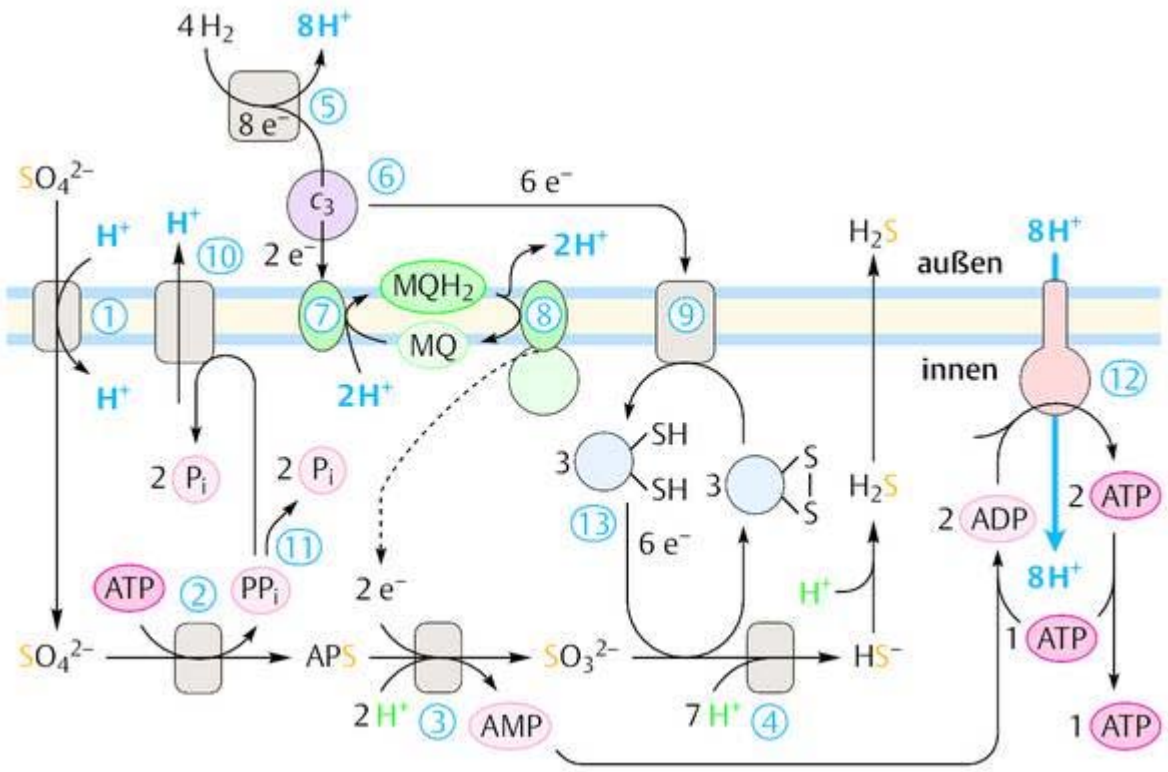
Microbiology II

Sulfate reduction



aus Fuchs, Allgemeine Mikrobiologie, 9. Auflage, Thieme

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**

Redox potentials

Tabelle A 1.2: Redoxpotenziale[a], die für die Mikrobiologie von Bedeutung sind

Redoxpaar	E_0' (V)
SO_4^{2-}/HSO_3^-	–0,52
CO_2/Formiat	–0,43
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(Fortsetzung nächste Spalte)

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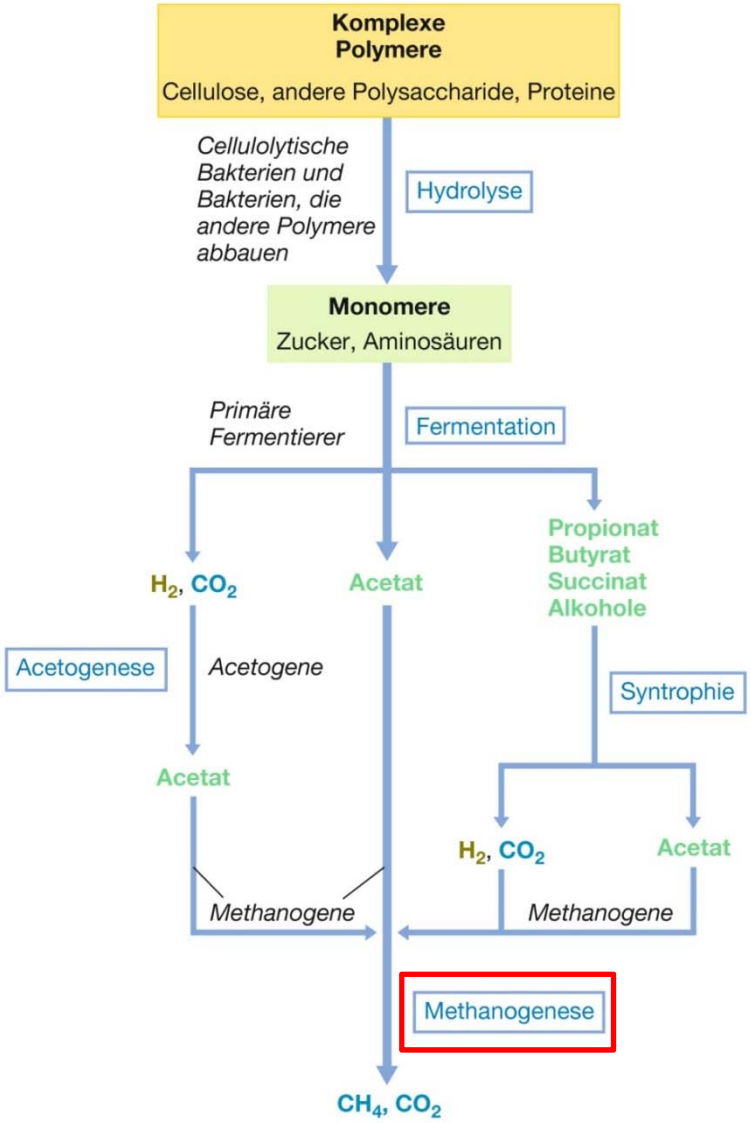
Anaerobic food chain



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.

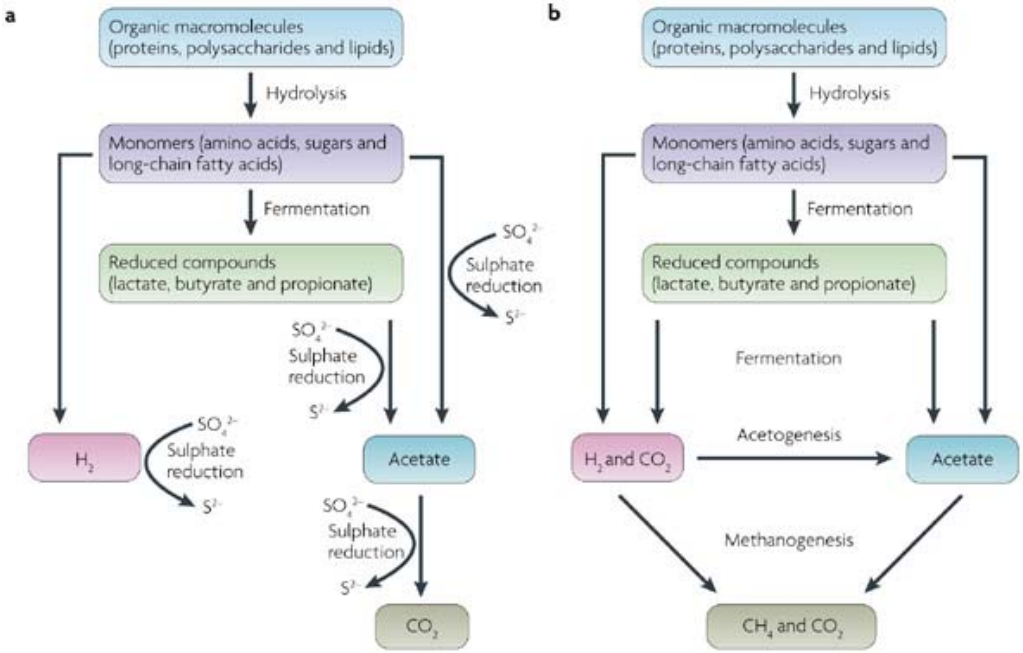
(Thauer RK et al. (2008) Nature Reviews Microbiology 6, 579-591)

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.

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

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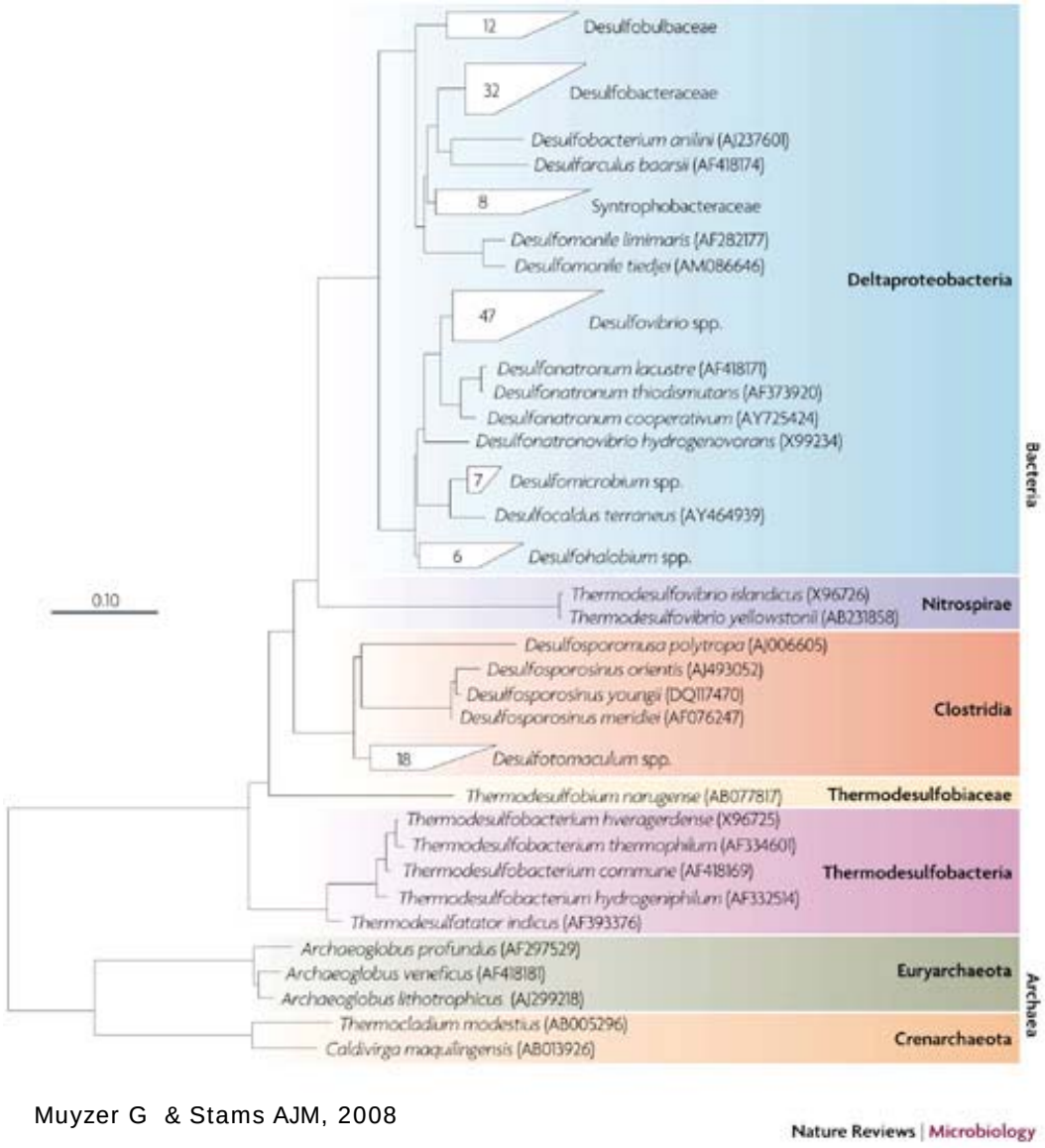
Sulfate reducers



- In five bacterial lineages, two archaeal
- anaerobic
- Ubiquitous in habitats where SO_4^{2-} is present
- Seawater ~28 mM SO_4^{2-}

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Sulfate reducers

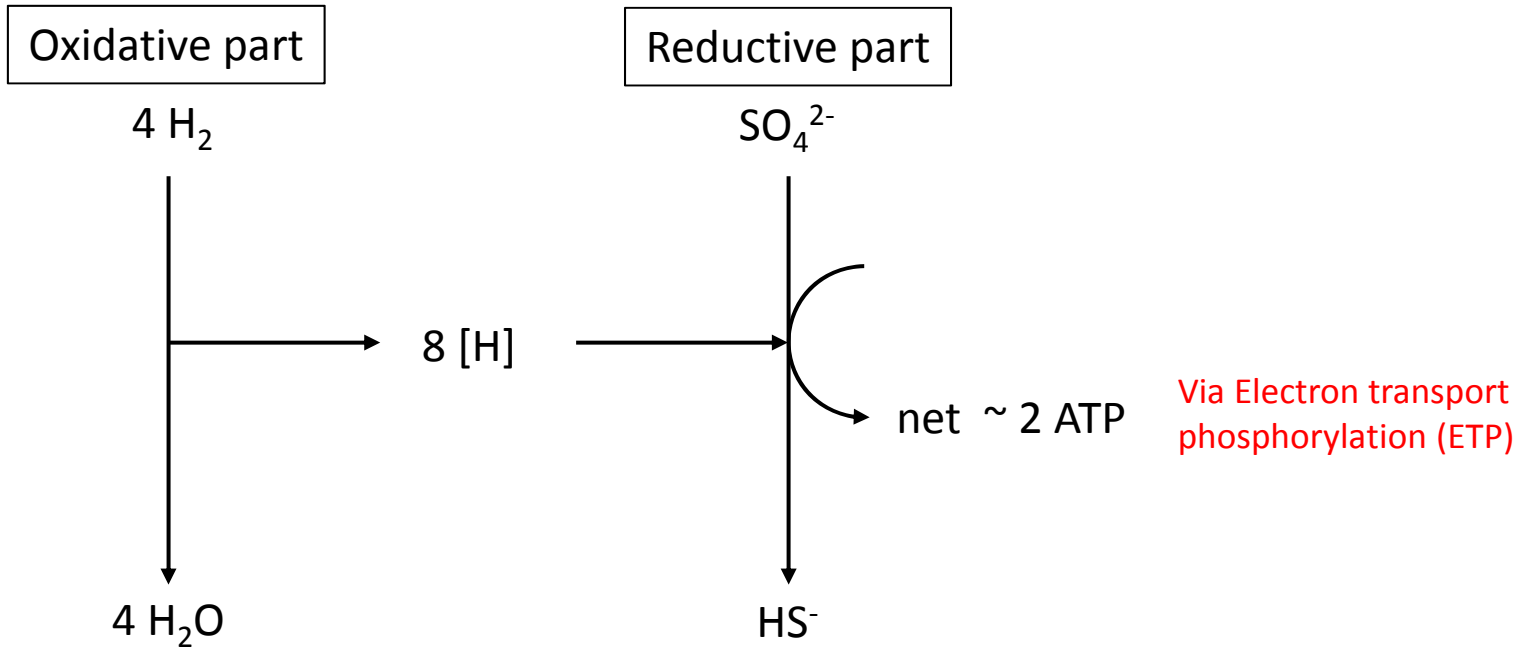
Three main physiological groups:

Sulphate reducing reaction	$\Delta G^{0'}$	group	
$SO_4^{2-} + 4\ H_2 + H^+ \rightarrow HS^- + 4\ H_2O$	-151.9	autotrophs	Many sulfate reducers, e.g. Desulfobacteriaceae (proteobacteria) Desulfotomaculum (gram positive) Archaeoglobus (archaea)
SO_4^{2-} + Acetate $\rightarrow HS^- + 2\ HCO_3^-$ (also: fatty acids, hydrocarbons, aromatic compounds → Acetyl-CoA)	-47.6	Complete oxidizers (heterotrophs)	Desulfobacteriaceae (proteobacteria) Desulfotomaculum (gram positive) Archaeoglobus (archaea) Many others
0.75 SO_4^{2-} + propionate → 0.75 HS^- + 2 acetate + HCO_3^- +0.25 H^+ 0.5 SO_4^{2-} + butyrate → 0.5 HS^- + 2 acetate + 0.5 H^+ 0.5 SO_4^{2-} + lactate → 0.5 HS^- + acetate + HCO_3^-	-37.7 -27.8 -80.2	Incomplete oxidizers (heterotrophs)	Desulfovibrio

Sulfate reduction

(chemolitoautotrophic)

$4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$ $\Delta G^{0'} = -152$ kJ/mol SO_4^{2-} red.



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Sulfate activation
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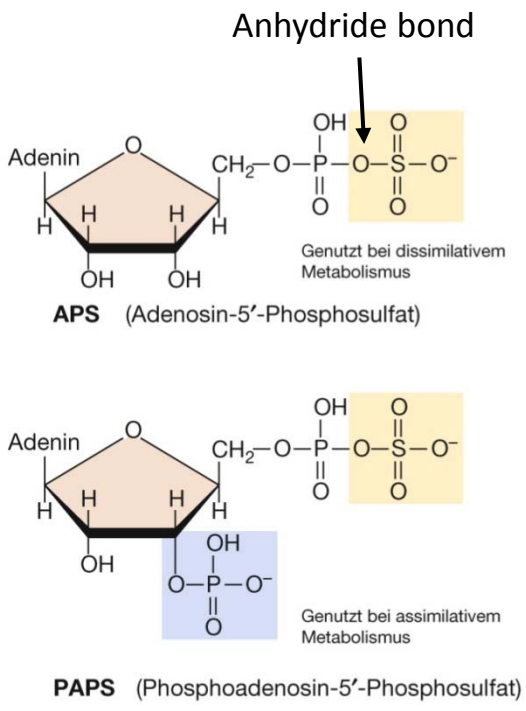
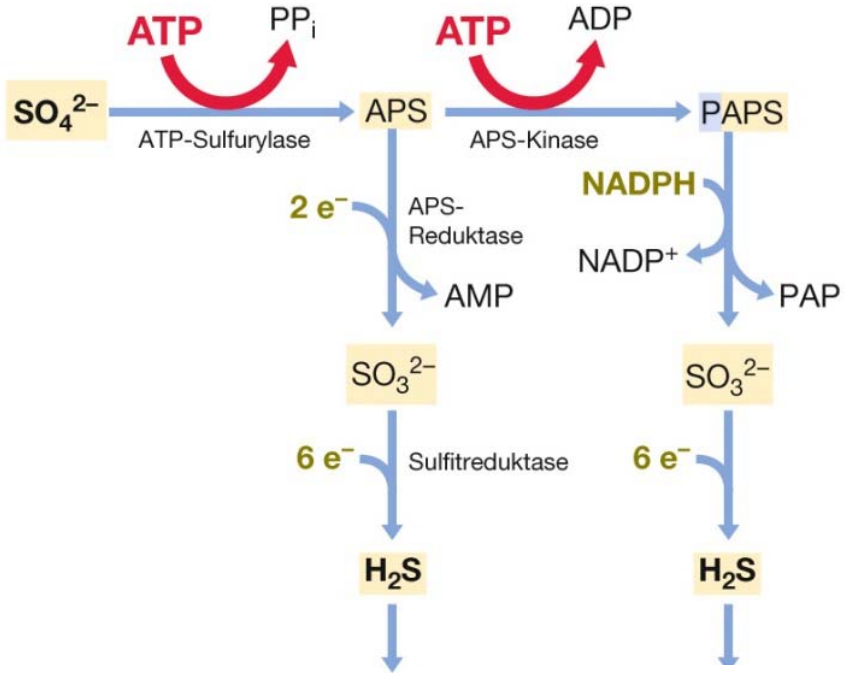




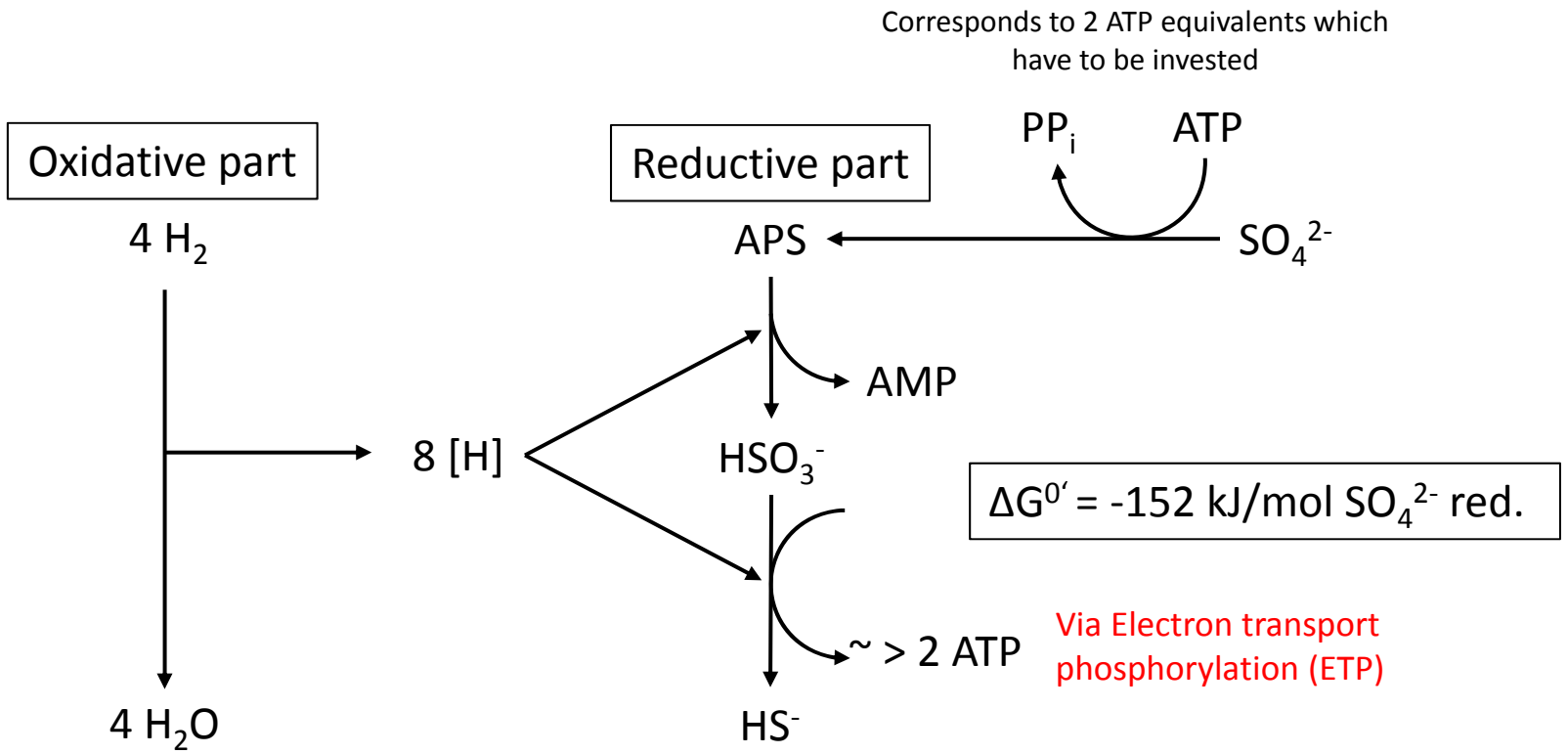
Dissimilatory sulphate reduction – excretion
Organic sulfur (Cystein, Methionine, etc.) – *B. subtilis*
Sulphate esters

Organic sulfur (Cystein, Methionine, etc.) – *E. coli*
Sulphate esters

$\underline{SO_4^{2-}}$ activation energy consuming

Sulfate reduction

(chemolitoautotrophic)



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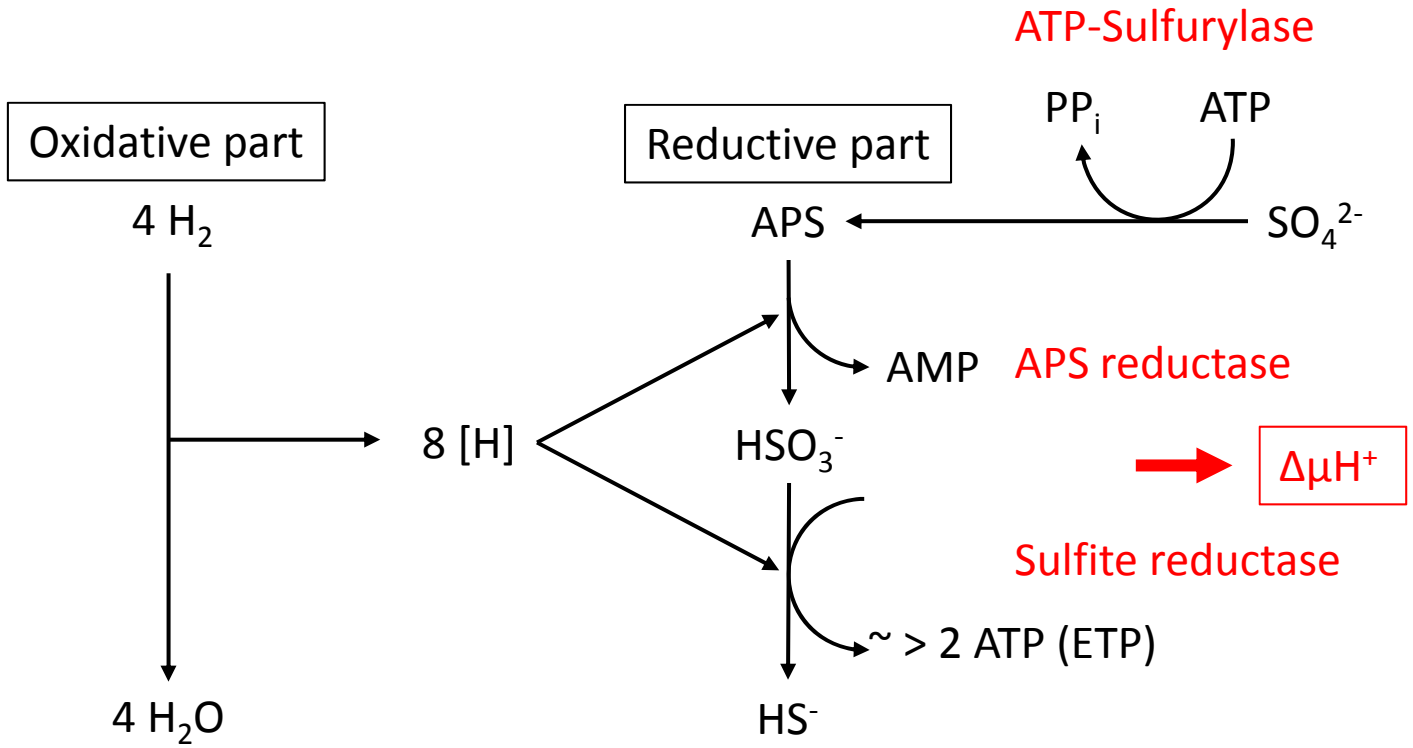

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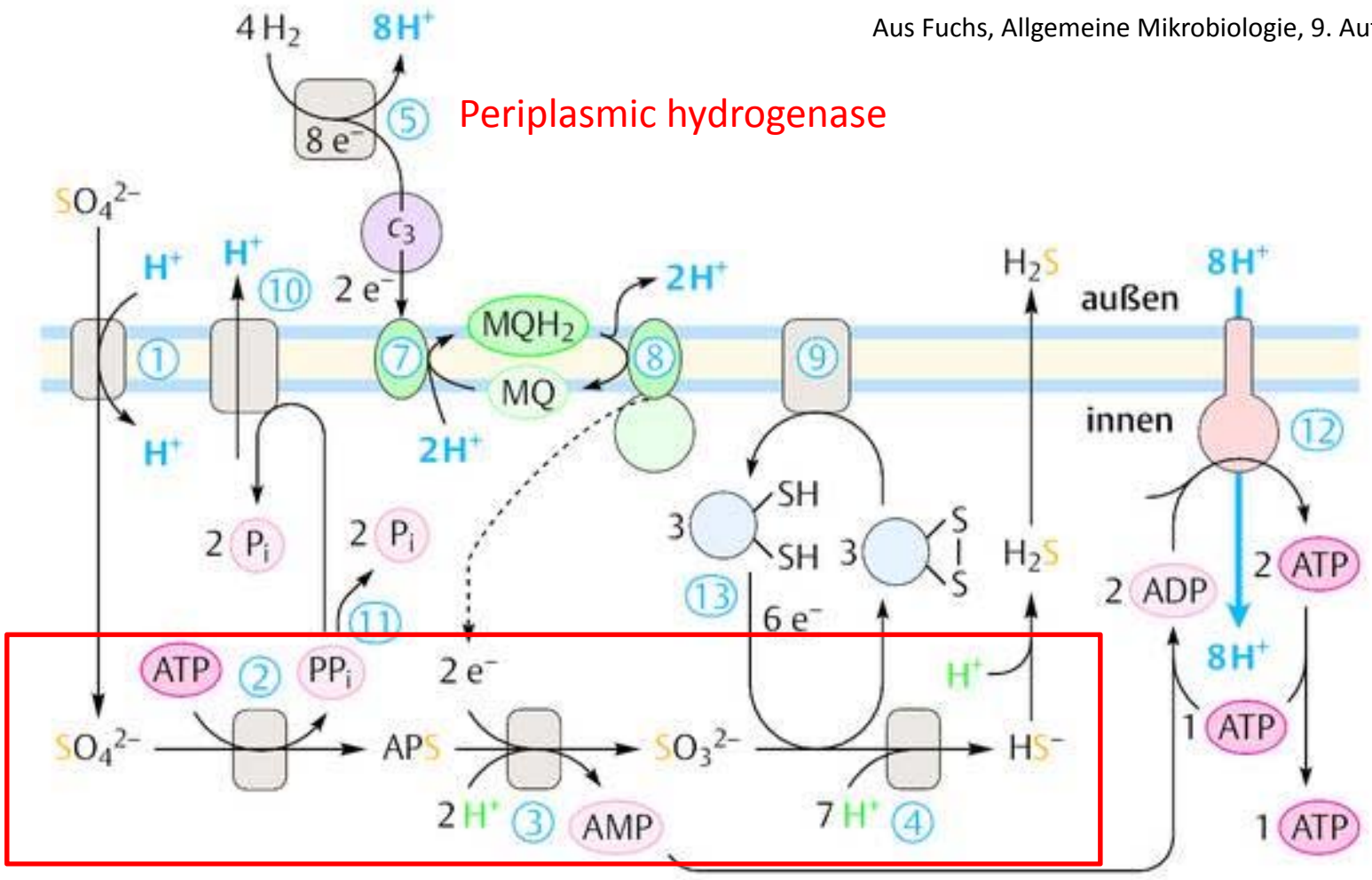

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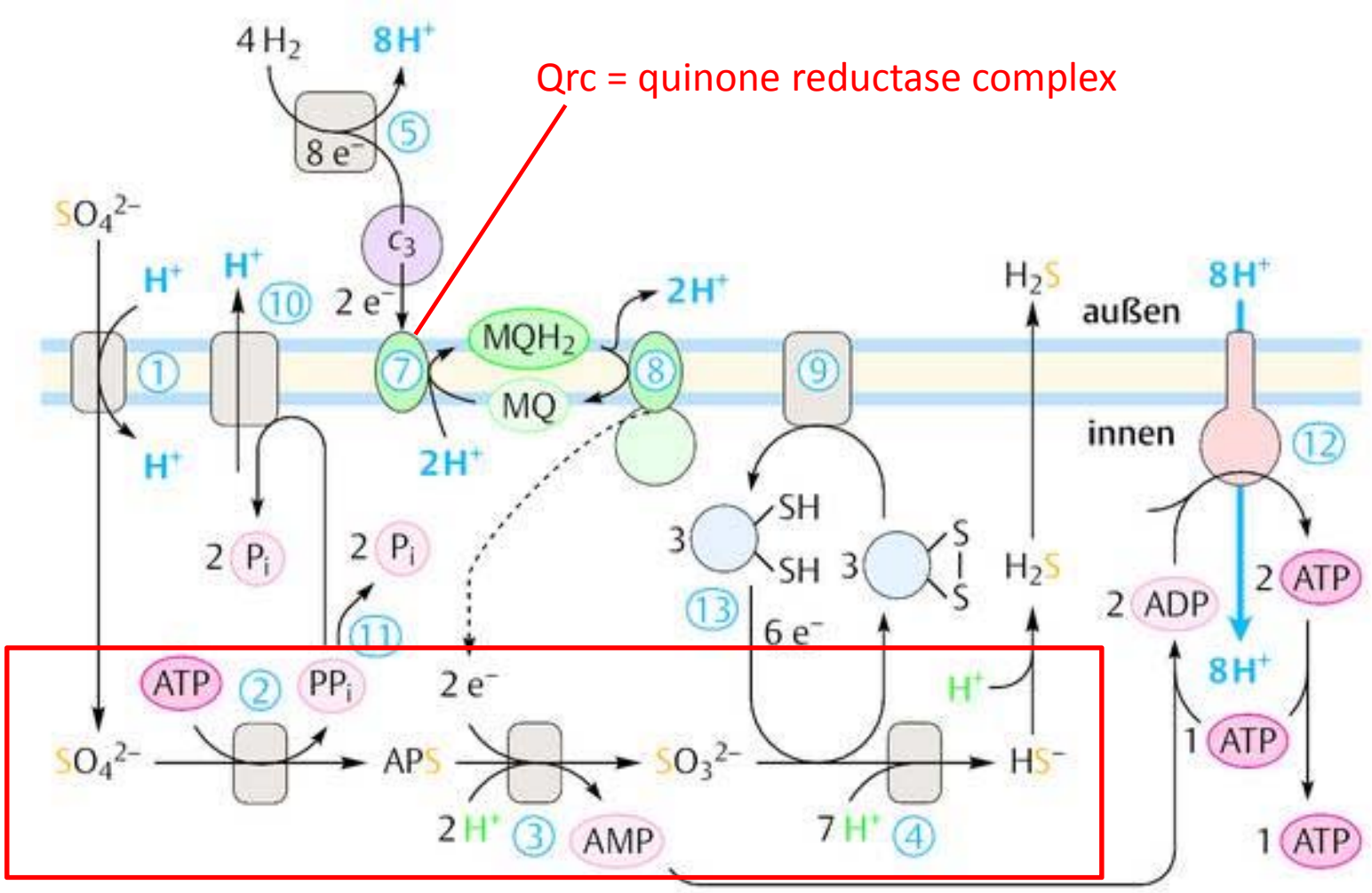

ATP-Sulfurylase APS reductase Sulfite reductase

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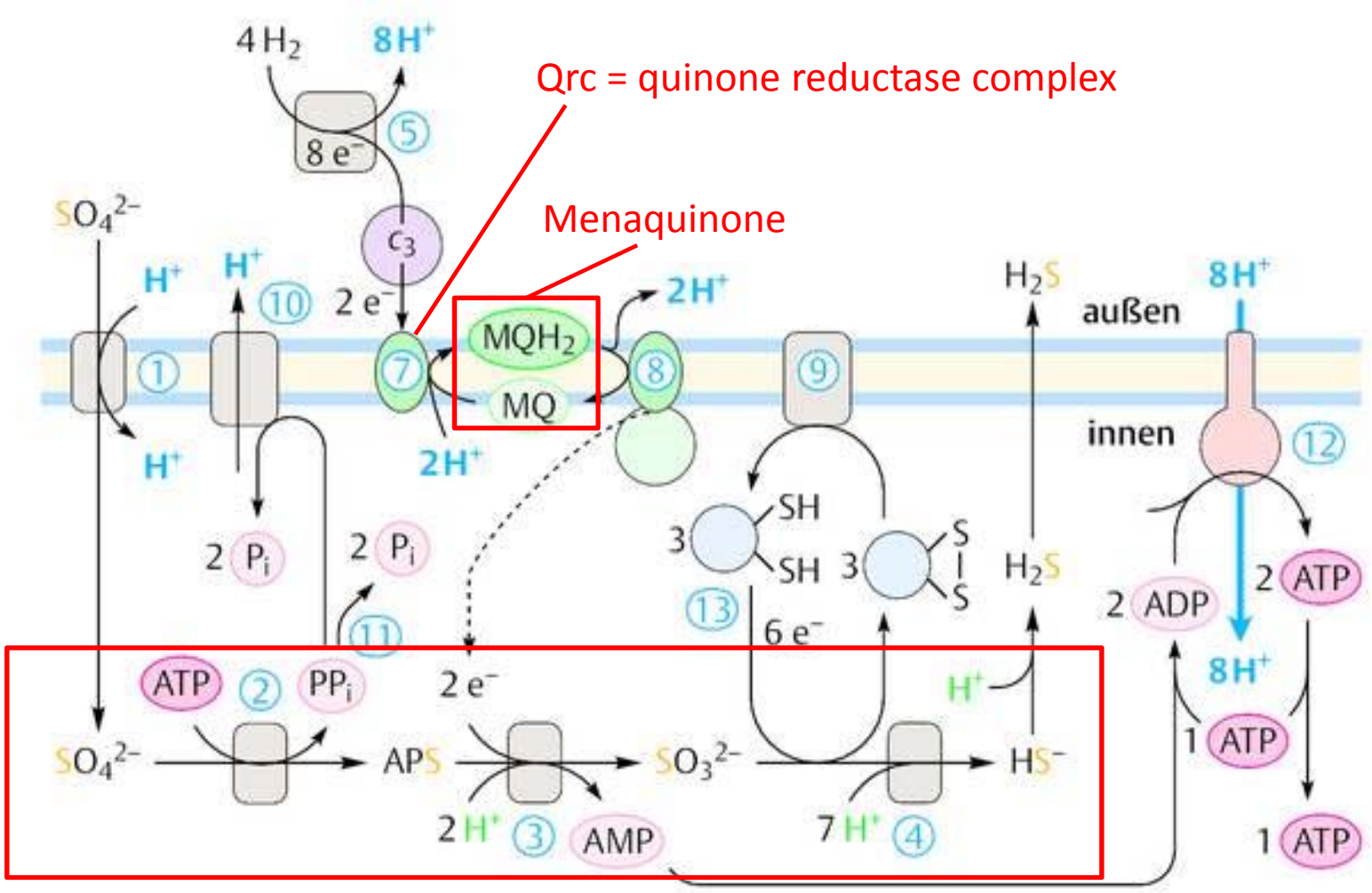

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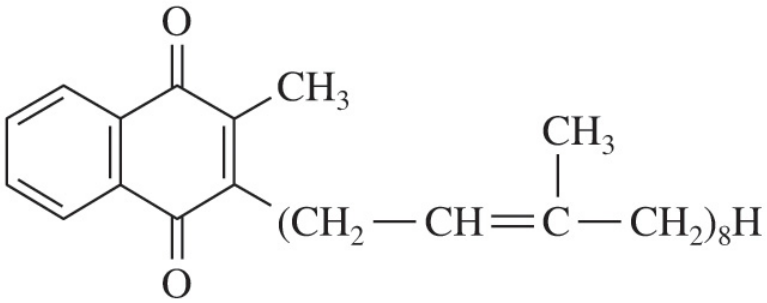
Menaquinone

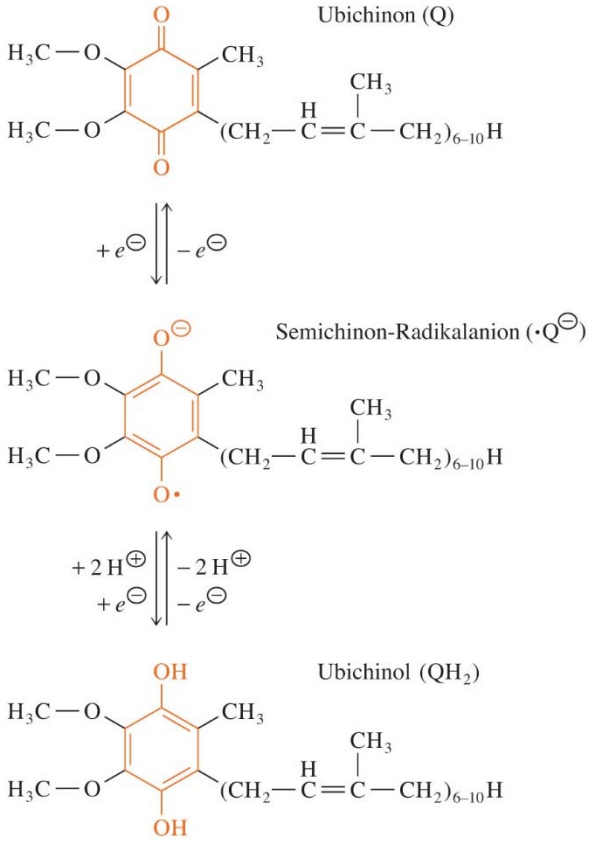
Menaquinone **-75 mV**



Menachinon

Abbildung 14.20: Struktur von Menachinon (MQ).

Ubiquinone **+113 mV**



Horton et al., Biochemie, 4. akt. Auflage

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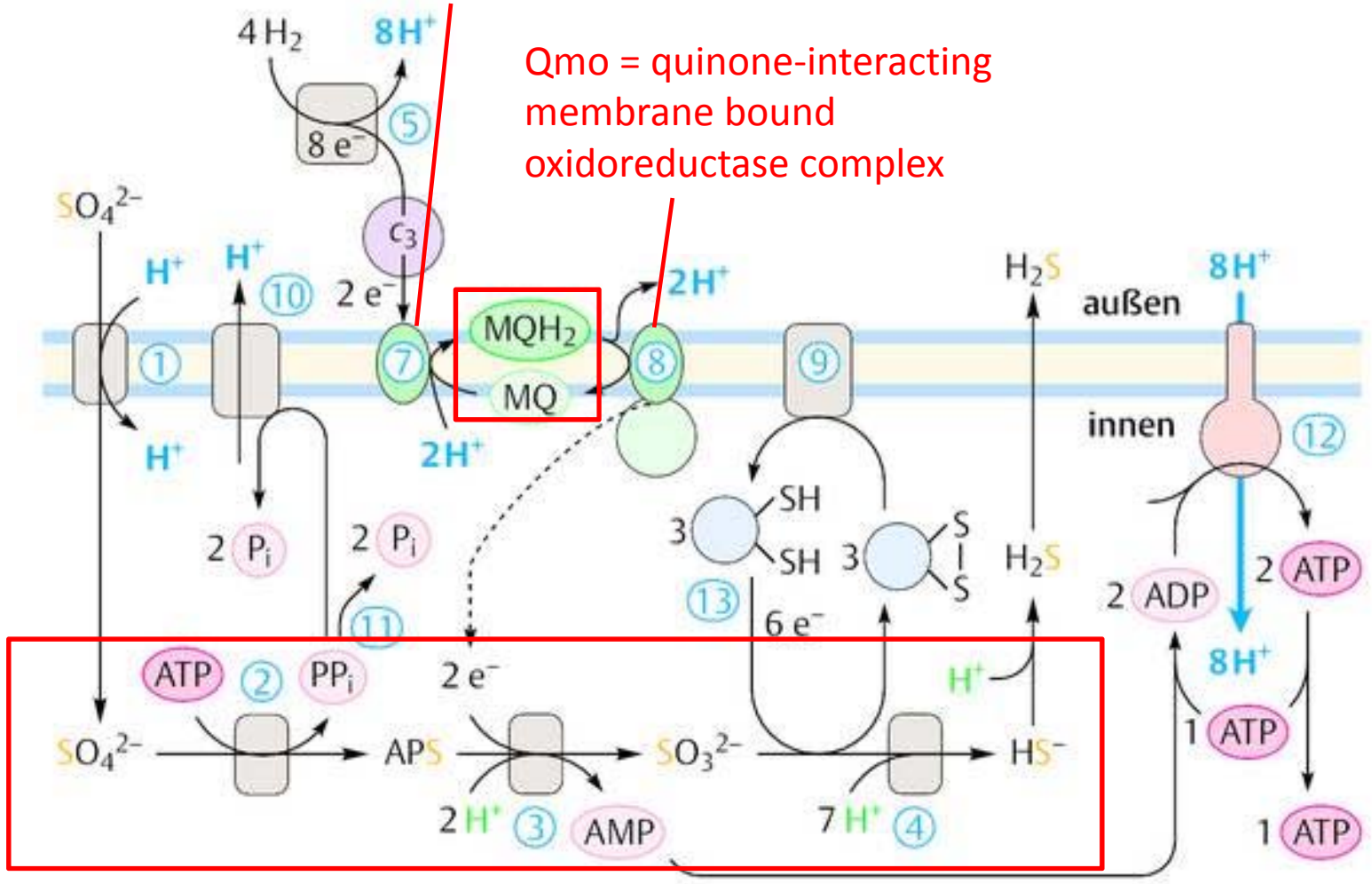

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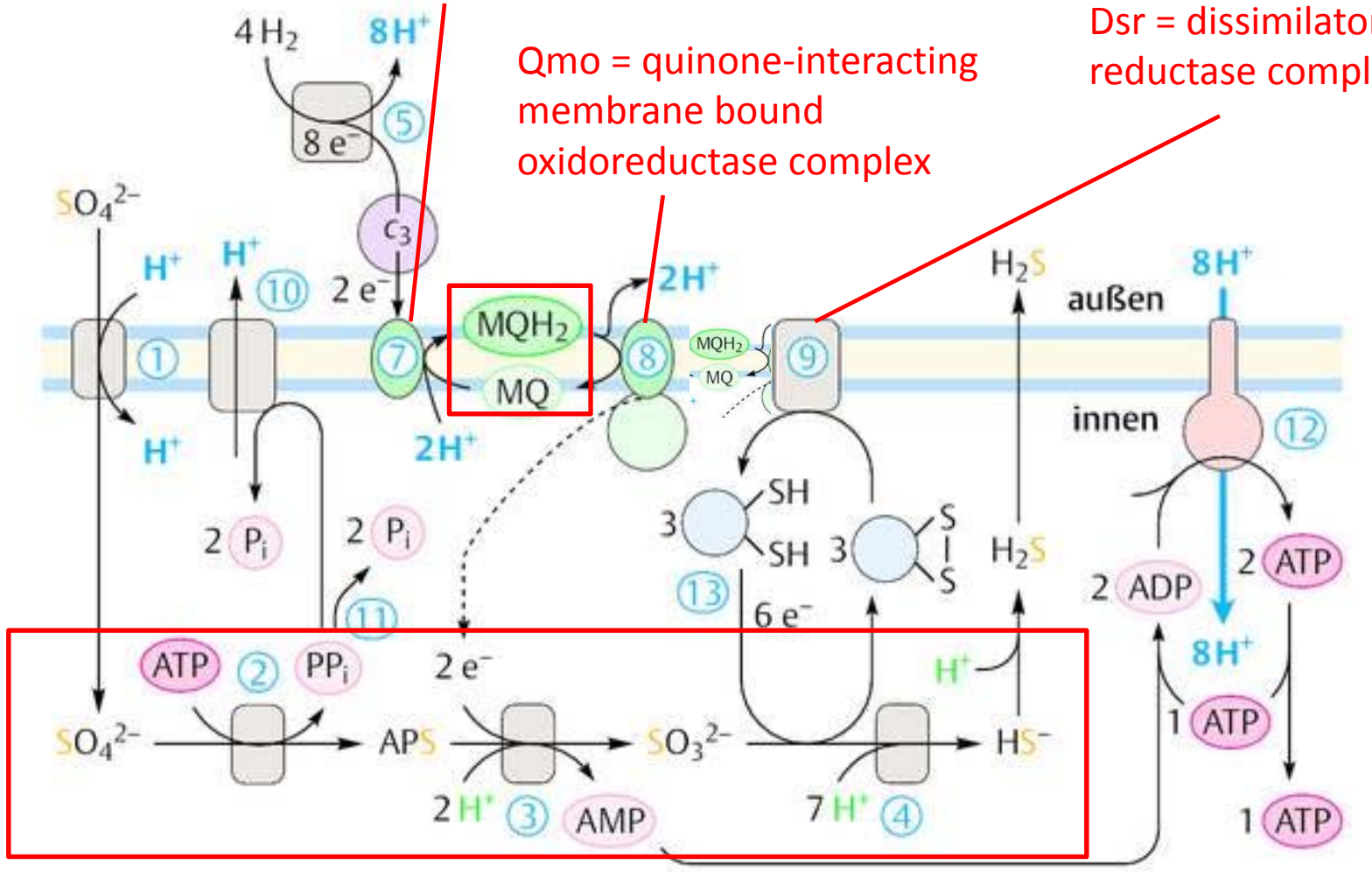
Qmo = quinone-interacting membrane bound oxidoreductase complex

Dsr = dissimilatory sulfite reductase complex



ATP-Sulfurylase APS reductase Sulfite reductase

Aus Fuchs, Allgemeine Mikrobiologie, 9. Auflage, Thieme

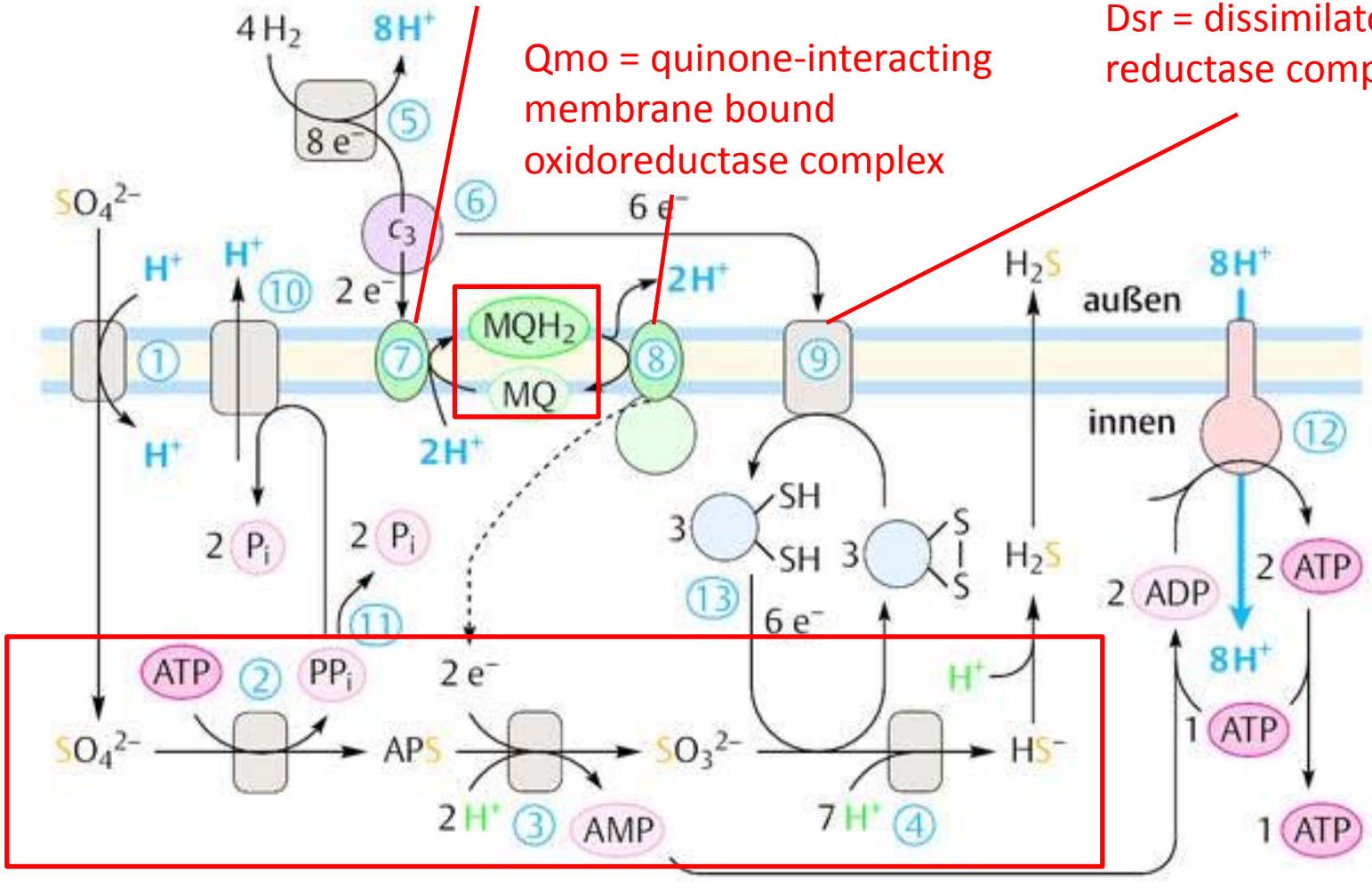
Sulfate reduction

(chemolitoautotrophic)

Qrc = quinone reductase complex

Qmo = quinone-interacting membrane bound oxidoreductase complex

Dsr = dissimilatory sulfite reductase complex



ATP-Sulfurylase APS reductase Sulfite reductase

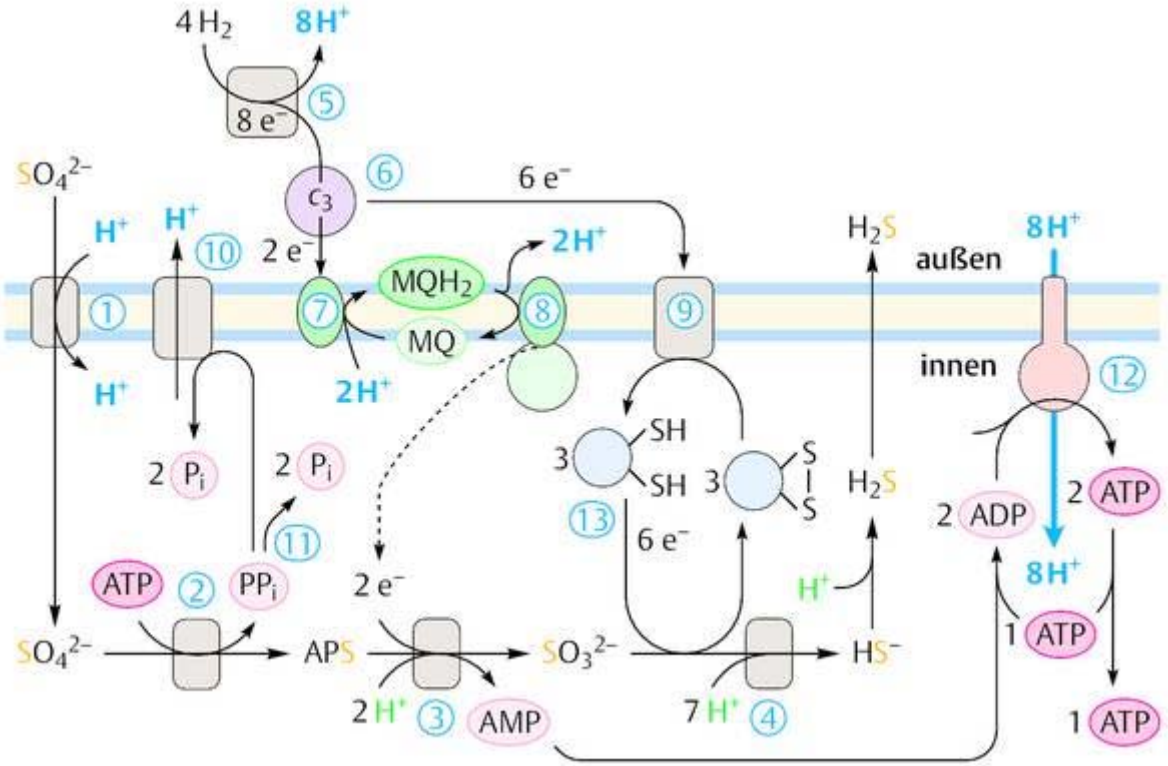
Aus Fuchs, Allgemeine Mikrobiologie, 9. Auflage, Thieme

Sulfate reduction

(chemolitoautotrophic)

Problems:
Sulfate transport
Sulfate activation

Energy consuming processes



1. Transport system
2. ATP-Sulfurylase
3. APS-Reductase (AprBA)
4. Sulfite reductase (DsrAB)
5. Hydrogenase
6. Cytochrome c3
7. Cytc3: Menaquinone Oxidoreductase/quinone reductase (Qrc Komplex)
8. Menaquinol dependent membrane Oxidoreductase (Qmo Komplex)
9. Dsr Komplex (coupled to dissimilatory Sulfite reduction)
10. Energy conserving Pyrophosphatase
11. Pyrophosphatase
12. ATP-Syntetase
13. Dithiol/Disulfide protein DsrC

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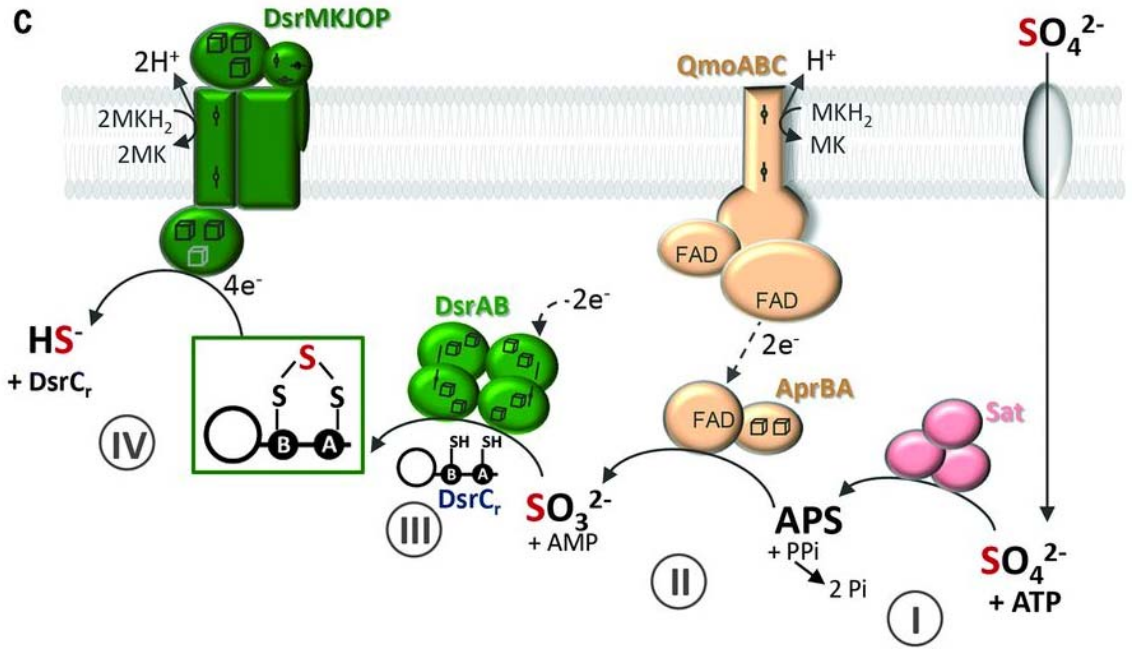
Sulfate reduction

(chemolitoautotrophic)



André A. Santos et al. Science 2015;350:1541-1545

Science
AAAS

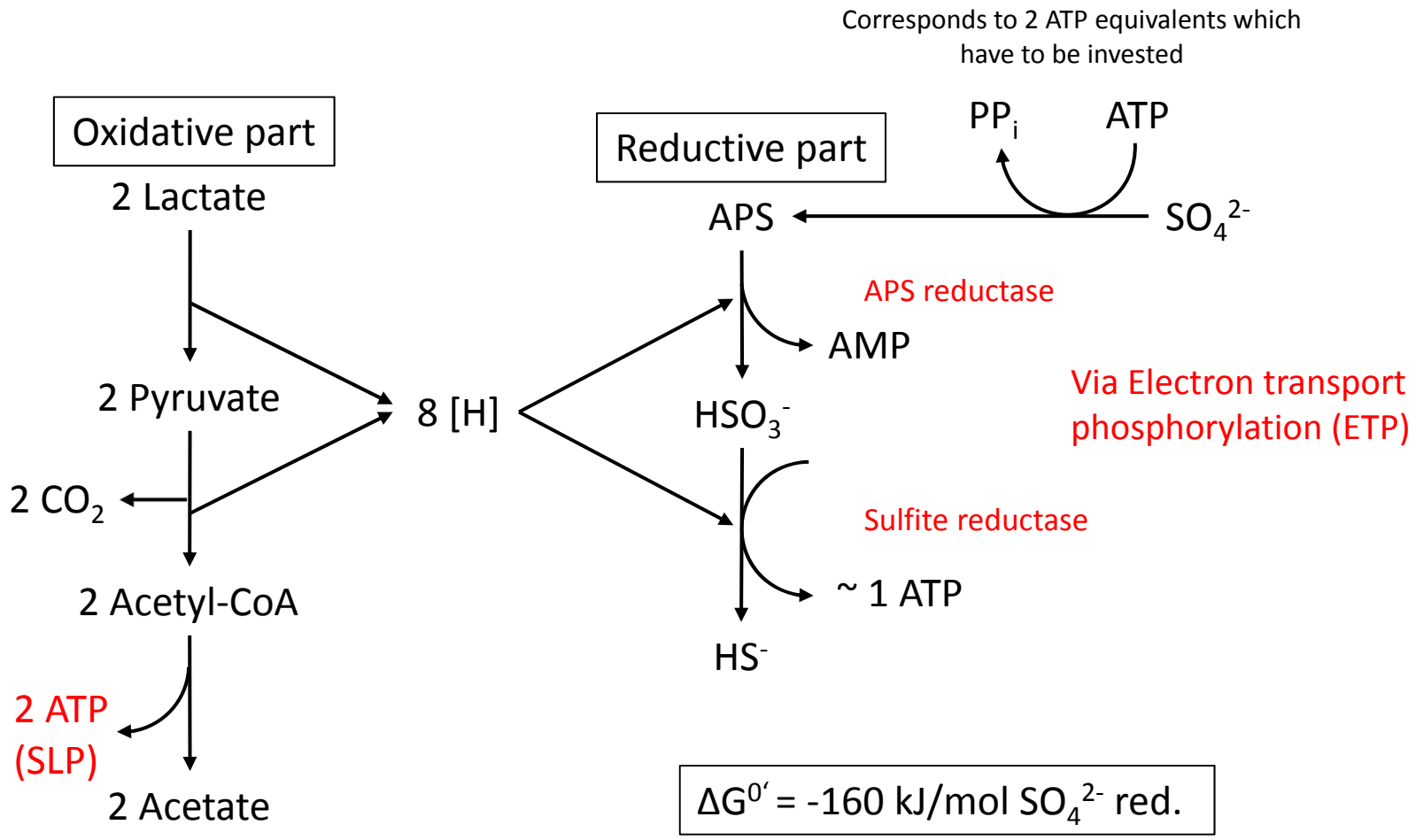
Sulfate reducers

Three main physiological groups:

Sulphate reducing reaction	$\Delta G^{0'}$	group	
$SO_4^{2-} + 4\ H_2 + H^+ \rightarrow HS^- + 4\ H_2O$	-151.9	autotrophs	Many sulfate reducers, e.g. Desulfobacteriaceae (proteobacteria) Desulfotomaculum (gram positive) Archaeoglobus (archaea)
SO_4^{2-} + Acetate $\rightarrow HS^- + 2\ HCO_3^-$ (also: fatty acids, hydrocarbons, aromatic compounds → Acetyl-CoA)	-47.6	Complete oxidizers (heterotrophs)	Desulfobacteriaceae (proteobacteria) Desulfotomaculum (gram positive) Archaeoglobus (archaea) Many others
0.75 SO_4^{2-} + propionate → 0.75 HS^- + 2 acetate + HCO_3^- +0.25 H^+ 0.5 SO_4^{2-} + butyrate → 0.5 HS^- + 2 acetate + 0.5 H^+ 0.5 SO_4^{2-} + lactate → 0.5 HS^- + acetate + HCO_3^-	-37.7 -27.8 -80.2	Incomplete oxidizers (heterotrophs)	Desulfovibrio

Sulfate reduction

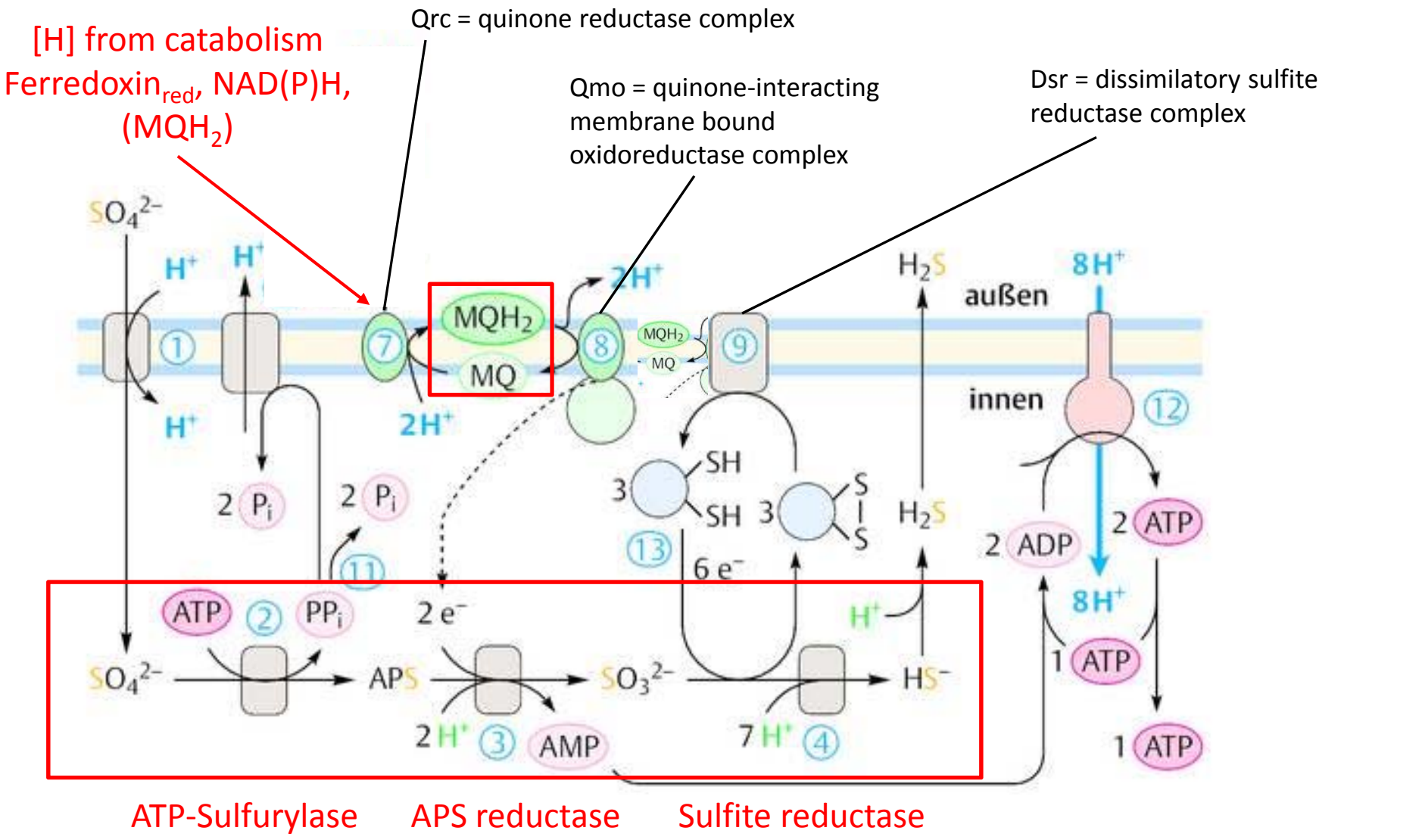
incomplete oxidation of organic electron donors



Incomplete oxidation of organic compounds to acetate allow for ATP formation via SLP

Sulfate reduction

(chemoorganoheterotrophic)



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Sulfate reducers

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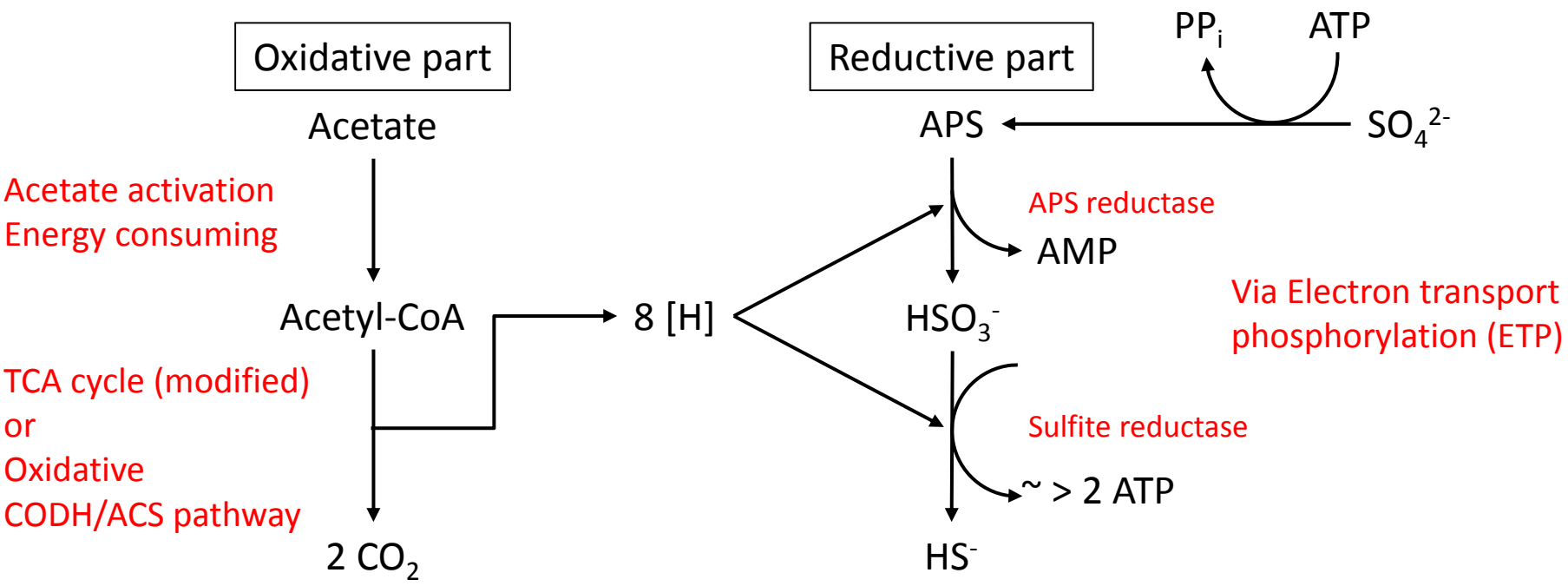
Sulfate reduction

complete oxidation of acetate as organic electron donor

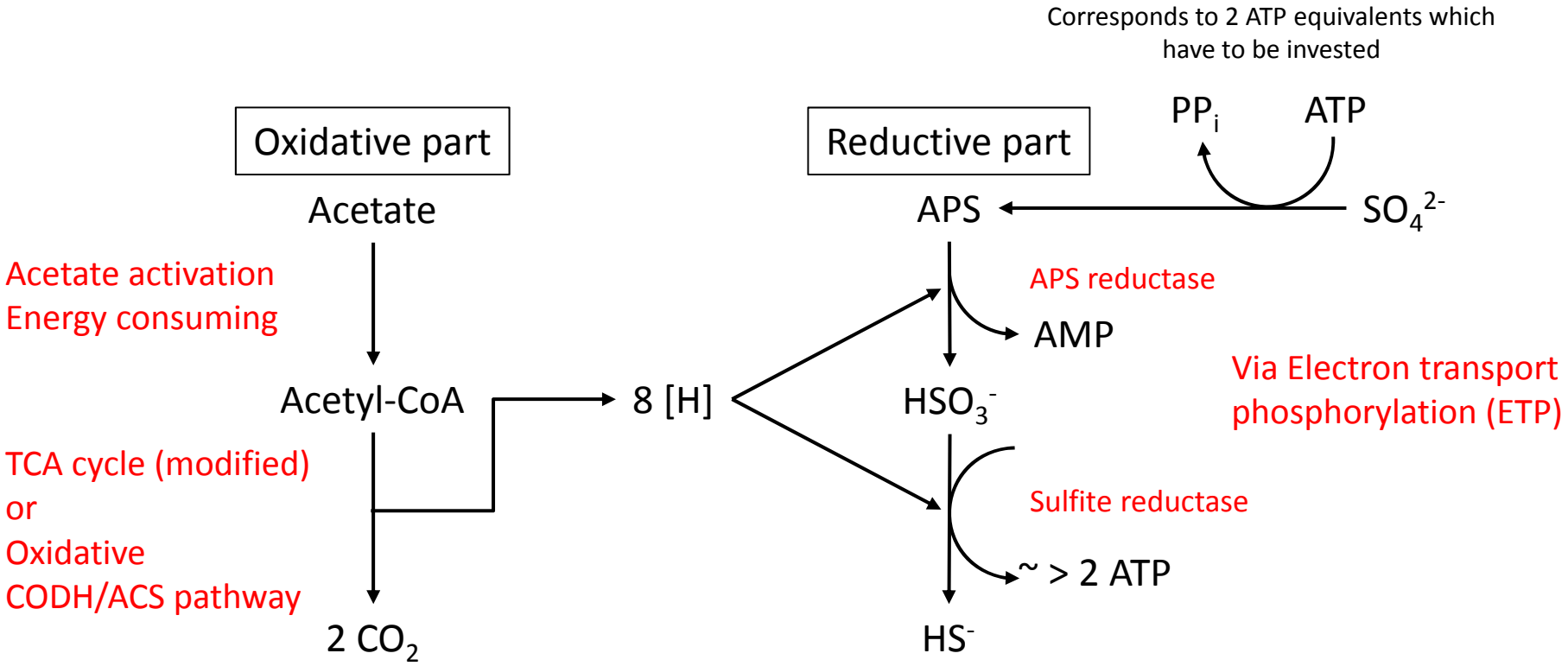
$$Acetate + SO_4^{2-} + 2\ H^+ \rightarrow 2\ CO_2 + HS^- + 2\ H_2O \qquad \Delta G^{0'} = -41\ kJ/mol\ SO_4^{2-}\ red.$$

Corresponds to 2 ATP equivalents which have to be invested



Sulfate reduction

complete oxidation of acetate as organic electron donor



No ATP via substrate level phosphorylation

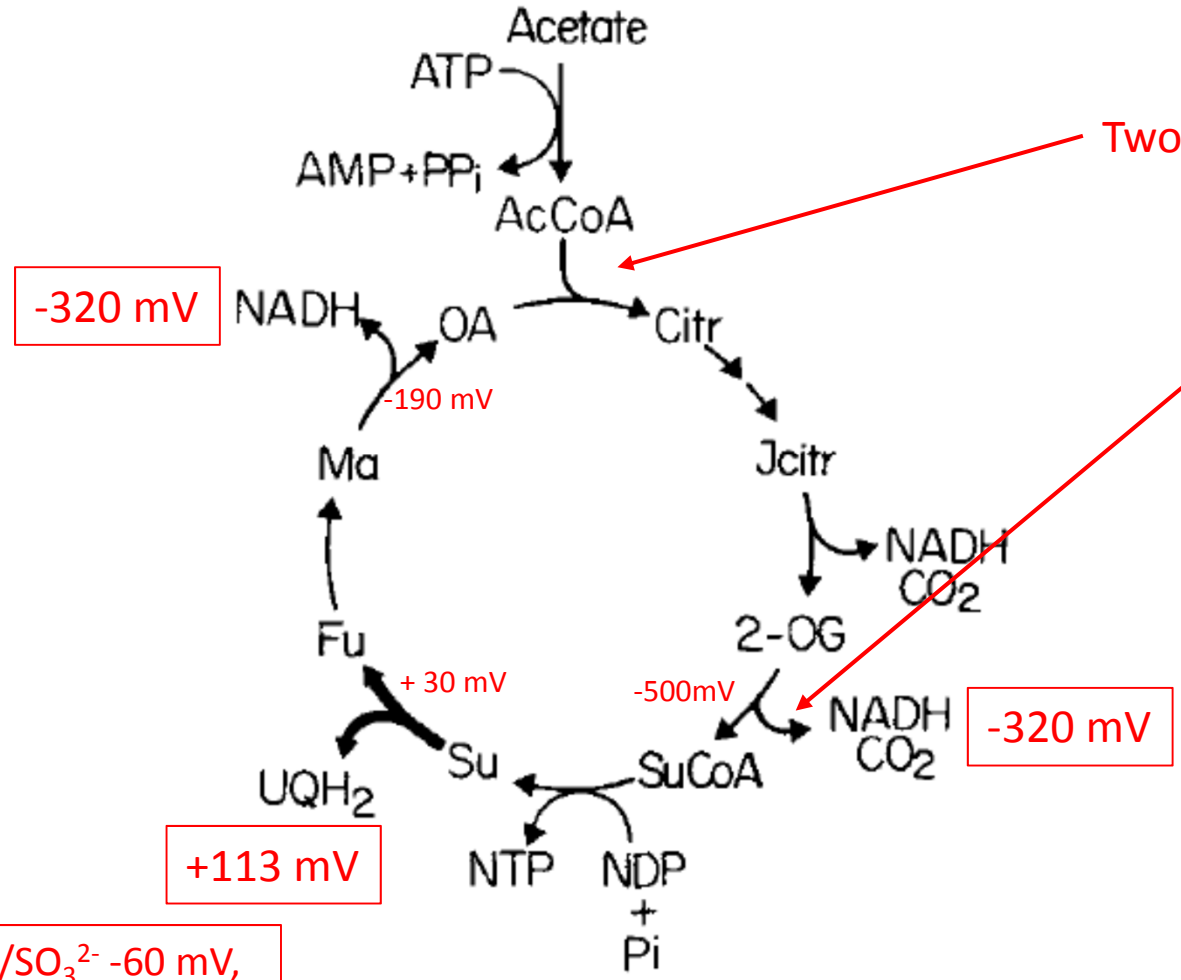
Reduction equivalents: ferredoxin (-400 mV), NAD(P)H (-320 mV) and menaquinone (-75 mV);

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

The Citric acid cycle for complete acetate oxidation

in arobic 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

Redox potentials

Tabelle A 1.2: Redoxpotenziale[a], die für die Mikrobiologie von Bedeutung sind

Redoxpaar	E_0' (V)
SO_4^{2-}/HSO_3^-	–0,52
CO_2/Formiat	–0,43
$2\ H^+/H_2$	–0,41
$S_2O_3^{2-}/HS^- + HSO_3^-$	–0,40
Ferredoxin ox/red	–0,39
Flavodoxin ox/red[b]	–0,37
NAD^+/NADH	–0,32
Cytochrom c_3 ox/red	–0,29
CO_2/Acetat	–0,29
S^0/HS^-	–0,27
CO_2/CH_4	–0,24
SO_4^{2-}/HS^-	–0,217
Acetaldehyd/Ethanol	–0,197
$Pyruvat^-$/$Lactat^-$	–0,19
FMN/FMNH	–0,19
Dihydroxyacetonphosphat/Glycerinphosphat	–0,19
$HSO_3^-/S_3O_6^{2-}$	–0,17
Flavodoxin ox/red[b]	–0,12
HSO_3^-/HS^-	–0,116
Menachinon ox/red	–0,075
APS/AMP + HSO_3^-	–0,060
Rubredoxin ox/red	–0,057
Acrylyl-CoA/Propionyl-CoA	–0,015

(Fortsetzung nächste Spalte)

Tabelle A 1.2: Redoxpotenziale[a], die für die Mikrobiologie von Bedeutung sind (Fortsetzung)

Redoxpaar	E_0' (V)
Glycin/$Acetat^-$ + NH_4^+	–0,010
$S_4O_6^{2-}/S_2O_3^{2-}$	+0,024
$Fumarat^{2-}$/$Succinat^{2-}$	+0,033
Cytochrom *b* ox/red	+0,035
Ubichinon ox/red	+0,113
AsO_4^{3-}/AsO_3^{3-}	+0,139
Dimethylsulfoxid (DMSO)/Dimethylsulfid (DMS)	+0,16
$Fe(OH)_3 + HCO_3^-/FeCO_3$, Fe^{3+}/Fe^{2+}, pH7	+0,20
$S_3O_6^{2-}/S_2O_3^{2-} + HSO_3^-$	+0,225
Cytochrom c_1 ox/red	+0,23
NO_2^-/NO	+0,36
Cytochrom a_3 ox/red	+0,385
$Chlorbenzoat^-$/$Benzoat^-$ + HCl	+0,297
NO_3^-/NO_2^-	+0,43
SeO_4^{2-}/SeO_3^{2-}	+0,475
Fe^{3+}/Fe^{2+}, pH2	+0,77
Mn^{4+}/Mn^{2+}	+0,798
$\frac{1}{2}\ O_2/H_2O$	+0,82
ClO_3^-/Cl^-	+1,03
NO/N_2O	+1,18
N_2O/N_2	+1,36

[a] Daten von Thauer, R. K., K. Jungermann und K. Decker, 1977. Energy conservation in anaerobic chemotrophic bacteria. *Bacteriol Rev* 41: 100–180.

[b] Für jeden Elektronentransfer sind bei diesem potenziellen Transfer von zwei Elektronen getrennte Potenziale angegeben.

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

The Citric acid cycle

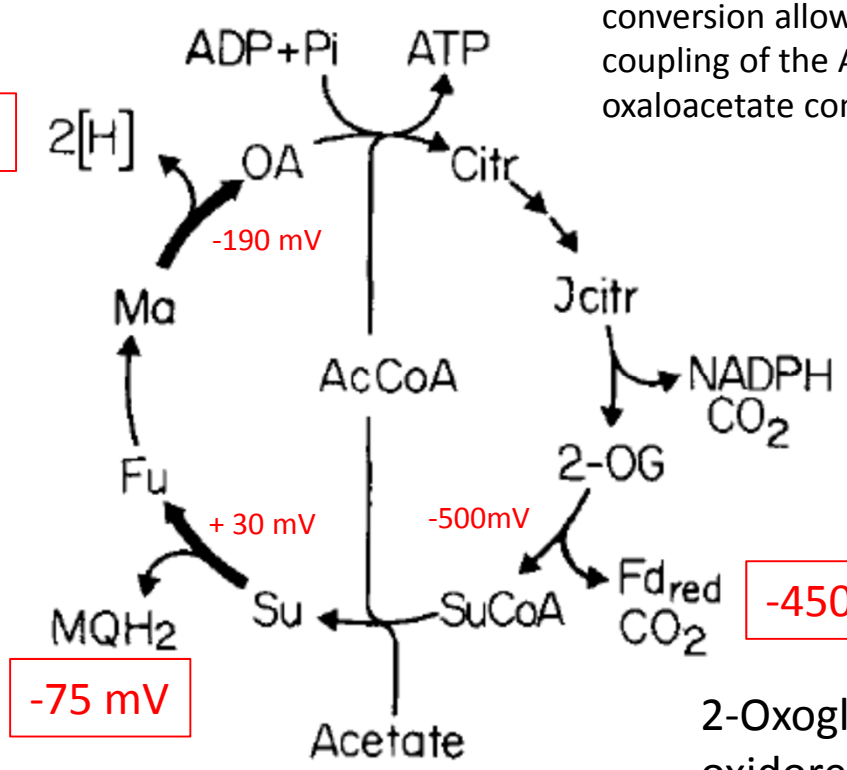
complete oxidation in *Desulfobacter postgatei*

→ with modifications

Menaquinone
Electron acceptor with more positive redox potential than NAD^+ moves equilibrium towards Oxaloacetate formation

-75 mV

Menaquinone (more negative redox potential than ubiquinone), redox difference bridged via concentration (or confurcation), enables electron transfer to APS and SO_3^{2-}



-450 mV

ATP-Citrate lyase (reversible);
the exergonic malate-oxaloacetate conversion allows for energetic coupling of the Acetyl-CoA-oxaloacetate condensation

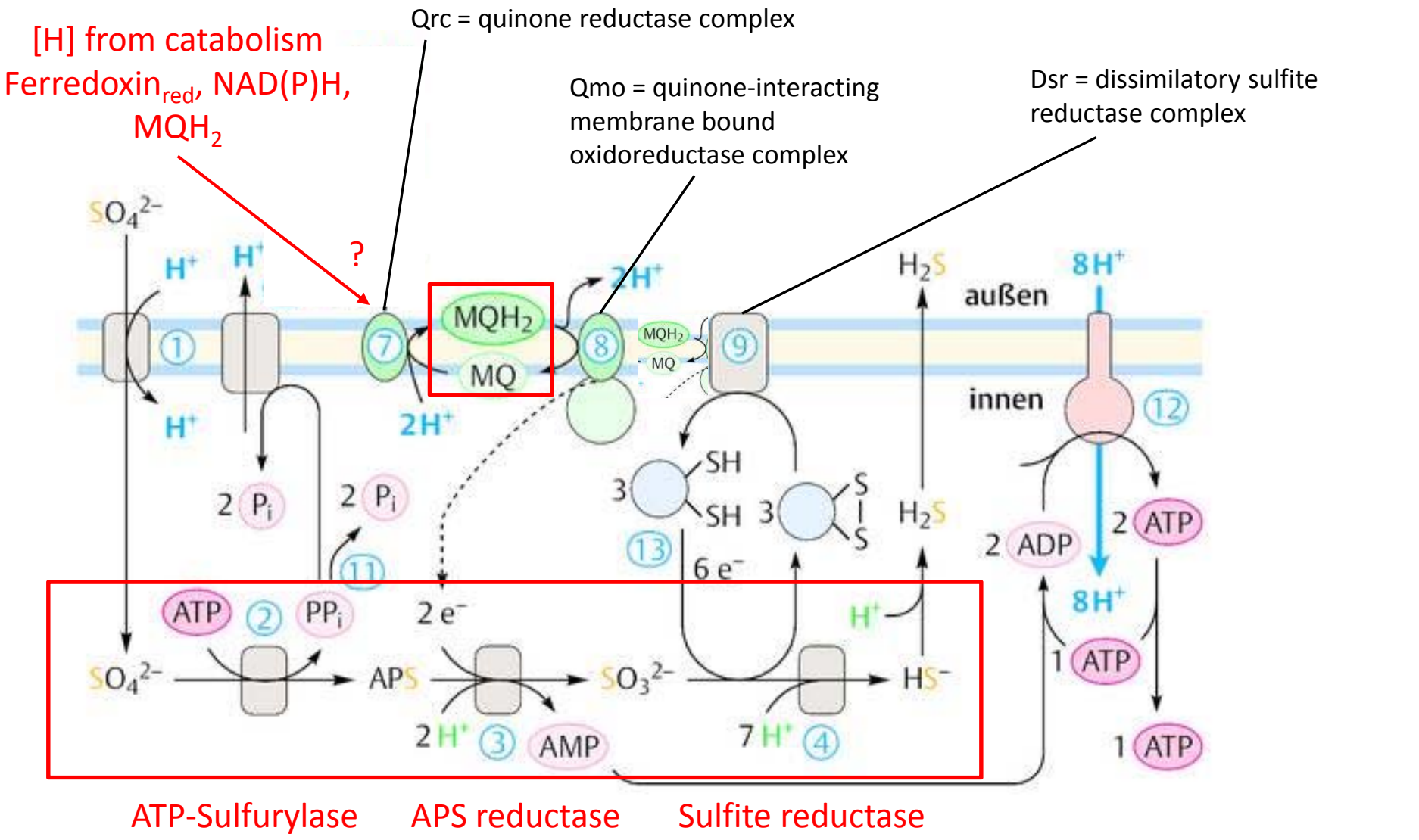
Acetate activation via Succinyl-CoA: acetate CoA-transferase (together with ATP-Citrate Lyase makes the acetate activation energetically neutral)

2-Oxoglutarate: ferredoxin oxidoreductase (reversible) Use of ferredoxin renders reaction reversible and allows for optimized energetic coupling via ETP

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

Sulfate reduction

(chemoorganoheterootrophic)



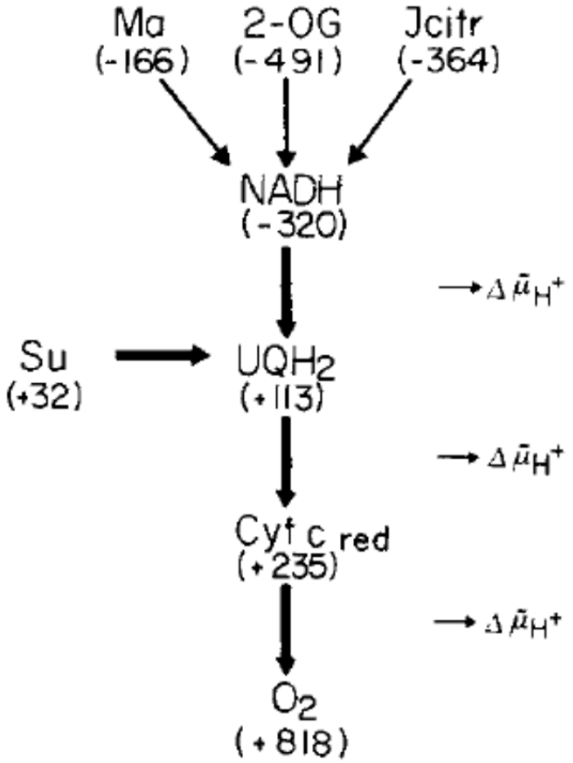
Aus Fuchs, Allgemeine Mikrobiologie, 9. Auflage, Thieme

Respiratory chain

complete oxidation in *Desulfobacter postgatei*

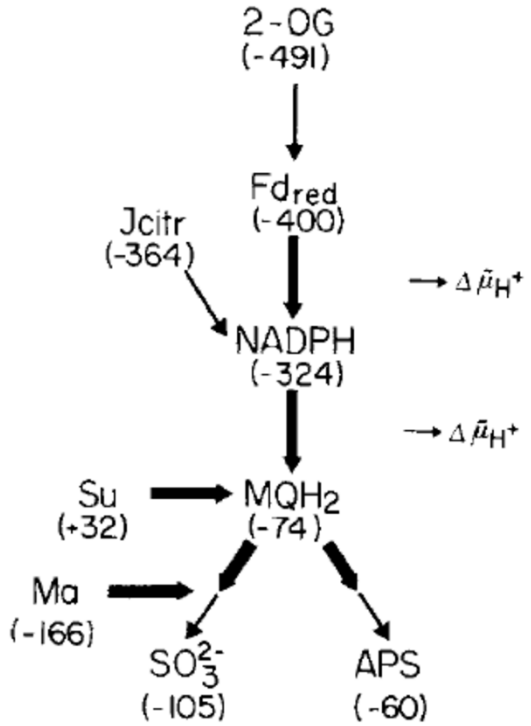
Proposed electron flow in the respiratory chain of *Desulfobacter postgatei* in comparison to that in mitochondria and aerobes e.g. *Paracoccus*

Aerobic organisms



$\rightarrow \Delta\mu H^+$ = generation of electrochemical proton potential

Desulfobacter postgatei
(proposed)



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

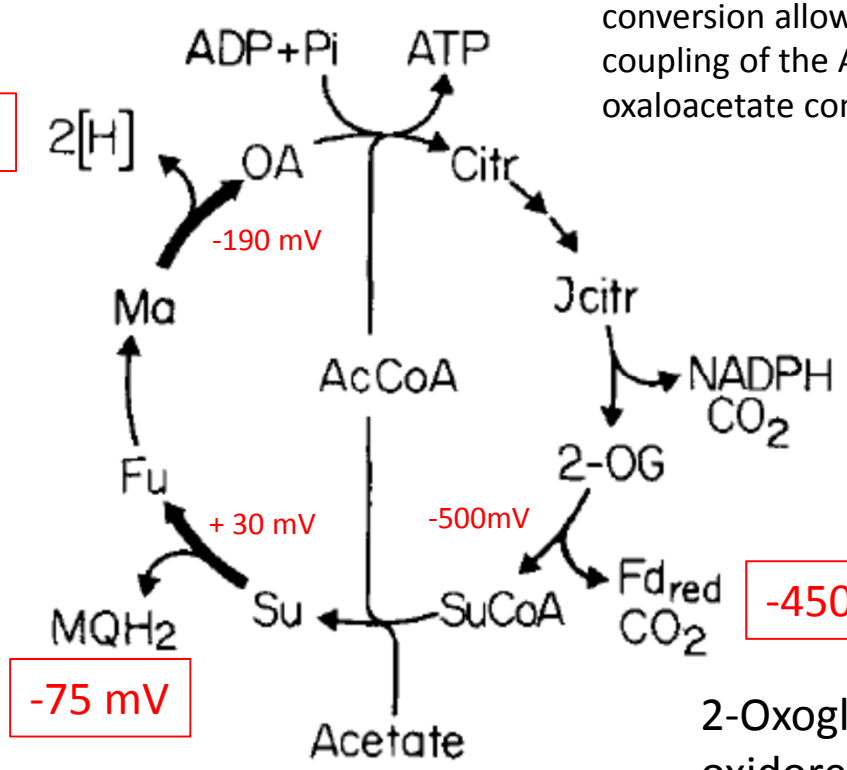
The Citric acid cycle

complete oxidation in *Desulfobacter postgatei*

→ with modifications

ATP-Citrate lyase (reversible); the exergonic malate-oxaloacetate conversion allows for energetic coupling of the Acetyl-CoA-oxaloacetate condensation

-75 mV



-450 mV

-75 mV

Especially the substitutions of irreversible Citrate synthase and 2-Oxoglutarate dehydrogenase by the reversible ATP-Citrate lyase and Oxoglutarate: ferredoxin oxidoreductase, respectively, make the whole cycle reversible!

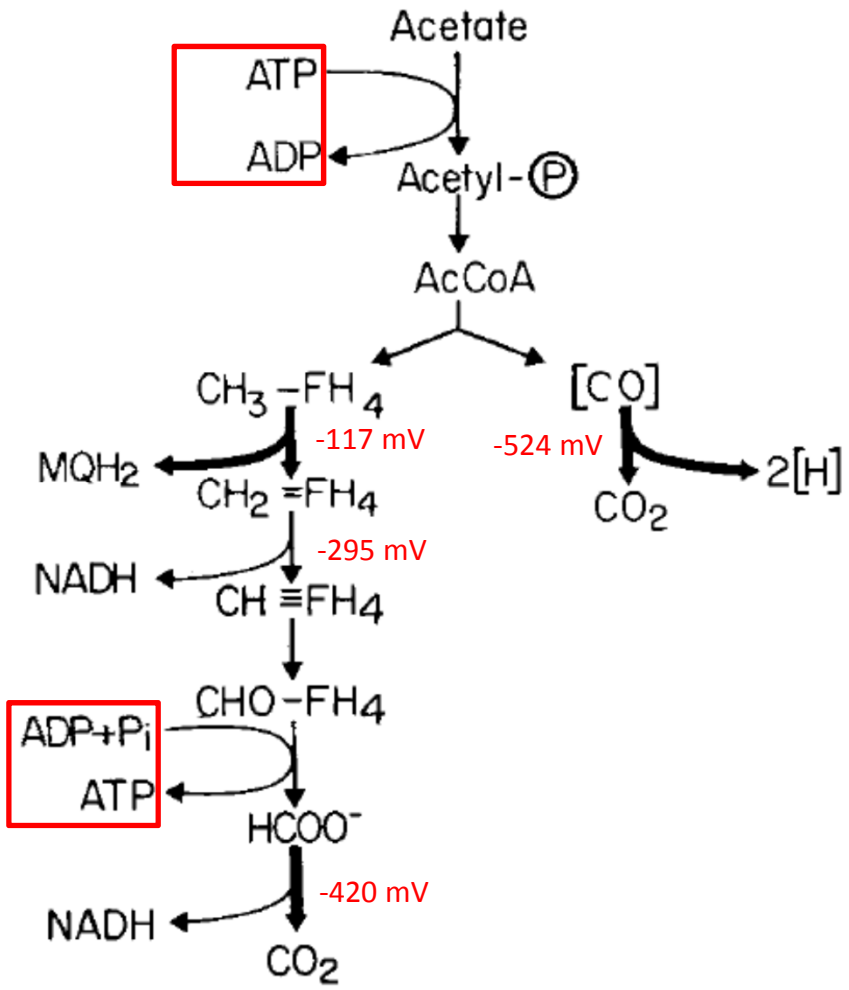
→ reversed pathway used for CO_2 fixation during autotrophic growth

2-Oxoglutarate: ferredoxin oxidoreductase (reversible) Use of ferredoxin renders reaction reversible and allows for optimized energetic coupling via ETP

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

The oxidative CODH/ACS pathway

complete oxidation in *Desulfotomaculum acetoxidans*



- Acetate activation via acetate kinase and phosphotransacetylase → 1 ATP instead of two for acetate activation, regained in CHO-FH_4 to formate conversion
- Menaquinone instead of NAD^+ makes the CH_3-FH_4 to CH_2=FH_4 conversion reversible

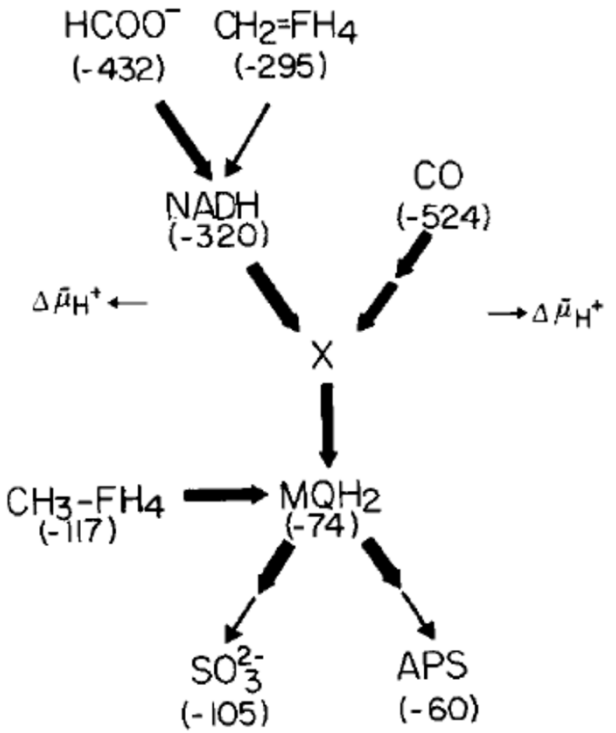
→ pathway is reversible and acts – in the reversed direction – in CO_2 fixation during autotrophic growth

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

Respiratory chain

complete oxidation in *Desulfotomaculum acetoxidans*

Proposed electron flow in the respiratory chain of *Desulfotomaculum acetoxidans*



→$\Delta\mu H^+$ = generation of electrochemical proton potential

X = so far unidentified electron carrier

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

Sulfur reduction

- Several anaerobes use elemetal sufur S^0 as electron acceptor
- Some of them are obligate sulfur S^0 reducers (e.g. some hyperthermophilic archaea like *Thermoproteus*, *Pyrodictium*)
- Redox potential S^0/HS^- -270 mV
- Others also utilize other electron acceptors e.g. *Desulfuromonas acetoxydans*, they only induce the sulfur respiratory enzymes on demand

Questions 6

- Describe the three physiological groups of sulfate reducers. What are the substrates, what the products?
- What are the (energetic) difficulties in sulfate reduction? Why has sulfate to be activated and how much ATP does it require? What is the product of sulfate activation? How does sulfate enter the cell?
- How is energy gained in the incomplete oxidation of lactate?
- Which pathways lead to complete oxidation of acetate? What is additionally challenging with respect to energetics during growth on acetate?
- Why does sulfate reducers outcompete methanogens in the presence of SO_4^{2-}?
- How does sulfate reducers fix CO_2 during autotrophic growth with H_2?
- Name at least 3 pathways of CO_2 fixation!

Questions 6

- Welches sind die drei physiologischen Hauptgruppen der Sulfat Reduzierer? Was sind die Substrate, was die Produkte?
- Was sind die energetischen Hürden bei der Sulfat Reduktion? Warum muss Sulfat aktiviert werden und wieviel ATP wird dazu benötigt?Was ist das Produkt der Sulfat Aktivierung? Wie wird Sulfat in die Zelle aufgenommen?
- Wie wird bei der unvollständigen Oxidation von Laktat Energie gewonnen?
- Über welche Stoffwechselwege wird Acetat vollständig zu CO2 oxidiert? Was stellt beim Wachstum auf Acetat eine zusätzliche energetische Hürde dar?
- Warum sind die Sulfat Reduzierer gegenüber Methanogenen in Gegenwart von SO_4^{2-} im Vorteil?
- Wie fixieren Sulfat Reduzierer CO_2 bei autotrophem Wachstum mit H_2?
- Benennen Sie wenigstens 3 CO_2-Fixierungswege.

Übung: Mikrobielles Wachstum II

frontiers in Microbiology

REVIEW
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Microbial growth and physiology: a call for better craftsmanship

*Thomas Egli**†

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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?

Übung: Mikrobielles Wachstum II

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

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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

20 mM Glucose → 0,12 mol C (Glucose 6 C-Atome)
→ 1,44 g C (C 12 g/mol)
→ 1,44 g TZ

Man benötigt also mindestens 0,175 g N (1,44 x 0,12 (12% TZ sind N)
→ 0,175 / 14 (N 14 g/mol) =12,5 mM NH_4Cl (=0,66 g/l)

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**	**Excess factors recommended for C-limited medium**
C	50	45[c]–58[d]	1	Limiting
O	21	18[e]–31[f]	–	–
N	12	5[d]–17[g]	8	3–5
P	3	1.2[h]–10[i]	33	5–10
S	1	0.3–1.3	100	5–10
K	1	0.2[j]–5[k]	100	5–10
Mg	0.5	0.1[l]–1.1	200	5–10
Fe	0.5	0.01–0.5	200	10–20

20 mM Glucose → 0,12 mol C (Glucose 6 C-Atome)
→ 1,44 g C (C 12 g/mol)
→ 1,44 g TZ

Man benötigt also mindestens 0,175 g N (1,44 x 0,12 (12% TZ sind N)
→ 0,175 / 14 (N 14 g/mol) =12,5 mM NH_4Cl (=0,66 g/l)
→ ~40 -60 mM NH_4Cl