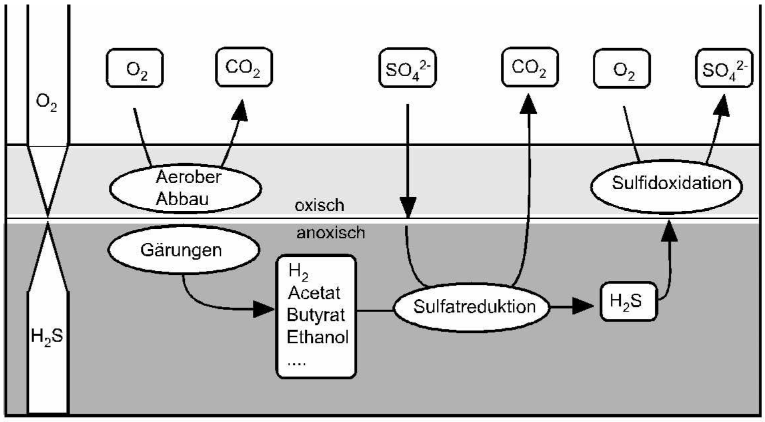
Anaerobic processes



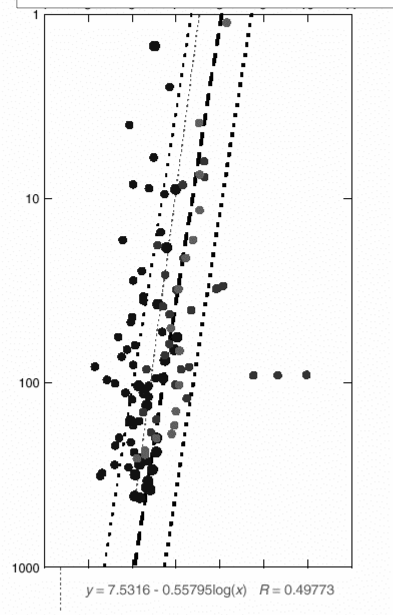
Where are they?

Motivation

Number of prokaryotes on earth	4 - 6 * 10^{30}
Cells in open ocean	1.2 * 10^{29}
in marine sediments	3.5 * 10^{30}
in soil	2.6 * 10^{29}
sub-terrestrial	0.5 – 2.5 * 10^{30}
Annual production of cells	1.7 10^{30} a^{-1}
Mean generation time in sediments	1 - 2 * 10^3 a

Whitman WB, Coleman DC, Wiebe WJ (1998) Prokaryotes: The unseen majority. Proc Natl Acad Sci USA 95:6578-6583

Bacterial counts in marine sediments



log (microscopic count) =
8.06 - 0.715 log (depth in mbsf)

(Parkes et al. 1994)

Integrated bacterial counts (cm^{-2})

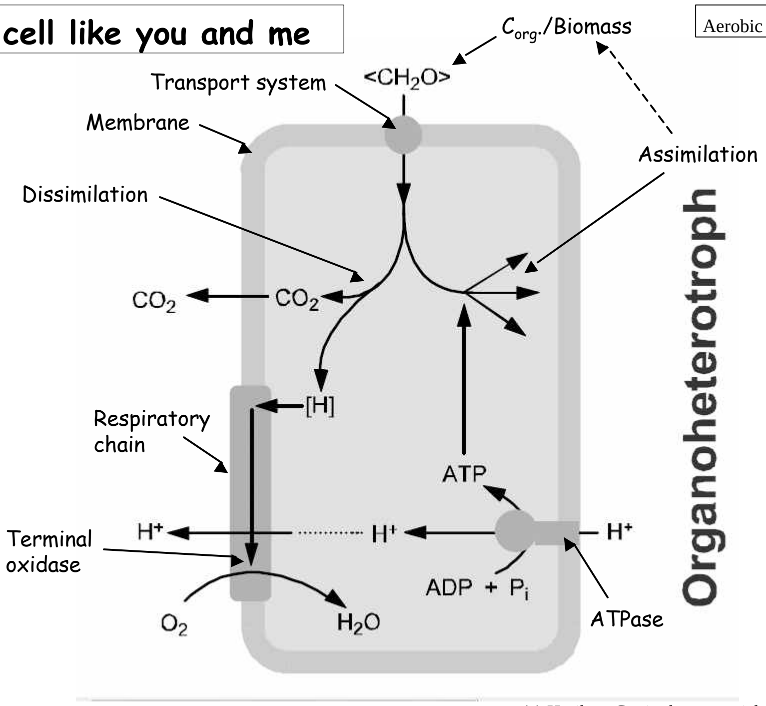
0 - 10 cm:	$162 * 10^9$
10 cm - 1 m:	$316 * 10^9$
1 - 10 m:	$613 * 10^9$
10 - 100 m:	$1194 * 10^9$

Themes

- **What is the difference between aerobic and anaerobic respiration?**
- **What is the difference between respiration and fermentation?**
- **In which sequence can we bring these processes (with repect to space, time and energetics)?**
- **How are fermentation and respiration coupled to lithotrophic processes?**
- **Which products are formed, which resources consumed?**
- **Which role had theses processes during early life history, and which do the have in present-day sediments?**

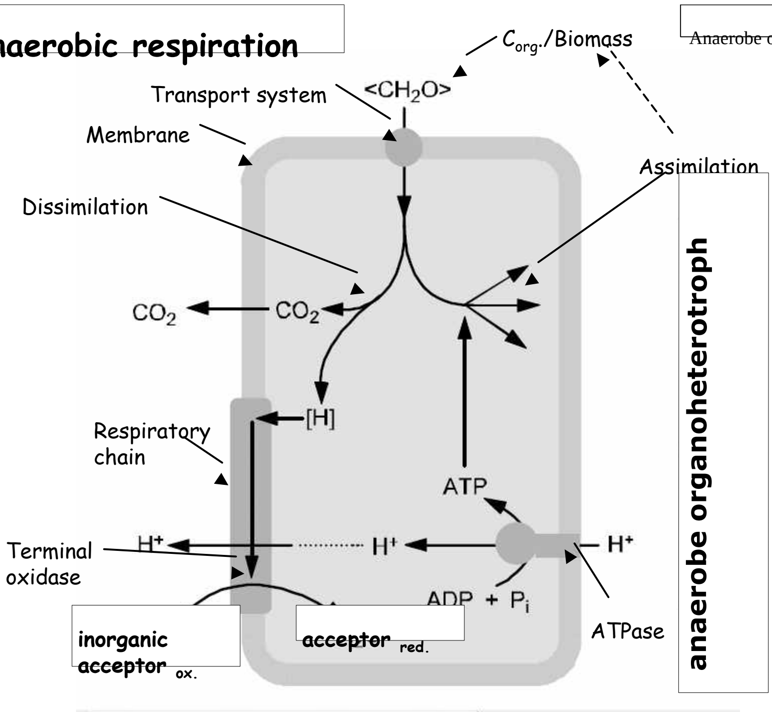
A cell like you and me

Aerobic organoheterotroph



Anaerobic respiration

Anaerobe organoheterotroph



What is different when oxygen is replaced by another inorganic electron acceptor?

Aerobe vs. anaerobe

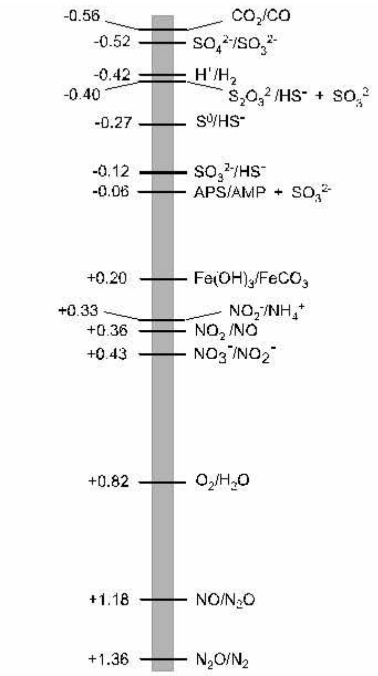
- There is less free energy (ΔG) available.
- Dissimilation will increase.
- There might be the necessity for transport of the acceptor or the reduced product.
- There might be insoluble or charged products or educts.
- The reduction is not a one-step reaction in most cases (i.e. we have several intermediate electron acceptors)
- The product might be toxic and will have to be recycled anyhow (lithotrophic processes).
- Oxygen might be lacking as reaction partner for the attack of some substrates (by oxygenase reactions).
- Several groups of anaerobes utilize a limited spectrum of substrates.

Respiration processes

Respiration processes

$4\,[H] + O_2 \rightarrow 2\,H_2O$	Aerobic respiration
$5\,[H] + NO_3^- + H^+ \rightarrow \frac{1}{2}\,N_2 + 3\,H_2O$	Denitrification
$8\,[H] + NO_3^- + 2\,H^+ \rightarrow NH_4^+ + 3\,H_2O$	Nitrate ammonification
$[H] + Fe^{3+} \rightarrow Fe^{2+} + H^+$	Iron reduction
$2\,[H] + Mn^{4+} \rightarrow Mn^{2+} + 2\,H^+$	Manganese reduction
$8\,[H] + SO_4^{2-} + 2\,H^+ \rightarrow H_2S + 4\,H_2O$	Sulfate reduction
$2\,[H] + S \rightarrow H_2S$	Sulfur reduction
$8\,[H] + CO_2 \rightarrow CH_4 + 2\,H_2O$	CO_2 reduction to methane
$8\,[H] + 2\,CO_2 \rightarrow CH_3COOH + 2\,H_2O$	CO_2 reduction to acetate
$2\,H^+ + e^- \rightarrow H_2$	Proton reduction (by fermenters without energy conservation)

Redox tower



Redox potential of some inorganic compounds that can be reduced and oxidized biologically.

Further metal ions: Mn^{4+}, Sb^{5+}, U^{6+}, chromate, selenate...

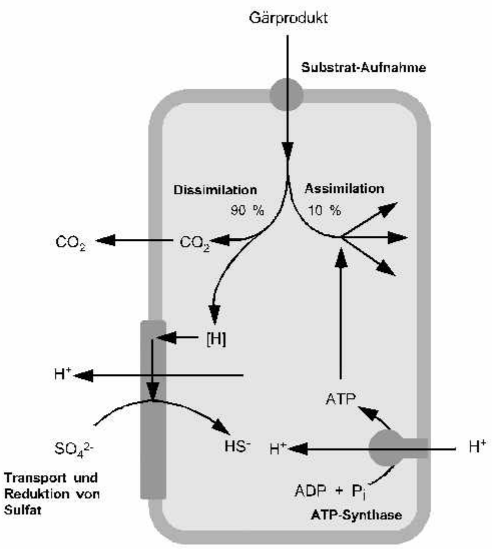
Maximum free energy:

$\Delta G = -z \cdot F \cdot \Delta E$

(c) Heribert Cypionka, www.icbm.de/pmbio

Sulfate-reducing bacterium - overview

SRB - overview



- **Mainly fermentation products as substrates (acetate and H_2 most important)**
- **Little ΔG, little assimilation, much dissimilation**
- **Most important final step as long as sulfate is available**
- **Some SRB carry out incomplete oxidation of their substrates to acetate, some grow litho-, or even autotrophically**

(c) Heribert Cypionka, www.icbm.de/pmbio

Sulfate reduction in detail

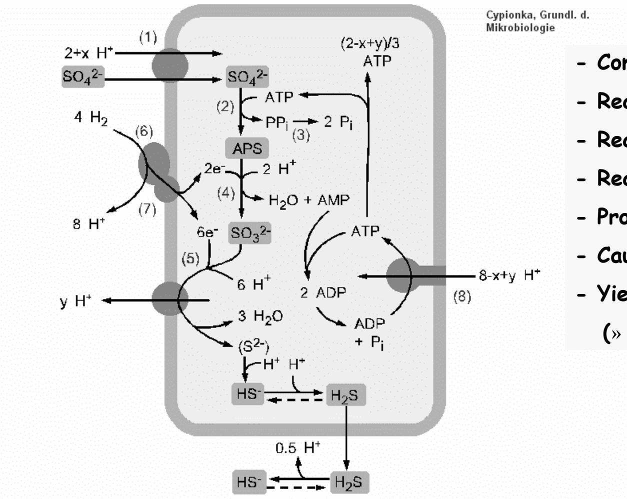
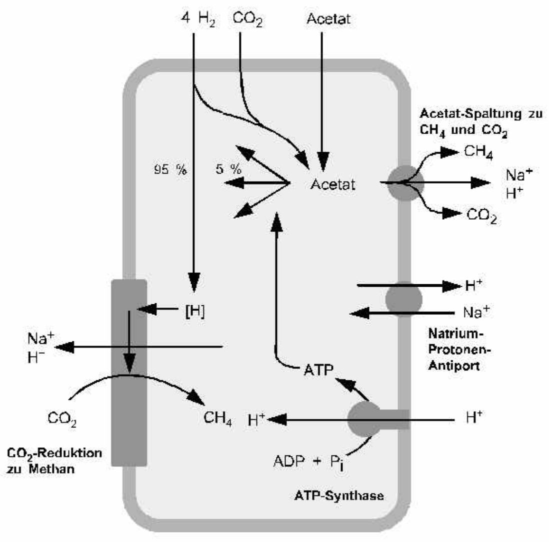


Abb. 15.6. Schritte der Sulfat-Reduktion mit Wasserstoff und der daran gekoppelten chemiosmotischen Energiekonservierung. (1) Transportsystem, (2) ATP-Sulfurylase, (3) Pyrophosphatase, (4) APS-Reduktase, (5) Sulfitreduktase mit Kopplung an Protonen-Translokation, (6) Hydrogenase, (7) Cytochrom c, (8) ATP-Synthase

- **Compare to: 2 H_2 + O_2 ® H_2O**
- **Requires transport of ions**
- **Requires activation by 2 ATP**
- **Requires several steps**
- **Produces toxic H_2S**
- **Causes alkalinization**
- **Yields little free energy (» $^1/_6$ compared with O_2)**

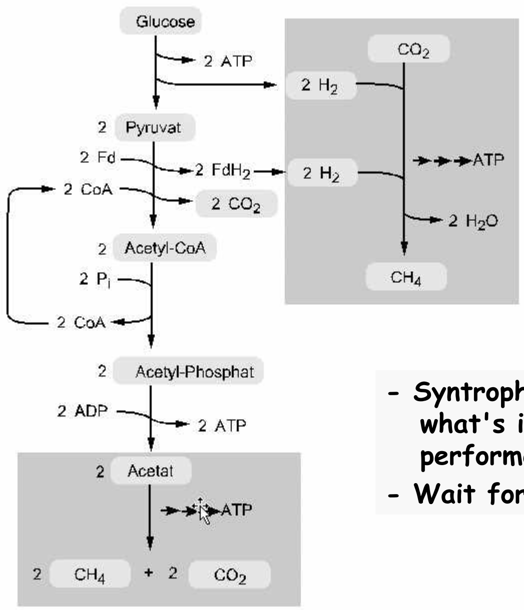
Methanogens - overview



- **Only acetate and H_2 are utilized as substrates (carbonate respiration and acetate fermentation)**
- **Archaea with special pathways and coenzymes**
- **Very little DG, little assimilation, much dissimilation**
- **Most important final step, if sulfate is not available**
- **Methane may be oxidized with sulfate as electron acceptor aerobically and anaerobically**

Methanogenic glucose degradation

Glucose -> CH_4



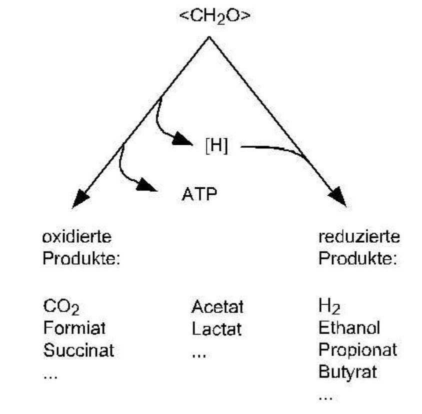
- Syntrophic process! Only what's in the gray boxes is performed by methanogens
- Wait for the Clostridia...

What is different when there is no inorganic electron acceptor at all?

No electron acceptor

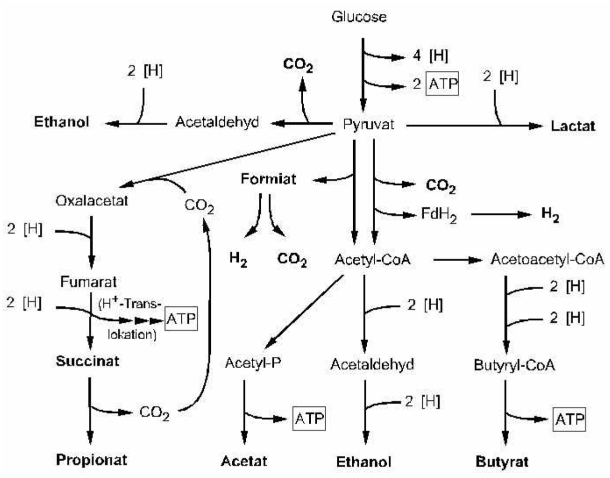
- There is even less free energy (DG) available.
- Dissimilation will increase.
- The only energy-yielding process is fermentation.
- Some substrates are easily fermentable. Others require the symbiotic cooperation with symbiotic partners.

Principle of fermentations



- **Most important fermentation products: acetate and hydrogen**
- **Organic compounds function as both, electron donor and acceptor**
- **Substrate phosphorylation instead of chemiosmotic energy conservation predominant**

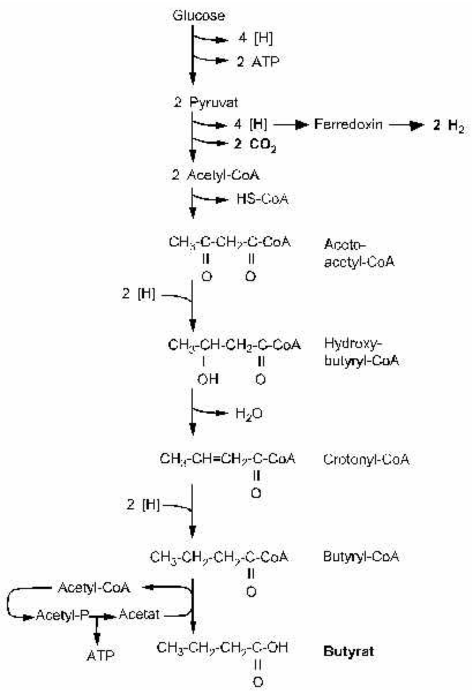
Most important fermentation pathways



In natural environments fermenters are rarely studied!

- **Excess electrons may be a problem**
- **As long as sulfate or CO_2 (+SRB/methanogens) are present the problem is solvable**
- **Alternatively, two-substrate fermentation may be possible (one organic compound as donor another one as electron acceptor)**

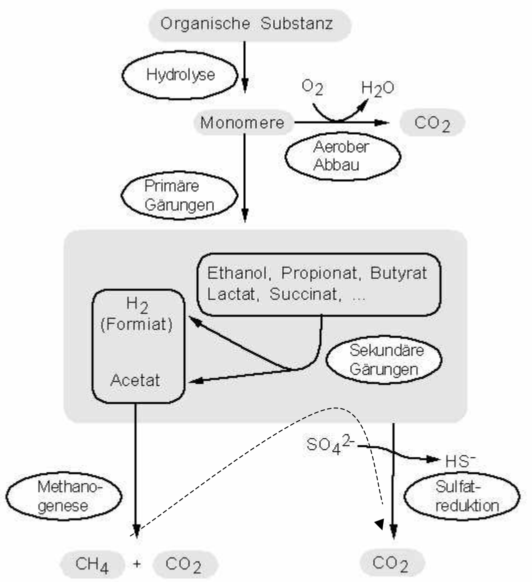
Why does a Clostridium form butyric acid?



It has to get rid of excess electrons.

Under steady state conditions in natural environments acetate and H_2 are formed almost exclusively.

Main steps involved in the degradation of organic matter



- **Aerobic degradation: One organism carries out the whole process completely**
- **Anaerobic degradation: Primary fermenters are fast and form the substrates of SRB**
- **In the absence of sulfate secondary fermenters can form the substrates of methanogens**
- **Steady state conditions result in the formation of acetate and H_2, almost exclusively**
- **What happens to the methane and sulfide formed?**

What happens to the reduced products of the anaerobic respirations?

Dogma of the biological infallibility:

What has been formed biologically can be degraded biologically.

- Holds true without any exceptions, but not without preconditions and loopways
- Is fulfilled mainly by microorganisms

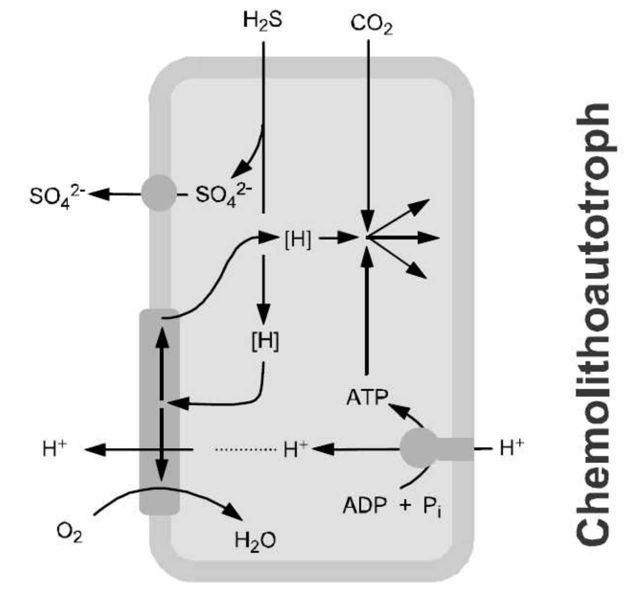
Lithotrophic processes

Electron donor		Oxidized end product	Process (example)
H_2	$\rightarrow$	H^+	Hydrogen oxidation (Knallgas bacteria)
CH_4	$\rightarrow\rightarrow\rightarrow$*) O_2!	CO_2	Methane oxidation (oxygenase!) (Methylo- or methanotrophs)
H_2S	$\rightarrow\rightarrow\rightarrow$	SO_4^{2-}	Sulfurication (Thiobacillus or phototrophic sulfur bacteria)
Fe^{2+}	$\rightarrow$	Fe^{3+}	Iron oxidation (Gallionella)
N_2	$\rightarrow\rightarrow\rightarrow$	NO_3^-	unknown (via loopway, only)
NH_4^+	$\rightarrow\rightarrow\rightarrow$	NO_3-	Nitrification, stepwise by 2 Bacteria
NH_4^+	$\rightarrow\rightarrow\rightarrow$ O_2!	NO_2^-	Nitrosomonas (Oxygenase!)
NO_2^-	$\rightarrow$	NO_3^-	Nitrobacter
H_2O	$\rightarrow$	O_2	Oxygenic photosynthesis (Cyanobacteria, chloroplasts)

*)More than one arrow indicates multi-step processes.

Metabolism of an aerobic sulfur oxidizer

Sulfur oxidizer



Lithotrophy

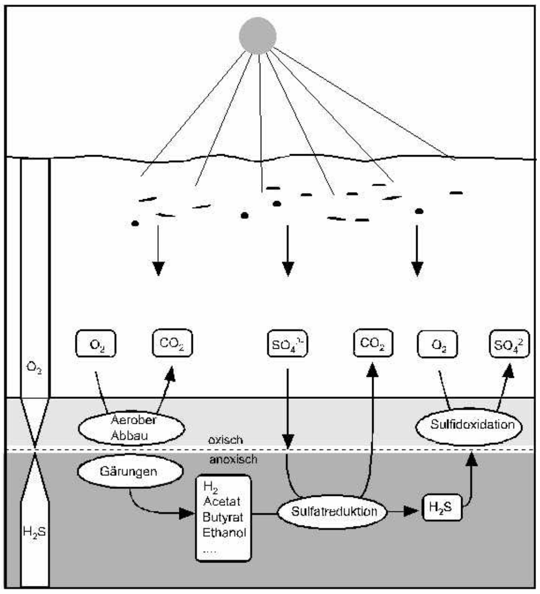
Lithotrophy

- Only found at Prokaryotes
- Good electron acceptors form bad electron donors for lithotrophic processes - and vice versa
- Inorganic electron donors can be oxidized with different acceptors, if the redox potential is appropriate
- Exceptions with inert molecules (alkanes, aromatic compounds, NH_3): Oxygen functions as reactant in oxygenase reactions, not as terminal acceptor
- New oxygen-independent pathways in several anaerobes detecte (hexadecane, methane...)

(c) Heribert Cypionka, www.icbm.de/pmbio

Role of lithotrophic processes in a sediment

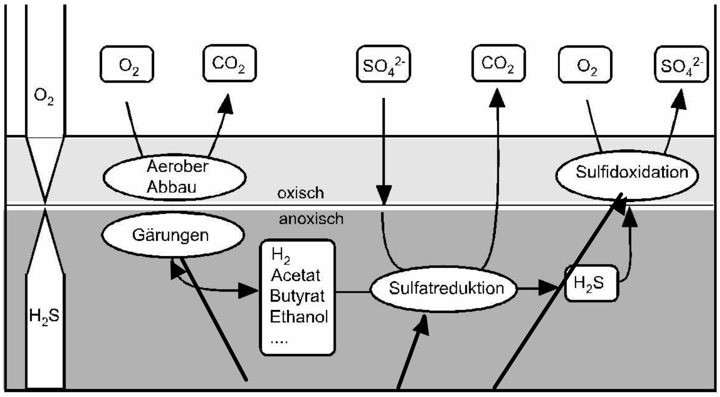
Marine sediment



(c) Heribert Cypionka, www.icbm.de/pmbio

Processes in a sediment

Sediment lithotrophs



Biological loopways...

- **Only half of the oxygen is consumed directly for the oxidation of organic matter**
- **Half of the organic matter is fermented and oxidized with sulfate and CO_2 as electron acceptor**
- **The sulfide (and methane) formed has to be reoxidized by lithotrophic (or methanotrophic) bacteria**

(c) Heribert Cypionka, www.icbm.de/pmbio

Scenario (1)

A plausible scenario - how life could have evolved

- Abiotic formation of organic matter (Miller and Urey 1953)
  - on earth or extraterrestrial (but not supernatural)
  - low amounts, little reducing power of the (2nd) atmosphere

- Energy: Irridiation (light, UV, no ozone layer), chemical Energy (hydrolysis, redox processes)

- Evolution of the first cells by cyclic recursive processes (Hypercycle?, many open questions) $^{13}C/^{12}C$ Isotope fractioniation und fossil cell structures prove: At least 3.8 Ga before present life had developed: prokaryotic cells with membrane, DNA, protein, ATP...

- Lines with hyperthermophilic prokaryotes deeply branching, (heat cannot be an energy source!), no (true) phototrophs among the Archaea

Scenario (2)

- Life mode: Anaerobe, not phototrophic, however: Growth on the 'primordial soup' would leave tar as residue and cause net mineralisation, no isotope fractionation would have been expected

- Autotrophy (CO_2 fixation) causes early enrichment of $^{12}C/^{13}C$

- Chemosynthesis (Lithotrophie) with H_2, S, $S_2O_3^{2-}$

- CO_2 as universal electron acceptor and carbon source (transformed to CH_4 or acetate)

- Lithotrophy (and phototrophy) always coupled to electron transport
- Primitive fermenters? - chemiosmotic energy transformation coupled to any transport and electron transport

- Dissimilatory sulfur metabolism found in many archaea
- Photosynthesis primarily anoxygenic (purple or green bacteria), one photosystem, which does not oxidize water (but possibly Fe^{2+})
- Oxygenic photosynthesis later, invented by cyanobacteria (development of a second photosystem as a copy of photosystem I)
- Aerobic respiration concomintantly wit O_2 formation, new structure of the carbon cycle
- Few life modes in eukaryotes

Scenario (3)

Life modes

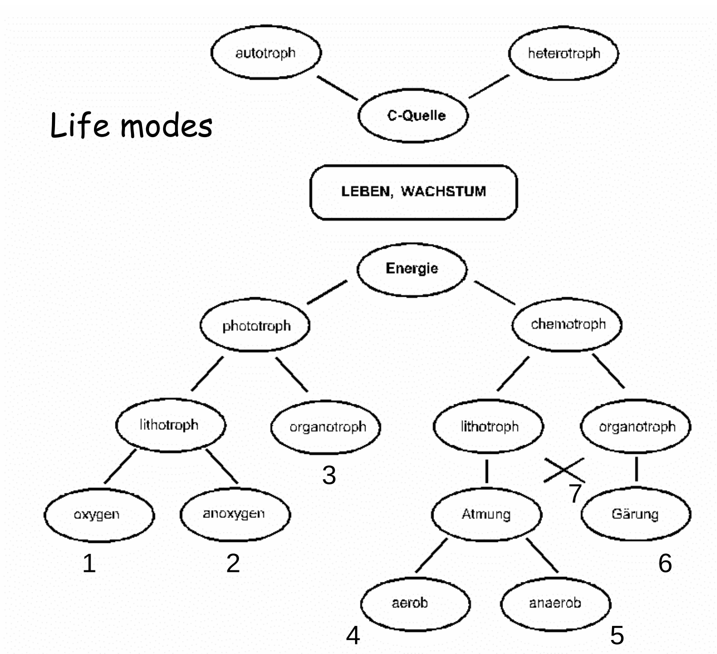


Abb. 18.1. Lebensweisen und Möglichkeiten biologischer Energiekonservierung

Cypionka, Grundlagen der Mikrobiologie

1: Green plant

2: Purple sulfur bacterium

3: Non-sulfur purple bacterium

4: Man, fungus

5: Sulfate-reducing bacterium

6: Enterobacterium

7: Desulfovibrio sulfodismutans