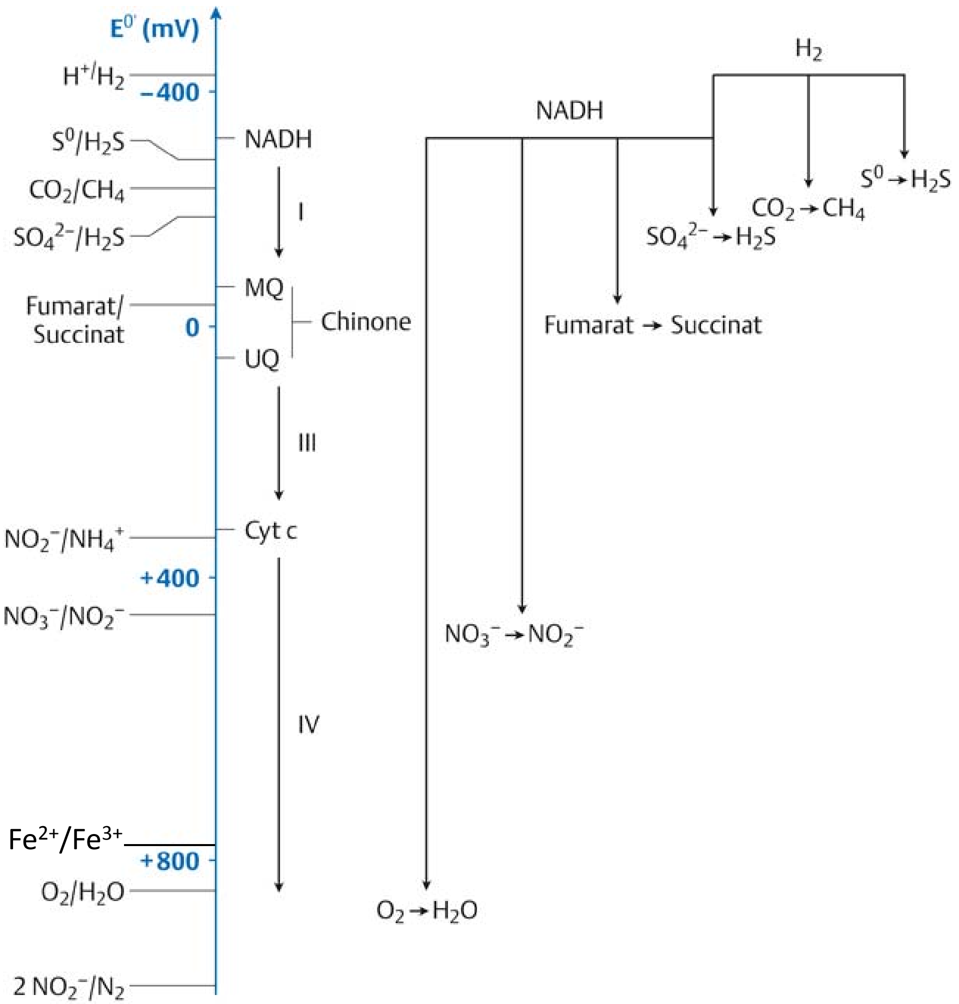
What means thermodynamic hierarchy of electron acceptors?

Was bedeutet „Thermodynamische Hierarchie“ der Elektronen-Akzeptoren?

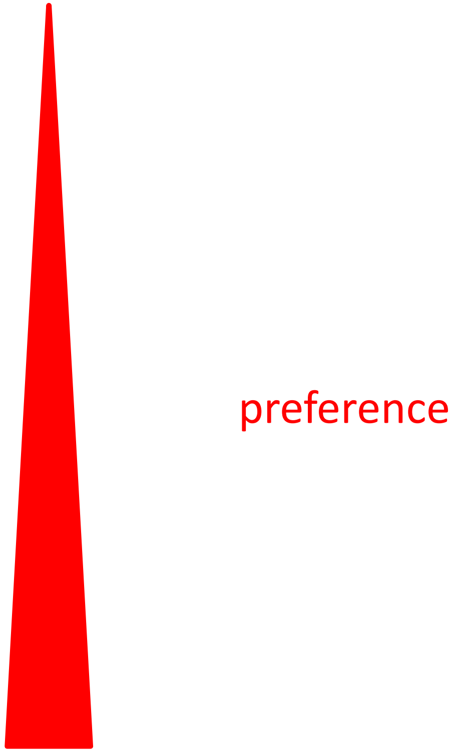
a. H_2 is always the acceptor
b. Preferred utilization with increasing redox potential.
c. Preferred utilization with decreasing redox potential.
d. There are no alternatives to O_2

Respiration without O_2
Redox potentials – thermodynamic hierarchy

$$\Delta G^{0'} = - n\ F\ \Delta E^{0'}$$
$$\Delta E^{0'} = (E^{0'}_{[Akzeptor]} - E^{0'}_{[Donor]})$$



Georg Thieme Verlag, Stuttgart
Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage · 2006



Questions 3

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Questions 3

What are the main mechanisms of nitrate respiration?

Was sind die Hauptmechanismen der Nitratatmung.

a. Nitrification
b. Dissimilatory Nitrate reduction to ammonia
c. Nitrogen fixation
d. Denitrification

Questions 3

What are the main mechanisms of nitrate respiration?

Was sind die Hauptmechanismen der Nitratatmung.

a. Nitrification

b. Dissimilatory Nitrate reduction to ammonia = Nitrate ammonification

c. Nitrogen fixation

d. Denitrification

Questions 3

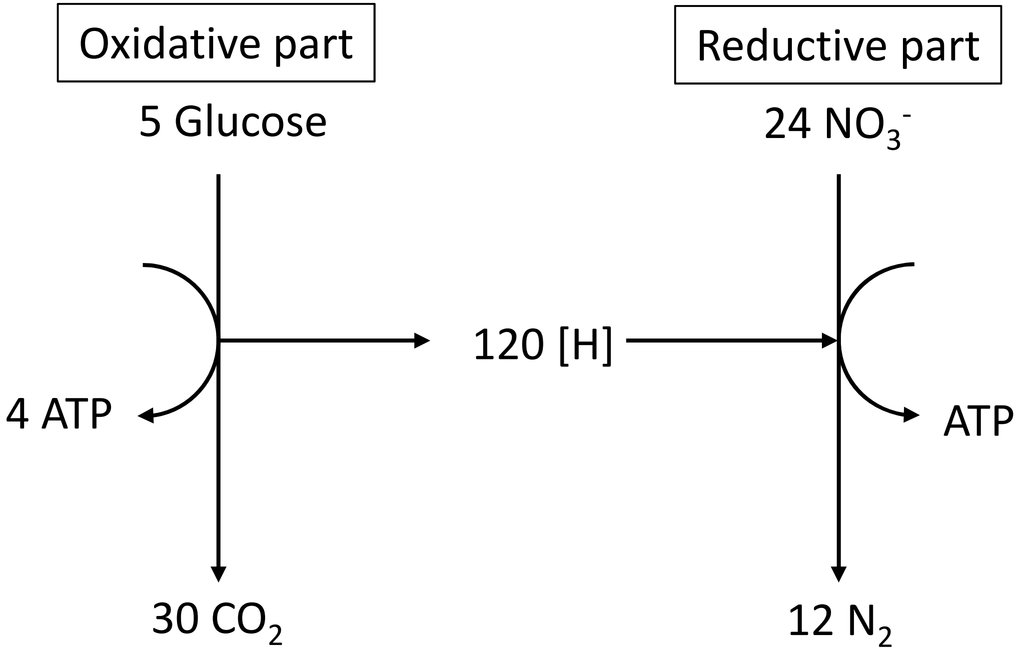
What is the energy gain of both nitrate respiring mechanisms (compared to O_2)?.

Wie hoch ist die Energieausbeute im Vergleich zu O2?

a. Higher (~42 ATP)
b. Lower (~21-26 ATP)
c. Same (~38 ATP)
d. Lower (~34 ATP)

Denitrification

e.g. *Paracoccus denitrificans*



Oxidative part

5 Glucose

4 ATP

$30\ CO_2$

120 [H]

Reductive part

$24\ NO_3^-$

ATP

$12\ N_2$

ATP synthesis via Substrate level phosphorylation (SSP)

ATP synthesis via Electron transport phosphorylation (ETP)

$E^{0'}(6\ CO_2/\text{glucose}) = -0{,}43\ V$

$E^{0'}(NO_3^-/N_2) = +0{,}75\ V$

$\Delta G^{0'} = -\ n\ F\ \Delta E^{0'}$

$\Delta E^{0'} = (E^{0'}_{[Akzeptor]} - E^{0'}_{[Donor]})$

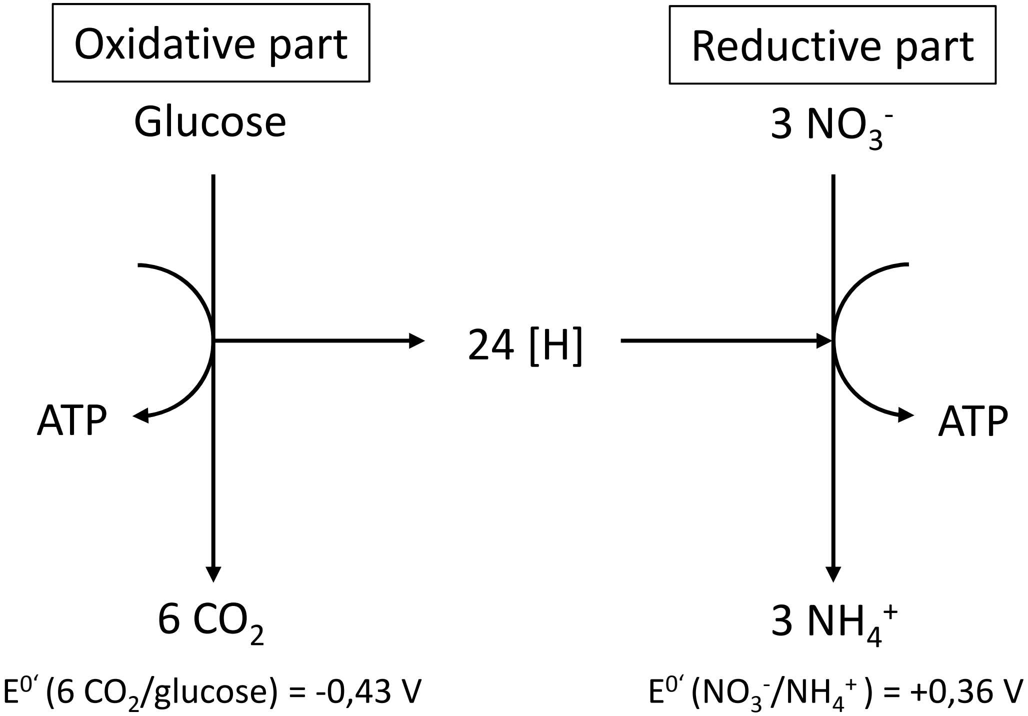
$$\Delta G^{0'} = -\ 24 \times 96.5\ \text{kJ/mol V} \times (+\ 0.43 - (-0.43)) = \underline{\underline{-\ 2732\ \text{kJ/mol}}}$$

2732/75 → <u>36 ATP (per mol glucose) would be possible -- BUT: only **<~26 ATP** are gained</u>

Dissimilatory NO_3^- reduction to ammonia

Heterotrophic metabolism $-O_2$, anaerobic respiration, Nitrate as alternative electron acceptor

e.g. E. coli, some other Enterobacteriaceae,
Some gram +, e.g. Staphylococcus aureus



Oxidative part

Glucose

24 [H]

ATP

6 CO_2

$E^{0'}$ (6 CO_2/glucose) = -0,43 V

Reductive part

3 NO_3^-

ATP

3 NH_4^+

$E^{0'}$ (NO_3^-/NH_4^+) = +0,36 V

ATP synthesis via
Substrate level
phosphorylation
(SSP)

ATP synthesis via
Electron transport
phosphorylation
(ETP)

$$\Delta G^{0'} = - n\,F\,\Delta E^{0'}$$
$$\Delta E^{0'} = (E^{0'}_{[Akzeptor]} - E^{0'}_{[Donor]})$$

$$\Delta G^{0'} = -24 \times 96.5\ \text{kJ/mol V} \times (+0.36\ \text{V} - (-0.43\ \text{V})) = \underline{\underline{-1829\ \text{kJ/mol}}}$$

1829/80 → **22 ATP**

Questions 3

What is the energy gain of both nitrate respiring mechanisms (compared to O_2)?.

Wie hoch ist die Energieausbeute im Vergleich zu O2?

a. Higher (~42 ATP)

b. Lower (~21-26 ATP)

c. Same (~38 ATP)

d. Lower (~33 ATP)

Questions 3

What is different between denitrification and dissimilatory nitrate reduction to ammonia?

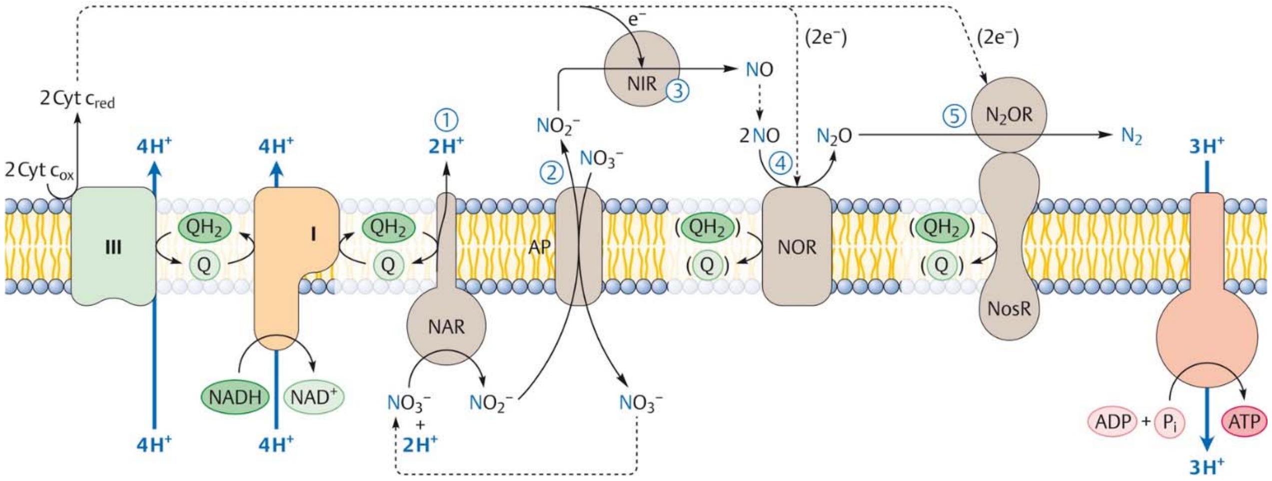
Was sind Unterschiede zwischen Dentrifikation und der dissimilatorischen Nitratreduktion zu Ammonium.

a. NO as intermediate
b. Nitrate reduction to nitrite
c. The endproduct
d. Carried out by *E. coli*

Denitrification

Widespread in bacteria, especially proteobacteria, some gram+
e.g. *Paracoccus denitrificans*,
Pseudomonas stutzeri
Bacillus spec.

- Reduction of NO_3^- to molecular nitrogen N_2
- Most important route of N_2 generation in nature



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Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage · 2006

$NO_3^- + 2\,e^- + 2H^+ \rightarrow NO_2^- + H_2O$ +0,43 V nitrate reductase → $\Delta\mu H^+$
$NO_2^- + e^- + 2H^+ \rightarrow NO + H_2O$ +0,35 V nitrite reductase
$2\,NO + 2\,e^- + 2H^+ \rightarrow N_2O + H_2O$ +1,18 V (NO) reductase
$N_2O + 2\,e^- + 2H^+ \rightarrow N_2 + H_2O$ +1,36 V N_2O reductase
(nitrite, NO and N_2O reductase) → $\Delta\mu H^+$

$$\boxed{NO_3^- + 5\,e^- + 6\,H^+ \rightarrow \tfrac{1}{2}\,N_2 + 3\,H_2O}$$

$E^{0\prime}$ + 0.75 V
$\Delta G^{0\prime} = -\,24 \times 96.5\ \text{kJ/mol V} \times (+0.75 - (-0.43)) = -2732\ \text{kJ/mol}$

Dissimilatory NO_3^- reduction to ammonia

Reductive part: $NO_3^- + 2\,e^- + 2H^+ \rightarrow NO_2^- + H_2O$ ($E^{0\prime}$+430 mV)

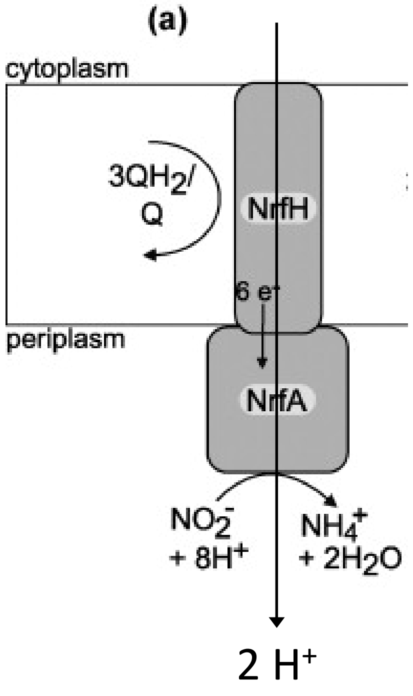
→ dissimilatory **Nitrate reductase** – molybdenum containing membrane protein, repressed by O_2, membrane bound, generates proton gradient, ATP via ETP

in nitrate ammonification nitrite is further oxidized to ammonia

Reductive part: $NO_2^- + 6\,e^- + 8\,H^+ \rightarrow NH_4^+ + 2\,H_2O$ ($E^{0\prime}$+340 mV)

Two dissimilatory **nitrite reductase** enzyme systems known:

1. Cytochrom c containing membrane bound enzyme complex, generates proton motive force, quinone as electron donor
2. Cytoplasmic siroheme containing enzyme, utilizes NADH as electron donor, does not couple reduction to proton export → detoxification of nitrite



Kraft et al., J Biotech. 2011

Questions 3

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Questions 3

What are differences between nitrate assimilation and dissimilatory nitrate reduction to ammonia?

Was sind Unterschiede zwischen Nitratassimilierung und der dissimilatorischen Nitratreduktion zu Ammonium.

a. Soluble cytoplasmic enzymes
b. Regulation, repression by oxygen
c. Nitrite reduction to ammonia in a single 6 e^- transferring step
d. Energy production

Assimilatory nitrate reduction

Nitrate is taken up by cells and reduced to NH_3 = assimilatory nitrate reduction:

Nitrate reductase

$\boxed{HNO_3 + 2\,[H] \rightarrow HNO_2 + H_2O}$ Molybdenum cofactor

Nitrite reductase

$\boxed{HNO_2 + 6\,[H] \rightarrow NH_3 + 2H_2O}$ Siroheme cofactor

- Soluble, cytoplasmatic enzymes
- Repressed by ammonia
- Induced by nitrate
- NAD(P)H or reduced ferredoxin as electron donors
- Nitrate reductase Molybdenum cofactor
- Nitrite reductase siroheme cafactor

Questions 3

What are differences between nitrate assimilation and dissimilatory nitrate reduction to ammonia?

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a. Soluble cytoplasmic enzymes
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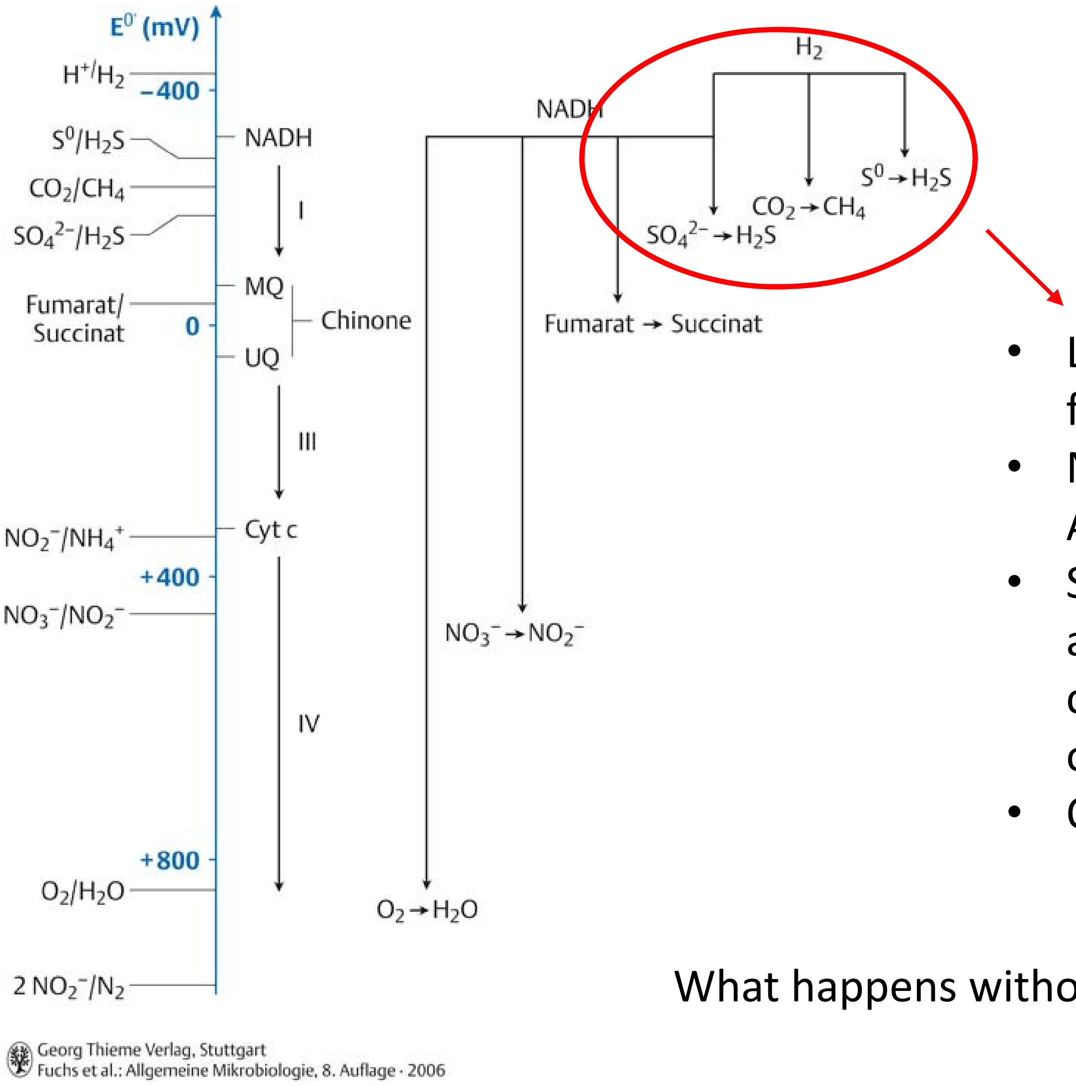
Redox potentials



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Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage · 2006

- Less energy than fermentation processes
- Not used by facultative Anaerobes
- Special adaptations which allow the degradation of certain substrate which otherwise can‘t be utilized
- Only by strict anaerobes

What happens without external electron acceptors?

Life style

Which life styles do you know?

Life style

Which life styles do you know?

Energy source?
Electron donor?
Carbon source?

Life style

Energiequelle	Licht	Photo-			-trophie
	Redoxreaktion	Chemo-			
Elektronendon(at)or	anorganischer Stoff		Litho-		
	organischer Stoff		Organo-		
Kohlenstoffquelle	anorganischer Stoff			Auto-	
	organischer Stoff			Hetero-	

- Microorganisms show a high metabolic diversity
- Play important roles in the biogeochemical cyles of elements

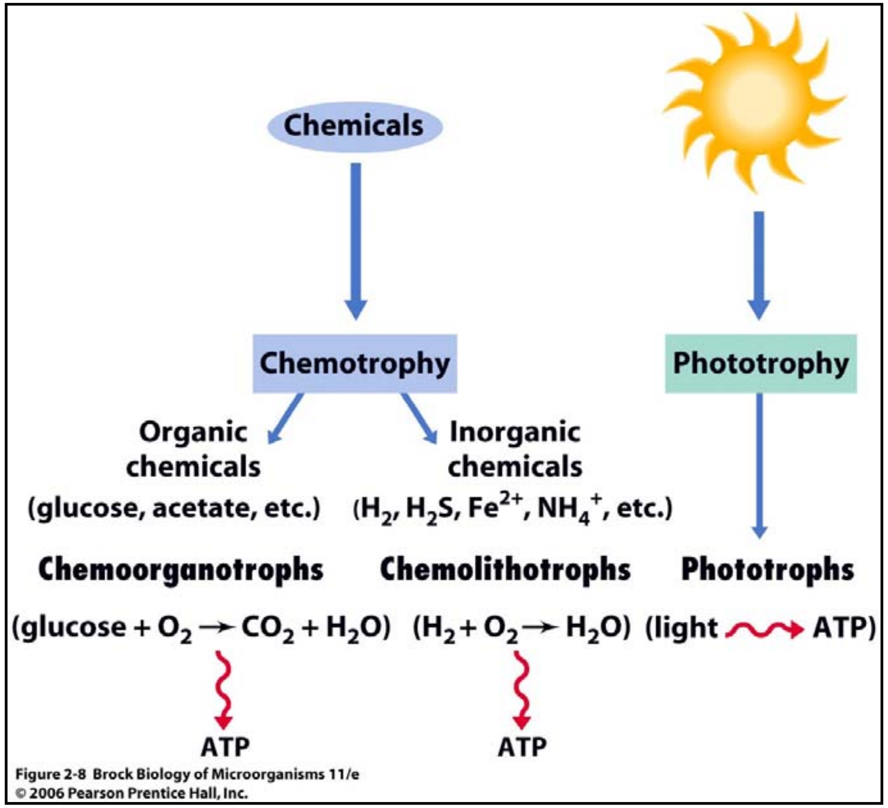

Figure 2-8 Brock Biology of Microorganisms 11/e
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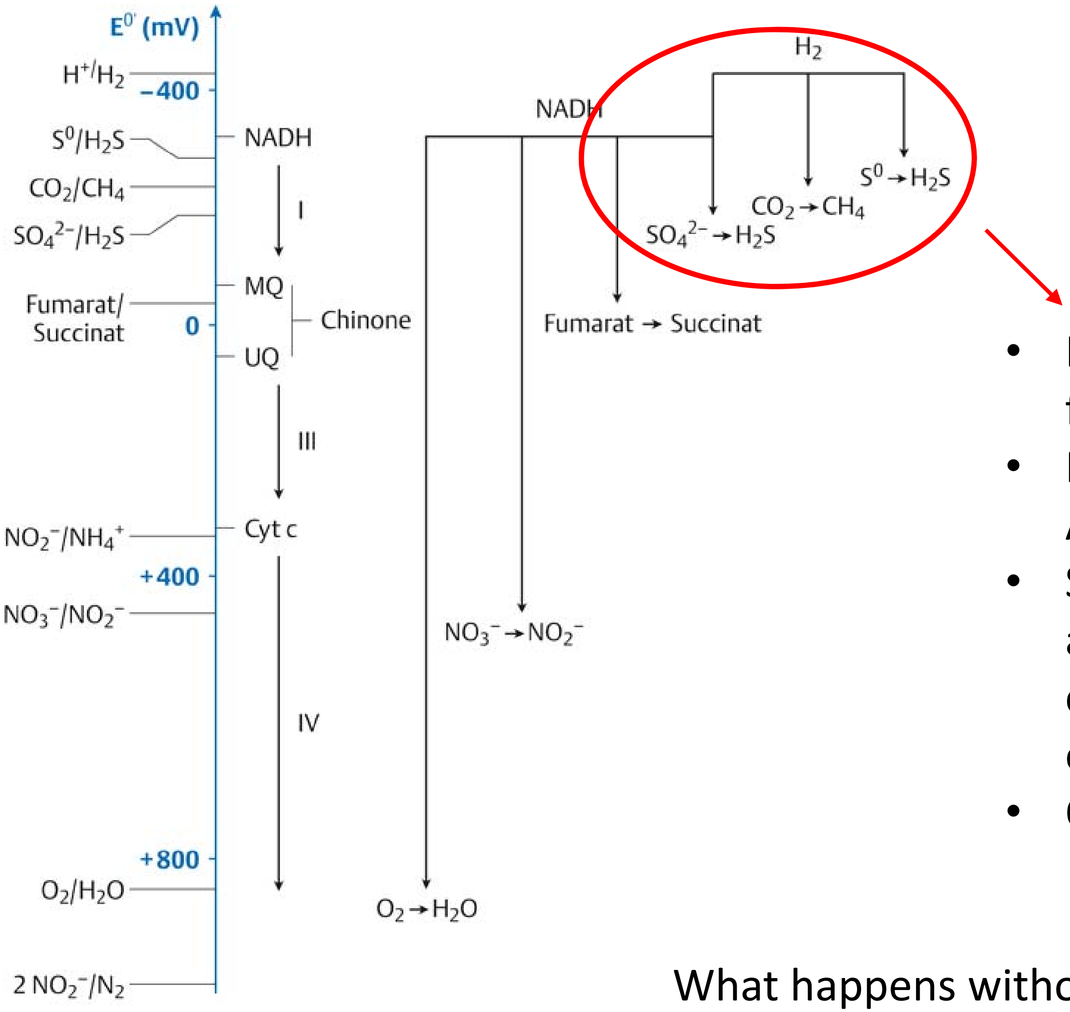
Redox potentials



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What happens without external electron acceptors?

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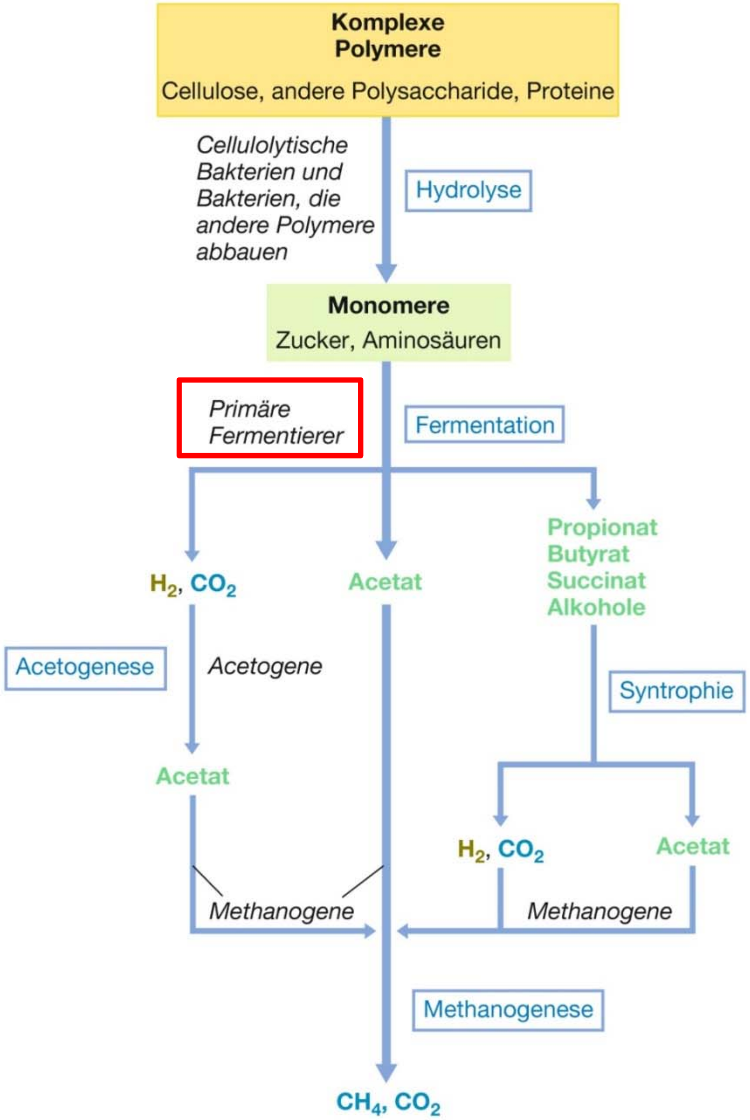
Anaerobic food chain



Komplexe Polymere

Cellulose, andere Polysaccharide, Proteine

Cellulolytische Bakterien und Bakterien, die andere Polymere abbauen

Hydrolyse

Monomere

Zucker, Aminosäuren

Primäre Fermentierer

Fermentation

H_2, CO_2

Acetat

Propionat
Butyrat
Succinat
Alkohole

Acetogenese

Acetogene

Syntrophie

Acetat

H_2, CO_2

Acetat

Methanogene

Methanogene

Methanogenese

CH_4, CO_2

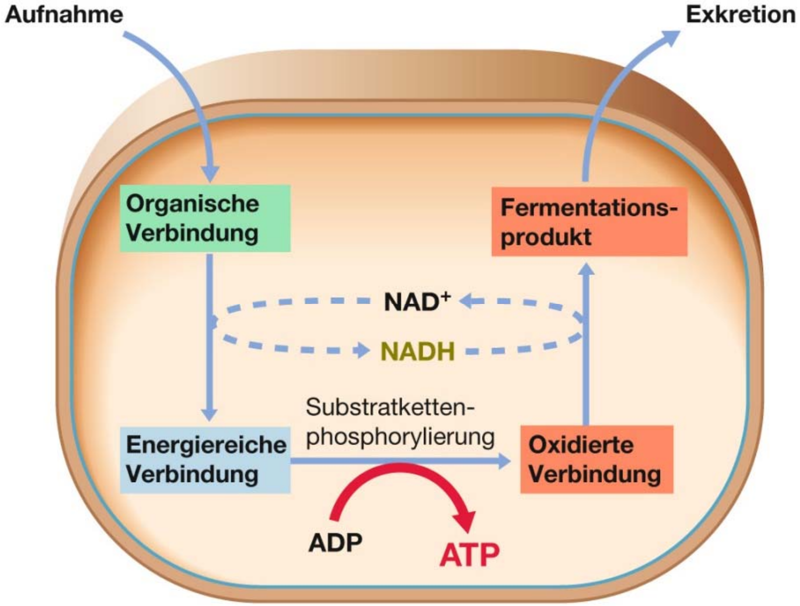
Abbildung 24.5: **Anoxischer Abbau.** Beim anoxischen Abbau kooperieren bei der Umwandlung komplexer organischer Substanzen von CH_4 zu CO_2 verschiedene Gruppen fermentativer Anaerobier. Diese Darstellung trifft auf Lebensräume zu, in denen sulfatreduzierende Bakterien eine untergeordnete Rolle spielen, zum Beispiel in den Sedimenten von Süßwasserseen, Klärschlammbioreaktoren oder dem Pansen.

Fermentation - Heterotrophic metabolism -O_2

without alternative electron acceptor e.g. in *E. coli*

Growth by oxidation of organic substrates without external electron acceptor

(Pyr-DH-Komplex)
TCA (α-KG-DH)*
(ETP)
} repressed



1. Low energy yields (~200-300 kJ/mol glucose → 2-4 ATP via SLP)
2. Max. ATP-Synthesis: oxidative part Aldehyde → carboxylic acid (ATP über SLP)
3. Reoxidation of reduction equivalents [H] → endegenous formation electron acceptors (often carbonyls like pyruvate, acetaldehyde or activated acids like acetyl-CoA)
4. Only a small portion of the organic substance is converted to cell mass

* TCA cycle only present in anabolic reactions

Fermentations

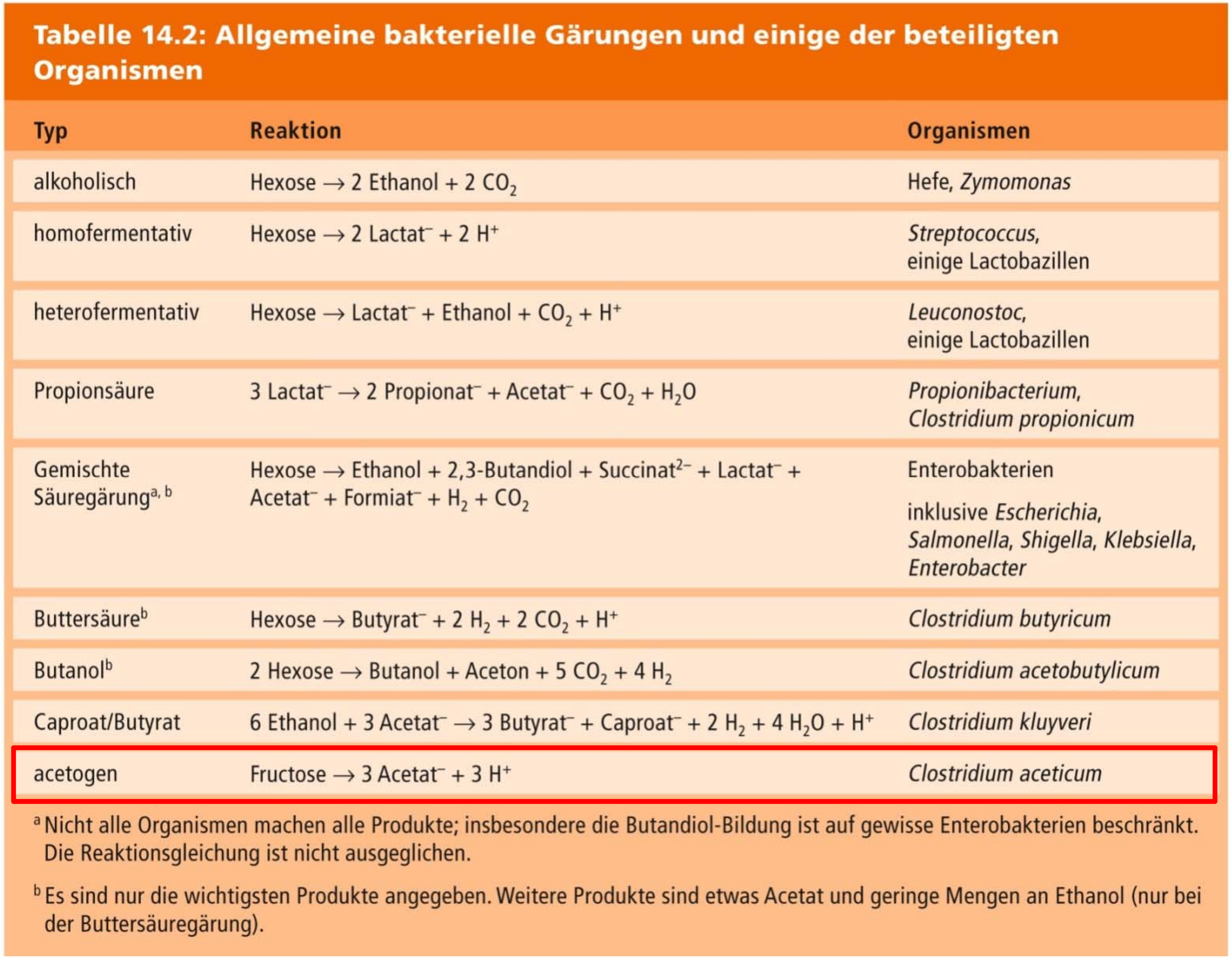


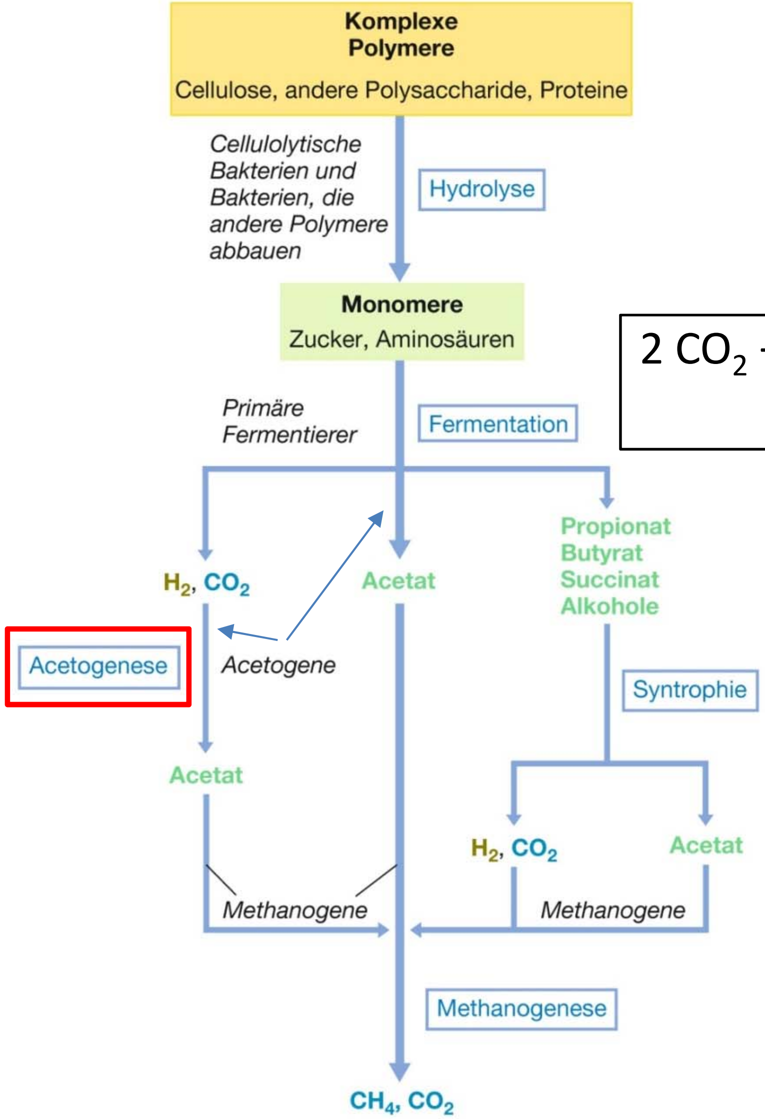
Tabelle 14.2: Allgemeine bakterielle Gärungen und einige der beteiligten Organismen

Typ	Reaktion	Organismen	
alkoholisch	Hexose → 2 Ethanol + 2 CO_2	Hefe, *Zymomonas*	-218 kJ/mol
homofermentativ	Hexose → 2 $Lactat^-$ + 2 H^+	*Streptococcus*, einige Lactobazillen	-198 kJ/mol
heterofermentativ	Hexose → $Lactat^-$ + Ethanol + CO_2 + H^+	*Leuconostoc*, einige Lactobazillen	-208 kJ/mol
Propionsäure	3 $Lactat^-$ → 2 $Propionat^-$ + $Acetat^-$ + CO_2 + H_2O	*Propionibacterium*, *Clostridium propionicum*	-162 kJ/mol
Gemischte Säuregärung[a, b]	Hexose → Ethanol + 2,3-Butandiol + $Succinat^{2-}$ + $Lactat^-$ + $Acetat^-$ + $Formiat^-$ + H_2 + CO_2	Enterobakterien inklusive *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Enterobacter*	-200 to -260 kJ/mol
Buttersäure[b]	Hexose → $Butyrat^-$ + 2 H_2 + 2 CO_2 + H^+	*Clostridium butyricum*	-247 kJ/mol
Butanol[b]	2 Hexose → Butanol + Aceton + 5 CO_2 + 4 H_2	*Clostridium acetobutylicum*	
Caproat/Butyrat	6 Ethanol + 3 $Acetat^-$ → 3 $Butyrat^-$ + $Caproat^-$ + 2 H_2 + 4 H_2O + H^+	*Clostridium kluyveri*	
acetogen	Fructose → 3 $Acetat^-$ + 3 H^+	*Clostridium aceticum*	-311 kJ/mol

[a] Nicht alle Organismen machen alle Produkte; insbesondere die Butandiol-Bildung ist auf gewisse Enterobakterien beschränkt. Die Reaktionsgleichung ist nicht ausgeglichen.

[b] Es sind nur die wichtigsten Produkte angegeben. Weitere Produkte sind etwas Acetat und geringe Mengen an Ethanol (nur bei der Buttersäuregärung).

Anaerobic food chain



$$\text{Glucose} \xrightarrow[\sim 4\ \text{ATP}]{} 3\ \text{Acetate} + 3\ H^+ \qquad \Delta G^{0'} = -311\ \text{kJ/mol}$$

(„Homoacetatgärung“)

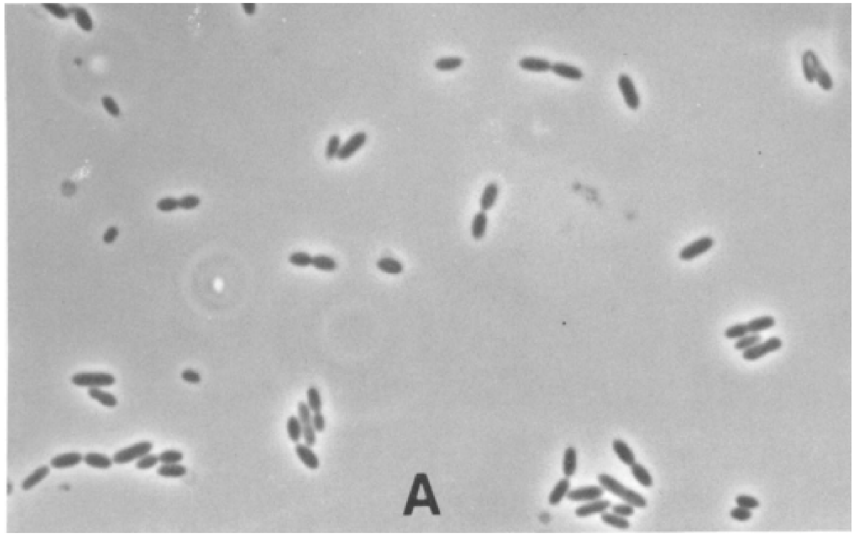
$$2\ CO_2 + 4H_2 \xrightarrow[\sim 1/3\ \text{ATP}]{} \text{Acetate} + 2\ H_2O + H^+ \qquad \Delta G^{0'} = -95\ \text{kJ/mol}$$

Abbildung 24.5: Anoxischer Abbau. Beim anoxischen Abbau kooperieren bei der Umwandlung komplexer organischer Substanzen von CH_4 zu CO_2 verschiedene Gruppen fermentativer Anaerobier. Diese Darstellung trifft auf Lebensräume zu, in denen sulfatreduzierende Bakterien eine untergeordnete Rolle spielen, zum Beispiel in den Sedimenten von Süßwasserseen, Klärschlammbioreaktoren oder dem Pansen.

Acetogens

- present in 23 different bacterial genera (acetogenesis is not a phylogenetic trait)
- Most acetogens found in the phylum Firmicutes (low GC Gram-positive)
- Several genera such as *Clostridium* contain acetogenic as well as non-acetogenic species, whereas other genera such as *Acetobacterium* or *Sporomusa* only contain acetogens
- Most known acetogens belong to the genera *Clostridium* and *Acetobacterium*
- three model organisms : *Moorella thermoacetica, Acetobacterium woodii* and *Clostridium ljungdahlii*.

Acetobacterium woodii

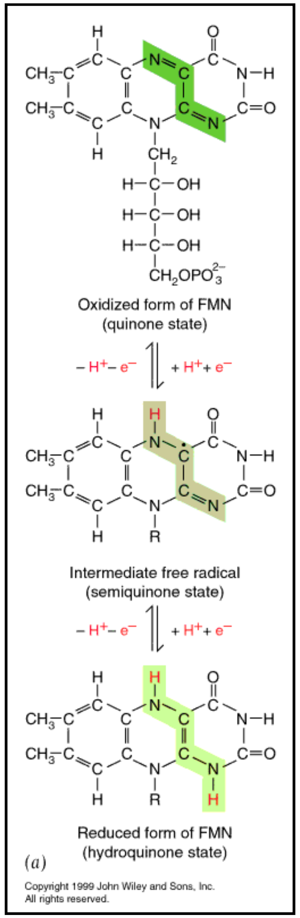


- Gram-positive
- Motile
- mesophilic
- non spore-forming
- grow on CO, H_2 and CO_2, formate, methanol, hexoses, pentoses a.o.

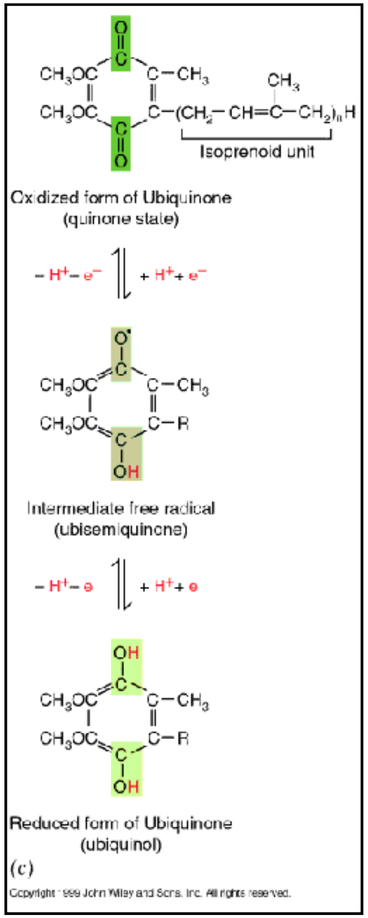
Electron transfer coenzymes

Je 2 H^+ und e^-

Flavin-Mononukleotid

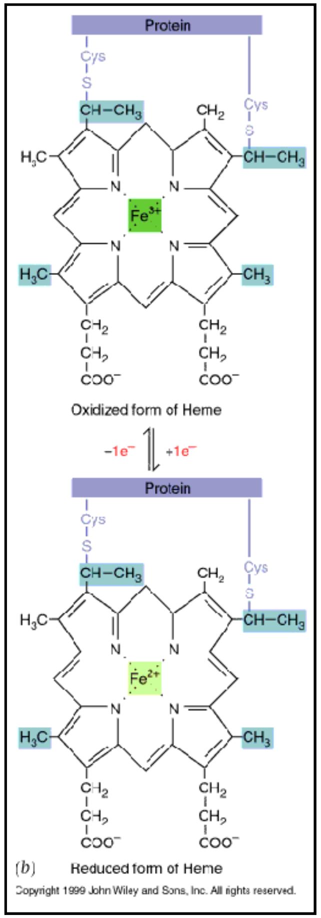


Chinon



nur e^-

Cytochrome



Eisen-Schwefel-Zentren

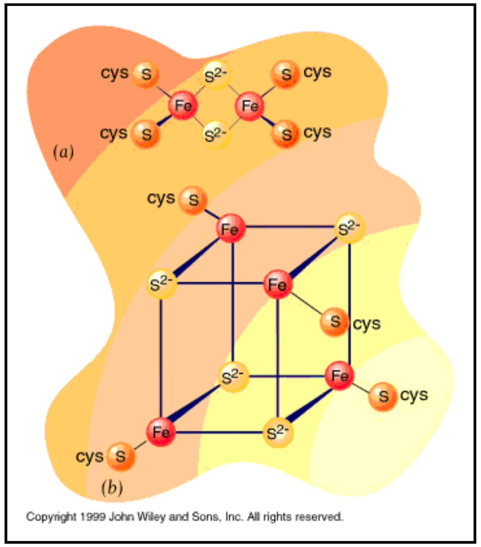
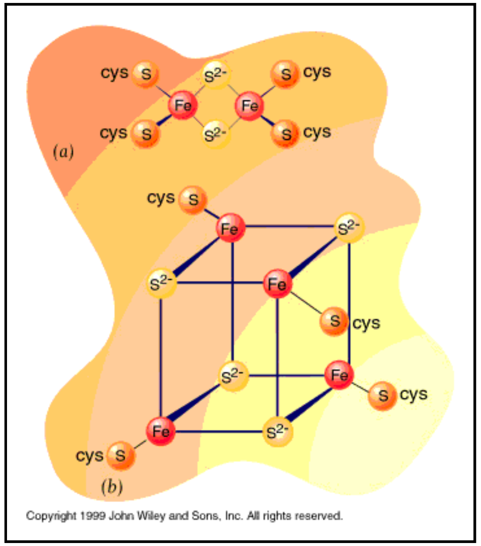


Fig. 14.22 Essential Cell Biology (2nd edition, Alberts, Bray et al.)
Fig. 5.12 Cell & Molecular Biology (4th edition, Karp)

Ferredoxin

- Small non-heme Fe/S protein
- Soluble, cytoplasmic
- One 2Fe2S or two 4Fe4S cluster
- $E^{0'}$ Fd_{ox}/Fd_{red} ~-400 mV (E' -450 mV)

Eisen-Schwefel-Zentren



“(…)involved in the energy metabolism of many anaerobes e.g. clostridia, acetogenic and sulfate reducing bacteria as well as methanogenic archaea.” (Buckel W, Thauer RK (2013), Biochimica et Biophysica Acta 1827, 94–113

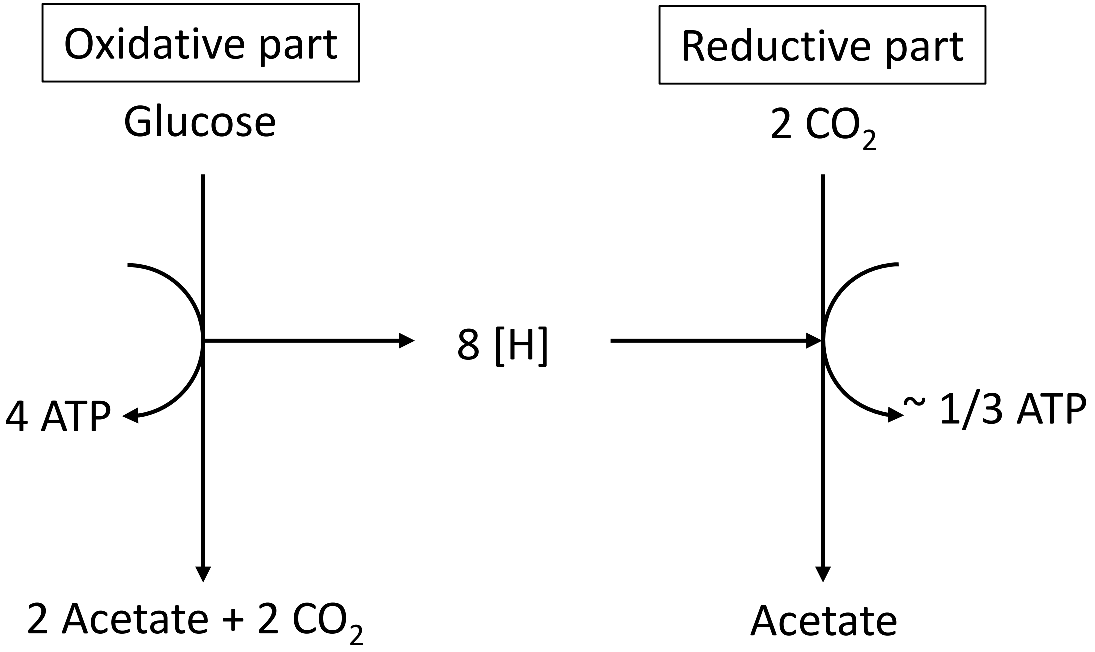
Acetogenesis

chemoorganoheterotrophic – e.g. *Acetobacterium woodii*

$$\text{Glucose} \xrightarrow[\sim 4\ \text{ATP}]{} 3\ \text{Acetate} + 3\ H^+$$

$\Delta G^{0'} = -311$ kJ/mol

(„Homoacetatgärung“)



Oxidative part

Glucose

4 ATP

8 [H]

2 Acetate + 2 CO_2

Reductive part

2 CO_2

~ 1/3 ATP

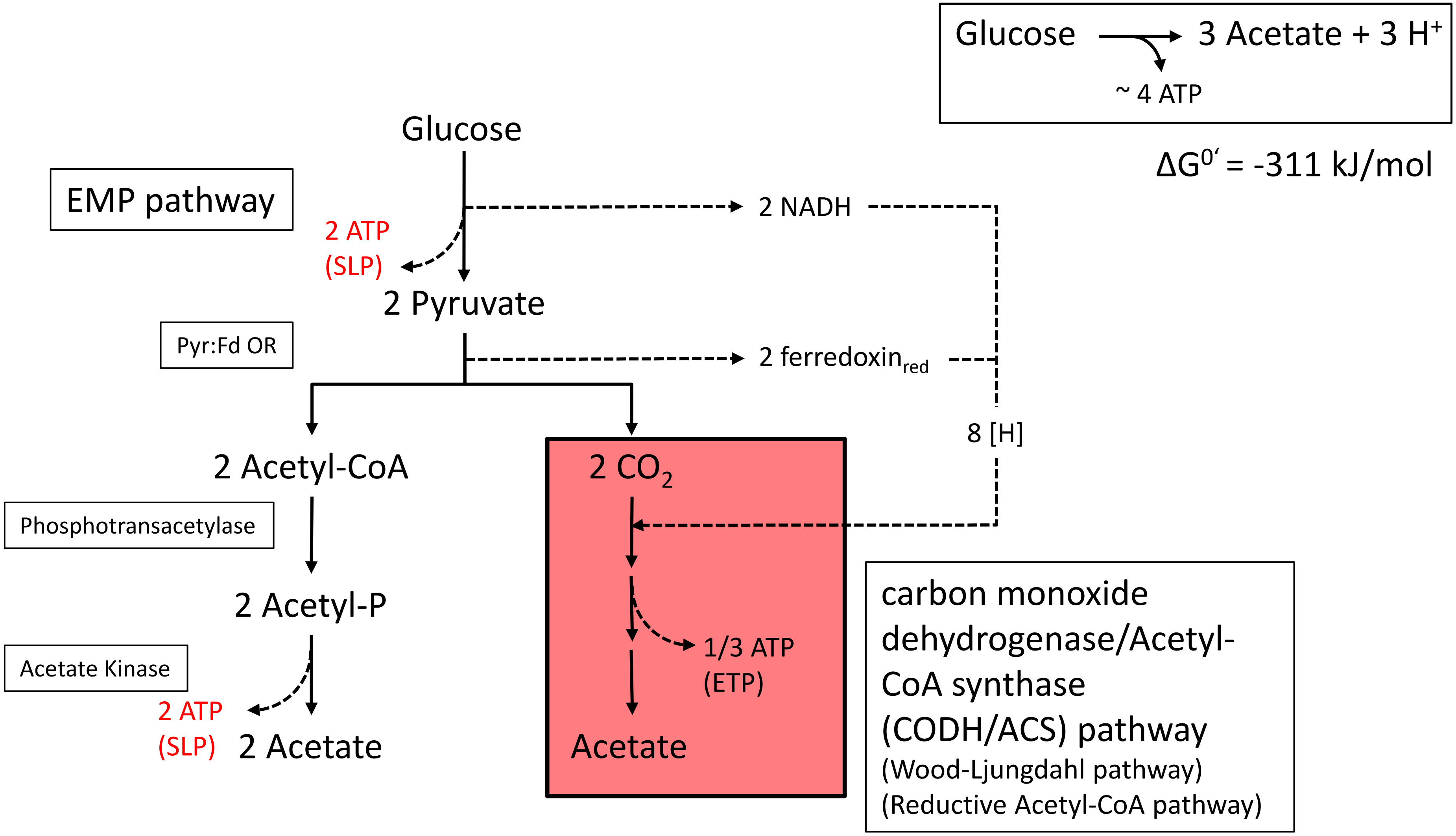
Acetate

Via Substrate level phosphorylation (SSP)

Via Electron transport phosphorylation (ETP) and substrate level phosphorylation (SLP)

Acetogenesis

chemoorganoheterotrophic – *Acetobacterium woodii*



Glucose → 3 Acetate + 3 H^+

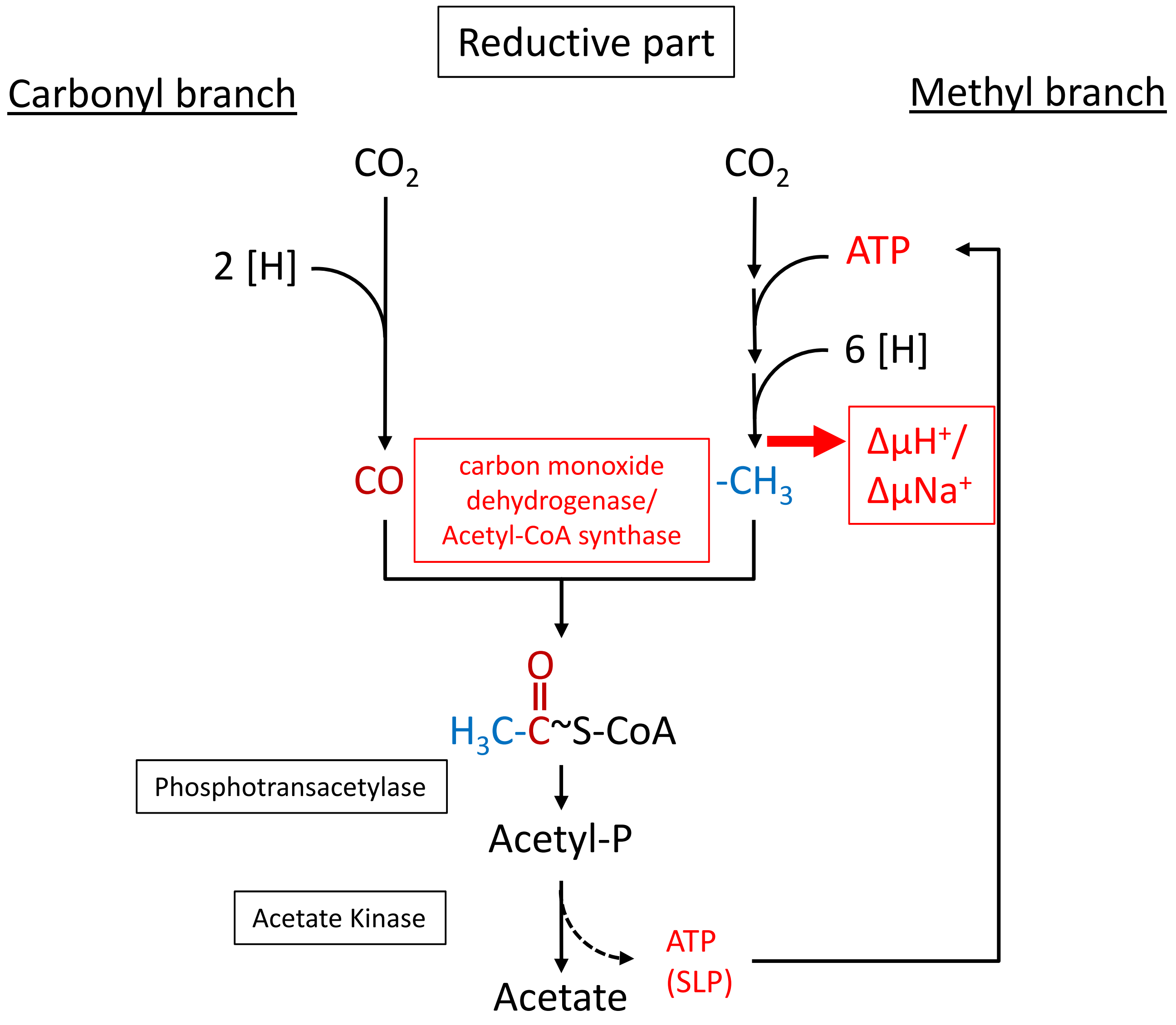
~ 4 ATP

$\Delta G^{0'} = -311$ kJ/mol

carbon monoxide dehydrogenase/Acetyl-CoA synthase (CODH/ACS) pathway
(Wood-Ljungdahl pathway)
(Reductive Acetyl-CoA pathway)

Acetogenesis

chemoorganoheterotrophic – *Acetobacterium woodii*



Reductive part

Carbonyl branch

Methyl branch

CO_2

2 [H]

CO

CO_2

ATP

6 [H]

$-CH_3$

$\Delta\mu H^+/$
$\Delta\mu Na^+$

carbon monoxide
dehydrogenase/
Acetyl-CoA synthase

$H_3C-\overset{\overset{O}{\|}}{C}{\sim}S\text{-}CoA$

Phosphotransacetylase

Acetyl-P

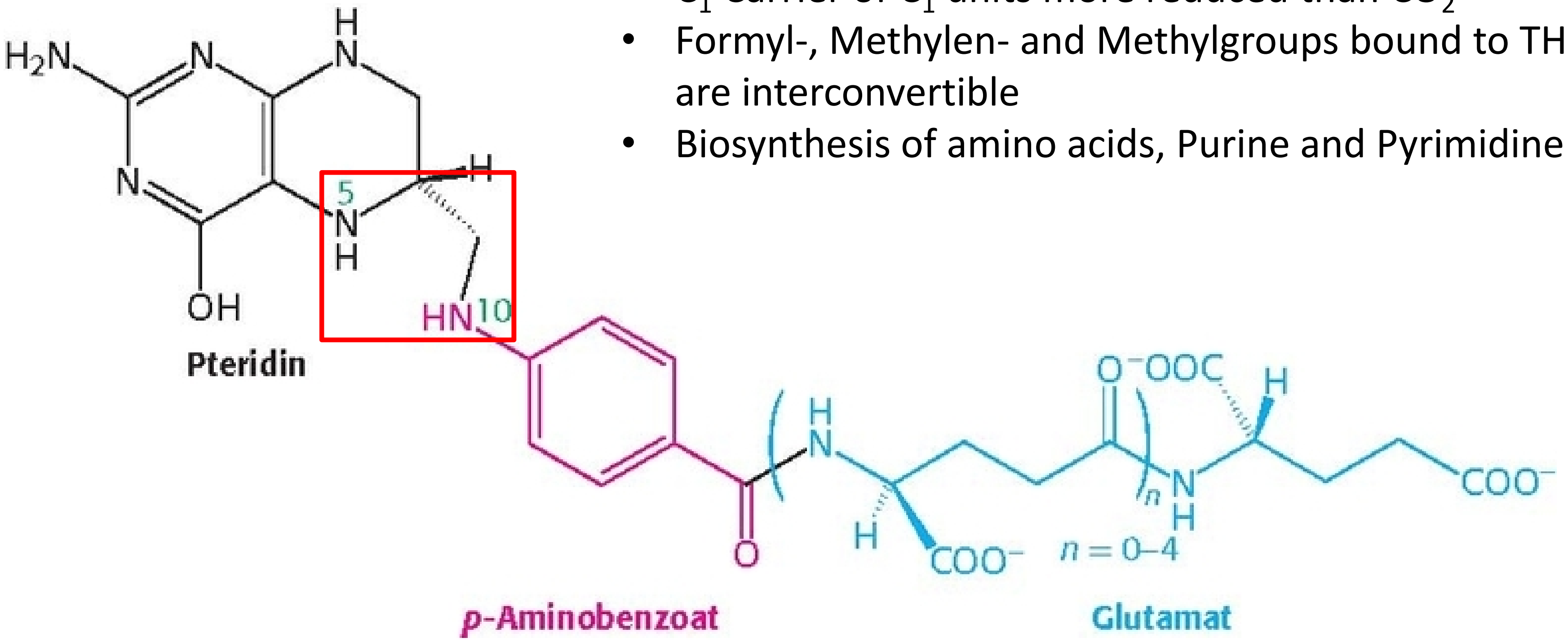
Acetate Kinase

Acetate

ATP
(SLP)

Tetrahydrofolate

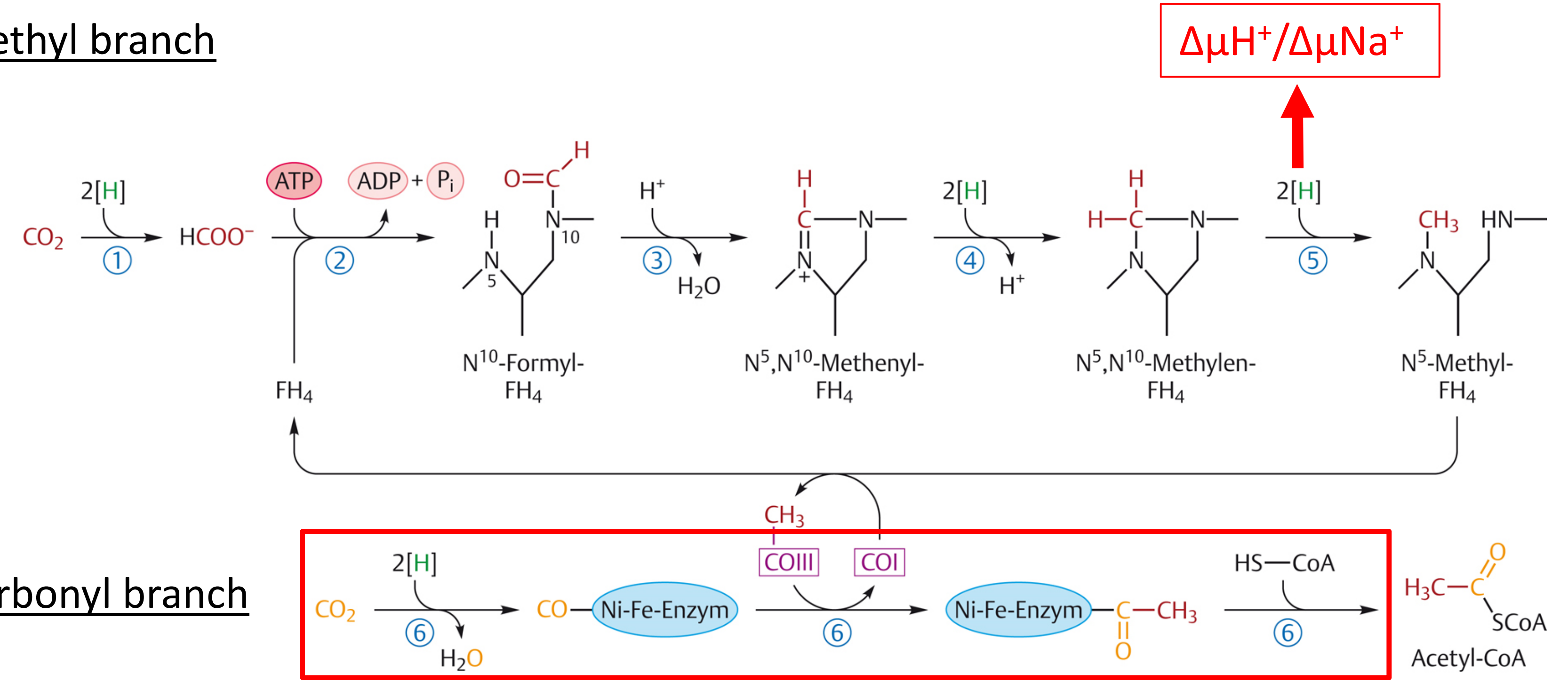
- C_1 Carrier of C_1 units more reduced than CO_2
- Formyl-, Methylen- and Methylgroups bound to THF are interconvertible
- Biosynthesis of amino acids, Purine and Pyrimidine



Aus: Berg, Stryer, Tymoczko, Gatto: *Biochemie*, Springer Spektrum © Springer-Verlag Berlin Heidelberg 2013

CODH/ACS pathway = Reductive acetyl-CoA pathway

Wood-Ljungdal pathway



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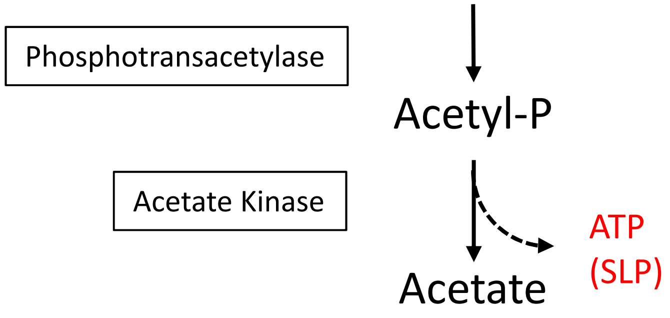
CODH/ACS enzyme complex


Phosphotransacetylase

Acetyl-P

Acetate Kinase

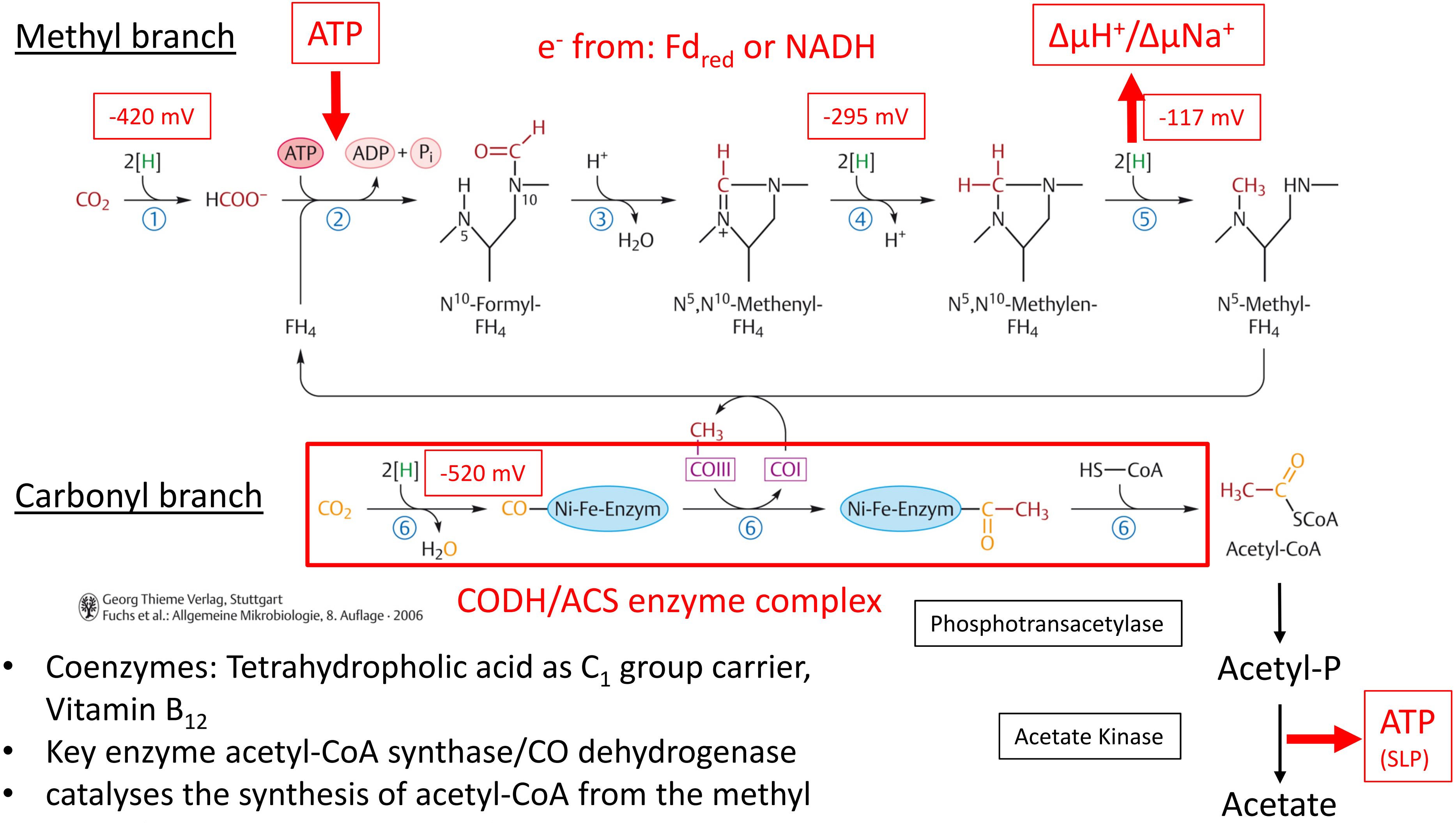
Acetate

ATP (SLP)

- Coenzymes: Tetrahydropholic acid as C_1 group carrier, Vitamin B_{12}
- Key enzyme CO dehydrogenase/acetyl-CoA synthase
- catalyses the synthesis of acetyl-CoA from the methyl group (from the methyl branch), the carbonyl group (from the carbonyl branch) and CoA.

CODH/ACS pathway = Reductive acetyl-CoA pathway

Wood-Ljungdahl pathway



Georg Thieme Verlag, Stuttgart
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- Key enzyme acetyl-CoA synthase/CO dehydrogenase
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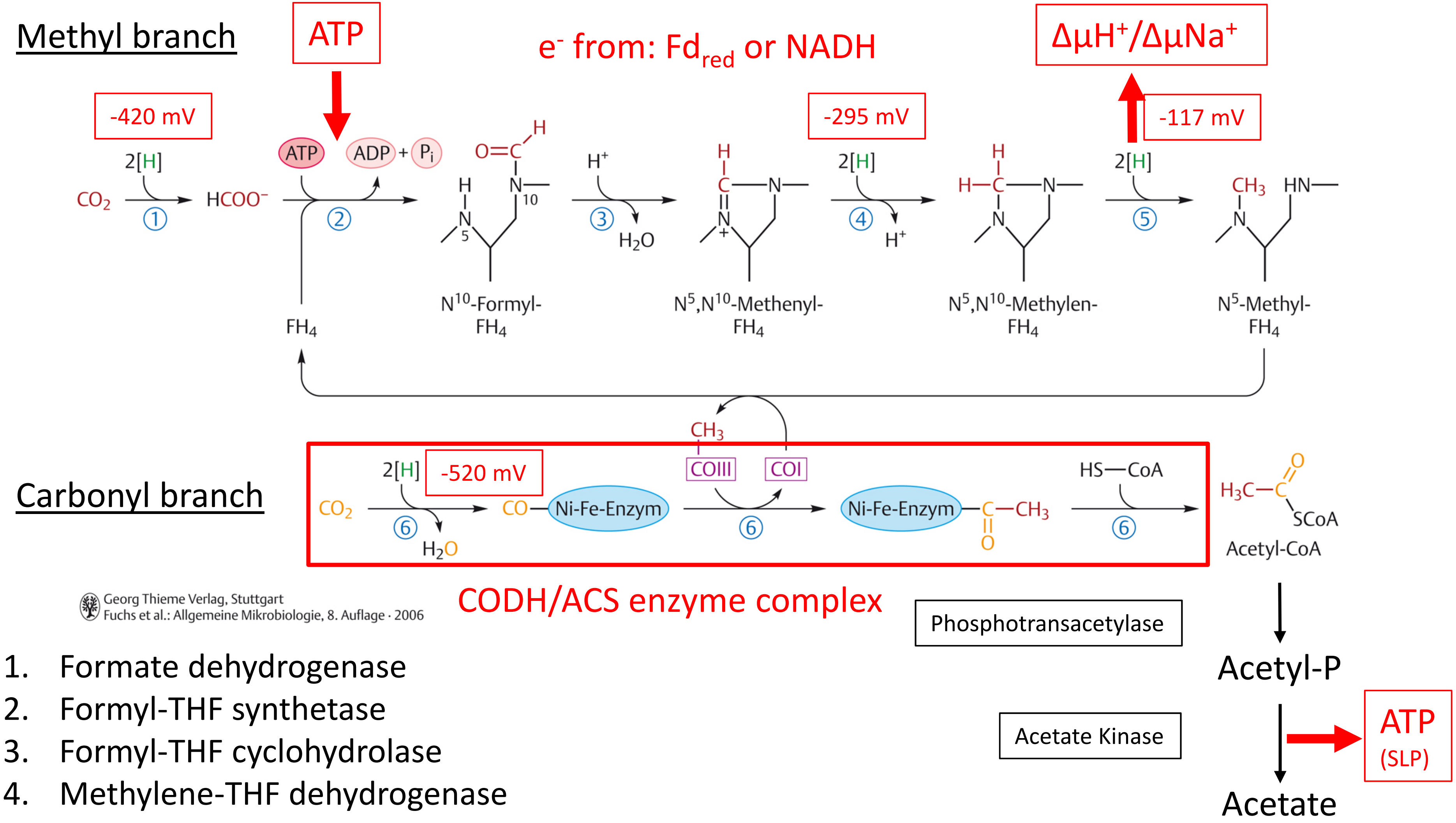
CODH/ACS pathway = Reductive acetyl-CoA pathway

Wood-Ljungdahl pathway



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Fuchs et al.: Allgemeine Mikrobiologie, 8. Auflage · 2006

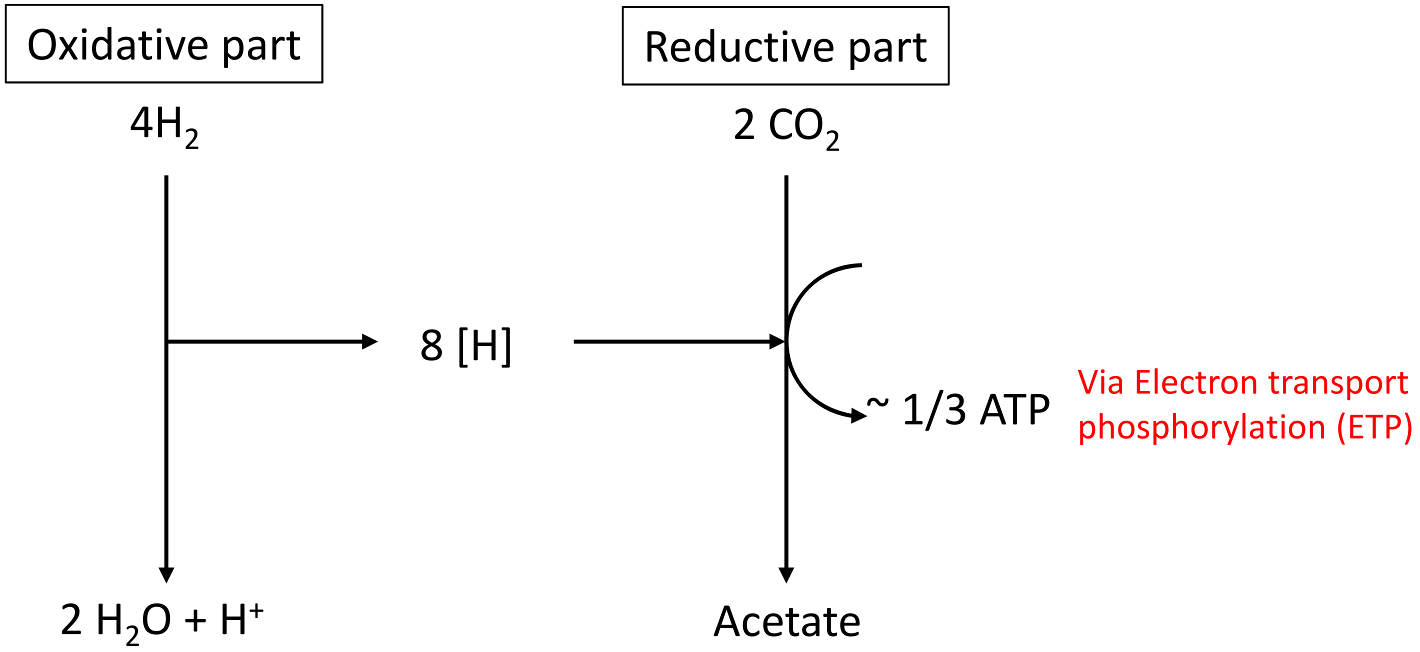
1. Formate dehydrogenase
2. Formyl-THF synthetase
3. Formyl-THF cyclohydrolase
4. Methylene-THF dehydrogenase
5. Methylene-THF reductase
6. Carbon monoxide dehydrogenase/acetyl-CoA synthase

Acetogenesis

chemolithoautotrophic – *Acetobacterium woodii*

$$\boxed{2\,CO_2 + 4H_2 \xrightarrow[\sim 1/3\ ATP]{} \text{Acetate} + 2\,H_2O + H^+}$$

$\Delta G^{0'} = -95$ kJ/mol



Oxidative part

$4H_2$

$8\,[H]$

$2\,H_2O + H^+$

Reductive part

$2\,CO_2$

~ 1/3 ATP

Via Electron transport phosphorylation (ETP)

Acetate

Acetogenesis

chemolithoautotrophic – *A. woodii*

$$\boxed{2\,CO_2 + 4H_2 \xrightarrow[\sim 1/3\ ATP]{} \text{Acetate} + 2\,H_2O + H^+}$$

$\Delta G^{0'} = -95$ kJ/mol
(pH_2 1 atm)

What happens when the H_2 partial pressure drops to 0.001 or 0.0001 atm?

$$\Delta G' = \Delta G^{0'} + RT \ln \frac{[P1]^a \times [P2]^b}{[S1]^c \times [S2]^d} = \Delta G^{0'} + 2{,}302\ RT \lg \frac{[P1]^a \times [P2]^b}{[S1]^c \times [S2]^d}$$

R = 8.315 J/mol K; T (25°C) = 298 K; ln Y = log Y x ln 10 = 2.302 logY

Acetogenesis

chemolithoautotrophic – *A. woodii*

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$\Delta G' = -95$ kJ/mol + 5.7 kJ/mol x lg $([P1]^a[P2]^b)/([S1]^c\mathbf{[10^{-3}]}^4)$
= -95 kJ/mol + 5.7 kJ/mol x 12
= ~-25 kJ/mol (pH_2 10^{-3} atm) → ~1/3 ATP
= ~-4 kJ/mol (pH_2 10^{-4} atm) → kein Wachstum

→ H_2 concentrations are crucial for energy generation

Acetogenesis

chemolithoautotrophic – *A. woodii*

$$\boxed{2\,CO_2 + 4H_2 \xrightarrow[\sim 1/3\ \text{ATP}]{} \text{Acetate} + 2\,H_2O + H^+}$$

$\Delta G^{0\prime} = -95$ kJ/mol
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R = 8.315 J/mol K; T (25°C) = 298 K; ln Y = log Y x ln 10 = 2.302 logY

$$\frac{-18\,\frac{kJ}{mol} - (-95\,\frac{kJ}{mol})}{5.7} = \lg\frac{[P1]^a\times[P2]^b}{[S1]^c\times[S2]^d} \rightarrow 13.5 = \lg\frac{1}{[H_2]^4} \rightarrow 13.5 = -4\,\lg[H_2] \rightarrow \sim\!-3.5 = \lg[H_2]$$

$\Delta G' = -95\ \text{kJ/mol} + 5.7\ \text{kJ/mol} \times \lg([P1]^a[P2]^b)/([S1]^c\mathbf{[10^{-3}]}^4)$
= -95 kJ/mol + 5.7 kJ/mol x 12
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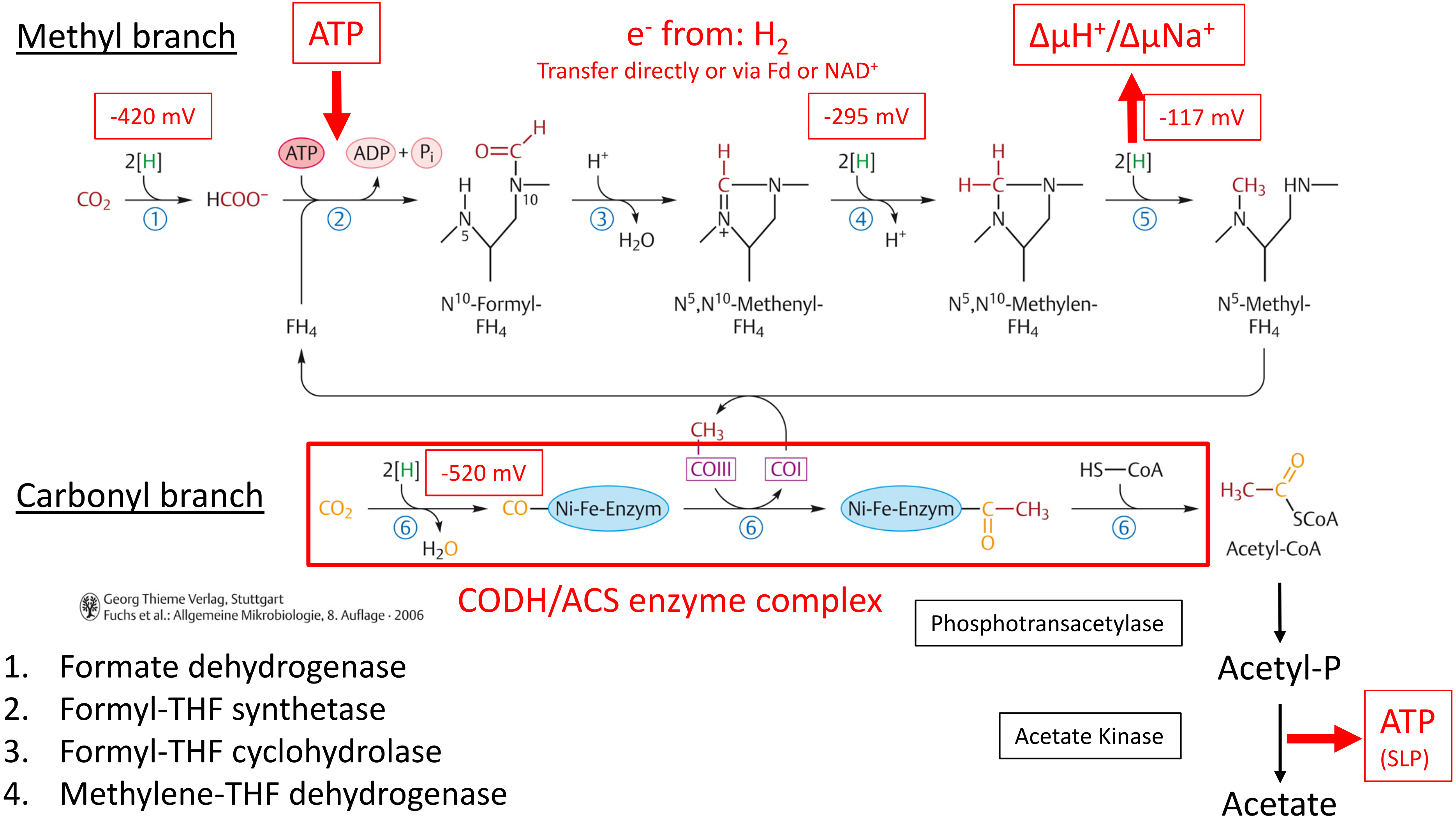
→ H_2 concentrations are crucial for energy generation

CODH/ACS pathway = Reductive acetyl-CoA pathway

Wood-Ljungdahl pathway



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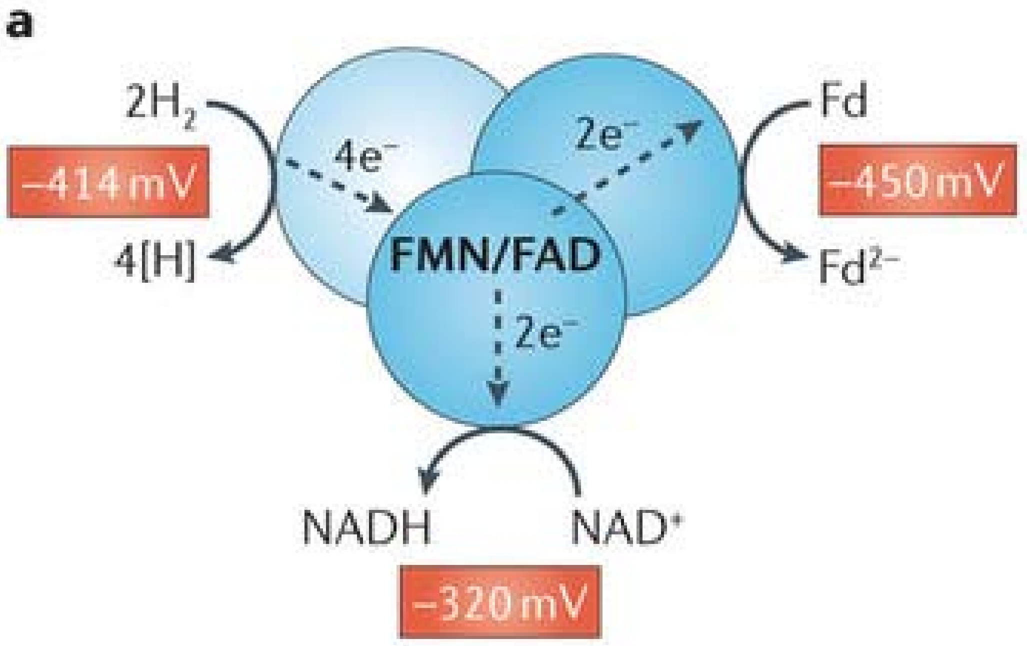
2. Formyl-THF synthetase
3. Formyl-THF cyclohydrolase
4. Methylene-THF dehydrogenase
5. Methylene-THF reductase
6. Carbon monoxide dehaydrogenase/acetyl-CoA synthase

Flavin-based Electron bifurcation



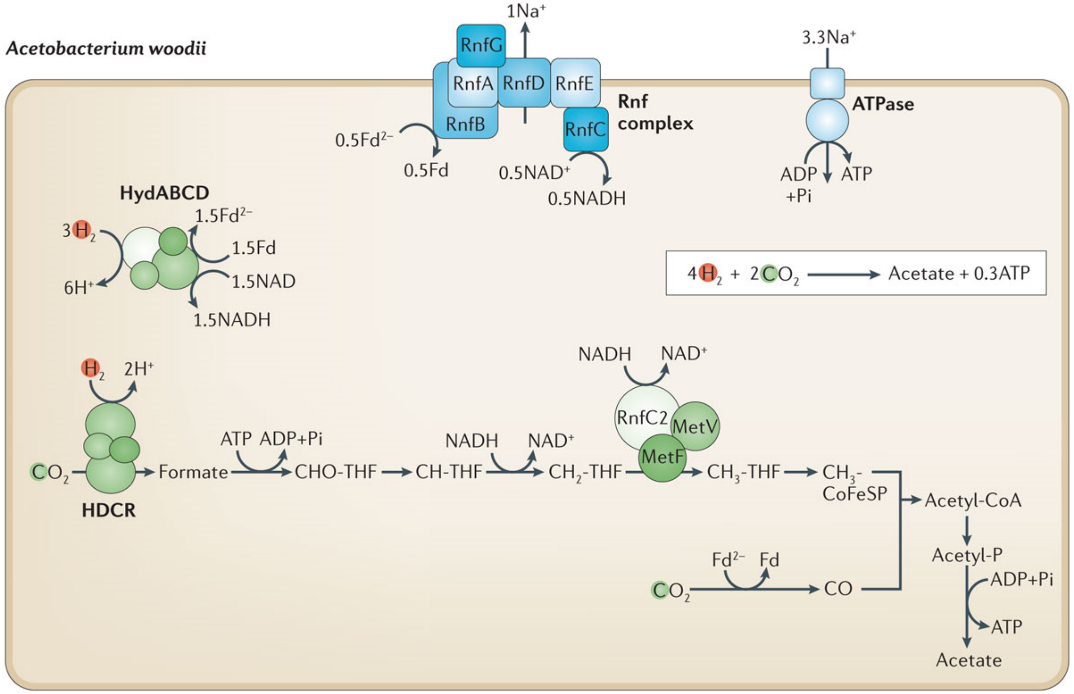
Nature Reviews | Microbiology

Schuchmann K, Müller V (2014),
Nature Reviews Microbiology 12, 809–821

Energy conservation *A. woodii*

autotrophic conditions

$$2\ CO_2 + 4H_2 \xrightarrow[\sim 1/3\ ATP]{} Acetate + 2\ H_2O + H^+$$



Nature Reviews | Microbiology

Rnf = multisubunit ferredoxin–NAD^+ oxidoreductase

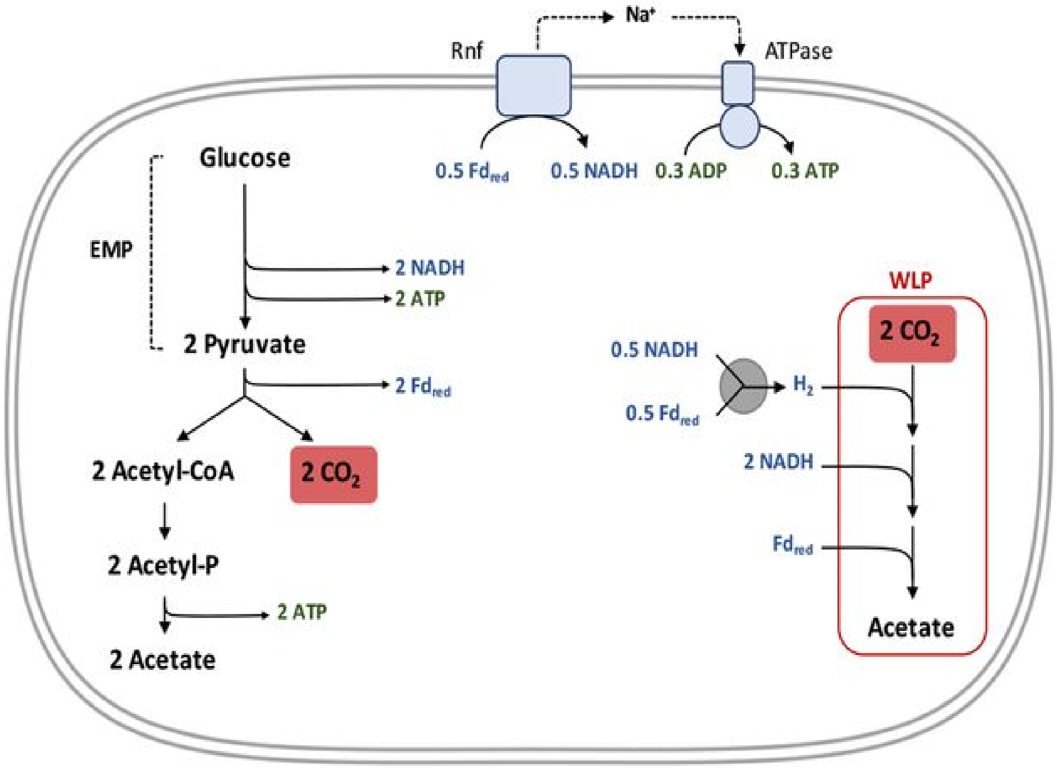
HDCR = hydrogen-dependent CO_2 reductase

HydABCD = electron-bifurcating hydrogenase

Schuchmann K, Müller V (2014),
Nature Reviews Microbiology 12, 809–821

Energy conservation *A. woodii*

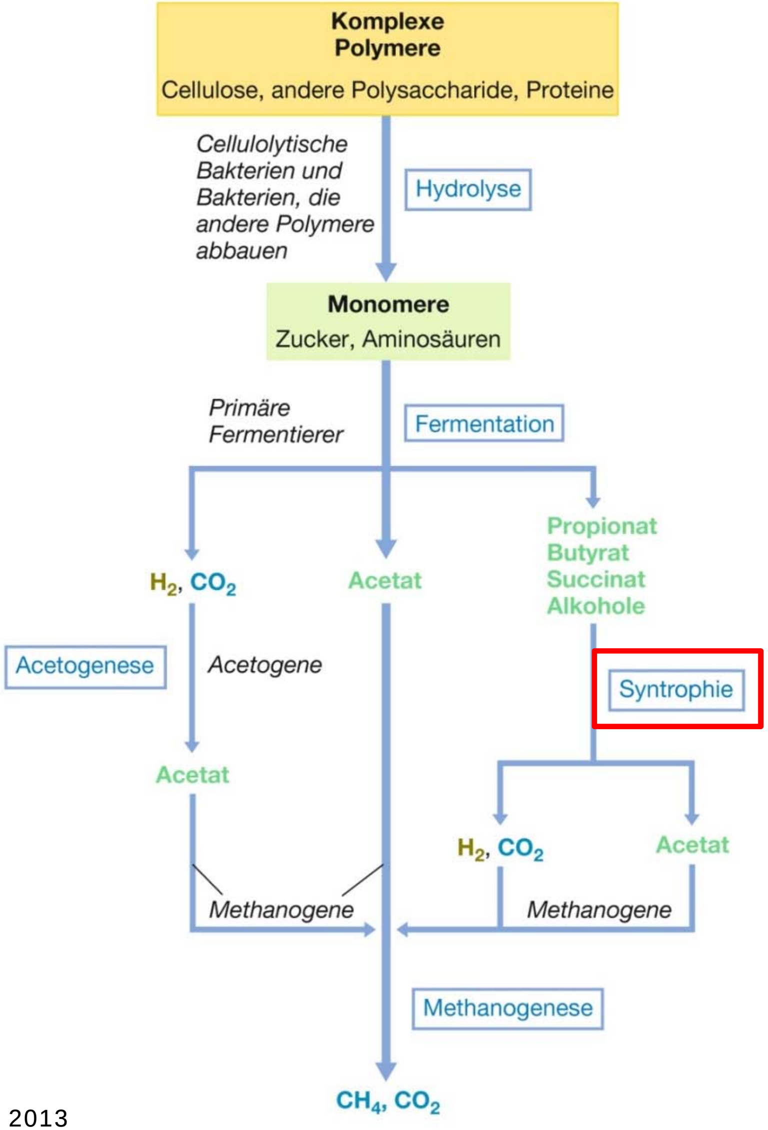
heterotrophic conditions



Rnf = multisubunit ferredoxin–NAD$^+$ oxidoreductase

Kai Schuchmann, and Volker Müller Appl. Environ. Microbiol. 2016;82:4056-4069

Anaerobic food chain



Komplexe Polymere
Cellulose, andere Polysaccharide, Proteine

Cellulolytische Bakterien und Bakterien, die andere Polymere abbauen

Hydrolyse

Monomere
Zucker, Aminosäuren

Primäre Fermentierer

Fermentation

H_2, CO_2

Acetat

Propionat
Butyrat
Succinat
Alkohole

Acetogenese

Acetogene

Syntrophie

Acetat

H_2, CO_2

Acetat

Methanogene

Methanogene

Methanogenese

CH_4, CO_2

Abbildung 24.5: Anoxischer Abbau. Beim anoxischen Abbau kooperieren bei der Umwandlung komplexer organischer Substanzen von CH_4 zu CO_2 verschiedene Gruppen fermentativer Anaerobier. Diese Darstellung trifft auf Lebensräume zu, in denen sulfatreduzierende Bakterien eine untergeordnete Rolle spielen, zum Beispiel in den Sedimenten von Süßwasserseen, Klärschlammbioreaktoren oder dem Pansen.

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Anaerobic food chain

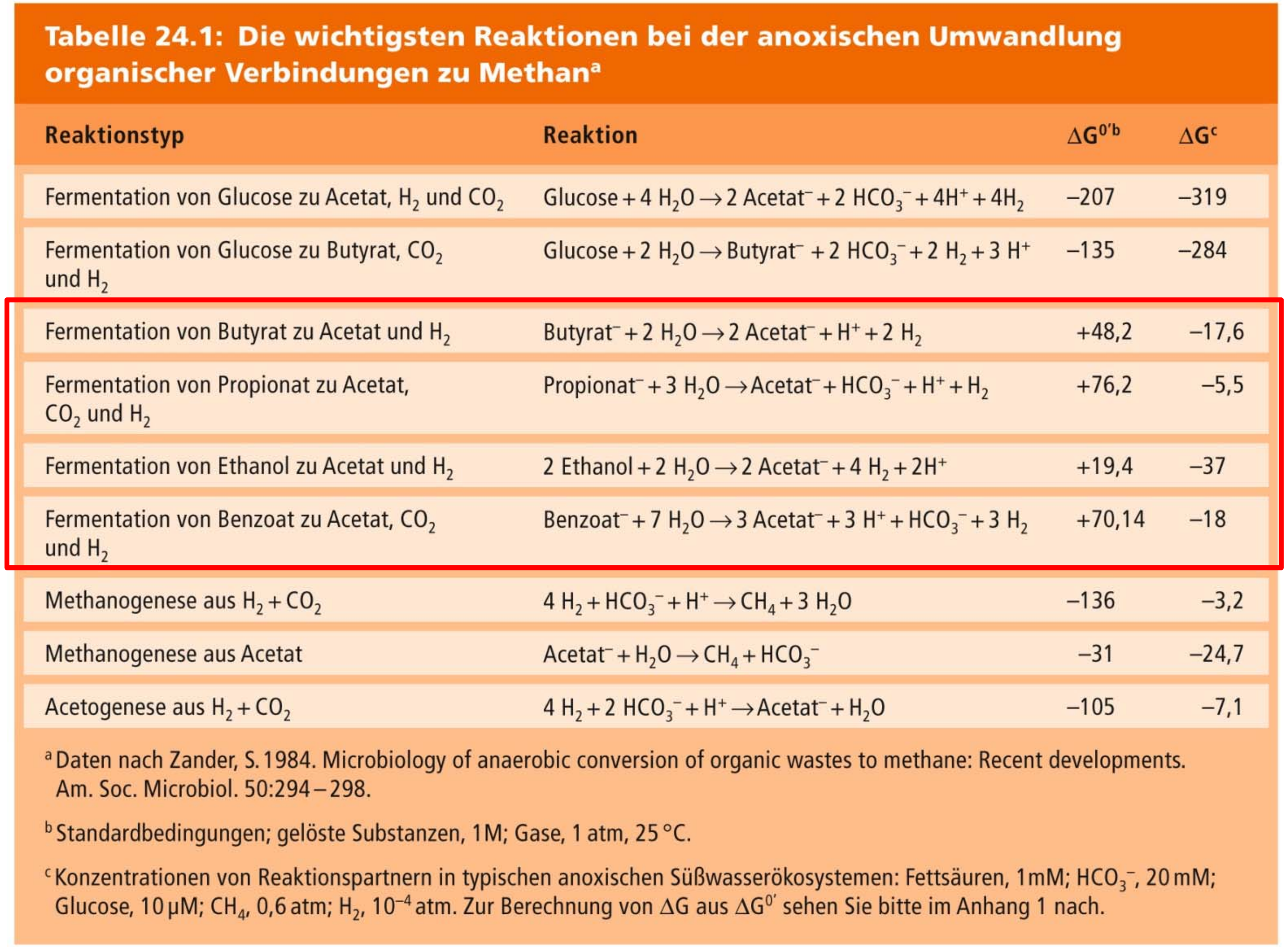


Tabelle 24.1: Die wichtigsten Reaktionen bei der anoxischen Umwandlung organischer Verbindungen zu Methan[a]

Reaktionstyp	Reaktion	$\Delta G^{0'b}$	ΔG^{c}
Fermentation von Glucose zu Acetat, H_2 und CO_2	$\text{Glucose} + 4\ H_2O \rightarrow 2\ \text{Acetat}^- + 2\ HCO_3^- + 4H^+ + 4H_2$	–207	–319
Fermentation von Glucose zu Butyrat, CO_2 und H_2	$\text{Glucose} + 2\ H_2O \rightarrow \text{Butyrat}^- + 2\ HCO_3^- + 2\ H_2 + 3\ H^+$	–135	–284
Fermentation von Butyrat zu Acetat und H_2	$\text{Butyrat}^- + 2\ H_2O \rightarrow 2\ \text{Acetat}^- + H^+ + 2\ H_2$	+48,2	–17,6
Fermentation von Propionat zu Acetat, CO_2 und H_2	$\text{Propionat}^- + 3\ H_2O \rightarrow \text{Acetat}^- + HCO_3^- + H^+ + H_2$	+76,2	–5,5
Fermentation von Ethanol zu Acetat und H_2	$2\ \text{Ethanol} + 2\ H_2O \rightarrow 2\ \text{Acetat}^- + 4\ H_2 + 2H^+$	+19,4	–37
Fermentation von Benzoat zu Acetat, CO_2 und H_2	$\text{Benzoat}^- + 7\ H_2O \rightarrow 3\ \text{Acetat}^- + 3\ H^+ + HCO_3^- + 3\ H_2$	+70,14	–18
Methanogenese aus $H_2 + CO_2$	$4\ H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3\ H_2O$	–136	–3,2
Methanogenese aus Acetat	$\text{Acetat}^- + H_2O \rightarrow CH_4 + HCO_3^-$	–31	–24,7
Acetogenese aus $H_2 + CO_2$	$4\ H_2 + 2\ HCO_3^- + H^+ \rightarrow \text{Acetat}^- + H_2O$	–105	–7,1

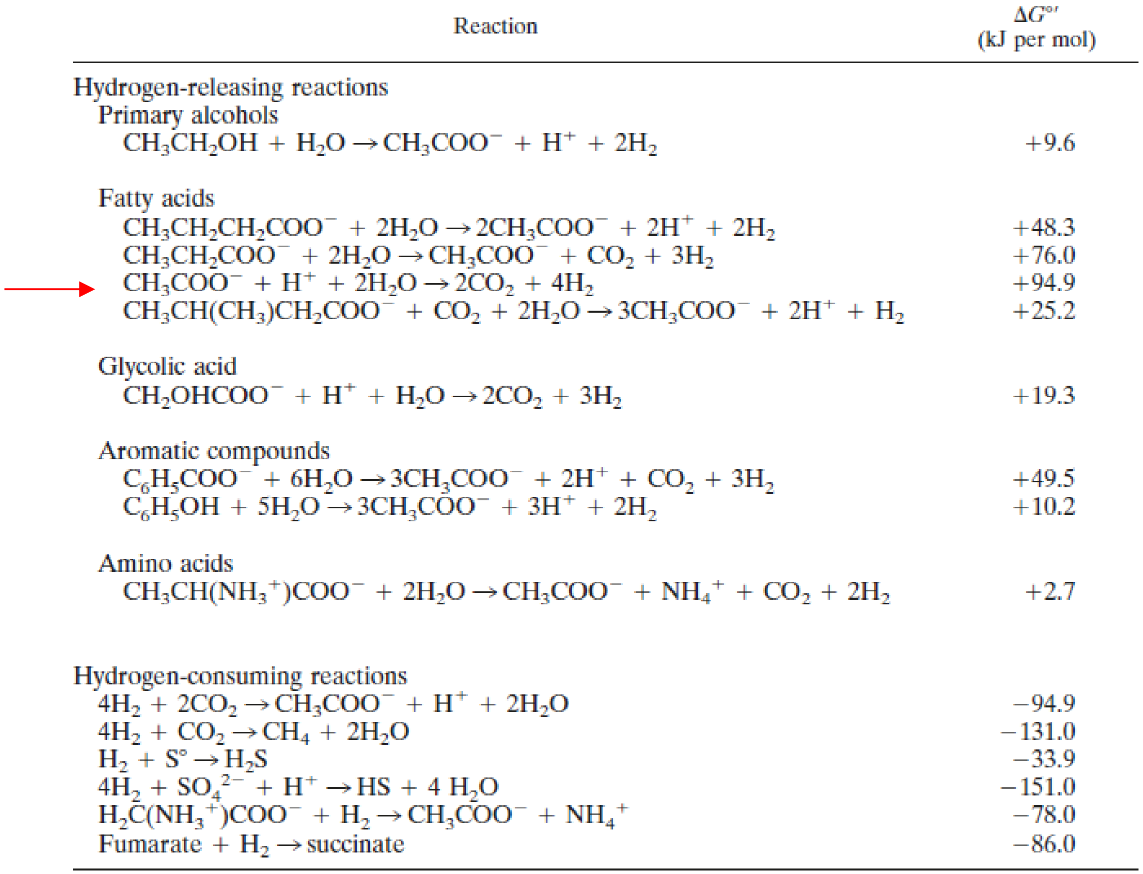
[a] Daten nach Zander, S. 1984. Microbiology of anaerobic conversion of organic wastes to methane: Recent developments. Am. Soc. Microbiol. 50:294–298.

[b] Standardbedingungen; gelöste Substanzen, 1 M; Gase, 1 atm, 25 °C.

[c] Konzentrationen von Reaktionspartnern in typischen anoxischen Süßwasserökosystemen: Fettsäuren, 1 mM; HCO_3^-, 20 mM; Glucose, 10 µM; CH_4, 0,6 atm; H_2, 10^{-4} atm. Zur Berechnung von ΔG aus $\Delta G^{0'}$ sehen Sie bitte im Anhang 1 nach.

Syntrophy

- Secundary fermentations = utilization of fermentation products (alcohols, fatty acids etc.) of other organisms to the finally yield acetate, CO_2 and H_2
- Under standard conditions endergonic processes
- Coupling of the endergonic H_2 producing secondary fermentations with H_2 consuming reactions like methanogenesis



Reaction	$\Delta G^{\circ\prime}$ (kJ per mol)
Hydrogen-releasing reactions	
Primary alcohols	
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6
Fatty acids	
$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + 2H^+ + 2H_2$	+48.3
$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2$	+76.0
$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2$	+94.9
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O \rightarrow 3CH_3COO^- + 2H^+ + H_2$	+25.2
Glycolic acid	
$CH_2OHCOO^- + H^+ + H_2O \rightarrow 2CO_2 + 3H_2$	+19.3
Aromatic compounds	
$C_6H_5COO^- + 6H_2O \rightarrow 3CH_3COO^- + 2H^+ + CO_2 + 3H_2$	+49.5
$C_6H_5OH + 5H_2O \rightarrow 3CH_3COO^- + 3H^+ + 2H_2$	+10.2
Amino acids	
$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2$	+2.7
Hydrogen-consuming reactions	
$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	−94.9
$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−131.0
$H_2 + S^\circ \rightarrow H_2S$	−33.9
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS + 4\ H_2O$	−151.0
$H_2C(NH_3^+)COO^- + H_2 \rightarrow CH_3COO^- + NH_4^+$	−78.0
$Fumarate + H_2 \rightarrow succinate$	−86.0

→ = Synthrophy

Schink, 1997

Syntrophy

- Secundary fermentations = utilization of fermentation products (alcohols, fatty acids etc.) of other organisms to the finally yield acetate, CO_2 and H_2
- Under standard conditions endergonic processes
- Coupling of the endergonic H_2 producing secondary fermentations with H_2 consuming reactions like methanogenesis

→ = Synthrophy, association of secondary fermenting organisms with H_2 comsuming organisms

$\boxed{\text{Butyrate} + H_2O \rightarrow 2\ \text{Acetate} + 2H_2 + 2H^+}$

$\Delta G^{0'} = +48$ kJ/mol
(pH_2 1 atm)

Acetate and H_2 formation from butyrate at **pH_2 10^{-4} atm, 1 mM butyrate and 0.1 mM acetate:**

$\Delta G' = +48 \text{ kJ/mol} + 5.7 \text{ kJ/mol} \times \lg([\mathbf{10^{-4}}]^2[\mathbf{10^{-4}}]^2)/([\mathbf{10^{-3}}])$
$= +48 \text{ kJ/mol} + 5.7 \text{ kJ/mol} \times -13$
$=$ **~-26 kJ/mol (pH_2 10^{-4} atm) → ~1/3 ATP**

Syntrophy

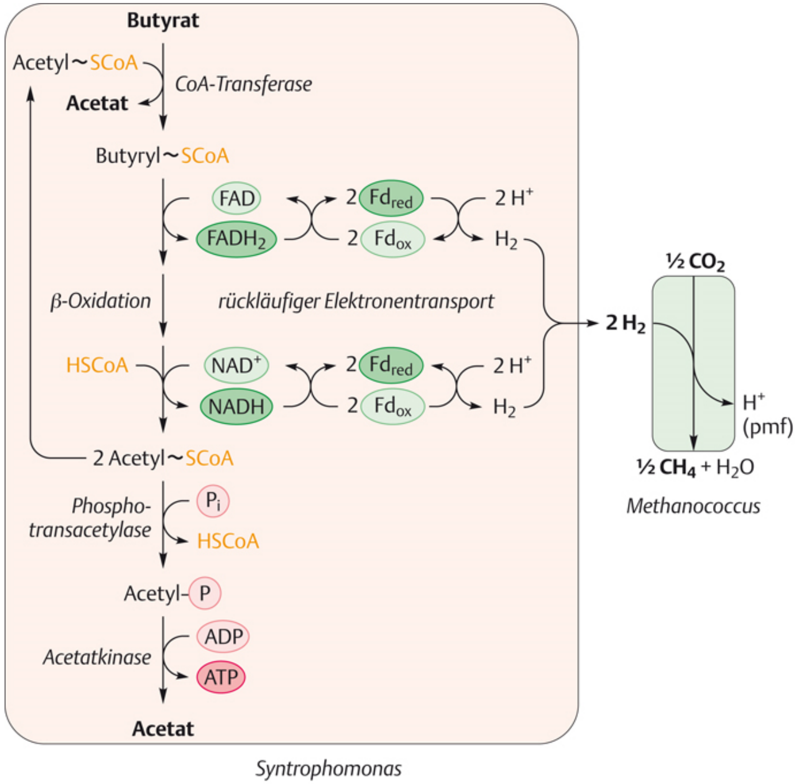
Butyrate + $H_2O \rightarrow 2$ Acetate + $2H_2 + 2H^+$

Acetate and H_2 formation from butyrate at **pH_2 10^{-4} atm, 1 mM butyrate and 0.1 mM acetate:**
$\Delta G' = +48$ kJ/mol + 5.7 kJ/mol x lg $([\mathbf{10^{-4}}]^2[\mathbf{10^{-4}}]^2)/([\mathbf{10^{-3}}])$
= +48 kJ/mol + 5.7 kJ/mol x -13
= **~-26 kJ/mol (pH_2 10^{-4} atm) → ~1/3 ATP**

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

Methanogenesis from CO_2 and H_2 at **pH_2 10^{-4} atm:**
$\Delta G' = -131$ kJ/mol + 5.7 kJ/mol x lg $([P1]^a[P2]^b)/([S1]^c[\mathbf{10^{-4}}]^4)$
= -131 kJ/mol + 5.7 kJ/mol x 16
= **~-39 kJ/mol (pH_2 10^{-4} atm) → ~1/3 – 1/2 ATP**

Syntrophy



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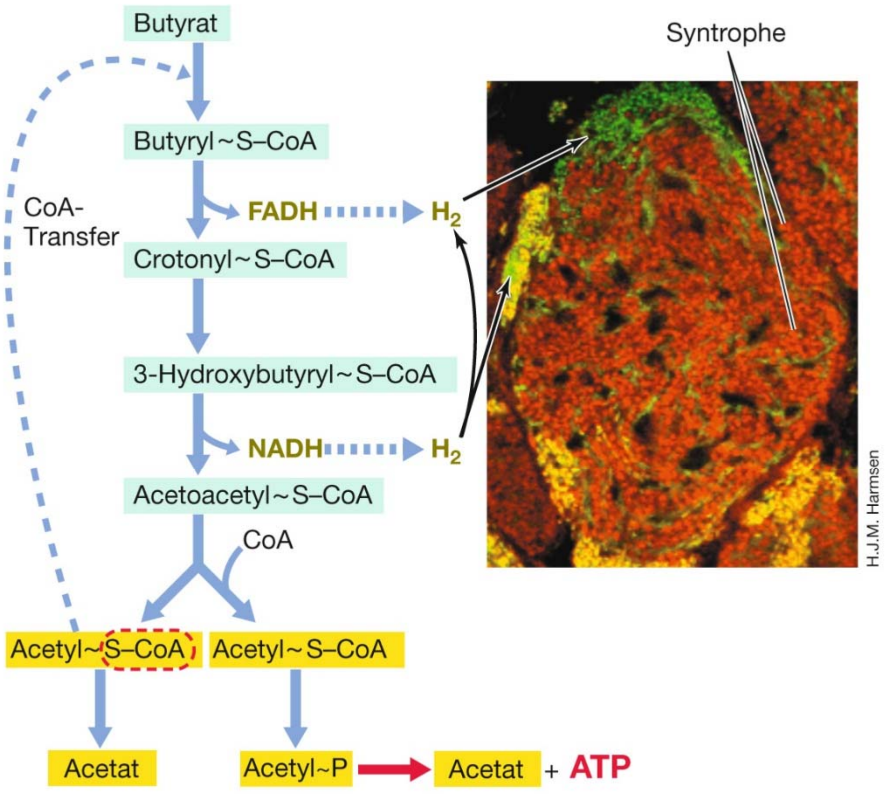

Summe: Butyrat$^-$ + H_2O $\longrightarrow$ 2 Acetat$^-$ + H^+ + 2 H_2 $\Delta G^{0\prime}$ = +48,2 kJ
(ΔG = –18 kJ)

(a) **Syntrophe Kultur**

Interspecies hydrogen transfer

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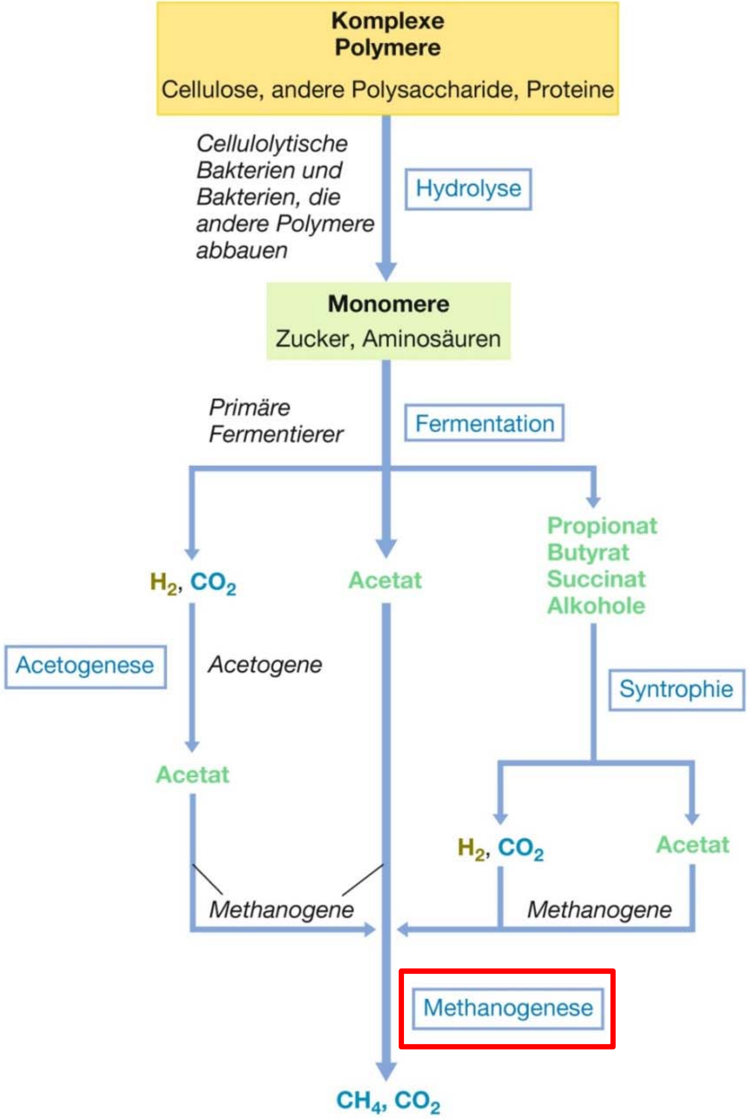
Anaerobic food chain



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Questions 4

- What is Acetogenesis, what are the substrates, what the products?
- Name the underlying pathway for CO_2 reduction and the two branches involved?
- What is the key coenzyme?
- What is the key enzyme and which reaction does it catalyze?
- How is energy gained? Which condition is crucial for autotrophic Acetogenesis?
- Outline the steps of the anerobic food chain! (remember also the different modes of primary fermentations, substrates, products etc.)
- What are the substrates and products of secundary fermentations? What is the key problem and how are these reactions driven?
- What is Syntrophy?

Fragen 4

- Was ist Acetogenese, was sind die Substrate, was die Produkte?
- Benenne den zugrunde liegenden Stoffwechselweg zur CO_2 Reduktion und dessen beiden „Zweige“, die daran beteiligt sind?
- Wie heißt das Schlüsselcoenzym dieses Weges?
- Wie heißt das Schüsselenzym und welche Reaktion katalysiert es?
- Wie hoch ist die Energieausbeute? Welche Bedingung ist für die autotrophe Acetogenese essentiell wichtig?
- Skizzieren Sie die Schritte der anaeroben Nahrungskette! (remember also the different modes of primary fermentations, substrates, products etc.)
- Was sind die Substrate und Produkte sekundärer Fermentationen? Was ist bei diesen Reaktionen das Grundproblem und wie werden sie angetrieben?
- Was ist Syntrophie?