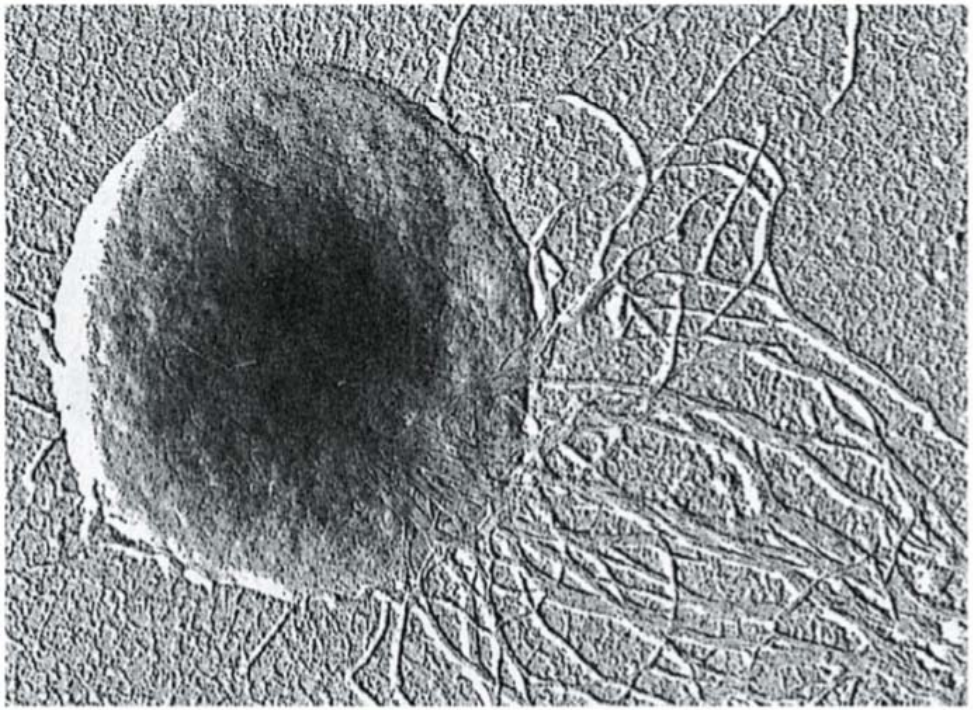
Microbiology II

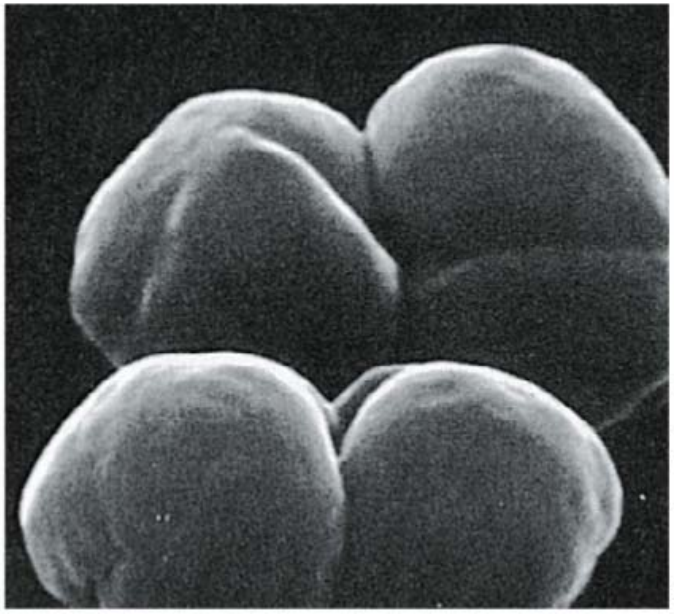
Methanogenesis



Helmut König und K. O. Stetter

(a)

Methanococcus jannaschii



Alexander Zehnder

(d)

Methanosarcina barkeri

Christopher Bräsen

Lecture Plan

17.10. 2017	Mikrobielle Physiologie I - Energetik	**Bräsen**
24.10. 2017	Mikrobielle Physiologie II – Einige Prinzipien und Mechanismen im zentralen Kohlenstoffmetabolismus	**Bräsen**
31.10. 2017	Keine Vorlesung	**Bräsen**
07.11. 2017	Mikrobielle Physiologie III – Nitrat-Atmung	**Bräsen**
14.11. 2017	Mikrobielle Physiologie IV – Acetogenese und der Acetyl-CoA/Kohlenmonoxid Dehydrogenase-Weg	**Bräsen**
21.11. 2017	Mikrobielle Physiologie V – Anaerobe Nahrungskette und Methanogenese	**Bräsen**
28.11. 2017	Mikrobielle Physiologie VI – Sulfate Reduktion	**Bräsen**
05.12. 2017	Antibiotika (Penicillium notatum)	**Meckenstock**
12.12. 2017	Mikroorganismen in der Umwelt (Geobacter metallireducens)	**Meckenstock**
19.12. 2017	Mikrobielles Wachstum (Elusimicrobium minutum)	**Meckenstock**
09.01. 2018	Mikrobielle Fortbewegung (Thioploca)	**Meckenstock**
16.01. 2018	Viren (T4)	**Meckenstock**
23.01. 2018	Geschichte der Mikrobiologie	**Meckenstock**
30.01. 2018	**Wrap up/Ausweichtermin**	**Meckenstock/Bräsen**

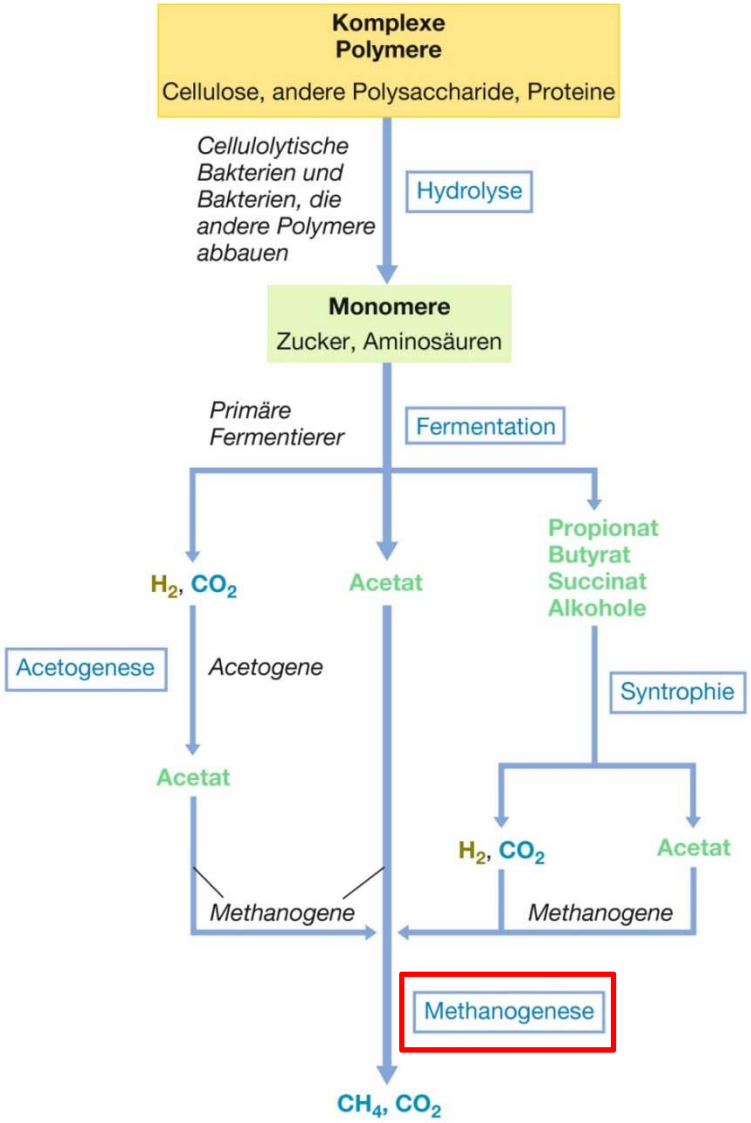
Questions 4

- 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 anaerobic 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?

Anaerobic food chain



Methane is a major end product of anaerobic biomass degradation only in anoxic environments where the concentrations of sulphate, nitrate, Mn(IV) or Fe(III) are low. In the presence of these electron acceptors, methanogenesis is out-competed by anaerobic respiration, mainly for thermodynamic reasons.

(Thauer RK et al. (2008) Nature Reviews Microbiology 6, 579-591)

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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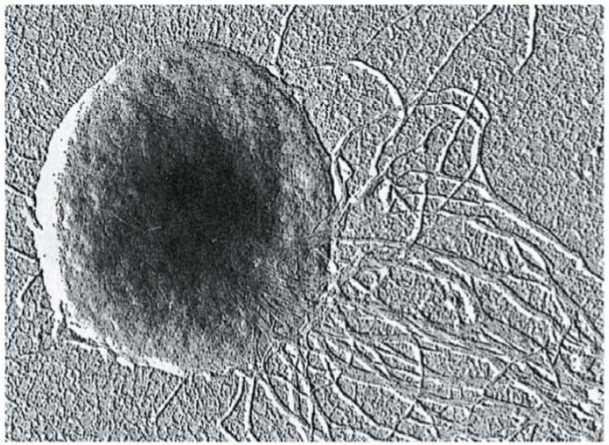
Methanogenesis



(a) *Methanococcus jannaschii*

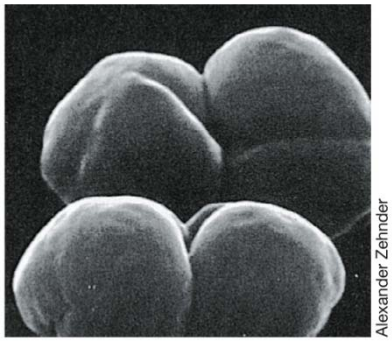
$CO_2 + 4H_2 \rightarrow CH_4 + 2\ H_2O$ (~ 1.5 ATP)

$\Delta G^{0'} = -131$ kJ/mol (pH_2 1 atm)

$Acetate + H^+ \rightarrow CH_4 + CO_2$ (0,5 – 1 ATP)

$\Delta G^{0'} = -36$ kJ/mol

- Strictly anaerobic Euryarchaea
- Methanobacteriales, Methanopyrales, Methanococcales, Methanomicrobiales, Methanosarcinales
- Most methanogens can grow with CO_2, formate and H_2
- Methanosarcinales also with acetate (methylamine, methanol)
- Unusual coenzymes
- 70% of the methane is formed from acetate



(d) *Methanosarcina barkeri*

Methanogenesis

Tabelle 19.4: Habitate der Methanogenen

Anoxische Sedimente: Marschland, Sumpf, Seesedimente, Reisfelder, feuchte Bodenanfüllungen
Tierische Verdauungsorgane: (a) Pansen von Wiederkäuern, zum Beispiel Rinder, Schafe, Elche, Rehe und Kamele (b) Caecum (Blinddarm) von Tieren mit Caecum wie Pferde und Hasen (c) Dickdarm von Tieren mit einem Magen wie bei Menschen, Schweinen und Hunden (d) Enddarm cellulolytischer Insekten (zum Beispiel Termiten)
Geothermale Quellen von $H_2 + CO_2$; Hydrothermalsysteme
Künstliche Einrichtungen zum biologischen Abbau: Klärschlammanlagen
Endosymbionten verschiedener anaerober Protozoen

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

~ 1.5 ATP

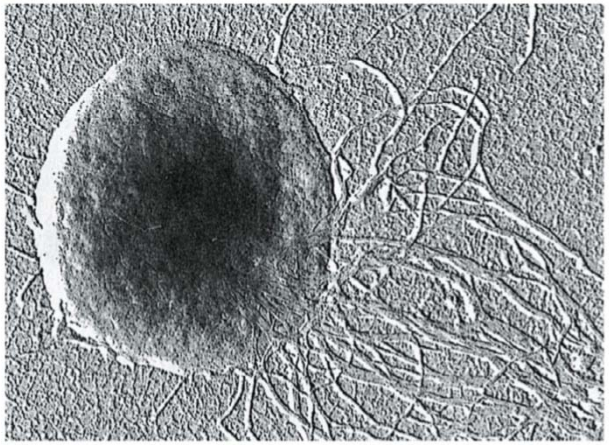
$\Delta G^{0'} = -131$ kJ/mol (pH_2 1 atm)

$$Acetate + H^+ \rightarrow CH_4 + CO_2$$

0,5 – 1 ATP

$\Delta G^{0'} = -36$ kJ/mol

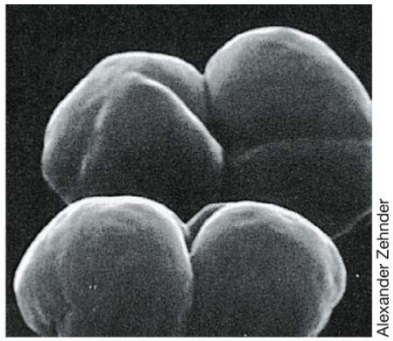
Methane is a very effective green house gas!



Helmut König und K. O. Stetter

(a)

Methanococcus jannaschii



Alexander Zehnder

(d)

Methanosarcina barkeri

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Methanogenesis from CO_2 and H_2

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

~ 1.5 ATP (1/2 ATP)

$\Delta G^{0'} = -131$ kJ/mol (pH_2 1 atm)

$\Delta G' = -131\ kJ/mol + 5.7\ kJ/mol \times lg\ ([P1]^a[P2]^b)/([S1]^c[\mathbf{10^{-5}}]^4)$

$= -131\ kJ/mol + 5.7\ kJ/mol \times 20$

$=$ **~-17 kJ/mol (pH_2 10^{-5} atm) → ~1/3 ATP**

- „Carbonate respiration“
- In natural habitats pH_2 is as low as 10^{-5} atm due to methanogenic activity
- This is the lower limit for (autotrophic) methanogenesis
- CO_2 fixation via the rCODH/ACS pathway (=reductive acetyl-CoA pathway)
- Allows however for syntrophic H_2 production

Methanogenesis from CO_2 and H_2

chemolithoautotrophic

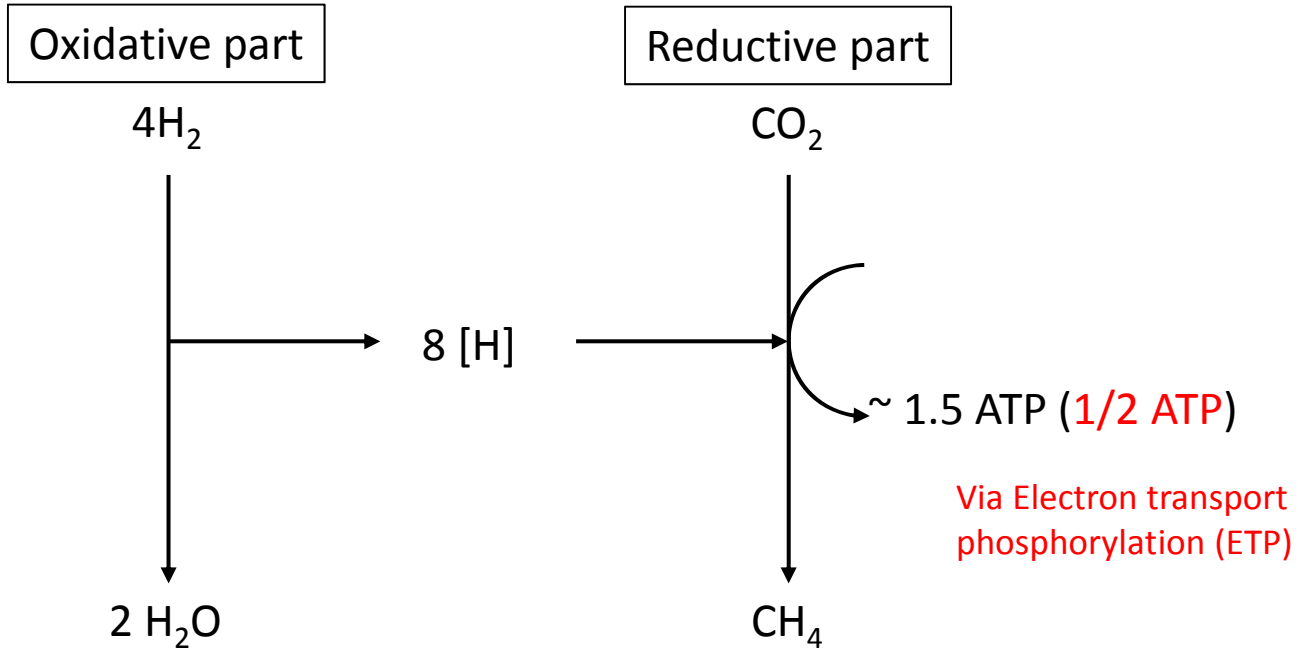
$CO_2 + 4H_2 \rightarrow CH_4 + 2\ H_2O$

~ 1.5 ATP (1/2 ATP)

$\Delta G^{0'}$ = -131 kJ/mol (pH_2 1 atm)

$\Delta G'$ = ~-17 kJ/mol (pH_2 10^{-5} atm)

→ ~1/3 ATP



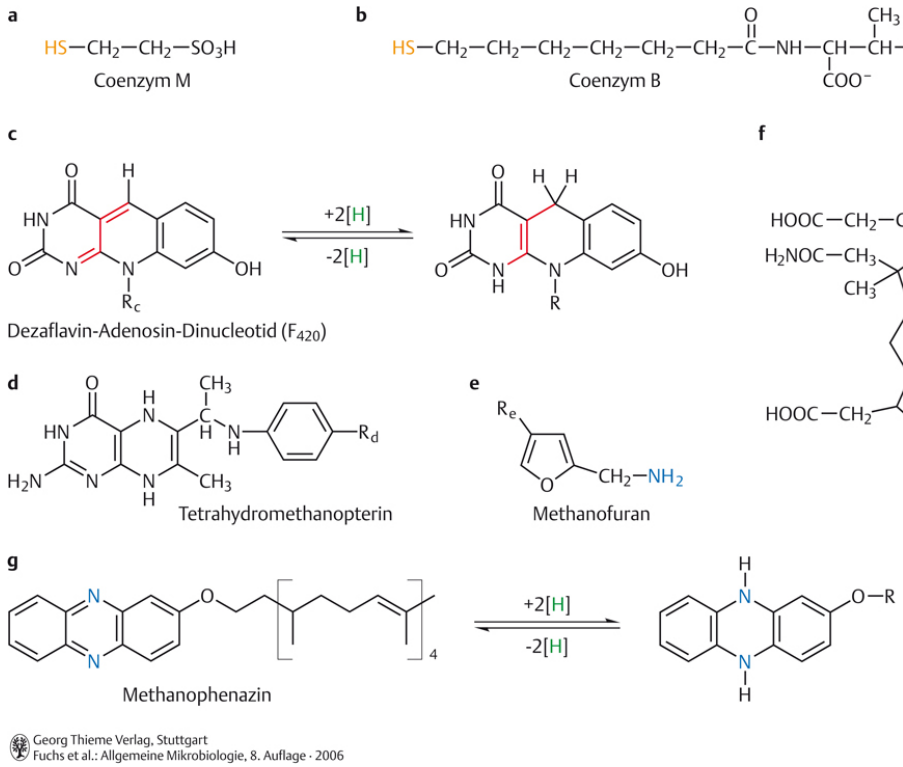
Methanogenesis

Electron transfer +
Energy generation

Electron transfer

C1 transfer

Quinone derivative
Electron transport



Methyl group reduction

- Strictly anaerobic Euryarchaea
- Methanobacteriales Methanopyrales, Methanococcales, Methanomicrobiales, Methanosarcinales
- Most methanogens can grow with CO_2, formate and H_2
- Methanosarcinales also with acetate (methylamine, methanol)
- Unusual coenzymes
- F_{420} absorbs light at 420 nm and fluoresce blue-green

Methanogenesis from CO_2 and H_2

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

~ 1.5 ATP (1/2 ATP)

$\Delta G^{0'}$ = -131 kJ/mol, (pH_2 1 atm)

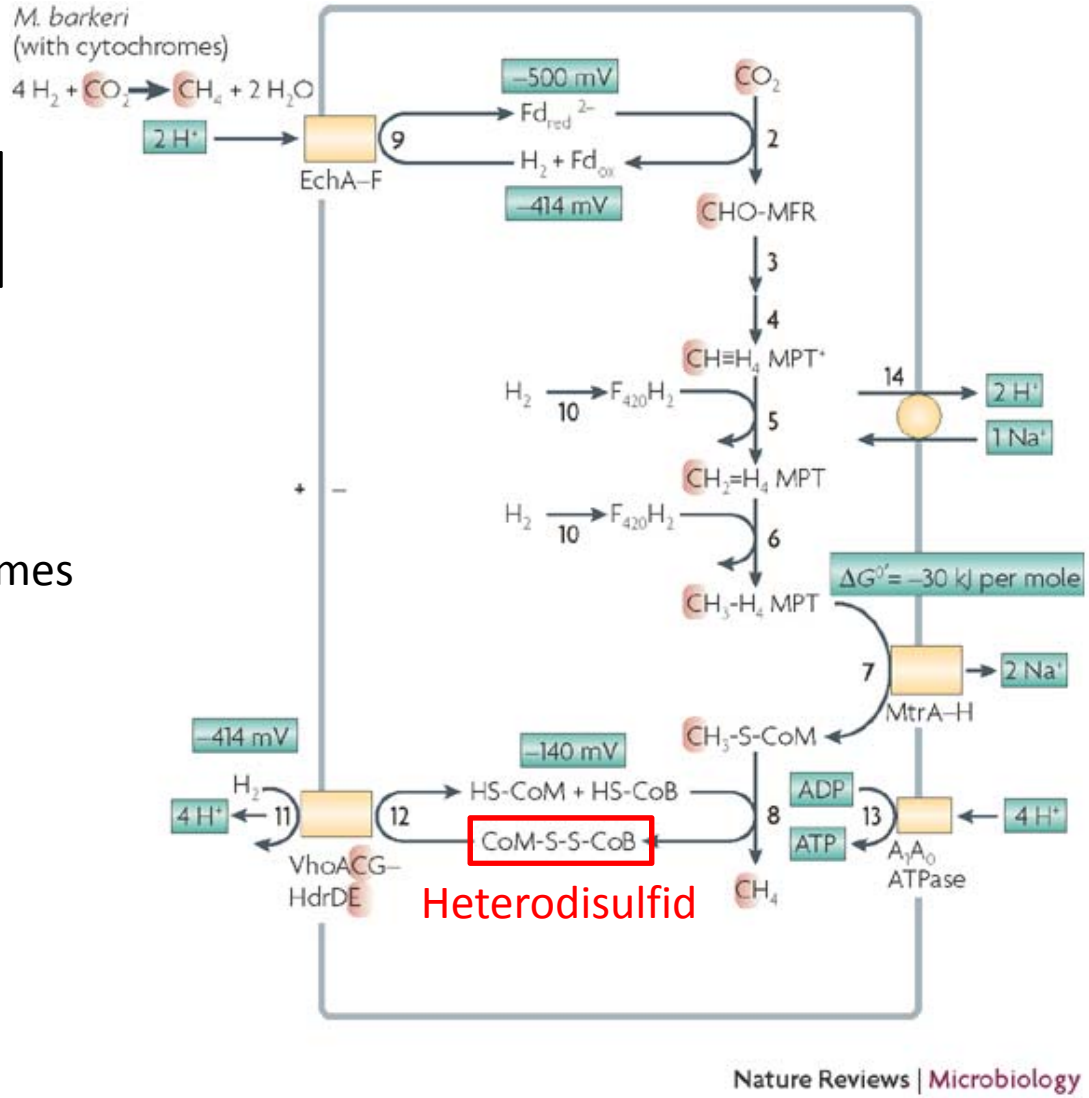
$\Delta G'$ = ~-17 kJ/mol (pH_2 10^{-5} atm)

Methanosarcinales with cytochromes and Methanophenazin!

→ higher growth yield

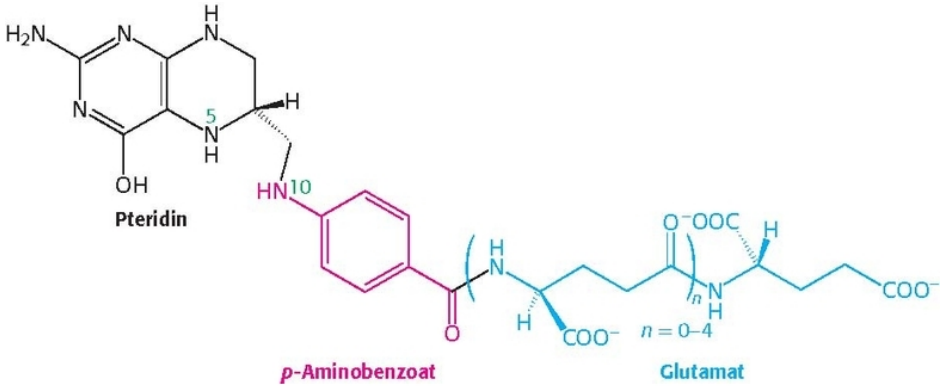
→ higher H_2 threshold

MFR = Methanofuran
H_4MPT = Tetrahydromenthanopterin
CoM-SH = Coenzym M
CoB-SH = Coenzym B

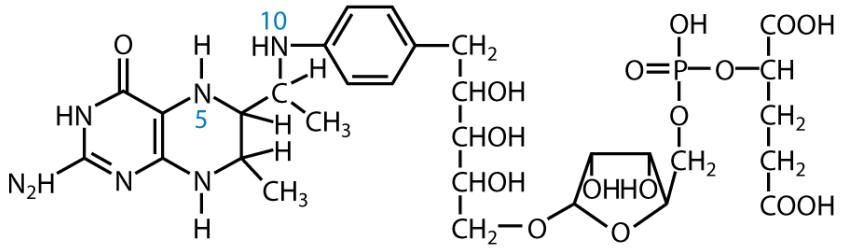


Thauer et al. (2008) Nature Reviews Microbiology 6, 579-591

Tetrahydrofolate - Tetrahydromethanopterin



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



5,6,7,8-Tetrahydromethanopterin (H_4MPT)

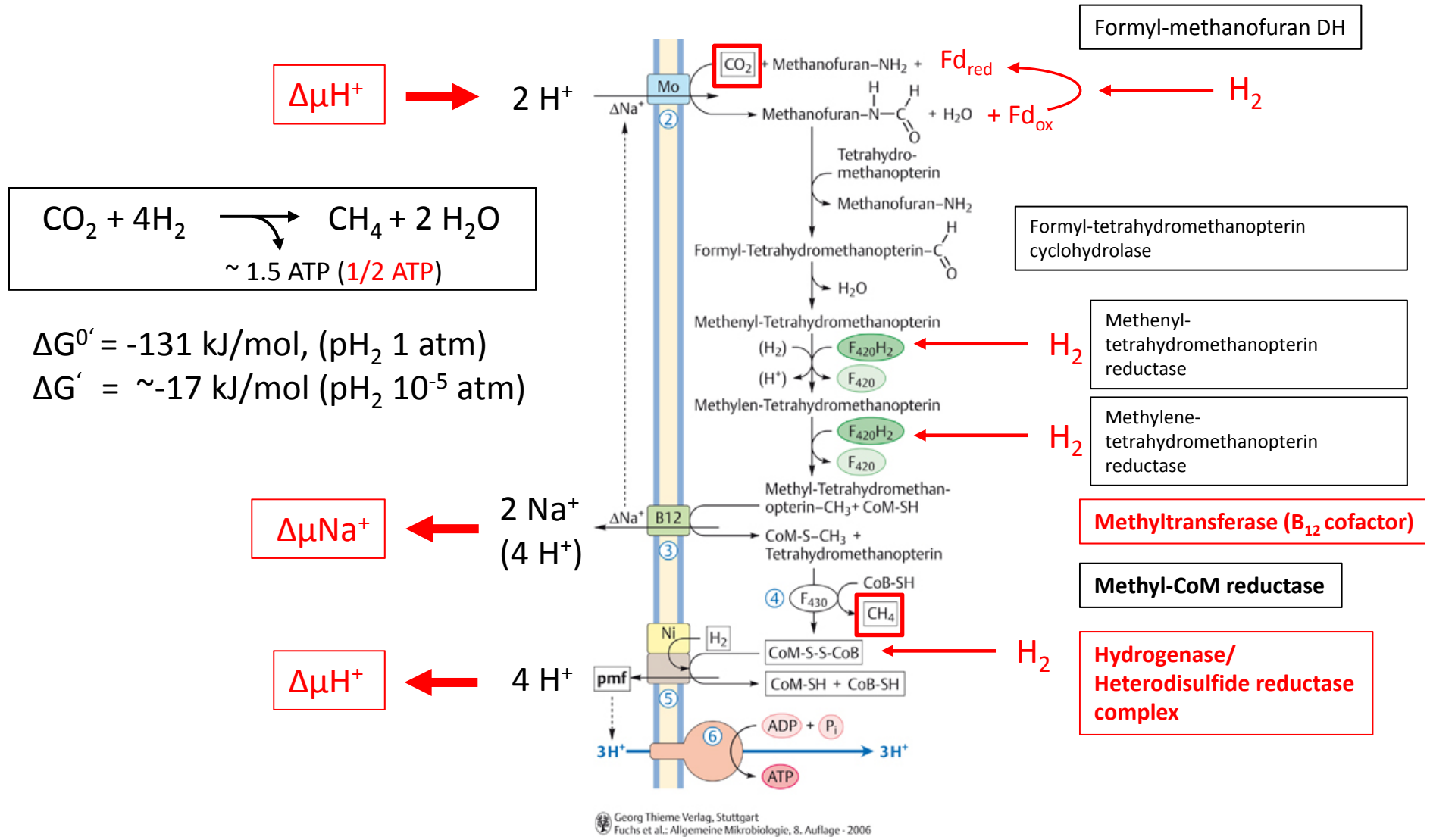
Methanogenesis from CO_2 and H_2

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

~ 1.5 ATP (1/2 ATP)

$\Delta G^{0'}$ = -131 kJ/mol, (pH_2 1 atm)

$\Delta G'$ = ~-17 kJ/mol (pH_2 10^{-5} atm)



Methanogenesis from CO_2 and H_2

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

~ 1.5 - 2 ATP (1/3 ATP)

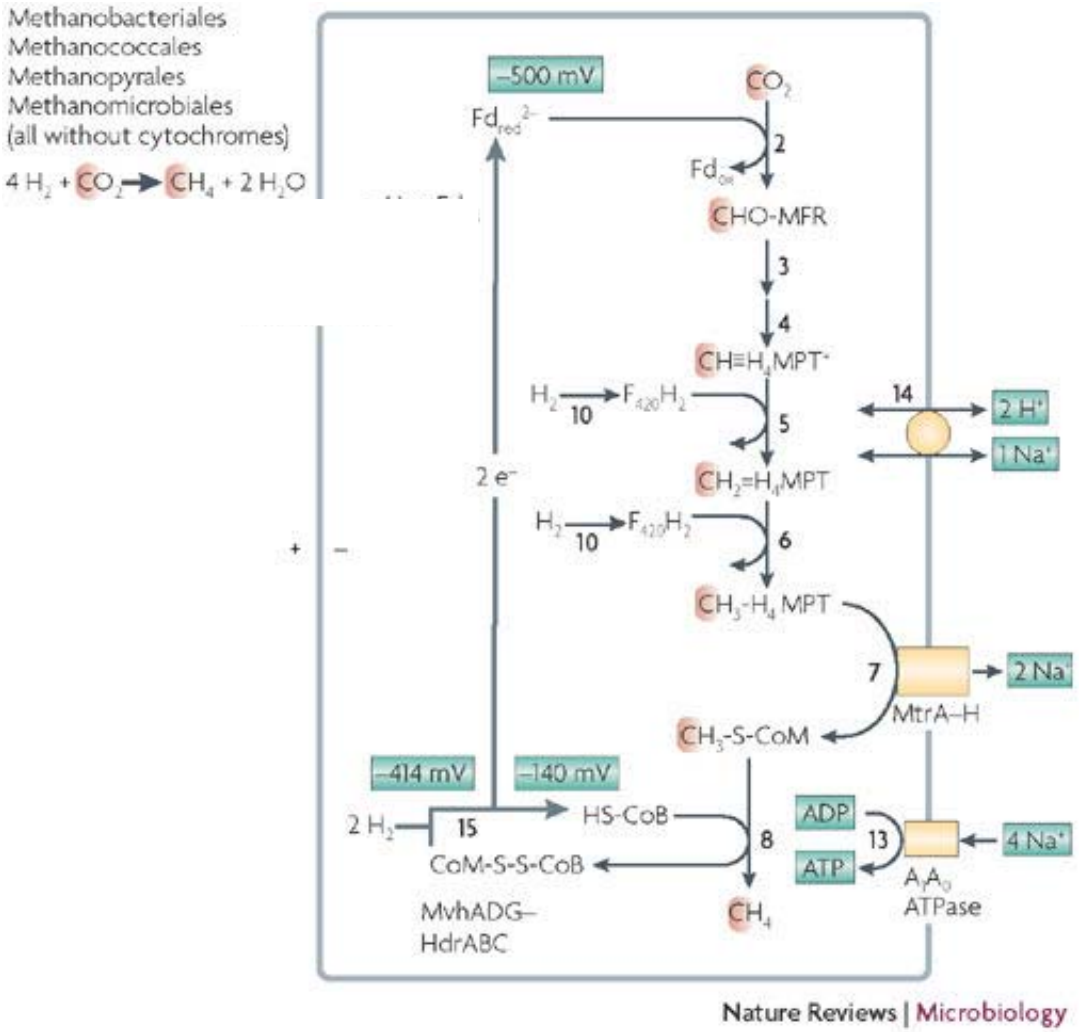
$\Delta G^{0'}$ = -131 kJ/mol, (pH_2 1 atm)

$\Delta G'$ = ~-17 kJ/mol (pH_2 10^{-5} atm)

Methanobacteriales, Methanococcales, Methanopyrales and Methanomicrobiales without cytochromes and Methanophenazin!

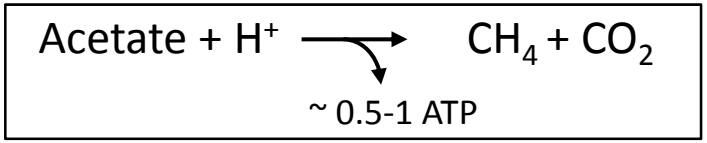
→ Lower growth yield

→ Lower H_2 threshold

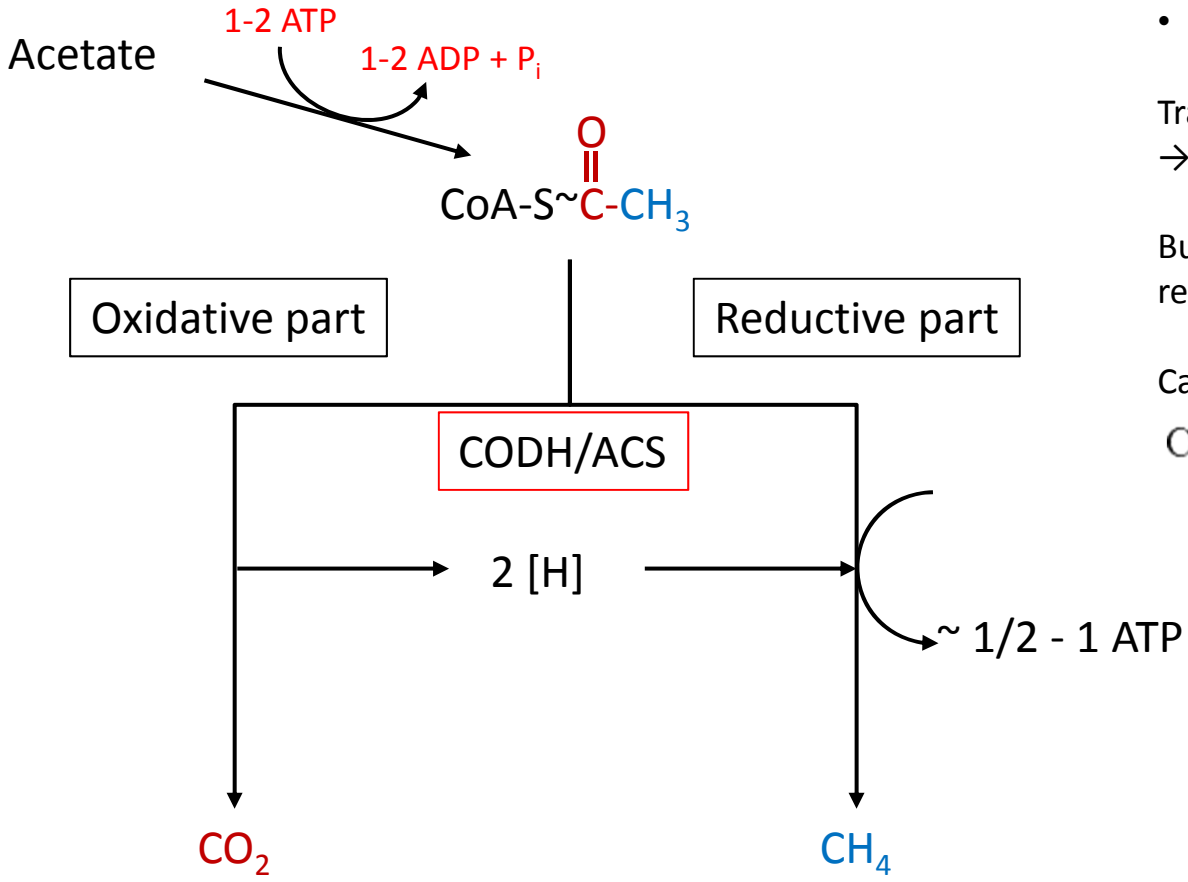


Thauer et al. (2008) Nature Reviews Microbiology 6, 579-591

Methanogenesis from acetate



$\Delta G^{0'} = -36$ kJ/mol



Disproportionation:

- Oxidation of the carboxyl group to CO_2
- Reduction of the methyl group to CH_4

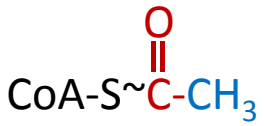
Transport of 7 H^+ (Na^+)/acetate
→ corresponding to ~2 ATP

But: Activation of acetate to acetyl-CoA requires at least 1 ATP

Carbonic anhydrase:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

Via Electron transport phosphorylation (ETP)



Methanogenesis from acetate

Acetate + H^+ → $CH_4 + CO_2$

~ 0.5-1 ATP

$\Delta G^{0'}$ = -36 kJ/mol

Disproportionation:

- Oxidation of the carboxyl group to CO_2
- Reduction of the methyl group to CH_4

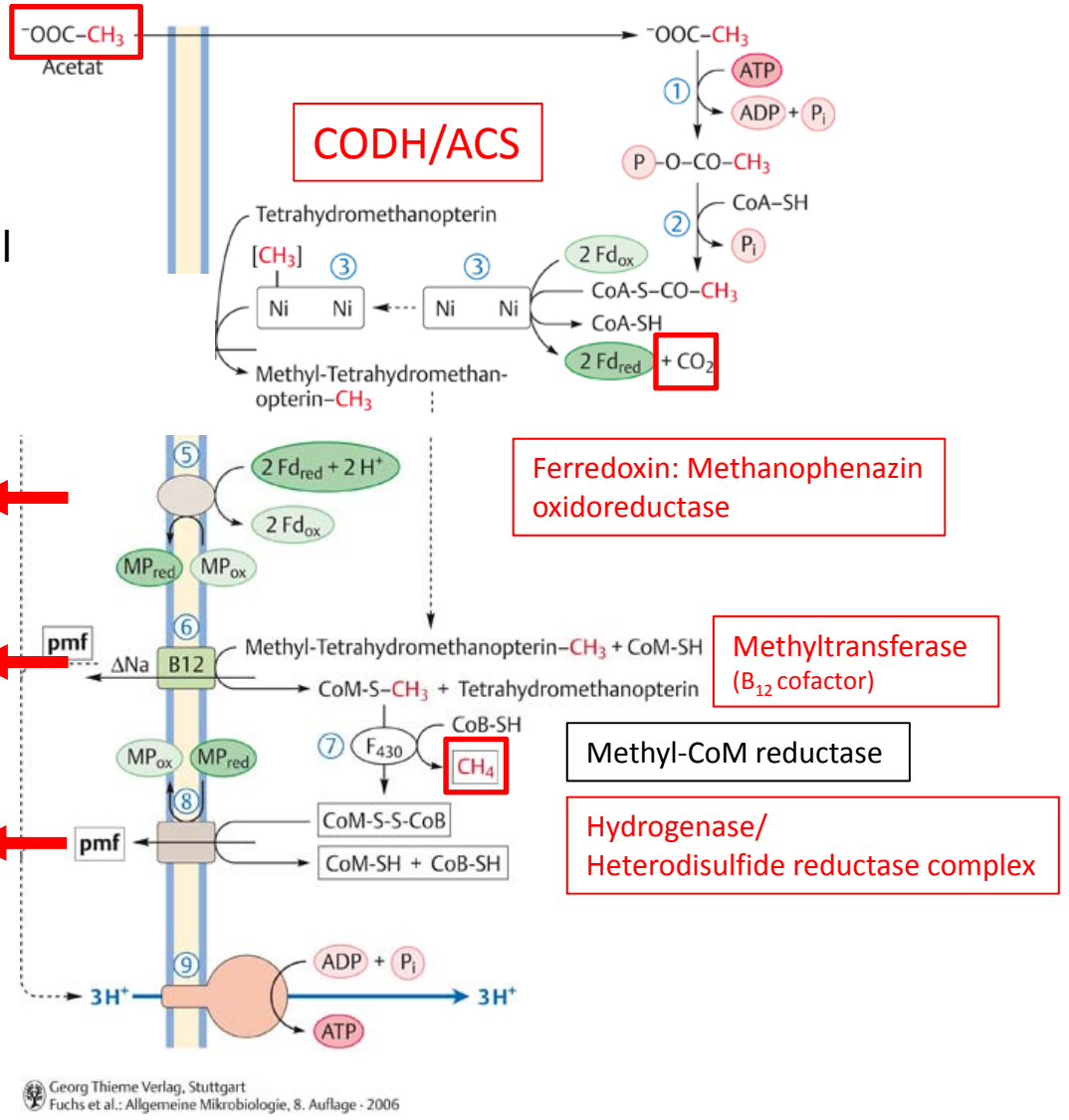
Transport of 6-8 H^+ (Na^+)/acetate

→ corresponding to 1,5 - 2 ATP

But: Activation of acetate to acetyl-CoA requires at least 1 ATP

Carbonic anhydrase:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$



Methanogenesis from acetate

Acetate + H^+ → $CH_4 + CO_2$

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Disproportionation:

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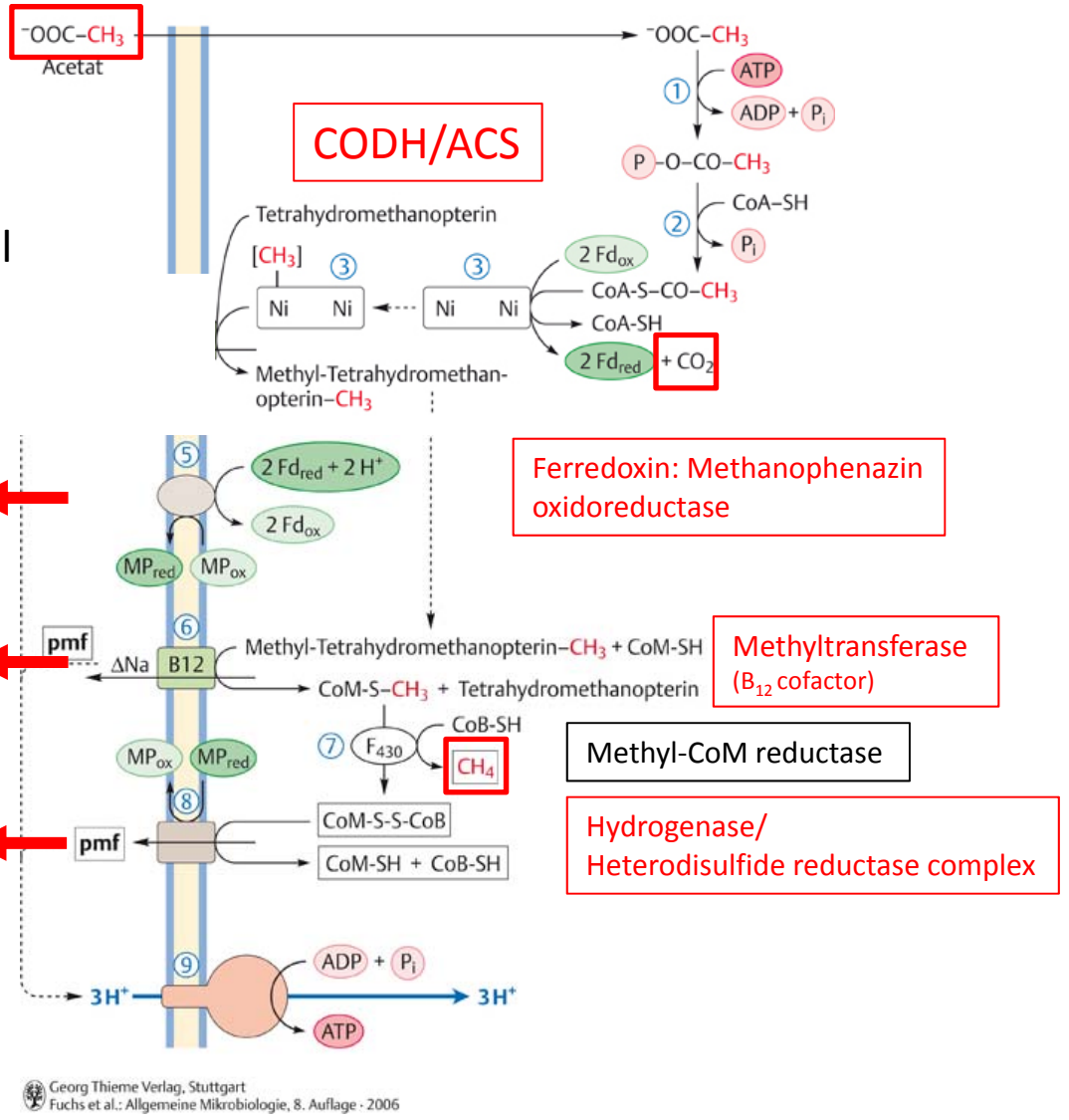

Anaerobic food chain

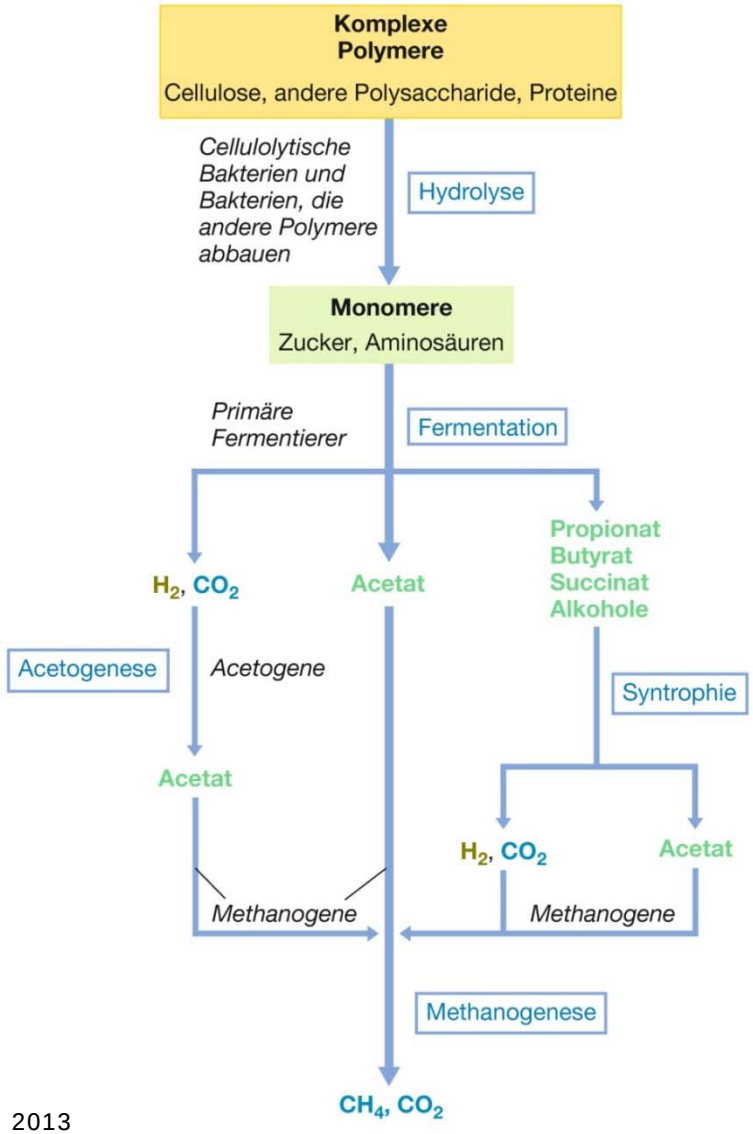

Abbildung 24.5: Anoxischer Abbau. Beim anoxischen Abbau kooperieren bei der Umwandlung komplexer organischer Substanzen von CH_4 zu CO_2 verschiedene Gruppen fermentativer Anaerobier. Diese Darstellung trifft auf Lebensräume zu, in denen sulfatreduzierende Bakterien eine untergeordnete Rolle spielen,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

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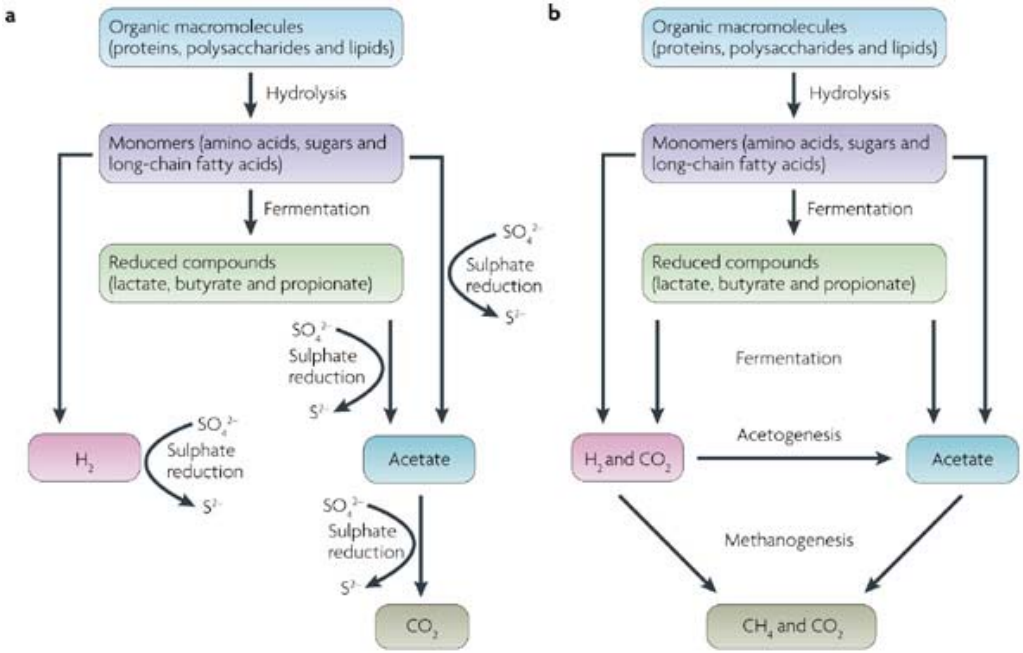
Sulfate reduction

- Competition of sulfate reducers with syntrophic methanogenic communities and acetogens for fermentation products (lactate, propionate, acetate, H_2 in anaerobic environments)
- Sulfate reducers with broader substrate spectrum than e.g. methanogens
- Higher affinity and lower threshold for H_2

→ sulfate reducers outcompete methanogens at elevated SO_4^{2-} concentrations e.g. in marine sediments



Muyzer G & Stams AJM, 2008

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

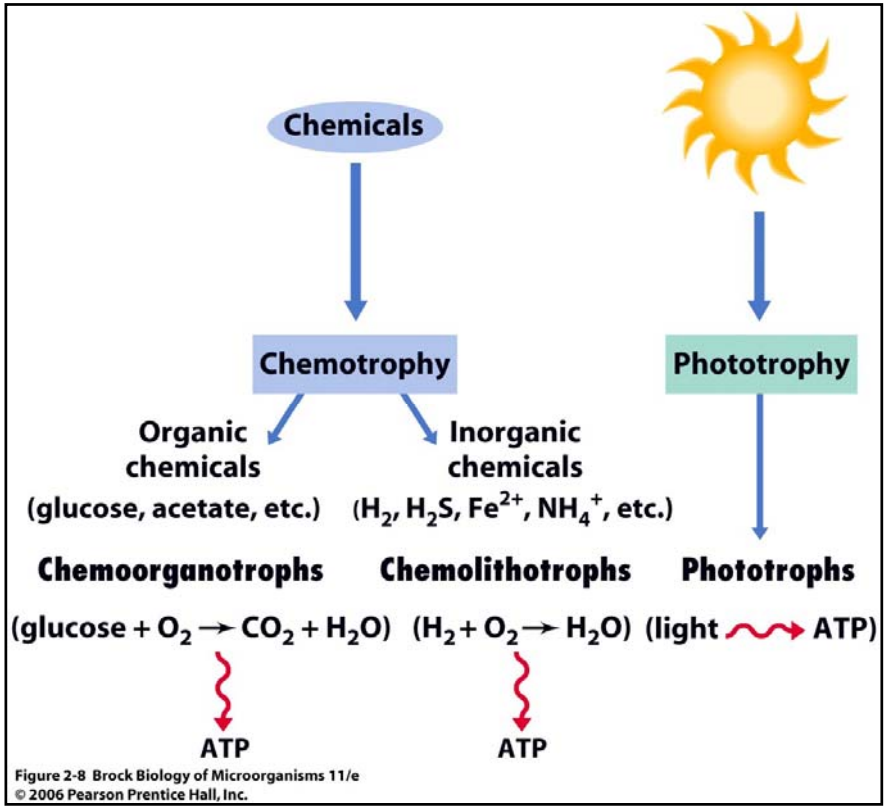


Figure 2-8 Brock Biology of Microorganisms 11/e
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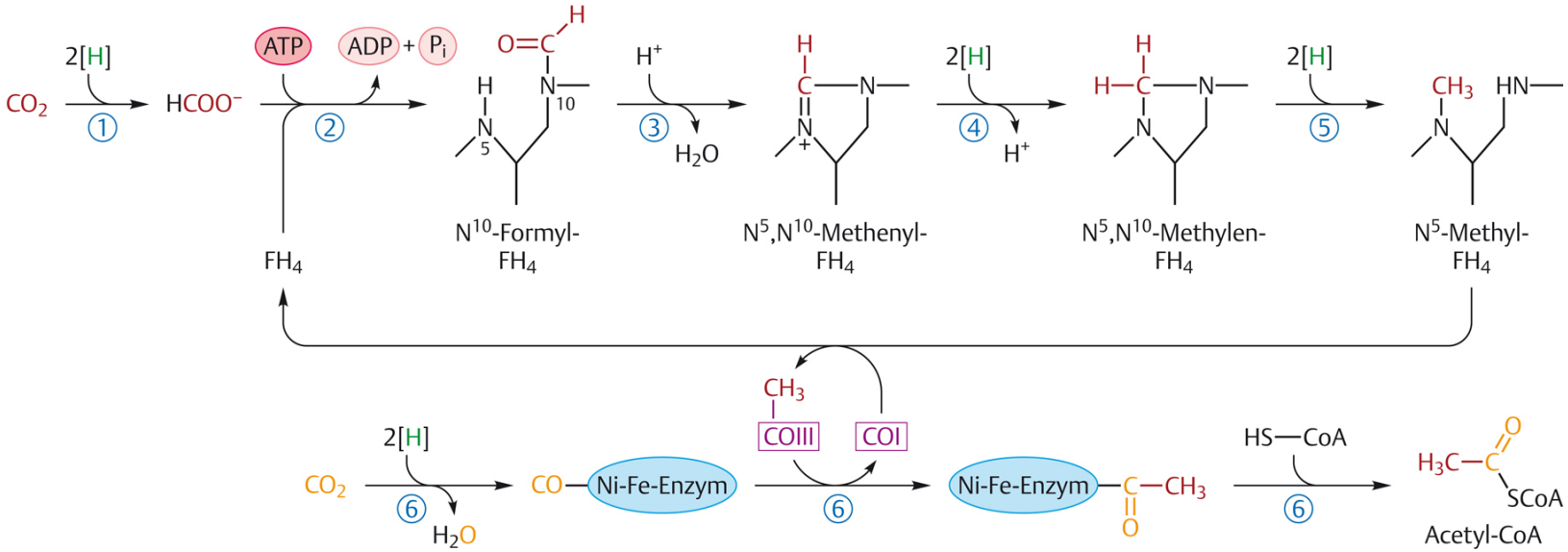
CO_2 Fixation

Reductive acetyl-CoA pathway

reductive CODH/ACS pathway = Wood-Ljungdal pathway

∑ ~ 1 ATP + 2 NAD(P)H ($F_{420}H_2$) + 3 ferredoxin/ pyruvate



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

Acetyl-CoA synthase/CO dehydrogenase

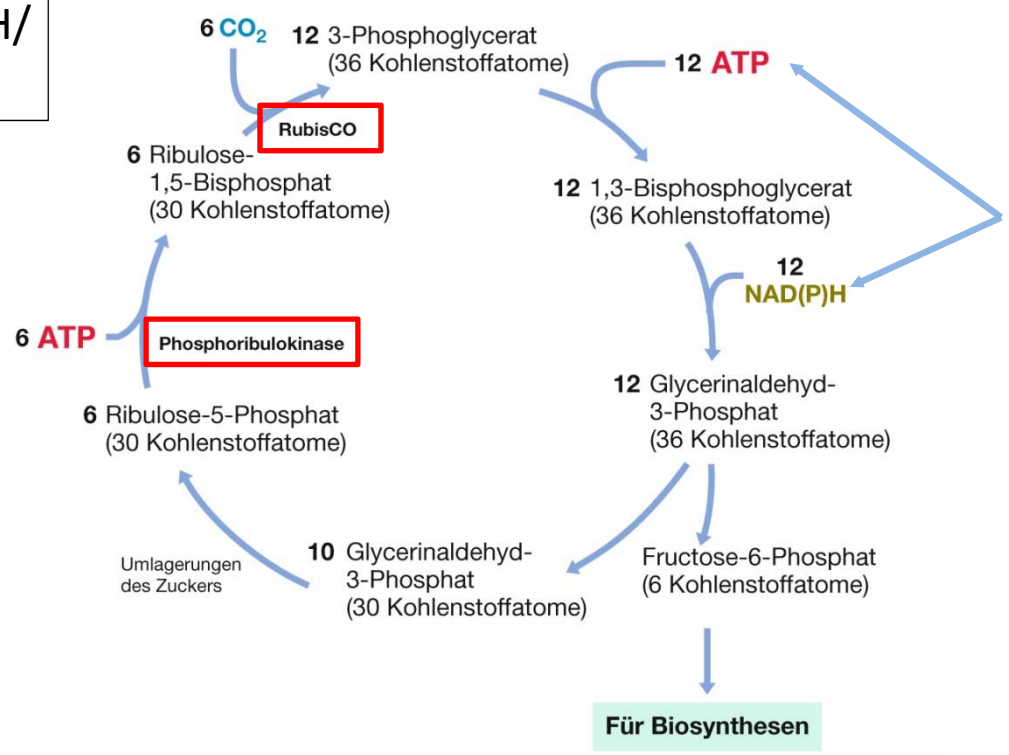
- Strict anaerobes: Methanogenic and sulfate reducing Euryarchaea, acetogens, some proteobacteria a.o., sulfate reducers
- Acetyl-CoA, pyruvate
- Well suited for the assimilation of C1 units

Reductive pentose phosphate cycle

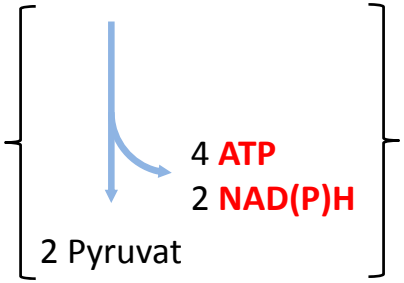
(Calvin-Benson cycle)

RubisCO = Ribulose-bisphosphate carboxylase/oxygenase

∑ 7ATP +5 NAD(P)H/ pyruvate



From light dependent reactions of photosynthesis
(ETP reactions in chemosynthesis)

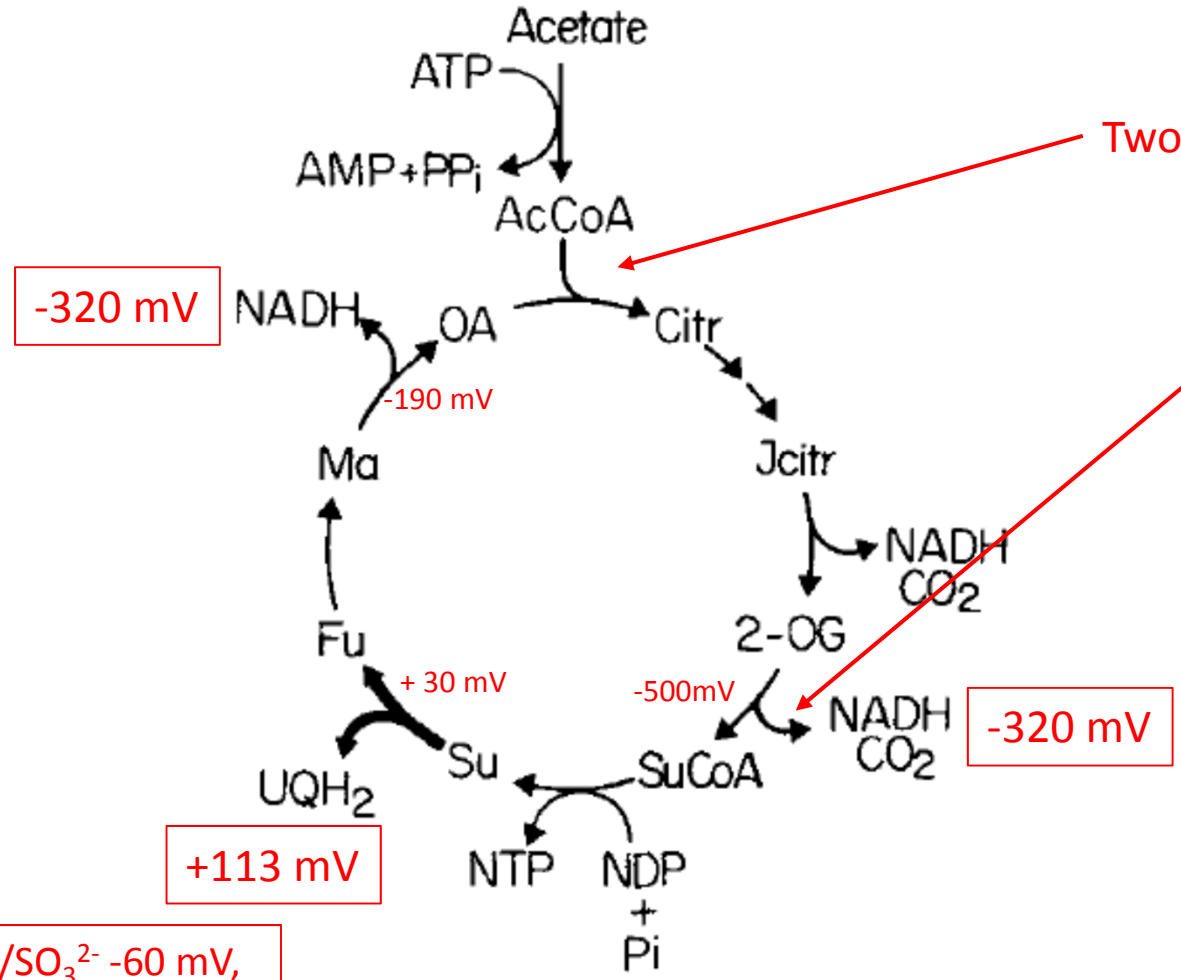


- Plants, algae, cyanobacteria, most aerobic and facultativ aerobic (chemolithoautotrophic) Bacteria, anoxygen phototrophic purple bacteria
- Triosephosphates, 3-phosphoglycerate, sugar phosphates as intermediates

The Citric acid cycle (oxidative)

in aerobic organisms

Acetate activation (two ATP equivalents)



Two irreversible steps <-30 kJ/mol

→ Energy dissipation

APS/SO_3^{2-} -60 mV,
SO_3^{2-}/HS^- -110 mV!

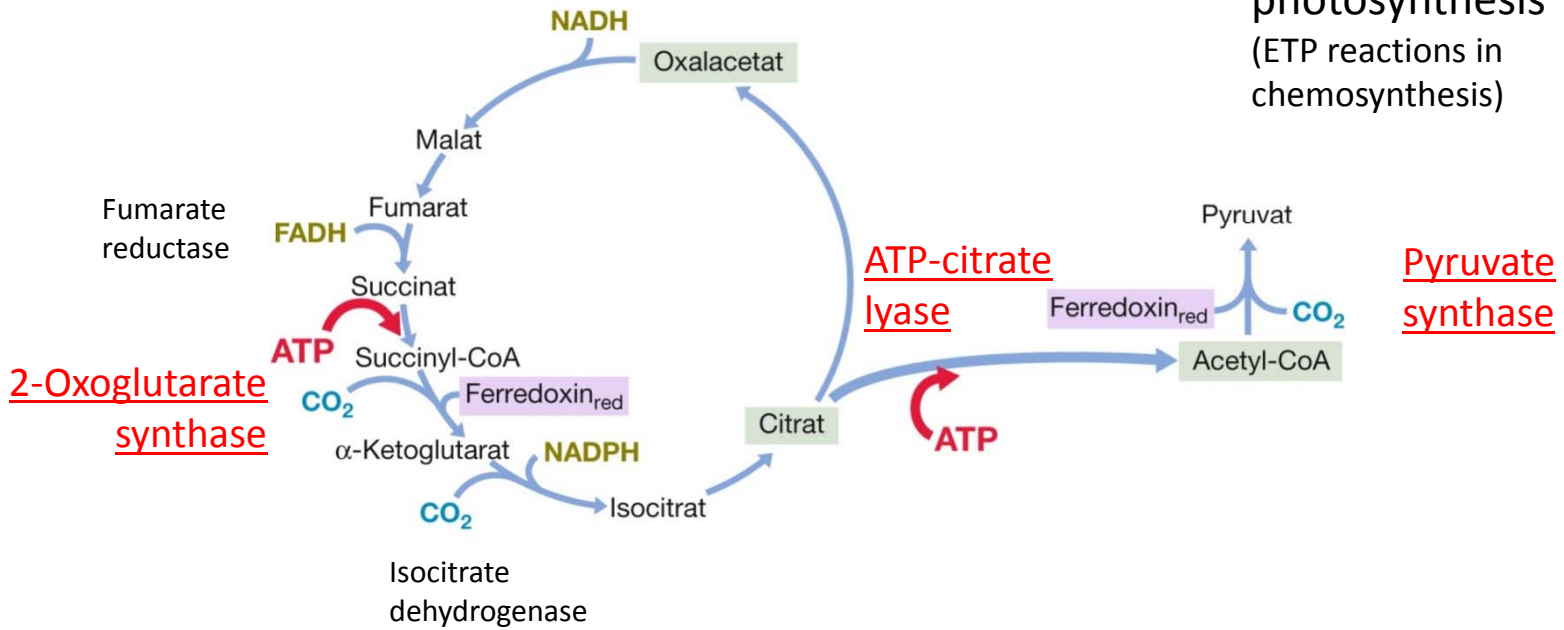
Thauer RK (1988) Citric-acid cycle, 50 years on. Eur J Biochem 176:497-508

Reductive citric acid cycle

(Arnon-Buchanan cycle)

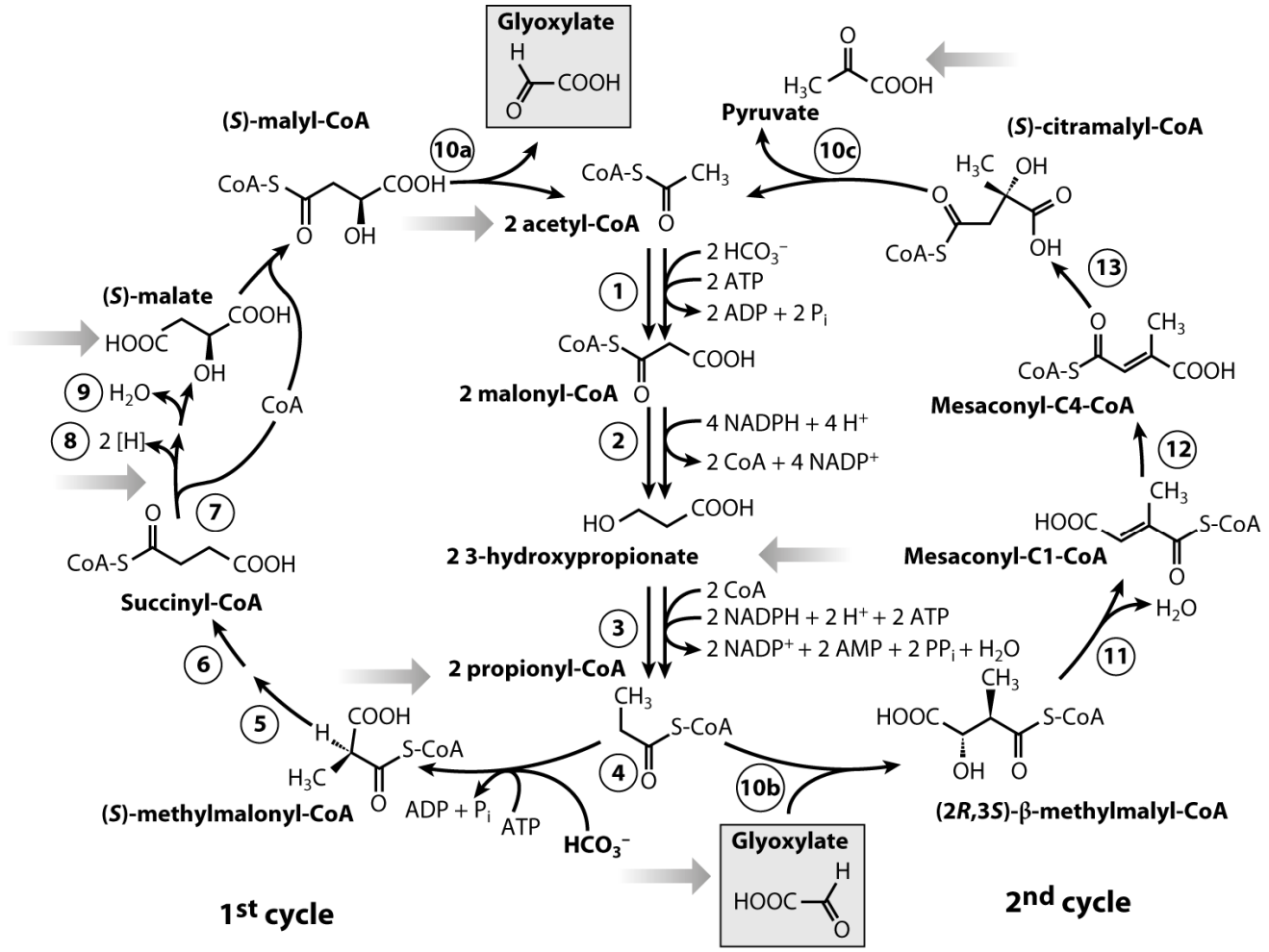
∑ 2 (-3) ATP + 3 NAD(P)H + 2 ferredoxin/ pyruvate

ATP and ferredoxin from light dependent reactions of photosynthesis
(ETP reactions in chemosynthesis)



- Anaerobic Green sulfur bacteria (Chlorobiales) and other Proteobacteria, Aquificales (microaerophilic)
- Acetyl-CoA, pyruvate, oxaloacetate, succinyl-CoA, 2-oxoglutarate, (PEP)
- Advantages under anaerobic, microaerophilic conditions

Hydroxypropionate bi-cycle

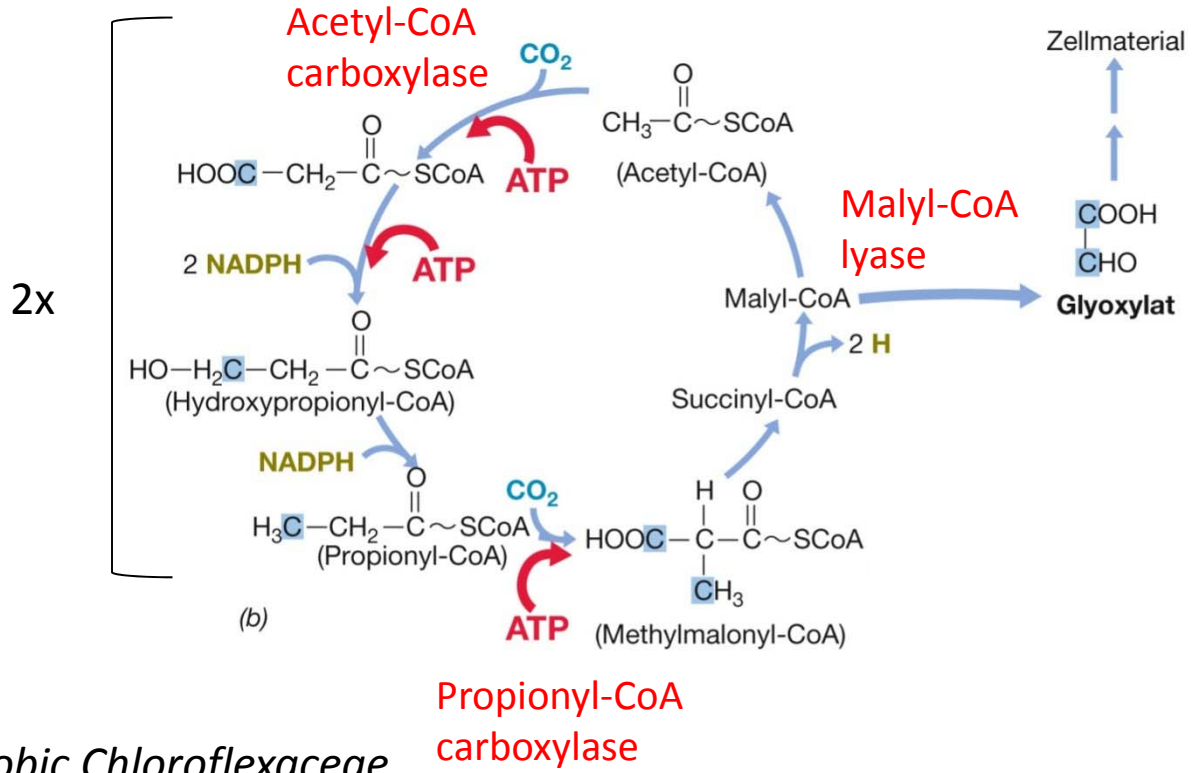


Fuchs G. 2011.
Annu. Rev. Microbiol. 65:631–58

$\sum$ ~ 7 ATP + 6 NAD(P)H (- 1QH_2) / pyruvate

3-Hydroxypropionate bi-cycle

∑ ~ 7 ATP + 6 NAD(P)H (- 1QH_2) / pyruvate

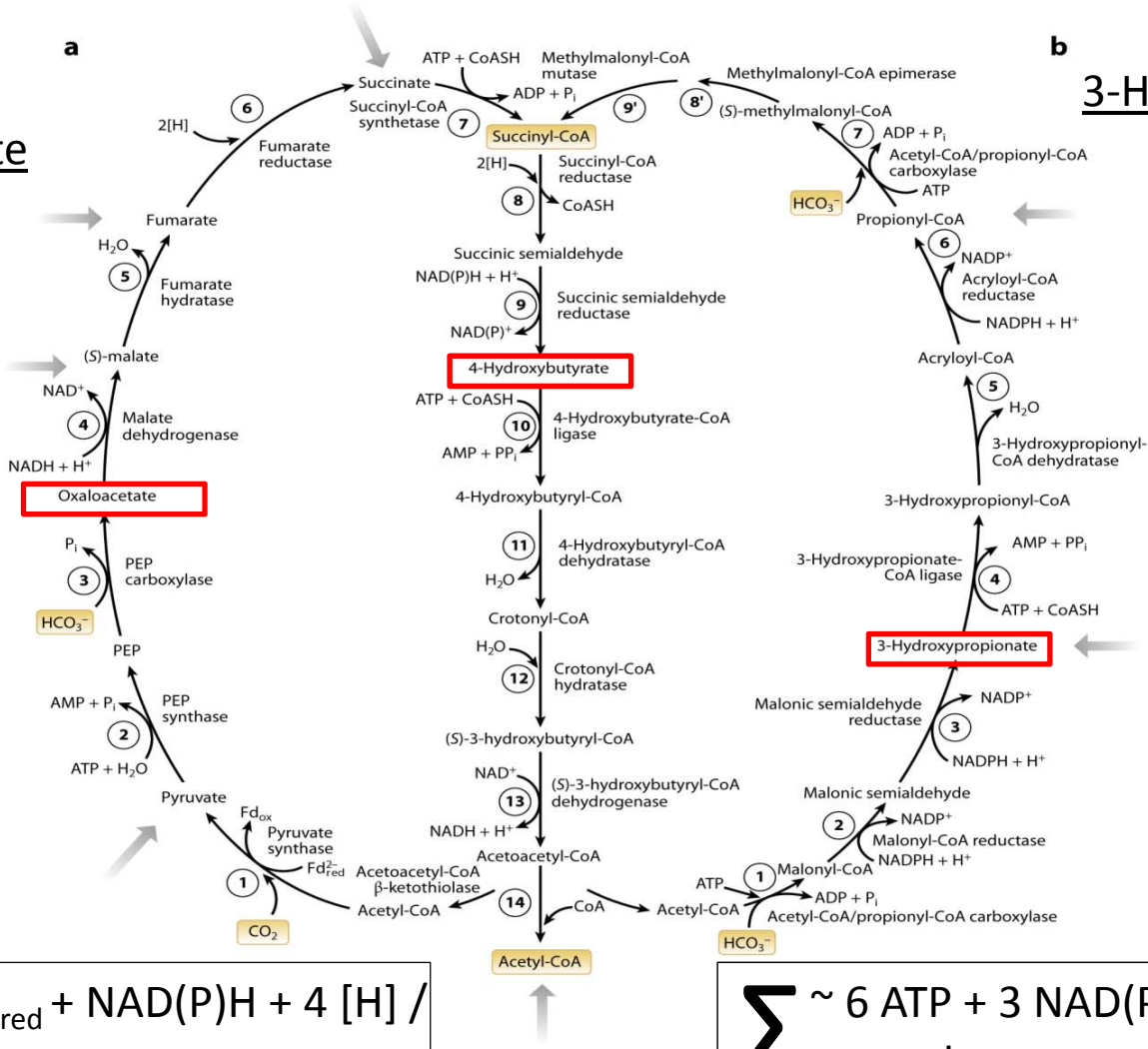


- *Anaerobic Chloroflexaceae*
- Acetyl-CoA, pyruvate, succinyl-CoA
- Suited for mixotrophic assimilation of fermentation products under oligotrophic conditions

Dicarboxylate/4-Hydroxybutyrate cycle
3-Hydroxypropionate/4-Hydroxybutyrate cycle

Dicarboxylat/ 4-Hydroxybutyrate cycle

Anaerobic Crenarchaeota (Thermoproteales, Desulfurococcales



3-Hydroxypropionate/ 4-Hydroxybutyrate cycle

Aerobic Crenarchaeota (e.g. *Sulfolobus*) and Thaumarchaeota (Thermoproteales, Desulfurococcales

$\sum$ ~ 5 ATP + 2 Fd_{red} + NAD(P)H + 4 [H] / pyruvate

$\sum$ ~ 6 ATP + 3 NAD(P)H + 4 [H] / pyruvate

Fuchs G. 2011.
Annu. Rev. Microbiol. 65:631–58

Questions 5

- What is methanogenesis? What are the substrates, what the products?
- Which organisms perform methanogenesis?
- Name a special feature of methanogens/methanogenesis.
- How does methanogenesis from acetate basically works? What is oxidized, what is reduced? What is especially challenging with respect to energetics?
- How does chemolithotrophic methanogens fix CO_2?
- Which are the energy gaining reactions in both types of methanogenesis?
- What is the lower pH_2 limit of methanogenesis and what does that mean for syntrophy?
- Name at least 3 pathways of CO_2 fixation!

Fragen 5

- Was ist Methanogenese? Was sind die Substrate, was die Produkte?
- Welche Organismen betreiben Methanogenese?
- Benennen Sie spezielle Eigenschaften der Methanogenen.
- Wie verläuft grundsätzlich die Methanogenese aus Acetat? Was wird oxidiert, was reduziert? Was ist im Hinblick auf die Energetik eine besondere Herausforderung?
- Wie fixieren chemolithoautotrophe Methanogene CO_2?
- Welches sind die energieliefernden Reaktionen der in beiden Formen der Methanogenese?
- Was ist das untere Limit des H_2 Partialdruckes für die Methanogenese und was bedeutet das für die Snytrophie
- Benennen Sie mindestens 3 Wege zur CO_2 Fixierung

Übung: Mikrobielles Wachstum I

Aufgabe:

1.) E. coli wurde auf eine OD_{600} von 0,1 aus einer exponentiell wachsenden Kultur auf das gleiche Medium (Minimalmedium mit 20 mM als C- & E-Quelle) überimpft und wächst exponentiell mit einer Verdopplungszeit von 40 min. Wie lange dauert es, bis die Kultur eine OD_{600} von 5,0 erreicht hat?

2.) Ihre Kurve soll in etwa alle 0.5 OD Einheiten einen Meßpunkt zeigen; zu welchen Zeitpunkten müssen Sie Probe nehmen?

Übung: Mikrobielles Wachstum I

Aufgabe:

1. *E. coli* wurde auf eine OD_{600} von 0,1 aus einer exponentiell wachsenden Kultur auf das gleiche Medium (Minimalmedium mit 20 mM als C- & E-Quelle) überimpft und wächst exponentiell mit einer Verdopplungszeit von 40 min. Wie lange dauert es, bis die Kultur eine OD_{600} von 5,0 erreicht hat?

Wachstumsrate μ:

$$\mu = \frac{\ln x \ - lnx_0}{t \ - t_0}$$

Verdoppelungszeit t_D:

$$t_D = \frac{\ln 2}{\mu}$$

Übung: Mikrobielles Wachstum I

Wachstumsrate μ:

$$\mu = \frac{\ln x \ - ln x_0}{t \ - t_0}$$

$$\rightarrow t \ - t_0 = \frac{\ln x \ - \ln x_0}{\mu}$$

$$\rightarrow t \ - t_0 = \frac{\ln 5 - \ln 0{,}1}{0{,}017 min^{-1}} = \frac{1{,}61 - (-2{,}30)}{0{,}017 min^{-1}} = \frac{3{,}91}{0{,}017 min^{-1}}$$

$$\rightarrow t \ - t_0 = \frac{3{,}91}{0{,}017 min^{-1}} = \underline{\mathbf{230\ min}}$$

Verdoppelungszeit t_D:

$$t_D = \frac{\ln 2}{\mu}$$

$$\rightarrow \mu = \frac{\ln 2}{t_D} = \frac{0{,}69}{40\ min} = 0{,}017\ min^{-1}$$

Übung: Mikrobielles Wachstum I

Wachstumsrate μ:

$$\mu = \frac{\ln x - ln x_0}{t - t_0}$$

$$\rightarrow t - t_0 = \frac{\ln x - \ln x_0}{\mu}$$

$$\rightarrow t - t_0 = \frac{\ln 0.5 - \ln 0{,}1}{0{,}017 min^{-1}} = \frac{-0{,}69 - (-2{,}30)}{0{,}017 min^{-1}} = \frac{1{,}61}{0{,}017 min^{-1}} = 95 \text{ min}$$

$$\rightarrow t - t_0 = \frac{\ln 1 - \ln 0{,}5}{0{,}017 min^{-1}} = \frac{0 - (-0{,}69)}{0{,}017 min^{-1}} = \frac{1{,}61}{0{,}017 min^{-1}} = 40 \text{ min}$$

$$\rightarrow t - t_0 = \frac{\ln 1{,}5 - \ln 1}{0{,}017 min^{-1}} = \frac{0{,}4 - 0}{0{,}017 min^{-1}} = \frac{1{,}61}{0{,}017 min^{-1}} = 23 \text{ min}$$

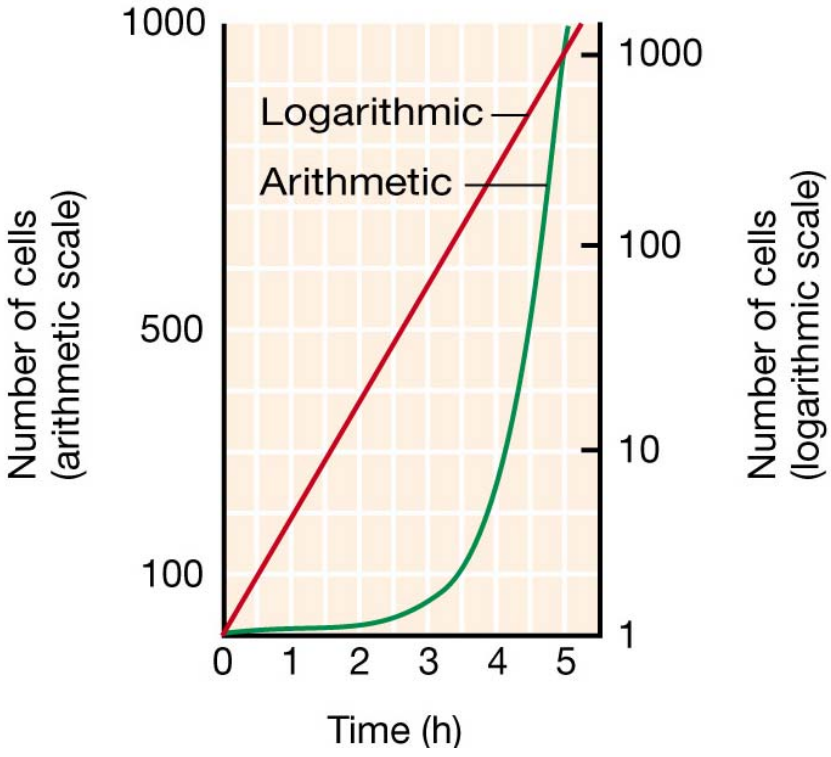
→ 17,2 min → 13,1 min → → → → 6,2 min (OD 4.5 – OD 5)

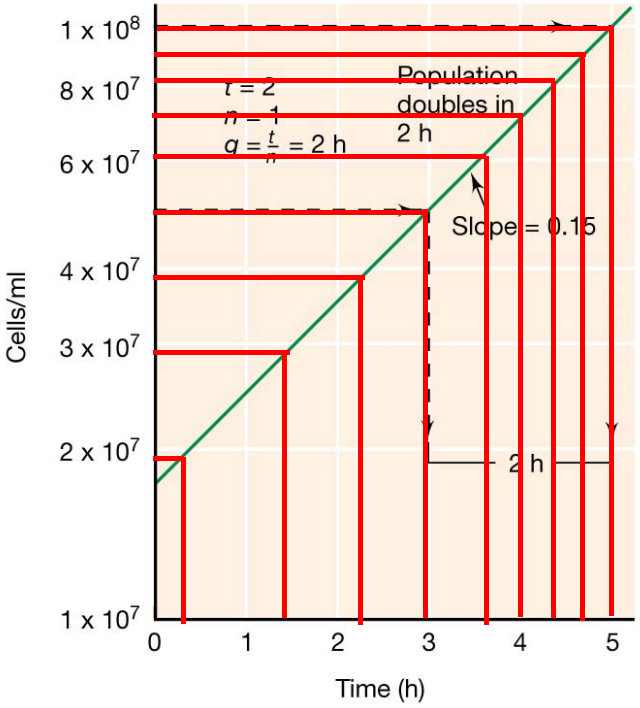
Verdoppelungszeit t_D:

$$t_D = \frac{\ln 2}{\mu}$$

$$\rightarrow \mu = \frac{\ln 2}{t_D} = \frac{0{,}69}{40\ min} = 0{,}017\ min^{-1}$$

Übung: Mikrobielles Wachstum I





Übung: Mikrobielles Wachstum II

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Microbial growth and physiology: a call for better craftsmanship

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Virtually every microbiological experiment starts with the cultivation of microbes. Consequently, as originally pointed out by Monod (1949), handling microbial cultures is a fundamental methodology of microbiology and mastering different cultivation techniques should be part of every microbiologist's craftsmanship. This is particularly important for research in microbial physiology, as the composition and behavior of microbes is strongly dependent on their growth environment. It has been pointed out repeatedly by eminent microbiologists that we should give more attention to the media and culturing conditions. However, this is obviously not adhered to with sufficient rigor as mistakes in basic cultivation principles are frequently found in the published research literature. The most frequent mistakes are the use of inappropriate growth media and little or no control of the specific growth rate, and some examples will be discussed here in detail. Therefore, this is a call for better microbiological craftsmanship when cultivating microbial cultures for physiological experiments. This call is not only addressed to researchers but it is probably even more important for the teaching of our discipline.

Keywords: cultivation, batch, continuous culture, growth media, nutrient limitation, physiology

"The study of the growth of bacterial cultures does not constitute a specialized subject or branch of research: it is the basic method of Microbiology"

Monod (1949).

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Wie entwickelt man ein Medium?

Übung: Mikrobielles Wachstum II

Minimalmedium (definierte Zusammensetzung), C limitiertes Wachstum (heterotroph)

(A)

Elemental constituents in dry biomass	Average[a] (% of DW)[b]	Range (% of DW)[b]	Average $Y_{X/E}$ for C-limited growth
C	50	45[c]–58[d]	1
O	21	18[e]–31[f]	–
N	12	5[d]–17[g]	8
P	3	1.2[h]–10[i]	33
S	1	0.3–1.3	100
K	1	0.2[i]–5[k]	100
Mg	0.5	0.1[l]–1.1	200
Fe	0.5	0.01–0.5	200

1 g TZ/ 1 g C

→ ~50% der C & E Quelle gehen in den Baustoffwechsel

Wie viel Stickstoff (NH_4Cl in mM) muss dem Medium mindestens zugegeben werden, damit der Stickstoff bei einer gegebenen C- & E-Quelle (20 mM Glucose) nicht limitierend wird?