Microbial methane metabolism

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KITP Forum

March 04, 2021

Let me introduce myself

2008 MSc in Biology (Microbiology/Biochemistry)

2011 PhD in Microbiology (Microbial Biochemistry)

2011-14 Postdoc at Bonn U (GER) and RU (NL)

2015-20 Assistant Professor RU

2021- Associate Professor RU

Head of the Microbial Interactions research group

I get excited about anaerobic methane cycling microbes and symbiosis between pest insects and microbes

Let me introduce myself

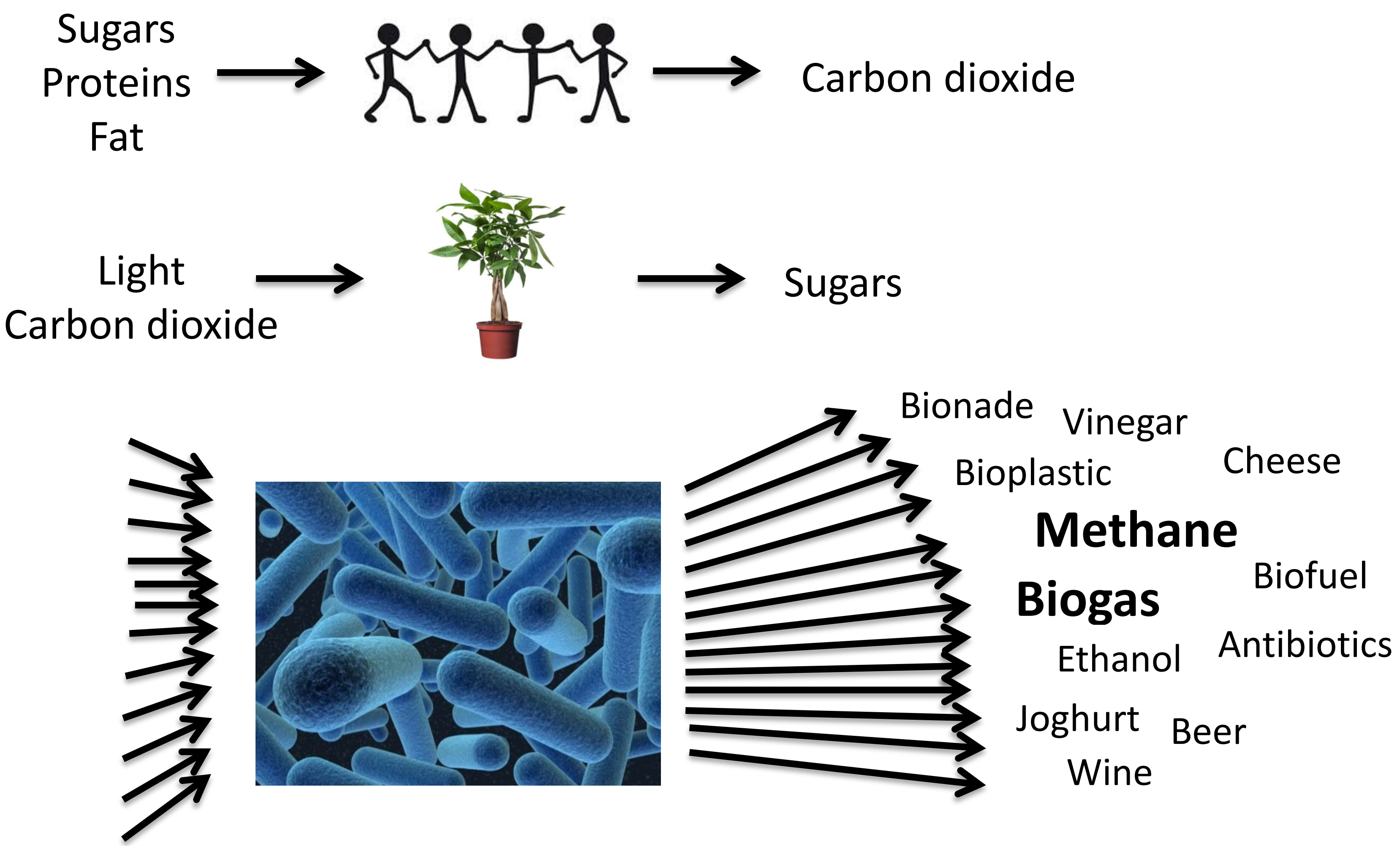
Sugars
Proteins
Fat

Carbon dioxide

Light
Carbon dioxide

Sugars

Let me introduce myself



ARTIS MICROPIA

Microworld

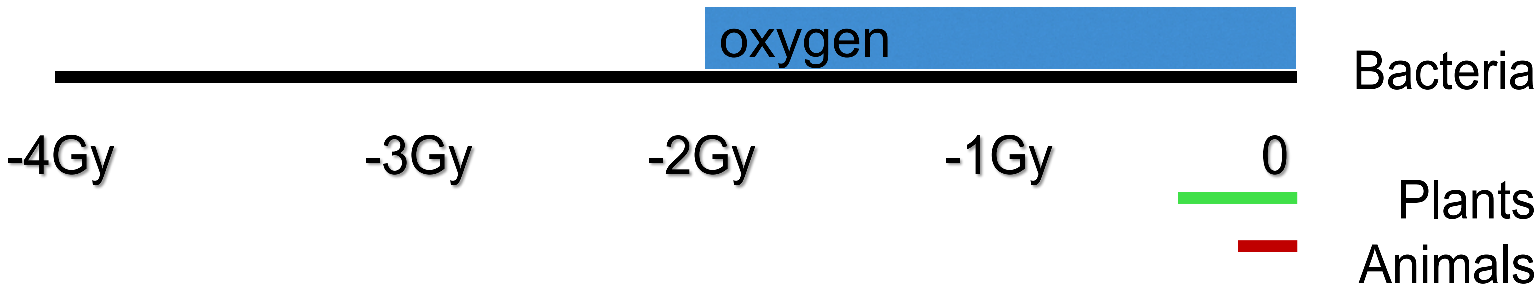
The most powerful life on earth

When you look from really close, a new world is revealed to you.
More beautiful and spectacular than you could ever have imagined.

Why care about anaerobic microorganisms?

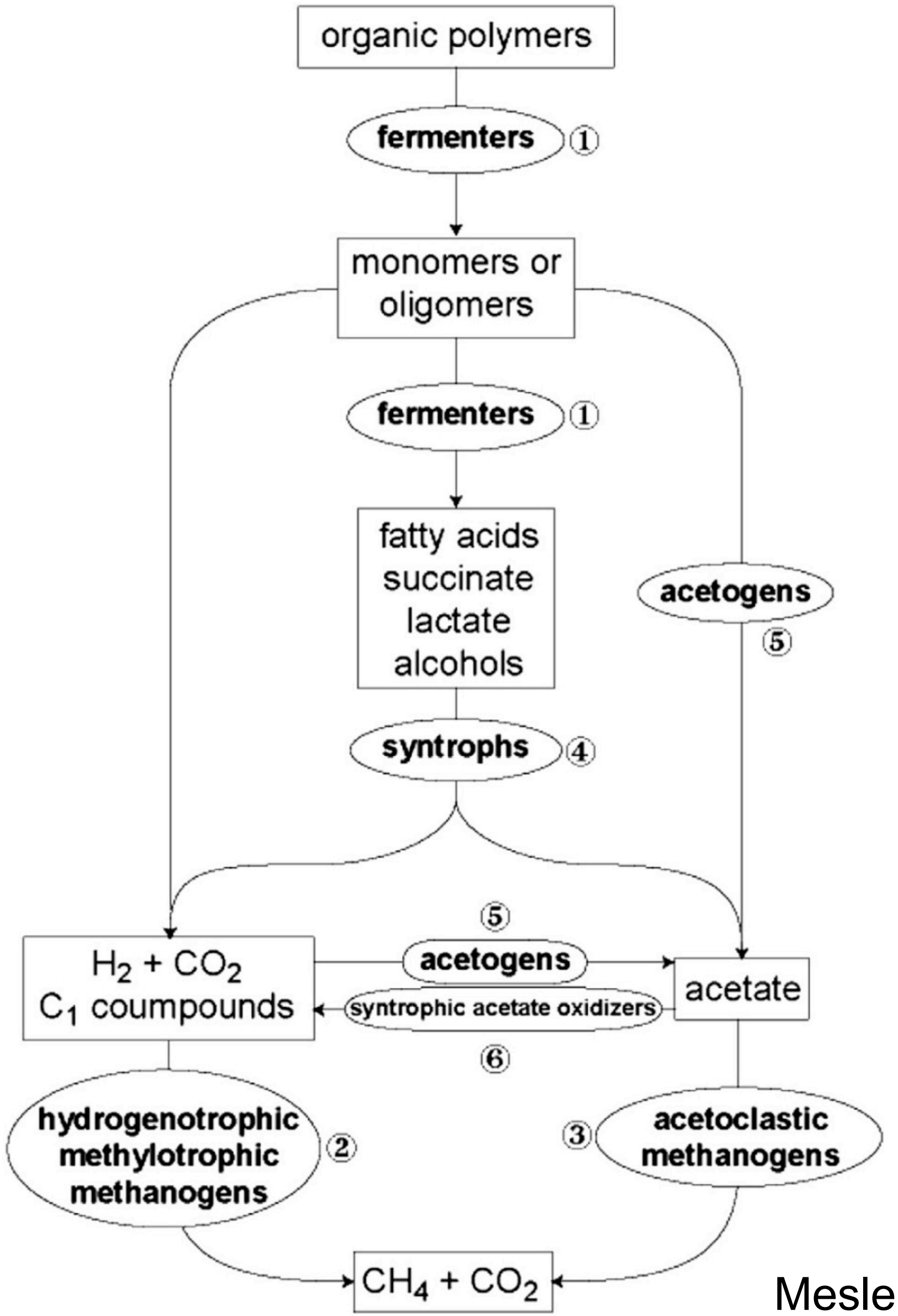
"The Earth is a microbial planet, on which macro-organisms are recent additions, highly interesting and extremely complex in ways that most microbes are not, but in the final analysis relatively unimportant in a global context."

Wheelis *et al.* (1998) PNAS 95:11043-11046



Anaerobic microorganisms had 4 billion years to evolve
A treasure trove for scientists and society!

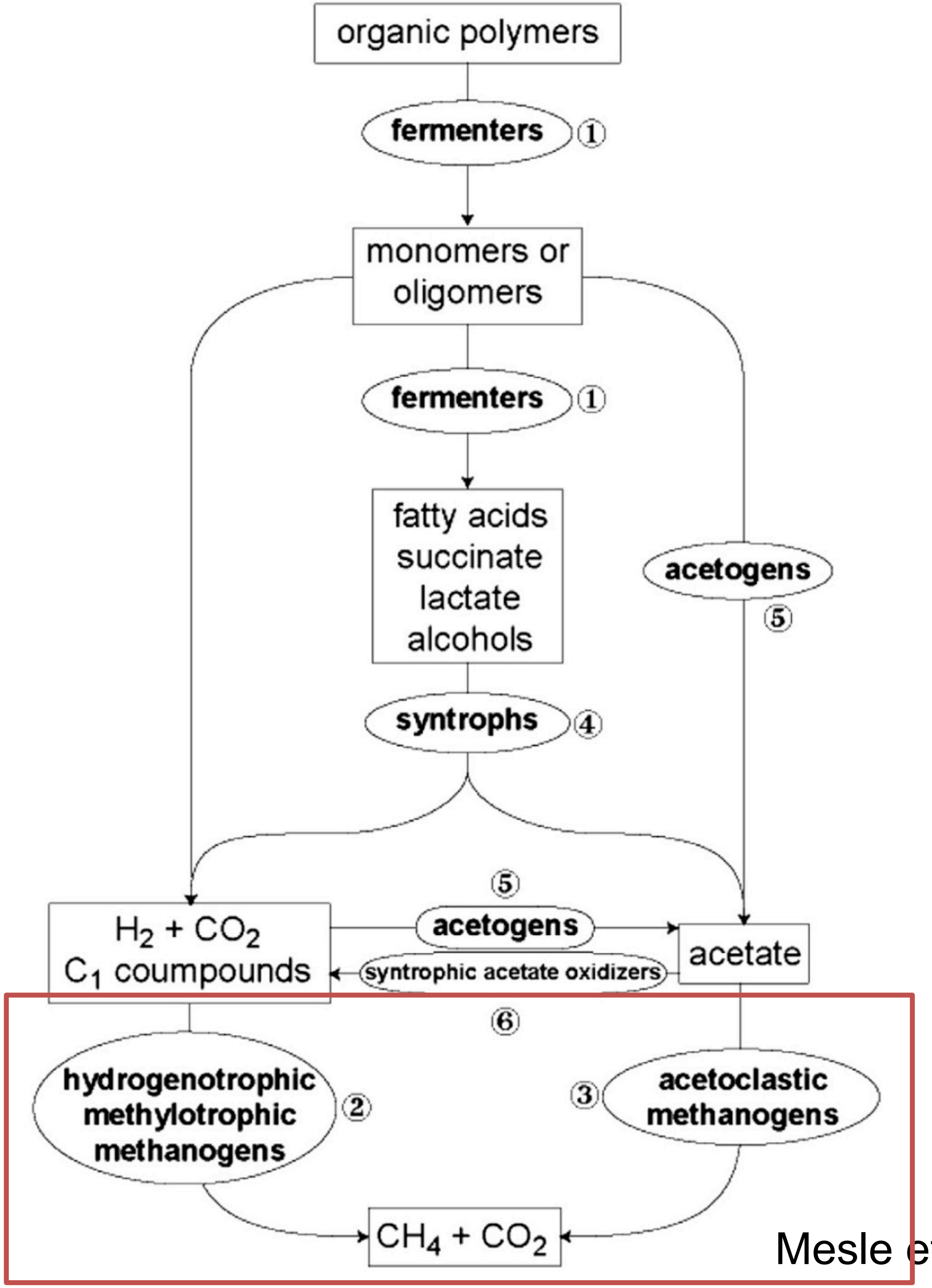
The anaerobic food chain



Happening in all kinds of anoxic environments:

- Intestinal systems
- Anaerobic digesters
- Anoxic ocean
- Sediments
- Sub-surface environments

Mesle et al 2013



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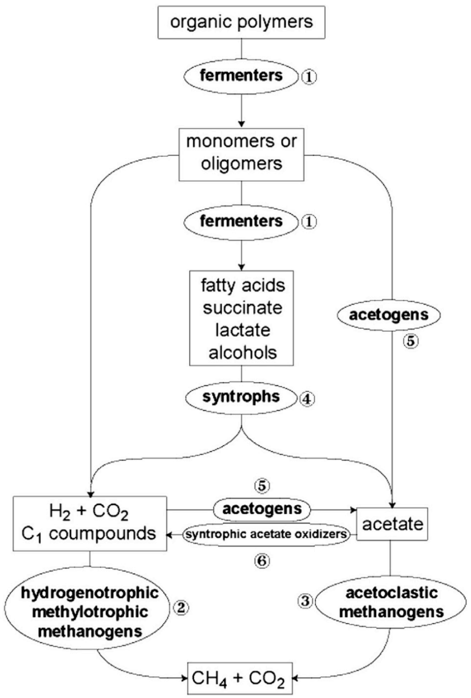
Mesle et al 2013

Thermodynamics of aerobes vs anaerobes

Anaerobes



Aerobes

Organics + O_2

↓

CO_2

Aerobes don’t have to share
ΔG <<< 0

Anaerobes need to cooperate
ΔG < 0
Many live at the thermodynamic limit
Often use “thermodynamic tricks”

Raoadmap for today

Part I: thermodynamics of methane generation and what it does (not) tell us

- Required knowledge: ΔG, ATP and pmf, respiration

Part II: the methanogenic metabolic pathway map and an anaerobic thermodynamic trick

Part III (Research Talk):

"How to revert a metabolic pathway and still make a living"

or

"Novel discoveries in anaerobic methane oxidation"

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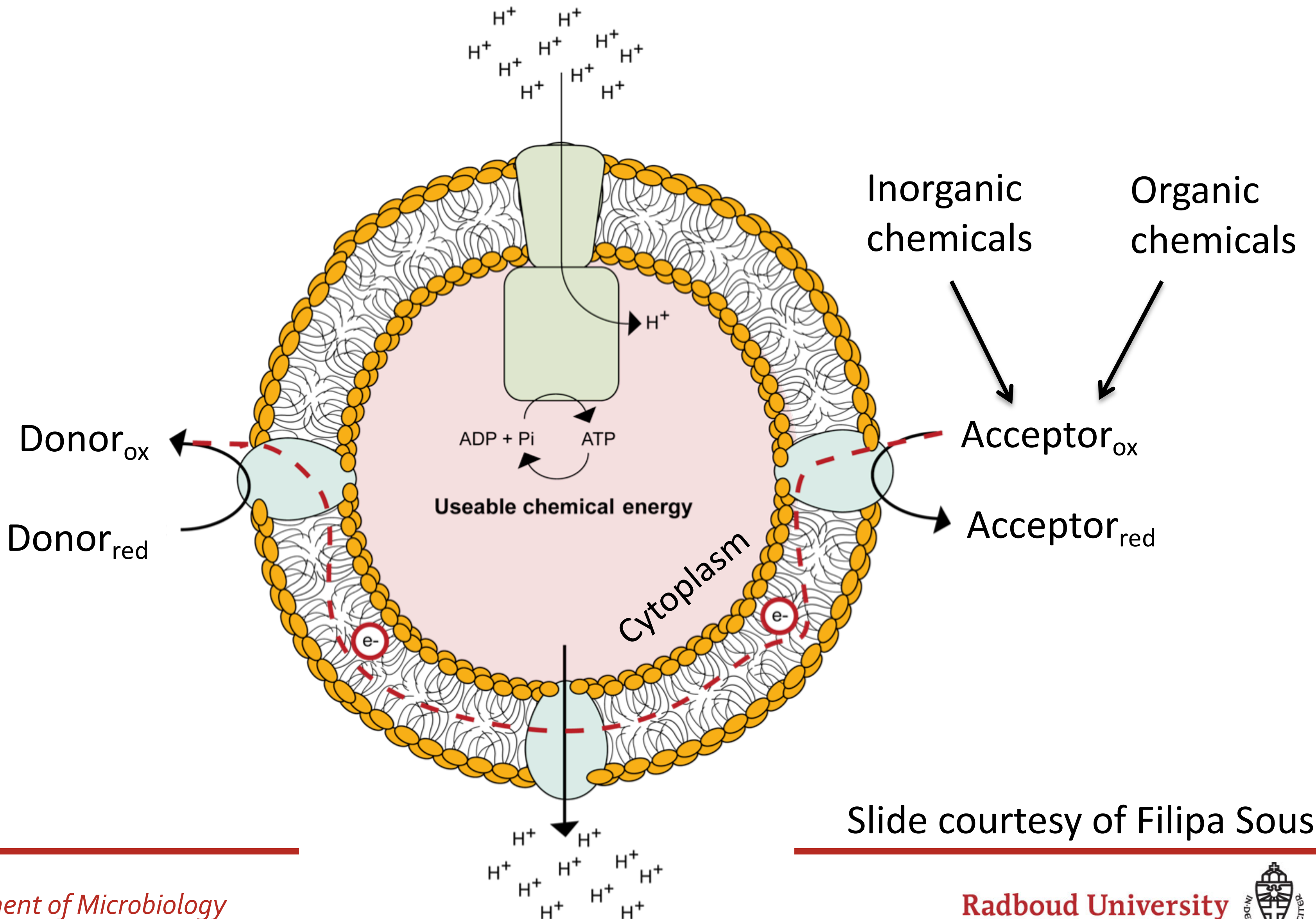
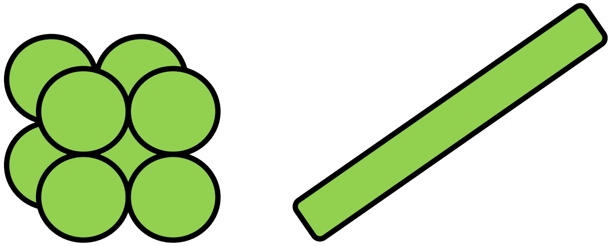
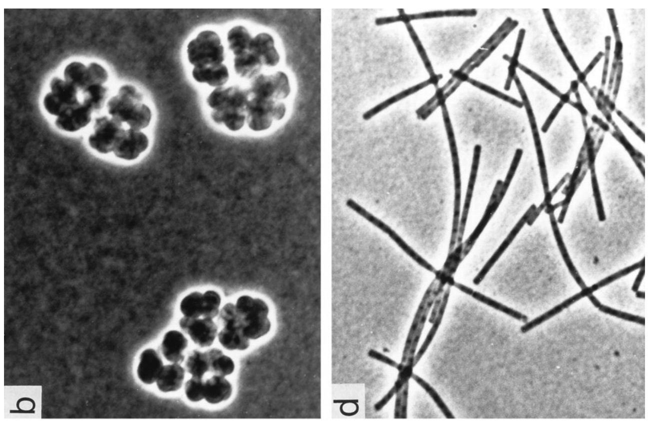
Principles of respiration



Slide courtesy of Filipa Sousa

Methanogens: ancient (simple?) metabolism



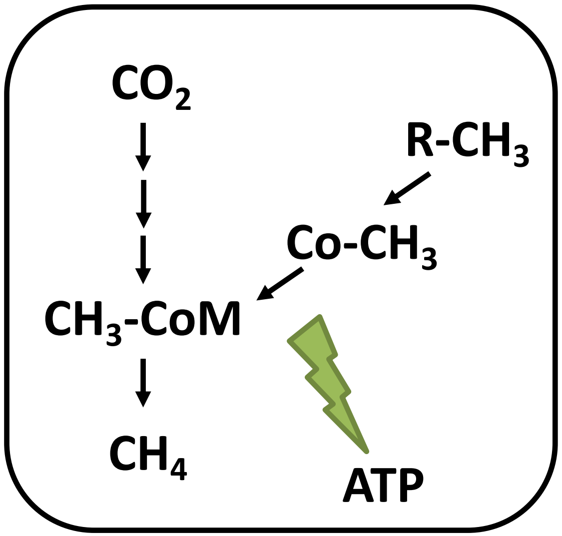


Grosskopf et al 1998

Acetate
Methanol
Methylated amines
Methyl sulfide
$H_2 + CO_2$

↓

CH_4



Methanogenesis is the energy conserving pathway of methane generating archaea (methanogens)

Methanogens: ancient metabolism

Table 2 Gibbs free energy values for different methanogenesis substrates

Substrate	Reaction equation	$\Delta G'^{\circ}$ (kJ/mol CH_4)
$H_2 + CO_2$	$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O$	− 131 (a)
$HCOO^-$	$4\ HCOO^- + 4\ H^+ \rightarrow CH_4 + 3\ CO_2 + 2\ H_2O$	− 145 (a)
$CH_3CH_2OH + CO_2$	$2\ CH_3CH_2OH + CO_2 \rightarrow 2\ CH_3COOH + CH_4$	− 121 (e)
$H_2 + CH_3OH$	$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	− 113 (e)
$CH_3OH + CH_3CH_2OH$	$2\ CH_3OH + CH_3CH_2OH \rightarrow 2\ CH_4 + H_2O + CH_3COOH$	− 100 (b)
$CH_3CHOHCH_3 + CO_2$	$4\ CH_3CHOHCH_3 + HCO_3 + H^+ \rightarrow 4\ CH_3COCH_3 + CH_4 + 3\ H_2O$	− 37 (c)
CH_3OH	$4\ CH_3OH \rightarrow CO_2 + 3\ CH_4 + 2\ H_2O$	− 107 (a)
CH_3-COOH	$CH_3COOH \rightarrow CO_2 + CH_4$	− 36 (a)
CH_3-SH (CH_3-S-R)	$4\ CH_3SH + 3\ H_2O \rightarrow 3\ CH_4 + HCO_3^- + 4\ HS^- + 5\ H^+$	− 49 (b)
Betaine (CH_3-N-R)	$4\ (CH_3)_3N^+CH_2COO^- + 2\ H_2O \rightarrow 4\ (CH_3)_2N^+CH_2COO^- + 3\ CH_4 + CO_2$	− 241 (c)
Choline (CH_3-N-R)	$4\ (CH_3)_3N^+CH_2CH_2OH + 6\ H_2O \rightarrow 4\ H_2NCH_2CH_2OH + 9\ CH_4 + 3\ CO_2 + 4\ H^+$	− 63 (d)
Trimethylamine (CH_3-N-R)	$4\ (CH_3)_3N + 6\ H_2O + 4\ H^+ \rightarrow 4\ NH_4^+ + 9\ CH_4 + 3\ CO_2$	− 31 (d)
2-methoxyphenol (CH_3-O-R)	4 2-methoxyphenol + 2 $H_2O \rightarrow$ 4 2-hydroxyphenol + CO_2 + 3 CH_4	− 90 (f)

$$C_6H_{12}O_6 + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O$$

$$\Delta G^{0\prime} = -2863\ \text{kJ/mol}$$

 Kurth, Op den Camp, Welte (2020) AMB

Three statements to think about:

Acetate-dependent methanogenesis has the **lowest Gibb's free energy change** of all methanogenic processes, yet it is the most relevant methanogenic process on our planet.

Low Gibb's free energy change of a microbial metabolic process **does not** equal low growth rate (= speed of cell division) of a microorganism.

The Gibb's free energy change **does not** usually determine the substrate threshold concentration, the minimal required concentrations for a microbe to grow.

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If $\Delta G<0$, a metabolic process can yield ATP

If $\Delta G>0$, a metabolic process cannot yield ATP

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However, **a minimal ΔG is required**, equaling the amount to transport 1 H^+ across the cytoplasmic membrane (we'll get there in a minute)

Three statements to think about:

Low Gibb's free energy change of a microbial metabolic process **does not** equal low growth rate (= speed of cell division) of a microorganism.

Aceticlastic methanogenesis: $\Delta G = -36$kJ/mol
doubling time ~24h

Anaerobic methane oxidation with nitrate:
$\Delta G = -523$kJ/mol
doubling time ~10 days

Three statements to think about:

The Gibb's free energy change **does not** usually determine the substrate threshold concentration, the minimal required concentrations for a microbe to grow.

Aceticlastic *Methanosarcina*: grows > 1mM acetate
Aceticlastic *Methanosaeta*: grows < 1 mM acetate

What determines the substrate threshold?

Aceticlastic *Methanosarcina*: grows > 1mM acetate
Aceticlastic *Methanosaeta*: grows < 1 mM acetate

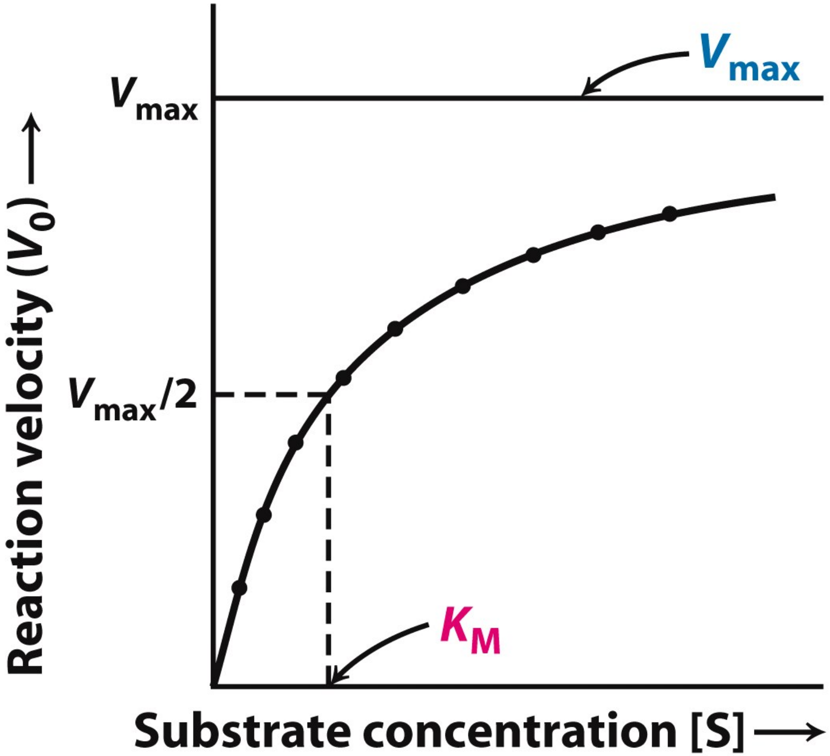
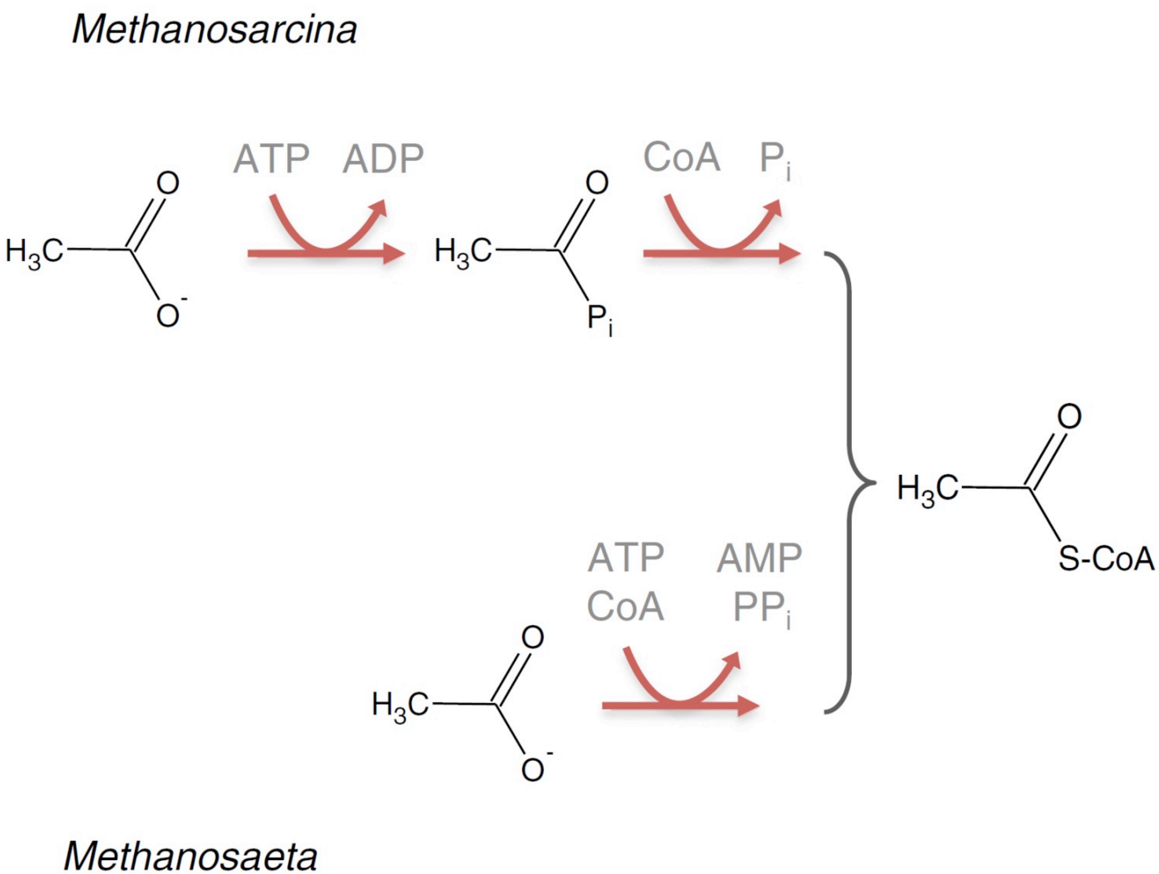


Figure 8.11
***Biochemistry*, Seventh Edition**

Contributing factors:
- Enzyme kinetics
- Enzymatic repertoire
- Substrate availability (gasses, membrane transport)

What determines the substrate threshold?

Methanosarcina: > 1mM acetate, uses 1 ATP for acetate activation
Methanosaeta: < 1 mM acetate, uses 2 ATP for acetate activation



YOU <u>CANNOT</u> HAVE IT ALL

Metabolic processes are **either** highly specific (low substrate threshold) **or** highly efficient (more ATP gain)

Three statements to think about:

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Thermodynamics provides you with a yes/no answer:
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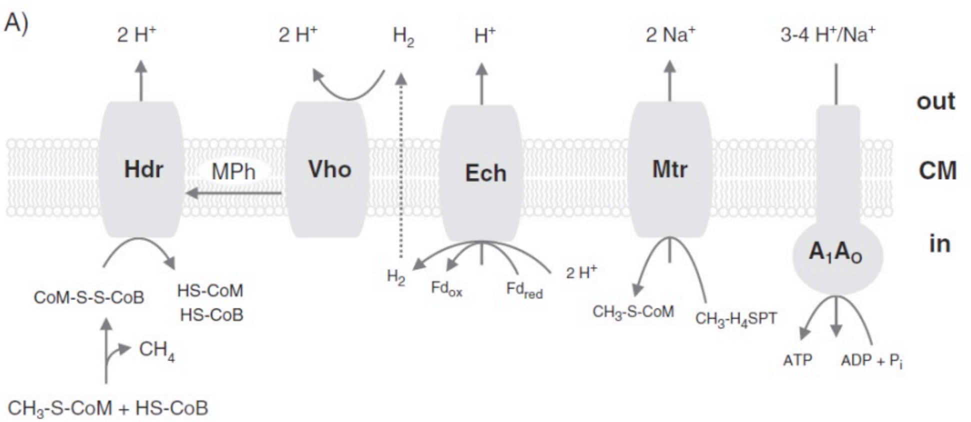
However, **a minimal ΔG is required**, equaling the amount to transport 1 H^+ across the cytoplasmic membrane

The minimal energy quantum

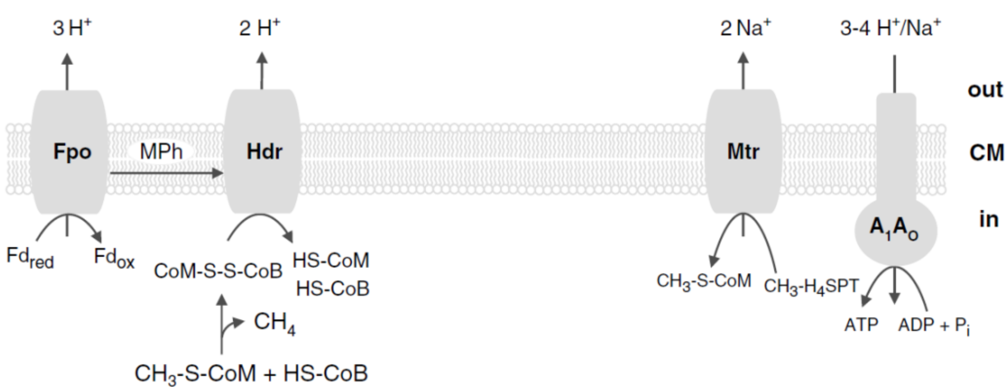
Methanosarcina



Aceticlastic respiratory chains:

7 H^+ per acetate translocated
Equals about 2 ATP
Minus 1 ATP invested
= 1 ATP net gain

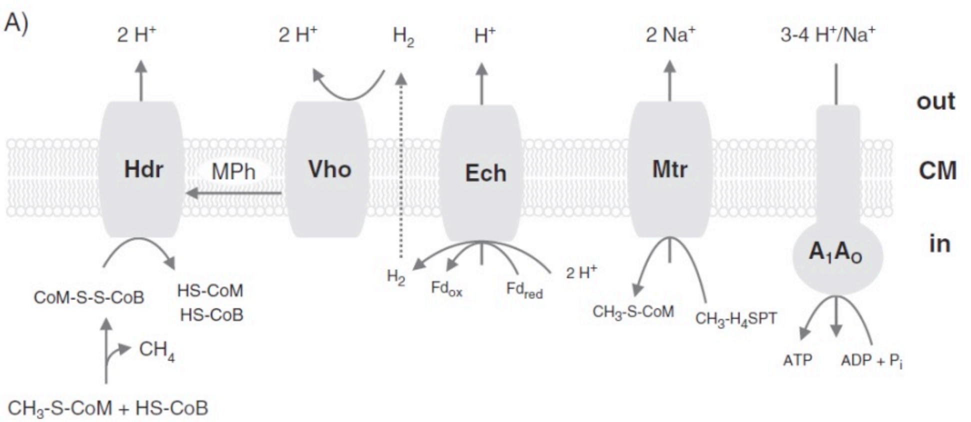
Methanosaeta



7 H^+ per acetate translocated
Equals about 2 ATP
Minus 2 ATP invested
= no net gain?!

The minimal energy quantum

Methanosarcina



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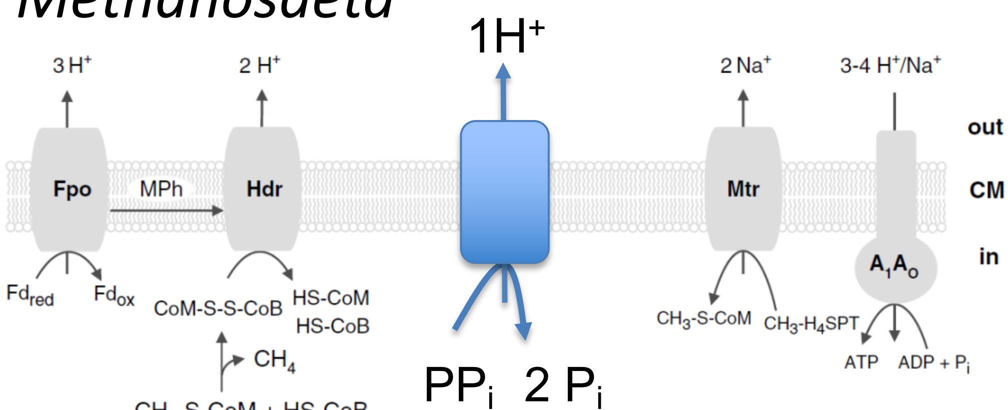


7 H^+ per acetate translocated
Equals about 2 ATP + 1 H^+
Minus 2 ATP invested
= 1 H^+ net gain
THE LIMIT OF LIFE

Take home points (part I) & questions

- Thermodynamics is important
- Thermodynamics is not directly quantitative
- Enzyme kinetics determines substrate thresholds
- High substrate affinity and high efficiency are mutually exclusive
- The minimal energy quantum is one translocated ion (H^+/Na^+)

Raoadmap for today

Part I: thermodynamics of methane generation and what it does (not) tell us

Part II: the methanogenic metabolic pathway map and an anaerobic thermodynamic trick

- **Required knowledge: ΔE and $E^{0\prime}$, reduction/oxidation**

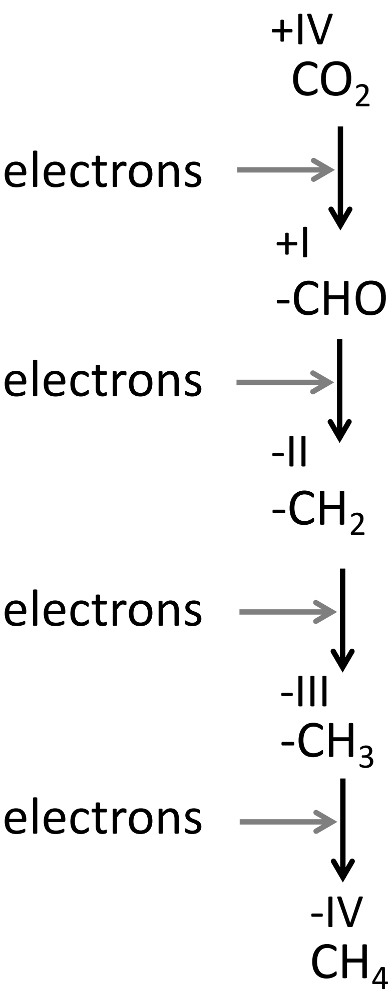
Part III (Research Talk):

"How to revert a metabolic pathway and still make a living"

or

"Novel discoveries in anaerobic methane oxidation"

The methanogenic pathway (from H_2+CO_2)



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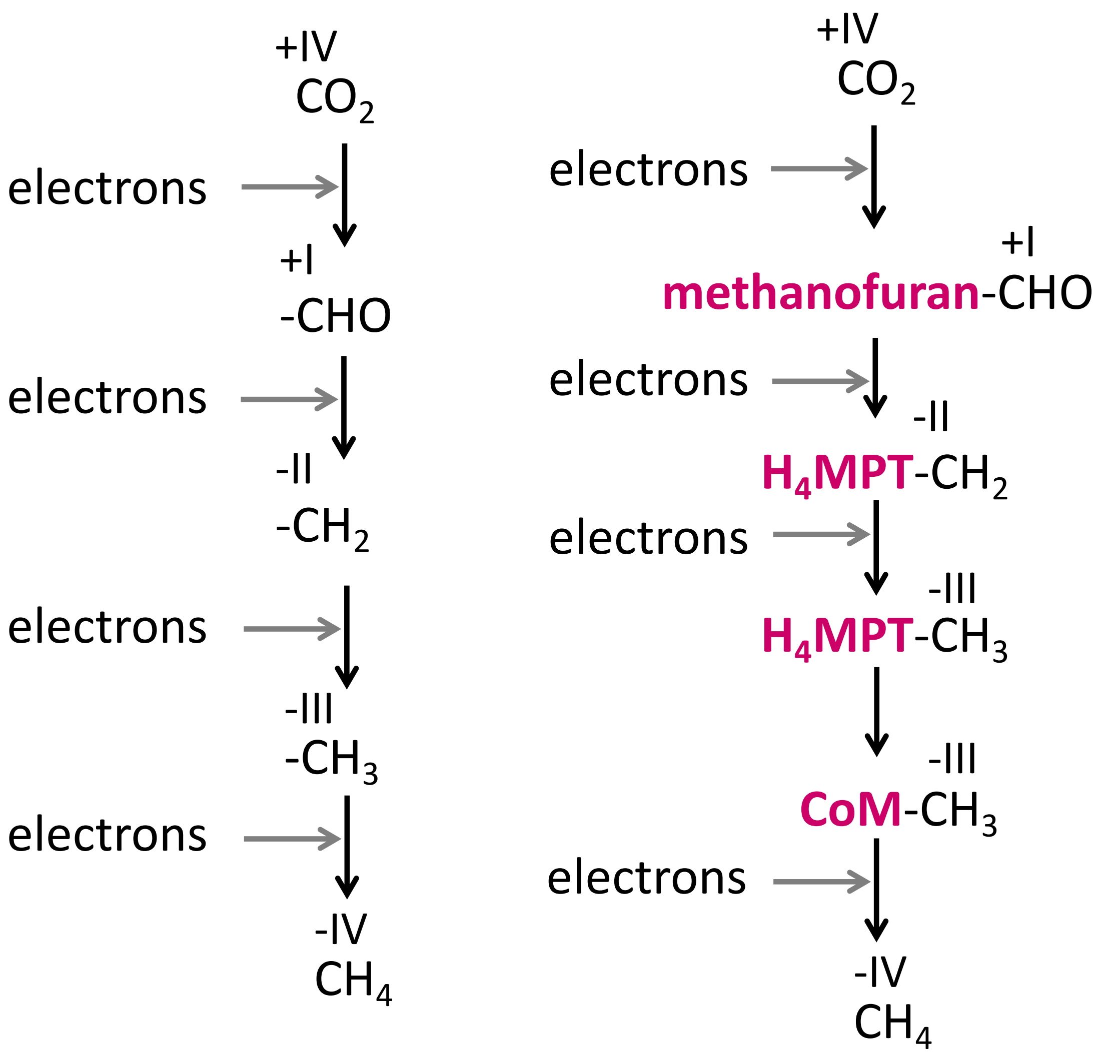

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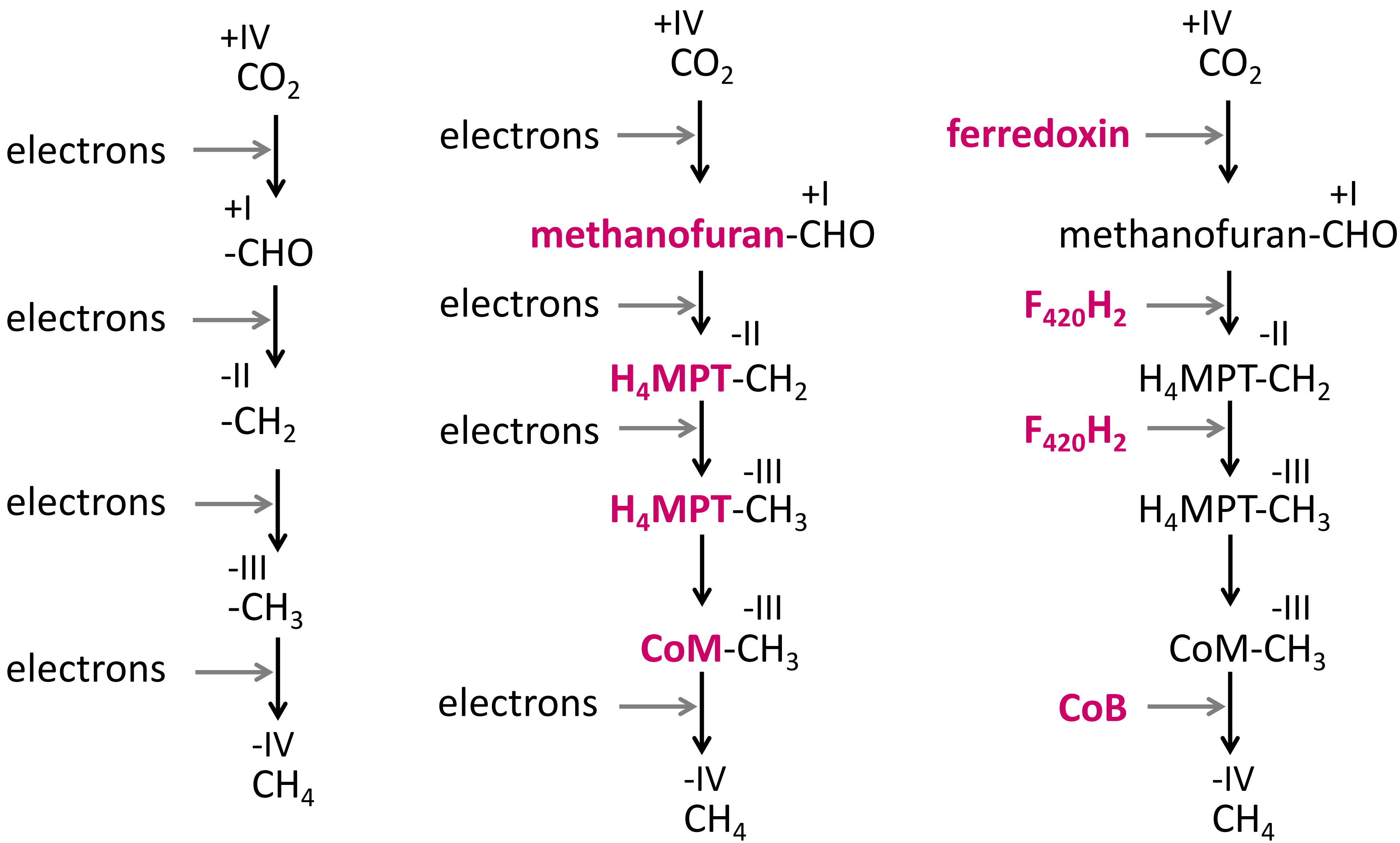


The methanogenic pathway (from H_2+CO_2)

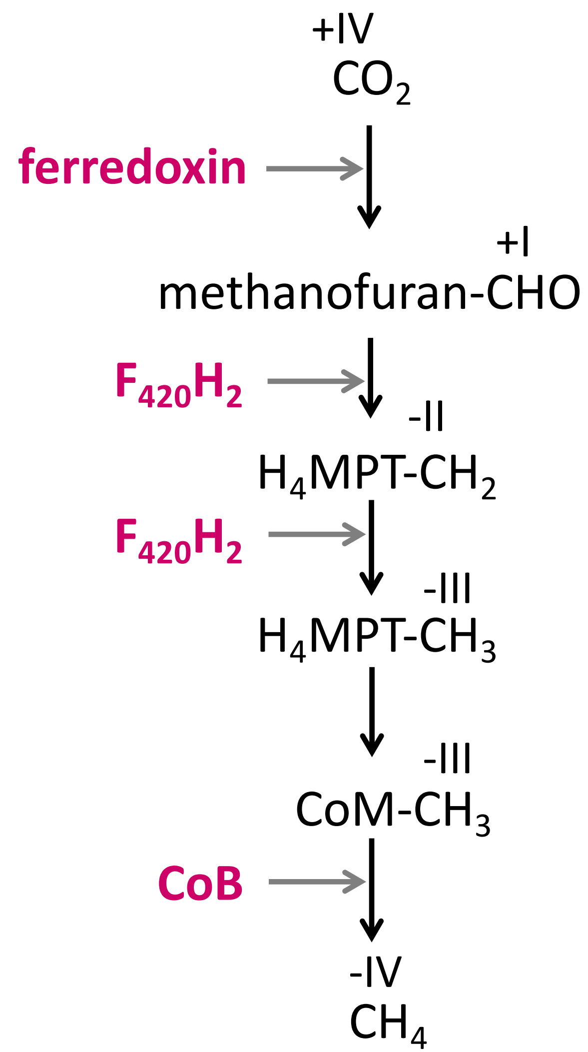
A few key questions:

Why are different cofactors used, and how are they regenerated?

How is energy conserved?

What is limiting the rate of methanogenesis?

How can CO_2 be fixed without the use of ATP?



The methanogenic pathway (from H_2+CO_2)

The $E^{0\prime}$ of ferredoxin is ~ -500mV

(if NADH (-320mV) is used, ATP is required to drive this reaction)

+IV CO_2

↓ **ferredoxin** →

+I methanofuran-CHO

↓ **$F_{420}H_2$** →

-II $H_4MPT\text{-}CH_2$

↓ **$F_{420}H_2$** →

-III $H_4MPT\text{-}CH_3$

↓

-III CoM-CH_3

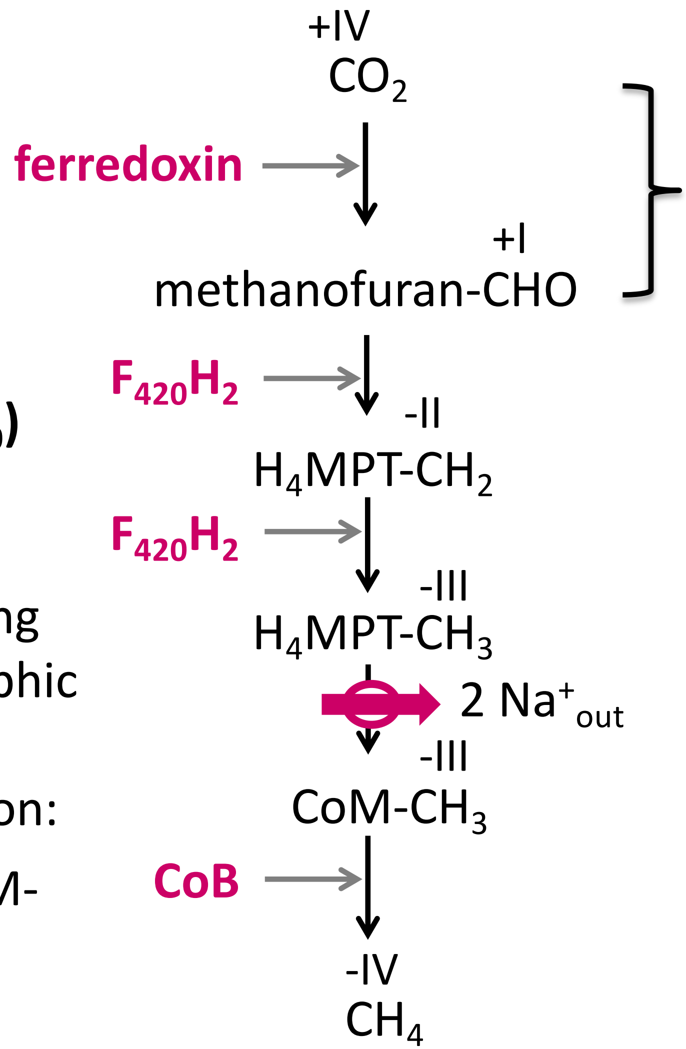
↓ **CoB** →

-IV CH_4

The CO_2/CO redox couple has a $E^{0\prime}$ ~ -500mV

The methanogenic pathway (from H_2+CO_2)

The $E^{0'}$ of ferredoxin is ~ -500mV



The CO_2/CO redox couple has a $E^{0'}$ ~ -500mV

$E^{0'}(F_{420}H_2/F_{420})$ = -360mV

The only energy conserving reaction in hydrogenotrophic methanogenesis is the methyl transferase reaction:

$H_4MPT-CH_3$ + CoM → CoM-CH_3 + H_4MPT

$\Delta G^{0'}$ = -30kJ/mol

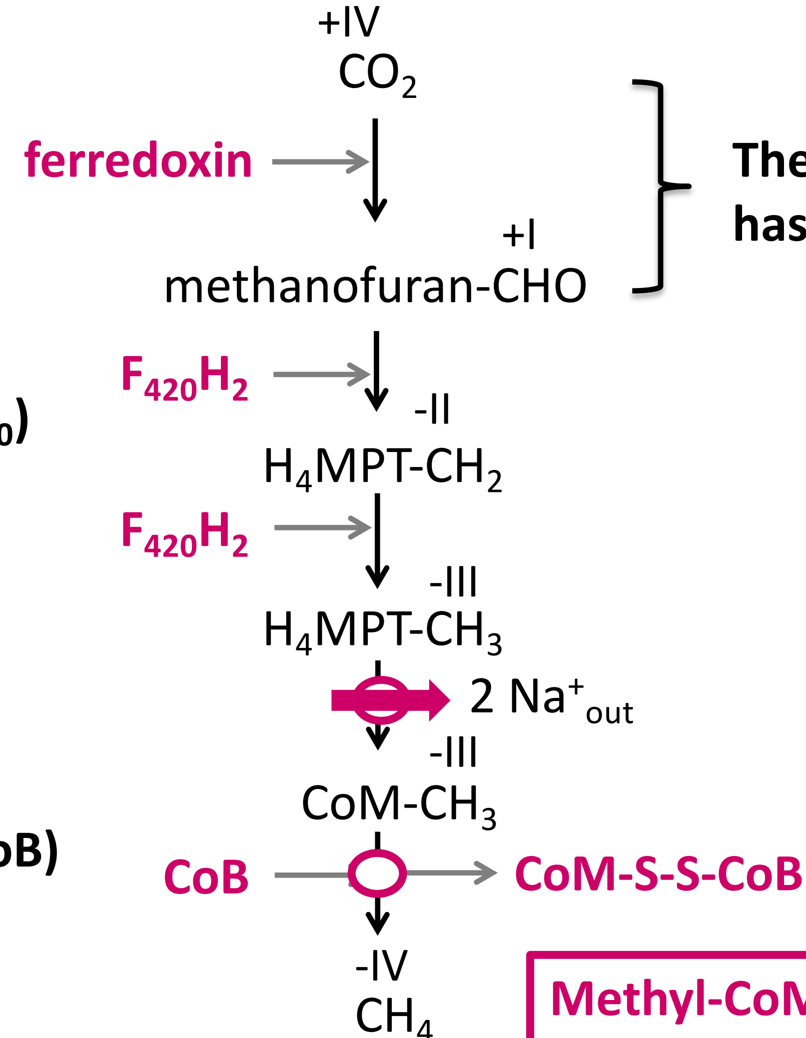
The methanogenic pathway (from H_2+CO_2)

The $E^{0\prime}$ of ferredoxin is ~ -500mV

The CO_2/CO redox couple has a $E^{0\prime}$ ~ -500mV

$E^{0\prime}(F_{420}H_2/F_{420})$ = -360mV

$E^{0\prime}$(CoB/CoM-CoB) = -120mV



Methyl-CoM reductase
Most abundant enzyme in the anaerobic world

The methanogenic pathway (from H_2+CO_2)

The $E^{0\prime}$ of ferredoxin is ~ -500mV

+IV
CO_2

CO_2/CO redox couple
$E^{0\prime}$ ~ -500mV

A few key questions:

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$E^{0\prime}$(F
= -36

$E^{0\prime}$(CoB
= -120mV

-IV
CH_4

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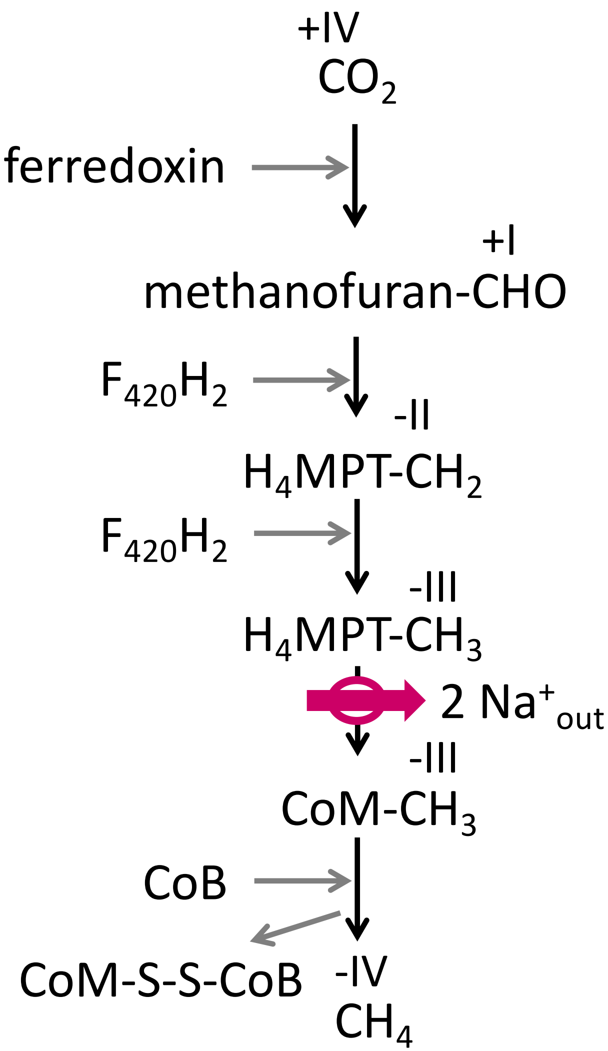
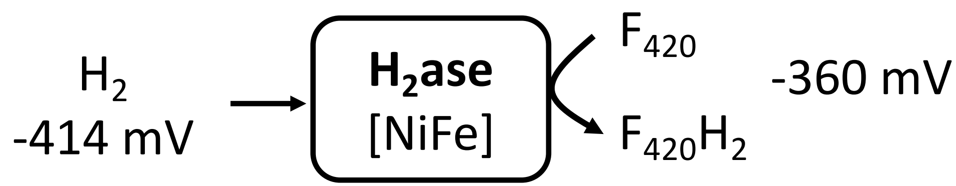
Most abundant enzyme in the anaerobic world

The methanogenic pathway (from H_2+CO_2)



Two hydrogenases regenerate cofactors



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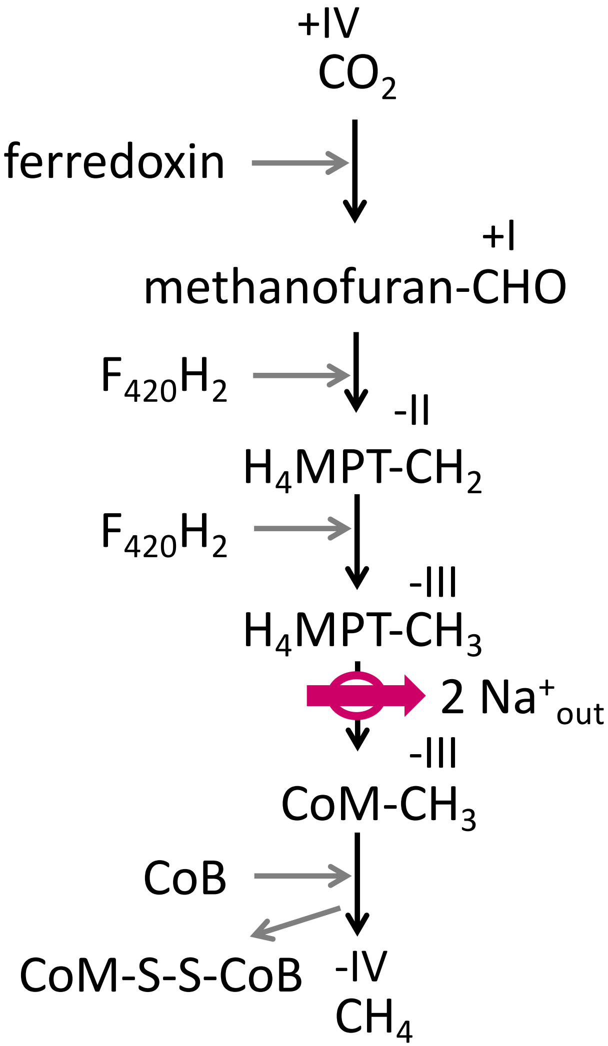

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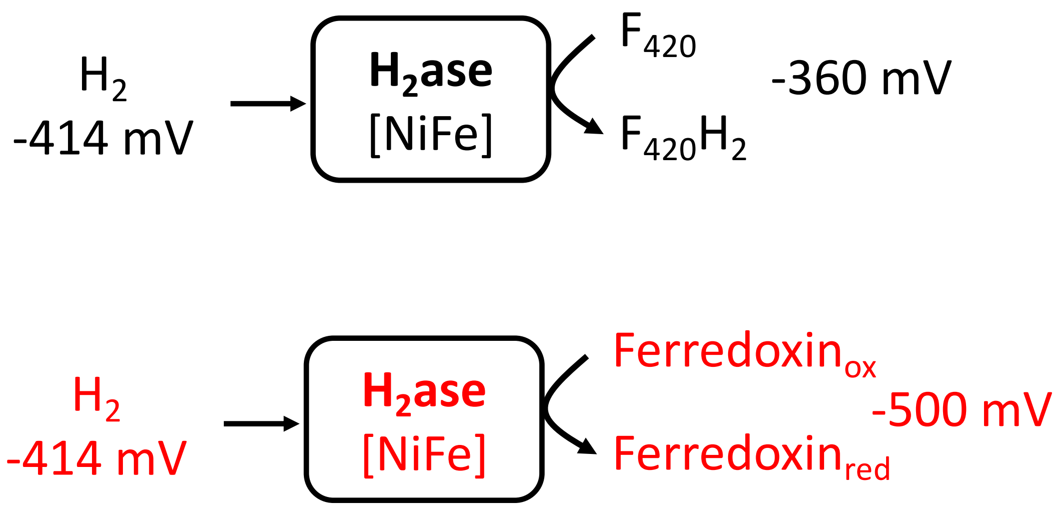
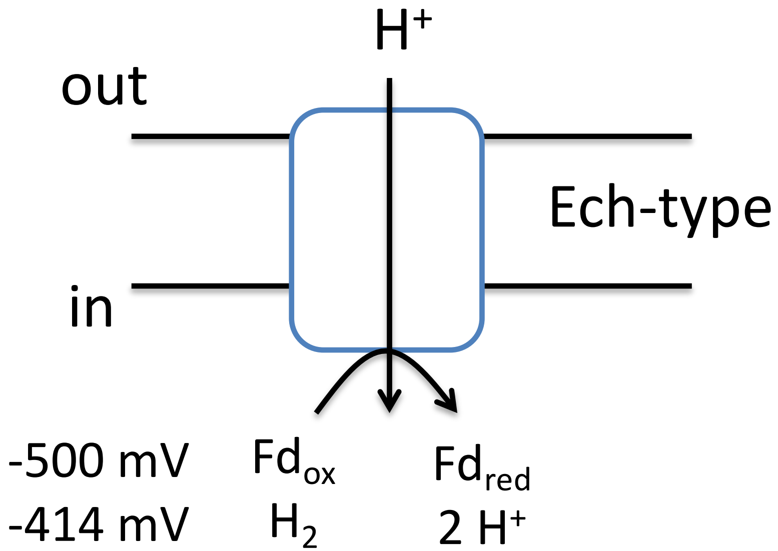


Thermodynamically unfavorable!!

Reducing ferredoxin with H_2 at the membrane

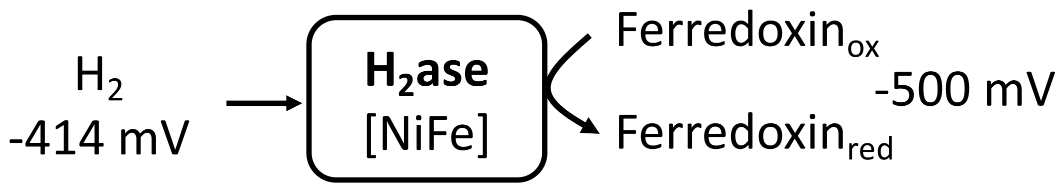


"Energy conserving hydrogenase"
[originally: ***E***. *c**oli*** **h**ydrogenase type 3]

Not an option for methanogenesis from H_2+CO_2:

- Only 1-2 Na^+ translocated per CH_4 produced
- 1-2 H^+ would be needed for ferredoxin reduction
- No net energy gain of the metabolism

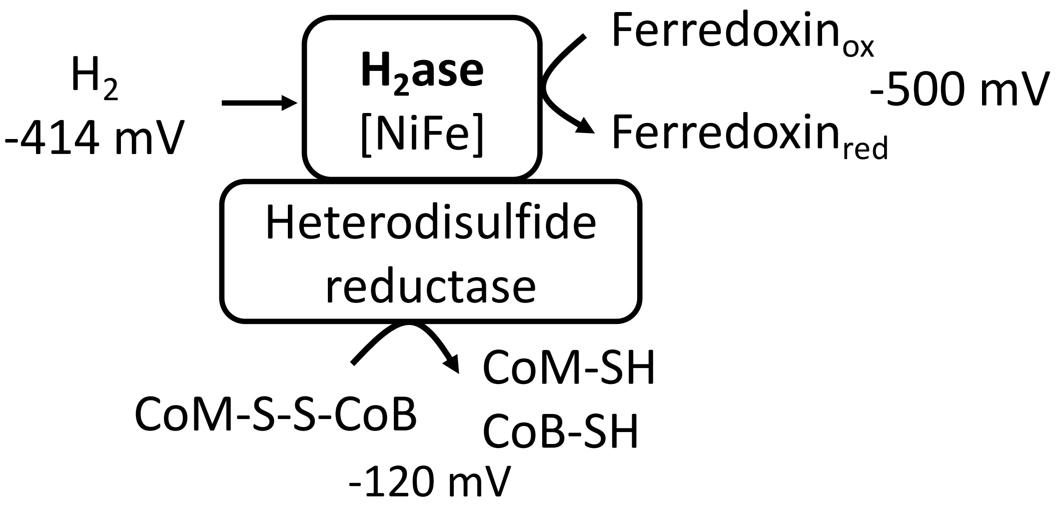
Reducing ferredoxin with H_2 in the cytoplasm



- **Thermodynamically unfavorable**
- **Input of ATP not possible: only 1-2 Na^+ translocated per ferredoxin required**

- **How can these methanogens obtain reduced ferredoxin?**

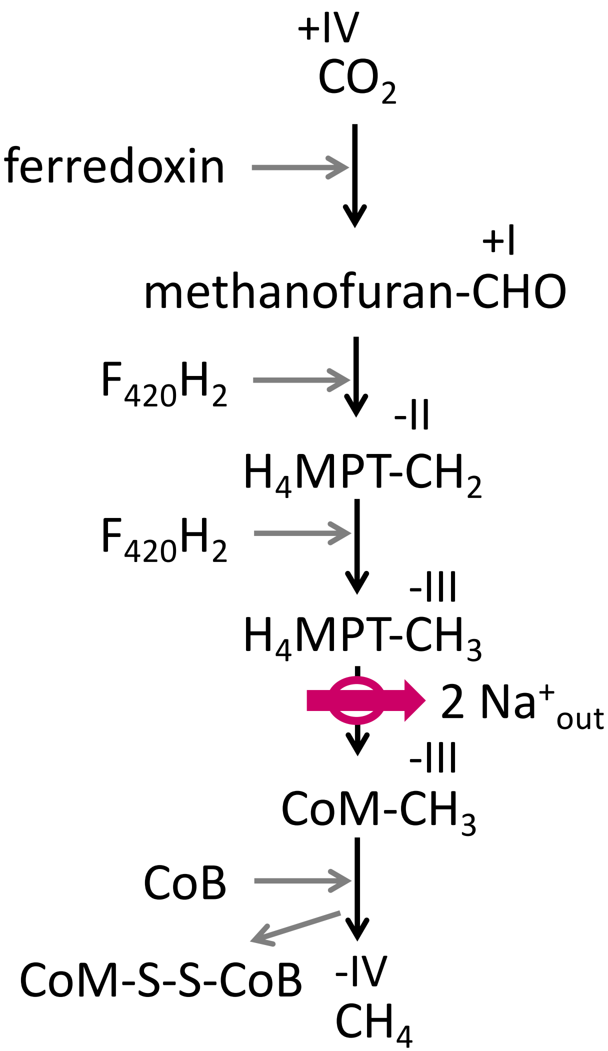
Reducing ferredoxin with H_2 in the cytoplasm



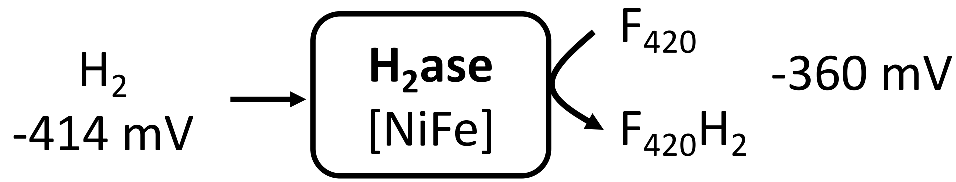
By using a thermodynamic trick: electron bifurcation

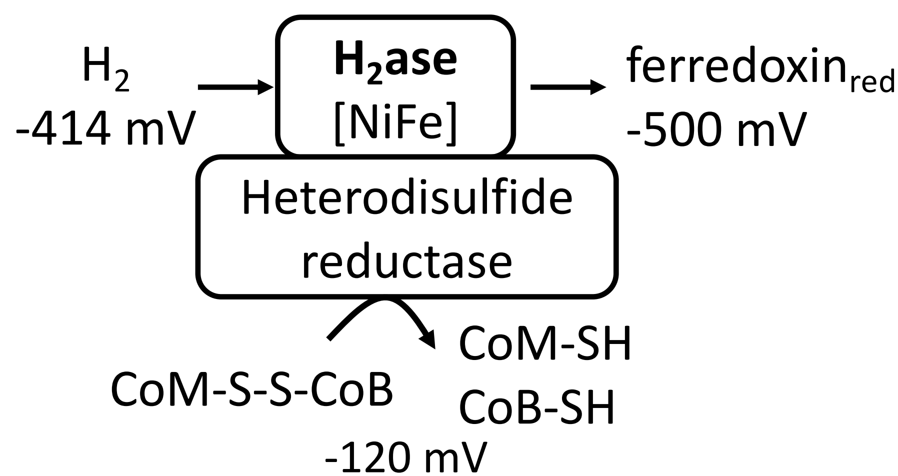
the exergonic reduction of CoM-S-S-CoB drives the endergonic reduction of ferredoxin

The methanogenic pathway (from H_2+CO_2)



Two hydrogenases regenerate cofactors





Take home points (part II) & questions

- Cofactors need to be regenerated (closed loop)
- After closing the loop, there needs to be a net gain of ATP/translocated ions
- Redox potentials of cofactors determine the way in which they can be regenerated
- Individual enzymes can be bottlenecks for entire pathways

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Novel discoveries in anaerobic methane oxidation

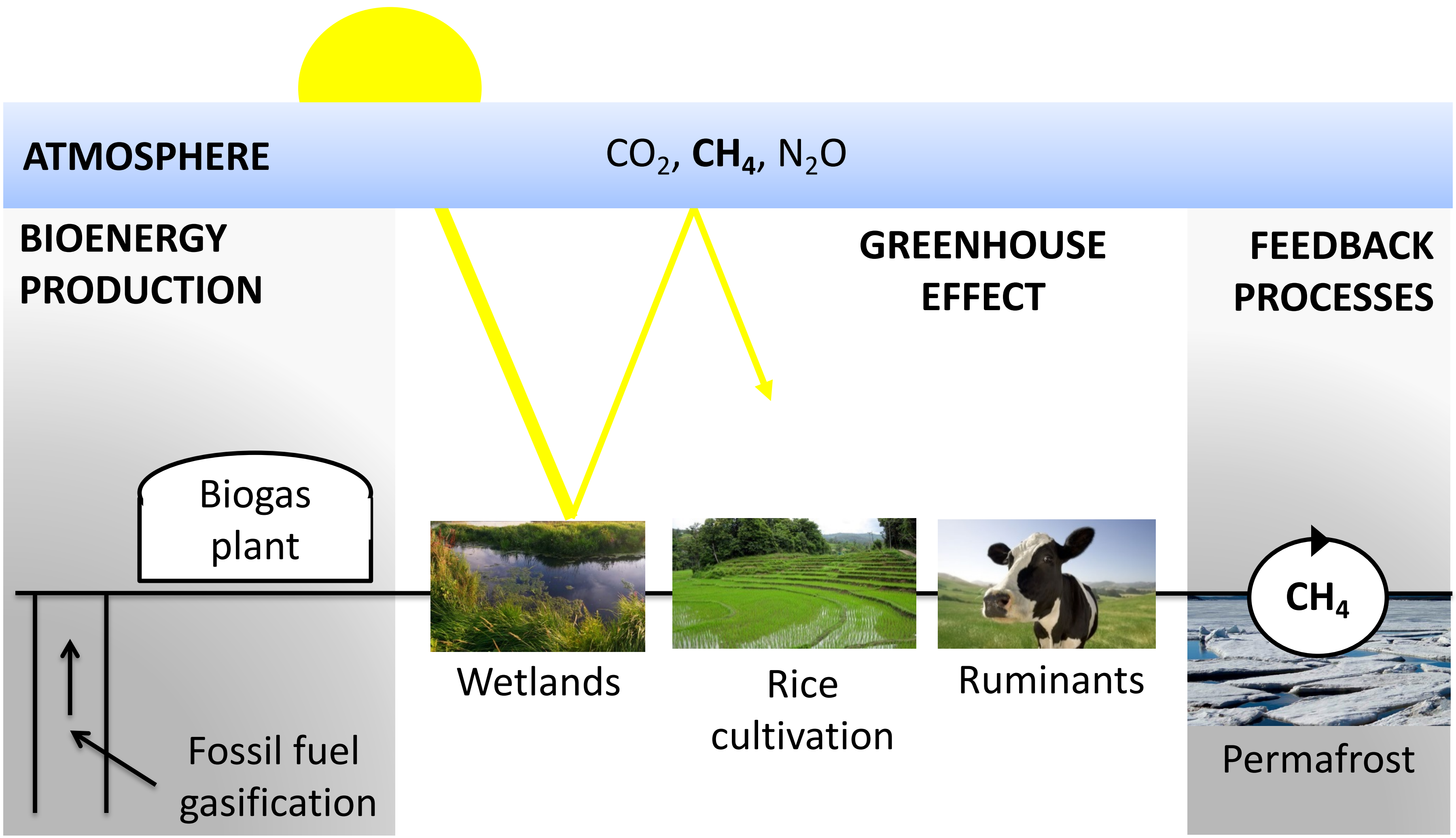
Cornelia Welte

Associate Professor

KITP Forum

March 04, 2021

The fascinating world of methane microbes



The fascinating world of methane microbes

CH_4

$\uparrow\downarrow$

methanogens

produce the biogenic methane

CO_2

methanotrophs

oxidize 50-80% of the produced methane

Understand the biochemistry, physiology and interactions between methanogens and methanotrophs

Biological conversion of methane

CH_4

methanogens ↑ ↓ methanotrophs

- (I) Aerobic methanotrophs (MMO)
- **(II) Anaerobic methanotrophs**

↓

(a) Consortia of ANME-1/-2abc/(3) & sulfate reducers
SO_4^{2-}-dependent, reverse methanogenesis

(b) NC10 bacteria (*Ca.* Methylomirabilis)
NO_2^--dependent, intra-aerobic pathway

(c) ANME-2d (*Ca.* Methanoperedens)
NO_3^--dependent, reverse methanogenesis
no obligate bacterial partner

(d) Coupled to metal reduction (Fe^{3+}, Mn^{4+})
ANME-2a, 2c, 2d

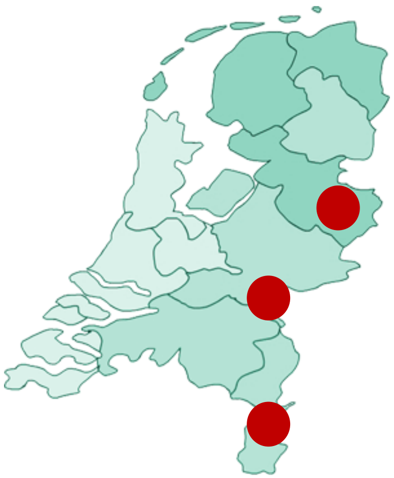
CO_2

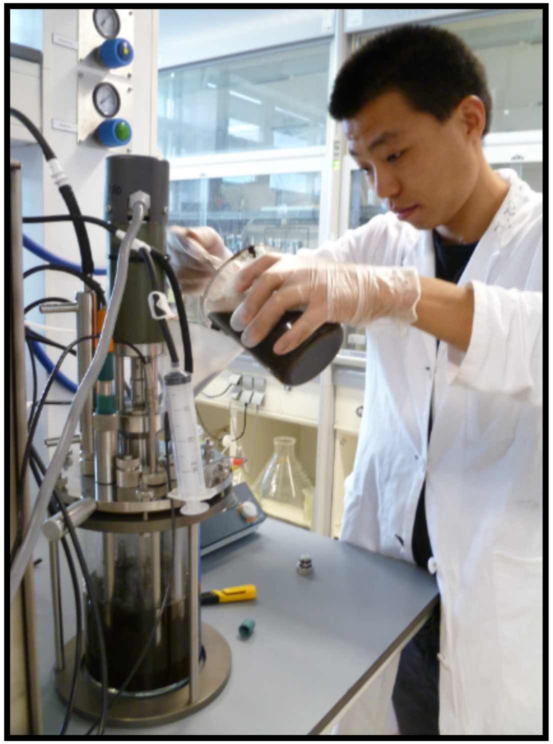
Sampling site nitrate/nitrite-dependent anaerobic oxidation of methane

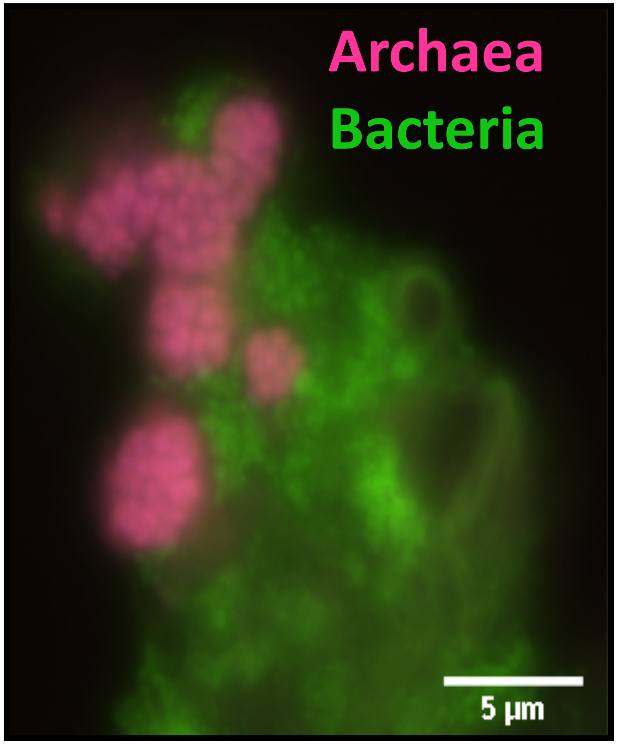
High NO_3^- due to agricultural run-off / ground water

High CH_4 production in the sediment

Characterization of the enrichment culture



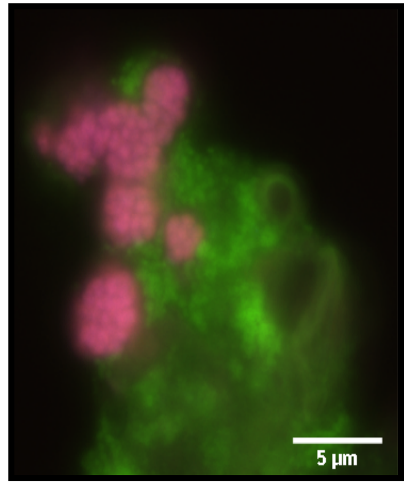




Culture coupled methane oxidation to nitrate and nitrite reduction

Raghoebarsing et al. (2006), Nature

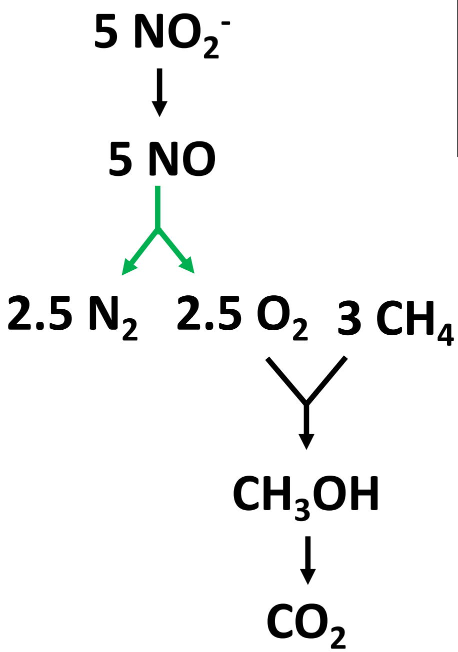
Initial characterization of the enrichment culture



+ Nitrite: Bacteria

Ca. **Methylomirabilis oxyfera**
Novel intra-aerobic pathway

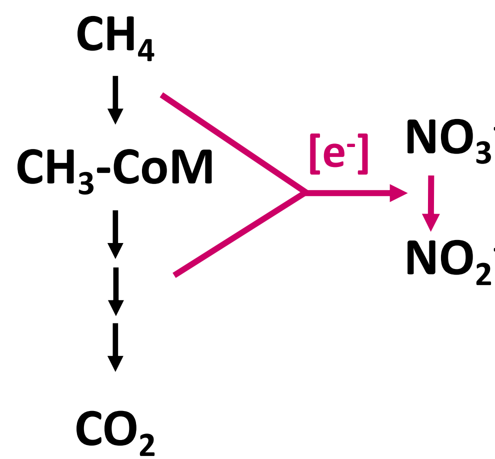
Novel NO dismutase



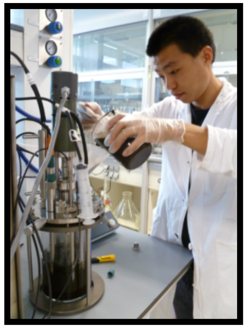
+ Nitrate: Archaea

Ca. **Methanoperedens nitroreducens**

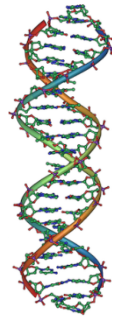
Mechanism?



Characterization of the enrichment culture

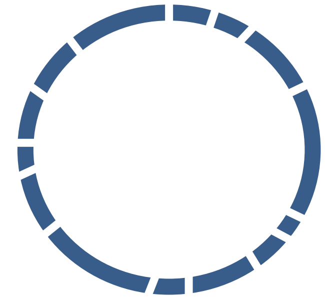


Reactor biomass



DNA
RNA

Metagenome
Metatranscriptome

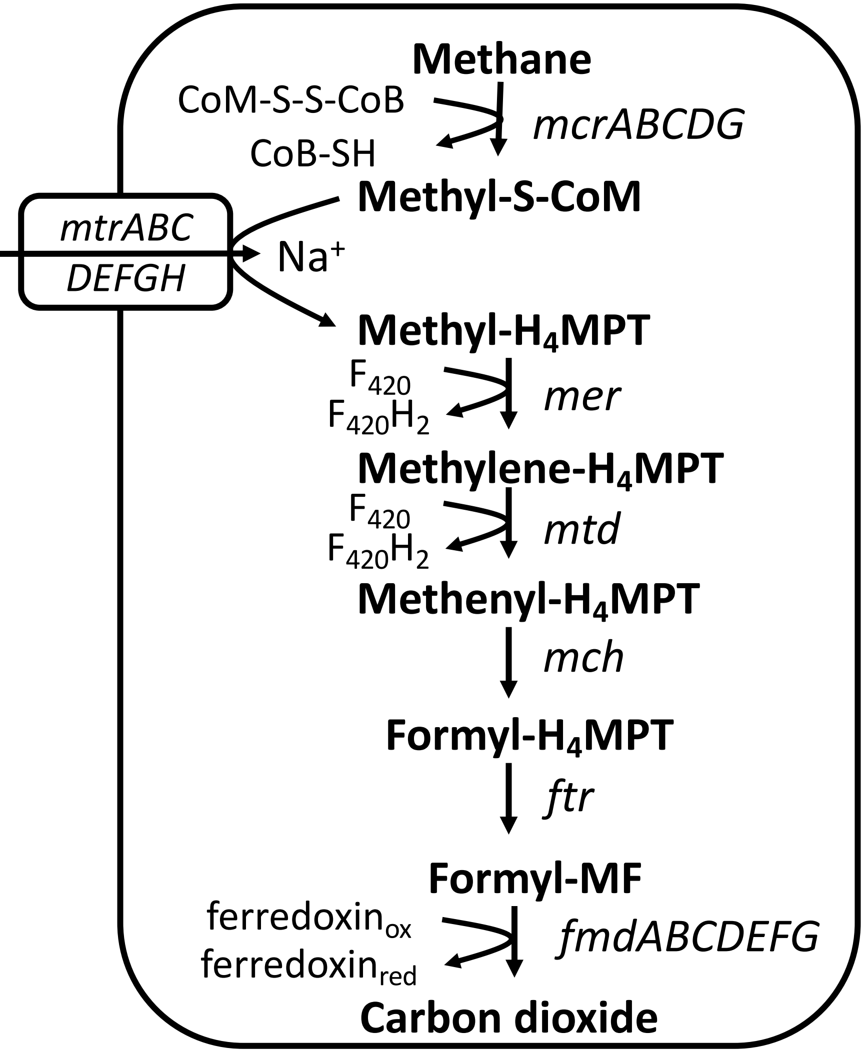


Binned environmental genome

A Metagenomics-Based Metabolic Model of Nitrate-Dependent Anaerobic Oxidation of Methane by *Methanoperedens*-Like Archaea

*Arslan Arshad, Daan R. Speth, Rob M. de Graaf, Huub J. M. Op den Camp, Mike S. M. Jetten and Cornelia U. Welte **

Central metabolic pathway of ANME archaea



A few considerations:

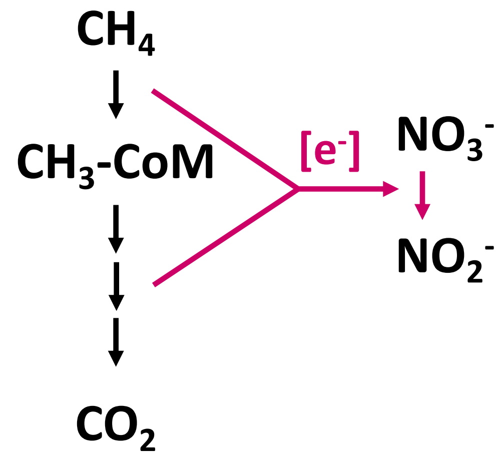
Just reverting a metabolic pathway leads to $\Delta G>0$

Redox-loops for the cofactors are not closed

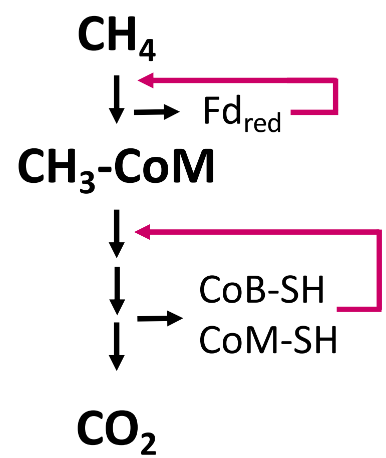
Nitrate reduction is not yet included => needs to conserve energy to compensate for Mtr

The genome contains ca 50% hypothetical proteins!

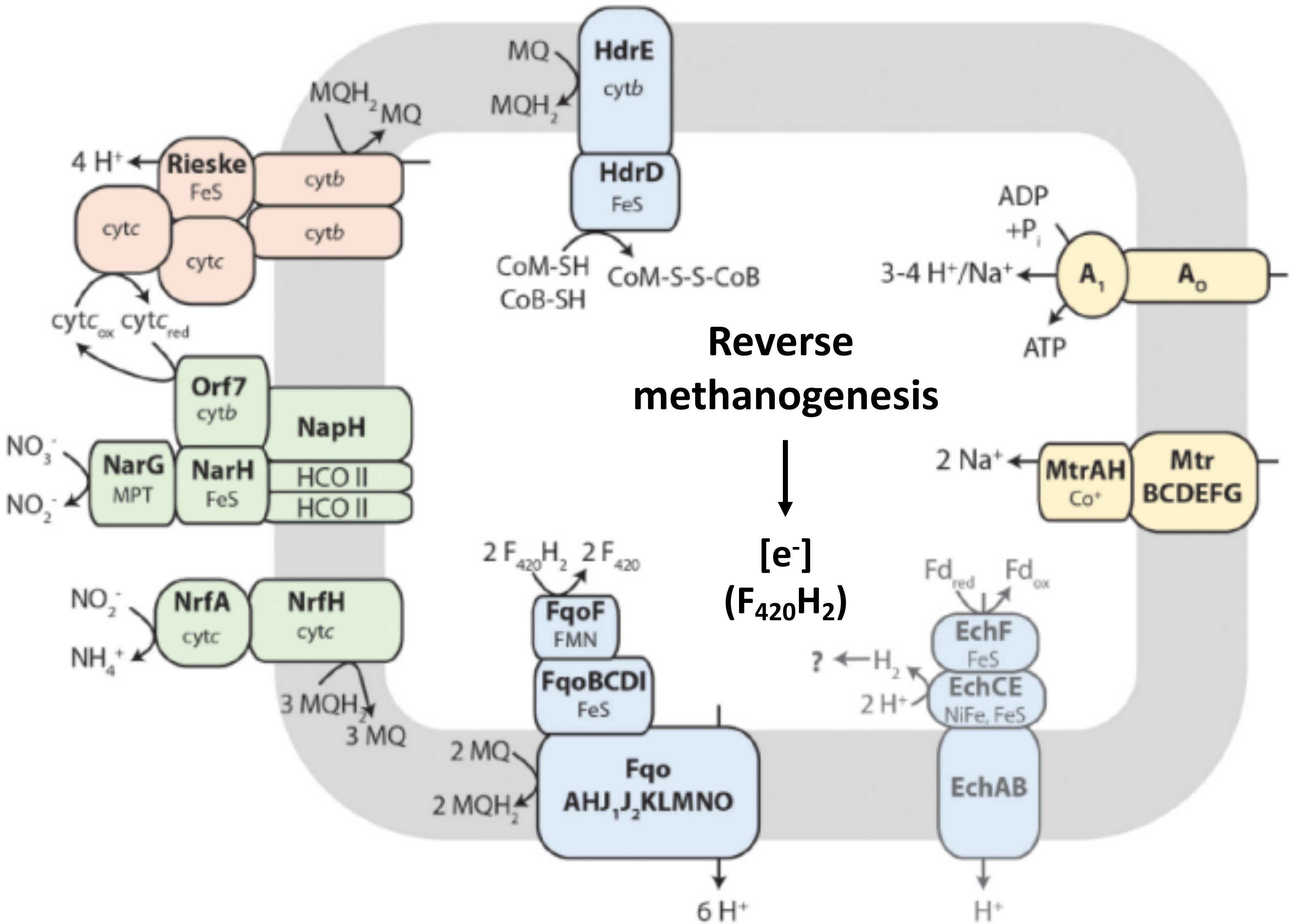
Central metabolic pathway of ANME archaea



How do electrons reach NO_3^-?



How are cofactors re-cycled?



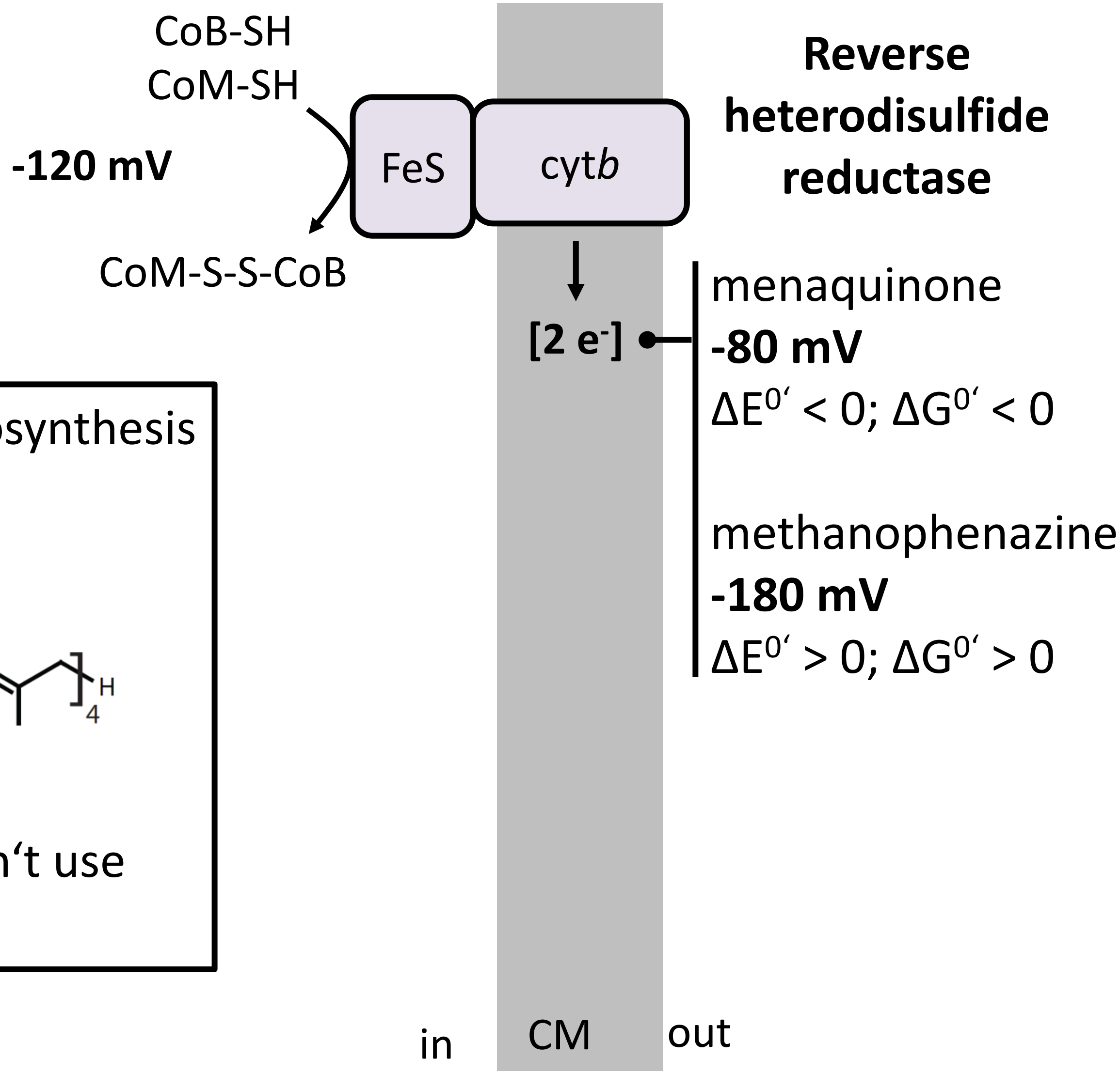
MQ
HdrE
cytb
MQH2
MQH2
MQ
4 H+
Rieske
FeS
cytb
cytc
cytc
cytb
HdrD
FeS
CoM-SH
CoB-SH
CoM-S-S-CoB
ADP
+Pi
3-4 H+/Na+
A1
Ao
ATP
cytcox cytcred
Orf7
cytb
NapH
NO3-
NO2-
NarG
MPT
NarH
FeS
HCO II
HCO II
2 Na+
MtrAH
Co+
Mtr
BCDEFG
NO2-
NH4+
NrfA
cytc
NrfH
cytc
3 MQH2
3 MQ
2 F420H2
2 F420
FqoF
FMN
FqoBCDI
FeS
2 MQ
2 MQH2
Fqo
AHJ1J2KLMNO
6 H+
Fdred
Fdox
EchF
FeS
? ← H2
2 H+
EchCE
NiFe, FeS
EchAB
H+
Reverse methanogenesis
[e-]
(F420H2)

How are cofactors recycled?

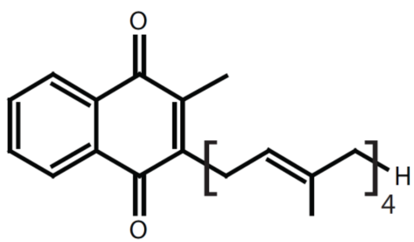
Reverse methanogenesis

↓

CoB-SH
CoM-SH

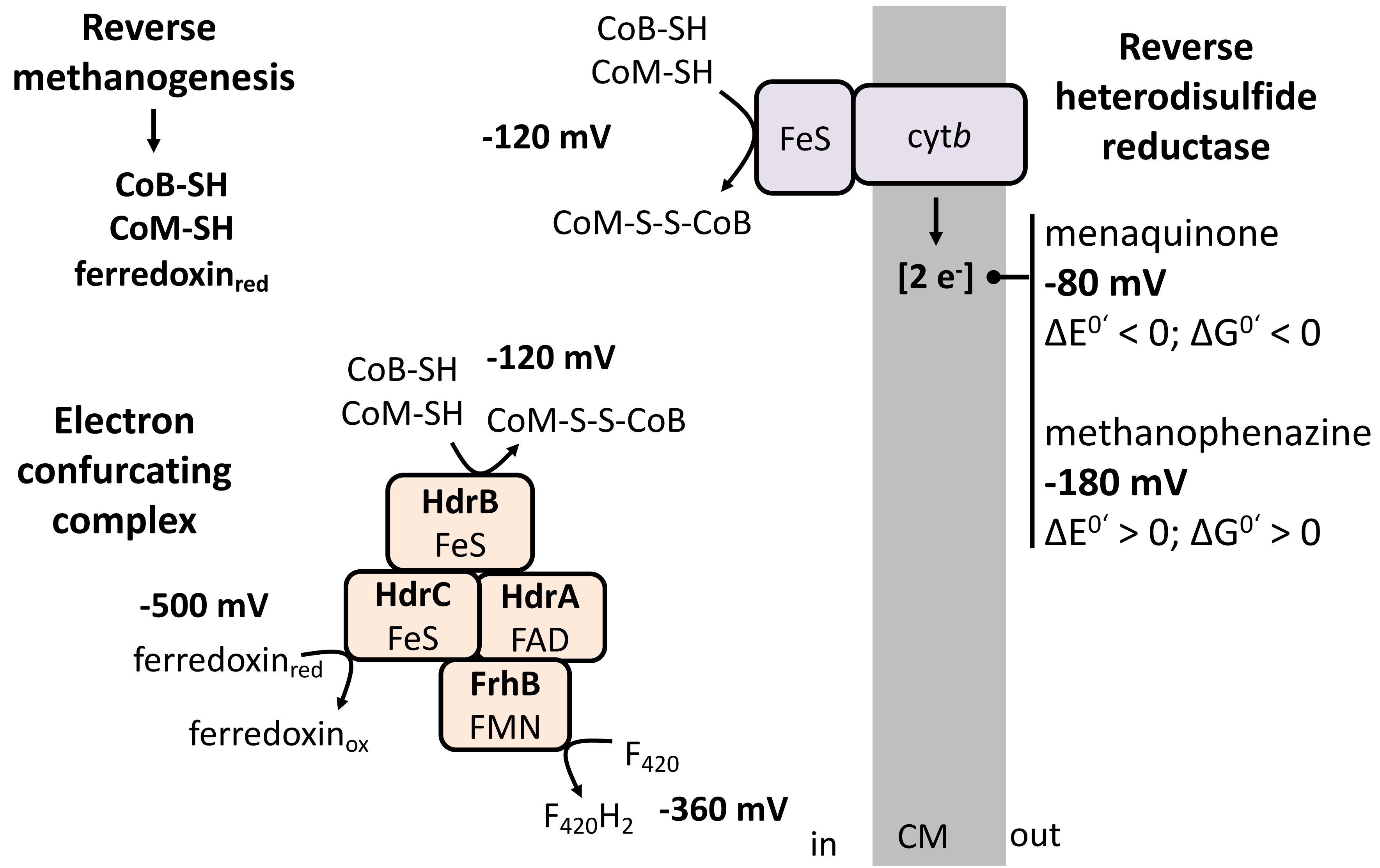


Menaquinone biosynthesis pathway present

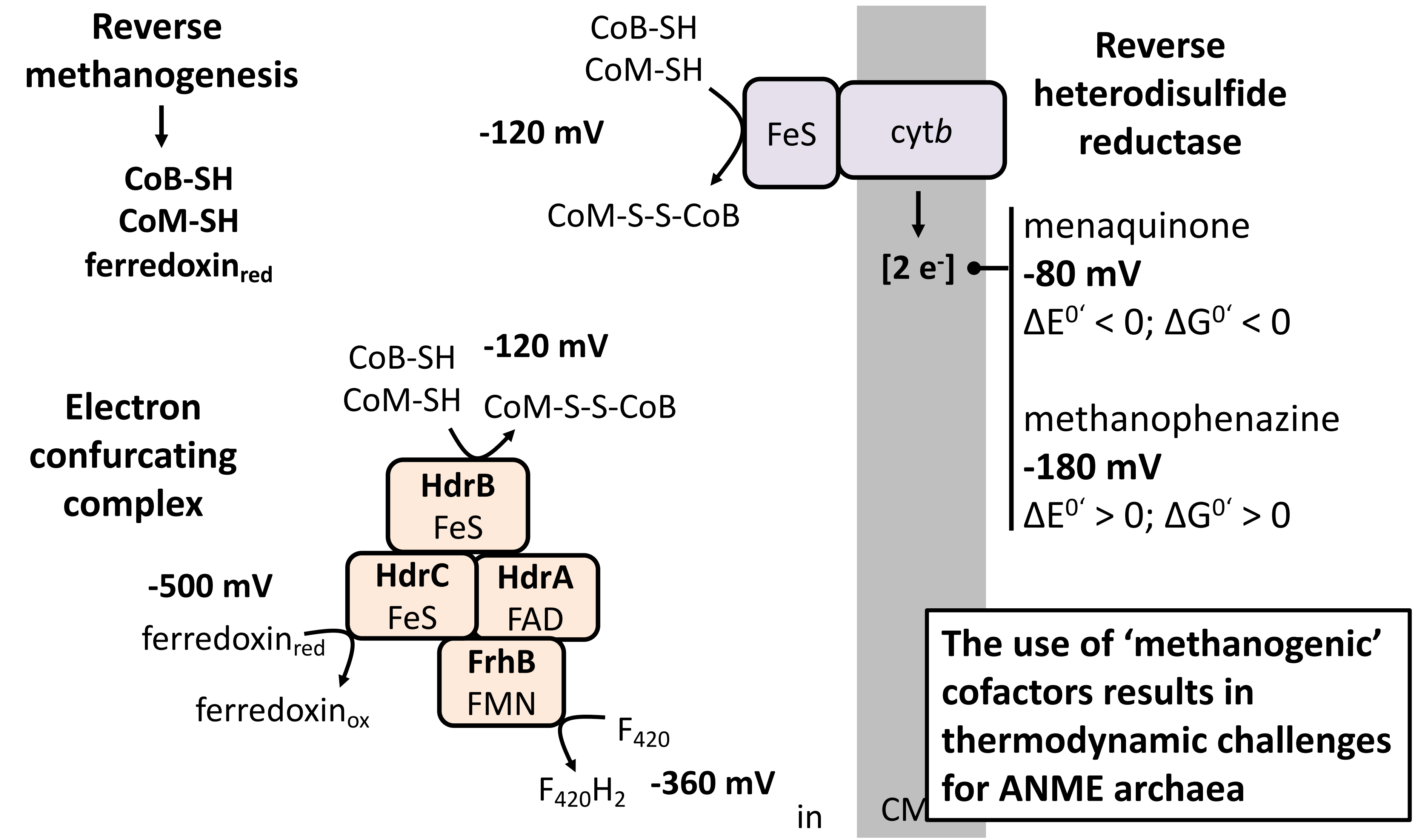


Closely related methanogens don‘t use menaquinones!

How are cofactors recycled?



How are cofactors recycled?



The use of 'methanogenic' cofactors results in thermodynamic challenges for ANME archaea

Metagenome sequencing: conclusions

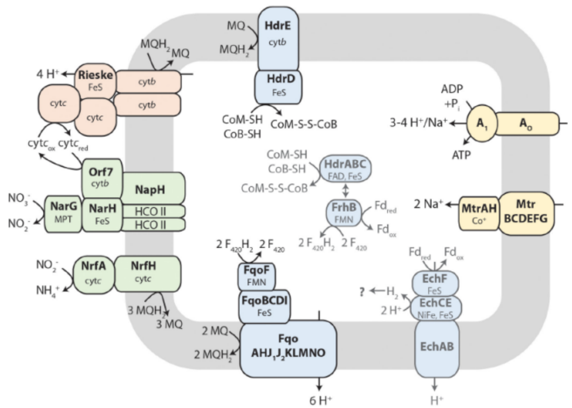
(I) Complete reverse methanogenesis pathway encoded

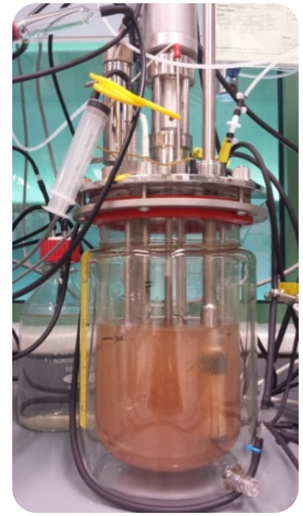
(II) Complex cytoplasmic and membrane-bound electron transport system

(III) Unusual high number of *c*-type cytochromes found in archaea

(IV) Methanotrophy is not just the reversal of methanogenesis!

Progress made on *Ca*. Methanoperedens archaea





Physiology

Microbial interactions

Maider Echeveste

Dr. Julia Kurth

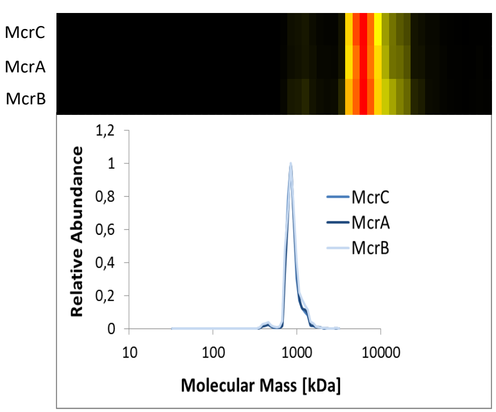
Dr. Paula Dalcin Martins

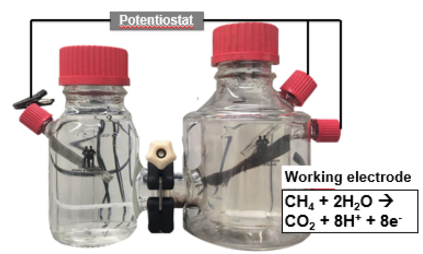
Dr. Martyna Glodowska

Genomic reconstruction

Dr. Arslan Arshad

Dr. Stefanie Berger





Biochemistry & complexome analysis

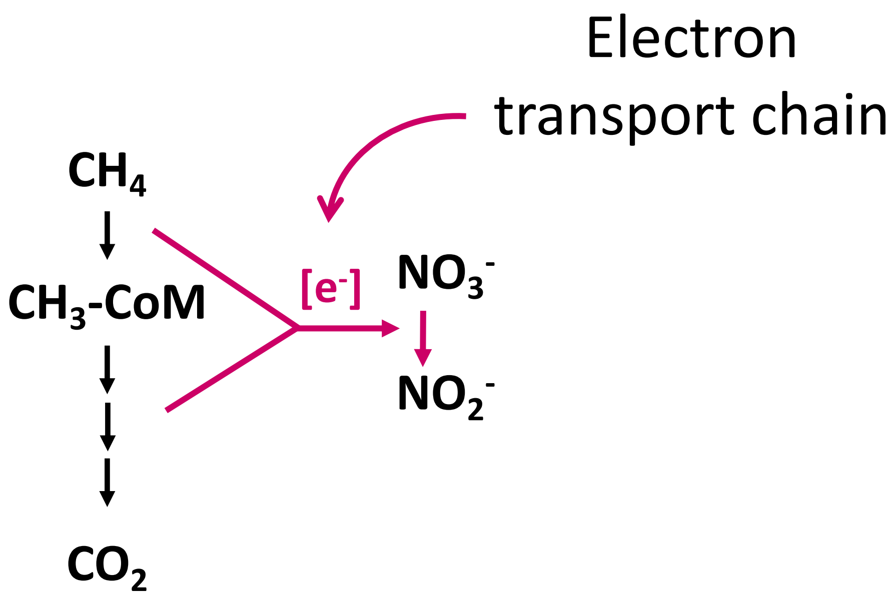
Dr. Stefanie Berger

Electricity production & Extracellular electron transfer

Heleen Ouboter

Application of ANMEs in wastewater treatment

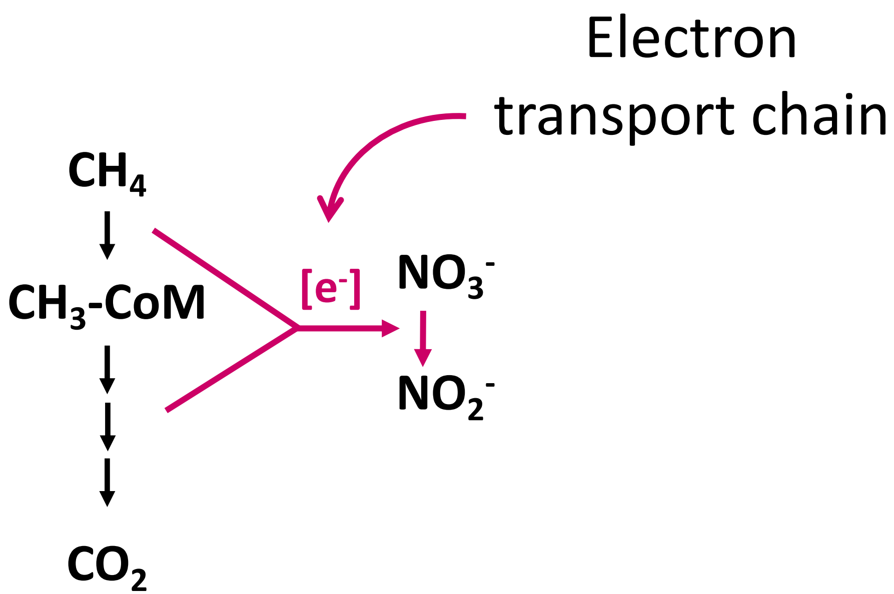
Extracellular electron transfer by *Ca.* Methanoperedens



Heleen Ouboter

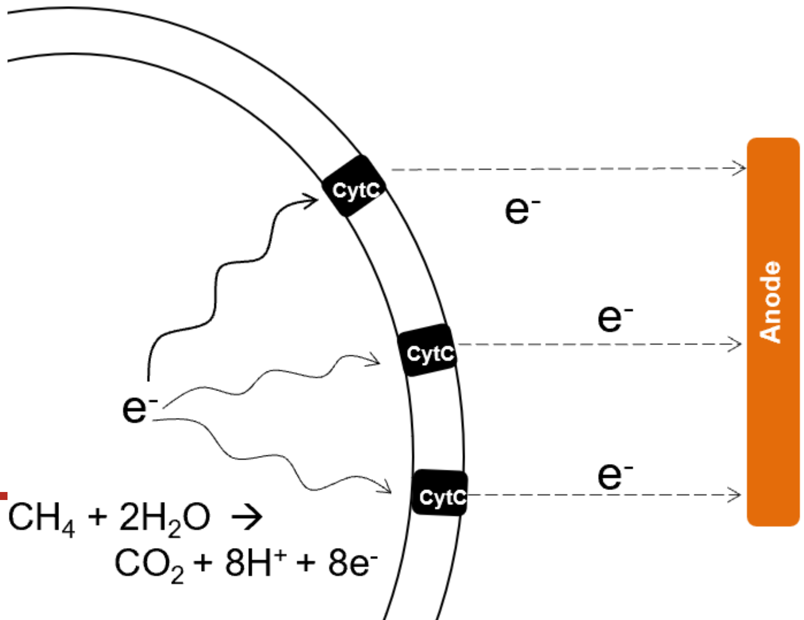
Can the electron acceptor be replaced by an electrode?

Extracellular electron transfer by *Ca.* Methanoperedens

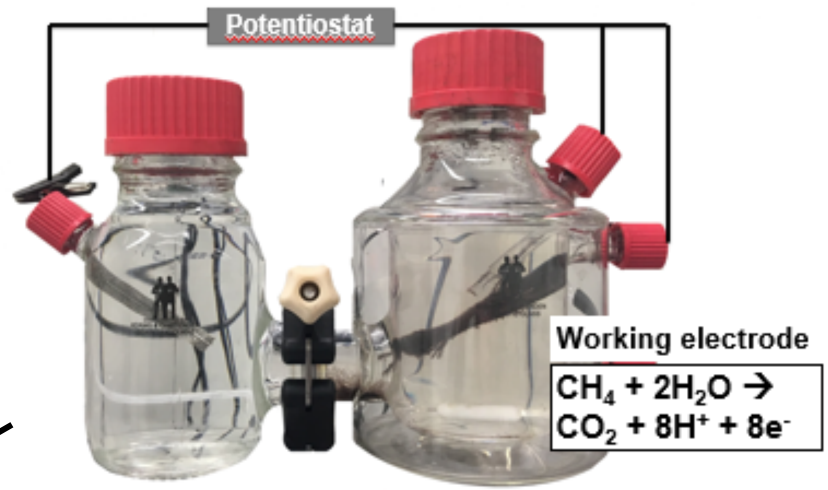



Heleen Ouboter

Can the electron acceptor be replaced by an electrode?



Growing *Ca*. Methanoperedens on a bioanode



Electrochemistry

Current production

Potential scans

Microbial community

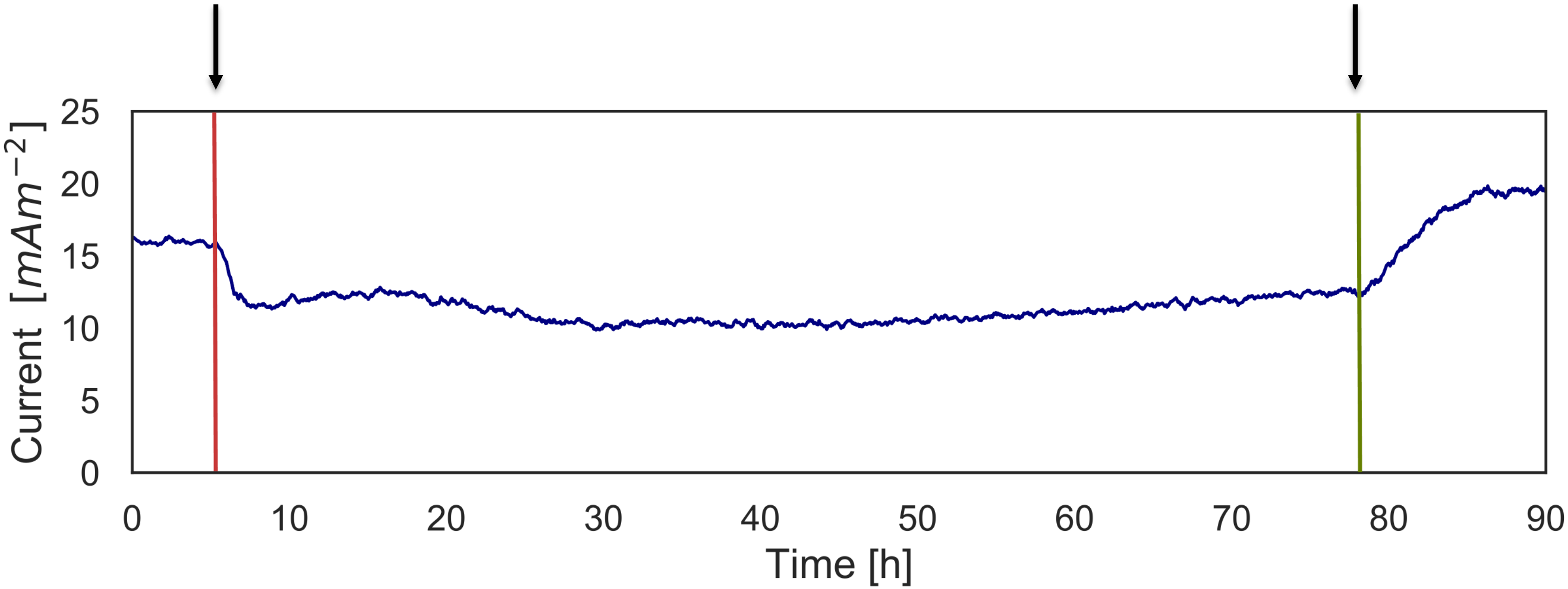
bioanode & inoculum

Growing *Ca*. Methanoperedens on a bioanode

Electrochemistry

Methane inflow stopped

Methane inflow re-started



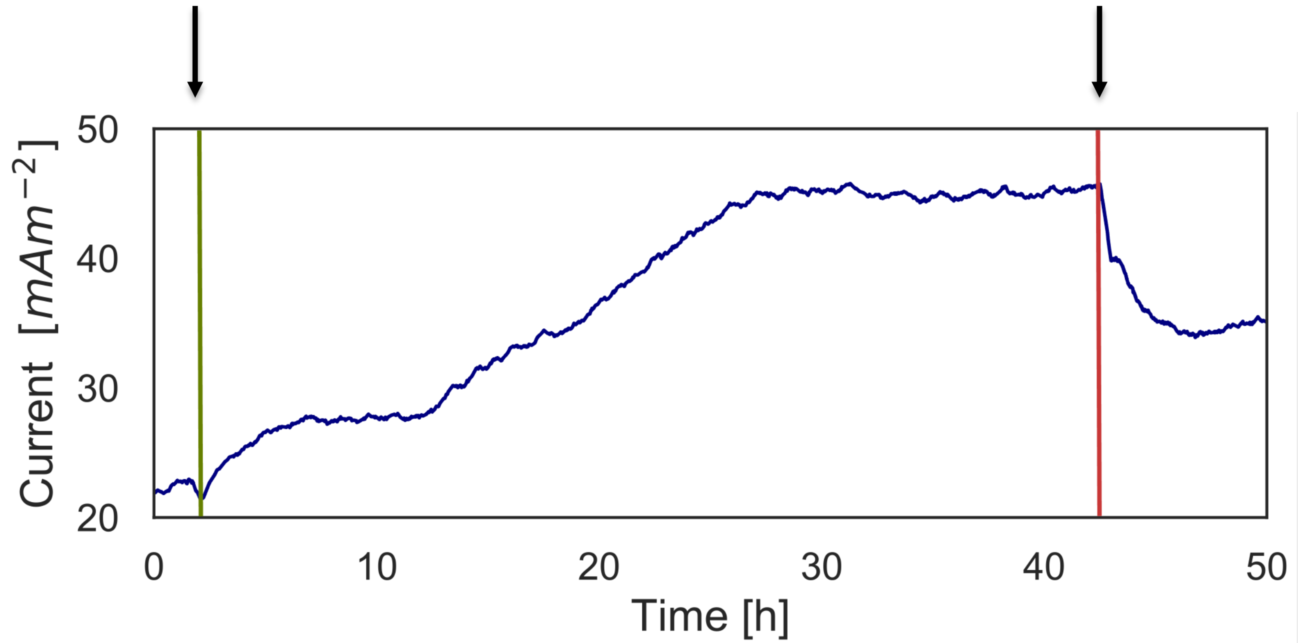
Current production is methane dependent

Growing *Ca*. Methanoperedens on a bioanode

Electrochemistry

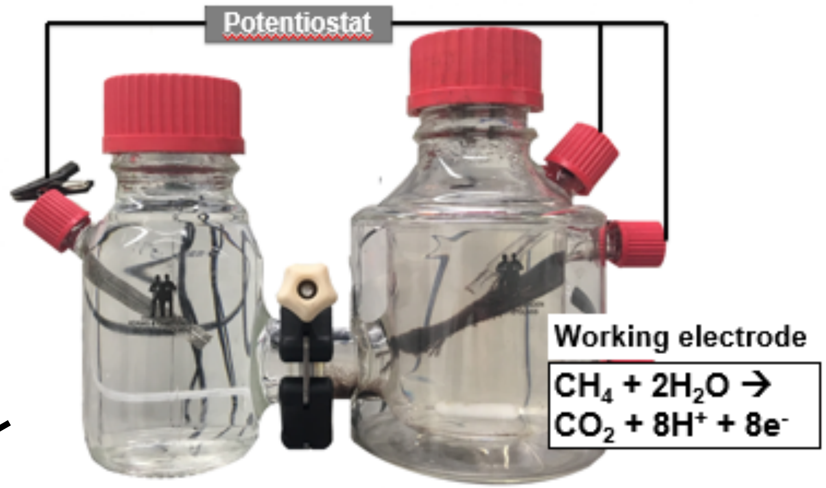
More methane added

System depressurized



Current production is methane dependent

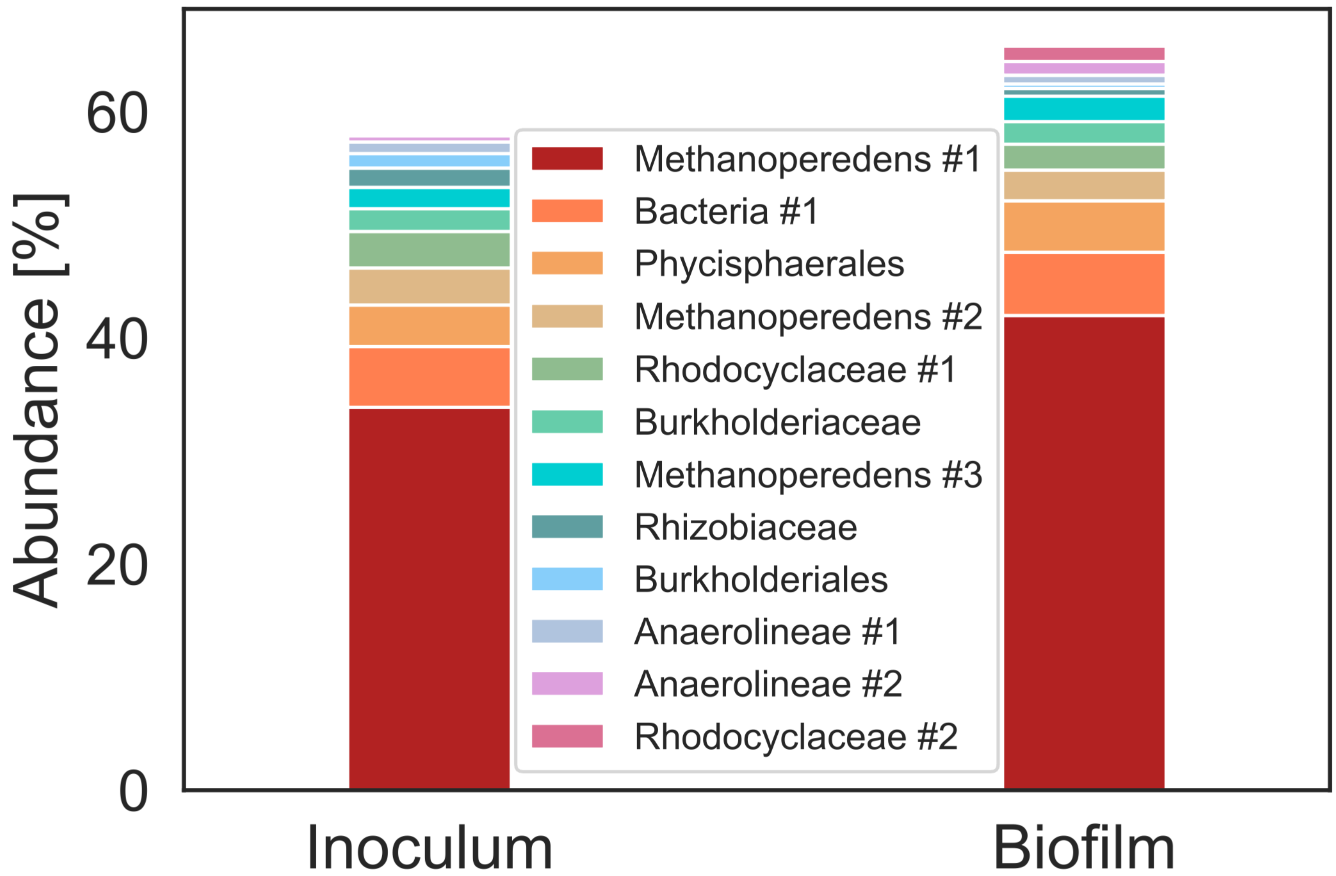
Growing *Ca*. Methanoperedens on a bioanode

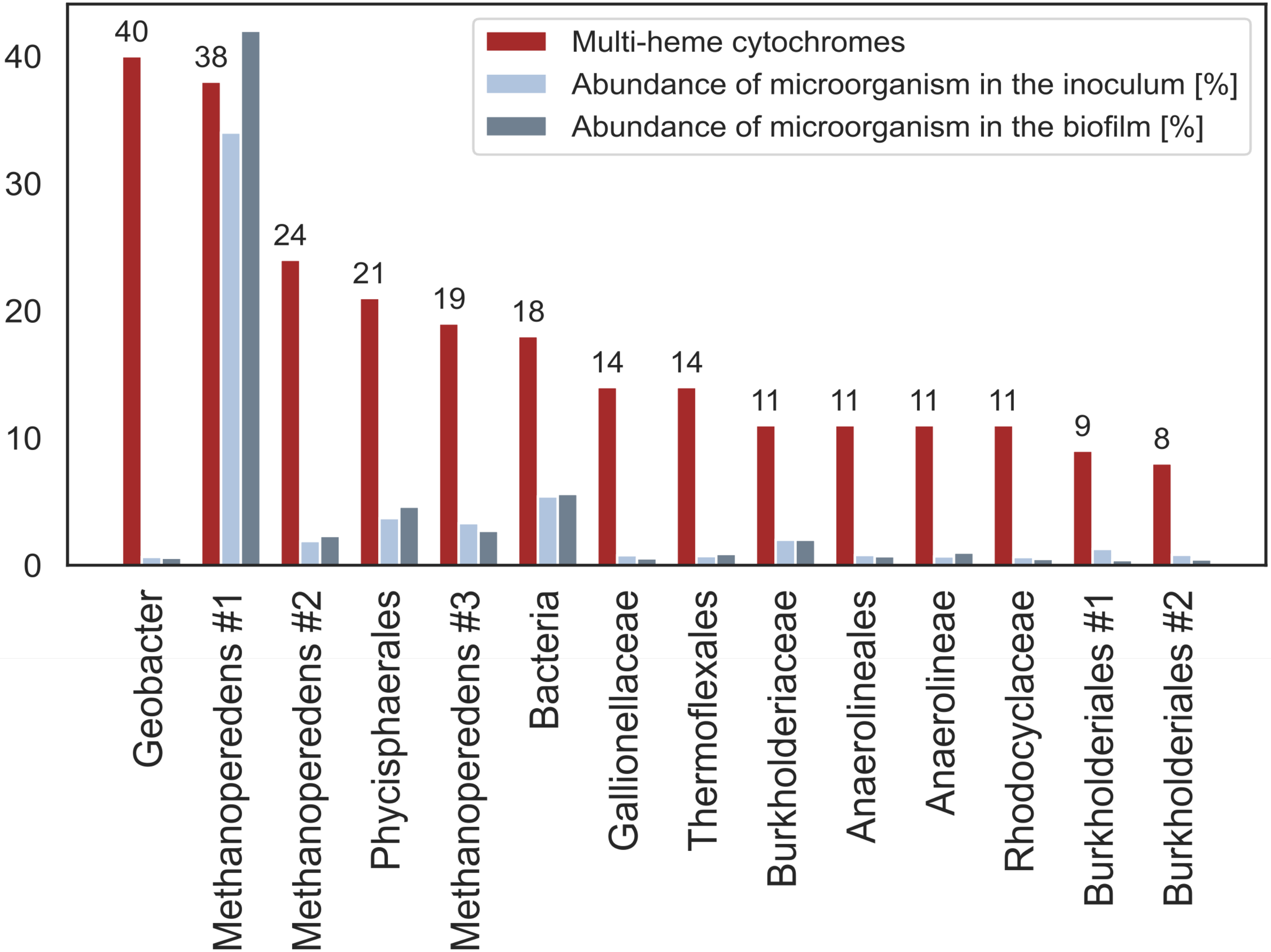


Electrochemistry
Current production
Potential scans

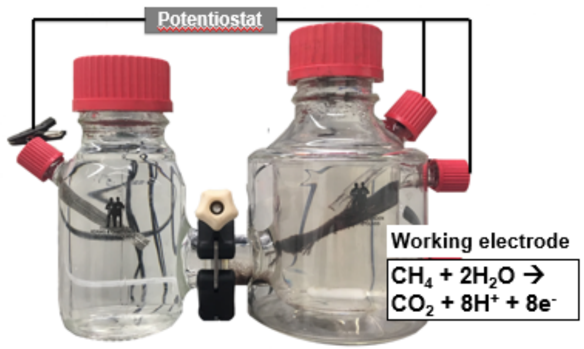
Microbial community
bioanode & inoculum

Metagenomics: *Ca*. Methanoperedens dominates both inoculum and bioanode




Multi-heme cytochromes
Abundance of microorganism in the inoculum [%]
Abundance of microorganism in the biofilm [%]
0
10
20
30
40
40
38
24
21
19
18
14
14
11
11
11
11
9
8
Geobacter
Methanoperedens #1
Methanoperedens #2
Phycisphaerales
Methanoperedens #3
Bacteria
Gallionellaceae
Thermoflexales
Burkholderiaceae
Anaerolineales
Anaerolineae
Rhodocyclaceae
Burkholderiales #1
Burkholderiales #2

Growing *Ca*. Methanoperedens on a bioanode



Proof-of-concept
Ca. Methanoperedens can be cultivated in bioelectrochemical system

Electrochemistry
Current production is methane dependent

Microbial community
Ca. Methanoperedens major player

Acknowledgements

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Koen Pelsma
Mike Jetten

MPI Bremen

Tristan Wagner

AIST Japan

Yoichi Kamagata
Masaru Nobu

Wageningen University

Annemiek ter Heijne

Wetsus

Tom Sleutels

King Abdullah University of Science and Technology (KAUST)

Dario Shaw

DFG Deutsche Forschungsgemeinschaft

SIAM

NESSC NETHERLANDS EARTH SYSTEM SCIENCE CENTRE

NWO

Thank you for your attention!

Bonus slides

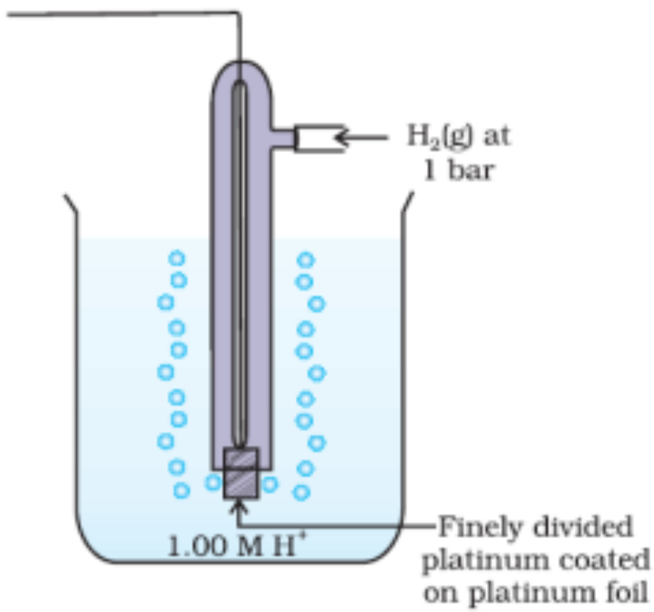
Redox potential

Wikipedia excerpt:

"Redox potential is a measure of the tendency of a chemical species to acquire electrons and thereby be reduced. Reduction potential is measured in volts (V), or millivolts (mV). Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced."

The standard hydrogen electrode

Chemical conditions – pH 0



- 1 mol/L H^+
- Continuous bubbling of Pt electrode
- Half cell / reference cell
- E^0 = 0 V

Biological conditions – pH 7

- pH is usually not 0 (even for extremophiles – intracellular pH!)
- $E^{0\prime}$ (H^+/H_2) = -420 mV
  - ⇒ The "standard hydrogen electrode" is not the standard for biological systems
- $E^{0\prime}$=0 has lost any meaning (if it ever had one); scale arbitrary but reference needed

- Whenever you think about gaseous electron donors/acceptors: constant bubbling over an electrode in a biological system…?

The redox-potential: real life
Displacement from standard conditions

$$E = E^\circ + 2.3\frac{RT}{nF}\log_{10}\left\{\frac{[\text{oxidised}]}{[\text{reduced}]}\right\}$$

Nernst equation

R Gas constant; 8.314 J K^{-1} mol^{-1}

F Faraday's constant; 96500 J mol^{-1} V^{-1}

n number of transferred electrons

Under equal concentrations of ox/red species, the second part of the equation is 0 and E = E^0

If the concentrations are very low, not thermodynamics but enzyme properties may become the limiting factor.

How to calculate Gibbs free energy

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

"Gibbs free energy"
$\Delta G < 0$ exergonic
$\Delta G > 0$ endergonic

Number of transferred electrons

Faraday's constant
96.5 kJ mol^{-1} V^{-1}

Redox potential difference

$E^{0'}$ ($2H^+/H_2$) = -414 mV
$E^{0'}$ ($\frac{1}{2}O_2/H_2O$) = +820mV

$\Delta E^{0'}$= +1234mV = +1.234 V

$$\mathbf{\Delta G^{0'} = -2 \cdot 96.5\ kJ\ mol^{-1}\ V^{-1} \cdot 1.234\ V}$$
$$\mathbf{= -238\ kJ\ mol^{-1}}$$