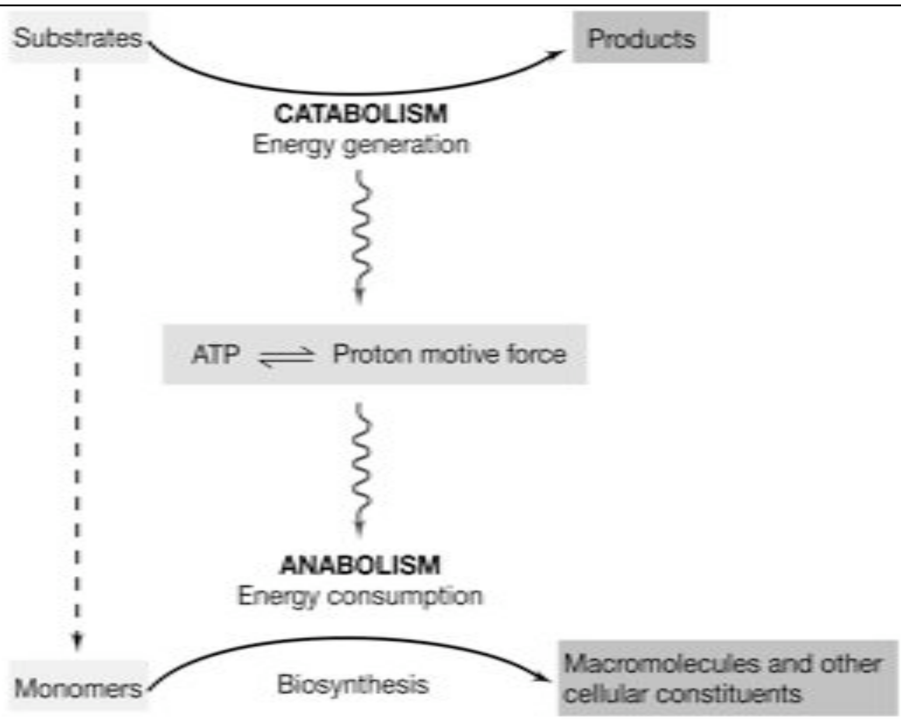
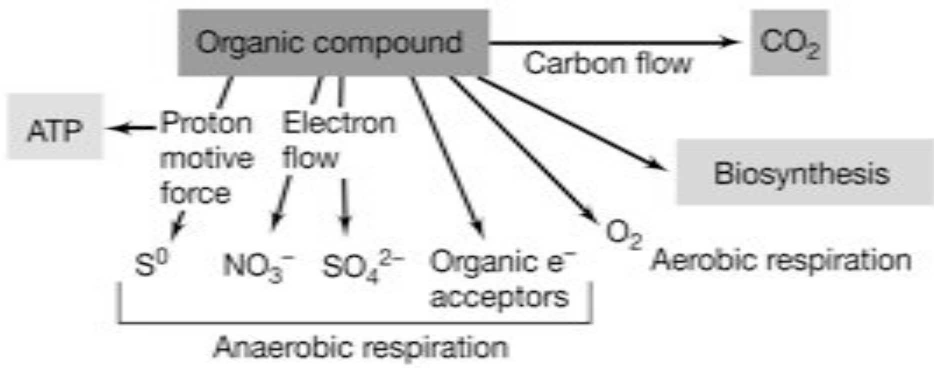
CHEMOLITHOTROPHS

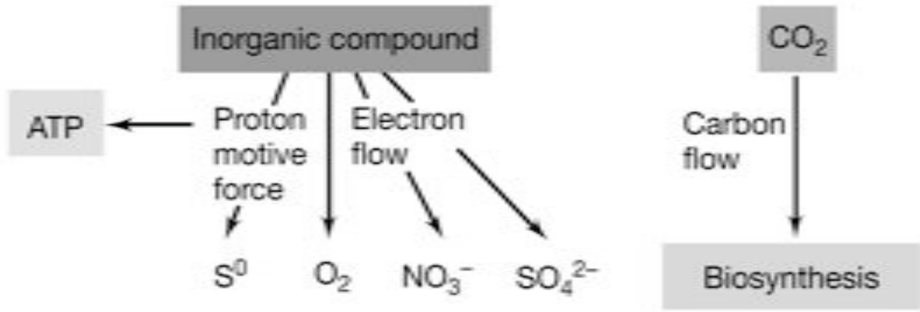
Martin Könneke



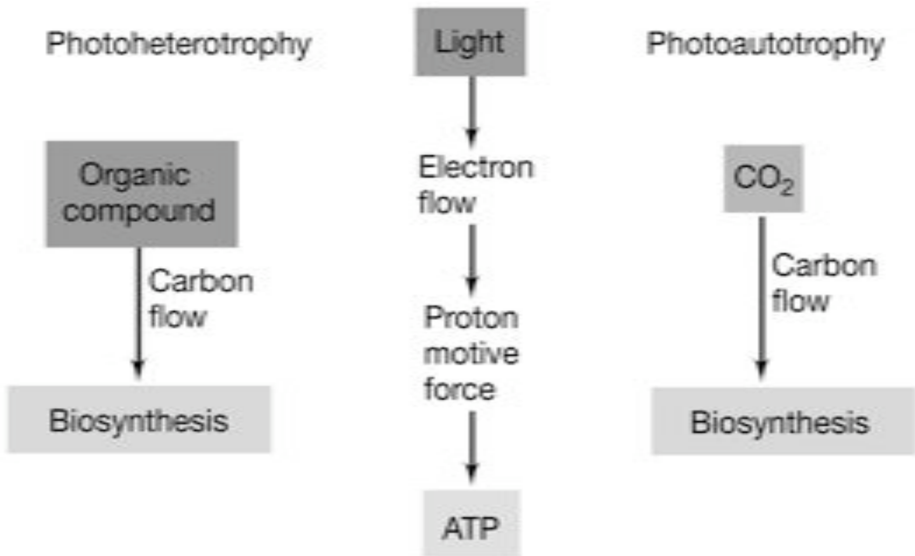
Energy source	**Electrondonor**	**Carbon source**
Chemo-	Organo-	heterotrophic
Photo-	**Litho-**	autotrophic



(a) **Chemoorganotrophic metabolism**



(b) **Chemolithotrophic metabolism**



(c) **Phototrophic metabolism**

Concept of lithotrophy (1886)

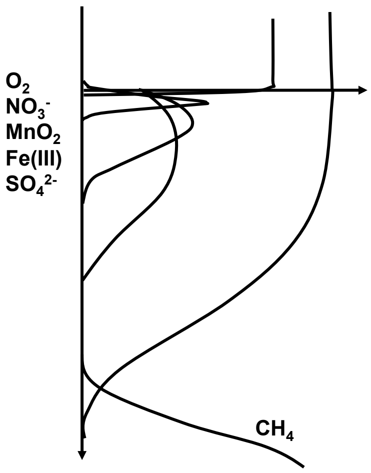
Ihre Lebensprozesse spielen sich nach einem viel einfacheren Schema ab; durch einen rein anorganischen chemischen Prozess...werden alle ihre Lebensbewegungen im Gange erhalten

Sergej Nikolaevitch Winogradsky (1857–1953)

Conversion of inorganic compounds for energy conservation:
- Nitrification (oxidation of ammonia to nitrate)
- Sulfur oxidation
- Iron oxidation

- Autotrophic bacteria
- Nitrogen fixing bacteria

Vertical profile of potential electron acceptors in sediments



	E_o' [mV]	
O_2/H_2O	+820	Aerobic respiration
NO_3^-/N_2	+751	Denitrification
NO_3^-/NH_4^+	+363	Nitrate ammonification
MnO_2/Mn^{2+}	+390	Manganese reduction
FeOOH	+150	Iron reduction
SO_4^{2-}/HS^-	-218	Sulfate reduction
S^o/HS^-	-240	Sulfur reduktion
CO_2/CH_4	-244	Methanogenesis

Lithotrophic processes are essential for the reoxidation of reduced electron acceptors!

All chemolithotrophes are prokaryotes!

Almost all known lithotrophes are autotroph!

Lithotrophic Processes

Elektronendonor	Oxidized product	Process/ organism
H_2	H^+ (H_2O)	Knallgas reaction/ *Ralstonia*
NH_4^+	NO_3^-	Nitrification (2 types)
NH_4^+	NO_2^-	Ammonia oxidizer (*Nitroso*-)
NO_2^-	NO_3^-	Nitrite oxidizer (*Nitro*-)
CH_4	CO_2	Methane oxidizer (*Methylo*-)
H_2S, S	SO_4^{2-}	Sulfur oxidizer/*Thiobacillus, Beggiatoa*
Fe^{2+}	Fe^{3+}	Iron oxidation/*Thiobacillus*
H_2O	O_2	Photosynthesis

‘Knallgas reaction’
Hydrogen-oxidizing bacteria:

Hydrogen as electron donor
A) Energy source
B) Reduction power for carbon fixation

Key enzyme:
Hydrogenase

Catalyses the reversible conversion
of hydrogen to protons and electrons

$H_2 \rightleftarrows 2H^+ + 2e^-$

Aerobic oxidation of hydrogen
“Knallgasbacteria”

$\mathbf{2\ H_2 + O_2 \rightarrow 2\ H_2O}$

Facultativ chemolithotrophes
(are also able to use organic compounds
as carbon source)

Microaerophilic (5-10% O_2)

$H_2 \rightarrow 2\ e^- + 2\ H^+$

Electron-donating half reaction

$\frac{1}{2} O_2 + 2\ e^- \rightarrow O^{2-}$

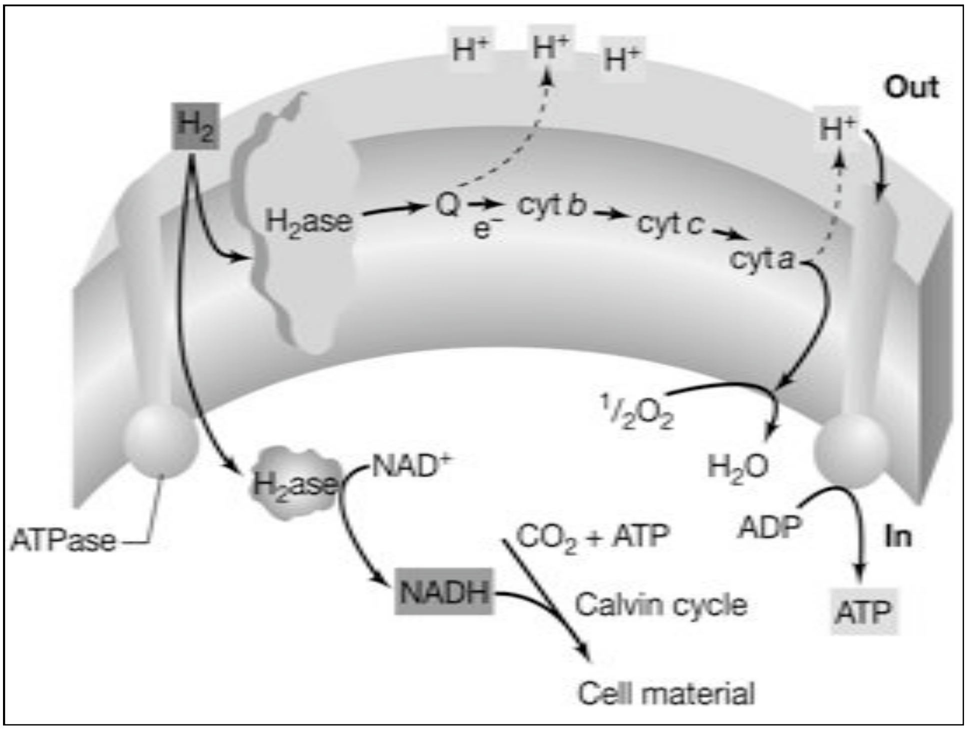
Electron-accepting half reaction

$2\ H^+ + O^{2-} \rightarrow H_2O$

Formation of water

Electron donor — $H_2 + \frac{1}{2} O_2 \rightarrow H_2O$ — Electron acceptor

Net reaction



Helicobacter pylori
Gram negative epsilonproteobacteria

The only known bacterium that can thrive in the acid environment of the stomach

Cause infections of the mucus lining of the stomach (gastritis)

Isolated by Robin Warren und Barry Marschall (Medicin Nobel Prize in 2005)

Containing hydrogenase as well as urease

Requires oxygen, but in lower levels than in atmosphere (microaerophilic)

Helicobacter pylori
(3 µm in length, 4-6 flagella)

Habitats of Knallgasbacteria

Hydrogen of biotic or abiotic origin

Habitats:
Boundary between oxic and anoxic conditions:
-Rhizosphere
Ralstonia eutropha
-Marine sediments
Hydrogenovibrio marinus
-Human gastrointestinal tract
Helicobacter pylori
-Hydrothermal systems
Aquifex pyrophilus (Bacteria, 85°C)
Pyrolobus spec. (Archaea, 106°C)

Anaerobic oxidation of hydrogen

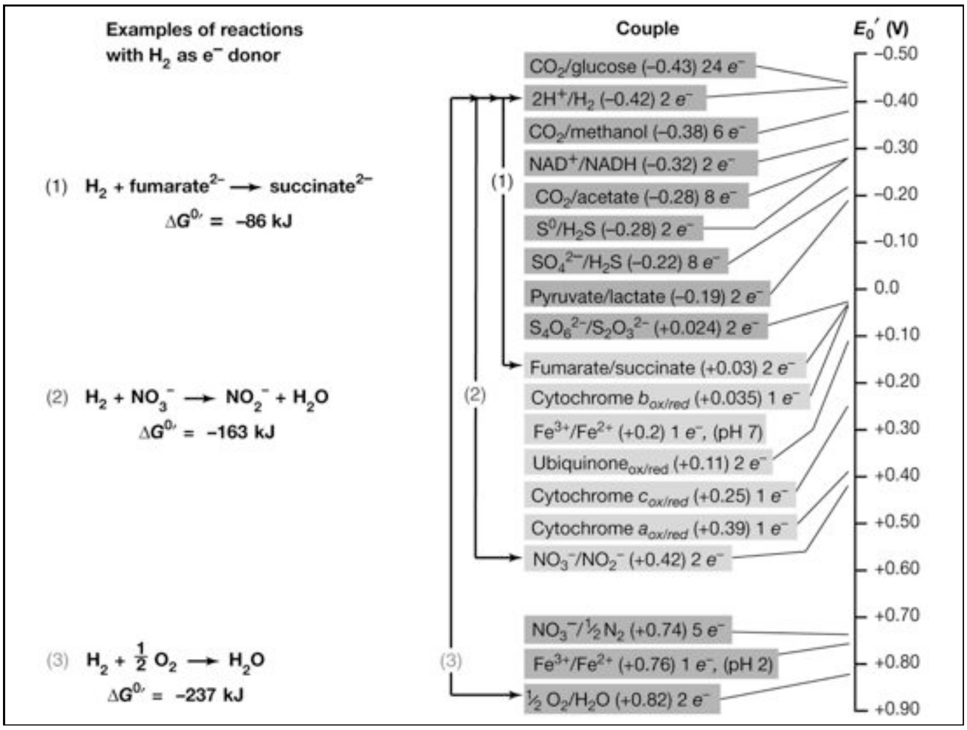
$5\ H_2 + 2\ NO_3^- + 2\ H^+ \rightarrow N_2 + 6\ H_2O$

$H_2 + 2\ Fe^{3+} \rightarrow 2\ H^+ + 2\ Fe^{2+}$

$4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$

$4\ H_2 + 2\ CO_2 \rightarrow$ acetate $+ 2\ H_2O + H^+$

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



Nitrification
Oxidation of ammonia to nitrate

Performed by 2 physiological distinct groups of microorganisms.

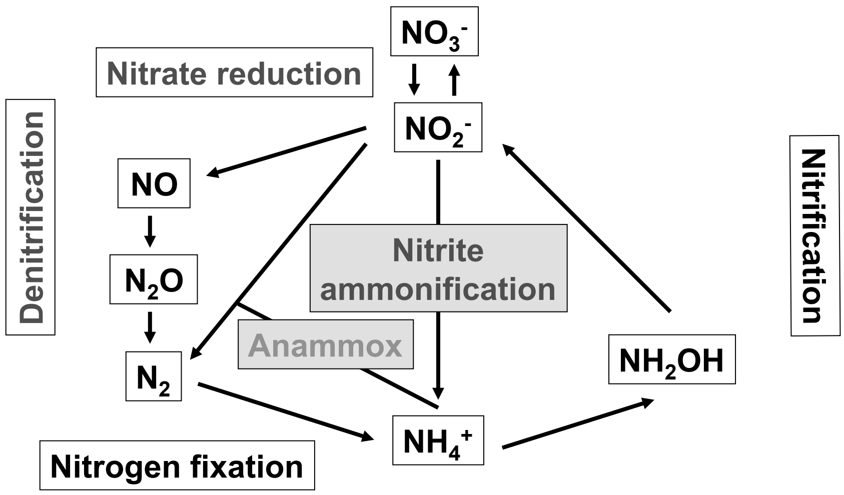
1.Ammonia oxidizer **(Nitroso-)**
z.B. *Nitrosomonas europaea*

$$\mathbf{2\,NH_3 + 3\,O_2 \rightarrow 2\,NO_2^- + 2\,H_2O + 2\,H^+}$$

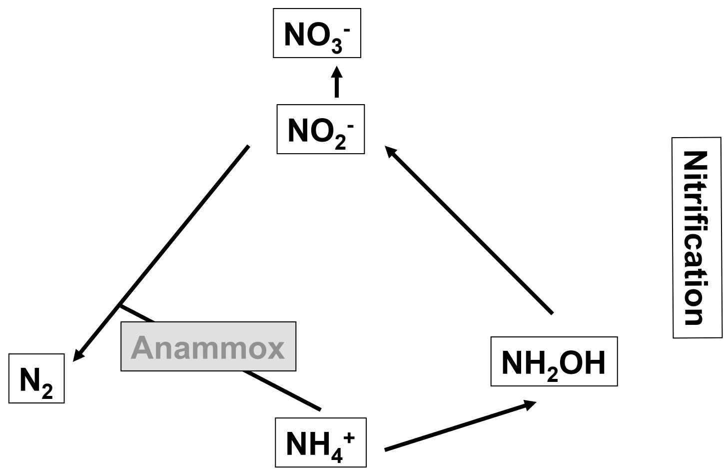
2. Nitrite oxidizer **(Nitro-)**
z.B. *Nitrobacter winogradskyi*

$$\mathbf{2\,NO_2^- + O_2 \rightarrow 2\,NO_3^-}$$

The biological nitrogen cycle

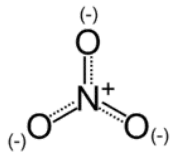


Oxidation of ammonium



Nitrification
Oxidation of ammonia to nitrate

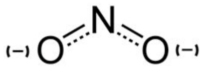
Nitrate (+ V)



Nitrite oxidizers

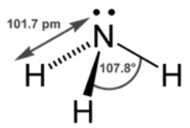
Nitrite (+ III)

Oxidation



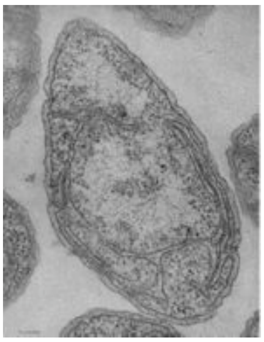
Ammonia oxidizers

Ammonia (- III)



Ammonia oxidizer

z.B. Nitrosomonas europaea



Activation of ammonia

“elemental oxygen as reactant”

1. **Ammonia monooxigenase (AMO)**

$NH_3 + 0.5\ O_2 \rightarrow NH_2OH$ (+17 kJ/mol)

2. **Hydroxylamine oxidoreductase (HAO)**

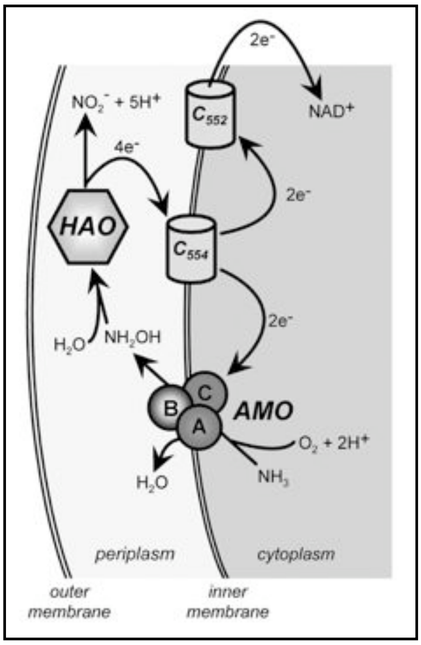
$NH_2OH + H_2O \rightarrow NO_2^- + 5\ H^+ + 4\ e^-$ (+23 kJ/mol)

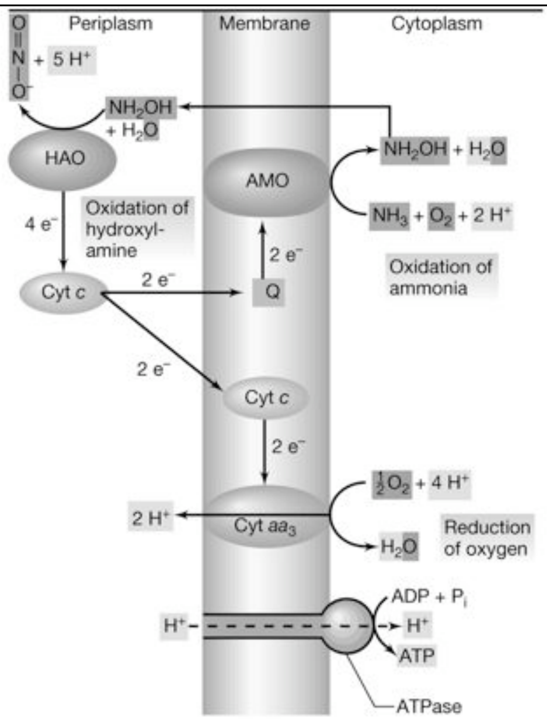
$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$ (-274 kJ/mol)

$NH_3 + 1.5\ O_2 \rightarrow NO_2^- + H_2O + H^+$ (-234 kJ/mol)

Ammonia oxidation in AOB

- Ammonia monooxygenase (AMO) catalyzes conversion of ammonia (NH_3) to hydroxylamine (NH_2OH)
- AMO is membrane associated and is composed of 3 subunits (AmoABC)





Nitrit-Oxidierer

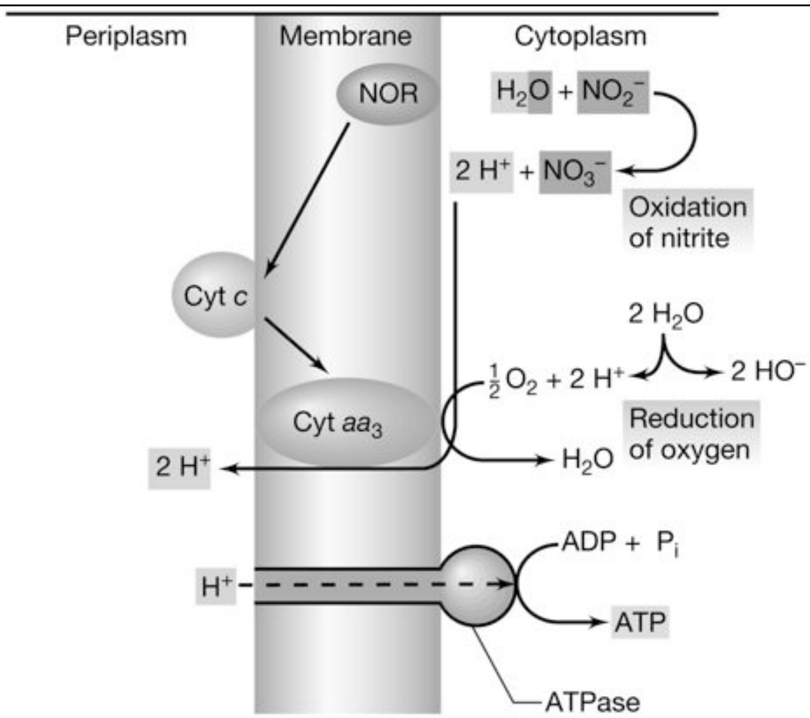
z.B. *Nitrobacter winogradskyi*

Nitrite oxigenasereductase (NOR)

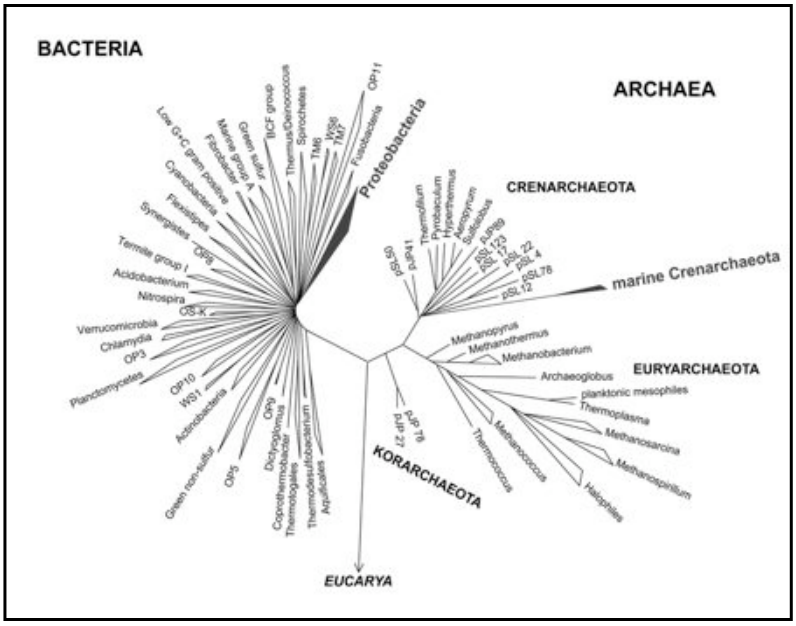
$NO_2^- + H_2O \rightarrow NO_3^- + 2\ H^+ + 2e^-$ (+83 kJ/mol)

$0.5\ O_2 + 2\ H^+ + 2e^- \rightarrow H_2O$ (- 137 kJ/mol)

$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$ (- 54 kJ/mol)



Ammonium-oxidizing microorganisms



Nitrosopumilus maritimus (Crenarchaeota)

a DAPI

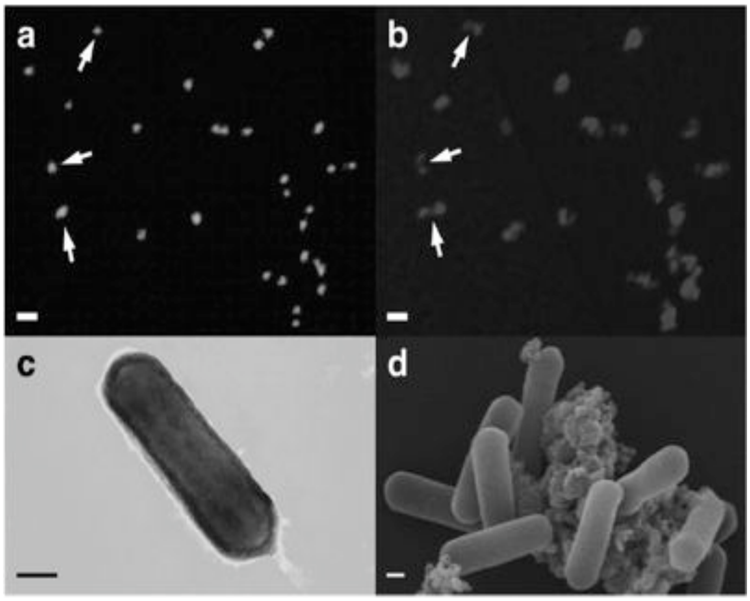
b FISH

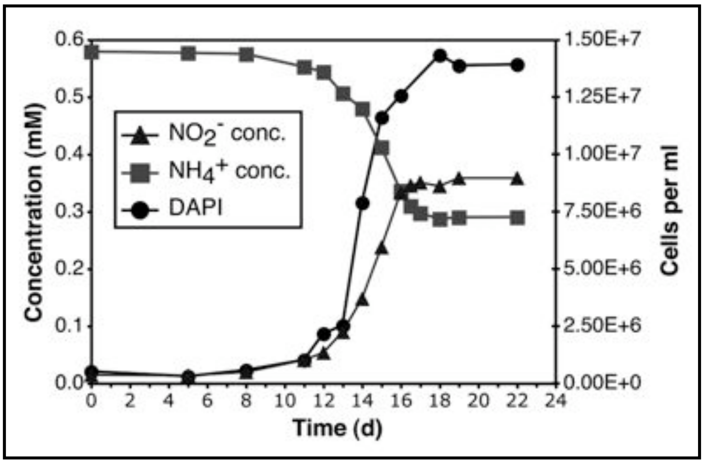
Scale: 1 µm

c TEM

b SEM

Scale: 0.1 µm

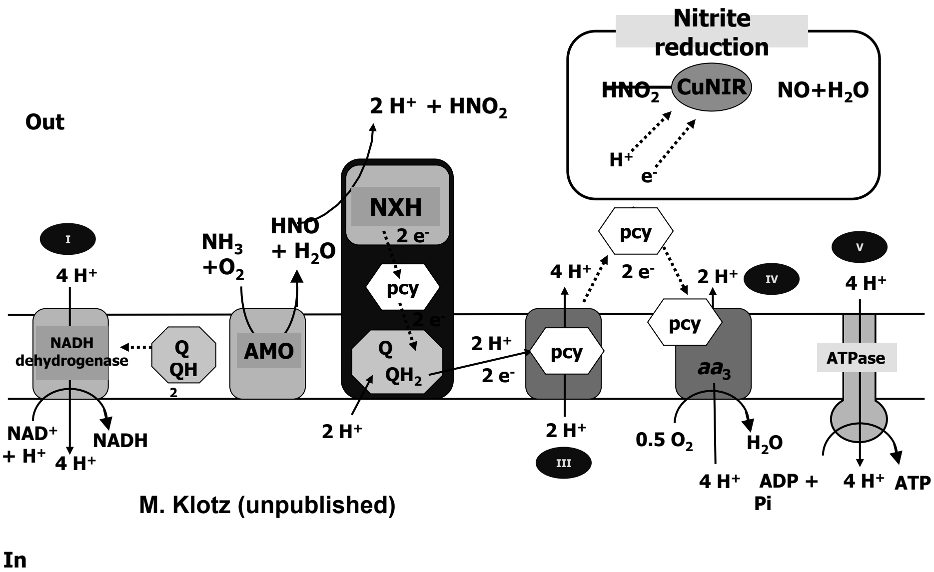




$NH_3 + 1.5\ O_2 \rightarrow NO_2^- + H_2O + H^+$ ($\Delta G^{0'}$ = - 235 kJ mol^{-1})

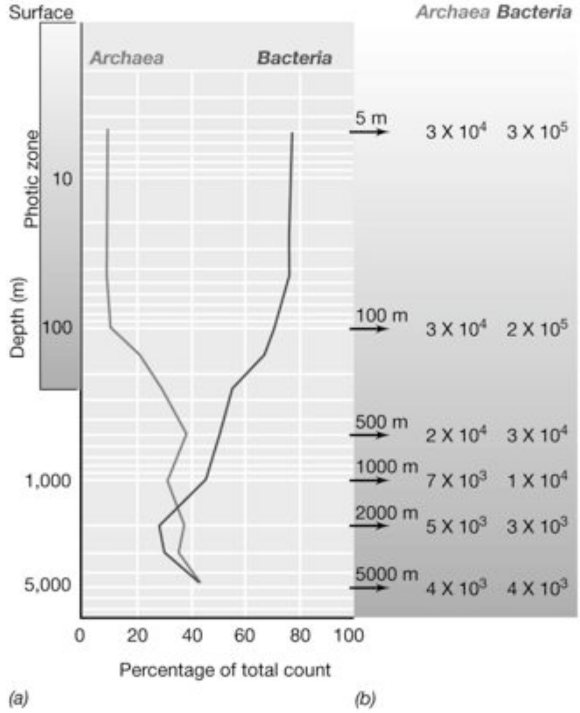
The first nitrifyer within the domain *Archaea*

Proposed ammonia oxidation pathway in ammonia-oxdizing archaea

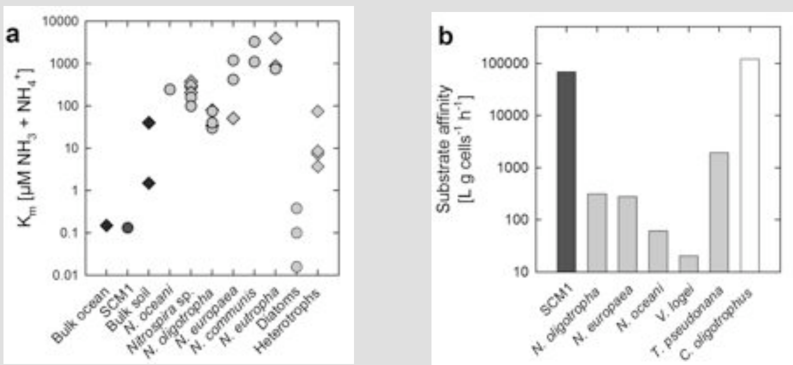


M. Klotz (unpublished)

Distribution of *Archaea* and *Bacteria* in North Pacific ocean water

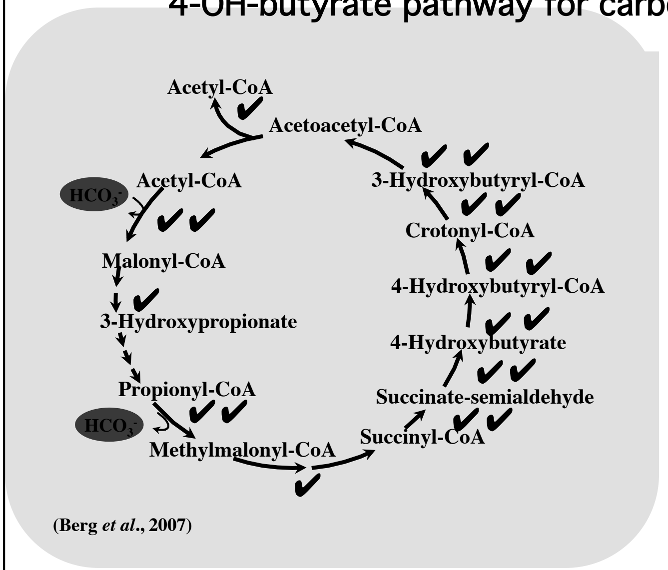


High substrate affinity and extremly low K_M values for ammonia



Martens-Habbena et al. (Nature, 2009)

N. maritimus uses the 3-OH-propionate/ 4-OH-butyrate pathway for carbon fixation



Whole genome analyses ✔

Biochemical analyses ✔

S. Standfest et al. (in prep.)

M. Könneke

Habitats of nitrifying microorganisms

- Open Ocean water and oxic marine sediments
- Freshwater habitats
- waste water treatment
- Aquaria
- Soils (forrest and agricultural)
- Surfaces of building material
- As symbionts in animals

At present all known nitrifyer are
obligat chemolithotrophautotroph!

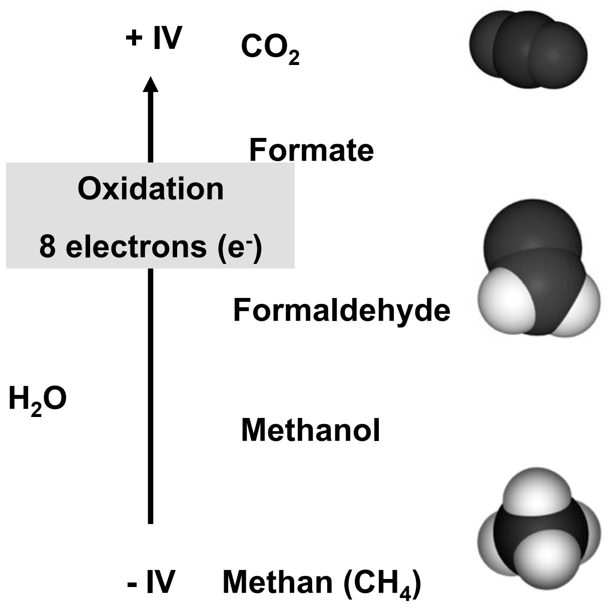
Nitrifying bacteria mainly fix carbon via the Calvin cycle (Calvin-Bassham-Benson-cycle)

Key enzyme: RubisCO

Ammonia-oxidizing archaea fix carbon via the 3 hydrroxypropionate/ 4 hydroxybutyrate cycle

Methane oxidation: $CH_4 \rightarrow CH_3OH \rightarrow CH_2O \rightarrow CHOO^- \rightarrow CO_2$

$CH_4 + 2\ O_2 \rightarrow CO_2 + 2\ H_2O$



Methane-oxidizing microorganisms (Methylotrophs)

Oxidize methane and few other C1 Compounds as electron donor for energy conservation and as sole carbon source.

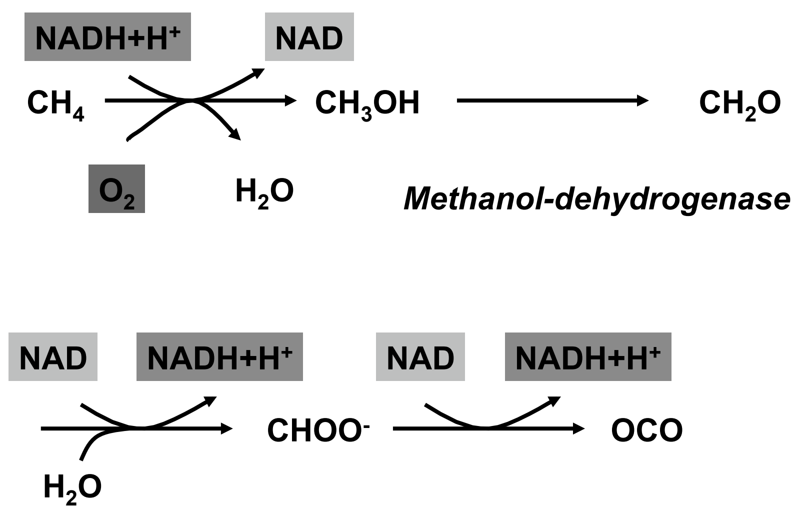
Methylotrophs synthesize all C-C bonds *de novo*

Key enzyme: **Methane monooxygenase**; catalyze the reaction of methane to methanol

Type I methylotrophes: C1 assimilation via **ribulose monophosphate pathway**

Type II methylotrophes: via **serine pathway**

Methane-monooxigenase



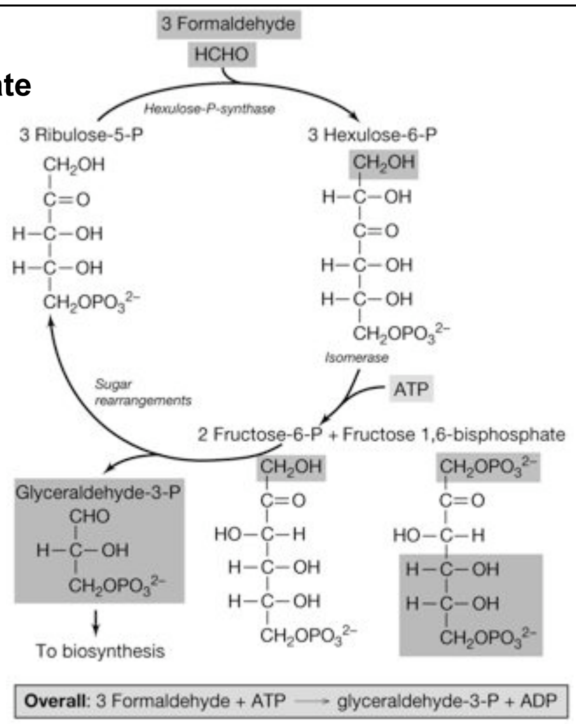
Type I methylotrophes:

Ribulose monophosphate pathway

Key enzyme:

Hexulose-P-synthase

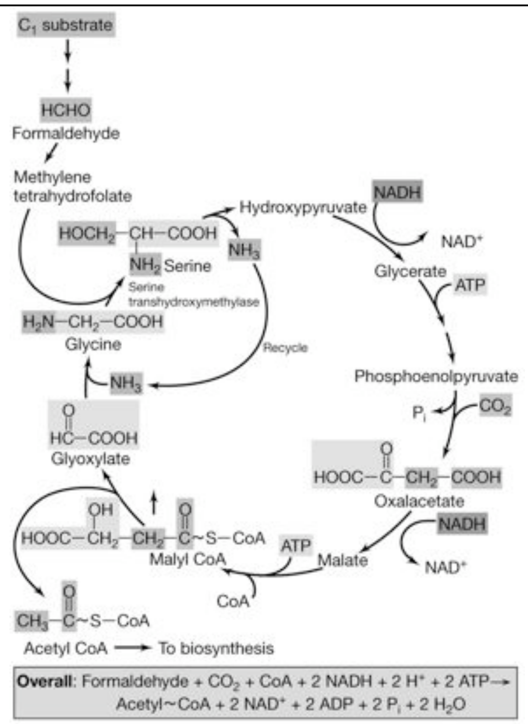
No reducing power required!



Type II methylotrophes:

Serine pathway

Key enzyme: Serine transhydroxymethylase



Oxidation of reduced sulfur compounds

Many reduced sulfur compounds can be used by 'Colorless sulfur bacteria'

Electron donor: Sulfide, sulfur, thiosulfate

Oxidation occurs in stages, resulting in formation of sulfur

Oxidation of red. sulfur compounds results in acidification (= sulfuric acid H_2SO_4)

Oxidation of sulfite to sulfate either via APS or sulfite oxidase

Sulfate (SO_4^-)

+ VI

Oxidation

8 electrons (e^-)

Sulfite (+IV)

Thiosulfate (av. +II)

Sulfur (0)

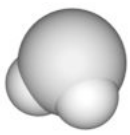
$HS^- + 2\ O_2 \rightarrow SO_4^{2-} + H^+$

$2\ HS^- + O_2 + 2\ H^+ \rightarrow 2\ S^0 + 2H_2O$

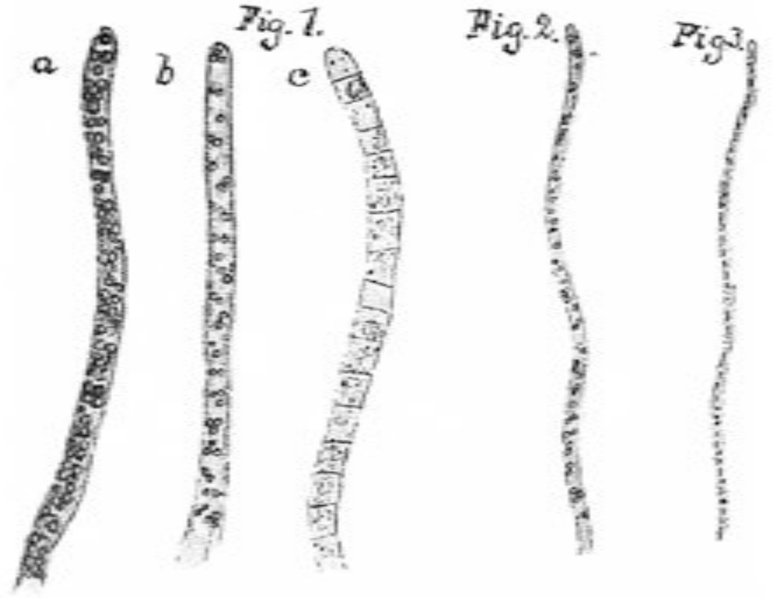
$2\ S^0 + 3\ O_2 + 2H_2O \rightarrow 2\ SO_4^{2-} + 4\ H^+$

$S_2O_3^{2-} + H_2O + 2\ O_2 \rightarrow 2\ SO_4^{2-} + 2\ H^+$

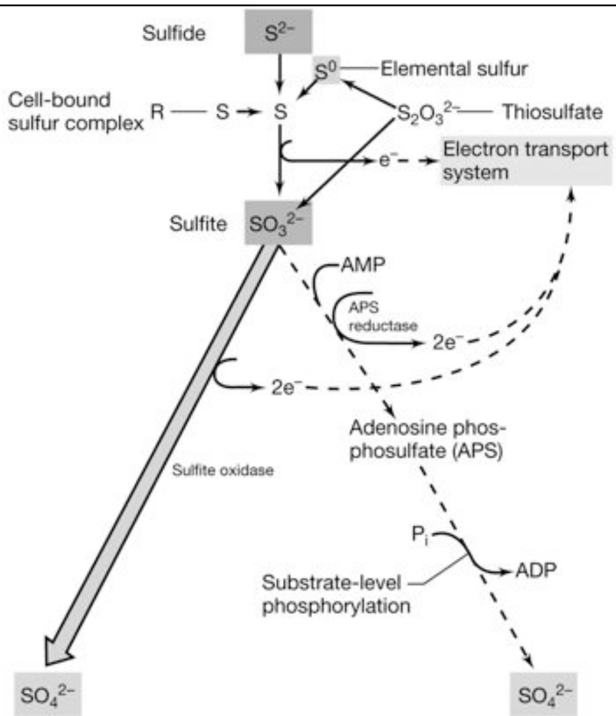
- II

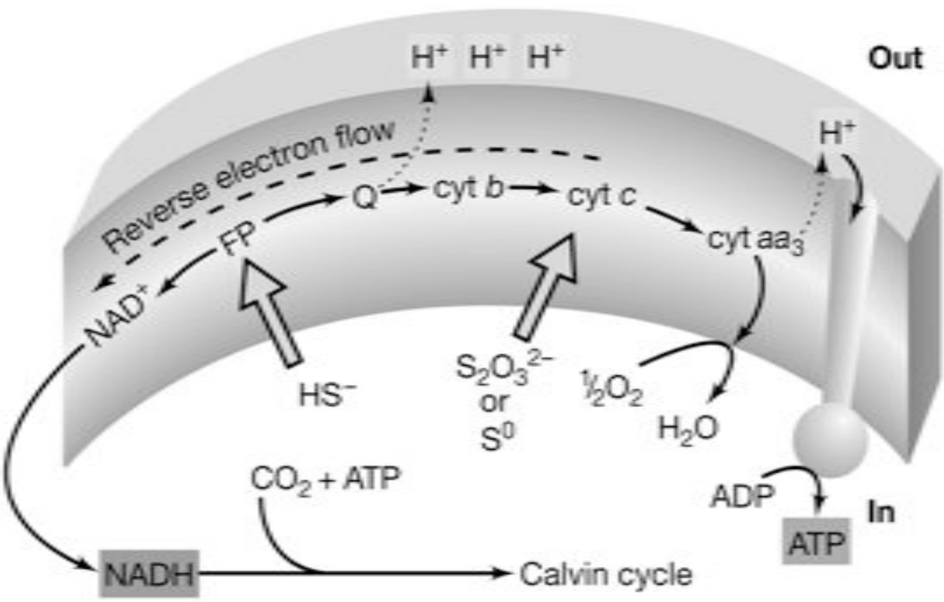


Hydrogen sulfide (H_2S)



Decrease of internal sulfur globules in *Beggiatoa* *(from Winogradsky)*





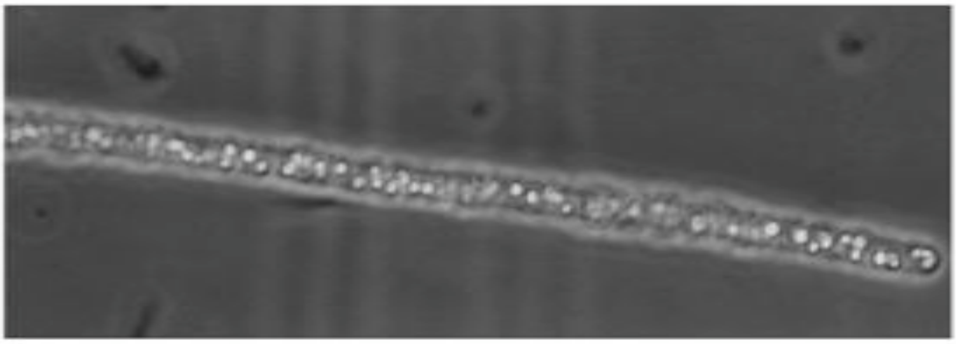
Sulfur oxidizing bacteria (archaea)

Archaea (thermophilic) : *Acidianus sp.*
Sulfolobus sp.

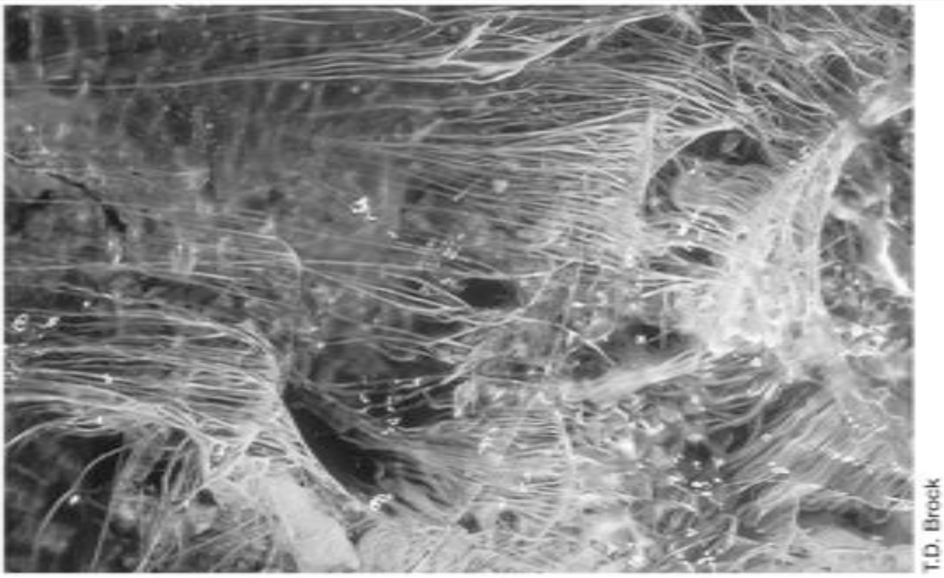
Bacteria: Thiomicrospira
Beggiatoa
Thioploca
Thiomargerita
Thiobacillus denitrificans

Most can also grow anaerobically by using nitrate as terminal electron acceptor

Carbon fixation via the Calvin-cycle

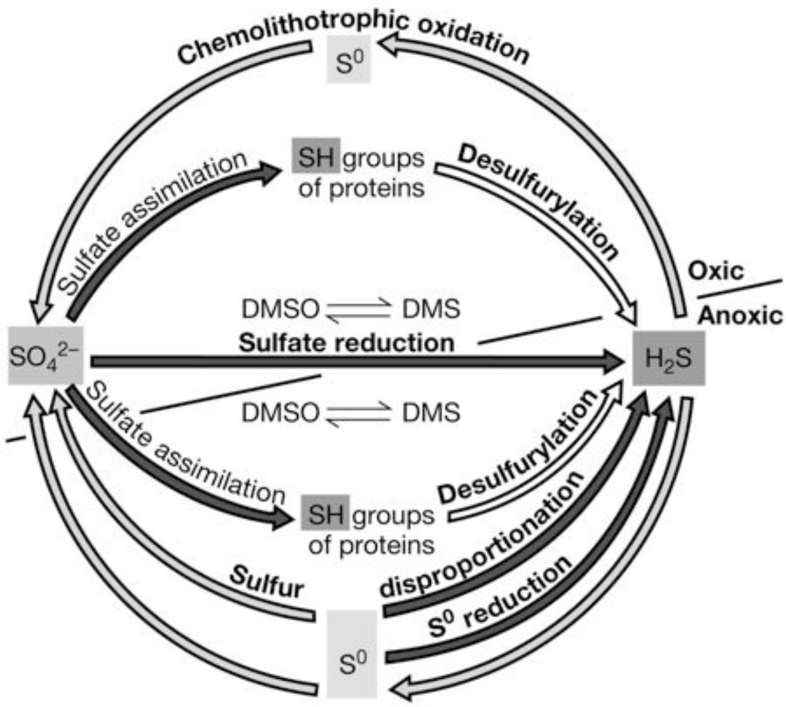


(a) **Sulfur globules in *Beggiatoa***



(b) **Filamente von Schwefel-oxidierenen Bakterien**

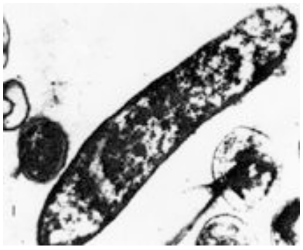
Size comparison:
Thiomargarita namibiensis - Drosophila



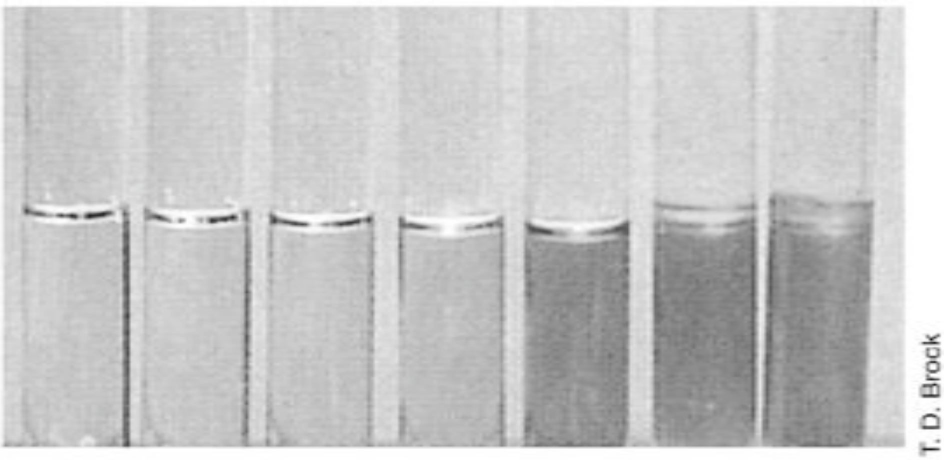
Aerobic oxidation of iron

$4\ Fe^{2+} + O_2 + 6\ H_2O \rightarrow 4\ FeOOH + 8\ H^+$

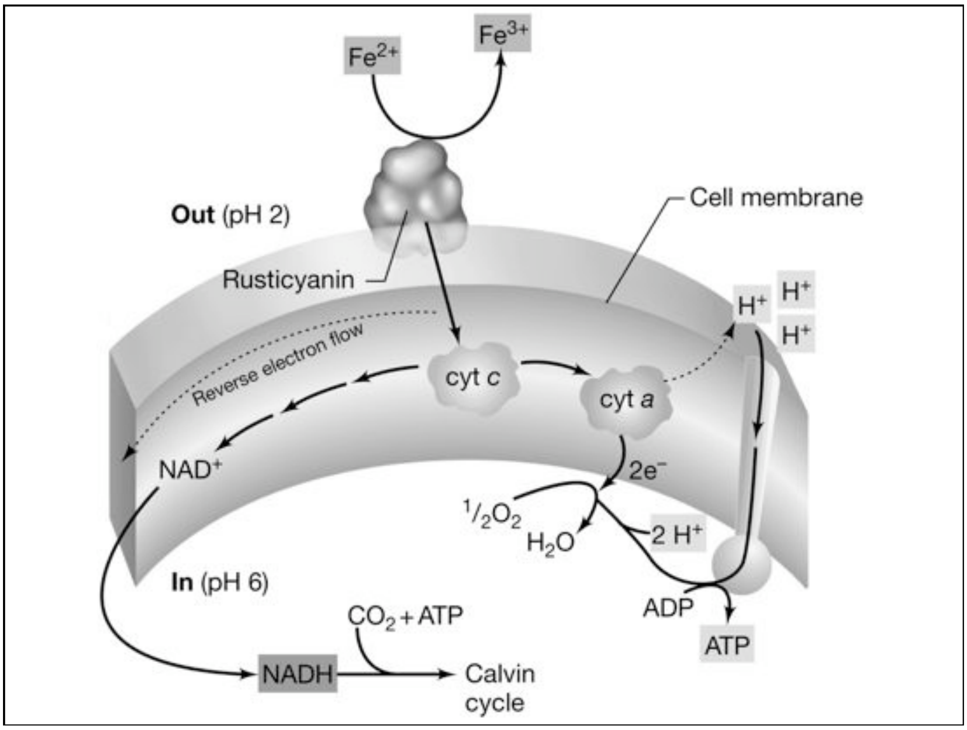
e.g. ***Acidithiobacillus ferrooxidans***
(former *Thiobacillus ferrooxidans*)



Can oxidize both iron and sulfur!



(b)



Lithotrophic Processes

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NH_4^+	NO_2^-	Ammonia oxidizer (*Nitroso*-)
NO_2^-	NO_3^-	Nitrite oxidizer (*Nitro*-)
CH_4	CO_2	Methane oxidizer (*Methylo*-)
H_2S, S	SO_4^{2-}	Sulfur oxidizer *Thiobacillus, Beggiatoa*
Fe^{2+}	Fe^{3+}	Iron oxidation (*Thiobacillus*)
H_2O	O_2	Photosynthesis

Lithotrophic processes are essential for the reoxidation of reduced electron acceptors!

All chemolithotrophes are prokaryotes!

Almost all known lithotrophes are autotroph!

Autotrophy in lithotrophic organisms

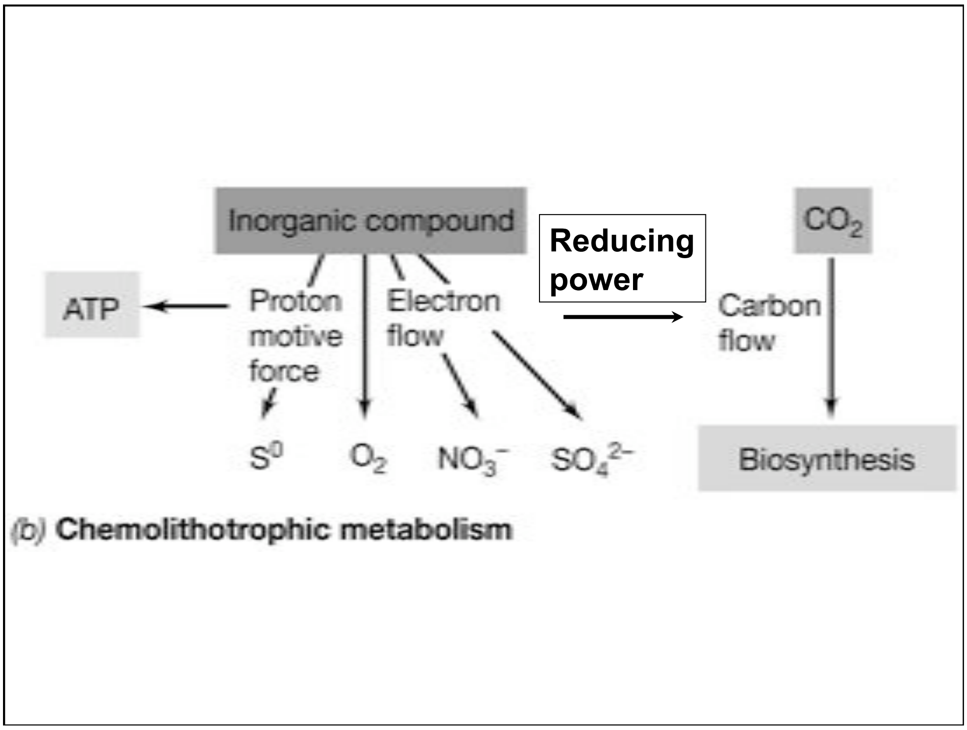
Most of the lithotrophs fix inorganic carbon via the Calvin cycle or pathways typical for anaerobes

Autotrophy requires much more energy than heterotrophy

Reduction of carbon dioxide requires additional reducing power (NADPH+H^+)

Electrons are introduced via energy consuming, reverse electron transport systems

Autotrophy and low energy yield results in low growth yield, but in high conversion rates.


Inorganic compound
Reducing power
CO2
ATP
Proton motive force
Electron flow
Carbon flow
S0
O2
NO3−
SO42−
Biosynthesis
(b) Chemolithotrophic metabolism