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Effects of Excessive Ultraviolet Irradiation on Assimilable Organic Carbon in Drinking Water

Master's thesis in the Nordic Master's Program Environmental Engineering: Urban Water

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Department of Architecture and Civil Engineering
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Abstract

Increasingly, drinking water treatment plants are implementing ultraviolet (UV) irradiation as a disinfection barrier in preference to traditional chemicals which can react with natural organic matter (NOM) and produce harmful disinfection by-products. Guidelines have been established for the minimum required UV dose but the effects of excessive UV irradiation have not thoroughly been studied.

A disadvantage of excessive UV irradiation is that dissolved compounds present in NOM can be cleaved by light into small bioavailable molecules. These bioavailable molecules provide food that fuels microbial regrowth, and the potential for regrowth can be measured as native Assimilable Organic Carbon (AOC). The objective of this thesis was to evaluate the effects of excessive UV irradiation on natural organic matter (NOM) and the availability of bioavailable molecules, measured as regrowth potential. As secondary objectives, optical measurements were used to examine whether regrowth potential could be estimated using fluorescence and absorbance spectroscopy.

Drinking water samples were collected from seven different treatment plants in Sweden that applied varying treatment processes to different kinds of source waters, including surface water and artificial ground water. Duplicate samples were collected from each treatment plant, one of which was irradiated with a UV dose ranging between 960 to 1655 mJ/cm^2. Both irradiated and non-irradiated samples were characterized at Chalmers in terms of DOC, pH, fluorescence and absorbance, high-performance size exclusion chromatography (HPSEC). The samples were then transported to the Norwegian University of Science and Technology (NTNU) and AOC was measured using a rapid bioassay method.

UV irradiation caused decreased molecular size as measured using HPSEC. Moreover, an increase in total average AOC concentration was observed after irradiation. High measurement variance was obtained using the bioassay method, contrasting with low variance in optical measurements, which highlighted the need for further studies and simpler methods for AOC quantification.

Keywords: Drinking Water, Assimilable organic carbon (AOC), Bioassay, Fluorescence, UV-treatment, HPSEC

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Megan Strand-Jordan, Göteborg, July 2018

Nomenclature

AIR	After Irradiation
AOC	Assimilable Organic Carbon
AR Plant	Artificial Groundwater Recharge Plant
BDOC	Biodegradable Dissolved Organic Carbon
BIR	Before Irradiation
CDOM	Colored Dissolved Organic Matter
CFU	Colony Forming Units
DNA	Deoxyribonucleic Acid
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
DWTP	Drinking Water Treatment Plant
EEM	Excitation-Emission Matrix
EPA	Environmental Protection Agency
FDOM	Fluorescent Dissolved Organic Matter
GAC	Granulated Activated Carbon
HPSEC	High-Performance Size Exclusion Chromatography
MQ	Milli-Q
NOX	Spirillum Strain NOX
NTNU	Norwegian University of Science and Technology
P17	Pseudomonas fluorescens Strain P-17
PARAFAC	Parallel Factor Analysis
PBS	Phosphate Buffered Solution
RNA	Ribonucleic Acid
UV	Ultraviolet
UV_{254}	UV Absorbance Measured at 254 nm

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1
Introduction

Minimizing bacterial growth in the drinking water distribution network is paramount to providing clean, safe drinking water to consumers. Bacterial regrowth potential measures the ability of bacteria to reproduce and pose a threat to consumers. In order for bacteria to multiply they must first survive the treatment process and then have a source of viable nutrients and bioavailable energy. Not all organic carbon compounds are able to be used by bacteria. The share of organic compounds which provide energy and carbon for potential bacterial growth are called biodegradable dissolved organic carbon (BDOC) or assimilable organic carbon (AOC) (APHA, 2012). Assimilable organic carbon concentrations can be measured to estimate regrowth potential.

Traditional methods for quantifying regrowth potential in a water supply employ a rapid bioassay method developed by LeChevallier et al. to determine AOC concentrations (LeChevallier et al. 1993). There are several disadvantages to this method, mainly the time and expense required to cultivate and maintain two bacteria colonies, Pseudomonas fluorescens Strain P-17 (P17) and Spirillum Strain NOX (NOX). Additionally, individual testing of samples by standard protocols requires 6 days of incubation. The method is therefore quite tedious and because of the inherent variability of bacteria growth, it also has relatively low precision around $\pm 17.5\%$ (APHA, 2012). An optical method of measuring AOC concentrations has not yet been developed as an alternative to the bioassay method.

Increasingly, drinking water treatment plants are implementing ultraviolet (UV) irradiation as a disinfection barrier in preference to traditional chemical disinfection which can react with natural organic matter (NOM) and produce harmful disinfection by-products (Långmark et al. 2007). Guidelines have been produced for minimum dosing required to provide an adequate disinfection barrier, however, there are not guidelines for maximum dosing (Ødegaard et al., 2014; US EPA, 2006). Previous studies have shown inconsistent results including either an increase or decrease in total AOC values after on-site UV treatment (Polanska et al., 2005; Thayanukul et al., 2013). Excessive UV dosing has rarely been tested for possible effects on NOM character or it's bioavailability to harmful microorganisms. (Bazri et al., 2012; Lehtola et al., 2003)

1.1 Aims and Objectives

The overall aim of this thesis was to investigate the effects of large ultraviolet (UV) doses on the biostability and chemical properties of treated drinking water. Samples were collected from several different drinking water treatment plants (DWTP) with differing source waters and treatment processes in order to answer the following research questions:

- Does a high UV dose split unreactive natural organic matter (NOM) into smaller, more bioavailable molecules?
- Is there a varried reaction between different types of NOM to a high UV dose?
- Does the source water of a DWTP influence the effect of irradiation on AOC levels?
- Can regrowth potential be estimated using fluorescence spectroscopy in combination with other chemical measurements?

2

Theoretical Background

2.1 Natural Organic Mater

Natural organic matter (NOM) is a complex, heterogeneous mixture of humic substances, humic and fulvic acids, as well as proteins, polysaccharides and other labile components (Uyguner et al., 2007). NOM is found in all natural surface and ground waters. It has varying characteristics depending on the geographical and physical nature of the source water as well as seasonal variations, including temperature, pH and variable flows. Human activities in a source catchment can also affect the NOM characteristics. NOM removal from source water is important as NOM compounds can reduce the effectiveness of several treatment processes including chemical precipitation, and can react with disinfectants to form harmful disinfection bi-products (Långmark et al. 2007).

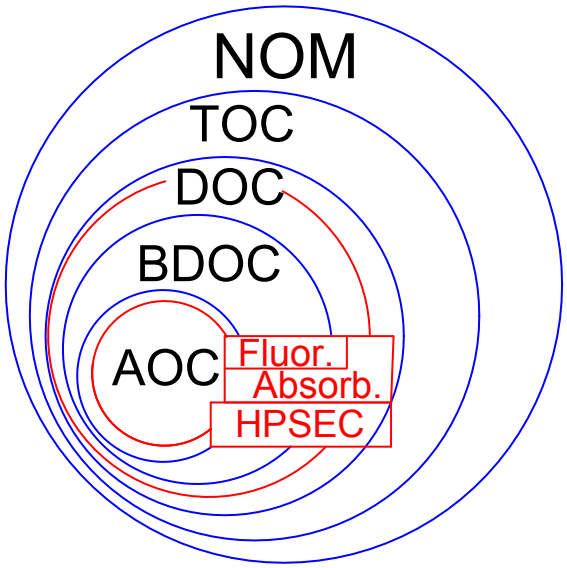

Figure 2.1: Conceptual representation of -containing compounds comprising NOM in drinking water. The measurement techniques used in this thesis are shown as red text and drawn to show which NOM fractions (black text) can be identified with each specific technique. Red circles are drawn around DOC and AOC to indicate there was specific measurement of those two fractions. This diagram is not drawn to scale.

NOM is comprised of several different fractions; a conceptual representation is presented in Figure 2.1. All NOM compounds contain carbon so NOM is often quantified as total organic carbon (TOC), which measures the total amount of soluble and insoluble organic carbon compounds present in a water sample. TOC is the most comprehensive measurement of organic mater in a water system, it is frequently used interchangeable with NOM (Uyguner et al., 2007). Dissolved organic carbon (DOC) is the soluble fraction of TOC, which may be found in water and can be estimated through analytical measurements. It is often defined as the fraction of NOM that passes through the 0.45 or 0.22 µm filter (Hansell and Carlson, 2014)

Biodegradable organic carbon (BDOC) is a fraction of DOC and consists of larger molecules that can be used as a substrate for bacterial growth. Previous study has shown that as an individual measurement BDOC cannot be used to predict heterotropic bacteria regrowth (Van der Kooij, 1992). Instead BDOC can be used to estimate a reduction in chlorine demand or disinfection by-product formation (Kaplan et al. 1994). An even smaller fraction of DOC is assimilable organic carbon, which is the fraction of BDOC that can be readily digested and used for growth by heterotrophic bacteria. AOC can represent between 0.1-9% of TOC (Van der Kooij, 1990). Source water, either surface or ground, has been shown to have a large influence on AOC concentrations (Kaplan et al. 1994). Kaplan et al. also observed a statistically significant correlation between AOC and DOC; however, data were limited and each individual water system should be evaluated (Kaplan et al. 1994).

2.1.1 Assimilable Organic Carbon

Assimilable organic carbon is a well-studied component in water. Different treatment processes have been shown to affect overall AOC concentrations. Ozonation has been shown in previous studies to significantly increase AOC (Escobar and Randall, 2001). Both granulated active carbon (GAC) and biological activated carbon (BAC) filters were effective at removing AOC (Yang et al, 2011). Past studies have shown inconsistent results of an increase or decrease in total AOC values after on-site UV treatment (Polanska et al., 2005; Thayanukul et al., 2013). AOC concentrations did not increase in the Stockholm water district after the implementation of UV irradiation at the Hässelby treatment plant (Långmark et al. 2007). It is important to note that this study did not specify the UV dose applied at the treatment plant; however, it can probably be assumed that the treatment plant applied at least the minimal recommended dose of 40 mJ/cm^2 (Ødegaard et al., 2014). Optimising the treatment processes train is therefore very important for reducing AOC since varying treatment steps may either increase or decrease AOC concentrations.

A significant seasonal variation has been shown in AOC concentration. This variation has been observed in Sweden and Belgium (Långmark et al. 2007; Polanska et al., 2005).

A study by Yang et al. in Taiwan, suggest that this effect is more prominent in wet seasons due to the resuspension of organic sediments during periods of high river flow (Yang et al., 2011). Additional treatment steps may need to be implemented in order to meet removal standards seasonally.

2.2 Ultraviolet Irradiation in Drinking Water Treatment

Ultraviolet light is characterized as having a wavelength between 100-400 nm. Within this range it is further separated into four different sub-types, UV-A, UV-B, UV-C and vacuum UV (Figure 2.2) (LIT Ultraviolet Technologies, 2018). Disinfection of drinking water by UV irradiation is process that results in the death or inactivation of microorganisms. Cell inactivation by UV light can be effective at a range of wavelengths between 205-320 nm. In drinking water treatment, UV-C is most effective at deactivating organisms, at a wavelength of approximately 264 nm. Many UV disinfection units utilize low pressure mercury or amalgam lamps which emit at a wavelength of 254 nm (Calgon Carbon Corporation, 2018; Trojan Technologies, 2018). The UV light acts as a disinfection barrier by penetrating the cell wall and cytoplasmic membrane of an organism and damaging its deoxyribonucleic (DNA) and ribonucleic acids (RNA). This makes the organism incapable of reproduction, so the organism is inactivated and effectively dead.

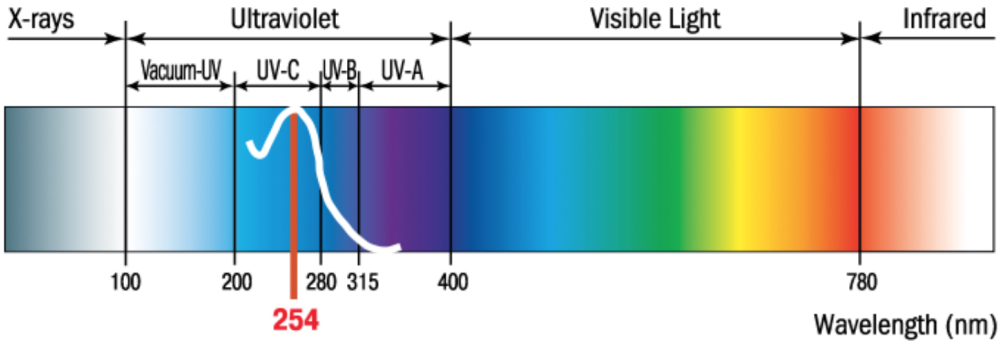

Figure 2.2: Electromagnetic waves arranged according to frequency and wavelength. The four ultraviolet wavelengths are denoted. Low-pressure mercury lamps emit at a wavelength of 254 nm (marked in red) and the spectral curve of cell inactivation (labeled in white). Figure is adapted from LIT Ultraviolet Technologies.

UV disinfection in drinking water is controlled by the UV dose, Equation 2.1 (Ødegaard et al., 2014). The intensity is determined by the lamp used in a disinfection unit and is equivalent to chemical concentration in traditional chemical disinfection. The exposure time is the amount of time that the water sample is in contact with the UV light, usually measured in seconds, which is equivalent to effective contact time in chemical disinfection (Ødegaard et al., 2014).

$$UltravioletDose[mJ/cm^2] = Intensity[mW/cm^2] * ExposureTime[s] \quad (2.1)$$

The United States Environmental Protection Agency (US EPA) has set forth guidelines that define minimum UV doses that are effective to treat different pathogens, namely Cryptosporidium, Giardia and several viruses including the common Rotovirus and Norovirus, as well as the very UV-resistant Adenovirus. These guidelines can be seen in Table 2.1. Log inactivation is the reduction of viable organisms by a factor of 10^x, where x is the number in the table (US EPA, 2006). The US EPA recommends a minimum log inactivation of Cryptosporidium and Giardia of 3 and a log inactivation of 4 for viruses (US EPA, 2006).

Table 2.1: US EPA UV Dose Requirements[mJ/cm^2] for various log inactivation of several common pathogens (US EPA, 2006)

Target Pathogens	**Log Inactivation**							
	0.5	**1.0**	**1.5**	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**
Cryptosporidium	1.6	2.5	3.9	5.8	8.5	12	15	22
Giardia	1.5	2.1	3.0	5.2	7.7	11	15	22
Virus	39	58	79	100	121	143	163	186

While the US EPA recommends UV dosing to reduce viable microorganisms by a factor of 10^4 for all viruses including the Adenovirus, Scandinavian countries do not view the Adenovirus as credible threat to the drinking water supply (US EPA, 2006; Ødegaard et al., 2014). For this reason the required minimum UV dose to achieve a four-log reduction in bacteria, viruses and parasites is between 25-40 mJ/cm^2. This UV dose range is effective against Cryptosporidium, Giardia, Rotovirus, Norovirus, as well as against bacterial spores and provides a log inactivation of 4 for bacteria and protozoa and 3.5 for viruses (Ødegaard et al., 2014).

2.3 DOM Optical Properties

Coloured, or chromophoric dissolved organic matter (CDOM), refers to naturally-occurring dissolved organic compounds in water that absorb UV light. These are mainly associated with the humic fraction of NOM. Absorbance spectroscopy is measured and used to track the abundance of CDOM in many drinking water treatment plants (Weishaar et al., 2003). Fluorescent dissolved organic matter (FDOM) is the fraction of CDOM that is able to absorb then emit light at specific frequencies, and

is measured using fluorescence spectroscopy. FDOM is associated with humic and fulvic compounds as well as protein-like compounds. Fluorescence spectroscopy is less precise but more sensitive than absorbance spectroscopy, so is useful for measuring low concentrations and small changes in concentration (Coble, 1996).

Fluorescence characterizes the relationship between absorbed and emitted photons at specified wavelengths. Most molecules at room temperature occupy the lowest vibrational energy level, and after absorbing light those molecules becomes excited to a higher level (Lakowicz, 1983). This is followed by a relaxation in vibrational energy to the lowest level of the excited state, then the fluorophore returns to the lowest energy level by emitting the excess energy as fluorescence (Figure 2.3 top) (Gooijer, 2000; Lakowicz, 1983).

By observing the location of fluorescence excitation and emission peaks (Figure 2.3 (bottom)), specific organic compound groupings of FDOM can be identified within NOM. Several fluorescence components that have been observed in drinking water samples are similar to those observed in diverse aquatic samples (Table 2.2). They include humic-like components with terrestrial or microbial origins, and protein-like components containing tryptophan-like fluorophores (Murphy et al., 2011; Murphy et al., 2014).

Table 2.2: Description and wavelength positions of peak excitation and emission for several PARAFAC components from literature (Murphy et al., 2011)[1] (Murphy et al., 2014)[2].

Component	**Ex. [nm]**	**Em. [nm]**	**Description**
C1^1	320	400	Microbial humic-like fluorescence
C2^2	370	460	Humic-like
C3^2	320	420	Humic-like
C4^1	370	464	Terrestrial humic-like fluorescence
C5^1	290	352	Protein, tryptophan-like

UV irradiation during disinfection could potentially modify organic matter structures, resulting in changes to absorbance and fluorescence (Liu et at. 2002). In fact, UV irradiation has been shown to increase the bio-availability of organic matter by increasing low molecular weight compounds like amino acids and carbohydrates (Sulzberger and Durisch-Kaiser, 2009). CDOM in combination with FDOM are frequently used in studying changes in DOM composition and concentration (Murphy et al., 2013). Although neither spectroscopic measurement is able to necessarily directly quantify the smallest fraction of bioavailable carbon, AOC, which can be used by heterotrophic bacteria (Heibati et al., 2017).

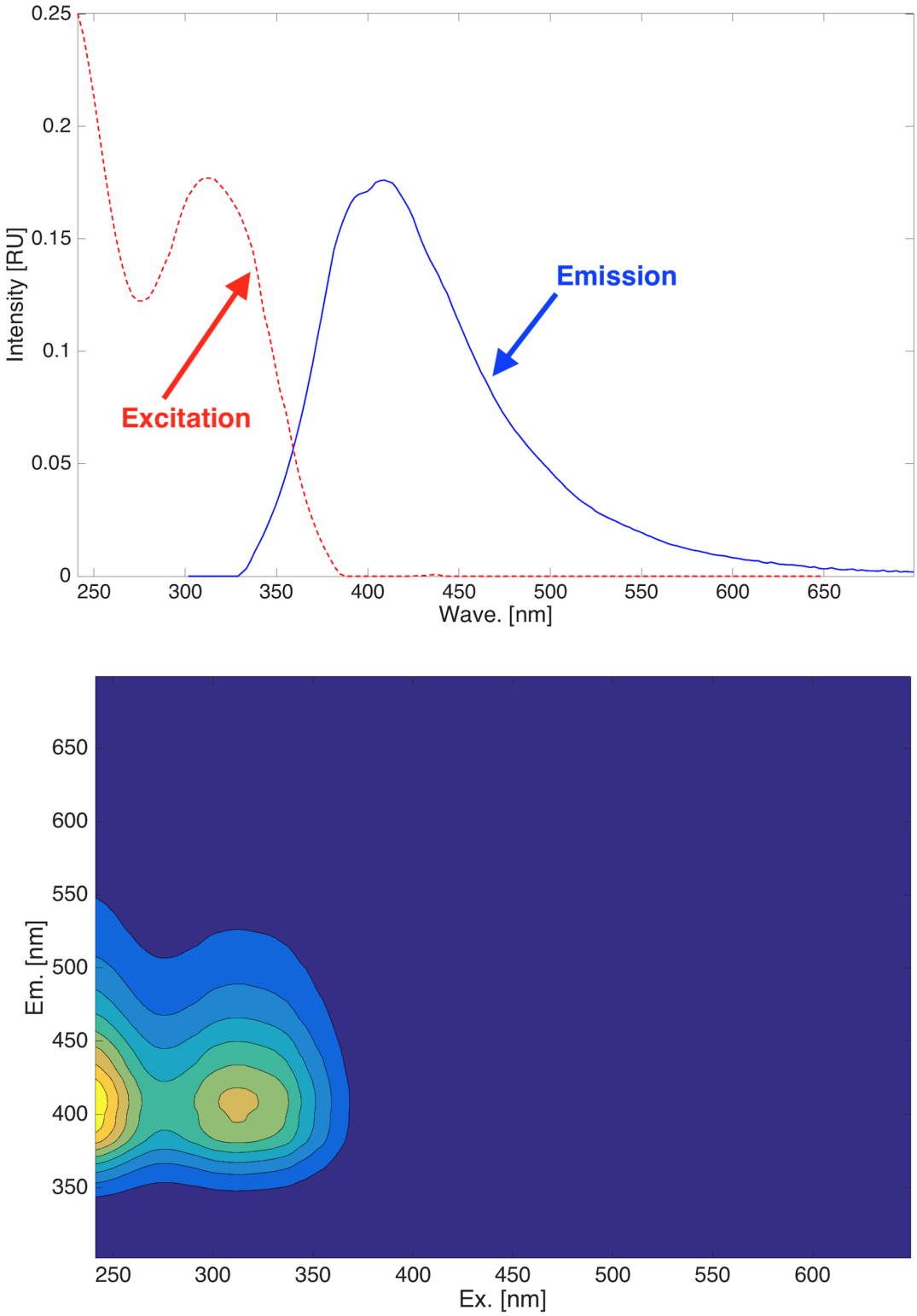

Figure 2.3: Top: Excitation (Red-dotted) and emission (Blue-solid) spectra of a FDOM component identified using parallel factor analysis. Bottom: Fluorescence EEM depiction of the same spectra illustrating a fluorescence peak (yellow) at 410 nm, traditionally interpreted as a humic-like component.

3

Materials and Methods

3.1 Study Sites

Seven treatment plants were used in this experiment, throughout the western coast of Sweden. The sites were chosen for their varied treatment processes and water sources. Two treatment plants, Lysegården and Dösebacka, use artificial ground water while the remaining sites utilize surface water as a source. Testing of DOC for the raw water from the plants ranged from 1-5 mg/L for all sites except for Marstrand which was an intake value of 13 mg/L.

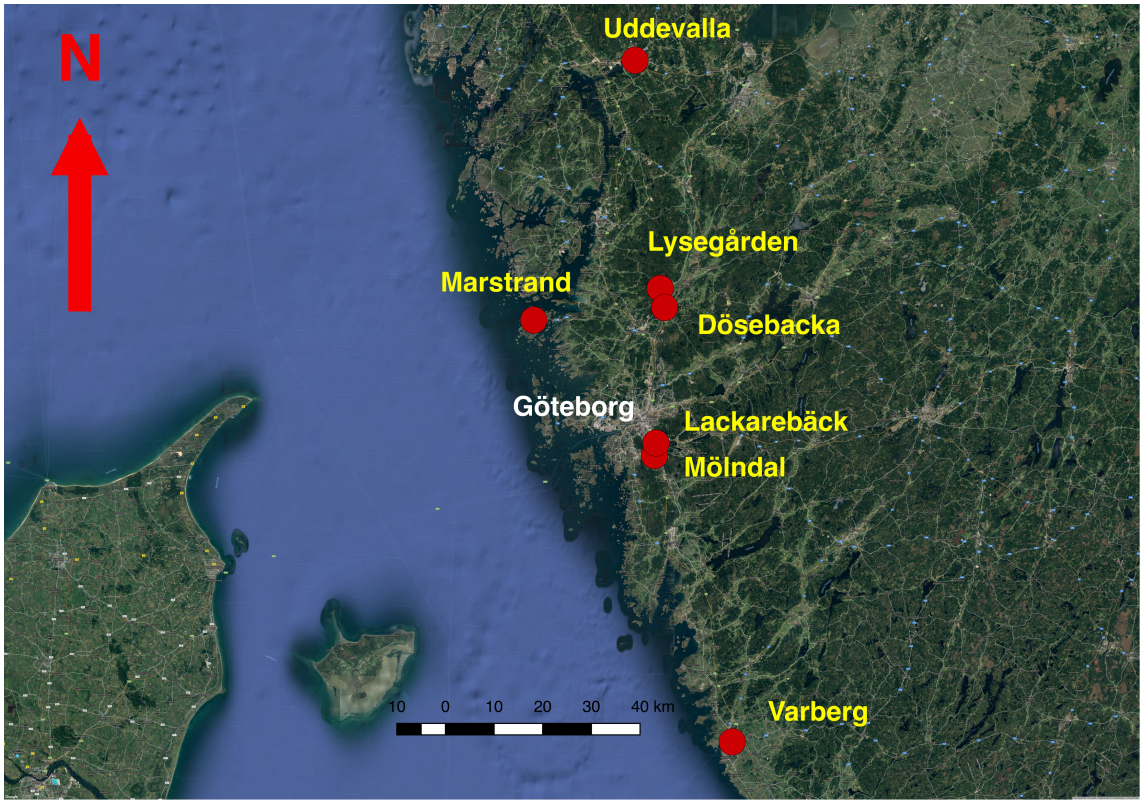

Figure 3.1: Study area and location of sample collection

3.1.1 Uddevalla

Marieberg is the primary DWTP located in Uddevalla, using Lake Köperödssjön as it's source water (Figure 3.1).Steps for treatment of the inflow include floculation with poly-aluminum chloride, rapid sand filtration (Dynasand), accompanied by granulated activated carbon (GAC) filters and finished with UV and chlorine disinfection (Moona et al., 2018).

3.1.2 Varberg

Kvarnagårde is the primary DWTP located in Varberg Sweden, owned by the private company Vatten & Miljö i Väst AB (Figure3.1). The plant utilizes a mixture of approximately 80% surface water from Lake Neden and 20% ground water as source water (VIVAB, 2018). Lime and carbonic acid are added to the intake water in a reservoir to adjust alkalinity, hardness and pH. This is followed by rapid sand filtration, afterward the water is then lead into three reservoirs. The water is then exposed to UV irradiation and chloramine is added for residual disinfection in the network (Lavonen et al., 2015).

3.1.3 Lackarebäck

Lackarebäck is the primary DWTP for the city of Göteborg (Figure 3.1). Water is pumped from the Göta Älv into the Delsjö lakes, which serve as reservoirs. The intake water is first pre-chlorinated (Cl_2) before aluminium sulphate is added for coagulation and accompanied with sedimentation. After rapid filtration with GAC filters, the water is then put through ultra filtration. This is followed with the final step of disinfection with chlorine and sodium hypochlorite (Moona, 2017).

3.1.4 Marstrand

The Marstrand DWTP sources it's surface water from the Pjäxedammarna dam located on Koön which is transported to the plant through pipelines (Figure 3.1). The first treatment step of the plant is a biological filter and then coagulation using alum and floculation, followed by sedimentation and then rapid sand filtration. For disinfection the water is treated with UV irradiation (Kungälvs Kommun, 2011).

3.1.5 Lysegården

The Lysegaården DWTP is an artificail groundwater recharge plant (AR-Plant), sourcing it's water from Drypesjön and Vallerån (Figure 3.1). After infiltration the water is treated with potassium permanganate and microfiltration.

The water is pH adjusted with sodium hydroxide for corrosion control within the distribution network and then for disinfection the water is irradiated with UV (Kungälvs Kommun, 2011).

3.1.6 Dösebacka

Dösebacka is a DWTP which is located approximately 5 km north of Kungälv, along the Göta Älv river (Figure 3.1). The treatment plant is an AR-Plant and contains one sedimentation basin, 9 infiltration basins and 15 abstraction wells. Water is pumped from the Göta Älv river to the sedimentation basin and is then transferred to the infiltration basins. The water is then abstracted through the 15 wells. Water in 2 wells can have high turbidity and because of this water from those wells is additionally treated with chemical precipitation (aluminum sulphate) and upflow sand filtration. The water is then pH adjusted with soda. Lastly, the water is dosed with UV for disinfection and pumped to a distribution reservoir.

3.1.7 Mölndal

Lake Rådasjön is the primary drinking water source for the town of Mölndal (Figure 3.1). The treatment plant uses coagulation (aluminium chlorohydrate) and Dynasand filters as it's first treatment steps. This is followed by rapid filtration through activated carbon filters. The final disinfection steps of the treatment are UV exposure and low dosing of sodium hypochlorite before being pumped to the distribution network.

3.2 Sample Collection and Preparation

Sampling for the experiment was conducted over the course of two weeks, March 21 to March 29 (Appendix A.1). Several samples were collected from each site at different points along the treatment process, the inflow, treated water before UV irradiation and chlorination and finally the outflow. Because the Läckareback treatment plant does not have UV disinfection sampling was taken instead before ultra filtration.

All samples and replicates were collected in carbon free ashed amber glass vials to minimize light exposure. Vials were cleaned using detergent and then rinsed twice in a 0.1 M HCl solution. The vials were then rinsed three times in MQ water, excess water was poured out of the vials before they were covered tightly with foil. The vials were then combusted for 5 hours in a 500°C furnace. The caps to the lids of AOC vials were submerged in a 10% sodium persulfate solution and heated in a 60°C for one hour.

The lids were rinsed in MQ water three times and placed back into the warm oven face down on a fresh sheet of foil to dry. The vials were capped with the cleaned lids, the same day of the cleaning.

All samples except for samples used in DOC testing were filtered through a pre-flushed 22 nm polypropylene filter, fitted on site. The filter was directly attached to collection pipes using plastic tubing which was soaked over night in a 0.1 M HCL solution. Ice packs and coolers were used to ensure samples stayed cold during transportation back to Chalmers University of Technology.

3.2.1 Sample Preservation

Water samples intended for bioassay AOC measurements were pasteurized in a water bath to 72°C for 30 minutes, followed immediately by a 30 min ice bath (Escobar and Randall, 2000). A tray of water was placed into an oven at the intended temperature for an hour before pasteurization to come to temperature. Samples were then placed in the tray ensuring the height of the water bath covered at least half of the height of the bottles. For the ice bath the bottles were again placed in a tray of water half the height of the bottles surrounded by ice packs which were placed in the water 5 min prior to the bottles.

Samples intended for non-irradiation AOC testing were pasteurized the same day of collection, sample which were irradiated were pasteurized the day of irradiation (Table A.1). The samples were then wrapped in foil to minimize light exposure and kept at 4°C until shipment from Chalmers University to the Norwegian University of Science and Technology (NTNU). Samples were at room temperature during the shipping process from April 5, 2018 to April 12, 2018. After preservation sample analysis began at NTNU on April 12, 2018. Samples waiting for analysis at NTNU were again kept at 4°C.

3.3 Laboratory Work

The preparation and testing of all parameters except for AOC took place at the Environmental Chemistry Laboratory of Chalmers University of Technology. Work was conducted between March 21 to March 30. AOC bioassay experiments were conducted at the Water Analysis Laboratory of NTNU, over the period of three weeks, April 3 to April 20.

3.3.1 UV Irradiation

At Mölndal, Uddevalla, Lackerbäck and Marstrand samples for irradiation were collected into 240mL vials. Due to a shortage of available total vials at Lysegården,

Dösebacka and Varberg, samples for irradiation and other testing were instead collected into 750 mL vials. Samples from each DWTP were all irradiated at Chalmers University of Technology. Non-irradiated water samples were individually transferred to an ashed, widemouthed carbon free jar (Figure 3.2 :a). The jar was then covered with foil to avoid contamination and the UV lamp (Figure 3.2 :b) was poked through the foil. The UV lamp was rinsed with MQ before each irradiation. The jar was placed on an agitator to ensure the water sample was fully mixed during the irradiation to ensure equal contact with the UV lamp (Figure 3.2 :c). The entire set up was covered with a black Styrofoam box to prevent light scattering when the lamp was irradiating (not pictured).

Figure 3.2: Experimental irradiation set-up, a) Carbon free amber ashed jar b) UV lamp c) Vial agitator

Two replicate samples were separately irradiated from each DWTP. After irradiation the sample was transferred to a clean 240 mL ashed glass bottle and labeled. The samples for AOC testing then went through the pasteurization method previously described in section 3.2.1. Testing for fluorescence, absorption, pH and DOC were collected directly from the irradiation vial.

Because past studies have shown UV irradiation could either increase or decrease AOC production each irradiated sample, from each treatment plant, was exposed to an excessive UV dose. Following minimum required dosing from both Norway and the US EPA, an overdose was considered to be above 200 mJ/cm^2 (US EPA, 2006; Ødegaard et al., 2014). The UV lamp used in the experiment emitted at a constant intensity of 1.6 mW/cm^2. The UV dose was then calculated by multiplying UV intensity with irradiation time (Appendix B.1). The time each sample was exposed to the UV lamp was determined by the initial transmittance at each sample site (Table 3.1). Transmittance is the amount of light which is able to pass through a sample without interference from absorption, scattering or reflection. This parameter was chosen as a good indication for the effectiveness of the irradiation.

Table 3.1: Transmittance and resulting irradiation time for each of the DWTP. *Lackerbäck was initially only irradiated for 10 minutes, then when the final UV dose was determined, the Lackerbäck samples were re-irradiated for 6 minutes, bringing the total irradiation time to 16 min.

DWTP	**Transmittance [%]**	**Irradiation Time [min]**
Lackerbäck	93.1	10, 6*
Mölndal	89.1	10
Varberg	90.8	12
Marstrand	80.2	5
Dösebacka	93.5	17
Lysegården	92.7	15
Uddevalla	89.1	10

3.3.2 Fluorescence and Absorbance

The Aqualog fluorescence spectrophotometer (Horiba Inc.) with a 10mm path length was used in CDOM fluorescence analysis. The EEMs were measured by scanning the excitation wavelengths from 240 nm to 650 nm in 3 nm increments and 2 second integration time. Scanning emission ranged from 240 nm to 800 nm with 2.33 nm increments (Heibati et al., 2017). Both fluorescence measurements were done at a constant 20°C using a cooling unit. Measurements were conducted using a quartz cuvette, which was rinsed with MQ water in between each sampling. To aquire blank EEMs ultra-pue water sealed in a quartz cell and MilliQ water in the sample cell were used. These blank scans were then followed by scans of MQ water from the Chalmers' laboratory for testing the cleanliness of the quartz cuvette. Duplicate fluorescence measurements were taken for all DWTPs before and after irradiation.

Data from the fluorescence spectrophotometer was analyzed using parallel factor analysis (PARAFAC) by Masoumeh Heibati using the N-way and drEEM toolboxes for MATLAB (Murphy et al., 2011). Before PARAFAC modeling, fluorescence EEMs were preprocessed as following. Raman and Reyleigh scatter bands were removed from EEMs and data were corrected for the concentration biases (inner filter effect) and converted to Raman unit (RU) using the raman area calculated from blank EEM. The fluorescence intensity of each component was calculated by multiplying the maximum loading of excitation and emission spectra by its score.

Absorbance was measured at least once for each DWTP before and after irradiation. The absorbance was only measured once before and after for the samples from Uddevala. It was measured once before and twice after irradiation for the samples from Lackerbäck and Mölndal. For the remaining treatment plants the absorbance was measure twice for both before and after irradiation. The absorbance was not measured using the fluorescence spectrometer but instead using a Shimadzu Spectrophotometer, UV-1800. MQ water from the Chalmers' laboratory was measured as a blank.

3.3.3 Dissolved Organic Carbon (DOC)

Samples for DOC were collected at two different points along the treatment process, the intake and before on-site UV exposure, or before ultra filtration at Lackarebäck. Samples for BIR and AIR were filtered on-site using a pre-flushed 22 mM polypropylene filter, fitted on site. Samples from the intake were collected without filtration; these samples were then filtered using a pre-flushed polypropylene 45 mM at Chalmers University of Technology on the same day as collection. Samples after irradiation were transferred to clean carbon free 45mL vials for DOC testing. DOC samples were acidified using 2M HCl to pH 3 on the day of sampling or irradiation. Samples after being acidified were stored at 4°C and analyzed within one week.

DOC was measured using a Shimadzu TOC-VCPH carbon analyzes with the TOC-ASI-V auto-sampler, using the non-purgeable organic carbon (NPOC) method. DOC mass concentration values were calculated using a five point calibration curve established by analyzing potassium hydrogen phathalate standard solutions, ranging from 0 to 20.0 mg C/L. This was followed by the subtraction of a MQ blank (CEN, 1997).

3.3.4 pH

The pH was measured once for each of the DWTP both before and after irradiation. Approximately 2 mL of each sample was poured into a clean 5 mL beaker for the measurements. Non-irradiated samples were poured directly from collection containers into the beaker. Irradiated samples were taken from storage vials after they had been transferred from the experimental set up. All measurements were taken using the same pH meter, a VWR pH110, which was calibrated each day. MQ water was used to rinse both the pH probe and the testing beaker before each sample was tested.

3.3.5 HPSEC

HP-SEC was run to see the distribution of apparent molecular weights of NOM at each of the sites and to see if irradiation changed this distribution. HP-SEC testing was conducted using a Shimadzu HPLC system with UV detectors. An Agilent Bio Sec-5 column was used in this experiment with a mobile phase of 100 mM NaCl, 8.3 mM KH_2PO_4 and 11.7 mM K_2HPO_4 pumped at 0.5 mL/min.The molecular sizes of the eluted molecules were calibrated against retention time using polyethylene glycol standards, PEG 10 calibration kit by Agilent (Moona et al., 2018).

3.4 Bio-assay Assailable Organic Carbon (AOC)

The protocol used in this experiment were established by LeChevallier et al. as a rapid way of measuring AOC. There are several steps to this procedure, first a colony count of both strains of bacteria, Pseudomonas fluorescens strain P-17 and Spirillum strain NOX (LeChevallier et al. 1993). The working suspension of the two bacteria, P17 and NOX, was made by Ingrid Johansen at NTNU following standard methods (APHA, 2012). Phosphate buffered solution (PBS) was created following standard methods, 9050C.1a, as a dilution solution (APHA, 2012). After which sample may be inoculated with a known volume and then AOC values for acetate and oxalate may by calculated.

R2A Agar plates were made in order to plate the samples following a slightly modified standard method, 9215A.6c (APHA, 2012). In a 1 L Erlenmeyer flask 9.1 g of Agar was combined and heated with 500 mL of MQ water to dissolved the powder. The flask was then covered and autoclaved, and then cooled for 15 min at room temperature. Under a fume hood, a new stack (33 plates) of carbon free petri dishes was opened. Approximately 15 mL of the agar solution was poured into each petri dish under a fume hod. A low intensity UV lamp was then turn on in the fume hood to remove any bacterial contamination on the outside of the plates. The plates were then allowed to harden in the fume hood for 24 hours. The plates were stacked back into their original plastic sleeve, which was taped shut to prevent contamination before use.

3.4.1 P17 and NOX Colony Count

In order to know how much inoculum of each bacteria was to be added to samples a colony count was done for the 2 working suspensions of both P17 (P1, P2) and NOX (N1, N3). Four dilutions, 10^{-1} - 10^{-4}, were plated 3 times for each of the suspensions, the method was the same for each. Each suspension of NOX or P17 were plated separately for the initial colony count.

The work station was sanitized using a 10% solution of alcohol. The prepared solution of PBS was agitated and then once opened, the rim was flamed before 180 μL of the solution was added to a well of a carbon free assay plate for each dilution using a autoclaved pipette tip. The lid to the assay plate was kept on-top to prevent any contamination while not in use. The vial of bacteria was then agitated for 30 seconds and again the rim of the container was flamed once opened. For the first dilution 20 μL of the inoculum was added to the first row. The opening to the vial of bacteria was then immediately flamed again and the lid was re-secured. The first dilution in the plate was then fully mixed 20 times using a 200 μL tip to ensure a successful dilution. From this first dilution 20 μL of sample was transferred to the second row to the assay plate to create the second dilution (10^{-2}). The same procedure was followed for all dilutions.

A new pipette tip was then used starting at the lowest dilution to transfer 10 μL of each dilution to a prepared and labeled R2A agar plate. The lid was then placed on top of the plate and it was held vertically in order to let the drops distribute down the plate without letting the rows touch or reach the bottom (Figure 3.3). The plate was set on the work bench until the liquid was completely absorbed into the agar, afterwards it was placed in an incubation box kept at room temperature, approximately 20°C.

Figure 3.3: Example of plated bacteria sample onto R2A agar petri dish, dilution 10^{-1} - 10^{-4} Figure is adapted from Michael Waak.

The incubation period for the plated bacteria counts was 3 days after which the plates were read. The dilution to count was selected based on readability, colonies should be distinct and easy to identify individually. The colony count of a dilution should be between 15-125, higher colony counts were preferred. Individual bacteria colonies were counted visually with the aid of a Gallenkamp colony counter. The colony counter which was back-lit and had a pressure plate which would record and update the count when activated. Plates would be placed in the counting space and a marker was used to indicated already counted colonies. This procedure was also used for sample testing. These plate counts were then converted to colony forming units per milliliter (CFU/mL) of stock inoculum and used in the following equation (3.1) to calculate the volume of inoculum.

$$Volume of inoculum = \frac{(500 CFU/mL)(40 mL/vial)}{CFU/mL stock inoculum} \tag{3.1}$$

There were two established stock incoulums available for P17 and for NOX, each was tested for the volume of inoculum required (Table 3.2). The colony count for both P17 solutions revealed very low CFU/mL values. This resulted in very large volumes of P17 required for the inoculation of each sample and because of this high volume both P17 stock solutions were used in the testing of samples (Table A.2). The NOX 3 strain was selected for all sample testing because of the lower required volume of inoculum.

Table 3.2: Calculated average volume of inoculum and standard deviation (n=4) for available stock strains of both NOX and P17.

Strain	Average Volume of Inoculum [μ L]	SD
NOX 1	26.05	2.45
NOX 3	22.53	3.16
P17 1	1281	110
P17 2	1654	98.9

3.4.2 Sample AOC Testing

Native AOC was examined during this experiment. Samples were specifically taken before the chlorination treatment step at each of the sample sites, because of this chlorine neutralization using sodium thiosulfate was not necessary for any of the samples examined. The protocol for native AOC testing have been laid out in Figure 3.4.

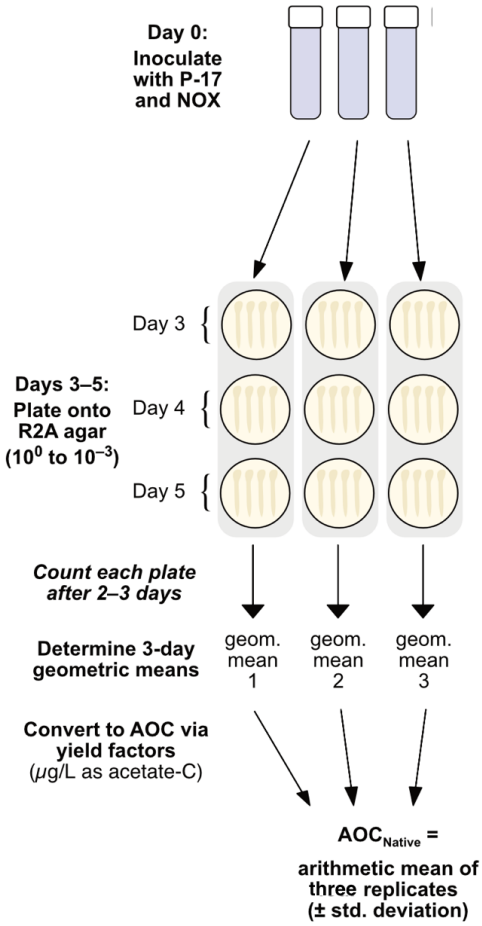

Figure 3.4: Protocol and time-line for AOC testing from inoculation to determination of native AOC concentrations. Figure is adapted from Michael Waak.

For colony counts, NOX and P17 are plated separately but for sample testing both bacteria are added simultaneously to the sample. Each sample set from a treatment plant had four individual samples within it. One sample was used as a negative control and the other three were inoculated with the known volume of inoculum of both NOX and P17 on day 0 (Table 3.2). Samples were agitated for 30 seconds after inoculation to ensure fully mixed solutions. The samples were then placed in a temperature controlled incubation unit, kept at 20°C. Three days after inoculation all samples were plated. Vials were shaken to ensure a fully mixed sample for 30 seconds before samples were transferred for dilution. The same dilution procedure was followed from the P17 and NOX colony count. Only three serial dilutions were done for the samples, however, 10^{-1} - 10^{-3}. An inoculated undiluted sample (10^{0}) was also plated. After the dilutions had been plated, a new pipette tip was then used to transfer 10 μL of undiluted inoculated sample to the same plate.

Plates could be counted 2-4 days after inoculation, in this study all petri dishes were counted 3 days after plating (LeChevallier et al. 1993). NOX and P17 can be counted at different dilutions, as seen in Figure 3.5. The counting procedure was the same for samples as it was for the P17 and NOX colony count, again importantly bacteria colonies needed to be distinct in order to be counted with an aim of between 15-150 colonies per plate, with a higher colony count being desired.

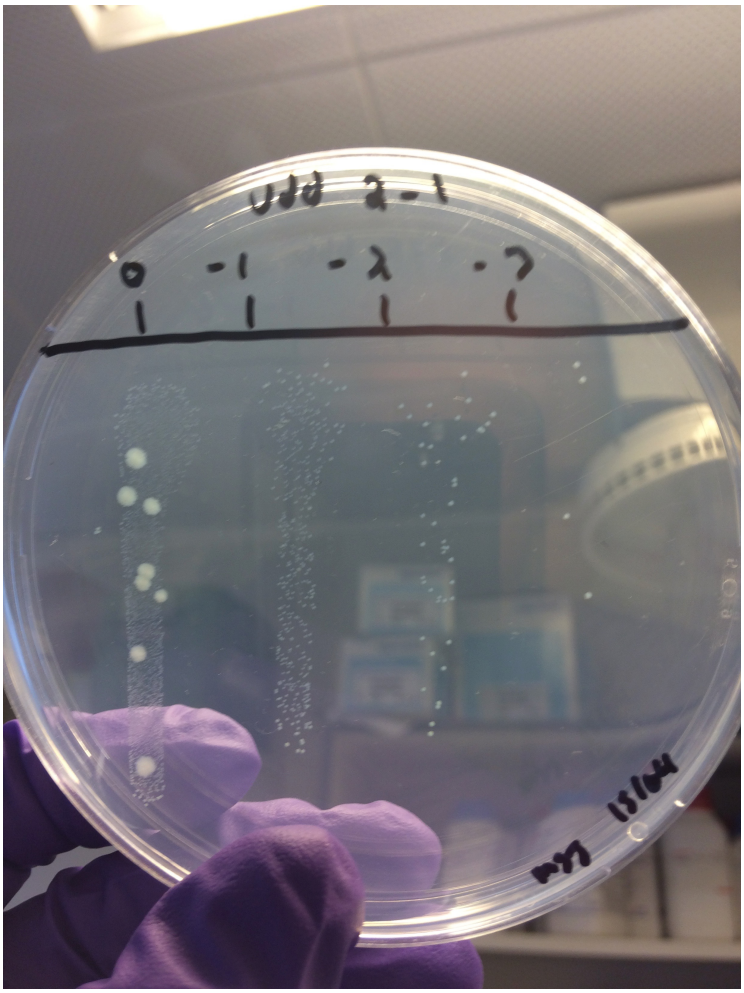

Figure 3.5: Growth for Uddevala BIR, three days after plating, before irradiation replicate #2, plate # 1. Colonies of P17 (larger cream dots) can be observed at the 10^0 concentration. Colonies of NOX (smaller white dots) are observed at all concentrations on the plate. While a count of NOX bacteria at concentration 10^{-1} would have yielded a higher colony count, it would have been too difficult to count distinct individual colonies. For this reason the NOX colonies were counted at the dilution 10^{-2}.

Non-irradiated sample sets from Marstrand were plated using the standard method, day 3,4 and 5 after inoculation. Several other non-irradiated sample sets from different treatment plants were also plated in addition to day 3 on day 5 (A.2). For samples which were plated on more than one day, geometric mean was taken for of a sample over the 2-3 days of plating. The geometric mean of each of the samples was

then used in the calculation of AOC concentrations, Equation 3.2. The arithmetic mean was then taken of the three samples, this is the value reported in this thesis.

$$\mu gAOC/L = [(meanP17CFU/mL)(\mu gacetate - c/4.1 * 10^6 CFU) + (meanNOXCFU/mL)(\mu goxalate - c/2.9 * 10^6 CFU)] * (1000mL/L) \quad (3.2)$$

3.4.3 Quality Control

A negative control was plated 4 times undiluted and read on day 3 for each set of samples which was inoculated and tested. Each DWTP has 1 for before irradiation and 2 after irradiation negative controls. This was done to ensure that no native bacteria was present in the samples, which would appear as growth on the plate (Figure 3.6). Derived empirical yield values were used in the is experiment for AOC calculations from the standard. Because of this a blank control, yield control and growth control were not tested or calculated. It was assumed that carbon was the limiting factor and because of this assumption these controls were deemed unnecessary (APHA, 2012).

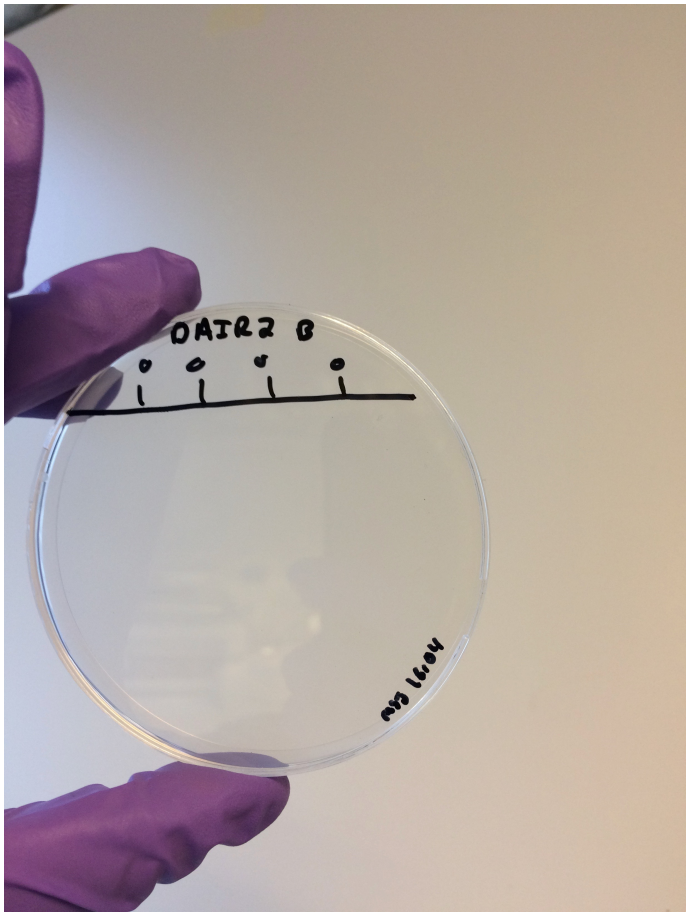

Figure 3.6: Negative control for Dösebacka after irradiation replicate #2, plated undiluted. No contamination can be seen.

4

Results and Discussion

4.1 DOC and pH

DOC before and after irradiation stayed consistent or decreased for most plants (Table 4.1). Generally DOC values ranged from 0.9-1.3 mg/L for the AR treatment plants and from 1.8-4.0 mg/L for the surface water treatment plants. High levels of dissolved molecules in the Marstrand samples increased the interference of light passing through the samples and therefore, decreased the transmittance. The DOC levels for most plants were consistent with values reported in past studies, <4 mg/l (Heibeti et al. 2017, Moona et al. 2018). The Marstrand samples also notably had raw water DOC levels which were at least twice as high as the other DWTPs'.

The consistency the DOC results shows that the carbon is not being added to the water samples. Any increase in AOC concentrations would be a result of the changing structure of the carbon and not the increasing quantity. Mölndal's treatment plant had a large increase in DOC after irradiation. It should be noted that Mölndal did not a have replicate sample for after irradiation. Because nothing was added to the samples during irradiation, the Mölndal AIR sample was eliminated from the data set because it was seen as an outlier. The variance of the DOC results was between 0-2% for all of the samples sites.

Table 4.1: Averaged DOC [mg/L] values with standard deviation (SD) from each treatment plant, before and after irradiation and raw untreated intake water. Average value was determined using 2 replicates unless denoted where only one sample was tested (1).

DWTP	**Before Irradiation**		**After Irradiation**		**Raw**	
	Mean	SD	Mean	SD	Mean	SD
Lackerbäck	1.8	0.02	1.7	0.02	4.8 [1]	—
Mölndal	2.7	0.30	3.4 [1]	—	4.0 [1]	—
Varberg	1.8	0.00	1.8	0.03	2.6	0.04
Marstrand	4.0	0.02	3.9	0.00	13.4 [1]	—
Dösebacka	1.3	0.06	1.3	0.02	4.0 [1]	—
Lysegården	0.9	0.14	1.0	0.00	1.0 [1]	—
Uddevalla	2.9	0.06	2.9	0.02	2.6 [1]	—

Samples were tested for pH before and after irradiation to see the affect of irradiation on the water set up. The pH of all samples decreased slightly after irradiation, with the exception of the Varberg sample which had a slight increase in pH (Table 4.2). All treatment plants have a similar pH value both before and after irradiation suggesting any differences seen in AOC values between the plants was not a result of differing pH values.

Table 4.2: pH for each treatment plant before and after irradiation

Drinking Water Treatment Plants	Before Irradiation	After Irradiating
Lackarebäck	7.42	5.50
Mölndal	7.20	7.13
Varberg	7.03	7.23
Marstrand	7.73	7.57
Dösebacka	7.97	7.71
Lysegården	7.74	7.72
Uddevalla	7.65	7.62

4.2 Fluorescence and Absorbance

Absorbance was measured for each of the samples before as well as after irradiation, the change in the absorbance was plotted (Figure 4.1) to show trends in the data. Uddevalla did not have a replicate available for absorbance testing. Replicates from the Marstrand and Varberg DWTPs had outlier data which was removed from analysis.

Marstrand had the highest initial absorbance, which again correlates to the high DOC values seen in both the raw and treated water from the plant. This also correlates with the lowest transmittance (Table 3.1). Lackarebäck and Varberg had similar DOC values for treated water (Table 4.1) however, when measuring absorbance Lackarebäck had a lower initial absorbance and a larger change in absorbance after irradiation than Varberg. The raw water of Lackarebäck had higher DOC values than Varberg which could explain the differences in changing absorbance. Varberg uses a combination of surface and ground water, which like the AR-plants, Lysegården and Dösebacka could explain it's low change in absorbance but slightly higher initial absorbance.

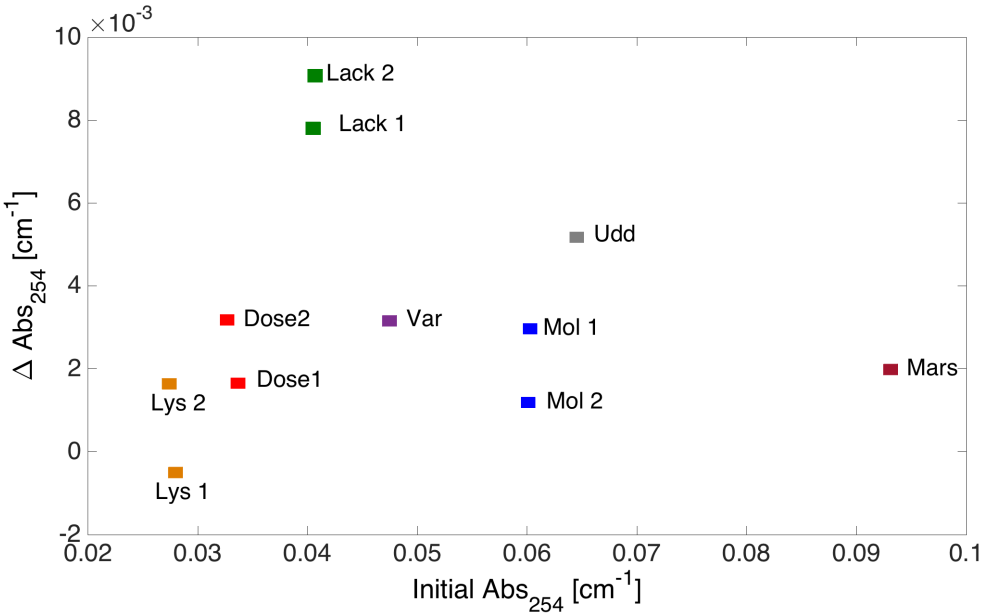

Figure 4.1: Change in Abs_{254} plotted against initial Abs_{254} for each treatment plant. Initial Abs_{254} are absorbance measurements taken before irradiation. Replicate samples for the DWTPs are denoted by 1 and 2 where available.

Interestingly, there is little variance both in the initial absorbance and in the change of absorbance for samples sites which have replicates available. The inter-replicate variance for before and after irradiation was 4% and 5% respectively. This differs from later methods, mainly the AOC bioassay method (Section 4.4) which has an overall precision of ±17.5% (APHA, 2012). This highlights the need for an optical method of estimating regrowth potential in drinking water samples.

A five-component PARAFAC model was calculated which explained 99% of the variation in fluorescence data. Spectral properties of PARAFAC components are represented in Figure 4.2 and 4.3. According to the published literature, the first four components C1, C2, C3 and C4 (a-d) are classified as humic-like dissolved organic matter (DOM) and C5 (e) is classified as protein-like DOM (Coble, 1996; Coble, 2007; Murphy et al., 2011).

The humic-like component C1 had peak emissions at 420 nm, and peak excitation at 322 nm. This is comparable to peak values found in literature of 448 nm for emissions and 340 nm for excitation (Coble, 1996). Additionally, the protein-like component C5 (Figure 4.2 e) showed similar peak emission, 340 nm, and excitation, 300 nm, to previous studies which showed global models ranged between 310-348 nm and 250-290 nm, respectively (Coble, 1996; Murphy et al., 2011).

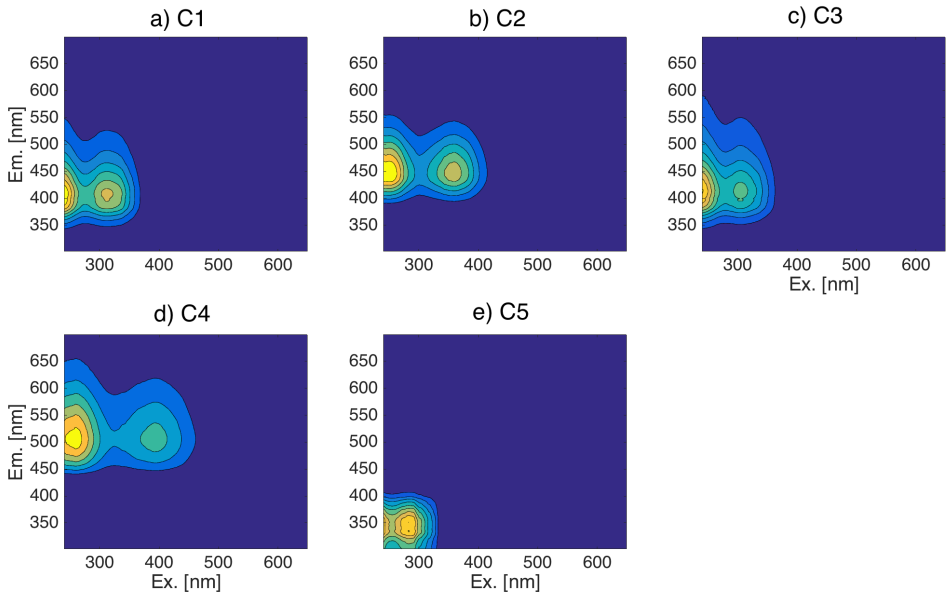

Figure 4.2: Spectral EEM representations PARAFAC components C1-C5 (a – e).

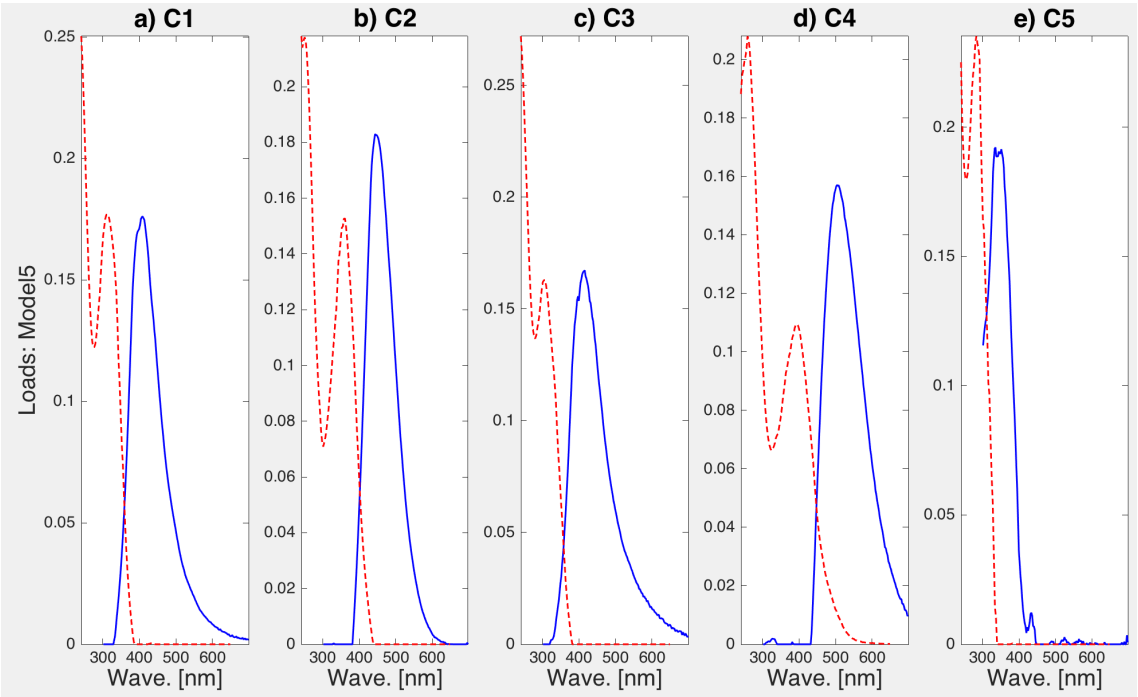

Figure 4.3: PARAFAC components C1-C5 (a-e) and their respective excitation (dashed red line) and emission (solid blue line) spectra.

Components C1 and C5 were selected to further examine at each of the treatment plants before and after irradiation. C1 (Figure 4.4) was selected as a representation of the humic-like DOM and C5 (Figure 4.5) the protein-like DOM.

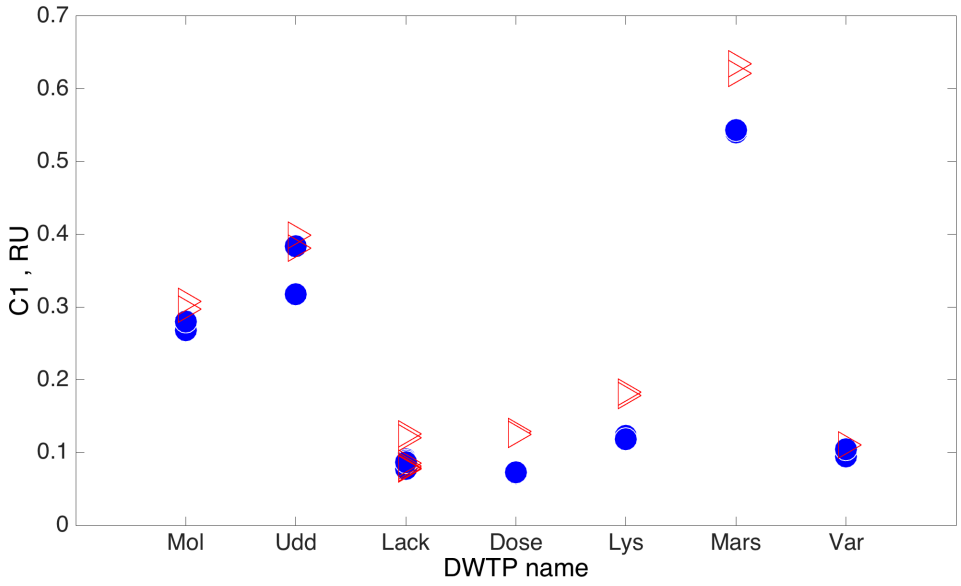

Figure 4.4: PARAFAC component C1 (humic-like) at each DWTP before (red unfilled) and after (blue filled) irradiation. Some treatment plants had multiple fluorescence measurements and all of which were included in the figure.

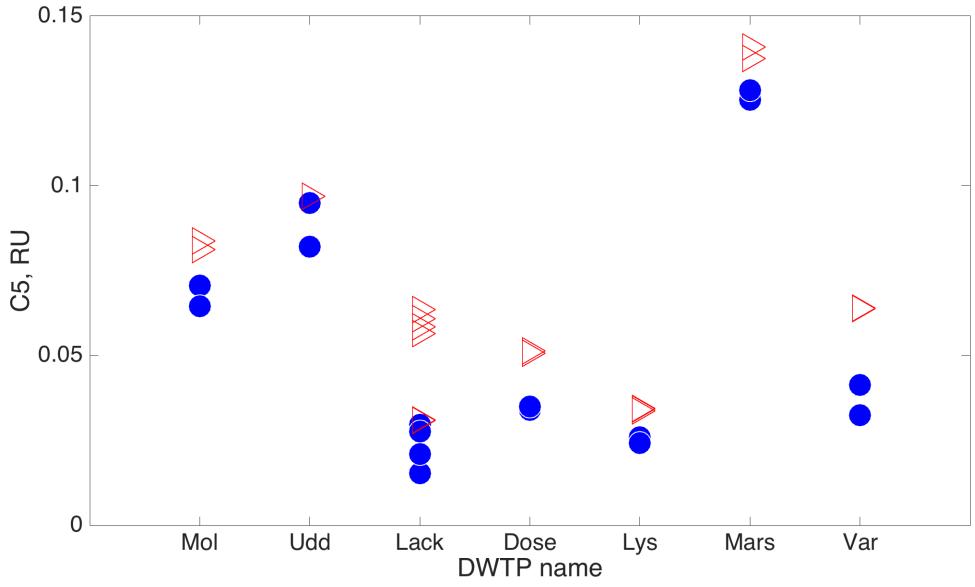

Figure 4.5: PARAFAC component C5 (protein-like) at each WTP before (red unfilled) and after (blue filled) irradiation. Some treatment plants had multiple fluorescence measurements and all of which were included in the figure.

All treatment plants saw an decrease in component C1 (humic-like) substances after irradiation. Marstrand had the greatest decrease, followed by Lysegården and Dösebacka, both of which had similar changes. Marstrand had the largest values both before and after irradiation for humic-like substances. Lackarebäck had simi-

lar initial values to Dösebacka, Lysegården and Varberg, which were the treatment plants utilizing 100% and 20% of ground water respectably. Mölndal, Uddevalla, Lackarebäck and Varberg showed statistically insignificant changes from before to after irradiation.

Similar to the C1 component, Lackarebäck had similar initial C5 (protein-like) values to Dösebacka, Lysegården and Varberg. Mölndal and Uddevalla have similar initial values which was also consistent with results of the humic-like substances. Both treatment plants use Dynasand filtration followed by rapid filtration through GAC filters, the plants however, differ on the coagulant used before the rapid filtration which could explain the slightly higher initial value of the Uddevalla sample. Marstrand utilizes a biological filter which differs from the other sample sites treatment processes. This could be the reason for Marstrand's consistently higher values seen in both component C1 and C5. It was been shown that pH can have an influence on fluorescence intensity, which differs between fluorophores. Tyrosine-like (protein-like, C5) fluorescence has been documented to be more sensitive to changes in pH than humic-like (C1) fluorophores (Reynolds, 2003). Based on the pH results (Section 3.3.4) this does not appear to influence the PARAFAC results.

4.3 HPSEC

HPSEC is an analysis of the distribution of molecular weights of NOM. From DOC results (Table 4.1), it was observed that for each of the samples, dissolved carbon did not show a significant increase. It was hypothesized then that any changes in molecular weight distribution would be from the irradiation of the different samples. Figure 4.6 on the following page shows, the apparent molecular weight distribution at four different apparent weights, 325, 850, 1260 and 1780 Da.

In general at the largest apparent molecular weight (1780 Da), there was a decrease after irradiation, except for Mölndal, Dösebacka and Lysegården which showed no significant change. Lackarebäck had the largest decrease. For the next two apparent molecular weights (1260, 850 Da) all of the DWTPs showed a decrease from before to after irradiation. The most significant decreases were observed at Lackarebäck for both apparent weights and Uddevalla and Mölndal for the respective apparent weights. At the lowest apparent weight (325 Da) there was an increase after irradiation observed at Lackarebäck and Uddevalla. It is possible that the decrease seen in the molecular apparent weights after irradiation for the remaining DWTPs which did not see an increase in the smallest size, had an increase at apparent weights which were below the detection measured in this experiment. A study by Lehtola et al. confirms that UV irradiaiton can reduce the fraction of larger molecules and increase the fraction smaller more bioavailable molecules (Lehtola et al. 2003). This is confirmed by a second study which indicates a decrease in larger molecular sizes and an increase in smaller molecular weights at UV doses between 500-1500 mJ/cm^2 (Bazri et al. 2012)

HPSEC results showed an increase in smaller apparent molecular weights for samples from Lackarebäck. As larger NOM molecules are broken into smaller molecules, they become more bio-available. Lackarebäck increase in total average AOC after irradiation could potentially be a result of the increase in bio-available carbon. The other treatment plants which showed an increase in AOC after irradiation also showed a general decrease in larger apparent molecular weights, although an increase in the smallest measured apparent molecular weight was not seen with the remaining DWTPs.

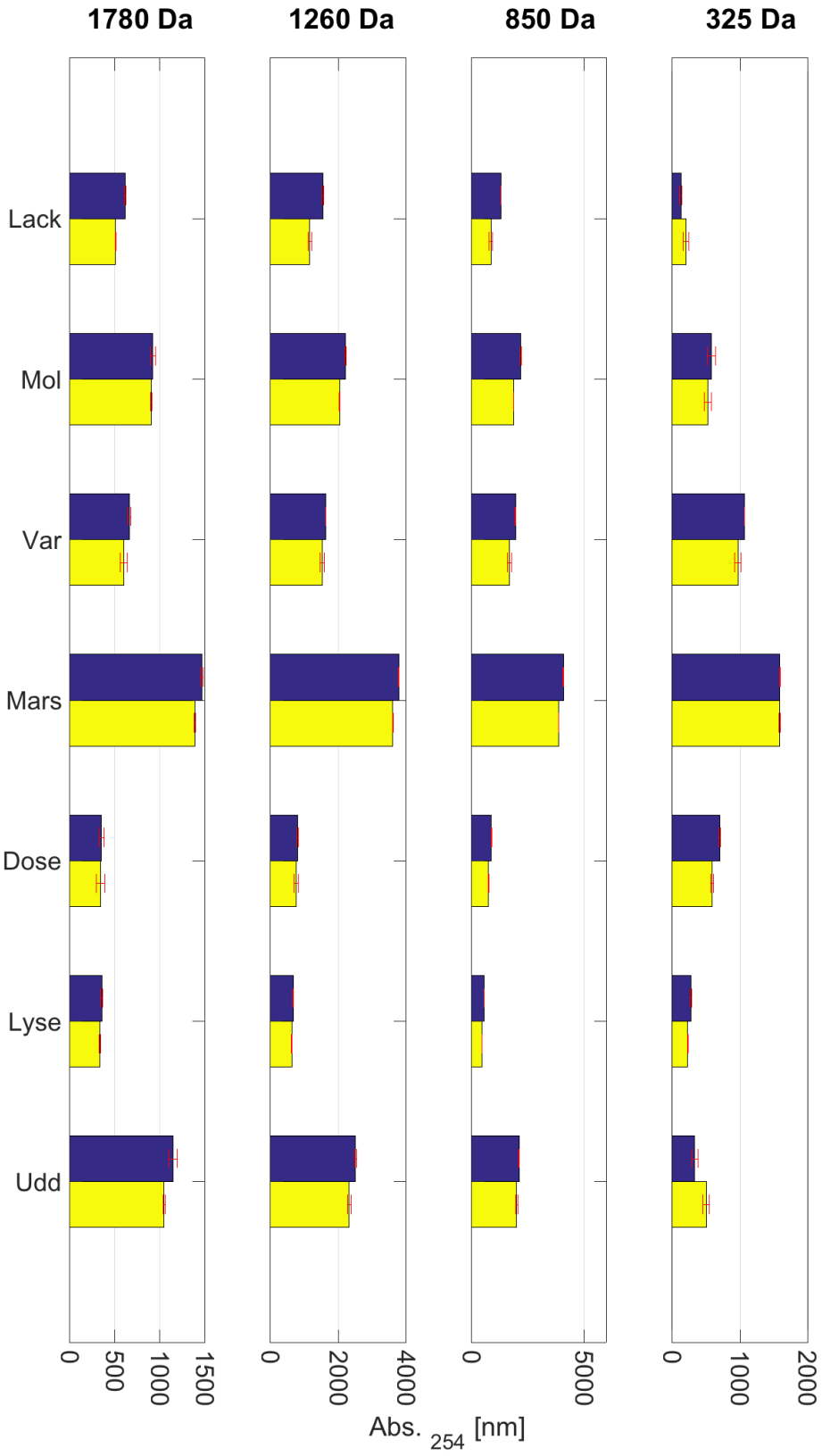

Figure 4.6: Averaged HPSEC molecular weight distribution before (blue) and after (yellow) irradiation for each DWTP with standard deviation (n=2). Two replicates were used in the average and standard deviation. Observe the changing y-axis scale.

4.4 AOC

Total average AOC concentrations increased generally for the treatment plants after irradiation (Figure 4.7). The changes from before irradiation to after were evaluated by calculating the percent change for the total average AOC concentration. The largest percentage increase was observed at the Lackarebäck WTP, a 2200% interest. For all treatment plants the majority of total AOC concentrations were a result of oxalate (NOX) rather than acetate (P17) both before and after irradiation. This is consistent with several previous studies which also report higher concentration of AOC resulting from NOX (Kaplan et al., 1993; Thayanukul et al., 2013; Yang et al., 2011)

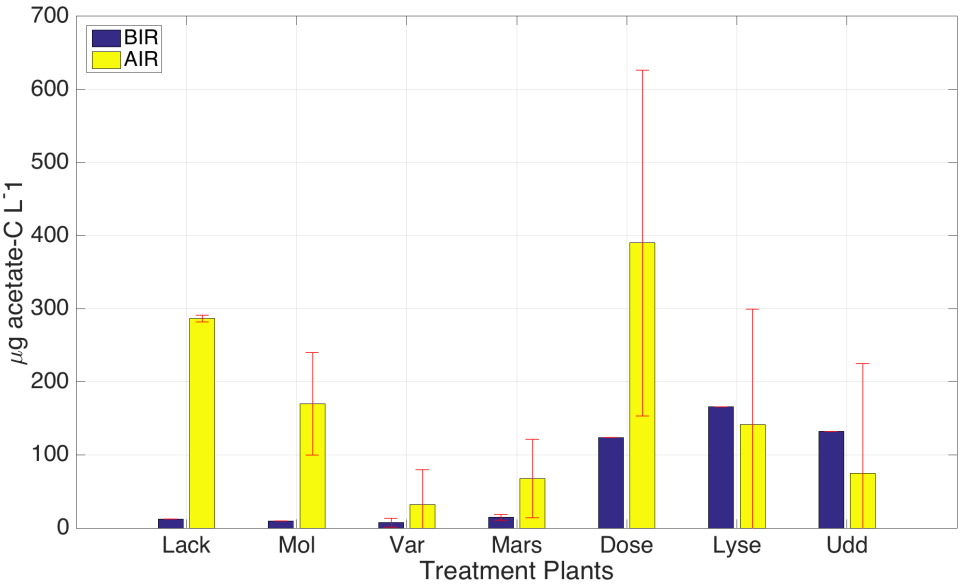

Figure 4.7: Total averaged AOC before and after irradiation. Average is calculated from two replicates for all of the after irradiation samples as well as the before irradiation for Varberg and Marstrand. Standard deviation (n=2) has been calculated and denoted in the error bar.

Initial averaged AOC concentrations for Lackarebäck, Mölndal, Varberg and Marstrand ranged between 2 to 12 µg/L. These surface WTP had lower AOC concentrations in caparison to averaged Flemmish drinking water values which ranged between 30 to 120 µg/L, averaging 72 µg/L (Polanska et al., 2005). Corresponding Swedish literature values, measured using only P17, reported AOC concentrations of 20-30 µg/L (Långmark et al. 2007). Uddevalla was the only WTP utilizing surface water which had a large initial averaged AOC value of 132 µg/L. This was much larger than previously reported values in Stockholm, Sweden (Långmark et al. 2007). Uddevalla averaged AOC values are still lower than reported AOC concentrations for surface water in Finnish treated drinking water, which can be as high as 400 µg/L (Miettinen et al., 1997). Uddevalla was the only surface water treatment plant which had

similar non-irradiated AOC values to the AR plants Dösebacka and Lysegården. For those treatment plants, while initial AOC concentrations were larger than those of the surface water treatment plants, 124 and 166 µg/L respectively, they are similar to findings in Finnish drinking water utilizing ground water as a source (Miettinen et al., 1997).

HPSEC results showed an increase in smaller apparent molecular weights for samples from Lackarebäck treatment plant. As larger NOM molecules are broken into smaller molecules, they become more liable. Lackarebäck increase in total average AOC concentrations after irradiation, an increase from 12 to 286 µg/L, could potentially be a result of the increase in liable carbon. The other treatment plants which showed an increase in AOC after irradiation also showed a general decrease in larger molecular weights, although an increase in the smallest measured molecular weight was not seen with the remaining DWTPs.

Dösebacka, a treatment plant with groundwater as its source and which had a larger initial AOC concentration, also saw an increase in values after irradiation. Assimilable organic carbon concentrations after irradiation were 390 µg/L. This closer to surface water value in Finnish drinking water and twice as high as the reported ground water values of 200 µg/L (Miettinen et al., 1997). Lysegården, had similar AOC values before irradiation however, this treatment plant saw a reduction in AOC values after irradiation, changing from 165 to 141 µg/L. These treatment plants had the lowest DOC values, which remained consistent before and after irradiation (Table 4.1). These treatment plants also had the lowest initial UV_{254} and minimal changes to absorbance before and after irradiation. Both treatment plants had overall decreases in molecular weight distributions (Figure 4.6). However, neither treatment plants saw an increase in the smallest measure molecular weight (325 Da). Changes to molecular weight could be smaller than the detection limit or could be to weights in between the specifically measure weights.

Mölndal had a larger initial absorbance but similar average change to Lysegården and Dösebacka. The Mölndal WTP total average AOC values however, had a percent increase of 1674% from before to after irradiation. After irradiation Mölndal had averaged AOC concentrations of 170 µg/L, which is higher than reported values in Stockholm, Sweden and in Belgium but lower than other surface water treated in Finland (Långmark et al., 2007; Miettinen et al., 1997; Polanska et al., 2005). Lackarebäck had the largest change in absorbance before and after irradiation, water from this treatment plant also had the largest percentage change in AOC values before and after irradiation. Uddevalla which had the next highest change in absorbance had a decrease in averaged AOC values after irradiation, from 132 to 75µg/L. It is difficult to correlate the available absorbance data with AOC data because of the few replicates available for AOC. No correlation between the absorbance and AOC data were found because of the few replicates available for AOC.

Lysegården and Dösebacka had the largest initial concentrations of AOC before irradiation. These treatment plants had the significant decreases to the humic-like components in the PARAFAC analysis. Marstrand which also had the largest C1 (humic-like) values had modest changes in AOC before and after irradiation, increasing from 3 to 68 µg/L. This suggest that change in humic-like substances are not indicators of concentrations of AOC after irradiation. Lackarebäck which showed the largest percentage increase in AOC concentrations had comparatively low values of the humic-like component, showing values similar to Lysegården and Dösebacka and Varberg. Mölndal had negligible changes to humic-like substances, however, had a 1674% increase in AOC concentrations after irradiation. Values from Mölndal were also most similar to the values from Uddevala treatment plant which had a decrease in AOC concentration after irradiation.

The largest change from before to after irradiation in component C5 (protein-like) substances, occurs in samples from Lackarebäck. This treatment plant also has the greatest percentage increase in AOC concentrations, before and after irradiation. Varberg also had a notable increase after irradiation in protein-like substances, as well as a moderate comparative increase in AOC concentrations after irradiation, increasing from 8 to 32 µg/L. The Varberg WTP had values which were similar to reported values in Stockholm, Sweden (Långmark et al., 2007). Dösebacka, Lysegården and Marstrand had similar decreases in component C5 after irradiation but showed varying changes after irradiation in AOC concentrations. Dösebacka had a significant increase, while Marstrand WTP had a smaller increase and Lysegården had a decrease after irradiation in average AOC concentrations.

The standard AOC method has a demonstrated day to day variation of 11-16% (APHA, 2012). Both Marstrand BIR sample sets were plated for the full three days recommended by literature (APHA 2012, LeChevallier et al. 1993). P17 counts showed a day to day average variation of 35%, while NOX day to day average variation was 18%. The NOX variation is similarly aligned with the literature. As previously stated, the volume of P17 inoculum was comparatively large to the NOX volume. This could be the reason for the much higher variation seen in the P17 colony counts and the only slightly elevated variation of the NOX inoculum.

All of the other DWTP, excluding Uddevalla, had a day 3 and a day 5 plating for their BIR samples. The average variation for the P17 counts for these sites was 41%, and for the NOX counts was 28%. These number are much higher than the literature estimates, and slightly higher than the Marstrand day to day variations. It is important to note that day 4 counts are missing for these sites, which could have lowered the day the day day variations, as there can be a 20% cell count increase over the course of the 3 day period (APHA 2012).

Between the replicates, large standard deviations can be seen in Figure 4.7. All of the WTPs had replicate AIR samples which were all plated three days after inoculation. The average variance in the P17 and NOX counts for inter-replicate samples were 127% and 59% respectively. A high degree of variability has been noted

in past studies measuring AOC after UV irradiation (Långmark et al. 2007). This differs from previous results in the change of absorbance which had very consistent replicate results for after irradiation and only a 5% variance (Figure 4.1). Results for DOC after irradiation also had lower variation amongst the replicates for AIR varying between 0-2%. Variance was not calculated for HPSEC results. It is clear from all of the sampling that the P17 counts constantly have the largest variations in comparison to the NOX counts.

This was a seasonally limited project, samples were only collected from each site once during the spring. Past studies in Belgium have shown an increasing trend in total AOC concentrations in the spring, peaking in the summer and a decreasing trend in the fall season, bottoming in the winter (Polanska et al., 2005). Studies in Stockholm using only P17 to measure AOC have found both a positive seasonal result at some sites as well as a negative seasonal effect (Långmark et al. 2007). This increasing trend could bring variability into the data set however, further study would need to be conducted using these samples to know for certain if the seasonal trend is also applicable to Swedish drinking water.

4.5 Previous Work

4.6 Limitations

During the scope of this thesis, AOC was investigated using the bioassay method. Limitations to the available volume of P17 and the colony count of the available material limited the amount of samples which could be inoculated and tested. The colony count performed on the two working suspensions of P17 (#1, #2), showed the volume of inoculum required to be 1.66 mL and 1.28 mL respectively (Table 3.2). Working suspensions are made in volumes of 100 mL and it was decided that three plates should be made from each sample location. Therefore, several samples and replicates were unable to be tested, while the experiment was performed at NTNU. Replicates for only two of the DWTP before irradiation were tested limiting conclusions which may be drawn. The 22 nm polypropylene filter was tested using fluorescence but not tested to see if it could be a potential source of AOC because of the limited supply of viable P17.

Delayed arrival of samples to NTNU also limited the testing. Standard method states that samples should be plated on day 3, 4 and 5 after inoculation to be read on day 6, 7 and 8. Limited time only allowed for one plating day, day 3 for most samples, with the exception of Marstrand before irradiation sample which was plated for both replicates on day 3, 4 and 5. The values used in AOC calculations were single day averaged values between the 3 plates and not a geometric mean over the course of 3 days of plating. Future comparisons between the AOC results are therefore limited.

5
Conclusion and Recommendations

5.1 Summary and Conclusion

- Two sets of drinking water samples with replicates were collected from 7 different water treatment plants in Sweden before on-site disinfection. The treatment plants had varying treatment processes as well as different source waters, including surface and artificial ground water. At Chalmers University of Technology one set of samples were then exposed to UV doses ranging from 960 to 1655 mJ/cm^2. Measurements were taken before and after irradiation for pH, DOC, HPCSEC and fluorescence and absorbance. Samples before and after irradiation were preserved and sent to the Norwegian University of Science and Technology for AOC testing using the rapid bioassay method.

- UV irradiation at doses ranging from 960 to 1655 mJ/cm^2 can split unreactive NOM into smaller more bio-available molecules. For the larger apparent molecular weights there was a general decrease for all DWTPs. Two DWTPs, Lackarebäck and Uddevalla, showed an increase at the smallest apparent molecular weight, 325 Da. Large molecules, however, could also have been split into compounds with smaller apparent weights than were able to be detected using the HPSEC method described in this thesis.

- For most samples, both NOX and P17 contributed to an increase in total averaged AOC concentrations after irradiation. The DWTPs which showed the largest total increases had a higher percentage of total average AOC resulting from oxalate (NOX) than from acetate (P17).

- Total average AOC values increased after irradiation for most treatment plants. The largest increases were seen in treatment plants which utilized surface water as their source. Treatment plants which used artificial groundwater had the smallest or negligible changes to total average AOC concentrations.

- Fluorescence and absorbance measurements only measure a subset of DOC, FDOM and CDOM, respectively. These measurements do not appear to correlate with AOC measurements and cannot be used as substitute measurements to the AOC concentrations before or after irradiation.

- Absorbance and fluorescence measurements had very little variance in comparison to AOC variance values seen in this experiment. The bioassay method of AOC testing has a known precision of ±17.5%. Further study could be done with more replicate samples from each treatment plant in order to lower the variance of the AOC measurements after irradiation. This would allow for a more statistically significant comparison.

- Individual components, C1 (humic-like) and C5 (protein-like) were further examined to see if there was a correlation between the change from before to after irradiation and the general increase in total averaged AOC after irradiation. All of the treatment plants saw and decrease in both components after irradiation. However, there was no trend in the data which indicated a clear correlation between the change in these two components and the percentage increase in total averaged AOC. Peak emission and excitation for the humic-like and protein-like components were constant with observed literature values.

5.2 Further Study and Recommendations

HPSEC testing and results appears to show that at high UV doses larger apparent molecules are split into smaller labile molecules. Future studies could investigate different UV doses, especially lower doses more similar to those typically used on-site at DWTPs, between 40 and 200 mJ/cm^2. Further testing could be done as well to measure smaller molecular weights in order to confirm that the decrease in large molecular weight molecules resulted in an increase in molecules with molecular weights smaller than 325 Da.

Lack of time and low P17 colony counts limited the number of samples that could be tested by the AOC method. For example, there was insufficient P17 to measure AOC blanks in Milli-Q water filtered through the pre-flushed 22 nm polypropylene filter. The filter was tested using using fluorescence to show it was not leeching, however, this study has shown little correlation between fluorescence measurements and AOC concentrations. It is possible that the filter may have contributed to AOC concentrations measured at each site. Further studies should attempt to replicate the results seen in this study and reduce the measurement variation by increasing the number of replicate samples. To have greater confidence in statistical tests, multiple replicates should be taken from each site, both before and after irradiation.

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A

Appendix A

A.1 Sample Collection and Testing Schedule

Table A.1: Sampling and irradiation date for each WTP

WTP	**Sample Collection**	**Sample Irradiation**
Lackerbäck	March 26, 2018	March 26, 2018
Mölndal	March 21, 2018	March 21, 2018
Varberg	March 29, 2018	March 30, 2018
Marstrand	March 27, 2018	March 30, 2018
Dösebacka	March 28, 2018	March 30, 2018
Lysegården	March 28, 2018	March 30, 2018
Uddevalla	March 22, 2018	March 22, 2018

Table A.2: Sample inoculation, platting and count date for each sample. Two different P17 strains were used for inoculations, these have also been denoted. Some samples were plated and counted over several days, all dates have been included. Two sets of samples were taken to test if the filter used on-site at the WTP was leaching AOC. Milli-Q water was run through the filter and collected unfiltered (MQF, MQ). Samples which were not tested due to limitations are marked NT (Section 4.6)

Sample	Inoculation	Platting	Bacteria Count	P17 Strain
MQF 1	NT	NT	NT	NT
MQF 2	NT	NT	NT	NT
MQ 1	NT	NT	NT	NT
MQ 2	NT	NT	NT	NT
Lack BIR 1	12/04, 14/04	15/04, 17/04	18/04, 20/04	P17 2
Lack BIR 2	NT	NT	NT	NT
Lack AIR 1	14/04/2018	17/04/2018	20/04/2018	P17 1
Lack AIR 2	13/04/2018	16/04/2018	19/04/2018	P17 1
Möl BIR 1	NT	NT	NT	NT
Möl BIR 2	12/04/2018	15/04, 17/04	18/04, 20/04	P17 2
Möl AIR 1	14/04/2018	17/04/2018	20/04/2018	P17 1
Möl AIR 2	13/04/2018	16/04/2018	19/04/2018	P17 1
Var BIR 1	12/04/2018	15/04, 17/04	18/04, 20/04	P17 2
Var BIR 2	12/04/2018	15/04, 17/04	18/04, 20/04	P17 2
Var AIR 1	14/04/2018	17/04/2018	20/04/2018	P17 1
Var AIR 2	13/04/2018	16/04/2018	19/04/2018	P17 1
Mar BIR 1	12/04/2018	15/04, 16/04, 17/04	18/04, 19/04, 20/04	P17 2
Mar BIR 2	12/04/2018	15/04, 16/04, 17/04	18/04, 19/04, 20/04	P17 2
Mar AIR 1	13/04/2018	16/04/2018	19/04/2018	P17 1
Mar AIR 2	14/04/2018	17/04/2018	20/04/2018	P17 1
Döse BIR 1	12/04/2018	15/04, 17/04	18/04, 20/04	P17 2
Döse BIR 2	NT	NT	NT	NT
Döse AIR 1	14/04/2018	17/04/2018	20/04/2018	P17 1
Döse AIR 2	13/04/2018	16/04/2018	19/04/2018	P17 1
Lyse BIR 1	12/04/2018	15/04, 17/04	18/04, 20/04	P17 2
Lyse BIR 2	NT	NT	NT	NT
Lyse AIR 1	14/04/2018	17/04/2018	20/04/2018	P17 1
Lyse AIR 2	13/04/2018	16/04/2018	19/04/2018	P17 1
Udd BIR 1	NT	NT	NT	NT
Udd BIR 2	12/04/2018	15/04/2018	18/04/2018	P17 2
Udd AIR 1	13/04/2018	16/04/2018	19/04/2018	P17 1
Udd AIR 2	14/04/2018	17/04/2018	20/04/2018	P17 1

B

Appendix B

B.1 Additional Calculations

Table B.1: UV irradiation time and calculated UV dose for each WTP

DWTP	**Irradiation Time [min]**	**UV Dose [mJ/cm^2]**
Lackerbäck	10, 6*	1548
Mölndal	10	960
Varberg	12	1143
Marstrand	5	500
Dösebacka	17	1655
Lysegården	15	1455
Uddevalla	10	960

C

Appendix C

C.1 AOC Raw Data

The following tables present the AOC raw data for each WTP. Each sample tested was labeled with two numbers. The first number in each sample is the replicate number, either 1 or 2. The second number is the sample number within that replicate between 1 and 3. Replicate sample sets were chosen at random. This data was used in the calculation of AOC concentrations following the method laid out in Section 3.4 using Equation 3.2. Final results were presented in Section 4.4.

Table C.1: Lackerbäck raw AOC data before and after irradiation.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Lack BIR 1-1	3	5	1	500	**Lack BIR 1-1**	3	43	0.1	43,000
Lack BIR 1-2	3	6	1	600	**Lack BIR 1-2**	3	35	0.1	35,000
Lack BIR 1-3	3	8	1	800	**Lack BIR 1-3**	3	46	0.1	46,000
			Average	621				**Average**	41,061
			SD	152				**SD**	5,686
Lack BIR 1-1	5	10	1	1,000	**Lack BIR 1-1**	5	32	0.1	32,000
Lack BIR 1-2	5	6	1	600	**Lack BIR 1-2**	5	25	0.1	25000
Lack BIR 1-3	5	7	1	700	**Lack BIR 1-3**	5	37	0.1	37,000
			Average	748				**Average**	30,933
			SD	208				**SD**	6,027
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox Count**	**Conc.**	**CFU/mL**
Lack AIR 2-1	3	3	1	300	**Lack AIR 2-1**	3	127	0.01	1,270,000
Lack AIR 2-2	3	4	1	400	**Lack AIR 2-2**	3	104	0.01	1,040,000
Lack AIR 2-3	3	2	0.1	2,000	**Lack AIR 2-3**	3	116	0.01	1,160,000
			Average	621				**Average**	1,152,829
			SD	953				**SD**	115,036
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox**	**Conc.**	**CFU/mL**
Lack AIR 1-1	3	49	0.1	49,000	**Lack AIR 1-1**	3	96	0.01	960,000
Lack AIR 1-2	3	31	0.1	31,000	**Lack AIR 1-2**	3	133	0.01	1,330,000
Lack AIR 1-3	3	33	0.1	33,000	**Lack AIR 1-3**	3	117	0.01	1,170,000
			Average	36,871				**Average**	1,143,149
			SD	9,865				**SD**	185,562

Table C.2: Mölndal raw AOC data before and after irradiation.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Mol BIR 2-1	3	17	1	700	**Mol BIR 2-1**	3	29	0.1	29,000
Mol BIR 2-2	3	12	1	1200	**Mol BIR 2-2**	3	32	0.1	32,000
Mol BIR 2-3	3	5	1	500	**Mol BIR 2-3**	3	26	0.1	26,000
			Average	749				**Average**	28,896
			SD	361				**SD**	3,000
Mol BIR 2-1	5	3	1	300	**Mol BIR 2-1**	5	22	0.1	22,000
Mol BIR 2-2	5	5	1	500	**Mol BIR 2-2**	5	27	0.1	27,000
Mol BIR 2-3	5	6	1	600	**Mol BIR 2-3**	5	29	0.1	29,000
			Average	448				**Average**	25,826
			SD	153				**SD**	3,606
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox Count**	**Conc.**	**CFU/mL**
Mol AIR 2-1	3	24	1	2,400	**Mol AIR 2-1**	3	47	0.01	470,000
Mol AIR 2-2	3	38	1	3,800	**Mol AIR 2-2**	3	38	0.01	380,000
Mol AIR 2-3	3	12	0.1	12,000	**Mol AIR 2-3**	3	29	0.01	290,000
			Average	4,783				**Average**	372,758
			SD	5,186				**SD**	90,000
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox**	**Conc.**	**CFU/mL**
Mol AIR 1-1	3	90	0.1	90,000	**Mol AIR 1-1**	3	21	0.01	210,000
Mol AIR 1-2	3	80	0.1	80,000	**Mol AIR 1-2**	3	14	0.001	1,400,000
Mol AIR 1-3	3	95	0.1	95,000	**Mol AIR 1-3**	3	114	0.01	1,140,000
			Average	88,109				**Average**	694,626
			SD	7,638				**SD**	625,646

Table C.3: Varberg raw AOC data before irradiation.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Var BIR 1-1	3	11	1	1,100	**Var BIR 1-1**	3	27	0.1	27,000
Var BIR 1-2	3	5	1	500	**Var BIR 1-2**	3	24	0.1	24,000
Var BIR 1-3	3	17	1	1,700	**Var BIR 1-3**	3	41	0.1	41,000
			Average	978				**Average**	29,839
			SD	600				**SD**	9,074
Var BIR 1-1	5	27	1	2,700	**Var BIR 1-1**	5	17	0.1	17,000
Var BIR 1-2	5	17	1	1,700	**Var BIR 1-2**	5	87	0.1	87,000
Var BIR 1-3	5	24	1	2,400	**Var BIR 1-3**	5	35	0.1	35,000
			Average	2,225				**Average**	37,269
			SD	513				**SD**	36,350
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox Count**	**Conc.**	**CFU/mL**
Var BIR 2-1	3	9	1	900	**Var BIR 2-1**	3	23	0.01	23,000
Var BIR 2-2	3	3	1	300	**Var BIR 2-2**	3	90	1	9,000
Var BIR 2-3	3	6	1	600	**Var BIR 2-3**	3	43	1	4,300
			Average	545				**Average**	9,619
			SD	300				**SD**	9,728
Var BIR 2-1	5	18	1	1,800	**Var BIR 2-1**	5	63	1	6,300
Var BIR 2-2	5	14	1	1,400	**Var BIR 2-2**	5	84	1	8,400
Var BIR 2-3	5	9	1	900	**Var BIR 2-3**	5	47	1	4,700
			Average	1,314				**Average**	6,289
			SD	451				**SD**	1,856

Table C.4: Varberg raw AOC data after irradiation.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Var AIR 2-1	3	9	0.1	9,000	**Var AIR 2-1**	3	46	0.01	460,000
Var AIR 2-2	3	15	0.01	150,000	**Var AIR 2-2**	3	74	0.01	740,000
Var AIR 2-3	3	13	0.1	13,000	**Var AIR 2-3**	3	81	0.1	81,000
			Average	25,987				**Average**	302,105
			SD	80,277				**SD**	330,737
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox**	**Conc.**	**CFU/mL**
Var AIR 1-1	3	19	1	1,900	**Var AIR 1-1**	3	46	0.01	46,000
Var AIR 1-2	3	27	1	2,700	**Var AIR 1-2**	3	62	0.01	62,000
Var AIR 1-3	3	28	1	2,800	**Var AIR 1-3**	3	44	0.01	44,000
			Average	2,431				**Average**	50,065
			SD	493				**SD**	9,866

Table C.5: Marstrand raw AOC data before irradiation, replicate 1.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Mar BIR 1-1	3	3	1	300	**Mar BIR 1-1**	3	50	0.1	50,000
Mar BIR 1-2	3	6	1	600	**Mar BIR 1-2**	3	47	0.1	47,000
Mar BIR 1-3	3	7	1	700	**Mar BIR 1-3**	3	40	0.1	40,000
			Average	501				**Average**	45,468
			SD	208				**SD**	5,132
Mar BIR 1-1	4	7	1	700	**Mar BIR 1-1**	4	30	0.1	30,000
Mar BIR 1-2	4	10	1	1,000	**Mar BIR 1-2**	4	27	0.1	27,000
Mar BIR 1-3	4	12	1	1,200	**Mar BIR 1-3**	4	52	0.1	52,000
			Average	944				**Average**	34,793
			SD	252				**SD**	13,650
Mar BIR 1-1	5	3	1	300	**Mar BIR 1-1**	5	48	0.1	48,000
Mar BIR 1-2	5	6	1	600	**Mar BIR 1-2**	5	85	0.1	85,000
Mar BIR 1-3	5	2	1	200	**Mar BIR 1-3**	5	40	0.1	40,000
			Average	330				**Average**	54,648
			SD	208				**SD**	24,007

Table C.6: Marstrand raw AOC data before irradiation, replicate 2.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Mar BIR 2-1	3	3	1	300	**Mar BIR 2-1**	3	36	0.1	36,000
Mar BIR 2-2	3	7	1	700	**Mar BIR 2-2**	3	55	0.1	55,000
Mar BIR 2-3	3	10	1	1,000	**Mar BIR 2-3**	3	49	0.1	49,000
			Average	594				**Average**	45,950
			SD	351				**SD**	9,713
Mar BIR 2-1	4	6	1	600	**Mar BIR 2-1**	4	32	0.1	32,000
Mar BIR 2-2	4	12	1	1,200	**Mar BIR 2-2**	4	38	0.1	38,000
Mar BIR 2-3	4	500	1	500	**Mar BIR 2-3**	4	56	0.1	56,000
			Average	711				**Average**	40,836
			SD	379				**SD**	12,490
Mar BIR 2-1	5	4	1	400	**Mar BIR 2-1**	5	30	0.1	30,000
Mar BIR 2-2	5	4	1	400	**Mar BIR 2-2**	5	38	0.1	38,000
Mar BIR 2-3	5	8	1	800	**Mar BIR 2-3**	5	35	0.1	35,000
			Average	504				**Average**	34,171
			SD	231				**SD**	4041

Table C.7: Marstrand raw AOC data after irradiation.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Mar AIR 1-1	3	9	0.1	9,000	**Mar AIR 1-1**	3	80	0.01	800,000
Mar AIR 1-2	3	14	0.1	14,000	**Mar AIR 1-2**	3	160	0.1	160,000
Mar AIR 1-3	3	9	0.1	9,000	**Mar AIR 1-3**	3	78	0.01	780,000
			Average	10,428				**Average**	463,911
			SD	2,887				**SD**	363,868
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox**	**Conc.**	**CFU/mL**
Mar AIR 2-1	3	13	1	1,300	**Mar AIR 2-1**	3	15	0.01	150,000
Mar AIR 2-2	3	11	1	1,100	**Mar AIR 2-2**	3	159	0.1	159,000
Mar AIR 2-3	3	8	1	800	**Mar AIR 2-3**	3	181	0.1	181,000
			Average	1,046				**Average**	162,825
			SD	252				**SD**	15,948

Table C.8: Lysegården raw AOC data before and after irradiation.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Lyse BIR 1-1	3	15	1	1,500	**Lyse BIR 1-1**	3	64	0.01	640,000
Lyse BIR 1-2	3	19	1	1,900	**Lyse BIR 1-2**	3	52	0.01	520,000
Lyse BIR 1-3	3	13	1	1,300	**Lyse BIR 1-3**	3	79	0.01	790,000
			Average	1,547				**Average**	640,624
			SD	306				**SD**	135,277
Lyse BIR 1-1	5	8	1	800	**Lyse BIR 1-1**	5	36	0.01	360,000
Lyse BIR 1-2	5	12	1	1,200	**Lyse BIR 1-2**	5	35	0.01	350,000
Lyse BIR 1-3	5	13	1	1,300	**Lyse BIR 1-3**	5	37	0.01	370,000
			Average	1,077				**Average**	359,907
			SD	265				**SD**	10,000
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox Count**	**Conc.**	**CFU/mL**
Lyse AIR 2-1	3	44	0.01	440,000	**Lyse AIR 2-1**	3	44	0.01	440,000
Lyse AIR 2-2	3	39	0.001	3,900,000	**Lyse AIR 2-2**	3	58	0.01	580,000
Lyse AIR 2-3	3	44	0.01	440,000	**Lyse AIR 2-3**	3	64	0.01	640,000
			Average	910,591				**Average**	546,622
			SD	1,997,632				**SD**	102,632
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox**	**Conc.**	**CFU/mL**
Lyse AIR 1-1	3	21	1	2,100	**Lyse AIR 1-1**	3	64	0.01	640,000
Lyse AIR 1-2	3	11	1	1,100	**Lyse AIR 1-2**	3	35	0.01	350,000
Lyse AIR 1-3	3	13	1	1,300	**Lyse AIR 1-3**	3	71	0.01	710,000
			Average	1,443				**Average**	541,796
			SD	529				**SD**	190,875

Table C.9: Uddevalla raw AOC data before and after irradiation.

Sample	Day	P17 Count	Conc.	CFU/mL	Sample	Day	Nox Count	Conc.	CFU/mL
Udd BIR 2-1	3	8	1	800	**Udd BIR 2-1**	3	43	0.01	430,000
Udd BIR 2-2	3	12	1	1,200	**Udd BIR 2-2**	3	36	0.01	360,000
Udd BIR 2-3	3	10	1	1,000	**Udd BIR 2-3**	3	36	0.01	360,000
			Average	986				**Average**	381,966
			SD	200				**SD**	40,415
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox Count**	**Conc.**	**CFU/mL**
Udd AIR 1-1	3	9	0.1	9,000	**Udd AIR 1-1**	3	102	0.01	1,020,000
Udd AIR 1-2	3	13	0.1	13,000	**Udd AIR 1-2**	3	93	0.01	930,000
Udd AIR 1-3	3	4	1	400	**Udd AIR 1-3**	3	100	0.01	1,000,000
			Average	3,604				**Average**	982,564
			SD	6,438				**SD**	47,258
Sample	**Day**	**P17 Count**	**Conc.**	**CFU/mL**	**Sample**	**Day**	**Nox**	**Conc.**	**CFU/mL**
Udd AIR 2-1	3	28	0.1	28,000	**Udd AIR 2-1**	3	95	0.1	95,000
Udd AIR 2-2	3	31	0.1	31,000	**Udd AIR 2-2**	3	87	0.1	87,000
Udd AIR 2-3	3	27	0.1	27,000	**Udd AIR 2-3**	3	88	0.1	88,000
			Average	28,617				**Average**	89,931
			SD	2,082				**SD**	4,359