

1. What is the major source of chlorine in seawater? How about sodium? How about sulfate?

Looking for specific mention of minerals that supply these major elements to the ocean and how they are delivered. Also, what do they have in common? These are all conservative ions, so they have high concentrations in the world's ocean today and their concentrations scale with salinity.

Weathering and delivery via rivers are the primary source, although additional possible sources such as hydrothermal vents and submarine groundwater discharge should be considered. Apatite, Na-plagioclase-feldspar, and oxidative weathering of pyrite are the main minerals that should be discussed as sources of these conservative ions. NaCl (halite) may be mentioned, but it's not a true source since it is the recycling of chloride and sodium from evaporites back to seawater. These elements had to originally get into seawater via another pathway. We discussed the origin of seawater sulfate in class by the oxidative weathering of pyrite, but weathering of gypsum will receive partial credit.

2. Discuss some processes through which global warming can influence the biological pump, including the concentration of oxygen in the water column, the production of CaCO₃ by phytoplankton (calcification), redox stratification and the production of SiO₂ by phytoplankton.

First, what is the biological pump? The biological pump refers to the creation of chemical gradients in the oceans as a result of biological processes, including primary production, export production, and remineralization. In recitation, we discussed several examples. Any type of nutrient depletion in the surface ocean and concentration increase at depth (~1000 m) would be a good example of the biological pump. Another example discussed in recitation is the dissolved inorganic carbon (DIC) concentration and $\delta^{13}\text{C}$ depth profiles. Carbon is fixed in the surface ocean and remineralized at depth, so there is a lower concentration of DIC in the surface ocean relative to the depths where remineralization is occurring (~1000 m). Similarly, photosynthesizers fix isotopically light carbon and the isotopically light carbon is shuttled to depth via export production. As a result, a carbon isotopic depth gradient is observed where DIC is isotopically heavier in the surface ocean compared to the deeper waters that have input from remineralized organic matter.

Now, that we have established what the biological pump means, how might global warming affect several aspects related to the biological pump? Marine oxygen concentrations are affected by a combination of physical and biological processes, specifically solubility, air-sea gas exchange, photosynthesis, and remineralization. Warming will decrease oxygen solubility, so the equilibrated surface ocean will hold less oxygen. This is particularly important at higher latitudes, which reportedly will experience the greatest degree of warming, because bottom waters sourced from high latitudes will start off with less oxygen (assuming similar ocean turnover rates). Ocean anoxia and expanded oxygen minimum zones (OMZs) are predicted for the future ocean owing to warming and are thought to have characterized past oceans in greenhouse conditions.

Expanded zones of anoxia will inherently change the biogeochemical redox cycling in the ocean. Nitrogen is a key nutrient that will likely be affected by expanded OMZs. Rates of anaerobic processes, such as anammox and denitrification, will likely increase in light of expanded OMZs.

The effects of global warming on production of CaCO₃ will depend on the source of global warming. Let's say that the global warming is driven by a pCO₂ increase on a geologically rapid timescale (100s to 1000s yrs), as in the modern. See Hönisch et al., 2012, particularly figure 3 for an illustration on the

results of this kind of perturbation. Ocean pH should decline as a result of adding more CO₂ (an acid). However, CO₂ must equilibrate amongst the different carbonate chemistry species (H₂CO₃^{*}, HCO₃⁻, CO₃²⁻). Recall the Bjerrum plot for the distribution of C at different pHs given a specified DIC concentration. The species shift left for lower pH. In other words, as pH decreases, less of the DIC will be in the form of carbonate ion (CO₃²⁻). The solubility product of calcium carbonate depends on Ca²⁺ concentrations and carbonate ion concentration. A lower concentration on carbonate ion concentration will drive equilibrium away from calcium carbonate precipitation. Therefore, calcification by organisms will become less energetically favorable. Some calcifying organisms may be more severely affected than other calcifying organisms by this pH driven equilibrium problem. Ongoing research is taking place to identify which calcifying organisms are more or less severely impacted by decreasing pH.

Use similar concepts to discuss effects for silicifying organisms. Remember most opal production occurs in the southern ocean by diatoms.

Microbial energetics

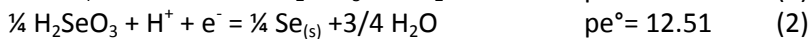
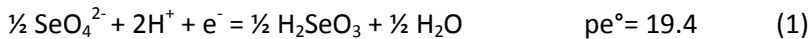
3. Selenate (SeO₄²⁻) is a highly toxic inorganic pollutant that occurs at micromolar concentrations in some agricultural wastewaters.

Can a microbe remove selenate from the water by reducing it to elemental selenium (Se) by coupling this reduction to the oxidation of acetate?

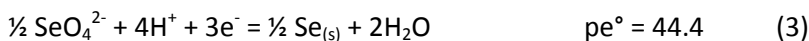
Address by:

- A) writing the balanced half-reaction for the selenate/Se couple

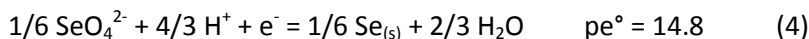
Use Morel and Herring for these problems (pg 432). Here's what's given in M&H:



Write equations to get rid of H₂SeO₃ since we need the reduction of selenite to selenium. We multiply the equation 2 describing the reduction of H₂SeO₃ to Se by 2 and add it to the first equation. Don't forget to multiply the corresponding pe° by 2 before adding it to pe° of equation 1. Add and simplify to get equation 3 and the corresponding pe°.



Multiply equation 3 by 1/3 to rewrite it as a single electron transfer.



So pe° of the SeO₄²⁻/Se_(s) redox couple (always expressed as the reduction reaction) is 14.8.

- B) writing the balanced half-reaction for the oxidation of acetate at pH = 8



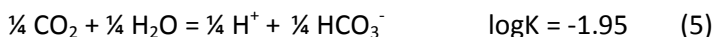
Need to adjust equation 1 for pH=8 where bicarbonate (HCO₃⁻) is the dominant species, not CO₂. Use the equations for carbonate equilibria and manipulate them to replace CO₂ by HCO₃⁻ as a reactant.



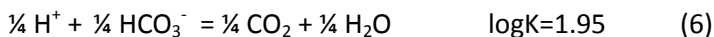
Add equations 2 and 3 and their corresponding logKs to yield equation 4:



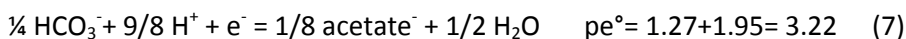
Divide by 4 so you can substitute into equation 1.



Multiply by -1 because you need reverse reaction:



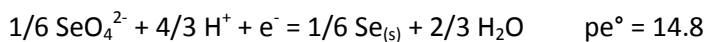
Add to equation 1 and simplify. Remember the definition for pe° (pe° = 1/n logK where n is the number of electrons which should be 1 since we are writing everything as a single electron transfer. Therefore, pe° = logK for a single electron transfer.)



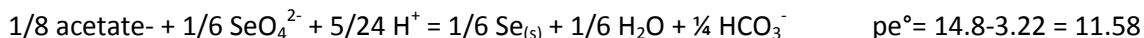
Write as reverse and multiply pe° by -1 for an oxidation reaction.

- C) writing the balanced redox reaction coupling the reduction of selenate to the oxidation of acetate and calculating its energy yield.

For clarification, energy yield means standard gibbs free energy (ΔG°). Remember, pe° and ΔG° are related. See Morel and Herring for equations that relate these two terms. We've written 2 half reactions in parts A and B. They are listed below with the calculated pe°.



The problem asks for "the reduction of selenate to the oxidation of acetate", so we need to reverse the HCO₃⁻/acetate⁻ redox half reaction and multiply the pe° by -1 so it's written as an oxidation reaction. Next, combine these two reactions for the following net reaction and identify the reductants and oxidants:



Check to make sure that your balanced net reaction is both charge and mass balanced. As long as it's charge and mass balanced, it is a valid reaction. Remember to be consistent with M&H:

$Ox_1 + Red_2 = Red_1 + Ox_2$ where $\Delta G = \Delta G^\circ + RT \ln \left(\frac{[Red_1][Ox_2]}{[Ox_1][Red_2]} \right)$. Here acetate related species are "species 2" and Se species are "species 1" according to which species are being oxidized and which are being reduced. Here we can only calculate ΔG° , not ΔG because we don't know any reaction condition specifics such as concentrations.

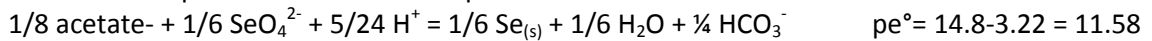
$$\Delta G^\circ = -2.3 \cdot n \cdot RT \cdot pe^\circ$$

Assume we are at standard conditions so $T=25^\circ C$:

$$\Delta G^\circ = -2.3 \cdot n \cdot RT \cdot pe^\circ = -2.3 \cdot 1 \cdot 8.314 \cdot 298.15 \cdot 11.58 = -66.02 \text{ KJ/mol electrons}$$

- D) discussing the effect of pH and the concentration of organics and selenate on the energetics of the reactions in C) per mole of acetate and per mole of selenite

Write out complete net reaction from part c:

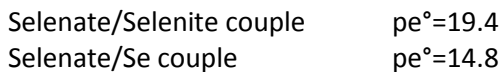


You can write an equilibrium expression for this reaction with the general form of $K = \frac{[Red_1][Ox_2]}{[Ox_1][Red_2]}$ which clearly affects the energetics of the reaction according to the thermodynamic equation $\Delta G = \Delta G^\circ + RT \ln(K)$. You can substitute as follows where the value for ΔG° is simply the answer from part c:

$$\Delta G = \Delta G^\circ + RT \ln \left\{ \frac{[Red1][Ox2]}{[Ox1][Red2]} \right\} = -66.02 \text{ kJ/mol} + RT \ln \left(\frac{[HCO_3^-]^{1/4}}{[acetate^-]^{1/8} [selenate]^{1/6} [H^+]^{5/24}} \right)$$

Note that water and selenium are not included in the K expression because they are liquids and solids, respectively. The coupled equation above shows that the increase in H^+ (lower pH) and the concentrations of acetate or selenite will drive ΔG more negative (more favorable for the reaction to proceed). More quantitatively, a quick comparison of exponents in the last term suggests that the pH and the concentration of selenite will have the largest influence on ΔG .

- E) discussing whether a microbe could also reduce selenate to selenite [i.e., not all the way to $Se(s)$] coupled with the oxidation of acetate



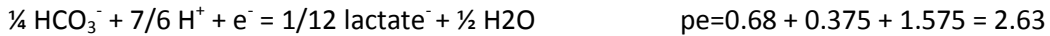
These values were calculated in part A or given in M&H. The selenate/selenite redox couple has a pe° that is even higher than the selenate/Se couple, so the reduction of selenate to selenite coupled with the oxidation of acetate would be energetically even more favorable than the reduction of selenate to Se. So the answer is yes.

- F) Would the microbe gain more or less energy by oxidizing lactate instead of acetate (coupled to the reduction of selenite/Se)?

As with acetate, we will need to do some adjusting for pH. The table in Morel and Hering gives:



Considering HCO_3^- as a reactant in the place of CO_2 by adding Eqs. 4 and 5 to Eq. 8 gives:



The $\text{HCO}_3^-/\text{lactate}$ – couple has a lower potential (2.63) than selenate/Se couple (3.22), so the former couple can donate electrons to the latter. The difference in the electron potentials is somewhat larger than the corresponding difference with acetate as the reductant, but not by much. Therefore, slightly more E would be gained by using lactate than acetate.

- G) Would you expect the reduction of selenate to selenite to Se to be carried out by one microbe or multiple microbes? Why? How would you test your hypothesis?

Compare the energy for selenite to Se calculated in part c to the energy for selenite to selenite in part e. The first reduction is energetically more favorable than the reduction of selenite to Se, so yes, if there's sufficient selenate around, some microbes may specialize in reducing selenate to selenite only. Selenite can be subsequently reduced to Se. You could test this hypothesis by enriching and isolating microbes that perform either reaction and comparing these microbes or by screening for the expression of genes that are used in one process, but not the other.



$$\Delta G = n 2.3RT(\text{pe}_2 - \text{pe}_1)$$

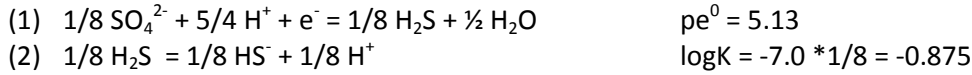
$$\Delta G = \Delta G^0 + RT \ln \left\{ \frac{[\text{Red1}][\text{Ox2}]}{[\text{Ox1}][\text{Red2}]} \right\}$$

4. A sulfate-reducing organism oxidizes formate to CO_2 while reducing sulfate to sulfide.

a. Write out the two half-reactions and the balanced complete reaction assuming the appropriate species at $\text{pH}=9.4$.

Hint: look up the dissociation constants for hydrogen sulfide, formate, carbonic acid and sulfuric acid from the table to determine the appropriate species at a pH of 9.4 (pK_a of formic acid \leftrightarrow formate is 3.74)

The half-reaction describing the reduction of sulfate to sulfide (with HS⁻ as the dominant species of S(-II) at pH 9.4 is derived by manipulating the half reaction equation from Table 7.1 and the equation describing the hydrolysis of H₂S.



The addition of Eqs. 10 and 11 gives:

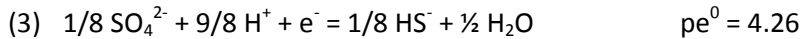
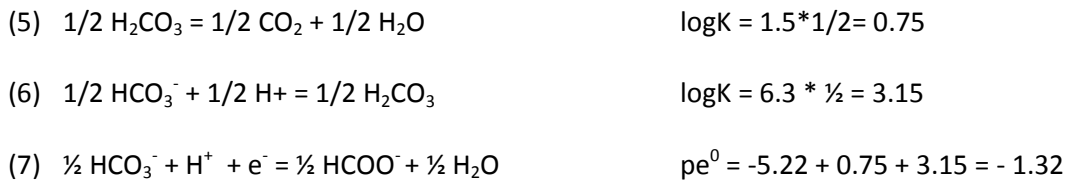


Table 7.1 gives:

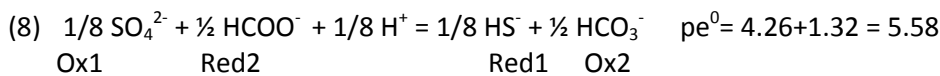


Again, we manipulate this to consider HCO₃⁻ as a reactant in the place of CO₂ (although at pH 9.4, the ratio of bicarbonate to carbonate is smaller than 10, so carbonate concentration becomes non-negligible). We add Eqs. 14 and 15 to Eq. 13:



b. Using reduction potentials (Table 7.1 from the Morel and Hering reading), calculate ΔG₀ for this metabolism.

The pe⁰ of the bicarbonate/formate couple is lower than the pe⁰ of the sulfate/sulfide couple, so formate donates electrons to sulfate. We combine the redox half-reactions (12) and (16) to describe the coupled oxidation of formate to the reduction of sulfate:



This means that bicarbonate/formate is the second redox couple and sulfate/sulfide is the first one. This gives:

$$\Delta G^0 = -2.3 * n * RT pe^0 = -2.3 * 1 * 8.314 * 298.15 * 5.58 \text{ J/mol} = -31.81 \text{ kJ/mol (electrons)}$$

c. Discuss how ΔG would change over the pH range 1-14. State your assumptions about the concentrations of other relevant species.

The relevant species at different pH will be those predicted by the pKa values. Sulfuric acid has two very low pKa values (for the hydrolysis to HSO₄²⁻ and SO₄²⁻, respectively), so between pH 1 and 3.74, sulfate will be the only dissociated species. Eq. (17) will then become:

(9) $1/8 \text{SO}_4^{2-} + 1/2 \text{HCOOH} + 1/4 \text{H}^+ = 1/8 \text{H}_2\text{S} + 1/2 \text{H}_2\text{CO}_3$ so a drop in pH or the increase in the concentration of sulfate and formate will make ΔG more negative (given the exponents, the change in formate concentrations and carbonic acid concentration will have the largest influence).

At $3.74 < \text{pH} < 6.3$, formate will be hydrolyzed as well:

(10) $1/8 \text{SO}_4^{2-} + 1/2 \text{HCOO}^- + 3/4 \text{H}^+ = 1/8 \text{H}_2\text{S} + 1/2 \text{H}_2\text{CO}_3$ so the drop in pH will have a larger influence on ΔG than the change in the concentrations of formate or carbonic acid.

At $6.3 < \text{pH} < 7$, bicarbonate will become the dominant species.

(11) $1/8 \text{SO}_4^{2-} + 1/2 \text{HCOO}^- + 1/4 \text{H}^+ = 1/8 \text{H}_2\text{S} + 1/2 \text{HCO}_3^-$ so changes in the concentrations of formate and bicarbonate will have the largest (and opposite) effects on ΔG .

At $7 < \text{pH} < 10.3$, HS^- and bicarbonate are the dominant species – this situation is described by Eq. (17). Changes in pH have a very small effect on ΔG in this range because of the smaller exponent (1/8).

At $\text{pH} > 10.3$, carbonate becomes the dominant species:

(12) $1/8 \text{SO}_4^{2-} + 1/2 \text{HCOO}^- = 1/8 \text{HS}^- + 1/2 \text{CO}_3^{2-} + 3/8 \text{H}^+$ so a drop in pH in this range actually makes ΔG more positive.

d. Would these organisms be heterotrophic or autotrophic?

Heterotrophic (they take up a small organic acid) and can use this C1 compound in biosynthesis.

5. Read Lavik et al. and answer the following questions:

a. What is annamox? Write the reaction for this metabolism.

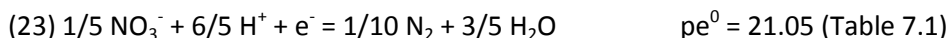
Annamox is anaerobic oxidation of ammonium by nitrate or nitrite.



b. Where in the water column would you expect annamox to occur?

In low oxygen zones where nitrate is available. Particularly in oxygen minimum zones (OMZs)

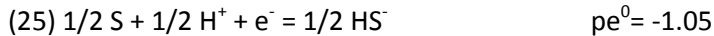
c. Calculate ΔG (correction ΔG°) for the reaction describing the oxidation of sulfide with nitrate.



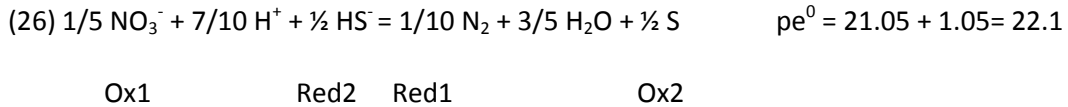
We need to manipulate equations from Table 7.1 to derive the half-reaction describing the reduction of sulfur to sulfide.



Subtracting Eq. 12 multiplied by 4/3 from Eq. 24 to cancel out sulfate (and multiplying everything by -3 to give a reduction reaction per 1e-) gives:

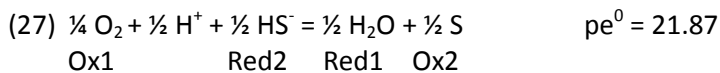


Clearly, the sulfur/sulfide couple has a lower potential than the nitrate/ammonium couple, so sulfide can donate electrons to nitrate. The coupled half-reactions give:



$\Delta G_0 = -2.3 n RT (pe^0) = 1 * -2.3 * 8.314 * 298.15 * (22.1) \text{ J/mol} = -126.0 \text{ kJ/mol}$ (electrons) or five times as negative per mol of nitrate.

d. Write the similar reaction describing the oxidation of sulfide with oxygen and calculate ΔG .



$\Delta G_0 = -2.3 * n RT (pe^0) = -2.3 * 1 * 8.314 * 298.15 * (21.87) \text{ J/mol} = -124.7 \text{ kJ/mol}$ (electrons) or four times as negative per mol of O_2 .

e. How do organisms performing metabolisms described in c. and d. acquire their carbon?

These organisms are autolithotrophic and fix carbon. Most have Rubisco.

f. Which genes are used as tracers of sulfur oxidation? What do these genes do?

The authors first use the 16S rRNA gene sequence to identify potential sulfide oxidizing organisms. They posit that comparative 16S rRNA gene analyses reveal some gamma and epsilon proteobacteria to be involved in sulfide oxidation with nitrate; such organisms had >95% gene identities to the 16S rRNA gene of the sulfide-oxidizing *Candidatus Ruthia magnifica*. In addition, the authors identified the gene sequences encoding alpha subunit of enzymes involved in the sulfur reaction (the adenosine 5' phosphosulfate reductase (AprBA) and the sulphate reductase (rDsrAB). They conclude that the 16S rRNA, AprBA, and rDsrAB genes can be used as potential indicators of sulfur oxidation and posit that GSO bacteria may be capable of sulfide oxidation. The 16S rRNA gene (in bacteria and archaea) codes for 16S rRNA which provides a structural component, binds to proteins which start protein synthesis, and makes up a portion of the ribosome (the small subunit). The AprBA and rDsrAB genes code for alpha subunits for enzymes involved in sulfur oxidation.

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Spring 2013

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