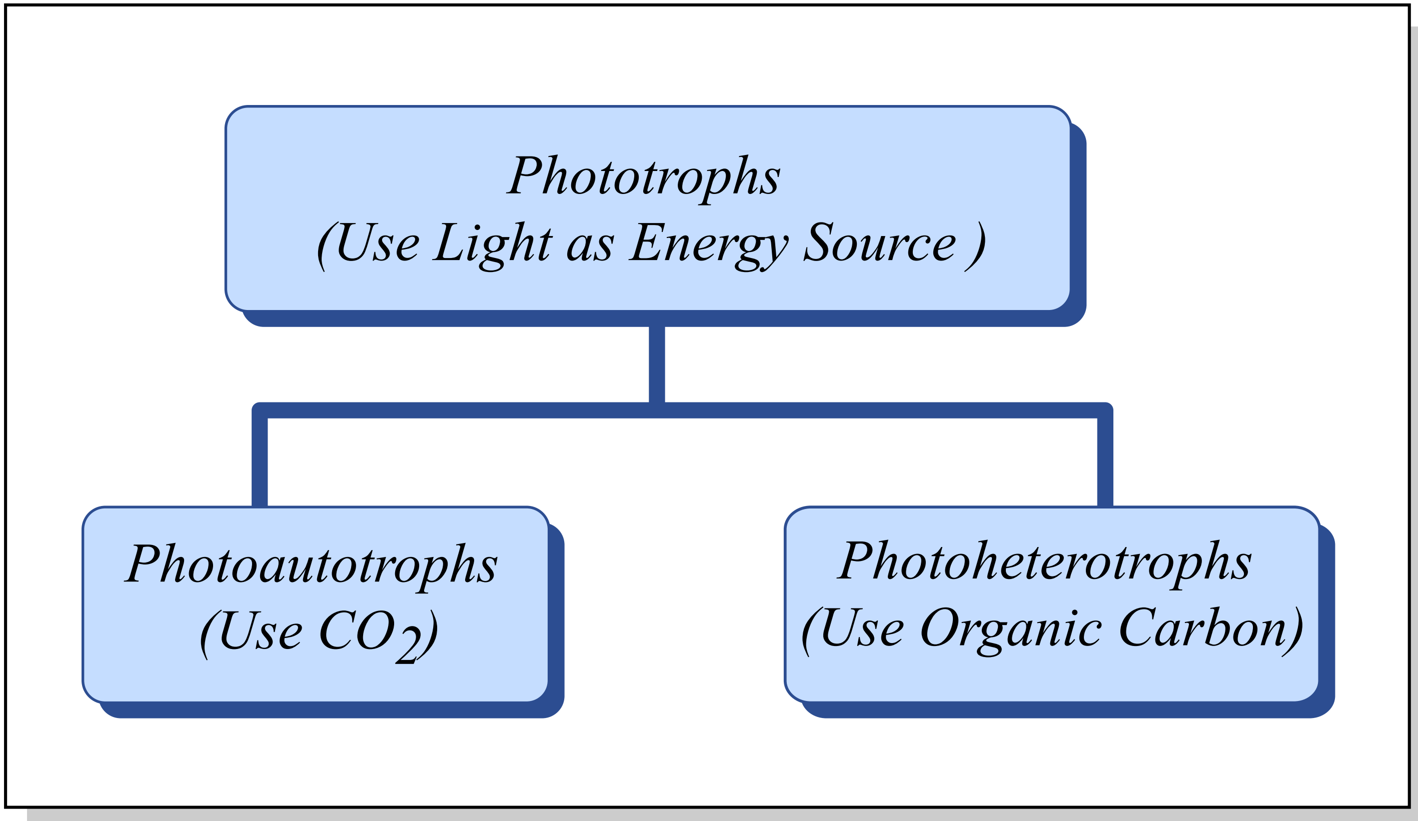
Systems Microbiology

Weds Sept 20 - Ch 5 & Ch 17 (p 533-555)

Bioenergetics & Metab.Diversity

- BASIC MODES OF ENERGY GENERATION
- THERMODYNAMICS OF GROWTH -cont'd
- APPLICATIONS of MICROBIAL CHEMOLITHOTROPHY & ANAEROBIC RESP



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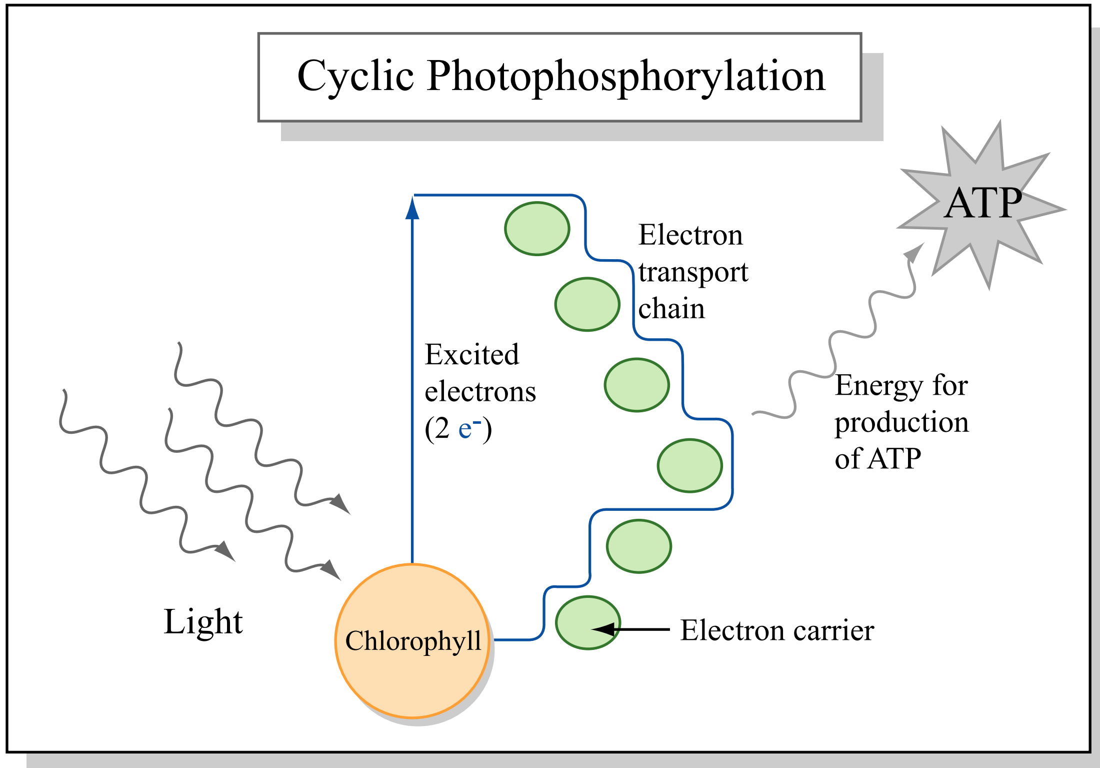
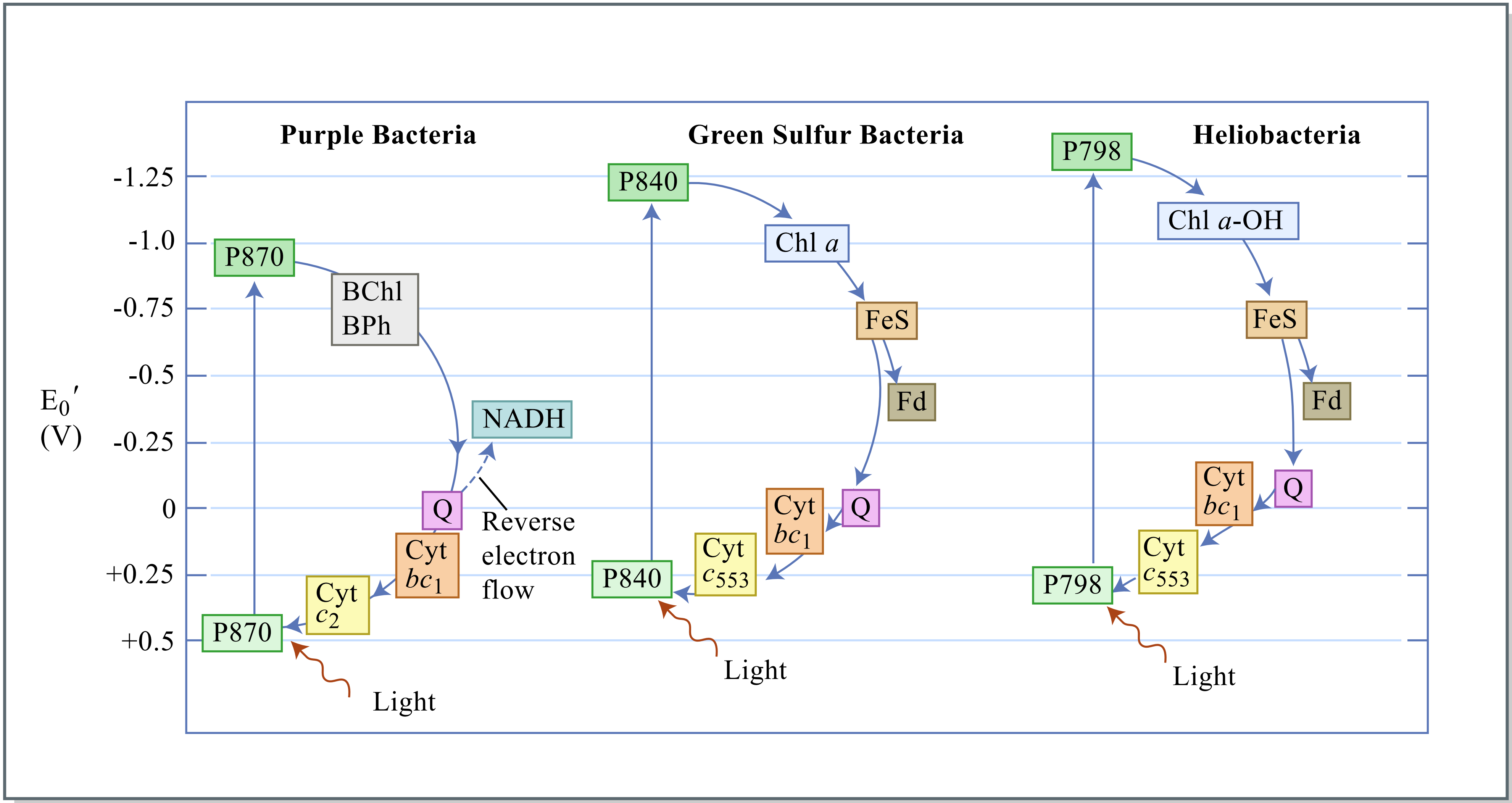


Figure by MIT OCW.

Anoxygenic photoautotrophs utilize cyclic photophosphorylation

LOTS OF DIVERSITY IN BACTERIAL ANOXYGENIC PHOTOTROPHS !



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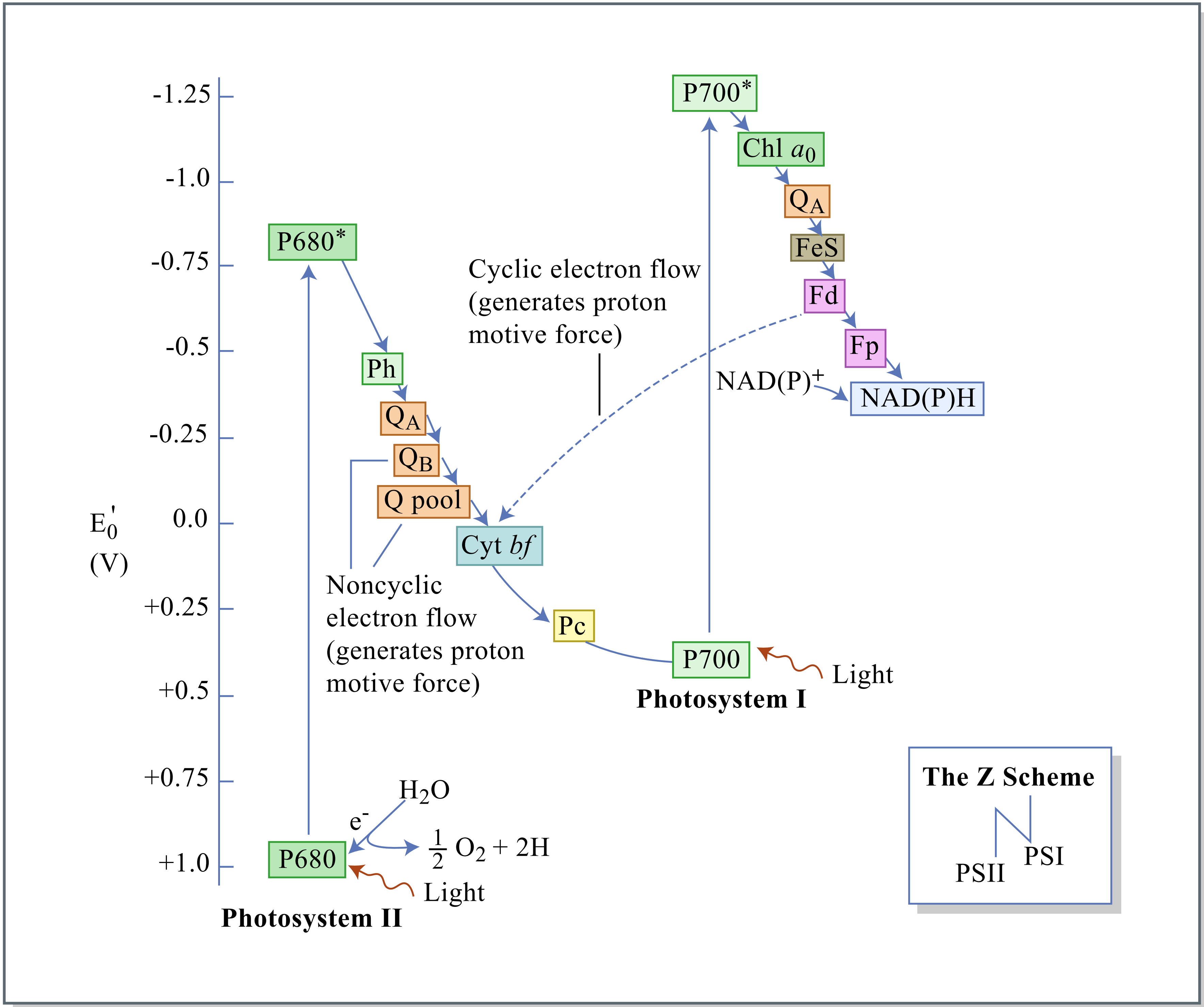


Figure by MIT OCW.

HALOARCHAEA
Live in hypersaline habitats

Aerial photograph of haloarchaea changing the colors of their saltwater habitats removed due to copyright restrictions.

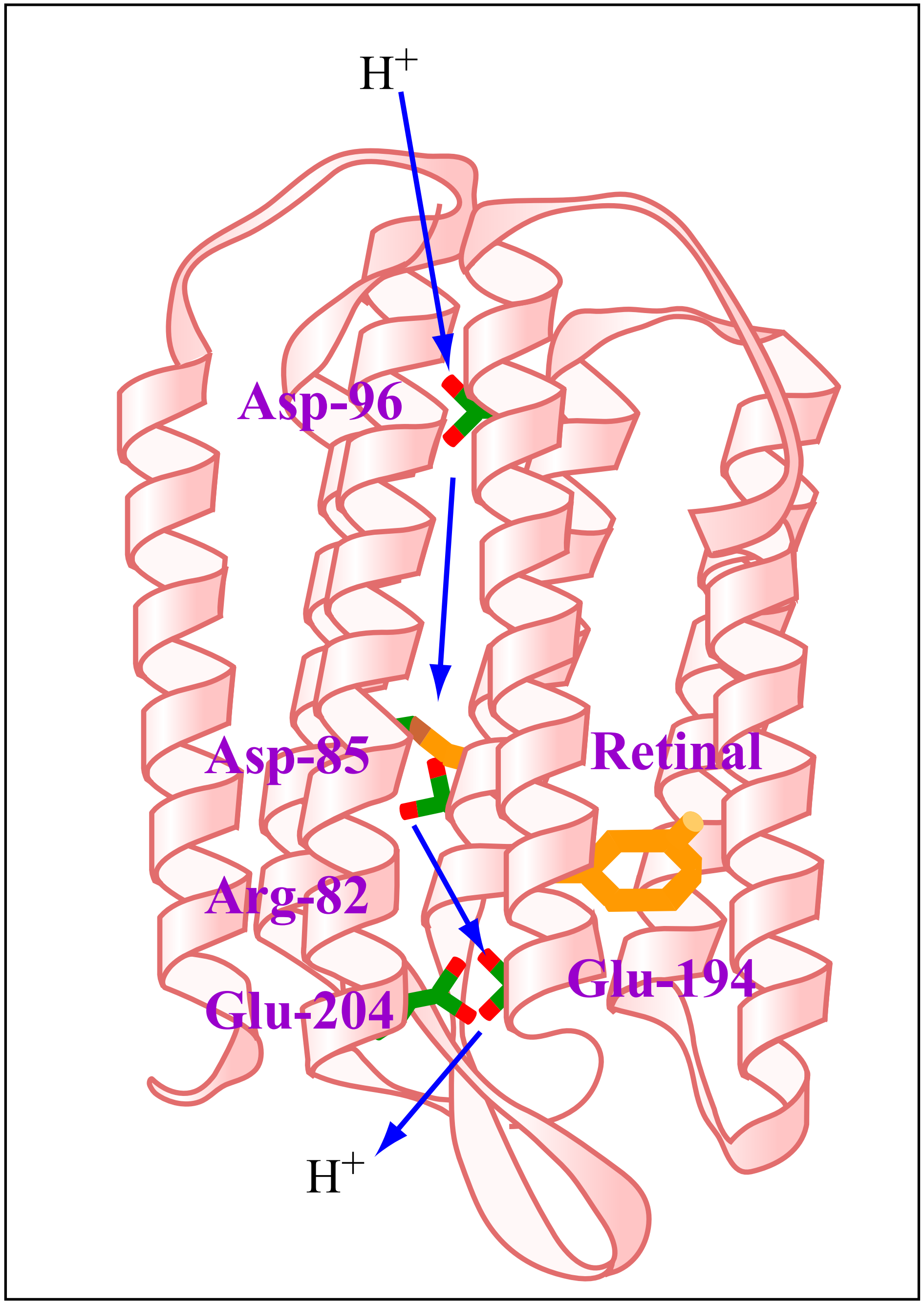


Figure by MIT OCW.

Microbial rhodopsins fall into two main functional classes

Light-driven ion pumps

Sensory rhodopsins

Bacteriorhodpsin

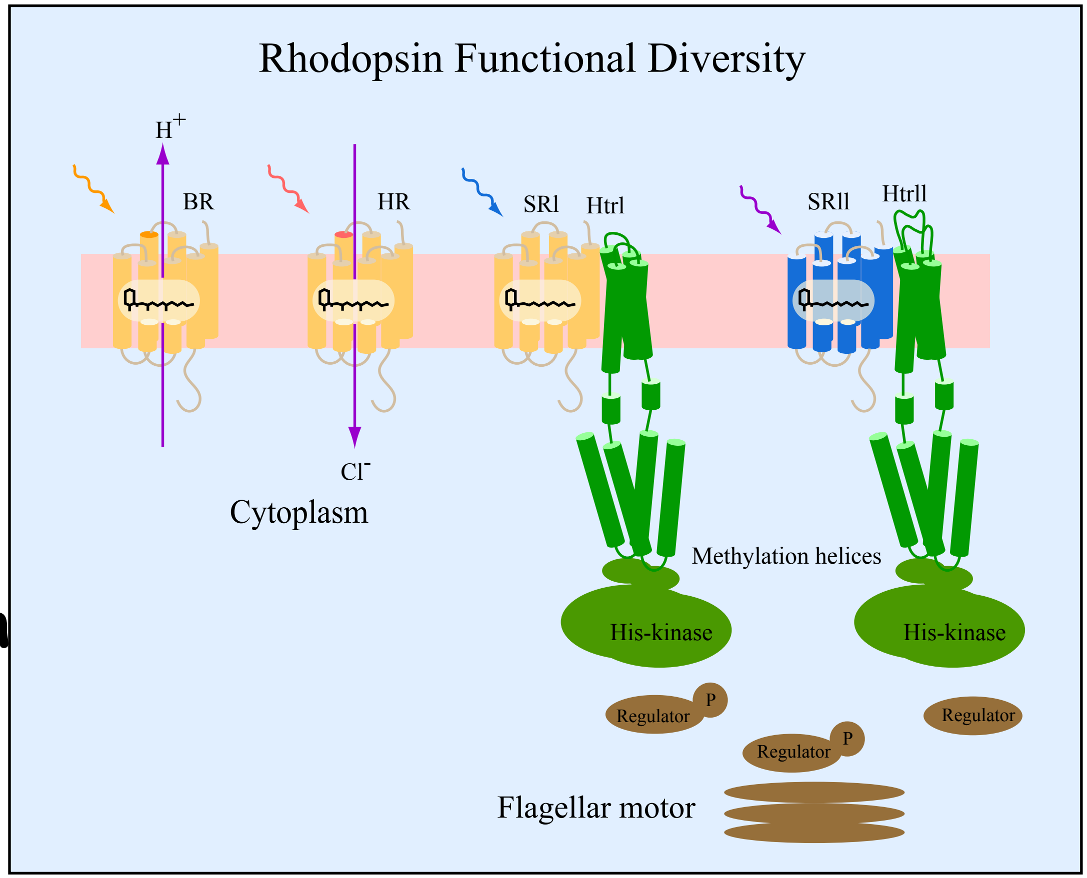
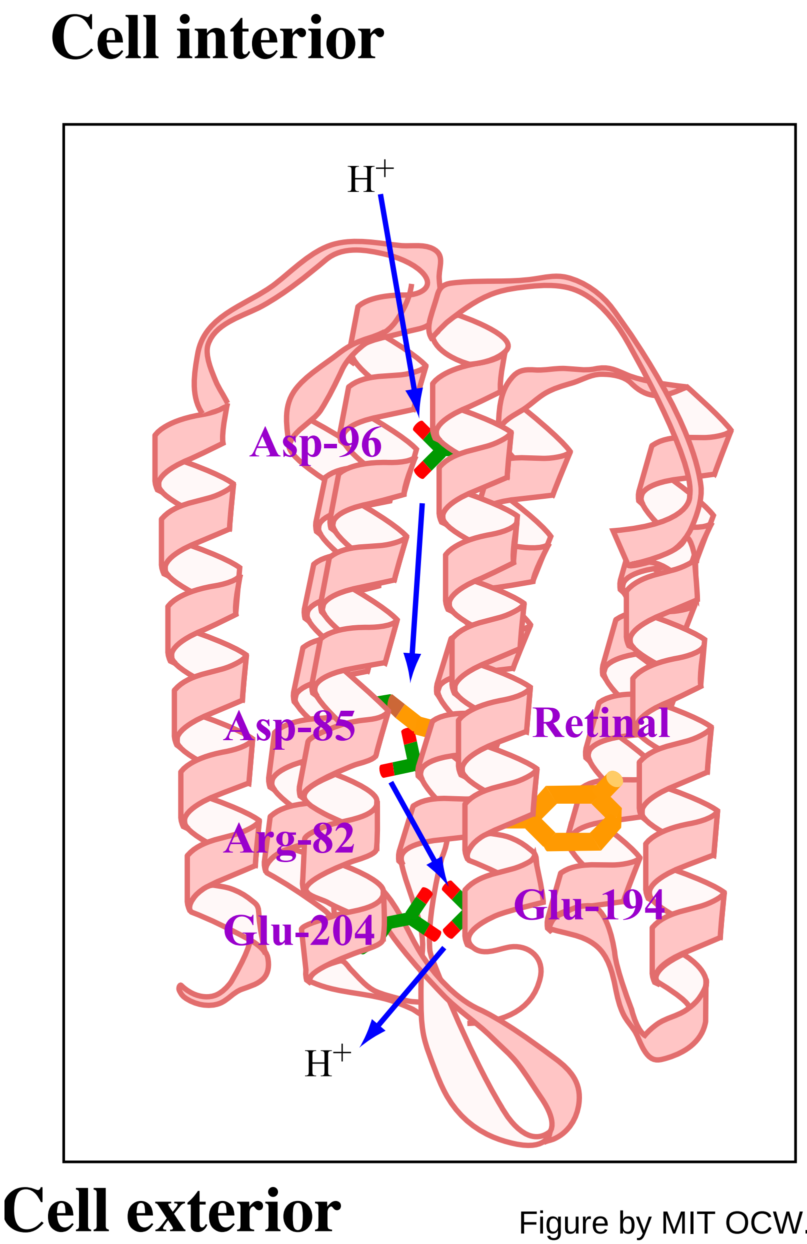


Figure by MIT OCW.

Bacteriorhodopsin and proteorhodospin
Light driven proton pumps



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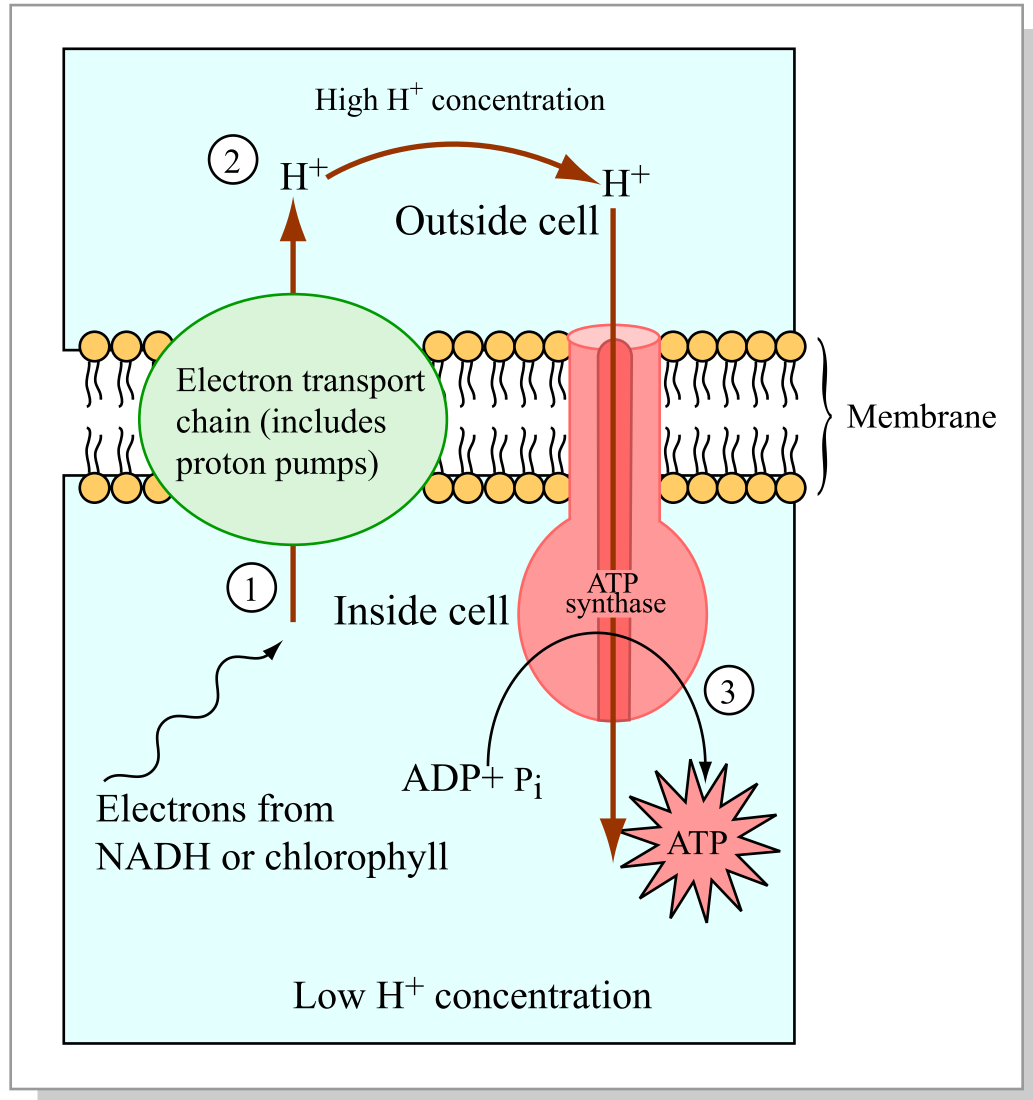


Figure by MIT OCW.

Diagram removed due to copyright restrictions.

Venter et al., Environmental Genome Shotgun Sequencing of the Sargasso Sea,

Science 394:66-74 (2004)

Many new bacterial proteorhodopsins discovered in environmetnal shotgun sequencing

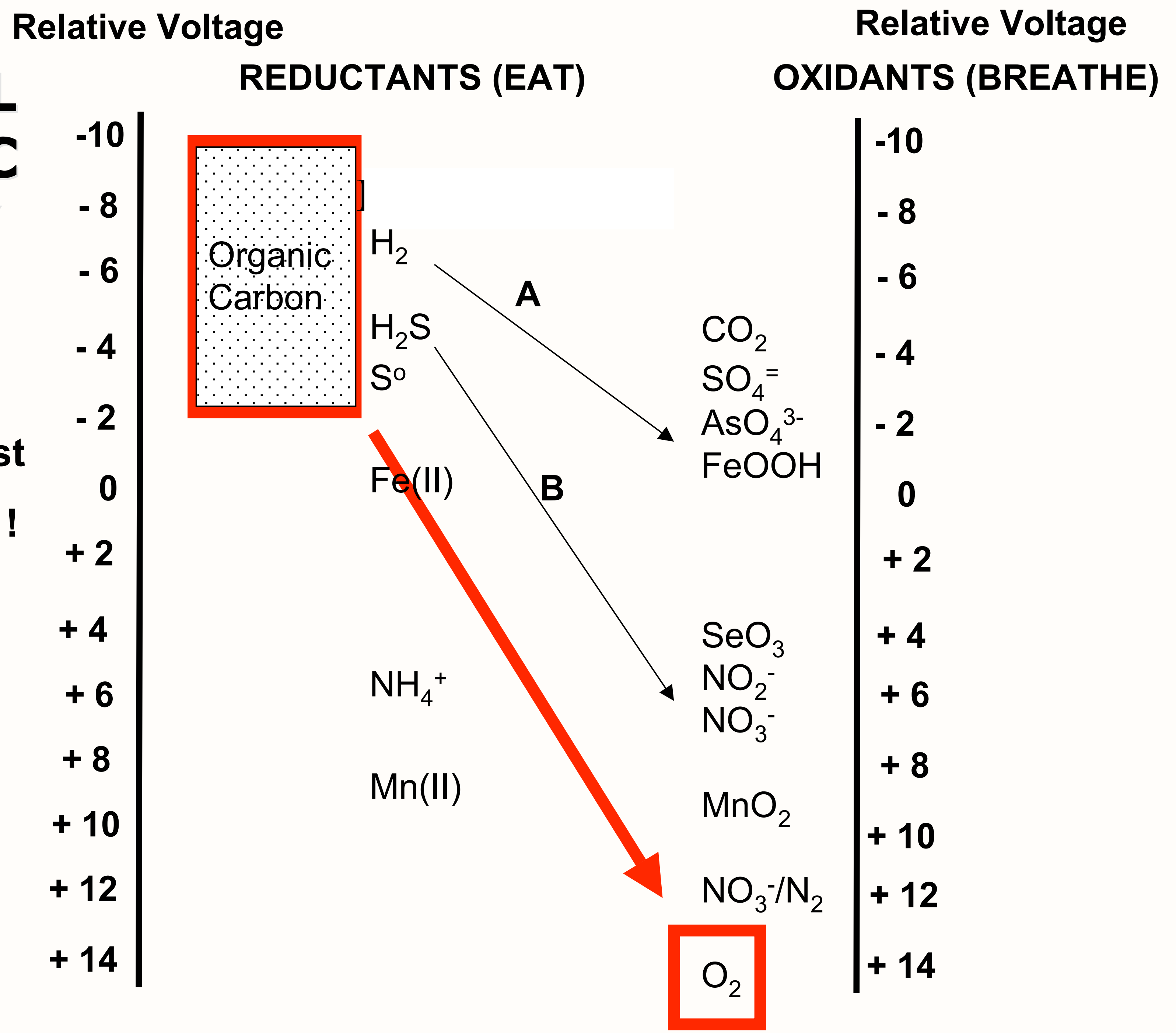
Images of a hybrid automobile, hydrocarbons, and electricity removed due to copyright restrictions.

Where do organisms get their energy?

- ALL ORGANISMS
  - chemotrophs
    - chemolithotrophs
      - **Oxidize inorganic compounds**
    - chemoorganotrophs
      - **Oxidize organic compounds**
  - phototrophs
    - **Derive energy from light**

MICROBIAL METABOLIC DIVERSITY

Microbes can eat & breathe just about anything !



METABOLIC DIVERSITY - Continued ….

Chemolithoautotroph (chemo [chemical], litho [rock], auto[self], troph [feeding])
Energy source: inorganic substrates (H2, NH3, NO2-, H2S, Fe2+)
Carbon source: CO_2
e- acceptor: O_2(aerobes), or S(some anaerobes), $Fe^{3+,}$ NO_3, SO_4

Chemolithoautotrophs can be grouped according to the inorganic compounds that they oxidize for energy:
Nitrifiers - Oxidize reduced Nitrogen compounds such as NH4+

Sulfur Oxidizers- Oxidize reduced Sulfur compounds such as H2S, S0, and S2O-

Iron Oxidizers- Oxidize reduced Iron-Fe2+ (ferrous iron)

Hydrogen Oxidizers-Oxidize Hydrogen gas-H2

Table of energy yields from the oxidation of various inorganic electron donors removed due to copyright restrictions.
See Table 17-1 in Madigan, Michael, and John Martinko. *Brock Biology of Microorganisms*. 11th ed.
Upper Saddle River, NJ: Pearson Prentice Hall, 2006. ISBN: 0131443291.

METABOLIC DIVERSITY
CHEMOLITHOAUTOTROPHS - Examples

Table 4. Groups of bacteria able to use inorganic electron donors for growth ("chemolithoautotrophs").

Bacterial group	Typical species	Metabolic process	Electron donor	Electron acceptor	Carbon source	Prod
Hydrogen-oxidizing bacteria	*Alcaligenes eutrophus*	H_2 Oxidation	H_2	O_2	CO_2	H_2O
Carbon monoxide-oxidizing bacteria	*Pseudomonas carboxydovorans*	CO oxidation	CO	O_2	CO_2	CO_2
Ammonium-oxidizing bacteria	*Nitrosomonas europaea*	Ammonium oxidation	NH_4^+	O_2	CO_2	NO_2^-
Nitrite-oxidizing bacteria	*Nitrobacter winogradskyi*	Nitrite oxidation	NO_2^-	O_2	CO_2	NO_3^-
Sulfur-oxidizing bacteria	*Thiobacillus thiooxidans*	Sulfur oxidation	S, $S_2O_3^{2-}$	O_2	CO_2	SO_4^{2}
Iron-oxidizing bacteria	*Thiobacillus ferrooxidans*	Iron oxidation	Fe^{2+}	O_2	CO_2	Fe^{3+}
Methanogenic bacteria	*Methanobacterium thermoautotrophicum*	Methanogenesis	H_2	CO_2	CO_2	CH_4
Acetogenic bacteria	*Acetobacterium woodii*	Acetogenesis	H_2	CO_2	CO_2	CH_3-COOI

CHEMOLITHOTROPHIC AMMONIA OXIDATION - AEROBIC

$$NH_4^+ + 3/2O_2 \longrightarrow NO_2^- + H_2O + 2H^+$$

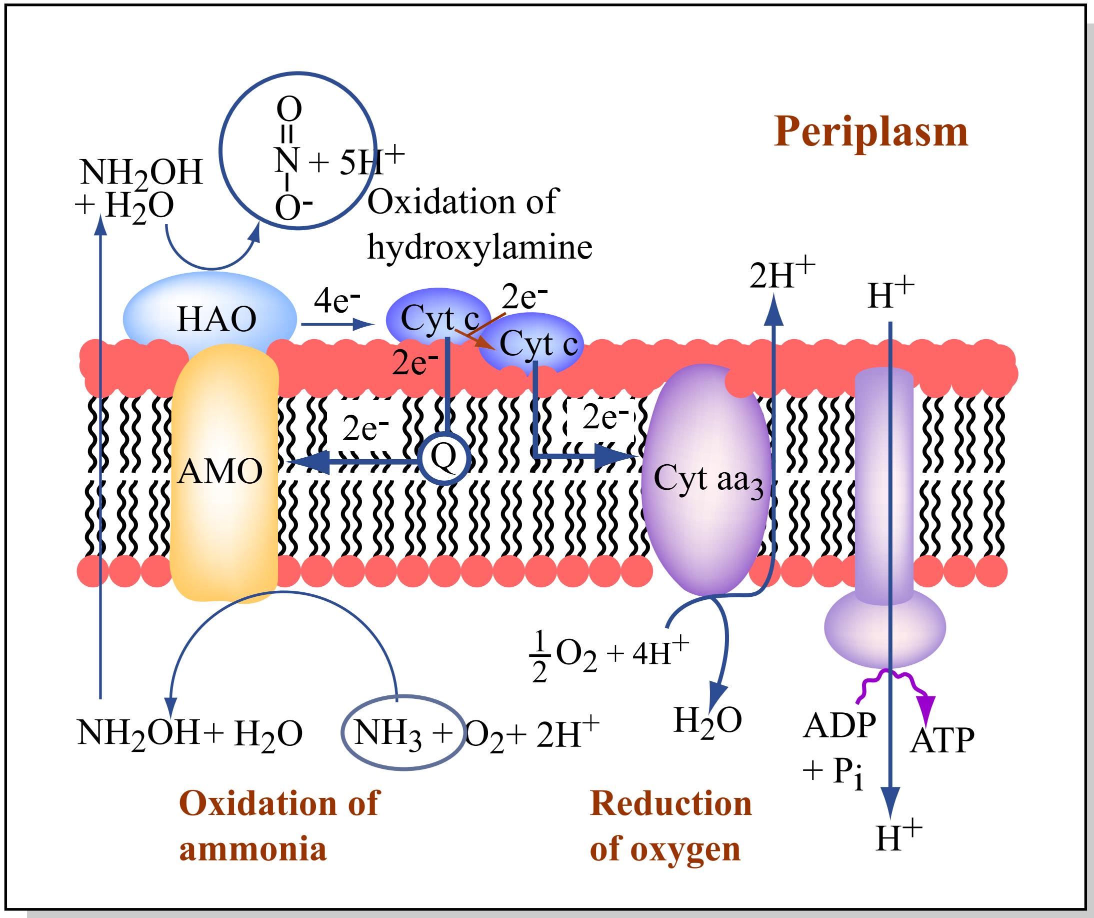


Figure by MIT OCW.

the online anammox resource
www.anammox.com
pioneering microbiology for a sustainable future

CHEMOLITHOTROPHIC AMMONIA OXIDATION - ANAEROBIC

Anammox means "anaerobic ammonium oxidation". Anammox is both a new low-cost method of N-removal in wastewater treatment, and a spectacular microbial way of life - **woo - woo !**

Courtesy of the Department of Microbiology at Radboud University Nijmegen. Used with permission.

Broda E: **Two kinds of lithotrophs missing in nature.** *Z Allg Mikrobiol* 1977, **17**:491-493.

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$$

$2NH_4^+/N_2$ half reaction (6e-) Eo = -0.277 V

$2NO_2^-/N_2$ half reaction (6e-) Eo = +0.956 V

$\Delta E_o = E_o$ (electron acceptor) - E_o (electron donor) = 1233 mV

$\Delta G_o = -nF\ \Delta E_o = -(3)\ (96.5\ kJ/Vmol)(1.233V) = -\ 357\ kJ/mol$

Broda predicted, based solely on the thermodynamics, that such microorganisms should exist. (And also the fact that if a bioenergetically favorable niche exists, a microbe will evolve to fill it !).

About a decade later, the ' bugs' were discovered in bioreactors started from waste water treatment plants.

Table 1 Current Opinion in Biotechnology 2001, **12**:283–288

Parameters of aerobic and anaerobic ammonia oxidation.

Parameter	Nitrification $NH_4^+ + O_2 \rightarrow NO_2^-$	Anammox $NH_4^+ + NO_2^- \rightarrow N_2$	Unit
Free energy	–275	–357	kJ/mol
Biomass yield	0.08	0.07	mol/mol C
Aerobic rate	200–600	0	nmol/min/ mg protein
Anaerobic rate	2	60	nmol/min/ mg protein
Growth rate	0.04	0.003	/h
Doubling time	0.73	10.6	days
K_s NH_4^+	5–2600	5	µM
K_s NO_2^-	N/A	<5	µM
K_s O_2	10–50	N/A	µM

Diagram removed due to copyright restrictions.

Partial nitrification/Anammox® is a new method for nitrogen removal from wastewater. It targets wastewater streams (or gases) high in ammonium (>0.2 g/l) and low in organic carbon (C:N ratio lower than 0.15). The two processes proceed as follows:

(partial nitrification)	$2NH_4^+ + 1.5O_2 = NH_4^+ + NO_2^- + H_2O + 2H^+$
(anammox)	$NH_4^+ + NO_2^- = N_2 + 2H_2O$
(total)	$2NH_4^+ + 1.5O_2 = N_2 + 3H_2O + 2H^+$

(the produced acid is balanced by the counter-ion of ammonium, usually bicarbonate or sulfide)

Compared to conventional nitrification/denitrification, this method saves 100% of the carbon source, & 50% of the required oxygen. This leads to a reduction of operational costs of 90%, a decrease in CO_2 emissions of more than 100% (the process actually consumes CO_2), and a decrease in energy demand.

Photograph removed due to copyright restrictions

[Anaerobic ammonium oxidation by anammox bacteria in the Black Sea](#)

Marcel M. M. Kuypers, A. Olav Sliekers, Gaute Lavik, Markus Schmid, Bo Barker Jørgensen, J. Gijs Kuenen, Jaap S. Sinninghe Damsté, Marc Strous and Mike S. M. Jetten. Nature **422**, *608-611 (10 April 2003)*

Graphs removed due to copyright restrictions.

ANAEROBIC RESPIRATION =

Dumping your electrons on something other than oxygen

Table 2. Physiological groups of bacteria able to grow under anaerobic conditions using external electron acceptors for electron transport ("erobic respiration").

Bacterial group	Typical species	Metabolic process	Electron acceptor	Reduction products(s
Denitrifiers	*Pseudomonas denitrificans*	Nitrate respiration	NO_3^-	N_2, N_2O, NO_2^-
Sulfate reducers	*Desulfovibrio vulgaris*	Sulfate respiration	SO_4^{2-}	S^{2-}
Sulfur reducers	*Desulfuromonas acetoxidans*	Sulfur respiration	S^0	S^{2-}
Methanogenic bacteria	*Methanobacterium thermoautotrophicum*	Carbonate respiration	CO_2	CH_4
Acetogenic bacteria	*Acetobacterium woodii*	Carbonate respiration	CO_2	CH_3—COOH
Succinogenic bacteria	*Wolinella succinogenes*	Fumarate respiration	Fumarate	Succinate
Iron reducers	*Pseudomonas* GS-15	Iron respiration	Fe^{3+}	Fe^{2+}

Denitrification = Use of NO_3^- as terminal electron acceptor, that results in complete conversion to N_2 gas.

Image of geobacter growing on iron oxides removed due to copyright restrictions.

Microbial redox interactions with uranium: an environmental perspective

INTERACTIONS OF MICROORGANISMS WITH RADIONUCLI
Miranda J. Keith-Roach and Francis R. Livens (Editors)

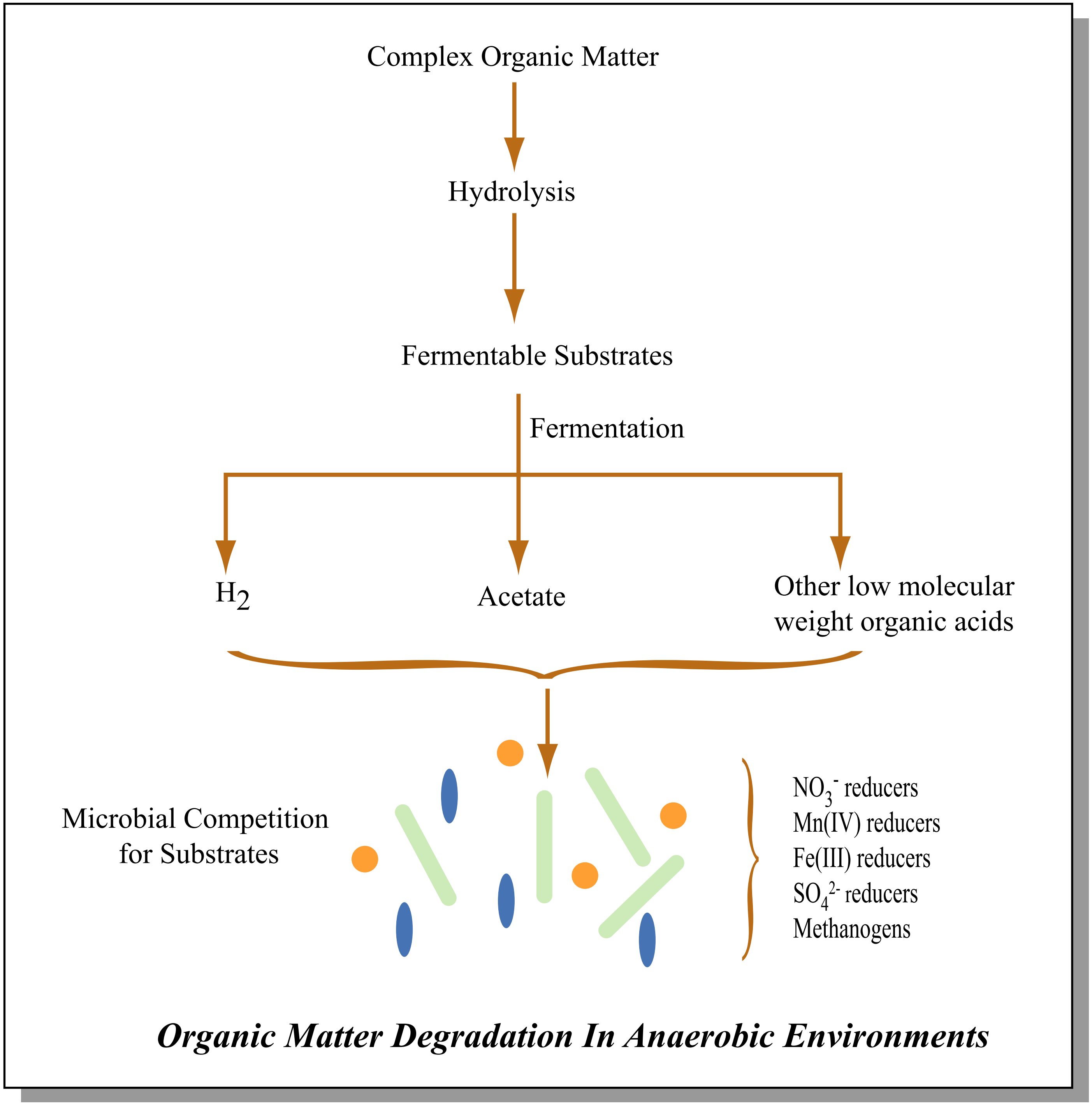


Figure by MIT OCW.

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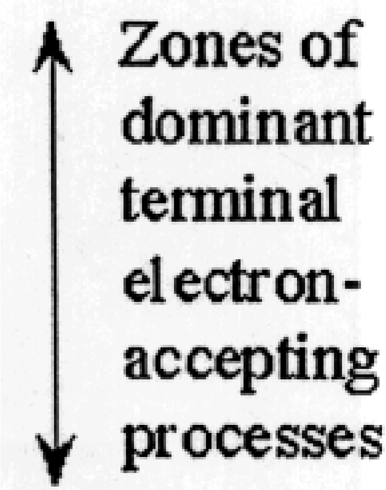
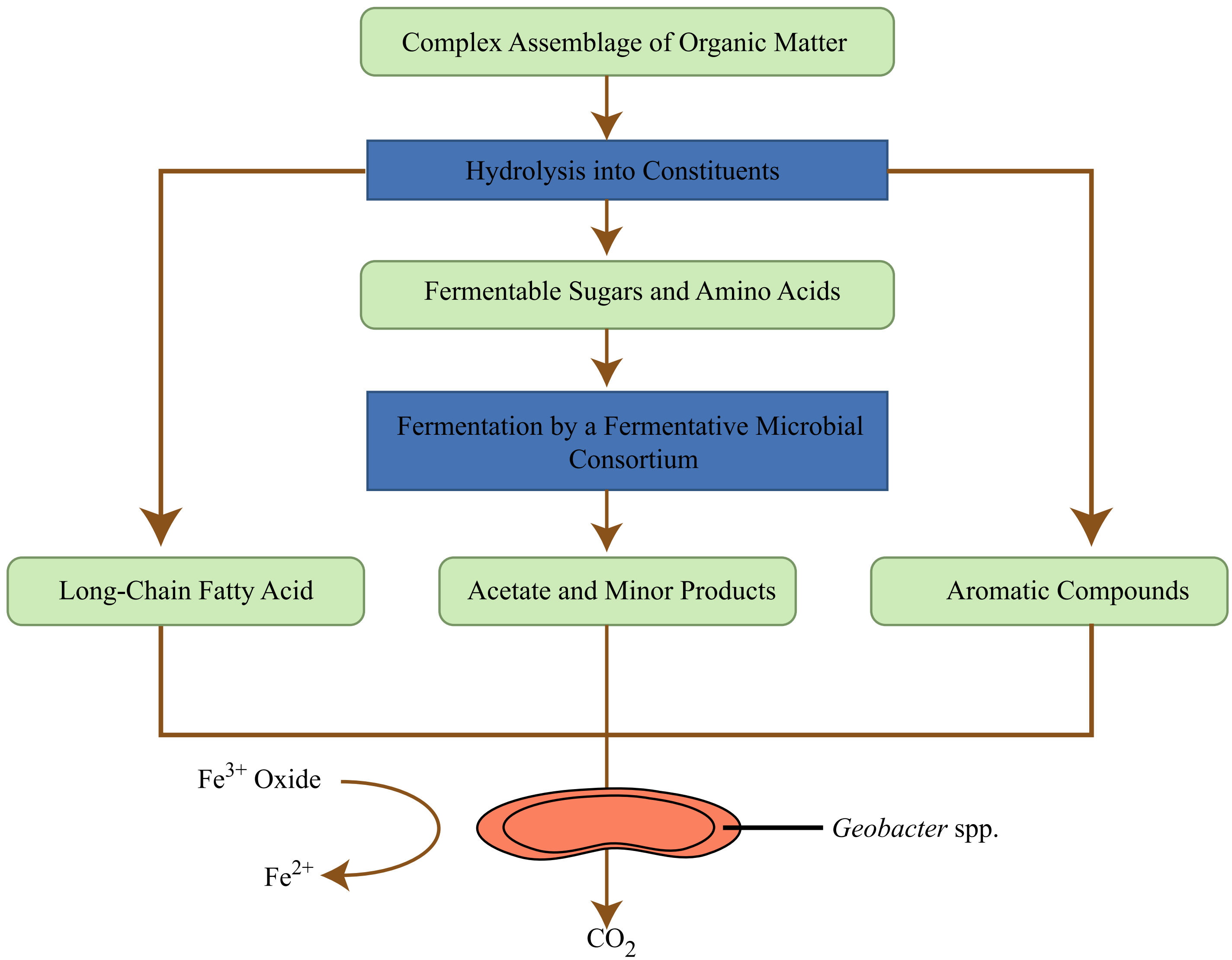


Fig. 1. The distribution of terminal electron-accepting processes (TEAPs) found with depth in aquatic and marine sediments.

Energetic explains order: not all e-acceptors are equal!

E- acceptor	$\Delta G^{o'}$ (using glucose)
Oxygen	-3190 kJ/mol
NO3-	-3030
Mn (IV)	-3090
Fe(III)	-1410
Sulfate	-380
CO2	-350

From Nealson and Saffarini 1994



Generalized pathway for the anaerobic oxidation of organic matter to carbon dioxide with Fe^{3+} oxide serving as an electron acceptor in temperate, freshwater and sedimentary environments. The process is mediated by a consortium of fermentative microorganisms and *Geobacter* species.

Figure by MIT OCW.

Microbial redox interactions with uranium: an environmental perspective

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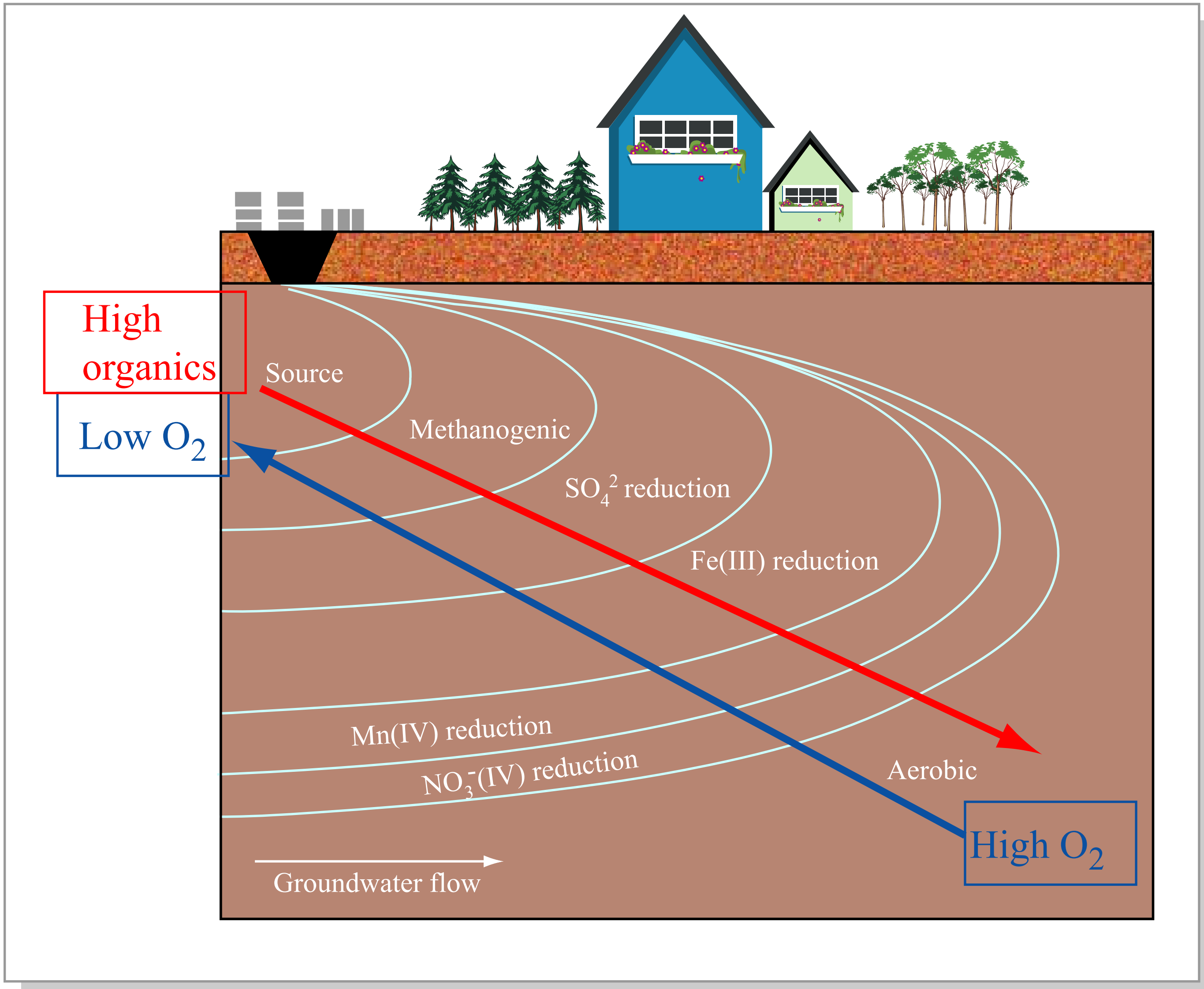

Figure by MIT OCW.

The distribution of terminal electron-accepting processes (TEAPs) observed within anaerobic portions of aquifers contaminated with organic compounds.

Images removed due to copyright restrictions.
See Lovley, D. R., E. J. P. Phillips, Y. A. Gorby, and E. R. Landa. "Microbial Reduction of Uranium." *Nature* 350 (1991): 413-416.

Anaerobic respiration to "clean up" of uranium pollution

Soluble= mobile   Insoluble, immobile

Acetate + U (VI) $\rightarrow$ **U (IV)$_s$** + CO_2

Photograph removed due to copyright restrictions.

CH_3C00- + 4 U(VI) $\rightarrow$ **U (IV)$_s$** + $2HCO_3$- + 9H+

Carried out by *Geobacter*

Example of "bioremediation"

Lovley DR, Phillips EJP, Gorby YA, Landa ER. Microbial Reduction of Uranium, 1991, Nature. 350(6317): 413-6.

Microbial redox interactions with uranium: an environmental perspective

INTERACTIONS OF MICROORGANISMS WITH RADIONUCLI
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n, *Derek R. Lovley*

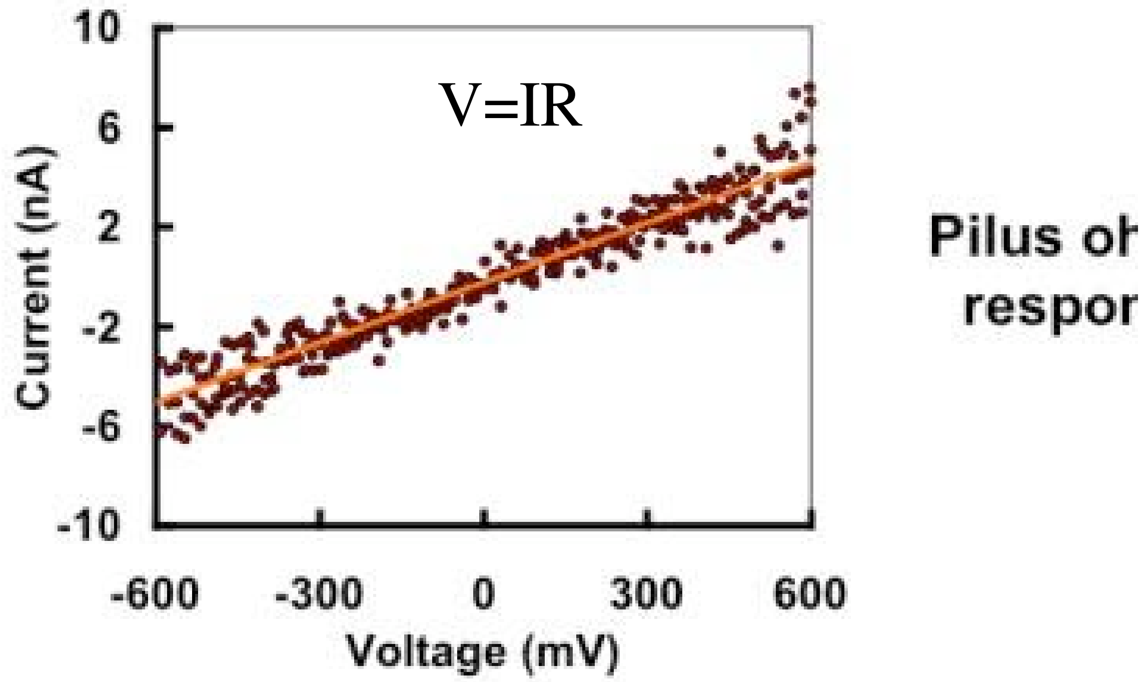
Diagram removed due to copyright restrictions.

Fig. 5. Stimulated U(VI) reduction in aquifers upon the steady addition of low concentrations of a suitable electron donor such as acetate. Fe(III) and U(VI) reduction are stimulated upon the depletion of O_2, NO_3^- and Mn(IV) as electron acceptors.

Diagram removed due to copyright restrictions.
See Bond, Holmes, Tender, and Lovley. *Science* 295 (2002): 483-485.

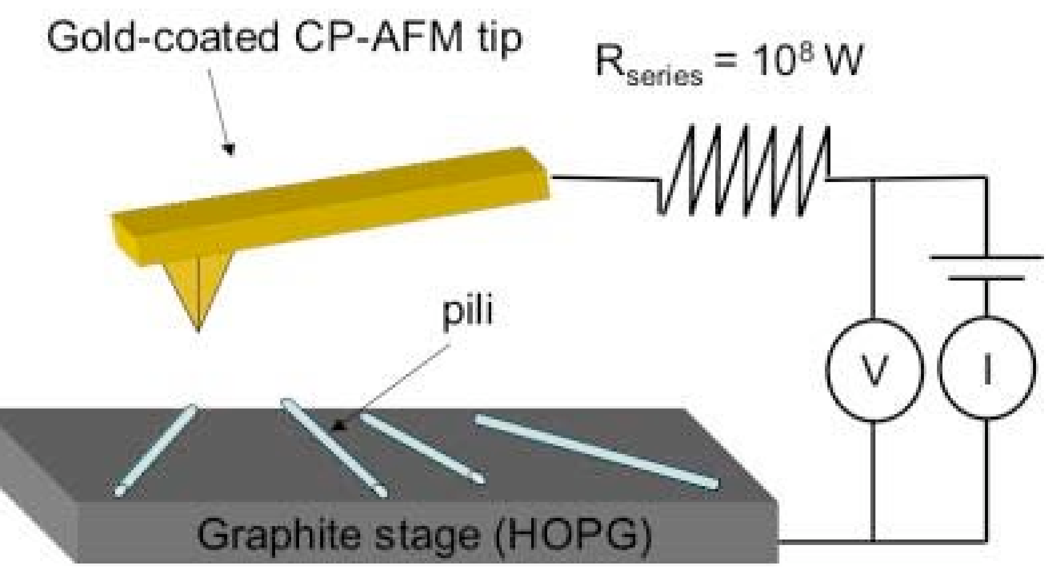
Diagram and photograph of a sediment microbial fuel cell removed due to copyright restrictions.

Figure 3 | **A sediment microbial fuel cell. a** | A schematic of a sediment microbial fuel cell. Organisms in the family Geobacteraceae can oxidize acetate and other fermentation products, and transfer the electrons to graphite electrodes in the sediment. These electrons flow to the cathode in the overlying aerobic water where they react with oxygen. **b** | An actual sediment fuel cell before deployment.



Correspondence between pilus current and applied voltage demonstrating the linear, ohmic, response characteristic of a true conductor.

Atomic force microscope stage



Schematic of the electronic connection of the AFM tip in a conducting probe atomic force microscope (CP-AFM). HOPG, highly oriented pyrolytic graphite.

Extracellular electron transfer via microbial nanowires.

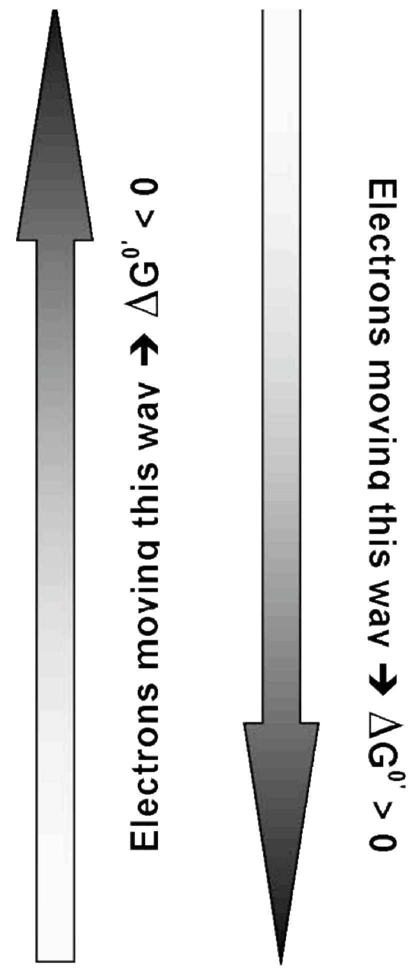
Nature. 2005 Jun 23;435(7045):1098-101.

Table 1. Standard reduction potential (E_0') values (at 25°C and pH 7)

Since e^- are being added to the reactants on the left sides of the equations, these reactions are showing **reduction** reactions.

Half-Reaction			E_o' (V)
_ $O_2 + 2\,H^+ + 2\,e^-$	⇒	H_2O	+0.816
$Fe^{3+} + e^-$	⇒	Fe^{2+}	+0.771
$NO_3^- + 6\,H^+ + 6\,e^-$	⇒	_ $N_2 + 3\,H_2O$	+0.75
$NO_3^- + 2\,H^+ + 2\,e^-$	⇒	$NO_2^- + H_2O$	+0.421
$NO_3^- + 10\,H^+ + 8\,e^-$	⇒	$NH_4^+ + 3\,H_2O$	+0.36
$NO_2^- + 8\,H^+ + 6\,e^-$	⇒	$NH_4^+ + 2\,H_2O$	+0.34
$CH_3OH + 2\,H^+ + 2\,e^-$	⇒	$CH_4 + H_2O$	+0.17
fumarate + 2 H^+ + 2 e^- ⇒	succinate		+0.031
$2\,H^+ + 2\,e^-$	⇒	H_2 (pH 0)	+0.00
oxaloacetate + 2 H^+ + 2 e^-	⇒	malate	-0.166
$CH_2O + 2\,H^+ + 2\,e^-$	⇒	CH_3OH	-0.18
pyruvate + 2 H^+ + 2 e^-	⇒	lactate	-0.185
acetaldehyde + 2 H^+ + 2 e^-	⇒	ethanol	-0.197
$SO_4^{2-} + 8\,H^+ + 6\,e^-$	⇒	$S + 4\,H_2O$	-0.20
$SO_4^{2-} + 10\,H^+ + 8\,e^-$	⇒	$H_2S + 4\,H_2O$	-0.21
FAD + 2 H^+ + 2 e^-	⇒	$FADH_2$	-0.219
$CO_2 + 8\,H^+ + 8\,e^-$	⇒	$CH_4 + 2\,H_2O$	-0.24
$S + 2\,H^+ + 2\,e^-$	⇒	H_2S	-0.243
$N_2 + 8\,H^+ + 6\,e^-$	⇒	$2\,NH_4^+$	-0.28
$NAD^+ + H^+ + 2\,e^-$	⇒	NADH	-0.320
$NADP^+ + H^+ + 2\,e^-$	⇒	NADPH	-0.324
$2\,H^+ + 2\,e^-$	⇒	H_2 (pH 7)	-0.414
$CO_2 + 4\,H^+ + 4\,e^-$	⇒	1/6 glucose + H_2O	-0.43
$Fe^{2+} + 2\,e^-$	⇒	Fe	-0.85

'Good' electron acceptors



'Good' electron donors

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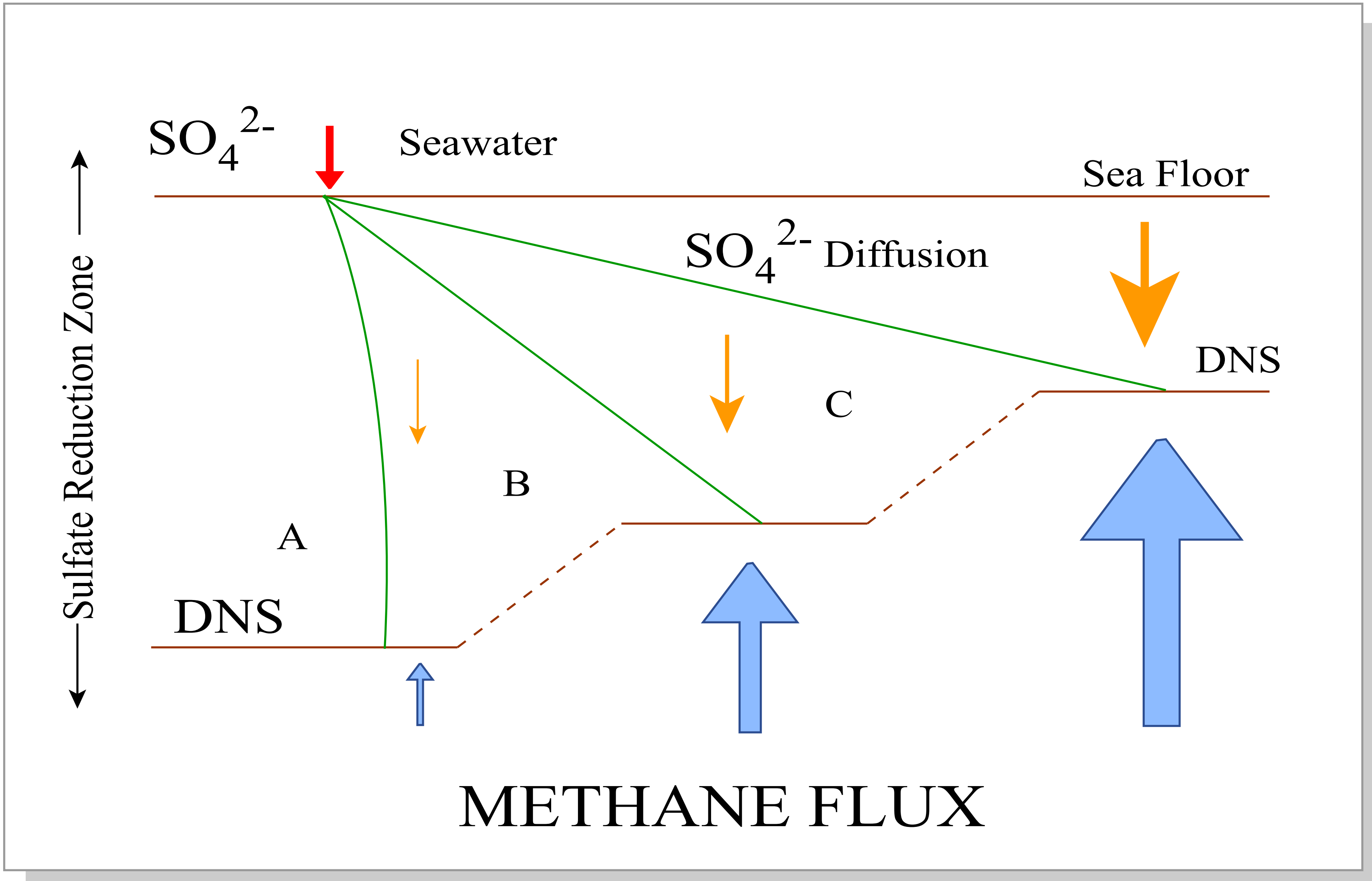

Figure by MIT OCW.

ANAEROBIC METHANE OXIDATION

Geochemical Observations:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$$

Microbiologically ???

$CH_4 + 2H_2O \rightarrow CO_2 + 4H_2$ "Reverse Methanogenesis" $\Delta G_o' = +131$ kJ/mol

$HSO_4^- + 4H_2 \rightarrow HS^- + 4H_2O$ Sulfate reduction $\Delta G_o' = -156$ kJ/mol

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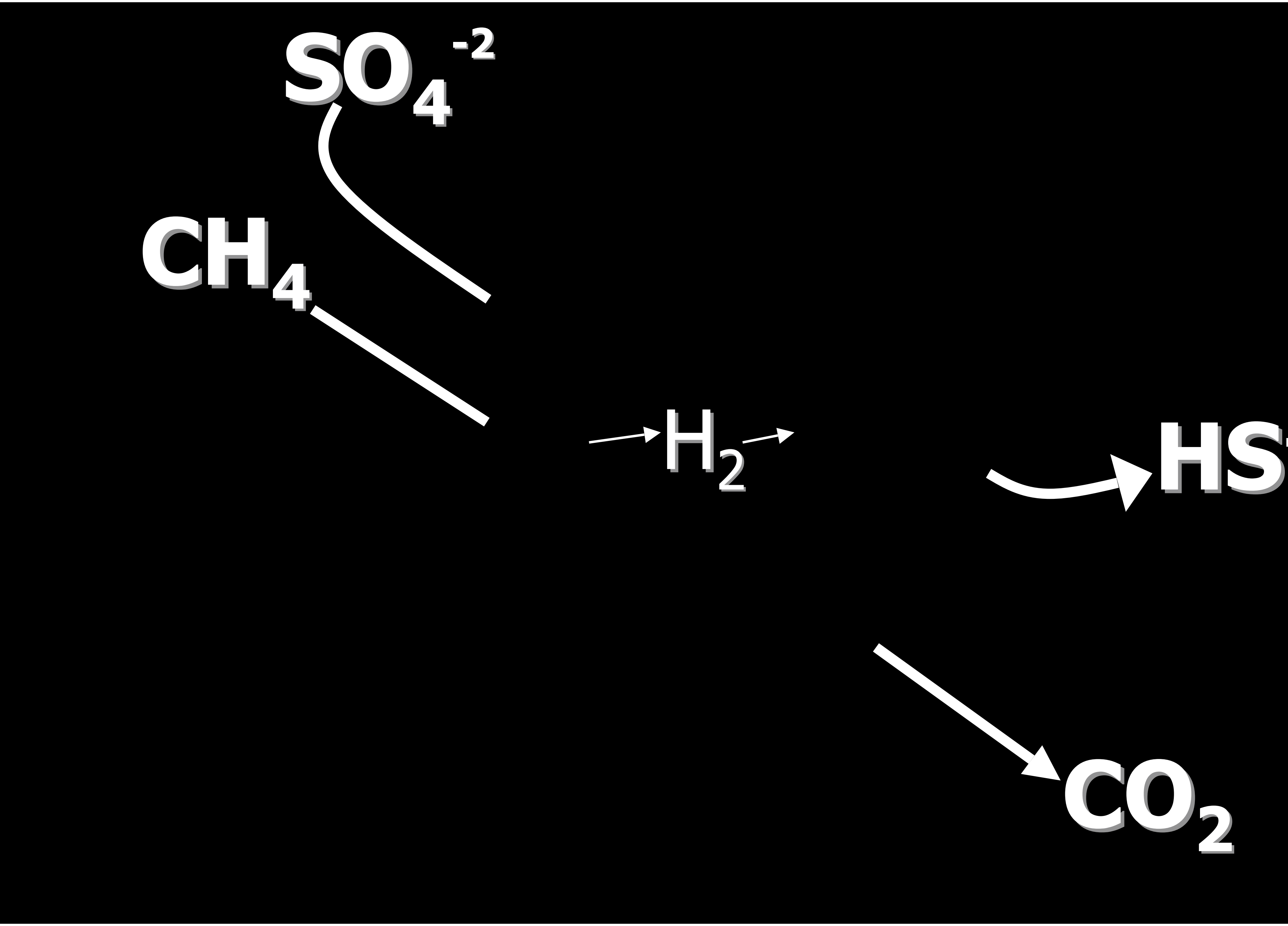
$$CH_4 + HSO_4^{2-} \rightarrow CO_2 + HS^- + 2H_2O$$

$\Delta G_o' = -25$ kJ/mol


SO_4^{-2}
CH_4
H_2
HS^-
CO_2

World maps showing global methane distribution removed due to copyright restrictions. See D'Hondt et al. *Science* 295 (2002): 2067.