

## BIO-413 "PLANETARY HEALTH" EXERCISES (WEEK 07)

Your Name : \_\_\_\_\_

**EXERCISE 1 "SUSTAINABLE AGRICULTURE - THE FUTURE OF FOOD"**      **Grade :** \_\_\_\_\_

1. What is the "Green Revolution"? Explain and discuss with your partner(s).

Characteristics of the "Green Revolution":

- > A shift from small family-owned farms to large industrial-scale agribusiness corporations.
- > A shift from human labor to mechanized work (mechanical tractors, threshers, harvesters, planters, etc.).
- > Increased use of genetically modified organisms (GMOs) as crop plants.
- > Increased use of artificial fertilizers, particularly nitrogen (in the form of ammonia,  $\text{NH}_3$ ) and phosphorus (in the form of inorganic phosphate,  $\text{PO}_4^{3-}$ ).
- > Increased use of pesticides to suppress bacterial, fungal, and insect pests.
- > Increased use of herbicides to suppress competition from "weed" plants.

Can you think of any other points? If so, please tell me so I can add them to the agenda for next year's class! 😊

2. What are the main advantages of the "Green Revolution"? List as many examples as you can remember and discuss with your partner(s).

- > The shift from small family-owned farms to industrial-scale agribusinesses brought big economies of scale and increased land productivity.
- > The shift from human labor to mechanization led to big increases in productivity per worker.
- > Increased use of genetically modified organisms (GMOs) as crop plants led to increased productivity per land unit by focusing on a small number of high-yield and pest-resistant crop plant varieties.
- > Increased use of artificial fertilizers allowed big increases in crop productivity per land unit while avoiding exhaustion of the soil.
- > Increased use of pesticides brought about big increases in crop productivity per land unit by preventing predation of crop plants, which was a major source of crop failure in the past.
- > Increased use of herbicides allowed big increases in crop productivity per land unit by suppressing resource competitors.

Can you think of any other points? If so, please tell me so I can add them to the agenda for next year's class! 😊

3. What are the main disadvantages of the "Green Revolution"? List as many examples as you can remember and discuss with your partner(s).

- > The shift from small family-owned farms to industrial-scale agribusinesses led to a sharp decrease in small-holder "private" land ownership, declining standards of land stewardship, and increased unemployment among farm workers.
- > Increased use of genetically modified organisms (GMOs) as crop plants led to a sharp decrease in the diversity of crops planted and loss of genetic diversity, resulting in

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potentially decreased resilience to new invasive species and pests or altered environmental conditions (for example, reduced rainfall or increased temperatures due to climate change).

> Increased use of artificial fertilizers led to widespread pollution of land and water sources, resulting in the buildup of toxic substances and "algal blooms" that deplete oxygen from the water, resulting in massive die-offs of other species (like fish).

> Increased use of pesticides resulted in pollution of land and water resources with chemicals that are often toxic to other species. (A well-known example is the impact of widespread DDT spraying on wild bird populations, the subject of Rachel Carson's classic book "Silent Spring". Don't worry, you don't need to know this last point for the exam, but it is an interesting historical example.)

> Increased use of herbicides resulted in pollution of land and water resources with chemicals that are often toxic to other species. A well-known example is glyphosate ("Roundup®"), which has been labelled as a probable carcinogen by the World Health Organization's International Agency for Research on Cancer (although this remains controversial).

Can you think of any other points? If so, please tell me so I can add them to the agenda for next year's class! 😊

**4. What is the so-called "Haber-Bosch process"? Why is it important? What are the main disadvantages of this process (hint: think about the reaction conditions)?**

The Haber-Bosch process is the industrial fixation of nitrogen via non-biological chemical processes:  $N_2 + 3 H_2 \rightarrow 2 NH_3$  (ammonia).

This was an important innovation because it permitted, for the first time, the industrial-scale production and distribution of fixed-nitrogen ( $NH_3$ ) fertilizer, which led to a huge increase in farmland productivity in terms of crop yields per hectare. This, in turn, led to impressive reductions in hunger and starvation worldwide and a surge in global human population growth. The Haber-Bosch process has often been called "the most important invention of the 20<sup>th</sup> century" for both its positive effects (reduction in human suffering) and its negative effects (explosive human population growth).

The main disadvantages of the Haber-Bosch process include the very high temperatures (circa 500°C) and pressures (circa 300 atm) required to make the reaction "go". This means that the process is highly energy-intensive and contributes a substantial amount of the total  $CO_2$  released each year by human processes, thus contributing to global climate change. Each year, globally, the Haber-Bosch process consumes nearly 2% of total global energy output and produces about 150 billion kg of  $NH_3$  and 500 billion kg of  $CO_2$  (which is nearly 2% of total global  $CO_2$  emissions).

**5. Why is runoff from fields fertilized with ammonia ( $NH_3$ ) and phosphorus ( $PO_4^{3-}$ ) a problem for sustainable agriculture?**

A large proportion (roughly half) of ammonia and phosphate fertilizers applied to farmlands is lost to rainwater runoff. The high concentrations of fertilizers that accumulate in water

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reservoirs stimulate overgrowth of algae, which results in depletion of oxygen from the water and die-off of other species (like fish, crustaceans, shellfish, etc.). Many species of algae also release toxins that are directly harmful or even fatal to other species, including aquatic mammals (like otters, beavers, etc.). The problem is compounded when algal cells die and sink down the water column, which stimulates explosive overgrowth of bacteria in the water column and water subfloor. These bacteria further deplete the dwindling oxygen supply and may also release bacterial toxins that are directly harmful to other aquatic life forms.

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**EXERCISE 2 "SUSTAINABLE AGRICULTURE - THE FUTURE OF FOOD"** Grade : \_\_\_\_\_

1. What is the practice of "crop rotation"? Why is it important for sustainable agriculture?

Crop rotation means planting different types of crops in different years, in a rotational cycle. This practice can have many benefits, including reduced accumulation of plant pests that prey on specific species of crop plants by periodically starving them. Probably the biggest benefit of crop rotation is the periodic planting of leguminous vegetables (like peas, beans, lentils, etc.). This is important because leguminous plants are uniquely capable of biological nitrogen fixation:  $N_2 + 3 H_2 \rightarrow 2 NH_3$  (ammonia). Other crop plants typically deplete fixed nitrogen from the soil, eventually leading to ever-declining farmland productivity, whereas leguminous plants add fixed nitrogen to the soil, thus rejuvenating it.

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  $\frac{1}{2}O_2/H_2O$  ( $E_0' +0.82$  V).

2. 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  $\frac{1}{2}O_2/H_2O$  ( $E_0' +0.82$  V). Thus, we can predict that  $NH_3$  will be the electron donor, being oxidized to  $NO_2^-$ .

3. Which molecule is the electron acceptor? Explain.

The redox couple  $\frac{1}{2}O_2/H_2O$  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  $H_2O$ .

4. 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 in your calculations!

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

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:  $96.5 \text{ kJ} * \text{V}^{-1} * \text{mole}^{-1}$  (about  $100 \text{ kJ} * \text{V}^{-1} * \text{mole}^{-1}$ , which is precise enough for us)

$\Delta E_0'$  = 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)

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

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

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where:

$$n = 2$$

$$F = 100 \text{ kJ} \cdot \text{V}^{-1} \cdot \text{mole}^{-1}$$

$$\Delta E_0' = (+0.82 \text{ V}) - (+0.34 \text{ V}) = +0.48 \text{ V}$$

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

$$\Delta G^{0'} = -96 \text{ kJ} \cdot \text{mole}^{-1} \text{ (approximately)}$$

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### EXERCISE 3 "SUSTAINABLE AGRICULTURE - THE FUTURE OF FOOD" Grade : \_\_\_\_\_

1. Leguminous plants have "root nodules". What are root nodules? What is their function? Explain.

Root nodules are a kind of benign "tumor" comprising cortical root cells (from the plant) and intracellular bacteria residing within membrane-bound compartments of the cortical cells. The function of the bacteria residing within the root nodules is to fix nitrogen by reducing dinitrogen gas ( $N_2$ ) to ammonia ( $NH_3$ ), which is then used to synthesize nitrogen-containing compounds, including amino acids. The bacteria transfer a fraction of the fixed nitrogen to the plant, which allows the plant to grow in soil that has been depleted of fixed nitrogen sources. In return, the plant uses photosynthesis to generate fixed carbon sources from atmospheric carbon dioxide ( $CO_2$ ), including sugars and organic acids. The plant transfers a fraction of the fixed carbon to the bacteria, which the bacteria use to fulfill their requirements for carbon "building blocks" and energy. In sum: the bacteria fix nitrogen (from atmospheric  $N_2$ ) and feed some of the fixed nitrogen to the plant, while the plant fixes carbon (from atmospheric  $CO_2$ ) and feeds some of the fixed carbon to the bacteria.

2. Root nodules are a great example of a "mutualistic symbiosis" between a eukaryotic host (leguminous plants) and a bacterial symbiont (rhizobia). What is a "mutualistic symbiosis"? Explain.

A mutualistic symbiosis is a long-term relationship between a host species (in this case, the leguminous plant) and a symbiont species (in this case, rhizobial bacteria) in which both species, the host and the symbiont, derive benefits from the relationship.

3. Consider the mutualistic symbiosis of leguminous plant and rhizobia. What is the role of the plant host? What is the role of the rhizobial symbiont?

The role of the host (leguminous plants) is to fix carbon via photosynthesis and to cross-feed fixed carbon sources (like sugars or organic acids) to the symbiont. The role of the symbiont (rhizobial bacteria) is to fix nitrogen and cross-feed fixed nitrogen sources (like ammonia or amino acids) to the host. Partitioning of these tasks is necessary because photosynthesis produces large amounts of molecular oxygen as a "waste product" and the nitrogenase that catalyzes nitrogen fixation is rapidly and irreversibly inactivated by exposure to oxygen. Thus, carbon fixation and nitrogen fixation cannot proceed in the same place at the same time. The root nodule symbiosis solves this problem by dividing these two incompatible tasks – carbon fixation and nitrogen fixation – between the plant host and bacterial symbiont, respectively.

4. What are currently the three main strategies for genetic engineering of non-leguminous plants to fix nitrogen? Can you think of other possible approaches (use your creativity)?

> Engineer non-leguminous plants (NLPs) to recreate the rhizobial root nodule symbiosis. So far, these efforts have been only partially successful. This is not too surprising, given the

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complexity of the rhizobia-plant symbiosis, which has co-evolved over millions of years. Efforts continue...

> Engineer NLPs to express bacterial nitrogenase from N<sub>2</sub>-fixing bacteria. So far, these efforts have been only partially successful due to poor production of nitrogenase proteins by the transgenic plants, poor assembly and activity of the nitrogenase proteins, etc. But this problem does not look insuperable. Efforts continue...

> Engineer NLPs to promote biofilms of N<sub>2</sub>-fixing soil bacteria on plant roots. In addition to the N<sub>2</sub>-fixing rhizobia that form root nodules on leguminous plants, there are free-living N<sub>2</sub>-fixing bacteria in the soil surrounding plant roots, which can also form biofilms on the surface of plant roots. The close proximity of N<sub>2</sub>-fixing bacterial biofilms to the plant roots facilitates cross-feeding of fixed nitrogen sources (like ammonia and amino acids) from the bacterial biofilm to the plant roots. Various strategies are being explored to increase the formation of N<sub>2</sub>-fixing biofilms on NLP roots by engineering either the plants or the bacteria themselves to enhance biofilm formation. We learned about one of these strategies in this week's class.

Can you think of any other strategies? If so, please tell me so I can add them to the agenda for next year's class! 😊

**5. Plant cells are a "hard target" for genetic transformation. Explain why. Explain how *Agrobacterium tumefaciens* can be used for genetic transformation of plant cells.**

Plant cells have a thick and tough cell wall that functions as an effective barrier to entry of molecules like DNA. Also, plant cells contain highly active digestive vacuoles. Unprotected DNA is shuttled to these vacuoles and digested. To get around these problems, the Ti plasmid of *A. tumefaciens* injects its DNA directly into the plant cell's cytoplasm using a specialized "type IV secretion system" (T4SS). The injected Ti DNA is coated with bacterial proteins that prevent the DNA from being digested and direct it to enter the plant cell's nucleus through the nuclear pores. Once the Ti DNA is in the nucleus, the associated Ti proteins facilitate the efficient integration of the Ti DNA into the plant cell's own genome, where the integrated DNA is now replicated and propagated along with the host cell's own DNA.