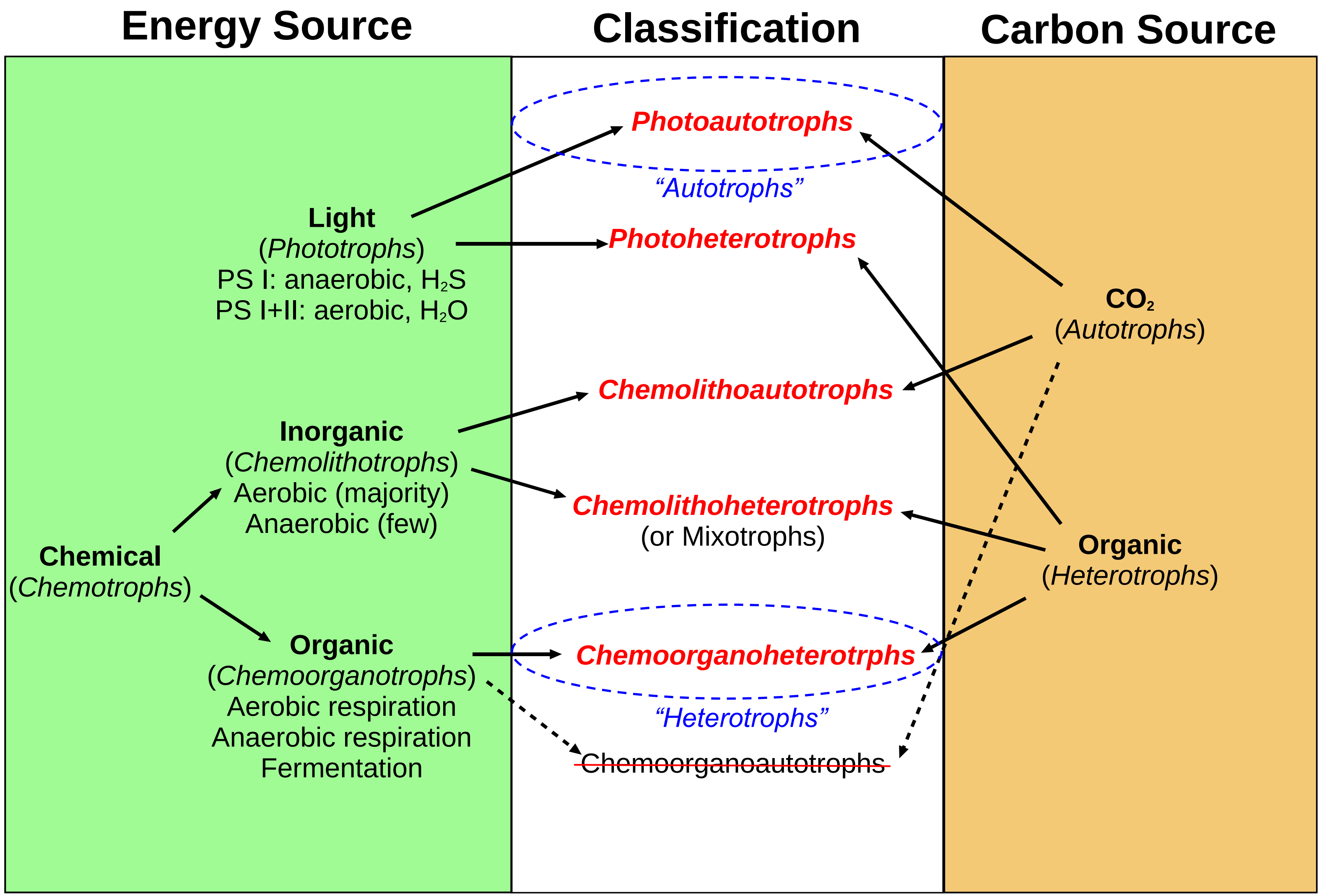
Microbial Biogeochemistry

Chemical reactions occurring in the environment mediated by microbial communities

Outline

- Metabolic Classifications.
- Winogradsky columns, Microenvironments.
- Redox Reactions.
- Microbes and Processes in Winogradsky column.
- Competition and Redox cascade
- Winogradsky column biogeochemistry.
- Lab work

Metabolic Classification of Life



Energy Source

Classification

Carbon Source

Light
(*Phototrophs*)
PS I: anaerobic, H_2S
PS I+II: aerobic, H_2O

Chemical
(*Chemotrophs*)

Inorganic
(*Chemolithotrophs*)
Aerobic (majority)
Anaerobic (few)

Organic
(*Chemoorganotrophs*)
Aerobic respiration
Anaerobic respiration
Fermentation

Photoautotrophs

"Autotrophs"

Photoheterotrophs

Chemolithoautotrophs

Chemolithoheterotrophs
(or Mixotrophs)

Chemoorganoheterotrphs

"Heterotrophs"

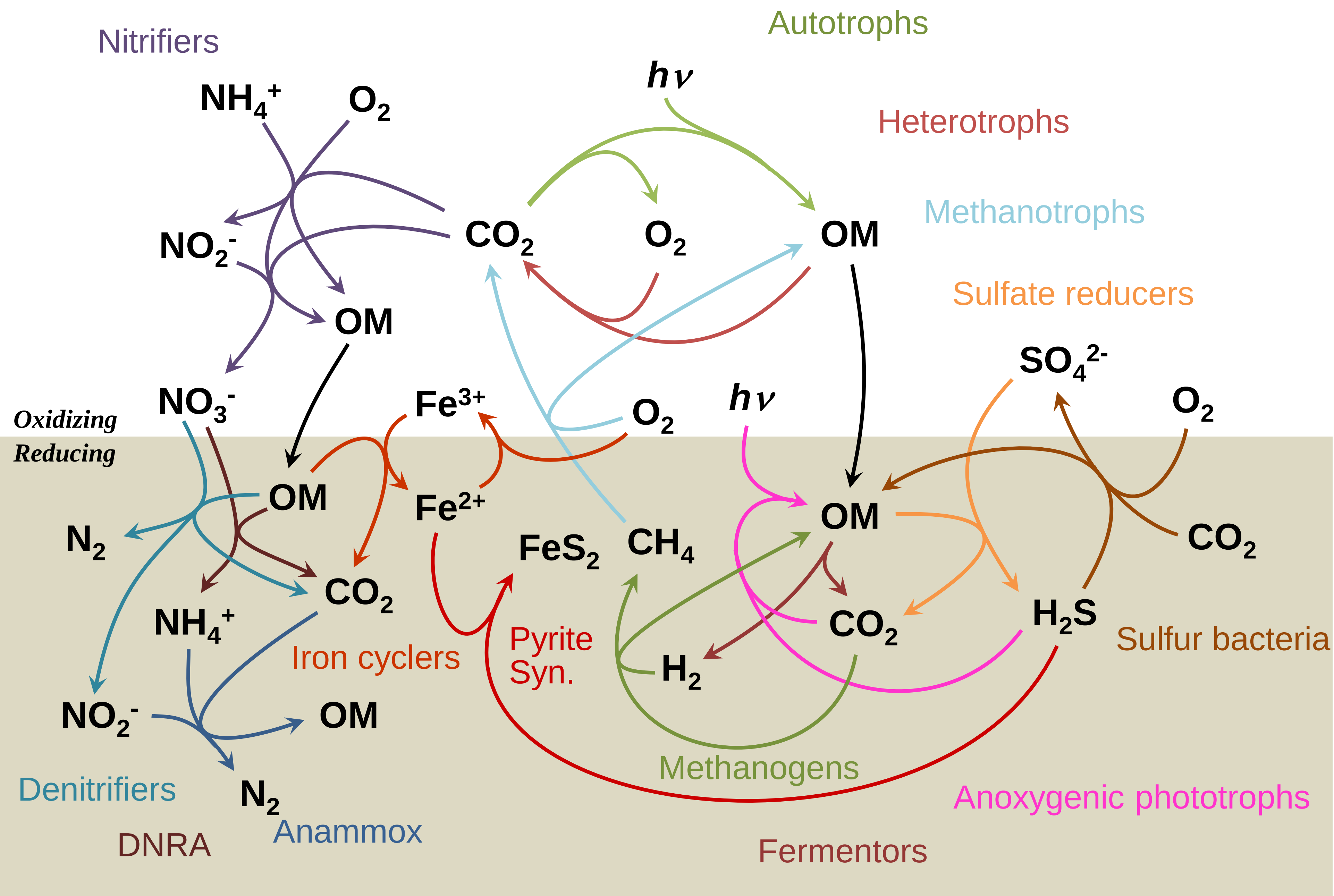
~~Chemoorganoautotrophs~~

CO_2
(*Autotrophs*)

Organic
(*Heterotrophs*)

Note, organisms that exhibit both autotrophy and heterotrophy are also called mixotrophs

Some Microbial Metabolic Redox Reactions



Nitrifiers

Autotrophs

$h\nu$

NH_4^+

O_2

Heterotrophs

NO_2^-

CO_2

O_2

OM

Methanotrophs

OM

Sulfate reducers

NO_3^-

SO_4^{2-}

Oxidizing

Reducing

Fe^{3+}

O_2

$h\nu$

O_2

OM

Fe^{2+}

OM

N_2

FeS_2

CH_4

CO_2

CO_2

NH_4^+

CO_2

H_2S

Sulfur bacteria

Iron cyclers

Pyrite Syn.

H_2

NO_2^-

OM

Denitrifiers

N_2

Methanogens

Anoxygenic phototrophs

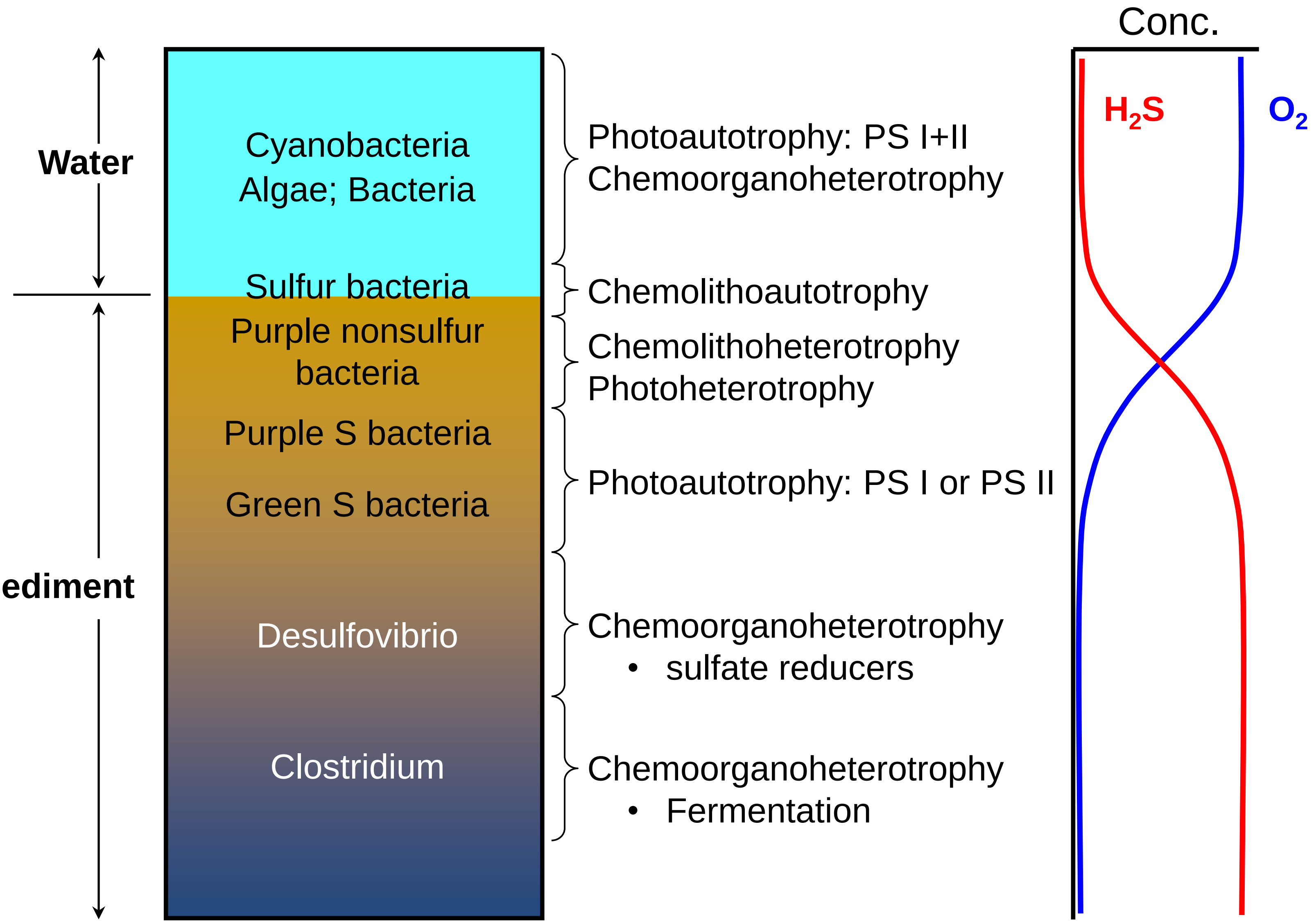
Anammox

DNRA

Fermentors

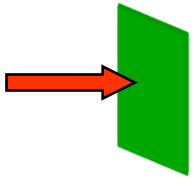
Winogradsky Column

Microenvironments generated by chemical gradients.



Transport Limitations; Advection

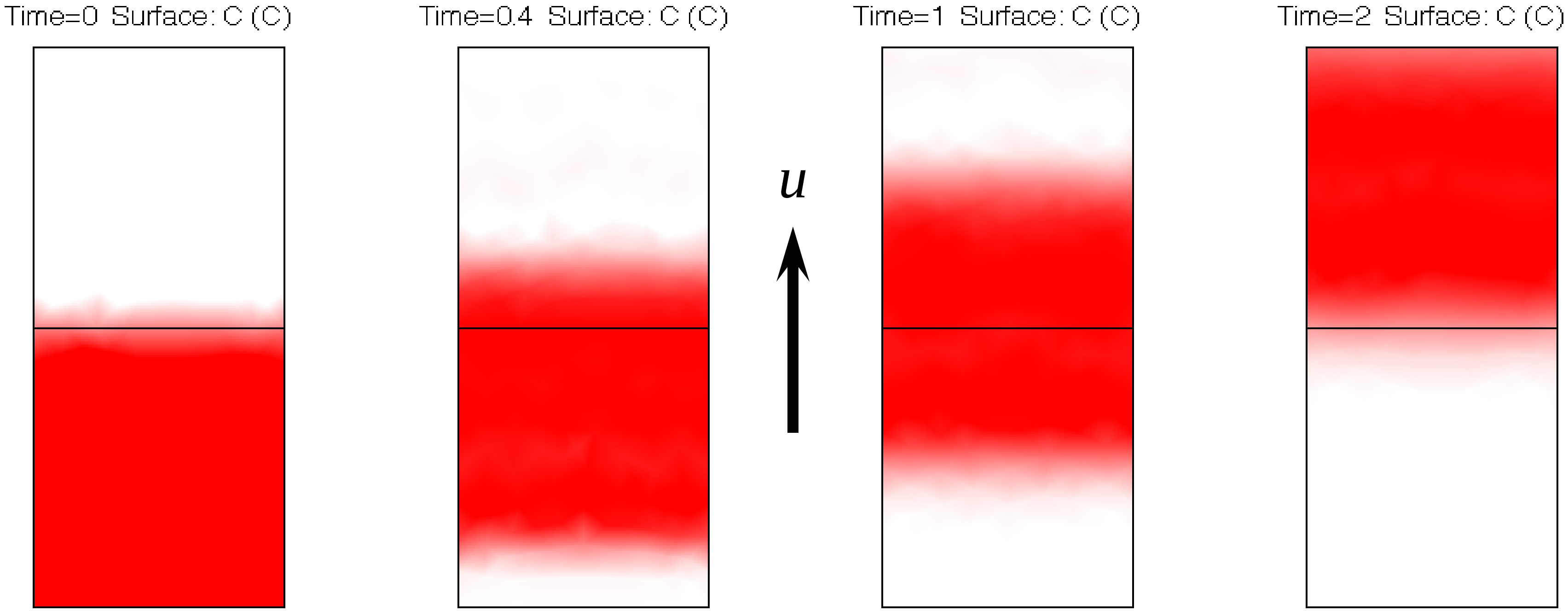
Advective transport:



$$Flux = uC \equiv \left[\frac{g}{m^2 s}\right]$$

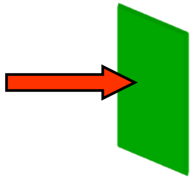
u: Fluid velocity [m s^{-1}]

$$\frac{\partial C}{\partial t} = -\frac{\partial}{\partial z}(uC)$$



Transport Limitations; Diffusion

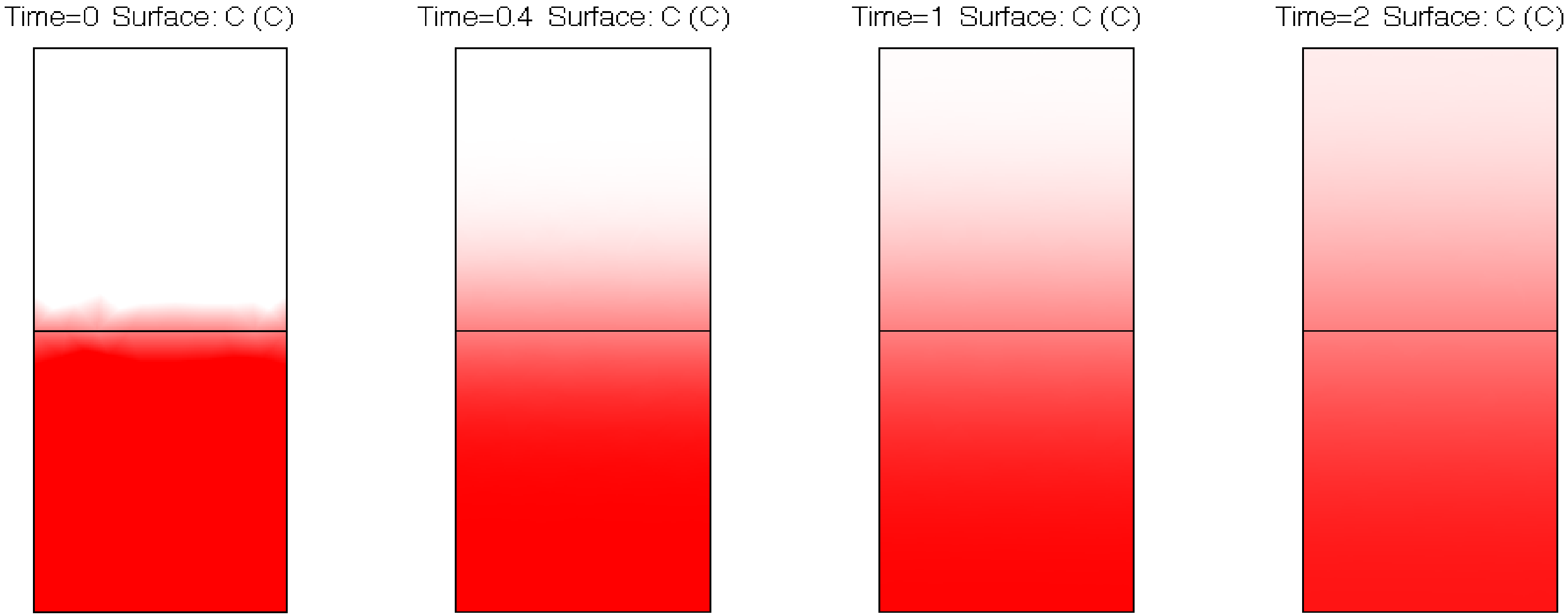
Fickian Diffusion:



$$Flux = -D\frac{dC}{dz} \equiv \left[\frac{g}{m^2 s}\right]$$

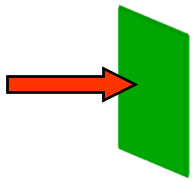
D: Diffusion Coefficient [$m^2\ s^{-1}$]

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial z}\left(D\frac{\partial C}{\partial z}\right)$$



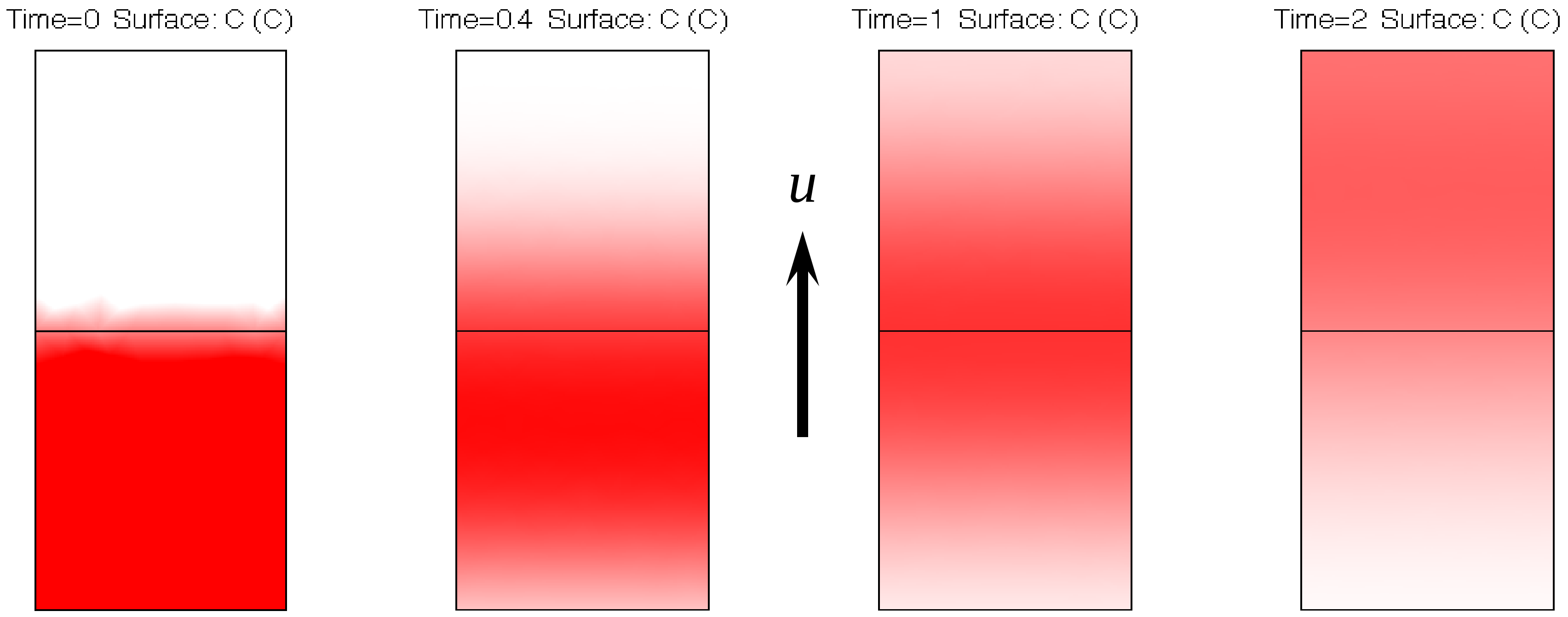
Transport Limitations; Advection-Diffusion

Transport by advection and diffusion:



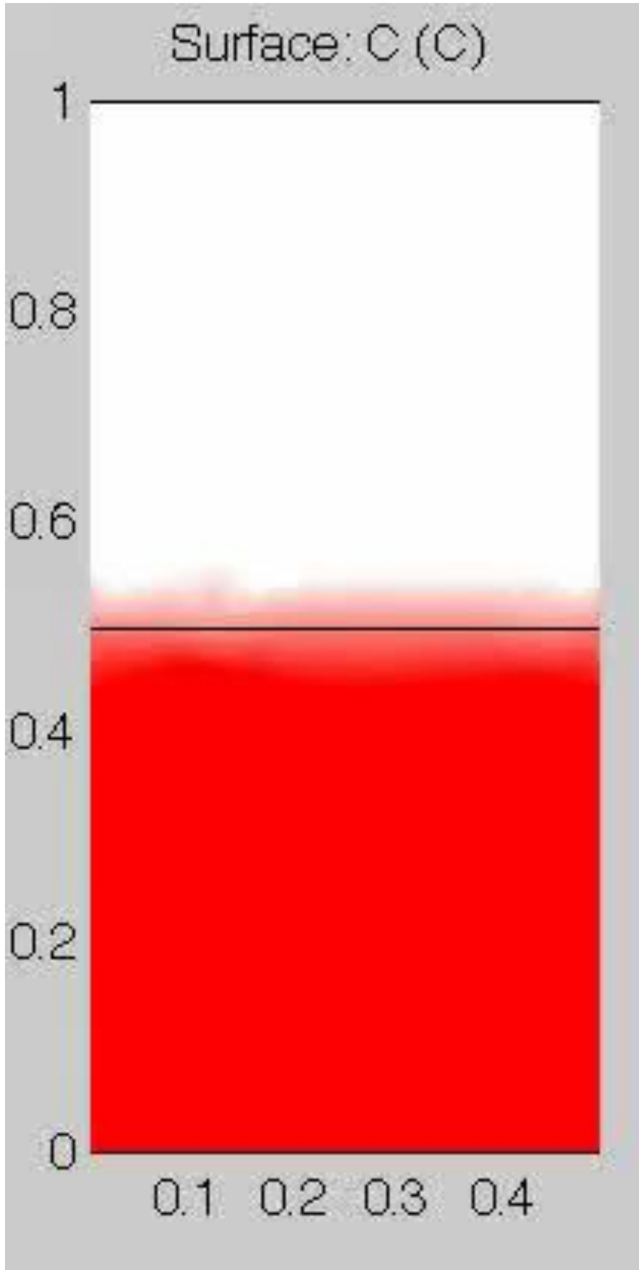
$$Flux = -D\frac{dC}{dz} + uC \equiv \left[\frac{g}{m^2 s}\right]$$

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial z}\left(D\frac{\partial C}{\partial z} - uC\right)$$

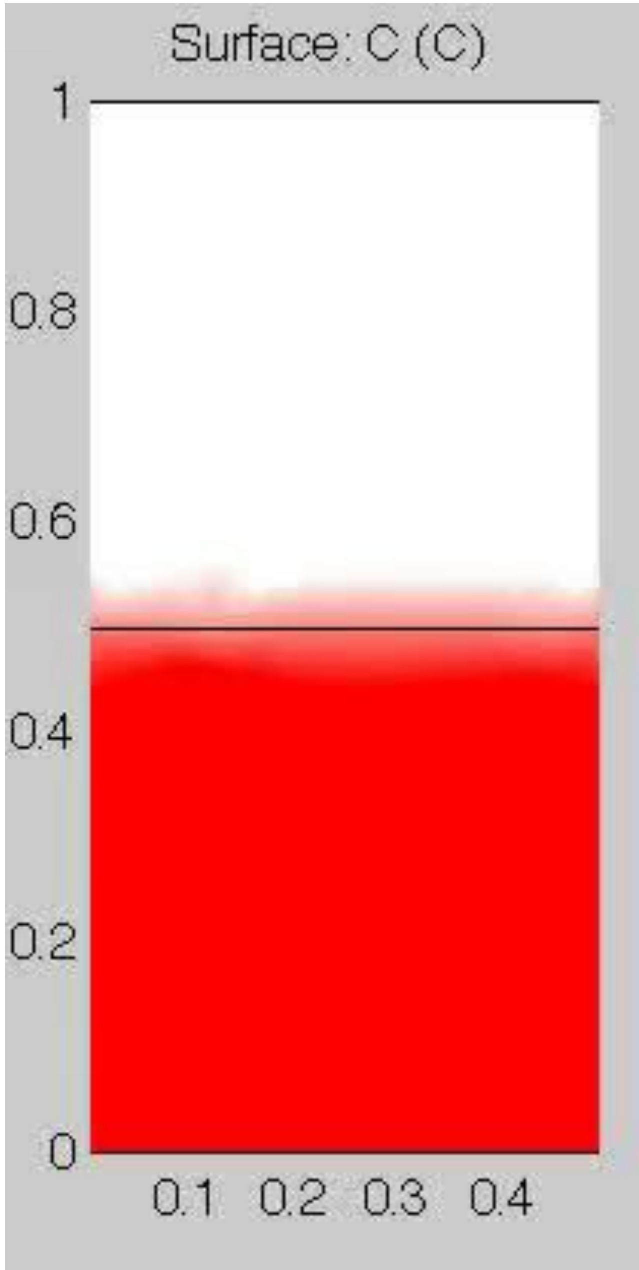


Simulations

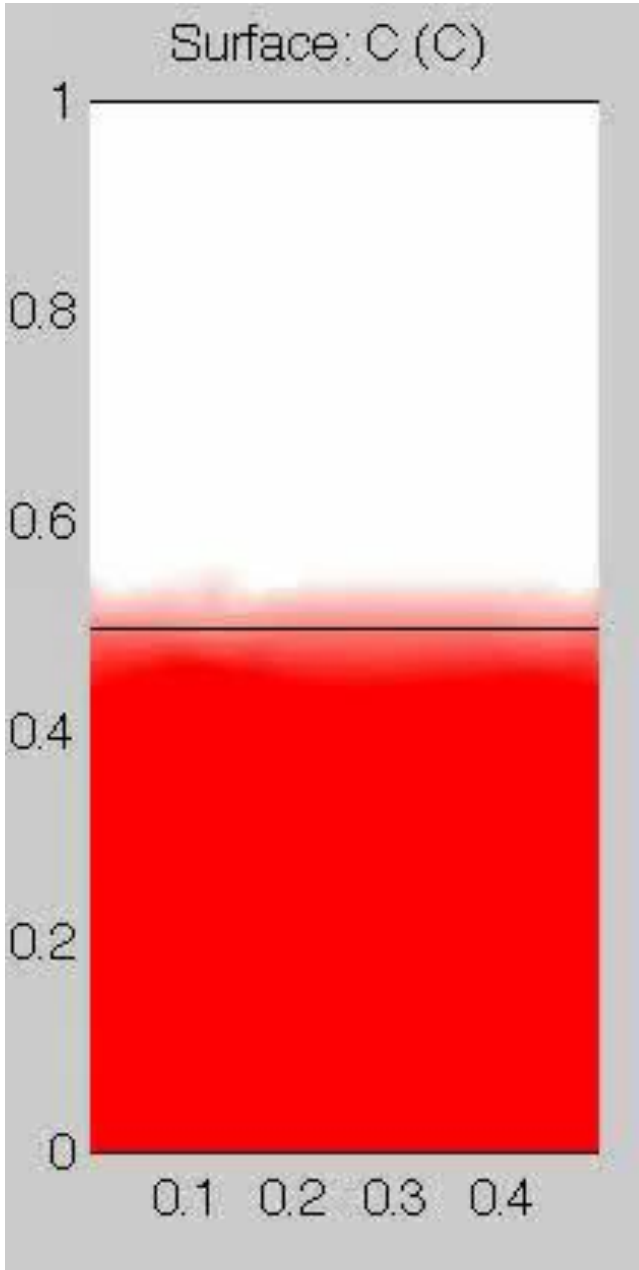
Advection



Diffusion

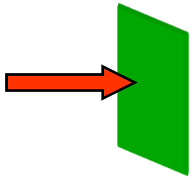


Advection & Diffusion



Transport Limitations; Advection-Diffusion-Reaction

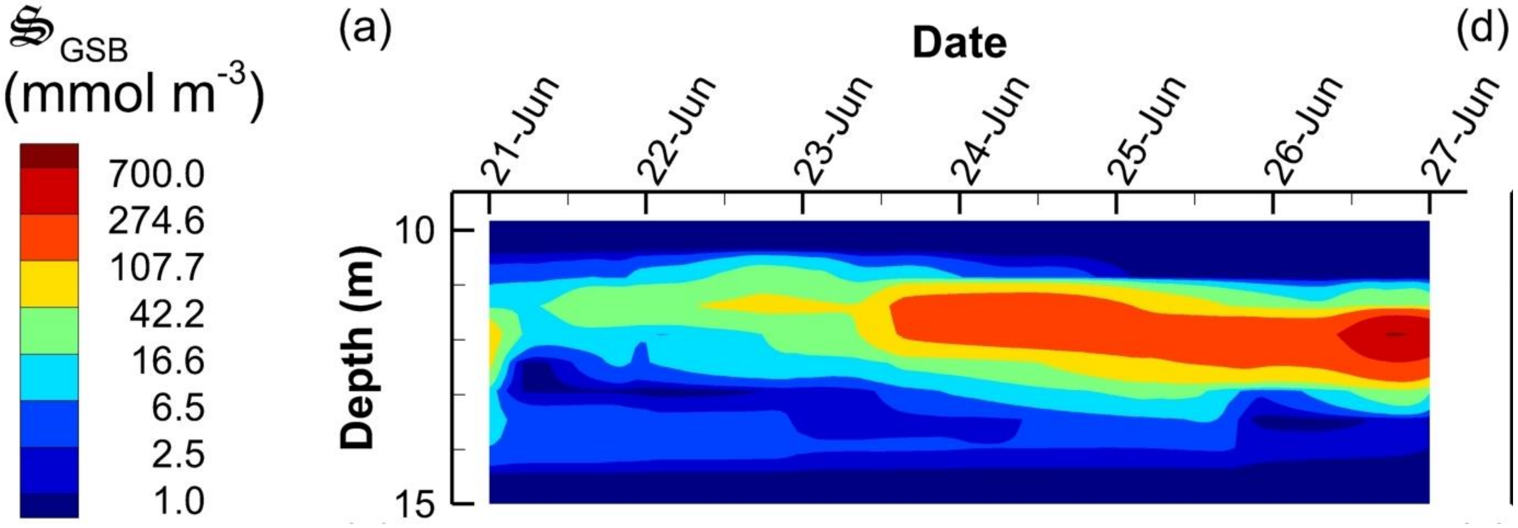
Transport by advection and diffusion:



$$Flux = -D\frac{dC}{dz} + uC \equiv \left[\frac{g}{m^2 s}\right]$$

Must also account for reactions!

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial z}\left(D\frac{\partial C}{\partial z} - uC\right) + r$$



(see Vallino & Huber 2018)

Redox Reactions

Reduction and Oxidation:

Half Reactions

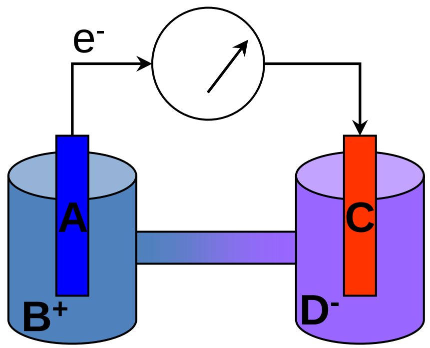
$A \rightarrow B^+ + e^-$ Oxidation

$C + e^- \rightarrow D^-$ Reduction

Complete Reaction

$A + C \rightarrow B^+ + D^-$

Redox Potential, Eº

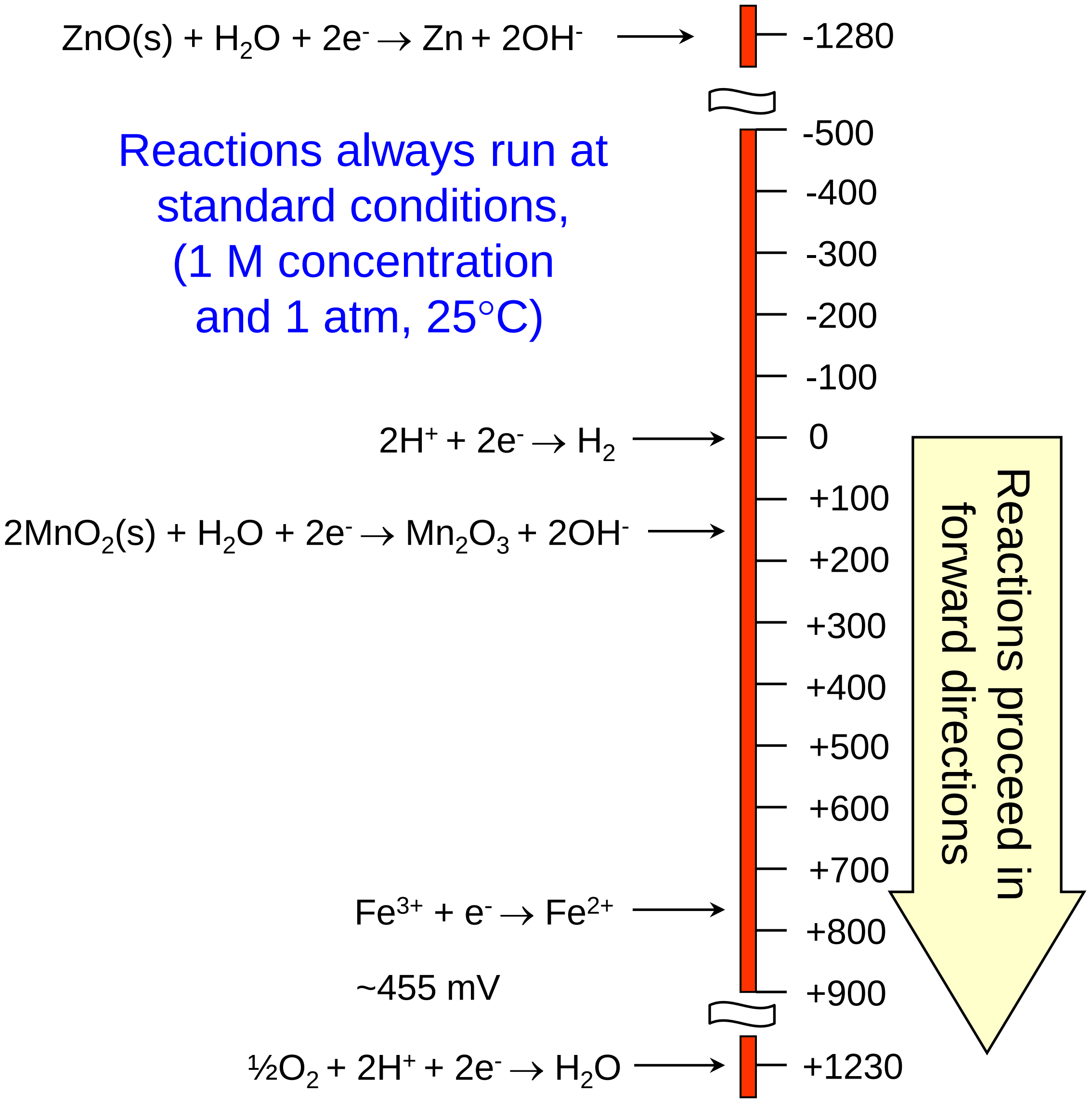


Reference Half Reaction:

$H_2(g) \rightarrow 2e^- + 2H^+$

Reference cell always at pH 0

Electron Tower (at pH 0) Eº (mV)



$ZnO(s) + H_2O + 2e^- \rightarrow Zn + 2OH^-$

Reactions always run at standard conditions, (1 M concentration and 1 atm, 25°C)

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

$2MnO_2(s) + H_2O + 2e^- \rightarrow Mn_2O_3 + 2OH^-$

$Fe^{3+} + e^- \rightarrow Fe^{2+}$

~455 mV

$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$

Alkaline Battery: $Zn(s) + 2MnO_2(s) \rightarrow ZnO(s) + Mn_2O_3(s)$: E° = 1.43 V

Redox Reactions-2

$$E_h = E^\circ - \frac{RT}{nF}\ln\frac{\prod_i[\text{Products}]_i^{\beta_i}}{\prod_j[\text{Reactents}]_j^{\alpha_j}}$$

$$E^{\circ\prime} = E^\circ - \frac{2.303RT}{nF}m\,\text{pH}$$

F = faraday (96493 Coulombs/mol)
R = gas const (8.314 J/K/mol)

m = no. of H^+ consumed in ½ rxn
n = no. of electrons in rxn.

Volt = J/(A·s)=J/C; C≡Coulomb

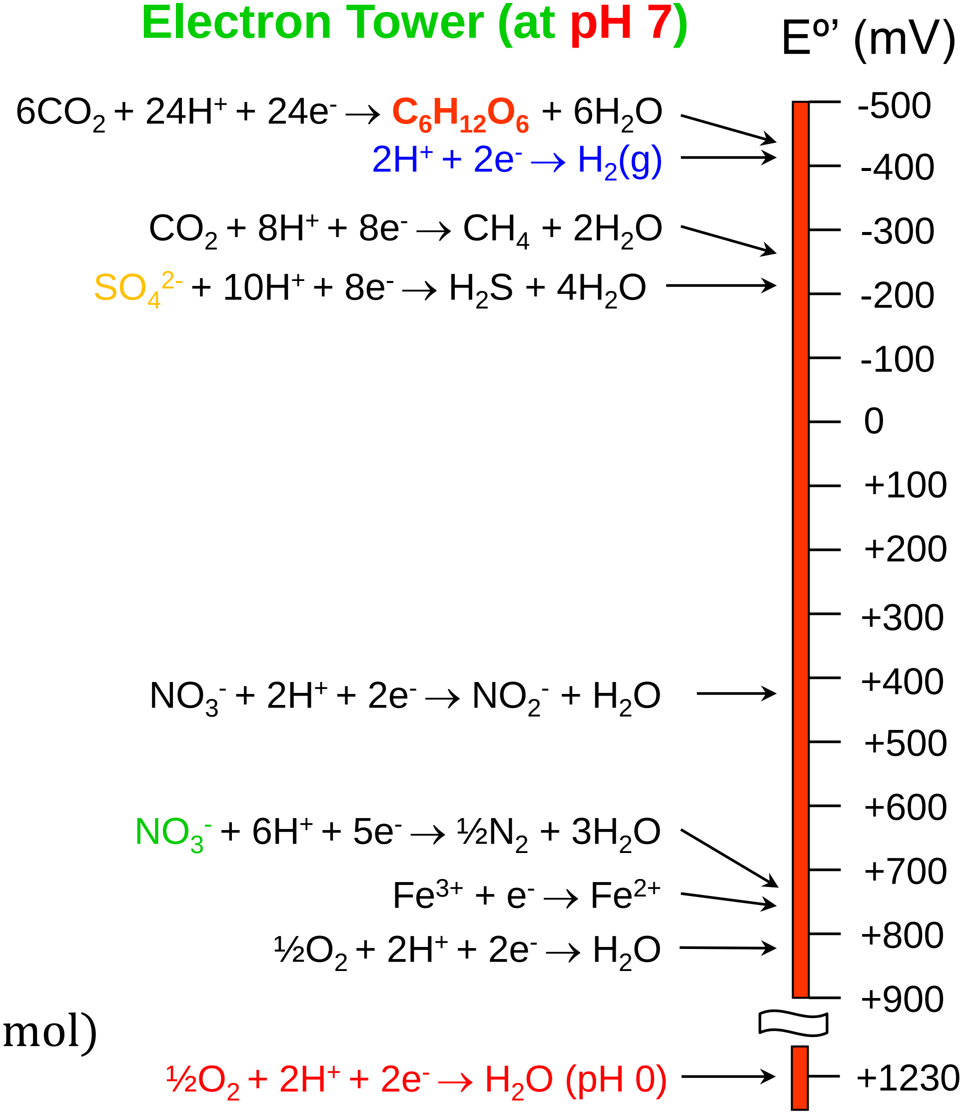
$$\Delta G^{\circ\prime} = -nE^{\circ\prime}F \text{ Gibbs Free Energy (kJ/mol)}$$

Reference Half Reaction:

$H_2(g) \rightarrow 2e^- + 2H^+$

Reference cell at pH 0

Electron Tower (at pH 7)



$6CO_2 + 24H^+ + 24e^- \rightarrow C_6H_{12}O_6 + 6H_2O$

$2H^+ + 2e^- \rightarrow H_2(g)$

$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$

$SO_4^{2-} + 10H^+ + 8e^- \rightarrow H_2S + 4H_2O$

$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$

$NO_3^- + 6H^+ + 5e^- \rightarrow \frac{1}{2}N_2 + 3H_2O$

$Fe^{3+} + e^- \rightarrow Fe^{2+}$

$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$

$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_2O$ (pH 0)

Note, the number of electrons transferred does NOT change the potential or voltage

Oxidation States and Fermentation

Oxidation states

- Some (many) elements have more than one stable electron configuration.
- Consequently, an element can exist in reduced or oxidized states; e.g., Fe^{3+} or Fe^{2+}.

Carbon, Nitrogen and Sulfur have several (assume H: +1; O: -2)

CH_4	-4	N_2	0	NH_3	-3	$S_2O_3^{2-}$	+2
CO_2	+4	NO_3^-	+5	H_2S	-2	SO_4^{2-}	+6

Fermentation and/or Disproportionation

- Organic carbon present, but no electron acceptors: O_2, NO_3^-, SO_2^{2-}, etc.
- Use organic carbon as both electron acceptor and donor:

$$C_6H_{12}O_6 \rightarrow 2\ CO_2 + 2\ C_2H_6O \searrow \left\{\begin{array}{l} 2/3C_6H_{12}O_6 + 8H^+ + 8e^- \rightarrow 2\ C_2H_6O + 2H_2O \\ 1/3C_6H_{12}O_6 + 2H_2O \rightarrow 2\ CO_2 + 8H^+ + 8e^- \end{array}\right.$$

$$4\ S + 4\ H_2O \rightarrow 3\ H_2S + SO_4^{2-} + 2\ H^+$$

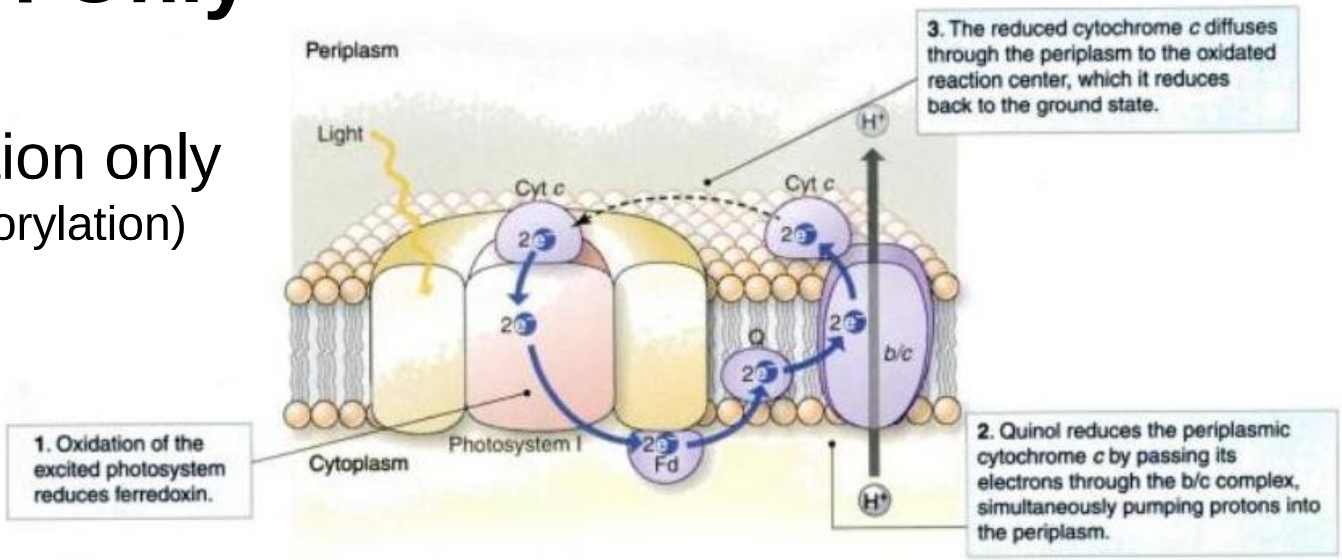
Autotrophy

$$6CO_2 + 24H^+ + 24e^- \rightarrow C_6H_{12}O_6 + 6H_2O$$

$H_2S \rightarrow 2\ H^+ + S + 2\ e^-$	PS I or PS II	Anoxygenic Photosynthesis
$H_2O \rightarrow 2\ H^+ + ½\ O_2 + 2\ e^-$	PS I and PS II	Oxygenic Photosynthesis

Photosystem I Only

Energy production only
(cyclic photophosphorylation)



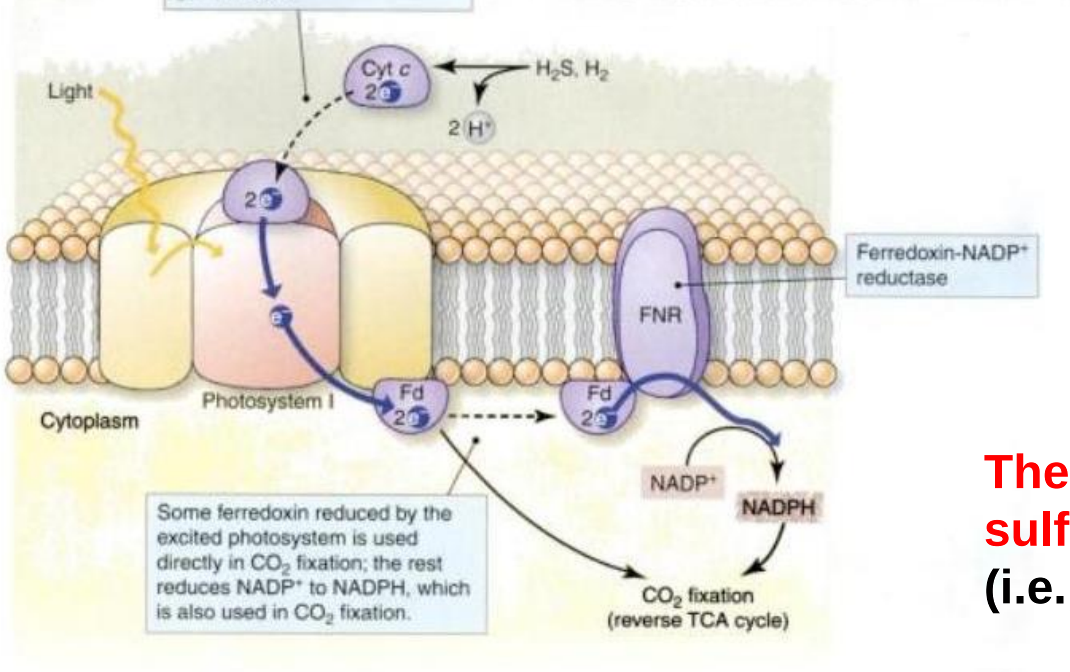
3. The reduced cytochrome *c* diffuses through the periplasm to the oxidated reaction center, which it reduces back to the ground state.

1. Oxidation of the excited photosystem reduces ferredoxin.

2. Quinol reduces the periplasmic cytochrome *c* by passing its electrons through the b/c complex, simultaneously pumping protons into the periplasm.

FIGURE 8.15 Cyclic photophosphorylation in type I photosynthesis.

The reduced cytochrome *c* diffuses through the periplasm to the oxidized reaction center, which it reduces back to the ground state.



Ferredoxin-NADP⁺ reductase

Some ferredoxin reduced by the excited photosystem is used directly in CO_2 fixation; the rest reduces NADP⁺ to NADPH, which is also used in CO_2 fixation.

FIGURE 8.16 Noncyclic photophosphorylation in type I photosynthesis.

NADPH production only needed to reduce CO_2

These occur in the green and purple sulfur bacteria
(i.e., your Winogradsky columns)

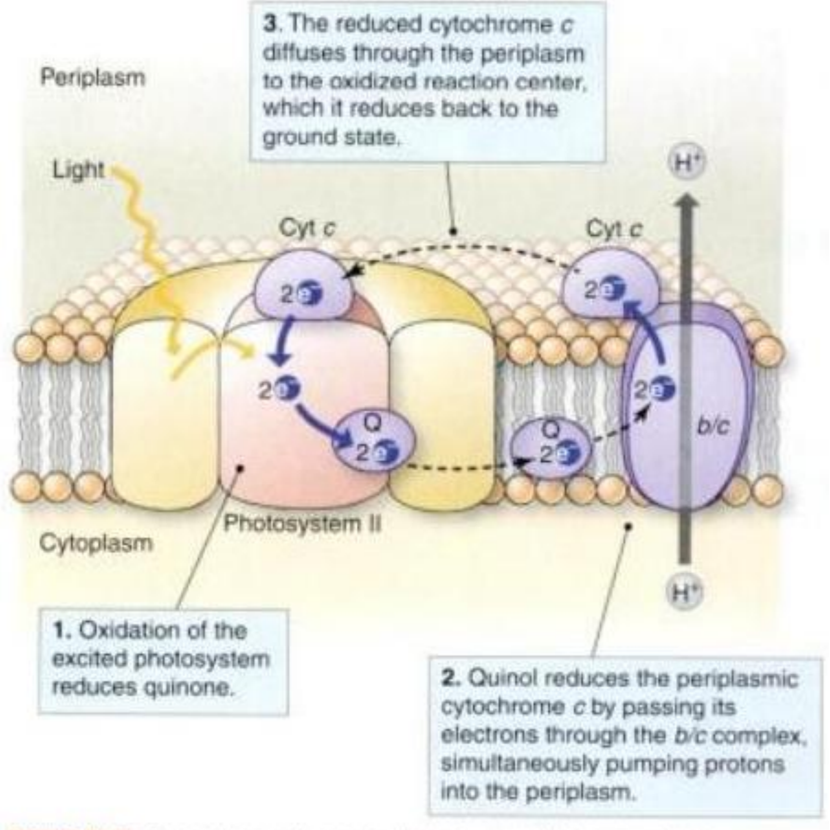
(Principles of Modern Microbiology, M. Wheelis)

Photosystem II Only

These occur in the green and purple **non**-sulfur bacteria

Energy production only

(cyclic photophosphorylation)

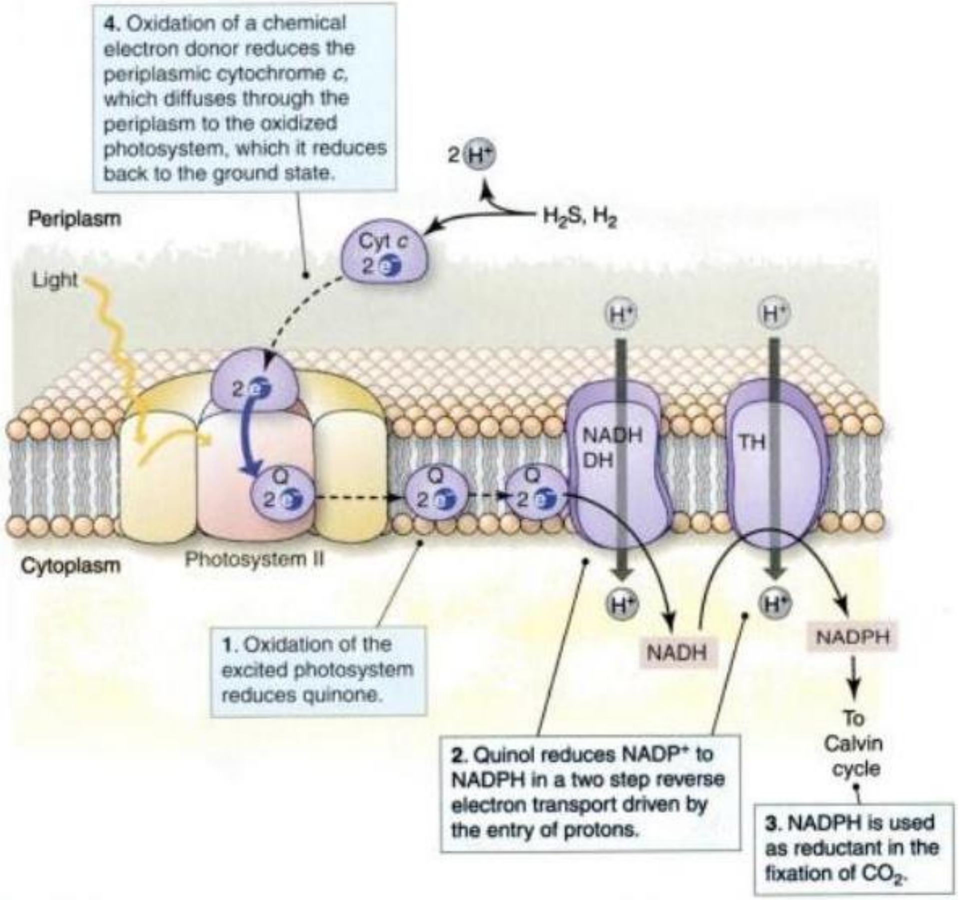


3. The reduced cytochrome *c* diffuses through the periplasm to the oxidized reaction center, which it reduces back to the ground state.

1. Oxidation of the excited photosystem reduces quinone.

2. Quinol reduces the periplasmic cytochrome *c* by passing its electrons through the *b/c* complex, simultaneously pumping protons into the periplasm.

FIGURE 8.17 Cyclic photophosphorylation in type II photosynthesis.



4. Oxidation of a chemical electron donor reduces the periplasmic cytochrome *c*, which diffuses through the periplasm to the oxidized photosystem, which it reduces back to the ground state.

1. Oxidation of the excited photosystem reduces quinone.

2. Quinol reduces NADP⁺ to NADPH in a two step reverse electron transport driven by the entry of protons.

3. NADPH is used as reductant in the fixation of CO_2.

FIGURE 8.18 Noncyclic photophosphorylation in type II photosynthesis.

NADPH production only needed to reduce CO_2

(Principles of Modern Microbiology, M. Wheelis)

Photosystem I+II

These occur in the cyanobacteria,
algae and plants.

Energy production only

(cyclic photophosphorylation)

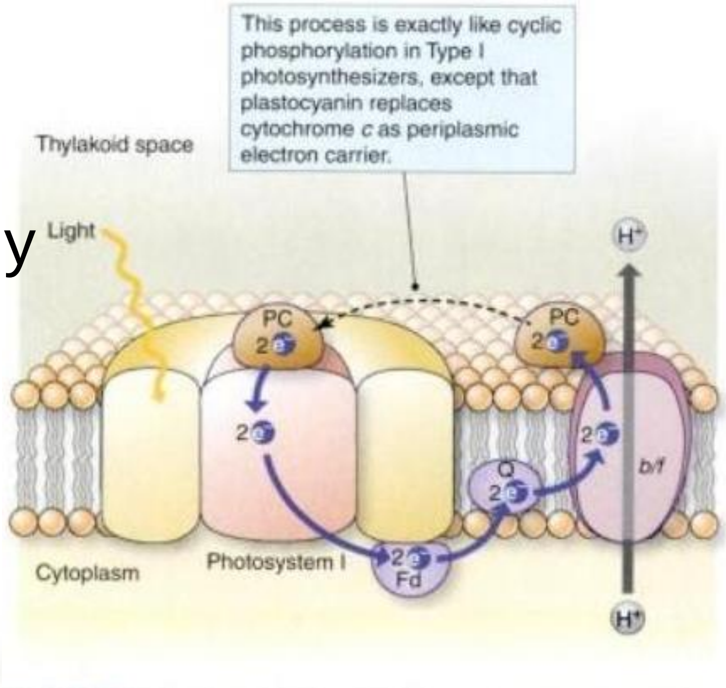
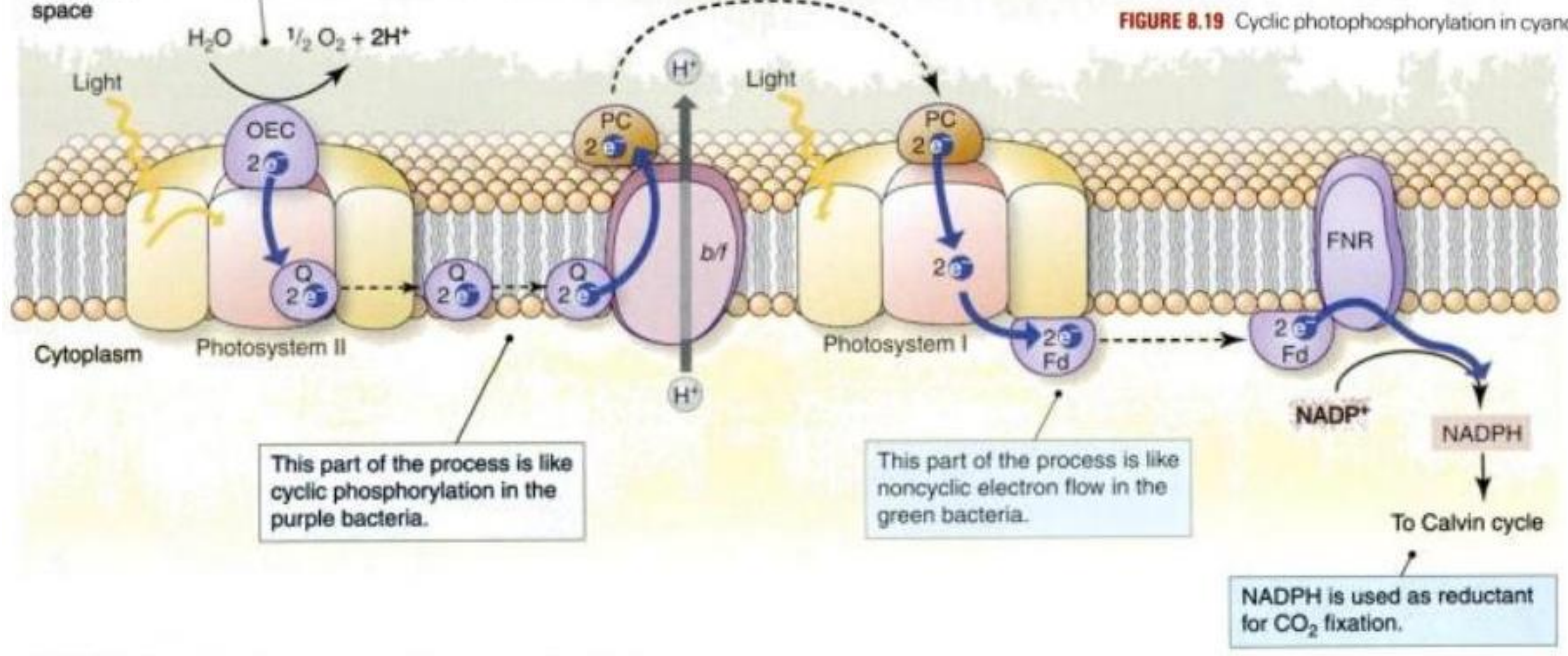

This process is exactly like cyclic phosphorylation in Type I photosynthesizers, except that plastocyanin replaces cytochrome *c* as periplasmic electron carrier.

FIGURE 8.19 Cyclic photophosphorylation in cyanobacterial photosynthesis.

NADPH production only needed to reduce CO_2

The oxygen-evolving complex allows H_2O to be used as reductant for the oxidized photosystem.



This part of the process is like cyclic phosphorylation in the purple bacteria.

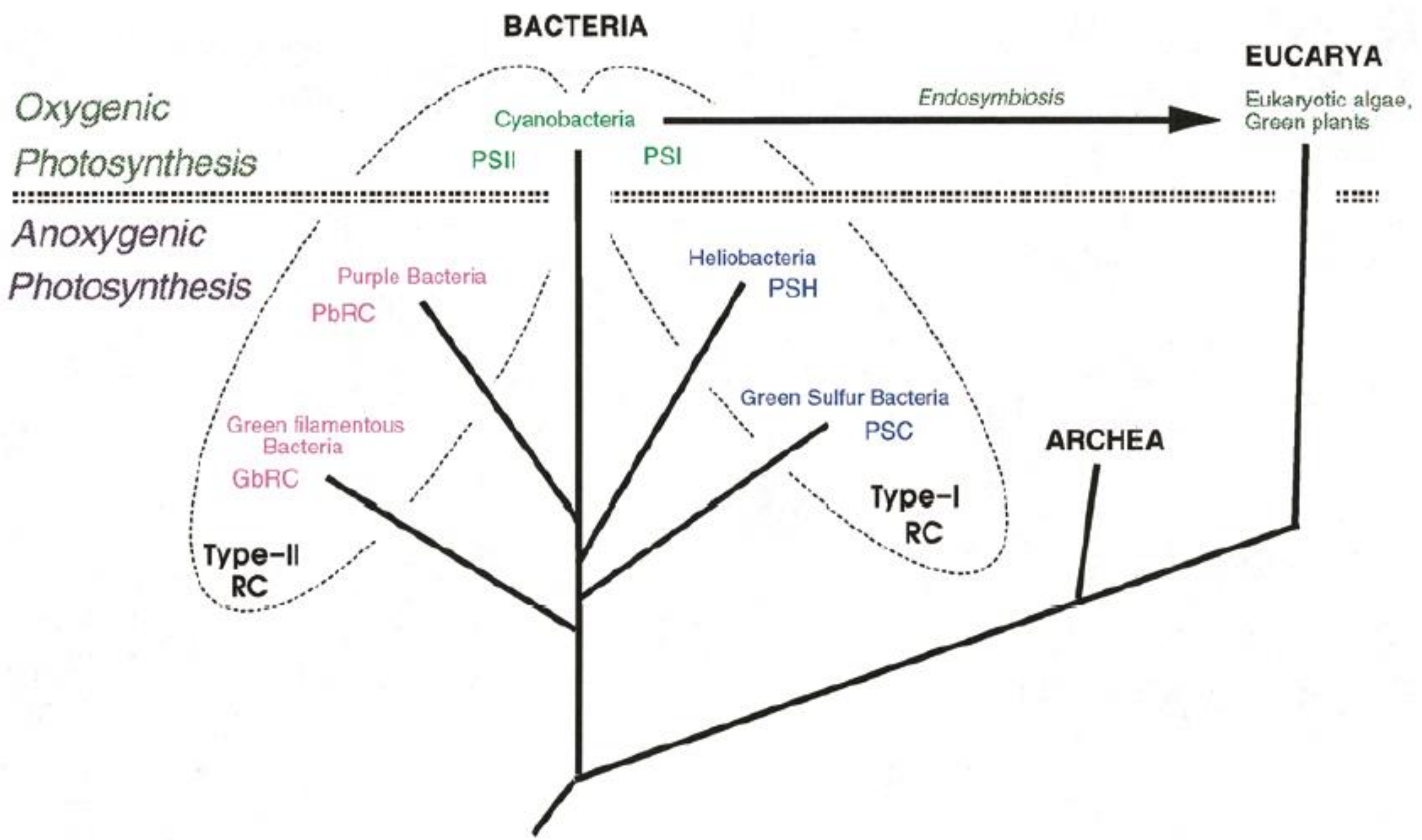
This part of the process is like noncyclic electron flow in the green bacteria.

NADPH is used as reductant for CO_2 fixation.

FIGURE 8.20 Noncyclic photophosphorylation in cyanobacteria.

(Principles of Modern Microbiology, M. Wheelis)

Phylogeny of PS I and II



Schubert et al. (1998) J. Mol. Biol. 280, 297-314

Microbes and Processes in Winogradsky column.

Aerobic Environment

- Algae and cyanobacteria (photoautotrophy using PS II)
- Bacteria and eukaryotes respiring (chemoorganoheterotrophy).
- Sulfide oxidizers (or sulfur bacteria): $H_2S + O_2 \rightarrow S$ or SO_4^{2-}
  - Some use CO_2 (chemolithoautotrophs), others use organic compounds (chemolithoheterotrophs)
  - Examples, *Thiobacillus* sp. And *Beggiatoa* sp.
- Methanotrophs: $CH_4 + O_2 \rightarrow CO_2 + 2H_2O$ (chemoorganoheterotrophs)
  - Example, *Ralstonia* sp., *Pseudomonas* sp.

Anaerobic Environment

Fermentors (chemoorganoheterotrophs)

- Break down cellulose, etc. and ferment sugars into:
  - alcohols
  - organic acids
  - acetate
  - hydrogen
- Many bacterial groups can conduct fermentation, but not all of these have the ability to decompose polymeric compounds such as cellulose.
- Example, *Clostridium* species

Anaerobic Environments, Continued

Sulfur Compounds

- Sulfate reducers: use sulfate, $SO_4^{2-} + e^- \rightarrow S$ or H_2S, to oxidize organic compounds produced by fermentors. (chemoorganoheterotrophs).
  - Many genera of bacteria. Example, *Desulfovibrio* sp.

- Phototrophic bacteria: Use light and H_2S as electron donor (PS I) (photoautotrophs).
  - Examples, purple and green sulfur bacteria.

Methanogens and Acetogens

- Methanogens: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ (chemolithoautotrophs)
  $Acetate^- + H_2O \rightarrow CH_4 + HCO_3^-$ (chemoorganoheterotrophs)
  - Example: *Methanobacterium* (Archaea)

- Acetogens: $2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ (chemolithoautotrophs)
  - Example: *Homoacetogens*

Other possible microbes

Aerobic Environments

Hydrogen

- Hydrogen oxidizers: $H_2 + ½O_2 \rightarrow H_2O$ (both chemolithoheterotrophs and chemolithoautotrophs). However, it is unlikely that H_2 will make it to the aerobic interface (it will be used in the anaerobic environment first)
  - Example, *Ralstonia eutrophus*

Iron

- Iron oxidizers: $Fe^{2+} + H^+ + ¼O_2 \rightarrow Fe^{3+} + ½H_2O$ (chemolithoautotrophs)
  Occurs only at low pH (~2)
  - Example: *Thiobacillus ferrooxidans*

Ammonium

- Nitrifiers: $NH_3 + 1½\ O_2 \rightarrow NO_2^- + H^+ + H_2O$
  $NO_2^- + ½\ O_2 \rightarrow NO_3^-$
  - Example: *Nitrosomonas* and *Nitrobacter*, respectively. Both chemolithoautotrophs.

Anaerobic Environments

Nitrate

- Denitrifiers: $NO_3^- + 6H^+ + 5e^- \rightarrow ½N_2 + 3H_2O$
  - Reaction combined with oxidation of organic matter.

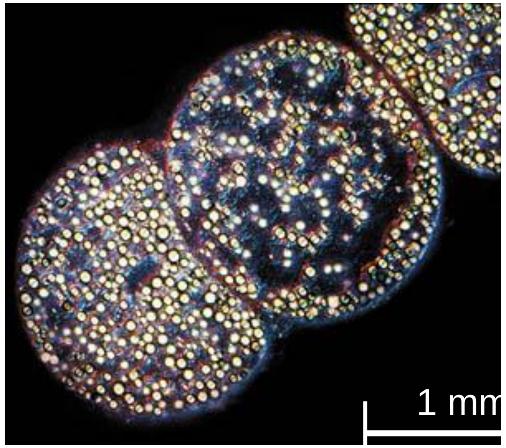
Iron

- Iron reducers: Many organisms can utilize Fe^{3+} as electron acceptor.

Chemical Potential Exploitation

H_2S oxidation by NO_3^-

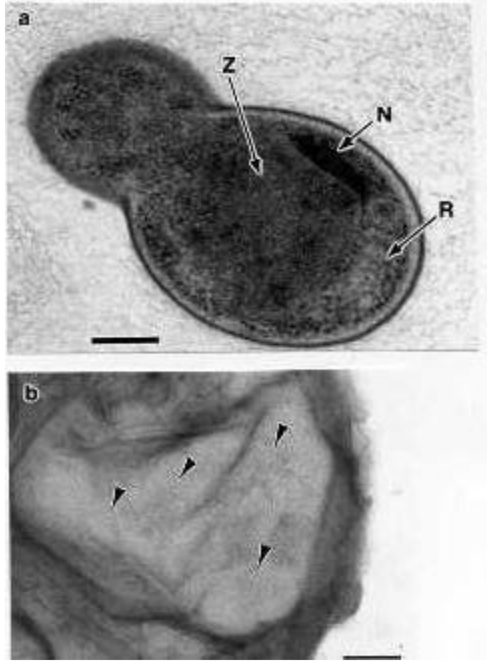
Schulz et al. 1999:
Thiomargarita
namibiensis



Anammox

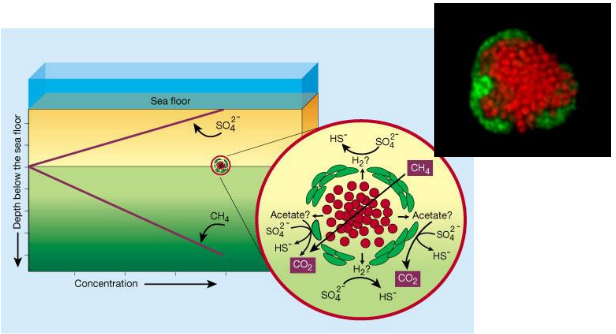
$NH_4^+ + NO_2^- = N_2 + 2H_2O$

Strous et al. 1999:
Planctomycete

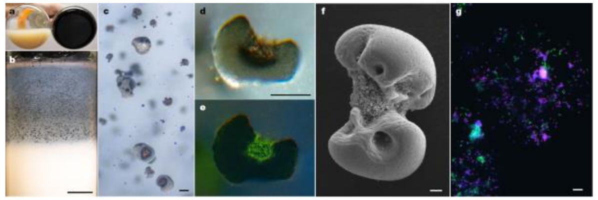


CH_4 oxidation by SO_4^{2-}

Boetius et al. 2000:

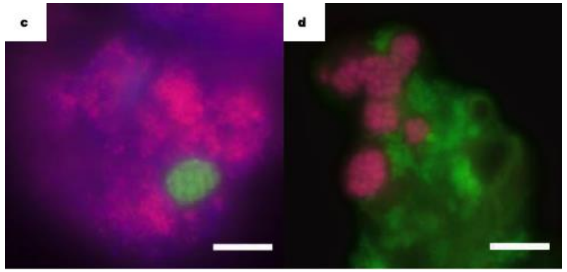


Manganese Oxidation:
Chemolithoautotrophy
(Yu & Leadbetter, 2020)



CH_4 oxidation by NO_3^- (Raghoebarsing et al. 2006)

$$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O$$



Competition and Redox cascade

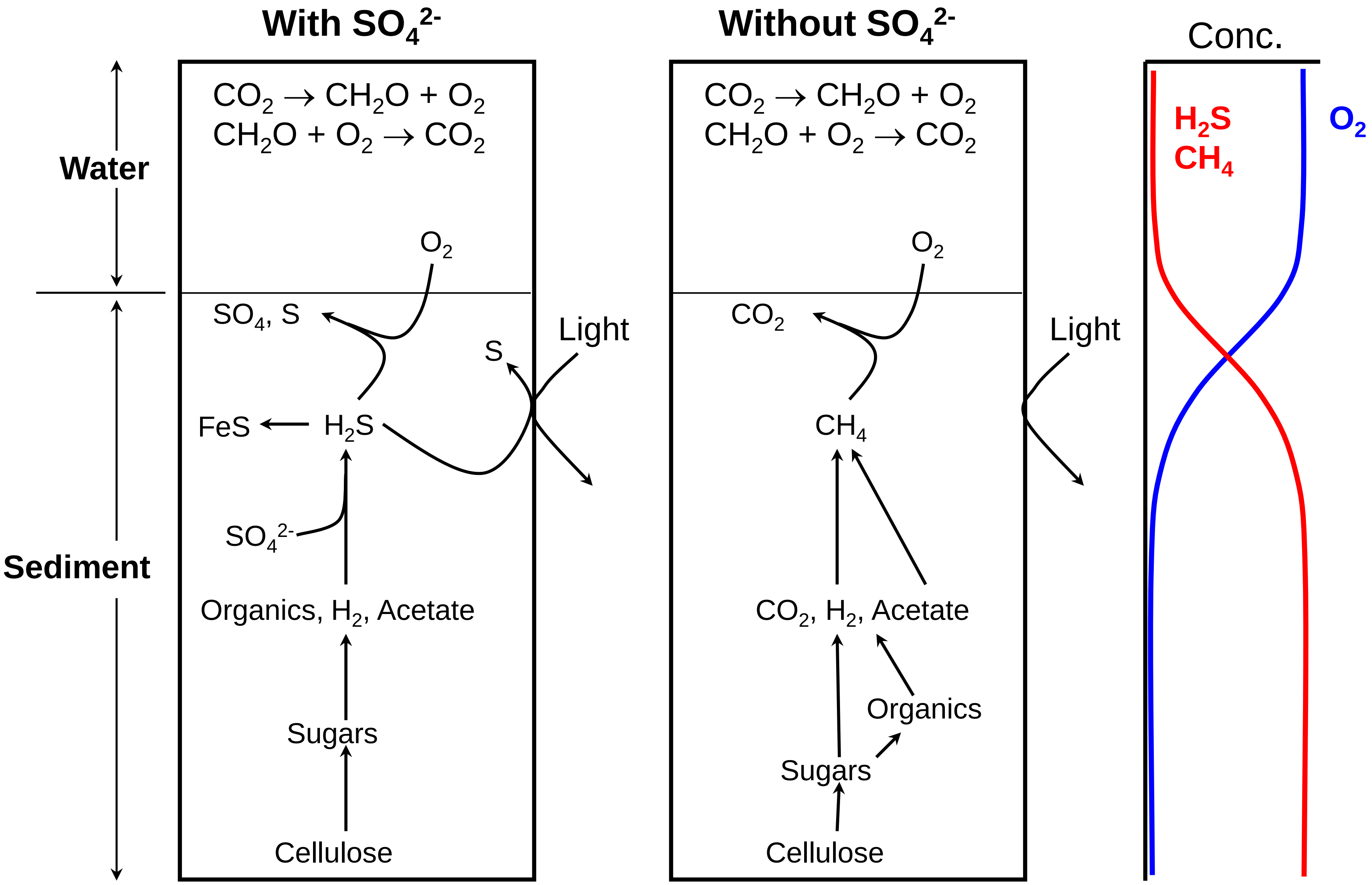
How do the chemical gradients arise in the Winogradsky column, or in natural environments?

Bacteria that are able to use the most energetic reactions in their surrounding environment will dominate that microenvironment. Transport combined with the microbial sources and sinks will determine the resulting chemical gradients. Chemical gradients can be transient as substrates are exhausted or products become toxic. This leads to succession.

Energetics are governed by the redox potentials of the possible reactions:

- Electron acceptors: $O_2 > NO_3^- > Mn^{4+} > Fe^{3+} > SO_4^{2-} > CO_2 >$ Fermentation

Winogradsky column biogeochemistry



With SO_4^{2-}

$CO_2 \rightarrow CH_2O + O_2$
$CH_2O + O_2 \rightarrow CO_2$

O_2

SO_4, S

S

Light

FeS ← H_2S

SO_4^{2-}

Organics, H_2, Acetate

Sugars

Cellulose

Without SO_4^{2-}

$CO_2 \rightarrow CH_2O + O_2$
$CH_2O + O_2 \rightarrow CO_2$

O_2

CO_2

Light

CH_4

CO_2, H_2, Acetate

Organics

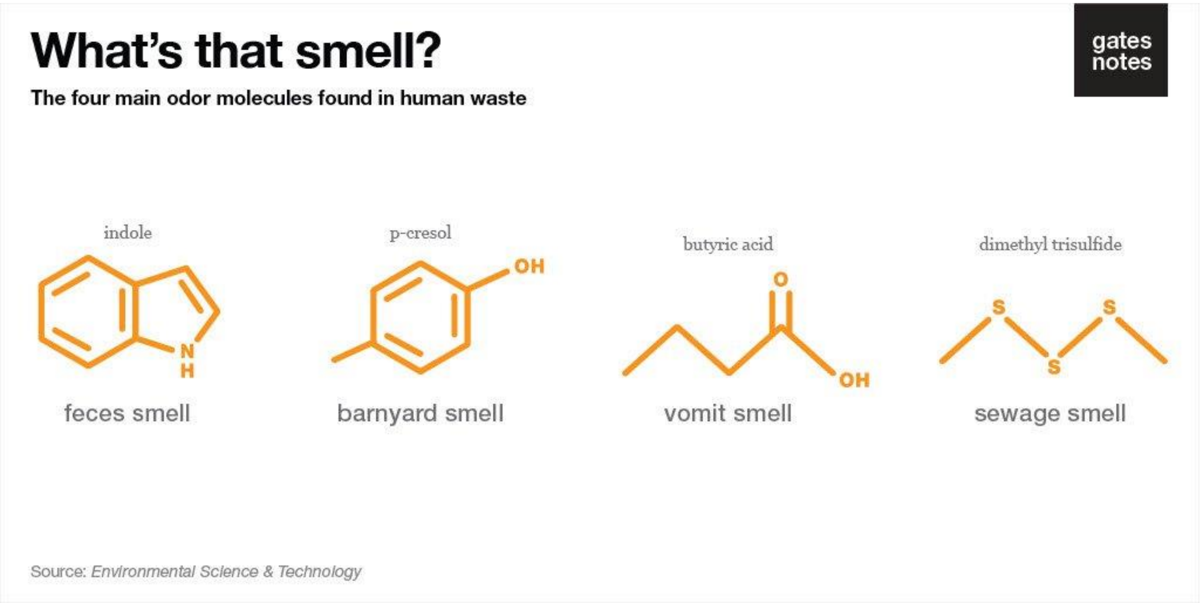
Sugars

Cellulose

Laboratory Work

Tuesday: Measure methane (1/2 class) and hydrogen sulfide (1/2 class) profiles in columns using gas chromatogram.

Thursday: Measure methane and hydrogen sulfide profiles in columns using spectrometer assay.



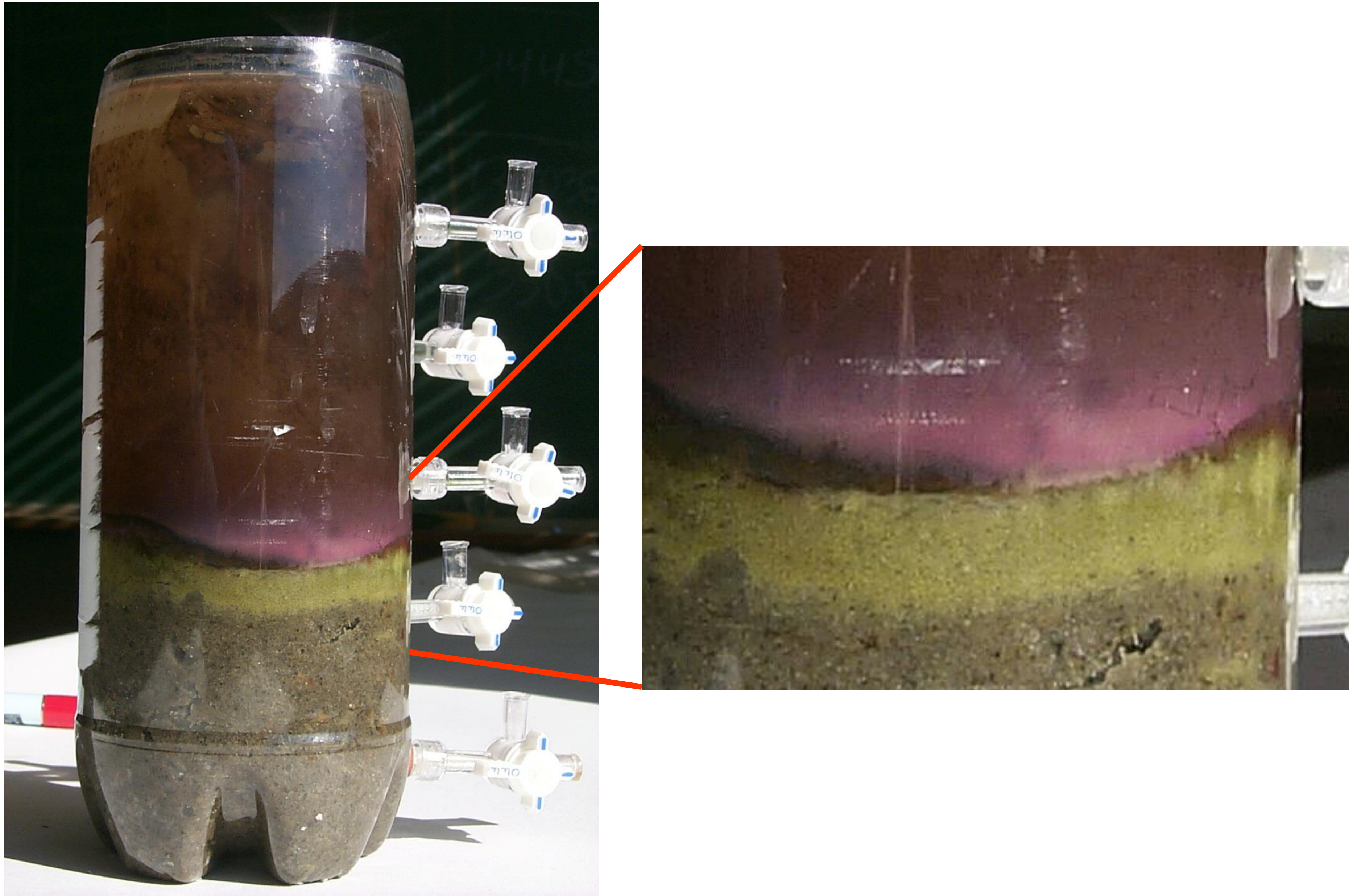
What's that smell?

The four main odor molecules found in human waste

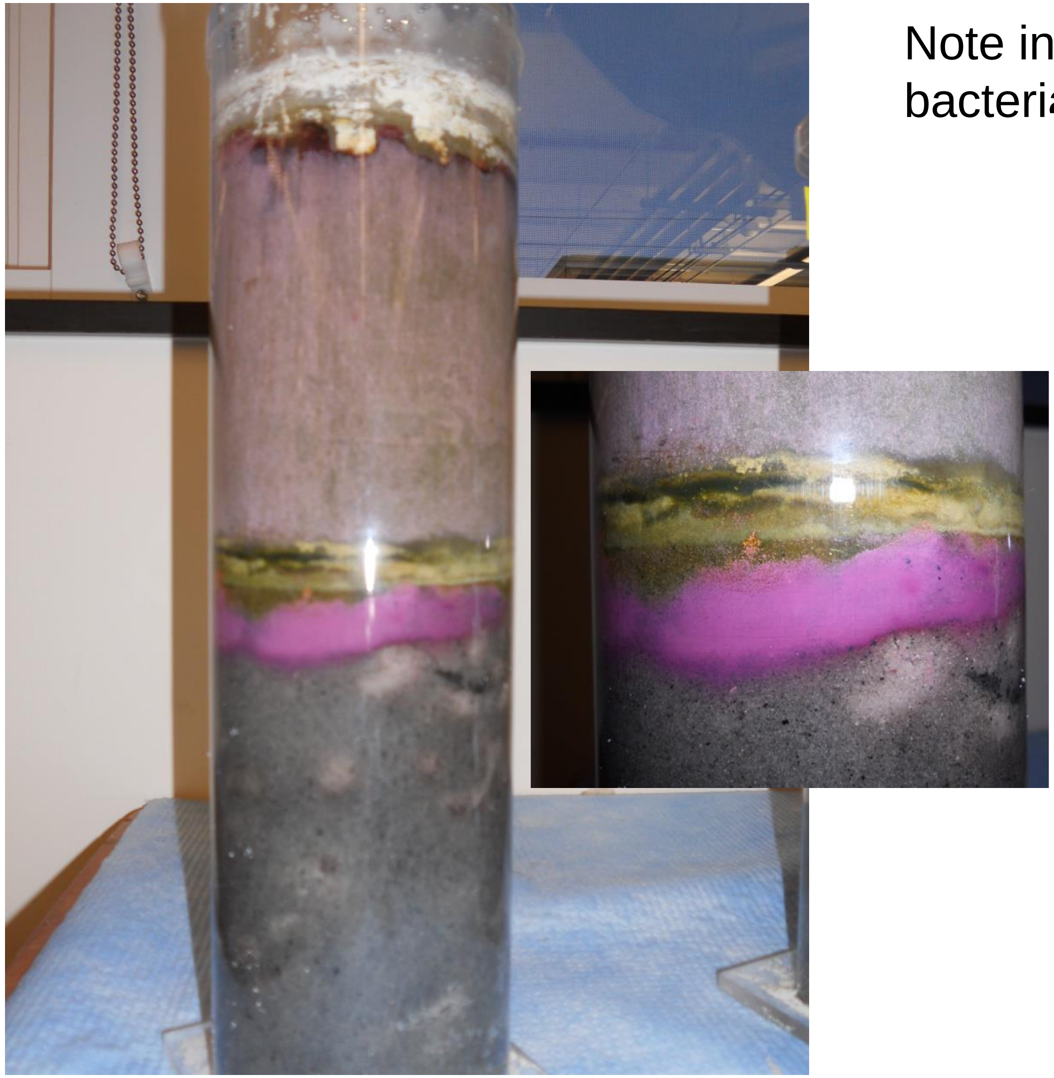
Source: *Environmental Science & Technology*

https://www.gatesnotes.com/Development/Smells-of-Success?WT.mc_id=20161202155430_Firmenich_BG-TW&WT.tsrc=BGTW&linkId=31842000

Winogradsky Column from 1999 Class



Winogradsky Column from 2011 Class



Saltwater with rice as carbon

Note inversion of green and purple sulfur bacteria