

# Chris Anthony

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## Electron transport in bacteria

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This section includes material on alternative electron transport chains in bacteria (Alternative oxidases; Methylotrophs, Chemolithotrophs).

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### Bacterial ATP synthesis

Bacterial biosynthetic pathways are similar to each other. The huge range of different types of bacteria is due to the differences in how they make ATP. And these differences determine the environments where they live and their ecological functions. This section is about bacterial ATP synthesis where electron transport chains (ETC) are involved (anaerobic fermentation is done separately).

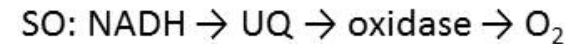
In principle ATP synthesis is the same as for mitochondria. The periplasm is equivalent to the mitochondrial inter-membrane space. The electron transport chain (ETC) uses same components and ATP synthesis by protonmotive force is same.

*NOTE:* most organic substrates are oxidised by way of acetylCoenzyme A and the Krebs' TCA cycle which produces NADH; exceptions include the methylotrophs and chemolithotrophs.

1. Typical mitochondrial systems. These are found in (eg) *Pseudomonas* species.
2. Special systems in bacteria. These include alternative oxidases, anaerobic systems using nitrate or sulphate instead of oxygen, special systems in methylotrophs and autotrophs (chemolithotrophs).

## Alternative oxidases

In *E. coli* and *Klebsiella pneumoniae*. These bacteria do not have cytochrome c or cyt bc1 complex.



The P/O ratio (ATP per atom of oxygen) is only 2.

The oxidase is a quinol oxidase. There are 2 types: cytochrome o and cytochrome d. These are important because these bacteria can grow anaerobically by fermentation (*Klebsiella* is also able to fix  $\text{N}_2$  in anaerobic conditions). Some of the enzymes for these processes are very sensitive to oxygen.

**Cytochrome o** is produced in aerobic conditions. It has a low affinity for oxygen but high rate of reaction. Its function is to be involved in efficient ATP synthesis in aerobic conditions.

**Cytochrome d** is induced as cultures become anaerobic. It has a very high affinity for oxygen. Its main function is to scavenge all oxygen from the culture so that the fermentation and N fixation enzymes are not damaged.

### Anaerobic respiration

**NOTE:** this is not the same as fermentation. It is electron transport phosphorylation in the absence of oxygen.

The oxidase in a typical ETC is replaced by an enzyme that reduces nitrate or sulphate instead of oxygen.

*Denitrification by Pseudomonas denitrificans:* Nitrate is reduced to nitrite, nitrous or  $N_2$

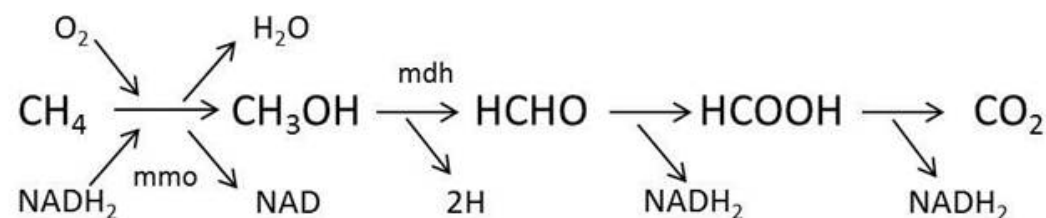
ETC:  $NADH_2 \rightarrow UQ \rightarrow Cytbc_1 \rightarrow Cyt\ c \rightarrow \text{nitrate reductase} \rightarrow \text{Nitrate}$

*Desulphurication by Desulphurivibrio:* Sulphate is reduced to sulphide

ETC:  $NADH_2 \rightarrow UQ \rightarrow Cytbc_1 \rightarrow Cyt\ c \rightarrow \text{sulphate reductase} \rightarrow \text{Sulphate}$

### Methylootrophs

These grow on methane, methanol or methylamine. These C1-compounds are oxidised direct to  $CO_2$ . The TCA cycle is not involved. Methane is oxidised by a monooxygenase (mmo) that uses  $NADH$  so no ATP is made in the first step.



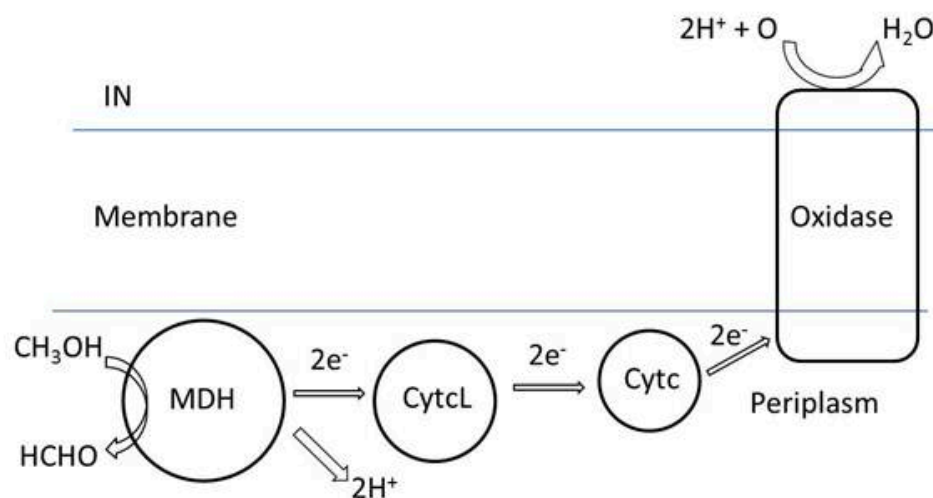
Note. [Click here for extensive discussions of methylootrophic energy metabolism on my website](https://www.chris-anthony.co.uk/mysvuteachingETC.html)

### Methanol oxidation

Methanol is oxidised by a methanol dehydrogenase (mdh) which is a special quinoprotein enzyme that uses PQQ as prosthetic group instead of FAD as in flavoproteins like succinate dehydrogenase. It passed electrons to a specific novel type of cytochrome c which then reduces typical cytochrome c that is oxidised by the oxidase.

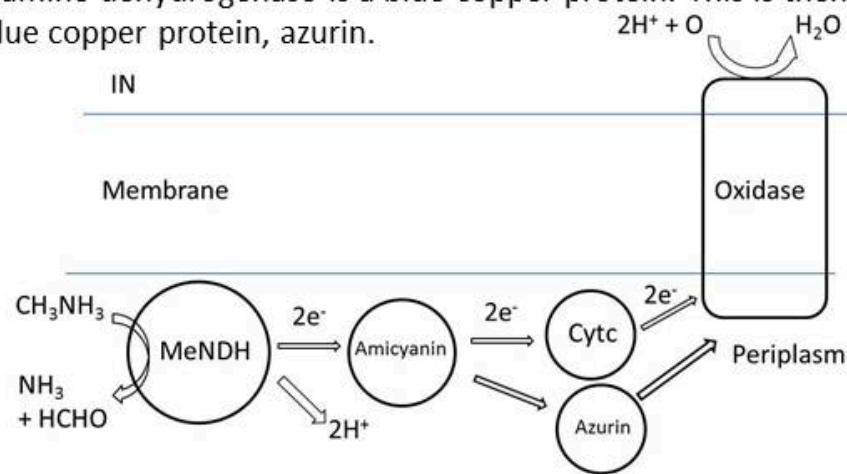
The ETC is very unusual. Except for the membrane oxidase the proteins are all soluble in the periplasm. Electrons are passed one at a time from methanol to the oxidase. When MDH reacts with cytL only the electrons pass to the cytochrome; the protons are released into the periplasm.

For each methanol oxidised 2 protons are produced outside and 2 are used by the oxidase inside. In effect 2 protons are translocated and a pmf is set up which drives ATP synthesis (about 0.7ATP/mol methanol).



### Methylamine oxidation

The methylamine dehydrogenase is a quinoprotein with TTQ prosthetic group. Its ETC is similar to that for methanol oxidation but the electron acceptor for the methylamine dehydrogenase is a blue copper protein. This is then oxidised by way of cytc or a 2<sup>nd</sup> blue copper protein, azurin.



### Electron transport systems using other quinoprotein dehydrogenases

Quinoproteins have a special quinone prosthetic group (eg PQQ in methanol dehydrogenase). They always catalyse reactions in the periplasm. They replace flavoprotein dehydrogenases which have FAD or FMN prosthetic group. They oxidise alcohols, amines or carbohydrates.

*Methanol dehydrogenase* (see above) has PQQ

*Methylamine dehydrogenase* (see above) has TTQ

*Alcohol dehydrogenase* (PQQ). In Acetic acid bacteria (eg *Acetobacter*); react with UQ;

*Pseudomonas*, reacts with cytochrome c as with methanol.

Glucose dehydrogenase (PQQ) in Acetic acid bacteria (eg *Gluconobacter*), *Pseudomonas* and enteric bacteria. These react with UQ.

**ATP synthesis in Chemolithotrophic bacteria**

These get their ATP and NADH by oxidation of inorganic molecules. They are usually strict Autotrophs, getting all their carbon from CO<sub>2</sub> which they assimilate by the Calvin RuBP pathway. Most of them have unusual electron transport chains because their substrates have high (positive) redox potentials. They are usually strict aerobes.

**Hydrogen bacteria** For example *Hydrogenomonas*.

These are exceptional chemolithotrophs because some can be anaerobic (using nitrate, or sulphate) and some can also grow on multicarbon compounds. They also have a conventional ETC for oxidation of reduced UQ which is produced by reaction of H<sub>2</sub> and UQ, catalysed by a specific hydrogenase.

**Sulphur bacteria** For example *Thiobacillus*, *Beggiatoa*, *Paracoccus*

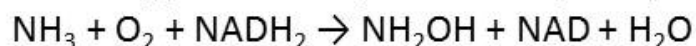
Sometimes called colourless Sulphur bacteria to distinguish for the coloured photosynthetic bacteria. *Thiobacillus denitrificans* can use nitrate instead of oxygen (rare). Sulphur has many oxidation states and so the metabolism of sulphur compounds is complex. These include hydrogen sulphide H<sub>2</sub>S, elemental S, sulphite SO<sub>3</sub><sup>2-</sup>, thiosulphate S<sub>2</sub>O<sub>3</sub><sup>2-</sup> and sulphate SO<sub>4</sub><sup>2-</sup>. Specific enzymes oxidise sulphide to elemental sulphur that can accumulate. This can be oxidised to sulphite, and thiosulphate to sulphate. The electrons are passed to cytochrome c and then to oxygen by a typical oxidase. So, as in methanol oxidation, there is a short periplasmic electron transport chain that gives a pmf for ATP synthesis.

**Nitrogen bacteria (nitrifying bacteria) *Nitrosomonas* and *Nitrobacter***

Very important in the nitrogen cycle as they convert ammonia to nitrate (nitrification). As in other chemolithotrophs the electron transport involves periplasmic cytochrome c but not UQ or the cytochrome bc1 complex.

*Nitrosomonas; oxidation of ammonia to nitrite*

Ammonia is oxidised by ammonia monooxygenase very similar to methane monooxygenase. The product is hydroxylamine:



Hydroxylamine is oxidised to nitrite  $\text{NH}_2\text{OH} + \text{H}_2\text{O} \rightarrow \text{NO}_2^- + 4\text{e}^- + 5\text{H}^+$ . The reaction is in the periplasm. The protons are released into the periplasm. The electrons reduce cytochrome c which is then oxidised by a typical oxidase which consumes protons on the inside. This leads to a protonmotive force and ATP synthesis.

*Nitrobacter; oxidation of nitrite to nitrate*

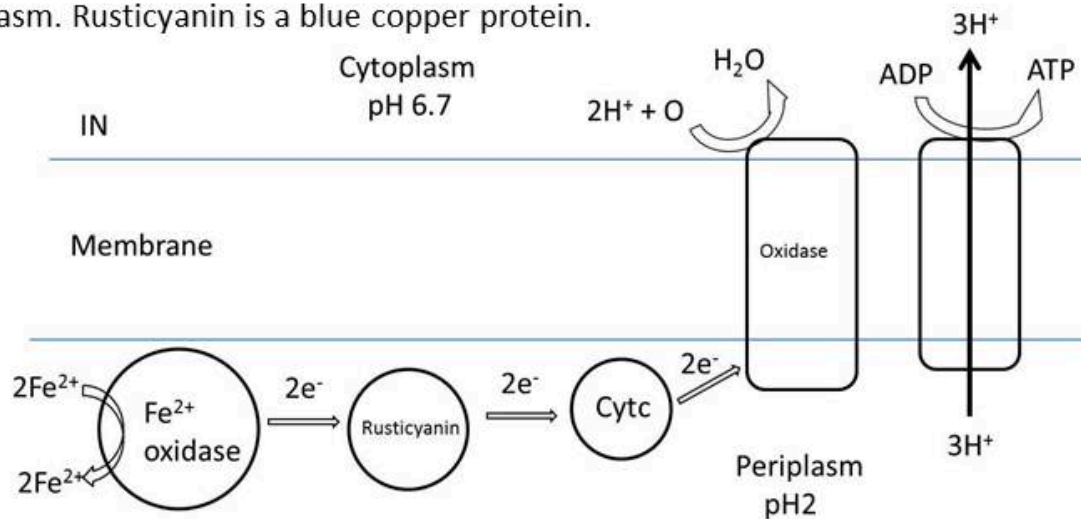
$\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{NO}_3^- + 2\text{H}^+$  As in the oxidation of hydroxylamine The protons are released into the periplasm. The electrons reduce cytochrome c which is then oxidised by a typical oxidase which consumes protons on the inside. This leads to a protonmotive force and ATP synthesis.

### Iron bacteria *Thiobacillus ferrooxidans*

These remarkable bacteria obtain all of their energy from the oxidation of ferrous ions to ferric:  $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ . The ferric ions are often deposited as insoluble orange hydroxides. These bacteria only grow at pH 2.

Because the cytoplasm is pH 6.7 and the external pH is pH2 there is already a large pH gradient to drive synthesis of ATP.

The function of the ETC is to provide electrons from  $\text{Fe}^{2+}$  to reduce oxygen. This process consumes protons that have entered by ATP synthase, so maintaining the pH level in the cytoplasm. Rusticyanin is a blue copper protein.

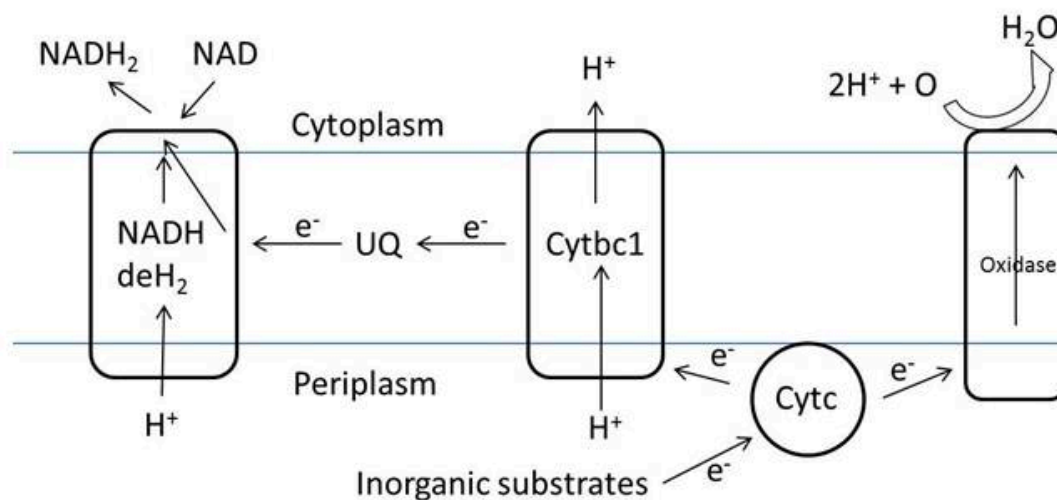


## Production of NADH in chemolithotrophs

All bacteria need NADH (or NADPH) for biosynthetic reactions. In typical heterotrophic bacteria this is produced by oxidation of organic substrates. In chemolithotrophs which have only inorganic substrates NADH is not produced during their oxidation. NADH must be produced by 'Reverse electron transport'.

### Reverse electron transport

It is called this because electrons flow from components with more positive redox potential to one with more negative redox potential. This requires energy which is provided by the protonmotive force produced by the oxidation of the substrate (nitrite, ferrous iron etc). This is why these bacteria have UQ and cytochrome bc1 even when these are not used for oxidation of substrates. Some electrons flow from cytochrome c to the oxidase to set up a pmf. Some electrons flow in the reverse direction to cytochrome bc1, and UQ to NADH; the energy is provided by proton flow into the bacteria in response to the pmf.



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