

To date you have covered microbial community sampling using molecular techniques to identify who is present in the environment. You have also looked at various genetic mechanisms to understand how organisms do what they do at the molecular level. Here we are going to try and identify why communities are structured the way they are and understand the constraints that define who is present in what environment.

Are microbially communities in the environment a haphazard collection of different microbial trophic groups or is there a structure? What trophic groups may be present and how are they defined.

Metabolic diversity is based on the **Electron donors, acceptors, and carbon sources** available - thermodynamics

Respiration v's fermentation

Is it appropriate to name an organism based on a metabolic trait (e.g *Desulfuromonas* species)

How is complex organic carbon degraded.

Different trophic groups which may be present

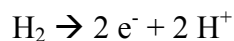
Sources of various electron donors/acceptors in the environment

Oxidation – reduction.

The conservation of energy for chemical reactions involves oxidation/reduction reactions also called redox. Chemically an oxidation is defined as the removal of electron(s) from a substance and reduction is the addition of electron(s) to a substance. In biochemistry redox reactions frequently involve the transfer of not just electrons, but whole hydrogen atoms. A hydrogen atom consists of an electron and a proton. When the electron is removed the hydrogen atom becomes a proton (hydrogen ion) or H^+ .

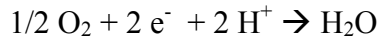
Electron donors/acceptors

Redox reactions involve electrons being donated by the electron donor and accepted by the electron acceptor E.g. H_2 can release electrons and hydrogen ions and become oxidized –



However, electrons cannot exist alone in solution; they must be part of atoms or molecules. The shown reaction represents a theoretical half reaction and must be coupled to a second half reaction. For any oxidation reaction to occur, a

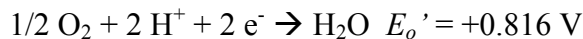
subsequent reduction reaction must also occur e.g. the above reaction could be coupled to the reduction of oxygen



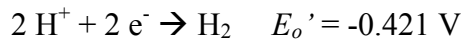
Thus H₂ is the electron donor and O₂ is the electron acceptor.

Reduction potentials

Substances vary in their tendency to become oxidized or reduced i.e. to donate or accept electrons. This tendency is expressed as the reduction (or redox) potential (E_o') of a substance. This potential is measured electrically in reference to a standard substance H₂ and is expressed in volts or millivolts. By convention redox potentials are expressed for half reactions written as reductions. Thus oxidized form + e⁻ → reduced form. When protons are involved in the reaction the reaction will to some extent be controlled by pH. By convention in biology, redox potentials are given for neutrality because the cytoplasm of most cells is circum neutral. Using this convention at pH 7 the redox potential of O₂ is



and



Redox couples

Many compounds can act as both electron donors or electron acceptors depending on the environment and other substances they react with. The same atom on either side of reaction represents a redox couple so the hydrogen redox couple is denoted as 2H⁺/H₂ and the oxidized form is always placed on the left.

In constructing complete redox reactions from constituent half reactions it is simplest to remember that the reduced substance of a redox couple whose reduction potential is more negative donates electrons to the oxidized substance whose potential is more positive. Thus in the couple 2H⁺/H₂ ($E_o' = -0.42 \text{ V}$) H₂ has a greater tendency to donate electrons while in the case of 1/2 O₂/H₂O with a potential of +0.82 V, H₂O has a very small tendency to donate electrons but O₂ has a great tendency to accept electrons.

The electron tower

Electron transfer between compounds can thus be viewed as a vertical tower representing the range of redox potentials with the most negative redox couples at the top to the most positive redox couples at the bottom. The reduced substance in the redox pair at the top has the greatest tendency to donate electrons and the oxidized substance at the bottom has the greatest tendency to accept electrons. As electrons from the e donor at the top fall

down the tower they can be caught by acceptors at various levels. The difference in the potential between the e donor and the e acceptor is expressed as $\Delta E_o'$. The farther the electrons fall the greater the energy that is released and available to the microorganism. Thus O_2 at the bottom of the tower is one of the most favorable e acceptors used by microorganisms.

Calculation of free-energy yield from reduction potential

The free energy of two reactions is the amount of energy that can be released. It can be calculated from the differences in reduction potentials of the two reactions and from the number of electrons transferred. The further apart the two reactions are on the redox tower, the more energy is released. The conversion of potential difference to free energy is given by

$$\Delta G^{\circ} = -nF \Delta E_o'$$

Where n is the number of electrons involved in the reaction and F is the faraday constant (96.48 kJ/V).

To convert ΔG° to $\Delta G^{\circ}'$ i.e. from standard conditions (pH 0) to biochemical conditions (pH 7) in reactions involving H^+

$$\Delta G^{\circ}' = \Delta G^{\circ} + mG'_{f}(H^+)$$

Where m is the net number of protons in the reaction (negative if more are consumed than produced) and $G'_{f}(H^+)$ is the free energy of formation of a proton at pH 7 = -39.83 kJ at 25°C

Examples

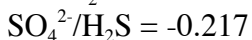
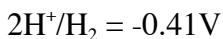


Thus the $2 H^+/H_2$ couple has a potential of -0.41 V and the $1/2 O_2/H_2O$ pair has a potential of +0.82V. Thus the potential difference is $(-0.41 - +0.82) = 1.23V$.

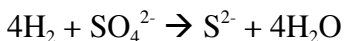
The $\Delta G^{\circ} = -2 (e^-) \times 96.48 \times 1.23 = -237.34$ kJ.

The $\Delta G^{\circ}' = -237.34 + 0(-39.83) = -237.34$ kJ

Or for H_2 oxidation coupled to SO_4^{2-} reduction it would be



Reaction is



The $\Delta G^{\circ} = -8(e^{-}) \times 96.48 \times 0.19 = -146.64 \text{ kJ}$.

Energy of Activation:

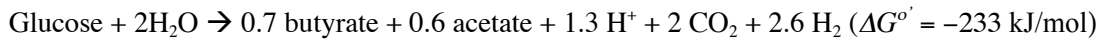
The importance of H_2 in anaerobic environments:

1. It accounts for 10 – 30% of electron flow in sulfate reducing systems
2. It accounts for 40% of electron flow in methanogenic environments
3. Low H_2 concentrations allow the activity of syntrophic bacteria
4. Low H_2 concentrations can alter fermentation end products

A low H_2 partial pressure (<10 Pa) allows electrons at the redox potential of NADH (-320 mV) to be released as molecular hydrogen, and fermentation patterns can shift to more oxidized endproducts i.e. acetate, CO_2 , and hydrogen rather than ethanol or butyrate formation, thus allowing additional ATP formation

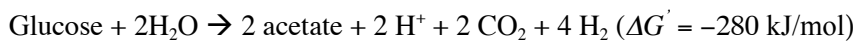
For Example:

Clostridium butyricum fermenting glucose



This metabolism yields 3.3 ATP units per glucose

At $[\text{H}_2] = 10 \text{ Pa}$ the overall reaction changes to:



Yielding 4 ATP units per glucose, two in glycolysis and two in the acetate kinase reaction

Hydrogen Threshold

Hydrogen is a key intermediate in aquatic sediments and when methanogenesis is the electron accepting process H_2 is an intermediate for *ca* 40% of the electron flow and between 10-30% (interspecies hydrogen transfer) with sulfate as the electron acceptor

H_2 uptake can be modeled by Michaelis Menten kinetics according to

$$v = (V_{\max}/(K + [H_2]) \times [H_2] \times B \quad 1$$

where

v is the rate of hydrogen uptake (units - moles per time)

V_{\max} is the maximum specific rate of hydrogen uptake (Units - moles per time per gram cells)

K is the hydrogen concentration at which $v = 0.5 V_{\max}$ (units - dissolved H_2 per liter)

B is the number (in grams) of H_2 consuming organism

$[H_2]$ is the concentration of dissolved hydrogen (moles per liter)

The growth of microorganisms on hydrogen can be defined by:

$$dB/dt = (v \times Y) - (b \times B) \quad 2$$

where

dB/dt is the rate of growth (grams of cells per time)

Y is the yield coefficient (grams cells formed per mole H_2 consumed)

b is the cell decay coefficient (per time)

If we assume steady state i.e. H_2 consumption equal H_2 production and also no net growth i.e. $dB/dt = 0$ then the H_2 concentration can be defined as (combine 1 & 2 above)

$$[H_2] = K/((V_{\max} \times Y/b)-1) \quad 3$$

Equation 3 indicates that under steady state conditions the hydrogen concentration in the environment depends solely upon the physiological characteristics of the organism consuming the hydrogen.

Or as stated by Perry McCarty (1972) “at steady state conditions in environments where there is no significant physical dilution of the microbial population, the steady-state concentration of microbial substrates can be predicted solely from a knowledge of the physiological characteristics of the organisms consuming the substrates” i.e. there is a specific threshold concentrations for different trophic groups.

Of the parameters in equ 3 least is known about the cell decay coefficient which includes all losses of cellular material including dilution, grazing, cell lysis, and cell death. However, it is assumed that this parameter is constant across the various metabolic types of organisms.

In addition, V_{\max} also appears to be constant amongst the various organisms regardless of the electron acceptor utilized and is probably a result of constraints on the maximum rate of electron transport.

In contrast to b and V_{\max} , both the K and Y parameters are strongly influenced by the energy yield that is available from hydrogen oxidation. K decreases as the potential energy for H_2 oxidation increases i.e.

Reaction	K value
$O_2 + 2H_2 \rightarrow 2H_2O$	67 nM
$2NO_3^- + 4H_2 \rightarrow N_2 + 6H_2O$	<1 μ M
$SO_4^{2-} + 4H_2 \rightarrow HS^- + 4H_2O$	1.4 μ M
$HCO_3^- + 4H_2 + H^+ \rightarrow CH_4 + 3H_2O$	4.7 μ M

Similarly, the cell yield Y also increases with the energy.

Given that K decreases and Y increases with the potential energy yield for H_2 oxidation equation 3 predicts that the steady state concentration of hydrogen in sediments will follow the order methanogenic > sulfate reducing > Fe(III) reducing > Mn(IV) reducing > nitrate reducing and each hydrogen consuming process will have a unique steady-state hydrogen characteristic that is characteristic of that reaction

Methanogenesis = 7-10 nM
 Sulfate reduction = 1-1.5 nM
 Fe(III) reduction = 0.2 nM
 Mn(IV) & Nitrate reduction < 0.05 nM

Factors controlling the environmental dominance of a give trophic group (e^- donor/acceptor ratio – the environment is always at steady state) –

TEAP determination

Geochemical v's H_2 conc v's ^{14}C -acetate – Problems associated with the method –

Geochemical analysis –

slow and expensive

Results are exclusive to each specific TEAP

Requires an observable loss or accumulation of specific metabolites (i.e. Fe(II), S^- , N_2 , NO_3 , SO_4 etc) which may not be possible – Black Creek Aquifer (Lovley, 1994 ES&T)

Ionic metabolites are soluble and diffuse thereby confusing delineation

H₂ concentration –

It is a bulk analysis

heterogeneous environments exist (Brown et al, 1999),
non-equilibrium environments i.e. transition points from one metabolism
to another

¹⁴C-acetate –

Solid sample collection is required

Requires supporting geochemical analyses for accuracy

Requires utilization of radioactive materials

Additional factors which control the population – relative population sizes (e.g. FeRB in marine), other nutritional sources and inhibitors, genetic regulation (ClO₄ v's NO₃), bioavailability of e acceptor (e.g. different iron forms)

Other nutritional requirements (nitrogen, sulfur – organic/inorganic sources)