Experiment B-45
Ammonium and Nitrate in an Aquarium

Objectives

- To learn about the nitrogen cycle in an aquarium with fish and in a pond.
- To examine the presence of ammonium and nitrate ions in an aquarium with fish, in different stages of the nitrogen cycle.

Modules and Sensors

- PC + NeuLog application
- USB-200 module
- NUL-240 Ammonium logger sensor NH_4^+
- NUL-241 Nitrate logger sensor NO_3^-

Equipment and Accessories

▪ Utility stand	1
▪ Right angle clamp	1
▪ Extension clamp	1
▪ 50 ml beaker	4
▪ Wash bottle	1
▪ Plastic container	1
▪ Pasteur pipette	4

- The items above are included in the NeuLog Utility accessories, UTL-KIT.

- For the electrode preparation process you may need more beakers.

Materials

For electrode preparation and offset:

- 200 ml distilled water
- Ammonium electrode ISA bottle (included with the sensor)
- Nitrate electrode ISA bottle (included with the sensor)
- Ammonium electrode 1000 ppm standard solution bottle (included with the sensor)
- Nitrate electrode 1000 ppm standard solution bottle (included with the sensor)

For the experiment:

- 30 ml of water from a fish aquarium
- 30 ml of tap water
- pH 4 buffer (not obligatory)
- Distilled water for the wash bottle

Introduction

Every new set up of an aquarium goes through a process of establishing bacterial colonies. These colonies also fluctuate during some periods of an older aquarium. When this process is not understood it can lead to a loss of fish.

An aquarium is a closed environment. A lot of waste exerted from the fish, from uneated food and decaying plants stays inside. Fortunately bacteria capable of converting wastes into safer by products begin to grow in the aquarium when the fish are added; still, for a period of several weeks the fish are at risk.

First stage: Ammonia is excreted from the fish which is highly toxic to them. Its unionized form, Ammonium is present when the pH is below 7 and is not toxic to fish.

Second stage: Nitrosomonas bacteria oxidize the ammonia into nitrite, which is also highly toxic to fish. They can begin rising by the end of the first week.

Third stage: Nitrobacter bacteria convert nitrites into nitrates which are not toxic to fish in moderate levels. Partial water changes keep the nitrate level low enough.

When testing the aquarium water, you will first see ammonia levels rising. A few weeks later you should see the nitrite levels rising and the ammonia level dropping. After another few weeks you should see the nitrate level rising and the nitrite levels dropping. On this stage it is safe to add to the aquarium tropical fish.

In this activity we will test an aquarium for ammonium and nitrate on different stages. Sample results for an aquarium that has already got to the last stage are included. A measurement of ammonium and nitrate in a pond or a lake is also recommended.

Procedure

Experiment setup

Caution:

It is recommended to wear personal protective equipment. Material Safety Data Sheets (MSDS) are available online.

1. Prepare the solutions listed in the materials part.

2. A water sample from an aquarium can be taken from each stage described in the introduction and the same experiment can be repeated for each of them in order to observe the nitrogen cycle in an aquarium.

3. For the sample experiment, a sample from an old aquarium (an aquarium that reached the third stage) was taken.

Electrode preparation - recommended to be done by the teacher beforehand)

4. Remove the protective cap encasing the ammonium and nitrate probe's electrode. Do not touch the PVC membrane at the end of the probe with your fingers.

5. Rinse the electrodes with distilled water and blot dry. Be sure not to rub it as this will harm the sensor.

6. Soak the electrodes in distilled water for 10 minutes, and then soak the ammonium electrode in a diluted ammonium standard solution (1 ppm) and the nitrate electrode in a diluted nitrate standard solution (1 ppm) for two hours until ready for use.

   For preparing the 1 ppm standard solution (a separate one for each electrode), mix one drop of the 1000 ppm standard solution (there is one for the nitrate electrode and one for the ammonium electrode) with 20 drops of ISA (there is one for the nitrate electrode and one for the ammonium electrode) and add distilled water up to a total of 40 ml solution (assuming one drop equals 0.04 ml). It is also possible to use a micropipette if available.

7. Prepare the offsetting solutions (a separate one for each electrode): Mix 1 ml of the ISA solution (there is one for the nitrate electrode and one for the ammonium electrode) with 0.5 ml of the 1000 ppm standard solution (there is one for the nitrate electrode and one for the ammonium electrode). Add distilled water up to a total of 50 ml. You will get two 10 ppm solutions, one for the nitrate electrode and one for the ammonium electrode.

- It is possible to add ISA to the test samples (with a 1:50 ratio) for a higher ionic strength, although for this fast measurement it is not necessary.

Sensor setup

8. Connect the USB-200 module  to the PC.

9. Check that the ammonium  and nitrate  sensors are connected to the USB-200 module in a chain.

Note:

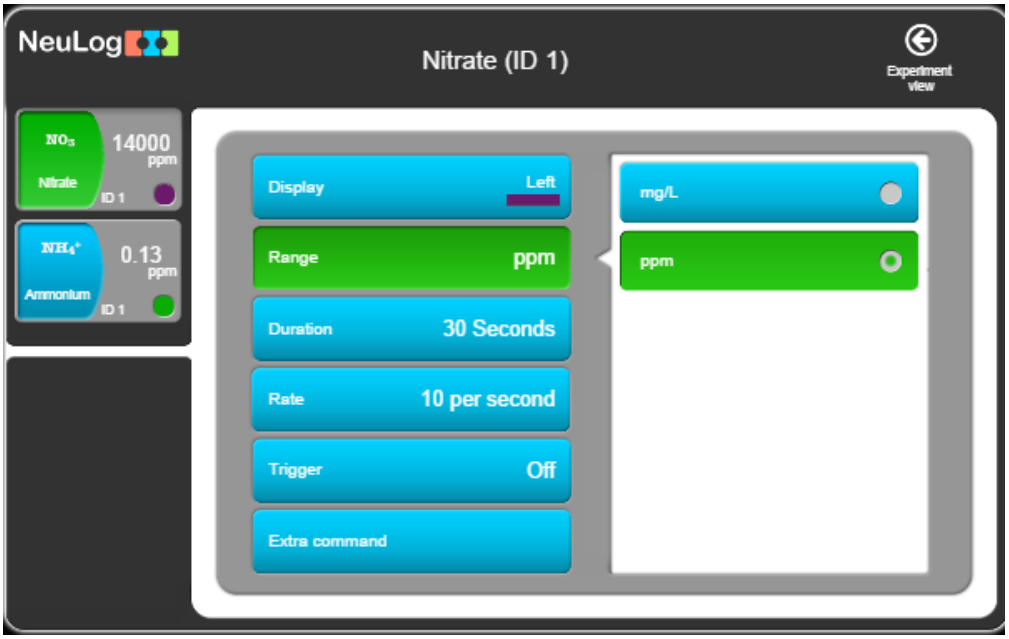
The following application functions are explained in short. It is recommended to practice the NeuLog application functions (as described in the user manual) beforehand.

10. Run the NeuLog application and check that the sensors are identified.

Settings

11. Click on each **Sensor's Module** box at a turn.

12. Select the "ppm" button to change the sensors' ranges.



13. This experiment is done in single step mode so the experiment duration and sample rate will not be set.

Testing and measurements

14. Sensor offset: Prepare the electrode as described in the "Electrode preparation" part. If you have already prepared it in the last three days and stored it in a 10 ppm standard solution (the same as the offsetting solution), you do not have to repeat it before offsetting the sensor.

15. Rinse each electrode with distilled water, blot dry and place it in the matching offsetting solution.

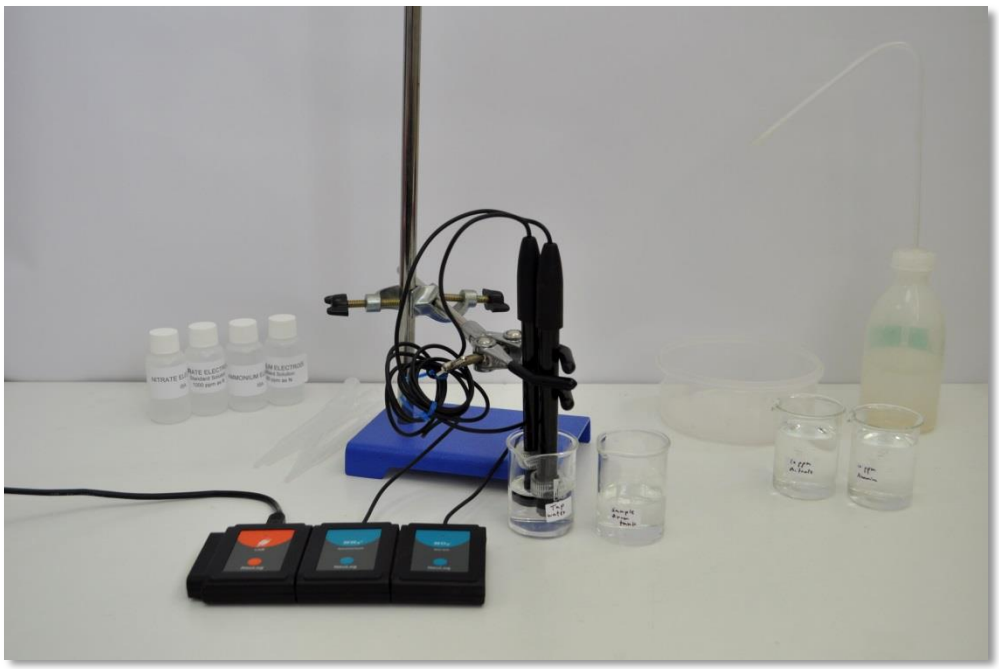
16. Press the "Start/Stop" button located on the sensors' faceplate for 4 seconds.

17. Rinse the electrodes once more with distilled water and blot dry.

18. Attach the two electrodes together to the extension clamp connected to the utility stand. This is for measuring ammonium and nitrate together. Another option is to connect first the nitrate electrode, make the measurements and then connect the ammonium electrode.

19. Ammonium can be detected better at a low pH. Adding some acidic buffer (not lower than pH 4) to the sample may help detect the ammonium better.

20. Place the beaker with the tap water underneath the electrodes and insert them into the beaker.

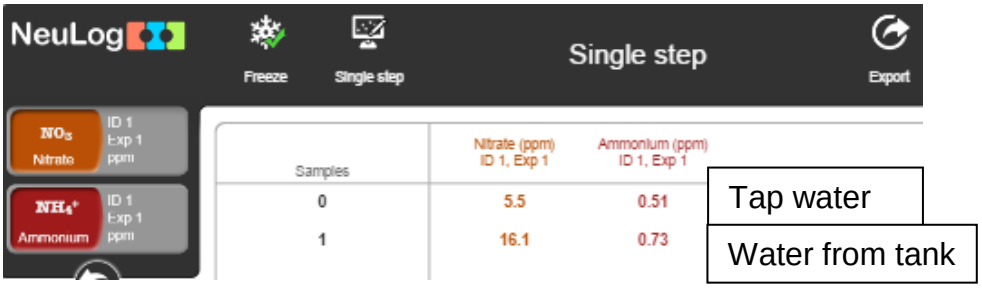


21. Click on the **Single step** icon and then on the **Table** icon .

22. Rinse the electrodes with distilled water and then replace the tap water beaker with the sample from the aquarium.

23. Click on the **Single step** icon .

24. The following table includes measurements of tap water and an old aquarium sample.



25. Click on the **Export** Icon  and then on the **Save value table (.CSV)** button to save your graph.

26. We can see that the aquarium has higher levels of ammonium and nitrate compared to tap water. Nitrate levels can reach 20 ppm before it is harmful for the fish. Ammonium levels are very low, and stayed low even after the pH 4 buffer was added (not shown). This means the ammonia levels in the aquarium are very low and the fish are at a good condition.

Challenge research

27. Repeat the experiment, this time outside at a pond or a lake, in offline mode (offline mode instructions can be found in the NeuLog user guide or in the NeuLog sensors general guide at www.NeuLog.com).

Summary questions

1. Describe your results and explain them in terms of the nitrogen cycle stages.

2. How would nitrate levels affect algae growth in an aquarium or a pond? How would that complete the nitrogen cycle that involves the fish?