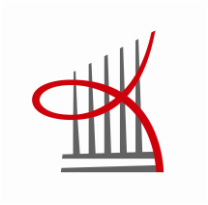

ALGAE CULTIVATION FOR WASTEWATER RECLAMATION

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Bachelor's thesis
April 2013
Environmental Engineering

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ABSTRACT

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The possibility of using algae to clean wastewater has recently gotten attention because wastewater is becoming a bigger problem all over the world. Many scientist and engineers are researching better ways to utilize the high potential of algae to clean these waters. By experimenting with algae we try to explore the potential of growing algae on a mechanical system called “algae turf scrubber” or “ATS” to absorb the excess nutrients for the production of biomass.

By knowing the amount of nutrients added we could see the results in the absorption productivity of our inoculated algae in the ATS. We also wanted to see the influence light and pH would have on the growing potential of the algae. The relationship between pH and total nitrogen were investigated while observing the influence of light.

In conclusion it was determined that if conditions are optimized the system would be well suited in Finland. The ATS could also be used in addition to conventional wastewater cleaning systems.

Key words: algae turf scrubber (ATS); pH; total nitrogen (TN); nutrients; waste water.

CONTENTS

ABBREVIATIONS OF TERMS

ATS – algae turf scrubber

BOD – biological oxygen demand

BOD_7 – biological oxygen demand taken over 7 days

DAQ – data acquisition

ml – millilitre

N – Number of mols

rpm – revolutions per minute

SYKE – Suomen ympäristökeskus / Finnish Environment Institute

TAMK – Tampere University of Applied Sciences

TN – total nitrogen

TP – total phosphorous

1 Introduction

Algae are very important bioremediation agents, and are already being used by many wastewater treatment facilities in United States and other parts of the world. The potential for algae in wastewater treatment is however much wider in scope than its current role since there is much more room for further research and development. (Oilgae, 2009)

Although algae are widely present in freshwater environments such as lakes, rivers and wetlands, they are typically present as micro-organisms visible only with the help of a light microscope. Although often overseen, they play a major import role in freshwater environments by absorbing excess nutrients. Therefore growing conditions should be optimized by controlling the amount of nutrients available (C.Sigee, 2010).

Algae have evolved into a diverse group of photosynthetic organisms, ranging from microscopic single cells to complex multicellular seaweeds (Sze, 1998). Algae are aquatic organisms that are photosynthetic oxygenic autotrophs, that are typically smaller and less structurally complex than land plants (Gualtieri, 2006).

As the world's energy need escalates, scientists and engineers try to come up with greener solutions to obtain more energy. Algae are one of the world's oldest plant forms. They are highly efficient and productive. Through research it has been determined that wastewater treating systems are able to generate biomass as a source of energy as well as a more environmentally friendly way of wastewater treatment.

The aim of this study was to test the efficiency the ATS system. We aimed to see the potential of total nitrogen and phosphorous removal while observing the amount of biomass that is produced as a result. Furthermore we utilized only natural light in the high latitudes of Finland. Finally it was our aim to compare this biomass growth with the algae grown suspended in water.

2 Background and History of ATS

The algae turf scrubber is not a new invention. Innovator and scientist Dr. Walter Adey established it in the late 1970's. It was displayed as a very successful public aquarium. In the 456 litre coral reef, he used an algae turf scrubber to control the chemical parameters such as excess nutrients, which in so many cases influence the water quality of small ecosystems. This system functioned extremely well with a coral growth similar to the top four wild reefs in the world. It recorded to have over 800 species which made it the most bio diverse reef ever measured.

Dr. Walter Adey is the Director of the Marine Systems Laboratory at the Museum of Natural History, Smithsonian Institution (Washington DC, USA). (Adey W.). He researched various types of algae, which led him to understand of how the ocean "recycles" its nutrients.

Recycling in this instance refers to the nutrients that go from plants to animals and then back to the plants again. On land, you see this recycling effect by following the oxygen flow: Green plants absorb carbon dioxide and in return release oxygen – animals use this oxygen, and release carbon dioxide. In oceans and lakes, nutrients go from algae to animals and then back to the algae again.

The picture below shows a simple yet practical illustration of the exchange in carbon sources in both terrestrial and aquatic environments (Wikipedia, 2012).

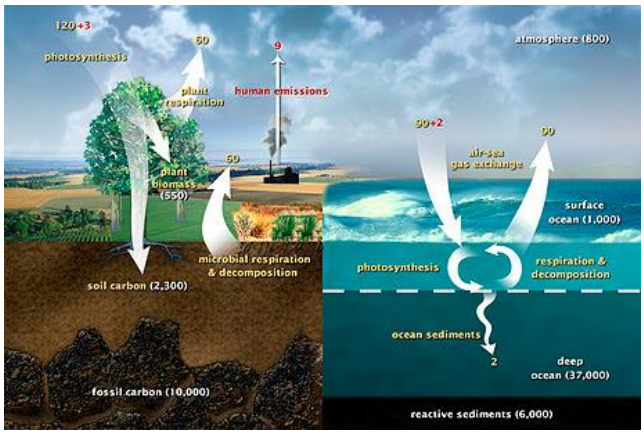


Image 1. An illustration of the carbon cycle as it is found in nature. This is the interaction between photosynthesis organisms on the water bodies and the also the ground.

Dr. Adey built several versions of algae scrubbers for aquariums at the Smithsonian to study the aquatic nutrient cycle. He called them "Algal Turf Scrubbers", because at the time it was believed that “turf” algae were the best type of algae to grow in a scrubber. Dr. Adey also created the first U.S. patent for an algae scrubber, which consisted of a complex dumping device that once it overflows, poured the wastewater onto a horizontal surface. This water would flow over the algae which would neutralize and absorb the nutrients within the wastewater. (Adey W. H., Oct 7, 1980).

After several years of development, he participated in testing a large ATS system on the Great Barrier Reef Aquarium. It was the first time that a system like this was used on a large scale aquarium, whereas before conventional purification methods such as bacterial filters were used. Traditional methods had nutrient levels measured in parts per million, while the ATS had improved concentrations measured in parts per billion even with heavy biological loads in the Reef Tank. The ATS was a great success which even as a result spawned *scleractinian* corals as well as many other organisms (Morrissey, 1988).

He licensed his patent to very few individuals, who for a short number of years sold a limited number of scrubbers to aquarium hobbyists. The complexity of the design, however, and the cost of the license, caused the scrubber units to be very expensive. This, combined with the fact that the units were noisy, splashy, and unreliable (the dumping mechanism would get stuck) caused the sales to be slow. The scrubbers were just starting to make headway into the aquarium hobby in the 1990’s when Adey decided to withdraw his license and no longer allow anybody to make or sell them. He turned his attention instead to commercial and industrial applications, and entered private business making large scale scrubber installations for lakes and rivers (Adey W. H., HydroMentia).

As the internet developed in the 1990’s, aquarium and pond hobbyists began discussing nuisance algae problems, and started noticing a trend: Aquariums and ponds with very high amounts of nuisance algae had no detectable nutrients in the water. This at first seemed odd, since the amount of nuisance algae usually increased as the nutrients in the water increased. How could there be a very large amount of nuisance algae, but no measurable nutrients in the water to support this?

Biologists then began pointing out that when the amount of nuisance algae became large enough, the algae actually consumed all the available nutrients from the water faster than new nutrients were added. Interest in using algae for nutrient control once again increased, this time in the form of keeping the algae in a "sump" or another small aquarium which was connected to the main aquarium through plumbing. With added lighting and flow, algae would grow in this area, and the algae would consume nutrients from the water, just as Dr. Adey's algae scrubber units did. Sumps or other small aquariums were used for this purpose and became known as "refugiums". (Anthony Calfo, 2009)

The name "refugium" was used because the growing algae provided a safe place for small and microscopic animals to breed and grow, and thus was a "refuge" from the large fish and invertebrates in the main aquarium that would otherwise consume them. While the refugiums did indeed consume nutrients from the water, they did not consume them fast enough in all situations; this caused many hobbyists to continue to have nuisance algae problems in their main aquariums.

2.1. Algae turf scrubbers in practice

Algae turf scrubbers are constructed ecosystems used for wastewater treatment. Algae Turf Scrubbing is a solar/algae technology that utilizes primarily string algae to capture the energy of sunlight and build algae biomass from CO_2. ATS is a highly efficient capturer of nutrients from fresh, brackish, and sea waters, as well as a wide variety of waste and industrially-polluted waters. It is thus used in waste water treatment ranging from aquariums to large scale landscapes. By combining algae-produced oxygen at supersaturated levels, with solar or artificial UV light, many toxic organic compounds can be degraded by the ATS systems (Kangas, 2012)**.**

ATS produces a low cost harvestable algae biomass at a faster rate than agricultural or forestry products of the same latitude. ATS is currently commercially used to retain nutrients while producing cattle feed and soil amendments. These algae products can be converted into paper and construction materials which absorb carbon and other heavy metals as well as to break down toxic hydrocarbons. ATS-produced algae can be converted into energy products such as biodiesel and methane (Adey D. W., 2012).

2.2. The algae turf scrubbers design and application

The Algae Turf Scrubber can be designed and constructed to meet a wide range of objectives such as controlling the nutrient concentrations. ATS can be applied on runoff water from agriculture and water management sites. The most important factor to take into consideration is sunlight and a sufficient growing environment for the algae. If the intention is to harvest the algae for a biomass, a harvesting method could be also implemented although the scale as well as easy access should be taken into account when making the designs.

The ATS consists of a suitably sloped surface on which the substrate can grow and the water can run off by means of gravity. Typically, it is advised in a small scale, to attach a grid-like material to your slope, upon which the algae can grow and attach themselves. This also makes it easier to harvest the algae at a later stage. In bigger scale applications, a well-constructed shallow pool should be constructed from a concrete composite which will provide as the growing surface for the algae. Methods may vary depending on the type of algae species used.

The ATS system can be setup with two main designs, namely a bucket pulse overflow (wave motion) or a continuous flowing system – which we used and described above. The wave motion system however works on a principle, where a bucket is slowly filled to capacity, and once this level is reached the bucket design lets the bucket tilt over, releasing the water thus creating a wave like motion.

This wastewater is therefore distributed in equal pulses from which the algae obtains its nutrients. It is believed that this pulse flow enhances the metabolism between the algae cells creating a better homogenisation of species on the growing surface. As a result, the algae turf consists of a dense mat of algae less than several centimetres in height.

As water travels down the ATS, pollutants are removed through biological and physical processes. Carbon dioxide (CO_2), nitrogen (N), phosphorus (P) and other elements necessary for algae growth are rapidly removed from the water column through biological uptake. This speed however is highly dependent on the amount of light available to the algae. The uptake rates will increase under favourable conditions including light, water

temperature, nutrient concentrations pH and correct flow rate. Removal of these compounds (CO_2, N, and P) results in water quality changes within the ATS, including elevation of pH concentrations.

Through control of operating parameters such as flow rates, micro and macro nutrients concentrations and biomass recovery rates, pollutants such as phosphorus are adsorbed onto the algae cell walls, and then recovered along with harvested biomass. This further enhances the phosphorus treatment capacity and reduces system treatment costs. Physical removal can also occur with particular species of algae by trapping substances within the web of algae filaments.

Increasing the performance of the ATS system can be simply done by routine harvesting. The more you harvest the more is able to grow however overharvesting will lead to lower performances causing a lower nutrient recovery. Therefore routine harvesting serves to optimize pollutant recovery.

Care should be taken to avoid letting the algae grow to big, as well as storing pollutants for too long before treatment. Algae always need space to grow and pollutants need to be absorbed otherwise the wetland effect occurs where pollutants will settle and decompose giving off a foul smell.

The Optimization of ATS for nutrient uptake and pollutant precipitation offers a lower-cost alternative for nutrient pollution control than either constructed wetlands or chemical treatment methods. Due to its small footprint and simple, cost–effective construction, the ATS can reduce nitrogen and phosphorus concentrations as well as reducing loads while requiring 90-99% less land than the constructed wetland systems (HydroMentia, 2012).

In addition to recovery of excess nitrogen and phosphorus, ATS systems offer treatment for a wide variety of toxic compounds including heavy metals and chlorinated compounds. Recovered biomass can be readily processed into high quality compost, organic fertilizer or livestock feed as long as there are no heavy metals present, however biofuels can still be easily obtained even with the presence of these heavy metals.

If high levels of heavy metals are present however, the end products should be treated similarly to ground remediation projects. Through recovery and management of the biomass, the ATS eliminates the responsibilities associated with storing the captured pollutants. With over two decades of research and commercial application, the ATS offers proven and sustainable treatment performance. (HydroMentia, 2012)

Below we can see some pictures taken from the system I build. It shows on the right the four lanes through which water would flow and algae would grow. In the middle we can see the netting I placed over the tank to prevent water loss through spillage. Finally the picture on the right shows the aeration stone in the main reservoir.



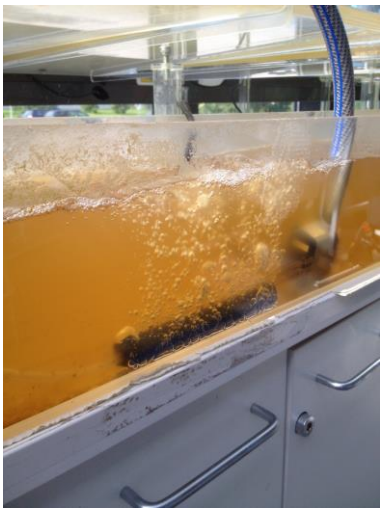


Image 2.The first image on the left shows the ATS system in lab scale.
Image 3.The image in the middle shows the netting placed over the tank.
Image 4.The picture on the right shows the aeration cube in the main reservoir.

2.3. Nutrients and light

2.3.1 Photosynthesis

This part deals with the photosynthesis reduction of inorganic carbon in algae. Whether or not the carbon concentration composition is a limiting factor for microalgae and weather it consist of a stress factor it will depend on a variety of factors. Carbon is the main component of most compounds essential for life i.e. carbohydrates, fats, proteins, nucleic acids, etc. It is the storage of chemical energy used by the living organisms. Carbon is the basis in organic compounds as well as the main element in all living organisms (Miller, 2000).

During the photosynthesis process the carbon from CO_2 and is converted into organic matter. It is this process that converts the inorganic carbon from the air into organic carbon found in the biomass of plants. (Gaur, 2001).

The sun is the primary source of energy for all algae types. In photosynthesis, the absorbed energy from sunlight is used to produce carbohydrates (also sugar) and other organic compounds such as lipids and proteins. The sugars are then used to provide energy for the organism. (Bailey, 2012). The chemical reaction occurring in plants known as photosynthesis is as follows:

$$6H_2O + 6CO_2 \leftrightarrow C_6H_{12}O_6 + 6O_2$$

Light is the primary physical source that regulates the growth of algae. Light is an electromagnetic radiation. It is characterized by its quality (wavelengths) and its intensity. (Wilcox, 2000).

2.3.2 Nitrogen

Nitrogen is one of the most important elements in organic organisms. It can be found in some of the basic structures in our cells (in protein, nucleic acids, etc.). Nitrogen is one of the most important nutrients for microorganisms. Some have the ability to choose between different nitrogen sources. Most commonly for algae, ammonia is considered to be the primary source of nitrogen. If there is no ammonia present in the nutrient source, the environment is considered to be slightly nitrogen deficient for algae. (Gaur, 2001).

2.3.3 Phosphorous

Like nitrogen, phosphorous is a really important part in all living cells. It is contained in the basic building blocks of our cells (nucleic acids, phospholipids, etc.), therefore necessary in the transmission of genetic information and protein synthesis. Phosphates are also necessary when converting ADP to ATP, which is the way of controlled energy storage that is released in living cells. Other vital importance of the phosphorus is that participates in the regulating essential cellar process, such as division, programmed cell death, growth etc.

Most of the inorganic phosphorous found in nature, is already used up in fertilizers or in the industrial application. The limitation of natural phosphorous resources is problematic since there is nothing that can be used as its substitute (Gaur, 2001).

2.3.4 pH for optimum for Growth

General studies show the optimum pH for photosynthesis of phytoplankton has a range from 8.2 to 8.7, while present study shows as 7.99±0.30. And when it becomes above 9.2 photosynthesis activities are inhibited. (LADU, 2012)

Although they can grow fastest at their optimum pH, some species can grow in a wide range of pH levels; however, they can make themselves suited to the environment by producing compounds in response to the environment.

Some species produce compounds at highest level at a certain pH which is different from optimum growth this however increasing their dry weight. This enables them to have tolerances in low or high pH. For example, the Chlorella ellipsoidea specie can grow fastest, in terms of dry weight content at a pH 10 and tolerant with wide range of pH (4~11). They get highest protein content at pH 4, B-carotene at pH 6 and Vitamin C at pH 7.5 and hydrocarbon at pH 9. (Khalil, Asker, El-Sayed, & Kobbia, 2009)

3 Methods and planning process

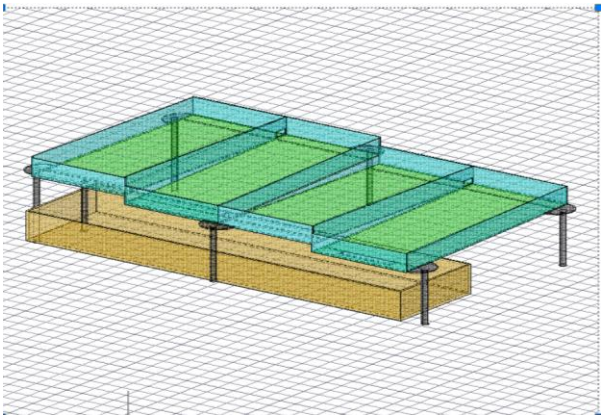
3.1. Planning and design of the project

After doing research about the processes of the algae turf scrubber and how it should function, the next step would be to design the system that would fit our purpose in the lab. One of the main factors or consideration of the design would be the working dimensions of the bench on which the ATS would rest on as the power source was directly behind the ATS.

Also the orientation to the sun was a factor as we need all the sun we could utilize for the algae as we would like to produce as much algae as possible on our small scale scrubber. Making the system as compact as possible would be an advantage.

The ATS dimensions were 700mm x 1000mm with a 50 mm high wall and a 5mm think acrylic. It was sloped at a one degree angle with a pathway of four tracks that are each linked to each other. The water was pumped onto the first track from the reservoir and flows along each track. The algae grew along each track by witch the water would flow down from the last track back into the reservoir. This was the ideal design as we placed the water reservoir underneath the growing surface.

The reservoir was a 30 L acrylic container. The overflow into the reservoir was lined with a rubber mesh that prevented water spills. The same material was used over the reservoir to prevent water spills by the air pump.



Images 5, 6. Above on the left is the simple design by AutoCad 2012. The dimensions are mentioned above. The green part on the sketch is the growing surface for the algae. The yellow is the water reservoir for the wastewater. On the right is a photo of the actual ATS system.

3.2. Artificial waste water used in our test

One of our considerations was to use real waste water from the local waste water treatment facility but to reduce smell as well as contamination from pathogens in the greenhouse we decided to make our own. We purchase a local organic fertilizer called Bio Back. The composition of the fertilizer was very close to the real wastewater; however we had to add some extra components to compliment the Bio Back. Urea, Sodium hydrogen phosphate and glucose were also added.

The recipe for our algae nutrient water is;

Bio Back organic fertilizer 25,416g

Urea 0,954g

Sodium hydrogen phosphate 0, 8/12g (Na2HPO4) M = 141,96g/mol

Glucose 20,2g (C6H12O6) M = 180,15g/mol

3.3. BOD 7 analysis

The biological oxygen demand (BOD) was measured with an Oxitop 110 “HACK” and over seven days. Mostly they are measured over either five or seven days and hence denoted as BOD5 or BOD7. For practical reasons I decided to use BOD7 system as it would allow me to do the measurements once a week without having to go to the lab during weekends. Converting between BOD5 and BOD7 is dependent on the characteristics of the wastewater. Experts within individual countries should be consulted to obtain appropriate conversion coefficient.

3.3.1 Calibration processes of the OXITOP heads

First you clean the OxiTop bottles and dry them. Fill them with magnets and add 164ml distilled water to the bottles. Next add the rubber inserts and place the bottles into the incubator. To reset the OxiTop heads - push the GLP button - maintenance - Reset / release - aim the device to the OxiTop head and push enter. To check the battery life of the OxiTop heads go to the GLP button - check - Info - hold against the OxiTop head and wait for the information screen to appear.

Next, for the calibration of the head itself - GLP button - check - Cal test - (an information screen will appear. Go to ID - Numbering and number the OxiTop heads accordingly.) - press START

Place the OxiTop heads in the incubator but not yet on the bottles. Wait for 4 hours and then place it back on the bottle. Wait 5 days and do a normal BOD5 reading. It is important to note that the incubator should be above 20^{o}C and no more than 22^{o}C.

3.3.2 Preparing the samples for the OxiTop analysis

At the first use of the analysis a calibration had to be done. All samples were taken from the influent water. As I had three systems that are identical from one another I used three samples per system, nine in total. I collected the waste water in identical beakers with a volume of 100ml. This was an ideal amount since 96 ml were needed for the tests on each OxiTop bottle. This amount was determined based on the range selected on the device which in our case was 800 mg/L.

3.3.3 Oxtitop procedure

I made sure that all the equipment is available with the right amounts. This included magnetic rods used for stirring, Oxitop heads, oxitop bottles, rubber fittings, the magnetic stirring bed, and the chemicals that would be needed for the BOD method. 12 bottles would be needed of each, nine samples and three blanks samples as the control group.

The bottles were arranged in a row. I inserted the magnets first into the bottles as they were most easily forgotten. I arranged the samples by number and marked the bottles to prevent future confusion.

Next the “DROPS” that would be added. Care needs to be taken to add the correct amount based on the range. In the case of 800mg/l I used two drops. Since the range was small enough and a large enough pipette was available, a calibrated pipette was used to transfer the samples into the bottles.

After this, the rubber inserts were fitted. The NTH tablets were added and the bottles closed up using the OxiTop heads.

The following step is likely the most important. The OxiTop heads need to be registered into the wireless reading device known as the OxiTop controller. After registering the heads, bottles are placed into the incubator onto the 12 spaced magnetic stirrers. It is very important to keep the samples at a stable temperature of 20–22^{o}C. The blanks would be treated in the same way however distilled water would be used instead of the wastewater.

3.4. Total nitrogen analysis

For nitrogen analysis we used the total nitrogen kit from HACH LANGE as it was easy and convenient. The kit can be used with the Hack reader and 12 samples can be digested at the same time. The whole process takes about an hour and results could be compared with other standard methods.

3.4.1 Sample preparation for total nitrogen

As the water sample was so thick with algae it had been problematic to filter even a small amount. I decided to use a centrifuge to first remove the algae and then take the samples. This technique seemed to work well and I was surprised to note that it hadn't been done in other studies. This process took 10 minutes at 3000rpm in 45ml centrifuge sample bottle. The disadvantage was that I could do only four tubes at a time; however I could prepare the following four samples in advance for the TN test.

Next I would gather the equipment for the analysis. The digester should be under the hood used for extracting toxic gases. The fin pipettes and pipettes tips should be close-by for easy access.

3.4.2 HACK method for total nitrogen

The method is described in a pamphlet found in the TN kit. It is rather easy to understand and contains 8 steps displayed by pictures. In the kit there should be 2 reaction tubes, 25 bar coded cuvettes and 4 reactant chemicals. These chemicals are labelled as A-D in the kit.

The first step is to insert 0.5ml of the sample into the reaction tube. In addition you should add 2.0ml of solvent A and one tablet of solvent B.
In the second step the reaction tube should be placed into the digester and digested for 60min. The digester thermostat will indicate when it's done with a beeping sound. Conveniently it heats the sample and then cools it to room temperature once the process is complete.

Third and fourth step describes adding the microcap C to the reaction tube and then to close the cap. Invert the tube back and forth until the microcap is fully removed.
Fifth step is to take 0.5ml sample out of the reaction tube and to insert it to the cuvette testing tube.
In the sixth and seventh step 0.2ml of solution D is added, and the cap should be closed and inverted again a couple of times. A timer should be set for 15 min.
The eighth and final step is to place the barcoded cuvettes into the Hack reader. The reader will automatically select the correct program and range then will proceed to do the reading. The sample range is at 40mg/l which is also the maximum reading range for the TN kit. If the value of your sample is more than that, then it should be diluted.

3.5. Total Phosphorus analysis

Total phosphorus is also measured using the Hack method 8190 and 8048 but requires a little more time and precision. This method contains two parts. The first part is the total Phosphorus digestion with an acid persulfate digestion. The second part is the actual Phosphorus reaction method with the PhosVer3 powder pillow (Ascorbic acid).

3.5.1 Sample preparation for total Phosphorus

I used a centrifuge to remove the algae and then take the samples. Samples were taken from the same samples used in the nitrogen analysis. This saved time from having to use new samples and having to place them in the centrifuge as well. The amount left over from the TN is sufficient for the TP test and could even be done at the same time as the TN.

It is good to have the equipment set out and ready for use, as this would increase the productivity. This included the Potassium Persulfate powder pillows, the 5.0N Sodium hydroxide solution, 5.25N Sulphuric acid solution, Deionized water, a 25ml graduated cylinder, a 125ml Erlenmeyer flask and a hotplate.

The digestion process goes as follows:

Step 1. Measure 20ml on the Erlenmeyer flask then mark it and top it with another 5ml. This is done to prevent faulty reading at later stages when the water is boiled and goes below that level.

Step 2. Add the content of the Potassium Persulfate powder pillow and swirl it until it is fully mixed.

Step 3 and 4. Use a 1ml calibrated dropper to add 2.0ml of 5.25N Sulphuric acid to the flask and place the flask onto the hot plate. Boil gently for 30min and make sure the sample is not boiled dry. To control this step it's important to add small amounts of de-ionized water.

Step 5. After boiling for 30min leave the samples to cool at room temperature.

Step 6. Use a 1ml calibrated dropper to add 2.0ml of 5.0 N Sodium Hydroxide solutions to the flask and swirl it until it is fully mixed.

Step 7. Pour the sample into a graduated cylinder and adjust the volume to 25ml with deionized water.

Step 8. Note that this step is identical to the second part of the TN methods. Gather nine cuvettes with a volume of 10ml and make sure that the cuvettes are clean and dry. Do not use any detergent to clean the cuvettes as it will leave a small filament in the cuvette which may give a faulty reading.

Step 9. Add the sample to the cuvette and add the PhosVer3 powder pillow. Make sure to mix it well. Samples should be left alone for 2min to allow the PhosVer3 to react.

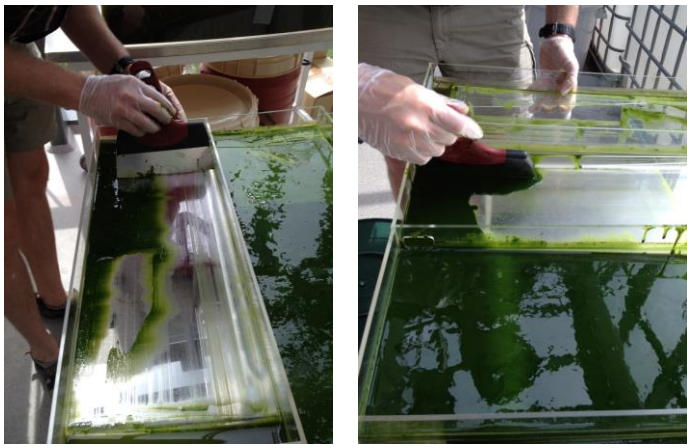
Step 10. As we are using the Hack reader it's important to use the appropriate program. Select the program (490 P React PV) with a consecration limit of 2.50mg/l. Insert the cuvettes and take the reading. If the reading is higher that the initial value it would be wise to dilute your sample and try again.

3.6. Harvesting method for the algae from the ATS

The total growth phase of the algae lasted three weeks. During this stage the algae would absorb nutrients from the wastewater, neutralizing the waste in the process. At the end of the three weeks the algae would be harvested.

Images 7, 8, 9 and 10. The images above show the growing sequence of the algae during a period of three weeks. The first image on the left shows the growth during week one. The next picture shows the grown during week two, followed by the next picture indicating the grown during week three. The final picture on the right was taken during harvesting two days after picture three at the end of week three.

The harvesting of samples was rather easy and I didn't need any special equipment for this process. As the algae are growing on the acrylic without any other growing surface it was very easy to harvest. I used a painter's tool to scrape it from the surface. This was a rather effective tool, as it would leave enough algae for the next generation to be cultivated.



Images 11,12. The images above show the process of harvesting the algae off the ATS.

I collected the algae in a centrifuge tube (45ml) as I used it in the centrifuge to remove excess water for the drying process. This also proved to be the fastest way comparing with the filtration method used before. It was interesting to note that all articles I researched filtered the algae rather than placing them in a centrifuge. Although smaller amounts of algae could be harvested this way to fit in the tube, and the process was more tedious, the time taken to dry was much faster than the previous method. Previously the algae were placed in a drying chamber which took three days. I removed the excess water from the tube so that there is as much algae left as possible. Next the algae were removed from the tube. I used a small spoon to remove as much as possible. There was a small amount of algae left in the tube however. This I removed using ethanol as it evaporated much fast during heating than water in the drying process.

The samples were put into a porcelain cup and then dried over a period of 24 hours at 65^{o}C to remove as much of the remaining moisture as possible. The dry sample was then taken and weighed.

Finally the samples would be placed into an incinerator for four hours at a temperature of 450^{o}C. The sample would be weighed again and compared with the weight measured before.

Images 13, 14. The image on the left above shows the samples in the dryer. The image on the right shows the samples after incineration.

3.7. The software used for the system.

As this thesis is done for the Finnish Environment Institute (SYKE), they suggested using an automated pH and light meter. All measurement equipment used was connected with an automated program on a laptop next to the ATS system that would record the data 24/7. It was important to make sure the equipment was well calibrated and checked regularly.

The only manual changes done were on the temperature to keep it regulated. All data was automatically saved onto a notepad then later I transferred it into excel for data processing. The recording interval was initially set to every second; however this gave too much data to be processed, so it was changed to an interval of every 300 seconds.

The system was programmed using the programming software by "National Instruments LabVIEW", which is a graphical programming environment commonly used in instrumentation and control applications. The software created with LabVIEW uses the DAQ device to read the measurements and converts the voltage signal to the corresponding pH reading with the following formula:

$$pH = 7 - \frac{2.5 - SensorValue}{0.257179 + 0.000941468 * \text{Temperature}}$$

Thus the pH reading is temperature dependent. The temperature is not measured by the system but instead the user needs to provide the correct temperature value. Also the 0-10V irradiation measurement is scaled to 0-2000 micromoles light quanta per second per m^2.

In addition to the measurement application (altubber.vi) there is a separate application to calibrate the electrodes. The measurement application uses calibration parameters to correct the measurement. These parameters are obtained by calibrating the electrodes with the calibration application.

This application calculates the parameters and saves them in a file. When the measurement application is started it reads the calibration parameters from the file. When the electrodes are recalibrated the parameters are also updated into the same file.

The measurement application shows the data on two graphs. All the data can also be saved in a text file.

3.8. The hardware used for measuring.

Since there were multiple ATS in the lab that each needed their own pH controller, I installed a special unit to control all at the same time. Utilizing a DAQ device, one can simultaneously and continuously measure and control the pH of 1-4 units. The DAQ is connected to the PAR which links the individual pH electrodes into the whole system. The system makes it possible to monitor the measurements in real time as well as save the data for later analysis. The system consist of 4 pH electrodes, 4 pH amplifiers, DAQ device, 2 power sources (1 for the amplifiers, 1 for the PAR sensor), a self-made electrical connection board, a measurement application and a calibration application that run on PC.

3.8.1 pH reader.

The Phidget pH Lab Electrode is a simple pH probe that does not compensate for temperature. This electrode was selected because it was cheap and seemed to be rather stable. The measurement signal given by the electrode has a quite small voltage that needs to be amplified.
To amplify the voltage signal the Phidget pH/ORP Adapter is used. It amplifies the millivolts signal to a 0-5V signal. The amplifier requires a 5V DC power input (phidgets, 2012).

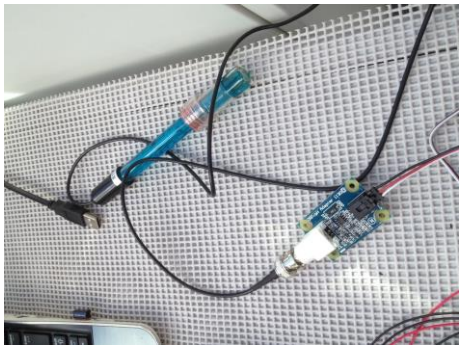


Image 15. The photo is the pH Lab Electrode at the top, and an amplifier below it

3.8.2 Light meter

The irradiation is measured with a LiCor Quantum sensor displayed below (LI-COR, 2012). The units most commonly used are micromoles of quanta per second per square meter (µmol s^{-1} m^{-2}).

Images,16, 17. The image on the left shows the quantum sensor which is connected to the PAR via the Li-Cor Trans conductor displayed in the image on the right.

3.8.3 The data acquisition (DAQ) device

To store the measurement data of the amplified signals on a PC, a data acquisition (DAQ) device is needed. In this system the "National Instrument's DAQ device model USB 6210" was used. The DAQ device conveniently connects to the PC through USB.

To make connecting the amplifiers to the DAQ device easier there's a self-made connection board again this was done by Susanna. The amplifiers are plug to the connectors on the board. The connection board has switches so that individual channel can be switched on and off. Also the 5V power source is plugged to the board to power them the amplifiers. This one common power source is used for all the amplifiers.

The measurements channels are wired to the DAQ device using differential measurement configuration. Hence each channel uses two wires that correspond to the positive and negative signals.

The PAR sensor is connected to the DAQ device in a similarly. In addition there is one ground wire. This connects all the measurement channels to the ground input of the DAQ device. (instruments, 2012)

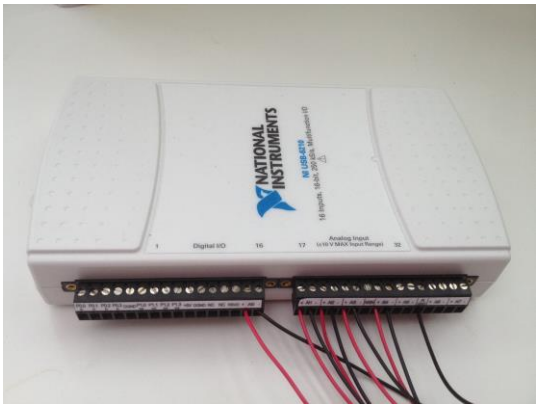
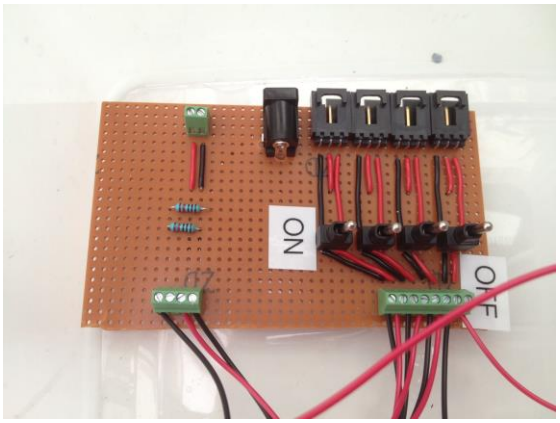


Image 18. The photo is the multifunction DAQ (multifunction data acquisition



Image, 19. This photo shows the PAR

4 Results

Due to unfortunate circumstances once the results had been collected and the system build down, all hard data results have been lost. So in this thesis I have tried to compile as much of the results as possible from averages and from memory. No accurate data can be given, however this section is aimed at giving an overview rather than exact account of the results obtained from this project.

Overall result of measurements assessment of purification performance of the system

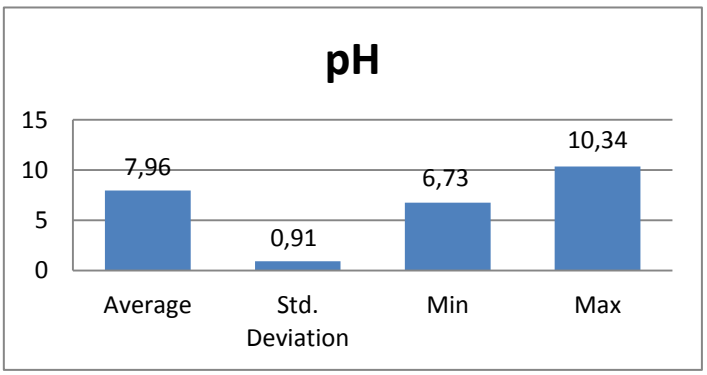
The table below is a general statistical view of the three test runs over the course of the project time. All measurements from each set of experiments over three weeks test runs are indicated in the values. It shows the amount of data for each variable as well as the average, median, standard deviation, minimum value, lower quartile, upper quartile, and the maximum value.

Table 1. Below is and statistical overview of the experiment and all values are in mg/L

	N	*Average*	*Median*	*Standard deviation*	*Min*	*Lower Quartile*	*Upper Quartile*	*Max*
pH	83	7,96	7,59	0,91	6,73	7,42	8,36	10,34
NO^{2-}	81	14,73	14,00	3,56	7,14	12,30	17,00	25,30
Total Nitrogen	81	8,69	8,26	2,09	4,21	7,25	10,03	14,92
PO_4^{3-}	72	1,12	1,15	0,71	0,15	0,39	1,83	2,27
Total phosphorus	74	0,36	0,36	0,24	0,00	0,12	0,59	0,74

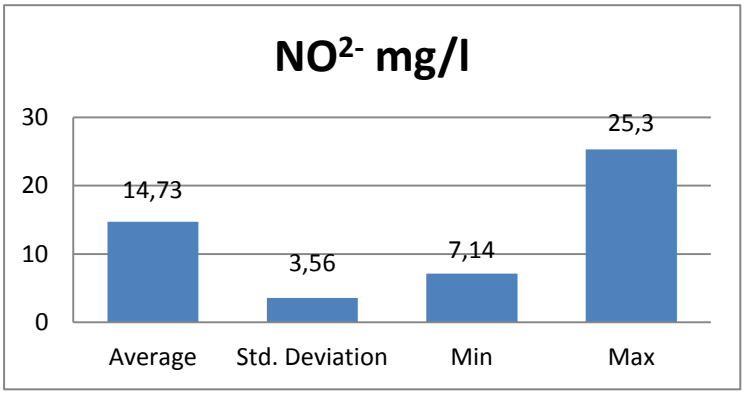
ASSES THE total N and total prediction based on the average and median values

Table 2. Statistical representation of the pH.



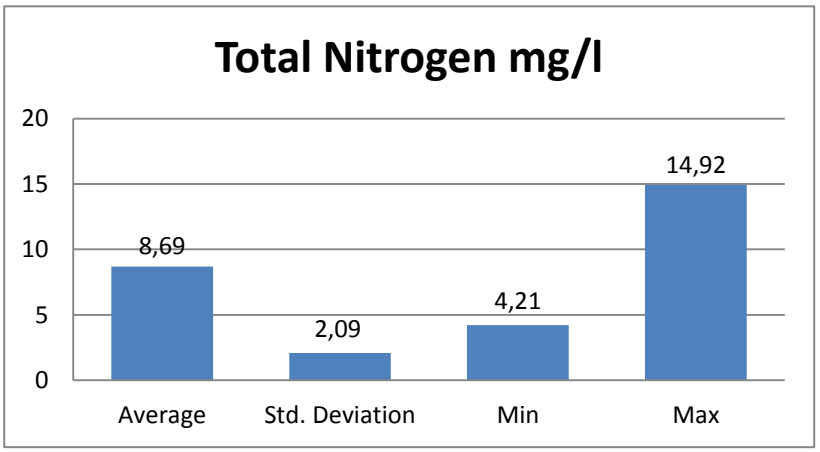
The next graph shows the statistical average for the total nitrate in the water. This gives us an understanding of how well the nitrate was absorbed. As we can see the max value is much larger than the min value. This indicates that the algae have definitely absorbed some of the nitrate.

Table 3. Statistical representation of the nitrate concentrations in the water.



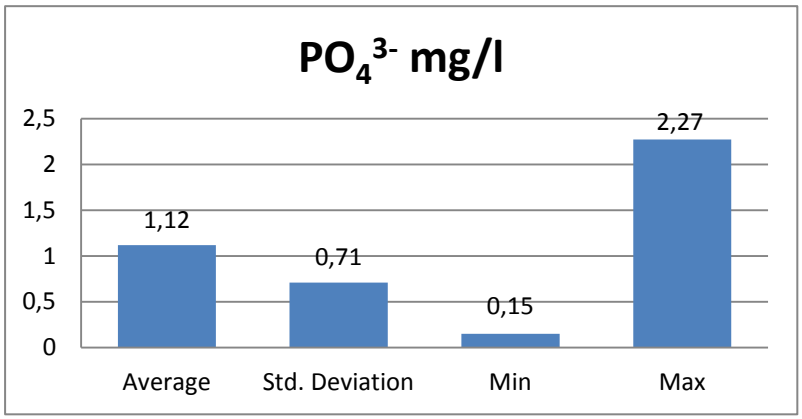
The graph below is a visual representation of the statistical averages for the total nitrogen over the period of the whole project. As we can see from the large fluctuation between min and max, there has been a definite decrease in total nitrogen showing that the algae has absorbed the nitrogen quite well.

Table 4. Statistical representation of the total nitrogen.



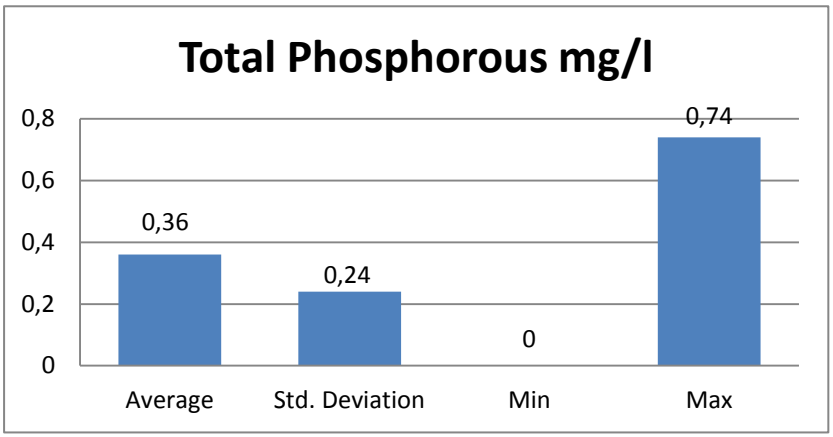
The graph below shows the phosphate concentration in the water. From there we can see that the phosphate has significantly decreased, however the standard deviation is quite high by comparison. The values here may be inaccurate due to calculation errors.

Table 5. Statistical overview of the phosphate concentrations in the water.



The graph below shows the total phosphorous in the water. The min and max values show a massive reduction. The results however may be inaccurate due to calculation errors.

Table 6. Statistical overview of the total phosphorous.



4.1. Nitrogen and phosphorus ratio

According to John Leju studies the optimum Nitrogen and phosphorus concentration for photosynthesis of phytoplankton has a range of 3858 ±1087.37µg/L (N), 340.83 ±12.44 µg/L (P) µg/L respectively. And at least 30µg/L (N) and 15µg/L (P) are needed for phytoplankton growth. (LADU, 2012)

Every kind of algae has its proper N/P ratio necessary for growth. So N and P don't restrict the growth at the same time. Study about algae on biofilm shows when N/P ratio is 10 : 1 the removal of Nitrogen and Phosphorous is the best. (Lizhen Xing, 2011)

4.2. Temperature

The temperature stayed rather stable throughout the duration of the project, increasing during the day as outside temperatures started to rise. Inside greenhouse the temperatures would stay more stable than on the outside.

The temperature ranged from 20-25^{o}C. Any changes in the temperature came from the environment surrounding the system and were not affected by the processes occurring in the tanks. The only possible internal influence would have been the water pump.

4.3. pH

The graph below is a visual representation of the statistical overview of the pH measurements taken over the duration of the project. As we can see from there, the pH varied during the experiment and being slightly basic mostly of the time

The pH varied quite a lot and would rarely stay stable. As we have seen from the statistical overview, the pH ranged from 6.73 – 10.34. The preferable pH was ranged from 7-8. The pH had a tendency to rise. The pH would stabilize for several days when adding water or nutrients, after which it would start to rise again.

In the graph below we can see the average of the results obtained each day when tests were done. From there we can see the fluctuations as well with a possible dependency on the nitrogen concentrations.

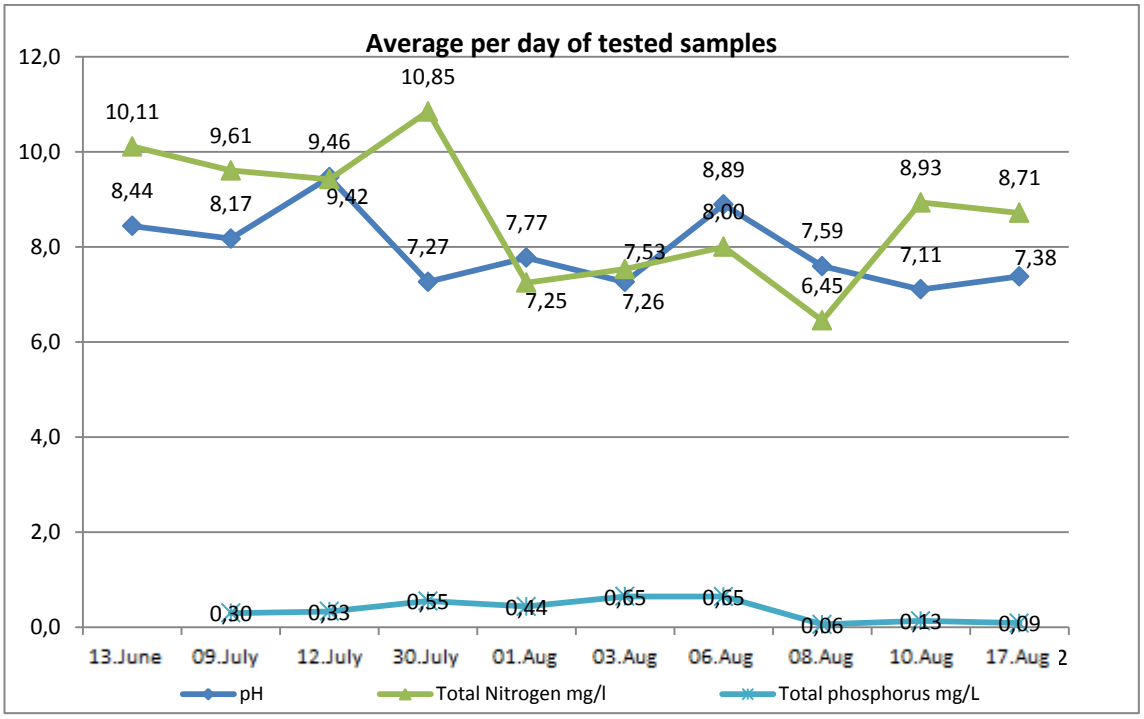


Figure 1. Average of results from each day of testing displaying pH, TN and TP.

4.4. Total Nitrogen

The total nitrogen varied quite significantly. Once nutrients were added, there was a definite break down in nitrogen. The NO_3 started at 40mg/l which was added for the first time on the 11th June. Two days later as we can see from the graph above the average values had already dropped to 17mg/l.

Nutrients were added several more times during the duration of the project. Unfortunately due to the loss of data, the amounts and dates that they were added are unknown. We can though see a trend of decreasing concentrations indicating a good amount of absorption by the algae.

4.5. Total Phosphorous

The total phosphorous concentrations gave some strange results. I determined that a calculation error occurred causing the results to be so low and hence unreliable. Further results obtained are also unfortunately lost similarly to those of the total nitrogen.

4.6. BOD_7

The BOD_7 results were never attained due to problems with the measuring equipment. The software used was out of date and could not update in time to transfer the results. During the course of the testing, they did seem to give acceptable values. They were however unexpected as they were far below the expected range.

4.7. Algae dry weight

At harvesting times, all of the algae attached to the growing surface was removed and weighed. There may however have been some remaining algae in the water since the colour of the water was still dark green.

The table below gives the results obtained from the first harvest. These results can't be compared to anything however to determine if the values are high or low.

	Dry Weight (mg)	Incineration Weight (mg)	Weight difference (mg)
Box 1	33,9	4,4	29,5
Box 2	29,2	5,1	24,1
Box 3	29,8	5,0	24,8

Table 7. Results of the weight of the algae, obtained from the first harvest.

5 Discussion

As this project had been done mostly during the summer time, results had been relatively good. This is due to the amount of daylight available during Finnish summers and taking into consideration that it had it been a cold and cloudy quite often. Despite some complications, in my opinion this project had overall been successful.

I noticed a trend in the absorption of the nutrients, due to a major decrease of the original added nutrients. It appears that the nutrient concentrations fluctuate. First a steep decrease, followed by a gradual decrease. Thereafter the concentrations rise, but fall again in a steep decline continued with the slower steady decline. This trend would repeat after every testing stage. Furthermore I noticed that the nutrients were not completely absorbed by the algae after the three week testing stage.

There seems to be an interesting dependency on the pH. Two conflicting trends were noticed. It appears at first that the pH and Total Nitrogen are inversely proportional to each other, since on each test when one increases, the other would decrease. However, it is possible that a delayed effect occurs, where if the pH changes, then in the next test the Nitrogen will have taken on the same change. More research is needed directly relating to changes in pH and the effects it has on the nutrients (specifically the total nitrate and the NO^{2-}).

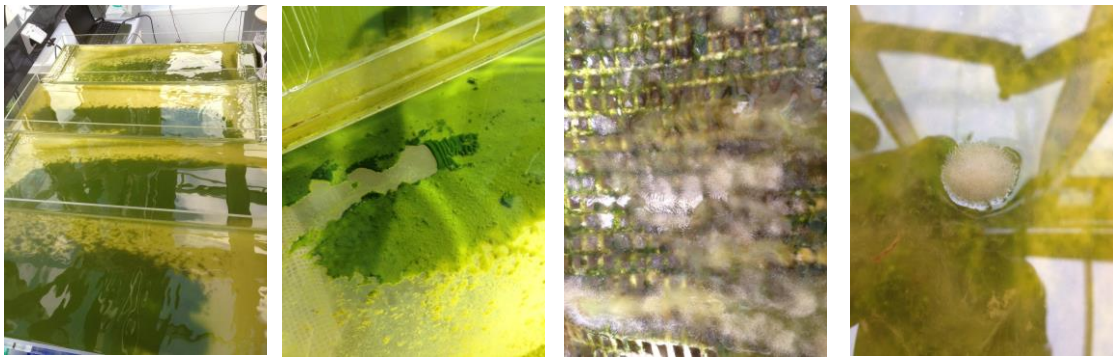
There is a definite dependence on the pH and therefore it is really important to have a constant continuous analysis of it. It is possible that the rapid growth of the algae had a significant effect on the change in parameters such as the pH, the nutrient levels, and the nutrient absorption rates. I observed from the results that the absorption rate was at its highest every time more nutrients were added.

While the nitrogen had definite fluctuations in its reading, indicating that some correspondence to changes in conditions, the phosphorous seemed unaffected. I can therefore deduce that the phosphorous testing was unsuccessful. After an initial decrease, the levels were permanently at a low level and remained relatively unchanged compared to the nitrogen and pH fluctuation. Reasons for this include possible errors in calculations, uneven heating due to uneven beakers and too large number of Erlenmeyer flasks on the stove, or even airflow under the hood that may have been too strong. Furthermore the

lab conditions may have been contaminated since the lab space was shared with students that could have been careless in cleaning the equipment after use. Although it's surprising that the conditions didn't affect the nitrogen readings in this way. To be on the safe side, I decided to make a quality control test.

After doing the quality control test, I found that the acid concentration used in this method for the digesting process had been wrongly calculated, making the samples less acidic and thus the results would be inaccurate. It is clear that therefore the three tests are unreliable and more attention should be paid during future tests. In the future it would be of most importance to do the quality control test before the actual test runs begin.

Other problems occurred with the growth of the algae showing that further investigations are required. Problems in absorption could have risen from wrong pH levels or lack in sunlight. As can be seen from the picture below on the left, growth was occasionally a bit stunted. To the right of that we can see some of the algae scratching off from the growing surface as the water flowed over it. It would seem that the growing surface should be changed a bit giving some resistance for the algae to attach its self to when facing the water current flow. The two pictures to the right indicate some mould growing on the algae. This shows that some impurities entered the lab. Care should be taken in the future to ensure sterile conditions.



Images 20,21,22,23. Stunted growth of the algae, algae tearing off of the surface, and mould growing on the algae.

One can however, despite of the complications and failures, note that there was a definite decrease in nutrient levels giving rise to good growth in algae. This was the basic aim of the experiment. I can deduce from this that the algae definitely neutralize the wastewater to some extent and that therefore the experiment as a whole was a success.

Algae turf scrubbers in Finland could work if conditions are optimised. Wastewater treatment plants release a lot of nutrients that could benefit algae growth. This is why it would be smart to use this in addition to conventional wastewater treatment plants to grow algae, which in turn will help to clean the water. From an economical point of view, this application may not even be farfetched. With careful planning and funding from investors this application could really make a different in the future. This would require more intensive studies, especially regarding winter times where conditions are far from optimal.

One of the challenges for this method for larger scale applications is the space requirement. ATS systems occupy relatively large amount of space. Since it is a horizontal system, it does not have the advantage of utilizing vertical space like other systems. Even though as a whole it is smaller than wetland constructions, Finland is a cold country and as such might have to be constructed in a greenhouse. Even though it would occupy a large land space, over the long run it would be cheaper than current wastewater treatment plants. According to (Kangas, 2012) it would take about 11ha of land to treat the daily manure excrement of 1000 cows in a hot to temperate climate. ATS systems would also give existing wastewater treatment companies an additional source of energy that would be beneficial from a sustainability point of view.

The fact that nutrients are significantly reduced, make this a method worth exploring. In Finland it should be attempted in conjunction to conventional treatment plants and not as a standalone process. Even in dairy farming or other similar applications such as piggeries, there is a problem of excess nutrients. Neutralizing these would dramatically reduce the environmental load. Small ponds and streams in the vicinity of these farms would carry their own loads better as a result, and give the farmer en extra source of income if well managed.

In conclusion I would advise further research in this area to be optimized for use in Finnish wastewater treatment plants, and even livestock farms.

6 Acknowledgment

I would like to express my very great appreciation to main supervisors Ms Hilda Szabo (TAMK) and Dr Kristian Spilling (SYKE) for their patient guidance, enthusiastic encouragement and useful critiques of this research work. Advice given by Both Ms Szabo and Dr Spilling has been a great help in understanding basic concepts in this study and their useful application.

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Assistance and Technical support provided by SYKE staff member, Ms Susanna Nenonen for Building of the electronics and programming was greatly appreciated. I wish to acknowledge the help provided by Dr Hanna Prokkola for oxitop calibration and technical assistance. I would also like to thank Tunkua Oy for building our equipment and their great costumer's service.

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