



Activity 5 - Nitrate Test

Introduction

Nitrogen is essential to the construction of protein in all living plants and animals. It can be found in many forms throughout aquatic ecosystems. Nitrogen is in fact a much more abundant nutrient than phosphorus in nature and is most commonly found in its molecular form (N_2), which makes up 79 percent of the air we breathe. Nevertheless, this form is useless for most aquatic plant growth.

Blue-green algae, the primary algae of algal blooms, are able to use N_2 and convert it into forms of nitrogen that plants can take up through their roots and use for growth: ammonia (NH_3) and nitrate (NO_3).

Aquatic animals obtain the nitrogen they need to form proteins using two methods. They can eat aquatic plants and convert plant proteins to specific animal proteins, as well as eating other aquatic organisms which feed upon plants. As aquatic plants and animals die, bacteria break down large protein molecules into ammonia. Ammonia is then oxidized (combined with oxygen) by specialized bacteria to form nitrites (NO_2) and nitrates (NO_3). These bacteria get energy for metabolism from oxidation.

Despite being rich in ammonia, the excretions of aquatic organisms do not contribute large quantities of nitrogen to waters. However, the excrement of duck and geese, contribute a heavy load of nitrogen. Nitrogen that was previously "locked up" is released through a reaction of the decomposition of dead plants and animals, and the excretions of living animals. Furthermore, there exists a bacteria that can transform nitrates (NO_3) into free molecular nitrogen (N_2). The **nitrogen cycle** begins again if this molecular nitrogen is converted by blue-green algae into ammonia and nitrates. Because nitrogen, in the form of ammonia and nitrates, acts as a plant nutrient, it also causes eutrophication. As you learned in the Total Phosphate Test, eutrophication promotes more plant growth and decay, which in turn increases biochemical oxygen demand. However, unlike phosphorus, nitrogen rarely limits plant growth, so plants are not as sensitive to increases in ammonia and nitrate levels.

Sources of Nitrates

Humans contribute nitrates to rivers and lakes in the form of sewage which enters in a number of forms. As a result of inadequately treated wastewater from sewage

treatment plants, in the effluent (outflow) from illegal sanitary sewer connections, and from poorly functioning septic systems.

The septic systems are usually found in rural areas, as apposed to the large, centralized urban sewer systems that collect waste from many households. Rural septic systems usually can only treat the waste from a single household.

In a septic system, household wastewater from toilets, sinks, bathtubs, and washing machines flows through a main pipe into a box called a septic tank. After larger waste materials settle and floating grease is skimmed off, the remaining liquid then flows through a grid of perforated pipes. The holes in these pipes allow the liquid to trickle out onto a layer of stone, gravel, and soil known as the "drain field".

When a septic system works correctly soil particles remove nutrients like nitrates and phosphates before they reach groundwater. However, this relies on two important factors. Firstly, septic systems must be properly located. When septic system drain fields are placed too close to the water table, nutrients and bacteria are able to percolate down into the groundwater where they may contaminate drinking water supplies. They may also find their way into lakes or rivers via groundwater flow.

Secondly, septic tanks must be regularly emptied. If the tank is full, household wastes go directly to the drain field instead of settling in the tank. When this happens, the drain field pipes may become plugged, and household sewage may start to pool on the ground and enter water through surface runoff.

Methemoglobinemia (met-hemo-glo-bin-emia) is a serious condition resulting from dangerous nitrate levels in water. When used for milk formula this can prevent the baby's blood from carrying oxygen; hence the nickname "blue baby" syndrome.

Fertilizers and the runoff from cattle feedlots, dairies, and barnyards are two other important sources of nitrates in water. As a result of excessive fertilizer use high nitrate levels have been discovered in groundwater beneath croplands, especially in heavily irrigated areas with sandy soils. Storm water runoff can carry nitrate-containing fertilizers from farms and lawns into waterways. Equally producing large amounts of wastes with high volumes of ammonia and nitrates are places where animals are concentrated, such as feedlots and dairies. If not properly managed, these can seep into groundwater or be transported in runoff into surface waters.

As discussed in the Total Phosphate section, people have created the eutrophication problem that threatens to limit organism diversity, recreational opportunities, and property values. Only we can reverse eutrophication through thoughtful action.



Equipment

- MultiLogPRO
- Nitrate selective sensor
- Two standard solutions that differ in concentration by a factor of ten. The standards should preferably be at the same temperature as the sample
- 150mL beaker
- 10mL and 100mL pipettes
- ISA solution
- Wash bottle with distilled water

MultiLogPRO Setup

Sensors

Input 1: ISE

Rate:

Every second

Recording time:

500 samples (500s)

Experimental Procedure

Sampling Procedure

Any sampling device might be used for this water quality test to obtain representative samples. It is important for the experiment that the glassware used is clean and has been rinsed with de-mineralized water. Always use de-mineralized water during the nitrate test, Distilled water contains ammonia (NH_3) ions that will interfere with the test. The water sample for the Nitrate test should be collected away from the river bank and below the surface. If possible, use an extension rod sampler.

The sample can be measured on site or in the lab. If sample cannot be measured within few hours, the sample should be placed on ice for storage

Testing procedure


Experiment setup

1. Slide the sleeve of the electrode cap down to uncover the fill hole
2. Rinse the electrode with DI water, blot dry. **Do not rub dry**
3. Condition the electrode in a 10ppm solution for 30 minutes
4. After the conditioning period, rinse the tip of the electrode with DI water
5. Turn on MultiLogPRO
6. Set MultiLogPRO up according to the setup specified above
7. Connect the electrode to the ISE amplifier, then connect the amplifier to input 1 (I/O-1) of MultiLogPRO

Preparing a calibration curve

1. Place 100mL of the more dilute standard into a 150mL beaker. Add 2mL of ISA. Stir thoroughly



2. Push Enter  on MultiLogPRO's keypad to begin recording
3. Rinse electrode with DI water, blot dry and place in the beaker. Wait for a stable reading, and then record the voltage reading
4. Measure 100mL of the more concentrated standard into a second 150mL beaker. Add 2mL of ISA and stir
5. Rinse electrode with DI water, blot dry and place in the second beaker. Wait for a stable reading, and then record the voltage reading of the second standard
6. On a semi-logarithmic graph paper, plot the voltage readings (linear axis) against the concentration (logarithmic axis)



Taking measurements

1. Pipette 100mL of sample into a 150mL beaker. Add 2mL of ISA. Stir thoroughly
2. Rinse electrode with DI water, blot dry and place in the sample beaker. Wait for a stable reading and record the voltage reading
3. Use the calibration curve to determine the sample's concentration
4. Record the sample's concentration in your data sheet