



Rapport

Diarienummer Projektnummer
NV Rapport 2021-10 Examensarbete, 15 hp

Feasibility Study for the Implementation of Algae Cell Monitoring in Raw Water Using an Optical Sonde at the Görväln Drinking Water Treatment Plant

Examensarbete, Högskoleingenjörsexamen Kemiteknik, KTH

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2021-06-23



DEGREE PROJECT IN CHEMICAL ENGINEERING,
FIRST CYCLE, 15 HP
STOCKHOLM, SWEDEN 2021

Feasibility Study for the Implementation of Algae Cell Monitoring in Raw Water Using an Optical Sonde at the Görväln Drinking-Water Treatment Plant

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DEGREE PROJECT

Bachelor of Science in
Chemical Engineering

Title: Feasibility Study for the Implementation of Algae Cell Monitoring in Raw Water Using an Optical Sonde at the Görvåln Drinking-Water Treatment Plant

Swedish title: Genomförbarhetsstudie för implementering av algövervakning i råvatten med optisk sond vid Görvålnverket

Keywords: Algae Monitoring, Chlorophyll, Fluorescence

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Date: 2021-06-21

Examiner: Susanna Höglund Lenninger

Abstract

Each year, algae blooms occur in lake Mälaren in Stockholm and can cause complications in drinking-water treatment plants. It is predicted that the risk for algae bloom in Mälaren will increase due to the effects of climate change. To monitor levels of algae can be time consuming but installing an on-line sensor for real-time monitoring can be beneficial and act as an early warning system. The aim of this project is to support Norrvatten in their work to implement an automated early warning system for the detection of cyanobacteria (aka blue-green algae), at their drinking-water treatment plant at Görväln.

In pursuit of this aim, the feasibility of using the EXO2 sensor was explored where chlorophyll was measured as a proxy parameter for total algae. The study took 10 weeks where on-site data collection was conducted, and validation of measurements was done by comparing data with laboratory results. The EXO2 has potential to be used as a monitoring instrument for an early warning system for algae bloom. It can measure chlorophyll concentrations above 10 µg/l in raw water at the Görväln plant with a 95% confidence when correction factors are applied. The level of chlorophyll can furthermore be translated to algae cell density which can be used as an indicator for total algae bloom. To maintain high confidence in the chlorophyll measurements, the sonde should be checked once a week with a new reference solution with known concentrations. This solution can be stored and used for one week, maximum two depending on the composition of the solution.

Sammanfattning

Varje år sker det algblooming i Mälaren i Stockholm och kan orsaka svårigheter i reningsprocesser hos vattenverk. Studier visar att risken för algblooming i Mälaren kommer att öka över åren på grund av temperaturökning från klimatförändringen. Att installera en analysteknik, där man med en on-linesensor detekterar alger kontinuerligt, skulle vara effektivare tidsmässigt än att räkna alger. En on-line sensor skulle även kunna fungera som ett tidigt varningssystem för algblooming i Mälaren. Syftet med detta projekt är att stödja Norrvatten i deras arbete med att implementera ett automatiserat system för tidig varning för cyanobakterier (blågröna alger) vid Görvålnverket.

Möjligheten att använda EXO2-sensorn undersöktes där klorofyllhalten mättes som proxy för totala alger. Studien tog 10 veckor där datainsamling genomfördes och validering av mätningar gjordes genom att jämföra data från laboratorieresultat. EXO2-sensorn har potential att användas som för ett tidigt varningssystem för algblooming. Det kan mäta klorofyllhalter över 10 µg/l i råvatten vid Görvålnverket med 95% konfidens när korrigeringsfaktorer tillämpas. Nivån av klorofyll kan dessutom översättas till densitet av algceller som kan användas som en indikator för total algblooming. För att upprätthålla högt förtroende för klorofyllmätningarna bör sonden kontrolleras en gång i veckan med en ny referenslösning med kända koncentrationer. Denna lösning kan lagras och användas i en vecka, högst två beroende på lösningens sammansättning.

Acknowledgments

This project has been a real pleasure to work on and it is all thanks to the people who made it possible. I want to first and foremost thank my supportive supervisor at Norrvatten, Ewelina Basiak-Klingspetz. From introducing me to the world of municipal drinking-water treatment to bouncing ideas and calculations with me. She was a great teacher who always made time for my questions and trusted me to work independently. Thank you, Ewelina. Then I want to thank Stephan Köhler who has been like a second supervisor for me. He has contributed with many ideas and suggestions during the project as well as shared his passion for water research, which I was inspired by. He was a great support and was always available to answer questions. Thank you, Stephan. I also want to thank Daniel Hellström who was my first point of contact at Norrvatten. He recognized my passion for water early on and has supported it throughout this project. I am grateful he took a chance on me, allowing me to do my project at Norrvatten. Thank you, Daniel. Then I want to thank my supervisor at KTH, Susanna Höglund Lenninger. Even with her busy schedule, she found time to support me by answering my questions, bouncing calculations with me and encouraging my decisions. She allowed me to work independently and I felt her trust and support throughout the project. Thank you, Susanna. Finally, I want to thank my friend and classmate Johnny Hagelin, our weekly chats about our respective projects gave me many ideas and motivation during the project. It was very rewarding to have a friend by my side, cheering me on.

Table of Contents

1	Introduction	1
1.1	Aim, Objective and Research Questions	2
2	Theoretical Background	3
2.1	Chlorophyll in Algae	3
2.2	Measuring Chlorophyll with Fluorescence	4
2.3	Calibration for Chlorophyll Fluorescence	5
2.4	Interferences and Other Parameters	6
3	Methodology	8
3.1	Data collection and Materials	8
3.1.1	Reference Solutions	9
3.1.2	Full Factorial Experiment	11
3.1.3	Raw Water Sampling and Measurements	12
3.1.4	UV measurement of chlorophyll sample	13
3.2	Data Processing and Analysis	13
3.2.1	Temperature and turbidity correction	14
3.2.2	Statistical analysis of collected data	14
3.2.3	Calculations for the full factorial experiment	15
3.2.4	Graphical comparison of collected data	15
3.3	Methods of Selection	16
3.4	Reliability and Validity	16
3.5	Ethical Considerations	16
3.6	Limitations of the Study	17
4	Results	18
4.1	Reference Solutions	19
4.2	Precision of Measurements	22
4.3	Full Factorial Experiment	22
4.4	Geometric Mean Regression	24
4.5	Algae Cells as a Function of Chlorophyll	26
4.6	Chlorophyll stabilization using a $MgCaO_3Mg(OH)_2$ solution	26
5	Discussion	28
5.1	Stability of Reference solutions	28
5.2	Precision of EXO ₂ Measurements	28
5.3	Variables with largest effect on chlorophyll concentration measurements	29
5.4	Accuracy of the EXO ₂ measurements	29
5.5	Quantifying of algae cells using chlorophyll concentrations	29
5.6	Chlorophyll stability	29
5.7	Sources of Error and Suggested Improvements	29
6	Conclusion	30
	References	31
	Appendix I. List of Materials	I
	Appendix II. Statistical calculations	II

Abbreviations

Colored Dissolved Organic Matter (cDOM)

Dissolved Organic Matter (DOM)

Drinking-Water Treatment Plants (DWTP)

Fluorescence Dissolved Organic Matter (fDOM)

Formazine Nephelometric Units (FNU)

Geometric Mean Intercept (GMI)

Geometric Mean Regression (GMREG)

Geometric Mean Regression Slope (GMS)

Inner Filter Effect (IFE)

Light-Emitting Diode (LED)

Parts Per Billion (ppb)

Quinine Sulfate Units (QSU)

Sustainable Development Goals (SDGs)

World Health Organization (WHO)

Water Tracer (WT)

1 Introduction

In 2015, all members states of the United Nations agreed on a global partnership for sustainable development. The partnership, also known as the 2030 Agenda, is grounded in the Sustainable Development Goals (SDGs) that act as aspirational goals for all UN member states to achieve. SDG number six, *Clean Water and Sanitation for All*, is arguably one of the most fundamental goals of the 2030 Agenda as it has strong links to poverty reduction, access to education, gender equality and more (WHO/UNICEF, 2017). SDG 6 highlights the importance of having access to safe drinking water but also warns UN member states to be aware that drinking-water quality is at risk due to the impacts of climate change such as the rise in temperature (UN-Water, 2018).

One of the recommended actions to maintain good water quality is to implement early warning systems for contaminants. This way, operational measurements can be taken before there is an accumulation that risk the efficiency of the water treatment. Some contaminants are more predicable than others, but contaminants caused by seasonal variability, such as algae, are highly important to monitor as climate patterns are changing and the global temperature is increasing (WHO, 2017).

General algae are not classified as toxic per se but where there is algae bloom, cyanobacteria (aka. blue-green algae) can also be present as they grow in similar conditions. Cyanobacteria can produce and release toxins into the water and cause health damage if ingested (Rapala *et al.*, 2002). The World Health Organization (WHO) recommend that drinking-water treatment plants (DWTP) monitor algae levels as an indicator for both general biomass and cyanobacteria. The monitoring should take place at the raw water intake as it can cause much damage to the primary steps in the treatment process (WHO, 2017).

Each year algae blooms occur in lake Mälaren in Stockholm. It is predicted that the lake's temperature will continue to rise as an effect of climate change which increases the risk for algae bloom (Ejhed, 2020). Algal blooms can cause filter blockages at DWTP and there is an increased risk of cyanobacteria accumulating in the process water if there are no preventative measures taken.

Quantifying algae in water can be done by measuring cell density [no. cells/ml] or measuring the chlorophyll concentration [$\mu\text{g/l}$] (Almuhtaram *et al.*, 2018). Cell density measurement is often done though a cell count using a microscope, which is time consuming and not very precise. Likewise, the method for determining chlorophyll concentration is commonly done by using spectrophotometry, which can also time consuming as the water sample needs to be filtered and processed (Kong *et al.*, 2014). A more time effective method of analysis is installing on-line monitoring for real-time data collection. The main issue regarding this method is that calibration and the reliability of the measurements are highly site specific and needs to be considered before installation (Bertone, Burford and Hamilton, 2018).

Norrvatten is a municipal association working with drinking water treatment and distribution. They distribute drinking water to 14 municipalities in northern Stockholm, covering nearly 700 000 people. Their treatment plant, Görväl, is located by lake Mälaren in Järfälla municipality. There they produce nearly 1600 liters per second resulting in roughly 50 million cubic meters per year (Norrvatten, no date). Norrvatten are part of a research project called DiCyano where universities, drinking-water treatment plants and companies have come together to create a digital platform for algae data. The focus is on cyanobacteria to better understand its growth due to climate change and evaluating how to implement early warning systems at DWTP (DiCyano, 2021).

1.1 Aim, Objective and Research Questions

The aim of this study is to support Norrvatten in their work to implement an automated early warning system for the detection of cyanobacteria, at their DWTP at Görvåln. The objective is to explore the feasibility of using the optical sonde called EXO2 as the detection device and chlorophyll as the parameter for the warning system. To explore this objective, the project is guided by the following research questions:

- How should the sonde be used for the detection and quantification of algae cells and how reliable are the measurements?
- How often does the EXO2 sonde need to be calibrated and which variables have the largest effect on the measurement of chlorophyll concentrations?

2 Theoretical Background

This chapter gives an overview of algae, why we measure chlorophyll-a, how to measure it and the technical workings of the measurements.

2.1 Chlorophyll in Algae

Algae is a group term of aquatic eucaryotic plants. There are different types of algae, such as red, green and blue-green, and one of the main properties they have in common is that they contain chloroplasts which give them the ability to photosynthesize (Andersen, 2021). The main molecule for photosynthesis inside the algae's chloroplast is chlorophyll (Tanaka and Tanaka, 2019), which can be divided into five categories of a,b,c,d and f. All categories of chlorophyll consist of a magnesium ligand structure but have different end groups. The differences in the end groups make certain chlorophylls more or less electronegative. These differences in chemical structure is what sets the categories apart and all categories have their own unique absorption spectra. Chlorophyll-a absorbs blue and red light (Chen, 2014) and is the least electronegative and most common of the chlorophylls in photosynthetic biomass (Hooper, Eggink and Chen, 2007). The structure of chlorophyll-a is shown in figure 1.

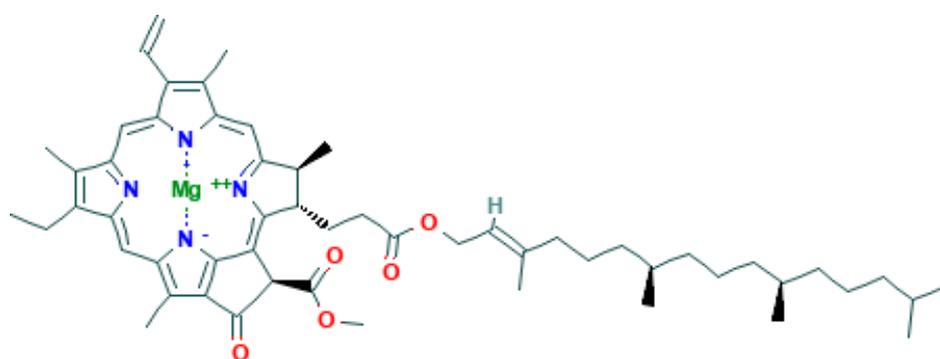


Figure 1. Chemical Structure of Chlorophyll a
(<https://pubchem.ncbi.nlm.nih.gov/compound/12085802#section=2D-Structure>)

Chlorophyll-a is the most common of the chlorophylls to measure when it comes to algae monitoring as it exists in all types of algae (Kong *et al.*, 2014). In regard to algae monitoring for the purpose of drinking-water quality, the main algae of interest is cyanobacteria (aka. blue-green algae), which is one of the many algae that contain chlorophyll-a (Rapala *et al.*, 2002). Cyanobacteria can produce toxins that can cause damage to the liver and gastrointestinal systems if swallowed. The WHO (2015) recommends that even though not all cyanobacteria produce toxins, all should be considered as toxic. Cyanobacteria can also, like any algae, cause filter clogging and prohibit coagulation in drinking-water treatment plants (WHO, 2017).

The risk of cyanobacteria in drinking-water treatment plants is when there is excessive growth, or so-called algal bloom (WHO, 2015). Blooms are a consequence of natural water circulation which impacts the temperature of the water but also of heightened levels of nitrogen and phosphorus from human activity, which cause eutrophication (Sellner, Doucette and Kirkpatrick, 2003). Climate change is also a contributing factor to algae bloom as it changes temperature patterns and therefore water patterns (Bertone, Burford and Hamilton, 2018). There are signs of this from lake Mälaren where there is an increase in run-off to the lake, causing an increase in temperature which is favorable for blooms. Each year, algae blooms occur during the summer season in lake Mälaren and it is predicted to become more frequent with climate change (Ejhed, 2020).

Monitoring of cyanobacteria can be done through measuring levels of chlorophyll-a, measuring levels total phosphorous, taking a sample under a microscope (WHO, 2015) or measuring levels of the pigment phycocyanin (Kong *et al.*, 2014). Phycocyanin is a pigment-protein that is uniquely produced by cyanobacteria and measuring it gives an indication of concentration of cyanobacteria, but not concentration of toxins that could be present (Bertone, Burford and Hamilton, 2018). Measuring chlorophyll-a will give a general measurement of total algal biomass as all photosynthetic algae contains chlorophyll-a. However, while it's a less specified indicator, this biomass also includes cyanobacteria and with that chlorophyll-a can be used as a parameter for early warning systems for algal blooms (WHO, 2015). Chlorophyll-a will hereafter only be referred to as chlorophyll.

2.2 Measuring Chlorophyll with Fluorescence

Chlorophyll can be measured with an optical fluorescence sensor (YSI, 2015). Fluorescence is a measurement of light being emitted from a molecule. All fluorescent molecules each fall into a specific emission spectrum, making it possible for quantitative and qualitative analysis. The principle of the measurement lies within the energy states of a molecule. When a molecule is met with a ray containing enough energy, it can transition from its normal orbital ground state to an excited state. The excitation itself is the absorption of the light source, which is a photon of energy exciting the electrons to a higher orbital energy state (Royer, 1995). This excitation does not last long and the molecule will quickly transition back between the energy levels to reach back to the ground state, so-called relaxation. During this transition, a photon is emitted at a certain wavelength depending on the “path” the excited molecules transition back on, see figure 2.

As the excitation and relaxation pathways are different, the photon absorbed compared to the photon released will have different wavelengths, or different energies, and that is what can be measured. In other words, a fluorescence sensor will measure the intensity of the emission (Harris, 2016). To measure chlorophyll in a water sample, the EXO2 uses a light-emitting diode (LED) to emit a blue light of 470 nm to excite the chlorophyll molecules. The emission from the excited chlorophyll is at 685 nm in the form of a red light, which is registered by the sonde (Smith, 2018).

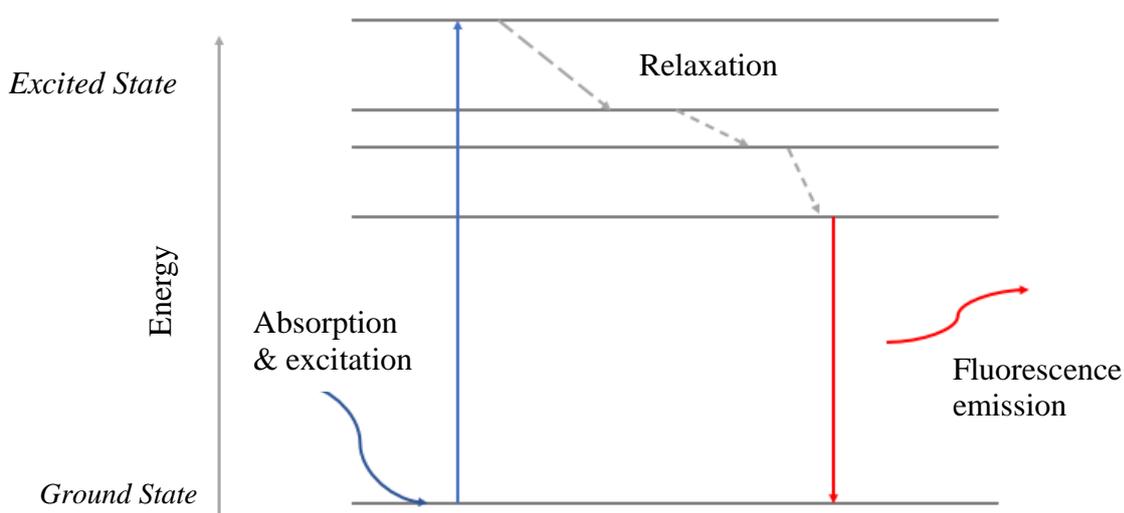


Figure 2. Fluorescence depicted through excitation and emission. Based on Harris (2016), p.446

2.3 Calibration for Chlorophyll Fluorescence

To calibrate the EXO2 for chlorophyll measurements, the red dye, Rhodamine Water Tracer (WT) (2,5%), is used as a secondary calibration standard (YSI, 2015). Like chlorophyll, Rhodamine is also a fluorescent molecule and is shown in figure 3.

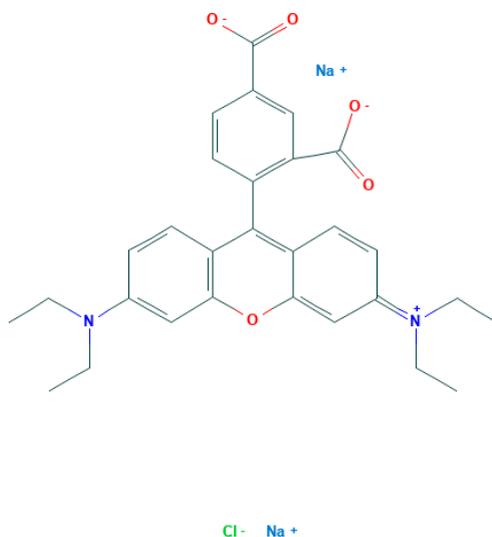


Figure 3. Chemical Structure of Rhodamine WT
(<https://pubchem.ncbi.nlm.nih.gov/compound/Rhodamine-WT#section=2D-Structure>)

Reason for using Rhodamine instead of a primary standard of the actual chlorophyll pigment is that Rhodamine WT is relatively more stable and not too costly (Smith, 2018). Rhodamine is soluble in water and a commonly used dye for fluorescent water measurements, the WT in the same even stands for “Water Tracer” (Smith, 2020). Rhodamine is easy to detect by sensors as well as falls in a unique fluorescence spectrum what covers only a select group of molecules normally found in water, narrowing the risk of interferences. The excitation spectrum is around 558 nm and emission around 582 nm se figure 4 (Wilson, Cobb and Kilpatrick, 1986). These spectra covers the same spectra of both chlorophyll and phycocyanin, where excitation is at 590 nm and emission at 685 nm, same emission as chlorophyll (Smith, 2018).

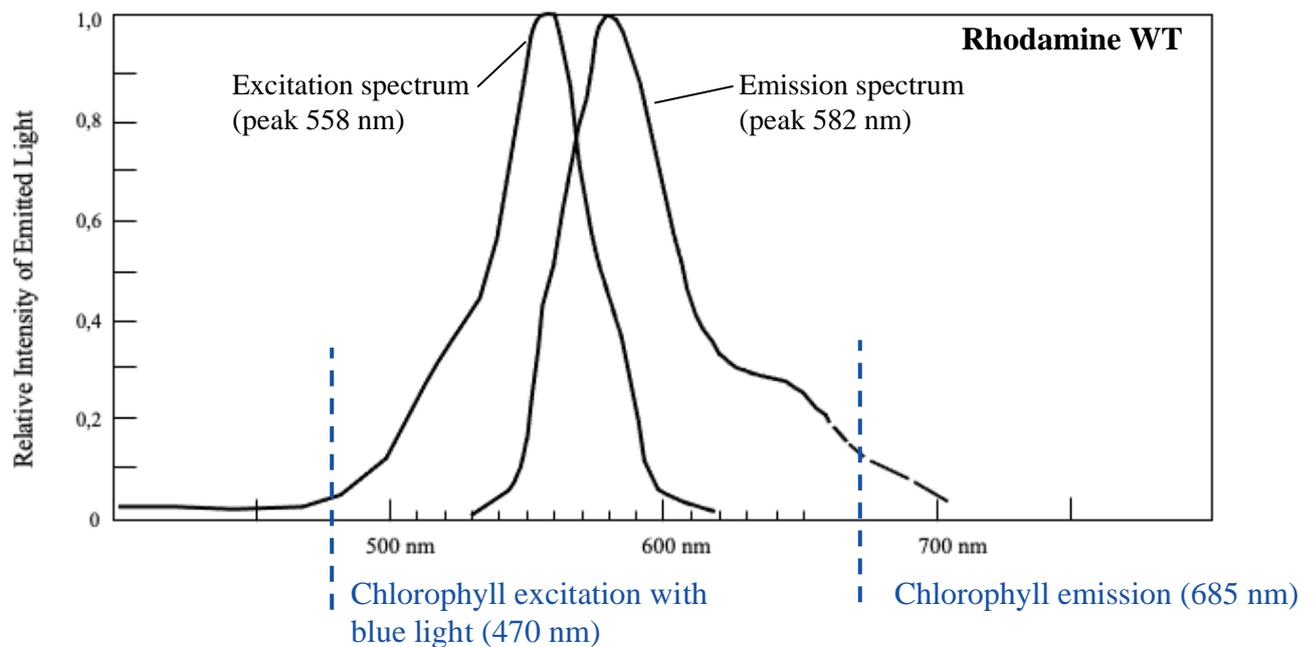


Figure 4. Excitation and emission spectrum of calibration solution, Rhodamine WT (Based on Wilson, Cobb and Kilpatrick, 1986), along with excitation and emission wavelengths for chlorophyll (Smith, 2018).

2.4 Interferences and Other Parameters

Known factors which can influence fluorescence measurements in water are turbidity, pH, quenching and so-called inner-filter effects (Brand and Johnson, 2000). Temperature has shown to change the fluidity of the membrane lipids of chloroplasts which consequently impacts fluorescence of chlorophyll inside. Reasoning behind this phenomenon is believed to be due to the hydrophilic end of the chlorophyll will have different mobility in the membrane when it's colder as the fluidity of the membrane is more crystalline (Murata and Fork, 1975). Temperature has also shown to have an inverse relationship with fluorescence, which also could be a consequence of the chlorophyll mobility, but it is said to also be due to general algae structure. In other words, as the temperature increases, the concentrations from the fluorescence readings decreases (Smith, 2018).

Turbidity can interfere with the light from the sensor as well as the light emitted from the excited molecule. The higher turbidity, the higher risk the light will scatter and interfere with the fluorescence. To mitigate this, the manufactures of the EXO2 sonde recommend to simultaneously measure the turbidity with an attached turbidity sensor as chlorophyll measurements are being made (Smith, 2018). Another interference is the Inner Filter Effect (IFE). It is similar to interference caused by turbidity, but instead of blocking the light, IFE is when other particles absorb the light, impacting the fluorescence measurement (Brand and Johnson, 2000). These particles that can absorb the emitted light from an excited molecule are called quenchers and they too can be fluorescent and give off their own emission. Therefore, it's recommended to verify measurement results with laboratory measurements as well (Smith, 2018).

Dissolved organic matter (DOM) exists in all raw water in various compositions of molecules but is mainly defined as dissolved organic carbon but also nitrogen, phosphorus and sulfur. DOM can be divided into two categories, separated by spectroscopic measurements where colored DOM (cDOM) which absorbs ultraviolet light and then there is fluorescence DOM (fDOM) which is the organic matter that is fluorescent (Hartnett, 2018). Both can be used for measuring DOM but in this project fDOM is the parameter used. To calibrate a fDOM sensor, quinine sulphate is used as its excitation wavelength is

365 nm and emission is around 480 nm, which covers the spectra for DOM. The unit for fDOM is most commonly expressed in Quinine Sulfate Units (QSU), which is defined as parts per billion (ppb) quinine sulfate (YSI, 2015).

A part from fDOM, turbidity is an essential parameter to measure as it can interfere with the chlorophyll measurements (Smith, 2018). Turbidity is a measurement of how light scatters due to suspended particles, in other words, it's a measurement of the transparency of the water (WHO, 2017). Turbidity is usually defined in formazine nephelometric units (FNU) and drinking-water in Sweden has a limit of 0,5 FNU, above that is no longer considered as potable water (Livsmedelsverket, 2017). A sensor can be calibrated by using a formazine standard solution but also polymer-based solutions are being used (YSI, 2015). Furthermore, measuring the water's pH is also important where NaHCO_3 is commonly used as a pH buffer (PubChem, no date). The EXO2 sensor uses an pH electrode containing a Ag/AgCl reference electrode and a glass electrode to measure the difference in potential (YSI, 2015).

3 Methodology

The study took 10 weeks and was approached from three different fronts: a literature review, on-site data collection and data analysis. All three components were addressed during a regular week where three days would be spent on literature review and data analysis and the remaining days would be for on-site data collection. The literature review consisted of a desk study based on evaluating scientific articles, published reports and technical briefs on research pertaining to algae monitoring, sensor optimization, validation of analysis and drinking-water quality. The research site for data collection took place at Norrvatten's DWTP, Görvåln. It is located in Järfälla municipality, right next to lake Mälaren, where the raw water samples were taken from. Data collection took place once to twice a week for five weeks. All collected data were quantitative measurements using the YSI EXO2 water quality optical sonde, for more details see 3.1 Data Collection and Materials.

The first research question pertains to the detection and quantification of algae cells using the sonde as well as the reliability and validity of the measurements. As the sonde does not quantify number of cells per se, the concentration of chlorophyll is measured as a proxy parameter. To test the validity of the chlorophyll measurements [$\mu\text{g/l}$], water samples were sent to an external laboratory, Eurofins, to confirm the concentration. Furthermore, the measurements were also compared to Norrvatten's own laboratory results where cell density [10^6cells/l] was counted. To test if the EXO2 data can act as an early warning system, the collected data was also uploaded to Norrvatten's data management system aCurve (Germit Solutions, Sweden) where alerts were put in place for when the chlorophyll concentrations in the raw water would reach certain limits.

The second research question focuses on the frequency at which the sonde needs to be calibrated. Calibration frequency is defined through the use of reference solutions. The intended purpose of the solutions is to act as verification of the sonde's calibration settings by simply measuring the chlorophyll concentration and comparing it to a standard solution, aka reference solution. The calibration frequency will then be defined by how long these reference solutions can be stable for, in other words, how long until the change in chlorophyll concentrations become statistically significant. To further explore how and why the reference solutions change over time, a full factorial experiment was done, for more details, see section Data Collection and Material.

3.1 Data collection and Materials

Data collection was divided into three parts;

1. Measuring reference solutions over time to evaluate the stability of the solutions;
2. A full factorial experiment to see which variable has the largest effect on the measurement of chlorophyll concentrations;
3. Measuring chlorophyll concentrations in raw water at the Görvåln DWTP;

The materials used for this study are listed in appendix I. The main analysis instrument was the EXO2 (YSI EXO2 599502-00, USA) which is a multiparameter sonde that can simultaneously measure pH, temperature, turbidity, chlorophyll and other water quality parameters. Measurement data is automatically stored in the sonde and can periodically be transferred in a CSV-file to a computer via Bluetooth for further interpretation and analysis (YSI, 2015). Figure 5 shows the EXO2 with the protective casing at the end. Underneath the casing are seven available ports to place different sensors, along with a wiper that can clean the tips of all the sensors. The sensor is roughly 70 cm long, with a 7,6 cm diameter and weights 3,6 kg.



Figure 5. EXO2 Multiparameter Sonde (YSI, 2021)

The EXO2 sonde that was used for this project was calibrated by Norrvatten in accordance with the user manual where 5 ml of the Rhodamine WT (2,5%) solution was taken out and filled to 1000 ml with distilled water, giving a concentration of 125 mg/L. In turn, 5 ml of this solution was taken out and filled to 1000 ml, giving a solution of 0,625 mg/L. This solution was then used for a calibration of chlorophyll [$\mu\text{g/l}$] using the relationship in table 1 (YSI, 2015).

Table 1. Temperature's [$^{\circ}\text{C}$] relationship to chlorophyll measurements [$\mu\text{g/l}$] at a rhodamine concentration at 0,625 mg/l.

Solution Temperature [$^{\circ}\text{C}$]	30	28	26	24	22	20	18	16	14	12	10	8
(Rhodamine 0,625 mg/l) Chlorophyll [$\mu\text{g/l}$]	56,5	58,7	61,3	63,5	66	68,4	70,8	73,5	76	78,6	81,2	83,8

3.1.1 Reference Solutions

The purpose of the reference solutions is to act as a calibration check for the EXO2 sonde and reason for testing them over time is to see how long a solution can be stored for. During the first week of data collection, three reference solutions were prepared and labeled REF 1, REF 2 and REF 3. The solutions contained different levels of rhodamine, bentonite, NaHCO_3 , Coca-Cola and distilled water. Rhodamine was for chlorophyll measurements, bentonite for turbidity, NaHCO_3 as a pH buffer and then to mimic fDOM in, decarbonated Coca-Cola was used (Köhler, 2021). For details see table 2.

Table 2. Composition of the three reference solutions (REF 1, 2, and 3).

Solution	Coca-Cola (diluted 0,2 times)	NaHCO_3 (1 M)	Bentonite (5 g/l)	Rhodamine (180 $\mu\text{g/l}$)	Distilled water	Total volume
REF 1	100 ml	30 ml	5 ml	75 ml	790 ml	1000 ml
REF 2	100 ml	10 ml	20 ml	75 ml	795 ml	1000 ml
REF 3	300 ml	30 ml	0 ml	75 ml	595 ml	1000 ml

3.1.2 Preparing and measuring the reference solutions

During the first week of data collection, 2000 ml of each reference solution was prepared. During the course of data collection phase, these bottles were stored in a refrigerator at 3°C and taken out once a week for measurements. Figure 6 shows the three reference solutions (REF 1, 2 and 3) as well as their ingredients.

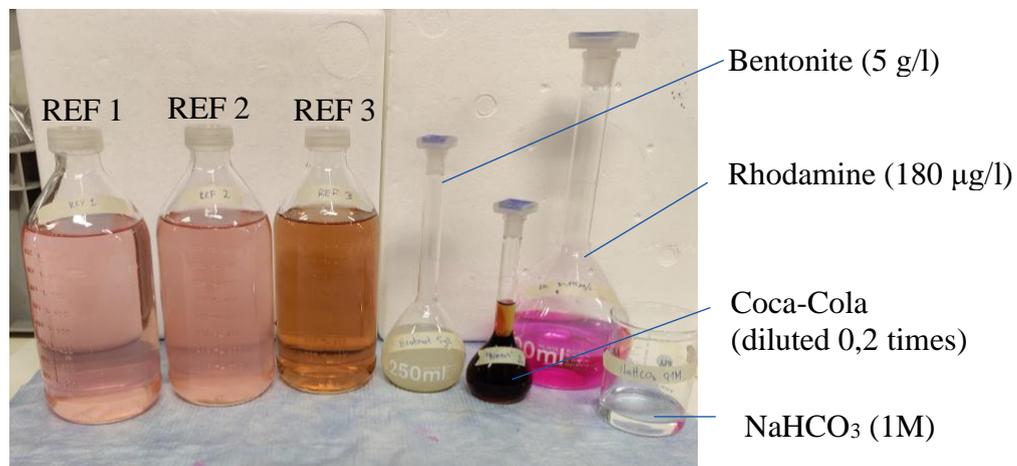


Figure 6. Reference solutions (REF 1, 2, and 3) were prepared with different concentrations of bentonite for turbidity, Coca-Cola for fDOM and NaHCO₃ as a pH buffer. Levels of rhodamine were constant in the three solutions for chlorophyll measurements.

The EXO2 was hung up by rope and each sample was measured using the sonde's own calibration cup. Reason for this is the calibration cup has a blue tint and may block out some of the light in comparison to a transparent, colorless beaker. Before each measurement, the sensors on the sonde and calibration cup were rinsed with distilled water and the sensors were wiped clean by the sonde's own brush system. A magnet was placed inside the protective casing and the sonde was placed over a stirrer, see figure 7 for the setup.

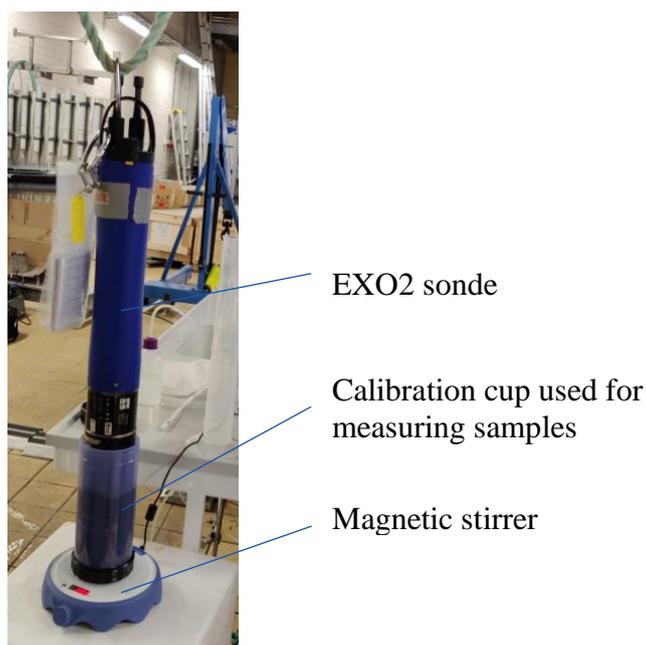


Figure 7. Measurement set-up with the EXO2 sonde, its blue calibration cup for samples and a magnetic stirrer.

The calibration cup was rinsed with the reference solution twice before the reference solution was poured in to the first line on the cup, which is roughly 400 ml. The magnet stirrer was set to 300 rpm and the sonde was left for one minute for the sensors to adjust to the sample before recording the data. For each reference solution, 15 measurements were recorded, taken at one second apart.

Each week, a new reference solution was prepared in the same way as the original solutions and labeled REF 1*, REF 2* and REF 3*. Measurements of these solutions was conducted using the same method as the original solutions made the first week of data collection. The data of both the original reference solutions and the weekly solutions were compared with each other to check the stability of the chlorophyll concentration over time and to see how the solution changes, see section 4 Results.

3.1.3 Full Factorial Experiment

To assess which variables have the largest effect on the measurements of the chlorophyll concentrations, a full factorial experiment was conducted. The purpose of a full factorial experiment is to evaluate selected variables individually as well as combined to see if there is an effect and how large it is. The outcome in this experiment is the concentration of chlorophyll. The selected variables are fDOM, pH and turbidity. The different trials, or sample solutions, consisted of high and low levels of these three variables, while the concentration of chlorophyll was kept constant in all trials. All four parameters were measured simultaneously using the same measuring method as for the reference solutions, se description above. As there were three variables with two levels, it's was a so-called 2^3 full factorial experimental design, meaning eight trials were needed as $2^3= 8$ (Blomqvist, 2017). The variables were fDOM, pH and turbidity and the eight trial solutions were based on the same “recipe” as the reference solutions where chlorophyll was measured to 75 ml of rhodamine and low/high levels of the variables were added and filled to 1000 ml with distilled water. Table 3 specifies the volume of each variable and level. Each trial sample was prepared and immediately measured and all samples were measured 14 times.

Table 3. List of variables and the volumes defining their low and high level.

Variable	Low level (-1)	High level (1)
A: fDOM	100 ml	300 ml
B: pH	10 ml	30 ml
C: Turbidity	5 ml	20 ml

The design matrix is presented in table 4, where “-1” signifies a low level of a variable and “1” a high level. With three variables at two levels, all combinations are covered in these eight trials.

Table 4. Design Matrix for the Full Factorial Experiment detailing levels for each trial (low -1 and high 1).

Trial	A	B	C
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1

3.1.4 Raw Water Sampling and Measurements

The raw water data was collected once a week where the EXO2 was submerged in the water overnight, recording chlorophyll concentrations every 20 minutes. The measurement site is called PP100 and is placed after a primary filtration of the raw water but it is still considered as raw water. The water samples were taken the following day, at the same location. Using two sample bottles of 500 ml each, water was collected from a tap pouring from the PP100 point, se figure 8.

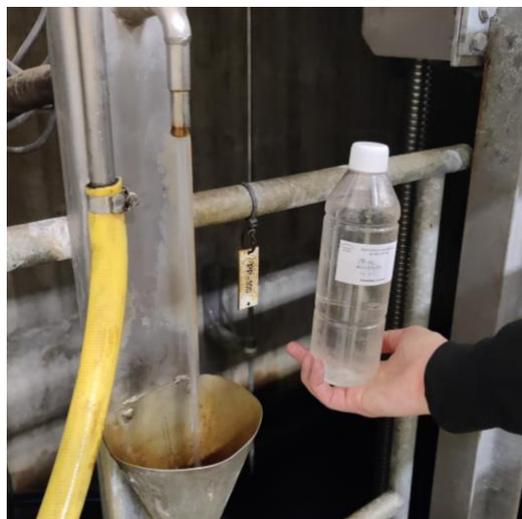


Figure 8. Sample bottle of 500 ml at sample collection site called PP100. Raw water coming out the tap for collecting raw water.

Each sample bottle was rinsed with the sample water twice before filling up the bottle. Immediately after sampling, the 1000 ml of the sample was measured out in a volumetric flask. Three milliliters of 1% $\text{MgCaO}_3\text{Mg}(\text{OH})_2$ solution was added to the 1000 ml sample. The water sample was then filtered through a suction filtration apparatus using a 47 mm filter (Whatman GF/C, VWR 513-5227) and a handheld pump, se figure 9. The filter was then removed from the filter head using plastic tweezers and folded once in the middle with the filtered particles on the inside. To remove excess water the folded filter was first placed on paper towels followed by a being placed in a plastic container containing silica beads. The sample was frozen until shipped to the Eurofins laboratory where the levels of chlorophyll would be measured in accordance with SS 028146-1. In this standardized spectrophotometric method, chlorophyll is first extracted with acetone followed by absorbance measurements at 664, 647 and 630 nm (SIS, 2004).



Figure 9. Suction filtration apparatus with handheld pump used to filter raw water samples to collect biomass on filter paper.

3.1.5 UV measurement of chlorophyll sample

The purpose of adding $\text{MgCaO}_3\text{Mg}(\text{OH})_2$ solution to the raw water sample is to stabilize the Mg-ligand of the chlorophyll molecules. To test the difference in stability, a quick UV-measurement was conducted. Two samples from PP100 were taken where one sample was processed as described above with adding three milliliters of $\text{MgCaO}_3\text{Mg}(\text{OH})_2$ solution, and the other sample was processed the same way but without the added solution. Each sample was run through a UV spectrometer (PerkinElmer LAMBDA 365, USA) at 254 nm at 1000 RPM for 400 seconds.

3.2 Data Processing and Analysis

Measurements collected at PP100 were stored in the EXO2 until transferred to a laptop via Bluetooth. The data first appears in the sonde's software, KorEXO, but can be downloaded as a CSV. file. All raw data files were transferred directly into the data management software, aCurve. A copy of the data was also made for analysis in Microsoft Excel. To make the files more manageable in Excel, the files were trimmed leaving only data of the following list:

- Date (MM/DD/YYYY)
- Time (HH:MM:SS)
- Site Name
- Chlorophyll ($\mu\text{g/l}$)
- fDOM (QSU)

- Turbidity (FNU)
- pH
- Temperature (°C)

All collected data was compiled in Excel where statistical calculations (see section 3.2.2) were conducted as well as compared graphically (see section 3.2.3) to identify patterns and potential correlations between the collected data of the parameters listed above.

3.2.1 Temperature and turbidity correction

The raw data of Chlorophyll and fDOM measurements were then processed in Excel with correction factors to account for temperature, turbidity and IFE. The correction factors and equations are based on previous research and empirical data (Köhler, 2021). Equation 1 shows the temperature correction for the chlorophyll measurements. Equations 2 corrects for fDOM absorbance which occurs at 254 nm. Equation 3 and 4 accounts for the temperature as well as the turbidity of the fDOM readings. Finally, equation 5 corrects for possible IFE, resulting in the final fDOM value used to data analysis. The reference temperature ($Temp_{Ref}$) is set to 25°C.

$$Chlorophyll_{Temp} = Chlorophyll_{EXO} + Chlorophyll_{EXO} \cdot 0,015(Temp_{EXO} - Temp_{Ref}) \quad (Eq. 1)$$

$$fDOM_{A254} = 1 + \frac{fDOM_{EXO}}{3,5} \quad (Eq. 2)$$

$$fDOM_{Temp} = fDOM_{EXO} + fDOM_{EXO} \cdot 0,012(Temp_{EXO} - Temp_{Ref}) \quad (Eq. 3)$$

$$fDOM_{Temp \& Turb} = \frac{fDOM_{Temp}}{0,7225 \cdot e^{-0,004687 \cdot Turbidity_{EXO}}} + 0,3041 \cdot e^{-0,0003624 \cdot Turbidity_{EXO}} \quad (Eq. 4)$$

$$fDOM_{Temp, turb \& IFE} = fDOM_{Temp \& Turb} + fDOM_{Temp \& Turb} \cdot 0,2508 \cdot \frac{fDOM_{A254}}{100} \quad (Eq. 5)$$

3.2.2 Statistical analysis of collected data

The purpose of the reference solutions was to see how long they can be stored for if they are stored in a dark refrigerator. Original reference solutions (REF, 1 2 and 3) were created first week of data collection and tested once a week for five weeks. They were also compared to newly made reference solutions (REF 1*, 2* and 3*). The hypothesis is a statistical analysis can be used to evaluate when the chlorophyll concentrations deviate too much from each other which indicates either the stability of the solutions is questionable or the EXO2 needs to be recalibrated. To statistically evaluate this, a F-test and a t-test were conducted. An F-test compares standard deviations between two measurements and a t-test is to see when the standard deviations become significantly different. The F-test was only done on the measurements that showed the greatest standard deviation in comparison to the other measurements. The F-test is based on two values, $F_{calculated}$ and F_{table} . If $F_{calculated} > F_{table}$ then the difference in measurements is significant and the measurement should be rejected. Equation 6 shows how $F_{calculated}$ was calculated. All statistical equations are from Harris (2016).

$$F_{calculated} = \frac{s_1^2}{s_2^2} \quad (Eq. 6)$$

In order to calculate $F_{calculated}$, one must first calculate the standard deviation, s . The formula “=STDEV” was used in Excel to where the equation 7 is used to calculate the standard deviation. Equation 8 shows the pooled standard deviation which is a term for a set of several standard deviations pooled together. The s_{pooled} was used in the F-test to compare one measurement with the rest of the set.

$$s_x = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}} \quad (\text{Eq. 7})$$

$$s_{\text{pooled}}^2 = \frac{s_1^2 + s_2^2 + s_3^2 + \dots}{N_{\text{set}}} \quad (\text{Eq. 8})$$

To get the F_{table} value, the formula “=F.INV.RT” was used with a 95% confidence interval and the corresponding degree of freedom to the data being analyzed. The Degrees of freedom is defined by equation 9.

$$\text{Degrees of freedom} = n_1 + n_2 + n_3 + \dots - N_{\text{set}} \quad (\text{Eq. 9})$$

As for the t-test, if $t_{\text{calculated}} > t_{\text{table}}$ it means the change is significant with a 95% confidence interval. For the t-test, equation 10 was used, along with equation 8 for s_{pooled} .

$$t_{\text{calculated}} = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{\text{pooled}}} \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}} \quad (\text{Eq. 10})$$

To evaluate the precision of the measurements, equation 11 was used. This equation accounts for errors by rejecting the 2,5th percentile, and putting the 95th percentile in relation to the median. This resulting in an understanding of the variance in the measurements.

$$95\% \text{ within range of the median} = \frac{(97^{\text{th}} \text{percentile} - 2,5^{\text{th}} \text{percentile})}{\text{Median}} \quad (\text{Eq. 11})$$

3.2.3 Calculations for the full factorial experiment

The purpose of a full factorial experiment is to determine the effects of the variables of interest. To calculate the main effects, β_1 , β_2 , and β_3 , as well as the interaction effects β_4 - β_7 , equation 12 was used where the +/- was determined on whether it was a high (+) or low (-) level and m= number of trails, which is eight.

$$\beta_i = \frac{\pm \bar{y}_1 \pm \bar{y}_2 \pm \bar{y}_3 \dots \pm \bar{y}_7}{m} \quad (\text{Eq. 12})$$

As there are multiple measurements, a pooled standard deviation was calculated using equation 8. A 95% confidence interval was calculated using equation 13.

$$\bar{x} \pm \frac{ts}{\sqrt{n}} \quad (\text{Eq.13})$$

3.2.4 Graphical comparison of collected data

In order to compare the EXO2 chlorophyll measurements in the raw water from the EXO2 with the results from the Eurofins laboratory, the data was first tabulated where differences and quotients were calculated. The data was then graphically compared doing a geometric mean regression (GMREG). The method is grounded in a cross-comparison where the variables on the x-axis are seen as independent and the variables on the y-axis as dependent. Two graphs were made titled adaptation 1 and 2 where the adaptation 1 had EXO2 results was on the x-axis and the Eurofins results on the y-axis and vice versa for adaptation 2. Using these two graphs, a combined linear relation could be calculated in the form of a

geometric mean regression slope (GMS) and geometric mean intercept (GMI) could be calculated using equation 14 and 15.

$$GMS = \left(\frac{k_{\text{adaptation 1}}}{k_{\text{adaptation 2}}} \right)^{1/2} \quad (\text{Eq.14})$$

$$GMI = \bar{y}_{\text{Eurofins}} - GMS(\bar{x}_{\text{EXO}}) \quad (\text{Eq. 15})$$

Using the GMS and GMI, a GMREG line can be defined by the equation 16 and was applied to the data from adaptation 1, for full GMREG graph, see section 4 Results.

$$GMREG = GMS \cdot x + GMI \quad (\text{Eq.16})$$

To compare the EXO2 measurements with the cell count from the laboratory, a t-test (Eq. 10) was done to compare the difference with a 95% confidence interval. The results were then put in a Log-Log plot to identify a potential correlation.

3.3 Methods of Selection

The location for the raw water sample, PP100, is the same location where Norrvatten's laboratory take their samples for algae cell count where they count number of cells under a microscope. As chlorophyll measurements were to be compared with laboratory results, PP100 therefore seemed like most appropriate location. The data collection (see detailed list in section 3.1.) took place once or twice a week for five weeks and these were weeks 14-18 (April 5-May 7, 2021). The weeks for data collection were selected in regard to the timeline for algae bloom as it usually starts around April-May in lake Mälaren (Basiak-Klingspetz, 2021). Then the compositions of the reference solutions were put forward by Norrvatten to mimic relevant parameters of the raw water.

3.4 Reliability and Validity

Validation of the PP100 measurements was done through sending a filter sample to the Eurofins laboratory in accordance with the Swedish Standard, SS 028146-1. The results took roughly 3 business days after arrival to the lab and the chlorophyll concentration was defined in µg/l. To determine the reliability of the collected data, a series of statistical calculations were conducted in Excel, using a 95% confidence interval. For list of calculations, see section Statistical Analysis.

3.5 Ethical Considerations

The project took place during the COVID-19 pandemic, which meant following the recommendations put forth by the Public Health Agency of Sweden as well as Norrvatten's own COVID-19 protocol. Compliance was of utter importance as data collection took place at the Görväln plant where they produce drinking-water for nearly 700 000 people (Norrvatten, no date). Being on-site also meant access to sensitive information regarding the plant and in certain areas photographs were not allowed to be taken. This was taken into consideration when photographing the data collection process and all photos have been approved by Norrvatten.

3.6 Limitations of the Study

The project is framed around chlorophyll measurements using the EXO2 sonde. All water sample data is location specific and limited to PP100 at the Görvåln plant. The data is also limited to the specific EXO2 sonde that was used during the project. If this project were to be replicated using another EXO2 sonde, it would preferably be calibrated the same way and measurement data would be compared to see that the signal is the same between the sondes.

The scope of the measurements was limited to investigate

1. How the chlorophyll concentration relates to number of algae cells;
2. How reliable the chlorophyll measurements are;
3. What factors impact the chlorophyll measurements the most and;
4. How often does a calibration solution need to be prepared.

4 Results

In the section, the results from the reference solutions and the full factorial experiment will be presented along with the evaluation of measurement accuracy, as well as the graphical comparisons between EXO2 with Eurofins and algae cell count. The chlorophyll and fDOM were corrected in accordance with equations 1-5 prior to any statistical calculations. All statistical calculations can be found in Appendix II and table 5 summaries the samples which have been studied along with their purpose and how the data was analyzed.

Table 5. List of all data collected, which parameters were recorded, during what time period as well as the purpose of the measurements and the method of data analysis.

Samples	Parameters recorded	Time period	Purpose of data	Method of data analysis	Results found in section
Reference solutions	Chlorophyll ($\mu\text{g/l}$) fDOM (QSU) Turbidity (FNU) pH Temperature ($^{\circ}\text{C}$)	5 weeks with measurements once a week.	Evaluate when the chlorophyll concentrations deviate too much from each other.	F-test and t-test (eq. 6-10)	4.1
			Evaluate the precision of the measurements.	Comparing the 95 th percentile in relation to the median (eq. 11)	4.2
Solutions for full factorial experiments	Chlorophyll ($\mu\text{g/l}$) Temperature ($^{\circ}\text{C}$)	Data for the full factorial experiment was collected in one day.	Evaluate which variable has the largest effect on the measurement of chlorophyll concentrations.	Calculating effect size and error bars (eq. 12, 13)	4.3
Raw water	Chlorophyll ($\mu\text{g/l}$) Temperature ($^{\circ}\text{C}$)	5 weeks with measurements once a week.	Evaluate accuracy of EXO2 data by comparing EXO2 data with laboratory results.	Geometric mean regression (eq. 14,15,16)	4.4
			Identify correlation between EXO2 data on Chlorophyll with number of algae cells.	Log-Log plot and t-test (eq. 10)	4.5
		Measured once using the UV-spectrometer	Test the stability of chlorophyll from the added MgCaO_3 solution.	Percentage difference	4.6

4.1 Reference Solutions

Chlorophyll, fDOM, turbidity and pH were observed over five weeks. Measurements were taken for all three reference solutions (REF 1, 2, and 3) along with the newly created solutions (REF 1*, 2*, and 3*). In REF 1, chlorophyll did not remain stable over time, as is shown in figure 10. The level of turbidity and pH also changed over time where pH steadily decreased by each week (Fig. 13) and turbidity fluctuated (Fig. 12). The only parameter that remained stable in comparison to the new weekly reference solution (REF 1*) was fDOM. The fDOM level dipped in the first week and then flattened out, see figure 11. Statistical calculations (Eq. 6-10) show that there is significant change (95% confidence) to the levels of chlorophyll already after one week meaning REF 1 cannot be stored over time. Measurement points that are statistically significant are marked with an asterisk, *.

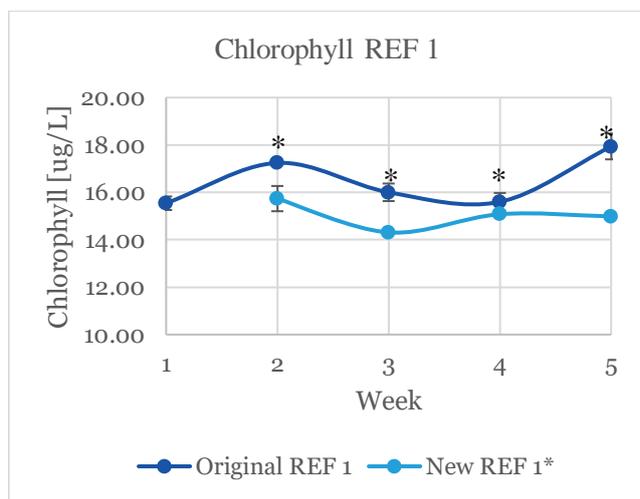


Figure 10. Chlorophyll concentration [µg/l] in solution REF 1 where points marked with * are statistically significant. Original REF 1 was prepared the first week of data collection and a new solution was prepared each week, labeled New REF1* . The concentration of chlorophyll fluctuates over time indicating the chlorophyll is not stable.

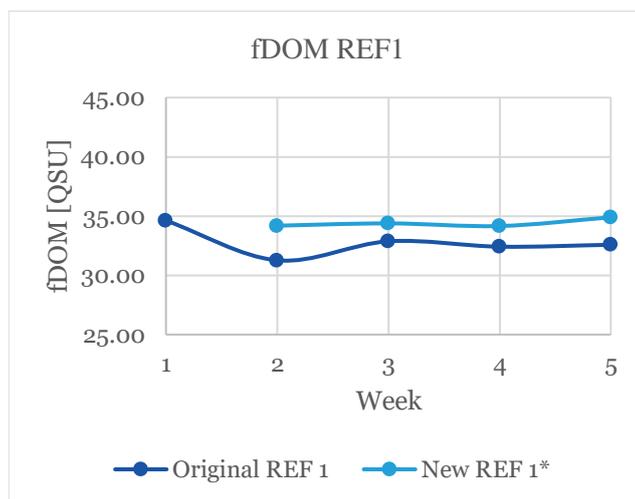


Figure 11. fDOM [QSU] in REF 1. Original REF 1 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF1* . The level of fDOM remains fairly constant over the weeks indicating this parameter can remain stable for four weeks.

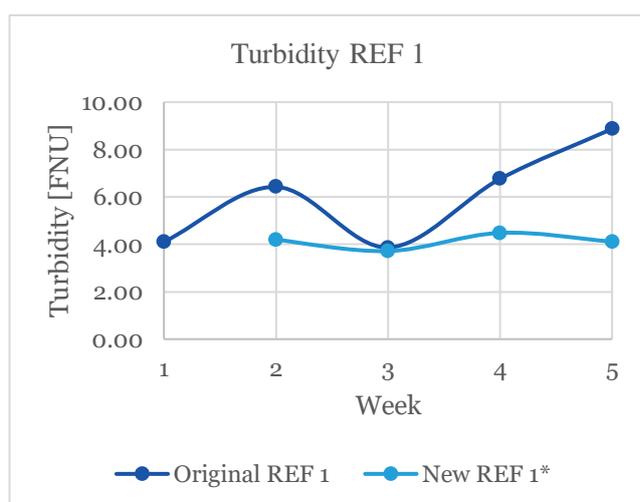


Figure 12. Turbidity [FNU] in REF 1. Original REF 1 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF1* . The turbidity fluctuates in the original solution, indicating it is not stable.

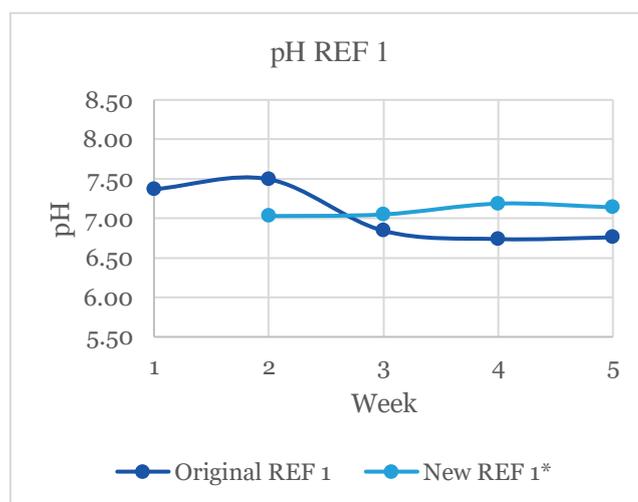


Figure 13. pH in REF 1. Original REF 1 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF1* . The pH drops after week 2, then stabilizes.

In REF 2, the chlorophyll levels remain stable from week 1 to week 3 before it starts decreasing (Fig. 14). Statistical testing (Eq. 6-10) shows that REF 2 can be stored for two weeks (week 1-3 was compared) without compromising the stability of the chlorophyll levels (95% confidence). Similar to REF 1, the fDOM measurements in REF 2 dip the first week then somewhat stabilize (Fig. 15). Turbidity levels fluctuate but it is not until week 4 the level rises (Fig. 16). The pH level in REF 2 steadily started to decrease after one week (Fig.17).

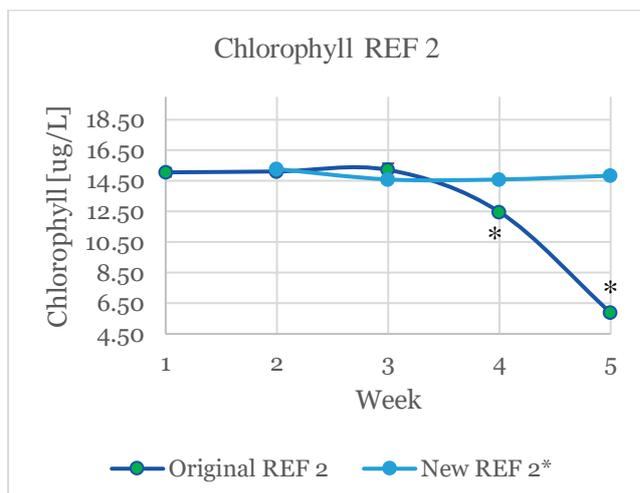


Figure 14. Chlorophyll concentration [µg/l] in REF 2 where points marked with * are statistically significant. Original REF 2 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF2*. The chlorophyll concentration remains steady until week three where the change becomes significant, marked by a *.

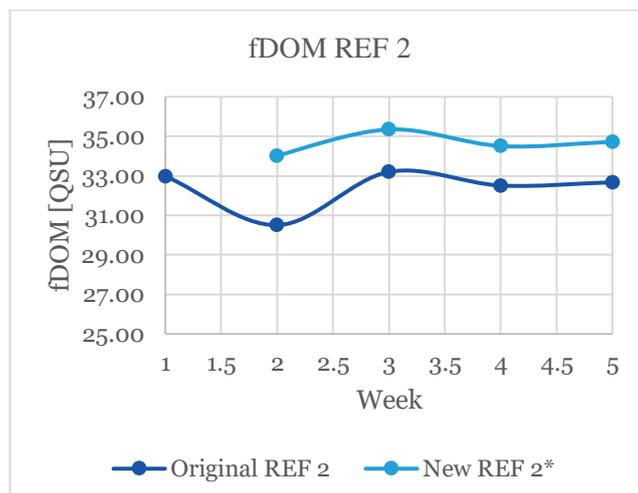


Figure 14. fDOM [QSU] in REF 2. Original REF 2 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF2*. Comparing the two solutions, they seem to follow the same pattern but with a slight difference of 1-2 units of measure.

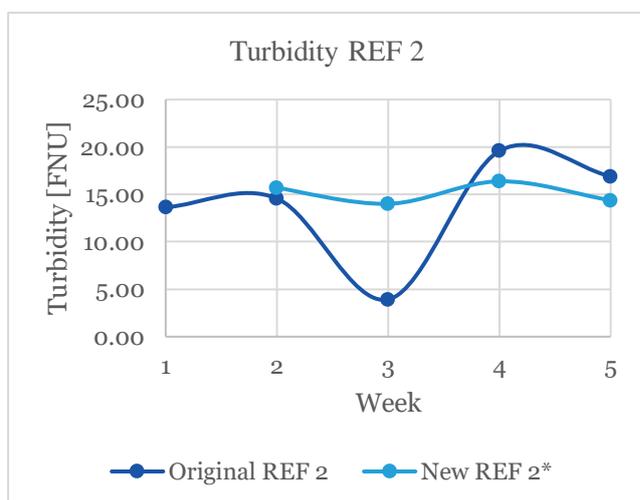


Figure 15. Turbidity [FNU] in REF 2. Original REF 2 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF2*. The turbidity drops at week 3, then rises above starting level. This could mean the Bentonite solution wasn't mixed well enough or there is something causing the fluctuation.

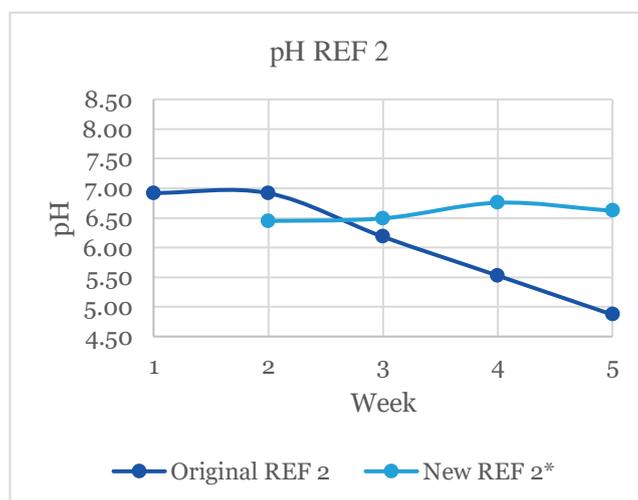


Figure 17. pH in REF 2. Original REF 2 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF2*. The pH remains the same for one week, followed by a steady decrease by each week.

The third solution, REF 3, shows a different pattern compared to REF 1 and 2. The levels of chlorophyll increase by week 3 before there is a large dip in the concentration (Fig. 18). Levels of fDOM and turbidity are stable for almost three weeks until both drastically increase from week 4 to week 5 (Fig. 19 and 20). The pH levels steadily decline by each week for this solution as well (Fig. 21). According to a statistical F- and t-test (Eq. 6-10), REF 3 cannot be stored without the chlorophyll levels changing significantly after one week (95% confidence interval). However, if the acceptance level is $\pm 10\%$ of the median for week 1, then REF 3 can be stored for one week, see calculation below.

Median(REF 3, Week 1)=14,5 \pm 1,45

Week 1: 14,5 $\mu\text{g/l}$ (falls within the 10%)

Week 2: 13,9 $\mu\text{g/l}$ (falls within the 10%)

Week 3: 18,4 $\mu\text{g/l}$ (does not fall within the 10%)

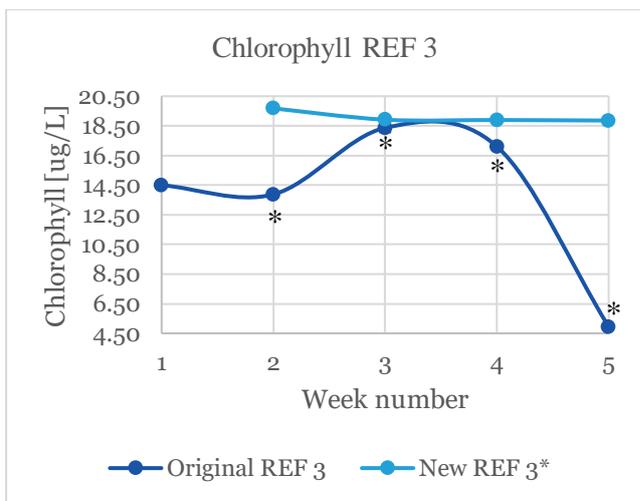


Figure 18. Chlorophyll concentration [$\mu\text{g/l}$] in REF 3 where points marked with * are statistically significant. Original REF 3 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF3*. The chlorophyll concentration changes significantly already at week 2.

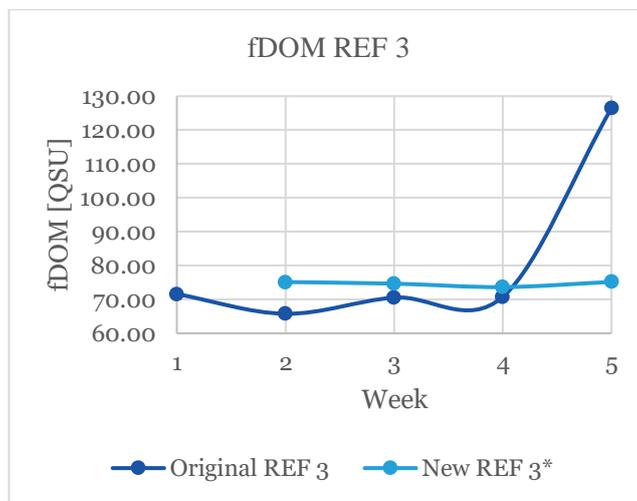


Figure 19. fDOM [QSU] in REF 3. Original REF 3 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF3*. The level of fDOM remains fairly unchanged until week 4 where there is an increase.

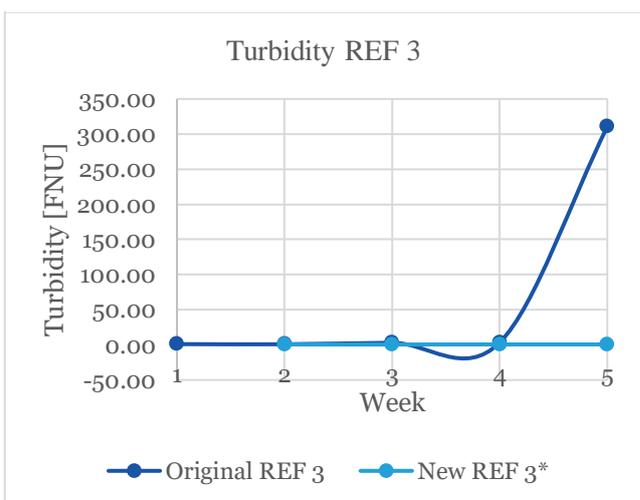


Figure 20. Turbidity [FNU] in REF 3. Original REF 3 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF3*. Similar to figure 20, the turbidity remains fairly unchanged until week 4 where there is an increase. As figure 20 and 21 follow a similar pattern, it indicates there is a correlation between them.

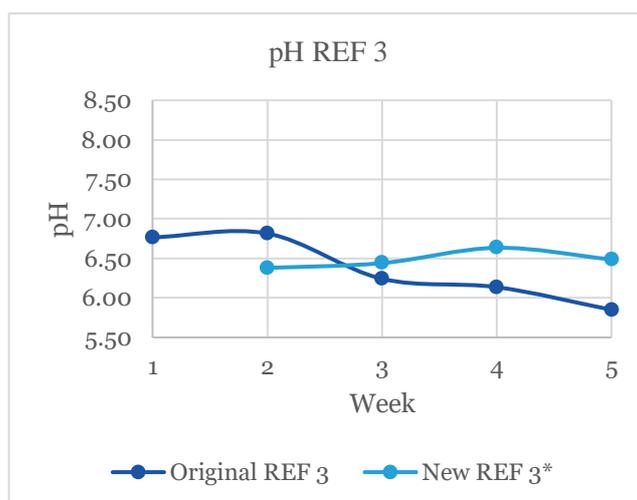


Figure 21. pH in REF 3. Original REF 3 was a solution prepared the first week of data collection and a new solution was prepared each week, labeled New REF3*. The pH level remains the same until week 2, followed by a steady decline each week.

4.2 Precision of Measurements

The precision of the measurements were calculated using equation 11, where the 95th percentile is compared the median. The percentages in table 6 show by how much percent in each direction of the median lies 95% of the sample. The precision of chlorophyll measurements seem to fluctuate, especially for the original REF-solutions. The weekly-made solutions (REF*) however, have a much higher precision in general compared to the original solutions, supporting the previous results in that the original solutions cannot be stored maximum two weeks.

Table 6. How much (%) of the 95th percentile is within the median

REF 1			REF 2		REF 3	
Week	fDOM	Chlorophyll	fDOM	Chlorophyll	fDOM	Chlorophyll
1	0,22%	5,73%	0,45%	6,32%	0,33%	2,50%
2	0,22%	0,89%	0,33%	1,39%	0,27%	1,35%
3	0,11%	6,58%	0,25%	10,44%	0,06%	4,93%
4	0,09%	8,30%	0,35%	2,05%	0,11%	1,38%
5	0,11%	10,60%	0,09%	3,72%	0,36%	6,77%
Week	REF 1*		REF 2*		REF 3*	
	fDOM	Chlorophyll	fDOM	Chlorophyll	fDOM	Chlorophyll
2	0,18%	1,37%	0,27%	1,43%	0,09%	0,62%
3	0,21%	11,34%	0,27%	1,96%	0,09%	0,79%
4	0,24%	1,39%	0,19%	0,90%	0,10%	1,11%
5	0,20%	1,12%	0,31%	0,69%	0,08%	0,62%

4.3 Full Factorial Experiment

The full factorial experiment shows that all three variables (i.e., fDOM, pH and turbidity) have a significant effect (β_i) on the outcome of the chlorophyll measurements to various degrees. They all contribute to interaction effects as well. Table 7 shows the full design matrix along with averaged Chlorophyll levels (n=17) and standard deviation for each trial.

Table 7. Design Matrix for full factorial experiment with averaged outcome (chlorophyll concentration) and the standard deviation for each trial. The levels are marked as -1 for low and 1 for high, for details, see table 3. The calculated effects (β_i) are presented under each variable.

Trial	A (fDOM)	B (pH)	C (Turbidity)	AB	AC	BC	ABC	\bar{y}_i [$\mu\text{g}/\text{l}$]	s_i
1	-1	-1	-1	1	1	1	-1	22,3	0,181
2	1	-1	-1	-1	-1	1	1	20,2	0,061
3	-1	1	-1	-1	1	-1	1	16,6	0,122
4	1	1	-1	1	-1	-1	-1	20,0	0,077
5	-1	-1	1	1	-1	-1	1	14,8	0,071
6	1	-1	1	-1	1	-1	-1	19,0	0,080
7	-1	1	1	-1	-1	1	-1	14,9	0,039
8	1	1	1	1	1	1	1	19,5	0,042
Effect	β_1	β_2	β_3	β_4	β_5	β_6	β_7	18,41	0,08
Size of effect	1,25	-0,66	-1,36	0,73	0,92	0,80	-0,63		

As the concentration of Rhodamine was the same in all trial samples but average concentration (\bar{y}_i) varies from 14,9 up to 22,3 [μg of chlorophyll/l] indicates the variables are causing some effect to the results. The effects β_1 and β_3 are the largest in size meaning they have the greatest effect on the outcome.

Figure 22 shows the size of each effect (β_i) and if it impacts the outcome positively or negatively. The error bars ($0 \pm 0,046$, 95% confidence, see appendix II) are relatively small in comparison to all the effects meaning all effects are significant. Each effect (β_i) is listed below:

- β_1 -Effect from fDOM
- β_2 -Effect from pH
- β_3 - Effect from turbidity
- β_4 - Interaction effect from fDOM and pH
- β_5 - Interaction effect from fDOM and turbidity
- β_6 - Interaction effect from pH and turbidity
- β_7 - Interaction effect from fDOM, pH and turbidity

Effect β_2 along with the interaction factors, β_4 , β_5 , β_6 and β_7 are smaller compared to β_1 and β_3 and can thus be seen as less significant. β_1 , representing the variable fDOM will give a more positive result meaning the Chlorophyll measurements will most likely show higher concentrations of the actual value in the presence of fDOM when using the EXO sonde. Similarly with turbidity, as described by β_3 , the results will show a lower concentration of Chlorophyll compared to the actual value.

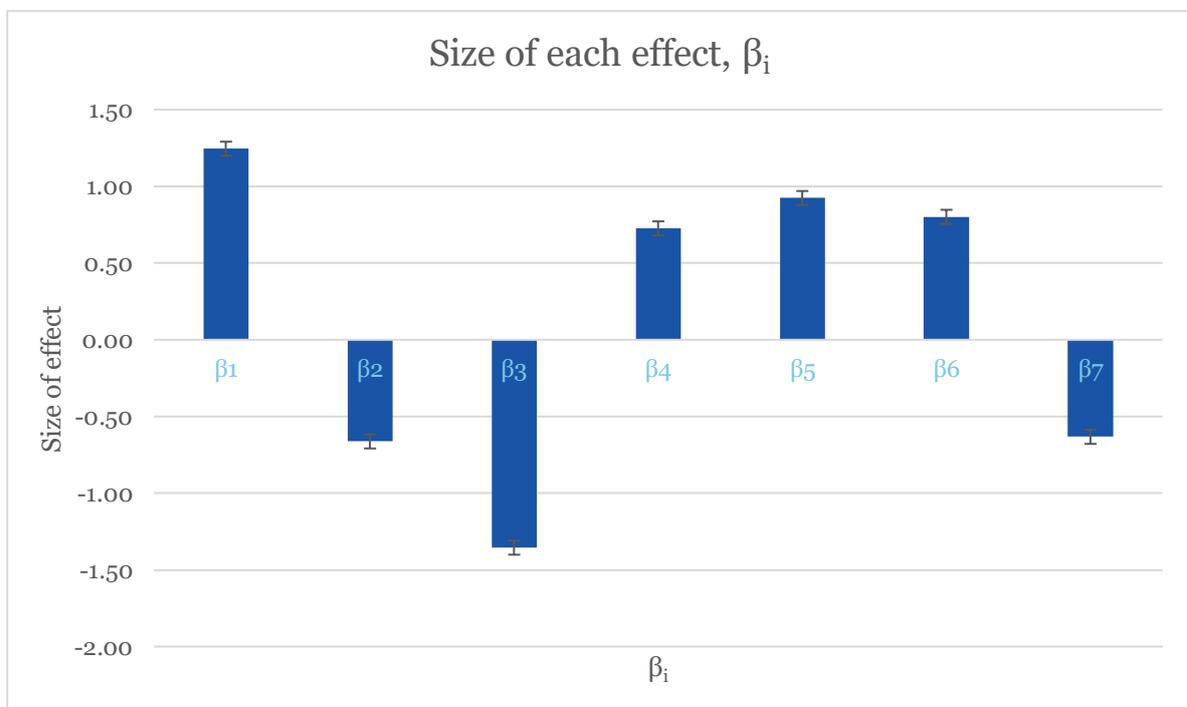


Figure 16. Size of each effect (β_i) and if it impact the outcome of the measurements of chlorophyll concentration is positive or negative. β_1 - fDOM, is one of the largest effects, along with β_3 -turbidity. β_2 - volume of pH buffer has a smaller effect on the outcome. The interaction effects, β_4 - β_7 also have an effect on the outcome.

The effects will also change depending on if it's a low or high level of the variable, as shown in figure 23. Effect B and C will increase as they go from low to high, while A, which is fDOM, has the inverse effect where a low level of fDOM will have a higher effect than a high level.

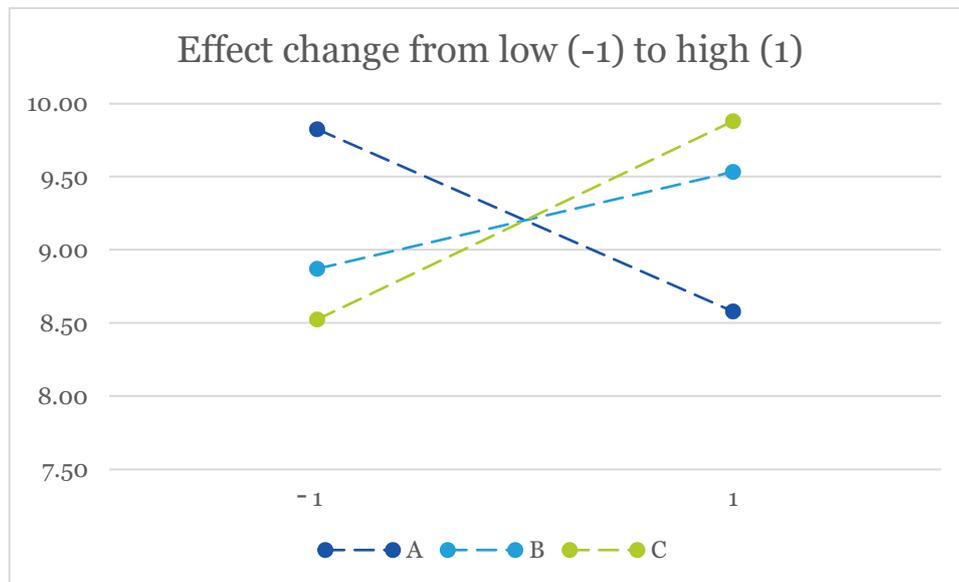


Figure 23. Change in effect size of A-fDOM, B-pH buffer, and C-turbidity when it goes from a low level (-1) to a high level (1). Meaning fDOM and pH buffer have a smaller effect at low levels, while fDOM has the inverse effect.

4.4 Geometric Mean Regression

A GMREG was conducted to compare the chlorophyll measurements [$\mu\text{g/l}$] from EXO2 with the results from Eurofins laboratory. It indicates a significant difference between the measurements, which was also confirmed by a statistical t-test where $t_{\text{calculated}} > t_{\text{table}}$, see appendix II for GMREG and t-test calculations. The differences in result had an average ratio of 2,4. When this ratio was applied to the EXO2 result, the difference between the measurements was no longer significant ($t_{\text{calculated}} < t_{\text{table}}$, see appendix II) meaning this correction factor of 2,4 should be applied. Figure 24 shows the GMREG without the correction factor. Note that the 1:1 line is far away from the GMREG line, this signifies that the measurements are significantly different from one another. In figure 25 on the other hand, the correction factor of 2,4 has been applied and the proximity of the 1:1 and GMREG lines is almost indistinguishable.

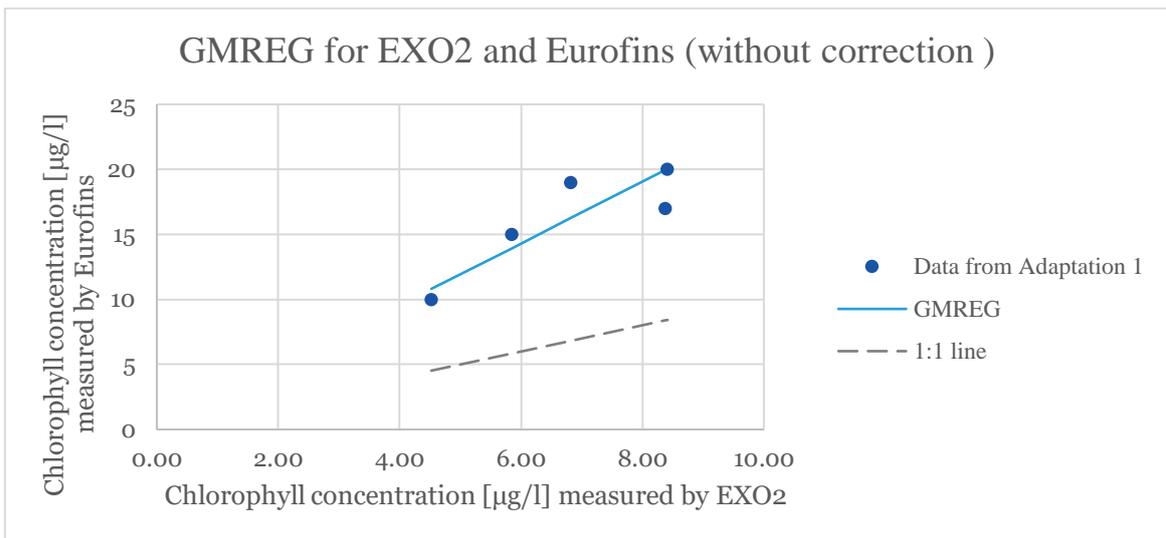


Figure 24. GMREG comparing chlorophyll measurements with EXO2 vs Eurofins lab, without correction factor. The GMREG line does not line up with the 1:1 line indicating the results from the two methods are significantly different from each other.

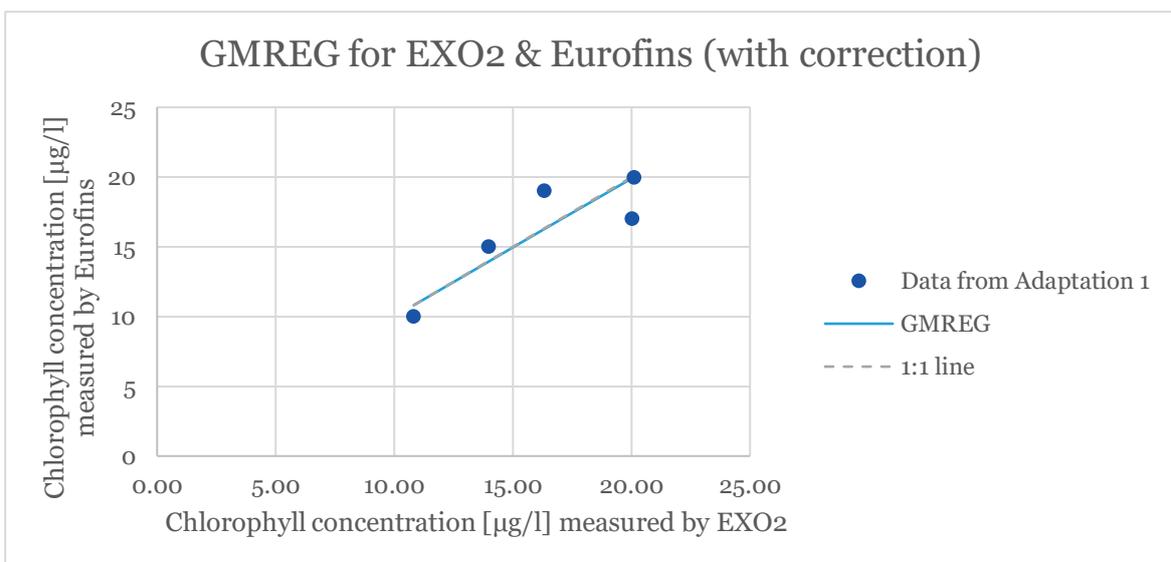


Figure 25. GMREG comparing chlorophyll measurements with EXO2 vs Eurofins lab, with correction factor. The GMREG line and the 1:1 line are almost indistinguishable from one another, indicating the results from the two methods are not significantly different from one another when the correction factor is applied.

4.5 Algae Cells as a Function of Chlorophyll

No clear relationship could primarily be identified when comparing the EXO2 measurements [$\mu\text{g/l}$] with the algae cell count [10^6 cells/L]. This was expected as they are defined in different units. However, after applying the identified correction factor of 2,4, the results were logarithmically compared in a graph and there was fairly linear relationship showing ($R^2=0,9402$), se figure 26. According to a t-test, the difference in resulting algae count is not statistically significant ($t_{\text{calculated}} < t_{\text{table}}$, see appendix II), meaning this relationship can be used to fairly predict number of cells with a 95% confidence.

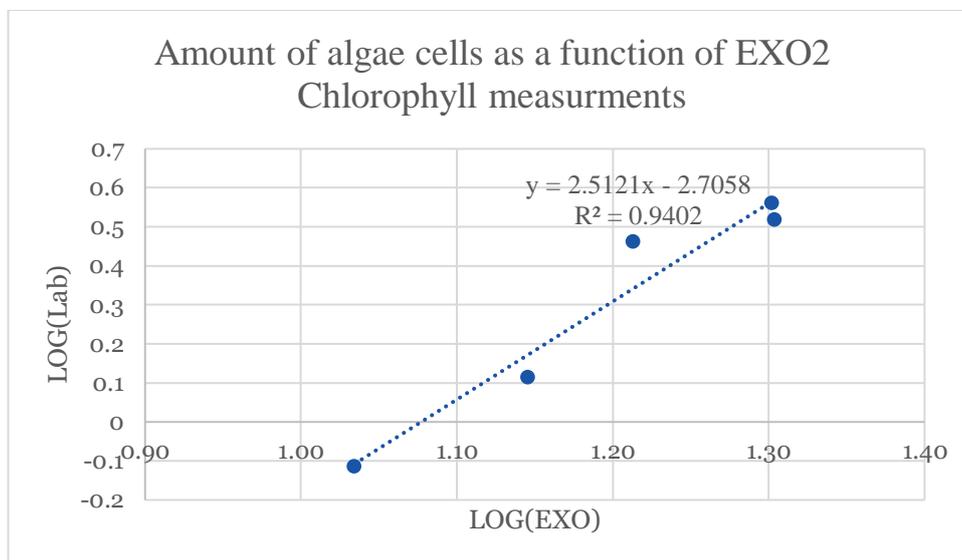


Figure 26. Number of algae cells as a function of EXO2 chlorophyll results

Apart from the statistical analysis in Excel as presented above, the data was also added to aCurve. The temperature correction equation and the identified correction factor was applied using the program's Calculator function. From there a detection limit was set by using the program's Alert Browser. The limits applied were 0-15 $\mu\text{g/l}$ is acceptable, 15-25 will raise an alert for attention and 25 $\mu\text{g/l}$ or higher will raise an alert for action and thorough follow-up. The limits were based on a recommendation by Basiak-Klingspetz (2021).

4.6 Chlorophyll stabilization using a $\text{MgCaO}_3 \text{Mg(OH)}_2$ solution

Before the raw water sample was filtered to be sent to Eurofins, three millilitres of $\text{MgCaO}_3 \text{Mg(OH)}_2$ solution were added to stabilize the Mg-ligand of the chlorophyll molecules. Results from the UV-vis measurement (254 nm) is presented in table 8 where the *Raw water* does not contain the $\text{MgCaO}_3 \text{Mg(OH)}_2$ solution and the *Eurofins water* did ($n=20$). There is a 4% difference in absorbance where the Eurofins water has a lower average, indicating that the $\text{MgCaO}_3 \text{Mg(OH)}_2$ does stabilize the chlorophyll.

Table 8. Absorption at 254 nm where three milliliters of $MgCaO_3Mg(OH)_2$ has been added to the Eurofins water and nothing has been added to the Raw water.

Sample	Raw water	Eurofins water
1	1,179	1,136
2	1,179	1,136
3	1,179	1,136
4	1,179	1,143
5	1,179	1,143
6	1,179	1,136
7	1,179	1,136
8	1,179	1,136
9	1,179	1,136
10	1,179	1,143
11	1,186	1,143
12	1,186	1,136
13	1,186	1,136
14	1,186	1,143
15	1,186	1,136
16	1,186	1,143
17	1,186	1,143
18	1,186	1,136
19	1,186	1,136
20	1,186	1,136
Average	1,183	1,138
% Difference		4%

5 Discussion

To address the research questions, data collection was conducted using the EXO2 sonde for the purpose of evaluating the following:

- The stability of the reference solutions to see how long they can be stored and used for;
- The precision of the EXO2 measurements;
- Which variables have the largest effect on the measurement of chlorophyll concentrations;
- The accuracy of the EXO2 measurement of chlorophyll concentrations;
- The possibility to quantify algae cells using EXO2 measurements of chlorophyll concentrations.

5.1 Stability of Reference solutions

The stability of the chlorophyll levels in the reference solutions were overall short-lived. If REF 1 is discarded due to too much fluctuation, then a reference solution can reliably be used as a calibration verification for one week if stored properly. If need be, this time limit can be pushed to two weeks if a measurement window of $\pm 10\%$ is allowed, as shown by REF 3. However, this is not recommended as it depends on the composition of the reference solution.

The levels of turbidity and pH indicate having an effect on the chlorophyll fluctuation in REF 1 as the levels follow a similar pattern. According to the full factorial experiment, turbidity has the largest effect on the chlorophyll levels, which could explain why the chlorophyll levels change with the turbidity. The interaction effect from turbidity and pH could also be a contributing factor to the fluctuation. Similarly in REF 2, the chlorophyll levels start to decrease after week 3, which is when turbidity rises. In week 3 however, turbidity is unexpectedly low in REF 2. Most likely assumption is bottle containing REF 2 was not mixed well enough that week before being poured into the EXO2 measurement cup leading not enough bentonite in the measurement sample. The pH in REF 2 on the other hand, is dropping steadily each week, somewhat following the pattern of chlorophyll. This is to be expected as low levels of pH have a greater effect on chlorophyll measurements than at higher pH, shown by the full factorial experiment.

The pH level decreases over time in all three reference solutions which indicates the buffer capacity of the NaHCO_3 does not hold over multiple weeks. The decrease can assume to be caused by the production of organic acids from the Coca-Cola. In REF 2, the pH continues to decrease whereas REF 1 and 3 are decreasing at a much slower rate. The level of Coca-Cola is equal in REF 1 and 2, but the volume of NaHCO_3 solution is lower in REF 2, indicating the buffer capacity is not strong enough to maintain a steadier pH level, like REF 1.

5.2 Precision of EXO2 Measurements

The precision of the chlorophyll concentration measurements from the EXO2 were fairly good when comparing the 95th percentile of the data to the median. The precision fluctuates in the original reference solutions that were prepared the first week of data collection. The weekly prepared solutions on the other hand generally had a higher precision throughout indicating the precision of the measurement wakens fairly quick with time. As mentioned previously, a reference solution is recommended to be stored for maximum two weeks and not more.

5.3 Variables with largest effect on chlorophyll concentration measurements

The full factorial experiment showed that fDOM and turbidity have the largest effect on chlorophyll levels, both on an individual level but also as an interaction effect. This is clearly demonstrated in REF 3 where levels of fDOM and turbidity are relatively stable until week 4 where both rise strongly. At the same time, the levels of chlorophyll drop significantly. This could be due to the fact the Coca-Cola being used during the project was not sugar-free and as the project was not conducted in a sterile environment, those sugars could have come in contact with bacteria or air-borne yeast and cause fermentation. Fermentation would contribute to a higher fDOM and turbidity value, increasing the interferences of the chlorophyll measurements.

5.4 Accuracy of the EXO2 measurements

For the detection of algae cells, chlorophyll was measured as a proxy parameter. The results show an average ratio of 2,4 needs to be applied to the EXO2 measurements to correspond with the results from the Eurofins laboratory with a minimum 95% confidence level. The laboratory results are treated as the correct value seeing as it is measured using a national standard. Therefore, it is safe to assume that the factor of 2,4 is correct. The application of data in aCurve along with an alert system shows that there is potential for the EXO2 to work as an early warning system. However, more time will be needed to make an adequate system as this project only covered a small application to aCurve, which has more potential.

5.5 Quantifying of algae cells using chlorophyll concentrations

The results show there is a logarithmic correlation between the chlorophyll measurements from the EXO2 and cell density put forward by the Norrvatten laboratory. While the correlation does not provide perfect linearity, it can still be used to predict cell density with no significant difference in result. Meaning, the EXO2 can be used for as early warning system to give a reliable indication of algae cell density in raw water. It should be noted that all results are site specific, meaning the identified correction factor and the algae cell correlation may vary if another sonde is used or if another location is studied

5.6 Chlorophyll stability

The UV-measurements on the filtered water showed roughly a 4% difference in absorbance when the $MgCaO_3$ $Mg(OH)_2$ solution was added. The difference in absorbance indicated that without the $MgCaO_3$ $Mg(OH)_2$ solution, chlorophyll is more soluble and can be filtered through the paper.

5.7 Sources of Error and Suggested Improvements

A potential source of error identified is handling of the bentonite solution. As bentonite sediments easily, it is important to mix the solution prior to pipetting or prior to measurements being taken. If this project was to be repeated, a recommendation is to use a magnetic stirrer when pipetting. Another source of error was the usage of regular Coca-Cola rather than a sugar free cola. This could potentially be useful as a sugar free beverage would not ferment as easily.

6 Conclusion

The EXO2 sonde has potential to be used as a monitoring instrument for an early warning system for algae bloom. It can measure chlorophyll levels in raw water at Görväln DWTP with a 95% confidence at concentrations of around 15 µg/l when correction factors are applied. The level of chlorophyll can furthermore be translated to algae cell density which can be used as an indicator for algae bloom. To maintain high confidence in the chlorophyll measurements, the sonde needs a new reference solution made once a week. This solution can be stored and used for one week, maximum two depending on the composition of the solution. While the EXO2 has shown to be a good monitoring tool, laboratory measurements should still be conducted with regular intervals to ensure the identified correlations last the whole algae blooming season.

As chlorophyll has shown to be a possible parameter for an algae bloom monitoring, it is still a measurement of total biomass and not specifically an indication of cyanobacteria density or concentration of toxins from cyanobacteria. Further research is therefore recommended to establish a correlation between total biomass and the amount of cyanobacteria or cyanotoxins.

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Appendix I. List of Materials

The following chemicals and materials were used for data collection.

List of Chemicals

- Rhodamine WT (125 mg/l)
- Pepsi (diluted 0,2 times with distilled water)
- Bentonite (5 g/L)
- NaHCO₃ (1 M)
- MgCaO₃ Mg(OH)₂ (1%)
- Distilled water

List of Material

- YSI EXO2 multiparameter optical sonde & calibration cup
- Rope, carabiner screw lock clip to hang EXO2
- Metal chain with attached carabiner screw lock clips to place at PP100
- Volumetric flasks (1000, 500, 250, 100 ml)
- Graduated pipettes (1,5, 10, 25, 100 ml)
- Beakers (250, 500, 1000 ml)
- Graduated laboratory bottles, glass, 1000 ml x9
- Sample bottle, plastic, 500 ml x2/week
- Suction filtration apparatus with handheld pump
- 47mm glass fiber filter (Whatman GF/C, VWR 513-5227)
- Plastic tweezers
- Sample container with yellow silica beads
- Wash bottle for distilled water
- Magnet and magnetic stirrer (300 rpm)

Appendix II. Statistical calculations

In this section, calculations regarding the F-test, t-test, precision, GMREG and full factorial are presented.

F-test

Solution: REF 1
 n 15
 SQRT(n) 3,873

The F-test was only done on the measurements that showed the greatest standard deviation in comparison to the other measurements. All solutions were treated in the same way, so only one example is demonstrated on how the calculations were conducted. Table 9 shows a summary of the average EXO2 measurements (n=15) for the first solution, REF 1. It also shows the standard deviation and the average standard deviation (=SQRT(n)/Stdev.). Using this table, figure 27 was created with the average standard deviation as error bars. As the last measurements for week 5 has a larger error bar, a F-test was conducted on that week to see if the difference is significant. The calculations of the F-test are listed in table 10, were the result showed $F(\text{calc}) > F(\text{table})$, meaning the measurements from week 5 should be rejected.

Table 9. Summary table of Chlorophyll measurements from EXO2 for F-Test

Week	Chl concentration (average) $\mu\text{g/l}$	Stdev.	Average Stdev.
1	15,54	0,29	0,074
2	17,24	0,05	0,013
3	16,00	0,37	0,095
4	15,60	0,37	0,097
5	17,94	0,54	0,140

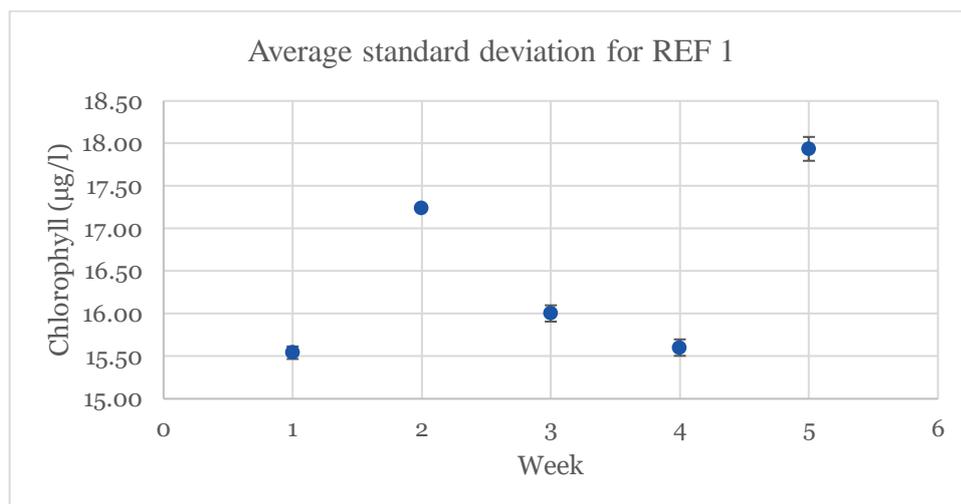


Figure 27. Chlorophyll concentration with average standard deviation for REF 1

Table 10. F-Test for REF 1, week 5

REF 1, Week 5	Equation used ($N_{set}=4$)	Calculated value
s^2	$=0,54^2$	0,30
s^2 pooled	$s_{pooled}^2 = \frac{s_1^2 + s_2^2 + s_3^2 + \dots}{N_{set}}$	0,090
F(calculated)	$F_{calculated} = \frac{s_1^2}{s_2^2}$	3,28
F(table)	=F.INV.RT(0,05;Degree of Freedom1;Degree of Freedom 2) Degree of Freedom1= n-1 =14 Degree of Freedom2= 52	1,89
Def. Of Freedom	<i>Degrees of freedom</i> = $n_1 + n_2 + n_3 + \dots - N_{set}$	52
F(calc)>F(table)		Reject the set

t-test

Solution: REF 2

Table 11 shows an example of how the t-tests were calculated to evaluate how long a reference solution could be stored for before the change became significant. First week 1 and week 2 were compared and showed no significant change with a 95% confidence. Second week 1 and 2 were averaged together to compare with week 3, also showed no significant change. By week 4 however there is a significant change where $t_{calculated} > t_{table}$, meaning the solution cannot be stored for that long without significant change.

Formula used for $t_{table} = T.INV.2T(0,05;Degree of Freedom)$

Equation to calculate $t_{calculated}$:

$$t_{calculated} = \frac{|\bar{x}_1 - \bar{x}_2|}{s_{pooled}} \sqrt{\frac{n_1 \cdot n_2}{n_1 + n_2}}$$

Table 11. t-test for comparing the weeks for REF 2

Week(s)	s pooled	Degrees of Freedom	t(calculated)	t(table)	Comment
1 & 2	0,213	13	0,74	2,16	Not significant
(1, 2) & 3	0,302	42	1,31	2,02	Not significant
(1,2,3) & 4	0,265	56	33,74	2,00	Significant

Precision of measurements

Solution: REF 3

Table 12 shows the calculations for percentiles in regard to the median value. The median is used to avoid fluctuating measurements which can distort the mean average. Percentiles were calculated with the following formula =PERCENTILE.INC(array;k), where array is the values in the observed sample and k is the percentile (2,5th or 97,5th). The 95th percentile within the range of the median is then calculated using the following equation:

$$95\% \text{ within range of the median} = \frac{(97^{\text{th}} \text{ percentile} - 2,5^{\text{th}} \text{ percentile})}{\text{Median}}$$

The calculated percentage then shows how much (%) of the 95th percentile will fall within the median value.

Table 12. Percentiles in regard to the median for REF 3, week 2

Parameter	REF3, week 2	
	fDOM	Chlorophyll
95 th percentile within the median	0,27 %	1,35 %
Median	65,79	13,87
97,5 th percentile	65,89	14,00
2,5 th percentile	65,71	13,82

Geometric Mean Regression (GMREG)

GMREG is a cross-comparison of the variables, grounded in two adaptations. Adaptation 1 had EXO2 results was on the x-axis and the Eurofins results on the y-axis and vice versa for adaptation 2, see table 13 for values, average, slope (=SLOPE) and intercept (=INTERCEPT). The correction factor of 2,4 has been applied to the EXO2 values.

Table 13. X and Y values for Adaptation 1 and 2

Adaptation 1	x	y	Adaptation 2	x	y
Week	EXO µg/l	Eurofins µg/l	Week	Eurofins µg/l	EXO µg/l
1	10,82	10	1	10	10,82
2	13,99	15	2	15	13,99
3	16,33	19	3	19	16,33
4	20,05	17	4	17	20,05
5	20,14	20	5	20	20,14
Average	16,27	16,20	Average	16,20	16,27
Slope (k1)	0,85		Slope (k2)	0,87	
Intercept (m)	2,395		Intercept (m)	2,233	

The adaptations were then the basis in calculating the geometric mean slope (GMS) and the geometric mean intercept (GMI) to create a GMREG line $y = GMS \cdot x + GMI$, where the calculated values of y are shown in table 14. The final figure is shown in Section 4 Results.

$$GMS = \left(\frac{k_{adptation\ 1}}{k_{adptation\ 2}} \right)^{1/2} = 0,99$$

$$GMI = \bar{y}_{Eurofins} - GMS(\bar{x}_{EXO}) = 0,1$$

Table 14. GMREG values

GMREG line (x from adaptation 1)			
y	GMS (k)	x	GMI (m)
10,81	0,99	10,82	0,1
13,95	0,99	13,99	0,1
16,27	0,99	16,33	0,1
19,94	0,99	20,05	0,1
20,03	0,99	20,14	0,1

Full factorial Experiment

All effects were calculated in the same way. Below is an example of how β_1 was calculated where the +/- was determined on whether it was a high (+) or low (-) level and m= number of trails, which is eight.

$$\beta_1 = \frac{\pm \bar{y}_1 \pm \bar{y}_2 \pm \bar{y}_3 \dots \pm \bar{y}_7}{m} = \frac{-22,3 + 20,2 - 16,6 + 20,0 - 14,8 + 19,0 + 14,9 - 19,5}{8} = 1,25$$

To determine the significance of the effects, error vars with 95% reference interval was added to figure 25.

Equation used	$\bar{x} \pm \frac{ts}{\sqrt{n}}$
Degrees of freedom	128
n	17
t _{table}	1,979
spooled	0,095
spooled/SQRT(n)	0,023
Error bars	±0,046

Algae Cells as a Function of Chlorophyll

Identifying a relationship between EXO2 chlorophyll measurements and the cell count from the laboratory was done by first adding the identified correction factor of 2,4 and then graphing them logarithmically (see figure 24 in Results). The linear equation ($R^2=0,9402$) from the graph was used to calculate the predicted value of the algae cells, as shown in table 13, under Calculated Log(y). The linear equation is: $y=2,5121x-2,708$. The difference in calculated predicted value and the real laboratory results were compared with a t-test, where it showed no significant difference in the results difference, see calculation under table 15.

Table 15. Values for establishing a relationship between EXO2 and algae cell count

EXO2*2,4 Chlorophyll [ug/l]	Laboratory Algae cells [10e6 cells/]	LOG(X)	Calculated LOG(Y)	10 ^(Y)	Difference
10,82	0,77	1,034	-0,107	0,781	-0,01
13,99	1,3	1,146	0,173	1,489	-0,19
16,33	2,9	1,213	0,342	2,196	0,70
20,05	3,64	1,302	0,565	3,675	-0,03
20,14	3,3	1,304	0,570	3,717	-0,42

Average difference 0,01
 Standard deviation 0,420
 n 5
 Degrees of freedom 4
 Confidence level 0,95
 t(table) 2,78
 t(calculated) 0,0564