2.4) THE NITROGEN AND PHOSPHORUS CYCLES

Objective: In this laboratory exercise you will compare the effectiveness of different conditions for the oxidation of ammonia by nitrifying bacteria. You will then take the nitrogen cycle one step further by using plants to remove nitrates from an aquatic environment. Phosphate assimilation by plant tissues will also be measured. Both of these nutrients are the main components of nutrient pollution.

Introduction: Have you ever purchased a new aquarium with fresh clean gravel, only to be disappointed upon seeing all the fish die over the next few days? More than likely this was a case of "new tank syndrome," the bane of many first-time aquarists. Even though the tank seemed "clean," the fish were literally poisoned by their own waste. The culprit is ammonia, the main nitrogenous waste produced by fish. Fish thrive better in "mature" tanks because these tanks usually have colonies of nitrifying bacteria that oxidize the ammonia and convert it to nitrate.

The brown slime you often find on the gravel or biofilter of a mature aquarium usually contains large concentrations of these valuable microbes. Even though it is a good idea to remove debris from your aquarium, experienced aquarists *never* disinfect the gravel or biofilter on a routine basis. If you need to do this in order to remove a disease, you need to "re-seed" the biofilter with nitrifying bacteria before re-introducing the fish.

Even though nitrate is much less toxic than ammonia, harmful levels of this ion can build up in a aquarium over a period of months. Mature aquaria have some amount of "denitrifying" bacteria that convert nitrate into nitrogen gas. But since these bacteria thrive only in regions where there is no oxygen, their activity in an aquarium is limited. Most aquarists simply change the water when nitrate levels get too high. In systems where water exchanges are expensive (such as those involving artificial seawater), foam fractionators are used to remove proteins and other organic compounds that serve as precursors to ammonia and nitrate. In larger systems, a tank is sometimes set aside exclusively for seaweeds for removing nitrates and phosphates by assimilating them into their biomass.

Part A: Nitrification of Ammonia

1) Compare the foam filter with the containers you will use in the experiment and note the height of the plastic tube extending from the foam filter (Fig. 1.).
2) If the height of the plastic tube is more than half that of the container (as is the case in Fig.1) remove it to ensure that the entire filtering system will be submerged when you add the water (Fig. 2).
3) Fill four containers with at least 500 mL of aquarium water (or dechlorinated tap water) that contains 1-2 ppm of ammonia. Label your containers 1-4.
4) To containers 1 and 2, insert an "activated" foam filter (foam filters from aquaria that have contained fish for at least 2 months).
5) To containers 3 and 4, insert "de-activated" foam filters. This is best accomplished by removing the filters from the aquarium and allowing them to dry for at least one week (nitrifying bacteria do not survive desiccation).
6) Aerate the water in containers 1 and 3 by connecting the foam filter to an active air line (Fig. 3).
7) You now have the following 4 treatments: #1-activated filter with aeration; #2-activated filter without aeration; #3-deactivated filter with aeration; #4-deactivated without aeration. Measure ammonia levels in each container daily for one week.
8) Organize your data into a table and use this information to generate a graph with independent variable (date) on the x-axis and the dependent variable (NH_3 ppm) on the y-axis. Your graph should have 4 lines corresponding to the 4 treatments indicated in Table A. *Note: The table below is just a guide for the table you need to make on your own.*

Table A: Ammonia Levels (ppm)

Date	Active filter, Aeration	Active filter, No aeration	Inactive filter, Aeration	Inactive filter, No aeration

Fig. 1

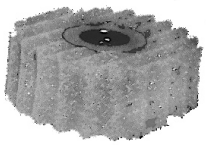

Fig. 2

Fig. 3

Part B: Nitrate and Phosphate Assimilation

1) Fill two containers with 600 mL of aquarium water (or aged tap water) that contains up to 40 ppm nitrate and 10 ppm phosphate (make sure they do not exceed the limit of your testing kit).
2) Aerate the water in both containers by placing an air stone in each (Fig. 4).
3) To one of your containers, add 6-10 grams of live *Elodea*. Be sure to record the initial biomass of the plants (Fig. 5)
4) Keep both containers near a light source that gives them at least 12-hours of light per day.

Fig. 4

Fig. 5

5) Measure nitrate and phosphate levels in each container every three days until nitrate and phosphate level become undetectable in at least one of the containers. This may take up to three weeks. Organize your data into a table and use this to generate two graphs, placing the independent variable (day) on the x-axis and the dependent variable (nitrate or phosphate level) on the y-axis. Each graph should have two curves corresponding to the treatments indicated in Tables B-1 and B-2.

Table B-1: Nitrate Levels (ppm)

Date	Plants	No plants

Table B-2: Phosphate Levels (ppm)

Date	Plants	No plants

6) At the end of the experiment, weigh the plants again and use the following equation to calculate the percent increase in biomass:
   {100% × (final biomass – initial biomass) ÷ initial biomass}= % increase
7) Calculate the total mg of nitrate and phosphate that was consumed by the plants. The equation below is based on the fact that ppm = parts per million = mg per liter of water:
   (initial ppm – final ppm) × total liters = total mg consumed

8) Use this to calculate mg of nitrate and phosphate consumption per gram of plant growth:

{mg $[NO_3]^-$ or $[PO_4]^{3-}$ consumed ÷ (final biomass – initial biomass)}= mg consumed per g biomass

Questions:

1. How does aeration without an activated filter affect the ammonia levels over time?

2. How does the use of an activated filter without aeration affect ammonia levels over time?

3. What is the combined effect of aeration and an activated filter?

4. What is the percent increase in plant biomass? Show work:

5. How many mg of nitrate was consumed? Show work:

6. How many mg of phosphate was consumed? Show work:

7. How many mg of nitrate was consumed per gram of plant growth? Show work:

8. How many mg of phosphate was consumed per gram of plant growth? Show work:

9. What kinds of problem might you encounter if you disinfect your aquarium to remove a disease? How might you address this problem? *Hint: Re-read the introduction.*

10. If you were involved in a commercial process that has a high potential for the release of nitrates and phosphates into the watershed (such as feedlot cattle), what trait would you look for in a plant in order to maximize the removal of these nutrients from the water?

Assignment Checklist:

1. Did you answer all the questions?
2. Did you organize your data in a table?
3. Did you graph your data for ammonia, nitrate, and phosphate?
4. Do all three graphs have titles, axis labels, and captions?
5. Are the graphs hand-drawn?