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)
b. How is rotational energy in the ATPase used to produce ATP? (Ch.5 RQ 15)

Problem 2.2

a. What are the differences in electron donor and carbon source used by *Escherichia coli* and *Acidithiobacillus thioparus* (a sulfur chemolithotroph)? (Ch.5 RQ 19)
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-} H_2/O_2
Fe^{2+}/O_2 NO^{2-}/Fe^{3+} H_2S/NO^{3-}

c. Explain the circumstances under which the same substance can be either an electron donor or an electron acceptor.
d. Consider the following reaction:

$$\mathbf{NADH + fumarate \Rightarrow\ succinate + NAD^+ + H^+}$$

   a. Using Table 1 calculate the $\Delta E_o'$ of the reaction. What is the $\Delta G_o'$? Does this reaction produce or consume energy?
   b. Does this look to you like a potential reaction in a respiratory pathway? Why, or why not?

Problem 2.3

a. Explain the following observation: cells of *E. coli* fermenting glucose grow faster when NO3- is suppled to the culture, and then grow even faster when the culture is highly aerated. (Ch.5 Application Question 4)
b. Discuss why energy yield in an organism undergoing anaerobic respiration is less than that of an organism undergoing aerobic respiration.

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?

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

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?
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?
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.

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.

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
$NO_3^- + 6\ H^+ + 6\ e^-$	⇒	$½\ N_2 + 3\ H_2O$	+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