Treatment of Wastewater from Mineral Processing by using Algae

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ABSTRACT

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Nowadays, the utilisation of algae in industrial processes to produce useful compounds or to treat waste streams is of great interest. Industrial wastewaters such as wastewater from mineral processing are becoming more and more an environmental issue. A lot of research projects all over the world are trying to find a sustainable, effective and cost-efficient way to treat these waste streams and deal with the usage of algae and Algae Turf Scrubbers (ATS).

In this Bachelor Thesis, the treatment of wastewater from mineral processing using algae in an Algae Turf Scrubber is presented. For this thesis project, wastewater from mineral processing from the Talvivaara Mining Company Plc. was used. The difficulties that this water creates for algal growth and viability is shown. Pollutants such as heavy metals (Ni & Zn), alkali metals (Na & K) and sulphates were analysed to investigate algal removal possibilities. The pH-level and illumination were monitored all the time in order to control for their effects.

For purposes of comparison, in addition to the ATS system, two other systems, a willow stack tower and an ebb and flow system, were used in a comparable way in this project to treat wastewater from mineral processing.

The first test runs in this research project show two ways in which it is not possible to treat wastewater from mineral processing with algae. Nevertheless, the tests gave good background information about which properties have to be adjusted to treat wastewater from mineral processing successfully.

Key words: algae turf scrubber (ATS); pH; heavy metals; wastewater

CONTENTS

ABBREVIATIONS AND TERMS

AAS	Atomic absorption spectroscopy
AS	Algae solution
ATP	Adenosine-5´-triphosphate
ATS	Algae Turf Scrubber
BOD	Biochemical oxygen demand
BOD_5	Biochemical oxygen demand measured over 5 days
DNA	Deoxyribonucleic acid
EBB	Ebb and Flow system – A system imitating ebb and flood, using willow branches as growing substrate for microorganisms to treat wastewaters.
HM	Heavy metals
MAPS	Managed Aquatic Plant Systems
MtIII	Metallothionein III
NWW	nutrient enriched wastewater from mineral processing
NS	nutrient solution
NW	nutrient enriched tab water
PLS	pregnant leaching solution
ppb	parts per billion
SYKE	Suomen ympäristökeskus / Finnish Environment Institute
TAMK	Tampere University of Applied Sciences
TOC	Total organic carbon
WST	Willow Stack Tower – A system for wastewater treatment, where water is spread over willow branches which are used as growing substrate for microorganisms.
WW	Wastewater from mineral processing

1 INTRODUCTION

Water is essential to life on our planet. A prerequisite of sustainable development must be to ensure uncontaminated streams, rivers, lakes and oceans. There is growing public concern about the condition of fresh water. Mining affects fresh water through heavy use of water in processing ore, and through water pollution from discharged mine effluent and seepage from tailings and waste rock impoundments. As mine technologies are developed to make it more profitable to mine low grade ore, even more waste will be generated in the future (Safe_drinking_water_foundation, 2009).

Through mineral processing metals and metal compounds tend to become chemically more available, leading to the generation of acid or alkaline drainage loaded with large quantities of dangerous substances, such as heavy metals. In many cases tailings are stored in large ponds, where they are retained by means of dams. The collapse of dams or heaps may have serious impacts on environment and human health and safety (European_Comission, 2012).

Conventional technologies, such as ion exchange or lime precipitation, are often ineffective and/or expensive, particularly for the removal of heavy metal ions at low concentrations (below 50 mg/L). Therefore cheap and sustainable treatment methods for wastewaters from mineral processes are required. Algae can provide an appropriate solution for this issue, because their ability to absorb heavy metals has been recognized for many years. Especially at low concentrations algae are effective in HM removal (Dwivedi, 2012).

2 BACKGROUND

In the following chapter the background to this project is given. The mining company Talvivaara is shortly introduced and the process where wastewater is produced simplified explained. Then the idea of an Algae Turf Scrubber and the utilized system is scarcely presented, before algae in common and especially the available species are introduced.

2.1 Talvivaara

Talvivaara Mining Company Plc. (further called Talvivaara) is a mining company located in Sotkamo in the east of Finland. Talvivaara is mining basically for nickel, but by-products like zinc, copper and cobalt are gained as well. To obtain the metals from ore a bioheapleaching process is used. In October 2008 the first metals had been produced at Sotkamo mine from the first industrial size heap (Talvivaara, 2009).

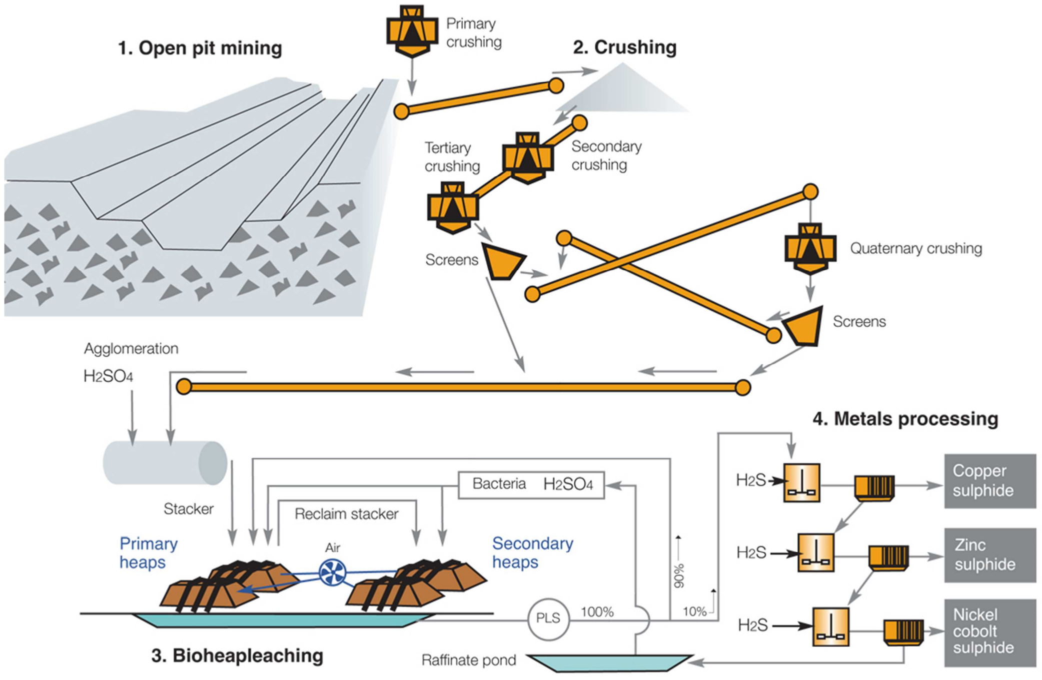


Figure 1. Process steps Talvivaara (Talvivaara, 2010)

At the mining area the production process consists of four main steps, shown in Figure 1: open pit mining, crushing, bioheapleaching and metal processing / metal recovery.

The wastewater from mineral processing analysed in this research project is produced during the last steps of this production process. A leaching solution is spread over an ore heap during 13-14 month. Additionally the heap is aerated, so that special bacteria convert slightly soluble metal ions in readily soluble ions, which are washed out by leaching solution. As a result the metal concentrations increase and a pregnant leaching solution (PLS) is generated. During the metal recovery process, shown in Figure 2, the metal ions are precipitated from PLS with hydrogen sulphide. A part of the arising water is treated in addition with limestone or lime and aerated to neutralize and precipitate metals and sulphates so it can be reused in the bioleaching process (Talvivaara, 2009). Residues like thickener underflows are sent to and stored in gypsum ponds (Net_Resources_International, 2012).

In November 2012 one of these gypsum ponds was leaking for around two weeks. The acidic and with heavy metals loaded water flew out of the gypsum ponds into safety areas on the mining area, but also into the environment (Talvivaara, 2009). This gave reason to think about a way to treat this wastewater from mineral processing.

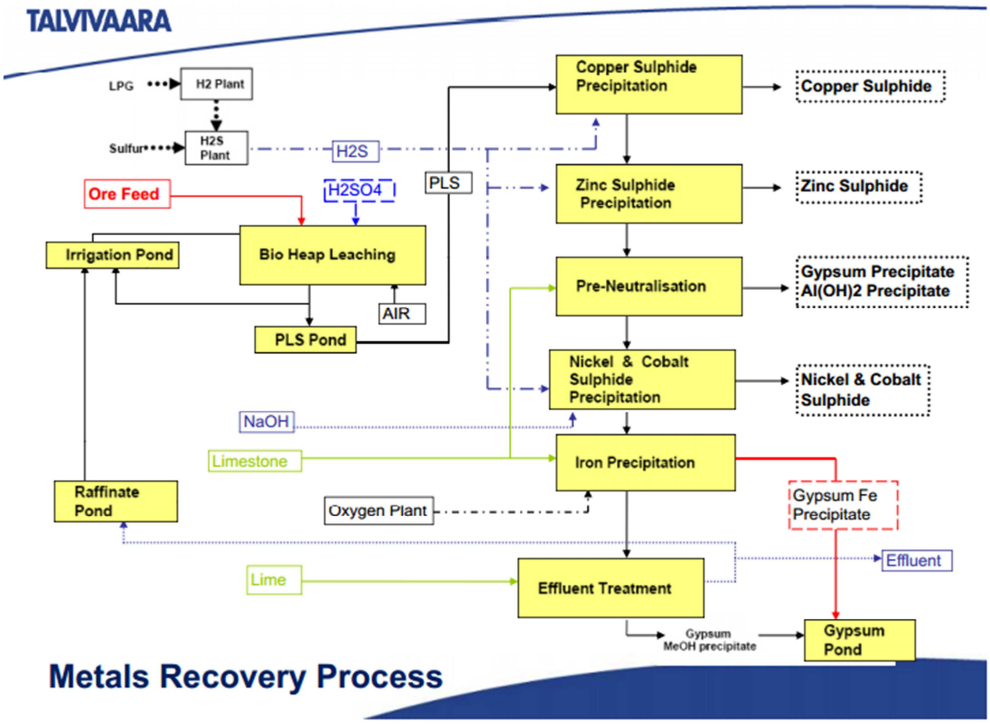


Figure 2. Metal Recovery Process at Talvivaara (Talvivaara, 2010)

The wastewater which is treated in this project is from the recovery process at Talvivaara area and already pre-treated. The pH-value is between 3 and 4, it contains lots of metals in different concentrations, which can be seen in Table 1, the sulphate content is between 15000-18000 mg/l and the nutrient content in form of nitrate and phosphate is very low.

Table 1. pH values, sulphate and metal concentrations measure by Talvivaara

Tank		**TAMK 3**	**TAMK 1**	**Average**
pH		3	3.9	3.45
SO_4^{2-}	**mg/l**	14900	17600	16250
Al	**mg/l**	175	207	191
As	**mg/l**	0.54	0.6	0.57
Ca	**mg/l**	318	378	348
Cd	**mg/l**	0.26	0.3	0.28
Co	**mg/l**	1.38	1.63	1.505
Cr	**mg/l**	<0.0121	<0.0121	<0.0121
Cu	**mg/l**	<0.0121	<0.0121	<0.0121
Fe	**mg/l**	1360	1595	1477.5
Mg	**mg/l**	1736	2045	1890.5
Mn	**mg/l**	1318	1559	1438.5
Na	**mg/l**	824	952	888
Ni	**mg/l**	64.6	77.7	71.15
Si	**mg/l**	13	15.4	14.2
Zn	**mg/l**	121	143	132
U	**mg/l**	0.77	0.82	0.795

2.2 Algae Turf Scrubber

Today ATS are already a quite old idea, but still not used very often in industrialized processes. The ATS was invented at the Smithsonian Institution, by scientist, Walter Adey in the 1980s for the application in highly diverse model ecosystems. Sometimes ATS are also called Managed Aquatic Plant Systems (MAPS) (Adey & Bannon, 2008). Today ATS are object of many wastewater treatment projects all over the world (Adey, Kangas, & Mulbry, 2011).

Usually benthic algal communities of common green algae (*Chlorella, Scenedesmus, Cladophora*), cyanobacteria (*Spirulina, Oscillatoria, Anabaena*) or consortia of both are used in ATS (Perales-Vela, Pena-Castro, & Canizares-Villanueva, 2006). They can grow attached to all kinds of surface like rocks, plants, wood items or even artificial materials (Adey & Bannon, 2008).

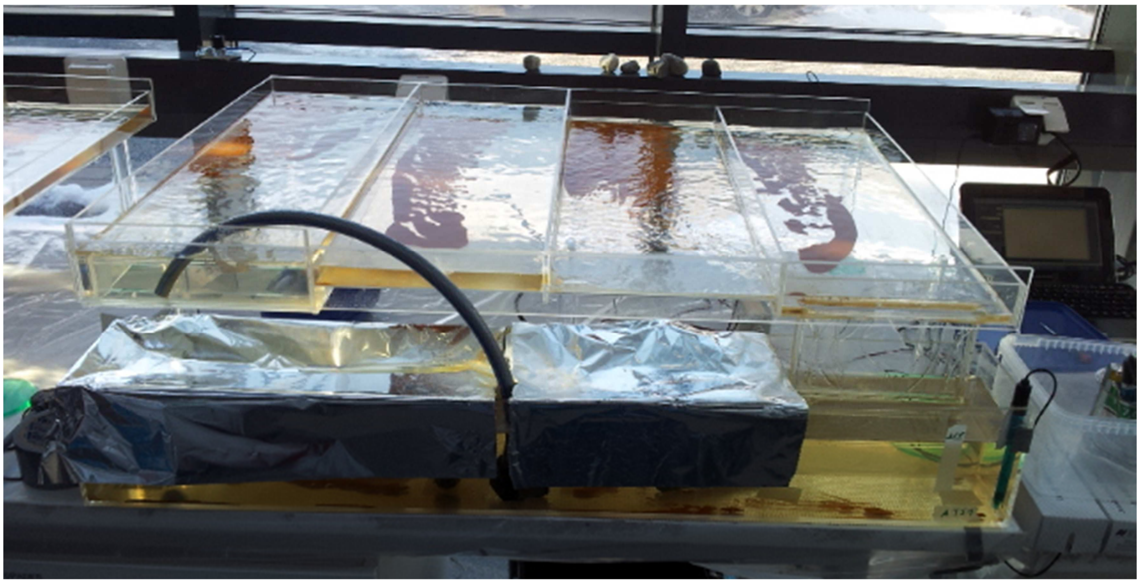
ATS have a lot of advantages in wastewater treatment. ATS systems use consortia of filamentous cyanobacteria and suspended green algae (Perales-Vela et al., 2006). They provide a wide biodiversity of local algae and thereby they are less affected by contamination or changing environments. Algae have a high efficiency capturing solar energy. Compared to other plants, algae are able to capture solar energy as much as 10 times that of agriculture and even as much as 50 times that of forestry. Thin layers of water in ATS support the capturing of solar energy and the high rates of mixing or continuous flow of water increase the chemical exchange with water ingredients. Therefore individual cells are able to take up carbon, nitrogen and phosphorus at fractions of ppb levels. Algae can also efficiently remove heavy metals to permitted levels or break down toxic hydrocarbons and in addition oxygenate waters. Another advantage in operation of ATS is the low energy and land requirement for production. Even in barren areas ATS can be setup (Adey & Bannon, 2008).

ATS can also play a role in energy production. Some algae have relatively high lipid contents and can be used to produce biofuel, while residues of biomass can ferment in digester or burned in incineration processes. ATS don`t compete with food production, cause it can be built in for agriculture useless areas and municipal wastewater and flue gases can be used as resources. In theory it is possible to combine wastewater treatment and flue gas clarification of a power plant with an ATS to produce fuel, methane or/and energy.

The ATS, which is present at TAMK was designed by G. Grobler and is made of polyacrylics. It has a basin with dimensions (LxB) of 1000mm x 700mm divided into 4 lines, where water flows with a height of 1-2 cm. In the whole system around 25l water fit in, a storage vessel included.

The basin is propped up on pedestals and water is pumped from the storage vessel into the basin. Then it runs through all 4 lines back into the storage vessel which is partly closed with aluminium foil to avoid splashing. In the storage tank water is aerated with aeration stones, usually used in aquatic systems, and a pH-meter is placed in one corner. The pH-meter is connected to a computer to record constantly the pH-value in the system. The computer is also connected with a light meter (upper left corner in PICTURE 1) recording the illumination. PICTURE 1 shows the system running with wastewater from mineral processing and the used netbook on the right site.

For more information about ATS, please have a look in the Final Thesis „Algae Cultivation for Wastewater reclamation“ (Grobler, 2013).



PICTURE 1. ATS system TAMK

2.3 Algae

In this chapter basic information about algae are given. The focus will be on freshwater algae, especially the used species of chlorophyla and cyanophyla/bacteria.

The world of algae is tremendous. Algae are (with numerous exceptions) aquatic organisms that (with frequent exceptions) are photosynthetic, oxygenic autotrophs that are (except for the kelps) typically smaller and less structurally complex than land plants. Algal taxonomists believe that there are from 36,000 -50,000 (John and Maggs, 1997) and possibly more than 10 million (Norton et. al., 1996) species of algae (Graham & Wilcox, 2000). They are divided into 11 divisions by morphological or phylogenetic differences, but since microscopic possibilities getting better and better and even DNA analyses are possible, the taxonomy is in a changing process.

As the world of algae is tremendous, there existing algae species adapted to completely different environments. There are species growing in marine or freshwaters, species growing in cold or hot, basic or acidic, nutrient rich or poor waters. It`s possible to find algae in all kind of extreme environments.

Most freshwater algae species grow in neutral lake waters with limited amounts of nutrients. In general the concept of limiting factors by Liebig is valid for algae as well as for plants. Often phosphorus is the limiting mineral nutrient for algal growth, but also nitrogen, carbon sources (carbon dioxide or hydrogen carbonate ions) or iron can get a limiting factor. The needed N/P ratios for algal growths can differ from species to species. Some species need more, some fewer nutrients to grow successfully (Graham & Wilcox, 2000).

In this study 10 different algae species from 4 phyla, provided by SYKE had been available. Most of these species are Chlorophyla or Cyanophyla/bacteria; species 5 belongs to the Chrysophyta and species 9 to the Ochrophyta. For the species 8, *Purpuraemus sp*., no information at all could be found.

Names of the 10 available algae species:

1) *Selenastrum capricornutum*
2) *Pediastrum simplex*
3) *Synechococcus sp.*
4) *Anabaena cylindrical*
5) *Fragilaria crotonenis*
6) *Scenedesmus sp.*
7) *Chlorphyta sp* (Pekari strain)
8) *Purpuraemus sp.*
9) *Navicula pelliculosa*
10) *Haematococcus pluvialis*

The algal species 1, 2, 6, 7 and 10 belong to the phylum of Chlorophyta. This phylum is with around 7000 species one of the biggest division of green algae. Especially *Selenastrum capricornutum* is known and commonly used as biomonitor, reacting sensitive to the presence of toxic substances. Other species of Chlorophyta are used as food in freshwater aquaculture systems for fish (Graham & Wilcox, 2000).

The algal species 3 and 4 belong to the Cyanophyta, also known as Cyanobacteria. These algae are the only one known to be capable of transforming molecular N gas into ammonia, which can be easier used by algae. They are also known for tolerance to high temperature differences. But most cyanobacteria are intolerant of low pH water, disappearing as the pH approaches 5 (Graham & Wilcox, 2000).

2.3.1 pH dependency

The pH value describes the concentration of protons in solution and has normally values between 1 and 14. A low pH describes a high H^+-concentration and a high pH a low H^+-concentration. It can be calculated with the negative logarithm to the base of 10 of the proton concentration.

$$pH = -\log(c(H^+))$$

The pH-value of an aqueous environment is very important for algae. Algae normally grow in alkaline medium of 7-9 with an optimum range between 8.2–8.7 and pH value exceeding 9.2 could inhibit the photosynthetic activity of phytoplankton (CELESTINO LADU, LUKAW, & Kenyi, 2012). Although there is a common pH algae normally grow, it has to be mentioned, that every algae species has its own optimum range of pH. A lot of algae are able to survive in a wide range of pH, like the acidotolerant species *Mougeotia*, which can survive in pH between 4 and 10. The reproduction might not work in optimum over the whole range, but these algae still survive in low or high pH environments (Graham & Wilcox, 2000).

All algae have to regulate their cytosolic pH, which is in most cases neutral. Several mechanisms have been observed, how algae can affect their inner pH. Figure 3 shows a general scheme of an acidophilic alga cell with essential parts of cell walls like carrier or channel (bold squares) and pumps (bold circles). Ion concentrations (like K^+, Na^+ or CL^-) are regulated through carrier and channels. Through these holes also protons get inside the cell. On the one hand proton pumps imbedded into cell walls carry needless protons outside of the cell. These pumps require energy, because they have to work against the concentrations gradient. This proton efflux can even lead to acidification of the surrounding, if efflux is higher than influx. On the other side a mechanisms is possible, which can change the composition of the cell wall. The acidophilic algae *Cyanidi-*

um caldarium and *Galdieria sulphuria* have developed mechanisms to make their cell membrane temporarily impermeable for protons. In *Dunaiella acidophila* a highly positive membrane potential and positive surface charge avoids entering of protons into the cell interior (Gross, 2000).

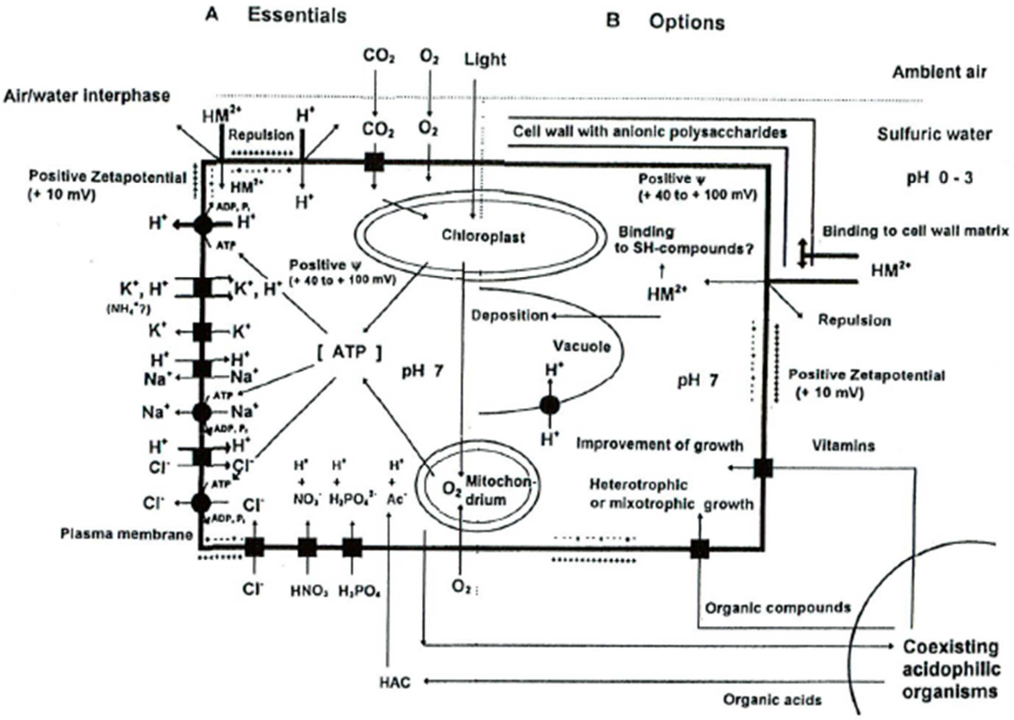


Figure 3. Summarising scheme of essential (A, left side) and optional properties (B, right side) of acidophilic algae. (Fig. 9.8 A.B.; reprinted from H. Gimmler (2001) Acidophilic and Acidotolerant Algae. In: L.C. Rai et al. (eds.), Algal Adaptation to Environmental Stresses, pp.259-290); with kind permission of Springer Science+Business Media

In highly acidic environments the biodiversity is very small. Under pH of 2.5, the species diversity is drastically reduced and often consists of just a handful of bacterial, fungal and algal species (Gross, 2000). Only acidophilic algae are able to survive in pH smaller than 3 and as low as 0.05, but they are unable to grow at neutral pH`s. Acidotolerant called algae have their optimum in pH 3-5 (Rai & Gaur, 2001).

A low pH-value has several impacts, that can effect algal growth more than the pH-value itself. Most metals have a higher solubility in low pH`s and concentrations can easily get toxic for organisms (Gross, 2000).

Carbon is especially soluble as CO_2 in acidic waters with low pH. Usually algae can take up hydrogen carbonate ions easier than carbon dioxide. At low pH the carbon source for algal growth is inhibited by absence of hydrogen carbonate ions. In acidic waters with a pH lower than 4-6 hydrogen carbonate ions are present only in small amounts, while most carbon is present as CO_2 as seen in Figure 4.

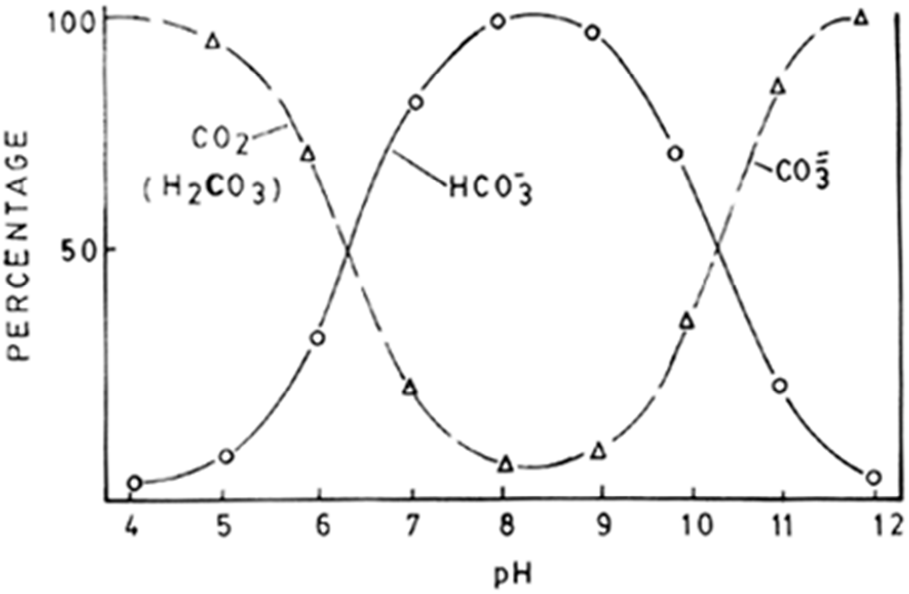


Figure 4. Dependence of CO_2, HCO_3^- and CO_3^{2-} solubility from pH (After Boyd, 1982) (M.N.Kutty, 1987)

Sulphate concentrations can increase as well and especially in mining areas, sulphuric acid is a prominent acid formed from decomposition of pyrite (Gross, 2000).

2.3.2 Heavy metals

It is already long well-known, that algae are able to remove HM from aqueous systems. In water bodies they play an important role regulating HM pollution. In all organisms trace amounts of HMs are essential as constituent e.g. of enzymes, but in high concentrations they can have toxic effects (Travieso, et al., 1999). Hence algae need adequate mechanisms to distinguish between essential and non-essential HM and mechanisms to regulate HM concentrations, but not all species have those. Therefore some algae species can be used as indicator for toxic environments like *Selenastrum capricornutum*. On the other hand some algae species are able to handle even relatively high concentrations of HM and bind or precipitate them. Again some other species are able to prohibit the uptake of HM into the cell cytoplasm under effort of energy and survive even in highly polluted areas (Perales-Vela et al., 2006).

The occurring mechanisms in algae to manage HM concentrations are not completely investigated and can differ from species to species, but some mechanisms should be presented here.

The first of these mechanisms is a combination of physical adsorption, absorption and sequestration called bioremediation. HM ions are adsorbed within seconds or minutes to the negative charged cell surface. Studies suggest that the constituents of algae cell walls such as alginate and fucoidan which have key functional groups are chiefly responsible for biosorption of heavy metal ions. Then it takes time, until the adsorbed HM ions are absorbed into the cell interior (Dwivedi, 2012). Inside microalgae have preferentially developed the production of peptides capable to bind heavy metals. One of the most important ones is the polypeptide named phytochelatin or metallothionein III (MtIII). The production of these polypeptides is activated by a wide variety of HM itself (Perales-Vela et al., 2006). In Figure 5 the general scheme of this mechanism is shown. Inside the cell HM ions are converted into MtIII complexes in low or high molecular weight forms. These molecules, as organometallic complexes, are further partitioned inside vacuoles to facilitate appropriate control of the cytoplasmic concentration of heavy metal ions, thus preventing or neutralizing their potential toxic effect (Perales-Vela et al., 2006). Species which are known for building metal binding proteins are *Fragilaria crotonensis* and *Navicula pelliculosa*, which are used in this project (Rai & Gaur, 2001).

The other mechanism of algae to survive in HM contaminated water bodies is preventing the entrance of HM inside the cell and active efflux of HM. Therefore the cell wall composition is changed to reduce the permeability (Rai & Gaur, 2001). Extra-cellular polymers, mainly carbohydrates are responsible for this change. The active efflux of HM is an energy consuming process, in form of ATP molecules, (Perales-Vela et al., 2006).

Next to these both mechanisms, also oxidation of HM inside algal cells and precipitation through elevated pH values by photosynthesis are mentioned in literature (Rai &

Gaur, 2001) & (Santas, Danielides, & Santa, 1993). The pH has an influence on HM concentrations, as described above and also on the adsorption to cell surfaces. With increasing pH the surface charge becomes more negative and the amount of free sites for HM-ions increase (Dwivedi, 2012). At small pH HM uptake is blocked, so that uptake rates and adsorption of HM by cells is significantly reduced (Gross, 2000).

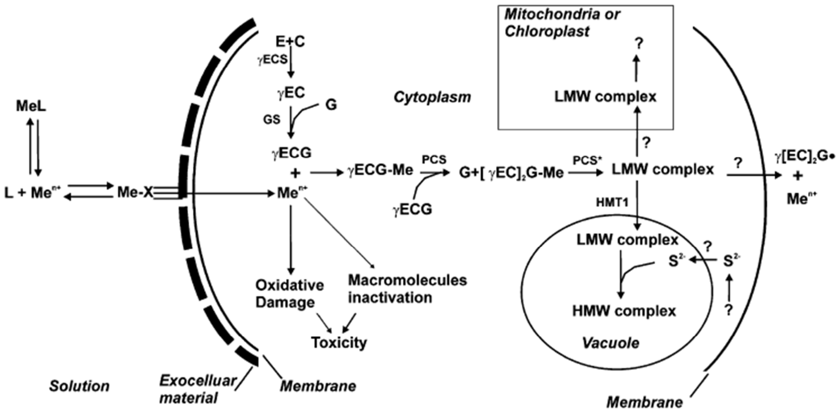


Figure 5. General scheme of HM detoxification mechanism mediated by class III metallothioneins in microalgae; reprinted from Chemosphere 64, Perales-Vela et al.. Heavy metal detoxification in eukaryotic microalgae, pp 1-10, (2006), with permission from Elsevier

All of these mechanisms have been observed for specific HMs in specific alga, but often algae have to tolerate more than one toxic metal, like in this project. It is possible that alga species are tolerant to several metals, but it is also possible that one HM interfere the uptake of another HM or even initiate the release of already adsorbed HM ions (Rai & Gaur, 2001).

The amount of HM algae are able to take up depends on the algal species and it`s cell composition as well as on HM concentrations and other properties of the surroundings. One example is given in (Travieso, et al., 1999) for *Euglena gracilis*, which could accumulate Zn until 5mg/g dry algae weight. Assumed that the used algae mix in this project could accumulate the same amount of Zn a dry algae biomass of 60g is required to accumulate the Zn amount of 1l of wastewater, which had an initial concentration of approximately 300mg/l.

3 AIM OF THE WORK

As mentioned before, industrial wastewaters contaminated with HM like wastewater from mineral processing are an actual environmental issue. At the moment there exists no treatment method for this kind of wastewater, which is affordable and environmental friendly, although wastewater contaminated with HM is produced at several places all over the world. Common treatment processes like ion exchange or lime precipitation are often ineffective and/or expensive. The reachable concentrations with payable amounts of chemicals are often still too high, so that algae are very useful particularly for the removal of HM ions at low concentrations (below 50mg/l) (Dwivedi, 2012).

Algae Turf Scrubbers showed in many research projects and applications, that they are able to remove nutrients like nitrate, ammonia and phosphate from municipal wastewater to permitted amounts. It is also known that algae are able to remove lots of metals from water bodies, especially in trace amounts. Research projects could illustrate that specific algae species are able to remove single metals from basic solutions.

In this project the purpose of an Algae Turf Scrubber for the treatment of complex wastewater from mineral processing is content of this investigation. In the beginning following questions had been of interest:

1. Are algae able to grow in wastewater from mineral processing and how much biomass can they generate?
2. Are there algae able to remove HM from this wastewater to reduce concentrations to permitted amounts?
3. Is it possible to remove sulphates with ATS from this wastewater?
4. Is a treatment of wastewater from mineral processing using ATS systems possible?
5. How high is the lipid and carbohydrate content of algae biomass produced in wastewater from mineral processing (if algae grow)?
6. What possibilities exist to use algae biomass from ATS?
7. Is it possible to raise the pH value through treatment in ATS?
8. Is a treatment for huge amounts of wastewater from mineral processing with ATS systems imaginable?

4 MATERIALS AND METHODS

In the following chapter first the methods and instruments of analyses are shortly explained. Then two small-scale experiments are described and evaluated, before the two test runs in ATS are described.

4.1 Methods of analyses

In the first part of this chapter the used methods and instruments of analyses are explained. Some of these methods had been already used in the former project on ATS and description of these methods can be found in the Final Thesis “Algae cultivation for wastewater reclamation” by (Grobler, 2013).

4.1.1 pH reader & light meter

During the complete test runs in ATS the pH-value and the illumination had been recorded constantly. Therefore Phidget pH Lab Electrodes and a LiCor Quantum sensor have been used. All pH-meters and the light meter are connected to a netbook, where all values are saved. It is the same kind of equipment, which has been used before by G. Grobler in his thesis. Cause of algae residues on the sensors of the pH-meters, completely new ones had been used. The detailed description can be found in his Final Thesis – Algae cultivation for wastewater reclamation.

4.1.2 TOC

To measure the total organic carbon content the Total Organic Carbon Analyzer TOC-5000A made by Shimadzu was used. In the laboratory of TAMK it is used together with the autosampler ASI 5000A also made by Shimadzu. The instruments are connected with a computer where the software TOC-Control (Version 1.05.01) is used to control and record the measurements. The measurement process is completely automated.

In the TOC-Control software the standards vesitc.cal and vesiic.cal are used. As method vesitoc.met is chosen. For each measurement 6 standard solutions of $Na_2CO_3/NaHCO_3$ and $C_3H_5KO_4$ have to be prepared with the concentrations of 0, 5 and 10 mg/l. The test tubes have to be put into the gadget beginning at St1 with the concentrations from 0 to 10 mg/l first of $C_3H_5KO_4$ and then 0 to 10 mg/l of $Na_2CO_3/NaHCO_3$. In the ASI 5000A up to 78 samples can be measured during one run. Every time two samples filled with distilled water have to be in the beginning and at the end.

4.1.3 AAS

The metal content of the wastewater from mineral processing has been measured with atomic absorption spectroscopy (AAS). Therefore the Atomic Absorption Spectrometer AAnalyst 400 made by PerkinElmer Instruments had been used. The content of Ni, K, Na and Zn had been analysed with wavelength and standard solution concentrations shown in Table 2. As calibration curve type, Linear through Zero has been used and samples had been diluted if necessary. On the computer for this instrument the software WinLab32 for AA (Version 6.0.0.0065) made by PerkinElmer is used to control the hardware.

Table 2. Used wavelengths and standard concentrations for AAS analyses

Metal		Ni	K	Na	Zn
Wavelength [nm]		341.48	766.48	330.24	307.59
Standard Solution [mg/l]	1	0	0	0	0
	2	5	0.1	5	10
	3	7.5	0.25	7.5	25
	4	10	0.5	10	50
	5	15	0.75	15	75
	6	20	1	20	100

4.1.4 BOD

The BOD was analysed with OxiTop system as BOD_5. The procedure is described in the Final Thesis “Algae cultivation for wastewater reclamation” by Grobler.

4.1.5 HACH

The HACH system was used to analyse sulphate, total nitrogen and phosphate concentrations. In the beginning it was planned to analyse also nitrate and total phosphorus, but a control of the methods showed that something interfered and therefore these results are not reliable.

The total nitrogen concentrations had been analysed with HACH Lange LCK 138 kits, providing a measurement range of 1-16mg/l. The sample preparation and instructions can be found in the Final Thesis “Algae cultivation for wastewater reclamation” by Grobler.

The sulphate content was analysed with the method 8051 and powder pillows, providing a measurement range of 2-70 mg/l

Phosphate concentrations were analysed with method 8048 and powder pillows, providing a measurement range of 0.02-2.50 mg/l PO_4^{3-}.

4.1.6 Spectroscopy

Spectroscopy analyses had been done with UV/VIS Spectrometer Lambda 20 made by Perkin Elmer and the software Perkin Elmer UV WinLab (version 6.0.3.0730) to assess the algal growth in small-scale experiment solutions, indicated by the absorbance rate. As wavelength program “Lambda 20” was used with the wavelength of 620nm.

4.2 Small-scale experiments

During this project small-scale experiments had been running to observe the growth of algae under different conditions. They had been more or less useful for the bigger test runs in ATS.

In the first small-scale experiment different pH values had been probed to find out the dependency of algae from pH-values. In the second small-scale experiment the second test run in ATS is imitated in Erlenmeyer flasks.

4.2.1 Algae species experiment with different pH values

The wastewater from Talvivaara has an initial pH value between 3 and 4. As described in the tremendous world of algae there exist species which are able to grow in low (<3) or high (>11) pH-values, but most algae species like slightly basic media. In this experiment the in chapter 2.3 listed 10 algae species provided by SYKE in Helsinki had been available.

Unfortunately no one of these algae species are known for acidophilic or acidotolerant characteristics. However a small scale test run was started with the wastewater to observe, if algae will grow in this water with different pH values. In this context also a stock solution for each species was created as comparison to the wastewater solutions and also as algae source for the ATS test runs. For this test a range from pure wastewater with a pH value around 4 to pH values of 5 and 6 had been chosen. To raise the pH value calcium hydroxide ($Ca(OH)_2$) and calcium carbonate ($CaCO_3$) had been used.

For each test row (named pH 4, 5 and 6) and also for the stock solution 11 Erlenmeyer flasks were prepared. Each row consists of a solution for each species and a "blank" solution without algae.

The algal growth was assessed spectroscopically by measuring the absorbance at 620nm.

The solutions were prepared as follows:

First the Erlenmeyer flasks were cleaned with ethanol and distilled water. To conserve the solutions of contamination the flasks were closed with cotton and aluminium foil. The flasks were filled with 250 ml of water according to the test row (pH 4, 5, 6 and distilled water with fertilizer) and 2.5 ml of algae solution (from SYKE).

The pH 4 test row was using pure wastewater from mineral processing. The pH value of test row pH 5 and 6 were raised with calcium hydroxide ($Ca(OH)_2$) and calcium carbonate ($CaCO_3$) to 5.01 (4.91 after 1 day) respectively 5.82 (6.02 after 1 day).

Every day the solutions were stirred a few times by hand. The spectroscopy was made in the beginning (15.03.2013) and on the 18.03.2013 and on the 22.03.2013.

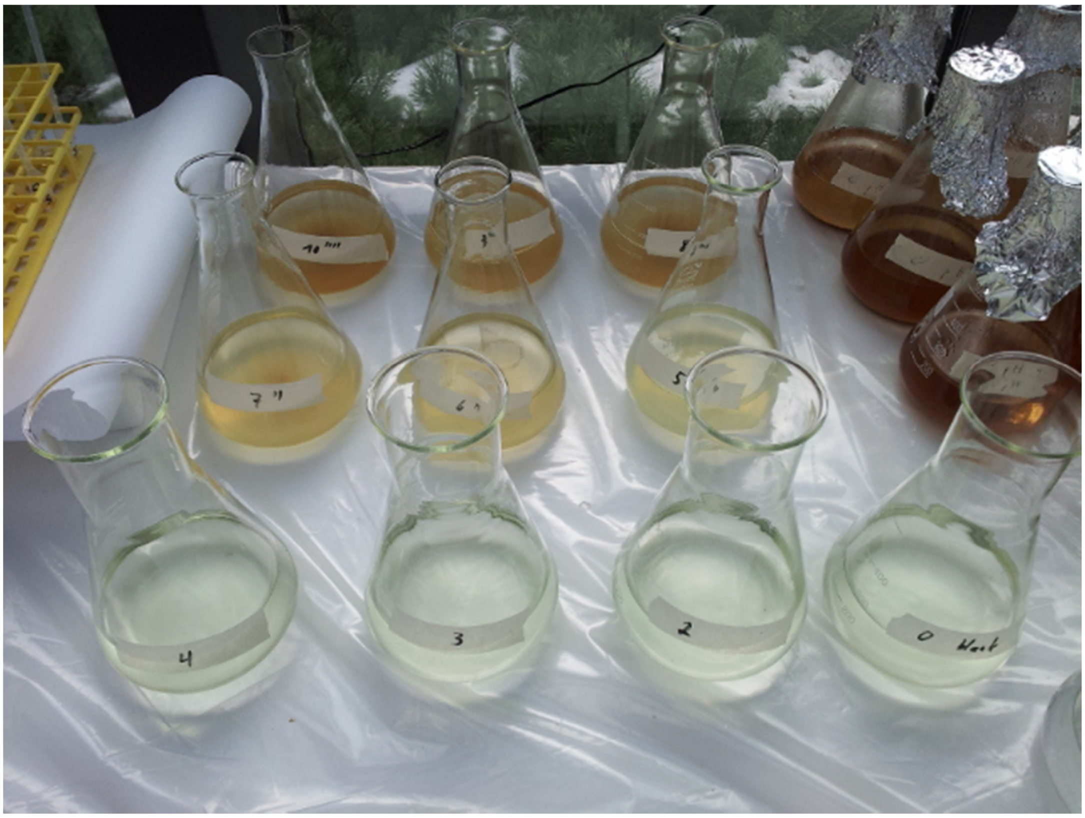
4.2.2 Algae adaptation experiment

In the algae adaptation test the process of the second test run in ATS should be imitated. Three different beginning conditions shall be tested. The basic idea behind this experiment is to have good conditions for algae growth in the beginning and change the water conditions slowly to wastewater from mineral processing by adding the amount of evaporated water. Another adjusted part is the amount of nutrients. Measurements during the first test run showed, that the amount of nutrients is low in wastewater from mineral processing, cause of pre-treatment at mining area. In this small-scale experiment and in the 2nd test run in ATS water will be enriched with nutrients. For this small-scale experiment a mixture of all algae species and the fertilizer "Substral" (PICTURE 2) used also for the stock solutions was used.
The scale of this experiment is 1/100 of ATS. In the ATS 25l fits in the system and storage tank together. Every day, depending on sunshine and temperature in the green house, about 2.5l of water evaporates. It is estimated, that 25 ml of 250ml in Erlenmeyer flasks will evaporate each day. After 2 weeks the complete amount is changed into wastewater.

PICTURE 2. Used fertilizer Substral

For this experiment three times three Erlenmeyer flasks were prepared in the same way as in the pH experiment before and one "blank" flask. Three different stages (0, 2 & 4 days ahead) of addition of wastewater were prepared as shown in PICTURE 3.

The Erlenmeyer flasks were filled with algae solution, nutrient enriched water and wastewater according to the plan, seen in Table 3. Each day the flasks were stirred by hand a few times. The original plan was to add every day 20 ml of wastewater and 5 ml of nutrient enriched distilled water. The pH-value was measured before and after each addition.



PICTURE 3. Algae adaptation test in the beginning

Soon we realised that the amount of evaporated water was smaller than 25 ml each day, so that in the beginning only once wastewater was added to all solutions. After that the pH was measured for a few days.

4.3 Test runs in ATS

During this project it was only possible to arrange two test runs in the ATS systems, cause of the short project time and the necessary growths time of a sufficient algae stock. For both test runs it was decided to run two of the three available ATS systems at TAMK with the same conditions. The first test run was done with pure and untreated wastewater from mineral processing, while the second test run started with comfortable conditions for algae without wastewater and a subsequent phase of adaptation to wastewater from mineral processing conditions.

Table 3. Water addition and pH values of algae adaptation experiment; AS=Algae solution, NS=nutrient solution, WW=wastewater from mineral processing

			control	0 days ahead			2 days ahead			4 days ahead			
		Sample	1	2	3	4	5	6	7	8	9	10	Temperature
	content	AS [ml]	50	50	50	50	50	50	50	50	50	50	23.5°C
		NS [ml]	200	200	200	200	160	160	160	120	120	120	
		WW [ml]	0	0	0	0	40	40	40	80	80	80	
	pH (initial)		5.47	5.25	/	/	3.35	3.24	3.26	3.22	3.25	/	
day		addition											
1		NS [ml]	25	5	5	5	5	5	5	5	5	5	25.3°C
		WW [ml]	0	20	20	20	20	20	20	20	20	20	
	pH (before adding)		5.89	5.55	5.5	5.5	3.54	3.53	3.53	3.37	3.38	3.38	
	pH (after adding)		/	3.55	3.56	/	3.47	3.47	/	3.31	3.32	/	
2		NS [ml]	0	0	0	0	0	0	0	0	0	0	28°C
		WW [ml]	0	0	0	0	0	0	0	0	0	0	
	pH		5.66	3.49	3.5	3.52	3.34	3.34	3.34	3.13	3.15	3.16	
4		NS [ml]	0	0	0	0	0	0	0	0	0	0	24°C
		WW [ml]	0	0	0	0	0	0	0	0	0	0	
	pH		5.6	3.61	3.59	3.57	3.15	3.15	3.14	2.96	2.98	2.97	

4.3.1 First test run (March 2013)

The first test run started on the 25th of March 2013. During this test run pure and untreated wastewater was used. It was quit already two weeks later on the 5th of April, because algae growth occurred neither in ATS nor in the earlier started pH test experiment.

Table 4. Water changes during test run 1 in ATS in l; WW= Wastewater from mineral processing, AM= Algae mix

Water changes					
Date/Time	**What?**	**ATS 1**	**ATS 1 sum**	**ATS 2**	**ATS 2 sum**
25.03.2013	WW	25	25	25	25
	AM	0.5	25.5	0.5	25.5
26.03.2013	Sample	-0.045	25.455	-0.045	25.455
27.03.2013	Sample	-0.05	25.405	-0.05	25.405
	WW	3	28.405	3	28.405
	Sample	-0.045	28.36	-0.045	28.36
28.03.2013			28.36		28.36
29.03.2013	WW	5	33.36	5	33.36
30.03.2013			33.36		33.36
31.03.2013	WW	5	38.36	5	38.36
01.04.2013			38.36		38.36
02.04.2013	Sample	-0.045	38.315	-0.045	38.315
	WW	5	43.315	5	43.315
03.04.2013	Sample	-0.045	43.27	-0.045	43.27
	Sample BOD	-1	42.27	-1	42.27
04.04.2013	Sample	-0.45	41.82	-0.18	42.09
	WW	5	46.82	5	47.09
05.04.2013		STOP		5	52.09
06.04.2013					
07.04.2013				STOP	
sum		**46.820**		**52.090**	

Before the test run could be started, the complete system and the used equipment and instruments like pH-meters, pumps and tubes had to be cleaned first with soap water and in the end with clean tab water, as far as they haven`t been new. Then the system is arranged in a horizontal level. The pH-meter is installed in the storage tank and the pump and aeration stones are inserted like in PICTURE 1. After the first 10-15 liter filled in the storage tank the pump and aeration can be launched and water can be filled into the system up to around 25l. It is important to mind that water is running over the whole surface of ATS and adjustments on the pedestals might be necessary. The software to record the pH and illumination values can already run during preparations.

In this test run the pure and untreated wastewater from mineral processing was used. The water was taken from container 2. Before it was taken it was stirred in the container to get a well-mixed sample. The same procedure had been done, when water had to be added later. Through evaporation and also by taking samples for measurements the amount of water decreased in the systems, so that water had to be added every couple of days. Table 4 shows the water amount changes in ATS 1 and 2.

During this test run the BOD_5, nutrients like nitrate and phosphate, TOC and metal contents of Ni, K, Na and Zn had been measured every week.

4.3.2 Second test run (April)

The second test run started on the 10th of April 2013. First a proper batch of algae grew under comfortable conditions in nutrient enriched tab water (NW) before wastewater from mineral processing was added gradually. The whole wastewater, which was added to the systems, had been enriched with nutrients like tab water before. The test run was quit on 10th of May, when the algae biomass went completely brown and looked dead.

The setup is equal with the setup in test run 1. First the systems had been cleaned and then arranged like described in 4.3.1. The used tab and wastewater was enriched with the following recipe of ingredients. All values are in g /l.

Bio Bact	1.29
Urea	0.0477
Glucose	1
Di-Sodium hydrogen phosphate	0.0445

In the beginning the systems had been filled with 25l nutrient enriched tab water and 1l of algae mixture. Over the first two weeks just the amount of evaporated water was replaced with nutrient enriched tab water. When we started to add wastewater from mineral processing it was decided to keep the amount of water in the ATS systems at 25l to add relatively the same amount of wastewater to all systems even to WST and EBB systems, to make it comparable. On the 23rd of April adding of wastewater began. Every day 1l of nutrient enriched wastewater was added. It was not stirred before taking it from container 2 to avoid adding of solids. The water changes can be seen in Table 5.

During this test run measurements have been done nearly the same way as in test run 1. In the beginning initial values have been measured for metals, nutrients, sulphates, BOD_5 and TOC. To keep the nutrient level constant, total nitrogen and phosphates have been analysed 2 times a week. The TOC and metal contents have been analysed 3 times at all. The amount of metals added to the systems is calculated with earlier measurements of pure wastewater from mineral processing.

Table 5. Water changes during test run 2 in ATS in l; NW=nutrient enriched tab water, AM=algae mix, NWW= nutrient enriched wastewater from mineral processing

Water changes							
Date/Time	**What?**	**ATS 1**	**ATS 1 day**	**ATS 1 sum**	**ATS 2**	**ATS 2 day**	**ATS 2 sum**
10.04.2013	NW	25	26	26	25	26	26
	AM	1			1		
11.04.2013	Sample	-0.1	-1.1	24.9	-0.1	-1.1	24.9
	Sample BOD	-1			-1		
12.04.2013	NW	2.5	2.5	27.4	2.5	2.5	27.4
13.04.2013				27.4			27.4
14.04.2013				27.4			27.4
15.04.2013				27.4			27.4
16.04.2013				27.4			27.4
17.04.2013				27.4			27.4
18.04.2013	NW	2.5	2.5	29.9	2.5	2.5	29.9
19.04.2013				29.9			29.9
20.04.2013				29.9			29.9
21.04.2013				29.9			29.9
22.04.2013	NW	1	1	30.9	1	1	30.9
23.04.2013	NW	10	15.5	46.4	10	15	45.9
	NWW	1			1		45.9
	NW	4.5			4		45.9
24.04.2013	NWW	1	2	48.4	1	2	47.9
	NW	1			1		47.9
25.04.2013	NWW	1	2	50.4	1	2	49.9
	NW	1			1		49.9
26.04.2013	NWW	1	1	51.4	1	1	50.9
				51.4			50.9
27.04.2013				51.4			50.9
28.04.2013				51.4			50.9
29.04.2013	NWW	1	3.6	55	1	3.6	54.5
	NW	2.6			2.6		54.5
30.04.2013	NWW	1	3.5	58.5	1	3.5	58
	NW	2.5			2.5		58
01.05.2013				58.5			58
02.05.2013	NWW	1	7	65.5	1	7	65
	NW	6			6		65
03.05.2013	NWW	1	2.5	68	1	2.5	67.5
	NW	1.5			1.5		67.5
04.05.2013				68			67.5
05.05.2013				68			67.5
06.05.2013	NWW	1	5	73	1	5	72.5
	NW	4			4		72.5
07.05.2013				73			72.5
08.05.2013				73			72.5
09.05.2013				73			72.5
10.05.2013				73			72.5

5 RESULTS AND DISCUSSION

In the following chapter observations during all tests, problems which occurred and results for the experiments and test runs are presented.

5.1 Results of the small-scale experiments

In the first small-scale experiment the results of spectroscopy analyse, shown in the appendix 3, showed algae did not grow. The values for almost all species and test rows are very small in the beginning, what was expected, because the amount of added algae mass (2.5ml) had been very small. But also the last measurement shows very small values. The algae growth was better seen by eyes than by spectroscopy. Within one week it was visible in some stock solutions (1, 6 and 9) that algae grew, because it coloured light green. In the wastewater solutions a change in species 8 was visible. The solution was in all test rows turbid and spectroscopy values increased accordingly. The other wastewater solutions did not change in colour and turbidity. Even the wastewater solutions of species 8 became clear by and by. In all wastewater solutions solids precipitated and Erlenmeyer flask walls got brown colour inside. In test row pH 6 precipitated solids were brown to grey in colour, because through addition of calcium probably gypsum ($CaSO_4$) precipitates.

After 3 weeks no changes occurred in the wastewater solutions, so that the test run stopped. The stock solutions had been kept alive until the end of this project time by adding nutrient enriched distilled water.

Already after 4 days the algal adaptation test experiment was stopped, because without the exception of solutions 2-4 on days 2 to 4 the pH value decreased after adding wastewater and stayed on a low level. The more wastewater was in test solution the darker got the colour of solution after adding wastewater and the more brown solids precipitated. Algal growth could not be observed.

During these experiments we realised several issues. The time range for spectroscopy measurements, done in the first small-scale experiment had been chosen badly. Measurements should have been done in the beginning and when in the stock solutions algae growth was visible.

Further occurred, that the process of taking samples for spectroscopy should have been done every time a certain time after stirring the samples. The first measurement results are higher, because solids couldn`t precipitate in the beginning. Some of them are even higher, because solution was mixed during pipetting the sample.

Another problem applying to both small-scale experiments is that the conditions in these test rows and the conditions in test runs in ATS had been different. Solutions had not been aerated at all and the water was not continuously flowing.

Beside the composition of nutrients used in the second small-scale experiment was another one than used later in ATS, because Substral was used instead of the recipe of nutrients.

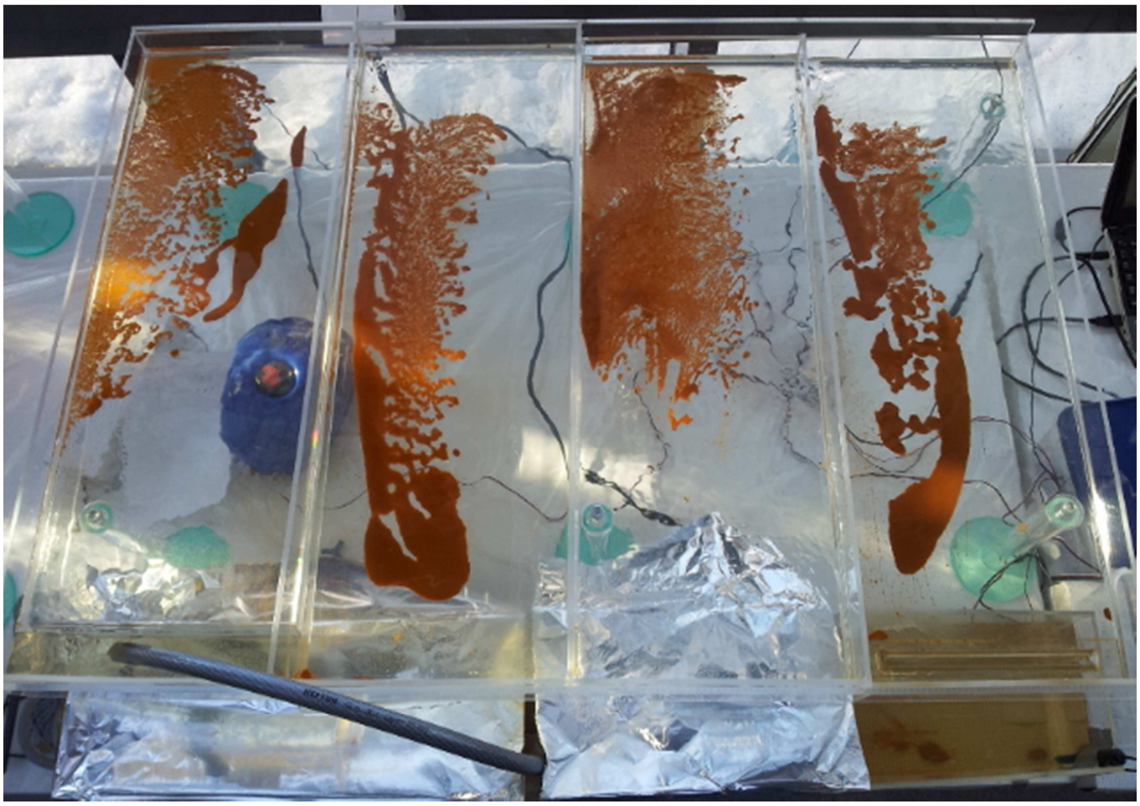
It is assumed neither elevation of pH nor addition of nutrients alone is the essential and significant change of water conditions for algal growth. No one of the 10 algae species grew in any of the solutions. As in Table 3 shown, the pH-value decreased even without adding of wastewater except in the solutions 2-4. It is not clear, if there algae had been able to raise the pH-value a little bit, because this experiment stopped after day 4 and measurements are connected with uncertainties.

But even if algae raised the pH-value, the results show, each pre-treatment on its own is not successful for algal growth. The next experiment should be a combination of both. Wastewater from mineral processing with elevated pH and added nutrients should be added gradually into a proper algae solution in different amounts. The pH and nutrient concentrations have to be monitored.

5.2 First test run ATS

5.2.1 Observations

In the beginning the used wastewater from mineral processing was relatively clear without any intensive colour. Solids precipitated on the bottom of ATS basins and storage tank as seen in PICTURE 4. By and by the colour changed into brown and more and more solids precipitated as seen in PICTURE 5. Probably due to the fact, that algae species arrived in small batches of algae, which haven`t been well grown, when they were added to the systems, no algae growth was visible.



PICTURE 4. ATS 1 on the 26.03.2013

The pH-value of both systems started between 3 and 3.5 and decreased over two weeks to around 2.5 as seen in Figure 7. At the first 2 test days there is a spiky profile in this figure, because the netbook turned off over night, while it was connected to the internet and therefore some measurements are missing. From the third test day on the pH curve goes on in a sinus profile due to temperature changes, which are not adjusted automatically through measurement and software. The illumination shows a slightly increase from maximum 580 µmol s^{-1} m^{-2} on the first day to around 700 µmol s^{-1} m^{-2} on the last days, as it is spring time.

In the end of testing time the aluminium foil started to break down like seen in PICTURE 6. This is a sign for acid water conditions. Furthermore this shows splashing of water. In addition to the water changes due to addition of water caused by evaporation, taking samples for measurements and inexact writing down of water changes makes it difficult in case of successful growth of algae to calculate the reduction of pollutants, particularly small reductions.

PICTURE 5. ATS 1 test run 1 colour change

PICTURE 6. Break down of aluminium foil

A white, slightly yellow solid, shown in PICTURE 7, appeared around the storage tank. The analysis of this solid with HACH showed that it consists of 40% sulphates.

PICTURE 7. Solid appearing at ATS after two weeks in test run 1

5.2.2 Results

In Figure 7 the recorded pH values and illumination are presented in a diagram over the test run time. During this test run the TOC was analysed two times. It increased within one week from around 4.7 to 10.6 mg/l in ATS I and 4.8 to 9.3 mg/l in ATS II.

In Table 6 the results of pollutant analyses are shown. As seen in Figure 6 all concentrations rose within one week. Every concentration increased with factor 1.5-2 except the zinc concentration which increased with factor 5.

The nutrient analyses of total nitrogen and phosphate had been difficult due to interferences. The available results of total nitrogen and phosphate analyses are not reliable during the first test run. Initial values had been measured with wastewater from mineral processing taken from a different container, so that only one result is available for the used water in ATS which is not comparable.

Table 6. Pollutant concentrations in mg/l test run 1

Pollutants							
Sulphate	ATS I	26.03.13			02.04.13		
		18000	18000	17000	35000	36000	33000
	ATS II	26.03.13			02.04.13		
		25000	25000	26000	35000	35000	38000
K 766.48 nm	ATS I	27.03.13			03.04.13		
		39.6	38.3	39.1	62.8	61.6	61.5
	ATS II	27.03.13			03.04.13		
		38.3	38.4	38.3	64.8	64.9	65.1
Na 330.24 nm	ATS I	27.03.13			03.04.13		
		1494	1496	1533	2364	2448	2266
	ATS II	27.03.13			03.04.13		
		1658	1665	1679	2256	2194	2154
Ni 341.48 nm	ATS I	27.03.13			03.04.13		
		92.12	91.88	92.25	138.0	137.7	138.4
	ATS II	27.03.13			03.04.13		
		92.64	92.49	91.97	136.3	135.8	135.6
Zn 307.59 nm	ATS I	27.03.13			03.04.13		
		347.6	357.7	362.1	1833	1857	1704
	ATS II	27.03.13			03.04.13		
		335.7	342.0	350.9	1602	2095	1870

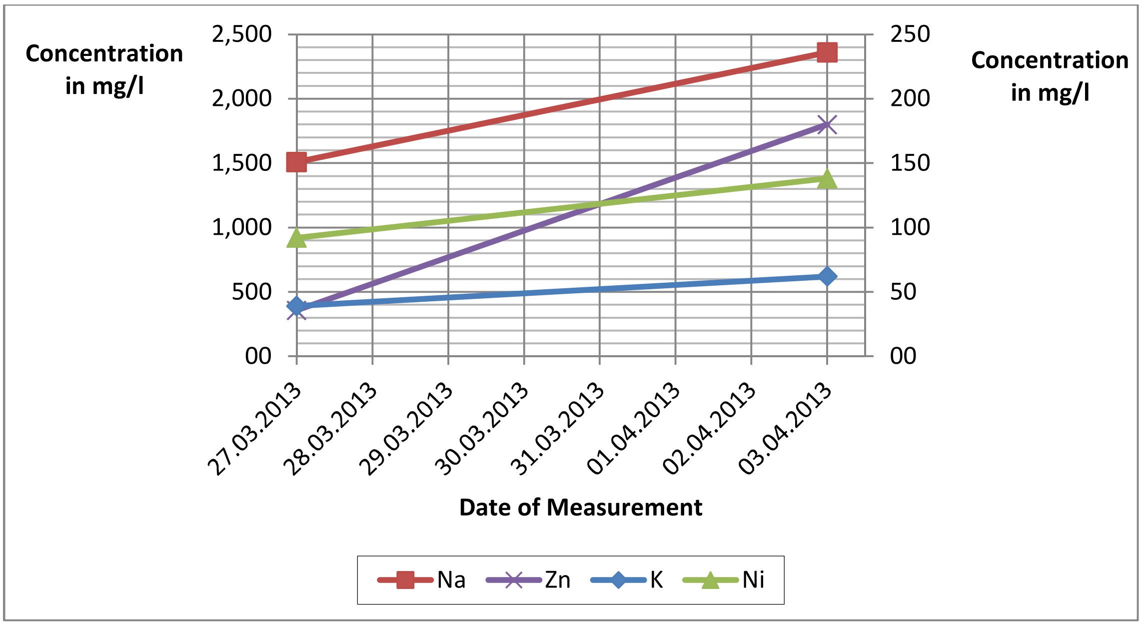


Figure 6. Trend of metal-concentrations in ATS 1 during first test run; Na & Zn plotted on left X-axis, K & Ni on right X-axis

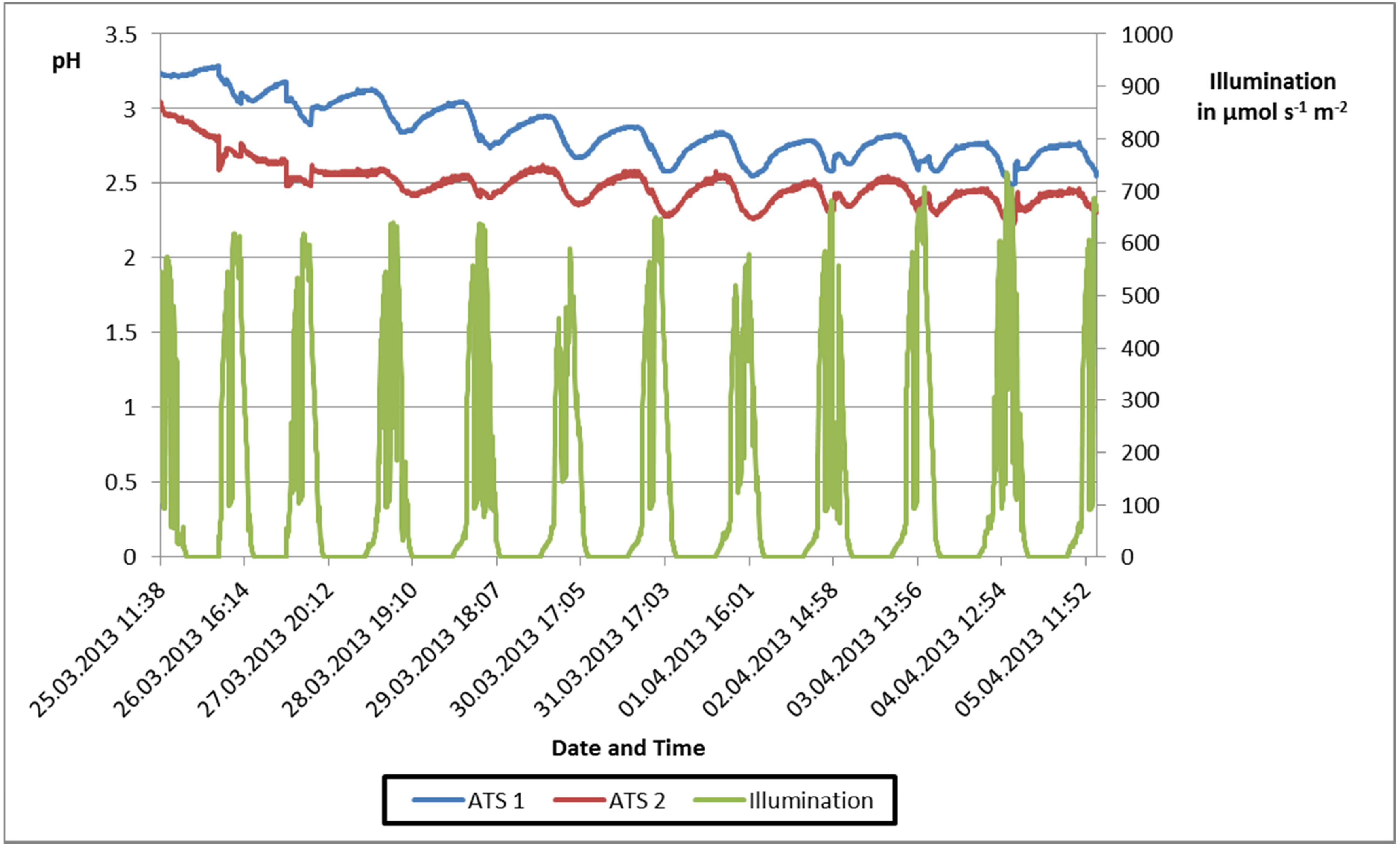
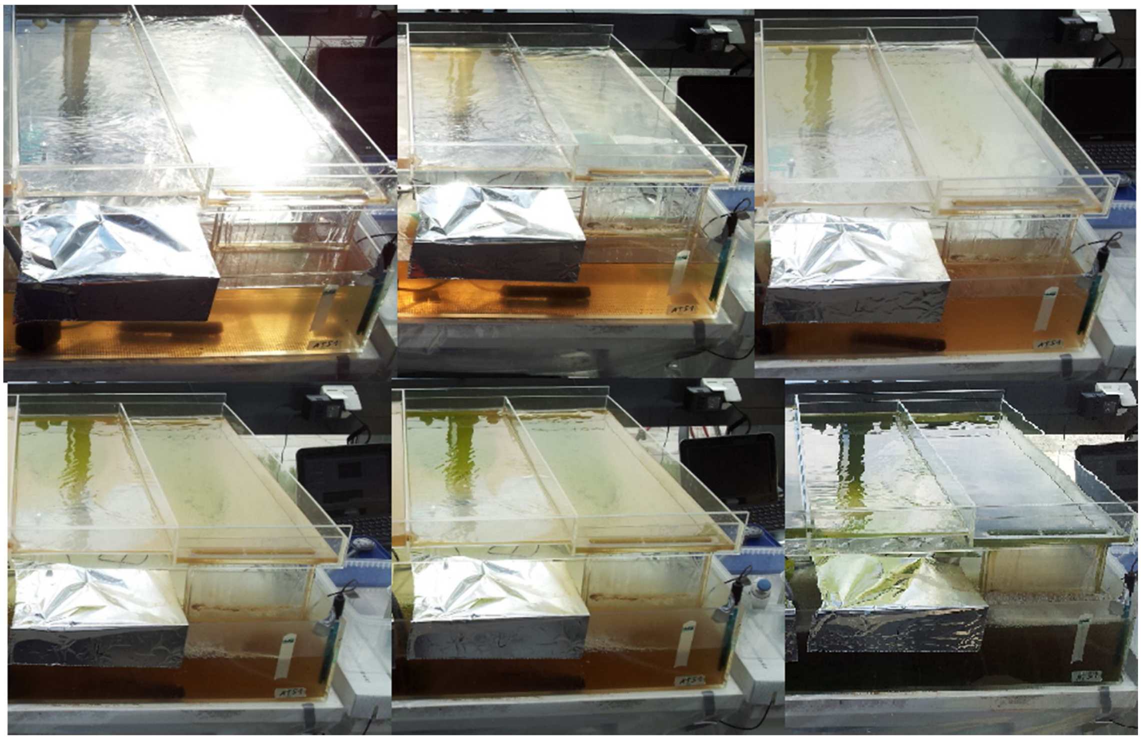


Figure 7. pH and illumination changes during test run 1

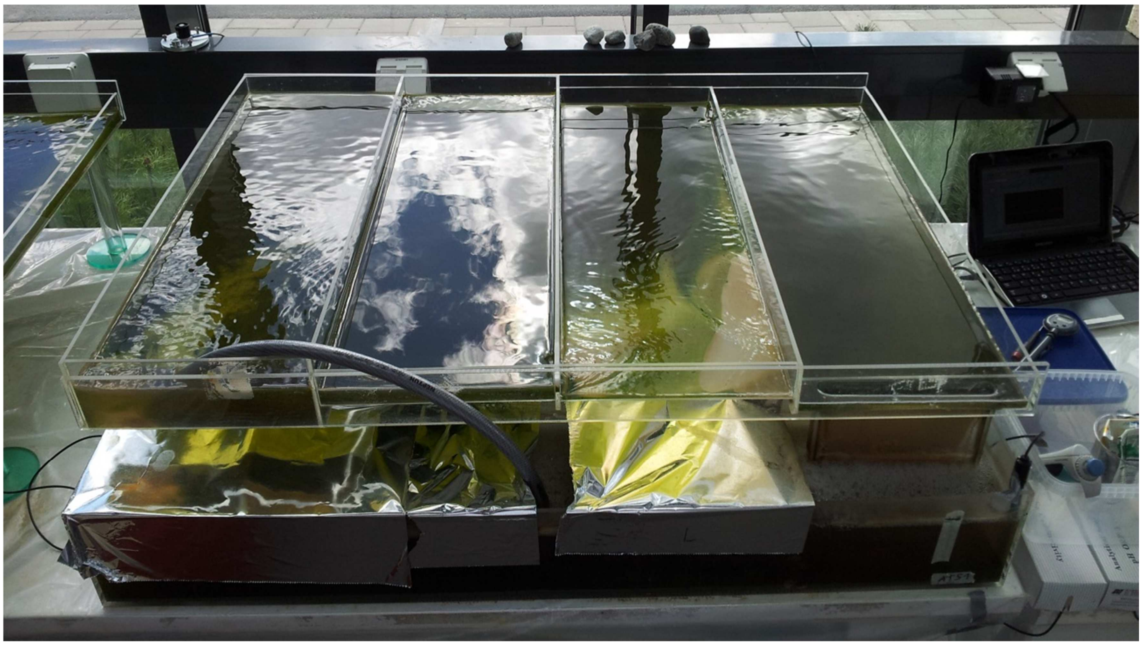
5.3 Second test run ATS

5.3.1 Observations

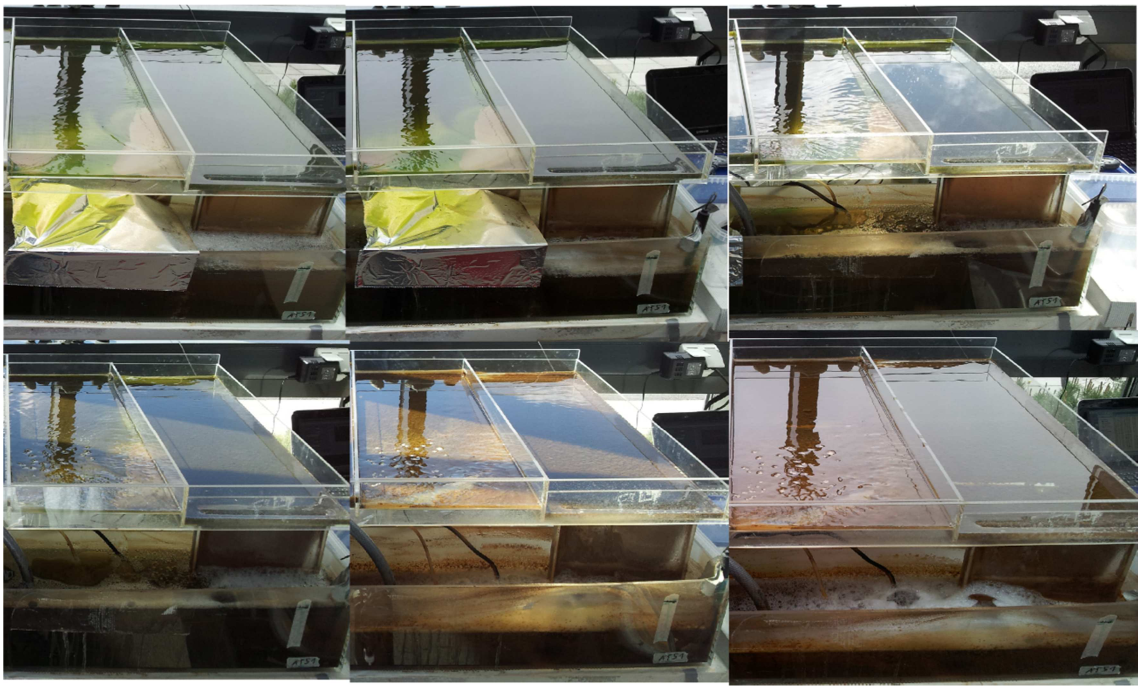
As shown in PICTURE 8 a proper batch of algae biomass grew in the first two weeks of test run 2 with nutrient enriched tab water. On the 25th of April, 2 days after adding wastewater algae had an intensive dark green colour, as shown in PICTURE 9. By and by the colour of algae biomass turned from green into brown and the systems got contamination by something looking like fungi, shown in PICTURE 11. In the end this fungi, or slime clogged often the sucking part of the pumps, so that they couldn`t work and the turf scrubbers got a little bit dry. It happened often directly after adding water, because that creates a sensation in storage tank and slime parts were sucked by the pump.



PICTURE 8. Algal growth in ATS 1 during the first two weeks of test run 2



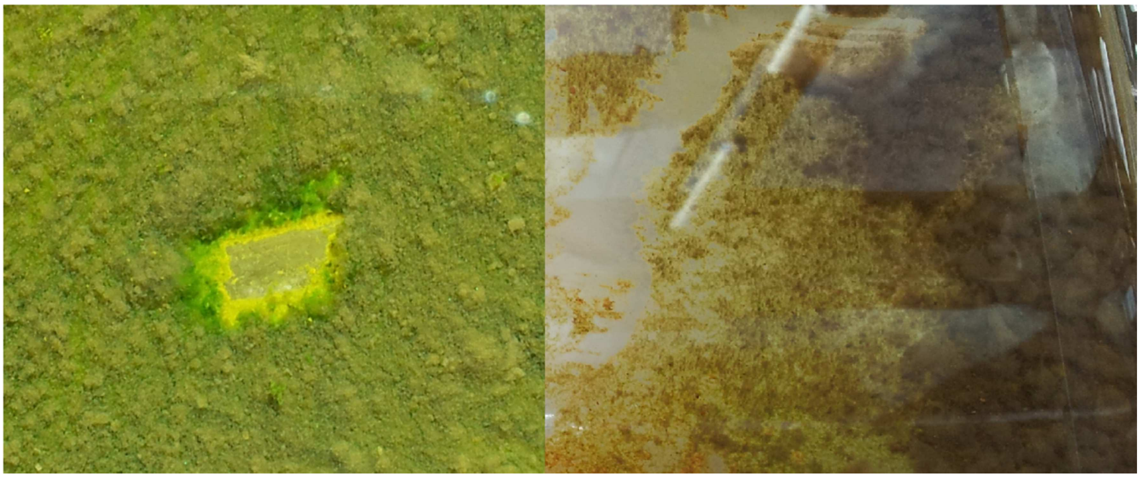
PICTURE 9. ATS I on 25.04.2013



PICTURE 10. ATS 1 after adding wastewater from mineral processing

After a few days, when it was visible that algae biomass is turning brown, it was possible to see, that the upper surface of algae is turning brown, but not the bottom layer. PICTURE 12 shows two different parts at different times, where it is visible that there are several layers. On the left side a small part of algae is removed with a finger. While the lower layer is light green, the upper layer is turning brown-grey. On the right side of this picture algae is darker, but the upper layer, seen on the right side, is removed on the left side. When algae are uncovered it starts to turn brown as well and on surfaces, where it`s removed it doesn`t grow again. On the right side of PICTURE 11 it can be seen, that algae biomass starts to lift from the surface through the higher flow rate, indicating algae are dying.

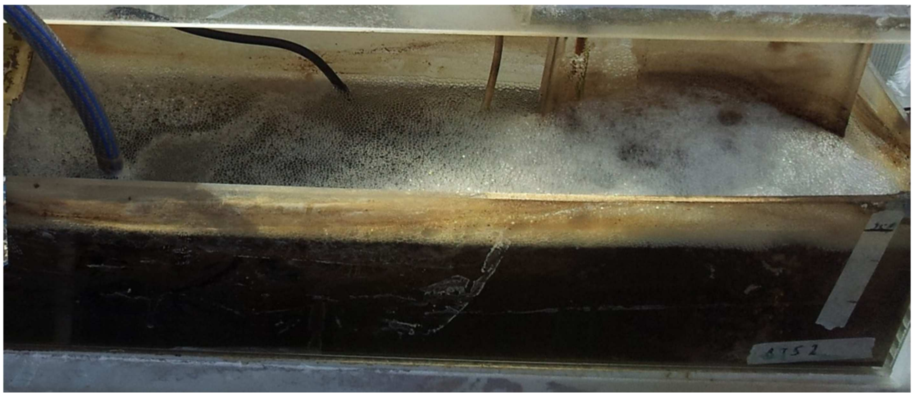
PICTURE 11. ATS with contamination of something looking like fungi



PICTURE 12. Different layers in algae biomass; left side: picture from the 29.04.2013 where a small part is removed by hand; right side: picture from the 14.05.2013 at the water entrance where upper layer is removed with water flow.

On the one side the preparation of nutrient enriched tab water goes relatively fast. All ingredients solved relatively quick. On the other side the preparation of nutrient enriched wastewater took much more time, especially when preparing a high concentrate for 50l in a 5l vessel it took several hours to solve everything. The colour of wastewater from mineral processing turned a little bit in green after adding all ingredients.

After the addition of nutrient enriched wastewater white foam (PICTURE 13) appeared on the surface of the storage tanks. It was every time heavier directly after adding nutrient enriched wastewater.



PICTURE 13. Foam on storage tank

5.3.2 Results

In Figure 8 the recorded pH values and illumination are presented in a diagram over the test run time from the 15th of April to 10th of May. Before the 15th the results haven`t been recorded, because the netbook turned off. The pH decreased from around 8 to 2.5. Although less wastewater from mineral processing was added to the systems in the end the pH was nearly as before in the first test run. As well as in the first test run a sinus curve appears, because temperature differences are not implemented. The illumination reached maximum values of 1010 µmol s^{-1} m^{-2} during the second test run time.

In Table 7 the results for total N, phosphates, BOD_5 and TOC are presented. As seen in Figure 9 the total nitrogen results are relatively constant and only slightly decreasing, while the phosphate concentrations decreased after adding wastewater from 32.4-36.5 mg/l to a minimum of 1.24 mg/l. This huge diminishment indicates together with the white foam, that something inside the wastewater reacts with phosphates. The BOD_5 was just measured in the beginning and in the end and decreased with a factor around 10 from 322 to 36.6 mg/l in ATS I and 370 to 36.6 mg/l in ATS II. Also the TOC decreased from 783 to 464 mg/l in ATS I and 800 to 441 mg/l in ATS II.

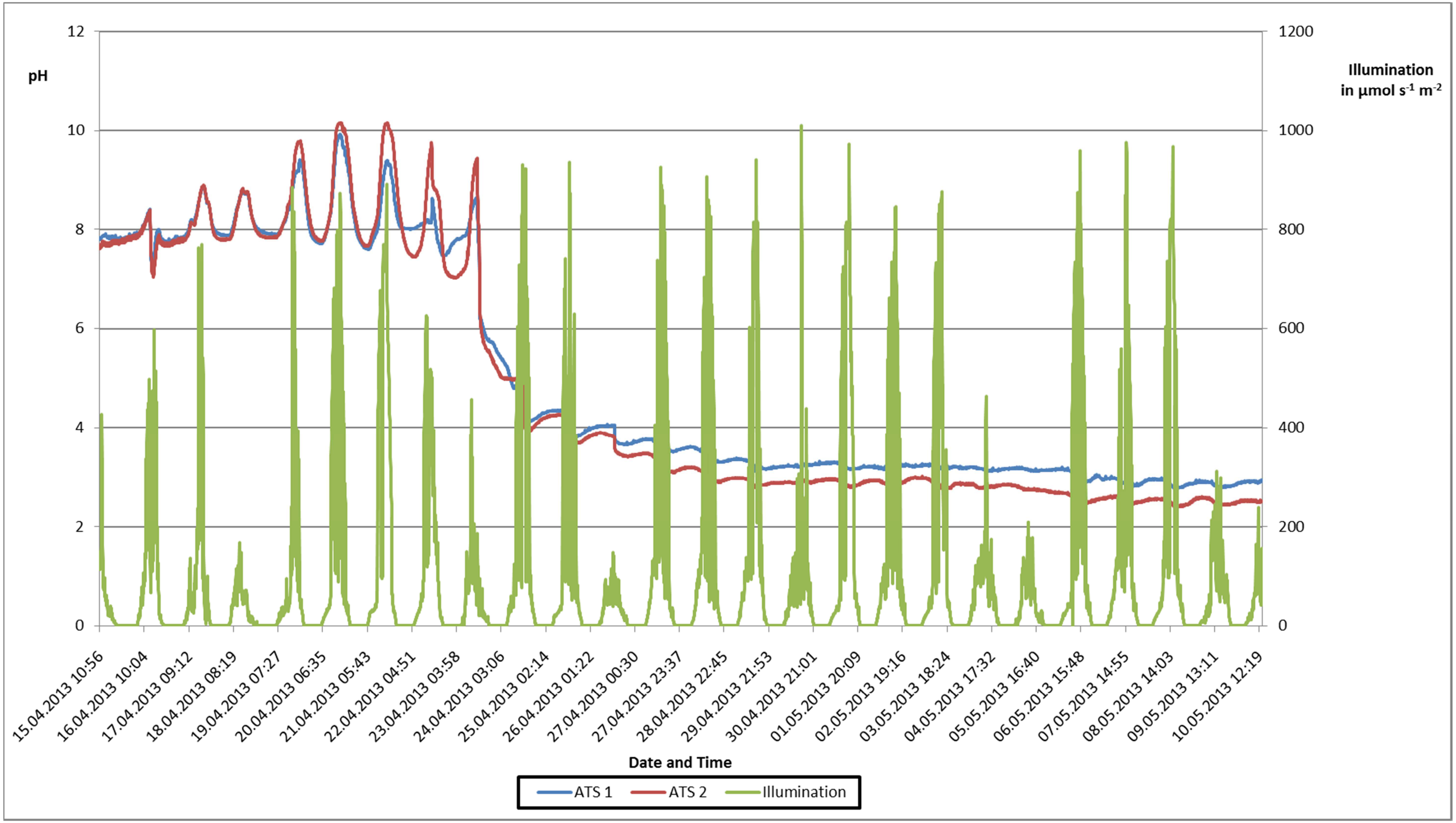


Figure 8. pH and illumination changes during test run 2

Table 7. Nutrient results test run 2

Experimental system Algae Turf Scrubber																
Analysis/test	Systems ID	**Week 1**			**Week 2**			**Week 3**			**Week 4**					
		(initial)			1L MW			5L MW			(Final)					
Nutrients	**DATE OF TESTING**	10-17.04			18-24.04			25.04-1.05			02.05-08.05					
Total N	ATS I	11.04.2013			24.04.2013			29.04.2013			03.05.2013			07.05.2013		
[mg/l]		40.3	41.5	41.8	37.6	28.8	40.8	35.0	25.2	33.4	29.0	29.4	30.9	34.4	35.7	36.9
	ATS II	11.04.2013			24.04.2013			29.04.2013			03.05.2013			07.05.2013		
		42.8	41.9	40.8	37.4	34.4	31.8	39.6	40.2	32.6	27.9	27.5	26.2	36.2	35.3	34.7
Phosphate	ATS I	11.04.13			24.04.2013			29.04.13			03.05.13			07.05.13		
[mg/l]		35.8	36.5	35.0	17.4	17.2	18.4	3.2	3.0	3.2	1.5	1.7	1.4	5.7	6.1	5.9
	ATS II	11.04.13			24.04.2013			29.04.13			03.05.13			07.05.13		
		33.4	32.4	33.2	13.4	13.8	15.2	2.2	2.6	2.8	1.2	1.3	1.3	7.1	7.2	7.0
BOD_5	ATS I	23.04.2013-29.04.2013												07.05.2013-12.05.2013		
[mg/l]		329.0	315.0											39.4	33.8	
	ATS II	23.04.2013-29.04.2013												07.05.2013-12.05.2013		
		374.0	366.0											33.8	39.4	
TOC	ATS I	11.04.2013			24.04.2013									08.05.2013		
[mg/l]		776.2	777.4	796.5	698.8	696.7	692.0							462.4	462.1	468.1
	ATS II	11.04.2013			24.04.2013									08.05.2013		
		793.9	801.0	804.0	668.8	668.2	658.3							440.6	444.9	439.3

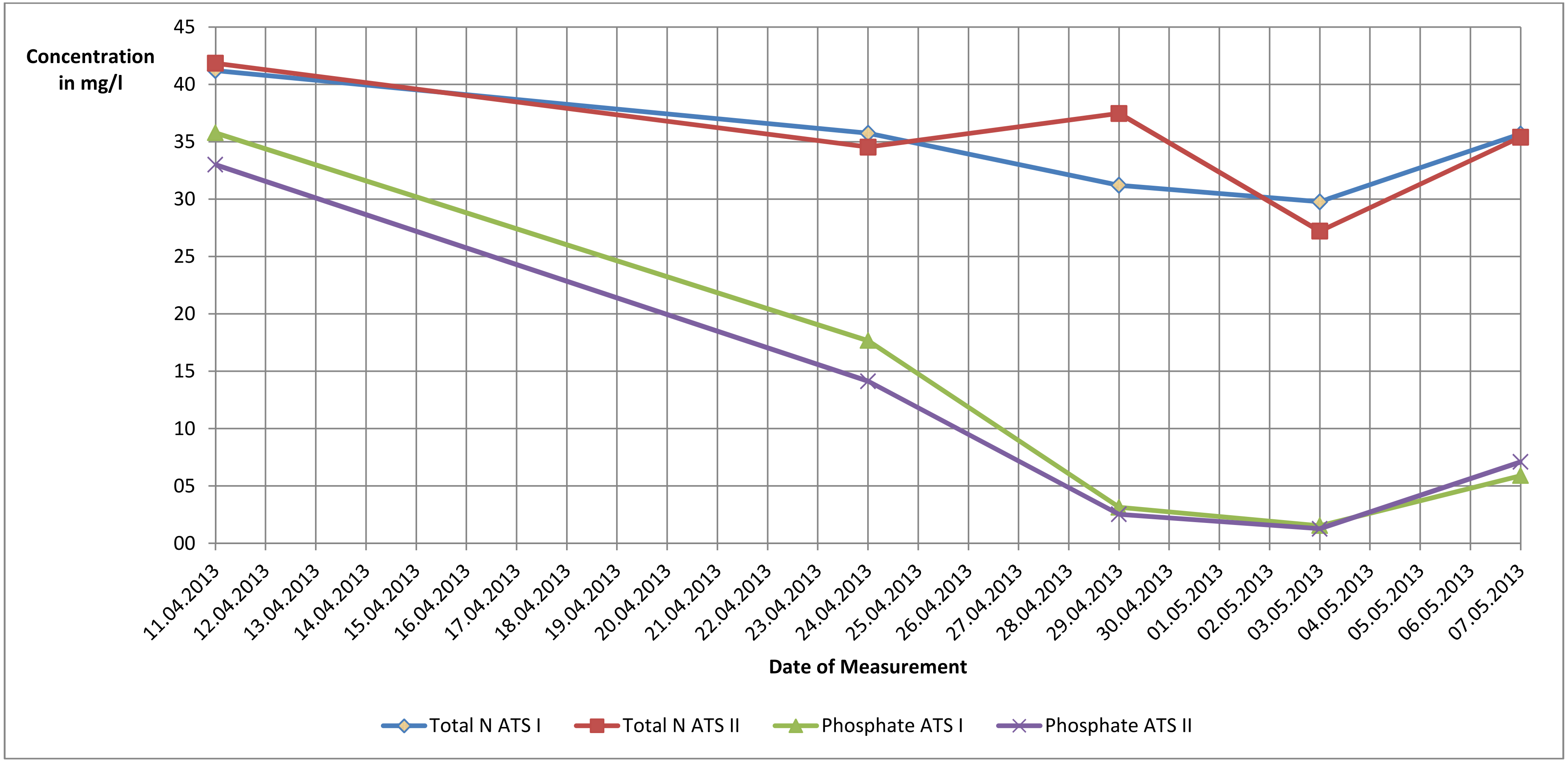


Figure 9. Total N and Phosphate concentrations in ATS 1 & 2 during second test run

Table 8. Measured pollutant concentrations in test run 2

Experimental system Algae Turf Scrubber										
Analysis/test	Systems ID	**Week 1**			**Week 3**			**Week 4**		
		(initial)			5L MW			9L MW (Final)		
Pollutants										
Sulphate [mg/l]	ATS I	11.04.13						07.05.13		
		67	63	64				12000	6000	4000
	ATS II	11.04.13						07.05.13		
		71	72	79				5000	5000	5000
K [mg/l] 404.41 nm	ATS I	11.04.13			30.04.13			08.05.13		
		45.6	46.8	46.9	79.0	78.8	80.0	134.4	130.4	134.0
	ATS II	11.04.13			30.04.13			08.05.13		
		47.2	47.6	48.1	83.4	79.3	80.2	124.2	117.8	120.0
Na [mg/l] 330.24 nm	ATS I	11.04.13			30.04.13			08.05.13		
		50.2	52.5	49.6	250.2	301.6	303.6	797.2	811.5	859.2
	ATS II	11.04.13			30.04.13			08.05.13		
		48.9	51.8	50.1	345.8	374.4	349.0	829.1	814.9	868.2
Ni [mg/l] 341.48 nm	ATS I	11.04.13			30.04.13			08.05.13		
		0.00	0.00	0.00	24.88	26.20	24.94	40.84	40.56	40.06
	ATS II	11.04.13			30.04.13			08.05.13		
		0.00	0.00	0.00	27.10	28.24	26.16	40.38	40.14	40.08
Zn [mg/l] 307.59 nm	ATS 1/1	11.04.13			30.04.13			08.05.13		
		13.0	15,5	14,1	2200.0	1656.2	2188.0	1126.8	1360.8	1033.2
	ATS 2/1	11.04.13			30.04.13			08.05.13		
		16.4	16.3	16.4	1881.6	2100.0	1767.2	1156.2	1335.8	1039.0

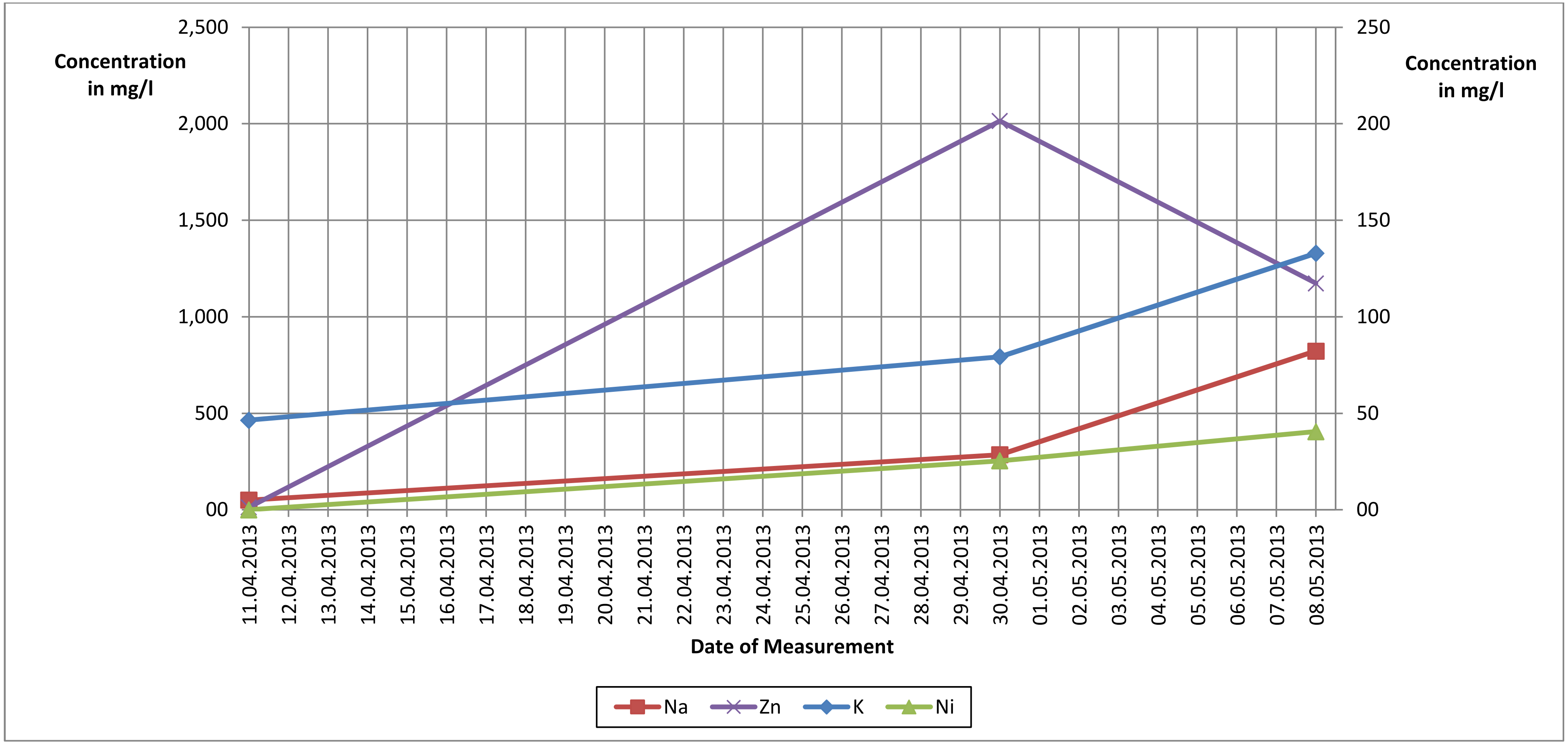


Figure 10. Trend of metal-concentrations in ATS 1 during second test run; Na & Zn plotted on left X-axis, K & Ni on right X-axis

The measured metal concentrations in the second test run can be seen in Table 8. Figure 10 shows that like in the first test run all metal concentrations increased. The expected concentrations without removal by algae are calculated with the initial concentrations in the beginning of test run 1 and the water changes during test run 2, shown in Table 5. The water amounts are only approximately, because unfortunately not all water changes had been written down. As initial concentrations for nutrient enriched tab water (NW) the initial concentrations of Table 8 are used. The concentrations of nutrient enriched wastewater from mineral processing (NWW) are estimated with the initial concentrations of the first test run plus the concentration of NW, because initial metal concentration of tab water can be neglected. The finally used concentrations can be seen in Table 9.

Table 9. Used concentrations for calculated metal concentrations

Metal	NWW	NW
K [mg/l]	85	46
Na [mg/l]	1650	50
Ni [mg/l]	92	0
Zn [mg/l]	365	15

The concentrations are calculated for the two points of time, when 5l respectively 9l NWW has been added. The first analysis (5l) was done after 50l of NW and 5l of NWW has been added to the systems. About 21.5l had been left in the system at that point. The second analysis (9l) was done after 64l NW and 9l NWW has been added. Approximately 20l had been left in the systems at that point.

The concentrations are calculated as shown in Equation 1.

$$c(M) = \frac{c(M_{NW}) \cdot V(NW) + c(M_{NMW}) \cdot V(NMW)}{V_{system}} \quad \text{Equation 1}$$

Table 10. Calculated and measured concentrations in test run 2

	5l		9l	
Metal	Measured	Calculated	Measured	Calculated
K [mg/l]	80.1	126.7	126.8	185.5
Na [mg/l]	320.8	500.0	830.0	902.5
Ni [mg/l]	26.3	21.4	40.3	41.4
Zn [mg/l]	1965.5	119.8	1175.3	212.3

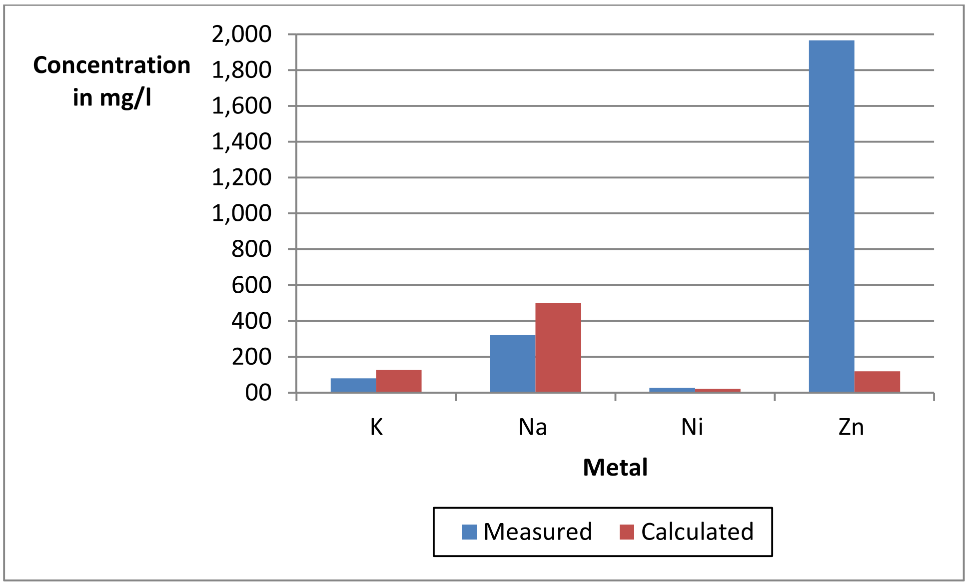


Figure 11. Measured and Calculated metal-concentration after addition of 5l wastewater

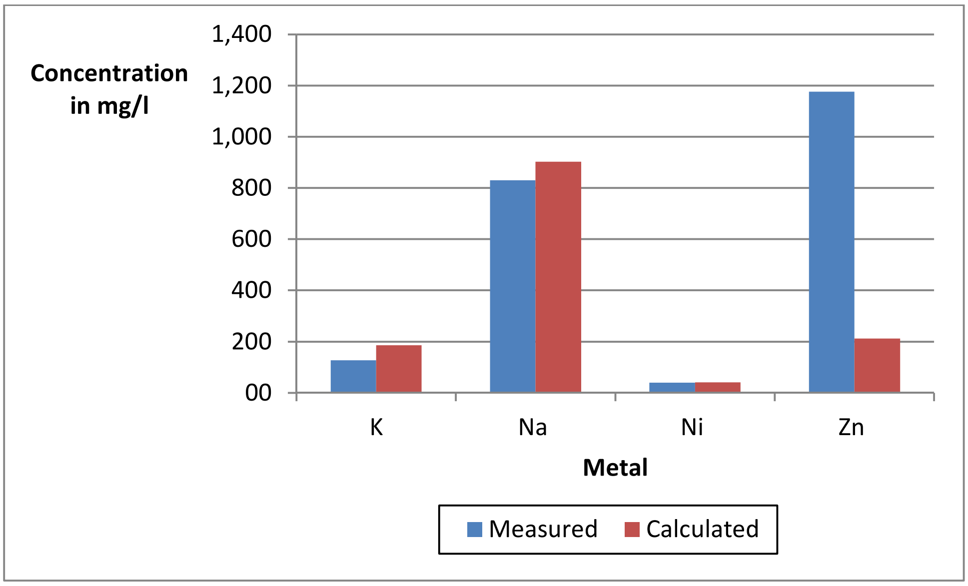


Figure 12. Measured and Calculated metal-concentrations after addition of 9l wastewater

As seen in Figure 11 & Figure **12** the calculated concentrations for K and Na are a bit higher than measured but quite equal, whereas Ni concentrations are more or less equal. The Zn concentrations are totally different and can`t be explained with inaccuracy.

6 CONCLUSIONS

The used algae species in this project are not especially known for acidic environments. However some of them are known for building polychelatin or metallothioneine III molecules which bind HM, like *Fragilaria crotonensis* and *Navicula pelliculosa* (Rai & Gaur, 2001).

In the application of ATS for the treatment of wastewaters from mineral processing, on the one side evaporation reduces water amounts, but on the other side already toxic pollutant concentrations concentrate and get even more toxic.

Obviously a removal of the analysed pollutants is not visible or measurable neither for metals nor sulphates. Different measured and calculated concentrations (Table 10) for K and Na, indicating a removal of this alkali metals. Unfortunately these results are associated with big uncertainties and further tests have to proof the removal. Ni as one of the main concerning HM is not removed at all.

In the first test run Zn-concentration increased in ATS with factor 5 while in EBB and WST Zn-concentrations increased just slightly and other metal concentrations increased just with factor 1.5-2. In the second test run factors of 10-20 are between measured and calculated Zn-concentrations, in ATS, EBB and WST. For other metal concentrations the differences are smaller. Changes between the test runs are different amounts of nutrients and wastewater additions. But neither in nutrient enriched tab water, Table 8 show only Zn-concentrations of 15mg/l, nor in wastewater Zn-concentration is high enough to explain these high factors by water addition. Possible are interferences in AAS analysis through built compounds or increased concentrations of other compounds added with nutrients.

The very high sulphate content of about 18000 mg/l is not removed. The results are connected with uncertainties, because samples had to be diluted with 1/1000.

The pH is as described in 2.3.1 a very important property for algal growth. Like pollutant concentrations also the proton concentration increased in all tests and was not changed by photosynthesis or algal growths. The pH decreased to around 2.5-3 and even elevation of pH to 5-6 during 1st small-scale experiment brought no successful algal growth. The pH value could be influenced already with small amounts of wastewater. In test run 2 the pH decreased from 8-9 to 4-5 after addition of 2l wastewater.

The influence of illumination on algal growth could not be investigated. Only the influence on water temperatures and evaporation can be seen in Figure 7 & Figure **8**.

As a result of pre-treatment at the mining area, the used wastewater from mineral processing has very small amounts of nutrients. For that reason an imitated municipal wastewater had been used to initiate and back up algal growth in the second test run.

In the beginning of test run 2 the concentrations of total nitrogen and phosphate had been quite high with around 40mg/l total nitrogen and 35 mg/l phosphate. The phosphate concentration decreased rapidly and white foam appeared after addition of wastewater. Explanation might be a reaction of phosphate and aluminium, which is used in acid mine drainage water to precipitate phosphates (Gross, 2000). This explanation applies also to EBB and WST systems, because wastewater contains aluminium.

In the end it is ambiguous, if algae removed any metals, because neither water nor algae biomass was analysed for all kind of metals. It`s also possible that the occurred slime in the end of test run 2 consists of polychelatins/metallothioneine III, which possibly bound some metals. Particularly in the first test run the change of colour into brown and the precipitated solids indicate oxidation of metals by aeration. But the amounts are small and they are not analysed.

In summary a treatment of wastewater from mineral processing using algae in an ATS is not possible in the applied way. In both test runs a similar development of all concentrations appeared and algae couldn`t grow or survive in this toxic wastewater. Hence this project can be continued with these algae species and water properties (pH, nutrients, pollutant concentrations) changed by chemical pre-treatment or other acidophilic or acidotolerant algae species, like *Mougeotia* or *Spirogyra*, which are known for acidic environments and also for HM uptake, can be used. In any case the toxic concentrations and properties of pollutants in this wastewater will be of interest. In progress of this project small-scale experiments should be used to keep wastewater amounts small.

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FIGURES

TABLES

PICTURES

APPENDICES

Appendix 1. Results of EBB

Table 11. Results of EBB system test run 1 in mg/l

Experimental system		**EBB**									
Analysis/test	Systems ID	**0 (initial)**	**Week 1**			**Week 2**			**Week 3**		
Nutrients											
Total N	EBB 1/1										
			5.45	5.91	5.89	5.37	5.76	5.98	5.69	6.07	5.86
Phosphate	EBB 1/1										
			0.32	2.82	/	0.99	/	/			
BOD$_5$	EBB 1/1		13-18.03.2013			18-23.03.2013			03-08.04.2013		
			/	/	/	13.2	12.9	11.8	4.2	4.5	4.5
TOC	EBB 1/1		13.03.2013			20.03.2013			27.03.2013		
		2.805	10.71	10.83	?	32.73	32.89	32.58	12.97	/	/
Pollutants											
Sulphate	EBB 1/1		12.03.13			18.03.13			26.03.13		
			18000	17000		13000	13000		20000	19000	19000
K	EBB 1/1	-	13.03.13			20.03.13			27.03.13		
404,41 nm			47.5	47.0	46.4	66.3	65.5	62.2	43.4	43.8	42.9
Ni	EBB 1/1	85	13.03.13			20.03.13			27.03.13		
341,48 nm			82.08	81.72	81.23	85.05	84.95	85.12	84.66	84.60	84.65
Na	EBB 1/1	1044.2	13.03.13			20.03.13			27.03.13		
330,24 nm			1216	1206	1194	1037	1074	1078	1360	1383	1360
Zn	EBB 1/1	291.15	13.03.13			20.03.13			27.03.13		
307,59 nm			281.1	307.4	290.2	312.5	320.9	315.4	335.7	342.0	350.9

Table 12. Results EBB system test run 2 in mg/l

Experimental system Ebb and flow system																	
Analysis/test	Systems ID		**Week 1**			**Week 2**			**Week 3**			**Week 4**			**Week 5**		
			(initial nutrient water)			**10L MW**			**50L MW**						**(Final)**		
Nutrients	DATE OF TESTING		10-17.04														
Total N	EBB 1		11.04.2013			24.04.2013			29.04.2013			03.05.2013			07.05.2013		
			35.0	34.9	34.2	40.0	23.6	37.0	44.0	41.8	44.2	62.9	57.1	52.1	36.0	35.2	38.0
Phosphate	EBB 1		11.04.13			24.04.2013			29/04/1/2013			03.05.13			07.05.13		
			9.30	8.40	8.80	0.32	0.34	0.36	1.20	0.80	1.00	1.97	1.78	1.77	2.30	1.50	1.60
BOD$_5$	EBB 1		23/04/2013-29/04/2013												07/05/2013-12/05/2013		
			59.1	53.5	56.3										29.6	29.6	29.6
TOC	EBB 1		11.04.2013			24.04.2013									08.05.2013		
			700.7	700.8	702.0	182.6	178.9	183.2							124.6	124.4	123.8
Pollutants																	
Sulphate	EBB 1		11.04.13						30.04.13						07.05.13		
			17	17	16										22000	10000	15000
K	EBB 1		11.04.13						30.04.13						08.05.13		
404,41 nm			43.5	43.2	43.4				63.5	61.1	63.0				106.6	94.6	96.0
Ni	EBB 1		11.04.13						30.04.13						08.05.13		
341,48 nm			0.07	0.07	0.07				19.48	21.28	22.14				33.44	32.96	32.88
Na	EBB 1		11.04.13						30.04.13						08.05.13		
330,24 nm			54.3	53.4	55.6				81.9	101.2	103.4				588.3	608.2	646.9
Zn	EBB 1		11.04.13						30.04.13						08.05.13		
307,59 nm			14.7	15.8	14.1				2092.0	2514.0	1823.6				1297.0	1584.6	1275.0

Appendix 2. Results of WST

Table 13. Results WST system test run 1 in mg/l

Experimental system		**Willow stack tower**								
Analysis/test	Systems ID	**0 (initial)**	**Week 1**			**Week 2**			**Week 3**	
Nutrients										
Total N	WST 1/1		12.03.2013			18.03.2013			26.03.2013		
			5.45	5.91	5.89	9.65	10.70	10.80	19.40	19.00	17.90
Phosphate	WST 1/1		12.03.2013			18.03.2013					
			0.32	2.82	/	1.81	/	/			
BOD$_5$	WST 1/1		13-18.03.2013			18-23.03.2013			03-08.04.2013		
			/	/	/	19.7	19.7	20.2	7.0	7.6	7.9
TOC	WST 1/1		13.03.2013			20.03.2013			27.03.2013		
		2.805	11.08	10.71	/	19.31	18.71	18.88	53.03	/	/
Pollutants											
Sulphate	WST 1/1		12.03.13			18.03.13			26.03.13		
			18000	17000	/	15000	15000	/	20000	21000	21000
K	WST 1/1	-	13.03.13			20.03.13			27.03.13		
766,48 nm			47.5	47.0	46.4	97.8	97.6	95.5	110.4	107.6	106.9
Ni	WST 1/1	85	13.03.13			20.03.13			27.03.13		
341,48 nm			82.08	81.72	81.23	87.85	88.03	88.16	92.97	91.92	92.73
Na	WST 1/1	1044.2	13.03.13			20.03.13			27.03.13		
330,24 nm			1216	1206	1194	1200	1181	1208	1381	1352	1399
Zn	WST 1/1	291.15	13.03.13			20.03.13			27.03.13		
307,59 nm			281.1	307.4	290.2	378.4	362.5	324.0	374.4	353.5	342.1

Table 14. Results WST system test run 2 in mg/l

Experimental system Willow stack tower																	
Analysis/test	Systems ID		**Week 1**			**Week 2**			**Week 3**			**Week 4**			**Week 5**		
			(initial)			**1L MW**			**5L MW**						**(Final)**		
Nutrients																	
Total N	WST 1		11.04.2013			24.04.2013			29.04.2013			03.05.2013			07.05.2013		
			33.5	34.0	34.2	48.4	54.6	55.6	47.8	40.4	36.8	44.1	35.8	42.8	48.6	46.5	47.4
Phosphate	WST 1		11.04.13			24.04.2013			29.04.13			03.05.13			07.05.13		
			1.80	2.50	0.90	3.40	3.60	3.60	0.60	1.00	1.20	1.01	1.02	1.02	1.43	1.42	1.42
BOD_5	WST 1		23/04/2013-29/04/2013												07/05/2013-12/05/2013		
			16.9	16.9	15.5										10.7	11.8	10.4
TOC	WST 1		11.04.2013			24.04.2013									08.05.2013		
			508.9	506.0	484.1	388.1	387.3	384.8							109.7	108.9	108.9
Pollutants																	
Sulphate	WST 1		11.04.13						30.04.13						07.05.13		
			140	160	150											2000	2000
K	WST 1		11.04.13						30.04.13						08.05.13		
404,41 nm			49.3	49.1	48.3				101.8	103.6					155.2	168.0	174.2
Ni	WST 1		11.04.13						30.04.13						08.05.13		
341,48 nm			0.18	0.16	0.17					16.98	17.20				28.80	27.38	27.36
Na	WST 1		11.04.13						30.04.13						08.05.13		
330,24 nm			56.5	55.5	54.7				242.4	183.1					507.2	490.0	494.6
Zn	WST 1		11.04.13						30.04.13						08.05.13		
307,59 nm			10.1	14.2	15.4				2254.0	2566.0	1790.0				1811.8	1572.2	1240.6

Appendix 3. Results of spectroscopy

Solution	Stock			
Algae species	15.03.2013	18.03.2013	22.03.2013	comment
1	0.009	0.004	0.007	
2	0.004	0.002	0.005	
3	0.004	0.002	0.008	
4	0.008	0.006	0.011	
5	0.006	0.003	0.006	
6	0.006	0.002	0.006	
7	0.006	0.004	0.01	
8	0.003	0.001	0.01	
9	0.005	0.005	0.028	Algae grow
10	0.004	0.001	0.009	
control 1	0.002	0.002	0.003	
control 2	0.002	0.003	0.003	

Solution	pH 4			
Algae species	15.03.2013	18.03.2013	22.03.2013	comment
1	0.1141	0.024	0.012	
2	0.0973	0.027	0.015	
3	0.09	0.06	0.026	
4	0.068	0.023	0.016	
5	0.099	0.029	0.017	
6	0.091	0.025	0.013	
7	0.1044	0.026	0.015	
8	0.1002	1.1357	0.018	???
9	0.111	0.024	0.013	
10	0.092	0.041	0.0211	
control 1	0.0159	0.03	0.009	
control 2	0.017	0.031	0.009	

Solution	pH 5			
Algae species	15.03.2013	18.03.2013	22.03.2013	comment
1	0.4943	0.0632	0.017	
2	0.1209	0.061	0.016	
3	0.1295	0.04	0.016	
4	0.3383	0.052	0.0152	
5	0.1129	0.061	0.0157	
6	0.1176	0.04	0.013	
7	0.1039	0.056	0.013	
8	0.3171	0.5703	0.015	???
9	0.1184	0.061	0.0166	
10	0.229	0.053	0.018	
control 1	0.2954	0.071	0.049	
control 2	0.2805	0.078	0.044	

Solution	pH6			
Algae species	15.03.2013	18.03.2013	22.03.2013	comment
1	0.2063	0.015	0.023	
2	0.1336	0.026	0.034	
3	0.1562	0.025	0.024	
4	0.1217	0.026	0.013	
5	0.1499	0.033	0.015	
6	0.1107	0.024	0.072	
7	0.091	0.032	0.0408	
8	0.1467	0.749	0.1071	???
9	0.1784	0.018	0.017	
10	0.1191	0.033	0.048	
control 1		0.013	0.011	
control 2		0.013	0.015	