Systems Microbiology 1.084J/20.106J PROBLEM SET #2 – Due Monday Oct. 2nd

Problem 2.1

    a.   What does *proton motive force* mean, and why is it important in biology? (Ch.5 Review Question [RQ] 14)
The porton motive force is an "energized" state of a membrane created by the expulsion of protons to the exterior face of the membrane creating a "charge separation" similar to a battery. The dissipation of this charge gradient through a membrane-bound ATPase complex provides energy for ATP synthesis.

    b.   How is rotational energy in the ATPase used to produce ATP? (Ch.5 RQ 15)
Rotational energy in ATPase is used to produce ATP via proton motive forse coupled to conformational changes in subunits of ATPase holoenzyme. Movement of protons through the F_o subunit drives rotation of the c proteins generating torque. Conformational changes in the β-subuint transfers this potential energy to the F_1 subunit.

Problem 2.2

    a.   What are the differences in electron donor and carbon source used by *Esherichia coli* and *Acidithiobacillus thioparus* (a sulfur chemolithotroph)? (Ch.5 RQ 19)
Electron transport chains with oxygen as the final electron acceptor are utilized by both organisms.
E. coli oxidizes organic compounds to produce energy, whereas *A. thioparus* oxidizes inorganic sulfur to use it as an electron donor.

    b.   The following is a series of coupled electron donors and electron acceptors. Using Figure 5.9, order this series from most energy-yielding to least energy-yielding. (Ch.5 RQ 9)

        H_2/Fe^{3+} H_2S/O_2 CH_3OH/NO_3^{3-} H_2/O_2
        Fe^{2+}/O_2 NO_2^-/Fe^{3+} H_2S/NO_3^{3-}

H_2/O_2, H_2/Fe^{3+}, H_2S/O_2, CH_3OH/NO_3^{3-}, H_2S/NO_3^{3-}, NO_2^-/Fe^{3+}, Fe^{2+}/O_2

    c.   Explain the circumstances under which the same substance can be either an electron donor or an electron acceptor.
The coupler pairing determines acceptor versus donor. The substance with a more positive E_o' is the e^- donor and the more negative E_o' is the e^- acceptor. Thus, depending on the available copule for reduction or oxidation, and the relative concentrations of each species (both e^- donor and acceptor) a substance will act as an e^- acceptor or donor.

    d.   Consider the following reaction:

$$\textbf{NADH + fumarate} \Rightarrow \textbf{succinate + NAD}^+ \textbf{+ H}^+$$
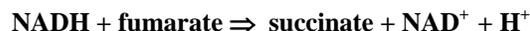
        a.   Using Table 1 calculate the ΔE_0' of the reaction. What is the ΔG_0'? Does this reaction produce or consume energy?
ΔE_0' = $[E_o$'(acceptor) - E_o'(donor)] = [+30mV/mol e^- - (-320mV/mol e^-)] = +350mV
ΔG = -nFΔE; n = 2 mol e^-; F = 96.5kJ/V*mol ΔG = -67.5kJ

        b.   Does this look to you like a potential reaction in a respiratory pathway? Why, or why not?
Answers will vary.

Problem 2.3

    a.   Explain the following observation: cells of *E. coli* fermenting glucose grow faster when NO_3^- is supplied to the culture, and then grow even faster when the culture is highly aerated. (Ch.5 Application Question 4)
When *E. coli* is growing in a fermentative mode, there is no external electron receptor and the energy yield is low. When NO_3^- is added to the culture, nitrate acts as an electron receptor. Under these respiratory conditions the energy yield is greater than fermentative conditions but still less than when O_2 is the final electron receptor (aerobic).

    b.   Discuss why energy yield in an organism undergoing anaerobic respiration is less than that of an organism undergoing aerobic respiration.
Answers will vary. Basically, O_2 has a more positive E_o', therefore in coupled redox reactions more free energy is available using O_2 as the terminal electron acceptor.

Problem 2.4

Explain our current understanding of molecular adaptations to the cytoplasmic membrane that are present in psychrophiles and describe their habitat. How about thermophiles?

Typically psychrophiles are found where the temperature is constantly cold opposed to seasonal winters. Their cytoplasmic membranes contain higher concentrations of unsaturated fatty acids and sometimes polyunsaturated fatty acids that maintain a semifluid state at low temperatures. Enzymes of psychrophiles are cold-active with greater amounts of α-helices and fewer β-sheets as well as greater numbers of polar amino acids. Thermophiles would be found in environments that are consistently hot. Therefore, thermophiles have enzymes and other proteins that are more stable and function optimally at high temperatures. Cytoplasmic membranes of thermophiles also contain lipids rich in saturated fatty acids, which allow form stronger hydrophobic environments (more stable membrane in heat).

Problem 2.5

a. Describe the growth cycle of a population of bacterial cells from the time this population is first inoculated into fresh medium. (Ch.6 RQ 6)

The four stages are: lag, log (exponential), stationary, and death phases. Measurements of total versus viable cell counts will be identical during log phase growth. However, during stationary phase the viable count may differ from total cell count since intact cells that are no longer viable will be included in the total cell count.

b. Describe a direct and an indirect method to measure microbial growth. (Ch.6 RQ 7)

<u>Direct counts</u> can be accomplished using a light microscope. Medium containing the cells is placed in a counting chamber, which is a microscope slide containing etched lines forming a grid of known size. Because the cover slip is a known distance above the slide, each grid contains a known voume of medium. By counting the cells in several grids, the number of cells in the culture can be calculated.
<u>Indirect counts</u> are based on turbidity of the medium (culture). The culture is placed in a spectrophotometer cell and the absorbance is measured. Under limited conditions (log growth), the number of cells present can be estimated fairly accurately using a standard curve generated by plate counts at the time of absorbance readings.

c. If a 1-liter culture of rich medium is inoculated with 3 bacterial cells per milliliter, how many cells will be in the culture after 2 hours, given that there is an hour lag phase and the generation time is 20 minutes? After 3 hours? After 4 hours if one of the initial 3 cells was dead? Calculate k for the 3-hour time period (assuming all initial cells were alive).

$N = N_0 2^n$; $n = t/g$; $k = 0.301n/t$; $N_0 = 3$ cells/ml
$n(2h) = 60min/20min = 3$ $N(2h) = 3*2^3 = 24$ cells/ml
$n(3h) = 6$ $N(3h) = 3*2^6 = 192$ cells/ml
$n(4h) = 9$ $N(3h) = (3-1)*2^9 = 1024$ cells/ml
$k(3h) = 0.301*6/2h = 0.903$ h^{-1}

Problem 2.6

As part of a UROP project, you get a chance to quantify bacterial numbers in environmental samples. The graduate student you are working with has used centrifugation to concentrate 10 1-liter samples of oligotrophic lake water 1000-fold (i.e. in a final volume of 1 ml). She asks you to determine the number of viable bacteria in the samples. By plating 10-fold serial dilutions on minimal media and incubating in ambient O_2 at 37°C, you get small, slow-growing colonies:

Starting dilution (1 ml)	Amount spread on plate	Number of colonies
Concentrated sample	100 µl	Too numerous to count
10^{-1}	100 µl	50
10^{-2}	100 µl	5
10^{-3}	100 µl	None
10^{-4}	100 µl	None
10^{-5}	100 µl	None

When you plate and incubate on rich media using the same conditions, you get no growth at all.

a. How could you distinguish between the presence of a growth inhibitor in the rich media and fatal oxidative stress leading to a failure to form colonies on the rich media?

Answers will vary. Could try lower O_2 concentrations.

b. The graduate student asks if you made a mistake with you dilutions, because she counted an average of 10^5 bacteria in the samples under the microscope. What might account for the discrepancy between your CFU counts and her direct total counts?

Non-viable cells are counted in total cell counts, but will not form CFUs.

c. One method for determining viability of bacteria employs direct microscopic visualization of cultures after incubation with nutrients in the presence of an antibiotic that inhibits DNA replication. Using this so called direct viable count (DVC) elongated bacteria are considered viable. What advantage does the DVC method have over CFU determination? Explain your reasoning.

Answers will vary.

Problem 2.7

For methyl-accepting chemotaxis proteins (MCP) that respond to chemoattractants, the "ground state" is high sensitivity, with decreaseing sensitivity as the organism traverses the gradient, and eventual re-set by demethylation (presumabley when the desirable chemoattractant is depleted). Do you think an MCP involved exclusively in responding to noxious stimuli that an organism tries to avoid would work the same or differently? Explain your reasoning.

MCPs responding to repellents work the opposite of those responding to attractants with regards to sensitivity and running with changes in stimulant (can be attractant or repellant) concentration. The proteins within the chemotaxis pathway all work the same. Repellent MCPs have highly methylated, insensitive 'ground states'. Upon activation, they activate CheA, which is the opposite of attractant MCPs. This situation is similar to the 'no stimulant/no prey' situation discussed in class. CheA is phosphorylated leading to phosphorylation of CheY and CheB. The presence of phosphorylated CheY causes tumbling more often. Phosphorylated CheB de-methylates the MCP, increasing sensitivity to repellent (less repellent required for activation). In turn, if the concentration of repellent decreases, the receptor is less 'active' causing less CheY phosphorylation leading to longer runs. This would coincide with less phosphorylated CheB, more CheR, and re-setting of the sensitivity to the 'ground state' of high methylation. The mechanisms of the pathways are the same, with the exception of the initial activation of the receptor. Upon stimulation with repellent, CheW inhibits CheA causing less phosphorylation and more methylation, whereas a repellent activates CheA causing more phosphorylation and de-methylation (less methylation).