BIO-372 "MICROBIOLOGY" EXERCISES (WEEK 10)

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EXERCISE 1 "GLOBAL NITROGEN CYCLE AND METABOLIC SYMBIOSIS"

Imagine a microbe that can perform a two-electron transfer reaction between the redox couples NO_2^-/NH_3 (E_0' +0.34 V) and $½O_2/H_2O$ (E_0' +0.82 V).

1. Which molecule is the electron donor? Explain.

The redox couple NO_2^-/NH_3 has a lower reduction potential (E_0' +0.34 V) than the redox couple $½O_2/H_2O$ (E_0' +0.82 V). Thus, we can predict that NH_3 will be the electron donor, being oxidized to NO_2^-.

2. Which molecule is the electron acceptor? Explain.

The redox couple $½O_2/H_2O$ has a higher reduction potential (E_0' +0.82 V) than the redox couple NO_2^-/NH_3 (E_0' +0.34 V). Thus, we can predict that O_2 will be the electron acceptor, being reduced to H_2O.

3. Write out the Nernst equation relating Gibbs free energy to the difference in reduction potential of an electron donor and an electron acceptor (redox pair) in a redox reaction. Define each term. Make sure you include the correct units, not just the numbers, in your calculations. Not including units was a source of many mistakes on the previous exams.

$\Delta G^{0'} = -n * F * \Delta E_0'$

where:
$\Delta G^{0'}$ is the Gibbs free energy change under standard conditions
n is the number of electrons transferred
F is the Faraday constant: 96.5 kJ * V^{-1} * $mole^{-1}$ (or about 100 kJ * V^{-1} * $mole^{-1}$, which is precise enough for our purposes here)
$\Delta E_0'$ = the difference in the reduction potential of the electron acceptor and the electron donor under standard conditions, that is: (E_0' acceptor) – (E_0' donor)

4. Calculate the amount of free energy liberated in the two-electron-transfer reaction between these redox couples. Show your work.

$\Delta G^{0'} = -n * F * \Delta E_0'$

where:
n = 2
F = 100 kJ * V^{-1} * $mole^{-1}$
$\Delta E_0'$ = (+0.82 V) – (+0.34 V) = +0.48 V

$\Delta G^{0'} = -2 * (100 \text{ kJ} * V^{-1} * \text{mole}^{-1}) * (+0.48 \text{ V})$

$\Delta G^{0'}$ = –96 kJ per mole (approximately)

5. Do you think this organism performs nitrification or denitrification? Do you think this organism lives in aerobic or anaerobic environments? Explain.

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This transformation is the first step in the **nitrification** process. Nitrification takes place exclusively in **aerobic** environments. Also note that the reaction itself involves molecular oxygen (O_2) as the electron acceptor.

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EXERCISE 2 "GLOBAL NITROGEN CYCLE AND METABOLIC SYMBIOSIS"

Imagine a microbe that can perform a two-electron transfer reaction between the redox couples NO_2^-/NO (E_0' +1.20 V) and N_2O/N_2 (E_0' +1.77 V).

1. Which molecule is the electron donor? Explain.

The redox couple NO_2^-/NO has a lower reduction potential (E_0' +1.20 V) than the redox couple N_2O/N_2 (E_0' +1.77 V). Thus, we can predict that NO will be the electron donor, being oxidized to NO_2^-.

2. Which molecule is the electron acceptor? Explain.

The redox couple N_2O/N_2 has a higher reduction potential (E_0' +1.77 V) than the redox couple NO_2^-/NO (E_0' +1.20 V). Thus, we can predict that N_2O will be the electron acceptor, being reduced to N_2.

3. Calculate the amount of free energy liberated in the two-electron-transfer reaction between these redox couples. Show your work.

$\Delta G^{0'} = -n * F * \Delta E_0'$

where:
n = 2
F = 100 kJ * V^{-1} * $mole^{-1}$
$\Delta E_0'$ = (+1.77 V) – (+1.20 V) = +0.57 V

$\Delta G^{0'}$ = (–2) * (100 kJ * V^{-1} * $mole^{-1}$) * (+0.57 V)

$\Delta G^{0'}$ = –114 kJ per mole (approximately)

4. Do you think this organism performs nitrification or denitrification? Do you think this organism lives in aerobic or anaerobic environments? Explain.

This transformation is the last step in the **denitrification** process, resulting in formation of N_2 (dinitrogen). Denitrification takes place exclusively in **anaerobic** environments.

5. On a different subject: what chemical transformation do "annamox" bacteria carry out? Why is this transformation important ecologically?

Anammox bacteria carry out anaerobic ammonium oxidation using ammonia (NH_3) or ammonium ion (NH_4^+) as the electron donor and nitrite (NO_2^-) as the electron acceptor. This transformation is important because it allows the direct removal of ammonia from **anaerobic** environments without requiring prior nitrification of ammonia to nitrate, which takes place only in **aerobic** environments. Without the anammox transformation, ammonia that made its way into anaerobic environments would get "trapped" there, as there would be no way to transform it back into dinitrogen gas (N_2), and the global nitrogen cycle would be interrupted. This is a major concern for agriculture, because farmers use large amounts of ammonia as fertilizer for their crops. Most of the ammonia that farmers spread on their fields runs off with rainwater and much of this runoff ammonia finds its way into anaerobic environments (such

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as the mud and silt in riverbeds). Without anammox bacteria, this "anaerobic ammonia" would gradually accumulate over time, poisoning these environments.

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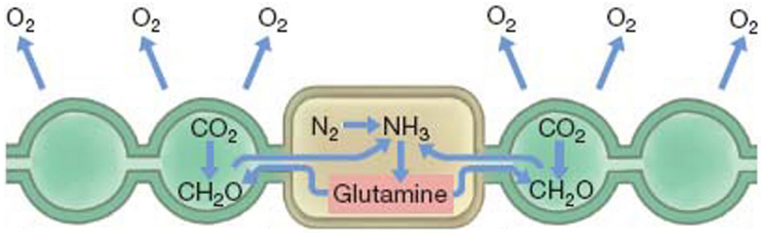
EXERCISE 3 "GLOBAL NITROGEN CYCLE AND METABOLIC SYMBIOSIS"

As depicted in the diagram, some species of cyanobacteria grow in linear chains consisting of two distinct cell types: *vegetative cells* (in green) and *heterocyst cells* (in brown).



1. What is the metabolic function of vegetative cells?

Vegetative cells are specialized for **carbon fixation** via oxygenic photosynthesis.

2. What is the metabolic function of heterocyst cells?

Heterocyst cells are specialized for **nitrogen fixation**.

3. What are the signals that cause vegetative cells to differentiate into heterocyst cells?

Nitrogen starvation leads to depletion of glutamine in the cell cytoplasm. As glutamine levels decrease, alpha-ketoglutarate levels increase because there is not enough glutamine to support the reaction catalyzed by glutamate synthase: 1 glutamine + 1 alpha-ketoglutarate = 2 glutamate. Rising cytoplasmic levels of alpha-ketoglutarate serve as the signal that triggers the differentiation of vegetative cells into heterocyst cells.

4. What changes must a vegetative cell undergo in order to become a heterocyst?

Nitrogen fixation is incompatible with oxygenic photosynthesis because O_2 rapidly and irreversibly poisons the enzyme (nitrogenase) that transforms N_2 into NH_3. When vegetative cells differentiate into heterocyst cells they destroy Photosystem II, which would otherwise generate O_2 by “stealing” electrons from water during oxygenic photosynthesis. Two new extracellular layers (a lipid layer and a polysaccharide layer) are added to the cell envelope to make heterocyst cells “airtight” to keep out oxygen. A special “junction” is created at the interface of the heterocyst cells with its neighboring vegetative cells; this junction controls the passage of materials (for example, glutamate) between the two cell types. Lastly, nitrogenase expression is switched on as the cell switches its metabolism from carbon fixation to oxygen fixation.

5. Why are both types of cells necessary for survival of the organism?

Bacteria that live in environments where sources of both fixed carbon and fixed nitrogen are scarce must make both types of compounds for themselves. For microbes that live in anaerobic environments and perform non-oxygenic photosynthesis, this is not a problem. However, it is a problem for microbes that live in aerobic environments and perform oxygenic photosynthesis because O_2 rapidly and irreversibly inactivates the key enzyme (nitrogenase) responsible for nitrogen fixation. This is why both processes (nitrogen fixation and oxygenic carbon fixation) cannot take place in the same cell. Consequently two cell types are required: one cell type specialized for carbon fixation (vegetative cells) and one

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cell type specialized for nitrogen fixation (heterocyst cells). These two cell types then cross-feed each other: vegetative cells feed fixed carbon (in the form of sugar) to heterocyst cells, and heterocyst cells feed fixed nitrogen (in the form of glutamine) to vegetative cells.

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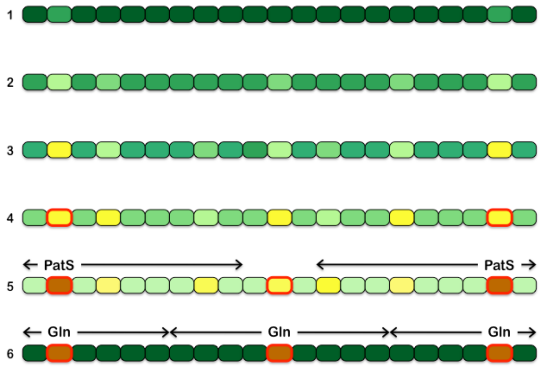
EXERCISE 4 "GLOBAL NITROGEN CYCLE AND METABOLIC SYMBIOSIS"

Some species of cyanobacteria grow in linear chains consisting of two distinct cell types: *vegetative cells* and *heterocyst cells*. The diagram illustrates how a chain of vegetative cells (Step 1) can differentiate into a mixed chain of vegetative cells (in green) with heterocyst cells (in brown) interspersed at regular intervals. Explain what is happening at each step of the differentiation process (steps 1-6).



1. Step 2:

Starvation. When environmental sources of fixed nitrogen are depleted, cytoplasmic levels of nitrogen-containing compounds begin to decrease. Some cells deplete their internal stores of fixed nitrogen faster than others (indicated by light green shading).

2. Step 3:

Initiation. Cells that run out of fixed nitrogen (indicated by yellow shading) initiate the processes required to differentiate into heterocyst cells. However, there is an interval of time between **initiation** of differentiation and **commitment** to differentiation. Cells that have initiated differentiation, but not yet committed to differentiation, can still "reverse direction" and revert back to being vegetative cells if they are returned to an environment with a plentiful source of fixed nitrogen.

3. Step 4:

Commitment. Cells that have committed to differentiate into heterocyst cells (indicated by yellow shading and red outlining) are no longer able to "reverse direction" and revert back to being vegetative cells, even if they are returned to an environment with a plentiful source of fixed nitrogen. At this point the differentiation process is irreversible.

4. Step 5:

Lateral inhibition. Cells that have differentiated into heterocyst cells begin releasing a differentiation-inhibiting peptide (called PatS, but the name is not important to remember). All cells in a chain of cyanobacteria are joined to each other by intercellular junctions and a single shared periplasmic space. Thus, PatS produced by heterocyst cells is able to diffuse along the chain in both directions to inhibit the differentiation of neighboring cells into heterocyst cells. The concentration of PatS diminishes with increasing distance from the source (heterocyst cell). Thus, vegetative cells close to the heterocyst cell are strongly inhibited, while vegetative cells far from the heterocyst cell are weakly inhibited (or not

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inhibited at all). This gradient of PatS-mediated inhibition determines the spacing between heterocyst cells along the chain.

5. Step 6:

Cross-feeding. Once a cell has fully differentiated into a heterocyst cell it can begin fixing nitrogen by transforming N_2 into NH_3, which can then be used to transform glutamate into glutamine (remember, compared to glutamate, glutamine carries an additional amine group on its side-chain). Some of this glutamine is used by the heterocyst cell for its own metabolic requirements (for example, for biosynthesis of amino acids and nucleotides). Some of this glutamine is exported from heterocyst cells to vegetative cells to be used by vegetative cells for their own metabolic requirements. Due to this "cross-feeding" of fixed nitrogen (glutamine) from heterocyst cells to vegetative cells, the entire chain of cells is restored to a condition of fixed nitrogen sufficiency (indicated by dark green shading).

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EXERCISE 5 "GLOBAL NITROGEN CYCLE AND METABOLIC SYMBIOSIS"

Imagine a species of cyanobacteria with an average spacing of 7 vegetative cells (V, in green) between heterocyst cells (H, in brown), which means that the average frequency of heterocyst cells is 1 in 8 (1 heterocyst per 7 vegetative cells). Imagine that one cell in the chain has already become a heterocyst cell, as depicted in the diagram (for simplicity, we ignore vegetative cells on the other side of the heterocyst cell).

1. In a chain of wild-type bacteria, which cell is most likely to become the next heterocyst (H): cell V4 or cell V8 or are they equally likely? Explain.

Cell V8 is more likely than cell V4 to become the next heterocyst. This is due to production of a differentiation-inhibiting peptide (PatS) by the heterocyst cell and its diffusion along the chain of cells, forming a gradient in which cells closer to the heterocyst cell are strongly inhibited while cells farther away from the heterocyst cell are weakly inhibited (or not inhibited at all).

2. In a chain of *patS* mutant (loss-of-function) bacteria, which cell is most likely to become the next heterocyst: cell V4 or cell V8 or are they equally likely? Explain.

In the absence of the PatS-mediated inhibition of differentiation, all cells in the chain have an equal probability of differentiating into heterocyst cells. However, for a cell to become the ***next*** heterocyst, two things must happen: (1) it must trigger the differentiation program (with, in this hypothetical case, a probability of 1 in 8 or $P = 0.125$), and (2) it must be the closest cell to the heterocyst cell of all the cells that trigger the differentiation process. For example, imagine that cells V4 and cell V8 both differentiate into heterocyst cells. Although in this scenario both cell V4 and cell V8 are heterocyst cells, only cell V4 is the ***next*** heterocyst cell moving along the chain from the original heterocyst cell (H).

3. If differentiation of vegetative cells into heterocyst cells is a completely random process, what is the probability that cell V5 will become a heterocyst cell?

The average frequency of heterocyst cells is 1 in 8. Thus, the probability that cell V5 will become a heterocyst cell is 1 in 8 (12.5%).

4. If differentiation of vegetative cells into heterocyst cells is a completely random process, what is the probability that cell V5 will become the **next** heterocyst cell?

For cell V5 to become the ***next*** heterocyst cell, two things must happen: (1) it must trigger the program to differentiate ($P = 0.125$); and (2) cell V1, V2, V3, or V4 must not differentiate into a heterocyst cell ($P = 0.875$ for each of cells V1, V2, V3, and V4). Thus, the probability that cell V5 will be the next heterocyst cell is given by:

(0.125) is the probability that cell V5 will become a heterocyst cell
X
(0.875) is the probability that cell V1 will not become a heterocyst cell
X

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(0.875) is the probability that cell V2 will not become a heterocyst cell
X
(0.875) is the probability that cell V3 will not become a heterocyst cell
X
(0.875) is the probability that cell V4 will not become a heterocyst cell
=
about 0.073 is the probability that cell V5 will be the ***next*** heterocyst cell along the chain

5. If you engineer a strain of cyanobacteria to overproduce PatS, would the average number of vegetative cells between heterocyst cells increase or decrease? Explain.

PatS peptide inhibits the differentiation of vegetative cells into heterocyst cells. PatS is produced exclusively by heterocyst cells. Thus, I would predict that overproduction of PatS would increase the number of vegetative cells between heterocyst cells by extending the "reach" of the PatS gradient farther along the chain of cells.

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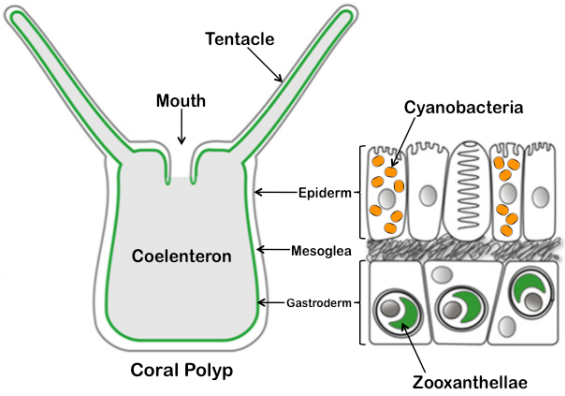
EXERCISE 6 "GLOBAL NITROGEN CYCLE AND METABOLIC SYMBIOSIS"

Coral polyps harbor two distinct types of symbionts: cyanobacteria (which are prokaryotic) in the polyp epiderm and zooxanthellae (which are eukaryotic) in the polyp gastroderm.



1. Would you classify these symbionts as parasites, commensals, or mutualists? Explain.

These symbionts are mutualists because both the symbiont and the host benefit from the partnership.

2. Would you classify these symbionts as epibiotic or endobiotic? Explain.

These symbionts are endobiotic because they actually live inside the host cells, in the host cell cytoplasm.

3. What is the specific metabolic role of each symbiont? Explain.

The cyanobacteria symbiont is specialized for nitrogen fixation. The zooxanthellae symbiont is specialized for carbon fixation via oxygenic photosynthesis. These two functions cannot take place in the same cell at the same time because the oxygen produced by oxygenic photosynthesis poisons the nitrogenase enzyme that does nitrogen fixation.

4. Which symbiont is active during the day? Explain why.

The zooxanthellae symbiont is active during the day. It does oxygenic photosynthesis, which requires light energy.

5. Which symbiont is active during the night? Explain why.

The cyanobacteria symbiont is active at night. It does nitrogen fixation, which cannot take place during the day because the oxygen produced by the zooxanthellae symbiont (due to oxygenic photosynthesis) poisons the nitrogenase enzyme that does nitrogen fixation.