Nutrient uptake by microalgae in tap water, wastewater and copper (Cu) amended wastewater

Belief Neupane

Bachelor's thesis
Bachelor's degree in
environmental engineering

TAMPEREEN AMMATTIKORKEAKOULU
Tampere University of Applied Sciences

ABSTRACT

Tampereen ammattikorkeakoulu
Tampere University of Applied Sciences
Bachelor's degree in Environmental Engineering

Belief Neupane
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In this work the ability of different strains of microalgae to remove nutrients from wastewater was studied. Ammonium, nitrate, phosphate concentration, pH and conductivity were measured throughout the experiments. Batch system was used for growing the eight different strains of algae in mono as well as combined culture by using Substral (an artificial fertilizer) as nutrient source. Algae strains that showed better growth rate were chosen from the growth culture and used in the first batch experiment run in which growth medium was changed from tap water to wastewater. The nutrient uptake decreased over the two weeks test period. In a second batch experiment run the wastewater was amended with Copper but otherwise had the same setting as the previous run. Nutrient concentrations for ammonium and nitrate and phosphate also showed a decreasing trend throughout the two week time of the experiment. In the last experiment run the Algae Turf Scrubber (ATS) system was used to test the nutrient removal ability of algae in a continuous process from a Copper amended wastewater. The wastewater used in the experiments was from the municipal wastewater treatment plant of Tampere. The ATS system removed efficiently –below detection limit- the P within two days of testing. Nitrogen concentration decreased as well within the first two days, with 40% but increased after that. The removal of nitrogen needs to be studied in further studies.

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GLOSSARY or ABBREVIATIONS AND TERMS

AAS	Atomic Absorption Spectrometer
ATS	Algae Turf Scrubber
B	Boron
Br	Bromine
C	Carbon
Ca	Calcium
Cd	Cadmium
Cl	Chlorine
Cu	Copper
Fe	Iron
H	Hydrogen
I	Iodine
K	Potassium
Mn	Manganese
Mo	Molybdenum
Mg	Magnesium
N	Nitrogen
Na	Sodium
O	Oxygen
P	Phosphorous
S	Sulphur
Si	Silicon
V	Vanadium
Zn	Zinc

1 INTRODUCTION

1.1 Nutrients in nature and their uses by microalgae

Chemicals such as carbon (C), nitrogen (N), phosphorous (P), sulfur (S), calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), manganese (Mn) and sodium (Na) are nutrients found in nature. They promote healthy growth in living organisms. Ionic forms of these nutrients contribute to the conductivity of water (Naiman & Bilby 2001).

In the case of observation of growth in different species of seaweed, the availability of nutrients is one primary factor regulating their growth, reproduction and biochemistry (Lobban & Wynne 1981). Biochemistry in seaweeds is the sum total of all the processes such as respiration, excrition, digestion, immune responses, hormon production and other processes. This determines and synthesizes seaweed's biochemical composition and their biochemical influences to their immediate surroundings.

In the book, the biology of seaweeds, seaweeds are described as photoautotrophic organisms. All phototrophic organisms green plants, photoautotrophic bacteria including algae, uses sunlight to produce organic matter from mineral ions and water. Some species of seaweed are also auxotrophic, i.e. they require small amounts of organic growth simulators such as vitamins (Lobban & Wynne 1981).

Further explanations on nutrient requirements in the book, the biology of seaweeds, suggest that plants (including all algae species) require 14-21 specific elements. Elements C, H, O, P, N, Mg, Fe, Cu, Mn, Zn, Mo, S, K and Ca are all required by algae, although S, K and Ca can be replace to some extent by other elements. In addition, Co (Cobalt), Na, I, Br, B, V, Cl and Si are probably required by one or more algae, by O'Kelly 1974, (Lobban & Wynne 1981).

Elements carbon (C), Nitrogen (N), Hydrogen (H) and Oxygen (O) are four building blocks of life. They are found in abundance in all living creatures on earth. Among these big four, element nitrogen carries very important properties and functions for all living creatures. In different algae species absorption of nitrogen (N) is unique. The heterogeneity or simply the diversity of algae species is one factor for it. There are algae species that are able to utilize atmospheric nitrogen directly, *Cyanophyta* for example

are atmospheric nitrogen fixers. This gives us an outlook of varying nutrient uptake mechanisms in algae.

In normal seawater, main source of nitrogen exists as inorganic nitrogen in dissolved state. Nitrate (NO_3^-), nitrite (NO_2^-) and ammonium (NH_4^+) are the three inorganic nitrogen compounds that are readily used by different species of algae. These inorganic forms of nitrogen usually occur in seawater at concentrations of 1-500μM(micro moles), 0.1-50μM and 1-50μM respectively (Riley & Chester 1971). Some seaweed also makes use of organic nitrogen compounds usually at concentration of less than 20μM, by Riley & Chester 1971 (Lobban & Wynne 1981).

The inorganic nitrogen compounds exist in dissolved state in seawater and also freshwater. Nitrate and ammonium ions are effective at promoting growth in algae. These inorganic ions are abundant in seawater. Ammonium ions stimulates healthy growth rate of algae (Lobban & Wynne 1981).

The importance of these compounds is further vilified by the storage mechanisms present in algae. Vacuoles in algae trap nitrates when it is supplied in abundance. These are stored in algal cell body and acts as reserves during the time of shortage of nitrogen (usually in summer months). The optimum N concentration in seawater is 120-2140μM (Lobban & Wynne 1981).

Elements phosphorous in seawater exists in different ionic state, mainly as HPO_4^{2-}, PO_4^{3-} & $H_2PO_4^-$ in dissolved state. These are limiting nutrients, meaning at lower concentration of these ions algae shows its highest activity (higher rate of absorption of these ions). Phosphorous in sea exists in the form of orthophosphate ions. Polyphosphate ions, occurs in coastal and estuarine water results from deposition of detergents. Algae, however, have not shown the use of these polyphosphate ions. Optimum phosphate concentration is 32-320 μM and similar to storage mechanism of nitrate in algae (vacuoles), phosphorous are likewise stored in vacuoles (Lobban & Wynne 1981).

Algae makes use of different metals such as zinc, iron, cobalt, arsenic, copper and others. These elements facilitate swift metabolic activity within the algae.

Let us take copper and its position in algae. It is generally accepted that all algae have a micronutrient, copper (Cu), requirement. Most algae are found sensetive to the concentration of copper and copper complexes. Copper is found in plastocyanin and amine oxidase. These molecules are photosynthetic enzymes found in the electron transport chain (Lobban & Wynne 1981).

Among all the array of elements that algae makes use of, the elements N,P and Cu carries important functions that helps algae grow well in suitable conditions. Presence of these elements are major determinant in monitoring of metabolic changes within and immediate environment (growing medium).

We now know that ionic forms of these elements such as Nitrate (NO_3^-), nitrite (NO_2^-) ammonium (NH_4^+), HPO_4^{2-}, PO_4^{3-} , $H_2PO_4^-$, Cu and other elements and ions are readily absorbed and utilized by growing algae (Lobban & Wynne 1981).

1.2 Nutrient uptake mechanisms in algae

Nutrient ions (cations & anions) diffuse along electrical potential gradient while uncharged molecules may diffuse along chemical potential gradients across the algal cell membrane (Lobban & Wynne 1981).

The fluxes in the ionic concentration outside the cell membrane (its immediate surrounding) and inside the cell membrane (cytoplasm) may result from passive diffusion, facilated diffusion, exchange diffusion or active transport. These are transport mechanisms occurring in all living organisms on earth. Passive diffusion of an ion is the difference of electrochemical potential between two points along the path. Facilated diffusion across the cell membrane includes facilitator molecules (gates). They are protein molecules. Sometimes these molecules are called charged pore or also they might be recognized as carrier proteins.

Among the diffusion mechanisms discussed in the previous paragraph, active transport is very important to algae during nutrient uptake. Active transport is the transport of material across a membrane against an electrochemical gradient, i.e. from area or low ionic concentration to higher concentration. In the case of transport of ions by exchange diffusion, a particular ion is required for the carrier membrane to function. For example, solute A is pumped against a concentration gradient by active transport. Solute B is then

allowed to cross the membrane through an exchange process with solute A (Lobban & Wynne 1981).

1.3 Nutrient uptake kinetics

In simple diffusion of ions, the rate of uptake of a particular ion is generally proportional to the external concentration. This means, higher the ionic concentration in the immediate environment the higher is the uptake rate. This however does not apply to facilitated diffusion and active uptake. In these processes the uptake rate exhibits 'saturation kinetics'. At low substrate concentrations, the rate of uptake increases sharply with increasing external concentrations but, at progressively higher concentrations, each added increment of concentration adds less and less increment in uptake rate until, at the highest concentration used, uptake is virtually independent of the concentration in the solution.

1.4 Environmental factors influencing the nutrient uptake

We have now understood at least one factor that affects nutrient uptake. It is the substrate or simply nutrient ion concentration at the immediate environment.

All algae when studied for their nutrient uptake behavior shows genetically controlled differences in the nutrient uptake capacities. There are several internal and external factors influencing nutrient uptake by cells.

Light is the foremost among all. Algae are photosynthetic organisms; this is the underlying statement that vilifies the importance of light. Light can and does influence nutrient uptake in many ways.

1. It provides the energy for active transport
2. It produces carbon skeletons which acts as a sink for incorporated ions
3. It provides energy for the production of charged polymers which establishes Donnan potentials
4. It stimulates growth which results in increased nutrient uptake and
5. Increase protein synthesis

Temperature on the other hand involves in the rate of diffusion, carrier mediated uptake and cell metabolism in general. The uptake of ions from the external medium will also be influenced by the concentration of competing ions and ions which may suppress carrier or enzyme systems.

Concentration of ions in the cytoplasm and in vacuoles may also influence the rate of ion uptake.

Water movement is another factor influencing the rate of uptake. The rate of uptake of nutrient by algae in water with low turbulence area is low than that of areas with high turbulent water flow. Seaweeds may grow profusely in very turbulent but low nutrient environments such as the areas along many rocky coastlines. The main reason why the nutrient uptake is high in turbulent water is the nutrient mixing although it may also damage seaweeds physically, limit predatory or competitive species and decrease available light.

1.5 Algae

Algae are simple microorganisms, they are generally found in both marine and freshwater ecosystems. There are various kinds of algae which can be differentiated physiologically and morphologically. Freshwater ecosystems, such as lakes, rivers, ponds generally have algae freely floating or attached in the benthic layer of such ecosystems. They are small; it requires a light microscope to observe them. The basic shapes of algae are well represented in picture 1 below. The characteristics of algae are also determined with the physical structure of these organisms. Some are motile; they use the aid of their tail for movement. These freely moving are the algae that are mostly available on the surface of the lake. (Bellinger, 2010)

‘Algae’, is Latin word for seaweed. The word ‘Algae’ is now applied to a broad group of simple organisms. Morphologically, algae are simple microorganisms; they are plants which cannot be divided into roots, stems, flowers and leaves-and their sexual organs are not enclosed within protective coverings. Algae are either prokaryotes (cells that lack membrane bound nucleus) or eukaryotes (cells with membrane bounded nucleus and organelles). Physiologically, algae generally falls under the trophic level photo autotrophs with exceptions of some heterotrophic algae (benthic). Photoautotrophs are producers; they trap the energy of the sun into organic molecules such as starch and lipids (photosynthesis) (Bellinger, 2010).

1.6 Grouping of the algae species: *Selenastrum capricornutum, Pediastrum simplex, Synechococcus sp., Anabaena cylindrical, Scenedesmus sp., Chlorphyta sp., Haematococcus pluvialis*

1.6.1 ***Selenastrum capricornutum*:** *Selenastrum capricornutum* falls under the phylum *Chlorophyta,* and belongs to the group green algae (A.Pascher, 2014).

1.6.2 ***Pediastrum simplex*:** Phylum of *Pediastrum simplex* is *Chlorophyta.* It belongs to the group green algae (algaebase, 2014).

1.6.3 ***Synechococcus sporangium*:** Phylum of *Synechococcus sporangium* falls under phylum *Cyanobacteria* and belongs to the group blue green algae (algaebase, 2014).

1.6.4 ***Anabaena cylindrical:*** *Anabaena cylindrical* falls under the group *Cyanophyta* (bioweb.uwlax.edu, 2014).

1.6.5 ***Chlorphyta sp.:*** *Chlorphyta sp*. falls under the group green algae.

1.6.6 ***Scenedesmus sp*.:** *Scenedesmus sp.* falls under the group green algae (www.britannica.com, 2014).

1.6.7 ***Haematococcus pluvialis*** Phylum of *Haematococcus pluvialis,* falls under phylum *Chlorophyta.* It belongs to the group green algae (www.algaebase.org, 2014).

2 Aim

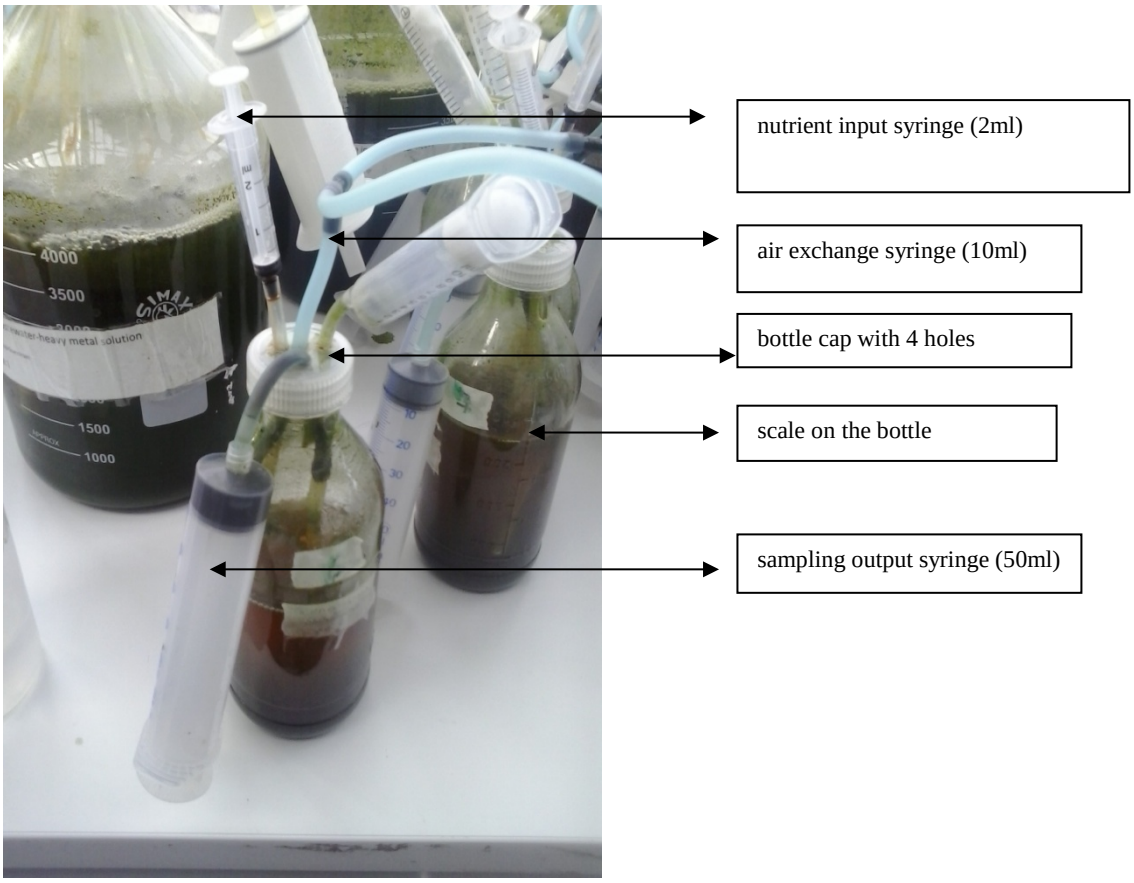
The aim of this work was to grow algae in tap water, wastewater and copper (Cu) amended wastewater and test how they remove nutrients.

3 Materials and methods

3.1 Preparation of the bottles for growing algae (Batch process)

Algae strains were cultured in bottles as shown in the picture below.

First step of the preparation of the algae culture bottles was the preparation of the bottle caps. A regular drill was used to drill four holes on each cap. Outcome of this step is represented in the picture below.



Picture 3.1.1 small bottles for growing algae

Holes in these caps serve as openings for:

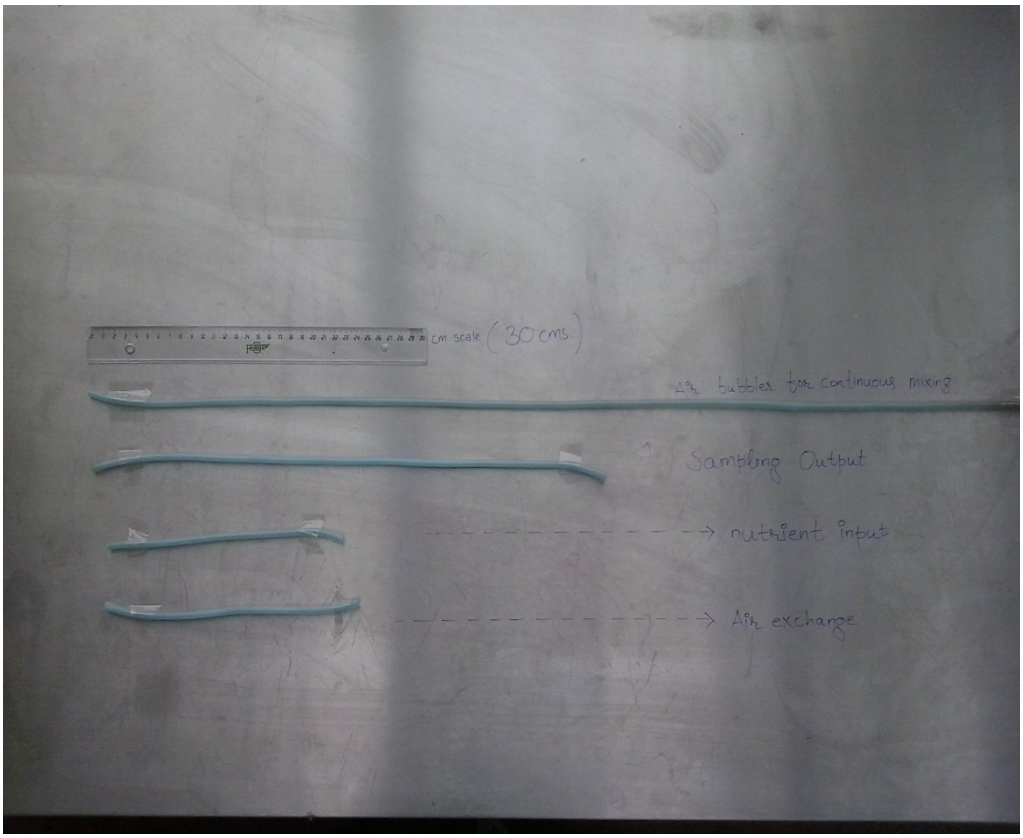
1. air exchange
2. nutrient input
3. air bubbler input for regular mixing and
4. sampling output

Eight bottle caps, shown in picture 3.1.3 for eight glass bottles (600ml), shown in picture 3.1.1. Each bottles had scale on it.

Three syringes were taken. Volumes of the syringes were 50ml, 10ml and 5ml respectively. These syringes served for the purpose of sampling, air exchange and nutrient input. Sampling of the algae was carried out for the measurement of nutrients, pH, and conductivity, total phosphorous and total nitrogen. Syringe with volume (50ml) was used for this purpose.

Similarly, the 10ml syringe was taken. This syringe was for the air exchange. Piston was removed from the syringe. The empty syringe (10ml) was filled with a ball of cotton. This step ensured that only air was coming out of the culture bottle.

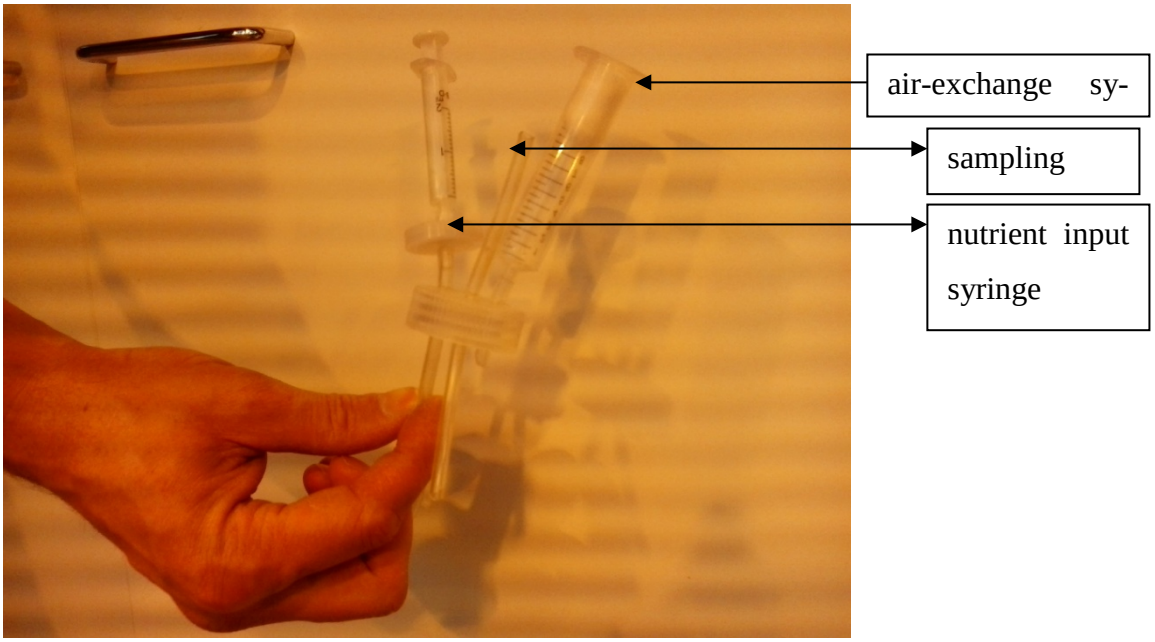
Lastly, the 2ml syringe was taken for the purpose of nutrient input.



Picture 3.1.2 pipe setup for small bottles

Four plastic pipes with dimensions 84 cm, 47 cm, 23 cm, 20 cm were cut accordingly as shown in picture 3.1.2. These pipes were fitted one by one to the holes in the bottle cap. The pipe with dimension 20 cm served for the purpose of air exchange. Air exchange syringe was attached to it. Similarly, nutrient input syringe was attached to the pipe with the measurement 23cm. Air bubbler was attached to the longest pipe of all four pipes (84cm). Lastly, the pipe with the length 47 cm was attached with the sampling output syringe.

Bottle cap after attaching the syringes looked like in the picture 3.1.3



Picture 3.1.3 bottle cap setup

Algae growth culture setup looked like in the picture 3.1.4

Picture 3.1.4 small bottle culture

This experimental setup for the preparation of the algae culture bottles were executed throughout the experiment. Design of this bottle made sampling easier. Moreover, it ensured sterile condition inside the bottle. Air exchange syringes allowed regular inter-change of oxygen and carbon dioxide inside the bottle. Feeding of the algae with the nutrient was carried out with the nutrient input syringe.

3.2 Growing the algae in the bottles

Following algae species were first ordered from SYKE.

1. *Selenastrum capricornutum*
2. *Pediastrum simplex*
3. *Synechococcus sp.*
4. *Anabaena cylindrical*
5. *Scenedesmus sp.*
6. *Chlorphyta sp.*
7. *Purpuraemus sp.(not described in the introduction)*
8. *Haematococcus pluvialis*

After getting these strains of algae, each species of algae was transferred to the 600ml glass bottle one by one.

Growth medium of these algae was tap water. All bottles were filled with 400 ml of tap water one by one. The bottles were then sealed with the prepared bottle caps (picture 3.1.3). Setup shown in the picture3.1.4 represents the outlook of this step. The bottles were labeled with the name of the algae species listed above in the first paragraph of this section.

7ml of substral (liquid fertilizer) was transferred into each bottle with the help of nutrient input syringe (5ml). This step was carried out to boost the algae growth. Addition of nutrient and the date of addition are shown below.

Date of addition	amount of nutrient (substral) in ml
17.02.14	2
24.02.14	2
28.02.14	1
26.05.14	2

Table 3.2.1 Addition of nutrient (substral) for boosting algal growth in the growth culture bottles

3.3 Preparation of mono and single culture

Mono and single culture of algae strains mentioned in 3.2 were carried out in big bottles. Six bottles with volume of about six liters were prepared for growing algae. The bottle corks for the culture were prepared with the same steps mentioned in section 3.1.

Growing medium for mono and single culture was tap water. 10ml of algae from the singular culture bottle were transferred to each bottle. The big bottles were then filled with 4 liters of water.

Liquid fertilizer, substral, was added to each bottle for boosting the growth of the culture. The amount of ‘substral’ added after the addition of 4 liters of water was 27ml.

Each bottle was labeled as SP1, SP2, SP3, SP4, SP5 and SP6 respectively. Strain of algae present were mentioned in the bottle labels.

Picture 3.3.1 algae culture in big bottles

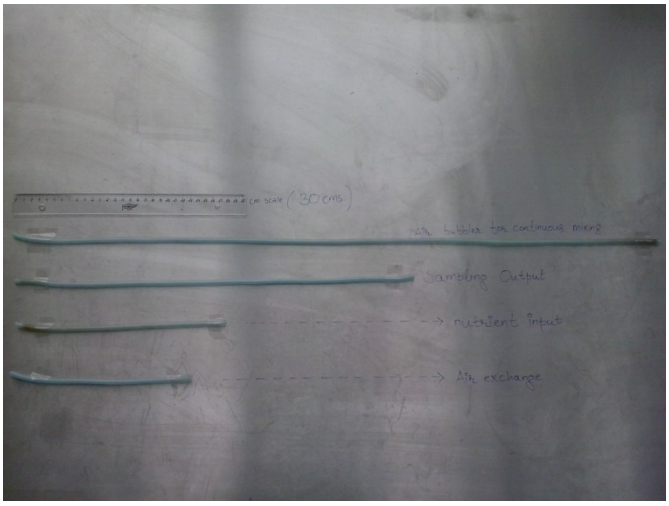
Table 3.3.1 gives information on the specific label and species present in each bottle.

Bottle name	Species of algae present
SP1	*Selenastrum capricornutum, Pediastrum simplex, Synechococcus sp., Anabaena cylindrical, Scenedesmus sp., Chlorophyta sp., Purpuraemus sp., Haematococcus pluvialis*
SP2	*Selenastrum capricornutum, Pediastrum simplex, Chlorophyta sp., Haematococcus pluvialis*
SP3	*Anabaena cylindrical, Scenedesmus sp.*
SP4	*Selenastrum capricornutum*
SP5	*Pediastrum simplex*
SP6	*Chlorophyta sp.*

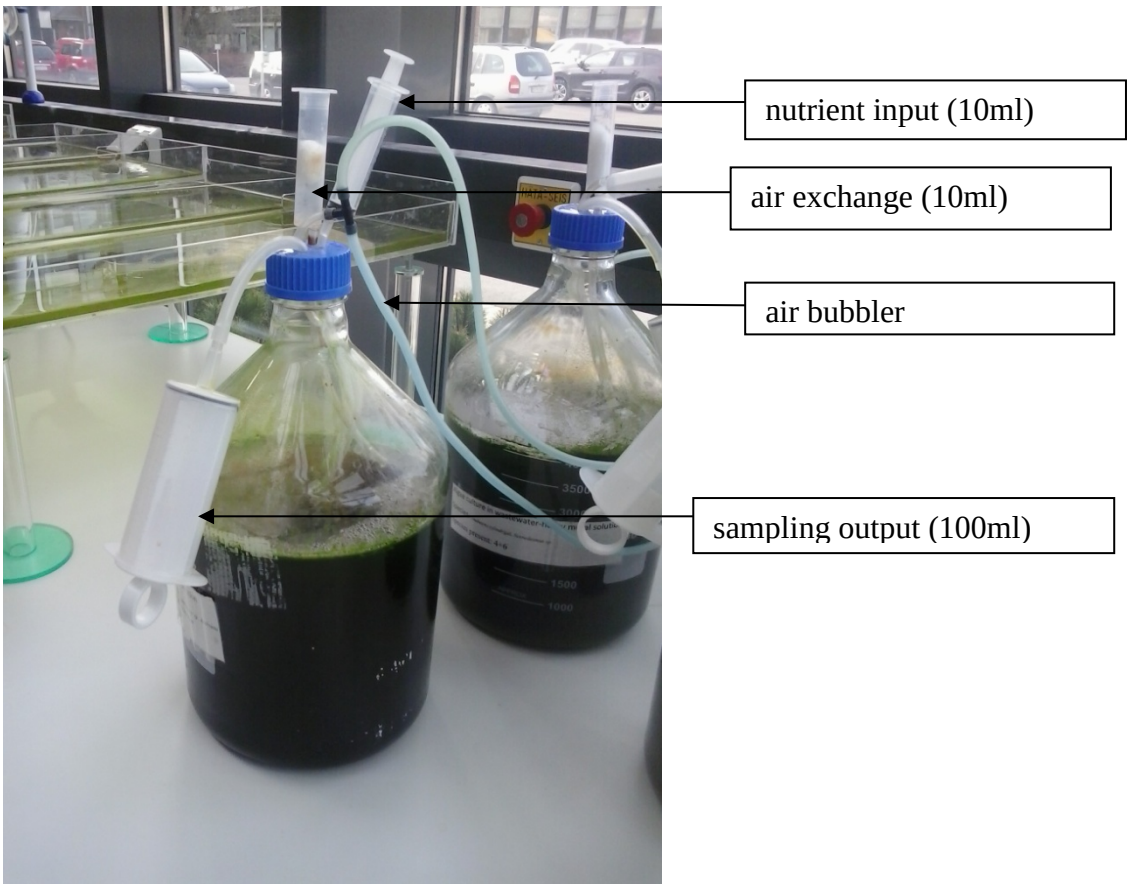
Table 3.3.1 bottle label name and the particular species present in each bottle

Note: Pipe setup for this step is slightly different compared to the singular strain culture in section 3.2. Dimensions (shown in picture 3.1.2) of the pipes were changed to 86 cm, 53 cm, 29 cm and 23 cm for air bubbler, sampling output, nutrient input and air exchange(shown in the picture 3.3.2) .

Picture 3.3.2 represents the outlook of this step.



Picture 3.3.2 big bottle pipes and its label



Picture 3.3.3 big growth bottle with syringes

Note: Volume of the syringes (with volumes shown in the picture 3.3.3) for sampling output, nutrient input and air exchange are (100ml, 10ml, 10ml respectively). Syringe setup corresponds to the syringe setup described in 3.1

3.4 Preparation of duplicate for each bottles (test run 1)

After 30 days, the mono and single culture were fully grown and ready to be used as seed. Algae seed was transferred to wastewater. Hilda Szabo, principle lecturer at TAMK University of applied sciences recommended the use of two bottles for each species (in mono as well as combined). The bottles were prepared by exactly following steps in 3.1. The picture 3.4.1 shows the setup in more detail. The amount of wastewater added to replica (B1-B7) is shown below.

Replica name	Nutrient (waste water added) (ml)	volume of algae add-ed
B1	400	40 ml from bottle SP1
B2	400	40 ml from bottle SP2
B3	400	40 ml from bottle SP3
B4	400	40 ml from bottle SP4
B5	400	40 ml from bottle SP5
B6	400	40 ml from bottle SP6

Table 3.4.1 preparation of blanks for test runs

Similarly, in replica (T1-T7), 10 ml of 33, 5 mg/l Cu was added.

Replica name	Nutrient (waste water added) (ml)	Cu (33,5 mg/l) add-ed	volume of algae added
T1	400	10ml/bottle	40 ml from bottle SP1
T2	400	10ml/bottle	40 ml from bottle SP2
T3	400	10ml/bottle	40 ml from bottle SP3
T4	400	10ml/bottle	40 ml from bottle SP4
T5	400	10ml/bottle	40 ml from bottle SP5
T6	400	10ml/bottle	40 ml from bottle SP6

Table 3.4.1 preparation of test bottles for test runs

3.5 Preparation of Algae Turf Scrubber (ATS)

Algae Turf Scrubber (ATS) was developed in the year 1980 by Walter Adey of Smith-sonian Institution. ATS is used in many wastewater treatment projects all over the world.

ATS, present at TAMK and was designed in TAMK laboratory (Grobler G., 2012). It is made of poly acrylics. Dimensions of ATS is (LxBxH), 1000mm X 700mmX 100mm. ATS basin has four equal section of dimension (250mm X 175mm). Each section is like steps in a ladder with height of 1-2 cm. Entire system has the capacity to hold 25 l; a storage vessel with dimension (1000mm X 200mm X 200mm) is included.

Basins of the ATS were propped up on pedestals and water was pumped from the vessel into the basin. Water fell from the top section to the bottom in steps. Wastewater in the ATS is continuously mixed with the help of air bubbler.

Picture 3.5.1 Algae Turf Scrubber (ATS)

Algae strain from the culture bottle SP2 was chosen as the test culture in the ATS. The reason behind the selection of the strain was the efficiency it showed in consuming Copper during test run 1 (small bottle experiment with wastewater). Rough calculation of the amount of algae sample to be transferred from bottle SP2 was made. Volume of algae added was 337,5ml.

Then, 225ml of copper was added to the system. Concentration of copper was 33,5mg/l. Twenty litres of wastewater was added to the system table 3.5.1 shows the addition of wastewater to the ATS system. Two ATS systems were used for the preparation of ATS Cu1 and ATS Cu2 respectively.

Third ATS system did not contain any copper. This was named Blank.

Date of Addition	Amount of waste water added to the ATSCu1, ATS Cu2 and ATS Blank
13/05/14	4l
14/05/14	4,5l
16/05/14	3l
20/05/14	15l
23/05/14	5l

Table 3.5.1 addition of wastewater to the ATS systems

3.6 Method of measurement of the nutrient in the growing medium

3.6.1 YSI Professional plus

YSI professional plus was used to measure the concentration of ammonium in mg/l, pH and conductivity. The analysis was carried out daily throughout February, 2014-June, 2014 time period.

(See Appendix 3, 4 and 5 for directions of use)

3.7 HACH

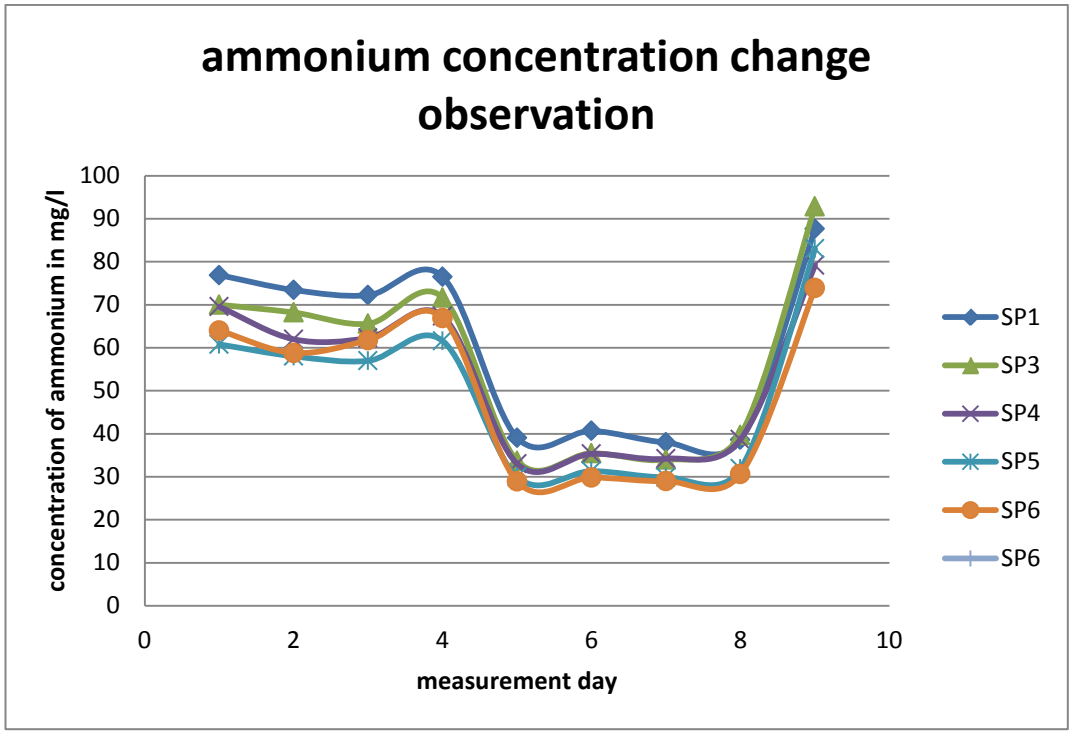
Nitrate-nitrogen and total phosphate-phosphorous was measured using HACH system. The analysis was carried out daily throughout February, 2014-June, 2014 time period.

The nitrate-nitrogen concentrations were analyzed by using nitra-ver5 powder willows. Moreover, the HACH machine was operated throughout February, 2014-June, 2014 time period to calculate phosphate concentrations. To calculate the phosphorous concentrations in the samples, phosphorous kit LCK-349 was used.

(See Appendix 1 and 2 for directions of use)

4 Results and Discussion

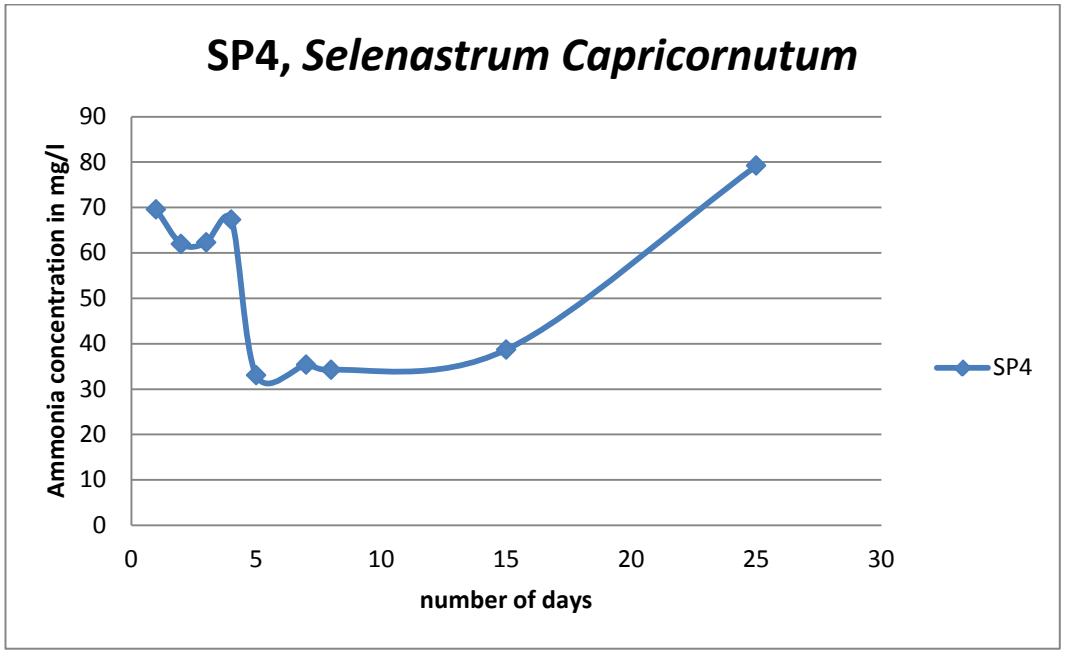
4.1 Ammonium concentration change in SP1-SP7



Graph 4.1.1 change of ammonium concentration in bottles SP1-SP7

Concentrations of ammonium in the big culture bottles are shown in graph 4.1.1. Measurements dates are between the dates 31.03.2014-24.04.2014. The graph above shows that ammonium concentration reaches its lowest level on the measurement day five.

4.2 Ammonium concentration change



Graph 4.2.1 change of ammonium concentration in bottle SP 4

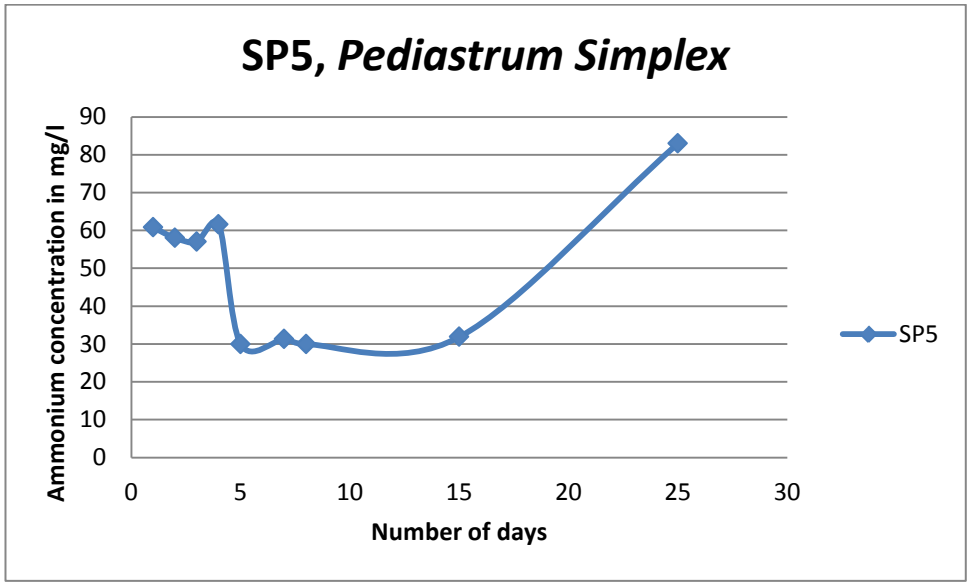
On the day 07/03/14, 27 ml of SUBSTRAL was added to the bottle. The algae strain *Selenastrum capricornutum* was left to grow. Measurements from the device, YSI professional plus was used to monitor the changes in ammonium concentration of the growth medium. The growth medium was tap water.

The changes in the ammonium concentration between the dates 31/03/14 to 05/05/14 is shown in graph 4.2.1. The rate of uptake of ammonium ions by *Selenastrum capricornutum* increases from 31/03/14 to 04/04/14. The graph shows the increased ammonium uptake until the fifth day (04/04/14). The highest uptake rate of ammonium is at 04/04/14.

The measurements taken from the date 04/04/14 to 14/04/14 shows the concentration of ammonium fluctuating between 33,01 mg/l and 38,71 mg/l. This is the plateau of the curve shown in the graph 4.2.1. The point where ammonium is taken best by the algae is at concentration is 38 mg/l for species SP4. The stable (ratio of birth:death is 1) growth phase is the point when the rate of growth of new cells is equal to the rate of cell deaths. The duration of the stable growth phase is just ten days. The explanation to this stable growth period is the exhaustion of growth nutrients in the bottle SP4. There was no addition of substral to the growing algae that eventually caused the decline of the algae cells.

The measurements taken from 14/04/14 till 24/04/14 in graph 4.2.1 give us the information that the ammonium concentration in the growth medium (tap water) is increasing. The algae cell started to decrease (die) from the day 14/04/14, this was the last day of the stable growth. The release of the ammonium ions in the bottle SP4 means that the algae cells started to break down into smaller compounds. There was no reproduction of algae occurring hence the tap water had growing ammonium molecules.

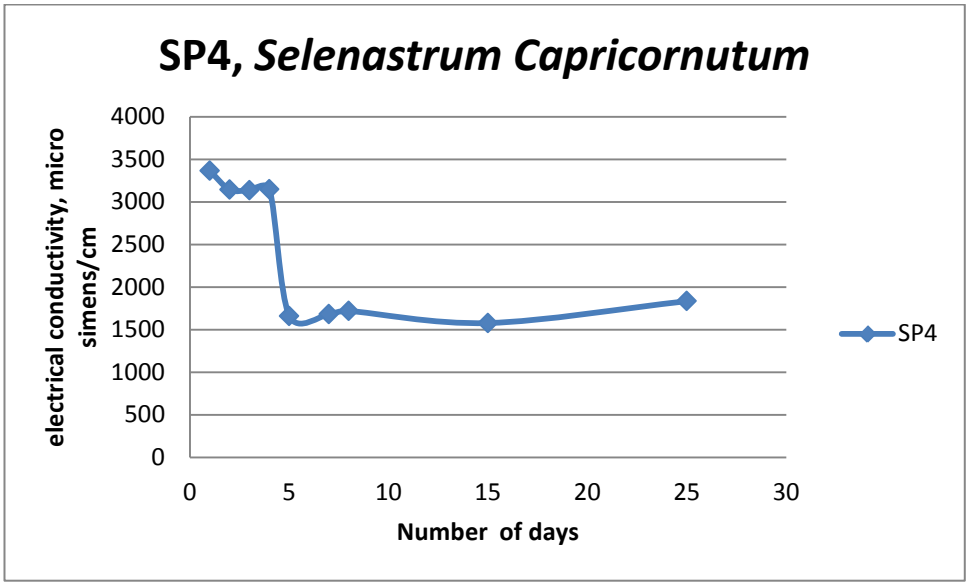
On the day 05/05/14, around 5 ml of substral was added to the bottle. This increased the ammonium concentration to 89 mg/l.



Graph 4.2.2 change of ammonium concentration in bottle SP5

Graph 4.2.2 shows changes in the ammonium concentration for bottle SP5. The similarity between the graph 4.2.1 and 4.2.2 is the nature of the curve. The curve shown in the graph 4.2.2 has maximum growth period similar to that of graph 4.2.1. The maximum uptake rate is at the fifth day. The concentration of ammonium at the fifth day is 29 mg/l. The stable growth phase is at concentration 30mg/l and lasts ten days. This is exactly the same number of optimal growth days in the bottle SP 4. The cell death starts to occur from 15/04/14. The explanation to the cell death is the exhaustion of growth nutrients.

4.3 Changes occurring in the conductivity of the growth water



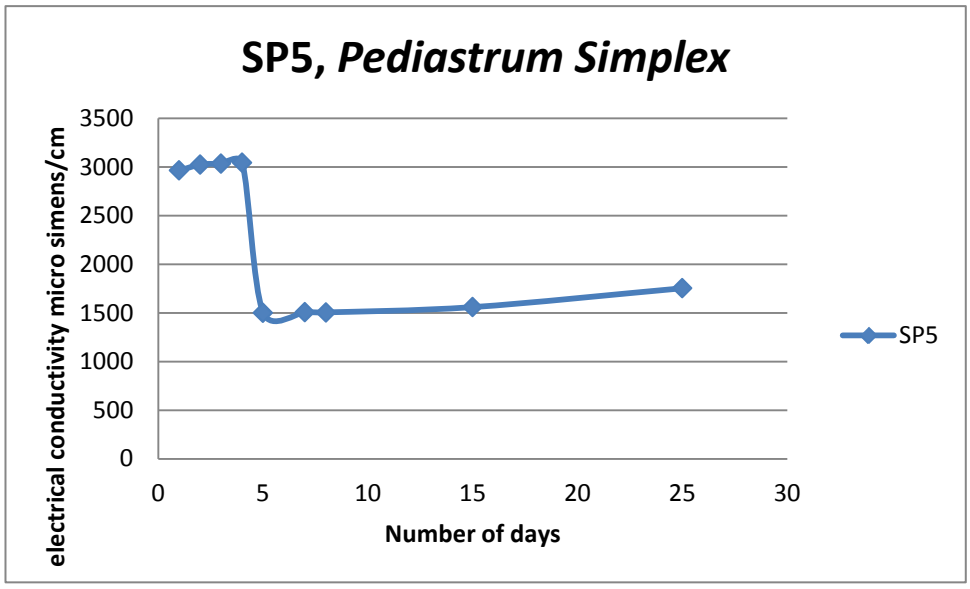
Graph 4.3.1 changes in conductivity in bottle SP4

The concentrations of total dissolved ions can be most easily measured as conductivity with units expressed as micro-simens per centimetre. The ability to use conductivity for the measurement arises from the fact that distilled water has a very high resistance to electron flow, and the presence of ions in water reduces that resistance (Sigee, 2004). Distilled water does not contain polar covalent molecules and ions. The polar covalent molecules give rise to Van Deer Wall's force. Similarly, distilled water is devoid of charged ions which are right condition for no conductance.

It is shown in the graph 4.3.1.; there is sharp decrease in the conductance of water between 03/04/14 and 04/04/14. The algae have rapidly exhausted essential ions during this phase. The growth of the algae is also highest during this phase as shown in the graph 4.2.1. The cell is at stress during this phase and is doing its best to keep the reproduction at the highest rate. At this period there may have been rapid storage of essential nutrient, let's say ammonium. The algae cells rapidly exhaust the ammonium ions decreasing the total conductance of the tap water. During this phase the transport medium such as active transport and facilitated diffusion occurs.

The specific conductance remains at the same position (plateau) phase between 04/04/14 and 24/04/14. This means that the cells have completely different biochemistry to that of the tap water. The cells at this moment are trying to survive using all the stored macromolecules and nutrients. The metabolism rate for the algae cells during this period is at its lowest. The duration, 20 days, for this lower metabolic activity can be an

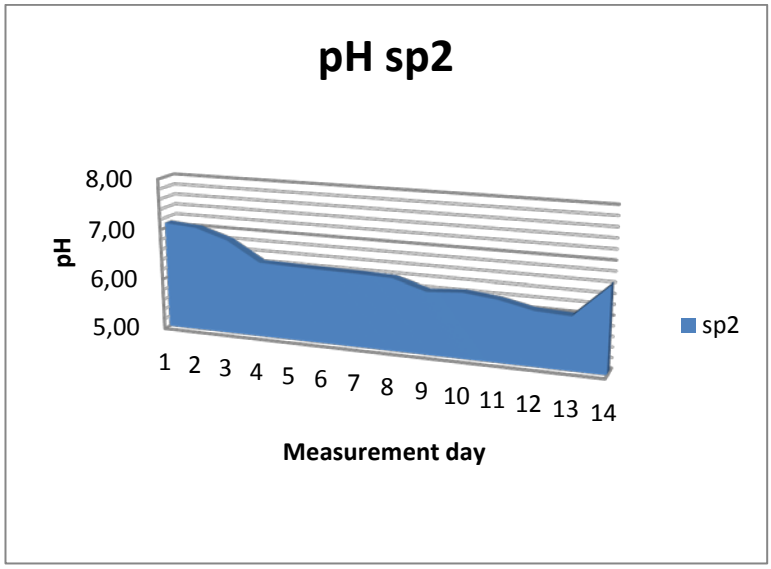
advantage. The algae are resistant and have stored so many nutrients that they are able to slow their degeneration for this period. The lower metabolism however does not remain for longer period of time as the cells are withering and releasing the nutrients.



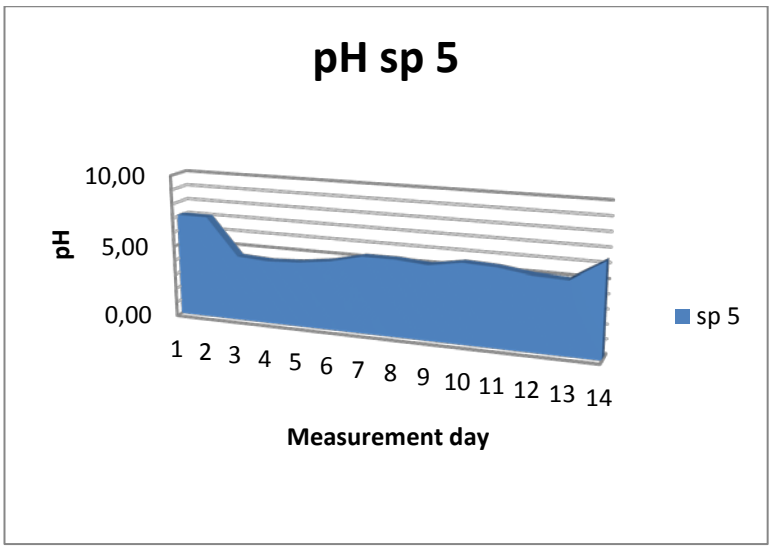
Graph 4.3.2 changes in conductivity in bottle SP5

The culture of the bottle SP 4 and SP5 was started in the same day. This explains the similarity in the changes in the conductance for both bottles. The changes in the ammonium concentration and specific conductance shows that they were observing similar storage mechanisms, metabolic activities, defence mechanisms in both of these bottles.

4.4 pH changes in SP2 and SP5



Graph 4.4.1 pH changes in SP2 from 05.03.14 to 31.03.14



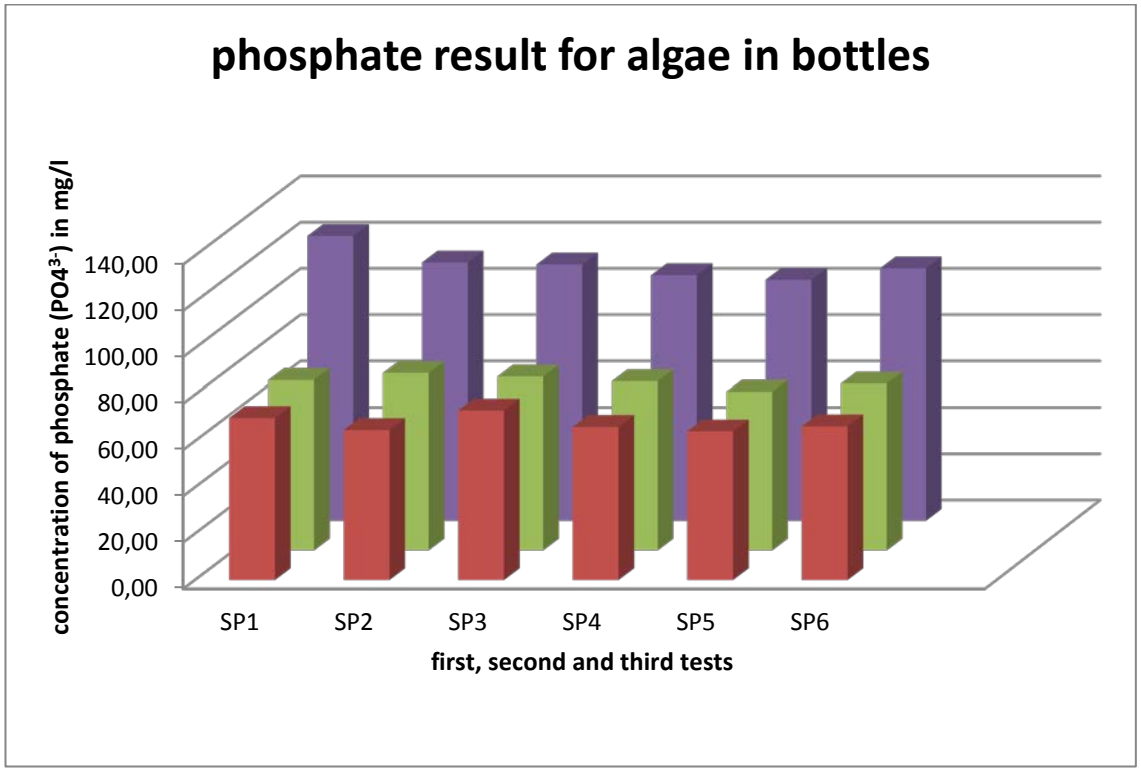
Graph 4.4.2 pH changes in SP5 from 05.03.14 to 31.03.14

The pH measurements were taken on a regular basis however, graph 4.4.1 and 4.4.2 gives the measurements of period 05/03/14-31/03/14. Graph 4.4.1 belongs to SP2 and graph 4.4.2 belongs to SP5. The period 05/03/14-31/03/14 was the initial growth phase of the algae in these bottles. The optimal pH level for bottle SP2 is 6,5 while algae in bottle SP5 have optimal growth at pH 5,0.

The difference between these two bottles is the influence of different algae species to its surrounding environment. Bottle SP2 has four different strains of algae; hence there is bigger influence of different algae that the bottle contains. The bottle SP5 however has only one strain of algae (*Pediastrum simplex*), hence, it is solely causing any changes around its immediate environment. The difference is also visible from the graphs shown above in this section. SP5 is much stable (it has pH at constant level of 5,0) than that of SP2. This gives us the answer for the favourable environment for growing *Pediastrum Simplex.*

The limiting factor during this period was the light. During the period the average lux is below the minimum required lux of 10,000 above.

4.5 Phosphate concentration changes in SP1-SP6



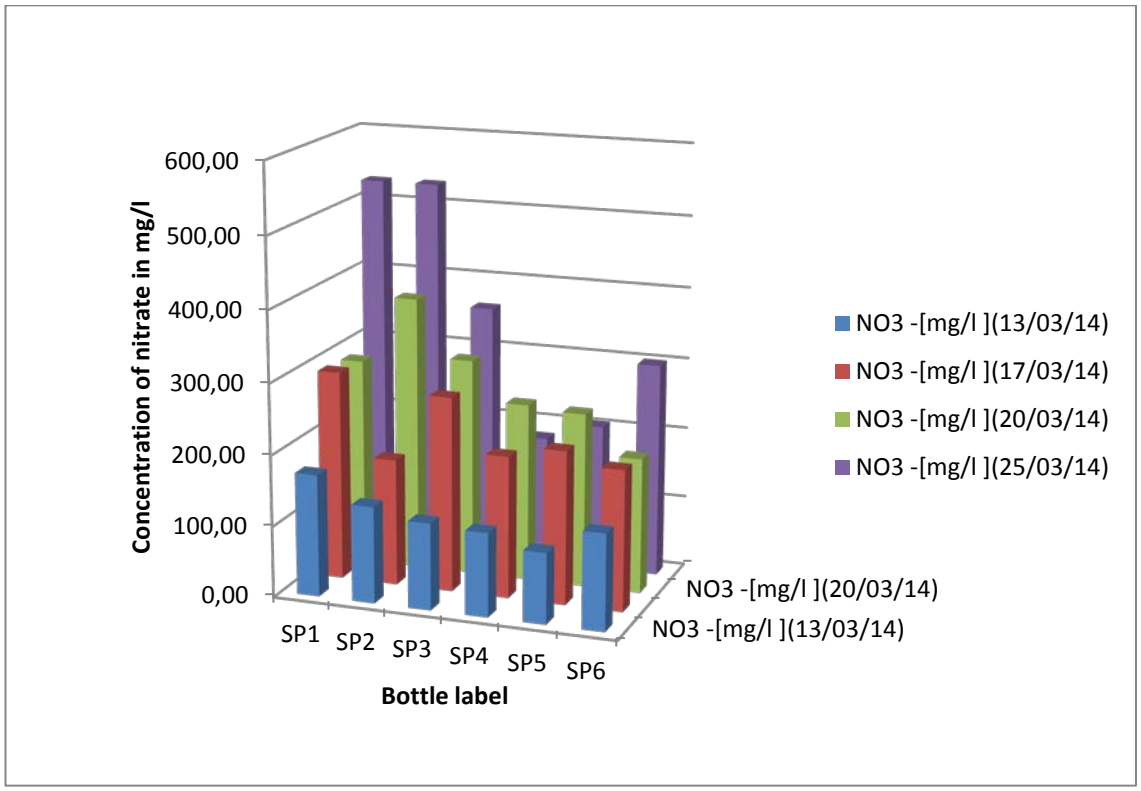
Graph 4.5.1 phosphate result for algae in bottles SP1-SP6

Phosphate is one of the limiting nutrients. The graph in this section shows the change in the concentration of phosphate in the algae culture bottles. The first measurement was taken on 17/03/14. The concentration of phosphate in all the bottle has an average of 67,47 mg/l. The second measurement was taken third day. The bottle cultures here have slightly higher average of concentration 73,08 mg/l. Lastly, the final measurement taken on after two weeks from the first measurement and the total average was 110,69 in the algae cultures.

The addition of 27ml substral was carried out on 07/03/14. The algae strains were growing rapidly consuming the nutrients added. The first total average for the growth culture as well as the second average for all growth culture is similar. Between 17/03/14-20/03/14 the stable uptake of the nutrient by the algae occurred. The nature of algae reproduction is such that at stress concentration of limiting nutrients it will try to uptake it with active diffusion. The algae have storage vacuoles in their cells that trap the limiting nutrients and store them for later consumption (Lobban, 1981). Since the date 07/03/14 the algae were devoid of essential nutrients. The two measurements, first and the second, were taken exactly when the active transport of this nutrient was occurring.
The third measurement however has a slightly different concentration average. This is because of the release of phosphate in the immediate environment by withering or dead

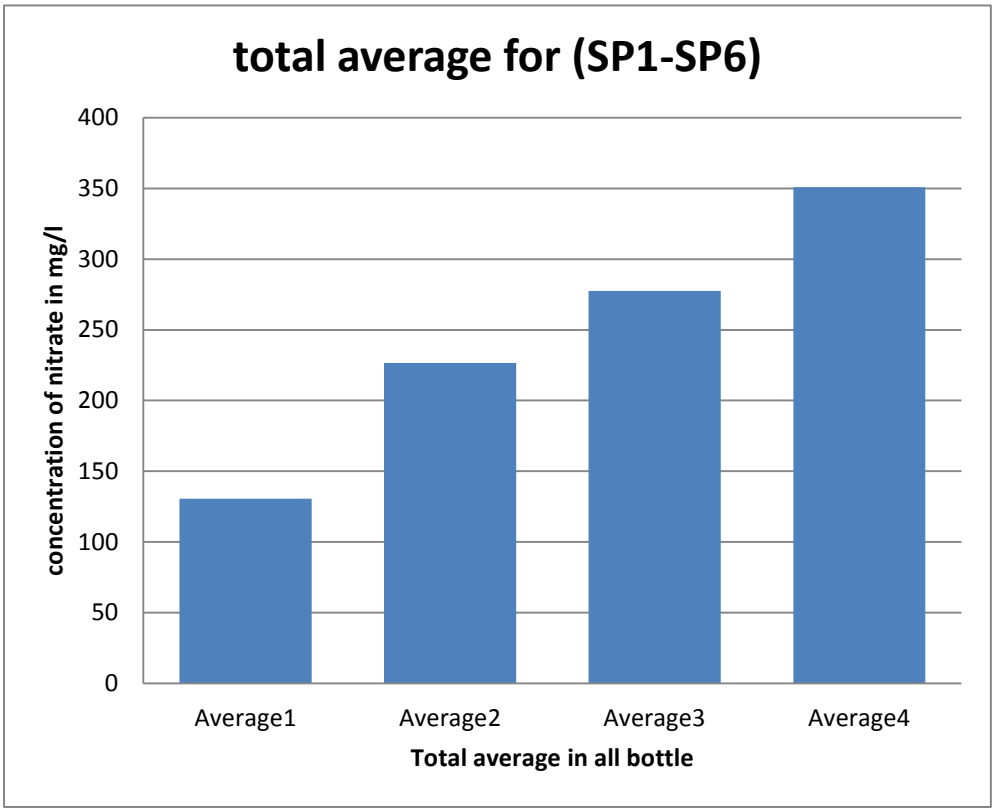
algae cells. The cells that exhausted their resource no longer survived releasing the nutrient in the immediate environment. The release was fairly equal in all the algae culture. This means that the growth phase in the bottles were quite similar.

4.6 Nitrate results for algae grown in bottles SP1-SP6



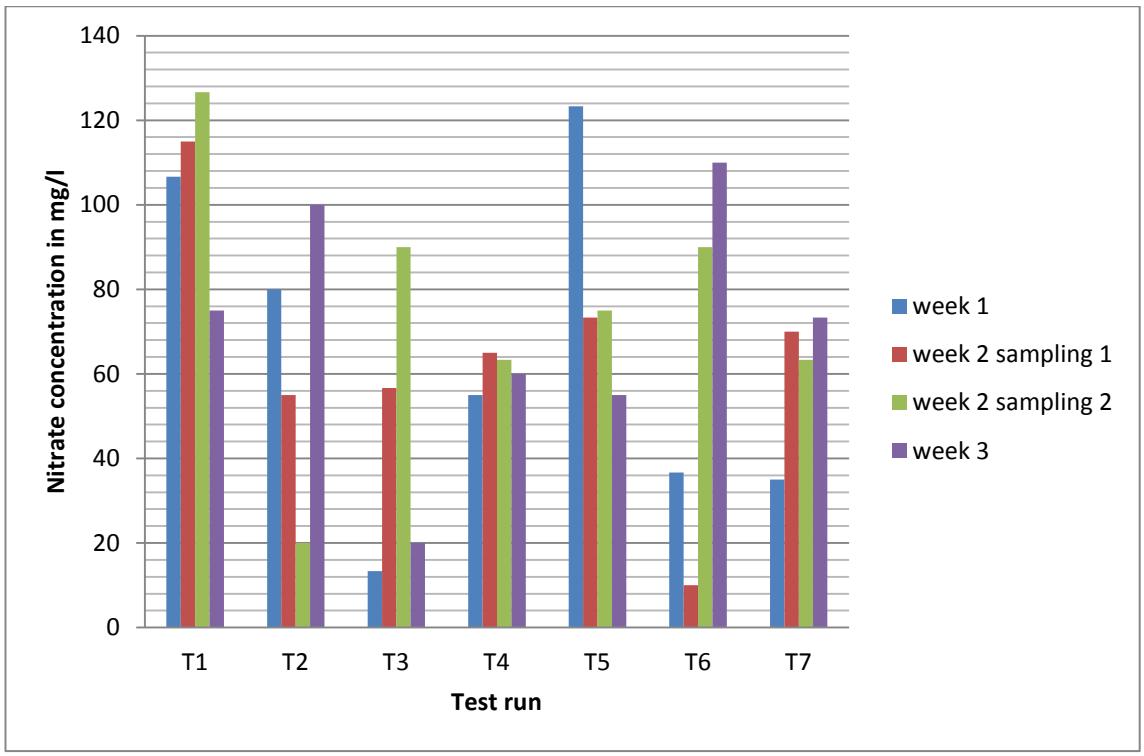
Graph 4.6.1 concentration of nitrate in the big bottle growth culture (SP1-SP6)

The measurements of nitrate concentration were carried out on the dates shown in the graph. The measurements at 17/03/14 and 20/03/14 have similar values of nitrate concentrations in all the growth bottles. The same trend was observed for phosphate concentration. This verifies that uptake of both nutrients, nitrate and phosphate was carried out intensively by the growing algae. The nutrient in all these bottles were being exhausted at the same rate and in these two dates the algae were storing nitrate as well as phosphate for further use. The average concentration of all the bottles respectively given in graph below makes the assessment of uptake easier.

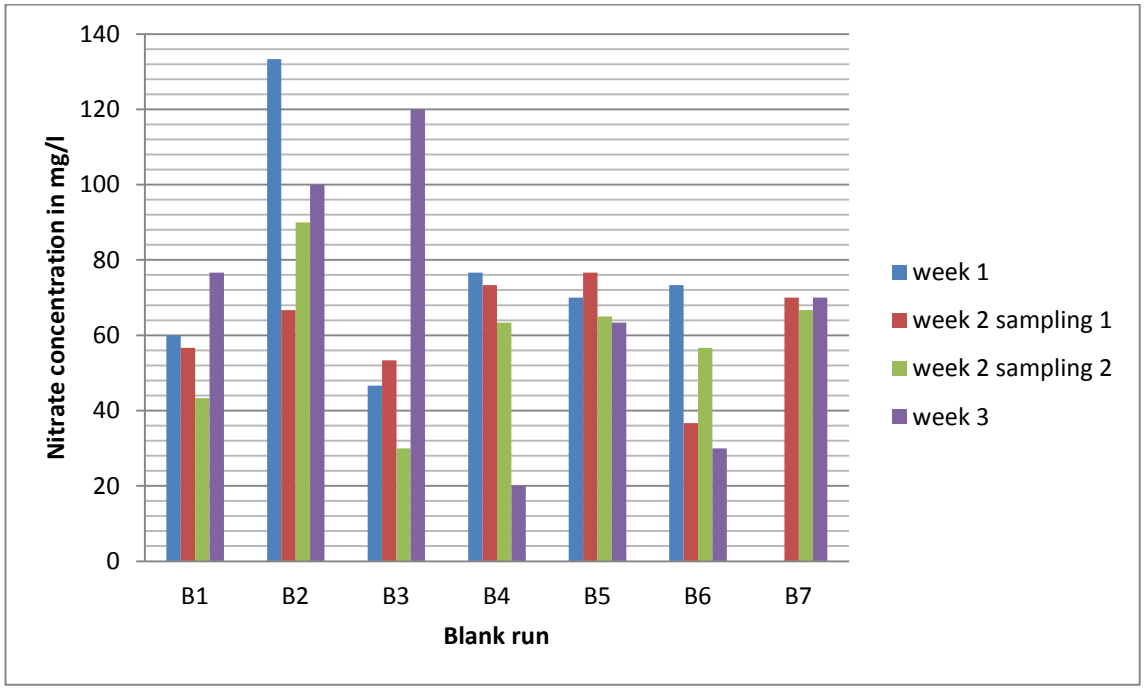


Bar chart 4.6.2 average nitrate concentration (mg/l) in the entire algal growth bottles (SP1+SP2+SP3+SP 4+SP5+SP6)/6

The bars on the graph correspond to the total average concentration in the entire growth bottle. The bars, average 2 and average 3 have close values. These bars correspond to the measurements taken in 17/03/14 and 20/03/14 respectively. The similarity in these bars is due to the addition of the nutrient substral. The date of the addition was same in the entire bottles (07/03/14). The algae in the growth culture bottles had similar growth phase and nutrient consumption. The conditions inside the laboratory allowed the different algae to have these similarities. The highest growth rate can be estimated to be on the 8^{th} day of the addition of substral. At this moment there was rapid multiplication of algae cells. The optimal phase started on the 9^{th} day and lasted for about a week. The two bars average 2 and average 3 have this constant concentration values (around 265mg/l). This was the moment when the active diffusion of these nitrate molecules occurred in the cell membrane (Lobban, 1981)

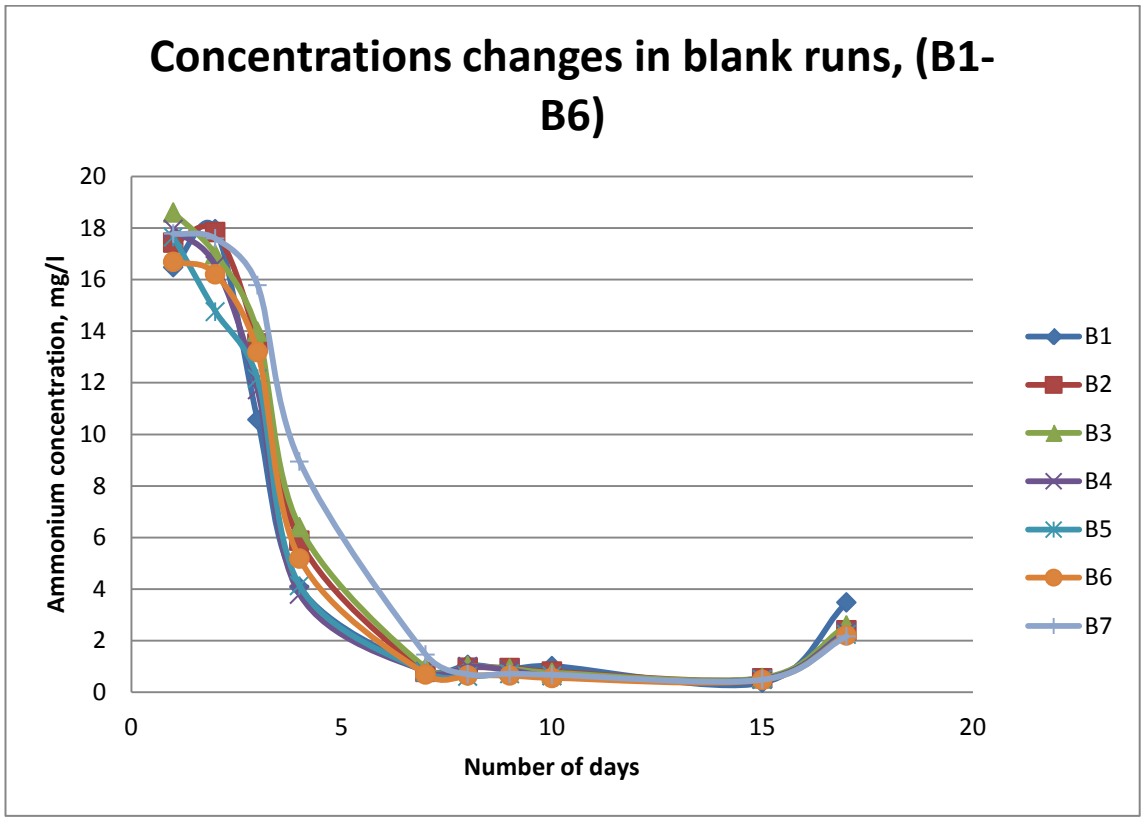


Histogram 4.6.1 nitrate concentrations in test run



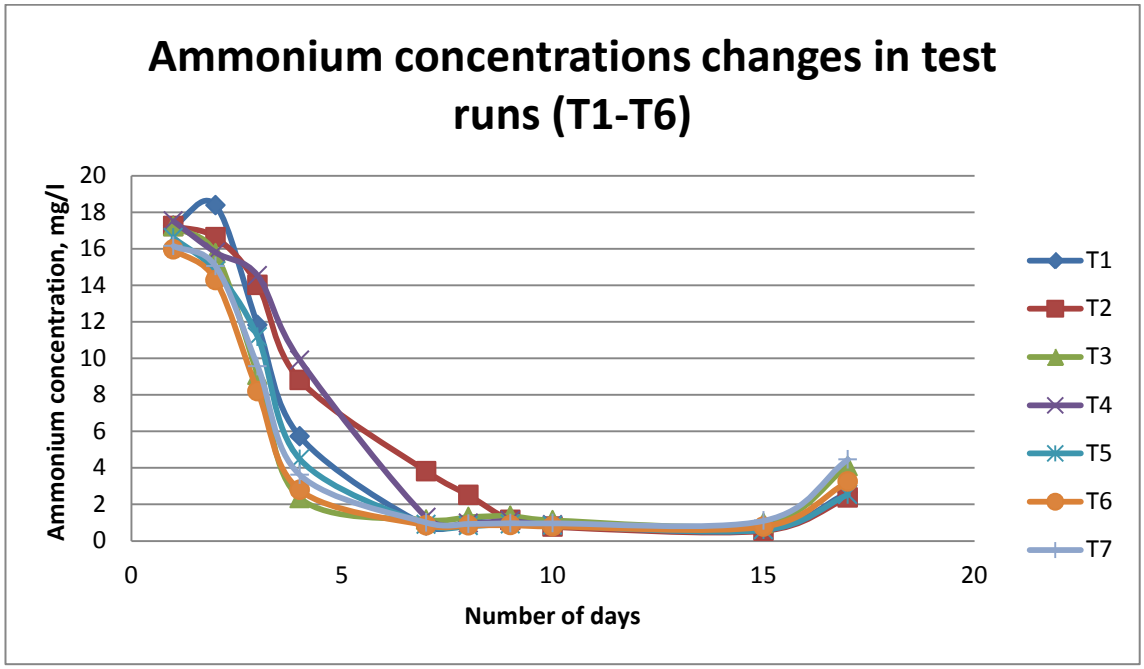
Histogram 4.6.2 nitrate concentrations in blank run

Ammonium concentration in blank test run (B1-B7)



graph4.6.1 ammonium concentration changes

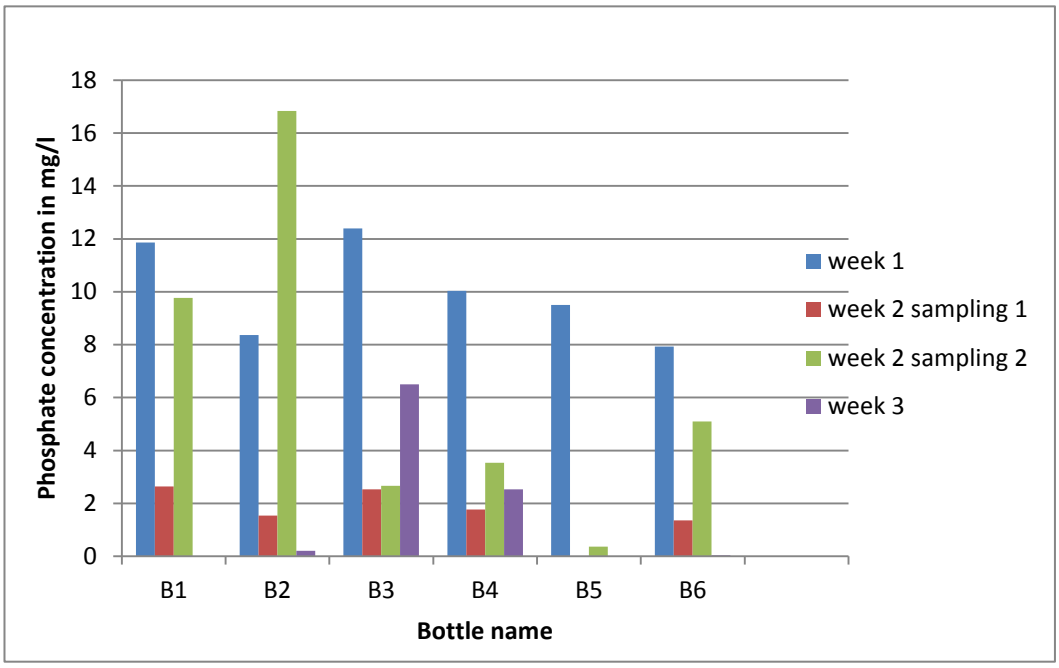
Ammonium concentration in test run (T1-T7)



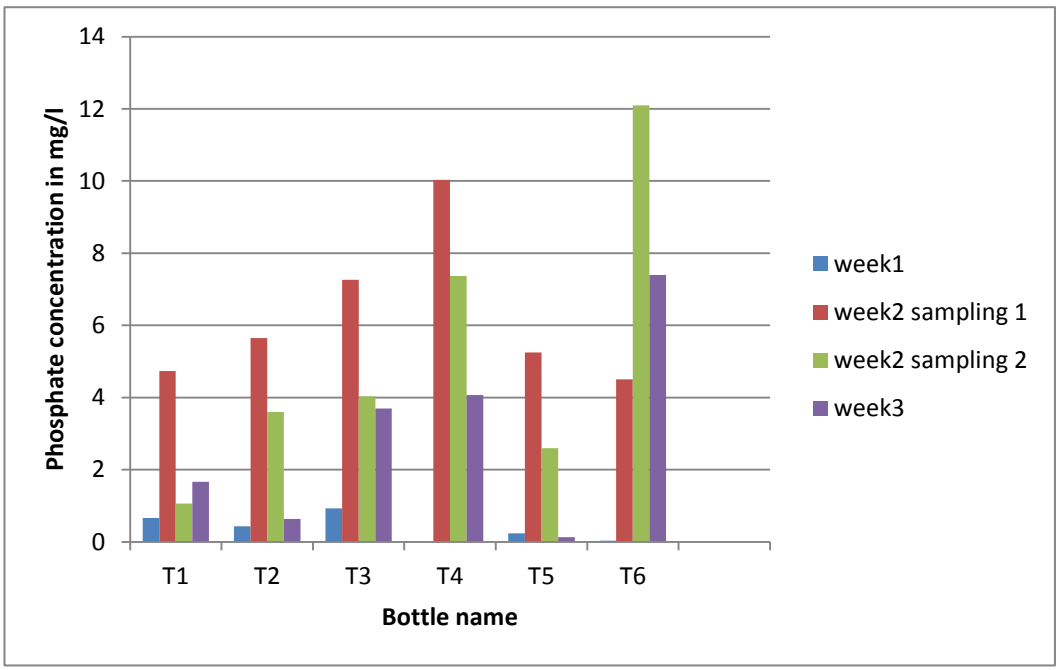
graph4.6.2 ammonium concentration changes

4.7 Phosphate concentration in blank sample and test sample

After the algae species grew they were let to grow in the wastewater medium for three weeks. The monitoring of the phosphate was carried out throughout the three weeks given in the histograms 4.7.1 and 4.7.2. The bar in the histogram represents the average phosphate concentrations in test run and blank run. In table 4.7.3 and 4.7.4 the actual concentrations are given. There were three replicates to each bottle during the sampling. The highest uptake of phosphate in blank samples occurred in week 2 sampling 1, one week after the test run commenced. The uptake of phosphate in test samples however was at its highest in week 2 sampling 2



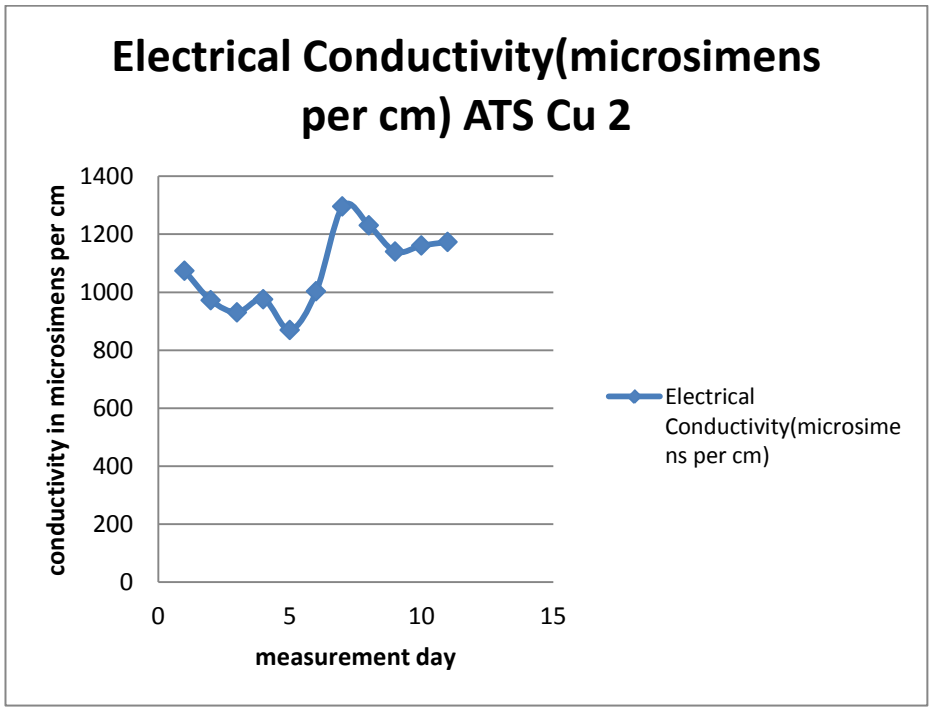
Histogram 4.7.1 phosphate concentration in blank samples



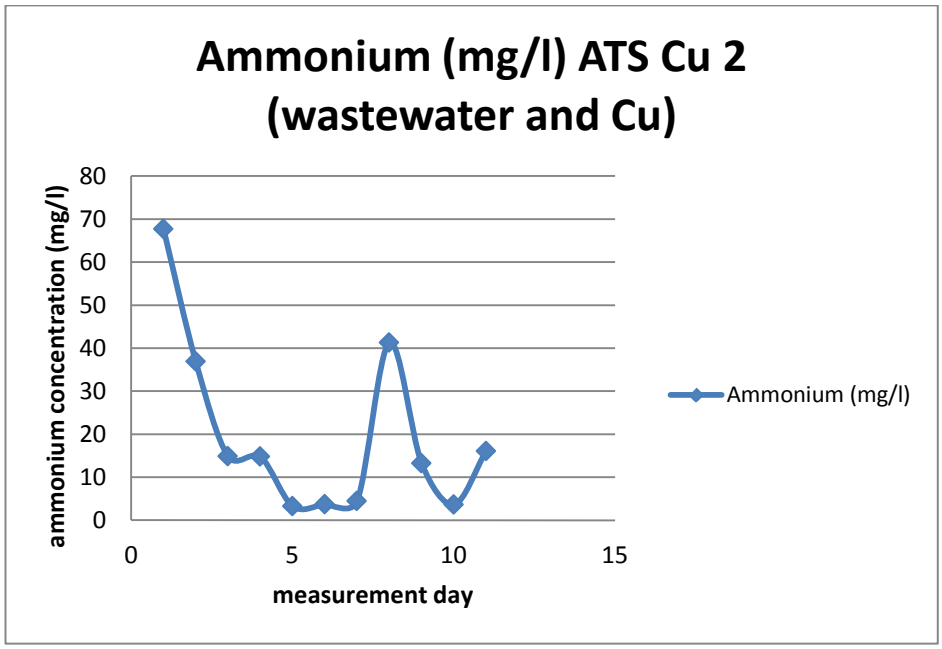
Histogram 4.7.2 phosphate concentration in test samples

The blank and the test samples were different from the growth bottles. The growing medium in these bottles was wastewater only for the blank samples and wastewater with copper in the test samples. The measurements carried out in the week 3 have elevated concentration of phosphate due to the nature of the culture. The culture was batch, meaning no replacement of wastewater was done after sampling. This significantly decreased the volume of water in the growth culture causing the high variations in the results shown in histograms above (4.7.1 and 4.7.2). The decrease of the water accounts to evaporation.

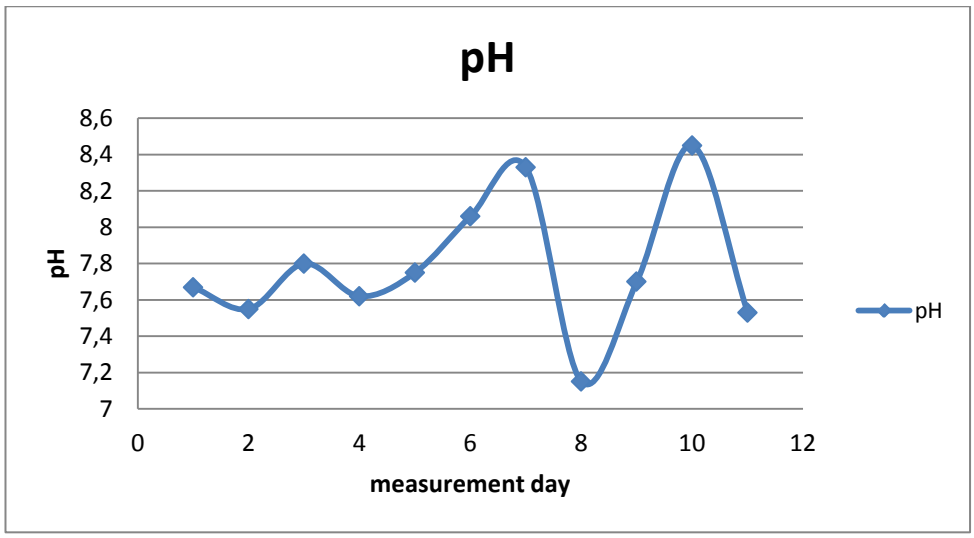
4.8 Results for ATS



Graph 4.8.1 electrical conductivity measurement for ATS Cu 2



Graph 4.8.2 Ammonium concentration measurement for ATS Cu 2



Graph 4.8.3 pH measurement for ATS Cu 2

4.9 Nitrate-N measurement for ATS

Nitrates conc. in mg/l	T1	T2	B
09.05.2014	100	60	90
	50	140	110
	100	120	90
Avg. mg/l	83,3333333	106,666667	96,6666667
14.05.2014	160	80	150
	120	120	80
	110	70	70
Avg. mg/l	130	90	100
20.05.2014	120	100	100
	80	100	80
	100	130	70
Avg. mg/l	100	110	83,3333333

Table 4.9.1 Nitrate measurements for ATS

4.10 Nitrate-N plus Ammonium-N for ATS with Copper (Cu)

Nitrate-N+ Ammonium-N (mg/l) for ATS Cu2

Date of measurement	Nitrate-N(mg/l)	Am-monium-N(mg/l)
9.05.14 (0wastewater added)	107	67,7
Nitrate-N+Ammonium-N(mg/l)	174,7	
14.05.14	90	14,89
Nitrate-N+Ammonium-N(mg/l)	104,89	
20.05.14 (waste water added)	110	41,29
Nitrate-N+Ammonium-N(mg/l)	151,29	

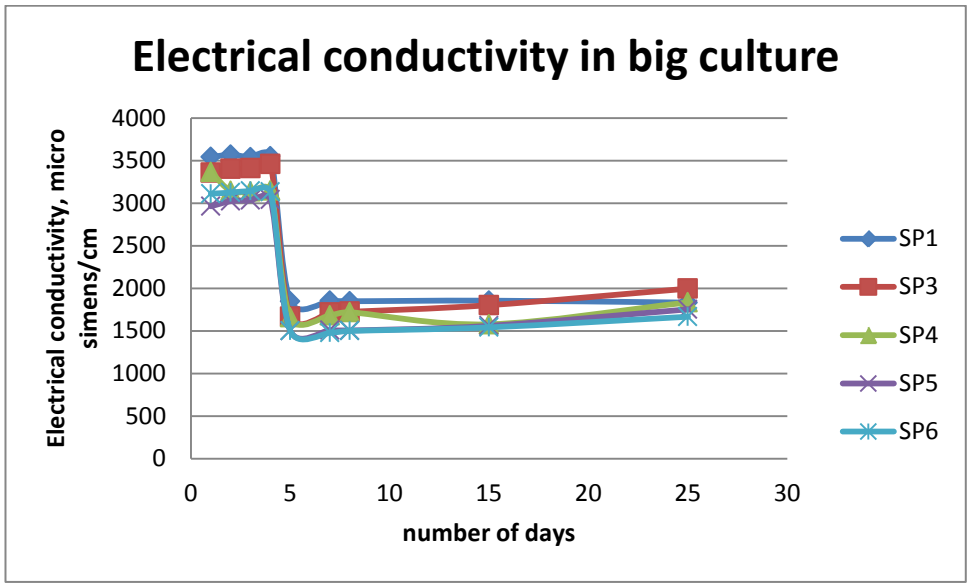
Table 4.10.1 Nitrate plus Ammonium-N for ATS Cu2

4.11 Phosphate-Phosphorous in ATS

Phosphate (mg/l)	T1	T2	B
09.05.2014	1,4	3	1,3
	1,3	4,2	2,2
	1,3	4	2
Average(mg/l)	1,33333333	3,73333333	1,83333333
14.05.2014	0	0	0
	0	0	0
	0	0	0
Average(mg/l)	0	0	0
20.05.2014	0	0	1,1
	0	0,1	0,5
	0	0	0,5
Average(mg/l)	0	0,03333333	0,7

Table 4.11.1 Phosphate-Phosphorous in ATS

4.12 Conductivity in for algae culture



Graph 3.12.1 Conductivity in algae cultures (SP1-SP7)

5 CONCLUSIONS

The major impediments to the measurement of the nutrients were very minimal. The total nitrogen measurement by the Kjeldahl experiment was full of risks, the data collected were not sufficient to draw any concrete evaluation hence the total nitrogen measurements were not taken into account. Algae prefer nitrate as the major source of nitrogen. Phosphorous is essential nutrient for the algae growth. ATS system removed efficiently –below detection limit- the P, within two days of testing. Nitrogen concentration decreased as well, within the first two days it decreased, 40%, but increased after that.

6 **References**

A.Pascher, 2014. *algaebase.* [Online] Available at: http://www.algaebase.org/browse/taxonomy/?id=6976 [Accessed 11 05 2014].

algaebase, 2014. *algaebase.* [Online] Available at: http://www.algaebase.org/search/species/detail/?species_id=L3ac9e8faeead728d&-session=abv4:C1A6E9610c7aa1AEA3hyljB8F0F9 [Accessed 11 05 2014].

algaebase, 2014. *algaebase.* [Online] Available at: http://www.algaebase.org/search/species/detail/?species_id=je94f9df8abefd792&sk=0&from=results [Accessed 11 05 2014].

algaebase, 2014. *algaebase.* [Online] Available at: http://www.algaebase.org/search/species/detail/?species_id=je94f9df8abefd792&sk=0&from=results [Accessed 11 05 2014].

base, a., 2014. *algaebase.* [Online] Available at: http://www.algaebase.org/search/species/detail/?species_id=Y42a648c73d1f272a&-session=abv4:C1A6E9610c7aa1AEA3hyljB8F0F9 [Accessed 11 05 2014].

Bellinger, E. S. D. D., 2010. *Freshwater Algae: Identification and Use and Bioindicators.* Hoboken, NJ, USA: Wiley.

bioweb.uwlax.edu, 2014. *bioweb.uwlax.edu.* [Online] Available at: http://bioweb.uwlax.edu/bio203/2010/towers_albe/ [Accessed 11 05 2014].

http://www.britannica.com/, 2014. *http://www.britannica.com/.* [Online] Available at: http://www.britannica.com/EBchecked/topic/526885/Scenedesmus [Accessed 11 05 2014].

Lobban, C. S., 1981. *The biology of seaweeds.* California: University of California Press.

Philip A. Lewis, D. J. K. a. J. M. L., 1994. *http://water.epa.gov/.* [Online] Available at: http://water.epa.gov/scitech/methods/cwa/bioindicators/upload/2007_07_10_methods_method_chronic-freshwater.pdf
[Accessed 11 05 2014].

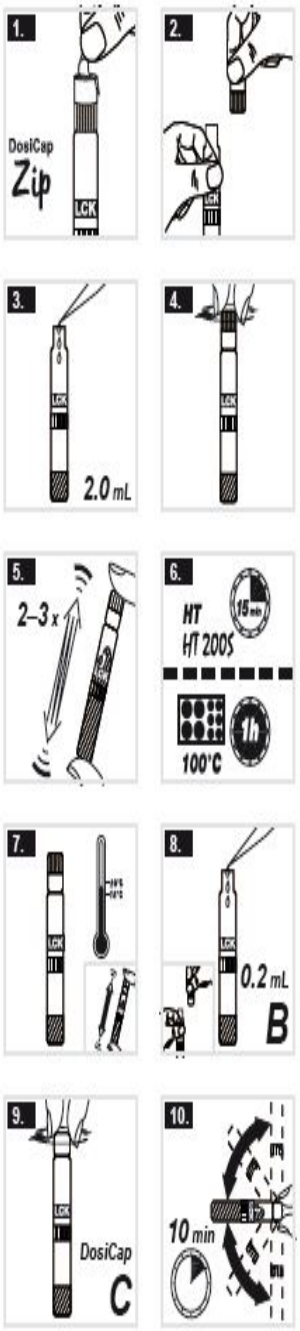
Robert J. Naiman, R. E. B., 2001. *River Ecology and Management, Lessons from the Pacific coastal ecoregion.* s.l.:Springer publication.

Sigee, D. C., 2004. *Freshwater Microbiology.* 1st ed. West Sussex: John Wiley & Sons Ltd..

6 Appendices

Appendix 1. HACH Phosphate-Phosphorous Kit LCK-349

LCK 349 – PO_4-P / PO_4 / P_2O_5 06/2013



1. – 10.
Gesamt-Phosphor
Phosphore total
Fosforo totali
Fosfor totaal
Total Phosphorus

3., 8. – 10.
Ortho-Phosphat
Orthophosphate
Ortofosfati
Orthofosfaat
Orthophosphate

DE

1. Siegelfolie von dem aufgeschraubten DosiCap® Zip *vorsichtig* abziehen.
2. DosiCap® Zip abschrauben.
3. *2.0 mL* Probe pipettieren.
4. DosiCap® Zip *fest* aufschrauben; Riffelung oben.
5. Kräftig schütteln.
6. Im Thermostaten erhitzen.
   HT 200 S: 15 min im Standardprogramm *HT*
   Thermostat: 60 min bei *100°C*
7. Auf Raumtemperatur abkühlen.
   Kräftig schütteln.
8. In erkaltete Küvette pipettieren:
   0.2 mL Reagenz B (LCK 349 B).
   Reagenz B nach Gebrauch *sofort* verschließen.
9. *Graues* DosiCap® C (LCK 349 C) auf die Küvette schrauben.
10. Küvette schwenken, dabei mehrfach auf den Kopf drehen. Nach *10 min* Küvette noch einmal schwenken, außen gut säubern und auswerten.

FR

1. Enlevez *délicatement* la feuille de protection du DosiCap Zip détachable.
2. Dévissez le DosiCap Zip.
3. Pipetter *2.0 mL* d'échantillon.
4. Vissez le DosiCap Zip *fermement*; dirigeant le cannelage vers le haut.
5. Secouer énergiquement.
6. Chauffer dans le thermostat.
   HT 200 S: 15 min avec le programme standard *HT*
   Thermostat: 60 min à *100°C*
7. Laisser refroidir à température ambiante.
   Secouer énergiquement.
8. Pipetter dans la cuve une fois refroidie:
   0.2 mL de réactif *B* (LCK 349 B).
   Fermer *immédiatement* le réactif B après emploi.
9. Visser un DosiCap C (LCK 349 C) *gris* sur la cuve.
10. Mélanger le contenu de la cuve en la retournant plusieurs fois de suite. Attendre *10 min*, mélanger de nouveau, bien nettoyer l'extérieur de la cuve et mesurer.

IT

1. Rimuovere *con attenzione* il foglio di alluminio.
2. Svitare il DosiCap Zip.
3. Pipettare *2.0 mL* di campione.
4. Avvitare *saldamente* il DosiCap Zip; scanalatura esterna verso l'alto.
5. Agitare energicamente.
6. Riscaldare nel termostato.
   HT 200 S: 15 min nel programma standard *HT*
   Termostato: 60 min a *100°C*
7. Fare raffreddare a temperatura ambiente.
   Agitare energicamente.
8. Pipettare nella cuvetta raffreddata:
   0.2 mL di reattivo *B* (LCK 349 B).
   Dopo aver prelevato il reattivo B, richiudere *immediamente*.
9. Avvitare un DosiCap C *(capsula grigia)* (LCK 349 C).
10. Mescolare capovolgendo la cuvetta più volte. Dopo *10 min* mescolare nuovamente, pulire bene la cuvetta esternamente e leggere.

NL

1. Afdekfolie *voorzichtig* verwijderen.
2. DosiCap Zip afschroeven.
3. *2.0 mL* monster pipetteren.
4. DosiCap Zip *stevig vast* opschroeven; geribbelde zijde naar boven.
5. Krachtig schudden.
6. In het thermostaat verhitten.
   HT 200 S: 15 min in standaard-programma *HT*
   Thermostaat: 60 min bij *100°C*
7. Laten afkoelen tot kamertemperatuur.
   Krachtig schudden.
8. In afgekoelde kuvet pipetteren:
   0.2 mL reagens *B* (LCK 349 B). De reagens B-fles na gebruik *onmiddelijk* dicht draaien.
9. Een *grijze* DosiCap C (LCK 349 C) op het kuvet schroeven.
10. Kuvet zwenken en daarbij meerdere malen op zijn kop houden. Na *10 min* het kuvet opnieuw zwenken, van buiten goed reinigen en meten.

EN

1. *Carefully* remove the foil from the screwed-on DosiCap Zip.
2. Unscrew the DosiCap Zip.
3. Pipette *2.0 mL* sample.
4. Screw the DosiCap Zip back *tightly*; fluting at the top.
5. Shake firmLy.
6. Heat in the thermostat.
   HT 200 S: in standard program *HT* for *15 min*
   Thermostat: 60 min at *100°C*
7. Allow to cool to room temperature.
   Shake firmly.
8. Pipette into the cooled cuvette:
   0.2 mL Reagent *B* (LCK 349 B).
   Close Reagent B *immediately* after use.
9. Screw a *grey* DosiCap C (LCK 349 C) onto the cuvette.
10. Invert a few times. After *10 min* invert a few times more, thoroughly clean the outside of the cuvette and evaluate.

Appendix 2. HACH Nitrate-Nitrogen with Nitra Ver 5 powder pillows

NitraVer 5 Nitrate Reagent Powder Pillows, 0.1-10mg/L NO_3-N

Order number: 1403499

Powder reagent in sealed packages for the determination of nitrate. For 25 mL sample, 100 pcs.

Technical Specifications

Description	NitraVer 5 Nitrate Reagent Powder Pillows, 25mL
Instrument	DR/3000, DR/2000
Measuring range	0.3 - 30.0 mg/L NO_3-N
Measuring range (2)	0.1 - 10.0 NO_3-N
Method	Cadmium Reduction
Method number	8171 MR
Method number	8039 HR
Number of tests	100
Parameter	Nitrate
Platform	PP
Sample size	25 mL
Storage conditions	10 °C - 25 °C
Volume / Package size	100 pk

Subject to change without notice.

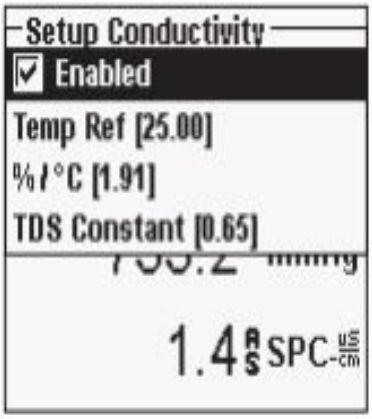
s

Appendix 3. YSI Conductivity

CONDUCTIVITY

Conductivity sensors are supplied with 60530-X, 6051030-X, 6052030-X , and Quatro cables. Conductivity sensors are built into the 60530-X, 6051030-X, and 6052030-X cables and are not replaceable. Conductivity/Temperature sensors are shipped with the Quatro cable, must be installed, and are replaceable.

SETUP - CONDUCTIVITY



Press Sensor, highlight **Setup,** and press enter. Highlight **Conductivity**, press enter.

Enabled allows you to enable or disable the conductivity measurement. Highlight **Enabled** and press enter to activate (☑) or deactivate (☐) conductivity. Disable conductivity if you do not have a conductivity sensor connected to the instrument.

> *If a sensor is Enabled that isn't connected to the instrument, the display will show an unstable, false reading next to the units.*

Temp Ref (Temperature Reference) is the reference temperature used for calculating temperature compensated Specific Conductance. This will be the

temperature all Specific Conductance values are compensated to. The default is 25 °C. To change the Reference Temperature, highlight **Temp Ref** and press enter. Use the numeric entry screen to enter a new value between 15.00 and 25.00 °C. Next, highlight <<<ENTER>>> at the bottom of the screen and press enter on the keypad to confirm.

%/°C (Percent per Degree Celsius) is the temperature coefficient used to calculate temperature compensated Specific Conductance. The default is 1.91% which is based on KCl standards. To change the temperature coefficient, highlight %/°C and press enter. Use the numeric entry screen to enter a new value between 0 and 4%. Next, highlight <<<ENTER>>> at the bottom of the screen and press **Enter** on the keypad to confirm.

TDS Constant is a multiplier used to calculate an estimated TDS (Total Dissolved Solids) value from conductivity. The multiplier is used to convert Specific Conductance in mS/cm to TDS in g/L. The default value is 0.65. This multiplier is highly dependent on the nature of the ionic species present in the water sample. To be assured of moderate accuracy for the conversion, you must determine a multiplier for the water at your sampling site. Use the following procedure to determine the multiplier for a specific sample:

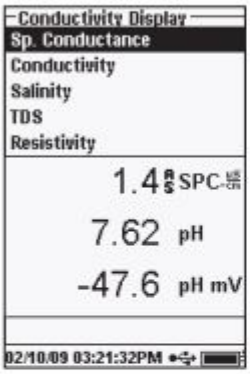
1. Determine the specific conductance of a water sample from the site;
2. Filter a portion of water from the site;
3. Completely evaporate the water from a carefully measured volume of the filtered sample to yield a dry solid;
4. Accurately weigh the remaining solid;
5. Divide the weight of the solid (in grams) by the volume of water used (in liters) to yield the TDS value in g/L for this site; Divide the TDS value in g/L by the specific conductance of the water in mS/cm to yield the conversion multiplier. Be certain to use the correct units.

> ⓘ *If the nature of the ionic species at the site changes between sampling studies, the TDS values will be in error. TDS cannot be calculated accurately from specific conductance unless the make-up of the chemical species in the water remains constant.*

To change the multiplier, highlight **TDS Constant** and press enter. Use the numeric entry screen to enter a new value between 0 and 0.99. Highlight <<<ENTER>>> at the bottom of the screen and press **Enter** on the keypad to confirm.

DISPLAY - CONDUCTIVITY

Press Sensor, highlight **Display** and press enter. Highlight **Conductivity** and press enter. Highlight **Sp. Conductance** (Specific Conductance), **Conductivity**, **Salinity**, **TDS**, or **Resistivity**, and press enter to select the reporting units for each parameter. One reporting unit per parameter may be enabled. To disable a parameter, select **None**. You will not be able to display any of these parameters unless the Conductivity sensor is **Enabled** in the Sensor Setup menu first.



Sp. Conductance can be displayed in us/cm or ms/cm. Specific conductance is temperature compensated conductivity.

Conductivity can be displayed in uS/cm or mS/cm. Conductivity is the measure of a solution's ability to conduct an electrical current. Unlike specific conductance, conductivity is a direct reading without any temperature compensation.

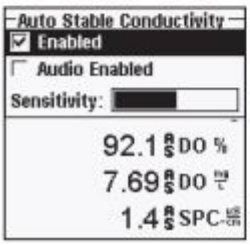
Salinity can be displayed in ppt (parts per thousand) or PSU (practical salinity units). The units are equivalent as both use the Practical Salinity Scale for calculation.

TDS can be displayed in mg/L (milligrams per liter), g/L (grams per liter), or kg/L (kilograms per liter).

Resistivity can be displayed in ohm-cm (ohms per centimeter), kohm-cm (kilo ohms per centimeter), or Mohm-cm (mega ohms per centimeter).

AUTO STABLE - CONDUCTIVITY

Press Sensor, highlight **Auto Stable** and press enter. Highlight **Conductivity** and press enter.

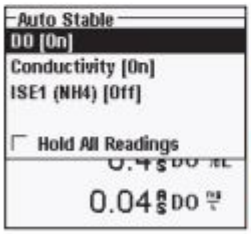


Auto Stable indicates when a reading is stable. Highlight **Enabled** and/or **Audio Enabled** (instrument will beep when the stability is achieved) and press enter enable (☑) or disable (☐). When Auto Stable is enabled, **AS** will blink next to the parameter until it is stable. Once the parameter is stable, **AS** will stop blinking.

The Auto Stable **Sensitivity** can be decreased or increased. Highlight **Sensitivity** and use the left and right arrow keys to slide the bar. The more sensitive you make it (larger black bar) the harder it is to achieve stability in a changing environment.

The **Auto Stable** system works by examining the previous 5 readings, computing the percent change in the data and comparing that change against a **%** threshold value. The % threshold value is determined by the **Sensitivity** bar setting. The following chart can be used as a guide when setting the Sensitivity bar.

Sensitivity selected by User	*% Data Variance Threshold*
100 - Most Sensitive, Sensitivity bar is set to the far right	0.025%
75	0.39375%
50	0.7625%
25	1.13125%
0 - Least Sensitive, Sensitivity bar is set to the far left	1.5%



Within the Auto Stable menu, you can also choose to **Hold All Readings** for as many parameters as you set for Auto Stable. For instance, if conductivity and DO have Auto Stable and Hold All Readings enabled, then the display will hold the readings once conductivity and DO have both reached their Auto Stable settings. You must press the **Esc** key to "release" the held display in order to take subsequent readings. **Hold All Readings** must be reactivated after each use!

Appendix 4. YSI professional plus pH probe

pH

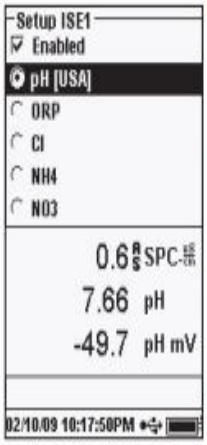
pH sensors can be used on 60510-X, 6051020-X, 6051030-X, 6051010-X, and Quatro cables.

If using a 605103 pH/ORP combination sensor on a 6051020 or 6051030 cable you can report both pH and ORP by configuring ISE1 as pH and ISE2 as ORP in the Sensor Setup menu.

The 605103 pH/ORP combination sensor is not recommended for use on a 6051010 or Quatro cable. If used on one of these cable, only pH will be reported and ORP will not be measured.

SETUP - pH

Press Sensor, highlight **Setup**, press enter. Highlight **ISE1** if using a 60510, 6051020, or 6051030 cable. If using a 6051010 or Quatro cable, highlight **ISE1** if the pH sensor is installed in port 1 or highlight **ISE2** if the pH sensor is installed in port 2(a sensor must be installed in port 1 for port 2 to operate). Press enter.



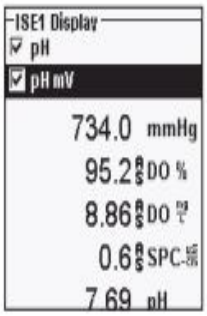
Enabled allows you to enable or disable the ISE function and select which ISE sensor is installed on the cable. Highlight **Enabled** and press enter to enable (☑) or disable (☐) the ISE you selected previously (either ISE1 or ISE2). Disable the ISE function(s) if you do not have a ISE sensor connected to the instrument.

After enabling the ISE function, ensure that it is set to pH as shown in the left screen shot. If necessary, highlight pH and press enter to set the ISE to pH.

Highlighting **pH[USA]** and pressing enter will also allow you to select the values for auto buffer recognition which are used during calibration. The buffer options are **USA** (4, 7, 10), **NIST** (4.01, 6.86, 9.18), and **User-Defined**. The selected option will be displayed in [brackets].

> *If a sensor is Enabled that isn't connected to the instrument, the display will show an unstable false reading, ?????, or ----- next to the units.*

DISPLAY - pH



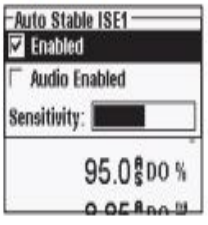
Press Sensor, highlight **Display** and press enter.

Highlight **ISE (pH)** and press enter. You will not be able to **Display** the sensor unless it is **Enabled** in the Sensor Setup menu.

Highlight **pH** and/or **pH mV**, press enter to enable (☑) or disable (☐). Both can be reported at the same time.

AUTO STABLE - pH

Press Sensor, highlight **Auto Stable** and press enter. Highlight **ISE (pH)** and press enter.

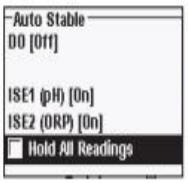


Auto Stable indicates when a reading is stable. Highlight **Enabled** and/or **Audio Enabled** (instrument will beep when the stability is achieved) and press enter enable (☑) or disable (☐). When Auto Stable is enabled, **AS** will blink next to the parameter until it is stable. Once the parameter is stable, **AS** will stop blinking.

The Auto Stable **Sensitivity** can be decreased or increased. Highlight **Sensitivity** and use the left and right arrow keys to slide the bar. The more sensitive you make it (larger black bar) the harder it is to achieve stability in a changing environment.

The **Auto Stable** system works by examining the previous 5 readings, computing the percent change in the data and comparing that change against a **%** threshold value. The **%** threshold value is determined by the **Sensitivity** bar setting. The following chart can be used as a guide when setting the Sensitivity bar.

Sensitivity selected by User	*% Data Variance Threshold*
100 - Most Sensitive, Sensitivity bar is set to the far right	0.025%
75	0.39375%
50	1.5%
25	1.13125%
0 - Least Sensitive, Sensitivity bar is set to the far left	0.15%



Within the Auto Stable menu, you can also choose to **Hold All Readings** for as many parameters as you set for Auto Stable. For instance, if ORP and pH have Auto Stable enabled and Hold All Readings is enabled, then the display will hold the readings once ORP and pH have both reached their Auto Stable settings. You must press the Esc key to "release" the held display in order to take subsequent readings. **Hold All Readings** must be reactivated after each use!

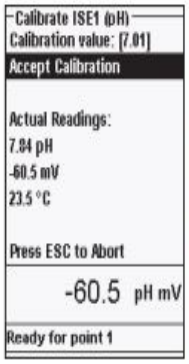
CALIBRATION - pH

> *Calibration can be accomplished in any buffer order. pH 7 buffer should be used regardless of how many calibration points you use but it does not have to be used first.*

Press Cal . Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. User ID will appear automatically. Select 'None' if you do not want a User ID stored with the calibration. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting your User ID and/or Probe ID if appropriate, highlight **ISE (pH)** and press enter. The message line will show the instrument is "Ready for point 1". The pH calibration allows up to six calibration points.

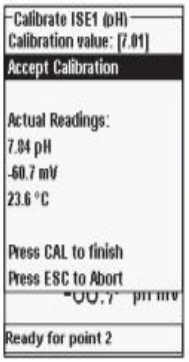
Place the sensor in a traceable pH buffer solution. The instrument should automatically recognize the buffer value and display it at the top of the calibration screen. If the calibration value is incorrect, the auto buffer recognition setting



in the Sensor Setup menu may be incorrect. If necessary, highlight the **Calibration Value** and press enter to input the correct buffer value.

Once the pH and temperature readings stabilize, highlight **Accept Calibration** and press enter to accept the first calibration point. The message line will then display "Ready for point 2".

If you do not wish to perform a second point, press Cal to finalize the calibration. Or, press Esc to cancel the calibration. If User Field 1 or 2 are enabled, you will be prompted to select these fields and then press Cal to finalize the calibration.



To continue with the 2nd point, place the sensor in the second buffer solution. The instrument should automatically recognize the second buffer value and display it at the top of the screen. If necessary, highlight the **Calibration Value** and press enter to input the correct buffer value. Once the pH and temperature readings stabilize, highlight **Accept Calibration** and press enter to confirm the second calibration point. The message line will then display 'Ready for point 3" and you can continue with the 3rd calibration point if desired.

If you do not wish to perform a 3rd calibration point, press Cal to complete the calibration. If User Field 1 or 2 are enabled, you will be prompted to select these fields and then press Cal to finalize the calibration.

Continue in this fashion until the desired number of calibration points is achieved (up to six).

> *Once you've achieved the desired number of cal points you must press Cal to finalize the calibration and to allow the instrument to update the pH offset and slope. The instrument will not take these cal values into account until Cal has been pressed.*

Appendix 5. YSI Professional Plus Ammonium-Nitrogen probe

AMMONIUM, NITRATE, CHLORIDE

Ammonium, Nitrate, and Chloride sensors can be used on 60510-X, 6051020-X, 6051030-X, 6051010-X, and Quatro cables. These cables also accommodate pH and ORP sensors so instrument setup is important.

WARNING: *Ammonium, Nitrate, and Chloride sensors should only be used at DEPTHS OF LESS THAN 55 FEET (17 METERS). Use of the sensors at greater depths is likely to permanently damage the sensor membrane.*

WARNING: *Ammonium, Nitrate, and Chloride sensors should only be used in FRESHWATER.*

SETUP - AMMONIUM, NITRATE, CHLORIDE

Install the Ammonium, Nitrate, or Chloride sensor in Port 2 if using in conjunction with pH or ORP sensor on a 6051010 or Quatro cable. See the **Getting Started Setup** section of this manual for a complete list of cable/sensor configurations.

Press Sensor, highlight **Setup**, press enter. Highlight **ISE1** if using an ammonium, nitrate, or chloride sensor on a 60510, 6051020, or 6051030 cable.

If using a 6051010 or Quatro cable highlight **ISE1** if the sensor is installed in Port 1 or highlight **ISE2** if the sensor is installed in Port 2. Press enter.

Enabled allows you to enable or disable the ISE function and select which ISE sensor is installed on the cable.

Highlight **Enabled** and press enter to enable (☑) or disable (☐) the ISE you selected previously (either ISE1 or ISE2).

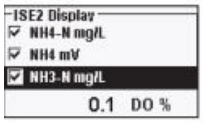
After enabling the ISE function, choose the parameter you want enabled for that ISE. In this example, NH4 is selected.

Cl - Chloride
NH4 - Ammonium
NO3 - Nitrate

> *If a sensor is Enabled that isn't connected to the instrument, the display will show an unstable, false reading next to the units.*

DISPLAY - AMMONIUM, NITRATE, CHLORIDE

Press Sensor, highlight **Display**, press enter. Highlight **ISE2(NH4)**, press enter. You will not be able to **Display** the sensor unless it is **Enabled**.



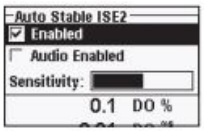
Highlight the value you wish to display and press enter to enable (☑) . Ammonium can be displayed as **NH4-N mg/L** (Ammonium), **NH3-N** (Ammonia) and/or **NH4 mV** (sensor signal).

The same steps would be followed to display nitrate or chloride.

Ammonia is calculated from the pH, salinity, and temperature readings. If a pH sensor is not in use, the instrument will assume the sample is neutral (pH 7) for the calculation. If a conductivity sensor (Salinity) is not in use, the instrument will use the salinity correction value entered in the Sensor Menu for the calculation (see Salinity Correction within the Dissolved Oxygen Setup section of this manual for more information).

AUTO STABLE - AMMONIUM, NITRATE, CHLORIDE

Auto Stable indicates when a reading is stable. When Auto Stable is enabled, **AS** will blink next to the parameter until it is stable. Once the parameter is stable, **AS** will stop blinking.

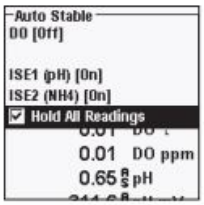


To enable Auto Stable, press Sensor, highlight **Auto Stable** and press enter. Highlight **ISE1** or **ISE2** and press enter.

Highlight **Enabled** and/or **Audio Enabled** (instrument will beep when the stability is achieved) and press enter to confirm. The Auto Stable **Sensitivity** can be decreased or increased. Highlight **Sensitivity** and use the left and right arrow keys to slide the bar. The more sensitive you make it (larger black bar) the harder it is to achieve stability in a changing environment.

The **Auto Stable** system works by examining the previous 5 readings, computing the percent change in the data and comparing that change against a **%** threshold value. The **%** threshold value is determined by the **Sensitivity** bar setting. The following chart can be used as a guide when setting the Sensitivity bar.

Sensitivity selected by User	*% Data Variance Threshold*
100 - Most Sensitive, Sensitivity bar is set to the far right	0.05%
75	0.62525%
50	1.275%
25	1.8875%
0 - Least Sensitive, Sensitivity bar is set to the far left	2.5%



Within the Auto Stable menu, you can also choose to **Hold All Readings** for as many parameters as you set for Auto Stable. For instance, if pH and Ammonium have Auto Stable enabled and Hold All Readings is also enabled, then the display will hold the readings once pH and Ammonium have both reached their Auto Stable settings. You must press the Esc key to "release" the held display in order to take subsequent readings. **Hold All Readings** must be reactivated after each use!

CALIBRATION - AMMONIUM, NITRATE, CHLORIDE

The 6051030 ISE/conductivity cable has a specialized calibration container that resembles a large test tube. This calibration chamber can be used to calibrate the ISE sensors with the conductivity sensor. A ring-stand should be used to support this chamber.

> *The ISE sensors can be calibrated at 1, 2, or 3-points.*
> **A 2-point calibration without chilling a third calibration solution is extremely accurate and is the preferred method.**
> *Greatest accuracy is achieved if the actual samples to be measured are within 10 °C of the calibration solutions.*

CALIBRATION TIP: Exposure to the high ionic content of pH buffers can cause a significant, but temporary, drift in the ammonium, nitrate, and chloride ISE sensors. Therefore, when calibrating the pH sensor, YSI recommends that you use one of the following methods to minimize errors in the subsequent readings:

- When calibrating pH, remove ISE sensors from the cable bulkhead and plug the ports. After pH calibration is complete, replace the ISE sensors and proceed with their calibration with no stabilization delay.
- Calibrate pH first, immersing all of the sensors in the pH buffers. After calibrating pH, place the sensors in 100 mg/L nitrate or ammonium standard or 1000 mg/L chloride standard depending on the sensor in use and monitor the reading. Usually, the reading starts low and may take awhile to reach a stable value. When it does, proceed with the calibration. This may take several hours.

Preparing Chloride Standards

The following recipes are provided for preparation of 10 and 1000 mg/L chloride reagents. Nitrate and Ammonium standards can be purchased from YSI or other laboratory supply companies.

It is important to note that some of the chemicals required for these solutions could be hazardous under some conditions. It is the responsibility of the user to obtain and study the MSDS for each chemical and to follow the required instructions with regard to handling and disposal of these chemicals.

You will need: Solid sodium chloride or a certified 1000 mg/L chloride solution from a supplier, magnesium sulfate, high purity water, a good quality analytical balance, 1000 mL volumetric flask, an accurate 10 mL measuring devices, and 1000 mL glass or plastic storage vessels.

1000 mg/L Standard: Accurately weigh 1.655 grams of anhydrous sodium chloride and transfer into a 1000 mL volumetric flask. Add 0.5 grams of anhydrous magnesium sulfate to the flask. Add 500 mL of water to the flask, swirl to dissolve all of the reagents , and then dilute to the volumetric mark with water. Mix well by repeated inversion and then transfer the 1000 mg/L standard to a storage bottle. Rinse the flask extensively with water prior to its use in the preparation of the 10 mg/L standard. Alternatively, simply add 0.5 grams of magnesium sulfate to a liter of a 1000 mg/L chloride standard from a certified supplier.

10 mg/L Standard: Accurately measure 10 mL of the above 1000 mg/L standard solution into a 1000 mL volumetric flask. Add 0.5 grams of anhydrous magnesium sulfate to the flask. Add 500 mL of water, swirl to dissolve the solid reagents, and then dilute to the volumetric mark with water. Mix well by repeated inversion and then transfer the 10 mg/L standard to a storage bottle.

AMMONIUM (NH4+) , NITRATE (NO3-), AND CHLORIDE CL-2-POINT

The calibration procedures for ammonium, nitrate, or chloride are similar to pH. The only differences are the calibration solutions. Recommended values for calibration solutions and the order of calibration are as follows:

Sensor	*1st Point*	*2nd Point*
Ammonium-nitrogen (NH4-N)	1 mg/L	100 mg/L
Nitrate-nitrogen (NO3-N)	1 mg/L	100 mg/L
Chloride (Cl-)	10 mg/L	1000 mg/L

Place the proper amount of 1 mg/L standard for Ammonium or Nitrate (10 mg/l for Chloride) into a clean, dry or pre-rinsed calibration cup. Carefully immerse the sensor into the solution. Allow at least 1 minute for temperature equilibration before proceeding.

Press Cal. Highlight Probe ID or User ID if you wish to add, select, edit, or delete an ID. Probe ID must be enabled in the System GLP menu to appear in the Calibrate menu. User ID will appear automatically. Select 'None' if you do not want a User ID stored with the calibration. When enabled, these IDs are stored with each calibration record in the GLP file.

After selecting your User ID and/or Probe ID if appropriate, highlight **Ammonium, Nitrate,** or **Chloride** to access the appropriate calibration, and press enter. The parameter you want to calibrate may appear under ISE1 or ISE2 depending on your cable type and setup. The message line will show the instrument is ready for the 1st calibration point.

The instrument will display the calibration value at the top of the screen. If necessary, highlight the **Calibration value** and press enter to input the correct value.

Once the readings stabilize, highlight **Accept Calibration** and press enter to accept the first calibration point. The message line will then display "Ready for point 2".

If you do not wish to perform a second point, press Cal (Cal) to finalize the calibration. If User Field 1 or 2 are enabled, you will be prompted to select these fields and then press Cal (Cal) to finalize the calibration. Alternatively, you may press Esc (Esc) to cancel the calibration.

To continue with the 2nd point, rinse the sensor with clean water, then dry it before placing it in the second calibration standard. Allow at least 1 minute for temperature equilibration before proceeding. The instrument will display the second calibration value at the top of the screen. If necessary, highlight the **Calibration value** and press enter to input the correct buffer value. Once the readings stabilize, highlight **Accept Calibration** and press enter to confirm the second calibration point. The message line will then display "Ready for point 3" and you can continue with the 3rd calibration point if desired.

If you do not wish to perform a 3rd calibration point, press Cal (Cal) to complete the calibration. If User Field 1 or 2 are enabled, you will be prompted to select these fields and then press Cal (Cal) to finalize the calibration. Alternatively, you may press Esc (Esc) to cancel the calibration.

AMMONIUM (NH4+) , NITRATE (NO3-), AND CHLORIDE CL- 3-POINT

A 2-point calibration without chilling a third calibration solution is extremely accurate and is the preferred method. If you must perform a 3-point calibration, the following procedure requires one portion of the high concentration calibration solution and two portions of the low concentration calibration solution. The high concentration solution and one of the low concentration solutions should be at ambient temperature. The other low concentration solution should be chilled to less than 10 °C prior to calibration.

> ⚠ **WARNING:** *The chilled calibration solution MUST BE CHILLED TO AT LEAST 5 °C COOLER THAN THE 1ST CALIBRATION POINT, otherwise the 1st point will be OVERRIDDEN.*

Follow the procedure for a 2-point cal. After the second calibration point is complete, the message line with state 'Ready for point 3". Place the proper amount of chilled 1 mg/L standard (10 mg/L for the chloride) into a clean, dry or pre-rinsed calibration cup. Carefully immerse the sensor into the solution. Allow for temperature equilibration. If necessary, highlight **Calibration value** and press enter to manually enter the 3rd buffer value. Once the readings are stable, highlight **Accept Calibration** and press enter to confirm. Press Cal (Cal) to complete the calibration. If User Field 1 or 2 are enabled, you will be prompted to select these fields and then press Cal (Cal) to finalize the calibration. Alternatively, press Esc (Esc) to cancel the calibration.